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Distribution patterns of soil entomopathogenic and birch symbiotic ectomycorrhizal fungi across native woodland and degraded habitats in Iceland

Edda S. Oddsdottir[1,2,4]
Charlotte Nielsen[3]
Robin Sen[2,5]
Susanne Harding[3]
Jørgen Eilenberg[3]
and
Gudmundur Halldorsson[1,6]

[1]Icelandic Forest Research, Mogilsa, IS 116 Reykjavik
E-mail: edda@skogur.is (corresponding author)

[2]Faculty of Biosciences, P.O. Box 56 (Viikinkaari 9), FI 00014 University of Helsinki

[3]Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsenvej 40, DK 1871 Frederiksberg C
E-mail: jei@life.ku.dk
E-mail: suha@life.ku.dk
E-mail: cn-49@hotmail.com

[4]Faculty of Life and Environmental Sciences, University of Iceland, Askja-Sturlugata 7, IS 101 Reykjavik

[5]Department of Environmental and Geographical Sciences, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, UK
E-mail: r.sen@mmu.ac.uk

[6]The Icelandic Soil Conservation Service, Gunnarsholt, IS 851 Hella
E-mail: gudmundur.halldorsson@land.is

ABSTRACT

In Iceland, extensive afforestation programmes have been initiated, often involving the outplanting of nursery seedling stock on eroded land. In some areas high seedling mortality, to a large extent due to root damage caused by *Otiorhynchus* spp. larvae, has been reported. Even though recent studies have shown that inoculation with entomopathogenic and ectomycorrhizal fungi may reduce the effects of *Otiorhynchus* spp. on seedling mortality, information on the occurrence and distribution of these key fungal species in Icelandic soils is limited. The present study reports findings of a targeted survey on the occurrence and distribution of entomopathogenic fungi and birch (*Betula pubescens*) root symbiotic ectomycorrhizal fungi in Icelandic soils from key habitats representing birch woodland, heathland and degraded/eroded land.

Entomopathogenic fungi were isolated from soil by baiting with *Tenebrio molitor* and *Galleria mellonella* larvae. Identification to species was achieved based on standard morphotyping of cultures that included conidiophores and conidia. Birch seedling root symbiotic ectomycorrhizal fungal distribution in eroded and birch woodland soil was determined following baiting with birch seedlings over 9 months and classification based on gross morphology. Significant lower frequency and diversity of both entomopathogenic and ectomycorrhizal fungi were detected in soil collected from eroded areas compared to soil from vegetated areas (birch and heathland). Three species of entomopathogenic fungi, *Beauveria bassiana, Metarhizium anisopliae* and *Isaria farinosa* were present in soil samples collected from the birch woodland and heathland sites. In contrast, no insect pathogenic fungi were found in soil collected from the eroded sites. *B. bassiana* and *M. anisopliae* were recorded for the first time in Iceland. The incidence of mycorrhizal root tips was higher on seedlings grown in soil from birch woodland than in soil from eroded land and a higher diversity of ectomycorrhizal morphotypes was found in birch soil. The importance of these findings is discussed in relation to afforestation in Iceland.

Keywords: Ectomycorrhiza, birch, *Betula pubescens*, entomopathogenic fungi, soil fungi

YFIRLIT

Útbreiðsla skordýrasníkjusveppa og svepprótarsveppa á birki í jarðvegi úr birkiskógi og af uppblásnum svæðum á Íslandi

Á sumum nýskógræktarsvæðum hefur borið talsvert á afföllum á trjáplöntum, sem orsakast af rótarskemmdum af völdum ranabjöllulirfa. Nýlegar rannsóknir benda til þess að hægt sé að draga úr slíkum skemmdum með því að smita plöntur með svepprót og skordýrasníkjusveppum. Hins vegar er lítið vitað um útbreiðslu slíkra sveppa í íslenskum vistkerfum. Því var útbreiðsla svepprótarsveppa á birki (*Betula pubescens*) og skordýrasníkjusveppa könnuð í þremur mismunandi vistkerfum; birkiskógum, lyngmóum og á rofnum svæðum. Lirfur tegundanna *Tenebrio molitor* og *Galleria mellonella* voru notaðar sem beita til að veiða skordýrasníkjusveppi úr jarðvegi. Sveppirnir voru síðan greindir til tegunda. Tegundagreining byggði á stöðluðum aðferðum við mat á útlitsgerð sveppagróa. Ungar birkiplöntur voru notaðar til að veiða svepprótarsveppi úr jarðvegi birkiskóga og jarðvegi af rofnum svæðum og sveppirnir flokkaðir gróflega eftir útlitsgerð. Tíðni og fjölbreytni sveppa var minni í jarðvegi af rofnum svæðum en af grónum svæðum (birkiskógur og lyngmói). Þrjár tegundir skordýrasníkjusveppa (*Beauveria bassiana, Metarhizium anisopliae* og *Isaria farinosa*) fundust í birki- og lyng vistkerfum en engir sníkjusveppir fundust í jarðvegi af rofnum svæðum. Tvær þessara tegunda, *B. bassiana* og *M. anisopliae* höfðu ekki áður fundist hér á landi. Tíðni og fjölbreytni svepprótarsveppa var hærri á birkiplöntum sem ræktaðar voru í jarðvegi af birkisvæðum en þeim sem ræktaðar voru í jarðvegi frá rofnum svæðum. Mikilvægi þessara rannsókna í tengslum við nýskógrækt á Íslandi er rætt.

INTRODUCTION

Iceland has been, and continues to be, subject to large scale soil erosion (Arnalds 1999). This severe land degradation is for the most part the result of the interaction of unfavourable natural events, such as vulnerable soils, low mean temperatures and frequent volcanic eruptions coupled with historic land use management that has driven intensive deforestation. Today, it is estimated that around 40% of the total land area has suffered from soil erosion and 35% of the land is now classified as Vitrisols with a low organic matter content (<3% C) and supporting poor vegetative cover. Soils covered with vegetation are typically Brown Andosol dominated by silt loam and <12% C (Arnalds et al. 2001, Arnalds 2004, Arnalds 2008). Forests have decreased from an estimated 25% of land cover at around 850 AD to the current level of approximately 1% (Traustason & Snorrason 2008). However, as a consequence of global warming, arctic and sub-arctic zones may support more growth of forest tree species (Juday et al. 2005). Downy birch (*Betula pubescens* Ehrh.), the only native forest-forming tree species in Iceland, is one of the most commonly used tree species in afforestation, especially on eroded sites (Gunnarsson 2005), but plantation establishment in Iceland has been hampered by poor growth and a high mortality rate during the early phase of establishment (Oskarsson & Ottosson 1990, Aradottir & Gretarsdottir 1995, Halldorsson et al. 2000). Several interacting factors have been identified that contribute to these high seedling mortality rates, namely, a lack of primary nutrients, frost heaving of soils, drought and insect damage on recently outplanted seedlings (Halldorsson et al. 2000, Oskarsson & Sigurgeirsson 2001).

Seedling establishment and productivity are highly dependent upon interactions with several different groups of beneficial soil organisms (Titus & Tsuyuzaki 2002, Tsuyuzaki et al. 2005, Fujiyoshi et al. 2006, Nara 2006). Symbiotic associations between the roots of forest trees and mycorrhizal fungi can greatly enhance nutrient and water availability for the plant hosts (Smith & Read 2008) and protect plants from attack by pathogenic fungi or insect herbivores (Gange et al. 1994, Halldorsson et al. 2000). Due to loss of mycorrhizal propagules during severe deforestation and subsequent soil erosion, association between naturally regenerated or transplanted seedlings and mycorrhizal fungi during the initial stage of revegetation and afforestation may be limited or non-existent (Kropp & Langlois 1990, Allen et al. 1992, Jumpponen et al. 2002). Both ecto- and arbuscular mycorrhizas are important for the development of vegetation, especially in volcanic (Titus & Tsuyuzaki 2002, Tsuyuzaki et al. 2005, Fujiyoshi et al. 2006, Nara 2006) and exposed or degraded areas, where stress factors such as low soil nutrient content and instability of the soil surface make seedling establishment difficult (Nara & Hogetsu 2004, Nara 2006). There remains, however, limited information on ectomycorrhizal (ECM) fungal diversity and particularly root symbiotic species richness in native and degraded Icelandic habitats (Aradottir 1991, Eyjolfsdottir 2007), which represents a serious impediment for their strategic incorporation into afforestation management.

Insect pathogenic fungi (IPF) may play a key role in regulating insect populations, including soil-dwelling herbivore insect pests, and several IPF of the order Hypocreales can be isolated from soil (Hajek 1997, Klingen & Haukeland 2006, Meyling & Eilenberg 2007). Numerous factors such as geographical location, climatic conditions, habitat type and soil properties affect the occurrence and distribution of IPF in soil (Vänninen 1995, Meyling 2005, Quesada-Moraga et al. 2007). High seedling mortality rates in Icelandic afforestation sites have, among other factors, been attributed to root herbivory by *Otiorhynchus* larvae (Halldorsson et al. 2000), which may reflect low densities of IPF and thus highlight the need for a systematic survey of IPF in Icelandic soils.

In Iceland, extensive afforestation programmes have been initiated, often involving outplanting of nursery seedling stock on eroded land (Gunnarsson 2005). Although beneficial soil biota, such as mycorrhizal fungi, have been shown to be necessary to ensure successful establishment of tree seedlings in such conditions (Nara & Hogetsu 2004, Nara 2006) and recent studies have shown that inoculation with IPF and ECM may reduce the damage caused by *Otiorhynchus* larvae on seedling roots (Halldorsson et al. 2000, Oddsdottir et al. 2010a, Oddsdottir et al. 2010b), there remains a lack of information from corresponding soil ecosystems in Iceland. Here, we test the working hypothesis that the occurrence of two key groups of beneficial soil fungi, ECM and IPF, is higher in the vegetated than in degraded non-vegetated areas. Novel data on the natural occurrence of ECM and IPF in Icelandic soil are elucidated by comparing the frequency and diversity of ECM and IPF in soils collected from old birch woodland, native heathland and eroded land.

MATERIALS AND METHODS

Study sites, soil properties and soil sampling

Soil was sampled at six different locations in Iceland (Figure 1) during two sampling expeditions in August 1999 and September 2001.

In 1999, soil was sampled from a natural birch (*B. pubescens*) woodland at Skaftafell and from heathland at Haukadalur. The birch woodland at Skaftafell covers 355 ha with an average stand height of 2-5 m. It was heavily impacted by grazing before 1967 and had earlier been logged for timber, firewood and charcoal production. The heathland site at Haukadalur was a fully vegetated heathland with dominance of dwarf shrubs (*Betula nana* L., *Salix* spp. and *Vaccinium uliginosum* L.), located close to an active erosion front. At each

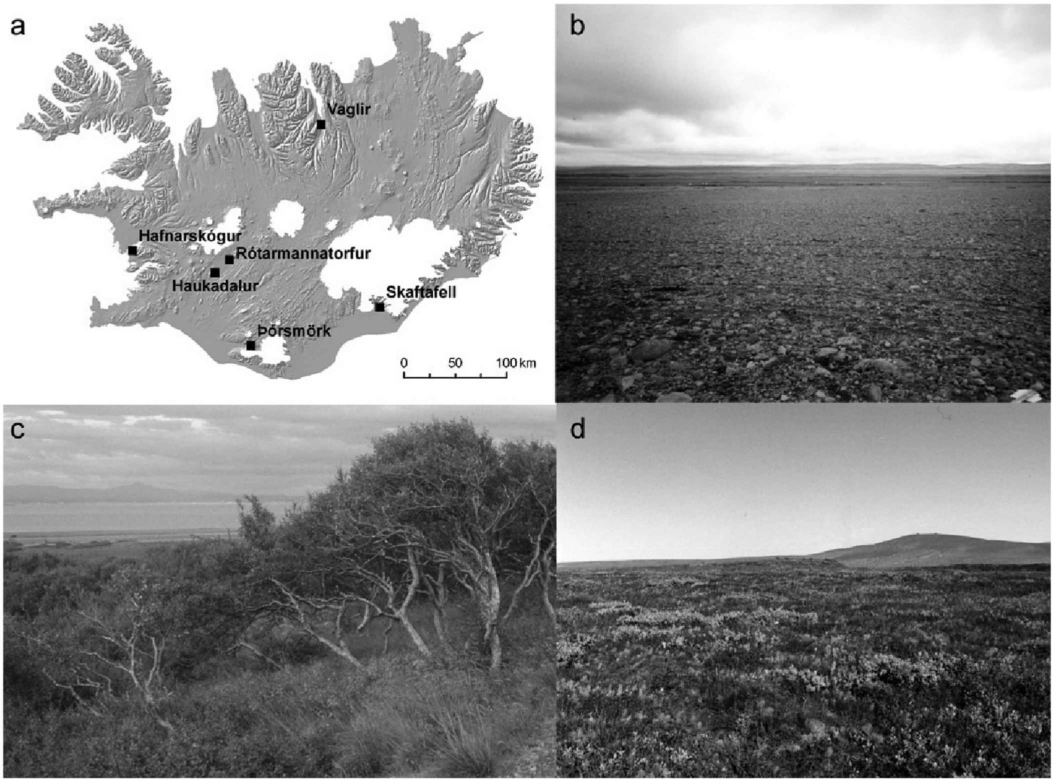

Figure 1. Sampling locations in Iceland, Hafnarskógur (N64°29', W21°57'), Haukadalur (N64°19', W20°16'), Rótarmannatorfur (N64°26', W19°59'), Þórsmörk (N63°41', W19°32'), Skaftafell (N64°00', W16°58') and Vaglir (N65°37', W18°05') (a), eroded site at Rótarmannatorfur (b), birch woodland at Hafnarskógur (c) and heathland at Haukadalur (d).

location, five soil samples were taken randomly from Brown Andosol for isolation and identification of IPF.

In 2001, soil was sampled from four locations around Iceland, Rótarmannatorfur, Vaglir, Þórsmörk and Hafnarskógur. At each location, soil was sampled from two paired habitats, situated within 0.5 km of each other: (i) birch woodland (Brown Andosol) and (ii) eroded land (Vitrisol). The surface of the eroded sites was composed mainly of gravel with a total vegetation cover of less than 10% at three locations (Þórsmork, Hafnarskógur and Vaglir) and around 20% at Rótarmannatorfur, while the vegetation cover at birch habitats was 100% at all locations. Within each habitat, 5 samples were taken randomly and each sample divided into three subsamples, one for isolation and identification of ECM, another for isolation and identification of IPF and the third for analysis of soil properties.

Soil properties

The soil samples were dried at 85°C for 48 h and analysed at the Centre of Chemical Analyses (ICETEC), Reykjavik Iceland. Total N was analysed by Kjeldahl wet combustion, total C by dry combustion, and the soil pH was measured by an electrode in a soil:water mixture (1:1v/v). The soil properties of the soil used in the present study are shown in Table 1.

Isolation and identification of insect pathogenic fungi

IPF were isolated from soil samples from all locations in accordance with IOBC guidelines (Zimmerman 1998). Soil samples were stored at 5°C 2-4 months before processing. For pro-

Table 1. Soil parameters measured in soil samples collected in 2001 from Hafnarskógur, Rótarmannatorfur, Vaglir and Þórsmörk.

	Hafnarskógur		**Rótarmannatorfur**		**Vaglir**		**Þórsmörk**	
	Birch soil	**Eroded soil**	**Birch soil**	**Eroded soil**	**Birch soil**	**Eroded soil**	**Birch soil**	**Eroded soil**
pH	5.2	5.5	5.1	5.3	5.4	6.1	6	6.3
%C	6.07	1.47	4.03	0.4	7.63	0.22	1.59	0.14
%N	0.35	0.1	0.28	0.04	0.46	0.02	0.09	0.01
C/N	17.6	15	14.6	11.4	16.5	11	18.5	12.7

cessing, the soil samples were air-dried at room temperature for 1-2 days and thoroughly mixed. Plastic containers (155 ml, ø 69 mm) were ¾ filled with the soil and six laboratory-reared bait larvae added. Each soil sample was baited twice with laboratory-reared larvae, once with *Tenebrio molitor* L. and once with *Galleria mellonella* L. These two bait insect species were chosen because they are known to exhibit differential susceptibility to IPF species (Meyling 2005). Thus, by using two bait species, we enhanced the chances of isolating several IPF species. The containers were incubated in constant darkness for 14 days at room temperature (20-22°C) and inverted daily for the first five days to maximize contact between larvae and the soil. Dead larvae were removed from the soil 7 and 14 days post-incubation, rinsed in sterile water and transferred to Petri dishes with moist filter paper. Fungi from mycosed bait larvae were identified based on careful examination and measurement of the morphology of conidiophores and conidia (Humber 1997).

Isolation and identification of ectomycorrhizas

ECM were isolated from Brown Andosol and Vitrisol from Þórsmörk, Rótarmannatorfur, Hafnarskógur and Vaglir using birch (*B. pubescens*) bait seedlings in two-dimensional Perspex® microcosms. The microcosm set-up was in accordance with Timonen et al. (1997), with some modifications. Icelandic birch seeds (prov. Embla, seed lot 950071), were water imbibed (o/n at 4°C), surface sterilized in 30% hydrogen peroxide and germinated on distilled water agar at room temperature. Germlings were aseptically grown in test tubes containing Leca® pellets (Optiroc OY, Finland), moistened with 100 ml 1/2 MMN (Marx 1969) and subsequently planted into microcosms following lateral root initiation.

The soil samples were sieved (4 mm mesh) before use and 20 x 20 cm Perspex® microcosms were prepared (Timonen et al. 1997), containing a 3-4 mm layer of soil from either the birch woodland or eroded sites. Birch seedlings (one seedling per microcosm) were planted in both soil types (5 replicates per type) from the four locations, giving a total of 40 microcosms. Microcosms were placed vertically in incubation chambers and arranged so that the roots were protected from light using white plastic spacers placed on the top surfaces of the microcosm stack. The growth chambers were equipped with a floor cooling system that maintained the soil temperature gradient from 12°C at the base to 14°C at the soil surface. A perspex lid was secured on the incubation chamber and the chamber transferred to a growth room (Timonen et al. 1997, Heinonsalo & Sen 2007). Seedlings were exposed to the following growth conditions: 19:5 h (day: night) photoperiod and day/night temperatures of 13-17/10°C and a photon fluence rate of 360 $\mu mol\ m^{-2}\ s^{-1}$. Soil moisture was maintained by regular spraying with distilled water.

Development and identification of ectomycorrhizas

Mycorrhizal colonization of birch roots was monitored using a stereomicroscope every 7 days over a 5-month period. The microcosms were then kept at growth conditions for a further 4-months before final mycorrhizal assessment and harvesting of seedlings. To compare the ECM distribution between soil types, the number of root tips colonized by ECM was recorded. Furthermore, mycorrhizas were morphologically classified according to shape, colour, size and outer mantle characteristics (Heinonsalo & Sen 2007).

Statistical analysis

Frequency of occurrence (% positive samples) of IPF was calculated for each soil type at each location. Overall differences between soil types were tested by a Kruskal-Wallis test by pooling the data from the two bait insects and testing all fungal species together.

The accumulated frequency of ECM root tips and morphotypes after 9 months in microcosms were used in the study and the average frequency per seedlings 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.

A regression analysis was performed between soil parameters (%C, %N, pH) from each habitat location^{-1} and average IPF occurrence habitat^{-1} location^{-1}, average frequency of ECM root tips and morphotypes habitat^{-1} location^{-1}.

Kruskal-Wallis analyses were carried out with the SAS 9.1 statistical program (SAS, 2004) and regression analysis in SigmaPlot 10 (Systat Software Inc. 2006).

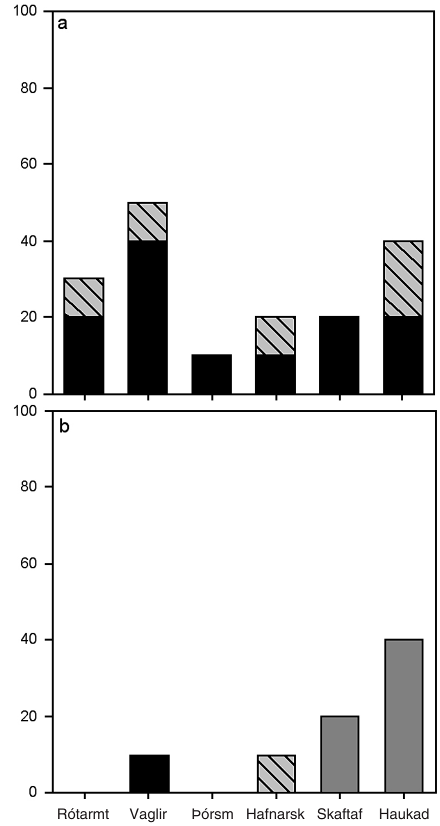

Figure 2. Percentage of soil samples with *Beauveria bassiana* ( ), *Metarhizium anisopliae* ( ) and *Isaria farinosa* ( ) in soil from birch woodland (Rótarmanntorfur (Rótarmt), Vaglir, Þórsmörk (Þórsm), Hafnarskógur (Hafnarsk) and Skaftafell (Skaftaf)) and in the heathland soil at Haukadalur (Haukad) when baited with *G. mellonella* (a) and *T. molitor* (b).

RESULTS

Occurrence of insect pathogenic fungi

IPF were found to occur in 17.7% (16 of 90) of soil samples. There was a clear effect of soil type: IPF were isolated from all birch woodland and heathland sites but from none of the soil samples originating from eroded land. In samples from vegetated habitats (birch woodland and heathland), IPF were found to occur in 32% (16 of 50) of soil samples.

Three species of IPF were identified in the study: *Beauveria bassiana* (Bals.-Criv.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokinand *Isaria farinosa* (Holmsk.) Fr. (formerly *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm.). From the birch woodland soil, the dominant species was *I. farinosa* followed by *B. bassiana.* In contrast, *M. anisopliae* was the most common species isolated from heathlands (Figure 2). *M. anisopliae* was only isolated when *T. molitor* was used as bait larvae whereas *I. farinosa* followed by *B. bassiana* were isolated more frequently when *G. mellonella* was used as bait larvae compared to *T. molitor.* However, when data from both experiments were pooled no statistically significant difference was detected between the vegetated sites

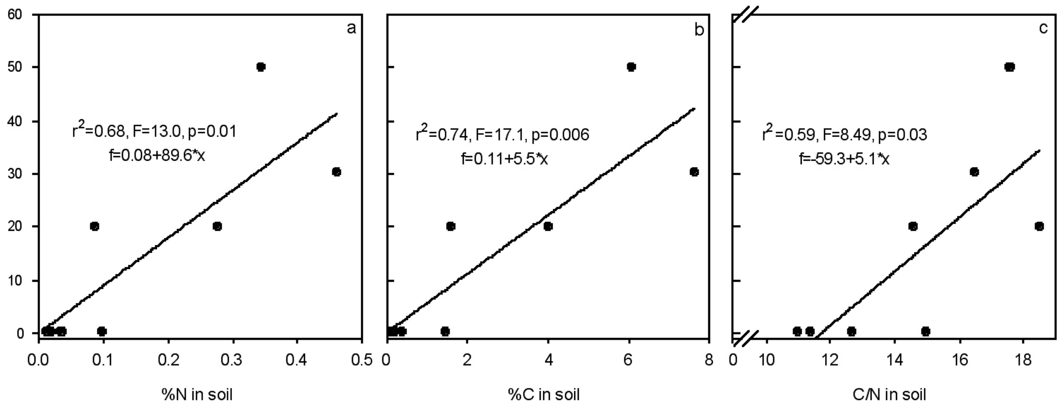

Figure 3. Correlation between %N (a), %C (b) and C/N ratio (c) in soil from birch woodland and eroded land from Rótarmannatorfur, Vaglir, Þórsmörk and Hafnarskógur, and average percentage of soil samples with IPF.

in the overall occurrence of IPF (χ^2=6.66, *p*=0.25, N=5;10).

There was a significant positive correlation between %C (*p*=0.006), %N (*p*=0.01) and C/N ratio (*p*=0.03), respectively, and occurrence of IPF fungi in the soil (Figure 3) but no significant correlation was found between pH and IPF occurrence (*p*=0.2).

Occurrence of ectomycorrhizas

The incidence of mycorrhizal root tips was higher on seedlings grown in soil from birch woodland than in soil from eroded land, the difference being statistically significant at three sites (Rótarmannatorfur: χ^2=6.9, *p*=0.009; Þórsmork: χ^2= 6.9, p=0.009; Hafnarskógur: χ^2= 7.0, *p*=0.008, N=5 in all cases) (Figure 4-a). A similar result was seen in the average number of morphotypes; seedlings in microcosms containing soil from birch woodland had higher numbers than those growing in soil from eroded land at three sites (Rótarmannatorfur: χ^2=5.3, *p*=0.021; Þórsmörk: χ^2= 7.4, *p*=0.007; Hafnarskógur: χ^2= 5.0, *p*=0.025, N=5 in all cases) (Figure 4-b). At one site, Vaglir, no significant differences were found in the incidence of mycorrhizal root tips or number of morphotypes.

Of the 6 most common morphotypes (Types 1, 2, 4, 6, 10 and 11), all were more frequent in microcosms containing birch soil than in microcosms containing eroded soil. (Table 2).

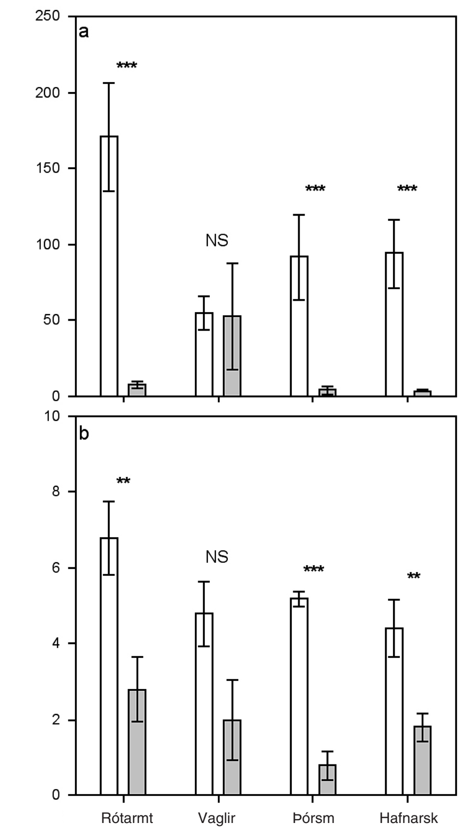

Figure 4. Average number of root tips colonized by ECM (a) and ECM morphotypes (b) on *Betula pubescens* seedlings grown in soil from birch woodland ( ) or eroded land ( ) from four locations in Iceland. Vertical lines show SE. Labels above bars show significant difference (** = p<0.05; *** = p<0.01; NS = no significant difference) between the paired birch woodland and eroded land soils.

Table 2. Gross ectomycorrhizal morphotypes (T=morphotype number), identified on birch bait seedlings in test soils from birch woodland and eroded soil habitats in microcosms during the study.

T	Gross features	Number of microcosm		Average number of root tips microcosms^{-1}	
		Birch soil	Eroded soil	Birch soil	Eroded soil
1	White mantle; white, long hyphae	18	10	47.50	23.10
2	Light yellow; long hyphae	9	4	21.44	3.50
3	White thick mantle; no hyphae	1	0	2.00	0.00
4	Black thick mantle; thick, short and stiff black hyphae	20	2	20.45	2.00
5	White, cotton-like mantle; short, white hyphae	3	1	6.00	26.00
6	Grey thin mantle; with few grey hyphae	20	12	17.90	3.25
7	Yellow thin mantle; light yellow hyphae	2	1	1.50	1.00
8	Dark grey; no hyphae	2	0	2.50	0.00
9	Brown thin mantle; brown hyphae	1	0	3.00	0.00
10	Yellow thin mantle, swollen root tip; yellow hyphae	17	2	9.00	4.00
11	Brown and white mantle; white hyphae	10	4	2.00	1.25
12	Dark brown mantle; white hyphae	1	0	1.00	0.00
13	Yellow cottonlike mantle, swollen root tip; yellow hyphae	1	0	29.00	0.00
14	White thin mantle; white, short and stiff hyphae	0	1	0.00	1.00

There was no significant correlation between %C, %N, C/N ratio or pH and the ECM root tips frequency (p=0.3, p=0.3, p=0.5, p=0.4, respectively) or the ECM morphotype frequency (p=0.2, p=0.1, p=0.3, p=0.2, respectively).

DISCUSSION

The entomopathogenic fungus *I. farinosa* has previously been documented in Icelandic soils (Eyjolfsdottir 1995, Elmarsdottir et al. 2008), whereas the present study documented *B. bassiana* and *M. anisopliae* for the first time in Iceland.

Based on the findings of this study, beneficial fungi appear to be unevenly distributed between different soil types in Iceland. At vegetated sites, such as birch woodland and intact productive heathland habitats, ECM and IPF were present, whereas fewer ECM root tips and morphotypes and no IPF were recorded from the eroded soil. This might reflect the soil quality, since high population densities of beneficial soil-borne organisms are characteristics of healthy soils (Magdoff 2001).

To our knowledge, the present paper is the first to study the occurrence of IPF in Icelandic soils using bait larvae, but fungi have been isolated from soil in different habitats in Iceland by other methods such as soil dilution and grown in pure culture (Eyjolfsdottir 1995 and references therein). Compared to studies on IPF in cultivated and natural soils in other countries at northerly latitudes, such as Canada (Bidochka et al. 1998) and Finland (Vänninen 1995), the occurrence of IPF in Icelandic soils is very low (17.7 % of soil samples in Iceland vs. 91% in Canada and 38.6% in Finland).

Three different IPF species were identified from Icelandic soil, whereas, eight species were isolated from soil in a UK study (Chandler et al. 1997) and four species in a study from northern-Norway (Klingen et al. 2002), Canada (Bidochka et al. 1998) and in New Zealand (Barker & Barker 1998). However, the results may not be fully comparable as the present study was conducted on a much smaller scale than the other studies, both as to number of soil sampling sites and samples per site. It must also be noted that even though most of the studies compared different vegetation habitats, the habitats included forests

(Vänninen 1995, Chandler et al. 1997, Bidochka et al. 1998) and/or cultivated land (Vänninen 1995, Chandler et al. 1997, Bidochka et al. 1998, Klingen et al. 2002) but not non-vegetated areas. Furthermore, the isolation procedures differed between studies. In the present study, we used two species as bait insects, *G. mellonella* and *T. molitor* but only *G. mellonella* was used as a bait insect in some studies (Chandler et al. 1997, Barker & Barker 1998, Bidochka et al. 1998) or insects other than *T. molitor* used with *G. mellonella* (Klingen et al. 2002). In the present study, *M. anisopliae* was only isolated via *T. molitor* but not *G. mellonella*, while the other two IPF species were isolated with both bait insects. This confirms the known differences in the sensitivity of these bait insects,

Several factors affect the occurrence of IPF in soil, such as temperature, moisture, land use and management and the physical properties of the soil substrate. *B. bassiana* appears to be more affected by cultivation since it was isolated more frequently from natural or undisturbed soil, whereas *M. anisopliae* was more common in agricultural habitats (Vänninen 1995, Bidochka et al. 1998). The soil samples in the present study were all from non-cultivated soil, so this does not explain the low occurrence of IPF in Icelandic soil, or the complete absence of IPF in eroded soil. However, eroded land is clearly not undisturbed land. According to Vänninen et al. (2000), the difference in persistence of IPF in Finnish agricultural and natural soil types was not solely caused by climatic conditions *per se*, but the soil type, together with soil moisture, temperature and biological characteristics, also affected the fungal persistence. In the present study, we did not measure soil temperature or moisture. However, the soil substrate classification and presumably also the biological characteristics such as insect occurrence and diversity greatly differed between the two habitats, the birch woodland a typical Brown Andosol with high organic content and water holding capacity, while the eroded soils were Vitrisols, sandy deserts dominated by poorly weathered volcanic tephra with low organic carbon and water holding capacity (Arnalds 2008). We assume that that these characteristics of the eroded soils hampered the occurrence of IPF but further research on the characteristics of Icelandic soils and their impact on IPF occurrence is needed.

The occurrence of IPF was positively correlated with %C, %N and the C/N ratio in the soil, which may simply relate to the fact that the eroded sites, where IPF was not found, are low in %C, %N and the C/N ratio. Other studies suggest that high biological activity and the presence of antagonistic organisms in soil with high organic matter content have negative effects on the occurrence of IPF in soil (Studdert & Kaya 1990, Vänninen et al., 2000, Kessler et al. 2003). On the other hand, soils with very low organic matter content, such as the eroded sites in Iceland, have low diversity and density of soil arthropods (Oddsdottir et al. 2008) which are the possible pathogen hosts, and might therefore hinder the occurrence of IPF.

Even though ECM was found in the soil from eroded land, there was a striking difference between the eroded land and birch woodland soil, with a higher number of ECM root tips and morphotypes in the birch woodland soil. Similar results were obtained by Aradottir (1991), who found that the ECM infectivity of birch soil was 25-35 times higher than that of eroded soil in South Iceland. Furthermore, Ruotsalainen et al. (2009) suggested that mountain birch (*B. pubescens* ssp. *czerepanovii*) maintained lower ECM diversity at sites with high abiotic stress, such as industrial barren sites, sites at high altitude or sandy coastal sites than low stress sites, including natural forests and sites at low altitude, in the Kola Peninsula in NW Russia, but the total colonization was highest at intermediate stress level. Disturbance and loss of vegetation and topsoil can reduce or alter the availability of ECM (Powell 1980, Perry et al. 1987, Brundrett et al. 1996), but usually not to the extent that ECM association is impossible, even though the formation of ECM is lower at disturbed compared to undisturbed sites (Helm et al. 1999). Therefore, successful ECM colonization can be problematic in some primary successional sites, especially in severely devastated volcanic deserts (Allen et al. 1992). This appears to be the case in Iceland, since the results presented in this paper show that ECM colonization of birch seedlings grown in soil from eroded sites at three locations (Rótarmannatorfur, Hafnarskógur and Þórsmörk) was very poor. This has also been shown on newly exposed substrate at the forefront of retreating glaciers, where the incidence of microbial propagules, including ECM, was low (Ohtonen et al. 1999, Jumpponen et al. 2002).

At one location, Vaglir in North Iceland, no significant statistical difference in the average number of ECM morphotypes or colonized root tips was detected between birch woodland and eroded soil. Even though the eroded site at Vaglir appeared similar to the other eroded sites with regard to distance from the birch woodland habitat, vegetation cover and soil nutrients at the time of soil sampling, a visit to the study location four years after soil sampling revealed a striking difference. While the eroded habitats at Þórsmörk, Rótarmannatorfur and Hafnarskógur were still characterized by low vegetation cover, the eroded habitat at Vaglir had achieved 60-80% vegetation cover, dominated by lupin (*Lupinus nootkatensis* Donn ex Sims) and scattered birch trees (personal observation). This suggests that the eroded habitat at Vaglir was in the initial phase of revegetation at the time of sampling, which might explain the high occurrence of ECM in soil classified as eroded. It is interesting that the only eroded habitat that had changed from eroded to vegetated habitat during these 4 years was the only habitat that showed some occurrence of ECM fungi.

The difference in number of ECM colonized roots between birch woodland and eroded soil was reflected in the distribution of morphotypes, since the most common morphotypes were more frequent in microcosms containing birch soil. ECM fungi belonging to type 6 were found in most microcosms but type 1 had the highest number of root tips within microcosms. In this study, we did not perform a full DNA analysis but a preliminary study showed that the white type belonged largely to different *Hebeloma* species and the black and grey types to ascomycetes of the order Helotiales group (Oddsdottir et al., unpublished results). Earlier studies have shown that fruit bodies around *B. pubescens* saplings during early establishment primarily belonged to a *Hebeloma* species (Mason et al., 1982), and species of this genus can easily colonize birch roots even if there are only few *Hebeloma* spp. propagules in the soil (Deacon et al., 1983). This indicates that *Hebeloma* spp. are important in the early ECM succession of birch seedlings.

Our results indicate that the requisite mycorrhizal fungal biota supporting seedling development and insect pathogenic fungi exist in old birch woodlands in Iceland, but are almost non-existing in eroded areas. In light of the fact that afforestation in Iceland is to some extent performed on areas that have suffered from large scale erosion and loss of vegetative cover, the lack of specific beneficial fungi may be an important factor contributing to the high mortality of young seedlings in eroded areas. Our earlier studies (Halldorsson et al. 2000, Oddsdottir et al. 2010a) have shown that inoculation with beneficial soil fungi can reduce survival of *Otiorhynchus* larvae feeding on roots and larval herbivory on roots in the field. Furthermore, inoculation with ECM has positive effects on plant performance (Enkhtuya et al. 2003). Therefore, inoculation of seedlings with specific beneficial ECM (for example *Hebeloma* spp.) and IPF fungi before planting might increase seedling survival and performance early in the establishment of tree seedlings.

In the present study *B. bassiana* and *M. anisopliae* were recorded for the first time in Iceland. However, as this and previous studies have been limited both spatially and temporally, there is an urgent need for further studies on the diversity and occurrence of indigenous soil fungi, utilising molecular rDNA barcoding techniques, both during early seedling establishment and during consecutive successional stages of forest development (e.g. Jonsson et al. 1999, Heinonsalo & Sen 2007)

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