Distribution and identification of ectomycorrhizal and insect pathogenic fungi in Icelandic soil and their mediation of root-herbivore interactions in afforestation

Edda Sigurdís Oddsdóttir

University of Iceland
Faculty of Life and Environmental Sciences
Askja, Sturlugötu 7
101 Reykjavík, Iceland

Rannsóknastöð skógræktar
Icelandic Forest Research
Mógilsá
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Research Supervisor

Dr. Guðmundur Halldórsson

Supervising Teacher

Prof. Kesara Anamthawat-Jónsson

PhD Advisory Committee

Prof. Kesara Anamthawat-Jónsson
Prof. Jørgen Eilenberg
Dr. Guðmundur Halldórsson
Dr. Robin Sen

Opponents

Prof. Alan Gange
Prof. Gísli Már Gíslason

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Abstract

The objectives of the studies in this thesis were to study the distribution and to identify ectomycorrhizal (ECM) and insect pathogenic fungi (IPF) in native Icelandic birch woodlands and eroded (non-vegetated) soils. Furthermore, to study the mediation of these two beneficial soil fungal groups of plant-herbivore interaction in a model system, which consisted of tree seedlings, *Otiorhynchus* larvae, IPF and ECM fungi.

The study was divided into three steps: 1) mapping the distribution and identifying these target soil beneficial fungi in Icelandic soils originating from birch woodlands, heath land and eroded areas, 2) studying the effects of ECM and IPF fungi on *Otiorhynchus sulcatus* larvae under controlled situations, and 3) studying the interaction among soil beneficial fungi, *Otiorhynchus* larvae and tree seedlings in representative afforestation sites.

Both ECM and IPF were identified in vegetated ecosystems, whereas no IPF were recorded in eroded ecosystems that also supported significantly lower occurrence of ECM. The low fungal occurrence was reflected in poorer birch seedling performance in eroded soil.

Three IPF species were identified: *Isaria farinosa*, *Beauveria bassiana* and *Metarhizium anisopliae*, the latter two species were not previously found in Icelandic soil. Preliminary phylogenetic analysis targeting internal transcribed spacer (ITS) sequences of the major ribosomal gene of mycorrhizal fungi from mycorrhizal birch root tips revealed three main phylogenetic groups: two Basidiomycetes (*Hebeloma* and *Cortinarius*) and one Ascomycetes group, of these *Hebeloma* and Ascomycetes were more common.

Inoculation with forest soil, IPF and certain ECM species had negative effects on survival of *O. sulcatus* under controlled conditions, thus highlighted the importance of careful selection of fungal species used as inoculants in the biological control of *Otiorhynchus*. The importance of inoculate selection and the need to understand the interaction between the inoculants and native soil biota were further demonstrated under field conditions, where habitat specific, differential root damage response to inoculation was detected.

Ágrip

Í þessu verkefni eru áhrif tveggja hópa hagnýtra jarðvegssveppa á rótarskemmdir af völdum ranabjöllulirfa rannsökuð. Verkefnið var framkvæmt í þremur skrefum: 1) kortlagning og tegundagreining hagnýtra jarðvegssveppa í mismunandi vistkerfum. Sveppahóparnir sem voru rannsakaðir voru skordýrasníkjusveppir og svepprótarsveppir á trjám, 2) rannsókn á áhrifum svepprótar og skordýrasníkjusveppa á lifun húskepps (*Otiorhynchus sulcatus*) í gróðurhúsum, og 3) rannsókn á samspili svepprótar, skordýrasníkjusveppa, ranabjöllulirfa (*O. arcticus*, og *O. nodosus*) og trjáplantna í mismunandi gróðurvistkerfum.

Bæði sníkjusveppir og svepprótarsveppir fundust í grónum vistkerfum (lyngmói og birkiskógur) en engir skordýrasníkjusveppir fundust í uppblásnum vistkerfunum og tíðni svepprótarsveppa var marktækt minni í uppblásnu svæðnum en þeim grónu.

Þrjár tegundir sníkjusveppa á skordýrum fundust, *Isaria farinosa*, *Beauveria bassiana* og *Metarhizium anisopliae*, en tvær síðustu tegundirnar höfðu ekki fundist fyrr hér á landi. Fyrstu niðurstöður DNA greiningar á ITS svæði svepprótarsveppa sýndu þrjá mismunandi hópa, tvo hópa basíðusveppa, ættkvíslirnar *Hebeloma* og *Cortinarius* og einn hóp asksveppa.

Smitun með skógarjarðvegi, skordýrasníkjusveppum og sumum svepprótarsveppum hafði marktæk neikvæð áhrif á lifun húskeppslirfa í gróðurhúsi. Enn fremur hafði smitun með svepprótarsveppum og skordýrasníkjusveppum marktæk áhrif á rótarskemmdir gróðursettra plantna í mörkinni en þau áhrif voru mismunandi eftir þeim vistkerfum sem gróðursett var í.

Þessar niðurstöður sýna að smitun með jarðvegsörverum getur dregið úr skemmdum af völdum ranabjöllulirfa á nýgróðursettum plöntum. Jafnframt undirstrika þær mikilvægi þess að velja vel þær sveppategundir sem smitað er með og hve nauðsynlegt er að rannsaka samspil þeirra tegunda sem smitað er með við jarðvegsvistkerfi viðkomandi gróðursetningastaða.

To my father

Oddur Eggertsson

(1949-1994)

whose immeasurable interest and love for nature

has inspired and encouraged me during this work

List of original papers

This thesis is based on the following papers:

I. Halldorsson, G., Sverrisson, H, Eyjolfsdottir, G.G. and Oddsdottir E.S. (2000). Ectomycorrhizae reduce damage to Russian larch by *Otiorhyncus* larvae. Scandinavian Journal of Forest Research. 15(3) 354-358.

II. Oddsdottir, E.S., Nielsen, C., Sen, R., Harding, S., Eilenberg, J. and Halldorsson, G. Distribution patterns of entomopathogenic fungi in soil and birch symbiotic ectomycorrhizal fungi across native woodland and degraded habitats in Iceland. (submitted to Icelandic Agricultural Sciences)

III. Oddsdottir, E.S., Eilenberg, J., Sen, R. and Halldorsson, G. (2010). The effects of insect pathogenic soil fungi and ectomycorrhizal inoculation of birch seedlings on the survival of *Otiorhynchus* larvae. Agricultural and Forest Entomology (in press)

IV. Oddsdottir, E.S., Eilenberg, J., Sen, R., Harding, S. and Halldorsson, G. (2010) Early reduction of *Otiorhynchus* spp. larval root herbivory on *Betula pubescens* by beneficial soil fungi. Applied Soil Ecology. doi:10.1016/j.apsoil. 2010.03.009

In addition, some unpublished data are presented.

Author's contribution

Paper I. The study was planned by Gudmundur Halldorsson, Halldor Sverrisson and Gudridur G. Eyjolfsdottir, Edda S. Oddsdottir participated in field work, analyzed the data and participated in writing the paper.

Paper II. Edda S. Oddsdottir designed the ectomycorrhizal part together with Robin Sen and Gudmundur Halldorsson. The insect pathogenic component was planned and carried out by Charlotte Nielsen, Susanne Harding and Jørgen Eilenberg. Edda S. Oddsdottir interpreted the results, wrote the paper together with co-authors and is the corresponding author.

Paper III. Edda S. Oddsdottir, in collaboration with Gudmundur Halldorsson, designed the experiment and was responsible for the experimental work. She interpreted the results, wrote the paper together with co-authors and is the corresponding author.

Paper IV. Edda S. Oddsdottir and Gudmundur Halldorsson designed the experiment and Edda was responsible for the study. She interpreted the results and wrote the paper together with co-authors and is the corresponding author.

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Abbreviations and definitions

AM Arbuscular mycorrhiza

ANOVA
Analysis of variance

C Carbon

DNA Deoxyribonucleic acid

ECM Ectomycorrhiza

ERM Ericoid mycorrhiza

IP Insect pathogenic

IPF Insect pathogenic fungi

K Potassium

LSD Fisher's Least Significant Difference test

Morphotype
In this study: Ectomycorrhizas which are grouped together on the basis of similar external morphological characteristics (color, shape, hyphal structure)

Mycorrhizosphere
The soil zone immediately influenced by the presence of the mycorrhizal hyphae and/or ectomycorrhizal mantle

N Nitrogen

P Phosphorus

PCR Polymerase chain reaction method, developed by Mullis and Faloona (1987) enabling the exponential amplification of target DNA sequences

RFLP Restriction fragment length polymorphisms

Rhizhosphere
The soil zone adjacent and influenced by plant roots

SE Standard error

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1 INTRODUCTION

In this thesis, the mediation by two fungal groups, ectomycorrhiza (ECM) and insect pathogenic fungi (IPF) of plant-herbivore interaction were studied, using the cultivation and regeneration of birch in Iceland as a model system. The system consisted of tree seedlings, *Otiorhynchus* spp. larvae, IPF and ECM fungi.

Over the last few decades, afforestation activity in Iceland has greatly increased, with large areas of poorly or non-vegetated land being planted with containerized conifer and birch seedling stock. Furthermore, as a result of climatic warming, expansion of the native birch (*Betula pubescens* Ehrh. and *Betula nana* L.) forests into non-vegetated sites is likely (Juday *et al.,* 2005). However, because of a range of negative impactors, such as poor nutrient status of the soil, frost heaving and root herbivory, seedling mortality is unacceptable, especially on exposed and/or degraded sites.

In extensive exposed or degraded sites, beneficial soil biota are important for the development of vegetation and it is possible that the lack of specific members in this class of biota hinders the establishment of tree seedlings in the degraded soil typical to Iceland. However, the identity and distribution of soil biota in Iceland, has been poorly studied and it is not clear if these groups exist in Icelandic soils or whether such biota affect planted tree seedling mortality.

In this study, the distribution of ECM and IPF was studied and the fungi identified. Furthermore, the interaction between the ECM, IPF and root herbivorous larvae (*Otiorhynchus* spp.) was investigated under both controlled and field conditions.

1.1 Forestry in Iceland

The vegetation cover of Iceland has changed dramatically since the settlement of the island in approximately 870 AD. Based on pollen analyses, historical records, soil remnants and relic vegetation (Thorarinsson, 1961; Hallsdottir, 1995; Kristinsson, 1995), it is estimated that large parts of Iceland were covered with vegetation at the time of human settlement. Forests or woodlands, comprising birch (*B. pubescens* and *B. nana*), *Salix* species

(especially *Salix phylicifolia* L.) and occasionally mountain ash trees (*Sorbus acuparia* L.) are believed to have covered at least 25% of the land area at this time (Blondal, 1987; Kristinsson, 1995). After settlement, the birch woodlands were a valuable resource for the Icelandic farming community, particularly as a source of charcoal and other fuel, building material, fodder and for livestock grazing (Thorarinsson, 1974; Kristinsson, 1995). The exploitation of the woodlands, combined with a cooling climate during medieval times, volcanic activity, the vulnerability of the forest ecosystem and underlying highly erodible Andosol soils led to forest destruction, resulting in massive soil erosion (Arnalds, 1987) and prevented efficient forest regeneration. Today the total forest cover of Iceland is approximately 1.5%, with birch forests covering 1.1% (Traustason & Snorrason, 2008).

Afforestation in Iceland began in the early 20th century, with planting at Thingvellir in southern Iceland. Since then, the number of planted seedlings has gradually increased, especially during the 1990´s when intensive afforestation programs were initiated, such as the Land Reclamation Forests and the Regional Afforestation Programs (Petursson, 1999; Eggertsson *et al.,* 2008). Although the primary goal of the programs was to positively influence rural development, other expected benefits were environmental (e.g. soil protection and carbon sequestration), social (for example forests for amenity and recreation), and economical (for instance timber production) (Eggertsson *et al.,* 2008). The Land Reclamation Forests program was initiated on the 60th anniversary of the Icelandic Forestry Association in 1990. Since then over 15 million trees have been planted within the program (Anon, 2009). At present, five Regional Afforestation Programs are operating, one in each part of the country. The primary goal of the Regional Afforestation Programs has been to have a positive influence on rural development, but other aims are less clear (Eggertsson *et al.,* 2008). In the most recent legislation passed by the Icelandic Government regarding afforestation, it was declared that at least 5% of lowland areas should be transformed into woodlands over the next four decades (Anon, 2006; Eggertsson *et al.,* 2008).

The use of introduced species, such as Siberian larch (*Larix sibirica* Ledeb. synom. *Larix sukaczewii* Dylis), lodgepole pine (*Pinus contorta* Dougl. ex Loud.), black cottonwood (*Populus balsamifera* var. *trichocarpa* Torr. & Gray.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) has increased in Icelandic forestry. However, native birch (*B. pubescens*)

comprises up to 30% of planted seedlings in some years and the species is widely used in afforestation projects (Eggertsson *et al.,* 2008).

1.1.1 *Betula* spp.

Birch species are an important ecological component in northern temperate and boreal forests. They are light-demanding pioneer species capable of rapid establishment and occupation of open areas (Hynynen *et al.,* 2009). Three birch species are recognized in Europe, silver birch (*Betula pendula* Roth.), downy or mountain birch (*B. pubescens*) and dwarf birch (*B. nana*) (Atkinson, 1992). Silver and downy birch has a wide-spread natural distribution ranging from the Atlantic to eastern Siberia. Both species grow throughout most of Europe (Hynynen *et al.,* 2009). Silver birch is more common in the southern part of the continent, while downy birch is widespread in northern and western Europe.

Dwarf birch is an arctic-alpine species, found in northern Europe often at high altitudes. Downy and dwarf birch co-exist in Iceland and hybridization between the species is common (Anamthawat-Jonsson & Thorsson, 2003; Thorsson *et al.,* 2007). Pollen analysis showed birch species colonized Iceland soon after the glaciers started to retreat and the distribution of birch was greatest 9000-7000 years ago (Hallsdottir, 1995; Eggertsson *et al.,* 2008). Recent studies on genetic variation in Icelandic birch showed a clear geographical division in haplotype distribution and indicated *Betula* arrived first in the Late Boreal and again in the latter half of the Holocene (Thorsson, 2008a).

The characteristics of Icelandic woodlands vary, reflecting site conditions, forest management and genetics (Aradottir *et al.,* 2001; Thorsson *et al.,* 2007). Birch trees are highly variable most often with multiple, crooked and leaning stems of variable sizes, although there are some provinces with single, relatively straight stems (Jonsson, 2004). Icelandic birch populations were classified by stature into three classes, shrubs (0-2 m), shrub-woodlands (2-4 m) and forests (4-12 m). However, it must be noted that in Iceland, tree height can be much lower than actual stem length (Jonsson, 2004). The ground vegetation of birch habitats is generally thick and commonly comprised of the shrubs *Vaccinum uliginosum* L., *Salix lanata* L. and *Empetrum nigrum* L., the forb *Rubus saxatilis* L. and the grasses *Deschampsia flexuosa* (L.) Trin. and *Agrostis capillaris* L. among others (Elmarsdottir & Magnusson, 2007).

1.1.2 Exotic tree species

Exotic tree species play an important role in the afforestation of Iceland and there has been quite an effort to establish forests of introduced conifers (Blondal, 1987). Early afforestation in Iceland began with the planting of exotic species at Thingvellir in 1899 (Bragason, 1995; Blondal & Gunnarsson, 1999). Since then, various species and many provenance trails of various species were conducted across the country in afforestation programs (Blondal & Gunnarsson, 1999). Today, the most important exotic species are Sitka spruce, lodgepole pine, black cottonwood and *Larix* spp. that combined comprise around 60% of planted seedlings (Gunnarsson, 2008).

Larch was first introduced in northern Iceland in 1900. The plantings of the early 20th century were unsuccessful, probably due to a poor understanding of the importance of appropriate species and perhaps more importantly appropriate provenance selection (Snorrason, 1987). Over 50 provenances of Siberian larch have been tested, most of them originating from Russia, Norway and Finland (Eysteinsson & Skulason, 1995; Eysteinsson, 2008). Larch species have exhibited acceptable growth rates on infertile and dry sites (Snorrason, 1987; Eysteinsson & Skulason, 1995). Because of this and other desirable characteristics *L. sibirica* is one of the most frequently planted species in some parts of the country and constituted approximately 30% of planted seedlings in 2007 (Gunnarsson, 2008).

Sitka spruce and lodgepole pine are among the most commonly planted conifer species in Iceland. Sitka spruce was first introduced to Iceland in the 1920's and again in the 1950's. Sitka spruce was planted extensively in Iceland and has shown relatively good growth around the country. The planting of lodgepole pine began in the 1950's and it was one of the most frequently planted species in forestry between 1982 to1985 and again from 1992 to 1995. The species is well suited for planting on barren sites (Sigurgeirsson, 1988; Blondal & Gunnarsson, 1999). To a large extent, both the Sitka spruce and lodgepole pine provenances used in Iceland originate from Alaska (Sigurgeirsson, 1988; Blondal & Gunnarsson, 1999; Blondal, 2004).

The first black cottonwood, *Populus trichocarpa,* in Iceland came from were cuttings that were planted in southern Iceland in 1944. Owing to its rapid growth and ease in propagation by cuttings, the species became popular for both forestry and gardening.

During the early years, clones from one site on the Kenai-peninsula of Alaska were cultivated (Blondal & Gunnarsson, 1999; Palsson, 2000).

Forestry in Iceland suffered a massive setback in 1963, when an extreme spring frost caused extensive damage and mortality of Sitka spruce, white spruce (*Picea glauca* (Moench) Voss) and black cottonwood in southern and western Iceland. After that, more emphasis was placed on collecting provenances with climate more similar to that of Iceland. The use of black cottonwood decreased following the spring frost. After 1990, the planting of this species has increased gradually (Petursson, 1999; Palsson, 2000) and the black cottonwood is now extensively used in afforestation and forestry.

In addition to the species mentioned above, over 20 other species were planted. However, the number of planted seedlings of these species is relatively low, and together they comprise approximately 10% of planted seedlings per year (Gunnarsson, 2005).

1.1.3 Survival of newly planted seedlings

Field observations, reports and studies from afforestation projects often revealed growth retardation and high mortality of seedlings during the first years after planting. Assessment of seedling survival in the Land Reclamation Forest project showed the average survival of 4-6 year old seedlings was 70% for birch, 63% for pine and 44% for larch. Survival was variable between planting sites. For example, the survival of birch seedlings ranged from 32% to 94% between sites, reflecting a range of factors such as climate conditions, vegetation cover and fertilization treatments (Aradottir & Arnalds, 2001). Generally, the mortality rate was highest in plantations on gravel sites with sparse vegetation cover. Planting on sites with dense grass or moss vegetation also had negative effects on seedling survival (Aradottir & Magnusson, 1992b; Aradottir & Gretarsdottir, 1995)

Some of the Regional Afforestation Programs evaluated seedling survival over the first years after planting. The survival of Siberian larch for Fljotsdalsherad, in eastern Iceland, evaluated both 1 and 2 years after planting, showed mortality rates varied between planting years and sites and survival rates ranged from 65-85% (Sigurjonsdottir, 1993). A later study from the same area, reported a 73.1% average survival rate for planted seedlings (Reynisson, 2007). In southern Iceland, the number of living seedlings was compared to the number of planted seedlings, 3 years after planting. The results showed an average seedling survival rate of 60%. Again there was considerable variability in survival between

sites and vegetation types. Planting in drained wetlands resulted with average 45% seedling survival, while survival was around 88% in sparsely vegetated areas (Eggertsson, 2005). In northern Iceland, a study from 2007 revealed the average survival of seedlings, planted in the period from 2000 to 2006, was 65-75%. As in other parts of the country, vegetation type was an important factor, with the highest survival rate in heathland, but lower in drained wetlands and areas covered with moss. However, no inter-species differences were detected. (Thorsson, 2008b).

Data on seedling survival in afforestation areas established after 1990 has been collected as part of the Icelandic Forest Inventory since 2005. The measurements were made on permanent plots, both in plantations and native birch woodlands (Snorrason, 2005). In 2007, data on seedling survival from the Icelandic Forest Inventory were analyzed and showed the average survival rate was 47%. A distinct difference between tree species and geographic location around the country was noted (Arnor Snorrason, personal communication, December 2009).

During the 1980´s, forest nurseries converted from producing bare-rooted to containerized seedlings. Soon after, the mortality rate of newly planted seedlings seemed to increase and several studies were conducted to determine measures that could decrease seedling mortality. A study on the effects of container-size on seedling survival showed the positive effects of using larger containers, especially when seedlings were planted in grasslands with high competition with other vegetation. Furthermore, seedling mortality from root herbivory of *Otiorhynchus* spp. larvae decreased when larger containers were used (Halldorsson *et al.,* 1999). Application of controlled-release-fertilizer or smaller amounts of readily soluble NP fertilizer had positive effects on seedling survival and growth, regardless of tree species. Large amounts of N, alone or in combination with P or K resulted in increased mortality during the first growing season (Oskarsson & Sigurgeirsson, 2001; Oskarsson *et al.,* 2006). In another study, Aradottir (2000) examined the effects of the density of lupines (*Lupinus nootkatensis* Donn ex Sims.) on birch survival. The survival of birch seedlings planted in open lupine stands and stand margins was higher than in non-lupine sites, probably due to N-fixing ability of root nodules in the lupine. On the other hand, seedling survival decreased with increasing density of lupine which effectively shaded and suppressed the lower birch seedlings (Aradottir, 2000).

Frost heaving of seedlings was identified as an important seedling mortality factor, especially when seedlings were planted on unvegetated sites (Aradottir & Magnusson, 1992a; Aradottir & Gretarsdottir, 1995). A study of different methods designed to reduce frost heaving, showed the application of fertilizer and sowing of annual grass seeds reduced frost heaving (Oddsdottir *et al.,* 1998) and other studies indicated the use of lupine as a nurse crop or mixing organic fertilizer with the soil before planting have similar effects (Aradottir & Arnalds, 2001). A comparison of the survival of birch and Sitka spruce seedlings planted at eroded sites with or without previous sowing of grass species showed that the grass supported plant survival, partly due to reduced frost heaving as a result of soil surface stabilization. A species specific difference was noticed between seedlings, with higher survival rates for the birch seedlings (Snorrason, 2004).

The studies mentioned above revealed several reasons for high seedling mortality. The results indicated that with appropriate choice of container size and fertilizers, seedling survival can be improved. Unfortunately biotic factors that might affect seedling mortality, especially those in the soil, have received less attention and little is known about the interaction between the planted tree seedlings and local micro- and meso- biota.

1.2 Soil

Soil forms a thin mantle over the Earth's surface and is a unique, heterogeneous environment. It provides a wide variety of niches for living organisms due to differences in physical, chemical and biological parameters. Soil is made of mineral and organic matter, water, gaseous atmosphere, living plant roots and soil biota (Bardgett, 2005; Lavelle & Spain, 2005). Originally, the parent material of mineral soil particles defines the chemical structure and physical texture of the soil (Bardgett, 2005), but vegetation, microbes and fauna alter the soil through various biological activities. Organic compounds, generated mainly by vascular plants, enter the soil *via* root exudation or litter fall providing energy to the soil ecosystems, and can also alter the chemistry of the soil directly (Grayston *et al.,* 1997).

Water and temperature are important factors in soil formation ultimately affecting soil biological activity and have a great influence on soil environments (Lavelle & Spain, 2005).

1.2.1 Icelandic soil

Icelandic soils are young, most of them are younger than the last glaciation (i.e. younger than 10,000 years old). Relatively thick deposits of tephra built up above the basal till deposited by receding glaciers (Arnalds *et al.,* 1995). All Icelandic soils have volcanic parent materials. These are a mixture of tephra layers and eolian sediments, consisting mostly of volcanic glass. Icelandic soils are placed into four classes, andic soils under vegetation, Vitrisols, Histosols and other soil types (for example Cyrosol and Leptosol). The majority of Icelandic soils fall into the first two classes (andic soils and Vitrisols) both belonging to Andosol. Worldwide, Andosols cover around 1-2% of dry-land whereof Iceland represents 5-7% (Arnalds *et al.,* 1995; Arnalds & Kimble, 2001; Arnalds, 2004; Arnalds & Oskarsson, 2009). Usually, Andosols are dark and rich in organic matter, but variable in their morphology. Andosol can be very friable and roots often extend far into this type of soil. Furthermore, they have low bulk density, high porosity and high water retention capacity (Arnalds, 2008a).

The Icelandic Andosols are unique, due to their young age, basaltic origin and large inputs of eolian additions from sandy deserts. Furthermore, they occur at low temperatures with variable precipitation (Arnalds, 2004). Icelandic Andosol can be classified into four classes:

1. Brown Andosols (Icelandic: Brúnjörð) are the classical Andosols found in vegetated areas of Iceland, often 0.5-2 m thick. The texture is dominantly silt loam and loam, with 3-4% organic C and pH around 6 (Arnalds, 2004; Oskarsson *et al.,* 2004).
2. Gleyic Andosols (Icelandic: Votjörð) include a range of wetland soils, with organic C of around 7% and Ferrihydrite generally between 25 – 28% (Arnalds, 2004; Oskarsson *et al.,* 2004).
3. Histic Andosols (Icelandic: Svartjörð) occur in poorly drained areas with sufficient eolian additions to decrease the organic C content to below the 20% limit for Histosols. (Arnalds, 2004; Oskarsson *et al.,* 2004).
4. Vitrisols (Icelandic: Glerjörð) include a range of infertile soils with limited vegetation cover and organic C content below 7. Because of frost heaving of coarse fragments, the soil surface is often covered by stones lifted from lower levels by

frost actions. Icelandic Vitrisols are unusual, in that they occur in the Boreal and Sub-Arctic climate with a moisture level and temperatures that elsewhere sustains vegetative growth (Arnalds & Kimble, 2001; Arnalds, 2004; Oskarsson *et al.,* 2004; Arnalds & Oskarsson, 2009).

Iceland has experienced long-standing and extensive soil erosion (Bjarnason, 1942; Thorarinsson, 1961; Arnalds, 1987) partly caused by the vulnerability of the Andosols (Arnalds, 1999), but the cumulative effect of cooler climate, increased eolian deposition and human land use has added to this vulnerability. It is estimated that around 40% of total land area has undergone considerable desertification (Arnalds *et al.,* 2001b) and presently sandy surfaces cover about 21% of the total area of Iceland (Arnalds *et al.,* 2001a). The surface of deserted soil has very different soil properties from the former Andosol cover. Nutrients are limited, the soil has very limited water retention capacity and vegetation cover is scarce (Arnalds, 1987; Arnalds, 2000; Oskarsson *et al.,* 2004).

An important characteristic of Andosols is their tendency to accumulate organic matter by forming allophone-organic matter complexes and meta-humus complexes (Arnalds, 2008a, 2008b). The climate in Iceland favors organic buildup, especially in saturated wetlands. Furthermore, the steady flux of eolian materials and tephra additions buries the organic rich surface, leading to a gradual increase in total organic matter content of the soils. However, the eolian influence reduces the proportion of organic matter that would have accumulated, especially on and near the active volcanic soil (Oskarsson *et al.,* 2004). There is a large difference in the amount of organic C between intact soils and soils of eroded or denuded areas in Iceland. The concentration of organic C is highest in Histosols and lowest in Vitrisols (Oskarsson *et al.,* 2004).

Another characteristic of Andosols is the high ability to fix phosphorous (P) shown in the Icelandic Andosols (Arnalds *et al.,* 1995). A relatively high proportion of the soil P is bound in the organic matter of the soil. The soil P is variable between regions, being lowest in areas close to active sources of eolian material (Helgason, 2002). Changes in land use also affect the soil organic carbon (SOC) and soil-P status, which is highly responsive to fertilization and cropping. Afforestation increased SOC, regardless of species used and there was a net increase in SOC in the top soil layer (0-10 cm) as the forest stand age increased (Ritter, 2007; Bjarnadottir 2009). Furthermore, available P in the soil top layer increased and calcium (Ca), magnesium (Mg) and sodium (Na) decreased (Ritter, 2009)

but concentration of N and total-P was not affected by different stand age of Siberian larch and downy birch (Ritter, 2007).

1.3 Soil biota

Roots are a principal biological component of the soil system, comprising a large part of the living soil biomass and exercising major control over processes such as soil formation, organic matter turnover, nutrient and water dynamics (Lavelle & Spain, 2005). Roots also enter into intimate relationships with free-living microflora and fauna, and form associations with other soil microorganisms, such as bacteria (for example N fixing bacteria) or fungi forming symbiotic organs called mycorrhiza (Smith & Read, 2008). The organisms inhabiting the soil are subjected to the spatial constraints of their habitat and have responded through a variety of adaptive strategies, where body size and respiratory patterns are the major characteristics. Soil organisms are classified into four functional groups based on their body width (Coleman & Crossley, 1996; Lavelle & Spain, 2005):

1. Microflora (fungi and bacteria)
2. Microfauna (for example protozoa and some nematodes)
3. Mesofauna (for example collembolan and mites) and
4. Macrofauna (for example isopoda and earthworms)

There are a number of ecosystem services provided by soil biota, including decomposition of organic matter, nutrient cycling, bioturbation and suppression of soil-borne diseases (Brussaard *et al.,* 1997). In addition, the rhizosphere may have the potential to contribute in C sequestration (Bailey *et al.,* 2002; Philippot *et al.,* 2009).

Microflora consists of viruses, fungi, bacteria, algae and archaea. The smallest soil invertebrates (<0.2mm), such as protozoa, nematodes and some of the mites belong to microfauna (Lavelle *et al.,* 1997). Micro-organisms in soil can have diverse functions and huge population densities and biomasses. The exponential growth of bacteria where nutrients are available leads to establishment of massive bacterial colonies or biofilms and they can colonize key soil microenvironments, such as root surfaces, the rhizosphere and mycorrhizosphere (Timonen & Sen, 1998; Heinonsalo *et al.,* 2000), in great numbers. A community of microorganisms can develop on each fragment of primary resources

(Lavelle & Spain, 2005), but generally with a limited number of species. The soil hosts various groups of micro-organisms responsible for several key processes in soil, such as decomposition of organic matters, nitrification, denitrification and N fixation (Lavelle & Spain, 2005). These micro-organisms belong to the primary trophic level of the soil food web. Alongside them, soil fauna (micro- and macrofauna) exist. The microfauna feed on other micro-organisms and/or microbial metabolites. (Lavelle *et al.,* 1997; Bardgett 2005).

With new research techniques, such as molecular DNA-based analysis, our knowledge of soil microbial diversity and function has and continues to increase. For instance, the phylum *Archaea* first recorded in boreal forest soil in 1997 (Jurgens *et al.,* 1997). Before this time, *Archaea* were thought to survive only under very extreme conditions. Further studies showed significant impacts of other soil microbes, such as mycorrhizal fungi, on the frequency of *Archaea* (Bomberg *et al.,* 2003; Bomberg & Timonen, 2007, 2009). The function of this group in soil is not fully understood. However, the two major soil functions that have been indicated are nitrification and methanogenesis (Timonen & Bomberg, 2009).

Roots attract large number of soil invertebrates, including many annelids, insects, collembola, crustaceans, myriapods, earthworms and other arthropod groups. These organisms function as litter transformers ingesting a mixture of organic matter and microbial biomass (Lavelle *et al.,* 1997). The soil fauna is also important in dispersing other organisms, such as the conidia of insect pathogenic fungi (IPF), either by carriage on the cuticle or by ingestion (Dromph, 2001, 2003). They have strong influence on litter breakdown rates, especially for more resistant substrates, even though decomposition *per se* is to a large extent due to microbial activities (Coleman & Crossley, 1996; Lavelle & Spain, 2005). Groups of soil animals, mainly nematodes and insect larva are root herbivores and can cause severe damage on plant roots (Lavelle & Spain, 2005).

Fungi are eukaryotic organisms that are divided into five major groups: Deuteromycetes (Fungi imperfecti), Oomycetes, Zygomycetes, Ascomycetes and Basidiomycetes. A major component of microbial biomass in soil is made of fungi, and, in forests this is to large extent ectomycorrhizal fungi (Hogberg & Hogberg, 2002). Fungi are also the dominating micro-flora of the litter system. They contribute to decomposition of organic matter, especially in the initial stage when they predominate, as they are more capable of degrading the sugars and polysaccharides of the primary resources than bacteria (Lavelle &

Spain, 2005). In addition to saprotrophic fungi (wood and litter decomposing) that contribute to decomposition, other functional groups such as parasitic, pathogenic (e.g. plant and insect pathogens) and symbiotic (e.g. mycorrhizal) fungi are known to exist in soil (Hajek, 2004; Smith & Read, 2008).

1.3.1 Soil biota in Iceland

Of the soil biota, earthworms and Collembola have received the most attention in Iceland. In an extensive study, Bodvarsson (1957) recorded 76 species of Collembola in Iceland and in a checklist compiled by Olafsson (1991) 77 Collembola species were reported. One of the outputs from the projects ICEWOODS and AFFORNORD was a new list with 149 Collembola species found in different habitats in Iceland (Fjellberg, 2007). The highest number of Collembolan species and proportion of habitat specialists were found in soil from sea shores, followed by remnants of old birch woodlands. Young and degraded sites had fewer species and a higher proportion of generalists (Fjellberg, 2007).

In the 1970's, a series of studies on the effects of different land-use practices on soil biota was performed in northern Iceland. The highest abundance of soil fauna, irrespective of faunal group, was found in soils were application of nutrients was in the form of manure (Sigvaldason, 1973). Application of inorganic fertilizer consisting of only P and K increased the soil fauna density in comparison to applications of fertilizer including N, P and K. A later study on the effects of the long-term use of fertilizer on soil surface invertebrates in hayfields showed that fertilization decreased the number of species collected, but increased the number of individuals, especially Acari and Collembola (Gudleifsson, 2002).

The comparison of hayfields, pastures, heathlands, mires and bare grounds in northern Iceland showed a distinct difference in soil animal density between bare ground and vegetated areas, while there was not a significant difference between vegetation types (Hallgrimsson & Sigvaldason, 1974; Hallgrimsson, 1975). All groups of soil animals showed a spring maximum in density in June and a still higher maximum in late August to late September (Hallgrimsson, 1976), which is in keeping with the findings of Gudleifsson and Bjarnadottir (2008) from a pit fall trap study in grassland habitats, also done in northern Iceland. In their study, Gudleifsson and Bjarnadottir (2002; 2008) found that Collembola and Acari comprised almost 90% of the specimens collected in the pit fall

traps. Furthermore, their study showed the Collembola density was higher in pastures than hayfields during summer, indicating hayfield cultivation had negative effects on Collembola density (Gudleifsson & Bjarnadottir, 2008). In addition spring grass burns in hayfields was shown to have negative effects on invertebrate density in soil and on the soil surface (Davidsson, 1996; Halldorsson, 1996).

The effects of afforestation and revegetation on soil fauna in Iceland has been reported in only a few papers. A study on the effects of land reclamation on density and group composition of soil arthropods showed the total number of Collembola was very low on eroded sites, but increased with revegetation regardless of the revegetation method used (Sigurdardottir, 1991; Oddsdottir *et al.,* 2008b). However, the soil arthropod community composition showed a differential response with respect to reclamation strategy, Acari were dominant in areas reclaimed with birch, Collembola in lupine reclamation, neither group dominated in areas reclaimed with a mixture of grass species (Oddsdottir *et al.,* 2008b).

Oskarsson (1984) compared three different habitats, old forest, young forest and non-forested land in eastern Iceland. His results indicated that even though there was no difference in the total abundance of decomposing organisms between habitats, the abundance of Collembola and Araneida was lower in the old forests than the other two habitats. The number of Phygotphages was highest in young forests and the number of Coleoptera larvae and Enchytraeida was highest in old forests (Oskarsson, 1984). A later study on the effects of afforestation on Acari and Collembola in an age chronosequence study of larch and birch in parallel sites as these of Oskarsson (1984) in eastern Iceland showed comparable results. Acari abundance was highest in the youngest larch stands and lowest in the oldest larch stands (Halldorsson & Oddsdottir, 2007). A similar, though insignificant trend was noted in Collembola abundance. No difference was detected between the exotic larch and the native birch (Halldorsson & Oddsdottir, 2007). In a related study in western Iceland, Oddsdottir *et al.* (2008a) showed afforestation with Sitka spruce or native birch increased both Collembola density and number of species, whereas afforestation with lodgepole pine only increased numbers of Collembola species when compared to non-forested heathland. A Twin-Span analysis showed a difference in Collembola species composition between forest stands, where younger forests contained

fewer species and specimens than older ones. No distinction between tree species was found (Oddsdottir *et al.,* 2008a).

In Iceland, 11 species of earthworms have been identified which is considerably fewer species than in other Nordic countries (Thorvaldsson & Sigurdardottir, 1998). The density and biomass of earthworms in natural biotopes was low on nutrient-poor sites, increasing as sites became more fertile (Bengtsson *et al.,* 1975). Land-use affected earthworm density and biomass. In general man-made biotopes, such as gardens or planted forests, had a higher number of earthworms than natural biotopes. An exception was hayfields, which were characterized by a low number of species and a varying species complex (Bengtsson *et al.,* 1975; Gudleifsson & Olafsson, 1981). However, a difference was detected between different types of hayfields. Those on sandy soils and wetland soils contained the lowest number of earthworms and an application of inorganic fertilizer did not affect the density (Sigurdardottir & Thorvaldsson, 1994). A clear difference in earthworm number, biomass and species composition was detected between different age classes of lupine (*L. nootkatensis*) patches. The highest values were found in the oldest lupine patch (12-20 year old) but no earthworms were found in eroded areas outside the lupine cover (Sigurdardottir, 2004). Afforestation with different tree species showed a tendency of decreasing earthworm biomass with the increasing age of forest stands, and higher biomass in native birch than in lodgepole pine or Sitka spruce stands. In addition, an increasing C/N ratio in forest stands affected the species composition of earthworms in the topsoil (Gudleifsson, 2007).

Measurement of microbial decomposition activity in different soil types *via* the Cotton Strip Assay (Latter & Walton, 1988), revealed a distinct difference between soil in eroded and vegetated areas. The higher decomposition activity at the vegetated sites indicated higher density of decomposition microbes in the soil (Sigurdardottir, 1998; Oddsdottir, 2002). A similar study of the decomposition rate in different forests showed decomposition was lowest in lodgepole pine stands, followed by Sitka spruce and Siberian larch and highest in birch forests. Generally, decomposition rate decreased with increasing forest age (Arneberg *et al.,* 2007). Guicharnaud (2009) found biological activity in soil was not only affected by climate and land use, but also by soil properties showing microbial activity was strongly related to soil temperature, physiochemical properties and availability of dead organic matter in soil (Guicharnaud, 2009).

In addition, some mycorrhizal and entomopathogenic fungal studies have been carried out in Iceland and will be described in the following sections.

1.4 Mycorrhizal fungi

Mycorrhiza is a symbiotic relationship between soil fungi and the roots of plants, where the fungal partner obtains energy in the form of photosynthetically fixed C compounds from the host plant. The plant receives nutritional benefits, especially by improved N and P uptake (Harley & Smith, 1983; Smith & Read, 2008), protection against plant pathogens (Suh *et al.,* 1991; Sen, 2001) and root herbivores (Gange *et al.,* 1994; Gange *et al.,* 2005), salt (Bandou *et al.,* 2006) and increased drought tolerance (Morte *et al.,* 2001; Marjanovic *et al.,* 2005). Furthermore, soil colonizing mycorrhizal fungal hyphae has a major positive influence on soil aggregation through the secretion of glycoproteins such as Glomalin (Rillig & Mummey, 2006).

There are several different types of mycorrhizal associations (Harley & Smith, 1983; Peterson *et al.,* 2004; Smith & Read, 2008). The main division is based on the nature of cellular contact between root and fungal cells and has resulted in two main subdivisions of mycorrhiza, endomycorrhiza and ectomycorrhiza (ECM). In endomycorrhizas, the fungal structures form within the plant cells and both herbaceous and tree species are involved, whereas trees are the predominant plant species involved in ECM. In ECMs, the fungi forms a mantle of fungal tissue, enclosing the root-tip, wherefrom hyphae penetrate both outwards into the surrounding soil and inwards, where they form a labyrinthine growth of hyphae between the root cells known as the Hartig net. However, the fungal cells in ECM do not puncture the cell membranes of root cells and are restricted to the inter-cellular space in cortical and epidermal layers of the fine roots. In the event of the penetration of healthy root cell by an ECM fungus (from the Hartig net or the mantle), the mycorrhiza is referred to as ectendomycorrhiza or arbutoid mycorrhiza (Harley & Smith, 1983; Peterson *et al.,* 2004; Smith & Read, 2008).

A relatively small number of seed plants are ECM, although their importance is increased by their economic value as the main producers of timber (Harley & Smith, 1983; Smith &

Read, 2008). In contrast to the relatively small number of plants involved in ECM formations, a great diversity of fungal species is involved (Molina, 1992; Smith & Read,

2008). In total, as many as 10,000 fungal species and 8,000 plant species may be involved on a global scale (Taylor & Alexander, 2005). However, these communities are often dominated by a few species of ECM fungi (e.g. Heinonsalo *et al.,* 2007). ECM fungi include members of three major divisions of true fungi: Zygomycota, Ascomycota and Basidiomycota (Bruns, 1995). The identification of the fungal species has improved progressively with new methodology, evolving from survey of sporocarp occurrence as indicator of ECM status during early observation (Mason *et al.,* 1982), to morphological analysis (Agerer, 1987-2006) and more recently molecular DNA (rDNA) methodology (Gardes *et al.,* 1991; Bruns *et al.,* 1998; Koljalg *et al.,* 2005). The rDNA-based methods have increased the understanding of fungi long known to be ECM and furthermore, revealed fungi not previously recognized as ECM as forming these associations (Koljalg *et al.,* 2000; Tedersoo *et al.,* 2003; Weiss *et al.,* 2004; Tedersoo *et al.,* 2009).

Mycorrhizal types are not randomly distributed on a geographical scale because vegetation types tend to be dominated with different mycorrhizal types. ECMs are widespread among the northern boreal forests and among the trees and shrubs, such as birch, pine and *Salix* species that constitute subalpine and subarctic forests. Arbuscular mycorrhizas (AM) are widespread in tropical forests and ericoid mycorrhizal (ERM) associations are common in arctic tundra regions, alpine and other heathlands (Read, 1991).

Members of the family *Ericaceae* form ERMs and beside arctic tundra and heathlands, ericoid mycorrhizal plants are commonly found as understorey vegetation in boreal and Mediterranean forests. In heathland communities, ericaceous shrubs can become dominant (Read, 1991; Perotto *et al.,* 2002), especially in habitats with limited mineral nutrients (Cairney & Burke, 1998). A unifying feature of plants, forming ERMs is the formation of hair roots. These are narrow lateral roots with simple anatomy and limited growth. The fungal hyphae colonize the epidermal cells forming a branched hyphal complex in each colonized cell (Peterson *et al.,* 2004). Until recently, ECM and ERM were thought to involve different sets of fungi. However, molecular studies have revealed closely related and even identical, genotypes in ERM and ECM (Vrålstad *et al.,* 2000; Vrålstad *et al.,* 2002a; Allen *et al.,* 2003; Cairney & Meharg, 2003),. Villarreal-Ruiz *et al.* (2004) demonstrated single fungal mycelium can develop an ERM and an ECM at the same time.

1.4.1 Roles of mycorrhiza

The improvement of mycorrhizal plants' nutrition by enhancing N, P and potassium (K) uptake, among other nutrients, has been known for decades (Smith & Read, 2008). There is intense competition for nutrients in the soil ecosystems, and other microorganisms in soil, such as wood decomposing fungi readily utilize the inorganic nutrients. Because of their direct access to plant photosynthates, mycorrhizal fungi have a competitive advantage in harvesting these nutrients, since they do not need to compete as much for primary carbon reserves.

An important feature of mycorrhizal fungi is the diameter of the fungal mycelium (approximately 15 μm) is significantly less than the diameter of the plant roots. The fast growing mycelium can access pores in the soil that are inaccessible to plant roots for nutrient and water uptake. Therefore the association of ECM with roots plays an important role in enhancing N, P and base cation uptake of plants by transporting inorganic nutrients through fungal hyphae from the bulk soil to the roots and rhizosphere. In addition to the ability of accessing inorganic N and P, directly or *via* mineral weathering (Hoffland *et al.,* 2004) some ECM fungi have the ability to access organic N and P pools by nutrient mobilization from natural organic substrate such as litter (Bending & Read, 1995, 1997; Perez-Moreno & Read, 2000). The pathway for transferring nutrients from dead soil animals to mycorrhizal plants has been described, indicating a significant proportion of the plant nutrients initially sequestered in the bodies of soil animals, can be effectively recycled by mycorrhizal fungi to the host plants, instead of being immobilized or transferred to other levels within the food web (Klironomos & Hart, 2001; Perez-Moreno & Read, 2001). These studies highlighted the likely importance of mycorrhizal fungi in the facilitation of nutrient processes in the soil through dead organic matter (Mulder, 2006). ECM fungi can also obtain P by inducing dissolution of phosphate rock and e.g. apetite, biotite and feldspars (Finlay & Rosling, 2005; Liu *et al.,* 2005; Rosling, 2009).

The often extensive below-ground ECM extrametrical mycelium networks can connect trees, of the same or different species (Newman, 1988; He *et al.,* 2006). These, networks allow allocation of nutrients from photosynthetically active individuals to other individuals, such as seedlings and shaded trees (Simard *et al.,* 1997). Furthermore, ECM associations are important in plant water uptake, especially in areas with low water availability (Morte *et al.,* 2001; Marjanovic *et al.,* 2005; Smith & Read, 2008). Similarly,

ECM associations can confer host plant tolerance to heavy metals and salt in areas exposed to salinization (Muhsin & Zwiazek, 2002; Bandou *et al.,* 2006; Smith & Read, 2008).

Mycorrhizas can protect host plants roots against pathogens (Fitter & Garbaye, 1994; Sen, 2001) and insect herbivory (Gange *et al.,* 1994; Rieske, 2001; Gehring & Whitham, 2002). The mechanisms involved in this protection can consist of physical barrier formation, especially where the ECM form dense mantles, chemical interactions, such as the production of antibiotics and phytoalexins, as well as the modification of the rhizosphere, for example by reducing pathogen's ability to invade the root through competition or by supporting associated antagonistic microorganisms (Fitter & Garbaye, 1994). So far, most of the research has focused on the relationship between above-ground herbivory and mycorrhiza (ecto and/or arbuscular) (Gange & West, 1994; Gehring & Whitham, 2002; Gange *et al.,* 2005) and the research available involving the effects of mycorrhiza on below-ground herbivory has focused on AM (Gange *et al.,* 1994). Less is known about the effects of ECM on root herbivores.

The ERM type is distinguishable from the ECM and AM types in that the ERM external mycelium only extends for few millimeters beyond the individual roots. Therefore, it is unlikely that the function of the mycelium is to capture and transport minerals (Read, 1991). Ericoid fungi can degrade complex and recalcitrant polymeric substrates (e.g. Varma & Bonfante, 1994; Bending & Read, 1996, 1997; Cairney & Burke, 1998) and the major benefits conferred to the ericaceous host plants by the mycorrhiza are the enzymatic degradation of organic polymers in the soil and the transference of the resulting products to the root (Smith & Read, 2008) enhancing the nutrient uptake of ericaceous plants in nutrient poor soils. Furthermore, plants that form ERM are often dominant in highly metal-contaminated sites (Meharg & Cairney, 2000). ERM are known to be metal resistant (e.g. Bradley *et al.,* 1981, 1982; Monni *et al.,* 2000a, 2000b; Cairney & Meharg, 2003) and are capable of conferring resistance upon their plant hosts (Cairney & Meharg, 2003).

1.4.2 Mycorrhiza in Iceland

In most cases, studies on mycorrhiza in Iceland were based on fruit body occurrence. Hallgrimsson (1962) reported ECM fungi accompanying pine (*Boletus luteus* Linn.) and larch (*Suillus grevillei* (Klotzsch) Singer) were common in Icelandic pine or larch plantations, even though these tree species were relatively newly introduced into the

country at that time. Furthermore, several species, including *Lactarius torminosus* (Schaeff.) Gray, *Leccinum scabrum* Bull. Gray, *Amanita muscaria* (L.:Fr.) Lam. and several *Hebeloma* and *Cortinarius* species, all known to form ECM on birch, are found in Icelandic birch forests (Hallgrimsson, 1962). Data is available on the occurrence of fungal fruit bodies that were sampled and stored in a fungal database at the Icelandic Institute of Natural History since 1992, but no attempt was made to classify the fruit bodies according to life strategies (e.g. litter decomposing *vs*. mycorrhizal fungi). Therefore, it is unclear how many ECM species are listed (Gudridur G. Eyjolfsdottir, personal communication, November 2009). As a part of the ICEWOOD study, the distribution of fungal fruit bodies in treeless heathland, downy birch and conifer forests of different ages was recorded during 2003-2004. Not surprisingly, the number of fungal species increased when Siberian larch or downy birch forests were grown on formerly treeless heathland in Fljotsdalsherad, eastern Iceland (Eyjolfsdottir, 2007). However, the majority of the fungal species in Siberian larch forests belonged to fungal groups other than ectomycorrhizal fungi, where litter decomposing and saprophytic fungi represented major groups, especially in older forest stands. An exception was identified in downy birch forests where the number of ECM species was higher than in unforested heathland (Eyjolfsdottir, 2007). A study of the same sites targeting ECM root tips on Siberian larch and downy birch seedlings in microcosms and Siberian larch roots from the field, showed the number of ECM root tips increased rapidly after afforestation until the forests reached the age of 20. After that, the number of ECM root tips decreased. In Siberian larch forests, the number of root tips in a 53 year-old forest was similar to a treeless heathland. The number of ECM root tips in a 100 year old downy birch forest remained significantly higher than in the heathland. Similar patterns were detected in the number of ECM morphotypes in larch forests, but the number of morphotypes in birch forests remained constant as the forest aged (Hrafnkelsdottir *et al.,* 2008; Hrafnkelsdottir, 2009). An earlier study on ECM formation on Siberian larch from similar sites in eastern Iceland showed that ECM can form soon after planting, but few morphotypes were recorded (Kristinsdottir, 1990).

A study of the distribution of fungal fruit bodies in downy birch, Sitka spruce and lodgepole pine stands of different age classes in Skorradalur, in western Iceland, showed similar results as those found in eastern Iceland, where the number of fruit bodies increased when forests were planted in treeless heathlands. In western Iceland, ECM

species were dominant in forest stands and no ECM fungi were recorded in unforested heathland (Eyjolfsdottir, 2007).

The mycorrhizal infectivity in soil from birch stands and eroded areas was compared by growing birch seedlings in pots containing the two soil types (Aradottir, 1991). The infectivity of the birch soil was 25-35 times higher than soil from eroded areas, indicating that mycorrhizal inoculum in eroded areas in Iceland is low. Furthermore, seedlings grown in soil from the birch stand, formed mycorrhiza earlier than seedlings grown in soil from eroded areas. No identification of ECM was attempted in this study. However, different forms of ECM indicated several fungal species were present in the soil (Aradottir, 1991).

There is limited research on the effects of ECM on afforestation in Iceland. Magnusson and Magnusson (2001) noticed increased growth of birch seedlings, located close to native willow (*Salix* spp.) plants and speculated that it might be caused by a mycorrhizal association between the willow and the birch seedlings. Even though they could not confirm this relationship, the largest birch seedlings were located in the same zone as *Laccaria laccata* Peck, *Hebeloma* spp. and *Inocybe* spp. fruit bodies, indicating an active mycorrhizal inoculum in the soil. Enkhtuya *et al*. (2003) showed that inoculation with the ECM fungi *L. laccata* and *Pisolithus arrhizus* (Pers.) Rauschert in combination with application of fertilizer before planting had significant positive effects on seedling growth. Inoculation with the ECM fungi further increased mycorrhizal colonization of seedling roots, compared to uninoculated seedlings or seedlings inoculated with soil from a natural birch forest. Similar results were obtained by Oskarsson (2007) were he showed inoculation with ECM had positive effects on mycorrhizal colonization of lodgepole pine and Siberian larch. Even though he did not detect a difference in seedling survival during the first year, a significant positive effect of ECM inoculum was detected on lodgepole pine plant height after 12 years. Interestingly, Oskarsson (2007) detected a difference between different inoculation methods. Plants that received commercial ECM inoculum were significantly smaller after 12 years, than plants inoculated with forest soil.

In the light of the positive effects, detected on plants when inoculated with ECM, research on how to establish ECM on roots was conducted by Oskarsson (2010). The results showed cultivation in *Sphagnum* peat with standardized fertilization hindered ECM colonization, while cultivation in sedge peat with escalating fertilization strength (increased NPK levels) had positive effects on the colonization. Seedling mycorrhization at

planting was positively correlated with seedling survival and growth in the field, but negatively correlated with shoot dieback, indicating biological conditions in the nursery are important for mycorrhizal development and the field performance of tree seedlings (Oskarsson, 2010).

In addition to the above research on ECM associations, limited research on AM associations has been conducted in Iceland, except on lymegrass (*Leymus arenarius* (L.) Hochst.) in sand dunes. Lymegrass is a native to Iceland and is important in controlling soil erosion and stabilizing sand drifts (Greipsson & Davy, 1994). Studies on the occurrence of AM fungi in natural and reclaimed sand dunes showed that no AM fungal spores were present in barren sand and few in young reclamation sites of lymegrass. However, the number of spores and AM root colonization was significantly higher in older lymegrass sites (Greipsson & El-Mayas, 2000; Greipsson *et al.,* 2002; Oskarsson, 2006) and spores from the genera *Glomus*, *Scutellospora*, *Acaulospora* and *Entrophospora* have been identified from eight sand-dune sites in Iceland (Greipsson *et al.,* 2002). This indicates lymegrass does not depend on AM association during establishment, but the importance of the association increases as the plants get taller and start forming seeds (Oskarsson, 2006). An interesting finding of Greipsson and El-Mayas (2000) was the effects of inoculation of lymegrass with AM fungi on plant growth varied between AM isolates, the indigenous AM fungal inoculum giving better results than their commercial counterparts. Furthermore, the effectiveness of native inoculum varied, where inland isolates had a larger impact on plant growth than coastal isolates. In addition, Greipsson and El-Mayas (2002) showed even though AM fungi protected the lymegrass against pathogenic soil fungi or nematodes, if one type of pathogen (fungi or nematodes) was present, a synergistic effect of pathogenic nematodes and soil fungi reduced this protection.

Regardless of the research mentioned above, there are many large gaps in the existing knowledge of mycorrhizal fungal diversity and functional ecology in Iceland. The current knowledge of the distribution and identity of ECM fungi is to a large extent based on fruit body survey. However, the above ground presence of fruit bodies, does not necessarily reflect ECM colonization on roots (Gardes & Bruns, 1996) and therefore, intensive morphological (Agerer, 1987-2006) and molecular DNA (Horton & Bruns, 2001) based research on ECM root tips remains a priority research area in the challenging Icelandic landscape conditions outlined above.

1.5 Insect pathogenic fungi

Insect pathogenic (IPF), or entomopathogenic, fungi parasitize and eventually kill their insect hosts (Hajek, 2004). These fungi play a key role in regulating insect populations, including soil-dwelling insect pests, and many IPF that are parasitic on above-ground insects inhabit the soil for a part of their life-cycle (Hajek, 1997; Klingen & Haukeland, 2006; Meyling & Eilenberg, 2007). Fungal spores attach to the external body surface of the insects where they germinate and the fungal hyphae colonize the insect's cuticle, eventually entering the body cavity. The fungus continues to grow in the host's body cavity/haemolymph, obtaining energy from their insect host by biotrophy, necrotrophy or hemibiotrophy, depending on fungal species (Vega *et al.,* 2009). The fungal capability to directly penetrate the body cavity *via* the insect cuticle, and not needing to be ingested in order to infect its host, makes the fungi unique among insect pathogens and important in controlling insects with sucking mouthparts (Hajek, 2004).

The IPF includes taxa from most taxonomic groups within the fungal kingdom, apart from the Basidomycetes. The most important taxa are of the order Entomophthorales, including the genera *Entomophthora*, *Zoophthora* and *Pandora* (Charnley & Collins, 2007) and Hypocreales, including *Beauveria*, *Metarhizium* and *Isaria* (formerly *Paecilomyces*). These Hypocreales are frequently isolated from the soil and the species *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorokin. are natural predators on many insect species from a range of orders and have been found worldwide. Both species have been applied as biological control agents in agriculture and forestry, especially in temperate regions (Meyling & Eilenberg, 2007).

Several factors affect the occurrence and distribution of IPF in soils, such as land-use and cultivation method (Vänninen, 1995; Bidochka *et al.,* 1998; Klingen *et al.,* 2002a; Keller *et al.,* 2003; Meyling & Eilenberg, 2006). However, the species seem to differ in their occurrence: *B. bassiana* and *Isaria farinosa* (Holmskiold) Fr. (formerly *Paecilomyces farinosus* (Holmsk.) A.H.S. Br& G. Sm.) seem to be more sensitive to cultivation than *M. anisopliae* (Vänninen, 1995; Bidochka *et al.,* 1998). Geographical location is another factor affecting IPF. The occurrence of *M. anisopliae* decreases in more northerly areas, whereas *B. bassiana* and *I. farinosa* are less sensitive to geographical location. For example the genus *Isaria* was the genus most commonly found in Greenland (Jørgen

Eilenberg, personal communication, March 2010). However *M. anisopliae* exhibited a greater persistence than *B. bassiana* in soil with northern climatic conditions (Vänninen *et al.,* 2000). There is also a difference in the occurrence of IPF genotypes in different habitats (Bidochka *et al.,* 2001; Bidochka *et al.,* 2002; Meyling *et al.,* 2009).

1.5.1 Roles of insect pathogenic fungi

The earliest studies with IPF were carried out in the early 1800s, by Agostino Bassi (1773-1856), who studied ways of managing diseases that were destroying the silkworm industry in France. These studies stimulated the idea of using fungal insect pathogens to manage insect pests (Vega *et al.,* 2009) and a future challenge for sustainable agricultural production to include IPF in pest control.

Fungi are important pathogens of many invertebrate pests, and over 700 fungal species are pathogens of arthropods. Most of the invertebrates targeted for biological control are pests in the terrestrial systems, although some aquatic pests have been targeted (Hajek, 2004).

With recently developed research tools, such as DNA sequence analysis, understanding of the phylogenetic classification and ecology of IPF has increased. Furthermore, it has been discovered that many IPF play additional roles to serving as biopesticides in nature. Examples include activity as plant endophytes (Meyling & Eilenberg, 2007; Vega, 2008), antagonists of plant pathogens (Askary *et al.,* 1998), beneficial rhizosphere-associates and possibly as plant growth promoters (Vega *et al.,* 2009).

Insect pathogens were also found in newly identified niches, for example, some species are common components of the surface mycota of arthropods, even in healthy individuals showing no disease symptoms (Greif & Currah, 2007), and there are indications that fungi not formerly classified as an insect pathogenic, may be pathogenic to insects (Klironomos & Hart, 2001). The development and spread of natural fungal diseases in insects are affected by several factors, such as characteristics of the host and the pathogen population, surrounding environment and, especially in agro ecosystems, the impact of human activities (Charnley & Collins, 2007).

1.5.2 Insect pathogenic fungi in Iceland

To date, studies on IPF in Iceland are very limited and further studies on identification, distribution and possible applications into Icelandic soil are urgently needed. The IPF

species *I. farinosa,* was documented as naturally occurring in Icelandic soil (Eyjolfsdottir, 1995), but other research on IPF have focused on Entomophthoralean fungi on aphids, especially the green spruce aphid (*Elatobium abietinum* Walker.) (Austarå *et al.,* 1997; Nielsen *et al.,* 2000, 2001). Austarå *et al*. (1997) sampled almost 5000 green spruce aphids from 25 locations around Iceland in 1994 and checked for IPF. Only one species, *Neozygites fresenii* Nowakowski. was found during the survey. In later research, Nielsen *et al*. (2000) confirmed the existence of *N. fresenii* on green spruce aphids and also found *Entomophthora planchoniana* Cornu. in a survey done in 1997 and 1998. Despite intensive sampling of green spruce aphids, only two more fungal species were found in Iceland on this host: *Pandora neoaphidis* (Remaud. & Hennebert) Humber. and *Entomophaga pyriformis* (Thoizon) Balazy (Jørgen Eilenberg, personal communication, March, 2010). On dipteral hosts, two species from Entomophthorales have been recorded from Iceland. These were *Entomophthora muscae* (C.) Fres. sensu lato, and *Strongwellsea castrans* Batko & Weiser. Both fungi were collected in 1999 (Jørgen Eilenberg, personal communication, March, 2010)

1.6 *Otiorhynchus* spp. and root damage

The adults and larvae of the insect order Coleoptera are a consistent feature of soil mesofaunal communities. They belong to different families, they differ in phenotypic characters and they play different ecological roles (Lavelle & Spain, 2005). The family *Curculionidae* (the true weevils) contains many species that cause damage on vegetation, including genera like *Hylobius* and *Otiorhynchus,* that are well known as pests in forestry and other types of agriculture (Day *et al.,* 2004).

The genus *Otiorhynchus* includes polyphagous pests both as larvae and as adults worldwide. The genus is considered to be of European origin, but now has a world-wide distribution (Moorhouse *et al.,* 1992; Cross *et al.,* 2001). The most important pest species in large parts of Europe is *Otiorhynchus sulcatus* Fabr. (black vine weevil). Other significant pest species in Europe include *Otiorhynchus singularis* L., *Otiorhynchus clavipes* Bonsd., *Otiorhynchus porcatus* Herbst., *Otiorhynchus ovatus* L. and *Otiorhynchus rugifrons* Gyll. (Moorhouse *et al.,* 1992). The black vine weevil is endemic in large part of Europe, from northern Italy to southern parts of the Baltic States (Moorhouse *et al.,* 1992).

It is a serious pest of fruit crops, especially strawberries, blackcurrants, grapes, cranberries and many hardy ornamental plants (Moorhouse *et al.,* 1992; Cross *et al.,* 2001).

The black vine weevil can cause a substantial degree of crop damage. Most of the damage reported is caused by the larva feeding on roots, but the adults also cause damage by feeding on the above-ground parts of plants. The damage threshold can be very low and has been reported between 1 to 8 larvae plant^{-1}, depending on plant species and location of the larvae within the root system. The pest status of the insect is intensified by the wide range of alternative hosts and high fecundity (Moorhouse *et al.,* 1992). and the difficulty of detecting its presence during the early stage of outbreak or before and during transportation (Hunter, 2001).

There are not many reports on damage due to *Otiorhynchus* in European forests, but in Central Europe, three *Otiorhynchus* species, *Otiorhynchus sensitivus* Scopoli., *Otiorhynchus niger* (Fabricius) Auctorum. and *O. ovatus,* have caused damage to some extent in forests but the damage caused by the *Otiorhynchus* has been judged as minimal (Wulf & Berendes, 1996).

1.6.1 *Otiorhynchus* in Iceland

In Iceland seven *Otiorhynchus* species have been recorded, four of which are indigenous and three introduced (Olafsson, 1991). Of the latter group, only one species, the black vine weevil, has become established and only under indoor conditions. Two of the indigenous species, *O. rugifrons* and *O. singularis*, are rare, whereas *Otiorhynchus arcticus* Fabr. and *Otiorhynchus nodosus* Müller are among the most common Coleopteran species in the country. There is a slight difference in habitat selection of the species; *O. arcticus* inhabits dry sites with sparse vegetation, whereas *O. nodosus* prefers more fertile sites, both species are found throughout the country (Larsson & Gigja, 1959).

Otiorhynchus nodosus and *O. arcticus* beetles were sampled in pit fall traps in five different vegetation types in eastern Iceland during the summer (June - August) 1992. The former one was more common in all vegetation types and exhibited the highest population at the end of June/beginning of July, whereas the population of *O. arcticus* was constant throughout the sampling time (Halldorsson, 1994).

Figure 1.6.1 Otiorhynchus *larvae on birch root in Haukadalur, Iceland.*

Based on these observations and other available information, it was concluded that *O. nodosus* adults emerge from pupae in late June or the beginning of July. The eggs are probably laid in late July to early August, while hatching and egg lying of *O. arcticus* is more dispersed over the summer (Larsson & Gigja, 1959; Halldorsson, 1994). Furthermore, *O. arcticus* differ from *O. nodosus* in that *O. arcticus* reproduces sexually, whilst *O. nodosus* reproduces parthenogenetically (Larsson & Gigja, 1959). Soon after the eggs are laid, the larvae hatch and burrow into the soil were they feed on the roots of various plants (Figure 1.6.1). The larvae are perennial and hibernate for two winters before metamorphosis takes place. The adults emerge on the surface were they feed on the green parts of various plant species (Figure 1.6.2) (Larsson & Gigja, 1959; Halldorsson, 1994).

During the 1980s, most nurseries in Iceland were converted to produce young containerized seedlings using *Sphagnum* peat as a growth substrate. Soon after, the mortality of newly planted seedlings seemed to increase and studies on root damage of young tree seedlings revealed a high mortality rate due to *Otiorhynchus* larvae (Halldorsson, 1994). The larvae feed both on fine and coarse roots of various tree seedlings, debarking the main root and can completely remove the fine root system, while causing extensive damage to the coarse roots. This can result in less (or no) water and nutrient uptake of seedlings from the soil and increased seedling mortality. In two studies in eastern and southern Iceland, a mortality rate of 10-35% was recorded during the first year after planting and up to 50% mortality in seedlings was recorded three years after planting. A difference between tree species and plant communities was detected. The highest mortality rate caused by *Otiorhynchus* larvae was detected where larch seedlings had been planted in heathland ecosystems. Application of insecticide reduced seedling mortality, but mortality remained fairly high at a rate of between 5-25%. However, insecticide application was not deemed feasible due to high cost and negative impacts on the environment (Halldorsson, 1994). These results highlighted the importance of finding

other solutions, including biotic factors that could increase seedling survival in afforestation in Iceland. However, knowledge on existing soil biota, especially beneficial organisms at afforestation sites, their interaction with plant seedlings and effects on plant performance is scarce and needs further investigation.

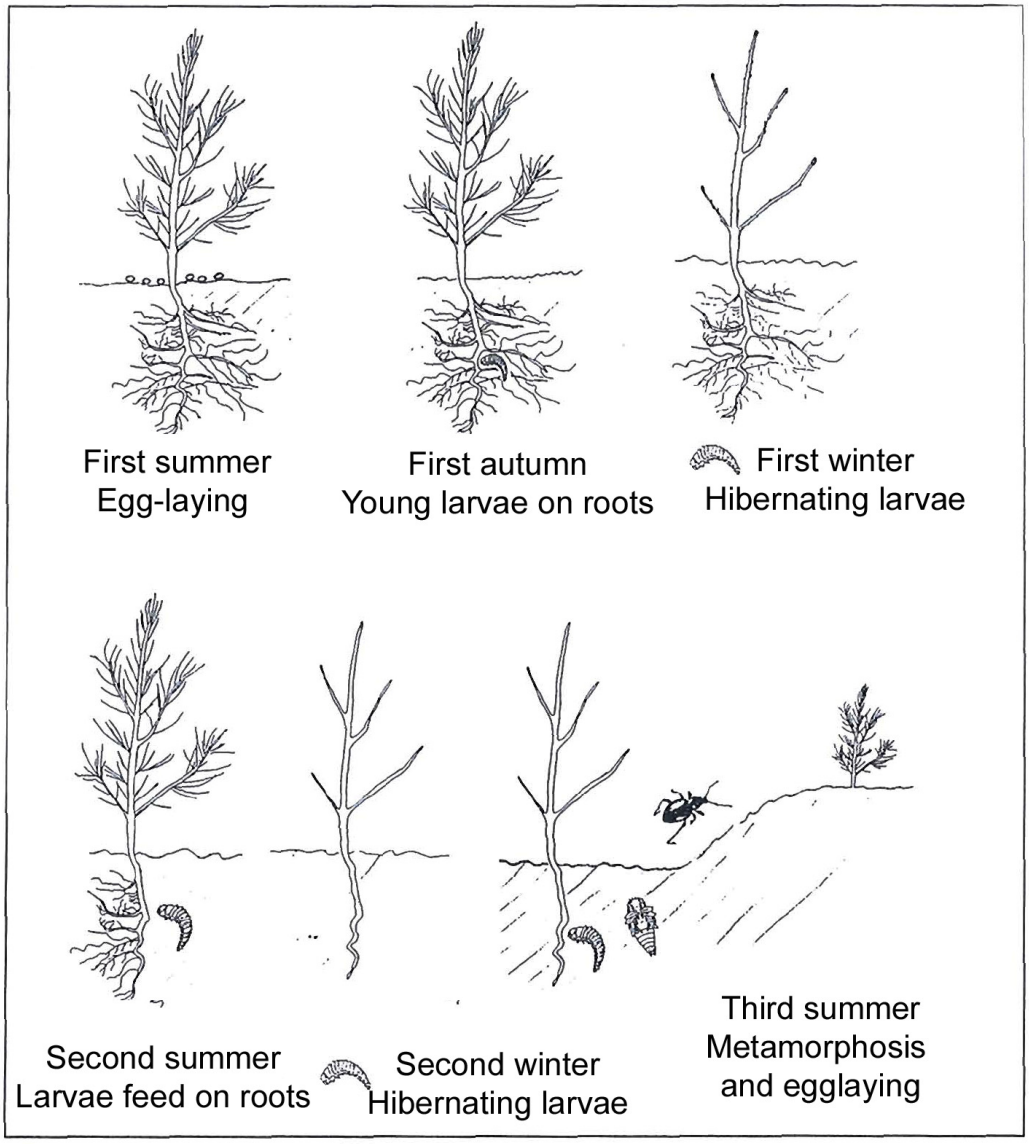

Figure 1.6.2. The life-cycle of Otiorhynchus *spp. in Iceland. Reprinted from Halldorsson (1994) with permission.*

1.6.2 Control of *Otiorhynchus* larvae

Otiorhynchus sulcatus larvae cause severe economical damage and several strategies have been employed to control or prevent larval infestation. These include breeding for plant resistance, selecting clones or cultivars more resistant to the *O. sulcatus* (Cowles, 2004), physical control, such as manually removing the adult weevils or inhibiting migration through the establishment of physical barriers (Bomford & Vernon, 2005) and use of insecticides (Moorhouse *et al.,* 1992; Hunter, 2001). There are two pathways to control the number of larvae, directly by treating the soil or indirectly by controlling the adults before egg-laying. Early attempts to control *O. sulcatus* involved application of arsenical insecticides, either in soil, mixed into baits or as foliar sprays (Moorhouse *et al.,* 1992). In the UK the pest was easily and effectively controlled by incorporating persistent insecticides, such as aldrin and DDT, into the soil before planting. However, concerns regarding the safety and toxicity of these chemicals resulted in the withdrawal of many of these insecticides from registration (Cross & Burgess, 1997).

In Iceland the insecticide Birlane was assessed to control the *Otiorhynchus* in field-trials in southern Iceland. The results showed the mortality rate of plants was reduced when the insecticide was applied soon after planting, but seedling mortality due to *Otiorhynchus* larvae was still between 5-25% (Halldorsson, 1994). However, because of environmental and safety concerns, the use of a biological control agent in Iceland is preferred.

Biological control (or biocontrol) is a strategy used to control pests and avoid economic damage to plants (Eilenberg, 2006). It has been defined a number of times, but a recent definition by Eilenberg *et al*. (2001) is most commonly accepted:

> *'The use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be'*

In this definition, living organisms such as predators, parasitoids, nematodes, bacteria, protozoa, viruses and fungi are included. However, metabolites produced by various organisms that are applied instead of the organisms itself, are not included in the definition of biological control (Eilenberg, 2006). Biological control can be achieved by direct or indirect methods. Indirect methods enhance the activity of indigenous microbial antagonists against a specific pathogen or the self-defense mechanisms of plants are stimulated by the use of organic soil amendments (Viterbo *et al.,* 2007). Direct methods

include the introduction of a specific microbial agent into soil or plant material that must establish itself to be active against the pathogen (Viterbo *et al.,* 2007).

The form of biological control used, greatly depends on the type of pests (invertebrates, vertebrates, plants or microorganisms). Therefore, biological control of weeds, plant pathogens or arthropods pests may differ in methodology and approach (Hajek, 2004). A great diversity of natural enemies has been used to control arthropod pests, including microorganisms such as bacteria and fungi. Since *O. sulcatus* is the most common insect pest of the *Otiorhynchus* family, most research on biological control has been carried out on this species. The species has a number of natural enemies, including mammals, birds, arachnids, insects and microorganisms, including nematodes, bacteria and fungi (Moorhouse *et al.,* 1992; Cross *et al.,* 2001). Insect pathogenic nematodes (*Rhabditida*: *Steinernematida* and *Heterorhabditidae*) are parasitic on many soil insects. Bacteria of the genera *Xenorhabdus* and *Photorhabdus* are symbiotically associated with the nematodes and the symbiotic bacteria are responsible for the death of the host. The infective juveniles of the nematodes persist in the soil until they enter a suitable host, mainly v*ia* the insect cuticle. Inside the host, toxic metabolites are produced by the nematode-bacterium complex that kills the insect. The nematodes then develop within the host cadaver were they feed on the host tissue and bacteria until new infective juveniles are produced and emerge in the soil (Kaya & Stock, 1997; Cross *et al.,* 2001). *Otiorhynchus* larvae have been controlled by insect pathogenic nematodes and effective control was achieved against *O. sulcatus* under greenhouse conditions (Vainio & Hokkanen, 1993; Cross *et al.,* 2001; Bruck *et al.,* 2005a; Lola-Luz & Downes, 2007). Outdoor biological control success was more variable, mainly due to low soil temperature, since many commercially available nematodes loose their efficacy below 14°C becoming ineffective at 10°C (Cross *et al.,* 2001). However, cold-storage of nematodes before application increased the number of nematodes invading *O. sulcatus* larvae and the consequent mortality rate of the larvae (Fitters *et al.,* 2001). The effects of insect pathogenic nematodes against *O. ovatus* and *O. nodosus* were also studied. Three *Steinernema* species proved to be an effective control against *O. ovatus* and *O. nodosus* in laboratory assays and also in strawberry fields in Finland. However, the persistence and activity of the nematodes were more dependent on the characteristics and frequency of the insect host than persistence and activity of IPF (Vainio & Hokkanen, 1993).

Control of *O. sulcatus* with IPF was conducted in a number of studies (Easterbrook *et al.,* 1992; Moorhouse *et al.,* 1992; Moorhouse *et al.,* 1993a; Vainio & Hokkanen, 1993; Moorhouse *et al.,* 1994; Cross *et al.,* 2001). Although *O. sulcatus* were susceptible at all life stages, but the best results were achieved against the larvae (Moorhouse *et al.,* 1992). Both *B. bassiana* and *M. anisopliae* were found to be pathogenic to *O. sulcatus* larvae, but *M. anisopliae* was the better prospect as a future biocontrol agent (Cross *et al.,* 2001), mainly because it was more persistent in the soil than *B. bassiana* (Vainio & Hokkanen, 1993; Vänninen *et al.,* 2000) and controlled *Otiorhynchus* larvae both under greenhouse and outdoor conditions (Easterbrook *et al.,* 1992; Moorhouse *et al.,* 1993a; Vainio & Hokkanen, 1993; Moorhouse *et al.,* 1994; Cross *et al.,* 2001). However, the virulence of *M. anisopliae* was negatively influenced by temperature (Moorhouse *et al.,* 1994) and low soil temperatures were the main limiting factor for using the fungus outdoors (Cross *et al.,* 2001).

Naturally occurring arbuscular mycorrhiza (AM) has been shown to protect plants against phytophagous insects (Gange & Bower, 1997) including *O. sulcatus* larvae (Gange *et al.,* 1994; Gange, 1996; Gange, 2001). When inoculated with one AM fungal species, the growth of larvae was reduced. However, when inoculated with two species the antagonistic effects on the insects disappeared (Gange, 1996; Gange, 2001), indicating that selection of inoculant fungal species for inoculation is crucial to successful eradication of the larval pest.

1.7 Interaction in the rhizosphere

In all ecosystems, no organism is isolated from its surroundings and all organisms interact with each other in some manner. These interactions can be classified into many different classes, according to mechanisms or effects of the interaction. The effects can range from predation (or parasitism), where one species has detrimental effect on other species, to mutualism, where species gain mutual benefits from the interaction (Begon *et al.,* 2006).

The rhizosphere has been described as the soil adjacent to and influenced by the plant roots (Lynch, 1994), and similarly, the mycorrhizosphere is the soil influenced by the mycorrhizal fungi colonizing the roots of a plant (Linderman, 1988). Plant roots play a major role in transferring nutrients from the soil to above ground parts of plants and are

important in the distribution of plant derived carbon into the soil. This makes the roots attractive sites to herbivores and a majority of soil invertebrates rely to a large extent on C inputs from roots (Bonkowski *et al.,* 2009). In addition to changing the soil near roots through secretion of a part of the C allocated to the roots as root exudates into the rhizosphere (Grayston *et al.,* 1997), plant roots also diffuse sugars, amino and organic acids, hormones and vitamins into the surrounding soil (Bertin *et al.,* 2003). This increases the food sources in the rhizo- and mycorrhizospheres and contributes actively to the heterogeneous structure of the soil. Therefore, the rhizosphere and mycorrhizosphere differ from the surrounding bulk soil, in for example pH, nutrient concentration and quality, moisture and oxygen levels (Nye, 1981; Wang & Zabowski, 1998). Apart from the surface layer, most biotic activity in soil occurs in the rhizosphere, because of the higher availability of nutrients than are found in bulk soil. Thus, a complex and variable interaction between soil organisms can be expected in the rhizospheres and mycorrhizospheres (Linderman, 1988).

The microbial community in the rhizosphere consists of highly diverse groups of organisms. These can be saprophytic, mutualistic or parasitic in regard to both plant hosts, and to each other. Both fungi and bacteria compete for the organic compounds exuded from plant roots. Many of the bacterial and fungal processes occurring in the rhizosphere have been studied in laboratories, but their identity and processes under field conditions is not well known. However, with the advantages of molecular methods, the knowledge of microbial communities in the mycorrhizosphere and rhizosphere is increasing (Buee *et al.,* 2009). For example, it was widely assumed that bacteria are the main decomposers of the easily degraded root exudates, but measurement of stable isotopes incorporated in biomarkers indicated fungi may make significant contributions to the decomposition of root exudates and there is an active competition between the bacterial and fungal inhabitants of the rhizosphere (Butler *et al.,* 2003; Treonis *et al.,* 2004; de Boer *et al.,* 2005).

Many of the microorganisms in the rhizosphere are important to the growth and fitness of their plant hosts, including mycorrhizas and plant growth promoting bacteria (PGPR) (Lugtenberg & Kamilova, 2009). Mycorrhizal fungi interact with an extensive range of other soil organisms. Inside the root the internal mycelium interacts with the root itself, but other organisms, such as pathogenic fungi, may invade the roots and interact with the

mycorrhizal fungi (Fitter & Garbaye, 1994). The external mycelium of the mycorrhiza interacts with a wide variety of soil organisms, from microorganism ranging from fungi and bacteria to mammals.

The highest bacterial abundance is closest to the plant roots, but the bacterial composition can differ between plant species, plant genotypes and even between different root zones (Hawkes *et al.,* 2007; Buee *et al.,* 2009). Fungal mycelium support bacteria (Nurmiaho-Lassila *et al.,* 1997; Sarand *et al.,* 1998; Timonen, 1998) and mycorrhizal helper bacteria are necessary for mycorrhizal formation (Garbaye & Bowen, 1987; Garbaye, 1994). Plant and ECM fungal species can have substantial influence on bacterial populations in the mycorrhizosphere (Smith & Read, 2008). The roles of bacterial communities in association with ECM fungi are not fully understood and require further investigation. It is possible that the interaction between bacteria and ECM fungi can increase available nutrients, by for example enhancing the weathering of minerals (Landeweert *et al.,* 2001) or supporting N_2-fixing bacteria (Paul *et al.,* 2007). The N fixing bacteria *Frankia*, whose activity is best known within the root nodules formed in symbiosis with actinorhizal plants, have also been found living freely in the rhizosphere of birch, pine and spruce, although their function there is unclear (Maunuksela *et al.,* 1999). Other microorganisms in soil, such as protozoa, can also affect nutrient uptake of plants and protozoa in the rhizosphere interact significantly with mycorrhiza (Jentschke *et al.,* 1995; Bonkowski *et al.,* 2001).

Results from Tiunov and Scheu (2005) showed the community structure of saprotrophic fungi was strongly affected by mycorrhiza. These effects may be mediated by plants, and involve nutrient competition or the mycorrhiza may exude chemicals affecting other organisms in the rhizosphere. However, these effects were more pronounced in the presence of fungivorous soil arthropods, indicating that fungal feeding soil invertebrates modulate the interactions (Tiunov & Scheu, 2005).

Another fungal group in the rhizosphere is IPF. Research on IPF in the rhizosphere is scarce (Bruck, 2010). In their study, Hu & St. Leger (2002) showed that *M. anisopliae* depended on the plant community and not necessarily on the presence of an insect host. Furthermore, Klingen *et al*. (2002b) suggested positive responses in the fungi to root exudates and Bruck (2005) showed *M. anisopliae* persisted longer in the rhizosphere than in the bulk soil. These results indicated that associations with plants may be important for the life cycle of IPF. Furthermore, different isolates of *M. anisopliae* responded differently

on various host species and all isolates increased their populations in the rhizosphere of *Picea glauca* (Moench) Voss., while only two isolates increased in the rhizosphere of *P. abies* (L.) Karst. and there were no increases in the rhizosphere of *Taxus baccata* L. (Bruck, 2004).

IPFs are clearly involved in tri-trophic interactions, with plant roots and insects in the soil. However, results concerning the effect of IPF on insect behavior are contradictory. Some results indicated insects were more attracted to soil treated with IPF than untreated soil (Kepler & Bruck, 2006; Bruck, 2010) while others found insects were repelled by the IPF (Kabaluk & Ericsson, 2007). Furthermore, the responses seemed to vary, between insect species, life stage (Villani *et al.,* 1994) and fungal isolate (Rath, 2000; Bruck, 2010). Kepler and Bruck (2006) studied the effects of inoculating *P. abies* roots with *M. anisopliae* on the behavior of *O. sulcatus* larvae in a two-choice soil olfactometer. The larvae preferred the roots growing in the *M. anisopliae* inoculated soil to the uninoculated counterpart. Unlike similar studies using insect pathogenic nematodes instead of fungi, it was not the insect pathogen that responded with altered behaviour (van Tol *et al.,* 2001), but the insect pest itself. It is unclear whether it is the IPF, the plant or the plant in association with the fungi that produces the attractive compounds (Bruck, 2010). St. Leger (2008) suggested *M. anisopliae* in the rhizosphere could form a repellent barrier around roots. This provides more protection for the plants *via* barrier effect than that caused by fungal induced disease in the insect, since there is always a time lag from infection until the death of the insect, during which the insect would continue feeding on the plant roots. However, a behavioral avoidance shown by certain insects against IPF, indicates recognition of the fungi by some insects, but the mechanisms for the avoidance are still unclear (Vega *et al.,* 2009). It is possible *M. anisopliae* and other IPF have multiple roles in plant protection (St. Leger, 2008) since some of them have dual activities against plant pathogens and some hypocrealean IPF produce secondary metabolites within their insect host, to help in outcompeting other opportunistic soil microbes (Strasser *et al.,* 2000).

In addition to the extensive microbial communities, the rhizosphere and mycorrhizosphere also host numerous soil invertebrates. Soil invertebrates are extremely diverse and may, according to Decaëns *et al*. (2006), represent up to 23% of the total diversity of all known living organisms. Even though fungi are an important part of the rhizosphere, and it is known that soil fauna contain many fungivorous species, the knowledge of faunal-

mycorrhizal interactions is inadequate (Bonkowski *et al.,* 2000). Soil arthropods may prefer saprophytic to mycorrhizal fungi (Klironomos & Ursic, 1998), but studies on the effects of fungal grazing by soil arthropods, such as Collembola and Acari, showed inoculation with Collembola can reduce mycorrhizal colonization (Warnock *et al.,* 1982; Hiol *et al.,* 1994), especially when the density of Collembola is high. The effects of soil arthropods grazing on mycorrhizal fungi is strongly density dependent, low or medium grazing either had no effect or increased extrametrical hyphae growth while intensive grazing reduced it (Ek *et al.,* 1994; Klironomos & Ursic, 1998). This could explain the reduction in plant biomass associated with high numbers of Collembola (Warnock *et al.,* 1982). These negative effects on plant biomass are not universal, as Setälä *et al*. (1995) reported higher above-ground biomass and higher N and P foliar concentration of birch and pine when Collembola were present, even though the ECM colonization on roots was reduced (Setälä, 1995). Furthermore, Steinaker and Wilson (2008) found that root and mycorrhizal production were greatest at intermediate collembolan densities. Cole *et al.* (2004) demonstrated that although certain individual collembolan species could influence the microbial community in the soil, they had no effects on microbial or plant uptake of N (Cole *et al.,* 2004). Soil arthropods may affect the fungal species composition in soils and on roots, through their feeding preferences for selective fungi (Hiol *et al.,* 1994; Schneider *et al.,* 2005) and the grazing of soil Collembola were shown to affect the interactions between mycorrhizal fungi and saprotrophic soil microorganisms (Tiunov & Scheu, 2005). There are also indications that the dynamics of soil fungi in forests are not only influenced by soil arthropods, but also by those found in forest litter (Krivtsov *et al.,* 2004).

As well as changing the density and composition of fungi in the rhizosphere, soil arthropods and earthworms may affect root morphology, perhaps via changes in nutrient availability and distribution (Endlweber & Scheu, 2006, 2007) and act as dispersal agents for soil microorganisms, such as mycorrhizal and IPF (Gange, 1993; Fitter & Garbaye, 1994; Klironomos & Moutoglis, 1999; Dromph, 2001; Lilleskov & Bruns, 2005). However, the dispersal range of microbes by soil fauna may be limited since neither earthworms, nor Collembola affected the spread of AM fungi over greater distances than 20 cm (Gormsen *et al.,* 2004) and fungivores seem to have more important roles as consumers than dispersing fungi in the rhizosphere (Bonkowski *et al.,* 2009).

Plant roots are attractive to many organisms, including herbivores, because of their high concentration of nutrients. Plant roots have evolved several direct defense compounds, such as terpenoids, and other indirect defenses against attacking herbivores and microorganisms. These indirect defenses include interactions with other plant roots, soil fauna and soil microorganisms (van Tol *et al.,* 2001; Rasmann *et al.,* 2005; Bonkowski *et al.,* 2009). Therefore, a successful root feeder must adapt and establish strategies to counteract the defensive systems of the plant roots. Two groups of soil organisms successful in such adaption are root-feeding nematodes and insect larvae (Bonkowski *et al.,* 2009). Plant roots are active in attracting pathogens of insect root herbivores, such as IP nematodes, into the rhizosphere by releasing a specific volatile when attacked by the insect larvae (van Tol *et al.,* 2001; Boff *et al.,* 2002; Rasmann *et al.,* 2005).

The above summary of the interactions in the rhizosphere does not account for all soil biota or interaction processes in the rhizosphere. Interactions in rhizospheres are complex and manifold and the fungal mycorrhizospheres are unique, extensive habitats supporting a variety of soil organisms, with ecologically important functions, which to a large extent remain a mystery. It must also be noted that below-ground interactions do not stop at the soil's surface, but rather affect plant performance and food-webs aboveground (Gange & Brown, 1997; Bonkowski *et al.,* 2000). To some extent, this vital part of the ecosystem has been neglected, even though the soil provides the foundation for human activities, including agriculture and forestry.

2 OBJECTIVES

The results presented in this thesis and the attached papers, forms part of the work carried out in the Nordic project "*Plant protection by beneficial soil organisms"*. Participants in the project were the Icelandic Forest Research, the University of Helsinki, the University of Copenhagen and the Forestry Service of the Faroe Islands. The overall goal of the project was to select, develop and apply functional guilds of beneficial soil microorganisms to improve plant production and forest establishment, using establishment of young tree seedlings (larch and birch) in Iceland and the Faroe Islands as model systems.

The current work focused on the Icelandic system and the more specific objects were to:

1. map the distribution of ECM and IPF in Icelandic soil (Paper II, Unpublished data)
2. compare the fungal distribution between different habitats (eroded and forest) (Paper II, Unpublished data)
3. study the effects of ECM and IPF on the performance of *Otiorhynchus* larvae (Paper III)
4. study the effects of inoculation with beneficial soil biota on root damage of newly planted tree seedlings (Papers I, III and IV)
5. estimate the persistence of soil beneficial fungi after inoculation under Icelandic field conditions (Paper IV, Unpublished data)
6. study the effects of inoculation with beneficial fungi on plant performance (Papers I, II and IV, Unpublished data)

3 MATERIALS and METHODS

3.1 Field sites (Papers I, II, IV, Unpublished data)

3.1.1 Mapping of beneficial soil fungi (Paper II, Unpublished data)

Soil was sampled from six locations around Iceland during two sampling expeditions, one in 1999 and the other in 2001 (Figure 3.1.1).

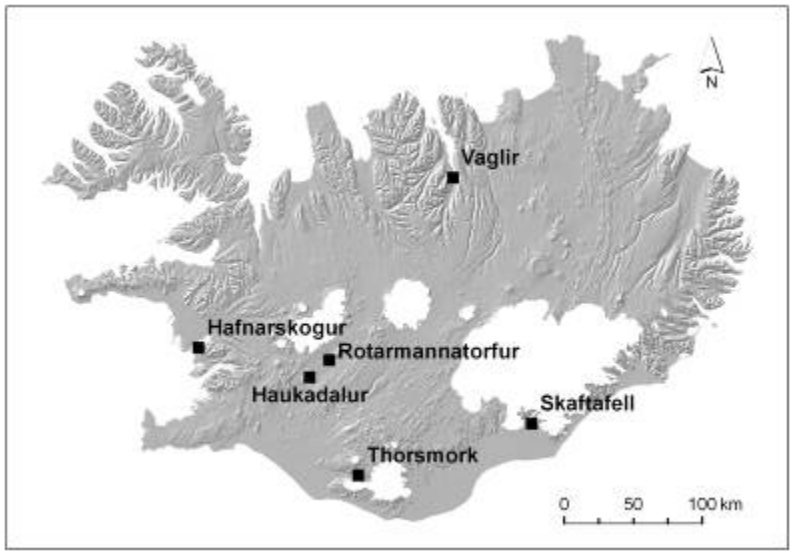

Figure 3.1.1 The location of soil sampling and field experimental sites in Iceland.

In 1999, soil was sampled from a heathland in Haukadalur (N64°19', W20°16') and birch woodland in Skaftafell (N64°00', W16°58') for analysis of entomopathogenic fungi. The heathland site in Haukadalur, southern Iceland was completely covered by vegetation, with dwarf shrubs dominating (*Betula nana*, *Salix* spp. and *Vaccinium uliginosum*); the birch woodland at Skaftafell was dominated with downy birch, with occasional *Salix* spp. shrubs (Figure 3.1.2)

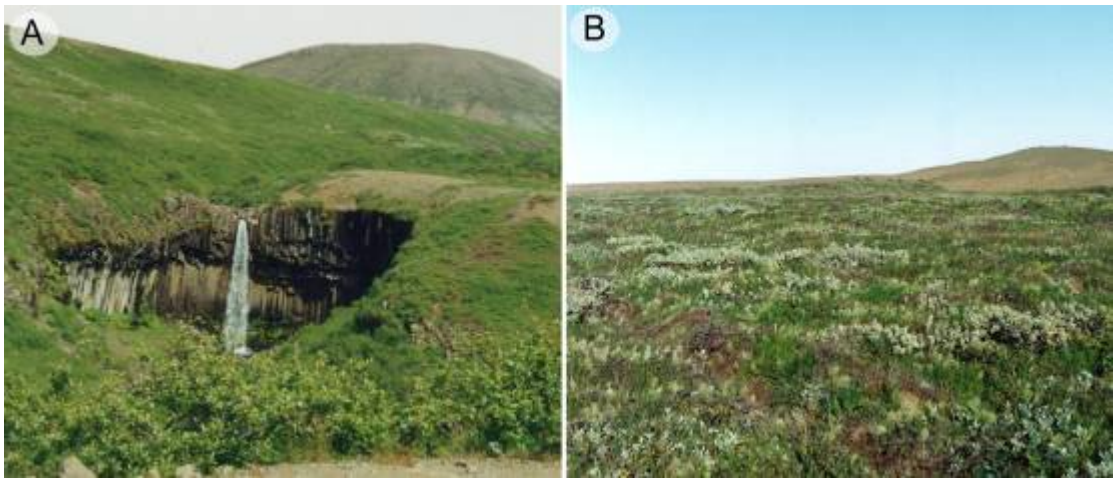

Figure 3.1.2 Soil was sampled from Skaftafell (A) and Haukadalur (B) in 1999.

In September 2001, soil was sampled from four locations: Rotarmannatorfur (N64°26' W19°59'), Vaglir (N65°37' W18°05'), Thorsmork (N63°41' W19°32') and Hafnarskogur (N64°29' W21°57') (Figure 3.1.3). At each of these four locations soil was sampled from two vegetative types, old birch woodland soil and eroded soil in the close vicinity (<500 m) of the woodland sites (Paper II, Unpublished data). All the birch woodlands had dense ground vegetation, dominated by *V. uliginosum*, *Geranium sylvaticum* L. and various grass species. The average canopy height was around 3-4 m, except at Vaglir were the average height was around 6-7 m. On the eroded sites, vegetation cover was between 5 to 15%, with species such as *Agrostis capillaries* L., *Trifolium repens* L., *Silene uniflora* Roth., *Alchemilla alpina* L. and *Armeria maritima* (P. Mill.) Willd. The soil at the eroded sites was sandy gravel, with low organic matter content, classified as Cambic or Gravelly Vitrosol. The soil at the birch woodland sites was Brown Andosol, containing higher organic matter (Table 1, Paper II).

Figure 3.1.3 Soil sampling sites at Rotarmannatorfur (A, B), Vaglir (C, D), Thorsmork (E, F) and Hafnarskogur (G, H). Birch forest sites are on the right (A, C, E, G) and eroded sites on the left (B, D, F, H).

3.1.2 Field experiments (Papers I, IV)

Tree seedlings were planted at Haukadalur, in southern Iceland (Figure 3.1.1, Figure 3.1.4) where earlier observations had revealed high mortality due to root damage caused by *Otiorhynchus* larvae. Three different habitats were used in the experiments that supported different vegetation cover and different land preparation methods were applied before planting:

1. Eroded (Paper IV). The eroded site has severe soil erosion, with the loss of almost all vegetation cover and topsoil. The soil was sandy with substantial signs of frost heaving. No ground preparation took place before planting (Figure 3.1.4 A).
2. Birch (Paper IV). The birch site was dense birch woodland with a canopy height of around 2-3 m and dense ground vegetation. Dominating species in the understorey vegetation were grass species and *G. sylvaticum*. Before planting, approximately 2 m wide corridors were cut through the birch stand and planting sites were scarified (Figure 3.1.4 B).
3. Heathland (Papers I and IV). The heathland site was fully vegetated, with of dwarf shrubs (*B. nana*, *Salix* spp. and *V. uliginosum*) dominating. The soil was cultivated by a dish harrow (TTS rotorator) before planting, leaving furrows 15-20 cm deep and 30-40 cm wide, into which seedlings were planted (Figure 3.1.4 C).

Figure 3.1.4 Field sites from Paper IV; eroded site (A), birch site (B) and heathland site (C).

3.2 Plant material and seedlings cultivation (Papers I, II, III, IV, Unpublished data)

Three tree species were used during the experiments, larch (*Larix sibirica*) (Paper I), downy birch (*B. pubescens*) (Papers II, III and IV, Unpublished data), and Scots pine (*Pinus sylvestris* L.) (Unpublished data).

Larch seedlings were grown for three months in a mixture of *Sphagnum* peat, organic soil from an old larch forest stand and pumice standard watering and greenhouse procedures were used, with the exception of fungicides application (Paper I).

To sterilize seedlings for microcosm experiments (Paper II, Unpublished data), birch seeds of Icelandic origin and Scots pine seeds, originated from Flatfanger, Norway, were water imbibed (o/n at 4°C), topically sterilized in 30% hydrogen peroxide and germinated in distilled water agar at room temperature. Germlings were aseptically grown in test tubes containing Leca® pellet (Optiroc OY, Finland). After lateral initiation, the germlings were planted into microcosms, containing soil from the sampling sites.

Seedlings for pot experimentation (Paper III) were grown for four months in multipots containing a thin layer of *Sphagnum* peat on top of a mixture of acid-washed expanded clay pellets (Leca) and *Sphagnum* peat (1:6 v/v). A 0.8g of slow release fertilizer (Osmocote; Scots Company, Marysville OH., USA) was added to the clay-peat mixture at the time of sowing and the seedlings were grown in a greenhouse. Each pot was fertilized at a respective N:P:K ratio of 2.5:1:5.6 mg and watered with untreated tap water as needed.

Birch seedlings for field experiments (Paper IV) were grown in *Sphagnum* peat-filled multipots for two years under standard nursery conditions before inoculation.

3.3 Inoculation with soil beneficial fungi and larval application (Papers I, III and IV)

3.3.1 Inoculation with forest soil (Papers I and III)

In paper I, half of the larch seedlings were grown in an autoclaved growth mixture of *Sphagnum* peat and soil from a mature larch forest to create an untreated control. The remaining seedlings were grown in un-autoclaved growth media.

In the pot experiment (Paper III), a layer of approximately 2 cm of soil from old birch forest was applied on top of the clay/peat mixture and the pots then filled up with *Sphagnum* peat.

3.3.2 Inoculation with ECM fungi (Papers III and IV)

Figure 3.3.1 Inoculation of birch seedlings with TerraVital-D slurry, containing four ECM species before planting in field in Iceland.

Inoculation with ECM was performed using the TerraVital-D inoculum from PlantWorks Ltd. (Sittingbourne, UK). In the pot experiment (Paper III), 1.5 ml of TerraVital-D inoculum, containing one ECM fungi (*Cenococcum geophylum* Fr., *Laccaria laccata* or *Phialophora finlandia* Wang & Wilcox.) was as applied on the clay/peat mixture and the pots subsequently filled with *Sphagnum* peat.

Birch seedlings for field experiment (Paper IV) were dipped into a TerraVital-D slurry (Figure 3.3.1), containing mixture of four ECM species (*Paxillus involutus* (Batsch: Fr.) Fr., *Telephora terrestris* Ehrh.:Fr., *P. finlandia* and *Hebeloma* sp.).

3.3.3 Application of *Otiorhynchus sulcatus* larvae (Paper III)

Otiorhynchus sulcatus larvae were obtained from Otiorhynchinae Supply & Advice (Herefordshire, UK). The larvae were late instar, but no attempt was made to identify the exact instar. Before application, 15 randomly selected larvae were weighed to establish pre-treatment larval weight, and subsequently 2 healthy larvae were introduced into each pot below the soil surface. The introduction was done four months after sowing and ECM inoculation and three days prior to IPF inoculation.

3.3.4 Inoculation with *Metarhizium anisopliae* (Papers III and IV)

Inoculation with *Metarhizium anisopliae* was done by spraying Triton-X solution (Sigma, UK) containing the fungus on the soil surface of pots receiving the IPF treatment. In the pot experiment, (Paper III) inoculation was done three days after application of *O. sulcatus* larvae. In the field experiment, (Paper IV) inoculation with *M. anisopliae* took place immediately after inoculation with ECM fungi.

3.4 Isolation and identification of soil beneficial fungi (Papers II, IV and Unpublished data)

3.4.1 Isolation and identification of ECM fungi (Papers II, IV and Unpublished data)

ECM was isolated from soil by using baiting seedlings in microcosms (Paper II and unpublished data). The microcosm set-up followed Timonen *et al.* (1997), with slight modifications. The soils were sieved (4 mm mesh) before use and 20x20 cm Perspex® microcosms were prepared (Figure 3.4.1), containing a 3-4 mm layer of soil from either Rotarmannatorfur, Vaglir, Thorsmork or Hafnarskogur (birch and eroded soil).

Figure 3.4.1 Birch seedlings after 147 days in microcosms containing birch woodland soil (A) and eroded soil (B) from Rotarmannatorfur.

Birch seedlings (Icelandic Forestry Research, Mogilsa: "Embla" seed lot 950071) were planted microcosms containing either birch or eroded soil from all sampling sites but Scots pine (Icelandic Forestry Research,: seed lot 990004), were only planted in microcosms containing soil (birch and eroded) from Hafnarskogur and Vaglir. Both birch and pine seedlings were grown under sterile conditions prior to planting in microcosms. The microcosms were placed vertically into growth chambers with transparent lids and arranged to protect roots from light (Figure 3.4.2). The seedlings were exposed to 19/5 hour (day/night) photoperiods and respective day/night temperatures of 13-17/10°C. The growth chambers were equipped with a floor cooling system and the soil temperature ranged from 12°C to 14°C at the soil surface and soil moisture was maintained by regular spraying with distilled water (Paper II and Unpublished data).

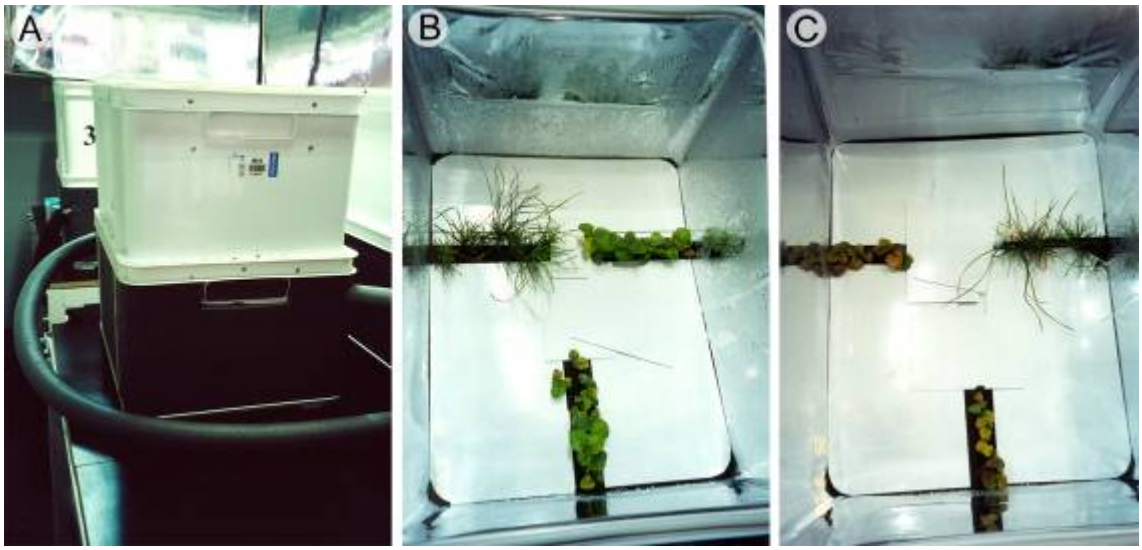

Figure 3.4.2 Growth chamber (A) used in microcosms experiment. A cooling unit circulates cold water through tubes in the insolated bottom part of the boxes. View from the top of chamber showing Scots pine and birch grown in microcosms containing birch forest soil (B) and eroded soil (C). Note the removable white plastic spacers resting on the upper surface of the microcosms that shield soils from light. Birch leaves are clearly discolored in the eroded soil.

The ECM colonization of birch and pine roots was monitored weekly under a stereomicroscope over a 5 month period. After that, the microcosms were maintained at growth conditions for further 4 months before final mycorrhizal assessment and harvesting of seedlings. The location (x-y coordinates) of individual mycorrhiza was marked on transparent films. Mycorrhizas were morphologically classified according to shape, color, size and outer mantle characteristics (Papers II, IV and Unpublished data). From each microcosm, three representatives of each morphotype were sampled, using micro scissors and scalpels. The samples were frozen in liquid N and stored at –20°C until DNA analysis (Paper II and Unpublished data).

ECM root tips were cut from roots and surface sterilized in 30% H_2O_2 for 10 seconds and subsequently rinsed with sterile water. The mycorrhizal root tip was then cut to small pieces and the pieces placed in $1/8^{th}$ Hagem's agar (Modess, 1941) with streptomycin (35mg/l) and benomyl (3mg/l), where it was cultivated in the dark at room temperature for a week. Mycelia from the fungal cultures was transferred to regular $1/8^{th}$ Hagem's agar to avoid mutations and grown further at the same conditions (Unpublished data).

DNA from ECM cultures, ECM root tips from birch and Scots pine seedlings, were extracted following the procedure described in Kåren *et al*. (1997) and Heinonsalo *et al*.

(2001), with slight modifications. Briefly, single ECM root tips or small bits of fungus from agar plates, were thoroughly ground with a micro pestles in 600 µl CTAB buffer (2% cetylammoniumbromide (CTAB), 20 mM EDTA, 100 mM Tris-HCl, 1.4 M NaCl) with quartz sand and incubated at 65°C for 60 min. The samples were then centrifuged (13,000 r.p.m.) for 5 min. The upper phase was extracted and mixed with an equal volume of chloroform and shaken before a 15 min centrifugation at the same r.p.m. before. The upper phase was then precipitated with 750 µl of isopropanol in -20°C for at least 1 hour and similarly centrifuged for 30 min. The supernatant was removed and 200 µl ice-cold (-20°C) 70% ethanol was added. After a 5 min. centrifugation (7,000 r.p.m.), ethanol was removed and the samples dried. The DNA pellet was redissolved in 50 µl of TE buffer (1 mM Tris-HCl and 0.1 M EDTA in distilled water, pH 8).

DNA from the ECM cultures and Scots pine roots were PCR amplified using the primers ITS1 and ITS4 (Gardes & Bruns, 1993), following the protocol described in Heinonsalo *et al.* (2001), with slight modifications. The DNA was amplified using the Ready To GoTM® PCR kit (comprising 1.5 units *Taq* DNA Polymerase, 10mM Tris-HCl, 50mM KCl, 1,5mM MgCl2, 200µM of each dNTP and stabilizers, including BSA (GE Healthcare)). The thermo cycling program was the same as described by Lanfranco *et al.* (1999) (3 min. at 95°C followed by 40 cycles: 45 s at 94°C, 45 s at 50°C, 45 s at 72°C and a final extension of 5 min. at 72°C). Negative (distilled water) and positive (known fungal species template) controls were included in each PCR batch to check PCR functioning and possible contamination by reagents. PCR products were checked in 1.5% agarose gels (1hour at 130 V) followed by ethidium bromide staining. Positive PCR products were subjected to RFLP fingerprinting with three restriction enzymes: *Hinf* I (Promega Corp., USA), *Mbo* I and *Taq* I (Finnzymes Inc., Finland) following Kåren *et al.* (1997) and Heinonsalo *et al*. (2001). Taxotron® software (Grimont, 1998) was used to produce matrices of RFLP fragments, allowing comparison of the fungal ITS sequencing to the existing dataset (Kåren *et al.,* 1997; Jonsson *et al.,* 1999b) and representative samples from each taxon sequenced by direct sequencing. Since attempts to obtain ITS-RFLP directly from the birch mycorrhizal root tip samples did not produce satisfactory results and the RFLP-dataset did not include close species to a number of the sequences obtained, samples from birch roots were further analyzed by cloning and sequencing (Unpublished data).

DNA from the ECM root tips from the birch seedling was amplified by nested PCR. The ITS region of birch roots representing each morphotype occurring on each plant was amplified using the nested PCR approach. The fungal specific primers ITS1F and ITS4 were used for primary reactions and the primers ITS1 and ITS4 then used as nested primers (Gardes & Bruns, 1993). In both cases, PCR reactions were performed using the Ready To GoTM® PCR kit. Since contamination was detected in the negative control when using the *Taq* DNA Polymerase, the restriction enzyme *Sau* 3AI was used to decontaminate the PCR as set forth by Carroll *et al.* (1999) with slight modifications. Prior to PCR amplification with outer primers (ITS1F and ITS4), one unit of *Sau* 3AI per *Taq* DNA polymerase unit was added to the master solution (containing water, 1.5 units *Taq* DNA Polymerase, 10mM Tris-HCl, 50mM KCl, 1,5mM MgCl2, 200µM each dNTP, and 0,5µM of each primer and stabilizer) and distributed in the PCR tubes. Consequently, the tubes were incubated for 30 minutes at 37°C in the thermocycler and the *Sau* 3AI was then deactivated by a 95°C step for two minutes before the DNA was added. The PCR protocol was then carried out as previously described. The nested PCR was then performed without any changes. The PCR products were checked in 1.5% agarose gels (1hour at 130 V) followed by ethidium bromide staining. Positive PCR products were cloned using the pGEM®-T Easy Vector System II (Promega Corp., USA) according to the manufacturer's instructions prior to sequencing. The clones were isolated from 5 ml. overnight cultures (Luria broth supplemented with ampicillin, 100µg ml^{-1}) using the Wisard® *Plus* SV Minipreps DNA Purification System (Promega Corp, USA). Isolated plasmids were precipitated with ethanol, dried and diluted in water to a concentration of 0.1-0.3µg μl^{-1} before sequencing (Unpublished data).

Sequencing was performed in both directions with the ITS1 and ITS4 (direct sequencing) or T7 and SP6 (sequencing of clones) primers, using Applied Biosystems 3700 DNA analyzer or 3100 Capillary DNA sequencer as in Heinonsalo and Sen (2007). The sequences obtained were aligned by ChromasPro or BioEdit and the alignment was manually optimized before the sequences were used to compare the samples with sequences in the UNITE and ISDN databases (Koljalg *et al.,* 2005). For identification, a minimum of 95% sequence identity to an ITS sequence of at least 450b.p. from a known specimen in the database was required. Samples with a 97-100% identity match to a known species were considered a match and identified to the species level. Samples with a 96% or lower identity were identified to the level of genus, family or order.

Preliminary analyses of the phylogenetic relationships were carried out using the Bayesian approach as implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003; Ronquist *et al.,* 2005). Default settings of the program were used which utilize a simple model of nucleotide substitutions (nucmodel=4by4, lst=1). In MrBayes single analyses of two parallel runs were carried out for 1,000,000 generations. Each run included four chains with a sample frequency of 100. A burn-in sample of 2,500 trees was discarded from the run and the remaining 7,500 trees were used to estimate posterior probabilities of different branches.

For the Ascomycota group, the aligned matrix contained 676 characters of which 400 were included in the analysis after exclusion of ambiguous regions. For the *Hebeloma* the aligned matrix contained 878 characters of which 662 were included in the analysis after the exclusion of ambiguous regions and for the *Cortinarius*, the aligned matrix contained 748 characters 556 of these were included in the analysis after the exclusion of ambiguous regions.

3.4.2 Isolation and identification of IPF (Papers II, III and IV)

IPF were isolated from soil samples in accordance with IOBC guidelines (Zimmerman, 1998). The samples were stored at 5°C before processing. For processing, the soil samples were lightly dried at room temperature for 1-2 days and thoroughly mixed. Plastic containers were filled to three fourths volume with the sampled soil and bait larvae (*Tenebrio molitor* L. or *Galleria mellonella* L.) added (Figure 3.4.3). The containers were incubated for 2-4 weeks at room temperature (20-22°C) and inverted on daily basis for the first five days to maximize the contact between larvae and the soil. Dead larvae were removed from the soil, rinsed in sterile water and transferred to petri-dishes with moist filter paper (Papers II and IV).

Figure 3.4.3 Larvae in soil from Icelandic birch woodlands

Baiting larvae (Papers II and IV) and *O. sulcatus* larvae from the pot experiment (Paper III) were examined for the occurrence of IPF and the fungi identified according to the presence of external features, followed by a morphological examination of conidiophores and conidia (Humber, 1997).

For comparison of radial growth, 16 strains of *M. anisopliae* were tested, three from Iceland, four from the Faroe Islands, from Denmark, one from Finland (Bipesco 6), one from Austria (Bipesco 5) and three from Panama. Three Sabouraud Dextrose Agar (SDA) Petri dishes were prepared for each strain and a piece of the fungal strain placed in the middle of the Petri dish. The Petri dishes were then incubated at 10°C or 15°C for 3-4 weeks and radial growth measured every second or third day.

3.5 Plant and larvae harvesting (Papers I, II, III and IV)

3.5.1 Plant performance parameters (Papers I, IV and Unpublished data)

The height and root collar diameter of seedlings were used as plant performance parameters in Papers I, IV and the microcosms experiment (Unpublished). The weight of the shoots and root systems was measured as part of the microcosms experiment.

3.5.2 Root damage assessment (Papers I, III, IV)

Root damage, caused by *Otiorhynchus* larvae, was assessed visually and plants divided into 5 damage classes, based on the proportion of main root debarked by the larvae, leaving the woody tissue exposed:

1) no damage
2) light damage (<25% of the main root debarked)
3) moderate damage (25-50% of the main root debarked)
4) serious damage (50-75% of the main root debarked)
5) severe damage (75-100% of the main root debarked)

3.5.3 Larval performance parameters (Paper III)

All insects harvested from pots in the pot experiment (Paper III) were weighed and their status recorded (larvae/pupae/adult/unidentified, living/dead).

3.6 Statistical analysis

The statistical methods used in different Papers are listed in Table 3.6.1

Table 3.6.1 Overview of statistical methods used in the papers. Detailed descriptions of the methods mentioned are given in the respective papers (I, II, III and IV).

Statistical methods	Paper I	Paper II	Paper III	Paper IV
Data transformation (logN or ArcSin)	X		X	
One-Way Anova followed by LSD			X	
Two-Way Anova followed by Tukey's HSD or LSD	X			X
Kruskal-Wallis followed by MW U		X		X
Regression analysis		X		
Wilcoxon signed-rank test				X

LSD = Fisher's Least Significant Difference test, Tukey's HSD = Tukey's Honestly Significant Difference test; MW U = Mann-Whitney U test

3.6.1 Statistical analysis of unpublished data

Radial growth of IPF

The mean (±SE) radial growth for each *M. anisopliae* strain was calculated from the three replicates. For each temperature, one strain from each country (the one that showed the highest radial growth) was selected for statistical analysis. The radial growth of the selected *M. anisopliae* strains was compared by using one-way ANOVA, followed by LSD posthoc test, with time as the second factor.

Distribution of ECM fungi on pine seedlings in microcosms

The accumulated frequency of ECM root tips and morphotypes after 9 months in the microcosms were used in the study and the mean number seedling^{-1} calculated. Since the data were not normally distributed, a Kruskal-Wallis test, with soil type as class, was used to compare ECM frequency between different soil types at each location.

Effects of inoculation on occurrence of ECM fungi on larch roots in the field

During the experiment, a small number of plants were lost, resulting in an unequal number of plants within blocks. To standardize the frequency data and make them comparable for statistical analysis, the frequency of non-inoculated and ECM root tips within each block was multiplied by the highest total number of root tips within blocks and divided by the actual total number of root tips tallied for each block. The data were then subjected to Kruskal-Wallis analysis.

The effects of beneficial fungi on birch and pine seedling performance in microcosms

A two-way ANOVA, with location and soil type as variables, was used to analyze plant performance data and test for differences in height, root column diameter, shoot and root weight between different soil types at each location.

A possible relationship between ECM colonization or IPF occurrence in soil *versus* plant performance in the microcosms was analyzed by regression analysis. When analyzing the relationship between ECM root tips or morphotype *versus* plant performance data, the data were for individual plants. When comparing the IPF occurrence frequency to other data, the average plant performance parameter of number of root tips and morphotypes in each soil type location-1 was used.

The statistical analysis was implemented in SPSS (Papers I and III), SAS (Papers II, IV and Unpublished data) and SigmaPlot 10 (Paper II and Unpublished data) (SPSS Inc., 1999; SAS Institute Inc., 2004; Systat Software, Inc., 2006).

4 RESULTS and DISCUSSION

4.1 Distribution of beneficial soil fungi in Iceland

4.1.1 Fungal species (Paper II and Unpublished data)

Ectomycorrhizal fungi (Paper II, Unpublished data)

The analysis of ITS-RFLP patterns from the ECM root tips with the Taxotron® and comparison with known dataset, identified three clearly defined clusters (see Appendix 1), whereof two included known mycorrhizal fungal species. The first cluster consisted of white morphotypes from pine (Figure 4.1.1) and black/grayish morphotypes from birch (Figure 4.1.2) or pine. The cluster included *Phialocephala fortinii* Wang & Wilcox. and *Piceirhiza bicolorata* and one strain was identified as *Phialophora finlandia* (Figure 4.1.3). The second cluster consisted of white morphotypes from pine roots and included *Suillus* species and the ECM fungus *Suillus luteus* (L.: Fries) Gray. was identified *via* direct sequencing (Figure 4.1.4). The third cluster did not include any known species from the database but consisted of various morphotypes from pine and birch roots. However, negative control samples subjected to nested PCR, gave positive results and were also included in this third cluster (Figure 4.1.5). The third cluster was therefore concluded to consist of contamination, which was cleaned by using the restriction enzyme *Sau* 3AI before cloning and sequencing.

Figure 4.1.1 ECM morphotype P1 (A-B) and P4 (C) on pine roots in microcosms.

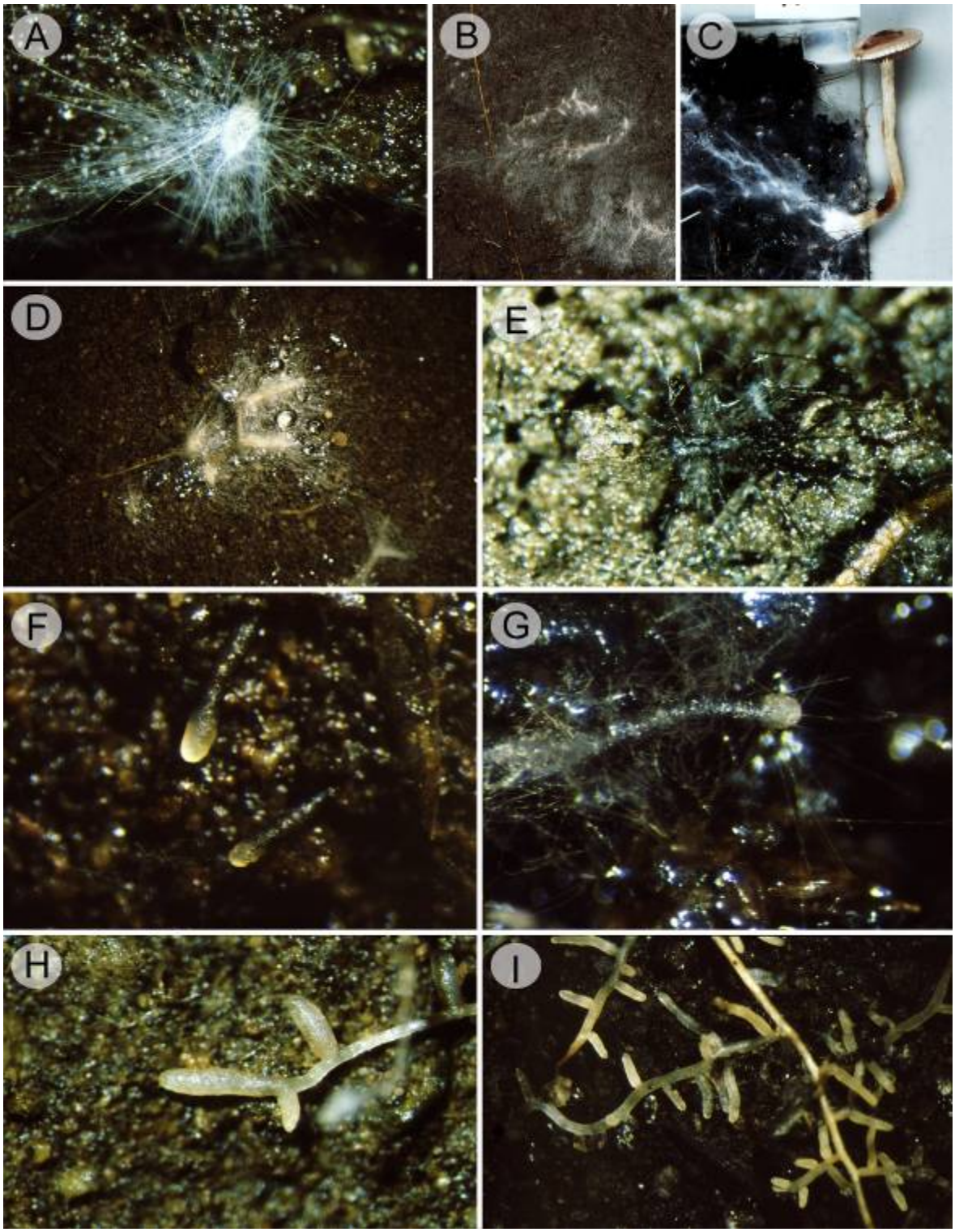

Figure 4.1.2 Examples of ECM morphotypes on birch roots in microcosms. A-B: morphotype B1, C: fungal fruit body growing from morphotype B1 in microcosms, D: morphotype B2, E: morphotype B4; F: young morphotype B6; G: mature morphotype B6; H-I: morphotype B10.

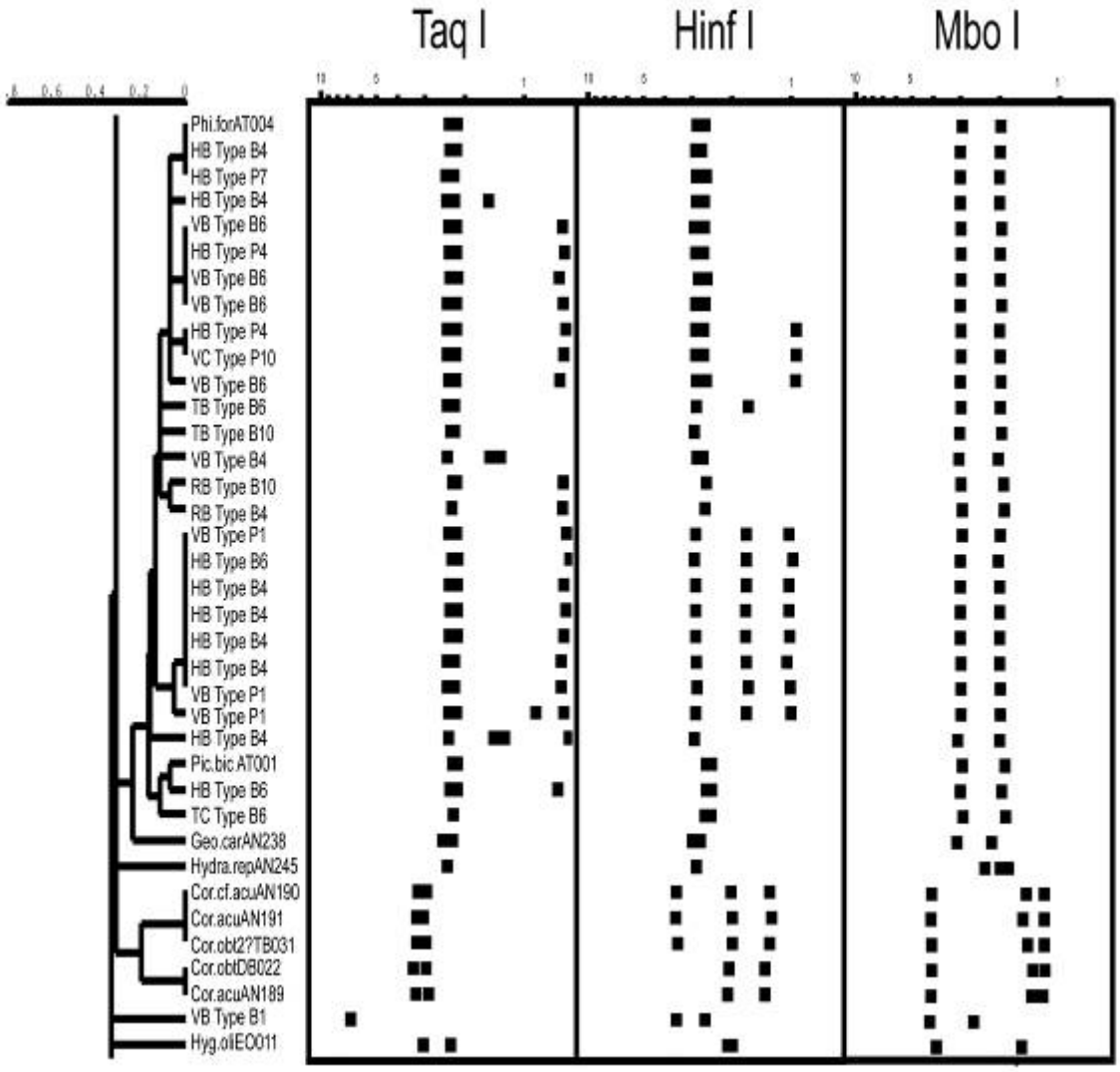

Figure 4.1.3 Dendrogram of single linkage cluster analysis of mycorrhizal fungal ITS-RFLPs representing Scots pine (P) and birch (B) mycorrhizal morphotypes, from different soil types in Iceland (HB=Hafnarskogur, birch forest soil; VB=Vaglir, birch forest soil; TB=Thorsmork, birch forest soil; RB=Rotarmannatorfur, birch forest soil; VC=Vaglir, eroded soil; TC=Thorsmork, eroded soil). See Appendix 1 for full ITS-RFLP dendrogram.

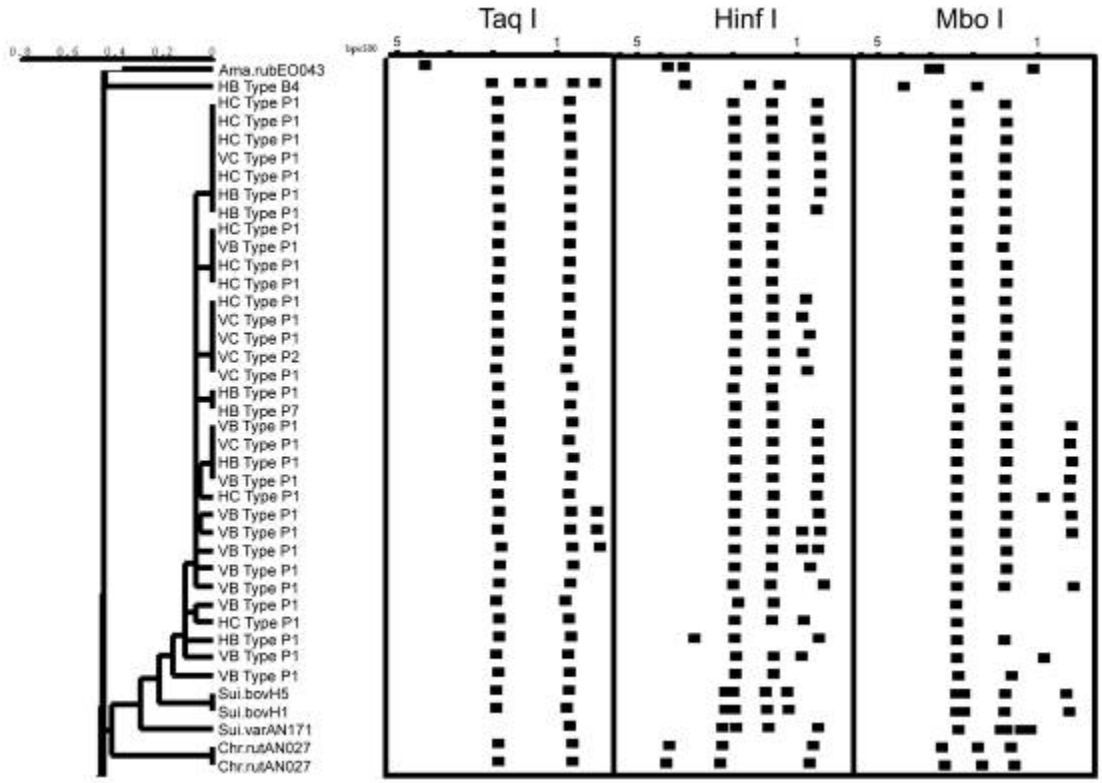

Figure 4.1.4 Dendrogram of single linkage cluster analysis of mycorrhizal fungal ITS-RFLPs representing Scots pine (P) mycorrhizal morphotypes. (HB=Hafnarskogur, birch forest soil; VB=Vaglir, birch forest soil; VC=Vaglir, eroded soil; HC=Hafnarskogur, eroded soil). See Appendix 1 for full ITS-RFLP dendrogram.

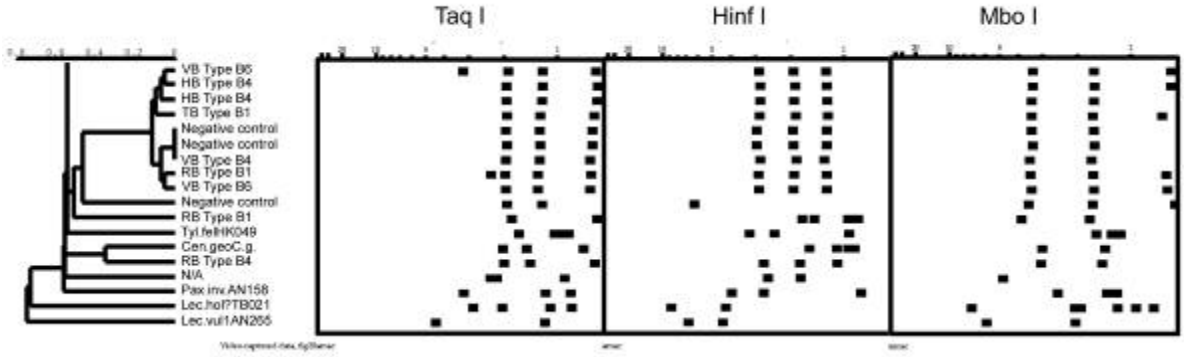

Figure 4.1.5 Dendrogram of single linkage cluster analysis of mycorrhizal fungal ITS-RFLPs representing negative control, Scots pine (P) and birch (B) mycorrhizal morphotypes, from different soil types in Iceland (HB=Hafnarskogur, birch forest soil; VB=Vaglir, birch forest soil; TB=Thorsmork, birch forest soil; RB=Rotarmannatorfur, birch forest soil; VC=Vaglir, eroded soil; TC=Thorsmork, eroded soil). N/A= No code available. See Appendix 1 for full ITS-RFLP dendrogram.

Furthermore, *Umbelopsis* sp. was identified from one pine root tip and *Acremonium strictum* W. Gams from two birch root tips (see Appendix 1).

In addition to the direct sequencing, 135 clones from 84 different birch root samples gave positive results from the sequencing (Table 4.1.1).

Table 4.1.1 Fungal species (according to the GenBank and UNITE databases (Koljalg et al., 2005) identified from birch root tips by ITS-PCR-RFLP-sequencing or ITS-PCR-sequencing. See Figure 4.1.2 for photos of morphotypes and Appendix 2 for detailed results.

Species	Number of root tips	Number of morphotypes	Morphotypes
Acremonium strictum	2	1	B1
Cortinarius erythrinus *	4	1	B1
Hebeloma crustuliniforme	1	1	B1
Hebeloma alpinum	1	1	B2
Hebeloma mesophaeum	6	4	B1; B2; B4; B5
Hebeloma pusillum	8	4	B1; B2; B4; B6
*Hebeloma leucosarx (*syn. *H. velutipes*)	3	2	B1; B4
Hebeloma sp.	16	1	B1
Phialocephala fortinii *	2	1	B6
Phialophora finlandia *	1	1	B6
Uncultured Ascomycota	1	1	B6
Uncultured Dothideomycetes	1	1	B4
Uncultured ectomycorrhizal fungus	3	2	B4; B6
Uncultured fungus	21	5	B1; B2; B4; B6; B10
Uncultured Helotiales	9	2	B4; B6
Uncultured Mucorales	3	2	B4; B6
Uncultured soil fungus	5	2	B4; B6

* not previously identified in Iceland

The variation of the ITS1 and ITS2 areas made it impossible to align the complete matrix containing all sequences isolated from the ectomycorrhizal roots. It was, however, possible to align the 5.8S area across all samples but the variation was insufficient for delimitation

of all the groups that could be recognized by the ITS-area. The Ascomycota and *Cortinarius* did, however, form distinct clades while sequences belonging to *Hebeloma* do not separate in similar way (Figure 4.1.6).

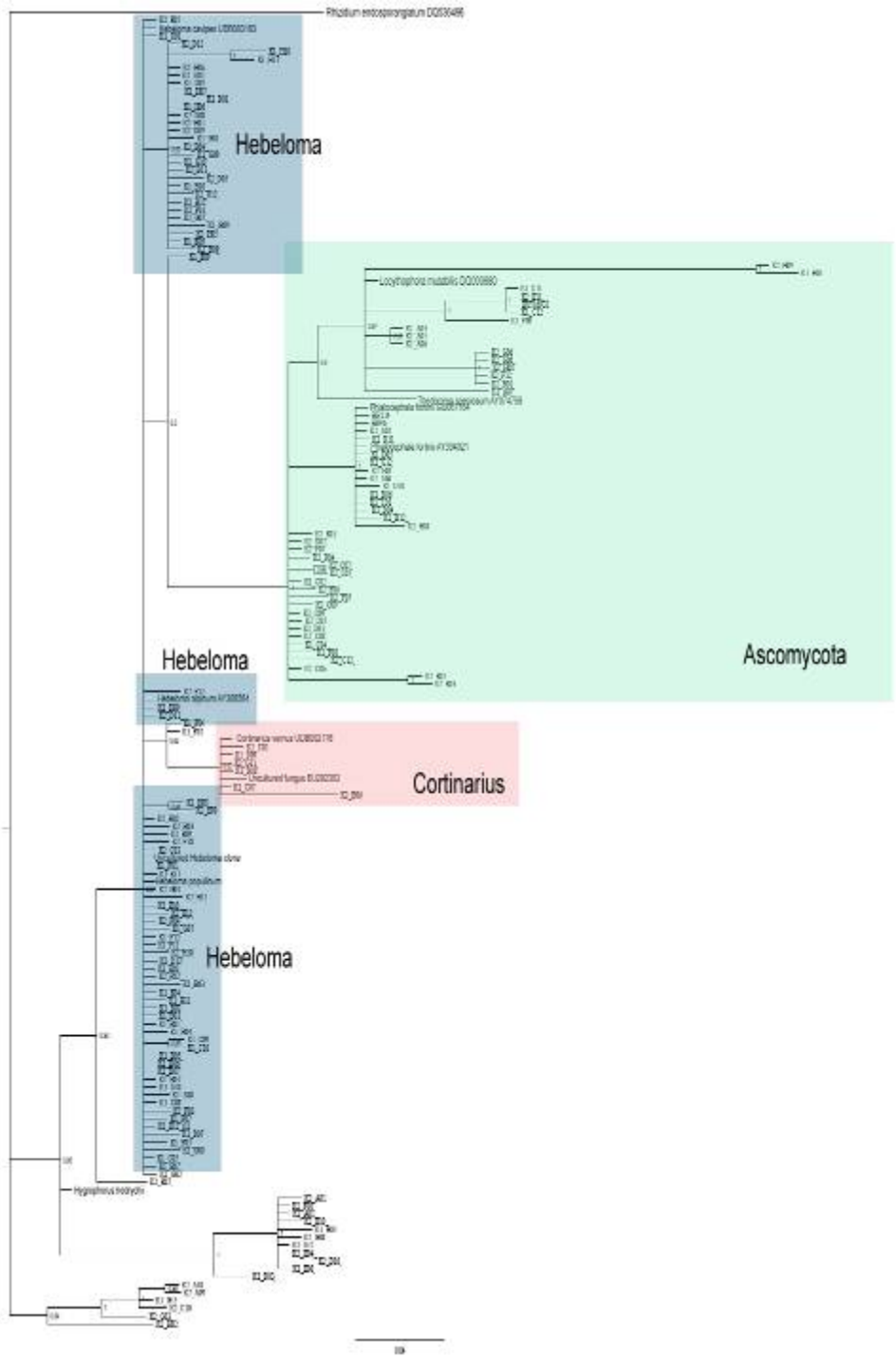

Figure 4.1.6 A phylogenetic tree based on Bayesian analysis of the ITS1-5.8S-ITS2 rRNA gene sequences of mycorrhiza sampled from birch root tips. Numbers above the branches refer to the Bayesian posterior probability of the node derived from 7500 MCMC sampled trees. Rhizidium endosporangiatum *was used as the out-group. The three main groups are color coded.*

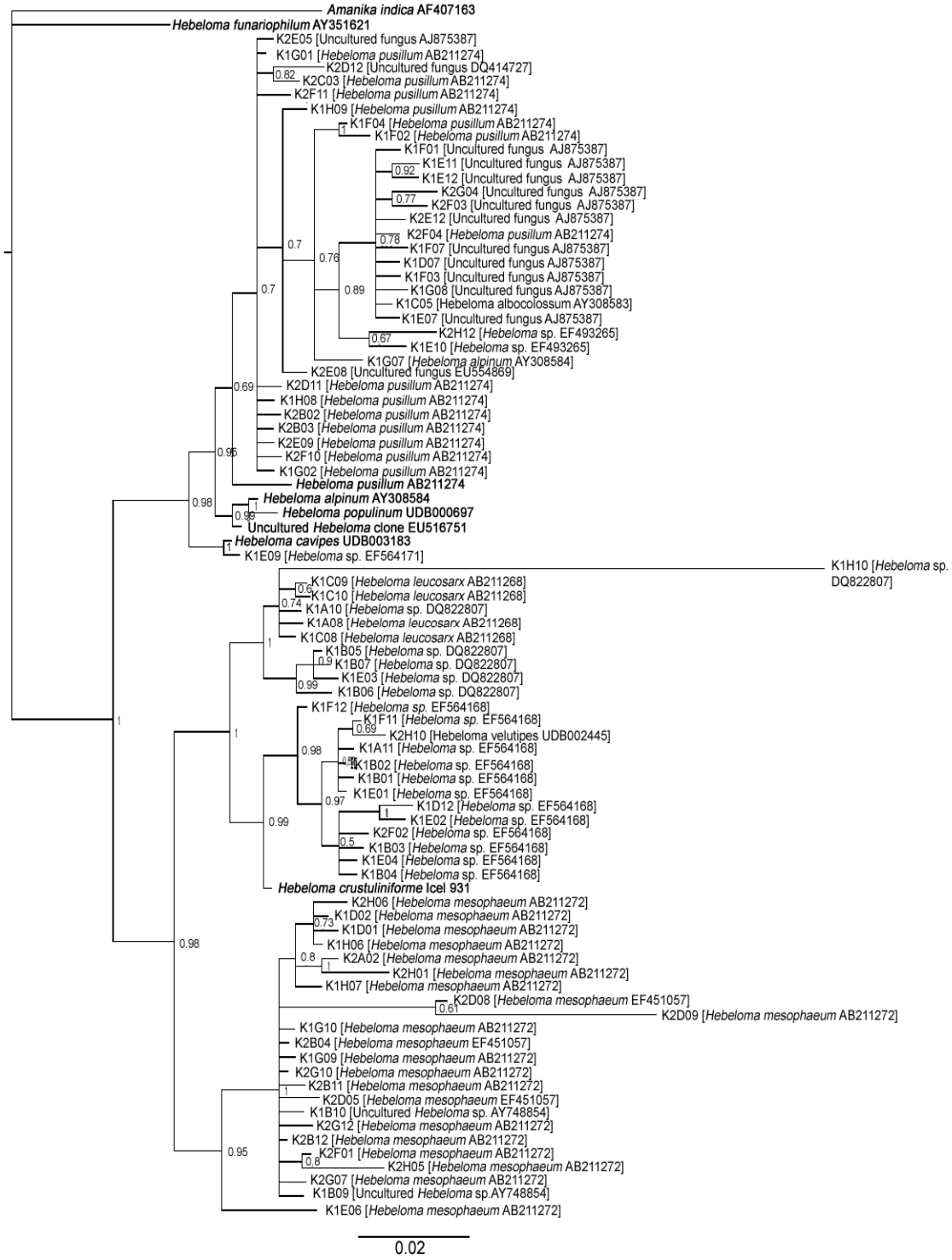

Figure 4.1.7 A phylogenetic tree based on Bayesian analysis of the ITS1-5.8S-ITS2 sequence of the Hebeloma*-cluster. Numbers above the branches refer to the Bayesian posterior probability of the node derived from 7500 MCMC sampled trees.* Amanika indica *was used as an out-group. The codes refer to clone number presented in Appendix 2. The closest Blast hits and accession number are given in brackets. Representative known species from UNITE and GenBank databases are written with bold letters.*

The most common ECM identified in this study was the genus *Hebeloma* (Figure 4.1.7). The genus occurs world-wide in the temperate zone and is known to form mycorrhiza with number of tree species, including birch (Mason *et al.,* 1982). *Hebeloma* spp. are frequent pioneer ECM on birch (Deacon *et al.,* 1983) and other tree species (Gryta *et al.,* 1997) and easily colonize birch roots, even if there are only few propagules in the soil (Deacon *et al.,* 1983). Since *Hebeloma* spp. are among the most commonly found fruit bodies in Icelandic birch forests (Hallgrimsson, 1962), the high occurrence of *Hebeloma* spp. on birch roots was not unexpected.

The species identification of *Hebeloma* spp., species concepts and classifications are controversial (Aanen *et al.,* 2000) and the ITS sequences of *Hebeloma* spp. differ in many cases only by few nucleotides, leading to the conclusion that the ITS (more specifically, ITS1-5.8S-ITS2) are not divergent enough to reflect the morphological specific difference (Boyle *et al.,* 2006). The preliminary phylogenetic studies on the *Hebeloma* spp. found in this study revealed two major groups, the pusillum/cavipes group and leucosarx/velutipes/mesophaeum group. The former group consists of one sequence grouped with *Hebeloma cavipes* Huijsman, but just over 30 sequences grouped around *Hebeloma pusillum* J.E. Lange. The second main group was divided into two subgroups, one around *Hebeloma leucosarx* P.D. Orton and *Hebeloma velutipes* Bruchet and the other around *Hebeloma mesophaeum* (Fries) Quélet (Figure 4.1.7). According to Boyle *et al.* (2006), who divided the *Hebeloma* genus into rather well-supported monophyletic groups, the *H. cavipes* and *H. pusillum* belong to the clade /Denudata, *H. leucosarx* and *H. velutipes* to the clade /Velutipes and *H. mesophaeum* to the clade /Indusiata. The preliminary phylogenetic analysis of the Icelandic *Hebeloma* species sampled in this study, therefore seem to be in keeping with the results of Boyle *et al.* (2006). However, Boyle *et al.* (2006) recorded more groups than were found in the present study. The genus *Hebeloma* shows limited host specificity since several species are known to form ECM with various host plants (Smith & Read, 2008). However, as discussed earlier, birch is the only native forest forming tree species in Iceland, which may impose a strong selection and explain the imitated diversity of *Hebeloma* species in the woodlands. This limitation was furthered by the geographic isolation of Iceland since the most common route of introduced tree species is in the form of seeds, not seedlings with their accompanying soil biota. *Hebeloma* species also show variable acclimation to low temperature. Some species are unaffected by freezing to -5°C, while other species showed significantly reduced post

freezing growth (Tibbett & Cairney, 2007). It is therefore likely that European *Hebeloma* species sensitive to low temperatures, do not exist in Iceland. Considering this, it is not surprising that there is less diversity of *Hebeloma* spp. in Iceland than in other parts of Europe.

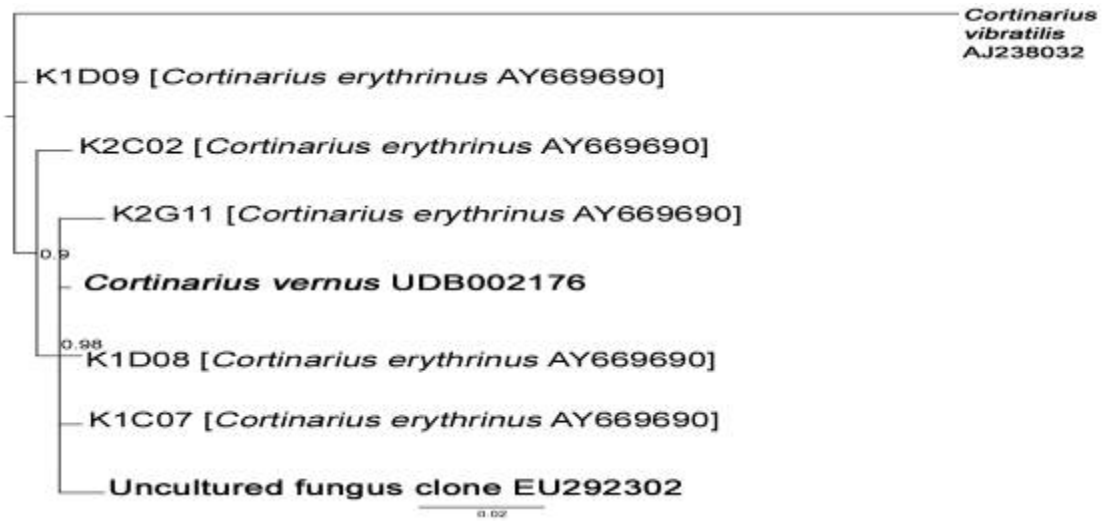

Figure 4.1.8 A phylogenetic tree based on Bayesian analyses of the ITS1-5.8S-ITS2 sequence of Cortinarius *from ectomycorrhizal birch root tips grown in Icelandic soil. Numbers above the branches refer to the Bayesian posterior probability of the node derived from 7500 MCMC sampled trees.* Cortinarius vibratilis *was used as the out-group. The codes refer to clone number in Appendix 2. The closest Blast hits and accession number are shown in brackets. Representative known species from UNITE and GenBank databases are in bold letters.*

According to the blast hits, only one *Cortinarius* species was recorded during this study. This may be unexpected since the genus is one of the most species-rich mycorrhizal genera in the world with approximately 2000 known species. The taxonomy of the genus was based largely on macro-morphology and is a matter of debate, with several major classification schemes proposed (see references in Garnica *et al.,* 2005). Analysis of ITS often provided limited phylogenetic resolution in the case of *Cortinarius* (Hoiland & Holst-Jensen, 2000) but Garnica *et al.* (2005) proposed a new basis for classification of *Cortinarius* into 8 major lineages, derived from phylogenetic relationships based on ITS and the D1-D2 region sequences. According to their proposal, the species found during this study, *Cortinarius erythrinus* (Fr.) Fr., (Figure 4.1.8) is located within the /Telamonia clade in the telamonoiod tax (Garnica *et al.,* 2005).

The low number of *Cortinarius* spp. found in this study is curious, since the genus is known to be one of the dominant fungal species forming ECM on arctic sites (Gardes *et*

al., 2000) and on successional sites, such as glacier forefronts (Mühlmann & Peintner, 2008) and it is known that several *Cortinarius* spp. are found in Iceland (Gudridur Gyda Eyjolfsdottir, personal communication, March 2010).

A relatively large proportion of the fungi identified belonged to *Ascomycetes* (Figure 4.1.6 and Figure 4.1.9), including the *Helotiales* group. In addition to approximately 2000 described species with different lifestyles, *Helotiales* includes a large number of unidentified root-associated fungi (Wang *et al.,* 2006). Various sub-groups of *Helotiales*, such as the *Phialocephala-Acephala* complex, have been identified from ECM and arbutoid mycorrhiza in forests and shrub lands of the Northern Hemisphere (Vrålstad *et al.,* 2002b; Rosling *et al.,* 2003; Tedersoo *et al.,* 2003; Heinonsalo & Sen, 2007; Heinonsalo *et al.,* 2007; Tedersoo *et al.,* 2008) and they most commonly form non-specific associations with various plant hosts (Vrålstad *et al.,* 2002b), although some host preference may occur (Grunig *et al.,* 2008).

With molecular methods, such as that used in this study, the identification of micro fungi identified from ECM root tips has increased and many ascomycete taxa, with unknown ecological roles have been revealed (Bergemann & Garbelotto, 2006; Smith *et al.,* 2007). In the present study, several species formed clusters with *P. fortinii* and *P. finlandia.* Both species have been shown to cluster together in the *Hymenoscyphus ericae*-aggregate (Vrålstad *et al.,* 2000; Vrålstad *et al.,* 2002b). *H. ericae* forms ericoid mycorrhiza with ericoid plants and has some ability to degrade chitin, lignin and tannin and hence has the potential to break down structural components of the organic matrices at the base of the litter layer (Read, 1991). For example, infection with ERM provided access to amino acids (Stribley & Read, 1980) and enabled utilization of peptides (Bajwa & Read, 1985) and proteins (Bajwa *et al.,* 1985), which non-ERM plants were unable to utilize. Furthermore, ERM are able to persist and maintain mycorrhizal ability in habitats lacking the host (Bergero *et al.,* 2003) and *P. finlandia*, isolated from ERM, formed ECM with *P. sylvestris* showing the same isolate of *P. finlandia* can act both as ERM and ECM (Villarreal-Ruiz *et al.,* 2004). Therefore, the relatively high occurrence of ERM-associated fungi, recorded on birch roots in this study may be important, due to the harsh conditions at the Icelandic non-vegetated and nutrient poor sites.

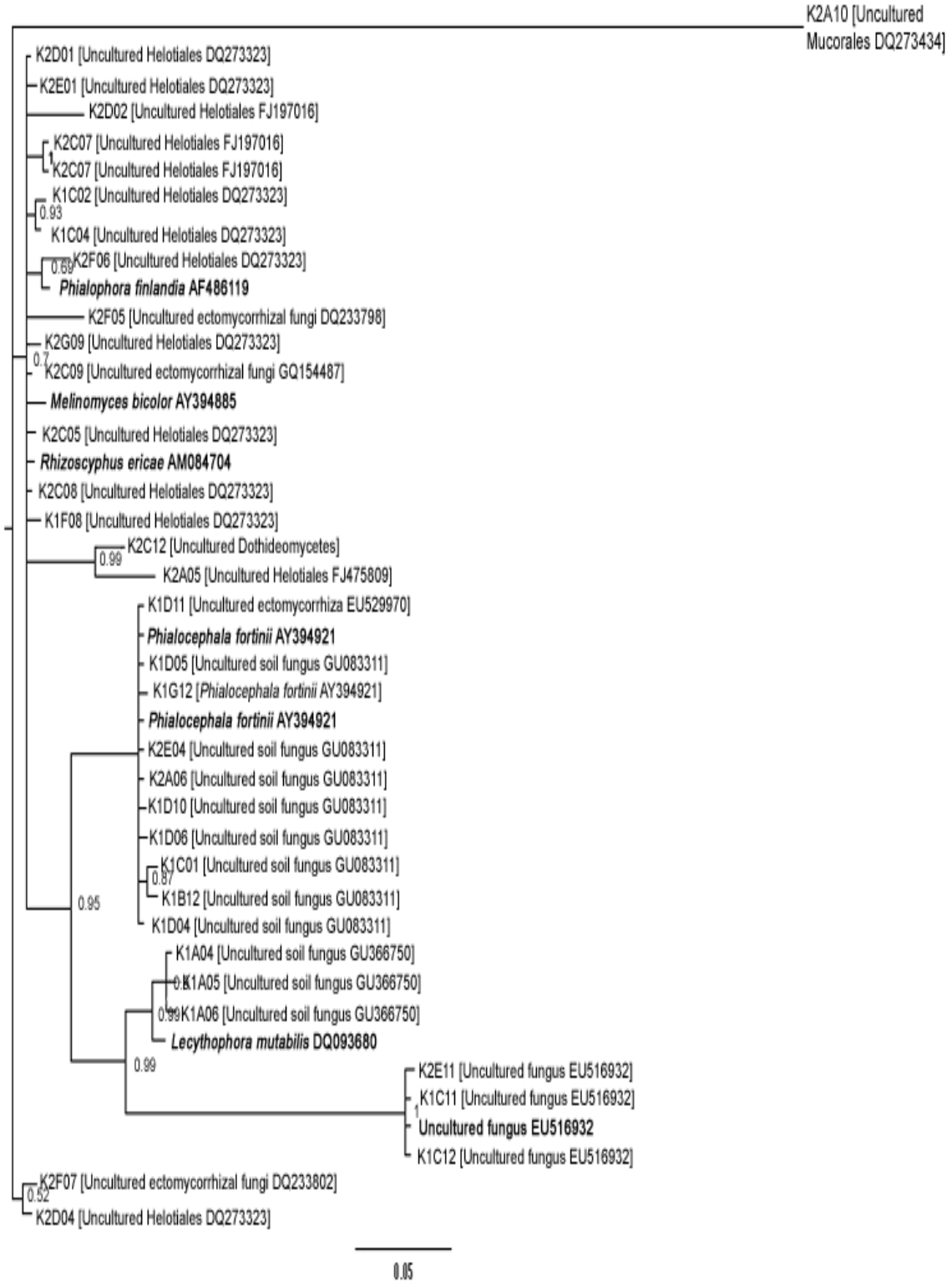

Figure 4.1.9 A phylogenetic tree based on Bayesian analyses of the Ascomycetes-group identified from birch roots. Numbers above the branches refer to the Bayesian posterior probability of the node derived from 7500 MCMC sampled trees. The codes refer to clone numbers presented in Appendix 2. The closest Blast hits and accession number are given in brackets. Representative known species from UNITE and GenBank databases are written in bold letters.

Insect pathogenic fungi (Paper II and Unpublished data)

Three IPF species were identified during this study. One of them, *Isaria farinosa* was previously recorded in Iceland (Eyjolfsdottir, 1995) but the other two, *Beauvaria bassiana* and *Metarhizium anisopliae* are new findings (Gudridur G. Eyjolfsdottir, personal communication, December 2009). This number of species is not much lower than in Finland (Vänninen, 1995), Canada (Bidochka *et al.,* 1998) or northern Norway (Klingen *et al.,* 2002a). However, the occurrence (number of soil samples with IPF) was lower in Iceland than in Finland or Canada. The soil sampling and the isolation procedures differed between studies and may account for the difference in occurrence between studies. In the Icelandic study, soils were sampled from a few sites, with defined vegetation cover (non-vegetated, heathland or birch woodland) whereas other studies involved more intense sampling and from variable vegetation cover. This may have limited the occurrence of species number of IPF recorded in the present study.

These results clearly show that IPF are present in Icelandic soil, but it is not clear how well these strains perform against soil-dwelling larvae. One of the factors limiting the use of IPF as biological control agents, especially in northern latitudes, is their requirement of a soil temperature in excess of 15°C (Cross *et al.,* 2001). When the radial growth of *M. anisopliae* strains isolated from Iceland and the Faroe Islands was compared to radial growth of other strains at 10 and 15°C, the ANOVA analysis showed a significant effect of isolates, time (days from start of incubation) and a significant interaction between isolate and time (Table 4.1.2), suggesting different isolates behaved differently at different times.

Table 4.1.2 Results of ANOVA analysis on the effects of different Metarhizium anisopliae *isolate and time on radial growth at 10°C and 15°C*

	Effect	df	F	*p*
10°C	*M. anisopliae* isolate	5	224.9	<0.0001
	Time (days)	9	229.8	<0.0001
	Isolate*time	45	12.0	<0.0001
15°C	*M. anisopliae* isolate	5	153.2	<0.0001
	Time (days)	8	2102.4	<0.0001
	Isolate*time	40	5.98	<0.0001

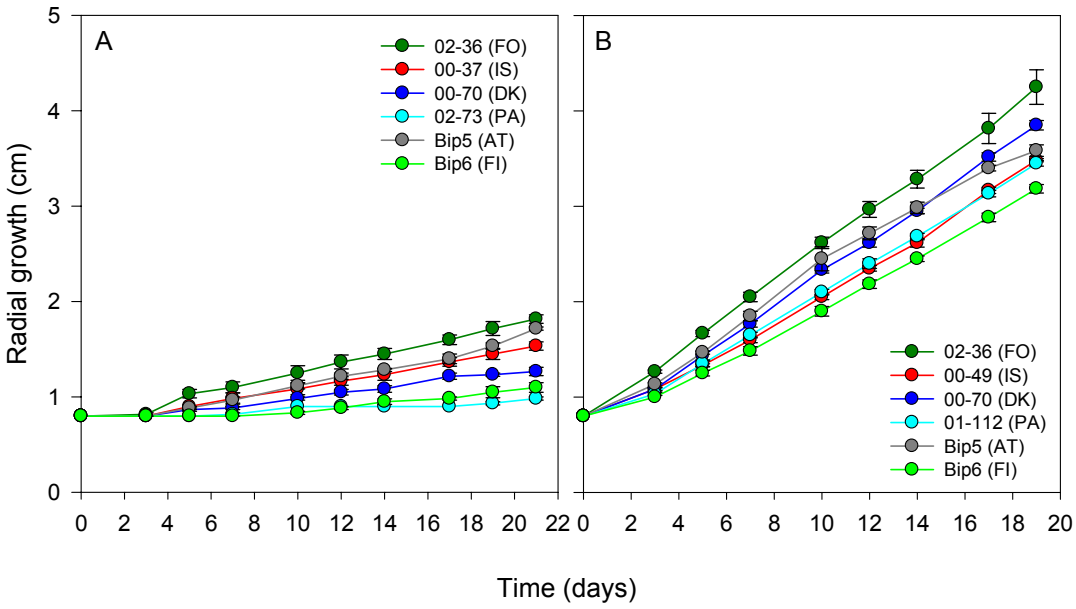

Figure 4.1.10 Mean (±SE) radial growth of Metarhizium anisopliae *at 10°C (A) and 15°C (B). Vertical lines show StE. FO= the Faroe Islands, IS= Iceland, DK= Denmark, PA= Panama, AT= Austria, FI= Finland. Numerical codes refer to the isolate collection of the University of Copenhagen.*

LSD posthoc tests revealed the isolates from the Faroe Islands (02-36) demonstrated significantly faster radial growth at 10°C than isolates from Iceland, Denmark, Austria, Finland and Panama from day 5 onwards (Figure 4.1.10 A; Table 4.1.3).

Table 4.1.3 Results of LSD posthoc test comparing radial growth (at 10°C) of Metarhizium anisopliae *isolate from the Faroe Islands (02-36) to other isolates. Significant p-values shown.*

	M. anisopliae isolate				
Day	00-37 (IS)	00-70 (DK)	02-73 (PA)	Bip5 (AT)	Bip6 (FI)
5	0.008	<0.001	<0.0001	0.003	<0.0001
7	0.02	<0.0001	<0.0001	0.008	<0.0001
10	<0.001	<0.0001	<0.0001	0.008	<0.0001
12	<0.0001	<0.0001	<0.0001	0.003	<0.0001
14	<0.0001	<0.0001	<0.0001	<0.001	<0.0001
17	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
19	<0.0001	<0.0001	<0.0001	<0.001	<0.0001
21	<0.0001	<0.0001	<0.0001	0.04	<0.0001

IS=Iceland, DK= Denmark, PA=Panama, AT=Austria, FI=Finland. Bip5= Bipesco 5, Bip6= Bipesco6. The numerical code refers to the isolate collection of the University of Copenhagen.

The Icelandic isolate (00-37), showed significantly faster radial growth than the Finnish isolate (Bip6) from day 5 and faster than the Danish isolate from day 7 (Figure 4.1.10 A; Table 4.1.4).

Table 4.1.4 Results of LSD posthoc test comparing radial growth (at 10°C) of Metarhizium anisopliae *isolate from Iceland (00-37) to Danish and Austrian isolates. Significant p-values shown, NS=non-significant.*

	M. anisopliae isolate	
Day	00-70 (DK)	Bip5 (AT)
5	NS	0.04
7	0.04	<0.001
10	0.04	<0.0001
12	0.02	<0.0001
14	0.003	<0.0001
17	0.003	<0.0001
19	<0.0001	<0.0001
21	<0.0001	<0.0001

DK= Denmark, AT=Austria, Bip5= Bipesco 5. The numerical code refers to the isolate collection of the University of Copenhagen.

At 15°C, the same isolate from the Faroe Islands showed faster radial growth than all other isolates from day 5, and faster than the isolates from Iceland, Denmark, Panama and Finland from day 3 (Figure 4.1.10 B, Table 4.1.5).

Table 4.1.5 Results of LSD posthoc test comparing radial growth (at 15°C) of Metarhizium anisopliae *isolate (02-36) from the Faroe Islands to other isolates. Significant p-values shown, NS=non-significant.*

	M. anisopliae isolate				
Day	00-49 (IS)	00-70 (DK)	01-112 (PA)	Bip5 (AT)	Bip6 (FI)
3	0.02	0.02	0.002	NS	<0.001
5	<0.0001	0.002	<0.0001	0.009	<0.0001
7	<0.0001	<0.001	<0.0001	0.009	<0.0001
10	<0.0001	<0.001	<0.0001	0.03	<0.0001
12	<0.0001	<0.0001	<0.0001	0.001	<0.0001
14	<0.0001	<0.0001	<0.0001	<0.001	<0.0001
17	<0.0001	<0.001	<0.0001	<0.0001	<0.0001
19	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

IS=Iceland, DK= Denmark, PA=Panama, AT=Austria, FI=Finland. Bip5= Bipesco 5, Bip6= Bipesco 6. The numerical code refers to the isolate collection of University of Copenhagen.

The Icelandic isolate 00-49 grew slower than the Danish and the Austrian isolate from day 7, but faster than the Finnish isolated from day 10 (Figure 4.1.10 B, Table 4.1.6).

Table 4.1.6 Results of LSD posthoc test comparing radial growth (at 15°C) of Metarhizium anisopliae *isolate from Iceland (00-49) to the Danish, Austrian and Finnish isolates. Significant p-values shown, NS=non-significant.*

	M. anisopliae isolate		
Day	00-70 (DK)	Bip5 (AT)	Bip6 (FI)
3	NS	NS	NS
5	NS	NS	NS
7	0.03	0.001	NS
10	<0.001	<0.0001	0.05
12	<0.001	<0.0001	0.03
14	<0.0001	<0.0001	0.03
17	<0.0001	0.002	<0.001
19	<0.0001	NS	<0.001

DK= Denmark, AT=Austria, FI=Finland. Bip5= Bipesco 5, Bip6= Bipesco 6. The numerical code refers to the isolate collection of University of Copenhagen.

Even though it is clear that more intensive studies on occurrence and performance of IPF are needed, these results indicate that IPF strains found in the soils of Iceland and the Faroe Islands may be limited to those that are cold tolerant.

4.1.2 Distribution in different habitats (Paper II and Unpublished data)

Ectomycorrhizal fungi (Paper II and Unpublished data)

When baited with birch seedlings in microcosms, a clear difference in frequency of ECMs between soil types was detected revealing higher number of ECM root tips and morphotypes in birch soil than in eroded soil at Rotarmannatorfur, Thorsmork and Hafnarskogur (Figure 4 in Paper II). At Vaglir, no difference was detected between the birch forest and eroded soils in the number of ECM root tips or morphotypes (Figure 4 in Paper II).

When birch and eroded soil from Hafnarskogur and Vaglir were baited by pine seedlings, no difference in the number of ECM root tips was detected between soil types for either location (Figure 4.1.11 A), but a significantly higher number of morphotypes were documented in birch forest soil than in eroded soil at Hafnarskogur (χ^2=5.6, 0.02) (Figure 4.1.11 B).

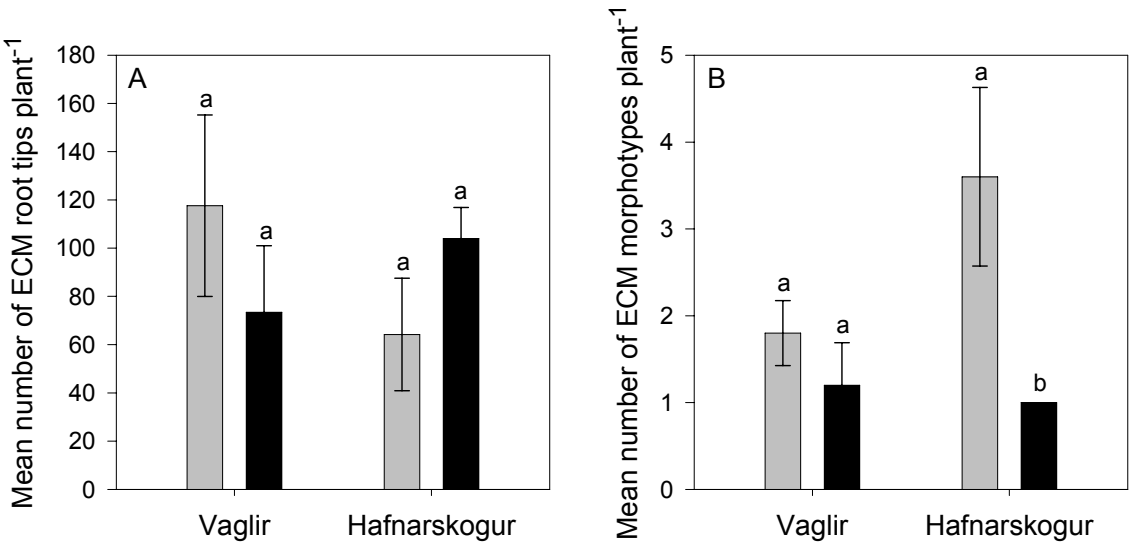

Figure 4.1.11 The mean (±SE) number of ECM root tips (A) and morphotypes (B) on pine seedlings grown in birch woodland soil (▭) and eroded (■) soil from Vaglir and Hafnarskogur. Different letters above bars indicate a significant difference ($p<0.05$) between the paired birch and eroded soil.

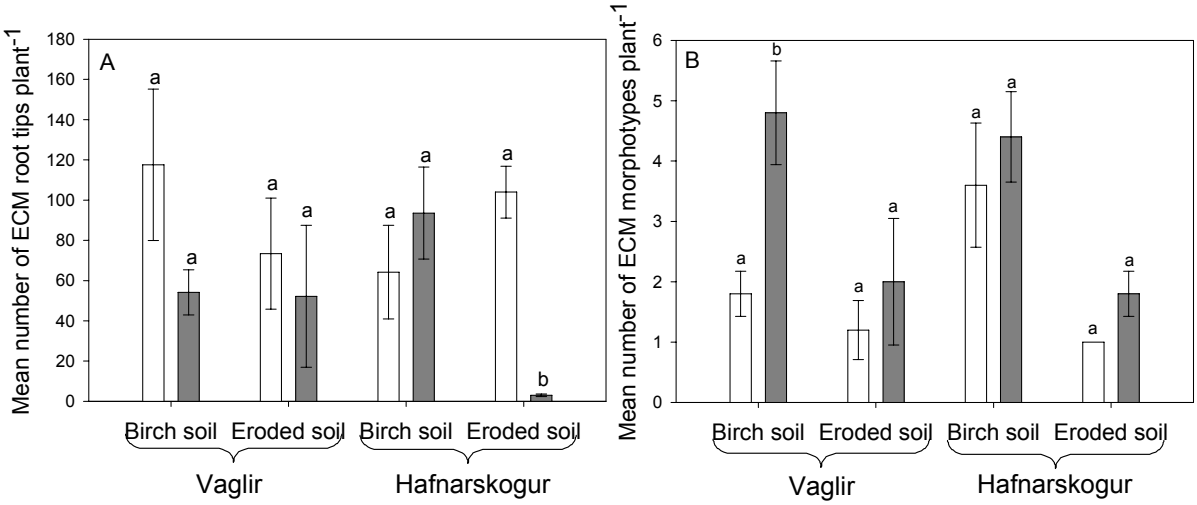

Figure 4.1.12 The mean (±SE) number of ECM root tips (A) and morphotypes (B) on pine (□) and birch (■) seedlings grown in birch woodland soil and eroded soil from Vaglir and Hafnarskogur. Different letters above bars indicate a significant difference ($p<0.05$) between the paired pine and birch seedlings.

The number of ECM root tips was higher on birch roots than on pine roots in birch forest soil from Hafnarskogur, but the difference was not significant (Figure 4.1.12 A). In three soil types (birch forest soil and eroded soil at Vaglir, eroded soil at Hafnarskogur) of four, the average number of ECM root tips plant^{-1} was higher on pine seedlings than on birch seedlings grown in same soil (Figure 4.1.12 A). This difference was significant in eroded soil from Hafnarskogur (χ^2=6.0, p=0.008).

When comparing the number of morphotypes, the average number of morphotypes was lower on pine seedlings than on birch seedlings in both birch forest soil and eroded soil at Hafnarskogur and Vaglir, even though the difference was only significant in birch forest soil at Vaglir (χ^2=2.4, p=0.04) (Figure 4.1.12 B). The most common morphotype on pine seedlings was P1 (Figure 4.1.13), identified as *S. luteus* by direct sequencing. In addition, pine mycorrhiza were in the same RFLP cluster as *P. fortinii* and *P. bicolorata* (Figure 4.1.4), even though it was not verified by sequencing. The latter two species were also identified on birch roots and are therefore part of the natural soil biota in Icelandic birch woodlands. However, even though it is known that *Hebeloma* spp. have little host restriction and can form mycorrhiza with pine (Dunstan *et al.,* 1998; Smith & Read, 2008), no *Hebeloma* spp. were detected on pine roots in this study, indicating that *S. luteus* is more aggressive in ECM formation on pine roots than *Hebeloma* spp.

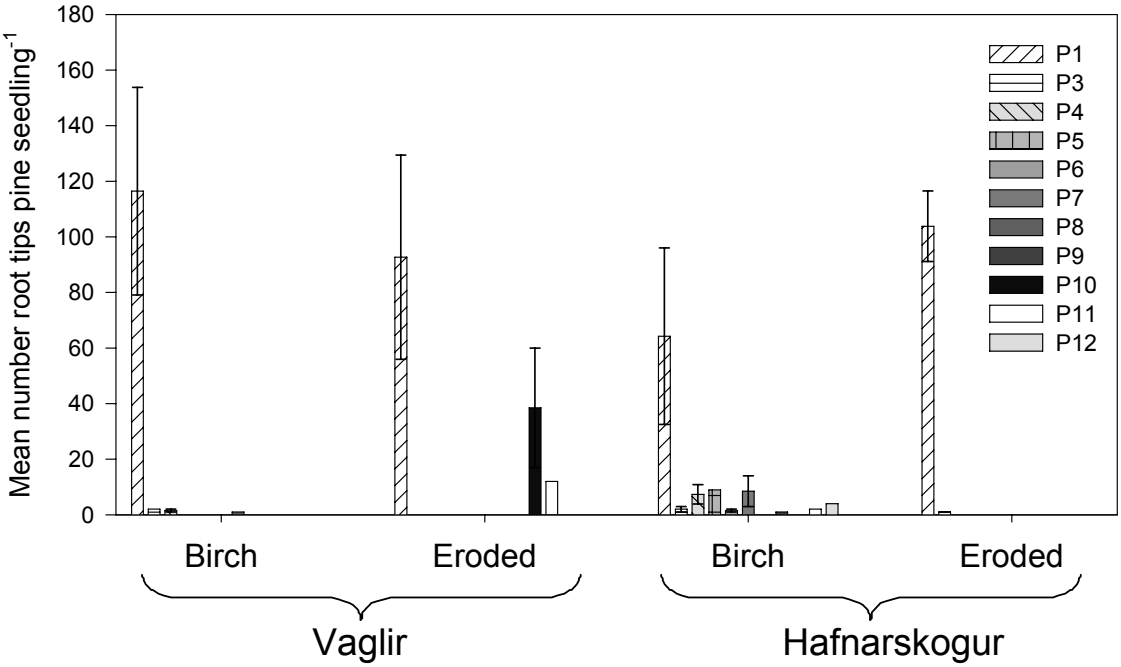

Figure 4.1.13 Mean (±SE) number of pine root tips grown in birch woodland and eroded soil in microcosms, colonized with ECM morphotypes. Note, if the morphotype was only found in one microcosms soil type^{-1} location^{-1}, no SE was calculated.

There are two main methods by which ECM inoculation take place, either by contact of the roots with existing ECM hyphae in the soil or by soil propagules (Smith & Read, 2008). Both birch and pine seedlings, grown in birch forest soil were colonized by mycorrhiza, indicating that adequate mycorrhizal inoculum is in the birch forest soil for ECM inoculation. In soil with low levels of vegetation cover, and no trees, such as the eroded soils in this study, it would be expected that the mycorrhizal inoculum is based on airborne fungal propagules. Since the eroded soil was sampled in the neighborhood of birch woodlands, the fungi known to form mycorrhiza on birch (such as *Hebeloma* species, *P. finlandia* and *P. bicolorata*) are likely to originate from the birch woodlands. However, since *S. luteus* does not form ECM with birch, the origin of the *Suillus* propagules must originate in pine stands, but no pine plantations were close to the soil sampling site although the exact distance was not measured. Since the soil was sampled in the autumn, when the fruit bodies are forming, an active spore distribution and relatively high ECM propagule availability is to be expected. However, the inoculation capability during other seasons, for example during spring and early summer, when high proportion of seedling planting is performed, remains unstudied.

The pine seedlings were more active in forming mycorrhiza in the eroded soil than the birch seedlings, even though the birch seedlings grown in eroded soil had higher numbers of morphotypes. The reasons for this difference are unclear, but as mentioned above, *S. luteus* seems to be very aggressive in ECM formation. *S. luteus* colonizes seedlings mostly through spores dispersed by wind or mammals. It is known to be an important symbiotic fungus on pine in pioneer situations, and increases plant tolerance to toxins in contaminated areas (Colpaert *et al.,* 2000; Adriaensen *et al.,* 2004; Colpaert *et al.,* 2004; Krznaric *et al.,* 2009). *S. luteus* therefore seems highly adapted to difficult areas, such as the eroded sites in this study. It is therefore possible that the difference in mycorrhizal colonization between pine and birch seedlings in soil from eroded sites reflects some difference in the adaptation between *Hebeloma* spp. and *S. luteus*. It is also possible, that the difference in mycorrhizal root colonization reflects a difference between the two tree species. Because pine seeds are larger than birch seedlings, the pine seedlings may have a larger nutrient supply than the birch seedlings, and therefore more readily form ECM. (Table 1, Paper II).

Insect pathogenic fungi (Paper II)

A clear difference between eroded and vegetated soil was detected in IPF occurrence, as IPF was only isolated from birch forests (Rotarmannatorfur, Vaglir, Thorsmork, Hafnarskogur and Skaftafell) and heathland soils (Haukadalur), but not from the soil from eroded sites. Compared to other studies in northerly latitudes, the overall occurrence of IPF in Icelandic soil (17.7%) is lower than that found in Canada (91%) (Bidochka *et al.,* 1998) or in Finland (38.6%) (Vänninen, 1995). The IPF occurrence in Icelandic woodlands and heathlands (32%) is also lower than the IPF occurrence in Finnish forests (52.3%) (Vänninen, 1995). The eroded soils are classified as Vitrisols and characterized by low organic carbon and water holding capacity (Arnalds, 2008b), in addition the annual mean soil temperature is relatively low (Arnalds & Kimble, 2001). Furthermore, eroded soils in Iceland support low numbers of soil arthropods (Oddsdottir *et al.,* 2008b), indicating a low biological activities. Since the persistence of IPF is known to be affected by soil type, soil moisture, temperature and biological characteristics (Vänninen *et al.,* 2000) the characteristics of the eroded soils in Iceland are likely to hinder the occurrence of IPF.

4.2 The effects of soil beneficial fungi on *Otiorhynchus* larvae (Paper III)

In order to estimate the effects of soil beneficial fungi on *Otiorhynchus* larvae, birch seedlings were inoculated with ECM and IPF (Paper III, Table 1) prior to introduction of *O. sulcatus* larvae. There was a significant difference in larval survival between inoculation treatments, with the highest in control pots and pots inoculated with *P. finlandia*. All other inoculation treatments showed higher rates of larval mortality (Figure 1 in Paper III). A significant effect of *M. anisopliae* inoculation on larval survival was not surprising, since *M. anisopliae* is highly a virulent pathogen against weevil and other soil inhabiting larvae (Moorhouse *et al.,* 1993a, 1993b, 1994; Bruck *et al.,* 2005b). Although the effects of AM on *O. sulcatus* larvae have been studied to some extent (Gange *et al.,* 1994; Gange, 2001; Gange & Brown, 2002), imitated studies on the effects of ECM inoculation are available. To the best of the author's knowledge, Paper III is the first paper to report the effects of ECM on root herbivorous larvae but the mechanisms involved in the protection provided by the ECM against the larvae remain unclear. However, *P. finlandia,* which in symbiosis forms a thin mantle and constricted external hyphae, did not have negative effects on the larvae, whereas ECM fungi that form thicker mantles and more extensive external hyphae (*Laccaria laccata* and *Cenococcum geophylum*), negatively affected larval survival. It is possible that the protection mechanisms involve physical barrier formation by the ECM. However, it must be noted that in Paper III, root damage was insignificant and there was no difference between the ECM species used, indicating the ECM effects larval survival through other means. Therefore, the existence and form of other possible mechanisms, such as chemical interactions or modification of the rhizosphere remains to be determined (Whipps, 2001).

No combined effects of the two fungi selected for combined inoculation (*P. finlandia* and *M. anisopliae*) was detected on larval survival. This is in contrast with other results, that have shown either synergistic (Ansari *et al.,* 2008) or antagonistic (Gange, 2001) effects of combined inoculation. It is possible that selection of another ECM fungus detected on birch roots in the present study, e.g. *H. mesophaeum*, may have resulted in more of a synergistic response, but inoculation with *P. finlandia* alone did not have significant effects

on the survival of *O. sulcatus*. Recent studies by St Leger (2008) suggest that it is important to take into consideration the rhizosphere competence of fungal strains when selecting IPF for biocontrol. The results from the present study indicate such competence was present. It is therefore clear that careful selection of species for inoculation is necessary, in either combined or single species inoculation.

It is unclear whether the negative effect of inoculation with birch forest soil on larval survival can be attributed to IPF, mycorrhizal fungi or the combined effects of the soil biota existing in the forest soil. It is clear that *O. sulcatus* is affected by inoculation with beneficial soil fungi, but the mechanisms by which ECM affect root-feeding insects remains unclear.

The fact that no difference in larval weight was detected between treatments, despite other studies having shown that AM can negatively affect larval weight (Gange *et al.,* 1994; Gange, 1996), are at least partially explained by the lack of larval feeding on roots in the pots (Paper III).

4.3 The effects of inoculation with beneficial fungi on root damage (Papers I, III and IV)

To estimate the effects of inoculation on root damage caused by *Otiorhynchus* larvae, two field experiments (Papers I and IV) and one pot experiment (Paper III) were designed, with different inoculation techniques. In Paper I, larch seedlings grown in mature forest soil prior to planting in heathlands had reduced root damage intensity compared to seedlings grown in customary *Sphagnum* peat (Figure 2, Paper I). It was concluded that this effect resulted largely from ECM inoculation, since a distinct difference was detected in the number of ECM root tips between treated and non-treated seedlings prior to planting (Gudmundur Halldorsson, unpublished data). However, these results do not reveal how the fungi affect the root damage, the value of the interaction between different soil biota groups or the importance of different functional group in the reduction of root damage.

In paper IV it was shown that inoculation of birch seedlings with known ECM and IPF, prior to planting in the field, had significant negative impact on root damage. The inoculation reduced root damage on the eroded site and within the birch forest, but the opposite affect when seedlings were planted in heathland (Figure 2, Paper IV).

The reduction of root damage intensity in eroded and birch forest sites, when inoculated with ECM or *M. anisopliae*, indicates the fungi influence the feeding behaviour and/or the survival of the *Otiorhynchus* larvae. The IPF *M. anisopliae* is known to increase the mortality rate of some *Otiorhynchus* species (Moorhouse *et al.,* 1993a), indicating the decreased root damage in treatments where *M. anisopliae* was applied, can to a large extent be explained by the efficacy of the IPF. The effects of mycorrhiza on root herbivorous larvae are less well studied than the effects of IPF. AM can reduce growth and survival of *O. sulcatus* larvae, feeding on plant roots (Gange *et al.,* 1994; Gange, 2001), but apart from the data presented in Paper III, few or no studies regarding the effects of ECM on root herbivory are available. Based on the results presented in this thesis, it is clear that ECM can affect the level of root damage, but it is not known whether ECM fungi affect the larvae directly, for example by improving the nutrient and defense status of the host plant, or indirectly, for example, by altering the mycorrhizosphere composition and functioning as indicated by Frey-Klett *et al*. (2005).

The difference in response to inoculation between planting sites (eroded and birch forest *vs.* heathland) may partly be explained by interaction between inoculated species and existing soil biota. Interestingly, the heathland sites in Paper I and Paper IV are similar. Both are located in Haukadalur within 1 km from each other, with vegetation dominated by dwarf shrubs (*Betula nana*, *Salix* spp. and *Vaccinum uliginosum*) and similar methods of soil preparation were used before planting. Still, the decrease in root damage, recorded when inoculated with forest soil (Paper I), was not present when seedlings were inoculated with ECM and/or *M. anisopliae* (Paper IV). Apart from the inoculation technique, other dissimilarities between these two experiments were the difference in tree species used (*Larix sibirica* vs. *Betula pubescens*). This might explain the differences between these two studies, since different tree species are likely to have different accompanying soil biota. Furthermore, inoculation with forest soil is more likely to ensure greater selection of beneficial soil biota, including adapted fungi, than inoculation with specific fungi.

Paper III reports a pot study originally intended to explore the effects of inoculation with single ECM fungal species, IPF and forest soil on root damage. However, very modest root damage due to larval feeding was detected on the seedling roots, probably because the larvae introduced were late instar. It was therefore impossible to draw any conclusions regarding root damage from the study.

4.4 The effects of inoculation on occurrence and persistence of soil beneficial fungi in the field (Paper IV and Unpublished data)

In an unpublished data set from the larch field study (Paper I), four ECM morphotypes were recorded at harvesting, but no attempt was made to identify the types on the genus or species level. After one growing season, the frequency of ECM root tips was higher in non-inoculated plots compared to inoculated ones (882 *vs*. 739 ECM root tips), but the difference was not significant (χ^2=0.5, p=0.46). Similar results, that were significant, were shown in Paper IV, were inoculation with ECM fungi appear to increase the number of non-mycorrhizal root tips on eroded, heathland and birch woodland sites (Figure 3, Paper IV). However, a slight tendency towards higher numbers of ECM morphotypes when inoculated with ECM was noted at the eroded site (Figure 4, Paper IV).

Even though an attempt to identify the ECM morphotypes on the genus or species level was not made, three morphotypes recorded in Paper IV were only found on pre-inoculated seedlings and it is likely they represent the inoculant species. However, further identification of inoculants and indigenous ECM fungi, based on detailed morphology (Agerer, 1987-2006) and/or DNA based methods (Horton & Bruns, 2001) are needed to assess the outcome of ECM inoculants.

The outcome of the IPF inoculum in Paper IV was estimated by analyzing soil samples taken from the planting sites after one growing season. The higher occurrence of *M. anisopliae* in *M. anisopliae* treatments show the fungi persist at least during the first summer. However, since no further samples were taken for analysis, it is not known if the fungi survived the winter.

4.5 The effects of beneficial fungi on plant performance (Papers I, IV and Unpublished data)

To estimate the effects of inoculation with beneficial fungi on plant performance, the seedling survival and height increment was measured in field experiments (Papers I and IV). Furthermore, seedling survival, height, shoot and root weight of pine and birch seedlings was measured in the microcosm experiments (Unpublished data).

In the microcosm experiments, birch and pine seedlings were grown in microcosms containing birch forest soil or eroded soil for 44 weeks before harvesting. By that time, a total of 13 seedlings had died, 10 seedlings grown in eroded soil (9 birches and 1 pine), but only three birch seedlings grown in birch soil died during that period. The soil type had a significant effect on birch (Table 4.5.1, Figure 4.5.1) and pine (Figure 4.5.2) performance parameters.

Table 4.5.1 Results of Two-way ANOVA on the difference of average shoot height, root column diameter, shoot and root weight between birch seedlings grown in birch woodland and eroded soil from four locations (Rotarmannatorfur, Vaglir, Thorsmork and Hafnarskogur) in Iceland.

		Height		Root column diameter		Shoot weight		Root weight	
	df	F	*p*	F	*p*	F	*p*	F	*p*
Location	3	0.8	NS	0.5	NS	1.0	NS	0.9	NS
Soil type	1	39.7	*<0.0001*	23.7	*<0.0001*	38.4	*<0.0001*	20.3	*0.0002*
Location * Soil type	3	0.5	NS	1.0	NS	1.4	NS	3.0	NS

DF = degrees of freedom; NS = no significant difference

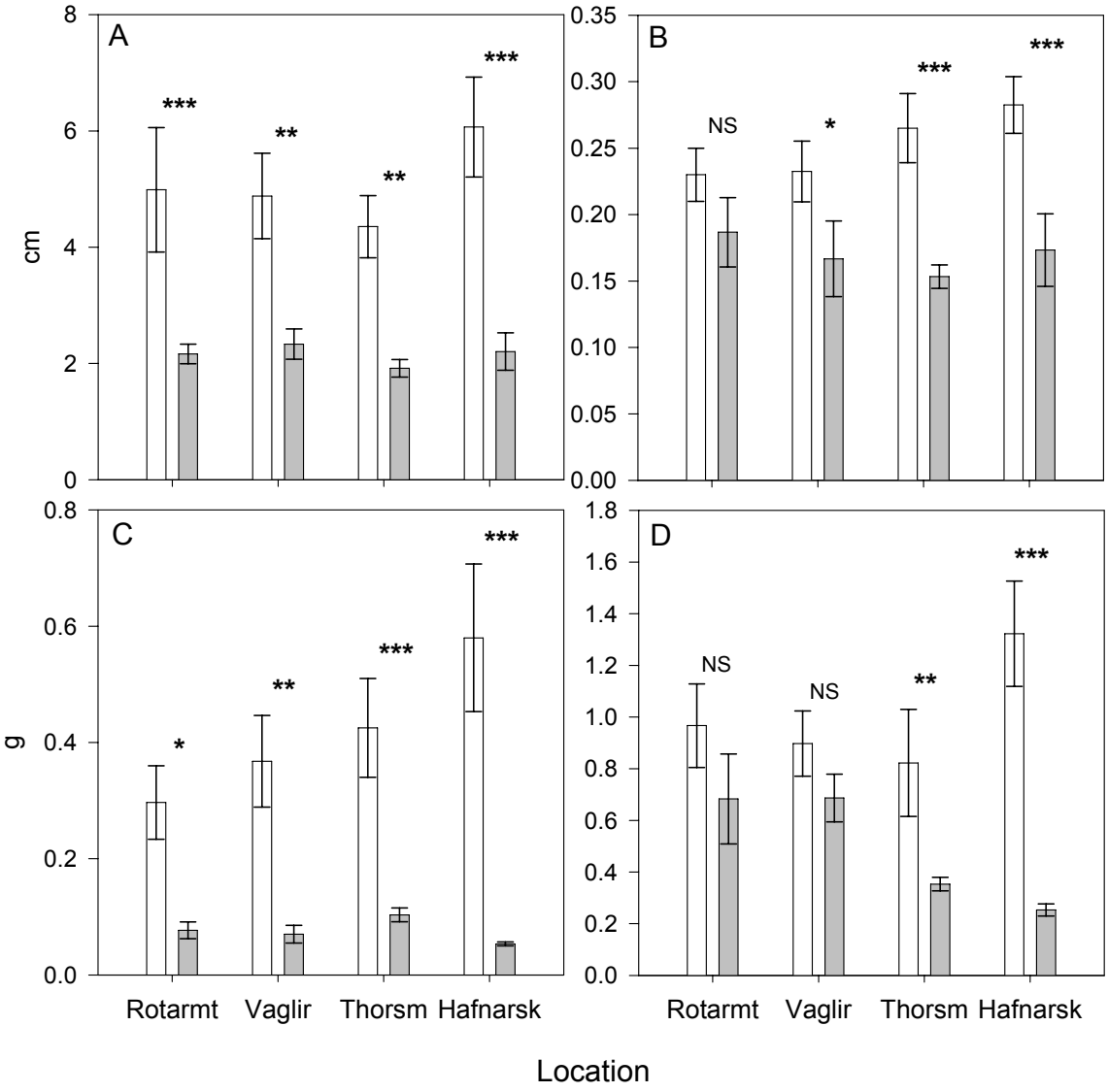

Figure 4.5.1 Mean (±SE) height (A), root column diameter (B), shoot weight (C) and root weight (D) of birch seedlings grown in birch woodland (□) and eroded (■)soil from Rotarmannatorfur (Rotarmt), Vaglir, Thorsmork (Thorsm) and Hafnarskogur (Hafnarsk). Labels above bars show significant difference (= p<0.1; ** = p<0.05; *** = p<0.01; NS = no significant difference) between the paired birch forest soil and eroded soil.*

Birch seedlings grown in birch soil were significantly taller with a greater shoot mass than birch seedlings grown in eroded soil for all soils (Table 4.5.1, Figure 4.5.1 A and 4.5.1 C). Root column diameter was significantly greater for seedlings grown in birch forest soil for three locations (Figure 4.5.1 B) and the root systems were heavier for two locations (Figure 4.5.1 D).

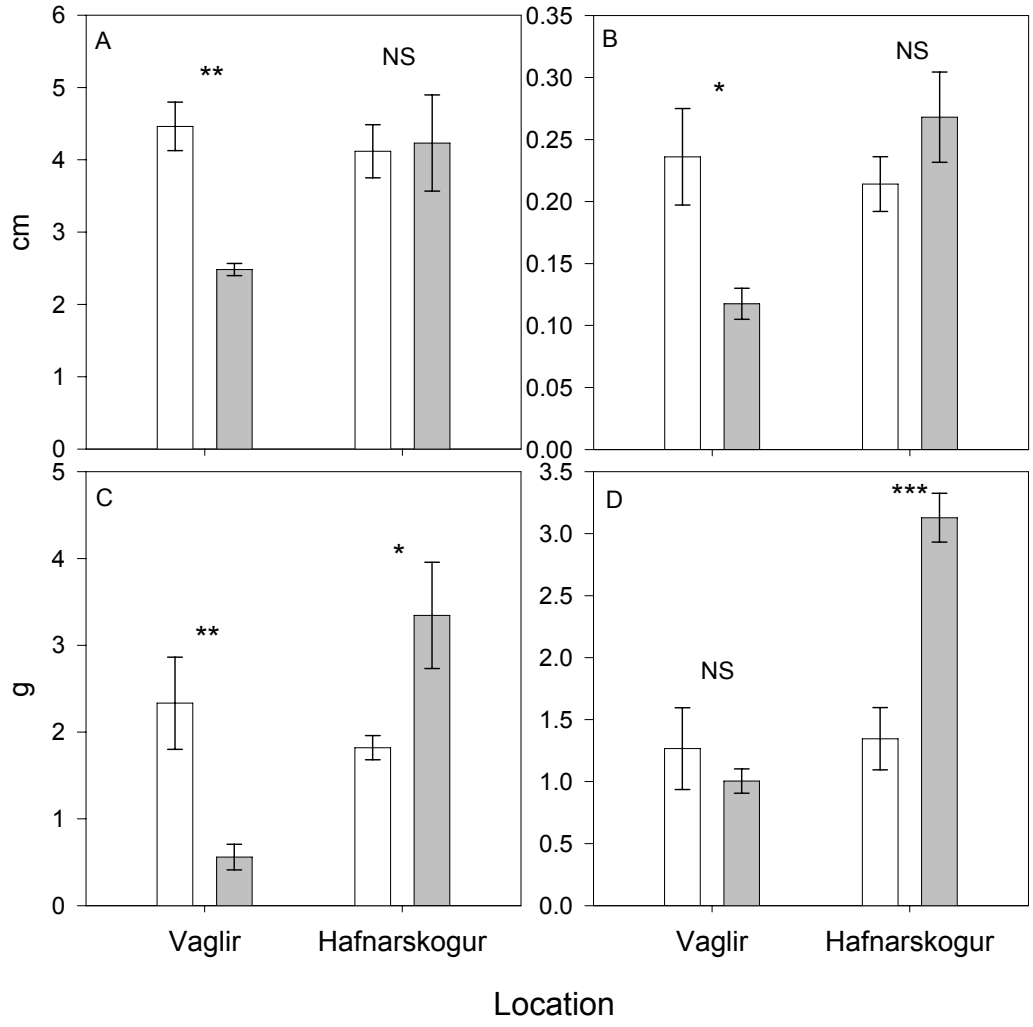

Figure 4.5.2 Mean (±SE) height (A), root column diameter (B), shoot weight (C) root weight (D) of pine seedlings grown in birch woodland (□) and eroded (■)soil from Vaglir, and Hafnarskogur. Labels above the bars show significant differences (= p<0.1; ** = p<0.05; *** = p<0.01; NS = no significant difference) between the paired birch forest soil and eroded soil.*

Pine seedlings grown in eroded and birch forest soils from Vaglir, showed similar tendencies as the birch seedlings. Seedlings were generally more robust when grown in birch forest soil than their eroded soil counterparts (Figure 4.5.2). On the other hand, pine seedlings grown in soil from Hafnarskogur showed the opposite tendency, i.e. seedlings grown in eroded soil had significantly greater shoot (Figure 4.5.2 C) and root mass (Figure 4.5.2 D).

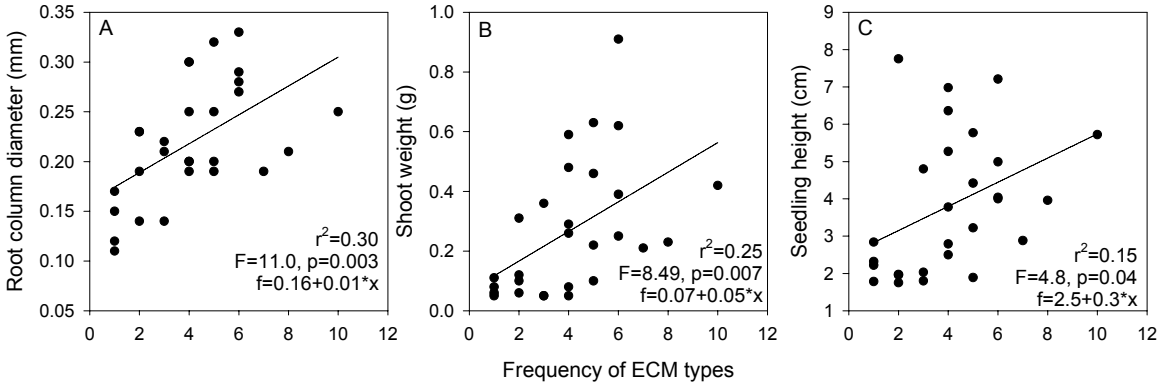

Figure 4.5.3 Regression analyses for the frequency of ECM types on birch seedlings grown in microcosms and birch root diameter (A), birch shoot weight (B) and birch height (C).

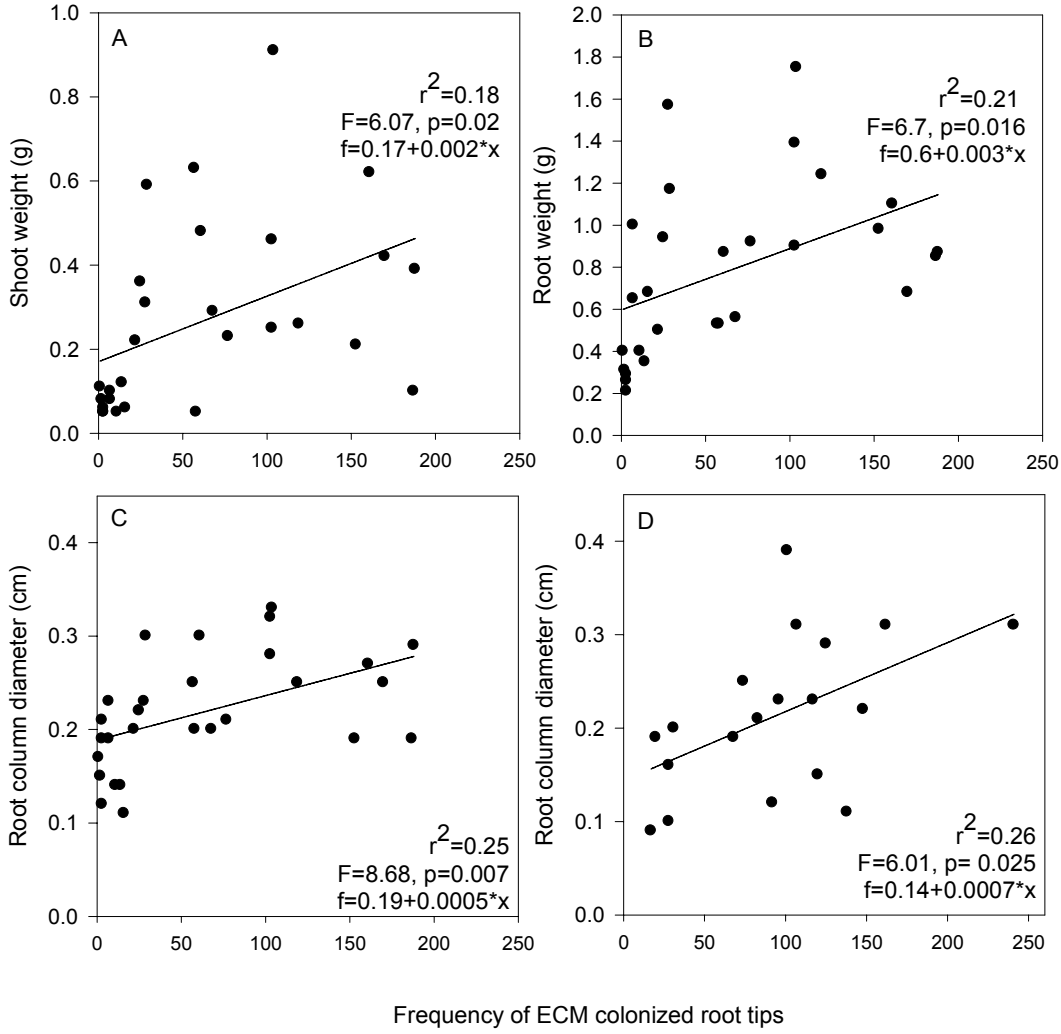

Figure 4.5.4 Regression analyses for the frequency of ECM colonized root tips on birch seedlings grown in microcosms vs. *birch shoot weight (A), birch root weight (B) birch root column diameter (C). Frequency of ECM colonized pine root tips* vs. *pine root column diameter (D).*

There was a weak positive correlation between the frequency of ECM types on birch roots and birch seedling root column diameter (Figure 4.5.3 A), shoot weight (Figure 4.5.3 B), and height (Figure 4.5.3 C). Furthermore, a weak positive correlation was found between the frequency of ECM colonized root tips on birch seedlings and birch shoot weight (Figure 4.5.4 A), birch root weight (Figure 4.5.4 B) and birch root column diameter (Figure 4.5.4 C). A weak positive correlation between the total number of ECM colonized pine root tips and root column diameter of pine (Figure 4.5.4 D) was also found. No other significant correlation was detected between ECM and pine or birch seedlings performance parameters. In the microcosm study, the focus was on early establishment of seedlings. ECM fungi are necessary to the plants uptake of soil nutrients (Smith & Read, 2008), especially during establishment on exposed areas (Obase *et al.,* 2008) as would be the case on the eroded sites in the present study. Therefore, it is not surprising that the poor ECM colonization on birch roots was reflected in birch performance, such as plant mortality, height and shoot weight, even though the correlation was weak.

Results from the two field studies are contradictory regarding seedling survival and growth. In Paper I, a distinct difference was found in seedling mortality between inoculated and non-inoculated seedlings in heathland and a considerable proportion of the non-inoculated seedlings died. Furthermore, the effects of inoculation were still visible in seedling mortality three years after planting (Paper I, Figure 2). In Paper IV however, no long term effect of inoculation on plant mortality was observed, but a significant difference was detected between different planting sites, with the highest mortality rates on the eroded site and the lowest on the heathland site. This site-dependant effect might be a sign of interaction between the introduced and native soil organisms, especially since eroded sites in Iceland seem to contain fewer soil organisms than vegetated areas (Aradottir, 1991; Oddsdottir, 2002; Oddsdottir *et al.,* 2008b; Paper II).

The results of the two papers also differed in seedling growth rates. In Paper I, larch seedlings grown in forest soil prior to planting in heathland, were significantly taller than the uninoculated seedlings during the first growth season (Paper I, Figure 3), whereas no treatment effects were detected in height growth of birch seedlings discussed in Paper IV when planted in eroded, birch woodland or heathland sites (Paper IV, Figure 1). These results are similar to a Spanish study, where *Betula celiberica* Roth & Vasc. seedlings inoculated with *Leccinum scabrum* did not show significantly more growth than

uninoculated seedlings (Dunabeitia *et al.,* 2004). In Paper IV, the only treatment effect on plant performance detected was at the birch site, where inoculation with *M. anisopliae* reduced the root collar diameter in comparison to inoculation with only ECM (Paper IV, Figure 1).

There are several reasons that might explain the difference in seedling growth and survival between the experiments in Paper I and IV. The experiments differed in several ways, including different tree species (larch in Paper I vs. birch in Paper IV) and different inoculation strategies. In Paper I, larch seedlings were inoculated by growing them in soil from mature larch forests for three months before planting, while birch seedlings in Paper IV were inoculated by specific ECM and IPF species one week before planting. The inoculation with forest soil ensured larch seedlings a greater selection of soil organisms, including adapted IPF, ECM and other soil biota (e.g. antagonistic and/or mycorrhizal helper bacteria) (Garbaye, 1994) necessary for the successful establishment and function of IPF and ECM. Growth responses of trees may depend on the ECM fungal species available (Manninen *et al.,* 1999; Jonsson *et al.,* 2001; Nerg *et al.,* 2008) and ECM formation on birch seems to follow a highly ordered pattern (Mason *et al.,* 1982). Thus, some ECM species may be more important during the early establishment of birch than others. Even though early stage fungi as *Hebeloma* sp. was used for inoculation in Paper IV, the lack of other appropriate early stage ECM fungi or necessary helper bacteria might explain the difference in the results between the two papers. This is further supported by the fact that seedlings grown in birch forest soil in the microcosm experiment generally showed better performance than seedlings grown in eroded soil containing low amounts of fungal inoculum.

The different results may also reflect the difference in the time from inoculation until planting, since the beneficial soil organisms had a longer time to become established on larch seedling roots than on the birch seedlings. The initial establishment of ECM on birch roots in Paper IV may have demanded more plant resources than were available in the field and therefore hampered seedling growth, while the ECM establishment of larch was accomplished under richer resources in the greenhouse before planting.

4.6 Methodological considerations

4.6.1 Isolation of mycorrhizal and insect pathogenic fungi

Microcosms *vs*. field experiment setup

Microcosms have been used extensively for the experimental study of ecological systems (Read, 2002). The use of such standardized laboratory growth conditions can include unnatural variables that might result in biased observation and conclusions. Also, microcosms use a relatively small mass of homogenized soil and can therefore fail to simulate the complexity of the soil community and the experimental duration is relatively short, compared to the time scale of many ecological processes. Furthermore, the impact of biotic and abiotic factors, such as litter fall or precipitation, is minimized. However, field studies are not always a feasible option, since they may be too costly or laborious to carry out and it is often impossible to study detailed mechanisms in the field. Microcosms allow a greater degree of control and ease of replication and repeatability than is available in the field. In such cases, the microcosms approach may be more appropriate and produce important information.

In the present study, the occurrence and identification of mycorrhiza on birch and pine seedlings in different soil types was estimated by using the microcosm-seedling baiting setup. The diversity of ECM fungi in the root system of Scots pine in microcosms simulated fairly accurately conditions found in the field (Heinonsalo, 2004; Heinonsalo & Sen, 2007; Heinonsalo *et al.,* 2007) and Jonsson *et al.* (1999a) reported that bait seedlings reflects the ECM community structure of the adjacent trees as well. Similar results were reported by Hrafnkelsdottir (2009), but she compared ECM diversity (based on gross morphotyping) of larch forests by using bait seedlings in microcosms and analyzing roots harvested in the field. However, in all the previous studies, the focus was on species rich Scots pine (Jonsson *et al.,* 1999a; Heinonsalo, 2004) or larch (Hrafnkelsdottir, 2009) forests, not on degraded ecosystems as in the current study. Furthermore, soil was only sampled once during the present study that could limit the number of mycorrhizal species detected. However, as the main purpose of the study was to compare the different soil types, the microcosm approach was very useful.

Isolation of insect pathogenic fungi

In this study, IPF were isolated by baiting with laboratory reared larvae (*Galleria mellonella* and *Tenebrio molitor*) in accordance with IOBS guidelines (Zimmerman, 1998). The baiting of soil samples with *G. mellonella* is a widely applied tool for screening indigenous species of insect pathogenic fungi (Vänninen, 1995; Chandler *et al.,* 1997; Bidochka *et al.,* 1998; Klingen *et al.,* 2002a; Meyling & Eilenberg, 2006). There are few studies that evaluate the use of bait insects of different taxa, but Klingen *et al*. (2002a) reported that *Delia floralis* (Fallén) larvae were more effective in isolating the insect pathogenic fungi *Tolypocladium cylindrosporum* Gams than *G. mellonella*. In the present study, the main purpose was to compare the IPF occurrence in different soil types and production of native IPF inoculum for further studies. To improve the probability of isolating existing IPF in the soil, both *T. molitor* and *G. mellonella* were used as bait insects. As *M. anisopliae* was only isolated with *T. molitor* larvae, but not with *G. mellonella* this turned out to be an essential method. However, because of problems in acquiring and rearing *O. nodosus* or *O. arcticus* larvae*,* no selective bait study using those larvae was performed.

Another method for isolation of IPF from the soil is soil suspension on selective media. However, since only a small soil sample (1 g) is used in the isolation process the risk of missing the fungus during sampling is relatively high. Furthermore, hypocrealean IPF grow relatively slow compared to other fungi found in the soil and are therefore the IPF are often overgrown on the media (Meyling, 2007).

4.6.2 Identification of mycorrhiza

This study adopted a stratified sampling approach where mycorrhiza were classified by gross morphotyping that recorded general characteristics (color, shape, hyphae and mantle structure) of the mycorrhizal root tips. This is a good method to process large numbers of root tips and gives a good estimate of the diversity of the individual seedling or microcosms (Heinonsalo *et al.,* 2001; Heinonsalo *et al.,* 2007; Heinonsalo & Sen, 2007). However, this method does not itself allow for species comparison between individuals or microcosms. It was therefore important to standardize each morphotype group by using PCR-RFLP or sequencing. The PCR-RFLP fingerprinting was not rigorous enough for the identification of large parts of the mycorrhizal groups, especially those on birch seedlings.

Furthermore, difficulties in obtaining one PCR band from the birch mycorrhizal root tips led to the necessity of cloning and subsequently, sequencing.

4.6.3 Mycorrhizal inoculation

Inoculation with mycorrhiza have been performed in several studies (e.g. Rincon *et al.,* 2001; Enkhtuya *et al.,* 2003; Heinonsalo *et al.,* 2004; Gagne *et al.,* 2006; Quoreshi & Khasa, 2008) and there are numerous methods used to prepare and apply mycorrhizal fungal inoculum, with variable levels of technical sophistication (Schwartz *et al.,* 2006). In the present study, two different inoculation methods were used. The first one is a relatively simple and straight-forward method, growing the seedlings in soil from a mature forest and this method was used both in the field (Paper I) and in the laboratory (Paper III). By using this method, the inoculum is undefined, and a wider variety of soil organisms are present than just mycorrhizal or other beneficial fungi, possibly including pathogenic fungi, soil invertebrates and prokaryotes. The results presented in this thesis indicate that inoculation with soil from mature forests, may be successful in promoting seedling growth and decreasing root damage caused by *Otiorhynchus* spp., and even more successful than inoculation with specific mycorrhizal species. However, due to the undefined nature of the forest soil inoculum, it is difficult to analyze the effects of individual functional groups in the soil on the host plant. Furthermore, since the undefined soil inoculum very probably contains plant pathogens that can outcompete the beneficial organisms under favorable conditions, the use of unsterile soil is not feasible in greenhouses.

Another approach to inoculating with mycorrhiza is the use of specific mycorrhizal inoculum. The production of such inoculum has evolved in recent years and now several companies produce mycorrhizal inoculum (Gianinazzi & Vosatka, 2004) and inoculation of birch seedlings with Terra Vital-G Ecto Mix from PlantWorks Ltd. (Sittingbourne, UK,) has been used successfully in Iceland (Enkhtuya *et al.,* 2003). There are several factors that are important to consider before inoculation (Schwartz *et al.,* 2006), one being the selection of inoculum species and even specific genotypes. It is clear that mycorrhizal species and genotypes used in studies on the effects of mycorrhiza must be carefully selected, with regard to both the host plant and ultimate habitat (Read, 2002). In the present study, inoculation with specific mycorrhizal inoculum was carried out in two papers, the pot experiment (Paper III) and one of the field experiments (Paper IV). In the pot

experiment (Paper III), the main goal of the study was to investigate the effects of mycorrhiza on the survival of *O. sulcatus* and for that purpose three mycorrhizal fungi were selected, *P. finlandia*, *C. geophylum* and *L. laccata*. The mycorrhizal fungus *P. finlandia* was the only indigenous mycorrhizal strain available and the other two were selected because the species have been found in Iceland and are known to form mycorrhiza on birch. In the field experiment (Paper IV), a mixture of four mycorrhizal species known to form mycorrhiza on birch, was used. Two of species were Icelandic isolates, *P. finlandia* (the same isolate used in Paper III) and *Hebeloma* sp. The other two were *T. terrestris* and *P. involutus* from PlantWorks Ltd's. Both species form ECM with birch, but have not been documented in Iceland.

5 SUMMARY and CONCLUSIONS

The main findings of this thesis are:

- There was a distinct and significant difference in the distribution of soil beneficial fungi (ECM and IPF) between vegetated and eroded sites in Iceland.
- IPF were found in vegetated ecosystems, but not in eroded soil.
- Particular fungal species were recorded for the first time in Iceland during the study.
- Inoculation with ECM or IPF reduced the number of living *O. sulcatus* larvae under controlled conditions. However, there was a difference between different ECM species.
- Inoculation of birch seedlings before planting significantly reduced root damage intensity in the field when planted in eroded or birch habitat.
- Inoculation of birch seedlings planted in heathland increased root damage.
- No synergistic effects were detected on the survival of larvae or root damage intensity when inoculated with both ECM and IPF.
- Low levels of beneficial soil organisms on eroded sites are indicated as cause of high levels of root damage and seedling mortality.

Eroded sites in Iceland are recalcitrant to afforestation (Aradottir & Magnusson, 1992a; Aradottir & Gretarsdottir, 1995), possibly due to lack of soil biota as seen in Paper II. Therefore, inoculation with beneficial soil biota might be beneficial to the enhancement of seedling survival in afforestation on poor sites in Iceland, as indicated by the results from the present study. However, this thesis also highlights the importance of the careful selection of inoculants, as shown both under controlled situations (Paper III) and field conditions (Paper IV). This is particularly apparent when comparing results from inoculating with soil from mature forests (Papers I and III) to inoculation with specific fungal species (Papers III and IV). While the forest soil inoculation had positive effects on seedling performance (Paper I), reduced root damage caused by *Otiorhynchus* spp. (Paper I) and increased *Otiorhynchus sulcatus* larval mortality (Paper III), no synergistic effects of inoculation with the IPF *Metarhizium anisopliae* and ECM *Phialophora finlandia* were

detected on larval mortality (Paper III) or plant performance (Paper IV). This indicates that some important functional guilds from the forest soil ecosystem are missing when inoculation is done using specific fungi. Therefore, studies on the spatiotemporal dynamics of the forest soil biota in Iceland, enabling the selection of appropriate inoculants, are necessary. This need is further strengthen by the fact that the effects of inoculating birch seedlings with ECM and/or IPF before planting, were dissimilar between different habitats (Paper IV), indicating interactions between the inoculants and native soil biota may affect the outcome of the inoculation.

In this thesis, at least 5 fungal species were documented for the first time in Iceland, three ECM species (*Cortinarius erythrinus, Phiaolocephala fortinii* and *P. finlandia*) and two IPF species (*Beauveria bassiana* and *Metarhizhium anisopliae*). Comparison of radial growth of *M. anisopliae* isolates from Iceland and the Faroe Islands (isolated in a related study) with isolates from Denmark, Finland, Austria and Panama, showed isolates from the Faroe Islands had significantly faster growth at lower temperature than the others, including the commercial isolates. Since the use of IPF is hampered by its inactivity at temperatures below 15°C, these results calls for further studies and isolation of IPF fungi suitable for use North-Atlantic soils.

IPF have been used against soil dwelling pests and their ecology and interaction with insects are relatively well documented (e.g. Hajek, 2004; Roy *et al.,* 2006; Meyling & Eilenberg, 2007). Furthermore, the effects of AM on insect herbivory both above and below ground level (e.g. Gange *et al.,* 1994; Gange, 1996; Gange & Bower, 1997; Gange, 2001; Gehring & Whitham, 2002; Gange *et al.,* 2005) and the effects of ECM on above-ground herbivory have been documented (e.g. Gehring & Whitham, 2002; Gange *et al.,* 2005). However, the impacts of ECM on root herbivory and root herbivorous larvae are poorly studied. In this thesis, new results on the effects of ECM on root damage due to insect herbivory and the insect itself are presented. It is indicated, that ECM fungi affect the mortality of *Otiorhynchus* spp. larvae (Paper III) and has the ability to reduce root damage caused by the larvae (Papers I and IV). However, as mentioned earlier, results were variable, both between the ECM fungal species used and the different planting habitats. It is therefore essential to continue these studies to gain a better understanding of the nature of these interactions.

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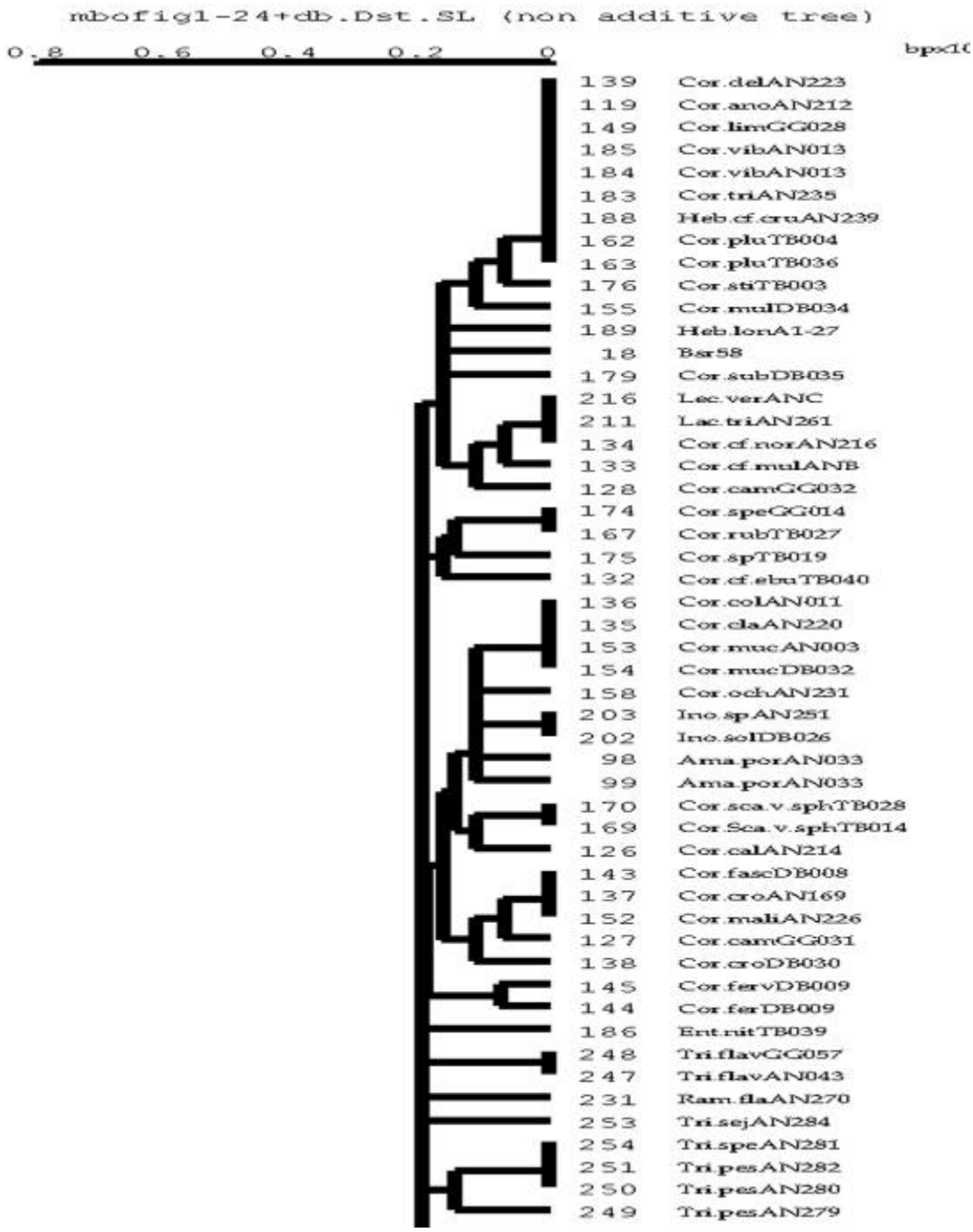
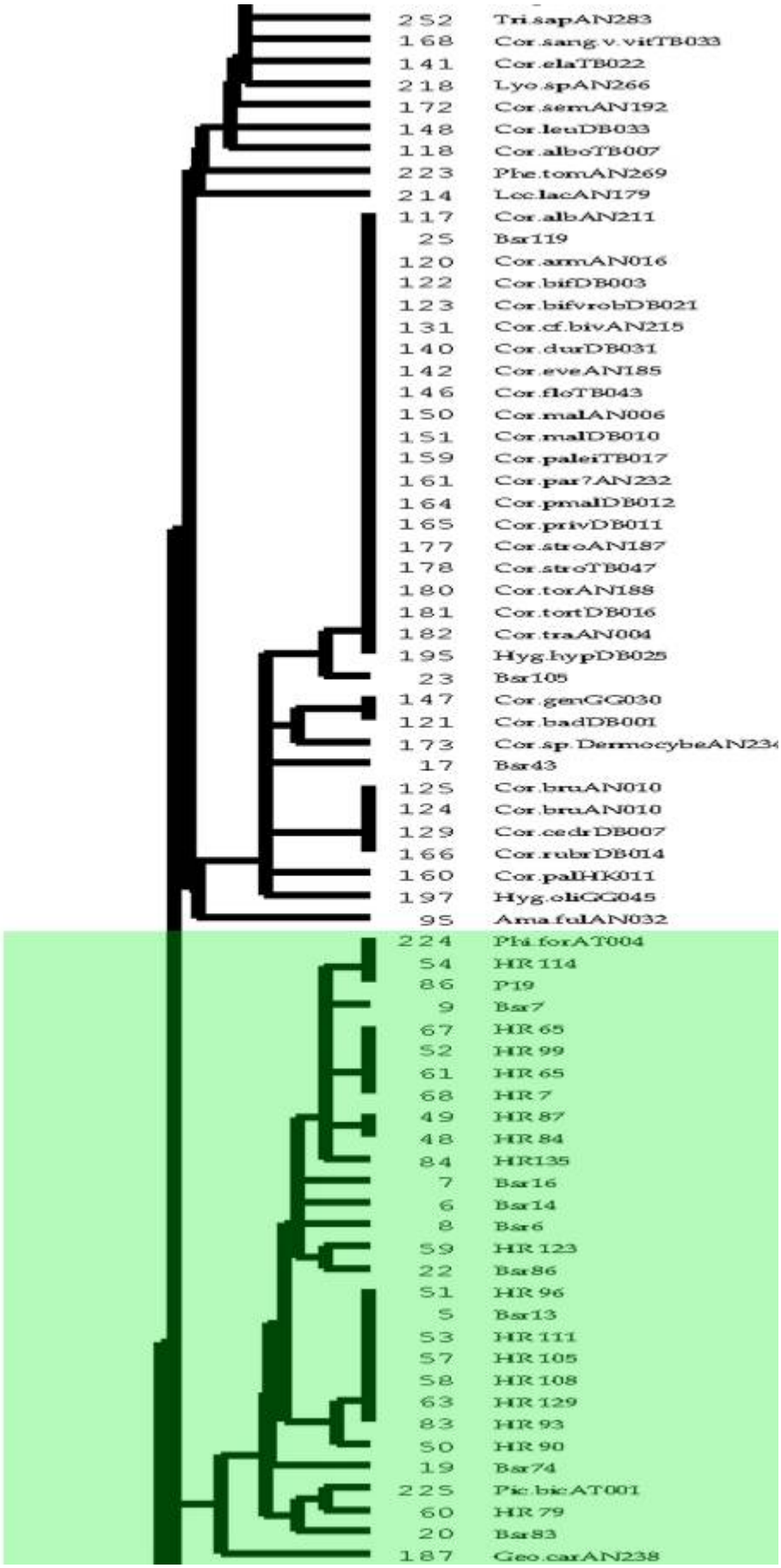
Appendix 1

Dendrogram depicition of single linkage cluster analysis of mycorrhizal fungal ITS-RFLPs representing samples from pure cultures (HR), birch (Bsr) and Scots pine (P) mycorrhizal root tips. T=negative control




252 Tri.sapAN283
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141 Cor.elaTB022
218 Lyo.spAN266
172 Cor.semAN192
148 Cor.leuDB033
118 Cor.alboTB007
223 Phe.tomAN269
214 Lec.lacAN179
117 Cor.albAN211
25 Bsr119
120 Cor.ammAN016
122 Cor.bifDB003
123 Cor.bifvrobDB021
131 Cor.cf.bivAN215
140 Cor.durDB031
142 Cor.eveAN185
146 Cor.floTB043
150 Cor.malAN006
151 Cor.malDB010
159 Cor.paleiTB017
161 Cor.par?AN232
164 Cor.pmalDB012
165 Cor.privDB011
177 Cor.stroAN187
178 Cor.stroTB047
180 Cor.torAN188
181 Cor.tortDB016
182 Cor.traAN004
195 Hyg.hypDB025
23 Bsr105
147 Cor.genGG030
121 Cor.badDB001
173 Cor.sp.DermocybeAN23
17 Bsr43
125 Cor.bruAN010
124 Cor.bruAN010
129 Cor.cedrDB007
166 Cor.rubrDB014
160 Cor.palHK011
197 Hyg.eliGG045
95 Ama.fulAN032
224 Phi.forAT004
54 HR 114
86 P19
9 Bsr7
67 HR 65
52 HR 99
61 HR 65
68 HR 7
49 HR 87
48 HR 84
84 HR135
7 Bsr16
6 Bsr14
8 Bsr6
59 HR 123
22 Bsr86
51 HR 96
5 Bsr13
53 HR 111
57 HR 105
58 HR 108
63 HR 129
83 HR 93
50 HR 90
19 Bsr74
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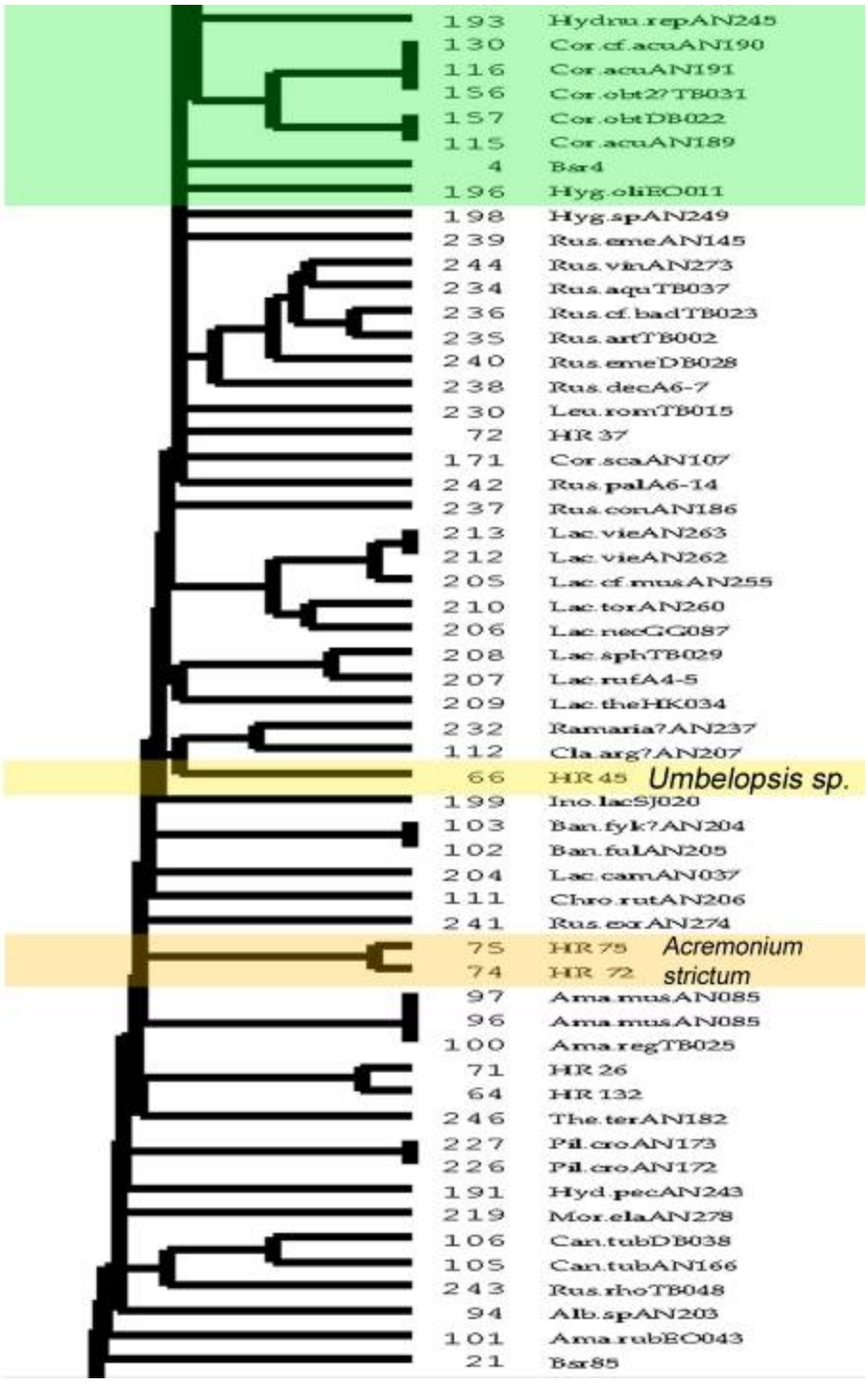
60 HR 79
20 Bsr83
187 Geo.carAN238

Figure 4.1.1

Figure 4.1.1


193 Hydnu.repAN245
130 Cor.cf.acuAN190
116 Cor.acuAN191
156 Cor.obt2?TB031
157 Cor.obtDB022
115 Cor.acuAN189
4 Bsr4
196 Hyg.oliEC011
198 Hyg.spAN249
239 Rus.emeAN145
244 Rus.vinAN273
234 Rus.aquTB037
236 Rus.cf.badTB023
235 Rus.artTB002
240 Rus.emeDB028
238 Rus.decA6-7
230 Leu.romTB015
72 HR 37
171 Cor.scaAN107
242 Rus.palA6-14
237 Rus.conAN186
213 Lac.vieAN263
212 Lac.vieAN262
205 Lac.cf.musAN255
210 Lac.torAN260
206 Lac.necGG087
208 Lac.sphTB029
207 Lac.rufA4-5
209 Lac.theHK034
232 Ramaria?AN237
112 Cla.arg?AN207
66 HR 45 Umbelopsis sp.
199 Ino.lacSJ020
103 Ban.fyk?AN204
102 Ban.fulAN205
204 Lac.camAN037
111 Chro.rutAN206
241 Rus.exrAN274
75 HR 75 Acremonium
74 HR 72 strictum
97 Ama.musAN085
96 Ama.musAN085
100 Ama.regTB025
71 HR 26
64 HR 132
246 The.terAN182
227 Pil.croAN173
226 Pil.croAN172
191 Hyd.pecAN243
219 Mor.elaAN278
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105 Can.tubAN166
243 Rus.rhoTB048
94 Alb.spAN203
101 Ama.rubEC043
21 Bsr85

Figure 4.1.2

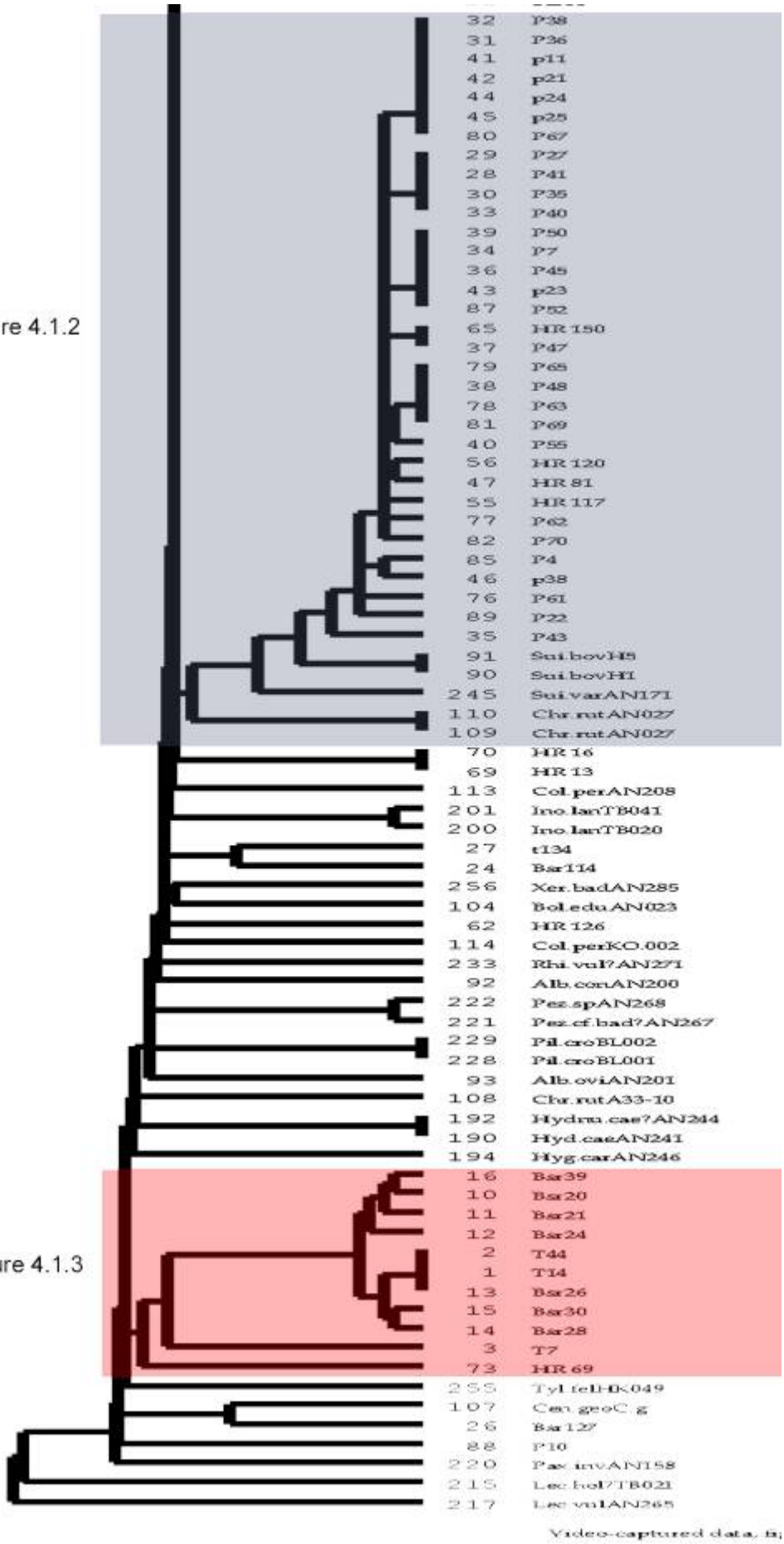

Figure 4.1.3

Video-captured data. fi

Appendix 2

Fungal species (according to the UNITE (Koljalg et al. 2005) and INSD databases) identified from birch root tips.

Clone number		Sample number	Loc.[1]	Soil type[2]	MC[3]	Type[4]	BLAST results				
							Accession number	Name	Score	Identity	E-value
K1	C07	38	T	B	7	1	AY669690	*Cortinarius erythrinus*	1092	601/606 (99%)	0.0
K1	D08	70	H	B	9	1	AY669690	*Cortinarius erythrinus*	1083	596/601 (99%)	0.0
K1	D09	70	H	B	9	1	AY669690	*Cortinarius erythrinus*	1086	600/606 (99%)	0.0
K2	C02	119	H	B	9	1	AY669690	*Cortinarius erythrinus*	1081	599/606 (98%)	0.0
K2	G11	105	H	B	9	1	AY669690	*Cortinarius erythrinus*	1081	599/606 (98%)	0.0
K1	C05	38	T	B	7	1	AY308583	*Hebeloma albocolossum*	1171	651/659 (98%)	0.0
K1	G07	122	T	B	7	2	AY308584	*Hebeloma alpinum*	1144	648/662 (97%)	0.0
K1	A08	22	T	B	1	1	AB211268	*Hebeloma leucosarx*	1208	680/691 (98%)	0.0
K1	C08	40	T	B	1	1	AB211268	*Hebeloma leucosarx*	1256	690/695 (99%)	0.0
K1	C09	40	T	B	1	1	AB211268	*Hebeloma leucosarx*	1260	691/695 (99%)	0.0
K1	C10	40	T	B	1	1	AB211268	*Hebeloma leucosarx*	1255	690/695 (99%)	0.0
K1	D01	42	R	B	8	2	AB211272	*Hebeloma mesophaeum*	1229	690/701 (98%)	0.0
K1	D02	42	R	B	8	2	AB211272	*Hebeloma mesophaeum*	1230	691/702 (98%)	0.0
K1	G09	123	R	B	1	5	AB211272	*Hebeloma mesophaeum*	1260	692/697 (99%)	0.0
K1	G10	123	R	B	1	5	AB211272	*Hebeloma mesophaeum*	1260	692/697 (99%)	0.0
K1	H06	36	R	B	2	2	AB211272	*Hebeloma mesophaeum*	1240	692/701 (98%)	0.0
K1	H07	36	R	B	2	2	AB211272	*Hebeloma mesophaeum*	1234	688/697 (98%)	0.0
K2	A02	2	R	B	2	4	AB211272	*Hebeloma mesophaeum*	1229	690/701 (98%)	0.0
K2	B04	35	R	B	1	2	EF451057	*Hebeloma mesophaeum*	1068	578/578 (100%)	0.0
K2	B11	90	R	B	1	2	AB211272	*Hebeloma mesophaeum*	1236	692/702 (98%)	0.0
K2	B12	90	R	B	1	2	AB211272	*Hebeloma mesophaeum*	1266	694/698 (99%)	0.0
K2	D05	46	R	B	1	5	EF451057	*Hebeloma mesophaeum*	1162	651/661 (98%)	0.0
K2	F01	46	R	B	1	5	AB211272	*Hebeloma mesophaeum*	1234	694/705 (98%)	0.0
K2	G07	46	R	B	1	5	AB211272	*Hebeloma mesophaeum*	1229	694/706 (98%)	0.0

[1]Loc.= Soil sampling location (T=Thorsmork, H=Hafnarskogur, V=Vaglir, R=Rotarmannatorfur), [2]B=birch soil; C=eroded soil,
[3]MC=microcosms number, [4]Type=Type according to gross morphotyping.

Appendix 2. Fungal species identified from birch root tips (cont.).

Clone number		Sample number	Loc.[1]	Soil type[2]	MC[3]	Type[4]	BLAST results				
							Accession number	Name	Score	Identity	E-value
K2	G10	90	R	B	1	2	AB211272	*Hebeloma mesophaeum*	1262	694/699 (99%)	0.0
K2	G12	107	R	B	1	5	AB211272	*Hebeloma mesophaeum*	1245	691/699 (98%)	0.0
K1	H08	77	V	B	5	1	AB211274	*Hebeloma pusillum*	1267	683/695 (98%)	0.0
K1	H09	77	V	B	5	1	AB211274	*Hebeloma pusillum*	1229	680/696 (97%)	0.0
K2	B02	24	T	B	7	1	AB211274	*Hebeloma pusillum*	1261	683/696 (98%)	0.0
K2	B03	24	T	B	7	1	AB211274	*Hebeloma pusillum*	1210	684/697 (98%)	0.0
K2	D11	94	R	B	2	2	AB211274	*Hebeloma pusillum*	1259	682/695 (98%)	0.0
K2	E08	95	V	B	5	6	AB211274	*Hebeloma pusillum*	1182	682/701 (97%)	0.0
K2	E09	93	R	B	2	4	AB211274	*Hebeloma pusillum*	1205	688/704 (97%)	0.0
K2	F10	93	R	B	2	4	AB211274	*Hebeloma pusillum*	1201	683/698 (97%)	0.0
K2	F11	104	T	B	7	2	AB211274	*Hebeloma pusillum*	1239	682/697 (97%)	0.0
K2	D09	107	R	B	1	5	AB211272	*Hebeloma* sp.	1096	692/733 (94%)	0.0
K2	H01	2	R	B	2	4	AB211272	*Hebeloma* sp.	1129	689/722 (95%)	0.0
K2	C03	89	T	C	4	6	AB211274	*Hebeloma* sp.	1134	682/711 (95%)	0.0
K2	F04	124	H	B	3	4	AB211274	*Hebeloma* sp.	1144	682/710 (96%)	0.0
K2	H05	26	V	B	3	4	AB211272	*Hebeloma* sp.	1146	685/712 (96%)	0.0
K2	H06	26	V	B	3	4	AB211272	*Hebeloma* sp.	1179	690/712 (96%)	0.0
K1	A10	22	T	B	1	1	DQ822807	*Hebeloma* sp	1255	690/695 (99%)	0.0
K1	E06	80	R	B	1	1	AB211272	*Hebeloma* sp.	1146	675/700 (96%)	0.0
K1	A11	23	T	B	8	1	EF564168	*Hebeloma* sp	1271	697/701 (99%)	0.0
K1	B01	23	T	B	8	1	EF564168	*Hebeloma* sp	1269	696/700 (99%)	0.0
K1	B02	27	V	B	3	1	EF564168	*Hebeloma* sp	1282	698/700 (99%)	0.0
K1	B03	27	V	B	3	1	EF564168	*Hebeloma* sp	1267	697/702 (99%)	0.0
K1	B04	27	V	B	3	1	EF564168	*Hebeloma* sp	1273	698/702 (99%)	0.0
K1	B05	31	R	B	5	1	DQ822807	*Hebeloma* sp	1266	696/702 (99%)	0.0
K1	B06	31	R	B	5	1	DQ822807	*Hebeloma* sp	1236	692/704 (98%)	0.0
K1	B07	31	R	B	5	1	DQ822807	*Hebeloma* sp	1260	695/702 (99%)	0.0
K1	D12	75	V	B	3	1	EF564168	*Hebeloma* sp	1225	689/701 (98%)	0.0
K1	E01	75	V	B	3	1	EF564168	*Hebeloma* sp	1230	678/683 (99%)	0.0

Appendix 2. Fungal species identified from birch root tips (cont.).

Clone number		Sample number	Loc.[1]	Soil type[2]	MC[3]	Type[4]	BLAST results				
							Accession number	Name	Score	Identity	E-value
K1	E02	75	V	B	3	1	EF564168	*Hebeloma* sp	1234	689/699 (98%)	0.0
K1	E03	79	R	B	5	1	DQ822807	*Hebeloma* sp	1242	692/702 (98%)	0.0
K1	E04	79	R	B	5	1	EF564168	*Hebeloma* sp	1269	697/702 (99%)	0.0
K1	E09	99	T	B	1	1	EF564171	*Hebeloma* sp	1366	692/693 (99%)	0.0
K1	F12	113	H	B	3	4	EF564168	*Hebeloma* sp	1277	697/700 (99%)	0.0
K1	H10	100	T	B	1	1	DQ822807	*Hebeloma* sp	830	638/721 (88%)	0.0
K2	F02	108	V	B	3	1	EF564168	*Hebeloma* sp	1188	699/722 (96%)	0.0
K2	H12	99	T	B	1	1	EF493265	*Hebeloma* sp	652	368/375 (98%)	0.0
K2	H10	113	H	B	3	4	UDB002445	*Hebeloma velutipes*	1240	692/701 (98%)	0.0
K2	G06	21	H	B	8	4	AF380354	*Penicillium minioluteum*	1057	556/560 (99%)	0.0
K2	B01	21	H	B	8	4	AB505424	*Penicillium* sp.	1154	582/582 (100%)	0.0
K2	F12	21	H	B	8	4	AB505424	*Penicillium* sp.	1140	582/583 (99%)	0.0
K2	H08	12	R	B	8	1	AB505424	*Penicillium* sp.	1118	578/580 (99%)	0.0
K1	G12	125	V	B	2	6	AY394921	*Phialocephala fortinii*	1074	561/566 (99%)	0.0
K1	D11	71	H	B	3	6	EU529970	*Phialocephala fortinii*	1090	550/550 (100%)	0.0
K2	E02	129	H	B	3	6	AM901892	Uncultured Ascomycota	965	522/522 (100%)	0.0
K2	E03	129	H	B	3	6	AM901892	Uncultured Ascomycota	944	520/524 (99%)	0.0
K2	C12	91	V	B	3	4	DQ273310	Uncultured Dothideomycetes	714	366/368 (99%)	0.0
K2	C09	84	H	B	10	4	GQ154487	Uncultured ectomycorrhizal fungus	775	454/463 (98%)	0.0
K2	F05	28	V	B	2	6	DQ233798	Uncultured ectomycorrhizal fungus	826	453/463 (97%)	0.0
K2	F07	20	H	B	8	4	DQ233802	Uncultured ectomycorrhizal fungus	991	513/516 (99%)	0.0
K1	A01	3	T	B	7	1	EU554869	Uncultured fungus	1197	607/608 (99%)	0.0
K1	D03	42	R	B	8	2	DQ054559	Uncultured fungus	1122	629/646 (97%)	0.0
K1	E08	82	H	B	10	10	EU554869	Uncultured fungus	1197	607/608 (99%)	0.0
K1	H05	36	R	B	2	2	EU554869	Uncultured fungus	1181	605/608 (99%)	0.0
K2	A12	14	T	B	1	10	EU554869	Uncultured fungus	1181	602/604 (99%)	0.0
K2	B10	26	V	B	3	4	EU554869	Uncultured fungus	1189	606/608 (99%)	0.0
K2	E10	56	H	B	10	10	EU554869	Uncultured fungus	1197	607/608 (99%)	0.0
K2	F08	56	H	B	10	10	FJ626934	Uncultured fungus	1034	559/565 (98%)	0.0

Appendix 2. Fungal species identified from birch root tips (cont.).

Clone number		Sample number	Loc.[1]	Soil type[2]	MC[3]	Type[4]	BLAST results				
							Accession number	Name	Score	Identity	E-value
K2	G08	56	H	B	10	10	FJ626934	Uncultured fungus	1201	609/610 (99%)	0.0
K2	H04	14	T	B	1	10	EU554869	Uncultured fungus	1191	608/609 (99%)	0.0
K1	E11	101	R	B	1	2	AJ875387	Uncultured fungus	1179	684/706 (96%)	0.0
K1	F01	101	R	B	1	2	AJ875387	Uncultured fungus	1182	681/701 (97%)	0.0
K2	A01	2	R	B	2	4	EU554931	Uncultured fungus	1084	559/563 (99%)	0.0
K2	E12	58	T	B	7	2	AJ875387	Uncultured fungus	1177	680/701 (97%)	0.0
K2	G04	58	T	B	7	2	AJ875387	Uncultured fungus	1166	680/703 (96%)	0.0
K1	A04	15	V	B	2	4	GU366750	Uncultured fungus	1080	557/561 (99%)	0.0
K1	A05	15	V	B	2	4	GU366750	Uncultured fungus	1031	556/564 (98%)	0.0
K1	A06	15	V	B	2	4	GU366750	Uncultured fungus	1057	555/561 (98%)	0.0
K1	C11	41	T	B	3	4	EU516932	Uncultured fungus	1005	510/511 (99%)	0.0
K1	C12	41	T	B	3	4	EU516932	Uncultured fungus	989	509/511 (99%)	0.0
K2	D12	94	R	B	2	2	DQ414727	Uncultured fungus	1203	680/703 (96%)	0.0
K2	E05	104	T	B	7	2	AJ875387	Uncultured fungus	1211	685/703 (97%)	0.0
K2	E11	57	T	B	3	4	EU516932	Uncultured fungus	997	509/511 (99%)	0.0
K1	D07	70	H	B	9	1	AJ875387	Uncultured fungus	1182	681/701 (97%)	0.0
K1	E07	80	R	B	1	1	AJ875387	Uncultured fungus	1182	681/701 (97%)	0.0
K1	E12	101	R	B	1	2	AJ875387	Uncultured fungus	1164	673/694 (96%)	0.0
K1	F03	103	T	B	3	4	AJ875387	Uncultured fungus	1171	680/703 (96%)	0.0
K1	F07	109	V	B	7	4	AJ875387	Uncultured fungus	1177	680/701 (97%)	0.0
K1	G08	122	T	B	7	2	AJ875387	Uncultured fungus	1182	681/701 (97%)	0.0
K2	F03	95	V	B	5	6	AJ875387	Uncultured fungus	1157	679/704 (96%)	0.0
K1	B09	33	R	B	1	1	AY748854	Uncultured Hebeloma	1260	687/690 (99%)	0.0
K1	B10	33	R	B	1	1	AY748854	Uncultured Hebeloma	1260	687/690 (99%)	0.0
K1	C02	37	R	B	2	4	DQ273323	Uncultured Helotiales	1063	551/556 (99%)	0.0
K1	C04	37	R	B	2	4	DQ273323	Uncultured Helotiales	1070	552/556 (99%)	0.0
K1	F08	112	H	B	9	4	DQ273323	Uncultured Helotiales	1033	549/557 (98%)	0.0
K2	A05	7	H	B	10	4	FJ475809	Uncultured Helotiales	741	386/390 (98%)	0.0
K2	C05	86	R	B	5	4	DQ273323	Uncultured Helotiales	1070	552/556 (99%)	0.0

Appendix 2. Fungal species identified from birch root tips (cont.).

Clone number		Sample number	Loc.[1]	Soil type[2]	MC[3]	Type[4]	BLAST results				
							Accession number	Name	Score	Identity	E-value
K2	C07	85	H	B	3	4	FJ197016	Uncultured Helotiales	940	496/502 (98%)	0.0
K2	C08	85	H	B	3	4	DQ273323	Uncultured Helotiales	1067	550/554 (99%)	0.0
K2	D01	88	H	B	3	6	DQ273323	Uncultured Helotiales	1070	552/556 (99%)	0.0
K2	D02	88	H	B	3	6	FJ197016	Uncultured Helotiales	908	535/558 (95%)	0.0
K2	D04	20	H	B	8	4	DQ273323	Uncultured Helotiales	1047	549/556 (98%)	0.0
K2	E01	16	T	B	9	6	DQ273322	Uncultured Helotiales	1053	549/555 (98%)	0.0
K2	F06	16	T	B	9	6	DQ273322	Uncultured Helotiales	932	531/542 (97%)	0.0
K2	G09	84	H	B	10	4	DQ273323	Uncultured Helotiales	1063	551/556 (99%)	0.0
K2	H03	13	H	B	8	6	DQ273434	Uncultured Mucorales	585	311/315 (98%)	e-166
K2	A10	13	H	B	8	6	DQ273434	Uncultured Mucorales	1180	601/603 (99%)	0.0
K1	G11	125	V	B	2	6	DQ273434	Uncultured Mucorales	1189	600/600 (100%)	0.0
K2	A09	13	H	B	8	6	DQ273434	Uncultured Mucorales	1172	600/603 (99%)	0.0
K2	C10	84	H	B	10	4	DQ273434	Uncultured Mucorales	1180	601/603 (99%)	0.0
K1	B12	34	H	B	8	6	GU083311	Uncultured soil fungus	1080	561/565 (99%)	0.0
K1	C01	34	H	B	8	6	GU083311	Uncultured soil fungus	1080	561/565 (99%)	0.0
K1	D04	47	H	B	9	4	GU083311	Uncultured soil fungus	1096	560/561 (99%)	0.0
K1	D05	47	H	B	9	4	GU083311	Uncultured soil fungus	1104	564/565 (99%)	0.0
K1	D06	47	H	B	9	4	GU083311	Uncultured soil fungus	1096	563/565 (99%)	0.0
K1	D10	71	H	B	3	6	GU083311	Uncultured soil fungus	1082	563/566 (99%)	0.0
K2	A06	7	H	B	10	4	GU083311	Uncultured soil fungus	1078	564/569 (99%)	0.0
K2	E04	124	H	B	3	4	GU083311	Uncultured soil fungus	1082	563/566 (99%)	0.0
		HR72*	H	B	5	1	GQ450275	*Acremonium strictum*	797	402/402 (100%)	0.0
		HR75*	R	B	5	1	GQ450275	*Acremonium strictum*	757	382/382 (100%)	0.0
		HR79*	H	B	9	6	EU557316	*Phialophora finlandia*	700	353/353 (100%)	0.0

*samples from direct sequencing.

I

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II

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