MICROBIAL ASSEMBLAGES OF ICELANDIC SOILS UNDER DIFFERENT REVEGETATION METHODS

Enock Ssekuubwa
Makerere University
Department of Forestry, Biodiversity and Tourism
P. O. Box 7062 Kampala
ssekuubwa@forest.mak.ac.ug

Supervisor
Halldór Sverrisson
Agricultural University of Iceland
halldors@skogur.is

ABSTRACT

In this study of microbial assemblages of Icelandic soils under different revegetation methods, the objectives were to: (1) identify groups of microorganisms in soils under different revegetation methods, (2) assess the relative abundance of microbial groups in soils under different revegetation methods, and (3) assess the effect of time of revegetation on microbial assemblages. Soil samples were collected at a depth of 0-8 cm systematically along a transect at Geitasandur, Hafnarmelar, Mogilsa and Keldnaholt where different revegetation methods existed. Samples were analysed for microbial groups and relative abundance using the plate count method. Differences in abundance were tested using one-way analysis of variance, the independent samples t-test, Kruskal-Wallis and Mann-Whitney tests. The results showed that Icelandic soils under revegetation contain bacteria, fungi and actinomycetes, with different growth rates and colours. The white and yellow bacteria, and white fungi were widespread, while actinomycetes were only detected in Geitasandur. Slow growing groups were more abundant than fast growing groups. At Geitasandur, bacteria were more abundant in *grass, fertilizer, birch and willows,* while fungi and actinomycetes were more abundant in *grass and fertilizer.* At Hafnarmelar, bacteria and fungi were significantly more abundant in *alder only* than at the untreated site. At Mogilsa, the abundance of bacteria and fungi in *birch only* was not significantly greater than at the untreated site. At Keldnaholt, there was no significant difference in abundance of fungi and bacteria between *grass and fertilizer* and *lupine only*. Unlike bacteria, young revegetation methods had more groups and a higher relative abundance of fungi than old methods.

Key words: microbial groups, relative abundance, bacteria, fungi and actinomycetes.

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Thesis and connection of the study to challenges in Uganda

Soil contains a large number of niches that may be separated in space and time, thereby supporting a high diversity of microorganisms (Sims 1990). The major groups of soil microorganisms include eubacteria (cyanobacteria and actinomycetes), archaebacteria, fungi, algae, protozoa, nematodes, viruses, and sometimes viroids, and prions are present (Sims 1990; Brady & Weil 2008). Soil microorganisms play a fundamental role in biogeochemical cycles through mineralisation (Poulsen 2011), a process by which immobile nutrients, found in litter, are released into soil and become available for plant growth (Greipsson 2011). Mineralisation takes place through processes like ammonification, nitrification, denitrification, phosphorylation, and decarboxylation (Sims 1990; Greipsson 2011). Thus, soil microorganisms are important in soil formation, energy transfer, nutrient cycling, vegetation reestablishment, and long term ecosystem stability (Moynahan et al. 2002). They reduce the erodibility of soil by improving aggregation of its particles (Greipsson 2011). In addition, they influence the quality of surface and ground waters and maintain environmental quality through detoxification of pollutants (Sims 1990).

Degradation of ecosystems influences the function of microbial communities in different ways. The ecological equilibrium of microbial communities can easily be perturbed by human activities, and may reduce its natural quality (Izquierdo et al. 2005). Changes in the quality and quantity of soil organic matter as a result of ecosystem degradation affect soil microorganisms and processes (Kandeler et al. 1999). Alteration of soil topography, water distribution, organic matter content, soil structure, and pH potentially affects the soil environment and the behaviour of microbial communities (Greipsson 2011). Changes in plant community composition and spatial distribution as a result of ecosystem degradation affect soil microbial communities by reducing the addition of root exudates and dead plant matter to soil (Greipsson 2011).

Alteration of the composition of microbial communities has consequences for their ability to perform ecosystem services (Chapin III et al. 2000; Greipsson 2011). For example, the composition of microbial communities in soil influences the decomposition of plant materials. Land degradation also reduces the ability of microbial communities to store carbon in microbial biomass, which in turn, can have consequences for global climate change by influencing both greenhouse gas production and carbon storage (Bardgett et al. 2008; Greipsson 2011). Land restoration can help to repair and establish properly functioning soil microbial communities by removing threshold impediments to recovery of degraded ecosystems (Whisenant 1999). The process often starts with revegetation, with the aim of establishing vegetation cover on a degraded site. Revegetation may be accomplished by use of commercial plants, some of which may be non-native. It may involve establishment of grass cover on denuded sites (Bainbridge 2007). It may also involve seeding or transplanting hardy shrubs and trees, and consideration of ecological and socioeconomic implications (Bainbridge 2007; Greipsson 2011).

In Iceland revegetation has been conducted by two state institutions which work on eroded or deforested lands (Eysteinsson 2009). The Soil Conservation Service of Iceland has carried out revegetation using grass and fertilizer spread with the aid of tractors and formerly aeroplanes, sowing nootka lupine (*Lupinus nootkatensis*), and sowing and planting birch (*Betula*

pubescens) and willow species, especially tea-leaved willow (*Salix phylicifolia*) and woolly willow (*S. lanata*) (Crofts 2011). The Iceland Forest Service has carried out revegetation by planting lodgepole pine (*Pinus contorta*), larch (*Larix sibirica*), alder (*Alnus sinuata*), birch and willow, and sowing birch and nootka lupine (Eysteinsson 2009). Revegetation in Iceland has also been conducted by Regional Forestry Associations (Crofts 2011), energy companies and NGOs (Halldórsson et al. 2011). The Iceland Forest Association is an umbrella organisation for many local associations which use various revegetation methods across the country (Eysteinsson 2009).

In Uganda revegetation has mainly focused on degraded forest reserves and sometimes agricultural land. The National Forest Authority oversees revegetation of degraded forest reserves by private companies and individuals. Revegetation of forest reserves has been dominated by planting of non-native commercial tree species (such as *Pinus caribaea* var. *hondurensis*, *Eucalyptus grandis*, *Maesopsis eminii*, *P. patula, P. oocarpa, Cupressus lusitanica, Auraucaria* spp., *Terminalia* spp. and *Tectona grandis)* (SPGS [Sawlog Production Grant Scheme] 2009), and to a lesser extent natural regeneration. Revegetation of agricultural lands involves the planting of multipurpose tree species, such as *Leucaena leucocephala* and *Calliandra calothyrsus* (Gutteridge & Shelton 1994). There are also reforestation programmes under the Clean Development Mechanism but still under pilot projects (Tennigkeit & Kallweit 2007). It is important to understand microbial succession during ecosystem development following revegetation, and how it varies with different revegetation methods.

1.2 Statement of the research problem

Iceland has experienced large scale ecosystem degradation resulting in a major loss of vegetation cover and soil since the settlement of the island in 874 AD (Crofts 2011; Greipsson 2012). The main drivers of degradation have been; clearing of large tracts of birch woodlands by humans, overgrazing by livestock (sheep and horses) especially during winter and spring when the soil is vulnerable, and the effects of volcanic eruptions (Ólafsdóttir et al. 2001; Crofts 2011; Greipsson 2012), exposing the soil to catastrophic wind and water erosion (Thorarinsdottir & Arnalds 2012), and snowmelt runoff processes (Arnalds 2000; Ólafsdóttir & Gudmundsson 2002).

Uganda has also experienced land degradation, manifested in the form of soil erosion, soil nutrient mining, soil compaction, and soil surface crusting (Olson & Berry 2003). The main causes of soil erosion in Uganda include; clearing of forests and woodlands, overgrazing, bush burning, and very intense rains especially in the south-western highlands, accounting for degradation of 60 to 90% of the total land area in the affected parts (Nkonya et al. 2004). Soil nutrient mining, estimated at 70 kg of nitrogen, phosphorus and potassium per hectare, is caused by declining fallow periods, and limited use of inorganic and organic fertilizers (Stoorvogel & Smaling 1990; Pender et al. 2004). Soil compaction and surface crusting are caused by overgrazing of rangelands, and use of heavy machinery for ploughing of drained wetlands (Sserunkuuma et al. 2001).

To curb the problem of ecosystem degradation, restoration mainly focusing on revegetation has been carried out in Iceland and Uganda but to varying extents. In Iceland, unlike Uganda, studies have been conducted to follow up ecosystem development under different revegetation methods. The studies have mainly focussed on trends in carbon accumulation (e.g. Arnalds et

al. 2013), soil arthropods (e.g. Oddsdóttir et al. 2008), and vegetation dynamics (e.g. Gretarsdottir et al. 2004). Previous studies on soil microorganisms and revegetation in Iceland have targeted: one microbial group, i.e. fungi, and revegetation with single plant species, such as ectomycorrhizal and insect pathogenic fungi in native Icelandic birch woodlands and eroded soils (Oddsdóttir 2010); arbuscular mycorrhizae of *Leymus arenarius* (Greipsson & El-Mayas 2000); and mycorrhization and field performance of birch seedlings (Óskarsson 2010). The development of microbial assemblages in different revegetation methods has not been attended to. This implies that the effect of different revegetation methods on microbial communities of Icelandic soils is not clearly understood. Therefore, this study assessed microbial assemblages of Icelandic soils under different revegetation methods.

1.3 Goal, objectives and research questions of the study

The goal of the study was to assess the microbial assemblages in Icelandic soils under different revegetation methods and to document how the assemblages may change with time following revegetation. The objectives were:

1. To identify the groups of microorganisms in Icelandic soils under different revegetation methods.
2. To assess the relative abundance of the microbial groups in Icelandic soils under different revegetation methods.
3. To assess the effect of time of revegetation on microbial assemblages of Icelandic soils.

The research questions question were:

1. Which groups of microorganisms are found in soils under different revegetation methods?
2. What is the number of individuals in one microbial group relative to other groups?
3. Do soils under revegetation methods established at different times contain different microbial groups?
4. Do soils under revegetation methods established at different times differ in the relative number of individuals in microbial groups?

1.4 Importance of the study

The study documented microbial communities in soils under different revegetation methods. The information from the study provides an understanding of the effects of different revegetation methods on microbial populations. This is important when selecting appropriate revegetation methods for restoration of soils of different characteristics. The study also provides an insight into how revegetation methods used in Uganda may influence microbial communities, and the implications they may have on soil quality.

In addition, the methods and knowledge obtained from this study in Iceland were intended to increase my capacity to conduct similar studies in Uganda. Such studies provide an objective measure of the status of systems under restoration (Harris 2003) and a basis for justifying the cost of restoration of many wildlands and communal lands in Uganda, upon which both women and men depend for production of food crops, and livestock and cash crops, respectively.

2. LITERATURE REVIEW

2.1 Groups of microorganisms found in soil

The groups of soil microorganisms include nematodes, protozoa, algae, fungi, prokaryotes, cyanobacteria and actinomycetes. Nematodes are unsegmented round worms, highly mobile and about 4 to 100 µm in cross section and up to several millimetres in length (Brady & Weil 2008). They stimulate the release of plant-available nitrogen in soil by feeding on bacterial cells (Brady & Weil 2008). Protozoa are mobile single-celled organisms, which include amoeba, ciliates, and flagellates (Brady & Weil 2008). Their diameter is considerably larger than that of bacteria and ranges from 4 to 250 µm. Most protozoa prey upon bacteria, thereby influencing their populations, and consequently affecting mineralisation of organic matter (Brady & Weil 2008).

Algae contain chlorophyll and are capable of photosynthesis (Brady & Weil 2008). Being autotrophs, they are found near the soil surface to be able to trap light (Brady & Weil 2008). However, some are capable of heterotrophism in the dark. Some algae form symbiotic associations, called lichens, with fungi. The associations are beneficial during primary succession on bare rocks. Algae also form microbiotic crusts in unvegetated patches of desert areas (Brady & Weil 2008). Fungi are heterotrophs, deriving carbon and energy from living or dead organic materials (Brady & Weil 2008). They are aerobic, but capable of surviving at low oxygen concentrations in wet and compacted soils. The main groups of fungi include yeasts and filamentous fungi, i.e. moulds and mushroom fungi (Brady & Weil 2008). Fungi play an important role in the formation of humus by breaking down organic compounds such as proteins, sugars, cellulose, starch, gums, and lignin, and carry out the largest percentage of decomposition in cultivated soils. Certain fungi also form a mutually beneficial association with roots of higher plants.

Prokaryotes include bacteria and archaea. Archaeans differ from bacteria in that their cell membranes are built by isoprene derivatives whilst those of bacteria are based on fatty acids. Like bacteria, archaeans are common in the more widespread environments (Brady & Weil 2008). The prokaryotes are either autotrophic or heterotrophic. The autotrophs obtain energy from sunlight (photoautotrophs) or from the oxidation of inorganic constituents such as ammonium, sulphur and iron (chemoautotrophs). Through biochemical oxidation and reduction reactions, prokaryotes improve environmental quality and provide plant nutrition (Brady & Weil 2008). Cyanobacteria contain chlorophyll for synthesis of organic compounds in the presence of sunlight (Brady & Weil 2008). They are important in forming microbiotic crusts on desert soils, and fixing atmospheric nitrogen in flooded rice paddies and wetland soils (Brady & Weil 2008). Actinomycetes are filamentous, often profusely branched and reproduce by breaking up into spores (Brady & Weil 2008). They are important for decomposition of soil organic matter, cellulose, chitin, and phospholipids during the final stages of compositing. They are more abundant than bacteria, especially in soils high in humus (Brady & Weil 2008).

2.2 Relative abundance of microbial groups in soil

The number of individuals in the soil, biomass per unit volume of soil and metabolic activity (based on the amount of carbon dioxide produced during respiration) is used to determine the importance of soil microorganisms (Brady & Weil 2008). Table 1 shows the relative

contributions of microorganisms to the total microbial biomass of soils (Sims 1990; Brady & Weil 2008).

Table 1. *Relative numbers and biomass of microorganisms commonly found in soil surface horizons (ᵃ for fungi the number column represents meters of hyphal length). (Source: Sims 1990; Brady & Weil 2008).*

Organism	Number		Biomass based on live weight	
	Per m²	Per gram	Kg/ha	g/m²
Microflora				
Bacteria and Archaea	10^{14}–10^{15}	10^{9}–10^{10}	400–5000	40–500
Actinomycetes	10^{12}–10^{13}	10^{7}–10^{8}	400–5000	40–500
Fungi [a]	10^{6}–10^{8} m	10–10^{3} m	1000–15000	100–1500
Algae	10^{9}–10^{10}	10^{4}–10^{5}	10–500	1–50
Microfauna				
Protozoa	10^{7}–10^{11}	10^{2}–10^{6}	20–300	2–30
Nematodes	10^{5}–10^{7}	1–10^{2}	10–300	1–30

The microflora dominate the microbial activity of soil and thus have a relatively higher metabolic activity, accounting for about 80% of the soil metabolism (Sims 1990; Brady & Weil 2008). The relatively small total biomass of microfauna notwithstanding, they play a vital role in nutrient cycling by preying on bacteria and fungi (Brady & Weil 2008).

2.3 Conditions affecting the growth of soil microorganisms

2.3.1 Organic matter requirements

Microbial growth is stimulated by the increase or addition of energy rich organic compounds. Certain bacteria and fungi require special types of amino acids and growth factors in the rhizosphere (Sims 1990). Actinomycetes dominate when materials are rich in cellulose, in the organic matter on the soil surface (e.g. forest litter) fungi dominate microbial activity, but where substrates are mixed into soil bacteria dominate microbial biomass (Brady & Weil 2008).

2.3.2 Oxygen requirements

Aerobic forms using oxygen as the electron acceptor in their metabolism are more active at optimum oxygen concentrations (Sims 1990). The anaerobic forms (some prokaryotes) using nitrate and sulphate ions as acceptors are active at very low oxygen concentrations (Sims 1990).

2.3.3 Moisture

The optimum moisture content for higher plants is usually suitable for soil microbes, especially aerobes. High moisture content limits oxygen supply and favours anaerobic microorganisms (Brady & Weil 2008).

2.3.4 Temperature

Most microbes cease metabolic activity below 3-5ºC (a temperature called biological zero) except certain psychrophilic species (Brady & Weil 2008).

2.3.5 Exchangeable calcium and pH

Most diverse bacterial populations exist at high calcium and near-neutral pH. When other soil conditions are favourable, bacterial diversity increases with pH from acidic to slightly alkaline. Fungi become dominant at low pH (Brady & Weil 2008).

2.4 Recovery of soil microorganisms from disturbance

Measurement of the recovery of microbial communities provides an early indication of whether the restoration process is on the desired trajectory (Harris 2003). Recovery of soil microorganisms (for example, arbuscular mycorrhizal fungi) in severely disturbed sites can take several decades (Greipsson 2011). Management history is one of the factors that influence the recovery of soil microbes. Buckley and Schmidt (2003) found that microbial composition in cultivated fields was different from that of fields which had never been cultivated.

Since soil microbial communities require a long time to recover from the effects of cultivation, they are sensitive to the characteristics of the soil (Buckley & Schmidt 2003). Depletion of carbon and nitrogen by long term cultivation affects the recovery of microbial communities by affecting the distribution of resources and soil characteristics (Buckley & Schmidt 2003). Thus, recovery of soil microbial communities to pre-disturbance conditions is influenced by recovery of the soil characteristics after disturbance.

2.5 Effects of re-vegetation methods on microbial development

The severity and scale of disturbance and prevailing environmental conditions influence the rate of recovery of the soil (Galatowitsch 2012). Recovery of ecosystems may take a relatively a short time where problems are not severe, unlike in areas with threshold impediments to ecosystem development (Galatowitsch 2012). Plant species that are tolerant to environmental stress and low soil fertility are used to rebuild carbon pools and support microbial activity (Galatowitsch 2012). Such species usually have low nutrient requirements, extensive root systems to acquire nitrogen from very large areas, and may host nitrogen fixing bacteria (Galatowitsch 2012).

The type of plant species, and procedure and time of sowing/and or planting define the re-vegetation method used. By influencing the requirements for microbial growth, revegetation methods are presumed to affect the development of microbial populations. Insam and Haselwandter (1989) found steady increases in microbial biomass with time while investigating changes in soil microbial carbon along a transect taken from a retreating glacier. Soil microorganisms and microbial processes respond to changes in the quality and quantity of organic matter (Kandeler et al. 1999), implying that the use of different plant species in revegetation is likely to impact trends in microbial development by influencing organic matter composition. Izquierdo et al. (2005) found that microbial communities vary with trees species used in revegetation. Areas under native vegetation differed significantly in microbial communities from those revegetated (Izquierdo et al. 2005).

3. MATERIALS AND METHODS

3.1 Study areas

This study was conducted from July to August 2013, in four areas of Iceland, i.e. Geitasandur, Hafnarmelar, Mogilsa and Keldnaholt. Two to five sites in each area were studied. The sites had either been revegetated with different methods or left untreated; they will be referred to as revegetation methods. The appendix shows the location of the study areas in Iceland.

3.1.1 Sites at Geitasandur

Geitasandur is a restoration research area near Gunnarsholt in South Iceland, the headquarters of the Soil Conservation Service of Iceland. Geitasandur is described as a sandy desert where the soils are predominantly volcanic glass and described as Andosols, with about 0.2% organic carbon in the top 10 cm and with a relatively low water holding capacity (Arnalds et al. 2013). The gravelly surface of the soil is maintained by frost heaving during the winter season (Arnalds et al. 2013). The black colour of the basalt parent materials of the soil may maintain a relatively high soil temperature (Arnalds et al. 2013). The mean annual air temperature is about 3.7 $^{\circ}$C and mean annual rainfall 1253.4 mm yr^{-1} (Icelandic Meteorological Office; 1964–1995 averages at the Hella weather station).

The restoration research consists of ten treatments, each replicated four times, giving 40 (1 ha) treatment plots. One (1 ha) treatment plot was sampled for five revegetation methods. According to Helgadóttir (2010) and Arnalds et al. (2013) fertilization and seeding of the plots started in the autumn of 1999 except the lupine plot which was seeded in the spring of 2000. The plots were fertilized (except lupine) with 50 kg N and 27 kg P$_2$O$_5$ per ha each time and fertilization was repeated in 2001, 2003 and 2005. Birch trees and willows were planted in 2001-2003. The treatments studied are summarised in Table 2. Figure 1 shows the characteristics of the sites for each revegetation method.

Table 2. *Revegetation methods at Geitasandur. The first letter 'G' in the code represents Geitasandur, and the rest of the letters correspond to the treatments in each revegetation method.*

Code for revegetation method	Treatment
GGF	Seeded with grasses (*Festuca rubra*, *Poa pratensis*) and fertilized
GF	Only fertilized
GU	Control; untreated, eroded land
GL	Seeded with lupine (*Lupinus nootkatensis*)
GGFBW	Seeded with grasses (*Festuca rubra*, *Poa pratensis*), fertilized and planted with clusters of birch (*Betula pubescens*) and willows (*Salix phylicifolia* and *S. lanata*)

(a) Grass, fertilizer, birch and willows (GGFBW). (b) Lupine only (GL).

(c) Untreated (GU).   *(d) Fertilizer only (GF).*

(e) Grass and fertilizer (GGF).

Fig. 1. *The study sites sampled at Geitasandur. (Photos: H. Sverrisson, 4 July 2013).*

3.1.2 Sites at Hafnarmelar

Two sites were studied at Hafnarmelar in West Iceland, an untreated site and a site planted with alder. The untreated site (coded HU) is an eroded site with a gravelly surface. The gravel is larger than that at Geitasandur, and there was sparse vegetation at the site. The other site was planted with alder (*Alnus sinuata*) in 2000, in a row about 3 m wide and 50 m long. Hafnarmelar is believed to be one of the windiest areas in Iceland which makes it hard for vegetation to establish naturally. Alder was planted as an experimental site for testing revegetation with a tree species which has nitrogen fixing actinomycetes in root nodules (H.

Sverrisson, 27 July 2013, Agricultural University of Iceland, personal communication). The site is believed to have been formerly a birch shrubland, and by the time alder was planted the site had been eroded. At Hafnarmelar, the mean annual temperature is about 3.3 °C and mean annual rainfall 929.2 mm yr^{-1} (Icelandic Meteorological Office; 1964–1995 averages at the Hvanneyri weather station). Figure 2 shows the characteristics of the two sites at Hafnarmelar.
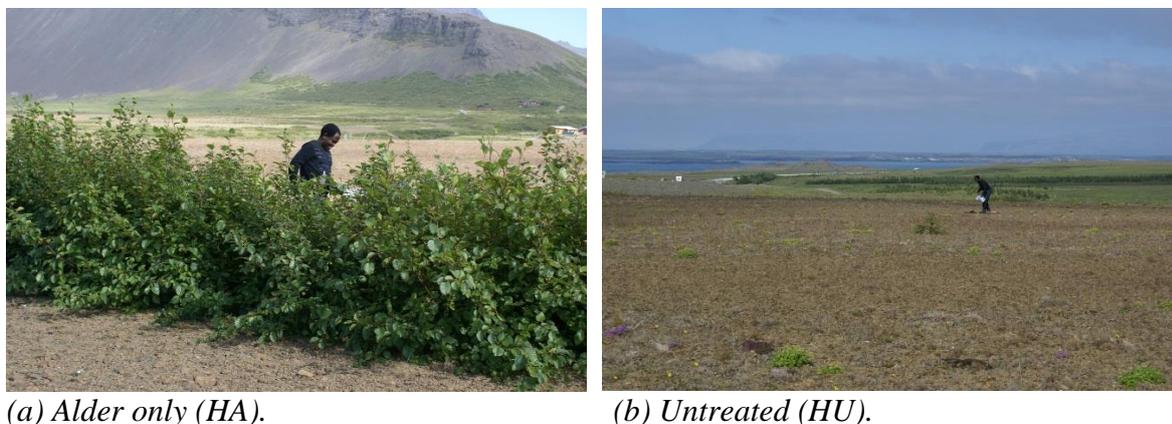
(a) Alder only (HA).   *(b) Untreated (HU).*

Fig. 2. *The study sites sampled at Hafnarmelar. (Photos: H. Sverrisson, 27 July 2013).*

3.1.3 Sites at Mogilsa

Two sites were studied at Mogilsa in South-West Iceland near the Icelandic Forest Research Station, a branch of the Icelandic Forest Service. One site, a birch forest planted around 1980 (H. Sverrisson, 27 July 2013, Agricultural University of Iceland, personal communication), had some pockets of a closed canopy and thick litter, which is characteristic of birch woodlands. This site was coded 'MB'. The untreated site (coded MU) had some plant growth, especially grasses, moss and some shrubs (such as birch and willows). There were also birch, pines and lupine growing in the neighbourhood (about five metres away). At Mogilsa the mean annual temperature is about 4.4 °C and mean annual rainfall 819.3 mm yr^{-1} (Icelandic Meteorological Office; 1964–1995 averages for the Reykjavik area). Figure 3 shows the characteristics of the two sites at Mogilsa.

(a) Birch only (MB).   *(b) Untreated (MU).*

Fig. 3. *Characteristics of sites sampled at Mogilsa. (Photos: H. Sverrisson, 27 July 2013).*

3.1.4 Sites at Keldnaholt

Two sites were studied at Keldnaholt in South-West Iceland, near the Agricultural University of Iceland. One site had lupine sown in 1997 (coded KL). The other site (coded KGF) was fertilized and planted with grass (*Festuca* sp.) around 1980 (H. Sverrisson, 27 July 2013, Agricultural University of Iceland, personal communication). The site was further fertilized in the first years of establishment but later fertilizer application was abandoned (about 20 years ago). The grass is cut every summer, which means that there is a seasonal nutrient removal from the soil without replacement (H. Sverrisson, 27 July 2013, Agricultural University of Iceland, personal communication). The mean annual temperature at Keldnaholt is about 4.4 °C and mean annual rainfall 819.3 mm yr^{-1} (Icelandic Meteorological Office; 1964–1995 averages for the Reykjavik area). Figure 4 shows the characteristics of sites for the two revegetation methods.

(a) Lupine (KL).   *(b) Grass and fertilizer (KGF).*

Fig. 4. *Study sites sampled at Keldnaholt. (Photos: H. Sverrisson, 27 July 2013).*

3.2 Soil sampling

For each revegetation method, five point samples were collected along a 10 m transect in a systematic sampling procedure with a distance of 2 m between adjacent point samples. At Mogilsa where the elevation was not uniform, samples were collected across the slope. At Geitsandur, the transect for the *grass, fertilizer, birch and willows (GGFBW)* treatment was established along the clusters of birch and willows, while at Hafnarmelar, the transect for *alder only (HA)* was established in the middle and along the direction of the row of alders. Soil samples were collected at a depth of 0-8 cm and sealed in clear polythene bags for subsequent analysis. For each revegetation method a GPS point was recorded. The Appendix shows the GPS coordinates for each site sampled.

3.3 Laboratory analysis of soil samples

Samples were analysed for microbial groups from the laboratory of the Soil Conservation Service of Iceland at Gunnarsholt. The plate count method of measuring soil productivity was used to assess the soil samples for microbial groups (Reynolds & Farinha 2005; Case n.d.). In the plate count, the number of colony-forming units (CFU) is determined. It is a direct method used to count the number of viable cells in a soil sample. Each colony may arise from a group of cells rather than from one individual cell (Reynolds & Farinha 2005; Case n.d.). The following procedure was used for laboratory analysis:

1. For each of the five samples collected from each revegetation method, a suspension was prepared by weighing out 1 g of soil and adding it to 100 mL of sterile water in a conical flask. The soil and water were stirred to form a mixture. The suspensions were sealed with aluminium foil to prevent contamination by bacteria from the air (Fig. 5).

Fig. 5. *Preparation and sealing of suspensions for subsequent serial dilutions. (Photo: M. Woldu Bezabeh, 16 July 2013).*

2. A 1:10 (10^{-1}) dilution was prepared by pipetting 1 mL of the undiluted suspension to another 9 mL of sterile water in a test tube. The contents were mixed thoroughly by pipetting up and down. A 1:100 (10^{-2}) dilution was prepared by pipetting 1 mL of the 10^{-1} dilution to another 9 mL of sterile water in a test tube. The contents were mixed thoroughly by pipetting up and down.

3. A 1:1000 (10^{-3}) dilution was prepared by pipetting 1 mL of the 10^{-2} dilution to another 9 mL of sterile water in a test tube. The contents were mixed thoroughly by pipetting up and down. A 1:10,000 (10^{-4}) dilution was prepared by pipetting 1 mL of the 10^{-3} dilution to another 9 mL of sterile water in a test tube. The contents were mixed thoroughly by pipetting up and down. 0.2 mL of the 1:10,000 dilution was aseptically pipetted onto the surface of the nutrient agar.

4. A spreading rod was disinfected by dipping in alcohol, quickly igniting the alcohol in a Bunsen burner flame, and letting the alcohol burn off. The spreading rod was left to cool. The 0.2 mL liquid was spread over the surface of the agar, and the spreading rod disinfected for use in subsequent mixtures.

5. Inoculation was repeated for the 1:1000, 1:100 and 1:10 dilutions and the plates were incubated inverted for 48 hours at room temperature. Figure 6 shows the plates being incubated for microbial growth.

Fig. 6. *Incubation of plates for microbial growth. (Photo: H. Sverrisson, 19 July 2013).*

6. After incubation the microbial groups on all the plates were identified with the aid of a microscope, and the number of colonies counted. The number of microorganisms (bacteria, fungi or actinomycetes) in 1 g of soil was calculated using the equation:

$$Number\ of\ microorganisms\ per\ gram\ of\ soil = \frac{Number\ of\ colonies}{Amount\ plated \times Dilution}$$

The number of microorganisms per gram of soil was used for calculation of the relative abundance of microorganisms. To identify the microbial groups, the nature of colonies was assessed using a light microscope and colonies identified as bacteria, fungi, or actinomycetes. The growth rate of colonies (of bacteria and fungi) was measured as a function of colony diameter; thus large colonies (diameter $\geq$ 0.5 cm) were categorised as fast growing subdivisions, while small strains (diameter $\leq$ 0.5 cm) as slow growing subdivisions of the microbial groups. The actinomycetes seemed to have the same colony diameter, and were not subdivided based on growth rate. Colonies (of bacteria, fungi, or actinomycetes) with different colours were also categorised as different subdivisions of the same.

3.4 Data analysis

For each point sample, 4 plates were inoculated giving a total of 20 plates (from the five point samples), hence 20 subsamples for each re-vegetation treatment. The number of colony forming units (CFU/g of soil) for each sample was calculated as the average number of units from the four serial dilutions. Descriptive statistics were used to determine the relative abundance of soil microbes for the microbial groups and revegetation method, as the mean or median of the CFU/g of soil.

To test for differences in relative abundance, all data were subjected to the Anderson-Darling test for normality, and the test for homogeneity of variances and where data conformed to a normal distribution and equality of variances ($p \geq 0.05$), one-way analysis of variance (ANOVA) and the independent samples t-test were used to test data for differences (Townend 2002; Dytham 2011). Non-parametric Kruskal-Wallis and Mann-Whitney tests were used

where data did not conform to normality even after square root transformation (Townend 2002; Dytham 2011). All tests were computed using Minitab 14 Statistical Software (2004).

4. RESULTS

4.1 Microbial groups in Icelandic soils under different revegetation methods

Three microbial groups, that is, bacteria, fungi and actinomycetes were identified in the revegetation methods studied. Table 3 shows subdivisions of microbial groups based on the colour of colonies. The white and yellow bacteria were recorded in all the revegetation methods while the pink bacteria were only recorded where *grass, fertilizer, birch and willows (GGFBW), alder only (HA)*, and *birch only (MB)* were used. The purple and green bacteria were restricted to where *birch only (MB)*, and *lupine only (KL)* were used. The brown bacteria were recorded at the *untreated site (GU)* in Geitasandur, and where *alder only (HA)* and *birch only (MB)* were used for revegetation (Table 3).

The white fungi were widespread, while the purple fungi were restricted to where *alder only (HA)* was used for re-vegetation. The yellow fungi were recorded in all revegetation methods except *grass and fertilizer* at Geitasandur and Keldnaholt (Table 3). The blue-black fungi were recorded in *grass and fertilizer (GGF)*, *only fertilizer (GF), lupine only (GL)* and the *untreated site (GU)* in Geitasandur, and *alder only (HA)* in Hafnarmelar. Actinomycetes were restricted to Geitasandur, with the blue/white actinomycetes being present in all the revegetation methods while the yellow actinomycetes were present in *grass and fertilizer (GGF)* and the *untreated site (GU)* (Table 3).

Table 3. *Microbial groups in Icelandic soils under different revegetation methods. The first letter in each code represents study area, i.e. G = Geitasandur, H = Hafnarmelar, M = Mogilsa, K = Keldnaholt, and the rest of the letters correspond to the treatments in each revegetation method. (x = presence of microbial group at site).*

Re-vegetation method and code	Bacteria						Fungi					Actinomycetes	
	White	Yellow	Pink	Purple	Green	Brown	White	Blue-black	Yellow	Brown	Purple	Blue/white	Yellow
Grass seeding and fertilizer (GGF)	x	x					x	x		x		x	x
Fertilizer only (GF)	x	x					x	x	x			x	
Untreated (GU)	x	x				x	x	x	x			x	x
Lupine only (GL)	x	x					x	x	x	x		x	
Grass, fertilizer, birch and willows (GGFBW)	x	x	x				x		x	x		x	
Untreated (HU)	x	x					x		x	x			
Alder only (HA)	x	x	x			x	x	x	x	x	x		
Untreated (MU)	x	x					x		x				
Birch only (MB)	x	x	x	x	x	x	x		x	x			
Grass and fertilizer (KGF)	x	x					x						
Lupine only (KL)	x	x		x	x		x		x				

4.2 Relative abundance of bacteria and fungi with different growth rates in Icelandic soils under different revegetation methods

In all the revegetation methods, slow growing microbial groups were more abundant than the fast growing groups (Fig. 7). There were no fast growing fungi in the *grass and fertilizer (KGF)* at Keldnaholt.
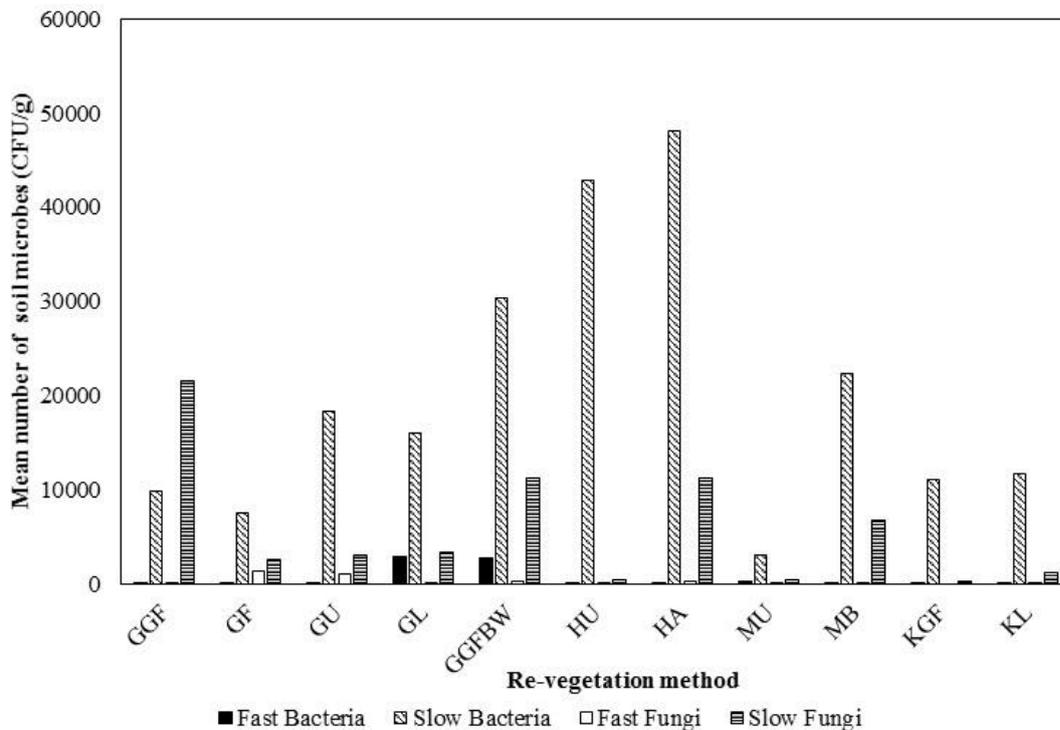

Fig. 7. Relative abundance of bacteria and fungi with different growth rates in Icelandic soils under different revegetation methods. (Geitasandur: GGF = grass and fertilizer, GF = fertilizer only, GU = untreated, GL = lupine only, GGFBW = grass, fertilizer, birch and willows; Hafnarmelar: HU = untreated, HA = alder only; Mogilsa: MU = untreated, MB = birch only; Keldnaholt: KGF = grass and fertilizer, KL = lupine only).

4.3 Relative abundance of bacteria, fungi and actinomycetes in Icelandic soils under different revegetation methods at Geitasandur

Table 4 shows that, generally, bacteria were more abundant than fungi and actinomycetes at Geitasandur, except in *grass and fertilizer (GGF)* where the fungi were more abundant than the bacteria. The Kruskal-Wallis test showed that the difference in relative abundance was statistically significant for bacteria, fungi and actinomycetes, amongst different revegetation methods (Table 4).

15

Table 4. *Relative abundance of bacteria, fungi and actinomycetes in revegetation methods at Geitasandur and results for Kruskal-Wallis test (H = Kruskal-Wallis test, df = degrees of freedom, p-value significant at ≤ 0.05). Different letters within columns show significant differences among revegetation methods according to the Mann-Whitney test.*

	Bacteria H = 10.1 df = 4 p = 0.03	Fungi H = 12.5 df = 4 p = 0.01	Actinomycetes H = 11.5 df = 4 p = 0.02
Revegetation method and code	**Median (CFU/g of soil)**		
Grass and fertilizer (GGF)	10175[a]	20725[a]	1350[a]
Untreated (GU)	6650[a]	4325[b]	325[ab]
Fertilizer only (GF)	5575[a]	3600[b]	150[b]
Grass, fertilizer, birch and willows (GGFBW)	19025[b]	10375[a]	150[b]
Lupine only (GL)	19025[b]	3088[b]	100[b]

Pairwise comparisons using the Mann-Whitney test showed that the difference in relative abundance of bacteria was statistically significant for two revegetation combinations, namely, *fertilizer only (GF)* and *lupine only (GL)* (p = 0.01), and *fertilizer only (GF)* and *grass, fertilizer, birch and willows (GGFBW)* (p = 0.04). The difference in relative abundance of fungi was statistically significant for four revegetation combinations, namely, *grass and fertilizer* (*GGF)* and *fertilizer only (GF)* (p = 0.04); *grass and fertilizer* (*GGF)* and *untreated (GU)* (p = 0.04); *grass and fertilizer* (*GGF)*, and *lupine only (GL)* (p = 0.02); and *lupine only (GL)* and *grass, fertilizer, birch and willows (GGFBW)* (p = 0.04).

In addition, pairwise comparisons of the relative abundance of actinomycetes showed that the difference was statistically significant for three revegetation combinations, namely, *grass and fertilizer* (*GGF)* and *fertilizer only (GF)* (p = 0.04); *grass and fertilizer* (*GGF)* and *lupine only (GL)* (p = 0.01); and *grass and fertilizer* (*GGF)*, and *grass, fertilizer, birch and willows (GGFBW)* (p = 0.03).

4.4 Relative abundance of bacteria and fungi in revegetation methods at Hafnarmelar and Mogilsa

Table 5 shows the relative abundance of bacteria and fungi in revegetation methods at Hafnarmelar and Mogilsa. The abundance of bacteria and fungi in *alder only (HA)* at Hafnarmelar was significantly higher than at the *untreated site (HU)*. There was no significant difference in relative abundance of bacteria and fungi in *birch only (MB)* and the *untreated site (MU)* at Mogilsa.

Table 5. *Relative abundance of bacteria and fungi (as median) in revegetation methods at Hafnarmelar and Mogilsa and test for difference between microbial groups. (In the codes H = Hafnarmelar and M = Mogilsa). The p-value is significant at ≤ 0.05.*

Microbial group	Revegetation method and code	Median CFU/g of soil	Mann-Whitney Test (W)	p
Bacteria	Untreated (HU)	287.5	10.0	0.03
	Alder only (HA)	5362.5		
Fungi	Untreated (HU)	537.5	15.0	0.01
	Alder only (HA)	2275.0		
Bacteria	Untreated (MU)	2988.0	20.0	0.14
	Birch only (MB	11675.0		

Fungi	Untreated (MU)	250.0	20.0	0.14
	Birch only (MB	3675.0		

4.5 Relative abundance of bacteria and fungi in revegetation methods at Keldnaholt

Table 6 shows the relative abundance of bacteria and fungi in revegetation methods at Keldnaholt. There was no significant difference in the abundance of bacteria and fungi between *grass and fertilizer (KGF)* and *lupine only (KL).*

Table 6. *Relative abundance (mean and standard deviation) of bacteria and fungi in revegetation methods at Keldnaholt and test for difference between microbial groups. The p-value is significant at ≤ 0.05. (SD = standard deviation, T = independent sample t-test, df = degrees of freedom, K = Keldnaholt).*

Microbial group	Revegetation method and code	Mean ± SD	T	*p*	df
Bacteria	Grass and fertilizer (KGF)	96.2±51.0	0.05	0.96	8
	Lupine only (KL)	94.4±61.3			
Fungi	Grass and fertilizer (KGF)	18.02±6.62	- 1.89	0.09	8
	Lupine only (KL)	32.7±16.1			

4.6 Effect of time of revegetation on microbial assemblages of Icelandic soils

The effect of time on microbial assemblages was based on the microbial groups and their relative abundance in the old and young treatments. Table 7 shows that generally there were more microbial groups in young treatments than old ones. There were similar bacterial groups in old and young treatments for *grass and fertilizer*. For *lupine only* there were more bacterial groups in old treatments than young treatments. There were more fungal groups in the young treatments than old treatments for both *grass and fertilizer* and *lupine only*. Actinomycetes were restricted to the young treatments of both *grass and fertilizer* and *lupine only*.

Table 7. *Microbial groups in revegetation methods established at different time. (Geitasandur: GGF = grass seeding and fertilizer, GL = lupine only; Keldnaholt: KGF = Grass and fertilizer, KL = lupine only, x = presence of microbial group at site).*

		Revegetation method (year of establishment in parentheses)			
Microbial groups		**GGF (1999)**	**KGF (1980)**	**GL (2000)**	**KL (1997)**
Bacteria	White	x	x	x	x
	Yellow	x	x	x	x
	Purple				x
	Green				x
Fungi	White	x	x	x	x
	Blue-black	x		x	
	Yellow			x	x
	Brown	x		x	
Actinomycetes	Blue/ white	x		x	
	Yellow	x			

Table 8 shows the relative abundance of bacteria and fungi in the young and old treatments of *grass and fertilize* and *lupine only*. There was no significant difference in relative abundance of bacteria between the young and old treatments of *grass and fertilizer*. Similarly, there was

no significant difference in relative abundance of bacteria between the young and old treatments of *lupine only*.

The relative abundance of fungi in the young treatment of *grass and fertilizer* was significantly higher than in the old treatment. Similarly, the relative abundance of fungi in the young treatment of *lupine only* was significantly higher than in the old treatment.

Table 8. *Relative abundance of bacteria and fungi in revegetation methods established at different times. Geitasandur: GGF = grass and fertilizer, GL = lupine only. Keldnaholt: KGF = grass and fertilizer, KL = lupine only. The p-value is significant at ≤ 0.05. (SD = standard deviation, df = degrees of freedom, F = F-test, W = Mann-Whitney test).*

Microbial group	Revegetation method	Time of establishment	One-way ANOVA (df = 9)			Mann-Whitney Test		
			Mean±SD	F	*p*	Median	W	*p*
Bacteria	GGF	1999	107.08±23.04	0.19	0.68			
	KGF	1980	96.20±50.97					
	GL	2000				137.9	35.0	0.14
	KL	1998				71.2		
Fungi	GGF	1999				144.0	40.0	0.01
	KGF	1980				13.7		
	GL	2000	56.79±18.47	4.83	0.05			
	KL	1998	32.73±16.05					

5. DISCUSSION

5.1 Microbial groups in Icelandic soils under different revegetation methods

Although three microbial groups, that is, bacteria, fungi and actinomycetes, were identified, the study does not rule out the presence of other microbial groups like viruses, protozoa and algae. White and yellow bacteria and white fungi were recorded in all the revegetation methods including the untreated sites (Table 3). These bacteria and fungi are more likely to be naturally present and characteristic of Icelandic soils, surviving under all conditions, and revegetation would not affect their presence in the soils.

The pink bacteria were only recorded in the *grass, fertilizer, birch and willows (GGFBW)*, *alders only (HA)*, and *birch only (MB)* treatmens (Table 3). This probably implies that the pink bacteria have a preference for Icelandic tree habitats. Pink-red pigmented colonies are formed by bacteria of the genus *Methylocystis,* for which a large number of strains is known to inhabit several different environments (Wise et al. 1999; Dunfield et al. 2002; Wartiainen et al. 2006). However, there is no specific mentioning of the pink bacteria and the tree species in microbial literature, which calls for more sophisticated methods to identify the strain of bacteria inhabiting the birch and alder habitats and its potential effects on other Icelandic biota.

The purple and green bacteria were restricted to *birch only (MB)* and *lupine only (KL)* (Table 3). According to Imhoff (1995), purple and green bacteria are known to inhabit anaerobic environments different from the sites assessed for this study. Although the litter layer of birch and lupine was observed to be thick, it does not render soils anoxic. The aerobic forms of

purple and green bacteria are limited to marine environments (Imhoff 1995). Thus, more sophisticated and long term studies of these bacterial strains should be conducted to fill this disparity in the microbial literature.

The brown bacteria were recorded at the *untreated site (GU)* at Geitasandur, and where *alder only (HA)*, and *birch only (MB)* were used for revegetation (Table 3). Since the untreated site is a different environment from the alder and birch, the strain inhabiting the untreated site may be different from that inhabiting soils under the trees. There were more bacterial groups in soils under *birch only (MB)* than in other revegetation treatments. The birch forest being 33 years old, its microclimate probably makes conditions suitable for more bacterial groups than the other revegetation methods. Priha et al. (2001) noted that soils under birch forests are generally suitable for microbial activity. In addition, birch being a native species in Iceland, more bacterial groups have probably evolved and formed associations with it.

The purple fungi were restricted to where *alder only (HA)* was used for revegetation (Table 3). Most likely the purple fungi have a distinct preference for alder species, and planting alder may increase their contribution to microbial biomass. One example of purple fungi is the *Paecilomyces lilacinus* which infests phytoparasitic species of nematodes (Kerry 1988; Olivares-Bernabeu & López-Llorca 2002; Morton et al. 2004). This presumed alder–*P. lilacinus* association should be investigated further and if proven, severely degraded sites where phytoparasitic nematodes are impediments to plant establishment can be rehabilitated by planting alder.

The yellow fungi were recorded in all revegetation treatments except *grass and fertilizer* at Geitasandur and Keldnaholt (Table 3). The grass species used at Geitasandur (*F. rubra*, *P. pratensis*) and Keldnaholt (*F. rubra*) may have an inhibitory effect on the yellow fungi. Yellow fungal colonies are associated with the aflatoxin producing ability of *Aspergillus* sp. which is reported to inhibit seed germination (Crisan 1973; Lin & Dianese 1976). Thus, these grass species may be used as biological control agents of the inhibitory effects of *Aspergillus* on seed germination. However, sophisticated methods should be used to confirm the identity of the yellow fungi before the use of the grass species in its control programmes is explored.

The blue-black fungi were recorded in *grass and fertilizer (GGF)*, *only fertilizer (GF)*, *lupine only (GL),* the *untreated site (GU),* and *alder only (HA)*. This probably implies that two strains of the blue-black fungi were recorded, one which prefers the desertified environment at Geitasandur (without birch trees) and another preferring *alder only.*

Actinomycetes were restricted to Geitasandur (Table 3), implying that the subdivisions recorded are favoured by the desertified environment. Studies of actinomycetes in Iceland have been limited to thermophiles (Fields & Lee 1974) and the volcanic island of Surtsey (Henriksson & Henriksson 1974). Thus, it is impossible to rule out the presence of actinomycetes in other Icelandic biomes based on the results of this study and previous studies. Absence of actinomycetes at other sites studied may be attributed to misidentification since their exact identification, composition and boundaries remain open to question and modification (Goodfellow & Williams 1983).

5.2 Relative abundance of bacteria and fungi with different growth rates in Icelandic soils under different revegetation methods

Slow growing microbial groups were more abundant than fast growing groups (Fig. 7). Icelandic soils probably contain more small microbial strains than large ones. The low abundance of large microbial strains may be linked to predation by flagellates. Hahn and Höfle (1999) found size-selective grazing by flagellates to be the major force controlling the morphological structure of bacterial communities. The flagellate composition of Icelandic soils should be investigated to ascertain whether it is not dominated by microbial predators, which may sabotage the ecosystem functions of beneficial microbes and, consequently, revegetation efforts.

The difference in the abundance of different fungal groups, and the absence of fast growing fungi in *grass and fertilizer (KGF)* may be attributed to predation by edaphic Collembola. Different species of Collembola feed on many species of fungi (Hanlon 1981; Jonas et al. 2007), and are known to occur in Iceland (Gudleifsson & Bjarnadottir 2008). However, since there is no specific mention of fungal size preference by edaphic Collembola in microbial literature, the relationship needs to be studied further.

The total number of colony forming units (CFU) recorded during the study is generally lower than seen in a study by Adesina et al. (2007) of soils of other European countries. The lower number of CFUs in Iceland may be attributed to the cold climate and the presence of coarse grained tephra layers in the soils which disturb water relations (Arnalds 2008) and provide a small surface area for microbial activity. In addition, soil organic matter in the rhizosphere influences microbial activity (Brady & Weil 2008), but it was not assessed in this study. Studies of organic matter content of soil under different revegetation methods and the relation to microbial activity would contribute significantly to Icelandic microbial literature.

5.3 Relative abundance of bacteria, fungi and actinomycetes in Icelandic soils under different revegetation methods at Geitasandur

In general, bacteria were more abundant than fungi and actinomycetes in all the revegetation methods studied, except in *grass and fertilizer (GGF)* where the fungi were more abundant than the bacteria (Table 4). The results are in accordance with Sims (1990) and Brady and Weil (2008), who noted that although fungi dominate microbial biomass (kg/ha), the relative number (per gram of soil) of bacteria is generally higher than that of fungi. In addition, since the 0-8 cm depth of soil was used for this study, the results are indicative of the aerobic forms of microbial groups. It is not clear whether the results would have differed if the anaerobic forms had also been included in the assessment.

Fungi were more abundant than bacteria and actinomycetes in *grass and fertilizer (GGF)* (Table 4). Brady and Weil (2008) found fungi to dominate microbial activity at low pH. Thus, perhaps revegetation using *grass and fertilizer (GGF)* makes conditions more habitable for soil fungi by lowering soil pH. Although the basaltic nature of glassy materials in the soils of Icelandic deserts causes a relatively high pH, organic inputs from primary production of new plant cover after revegetation lower soil pH (Arnalds 2008; Arnalds et al. 2013). Furthermore, for this study, the relative abundance of fungi was measured as a function of colony forming units per gram of soil. The results may differ when the length of fungal hyphae are used to assess fungal composition.

In addition, *grass, fertilizer, birch and willows (GGFBW)* had a significantly higher relative abundance of fungi than *lupine only (GL)* (Table 4). The high relative abundance of fungi

may be attributed to the higher number of plant species in *grass, fertilizer, birch and willows (GGFBW)* than in *lupine only*. The high relative abundance of bacteria in *grass, fertilizer, birch and willows (GGFBW)* (Table 4) may also be attributed to the higher number of plant species in that revegetation method. The results confirm the findings by Zak et al. (2003) and Kowalchuk et al. (2002) that microbial biomass increases with number of plant species due to the increase in plant production. The relative abundance of bacteria in *lupine only (GL)* was significantly greater than in *fertilizer only (GF)*. Probably, there is more plant biomass at sites with *lupine only* than those with *fertilizer only,* which provides more substrates for bacterial growth.

The relative abundance of actinomycetes in *grass and fertilizer (GGF)* was significantly greater than in *fertilizer only (GF), grass, fertilizer, birch and willows (GGFBW),* and *lupine only (GL)* (Table 4). This probably implies that the use of *grass and fertilizer* for revegetation of degraded Icelandic soils induces more favourable conditions for actinomycetes. According to Crawford et al. (1993) actinomycetes can survive a wide range of pH. Therefore, the differences in relative abundance of actinomycetes in *grass and fertilizer (GGF), fertilizer only (GF), grass, fertilizer, birch and willows (GGFBW),* and *lupine only (GL)* may be attributed to changes in soil pH induced by primary production (Arnalds 2008; Arnalds et al. 2013) and other resource gradients caused by the different plant species.

The increased abundance of actinomycetes in *grass and fertilizer (GGF)* has implications for subsequent colonisation of revegetated sites by the native vegetation of Iceland. Certain actinomycetes like the genus *Streptomyces* have antifungal activities against some plant pathogenic fungi (Crawford et al. 1993; Lee & Hwang 2002) and thus increase survival of plants which aids colonisation by protecting plant roots against invasion by root pathogenic fungi. Therefore, areas where pathogenic fungi prevent plant establishment, rehabilitation using *grass and fertilizer* may precede planting of less hardy species.

5.4 Relative abundance of bacteria and fungi in revegetation methods at Hafnarmelar and Mogilsa

At Hafnarmelar, the significantly higher abundance of bacteria and fungi in *alder only (HA)* compared to the *untreated site (HU)* (Table 5) may be attributed to the high biomass from alder. Availability of plant litter from alder favours microbial activities by providing substrates on which the microbes thrive. In addition, alder species form symbiotic relationships with certain microorganisms. According to Molina (1981), Chatarpaul et al. (1989) and Baar et al. (2000), alder forms symbiotic associations with ectomycorrhizal and arbuscular mycorrhizal fungi and certain bacteria. This increases the availability of carbohydrates for the microbes which, in turn, enhances their activity and, hence, their relative abundance.

There was no significant difference in relative abundance of bacteria and fungi in *birch only (MB)* and the *untreated site (MU)* at Mogilsa (Table 5). This phenomenon could have several implications. First, some plant species select against certain groups of microorganisms (Long 2001; Berg & Smalla 2009; Raaijmakers et al. 2009) which may reduce microbial diversity. It is important to ascertain, using more comprehensive microbial methods, whether the microbial groups at the untreated site are similar to those in birch, to rule out possible microbial specificity of the birch trees which would make them uninhabitable by some microbes.

Second, since the untreated site was not far from the birch trees (about five metres), it is possible that microbial dispersal occurred from the birch to the untreated site, making the difference in relative abundance of bacteria and fungi not statistically significant. According to Telford et al. (2006), and Finlay and Clarke (1999), microorganisms have cosmopolitan distributions due to the ubiquitous dispersal over a geographical range.

Third, the bacterial and fungal groups at the *untreated site (MU)* may actually have a preference for such an environment, implying that they would be recorded in large numbers there. The microbial community may also be resilient and capable of returning to its pre-disturbance composition. Allison and Martiny (2008) found that certain microorganisms are resilient and recover from disturbance by altering their growth rates and physiology.

5.5 Relative abundance of bacteria and fungi in revegetation methods at Keldnaholt

There was no significant difference in the abundance of bacteria and fungi between *grass and fertilizer (KGF)* and *lupine only (KL)* (Table 6). Since the application of fertilizer to the grass ended around 20 years ago, the effect could be entirely due to the grass and lupine at the two sites. In this case, it can be presumed that the effect of lupine and grass species is more relevant to microbial groups (species and functional diversity) than relative abundance. This is consistent with the assessment of microbial groups which showed that there were more groups in *lupine only* than *grass and fertilizer only* (Table 3). Therefore the choice of any of these revegetation methods should be based on a comprehensive analysis of the microbial groups to identify the revegetation method with more desirable functional groups in light of plant growth requirements.

5.6 Effect of time of revegetation on microbial assemblages of in Icelandic soils

The bacterial groups in the young treatment of *grass and fertilizer* were similar to those in the old treatment, and there were more bacterial groups in old treatments of *lupine only* than in young treatments (Table 7). In addition, there was no significant difference in the relative abundance of bacteria in young and old treatments of *grass and fertilizer* and *lupine only* (Table 8). The results probably imply that for bacteria, a difference in time of establishment of *grass and fertilizer,* and *lupine only* in the range of 2-19 years does not affect relative abundance. The difference in time of establishment perhaps also does not affect bacterial groups in *grass and fertilizer,* except for *lupine only* where old treatments supported more groups. These results are not consistent with Marschner et al. (2002), who found more microbial activity in soils under young lupine.

There were more fungal groups in the young treatments than old treatments for both *grass and fertilizer* and *lupine only* (Table 7). In addition, the relative abundance of fungi in young treatments of *grass and fertilizer* and *lupine only* was significantly higher than in old treatments (Table 8). Similarly, actinomycetes were restricted to the young treatments. The results probably imply that fungi and actinomycetes prefer young sites to old sites. Marschner et al. (2002) found more microbial activity (including eukaryotic communities) associated with young lupine than old lupine. The differences in the microbial community structure may be attributed to high release rates of organic acids and phenolics by the roots of old lupine, which has strong negative effects on microbes in the rhizosphere (Marschner et al. 2002).

The young site of *grass and fertilizer (GGF)* had more groups and higher relative abundance of fungi and actinomycetes, probably because they have better quality of grass litter than the older sites (KGF). Unlike the young sites, the grass at the old site is cut every summer, causing a seasonal loss of nutrients from the soil. This lowers the quality of plant growth and litter produced, subsequently affecting microbial activity. According to Hobbie (1992), plants in low nutrient environments produce poor quality litter which reduces microbial activity. The differences in microbial groups and relative abundance may also be attributed to differences in location of the sites which may induce strong resource and environmental gradients in soil causing differences in microbial assemblages.

6. CONCLUSIONS

- Three microbial groups were recorded, i.e. bacteria, fungi, and actinomycetes, and several subdivisions of the same based on growth rate and colour of colonies. Based on growth rate, there were both fast and slow growing microbial groups. For colour, the bacterial groups recorded were white, yellow, pink, purple, green and brown. Amongst the fungi were white, blue-black, yellow, brown and purple subdivisions while actinomycetes included blue/white and yellow groups.

- In all the revegetation methods studied, slow growing microbial groups were more abundant than fast growing groups. In the desertified environment at Geitasandur, bacteria were generally more abundant than fungi and actinomycetes in all re-vegetation methods studied. A high relative abundance of bacteria is favoured by use of *grass, fertilizer, birch and willows,* while *grass and fertilizer* favours increased abundance of both fungi and actinomycetes in a desertified environment.

- The use of *alder only*, *birch only, lupine only* and *grass and fertilizer* for revegetation favours increased microbial groups, and relative abundance of bacteria and fungi, but eroded sites under natural regeneration may have comparable attributes of microorganisms.

- The time of establishment (in the range of 2-19 years) of a revegetation method affects the number of bacterial groups but not the relative abundance when *lupine only* is used. Young revegetation treatments of *grass and fertilizer* and *lupine only* favour more groups and relative abundance of fungi and actinomycetes compared to old treatments.

ACKNOWLEDGEMENTS

REFERENCES

Adesina, M. F., A. Lembke, R. Costa, A. Speksnijder, and K. Smalla. 2007. Screening of bacterial isolates from various European soils for in vitro antagonistic activity towards *Rhizoctonia solani* and *Fusarium oxysporum*: Site-dependent composition and diversity revealed. Soil Biology and Biochemistry **39:**2818-2828.

Allison, S. D., and J. B. Martiny. 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences **105:** 11512-11519.

Arnalds, O. 2000. The Icelandic 'rofabard'soil erosion features. Earth Surface Processes and Landforms **25:**17-28.

Arnalds, O. 2008. Soils of Iceland. Icelandic Journal of Earth Sciences (Jökull) **58:**409-421.

Arnalds, O., B. Orradottir, and A. L. Aradottir. 2013. Carbon accumulation in Icelandic desert Andosols during early stages of restoration. Geoderma **193:**172-179.

Baar, J., J. V. Groenendael, and J. Roelofs. 2000. Are ectomycorrhizal fungi associated with *Alnus* of importance for forest development in wet environments? Plant Biology **2:**505-511.

Bainbridge, D. A. 2007. A guide for desert and dryland restoration: New hope for arid lands. Island Press, Washington D.C., USA.

Bardgett, R. D., C. Freeman, and N. J. Ostle. 2008. Microbial contributions to climate change through carbon cycle feedbacks. The ISME Journal **2:**805-814.

Berg, G., and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology **68:**1-13.

Brady, N. C., and R. R. Weil. 2008. The nature and properties of soils. Pearson Education International, Upper Saddle River, NJ, USA.

Buckley, D. H., and T. M. Schmidt. 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environmental Microbiology **5:**441-452.

Case, C. L. n.d. Soil productivity: plate count method. Environmental microbiology, experiments for middle and high school. URL http://www.smccd.edu/accounts/case/envmic/plate_count.html [accessed on 14 May 2013].

Chapin III, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, and S. E. Hobbie. 2000. Consequences of changing biodiversity. Nature **405:**234-242.

Chatarpaul, L., P. Chakravarty, and P. Subramaniam. 1989. Studies in tetrapartite symbioses. Plant and Soil **118:**145-150.

Crawford, D. L., J. M. Lynch, J. M. Whipps, and M. A. Ousley. 1993. Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Applied and Environmental Microbiology **59:**3899-3905.

Crisan, E. V. 1973. Effects of aflatoxin on seedling growth and ultrastructure in plants. Applied and Environmental Microbiology **26:**991-1000.

Crofts, R. 2011. Healing the land: The story of land reclamation in Iceland. Soil Conservation Service of Iceland, Gunnarsholt, Iceland.

Dunfield, P. F., M. T. Yimga, S. N. Dedysh, U. Berger, W. Liesack, and J. Heyer. 2002. Isolation of a *Methylocystis* strain containing a novel *pmoA*-like gene. FEMS Microbiology Ecology **41:**17-26.

Dytham, C. 2011. Choosing and using statistics: A biologist's guide. Wiley-Blackwell, Oxford, UK.

Eysteinsson, T. 2009. Forestry in a treeless land. Updated from an article originally published in Lustgården 2004. URL http://www.skogur.is/english/forestry-in-a-treeless-land/ [accessed on 15 May 2013].

Fields, M., and P. C. Lee. 1974. *Bacillus stearothermophilus* in soils of Iceland. Applied and Environmental Microbiology **28:**638-640.

Finlay, B. J., and K. J. Clarke. 1999. Ubiquitous dispersal of microbial species. Nature **400:**828-828.

Galatowitsch, S., M. 2012. Ecological Restoration. Sinauer Associates, Sunderland, MA, USA.

Goodfellow, M., and S. Williams. 1983. Ecology of actinomycetes. Annual Reviews in Microbiology **37:**189-216.

Greipsson, S. 2011. Restoration ecology. Jones and Bartlett Learning, Sudbury, MA, USA.

Greipsson, S. 2012. Catastrophic soil erosion in Iceland: Impact of long-term climate change, compounded natural disturbances and human driven land-use changes. Catena **98:**41-54.

Greipsson, S., and H. El-Mayas. 2000. Arbuscular mycorrhizae of *Leymus arenarius* on coastal sands and reclamation sites in Iceland and response to inoculation. Restoration Ecology **8:**144-150.

Gretarsdottir, J., A. L. Aradottir, V. Vandvik, E. Heegaard, and H. Birks. 2004. Long-term effects of reclamation treatments on plant succession in Iceland. Restoration Ecology **12:**268-278.

Gudleifsson, B. E., and B. Bjarnadottir. 2008. Springtail (Collembola) populations in hayfields and pastures in northern Iceland. Icelandic Agricultural Sciences **21:**49-59.

Gutteridge, R. C., and H. M. Shelton. 1994. The role of forage tree legumes in cropping and grazing systems. Pages 14-20 in R. C. Gutteridge and H. M. Shelton, editors. Forage tree legumes in tropical agriculture. CAB International, Wallingford.

Hahn, M. W., and M. G. Höfle. 1999. Flagellate predation on a bacterial model community: Interplay of size-selective grazing, specific bacterial cell size, and bacterial community composition. Applied and Environmental Microbiology **65:**4863-4872.

Halldórsson, G., A. L. Aradóttir, O. Arnalds, and K. Svavarsdóttir. 2011. Restoration in Iceland. Pages 9-12 in A. L. Aradóttir and G. Halldórsson, editors. Vistheimt á Íslandi. Landbúnaðarháskóli Íslands og Landgræðsla ríkisins, Reykjavík, Iceland. URL http://pdfvef.oddi.is/landgraedslan/vistheimt_a_islandi/#/1/ [accessed on 20 August 2013]. (in Icelandic).

Hanlon, R. 1981. Influence of grazing by Collembola on the activity of senescent fungal colonies grown on media of different nutrient concentration. Oikos **36:**362-367.

Harris, J. 2003. Measurements of the soil microbial community for estimating the success of restoration. European Journal of Soil Science **54:**801-808.

Helgadóttir, Á. 2010. Krækilyng (*Empetrum nigrum*) í frumframvindu (Crowberry in primary succession). 12 ECTS thesis in partial fulfilment of a Baccalaureus Scientiarum degree in biology. Department of Life and Environmental Sciences, University of Iceland, Reykjavík, Iceland. URL http://skemman.is/stream/get/1946/5602/16896/1/Kr%C3%A6kilyng_%C3%AD_frumframvi ndu.pdf [accessed on 20 August 2013]. (in Icelandic).

Henriksson, L. E., and E. Henriksson. 1974. Occurrence of fungi on the volcanic island of Surtsey, Iceland. Acta Bot Islandica **3:**82-88.

Hobbie, S. E. 1992. Effects of plant species on nutrient cycling. Trends in Ecology and Evolution **7:**336-339.

Imhoff, J. F. 1995. Taxonomy and physiology of phototrophic purple bacteria and green sulfur bacteria. Pages 1-15 in R. E. Blankenship, M. T. Madigan and C. E. Bauer, editors. Anoxygenic photosynthetic bacteria. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Insam, H., and K. Haselwandter. 1989. Metabolic quotient of the soil microflora in relation to plant succession. Oecologia **79:**174-178.

Izquierdo, I., F. Caravaca, M. M. Alguacil, G. Hernandez, and A. Roldan. 2005. Use of microbiological indicators for evaluating success in soil restoration after revegetation of a mining area under subtropical conditions. Applied Soil Ecology **30:**3-10.

Jonas, J. L., G. W. Wilson, P. M. White, and A. Joern. 2007. Consumption of mycorrhizal and saprophytic fungi by Collembola in grassland soils. Soil Biology and Biochemistry **39:**2594-2602.

Kandeler, E., D. Tscherko, and H. Spiegel. 1999. Long-term monitoring of microbial biomass, N mineralisation and enzyme activities of a Chernozem under different tillage management. Biology and Fertility of Soils **28:**343-351.

Kerry, B. 1988. Fungal parasites of cyst nematodes. Agriculture, Ecosystems and Environment **24:**293-305.

Kowalchuk, G. A., D. S. Buma, W. De Boer, P. G. Klinkhamer, and J. A. Van Veen. 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie Van Leeuwenhoek **81:**509-520.

Lee, J. Y., and B. K. Hwang. 2002. Diversity of antifungal actinomycetes in various vegetative soils of Korea. Canadian Journal of Microbiology **48:**407-417.

Lin, M., and J. Dianese. 1976. A coconut agar medium for rapid detection of anatoxin production by *Aspergillus* spp. Phytopathology **66:**1466-1469.

Long, S. R. 2001. Genes and signals in the rhizobium-legume symbiosis. Plant Physiology **125:**69-72.

Marschner, P., G. Neumann, A. Kania, L. Weiskopf, and R. Lieberei. 2002. Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupine (*Lupinus albus* L.). Plant and Soil **246:**167-174.

Minitab 14 Statistical Software. 2004. Minitab student version 14 for Windows. Minitab Inc., State College PA, USA.

Molina, R. 1981. Ectomycorrhizal specificity in the genus *Alnus*. Canadian Journal of Botany **59:**325-334.

Morton, O. C., P. R. Hirsch, and B. R. Kerry. 2004. Infection of plant-parasitic nematodes by nematophagous fungi-a review of the application of molecular biology to understand infection processes and to improve biological control. Nematology **6:**161-170.

Moynahan, O., Seastone, C. A. Zabinski, and J. E. Gannon. 2002. Microbial community structure and carbon-utilization diversity in a mine tailings revegetation study. Restoration Ecology **10:**77-87.

Nkonya, E., J., P. J. Pender, D. Sserunkuuma, C. Kaizzi, and H. Ssali. 2004. Strategies for sustainable land management and poverty reduction in Uganda. Research report 133. International Food Policy Research Institute, Washington D.C., USA.

Oddsdóttir, E. S. 2010. Distribution and identification of ectomycorrhizal and insect pathogenic fungi in Icelandic soil and their mediation of root-herbivore interactions in afforestation. PhD dissertation, University of Iceland, Reykjavík, Iceland.

Oddsdóttir, E. S., K. Svavarsdóttir, and G. Halldórsson. 2008. The influence of land reclamation and afforestation on soil arthropods in Iceland. Icelandic Agricultural Sciences **21:**3-13.

Ólafsdóttir, R., and H. J. Gudmundsson. 2002. Holocene land degradation and climatic change in northeastern Iceland. The Holocene **12:**159-167.

Ólafsdóttir, R., P. Schlyter, and H. V. Haraldsson. 2001. Simulating Icelandic vegetation cover during the Holocene. Implications for long-term land degradation. Geografiska Annaler: Series A, Physical Geography **83:**203-215.

Olivares-Bernabeu, C. M., and L. V. López-Llorca. 2002. Fungal egg-parasites of plant-parasitic nematodes from Spanish soils. Revista Iberoamericana de Micología **19:**104-110.

Olson, J., and L. Berry. 2003. Land degradation in Uganda: Its extent and impact. URL http://earthmind.net/slm/docs/uganda/docs/olson-berry-2003-uganda-ld.pdf. [accessed on 10 May 2013].

Óskarsson, Ú. 2010. Potting substrate and nursery fertilization regime influence mycorrhization and field performance of *Betula pubescens* seedlings. Scandinavian Journal of Forest Research **25:**111-117.

Pender, J., S. Ssewanyana, K. Edward, and E. Nkonya. 2004. Linkages between poverty and land management in rural Uganda: Evidence from the Uganda National Household Survey, 1999/00. International Food Policy Research Institute, Washington D.C., USA.

Poulsen, R. 2011. The effect of fluoride pollution on soil microorganisms. BSc thesis, Department of Physical Sciences, University of Iceland, Reykjavik, Iceland.

Priha, O., S. J. Grayston, R. Hiukka, T. Pennanen, and A. Smolander. 2001. Microbial community structure and characteristics of the organic matter in soils under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Biology and Fertility of Soils **33:**17-24.

Raaijmakers, J. M., T. C. Paulitz, C. Steinberg, C. Alabouvette, and Y. Moënne-Loccoz. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant and Soil **321:**341-361.

Reynolds, J., and M. Farinha. 2005. Biology 2420 laboratory manual: Microbiology. Richland College, Dallas, USA. URL http://www.biotech.univ.gda.pl/odl/doc/numbers.pdf [accessed on 14 May 2013].

Sims, G. 1990. Biological degradation of soil. Pages 289-330 in R. Lal and B. A. Stewart, editors. Advances in Soil Science. Springer, New York, USA.

SPGS [Sawlog Production Grant Scheme]. 2009. URL http://www.sawlog.ug/index.php?option=com_content&view=article&id=46&Itemid=107 [accessed on 30 May 2013].

Sserunkuuma, D., J. Pender, and E. Nkonya. 2001. Land management in Uganda: Characterization of problems and hypotheses about causes and strategies for improvement. International Food Policy Research Institute, Washington, D.C., USA.

Stoorvogel, J., and E. Smaling. 1990. Assessment of soil nutrient depletion in Sub-Saharan Africa 1983-2000: Volume II: Nutrient balances per crop and per land use system. Winand Staring Centre, Wageningen, the Netherlands. Report 28, URL http://library.wur.nl/isric/fulltext/isricu_i00013990_001.pdf [accessed on 15 May 2013].

Telford, R. J., V. Vandvik, and H. J. B. Birks. 2006. Dispersal limitations matter for microbial morphospecies. Science **312:**1015-1015.

Tennigkeit, T., and K. Kallweit. 2007. Uganda Pre-feasibility Report II: Uganda Nile Basin Reforestation Project. Forest Industry Services, Kampala, Uganda. URL http://www.joanneum.at/encofor/casestudies/docs/prefeasibility%20reports/Uganda%20-%20Kasagala%20%20Prefeasibility_Report.pdf [accessed on 20 May 2013].

Thorarinsdottir, E. F., and O. Arnalds. 2012. Wind erosion of volcanic materials in the Hekla area, South Iceland. Aeolian Research **4:**39-50.

Townend, J. 2002. Practical statistics for environmental and biological scientists. John Wiley and Sons, Chichester, UK.

Wartiainen, I., A. G. Hestnes, I. R. Mcdonald, and M. M. Svenning. 2006. *Methylocystis rosea* sp. nov., a novel methanotrophic bacterium from Arctic wetland soil, Svalbard, Norway (78 degrees N). International Journal of Systematic and Evolutionary Microbiology **56:**541-547.

Whisenant, S. 1999. Repairing damaged wildlands: A process-orientated, landscape-scale approach. Cambridge University Press, Cambridge, UK.

Wise, M. G., J. V. Mcarthur, and L. J. Shimkets. 1999. Methanotroph diversity in landfill soil: isolation of novel type I and type II methanotrophs whose presence was suggested by culture-independent 16S ribosomal DNA analysis. Applied and Environmental Microbiology **65:**4887-4897.

Zak, D. R., W. E. Holmes, D. C. White, A. D. Peacock, and D. Tilman. 2003. Plant diversity, soil microbial communities, and ecosystem function: Are there any links? Ecology **84:**2042-2050.

APPENDIX

Location of study areas in Iceland, and GPS coordinates for study sites

Location of the four study areas in Iceland.

GPS coordinates for study sites (re-vegetation methods sampled).

Study area	Revegetation method	Code	Coordinates	
Geitasandur	Lupine only	GL	N63.82943	W020.21080
Geitasandur	Grass, fertilizer, birch and willows	GGFBW	N63.82774	W020.21650
Geitasandur	Untreated	GU	N63.82704	W020.21055
Geitasandur	Fertilizer only	GF	N63.82586	W020.20946
Geitasandur	Grass seeding and fertilizer	GGF	N63.83010	W020.20745
Hafnarmelar	Alder only	HA	N64.45908	W021.95886
Hafnarmelar	Untreated	HU	N64.45987	W021.95978
Mogilsa	Birch only	MB	N64.21194	W021.71745
Mogilsa	Untreated	MU	N64.21221	W021.71730
Keldnaholt	Grass seeding and fertilizer	KGF	N64.13847	W021.76974
Keldnaholt	Lupine only	KL	N64.13811	W021.76919