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ECTOMYCORRHIZAS OF NORWAY SPRUCE (*PICEA ABIES* (L.) KARST.) IN MANAGED FOREST STANDS OF LATVIA

DOCTORAL THESIS

Submitted for the degree of Doctor of Biology
Subfield: Ecology

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Riga, 2015

Abbreviations

AIC	**Akaike's information criterion**
ANOVA	**Analysis of variance**
CCA	**Canonical Correspondence Analysis**
DGGE	**Denaturing gradient gel electrophoresis**
DNA	**Deoxyribonucleic acid**
ECM	**Ectomycorrhizae / Ectomycorrhizal**
ECMf	**Ectomycorrhizal fungi**
GLMM	**Generalized linear mixed model**
ITS	**Internal transcribed spacer**
JSC	**Joint stock company**
LLC	**Limited Liability Company**
LSFRI Silava	**Latvian State Forest Research Institute "Silava"**
PCA	**Principal Component Analysis**
PCR	**Polymerase chain reaction**
rRNA	**Ribosomal ribonucleic acid**

Content

Summary

This thesis assembles six separate studies on ectomycorrhizal fungi (ECMf) of young and mature Norway spruce (*Picea abies* (L.) Karst.) under different management practices. The studies provide insight in ectomycorrhizal (ECM) species composition in relation to tree growth rate, soil parameters and forest management practices applied.

The main method used in this work was fine root analysis. Morphotyping was carried out in combination with sequencing of the fungal internal transcribed spacer (ITS) region of rRNA genes to identify fungal species. Stand or seedling growth rate and health status as well as soil parameters were taken into account for data analysis and interpretation.

In total, 148 fungal species were identified from sequences of root tips. Most of the species represented basidiomycetes, 105 species, mainly from the orders of ECMf *Atheliales*, *Agaricales*, *Russulales* and *Thelephorales*. Ascomycetes were represented by 43 species, mainly from the *Helotiales* and *Pezizales* orders. Many species recorded were new for Latvia as only few of these taxonomic groups had been studied before.

The results showed that fine root parameters were related to Norway spruce heath status and could serve as indicators of tree vitality. In stands with severe foliar damage symptoms, the proportion of living fine roots was reduced and greater abundance of saprotrophic species on fine roots were observed in comparison with less damaged stands. Overall, sites with high *Heterobasidion* root-rot incidence on fertile peat soils were characterized by ECM species *Amphinema* spp., *Inocybe* spp. and *Tylospora asterophora* and on peat soils with extremely low pH – with poor fine root mycorrhization.

Frequency of some ECM species differed between Norway spruce stands of different age (young vs. mature stands). In young tree plantings the ECM species *Thelephora terrestris, Amphinema byssoides* and *Wilcoxina* species were the main root symbionts of Norway spruce seedlings. In contrast, mature stands mainly on peat soils were dominated by ECMf from genera *Tylospora*, *Amphinema*, *Lactarius* and *Tomentella*. Members of *Helotiales* fungi and genera *Russula*, *Cortinarius*, *Inocybe* were commonly observed on fine roots. Some observed differences in species abundance could be attributed to soil conditions (soil moisture, acidity and fertility). For example, ECMf from genera *Amphinema*, *Tuber* and *Inocybe* were specific for wood ash fertilized plots with increased soil pH, while *Tylospora*, *Lactarius* and *Russula* species – in unfertilized forest stands on peat soil ($p < 0.05$).

Provenance of spruce seedlings, cultivation system and site preparation method were suggested to have impact on seedling mycorrhization and early growth. Stump removal in comparison to conventional trenching treatment showed positive effect on initial seedling

growth and on root development. However, some indications of reduced ECM inoculums in stump removal sites were noted. Seedling provenances with the best growth rate had diverse communities of ECMf. Abundance of ECM species *A. byssoides* and *Wilcoxina* sp. differed significantly among seedlings of different seed origin. Experiments with bare-rooted and containerized seedlings showed some differences between cultivation methods, which could be related to better adaptation to field conditions of bare root seedlings, either due to its more developed root system or mycorrhizal community.

The doctoral thesis was carried out at the Latvian State Forest Research Institute „Silava" in collaboration with the University of Latvia, Finnish Forest Research Institute „Metla" (now Natural Resources Institute Finland (Luke)) and Swedish University of Agricultural Sciences from 2009 to 2015.

Kopsavilkums

Disertācijā apkopoti sešu atsevišķu eksperimentu rezultāti par parastās egles (*Picea abies* (L.) Karst.) ektomikorizas sēnēm audzēs, kurās tiek veikta saimnieciskā darbība, un jaunaudzēs. Katrs pētījums sniedz ieskatu par ektomikorizu sugu sastāvu saistībā ar koku augšanas gaitu, augsnes parametriem un audzes apsaimniekošanas veidu.

Galvenās metodes šajā darbā ir saistītas ar sakņu analīzi – to morfotipēšanu un sēņu sugu identificēšanu pēc ribosomālā RNS gēnu ITS rajona sekvencēm. Papildus novērtēta arī audzes vai stādu augšanas gaita, vitalitāte un augsnes parametri, kas datu analīzē saistīti ar mikorizas sugu sastāvu un ekoloģiju.

Kopumā no analizētajiem sakņu paraugiem tika iegūtas 148 sēņu sugu sekvences. Lielākā daļa no izdalītajām sugām bija bazīdijsēnes (105 sugas), galvenokārt no *Atheliales*, *Agaricales*, *Russulales* un *Thelephorales* rindām. Askusēnes pārstāvētas ar 43 sugām, galvenokārt no *Helotiales* un *Pezizales* rindām. Tā kā vairākas no konstatētajām sēņu taksonomiskajām grupām Latvijā ir maz pētītas, daļa identificēto sēņu sugu ir jaunas Latvijai.

Iegūtie rezultāti liecina, ka īssakņu parametri (biomasa, vitalitāte, mikorizācija un mikorizas sēņu sugu sastāvs) ir saistīti ar parastās egles veselības stāvokli un var tikt izmantoti kā koku vitalitātes rādītāji. Audzēs ar nopietniem vainaga bojājumiem, salīdzinot ar veselām audzēm vai audzēm ar nelieliem vainaga bojājumiem, konstatēta samazināta īssakņu vitalitāte, kā arī biežāk uz īssaknēm bija sastopamas saprotrofās sēnes. Kūdras augsnēs ar sakņu trupi inficētās platībās raksturīgas ektomikorizas sēņu sugas *Amphinema* spp., *Inocybe* spp. un *Tylospora asterophora*, savukārt inficētās audzēs uz kūdras augsnēm ar ļoti zemu augsnes pH raksturīga vāja sakņu mikorizācija kopumā.

Atsevišķu ektomikorizas sēņu sastopamība atšķirās starp pieaugušām mežaudzēm un jaunaudzēm vai kokaudzētavu stādmateriālu. Jaunos stādījumos dominēja ektomikorizas sēnes *Thelephora terrestris, Amphinema byssoides* un *Wilcoxina* spp., savukārt pieaugušās audzēs, galvenokārt ar kūdras augsnēm ektomikorizu pamatā veidoja *Tylospora*, *Amphinema, Lactarius* un *Tomentella* ģints sugas; uz īssaknēm bieži konstatētas arī *Helotiales* rindas sēnes un *Russula*, *Cortinarius*, *Inocybe* ģints sugas. Atšķirības sugu sastopamības ziņā daļēji skaidrojamas arī ar augsnes faktoriem (augsnes mitrums, pH un barības vielu saturs). Piemēram, *Amphinema*, *Tuber* un *Inocybe* ģints sugas dominēja ar pelniem mēslotās mežaudzēs ar paaugstinātu augsnes pH, savukārt *Tylospora*, *Lactarius* un *Russula* ģints sugas vairāk ($p < 0,05$) pārstāvētas nemēslotās audzēs ar kūdras augsnēm.

Darbā secināts, ka stādmateriāla izcelsme, audzēšanas tehnoloģija un stādvietu sagatavošanas veids var ietekmēt stādu mikorizāciju un augšanu. Celmu izstrāde, salīdzinot ar tradicionālo augsnes sagatavošanu, pirmajā gadā pēc izstādīšanas veicināja gan stādu virszemes daļu, gan sakņu attīstību. Tomēr mikorizu veidojošo sēņu sugu sastāva analīze liecināja par iespējami samazinātu sakņu mikorizācijas potenciālu celmu izstrādes objektos. Stādu proveniencēs ar sekmīgāku virszemes daļu augšanu raksturīga daudzveidīgāka ektomikorizas tipoloģiskā struktūra. Konstatētas būtiskas atšķirības ektomikorizas sēņu sugu *Amphinema byssoides* un *Wilcoxina* sp. sastopamībā dažādu provenienču stādmateriāla. Mikorizācija un augšanas rādītāji atšķīrās starp kailsakņiem un ietvarstādiem, kas galvenokārt skaidrojamas ar kailsakņu labāku adaptāciju lauka apstākļiem, mikorizas sēņu sabiedrību vai arī labāk attīstītu sakņu sistēmu kopumā.

Promocijas darbs izstrādāts Latvijas Valsts Mežzinātnes institūtā "Silava", sadarbībā ar Latvijas Universitātes Bioloģijas fakultāti, Somijas Meža Pētījumu Institūtu "Metla" (tagad Somijas Dabas Resursu Institūts (Luke)) un Zviedrijas Lauksaimniecības universitāti laikā no 2009. līdz 2015. gadam.

1. Introduction

Application of new or more intense forest management methods such as new breeding technologies, reforestation of drained peatlands or previous agricultural land, intense forest cutting, stump removal etc. implies new risks for forest health and the need for more investigations in this field. Microbial-plant interactions in the soil play an essential role in successful tree growth, productivity and stability of forest ecosystems. Ectomycorrhizal fungi form an important part in these interactions and, therefore, it is of great importance to study ECMf and its community composition in relation with forest management practices. More field data about mycorrhizal interactions are needed, as most of the knowledge on these relations is based on laboratory experiments, which do not always reflect the situation in nature (Johnson *et al*., 1997). Field research about mycorrhizal symbiosis is important but complex. The results of this work are aimed to provide more comprehensive knowledge on ecology of ECMf and to indicate some possible risks or advantages in forest management practice from a belowground perspective. Since there has been no specialized research conducted previously in Latvia focusing on belowground communities of ECMf, the results of this work will provide new knowledge on ECM species in Latvia.

Objectives of the work are:

1) to evaluate the fungal community associated with fine roots of Norway spruce in stands with foliar damage and in stands with *Heterobasidion* root rot; 2) to assess impact of wood-ash fertilization on root vitality and on ECM communities in conifer stands on peat soils; 3) to evaluate impact of planting material and soil preparation technology on initial growth and mycorrhization of spruce seedlings; 4) to identify general patterns of association of the ECM community or particular species with forest growth stage, tree vitality and soil characteristics.

The work was based on six different studies dealing with communities of ECMf in managed forests.

1. Fine root abundance and mycorrhization in stands showing symptoms of foliar damage (Foliar damage study). The aim: to assess fine root status and fungal community in relation to foliar damage and soil characteristics.

2. Fine root abundance and ECMf in *Heterobasidion* root-rot infected vs. uninfected stands (Root-rot study). The aim: to assess the fine root status and its fungal community in relation to Norway spruce infection with *Heterobasidion* root-rot.

3. Impact of wood ash fertilization on fine root mycorrhization in mature conifer stands (Fertilization study). The aim: to compare fine root ECM community in wood ash fertilized and control sites 12 years after the treatment.

4. Impact of site preparation on mycorrhization and early growth of spruce seedlings (Stump removal study). The aim: to compare ECM communities of Norway spruce seedlings one growing season after their outplanting on clear-cuts of different forest types where two soil preparation techniques were used – stump removal and disc trenching.

5. Impact of seed origin on seedling growth and mycorrhization (Seed origin study). The aim: to investigate whether spruce seedlings representing different seed regions and provenances in Latvia differ in natural ECM colonization of roots (richness of morphotypes, ECM species diversity and composition) under nursery conditions.

6. Seedling cultivation system and mycorrhization (Cultivation system study). The aim: to assess ECM community of containerized and bare-root cultivated spruce seedlings following their outplanting on a forest clear-cut in Latvia.

Theses

1. Belowground parameters such as fine root vitality, biomass and mycorrhization can be associated with Norway spruce health status.

2. Occurrence of some ECM species differs between Norway spruce stands of different age (young vs. mature stands) and soil conditions (soil moisture, acidity and fertility).

3. Origin of spruce seedlings, their cultivation system and site preparation method can have an impact on seedling mycorrhization and growth.

2. Background

2.1. Ectomycorrhizal Symbiosis

2.1.1. General Description

Under natural conditions the root surface is a zone of continuous interaction among plants and various rhyzosphere organisms. One specialized group of these organisms is mycorrhizal fungi, which forms symbiotic (mainly mutual but sometimes weakly pathogenic) associations with plants (Kirk *et al*., 2001). This group includes great variety of fungi (approx. 8000 species), host plants (90–95% of vascular plants) and mycorrhizas as such.

A mycorrhiza is a symbiotic association essential for one or both partners, between a fungus (specialized for life in soils and plants) and a root (or other substrate-contacting structure) of a living plant, which is primarily responsible for nutrient transfer (Brundrett, 2004). Seven types of mycorrhizas are distinguished on the basis of root colonization nature, fungal and plant taxonomical group, fungal structures and functional characteristics of mycorrhizae (Cairney, 2000). Among these, ECM is one of the most widespread mycorrhizal types in forest ecosystems and the dominant type of mycorrhiza in the boreal and temperate zone of Northern Hemisphere.

Ectomycorrhizas consist of mantle – dense hyphae net around root external surface, a Hartig net – formed by hyphae surrounding the plant cells within the root cortex, and external mycelium formed by an extensive network within the soil and leaf litter. Fungi from different taxonomical groups form ECM symbioses – basidiomycetes, ascomycetes as well as zygomycetes. There are approximately 7 750 known fungal species forming ECM, but the predicted number of these species is speculated to be about 20 000–25 000 (Rinaldi *et al*., 2008) or approx. 23% of total soil fungi diversity (Tedersoo *et al*., 2014).

2.1.2. Role of Ectomycorrhizas

Ectomycorrhizal symbiosis has an impact on metabolism of both symbionts. Plant supplies for fungi are relatively stable and a direct carbohydrate source (glucose and sucrose) (Smith & Read, 1997). Plant benefits from fungi are water and mineral uptake, mainly because fungi have a large mycelium network exposed for absorption of water and minerals dissolved in it (Selosse *et al*., 2006). Fungi by their absorption capacity and enzyme activity can supply plants with phosphorous and nitrogen, especially in nitrogen- and phosphorous-limited ecosystems (Klironomos & Hart, 2001; Li *et al*., 2006). It has been shown that, due to

ECM symbiosis, seedlings can absorb greater amounts of N, P and K (Eltrop & Marshner, 1996), and also Ca and Mg uptake can be stimulated (Andersson *et al*., 1996).

Ectomycorrhizas have a positive effect on plant vitality also due to protection against environmental stress factors such as drought (Lehto, 1992; Garbaye & Churin, 1997; Ortega *et al*., 2004), pathogens (e. g. Morin *et al*., 1999) and alleviation of heavy metal toxicity (Mackova *et al*., 2006). Ectomycorrhizal fungi also improve soil stability and water storage capacity. On the other hand, for ECM, any factor causing changes in carbohydrate flux and amount in the roots and can have a potential stress effect on ECM symbiosis (Nylund, 1988). Environmental stress can have an impact on communities of ECMf and longevity of ECM. Fine root turnover of Norway spruce takes approximately two years (Leppälammi-Kujansuu *et al*., 2014), but environmental stress can promote aging and turnover rate of mycorrhizal root tips (e. g. Kottke *et al*., 1993).

Soil factors, especially soil physical and chemical traits, among all environmental factors are the most important for mycorrhizas and have influence on the richness of spruce ECM (Karliński *et al*., 2007). Most of the ECMf are sensitive to high pH and can grow *in vitro* under pH ranging between 3.2 and 6.5 with an optimum at pH 4.5 to 5.5 (Hung & Trappe, 1983).

As different ECMf appear to have different ecology (di Pietro *et al*., 2007; Lehto *et al*., 2008), it has been assumed that high diversity of ECMf is important in the dynamic functioning of woodland. As species differ in their functional and enzymatic activities (Pritsch & Garbaye, 2011; Velmala *et al*., 2014), some species may be better at helping the tree to take up particular nutrients by special enzyme production capacities; others may be specialized in protecting against pathogens etc. Although the importance of ECM has been widely acknowledged, the factors determining ECM community structure and species diversity are scarcely understood (e.g. Tedersoo *et al*., 2012).

2.1.3. Description Methods of ECM Communities

The identification of the fungi involved in mycorrhizal symbiosis is critical to understand ECM community composition, structure and ecology. Initially, knowledge of ECMf was based on observations or systematical monitoring of fungal fruiting bodies. Collection of such data is the least expensive and less time consuming method, but it requires great specialization to identify fungal species and there is great variability in fungal fruiting within a season and between different years (Tofts & Orton, 1998). To interpret fruitbody observation data as communities of ECMf, it was assumed that fruitbody production is related with relative abundance or impact of these species in soil (Vogt *et al*., 1992). However, at

least half of the species of a belowground ECM community can be comprised of species that did not produce conspicuous epigeous fruitbodies. This means that absence of fruitbodies of species should not be interpreted as absence of this species in soil (Dahlberg *et al*., 1997). Detailed studies combining morphological and molecular methods demonstrated that fruitbody data gives only a small insight into belowground communities of ECMf (Taylor & Alexander, 1990; Mehmann *et al*., 1995; Nylund *et al*., 1995, Peter *et al*., 2001), as many important ECMf from *Corticiaceae*, *Thelephoraceae*, and *Ascomycetes* produce easily overlooked or hypogeous sporocarps or do not form sporocarps at all.

Another method used to describe ECM communities is root morphotyping. This method is based on observation of morphology of ECM on fine roots of forest trees. It can reveal relatively stable differences among ECM and therefore is suitable for ECM community description. However, few ECMf can be visually identified beyond all doubt by certain morphological features (for instance, *Cenoccocum geophilum*); for some species it is possible to determine an affiliation with a particular genus (*Tuber*, *Hebeloma*, *Laccaria*, or *Inocybe* e. g. Igleby *et al*., 1990). As positive identification of ECM is restricted to certain species, an alternative classification into "mycorrhizal types" based on morphological features has been elaborated (Agerer, 1986–2006). The main characters used for identification of mycorrhizal types are colour, type of ramification and shape, structure of the mantle surface, and the presence and characteristics of hyphae and rhizomorphs, presence or absence of laticifers etc. However, classification based on these features can create problems of interpretation (Suz *et al*., 2008). There is no consensus on which characters are the most informative and how many of them must be recorded for a valid description. In many cases, not enough distinct morphological characters are available; also some often used characters as colour and ramification can vary depending on root age or environmental factors (Egli & Kalin, 1991). Similarly as for the method of fruitbody identification, the morphological description method depends on researcher skill and experience; also nomenclature of morphotypes is not standardized, which makes it difficult to compare different studies.

Knowledge about the ecology of ECM communities has increased dramatically since molecular methods were introduced in this research field (Horton & Bruns, 2001). These new methods allow using either morphotyping in combination with molecular methods or exclusively molecular tools in ECM community research. Different patterns of association between the morphotypes and fungal species have been observed: (i) one morphotype represents one species, (ii) one morphotype represents several species or (iii), several morphotypes represent one species.

Most ECM studies have been focused on genes encoding the small subunit of ribosomal RNA (18SS rRNA) and internal transcribed spacer (ITS) region, because polymorphism of this region allow identification between ECM species (Kårén *et al*., 1997). Initially, molecular methods were used to characterize strains isolated on laboratory media or material extracted from hyphae (Prosser, 2002). Techniques of DNA extraction and methods to extract total DNA from bulk samples without laboratory isolation of fungi have been widely used. Denaturing gradient gel electrophoresis (DGGE) analysis has been applied to characterize fungal communities in soil by identification of sequencing bands of interest. The two most classical methods for ECM studies are fragmentation (polymerase chain reaction-restriction fragment length polymorphism) and Sanger sequencing of the ITS region (Mehmann *et al*., 1995; Peter *et al*., 2001). The implementation of next generation sequencing technologies (e. g., 454 pyrosequencing, Ion Torrent) enables large-scale analyses of these complex and normally species-rich fungal communities in soil (e. g., Tedersoo *et al*., 2010; Wallander *et al*., 2010; Kauserud *et al*., 2012).

2.2. Norway Spruce Ecology and Mycorrhization

Norway spruce (*Picea abies* (L.) Karst.), a conifer tree species from the *Pinaceae* family, is one of the most important tree species in temporal and boreal forests in Europe (Larsson, 2001). It has wide distribution in boreal and temperate zones. The Norway spruce distribution in Latvia is not even and differences are mainly related with and topographical relief and climate: upland regions are more favourable for spruce forests than lowlands mainly due to soil conditions (better aerated and nutrient rich in slope forests) and a more continental climate (Laiviņš, 2005).

Norway spruce grows in oligotrophic and mesotrophic soils – mesic, loamy, medium rich in nutrients and with a relatively shallow depth of water table. Optimal pH for Norway spruce is 5.3–6.0 (tolerates pH values ranging from 3.4 to 6.7); in Latvia it normally ranges between 4.1 and 4.5 in spruce stands (Kāposts & Ošlejs, 1988; Modrzyński, 2007).

Norway spruce has a shallow root system formed by several lateral roots and a wide root net in the soil upper layer. A tap root is present only in the first year and then it becomes reduced (Lange *et al*., 1978). But, depending on soil type, some anchor roots or even a tap root can be formed also in later stages of stand development (Mangalis, 2004). The development of second order roots depends on soil conditions and determines the rooting depth (Modrzyński, 2007). On well-aerated, moist, and fertile soils, lateral roots arising from the first order roots extend to a depth of 1 to 2 m (Modrzyński, 2007). However, the fine roots

(less than 2 mm in diameter) have maximum density in the humus and the top mineral soil layers (Schmid *et al*., 2002).

Fine roots of spruce are usually mycorrhizal and functionally adapted to uptake of water and mineral nutrients (Kalliokoski, 2011). Ectomycorrhizal symbiosis is characteristic for several tree families (*Pinaceae*, *Betulaceae*, *Fagaceae* and *Salicaceae*) from boreal and temperate forests (Bruns & Shefferson, 2004), including also for Norway spruce. This species was among the first plants in which mycorrhiza was observed in 1885 by Albert Bernhard Frank (Rudawska, 2007).

Mycorrhizas are found up to a depth of 1.25 m in Norway spruce stands, but distribution of species can differ; some occur at various depths or horizons, others are specific to a soil depth or horizon (Rosling *et al*., 2003; Rudawska, 2007). However, most of the Norway spruce ECM fungal network is aggregated in the uppermost 20 cm of soil, where nutrient cycling is the most rapid. Decrease of abundance of mycorrhizas with increasing soil depth can be related with decrease of oxygen content and increase of CO_2 concentration in deeper soil layers, along with changes in microflora, composition of organic soil components, and the nutrient status of soil and roots (Meyer, 1973).

Norway spruce forms symbioses with a wide spectrum of ECM species. The ECM species richness of Norway spruce stands has been reported from various countries and it is quite variable (Rudawska, 2007). In Sweden, sporocarp data indicated that about 240 ECMf are restricted to Norway spruce forests and 130 species are strongly associated with this habitat type (Hallingback, 1994). A range of 42 to 124 ECMf have been found in sporocarp surveys conducted in old, oligotrophic spruce forests in Fennoscandia (Dahlberg, 1991; Brandrud, 1995).

Species that produce inconspicuous sporocarps or none at all can be abundant on root systems of Norway spruce (Fransson *et al*., 2000; Peter *et al*., 2001). Therefore, in studies of the ECM community on Norway spruce, macroscopic features of mycorrhizas in combination with molecular identification methods have been used (Agerer 1986–2006; Rudawska, 2007). Scandinavian data suggests that most forests are dominated by a few species with high underground densities, while most other species have low densities (Fransson *et al*., 2000). The inherent structure of most ECM communities, with a few common species and large number of rare species, severely limits ability to accurately assess species richness (Taylor, 2002). Information on Norway spruce ECM communities in Latvia is available mainly based on records of fruitbodies found in Norway spruce stands (Dāniele & Krastiņa, 2002) and several studies based on the root morphotyping approach (Gaitnieks *et al*., 2000; Gaitnieks,

2005). However, no ECM studies combining morphotyping and a molecular approach have been previously made in Latvia.

2.3. Norway Spruce Forest Management

Norway spruce has been the most favoured tree species in economic terms, because of low number of planted out seedlings for regeneration, easy tending, relatively short rotation period, and as the most demanded wood raw material (Mauer *et al*., 2008; Jansson *et al*., 2013). It is one of the most important commercially grown conifers in Europe (Jansson *et al*., 2013) and Latvia. The territory of Latvia is in the southern part of Norway spruce distribution range for this species. Norway spruce forests form 17% (5374 km^2) of the forest area in Latvia (Jansons, 2011).

Commercial nursery production in Latvia exceeds 23 million Norway spruce seedlings yearly (Seeds and Plants, Latvian State Forests JSC (Joint Stock Company), unpublished data). Among other factors, seedling cultivation regime, system and seed provenance may have significant effect on survival and growth of replanted tree seedlings (Ying, 1991). In Latvia, three major standardized cultivation systems for Norway spruce are used: i) containerized system – seedlings are grown in interconnected plastic pots in greenhouses; ii) Plug+1 system – seedlings are pre-grown for a year as containerized and then transplanted and cultivated as bare-root and iii) bare-root system – seedlings are grown in open field beds (Seeds and Plants, Latvian State Forests JSC, unpublished data). For seedling cultivation, use of local seed provenances is often recommended because they are thought to be better adapted to local site conditions (Rone, 1984; Gailis, 1993). There are three seed regions (Western, Central and Eastern) of Norway spruce in Latvia; each with established seed orchards of local provenances. For seedling production, spruce seeds are predominantly used from the Central region (53%) and to a lower extent – from the Eastern (23%) and Western (24%) regions (Seeds and Plants, Latvian State Forests JSC, unpublished data).

More than one half of Norway spruce stands in Latvia are on mineral soils (Laiviņš, 2005). However, as there are many water-saturated sites in Latvia, to obtain greater wood yield, active forest management was developed not only for mineral soils, but also on peatland. As spruce stands do not occur naturally in water-saturated sites, drainage is commonly used forestry practice to promote forest growth (Prescott *et al*., 2000; Zālītis, 2006). Presently, in Latvia, 2067 km^2 (38% of spruce forests) of forest occurs on drained soil (Jansons, 2011).

Norway spruce stands on drained organic soil are characterized by productive growth up to 40 to 60 years reaching wood yield more than 300 m^3/ha (Zālītis, 2006). Therefore,

plantings of Norway spruce are recommended not only in dry forest types (*Hylocomiosa*, *Oxalidosa*, *Aegipodiosa*), but also in drained forest types on mineral soil *(Myrtillosa mel*., Mercurialosa mel.) and organic soil (*Myrtillosa turf. mel*., *Oxalidosa turf. mel*.) (Lībiete & Zālītis, 2007). However, wood yield reduction usually is observed in drained stands of Norway spruce on organic soils after the active growth period has stopped at 40 to 60 years (Zālītis, 2006). Norway spruce forests on drained peatland areas can be more problematic than stands on mineral soils for reforestation due to changes in the water table, weather conditions and plant community succession after draining (Potila *et al*., 2007) as well as nutrient imbalance and pathogen activity (Kāposts & Ošlejs, 1988).

2.4. Some Problematic Aspects of Spruce Forest Management in Latvia

Among the dominant forest tree species in Europe, Norway spruce is the most susceptible to both abiotic and biotic stress (Schmidt-Vogt, 1989; Modrzyński, 2007). Spruce mortality has increased in Eastern and Northern Europe (e. g. Mäkinen *et al*., 2001; Forster & Meier, 2005; Mauer *et al*., 2008). Windstorms, snow and insects are the main agents causing disturbance and severe damage in spruce stands. A shallow root system is one of the factors that make Norway spruce susceptible to environmental disturbances (Mäkinen *et al*., 2001).

In spring and early summer of 2010, serious damage in spruce stands was observed in Latvia (Lazdiņš *et al*., 2011). Characteristic spruce damage symptoms were brownish and drying spruce top shoots and short new shoot increments. Foliar damage progressed until the last decade of July, when in several stands dieback symptoms were present also in the canopy, and even total defoliation was noted. After July, changes in damaged stands were minimal, with the exception that activity of the pest *Physokermes piceae* became significant. This species mostly colonizes weakened trees and thus is not considered as a typical and important pest on spruce (Meier *et al*., 2006). Most of the damaged stands in Latvia were even-aged spruce monocultures on drained soils (Lazdiņš *et al*., 2011). Such stands are characterized by low soil transpiration rate, unstable soil temperature and moisture regime due to accumulation of spruce litter (Mangalis, 2004). Impact of soil factors on tree damage was hypothesized because damage was mainly observed in stands on seasonally or permanently water-saturated soils and the damaged sites had a patchy distribution, with well defined borders along drainage ditches or stand edges. The study showed that soil pH, peat layer density, groundwater level and potassium concentration were significantly correlated with foliar damage intensity (Bardule *et al*., 2012).

On drained peatlands with sufficient aeration, tree growth is commonly limited by nutrient availability, especially by P or K, but sometimes also by N or B (Rütting *et al*., 2014). Continuous wood production on drained peatland may exhaust especially the K stores of the root layer (Finer, 1989 and references within it). It is likely that the peat layer gradually becomes thinner as a result of decomposition and tree harvesting, and tree roots penetrate into deeper peat layers or even down to the subsoil for nutrients. On deep peat mires this, however, does not increase the available potassium reserves, as they are mainly in the surface peat (Finer, 1989). The situation can be improved by K fertilization and by avoiding whole-tree harvesting. Therefore, fertilization in adequate dosage could be beneficial for wood production and stability in managed forest on peat soils (Aronsson & Ekelund, 2004).

At the beginning of the present century, several experiments on effect of wood ash fertilization in conifer forest on peat soils were established in Latvia (Indriksons *et al*., 2003; Gaitnieks *et al*., 2005). Wood ash supplies potassium (Pitman, 2006; Augusto *et al*., 2008), which could be deficient in forests on peat soils (Finer, 1989 and references within it). Some studies have studied the effects of wood ash application on forest growth (Aronsson & Ekelund, 2004). Long-lasting positive effects on tree growth have been observed on shallow peat rich in N (Hytönen, 2003; Aronsson & Ekelund, 2004) and on drained peatlands (Ferm *et al*., 1992; Moilanen *et al*., 2002; Haveraaen, 2014).

Other problematic aspect of spruce stands in Latvia is *Heterobasidion* root-rot (Arhipova *et al*., 2011); timber losses of this desease are estimated up to 20% in Latvia (Gaitnieks *et al*., 2008). It is one of the most important diseases in conifer stands of north temperate regions worldwide (Gonthier & Thor, 2013). In about 80% of cases of infected trees, root-rot is caused by *Heterobasidion annosum* sensu lato (Korhonen & Piri, 2003). High incidence of *Heterobasidion* spp. usually occurs on dry, sandy soils with high pH and low organic matter content, especially in stands occupying previously arable land (Stenlid & Redfern, 1998; Redfern *et al*., 2010). However, in Latvia *Heterobasidion* root-rot observed has been observed also on drained nutrient rich peatland (LSFRI Silava, unpublished data).

Heterobasidion annosum s.l. infection can be dispersed by mycelium through root contacts (Hodges, 1969), but it is not able to freely grow in the soil (Garbelotto & Gonthier, 2013). However, the incidence of *Heterobasidion* root-rot in some studies has been related to the soil microbial community and its antagonism to *H. annosum* (Gibbs, 1967; Mańka, 1970, Arhipova *et al*., 2008a; Arhipova *et al*., 2008b; Grantina-Ievina *et al*., 2013), including ECMf (Kowalski, 1974).

Numerous experiments have shown that stump removal is an effective method for controlling spread of *H. annosum* mycelium in heavily infected forest areas (Greig, 1980;

Stenlid, 1987; Vasaitis *et al*., 2008). Although this operation is expensive, the interest in this procedure has been resumed during the last years due to the possibility of using stumps as an energy source (Laitila *et al*., 2008; Walmsley & Godbold, 2010). Moreover, Saarinen (2006) showed that slash and stump removal will increase the work productivity and quality of mechanized forest regeneration. However, little is known about the possible ecological impact of stump removal (Walmsley & Godbold, 2010). Although the majority of available studies indicate improved seedling establishment and growth on stump removal sites, especially on dryer and sandy soils (Vasaitis *et al*., 2008 and references therein), some studies indicate that stump harvesting may lead to increased biomass removal, which may result in a significant loss of nutrients, leading to potentially negative effects on site productivity in the future (Palviainen *et al*., 2010; Egnell, 2011). There have been several trial experiments of stump removal done in Latvia in studies based on phytopathological, ecological and economical points of view (Lazdiņš *et al*., 2014). However, this forest management practice is not established and widely applied in Latvia.

2.5. Specific Characteristics of Conifer Mycorrhization in Forest Nurseries and after Outplanting

Ectomycorrhizal fungi provide nutritional benefits to their hosts (Parlade & Alvarez, 1993; Jonsson *et al*., 2001) and consequently may affect vitality and quality of the seedlings (Smith & Read, 1997). Well developed ECM can promote seedling survival and growth in the nursery (Hunt, 1992) and in the field (Kropp & Langlois, 1990; Le Tacon *et al*., 1994; Menkis *et al*., 2007; Menkis *et al*., 2012). Diversity and community structure of ECMf are commonly associated with the growth parameters of their hosts (Korkama *et al*., 2006). A recent study from Finland showed a statistically significant clone-specific effect on diversity of ECMf and abundance of dominating fungal taxa (Velmala *et al*., 2013).

Intensive management practices in forest nurseries may often result in both reduced ECM colonization of seedling roots and species diversity (Arnebrant & Soderstrom, 1992; Nilsson & Wallander, 2003), while promoting only a limited number of ECMf species that tolerate such growth conditions (Khasa *et al*., 2001; Menkis *et al*., 2005; Menkis & Vasaitis, 2011). Cultivation practices in the forest nursery have an impact on conifer seedling mycorrhization and fungal diversity (Menkis *et al*., 2005, Rudawska *et al*., 2006; Trocha *et al*., 2006; Flykt *et al*., 2008). Intense seedling fertilization with nitrogen increases biomass of spruce seedlings, but reduces root/shoot ratio, number of fine roots and their mycorrhization (Brunner & Brodbeck, 2001; Seith *et al*., 1996; Wallenda *et al*., 1996; Haug & Feger, 1990).

However, as planting material and nursery practices are different in various countries, local studies are useful and provide more transferable information for the local forestry sector.

The seedling mycorrhizal community from a nursery or artificial inoculum is replaced by other species quite fast after outplanting in a field (Menkis *et al*., 2007). Seedling roots are mainly colonized by early stage ECM species, since after forest clear cutting losses in ECM inoculum and large changes in its species composition are commonly observed (Jones *et al*., 2003). Soil preparation method before planting also can influence seedling mycorrhization (Heinonsalo *et al*., 2004; Pennanen *et al*., 2005). Stump removal in certain cases is associated with site disturbances, which may lead to alterations in chemical, physical and biological properties of soil (Hope, 2007). Few studies have looked at root mycorrhization and communities of ECMf in stump removal sites (e. g. Page-Dumroese *et al*., 1998; Menkis *et al*., 2010; Kataja-aho *et al*., 2012): thus, more studies are needed, especially covering a broader spectrum of forest types and geographical regions.

2.6. Specific Characteristics of Spruce Mycorrhization in Mature and Managed Forest Stands

In both seedlings and mature trees, well developed mycorrhiza can be beneficial for forest growth; it may be crucial for plants adapting to different site conditions, since fungi can increase host plant tolerance to both abiotic and biotic stress (Potila *et al*., 2007). Moreover, reduced mycorrhizal intensity and diversity can be related to stress factors causing forest decline (Mejstřík, 1989). Recently, interest about communities of ECMf in damaged forests and their possible role in plant protection is increasing (Blom *et al*., 2009; Corcobado *et al*., 2014). In spruce stands on drained peatland soils in Latvia, it has been observed that stands with low above-ground increment have less diverse ECM than stands with greater growth rate (Zālītis, 1989). Zālītis (2006) suggested that reduction of productivity on spruce stands on drained peat soils could be related with reduced activity or decline of fine roots or disturbance of mycorrhizae.

Many studies have shown that ECMf have negative impact on the incidence of diseases caused by root pathogens, such as by *Phytophtora*, *Phytium* and *Fusarium* species (Strobel & Sinclair, 1992; Megumi Kasuya *et al*., 1996; Whipps, 2004). Although *Heterobasidion annosum* is not a fine-root pathogen, there is evidence that in nurseries and young plantations coniferous trees can be infected through thin roots (Korhonen, 1978). In other, more recent studies, it has been shown that resistance factors of young nonsuberized, suberized and woody roots of Norway spruce can be overcome by different infection procedures (Asiegbu *et al*., 1993; Heneen *et al*., 1994a, 1994b). Unfavourable site conditions with negative impact on

host vitality also can make fine roots vulnerable to infection by *H. annosum* s.l. (Schoeneweiss, 1975). Under laboratory conditions, several ECM species have been observed to suppress mycelium growth of *H. annosum* (Hyppel, 1968a, 1968b; Hüppel, 1970; Červinkova, 1989, Napierała-Filipiak & Werner, 2000). Some studies have combined evaluation of mycorrhizal composition in infected stands with ECM species isolation and antagonism tests against *H. annosum in vitro* (Bücking, 1979; Napierała-Filipiak & Werner, 2000). In Latvia, fine root morphological parameters and ECM morphotype abundance were compared in *Heterobasidion* root-rot infected and health Norway spruce stands (Gaitnieks *et al*., 2000; Gaitnieks, 2005). These results showed some evidence of ECM differences between healthy and *Heterobasidion* infected stands.

Forest fertilization with wood ash can have an impact on development and distribution of fine roots of forest trees (Majdi & Viebke, 2004) and increase fine root biomass (Nowotny *et al*., 1998; Jonsson *et al*., 1999). The effect of wood ash fertilizer on fine roots largely depends on dosage applied (Clemensson–Lindell & Persson, 1995; Majdi & Viebke, 2004; Augusto *et al*., 2008) and site conditions. The greatest reported adverse ecological effect of wood ash fertilization has been to acidophilic ecosystems, particularly the constituent communities of bryophytes, soil bacteria and ECMf (Pitman, 2006; Augusto *et al*., 2008). Since wood ash fertilization has a strong liming effect on soil, and significant differences in ECM community in fertilized sites are mainly related with increased soil pH, as most of ECMf are sensitive to high pH (Hung & Trappe, 1983). *In vitro* studies, for example, have shown that mycelium of ECMf grows slower in limed peat substrate with increased pH than in unlimed peat (Erland *et al*., 1990). Wood ash may contain heavy metals (Augusto *et al*., 2008), and therefore differences in occurrence of ECMf can be related to different tolerance to heavy metals among ECMf.

3. Material and Methods

3.1. Studied Sites and Experimental Design

All study sites were located in the territory of Latvia, mainly in the central and eastern part of the country (Figure 1). Latvia is located in the transitional area between the temperate and boreal biomes, called the hemiboreal zone (Laiviņš *et al*., 2009). Its vegetation shares many similarities with the boreal biome. Latvia is largely covered by forest (approx. 50% of the total land area; LSFRI Silava, unpublished forest inventory data). The most common tree species are Scots pine (*Pinus sylvestris* L.), Norway spruce and silver birch (*Betula pendula* Roth). Latvia has a temperate climate, which is maritime (cool summers and mild winters) in coastal regions, especially along the western coast, and continental (warmer summers and harsher winters) in eastern parts. The climate is seasonal – the warmest month is July (mean air temperature +17.0ºC) and coldest months are January and February (mean air temperature -4.6 and -4.7ºC). Mean annual air temperature is +5.9ºC. Precipitation is common throughout the year with annual precipitation about 667 mm. Meteorological data given here and used for data interpretation in this thesis were obtained from the Latvian Environment, Geology and Meteorology Centre LLC (Limited Liability Company).

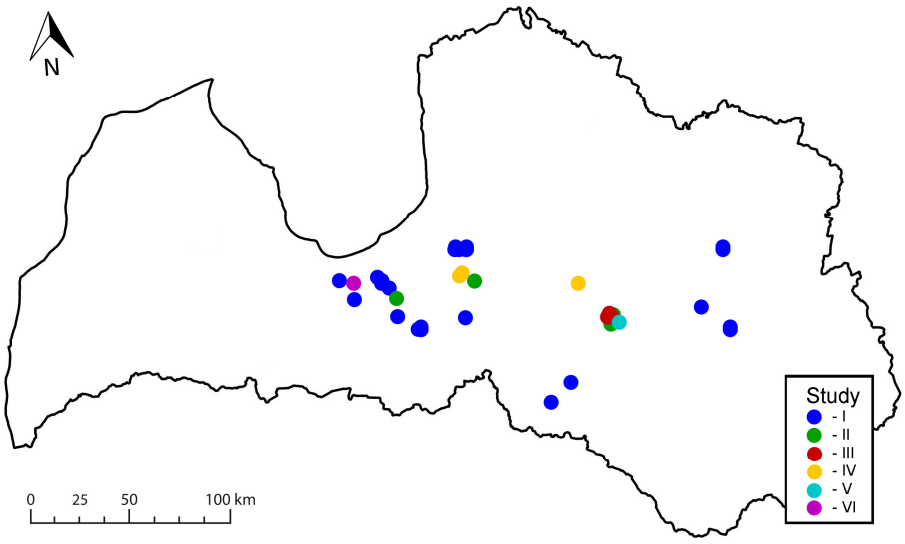

Figure 1. Location of sample sites in Latvia. Each circle represents one study (I – Foliar damage study; II – Root-rot study; III – Fertilization study; IV – Stump removal study; V – Seed origin study; VI – Cultivation system study) site that corresponds to colour in the legend.

The study sites and experimental design differed among studies, and therefore their descriptions are given separately. Geographical coordinates, abbreviations of different treatments, and brief descriptions of forest stands and experimental planting sites are given in Appendix 1. Forest classification used according to the Latvian classification system (Zālītis and Jansons 2013).

3.1.1. Foliar Damage Study

Twenty four sites where spruce foliar damage had been reported in spring 2010 were randomly chosen. The study sites were mostly located in Central and Eastern Latvia, as damaged stands were present only in those regions. All studied forest stands were managed by Latvian State Forests JSC or Riga Forests LLC.

Two sample plots were established in each site – one in a severely damaged part of the stand and the other in a minimally damaged part. Sample plots were circular (radius 12.62 m) with total area 500 m^2. Distance between plots was 50–150 m. Evaluation of crown damage was carried out in July 2010. Browning of the top and canopy basal part of spruce was considered as a damage symptom. Foliar damage of each individual spruce tree in sample plots was evaluated in four classes: i) healthy trees (no foliar damage); ii) weakly damaged trees with brown top shoots iii) heavily damaged trees with defoliation or dechromation more than 60%; and iv) dead trees. Data on stand foliar damage was used as background information for analysis of root parameters.

All sites were dominated by Norway spruce (more than one half were spruce monocultures and the others were spruce stands mixed with mainly silver birch or in some sites with other species – Scots pine, black alder (*Alnus glutinosa* L.) or grey alder (*Alnus incana* (L.) Moench). Most of the stands had age 40 to 50 years (range 20 to 70 years). All sites except three were on drained soils. Soil type was described from soil profile pits to 1.5 m depth. Soil type taxonomy was described according to Latvian nomenclature and linked to the international soil classification system according to Karklins *et al*. (2009).

3.1.2. Root-Rot Study

Four Norway spruce stands growing on drained peat soils were chosen. Stand age differed among sites (41 to 112 years); two stands were located in eastern Latvia and two in the central part. These stands were previously examined in a study on infection of *Heterobasidion* root-rot (LSFRI Silava, unpublished data) and the available detailed background data on health status for each individual tree and stump were the main criteria for

study site selection. All the trees and stumps in these stands were marked, numbered and mapped. Wood samples from each tree and stump were taken. Trees were sampled at height 30–40 cm from root collar using a Pressler increment borer or motor-saw. Wood samples from stumps were taken from stump base or roots with a motor-saw or axe. Each wood sample was sterilized by flame and placed on sterile malt-agar media, and after one week presence of *Heterobasidion* spp. was assessed. Two *Heterobasidion* species i.e. *Heterobasidion annosum* (Fr.) Bref. and *Heterobasidion parviporum* Niemelä & Korhonen were recorded from the study sites.

Based on these data, six to eight sample plots per site were selected. Three or four plots, each possessing a group of 3 – 5 infected trees, and a corresponding number of closely situated plots with a group of 3 – 5 uninfected trees, were selected in each site.

3.1.3. Fertilization Study

The experimental sites were located in the eastern part of Latvia in the Kalsnava forest district of the Forest Research Station. For this experiment, three mature conifer stands on drained peat soils dominated by Scots pine and Norway spruce were selected. The age of dominant tree species was similar among sites (Appendix 1). Sites belonged to different forest types (*Vacciniosa turf. mel*., *Myrtillosa turf. mel*. and *Caricoso-phragmitosa*) and consequently soil fertility and moisture regime differed among sites. Background data of tree growth rate after fertilization was available, which showed significantly higher stem growth rate in fertilized sites, except in one site (*Myrtillosa turf. mel./Caricoso-phragmitosa*) where no difference between fertilized and control trees was observed.

To determine long-term effects of fertilization with wood ash, plots for a groundwater quality study were established in spring 2002 (12 years prior to this study) as a part of the European Union project WOOD-EN-MAN (Indriksons *et al*., 2003). The experimental design was setup to measure nutrient flow from wood ash fertilized plots located at different distances to groundwater wells. In each site, wood ash was spread around three groundwater wells in 1-m wide circular zones, with inner circle edge located at 1, 2 or 3 m from the respective well. These three types of fertilized sample plots had area 9.4 m^2, 15.7 m^2 and 22.0 m^2, respectively. In addition, there was one control sample per forest type with a 5 meter radius around a control groundwater well (area approx. 78.5 m^2). Groundwater wells and consequently sample plots were set up along a transect at 25-m intervals away from a drainage ditch. After marking edges of plots, 5 kg m^{-2} of loose wood ash were applied, resulting in application of 215 kg PO_4^{-3} , 540 kg K^+ , 2820 kg Ca^{2+} , and 3820 kg of Mg^{2+} per

ha. Ash was obtained from a timber sawmill Vika Wood LLC (Talsi region, Latvia). Spruce bark was the main fuel used in the incinerator.

In the original design, experimental plots were located along a transect where the control plots were the most distant from a drainage ditch. Additional circular control plots were established with a 5 m radius paired with each wood ash fertilized plot equidistant from the drainage ditch. Distance between fertilized and control plots was at least 25 m. In total, 21 plots (three wood ash fertilized and four control plots on each of three experimental sites) were established.

3.1.4. Stump Removal Study

The study sites comprised three forest clear-cuts (3.8 ha, 2.7 ha and 1.5 ha in size), located about 10-km distance from Turkalne (Ikšķile district) in the central part of Latvia. All sites were managed by Riga Forests LLC. Each site represented a different forest type: *Hylocomiosa*, *Myrtilloso-sphagnosa* and *Myrtillosa-mel*. The soil of the *Hylocomiosa* forest type was a well-aerated dry podzol. The *Myrtilloso-sphagnosa* type was on poorly-aerated gleyic soil and the *Myrtillosa-mel*. type on a wet and drained (dry) podzol. All studied stands were clear-felled in winter 2010/2011. Before planting in spring 2012, in each site stumps were removed from half of the area using a New Holland tractor (v. 5215B) equipped with a stump extractor MCR-500 (LSFRI Silava and Orvi LLC, Salaspils, Latvia). For the other half of each site, disc trenching was used as a soil preparation treatment using a two-row disc trencher TTS Delta (LSFRI Silava, Salaspils, Latvia). Distance between trenching rows was ca. 2.5 m. In the *Myrtilloso-sphagnosa* and *Hylocomiosa* types, soil preparation (stump removal and trenching) was conducted in two replicates, while in the *Myrtillosa-mel*. type the treatments were not replicated. As a result, ten plots were established. Within each clear-cut, different treatments and replicates were separated from each other by a 20 m buffer zone.

Planting was conducted in April 2012 using two-year-old containerized seedlings of Norway spruce (Norupe forest nursery, Riga Forests LLC, Ikšķile district, Latvia). Seedlings were planted in rows at a density between ca. 918 and 2773 seedlings per hectare. Prior to planting, ECM status of the roots was not examined, but assumed to be similar for all seedlings due to similar growing conditions in the nursery. General information of seedlings used in this study is given in Appendix 2.

3.1.5. Seed Origin Study

Seedlings representing five different seed origins were analysed in this study. For seedling cultivation seeds of five different provenances representing Western (W1 and W2 provenances), Central (C1 and C2) and Eastern (E1) seed regions of Norway spruce in Latvia were used. Seeds were obtained from the seed storage centre of Latvian State Forests JSC. Seeds were collected from at least 50 trees in each commercial seed stand (provenances W1, C1, C2 and E1) and seed orchard (Remte seed orchard – W2). The Remte seed orchard represents the first generation clonal material of 54 clones.

In April 2006, seeds were sowed and bare-rooted seedlings were cultivated for three years in sandy soil at the Forest Research station in Kalsnava (56º67.97´N, 25º97.17´E). Before this study, conifer seedlings were regularly cultivated in the field and no substrate sterilization had been carried out. Seedling cultivation included fertilization twice a year using NPK fertilizer (Bayer CropScience, Monheim, Germany), application of pesticide Previcur 607 (Bayer CropScience, Monheim, Germany) at initial stage of cultivation and application of pesticide Actara (Syngenta, Basel, Switzerland) twice a year. No artificial ECM inoculation was carried out. In April 2009, three-year-old seedlings were separately replanted in 2-litre plastic pots (MCI 17, East Riding Horticulture Ltd., York, UK) filled with sphagnum peat substrate KKS-M1, pH 4.5 (Laflora LLC, Jelgava, Latvia). This separated root systems of the seedlings from each other. Plastic pots with seedlings of different seed provenances were labelled and randomly arranged in an open field, where they were grown for the next three years. At this stage of seedling cultivation NKP fertilizer was applied once seasonally (approx. 6 g per seedling) and extra fertilization with Vito-Silva mineral fertilizer (Spodrība JSC, Dobele, Latvia) in spring 2010. No pesticides were applied to the seedlings after transplanting to pots. In total, 50 spruce seedlings were produced from seeds of each provenance, with a total of 250 seedlings. General information on seedlings used in this study is given in Appendix 2.

3.1.6. Cultivation System Study

An experimental plantation 7500 m^2 in size was established in May 2006 in Tīreļi forest district (managed by Riga Forests LLC). The site was a previous northern temperate forest stand dominated by Norway spruce, which had been clear-cut in 2005. Soil had the following chemical composition (mg/l of soil): N 30, P 87, K 22, Ca 2550, Mg 213, S 28, Fe 850, Mn 6.5, Zn 1.6, Cu 0.2, Mo 0.06, B 0.1; soil KCl pH was 5.03 (data from the Laboratory of Plant Mineral Nutrition, University of Latvia, Institute of Biology).

Before planting, the site was ploughed in 20 rows at intervals of 1.5 m, and in each row seedlings of different treatments (one treatment per row) were planted. Different treatments (two Scots pine variants (not analysed in this thesis) and two Norway spruce variants) were arranged in four adjacent rows, resulting in a block, which was replicated five times throughout the plantation. Arrangement of different treatments within each block varied.

Seedling material used was produced in the Strenči forest nursery (Seeds and Plants, Latvian State Forests JSC), which is the largest seedling producer in Latvia. In the nursery, containerized seedlings were grown in 85 cm^3 pots in the greenhouse using sphagnum peat as a substrate, and bare-root seedlings were grown in mineral sandy soil in open field beds. Sphagnum peat (pH 3.6) was produced by Seda JSC (Seda, Latvia) and contained 70% of milled peat and 30% of block peat with addition of 0.8 g/m^3 PG Mix 14:16:18 (N, P, K) and 1.8 kg/m^3 lime (Nollendorfs, 2004). No pesticides were used in cultivation of seedlings. Seedlings used in the present study represented standard planting material of each respective cultivation system. General information of seedlings used in this study is given in Appendix 2.

3.2. Root Analysis

3.2.1. Root Sampling

For the **Foliar damage study,** root samples were collected in August 2010 under tree canopies (about 2 m from tree stem), 240 samples in total. Roots were sampled to 20-cm depth using a soil core (3.6 cm in diameter). Five replicate cores from each sample plot were collected.

For the **Root-rot study,** roots were sampled to 20-cm depth using a soil core (12 cm in diameter). Samples were taken from each tree group within a common rooting zone of selected trees resulting in 140 samples (4 stands x two tree health classes x 3 to 4 replicates plots x 5 samples). Sampling in different sites was carried at different times – July 2010, 2013 and August 2014.

For the **Fertilization study,** roots were sampled to 20-cm depth using a soil core (12 cm in diameter). Three replicate cores were collected from each sample plot. Sampling was done in August 2014. In total, 63 soil cores (27 from fertilized areas and 36 from control plots) were collected.

For the **Stump removal study**, in October 2012, 6 months after planting, 20 spruce seedlings were collected from each of ten plots, resulting in 200 sampled seedlings. In each plot, seedlings were sampled along the diagonal in consecutive order. Before washing, roots of each seedling were separated in two categories: roots originally present in a forest nursery

container ("old roots" present in a peat lump); and, current year roots ("new roots") produced during the growing season 2012 (growing out from the peat lump).

For the **Seed origin study**, root sampling was done six years after seed sowing in January 2012, i. e., at time of dormancy. Root samples were collected taking entire root systems out of the pots and cutting with a secateur ca. 10% of the entire root volume with the substrate, which was retained for study.

For the **Cultivation system study**, sampling of seedlings was carried out during four consecutive growing seasons (2006, 2007, 2008 and 2009) – 86 seedlings in total.

All root samples (either seedlings or soil cores) were carefully excavated to preserve fine roots, packed into plastic bags, labelled, transported to the laboratory and stored under +4˚C for a maximum period of three weeks before processing.

In the laboratory, fine roots were mechanically separated from soil and washed under tap water. Before root analysis, all woody roots with diameter more than 2 mm and roots of other plants were removed. Samples not analysed immediately were stored in Petri dishes with water at + 4ºC for a period shorter than a week. To facilitate analysis of root microscopic features, samples were cut into approx. 1-cm fragments.

3.2.3. Analysis of Root Morphology

In the **Foliar damage study,** the number of fine roots per growth stage was estimated. Grouping of fine roots in three growth stages was based on external features as described previously (Ritter *et al*., 1989; Clemensson-Lindell & Persson, 1995; Göbl, 1996): 1) fine roots with white turgid root tips; sometimes darkened but with well formed and undamaged mycorrhizal mantle; the stele is white and elastic (Young living root); 2) more suberized fine roots or fine roots with damages of external mantle surface; the stele is still elastic and light to slightly brown (Old living root); 3) fine roots, which normally are referred to as dead; the stele is brownish and easily broken and no elasticity remained; root tips are blackened and fragile (Dead root).

In the **Root-rot and Fertilization studies,** the numbers of living and dead fine roots were estimated. The distinction between live and dead fine roots was made by evaluating the colour and elasticity of the central cylinder of fine roots (Vogt & Persson, 1991). In the **Root-rot study**, all root fragments of a sample were spread out in a Petri dish. A metal construction was placed in the dish, which physically divided into 8 radial sectors with sides extending from centre to edge (Figure 2). Roots within one of these sectors were transferred to another Petri dish for analysis. In the **Fertilization study**, number of living and dead fine roots was assessed analysing the same root fragments as for root morphotyping.

Figure 2. Petri dish with metal construction used in **Root-rot study**.

Root biomass was determined in the **Root-rot, Fertilization** and **Stump removal studies**. Roots were dried at 50ºC for 12 h and weighed.

In the **Root-rot** and **Stump removal studies,** morphological parameters (length, volume, surface area, and number of root tips) of roots were determined using an Epson Perfection V750Pro scanner (Epson, Tokyo, Japan) and WinRHIZO 2005 C (Regent instruments Inc., Canada) software.

3.2.4. Ectomycorrhizal Morphotyping

ECM tips were recognized by the presence of mantle, external hyphae or rhizomorphs, and the absence of root hairs (Smith & Read, 1997). ECM root morphotyping was conducted in all studies. Sampled ECM root tips from seedlings and mature spruce were grouped into different morphotypes according to their morphological characteristics (colour, shape, mantle structure, patterns of rhizomorphs and extramatrical hyphae) (Agerer, 1986–2006) (Figure 3). A stereomicroscope (Leica MZ-7.5, Solms, Germany) was used for root morphotyping.

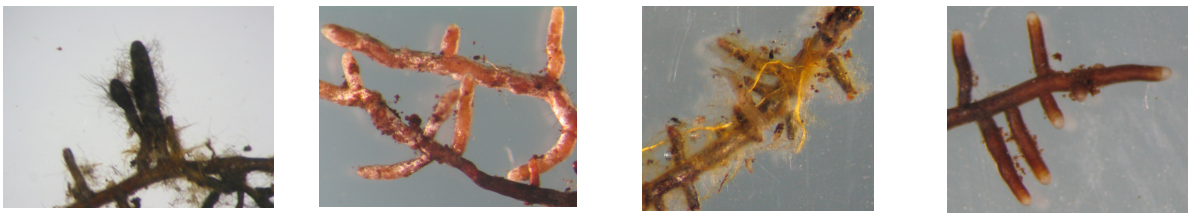

Figure 3. Different ECM morphotypes.

In **Foliar damage, Root-rot, Stump removal** and **Seed origin studies,** roots were evenly placed in Petri dish in water with a grid (mesh size 7x7 mm) on the bottom. In the **Foliar damage study**, number of root tips representing each root growth stage and dominant

ECM morphotypes were counted in 100 grid squares of each Petri dish, starting from the upper left corner. In the **Root-rot study**, presence of each distinct ECM morphotype was noted in 200 grid squares. In the **Stump removal study**, only new outgrown roots were taken for morphotyping. Single root tips were systematically sampled at the crossing points of the gridlines. In total, 100 single root tips were sampled from each root system and their mycorrhization and morphotype were determined. In the **Seed origin study**, for each seedling, 30 randomly selected squares of the grid were used to estimate the number of fine living roots, their mycorrhization and ECM morphotypes.

In the **Fertilization study**, 20 randomly chosen root fragments were taken and number of ECM root tips per morphotype and number of dead fine roots were counted.

In the **Cultivation system study**, 20 single ECM root tips from each seedling were randomly collected from different parts of the root system and morphotyped.

3.2.5. Molecular Identification of ECM Fungi

For molecular identification of fungal species of each morphotype, one to six root tips were separately placed in 1.5 ml centrifugation tubes and stored in 70% ethanol at -20ºC. Representatives of each distinct ECM morphotype were collected from each sample plot, forest site or seed provenance. Ethanol was removed and roots were dried on sterile filter paper immediately before molecular identification procedure.

A Phire Plant Direct PCR Kit (Thermo Fisher Scientific, Inc., USA) was used for direct polymerase chain reaction (PCR) from root tips (Velmala *et al*., 2014) in the **Foliar damage, Root-rot, Fertilization** and **Stump removal studies**. Polymerase chain reaction was performed in 20 µl reactions containing 0.5 µl dilutions of crushed samples. The thermal conditions were as follows: 5 min initial denaturation at 98ºC, then 40 cycles of 5 s denaturation at 98ºC, 10 s annealing at 57ºC and 20 s extension at 72ºC, and 1 min final extension at 72ºC. Amplification of the fungal ITS region of rRNA gene was performed using universal primer ITS4 (White *et al*., 1990) and fungal-specific primer fITS7 (Ihrmark *et al*., 2012) (in the **Foliar damage** and **Stump removal studies)** or primer ITS1F (Gardes & Bruns, 1993) (in the **Root-rot** and **Fertilization study**).

In the **Seed origin study**, isolation of DNA was done following the protocol described by Vainio *et al*. (1998). The internal transcribed spacer region of fungal ribosomal DNA was amplified by PCR using fungal specific primer ITS1F (Gardes & Bruns, 1993) with a 40 bp GC clamp (Muyzer *et al*., 1993) and universal primer ITS2 (White *et al*., 1990). The 50 µl PCR reaction was performed with Dreamtaq DNA polymerase (Fermentas, Thermo Scientific, Waltham, MA, USA) in an Arktik TM Thermal Cycler (Thermo Scientific, Waltham, MA,

USA). The cycling conditions were optimized according to manufacturer's instructions: 5 min initial denaturation at 95 ºC, followed by 35 amplification cycles (30 s denaturation at 95 ºC, 30 s annealing at 57 ºC and 60 s extension at 72 ºC) and 5 min final extension at 72 ºC (Thermo Scientific, Waltham, MA, USA). Success of PCR-amplification was verified on 1% agarose gels using electrophoresis. Denaturing gradient gel electrophoresis (DGGE) was performed using the D-GENE gel system (Bio-Rad, Hercules, USA) at 75 V, 60ºC for 16 h as described by Korkama *et al*. (2006). Based on DGGE analysis, all unique PCR amplicons, and between two and three amplicons of the same pattern were selected. Selected samples were amplified by PCR using original DNA extracts and a primer pair ITS1F-ITS4 (White *et al*., 1990, Gardes & Bruns, 1993).

For the **Cultivation system study**, DNA extraction and PCR using ITS1F and ITS4 primers (White *et al*., 1990) were done as described previously (Menkis & Vasaitis, 2011).

Amplification success in all studies was checked in 1% agarose electrophoresis gel in 1% TBE (Tris-Buffer-EDTA) stained with ethidium bromide and visualized under UV light.

Successful PCR products were Sanger-sequenced in one direction by Macrogen Inc. (Europe, The Netherlands (or Seoul, Korea in case of **Cultivation system study**)). Some samples from **Root-rot study** were sequenced in the Genetic Resource centre of LSFRI Silava.

Raw sequence data were analyzed using SeqMan Pro version 5.07 and 9.1.0 software from the DNASTAR package (DNASTAR, Madison, WI, USA) and BioEdit version 7.0.5.2 (Hall, 1999). Reference sequences from databases of GenBank (Altschul *et al*., 1997) and UNITE https://unite.ut.ee/ (Kõljalg *et al*., 2005; Kõljalg *et al*., 2013) were used to determine the identity of ITS rRNA coding sequences.

The criteria used for identification were: sequence coverage > 80%; similarity to species level 97–100%, similarity to genus level 94–96%. Sequences not matching those criteria or lacking taxonomic description in the reference sequences were considered unidentified and were assigned to a phylum. The names of closest species hypothesis (Kõljalg *et al*., 2013) was used to name sequences.

3.3. Background Data: Seedling Growth Evaluation, Needle and Soil Analysis

3.3.1. Tree Growth Evaluation, Needle Chemical Analysis

Seedling shoot height and root collar diameter were measured at time of root sampling in the **Stump removal** and **Seed origin studies**. Shoot height in the **Cultivation system study**

was measured at time of planting and after each growing season in October 2006, 2007, 2008 and 2009, when also survival of the seedlings was determined.

In the **Stump removal study**, each seedling shoot was dried for 12h at +60 ºC to determine dry biomass of shoots and needles. In addition, chemical analysis of the needles was conducted at the LSFRI Silava Laboratory of Forest Environment. Needles were separated from oven-dried shoots. From each seedling, 1 g of dry needles was taken and all sampled needles from each plot were pooled together, resulting in ten bulk samples. Sampled needles were finely grounded using an A-11 analytical mill (IKA-Werke GmbH, Staufen, Germany). Samples were dry-ashed in concentrated HNO_3 vapour and re-dissolved in 3% HCl. Nutrient concentrations in extracts were measured by atomic absorption spectrophotometry (K, Ca, Mg, Fe, Cu, Zn, Mn) and colorimetry (P). Total N was determined using Kjeldahl method (ISO 11261:1995). Total C and S were determined using an element analyzer (ELTRA CS530).

In the **Cultivation system study**, chemical composition of the needles and seedling root collar diameter were determined at the end of the experiment in October 2009. To determine chemical composition of needles, ten current-year needles per plant were taken from ten random plants in each treatment, pooled together within each treatment and analyzed as a bulk sample. Sampled needles were oven-dried at +60°C for 12 hours and then finely ground using a ball mill. Samples were prepared for analysis the same way as in the **Stump removal study**. Nutrient concentrations in extracts were measured by atomic absorption spectrophotometry (Ca, Mg, Fe, Cu, Zn, Mn), colorimetry (N, P, Mo, B) and flame photometry (K, Na). Chemical analyses were carried out at the Laboratory of Plant Mineral Nutrition, University of Latvia, Institute of Biology.

3.3.2. Soil Analysis

In the **Foliar damage study**, depth of organic layer to mineral soil was evaluated at least ten points in each sample plot using a metal probe. Groundwater level was determined from soil pits and also by the water level in ditches. Soil bulk density was estimated for 100 cm^3 soil samples taken from the upper soil layer (0–10 cm) of each sample plot. Samples were oven dried at 105ºC until stable mass. Soil bulk density was calculated as ratio between sample mass and volume. For pH determination, soil samples from each sample plot (taken at 0–20 cm depth) were dried for one week at room temperature and then sieved (mesh size 2 x 2 mm) to separate larger fractions and roots, which were discarded. Soil pH was determined in 1 M KCl (soil – extractant mixture 1:2.5) (Pāvule, 1978).

In the **Root-rot study**, after root extraction soil samples were separated for chemical analysis. Bulk samples from each sample plot (28 samples, in total) were taken. In the **Fertilization study**, soil chemical content was determined from samples collected in the plots in summer 2014 by colleagues from the LSFRI Silava Laboratory of Forest Environment. In the **Stump removal study**, soil samples used for chemical analyses comprised forest soil adhered to seedling roots produced during the growing season 2012 (new outgrown roots). Substrate from the nursery was excluded. In each of ten plots, 20 soil samples were collected and pooled together, resulting in a total of ten bulk samples.

Soil samples in the **Root-rot, Fertilization** and **Stump removal studies** were dried for a few days to one week at room temperature and sieved (mesh size 2 x 2 mm) to separate larger fractions, which were discarded. Soil pH in distilled water or 1 M KCl (**Stump removal study**) (soil – extractant mixture 1:2.5) was determined. Chemical elements present in the soil were extracted using 1 M HCl solution (soil – extractant mixture 1:5 as described by Rinkis *et al*. (1987) in the **Root-rot** and **Stump removal studies** or in 1M HNO_3 solution for K, Ca and Mg in the **Fertilization study**. The concentrations of Ca, Mg, K, Fe, Cu, Zn, and Mn were determined by atomic absorption spectrophotometer with an acetylene-air flame (Page *et al*., 1982). The concentration of total N was determined using Kjeldahl method (ISO 11261:1995) and P was assayed by colorimetry using ammonium molybdate spectrometric method (LVS EN ISO 6878:2004 A), and concentration of total C and S with an element analyser (ELTRA CS530).

Soil characterization in the field and/or chemical analyses under laboratory conditions was done by the LSFRI Silava Laboratory of Forest Environment.

3.4. Data Analysis

Relative abundance of ECM morphotypes **in all studies** was calculated as a proportion of living roots of each morphotype divided by total number of living fine roots observed in analysed grid squares or as a total. Based on molecular species identification, relative abundance of ECM morphotypes was determined for fungal species. If molecular data revealed that two or more species formed one morphotype, the estimated relative abundance was divided between the species.

Species richness, Shannon diversity index and qualitative (S_S) Sorensen similarity index were used to characterize diversity and similarity in composition of fungal communities among different treatments, sample plot groups or seedling variants **in all studies** (Shannon, 1948; Magurran, 1988).

In the **Foliar damage study,** foliar damage intensity in a sample plot was calculated on a scale from 0 to 4, where 0 referred to stands with no damage and 4 to stand where all trees were dead. Stand damage class was estimated as: $D = 4 \times (a + b \times 2 + c \times 3)/(n \times 3)$ (D – damage class; *a, b, c* – number of trees in 2nd, 3rd and 4th foliar damage class; *n* – total number of spruce trees in a sample plot). As foliar damage classes 2 (on mineral soils) and 4 (on organic soils) were represented only by 1 or 2 samples, they were merged to the closest class (1 or 3, respectively) for analysis. Site classification in organic and mineral soils was based on obtained soil types (histosols – organic soils; gleysols and podzoluvisols – mineral soils). In addition, as in three sites on mineral soils the organic layer was on average deeper than 25 cm, these were considered as organic soils. Soil parameters (organic layer depth, soil bulk density, groundwater depth, and soil pH) were tested for normality using the Shapiro and Wilk normality test (Royston, 1982). Response variables (organic layer depth and soil bulk density) not meeting the assumption of normality were analysed separately for mineral and organic soils, as normality was then obtained. One-way analysis of variance (ANOVA) was used to compare all soil parameters among foliar damage classes; organic layer depth and soil bulk density were analysed separately for mineral and organic soils. As foliar damage classes 2 (on mineral soils) and 4 (on organic soils) were represented only by 1 or 2 samples, they were merged to the closest class for one-way ANOVA and chi-square tests. To evaluate the effect of soil factors on relative abundance of fine root growth class (two classes – dead fine roots and older living fine roots were separately tested), generalized linear mixed models (GLMM) assuming a Binomial error distribution and link function logit were used. Models were calculated using the program package lme4 (Bates *et al.*, 2011). The full model included relative abundance of dead or older living fine roots as a response variable and soil type, organic layer depth, soil pH and groundwater level as explanatory variables with site as a random factor. The Full model was simplified with one-way ANOVA comparisons by excluding stepwise the most insignificant factor. The model with lower AIC value (Akaike, 1987) or the less complex model was considered as superior. Chi-square (χ^2) tests calculated from the actual number of observations (Mead & Curnow, 1983) were used to compare occurrence of fine roots of each root growth stage class and fungal species among stands groups based on soil types and foliar damage classes. Shannon diversity indices were calculated separately for all fungal species all ECMf species and all saprotrophic species. The Wilcoxon test (Hollander & Wolfe 1999) was used to compare species richness and Shannon diversity indices between sample plots grouped according to soil type and foliar damage. Fungal communities and their compositional relationship to soil parameters and foliar damage were analyzsed by canonical correspondence analysis (CCA). Species presence/absence data

and, as environmental factors, foliar damage and soil parameters, were used for analysis. Significance of environmental factors was tested using the Monte Carlo test under 499 premutations. If two or more variables were intercorrelated, only the most significant was used in analysis.

In the **Root-rot study**, Chi-square (χ^2) tests (Mead & Curnow, 1983) calculated from the actual number of observations were used to compare proportions of living root tips and occurrence of ECM species between infected with *Heterobasidion* root-rot and uninfected sample plots of each site. Mean values of root dry weight and its morphological parameters were compared between healthy and infected tree groups using one-way ANOVA within each site and among sites. Pearson correlation coefficients were calculated to evaluate correlation between all analyzed root morphological parameters and soil chemical composition. Fungal communities and their relationship to soil parameters were analyzed by CCA. Relative abundances of species and soil parameters as environmental data were used for analysis. Significance of the environmental variables was tested using a generalized linear model with Gaussian distribution.

In the **Fertilization study**, Chi-square (χ^2) tests (Mead & Curnow, 1983) calculated from the actual number of observations of morphotypes were used to compare the occurrence of ECM species in fertilized and control sites. The Wilcoxon test (Hollander & Wolfe 1999) was used to compare species richness and Shannon diversity indices between sample plots. Fungal community composition was analyzed using Principal Component Analysis (PCA).

In the **Stump removal study**, Chi-square (χ^2) tests (Mead & Curnow, 1983) calculated from the actual number of observations of morphotypes were used to compare richness of fungal taxa in different treatments of each site. Differences in seedling morphological parameters, number of ECM morphotypes, occurence of ECM morphotypes, chemical composition of needles and relative abundance of ECM taxa among seedling variants were analyzed by one-way ANOVA (Chalmers & Parker, 1989; Fowler *et al*., 1998). Each parameter was tested for normal distribution using the Shapiro and Wilk normality test (Royston, 1982). If data were not normally distributed, the Wilcoxon test was used instead (Hollander & Wolfe, 1999). Fungal community composition was analyzed using PCA.

In the **Seed origin study**, differences in seedling morphological parameters, number of ECM morphotypes, occurence of ECM morphotypes, and relative abundance of ECM taxa among seedling variants were analyzed by ANOVA and Tukey's tests (Chalmers & Parker, 1989; Fowler *et al*., 1998). Each parameter was tested for normal distribution using Shapiro and Wilk normality tests (Royston, 1982). If data were not normally distributed, the

Wilcoxon's tests were used instead (Hollander & Wolfe, 1999). Fungal community composition was analyzed using PCA.

In the **Cultivation system study**, Chi-square (χ^2) tests (Mead & Curnow, 1983) calculated from the actual number of observations of morphotypes were used to compare the impact of the cultivation system on richness of fungal taxa in seedling roots. Differences in seedling morphological parameters, number of ECM morphotypes and relative abundance of ECM taxa among seedling variants were analyzed by one-way ANOVA (Chalmers & Parker, 1989; Fowler *et al*., 1998). Fungal community composition was analyzed using PCA.

Data were statistically analyzed using the R program (Vienna, Austria) (R Development Core Team, 2011). Fungal community composition was analyzed using the CANOCO 4.5 program (Ter Braak & Smilauer, 1998). Species richness, Shannon diversity index and qualitative (S_S) Sorensen similarity index were calculated in ComEcoPaC (Drozd, 2010).

Statistical analyses were conducted at significance level α=0.05. Standard errors were used to represent data variation of mean values of soil parameters, seedling morphological parameters and number of ECM species or morphotypes in all studies.

4. Results and Discussion

4.1. Foliar Damage Study

4.1.1. Soil Parameters and Root Growth Stages

Soil analysis showed that foliar damage was concentrated in forest sites on semihydromorphic or hydromorphic soils (Histosols, Gleyic podzoluvisols and Gleysols). Highly damaged plots (damage class 3-4) were mainly located on transitional mire soils or fen peat soils, which are wet and rich in nutrients.

The values of soil parameters in sites grouped according to soil type and foliar damage classes are given in Table 1. Groundwater level and soil pH were generally higher in more damaged sites. No significant differences in soil parameters between foliage damage classes occurred on mineral soils. However, on organic soils, the severely damaged plots (foliar damage class 2 to 4) had a deeper organic layer and higher soil pH in comparison to minimally damaged plots (foliar damage class 0 to 1) (Table 1).

Table 1. Soil parameters in plots grouped according to tree foliar damage and soil type.

	Mineral soil		**Organic soil**			
Foliar damage classes	**0**	**1-2**	**0**	**1**	**2**	**3-4**
No. of sample plots	12	13	4	3	10	6
Organic layer, cm	3 ± 1	3 ± 1	21 ± 2a*	68 ± 17ab	77 ± 12b	80 ± 18b
Soil bulk density, kg/m^3	687 ± 78	636 ± 58	270 ± 40	253 ± 48	255 ± 23	271 ± 41
pH	3.6 ± 0.1a	3.5 ± 0.1a	3.7 ± 0.2a	4.2 ± 0.6ab	4.2 ± 0.2b	4.5 ± 0.3b
Groundwater depth, cm	123 ± 11 a	115 ± 12 a	109 ± 28ab	82 ± 21ab	75± 10b	59 ± 11b

* Different letters next to average values are given to indicate significant difference ($p < 0.05$) between samples representing sites of different foliar damage classes (data from different soil types were analyzed separately in cases of organic layer and soil bulk density).

In total, 229 911 fine root tips were examined. Dead fine roots were more frequent (55.5% of all root tips) than living fine roots (14.8% formed young fine roots and 29.7% – older fine roots) in the samples. As soil type varied strongly among sample plots, the level of damage was assessed separately for mineral and organic soils (Table 1).

Analysis showed that there were no significant differences in distribution of roots by growth stage classes between foliar damage groups in sample plots on mineral soils (Figure 4). On organic soil, the foliar damage groups of plots significantly differed in the amount of damaged roots ($p < 0.05$). The proportion of dead fine roots was significantly higher in

severely damaged sites (foliar damage class 2 to 4) in comparison to less damaged sites (foliar damage class 0 to 1). The number of older living fine roots was significantly lower in more damaged sites than in less damaged sites, and the proportion older living fine roots in samples also was lower (25.3% and 39.3%, respectively).

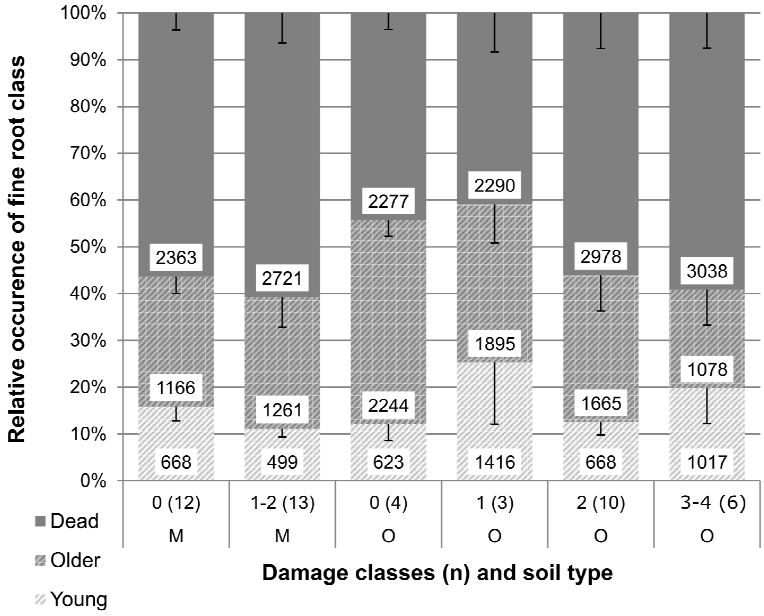

Figure 4. Abundance of fine roots by growth stage classes (relative values in percent and average numbers are shown) in spruce stands on mineral (M) and organic soils (O) with different foliar damage intensity. Number of sample plots representing each foliar damage class is given in parenthesis. Negative standard error is used to represent the means.

GLMM analysis indicated that the relative abundance of older living fine roots was was best explained by a model with only groundwater level ($p < 0.05$) as an explanatory variable. The best GLMM analysis for predicting relative abundance of dead fine roots included two factors – groundwater level ($p < 0.01$) and organic layer depth ($p < 0.05$). AIC values showed this simple model to be superior. The model predicts that relative abundance of dead fine roots increases with a shallower groundwater level (Figure 5).

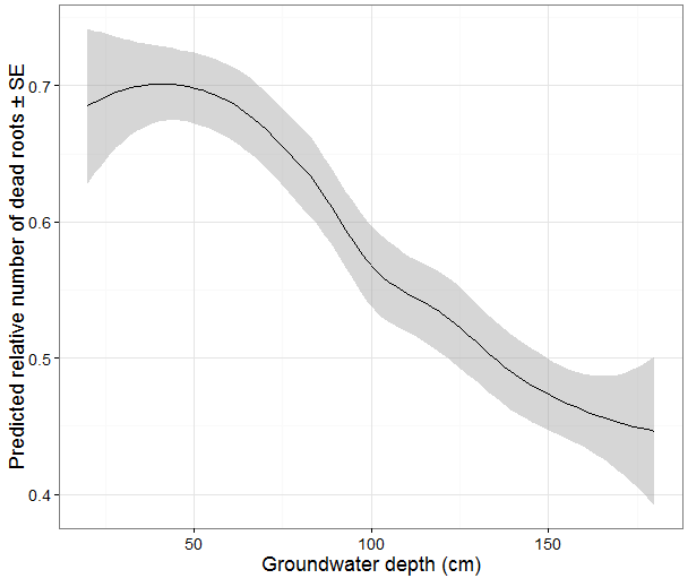

Figure 5. Predicted variation of relative abundance of dead fine roots in relation to groundwater depth based on the GLMM model including groundwater depth and organic layer thickness as fixed and location of sampling sites as random effects.

Characteristic indicators of tree-root system stress include decrease in number of living fine roots, living root tips and mycorrhizas (Clemensson-Lindell & Persson, 1995). Higher number of dead fine roots in severely damaged sites as well as reduced relative abundance of older living roots may indicate present tree-root system stress or past root disturbance followed by re-growth. As this study was a retrospective analysis of tree dieback, causal relationships of the origin of the damages cannot be made. Root samples were collected in late summer 2010, and thus the observed fine root mortality might have been a secondary response to foliar damage. However, the author is sceptical to this hypothesis as carbohydrates from foliage are largely allocated to roots at the end of the growing season. Being so, the effect of canopy damage to the roots should have occurred later than observed in this study. The fine root turnover of spruce is approximately two years (Leppälammi-Kujansuu *et al.*, 2014), and therefore the author considers that the timeframe was not wide enough to show secondary root response to foliar damage.

The damage in fine roots correlated strongly with high soil water level and depth of the organic layer. As moist soil holds more heat than dry soil, such soil conditions, particularly in hydromorphic peat soils, can promote more active root growth in autumn, resulting in reduced root hardening to sudden soil frost; also root distribution in the upper soil layer, typical of peatland trees to avoid flooding and for Norway spruce in general, can predispose roots to climatic factors (Kost *et al.*, 2007; Modrzyński, 2007). Meteorological data indicated that summer (June-July) 2009 and autumn (September-October) 2009 were 30% wetter in

comparison with the 10-year average (Appendix 3). September and November 2009 temperature was one degree above the norm and October 2009 was about two degrees colder than the norm (Appendix 3). In mid December 2009, the temperature fell very rapidly from above zero to -15°C (Appendix 3). At that time precipitation was low and there was little or no snow cover (Appendix 3). The last previous record of such a rapid fall in temperature was in 1938 (data from Riga and Gulbene meteorological stations) (data from Latvian Environment, Geology and Meteorology Centre LLC). High precipitation rate with mild temperature in autumn 2009 could have produced a late flush of fine roots growth (Montagnoli *et al*., 2014). This period in Latvia coincided with little protection from a snow cover (Appendix 3), which could have aided to maintain higher soil temperature (Swanson & Rideout, 2009).

Some studies on Norway spruce have shown that increased soil freezing can promote fine root mortality (e.g. Gaul *et al*., 2008). In contrast, a recent Finnish study (Repo *et al*., 2014) showed evidence of little effect of soil frost on fine roots. Since their study sites were on mineral soils without increased water-saturation, site differences could be a reason for the contrasting results with those in the present study. In the USA, fine root mortality of tree in hardwood forest was explained by mechanical damage to roots in frozen soil, not by the low soil temperature *per se* (Groffman *et al*., 2001; Tierney *et al*., 2001). Regarding the observed spruce dieback in Latvia, not only temperature, but also soil properties, soil drainage and soil moisture regime were important factors that probably contributed to mechanical damage and low root hardening. Recent studies in USA (Alaska) reported decline of yellow-cedar initiated by freezing injury of roots when soils froze during times of limited snowpack (Schaberg *et al*., 2011). Both limited root cold tolerance and shallow rooting are likely to contribute to the sensitivity to freezing injury and decline (Schaberg *et al*., 2011). In a review of previous studies (Hennon *et al*., 2012), yellow-cedar decline was explained by a cascade of interacting topographic, forest-structure, and microclimate factors that acted on unique tree vulnerability to fine-root freezing. The complex causing tree mortality in their study were reduced to two risk factors—snow depth and soil drainage.

4.1.2. Fine Root Fungal Community

Amplification and direct sequencing of fungal ITS region of the rRNA gene from 1051 root tips representing ECM morphotypes from each site resulted in 508 high-quality fungal sequences. The obtained sequences represented 88 root-associated fungal taxa (Table 2, Appendix 4). Basidiomycetes dominated among identified taxa in number of species (59) and in species abundance in root samples (68% of all root tips analyzed). Consequently,

Ascomycetes were less abundant – 29 species and 32% of all root tips. In total, the number of fungal species obtained from the stands was high, taking into account the limited number of root samples analyzed by molecular methods.

Seventy six ECM/root-endophytic fungal species were identified, as well as 12 saprotrophic fungi or fungi with other or unknown ecology. 34 species were relatively rare, as they were represented only by one sequence or found only in one sample plot. There were 2 to 11 fungal species per sample plot identified (mean 6.3±0.3) and most of them (1 to 10 per sample plot) were ECM species or root endophytes. Shannon diversity indices of fungal communities varied by even more than 10 times with values between 0.28 and 2.89 (mean 1.87±0.09).

Many common ECM genera, such as *Amphinema*, *Cortinarius*, *Inocybe*, *Piloderma*, *Russula*, *Lactarius*, *Tomentella*, and *Tylospora*, were observed. The most common ECM species was *Tylospora asterophora* (10.6% of all sequences, 11.6% of all root tips).

A role of the observed ectomycorrhizal genera *Lactarius, Russula, Piloderma* and *Tylospora* in transfer of nutrients as facultative decomposers has been disscused (DeBellis *et al*., 2006; Rajala *et al*., 2011). Dominance of *Tylospora asterophora* is typical in spruce forests, as this species is a common and abundant ECM fungus on spruce (Eberhardt *et al*., 1999; Korkama *et al*., 2006). The role of *Tylospora* spp. in degradation of remnants of decayed wood and soil humic polymers to acquire extra carbon and other nutrients has been suggested (Tedersoo *et al*., 2003). This function might make *Tylospora* species even more competitive on soils with a deep organic layer, as in most of the studied sites.

Two other species (*Oidiodendron maius* and yeast *Cryptococcus magnus*) were very common in the samples. *O. maius* sequences formed 9.1% of all sequences and *C. magnus* – 13.6%. Both species were also common on root samples – 12.7% and 14.5% of all root tips. *Oidiodendron maius* has been reported as a root-associated fungus with Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and its ECM ability *in vitro* has been demonstrated (Schild *et al*., 1988). However, *O. maius* is mainly known as a metal-tolerant endomycorrhizal fungus with ericaceous plants and typical on peatlands. It has the potential to degrade complex organic polymers in soil (Rice & Currah, 2006). Species from the genus *Cryptococcus* are commonly isolated from soil of managed forest areas (Yurkov *et al*., 2012). The high proportion of *C. magnus* found in fine root samples might indicate high activity of saprotrophic fungi on the fine root surface, which could be related with primary or secondary fine root dieback.

Only one weak pathogen species of Norway spruce (*Rhizosphaera kalkhoffii*) was sequenced from root samples, but only from two sample plots, both of which represented the low foliar damage (class 0 and 1). This species was observed also on needles in some of the

severely damaged sites (Lazdiņš, 2011). This species was commonly observed on needles in Norway spruce stands with similar foliar damage in Lithuania (Menkis *et al*. 2015). *Rhizosphaera kalkhoffii* infection in Norway spruce stands with "top-dying" symptoms was reported by Diamandis (1979), who concluded that the observed fungus infection was secondary and that the spruce dieback primarily was caused by climatic stress (summer and spring drought).

The impact of ECM on tree vitality depends on natural factors, such as soil properties, climate, and the fungal species (Kottke *et al*., 1993). Therefore fungal species data among different tree damage severity classes was analyzed taking into account soil type (Table 2).

Table 2. Occurrence (%) of ten dominant fungal species in analyzed plots grouped according to foliar damage and soil type of sampled layer.

	Mineral soils		**Organic soils**			
Foliar damage classes	**0**	**1-2**	**0**	**1**	**2**	**3-4**
No. of sample plots	12	13	4	3	10	6
Root tips analyzed	19409	15684	5783	6591	20896	10184
Cryptococcus magnus	5% a	5% a	22% c	18% b	25% d	19% b
***Helotiaceae* sp.**	1% a	2% b	-	>0.5% a	>0.5% a	13% c
Hygrophorus pustulatus	11% b	-	-	-	-	5% a
Lactarius deterrimus	1% a	7% b	-	14% c	-	-
Oidiodendron maius	12% c	12% c	7% b	20% d	18% d	3% a
Phialocephala fortinii	3% b	<0.5% a	-	20% d	5% c	2% b
Piloderma sphaerosporum	13%	-	-	-	-	-
***Piloderma* sp.**	-	-	2% a	-	6% c	4% b
Tylospora asterophora	6% a	20% d	20% d	11% b	5% a	18% c
***Wilcoxina* sp.**	7% a	6% a	-	-	11% b	-

* Different letters mean significant difference ($p < 0.05$) between mean values of each soil parameter or represented proportions in case of fungal species

Two dominant species *T. asterophora* and *O. maius* were present in both soil types and represented in all foliar damage classes. *C. magnus* also was common in all sample groups, but it showed higher abundance in samples from peat soils. In total, mean number of saprotrophic species in root samples from damaged sites (foliar damage classes 2 to 4) was 1.3±0.1, which was significantly higher than in less damaged stands (foliar damage classes 0 to 1) – 0.8±0.2 ($p=0.02$). The shift in fine root-associated fungi from a community with ECM dominance to greater abundance of saprotrophic species might indicate greater activity of

saprotrophic fungi in the soil or be a response to root dieback. However, soil type did not have an effect on richness of fungal species or ecological groups.

Species like *Helotiaceae* sp., *Lactarius tabidus* and *Amphynema* spp. were characteristic of severely damaged plots and *Phialocephala fortinii*, *Tomentellopsis echinospora* and *Lactarius necator* were more common in minimally damaged plots (Table 2, Appendix 4). The ecology of *Helotiaceae* sp. and *Phialocephala fortinii* of the Helotialean fungi group is still insufficiently understood (Rice & Currah, 2006). In this study, differences in occurrence of species among soil types and foliar damage classes indicated theirdifferent ecological spectrum. Species present in sites with severe foliar damage included common ECM species that prefer organic soils, such as *Amphinema* spp. (Korkama *et al.*, 2006), and species that prefer nutrient rich wet habitats (high N and P concentration), like *Lactarius tabidus* (Stankevičienė & Pečiulytė, 2004).

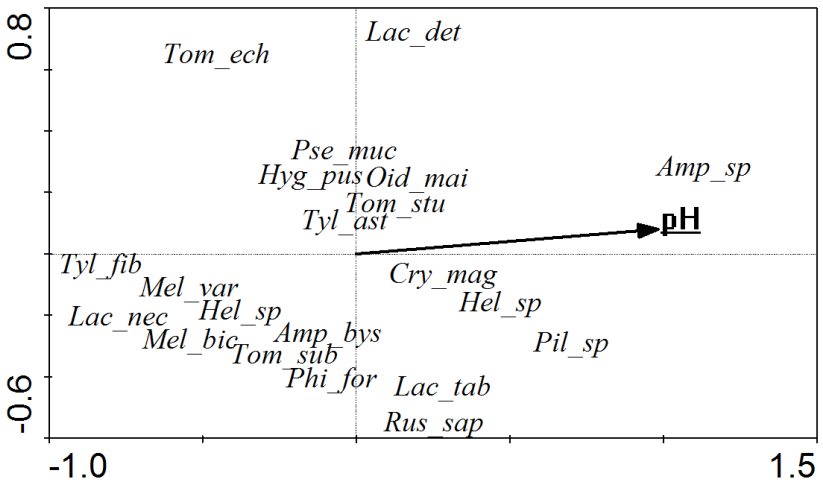

Figure 6. Canonical correspondence analysis (CCA) fungal species ordination in relation with soil factors. Presence/ absence data of most abundant fungal species (observed in at least four sample plots) was used. Only significant ($p < 0.05$) environmental factors are shown.

The first two axes of the CCA, explained only 8.6% of the species variance, which indicated that the studied soil factors and foliar damage intensity had low impact on fine root fungal communities, i.e. other factors not studied were more important. Of the studied soil factors, soil pH (p=0.002; F=2.59) (Figure 6) was a significant factor explaining fine root fungal community composition in the studied plots. Since Pearson correlation was significant between soil pH and organic layer depth only the most significant of these factors was included in CCA analysis. *Amphinema* sp. preferred higher soil pH and species like *Tylospora fibrillosa*, *Lactarius necator* and *Meliniomyces* spp. were associated with lower soil pH values.

4.2. Root-Rot Study

4.2.1. Soil Parameters and Fine Root Growth

Within each of the sites (Ogre, Strautiņi, and Misa), root morphological parameters were similar between *Heterobasidion* root-rot infected and uninfected plots. In the Kalsnava site, in contrast, root morphological parameters in root-rot infected plots were significantly lower than those in uninfected plots. The Kalsnava site had suffered severe defoliation because of tree damage by root-rot (LSFRI Silava, unpublished data) and reduction of root amount in samples from this stand might be a secondary effect of tree defoliation. On the other hand, reduced fine root length and biomass in rot infected tree groups might be directly related with *Heterobasidion* sp. infection and damage to the root system caused by it.

Soil chemical properties varied among the sites, but within each site they were generally similar between root-rot infected and uninfected plots. An exception was in the Kalsnava site, where pH and concentration of N and Ca were significantly lower in root-rot infected plots than in uninfected plots, and the C:N ratio was significantly higher in infected vs. uninfected plots.

Very low soil pH (less than 3) was found in the Misa stand. This stand had a higher carbon content and C:N ratio in comparison with the other stands. Some authors suggest that conifer infection with *H. annosum* is lower on peat soils because of low pH and presence of microflora antagonistic against *H. annosum* (Bendz-Hellgren *et al*., 1999). On the other hand, *Heterobasidion* sp. can directly colonize the bark of live roots in low-pH soils (Garbelotto & Gonthier, 2013). Low pH is an unfavourable abiotic factor causing stress to trees, making them weaker and more susceptible to pathogen infection. As growth of *Heterobasidion* sp. mycelium in roots depends on tree vitality, such high environmental stress might contribute to tree infection (Redfern & Stenlid, 1998).

Table 3. Root morphological parameters in healthy tree samples and those infected with *Heterobasidion* root-rot.

	Kalsnava site		**Ogre site**		**Strautiņi site**		**Misa site**	
	Root-rot	**Healthy**	**Root-rot**	**Healthy**	**Root-rot**	**Healthy**	**Root-rot**	**Healthy**
pH (KCl)	3.7 ± 0.1	4.2 ± 0.1 *	5.4 ± 0.1	4.2 ± 0.6	4.4 ± 0.2	4.5 ± 0.2	2.6 ± 0.1	2.6 ± 0.1
N	22 ± 1	26 ± 1 *	11 ± 3	15 ± 1	15 ± 4	12 ± 2	17 ± 1	16
C	472 ± 11	455 ± 15	217 ± 58	310 ± 44	274 ± 74	230 ± 42	526 ± 9	530 ± 4
C/N	21 ± 1 *	18 ± 1	19 ± 1	21 ± 2	19 ± 1	19	32 ± 1	34 ± 1
P, mg/kg	26 ± 9	47 ± 20	58 ± 8	37 ± 15	53 ± 10	84 ± 28	54 ± 3	53± 3
K, mg/kg	100 ± 19	130 ± 14	58 ± 7	94 ± 26	141 ± 31	136 ± 21	131 ± 10	133 ± 11
Mg, g/kg	0.7	0.9 ± 0.2	1.1 ± 0.4	0.9 ± 0.3	0.5 ± 0.2	0.5 ± 0.1	0.7	0.7
Ca, g/kg	17 ± 1	25 ± 2 *	15 ± 6	13 ± 4	10 ± 5	7 ± 2	6 ± 1	6
Dry mass fine root (g)	1.5 ± 0.2	2.3 ± 0.2 *	2.0 ± 0.3	2.7 ± 0.4	2.2 ± 0.2	1.7 ± 0.2	1.4 ± 0.2	1.5 ± 0.1
Fine root total length (m)	13 ± 1	20 ± 2 *	13 ± 2	18 ± 2	23 ± 2	28 ± 2 *	26 ± 4	27 ± 4

Comparisons were made within each site. Significantly greater values are denoted by: * - $p < 0.05$.

Soil pH and C:N ratio showed correlation with root morphological parameters, but this was mainly due to variation in soil chemical composition among sampling sites. However, potassium concentration in soil was positively correlated with fine root length (r=0.62). No significant correlations were observed among root parameters and phosphorous, nitrogen and magnesium content in soil.

4.2.2. Fine Root Fungal Community

Ectomycorrhizal morphotype identification resulted in 42 ECM or root endophyte species and three saprotrophic or weakly pathogenic fungal species (Table 4, Appendix 4). The saprotrophic or weakly pathogenic fungal species were *Cryptococcus magnus* and *Ilyonectria* sp. (Strautiņi, both sample groups) and *Trichosporon* sp. (Ogre, healthy tree samples). Within each site, richness of fungal species in root-rot infected and uninfected plots was similar and non-significant (chi-square test). The Shannon diversity index was also similar between root-rot infected vs. uninfected plots of each site.

Root mycorrhization was high in all sites and plots (ranging between 86.4 and 100%), and no significant differences were found between corresponding root-rot infected and

uninfected plots. However, significantly lower fine root mycorrhization (92.8% on average) was observed in the Misa stand than in other stands (97.0% (Strautiņi), 99.6% (Kalsnava) and 99.8% (Ogre) on average). This might indicate unfavourable conditions, such as low soil pH, for mycorrhization in the Misa site in general. Soil pH values close to 3 is not optimal for the majority of ECMf (Rudawska, 2007). Total number of ECM species was higher in the Misa stand compared with other sampling sites (Table 4). This might be due to either reduced competition among species because of soil acidity and consequently higher diversity of ECMf or with greater tree age in the Misa stand, since ECM diversity increases with stand age (Smith *et al*., 2002).

Table 4. Number of ECM species, Shannon diversity index and frequency of the most common fungal taxa, shown as percentage of ECM roots colonized in *Heterobasidion* infected and uninfected sampling plots in four Norway spruce stands.

	Kalsnava		**Ogre**		**Strautiņi**		**Misa**		**Total**
	Root-rot	**Healthy**	**Root-rot**	**Healthy**	**Root-rot**	**Healthy**	**Root-rot**	**Healthy**	
Amphinema byssoides	8.8	17.7	10.5	17.9	14.0	16.0	7.7	5.3	12.5
***Amphinema* sp.1**	-	-	-	-	13.6	5.9	-	-	3.1
***Amphinema* sp. 2**	-	-	-	-	-	-	23.8	16.9	4.8
***Clavulina* sp.**	-	-	-	22.4	-	-	-	-	2.5
Inocybe nitidiuscula	-	-	6.2	-	10.0	19.6	-	-	5.4
Russula sapinea	44.8	29.1	-	-	-	-	-	-	8.4
Sebacina epigaea	-	-	-	17.6	-	-	-	-	2.0
Tomentella stuposa	12.3	9.3	-	-	7.6	6.9	0.9	16.0	6.7
Tylospora asterophora	32.6	29.5	-	7.5	44.0	39.8	-	-	21.3
No. of species	11	12	6	8	9	9	18	20	51
Shannon diversity index	1.3	1.6	2.2	2.1	1.7	1.7	2.3	2.3	

The most common fungi were *Tylospora asterophora* (21.3%), *Amphinema byssoides* (12.5%) and *Russula sapinea* (8.4%). High abundance of *Amphinema byssoides* in the studied stands might be due to high soil pH values, which in general are favourable for this species and *H. annosum* development as well (Bendz-Hellgren *et al*., 1999; Jonsson *et al*., 1999). None of the dominant ECM species found in the studied sites (e. g. species from genera *Amphinema*, *Inocybe*, *Tylospora* and *Tomentella*) had previously been tested for antagonism against *H. annosum* under laboratory conditions (e. g. Napierała-Filipiak & Werner, 2000). There were some species that were common or exclusively found in healthy tree samples. Some of them

represented genera that had shown some inhibiting activity against *H. annosum* in experiments *in vitro* by other authors (e. g. Napierała-Filipiak & Werner, 2000). However, none of these species was dominant on fine roots in the studied sites.

The species *Tylospora asterophora* and *Tomentella stuposa* were characteristic in most of the studied stands and common in both healthy and infected trees (Table 4). Both species are common for spruce forests – *Tylospora asterophora* has been reported as one of the most constant and abundant ECMf on spruce (Eberhardt *et al*., 1999) and *Tomentella stuposa* has been described as one of the most commonly encountered tomentelloid fungi in spruce stands surveyed in Sweden (Kõljalg *et al*., 2000). *Amphinema byssoides* was also very abundant in studied stands. As *A. byssoides* is a lime-dependent species (Jonsson *et al*., 1999), its abundance in samples might be due to the high soil pH values, which in general are favourable for *H. annosum* development as well (Bendz-Hellgren *et al*., 1999).

Most of the dominant species of ECMf (*Amphinema*, *Inocybe*, *Tylospora* and *Tomentella*) have not been tested for antagonism against *H. annosum* under laboratory conditions (e.g. Hyppel, 1968a; Napierała-Filipiak & Werner, 2000). There were some significant differences in abundance of these genera between healthy and infected trees: greater abundance of *Amphinema byssoides* in healthy tree samples and in contrast, high abundance of *Amphinema* sp. in samples from infected trees, and high abundance of *Tylospora asterophora* in all samples and *Tylospora fibrillosa* mainly in samples from healthy tree groups. In the future, interaction of *Amphinema* and *Tylospora* species with *Heterobasidion* sp should be tested *in vitro*.

There were some species typical only to one stand (for example, *Russula sapinea* in the Kalsnava stand). Some species were more dominant or exclusively found in either healthy or infected tree samples. For example, in the Misa site, *Inocybe relicina*, *Cortinarius* sp.1 and *Tylopilus felleus* were observed only in healthy sample plots and *Cortinarius* sp. 2 and *Elaphomyces muricatus* only in roots of trees infected with *Heterobasidion* sp. In the Ogre site, *Clavulina* sp., *Amanita citrina*, *Xerocomus ferrugineus*, *Sebacina epigaea* and *Thelephora terrestris* were observed in the healthy site, while *Inocybe proximella* was found only in samples from trees infected with *Heterobasidion* root rot. *Cenoccocum* sp. was observed in both tree health classes in the Strautiņi and Misa stand, but its frequency was higher in samples from healthy trees.

In vitro experiments in Poland indicated that *Amanita citrina* and members of the *Xerocomus* genus can inhibit *H. annosum* growth (Napierała-Filipiak & Werner, 2000). *In vitro* experiments (Marx, 1972; Napierała-Filipiak & Werner, 2000) noted that also *Cenoccocum* sp. has some antifungal activity and it can inhibit growth of some *Heterobasidion* sp. isolates.

Some antifungal activity of *Thelephora terrestris* and *Lactarius necator* was suggested by Marx (1972), based on observation that these species were observed mainly in healthy tree samples of this study. Abundance of *Lactarius* species in samples might be indirectly related to absence of *Heterobasidion* sp. infection, as these species prefer more acid sites, which are not favourable for *Heterobasidion* sp. (Jonsson *et al*., 1999). However, *Lactarius* species were not dominant in the studied sites and probably they are generally rare due to strong competition among ECM species. Its low abundance thus does not provide support for its antifungal effect in healthy trees. However, abundance of ECM mycelium in soil can reduce *Heterobasidion* sp. development and ECM mycelium abundance in soil does not necessarily correspond to its abundance on root tips (Anderson & Cairney, 2007).

In the CCA ordination (Figure 7) of fungal communities, 26.5% of the variation on axis 1 and 24.5% on axis 2 could be explained by soil factors, the most important of which were C:N ratio ($p<0.002$), pH ($p<0.0001$) and K ($p<0.04$). Separation of root-rot infected (MR) and uninfected (MH) plots of Misa site (M) in the ordination was associated with differences in the C/N ratio, and of the Ogre site (O) by potassium. Sites K and S differed in soil pH.

The results demonstrated that *Heterobasidion* root-rot had little or no effect on fine root morphology and on composition of fungal communities in roots of Norway spruce. In the Kalsnava site, differences in root morphological parameters between root-rot infected vs. uninfected plots were likely due to differences in soil chemical properties. Moreover, it has been shown that pH and concentration of N in the soil may have major effect on root morphological parameters of Norway spruce (Helmisaari *et al*., 2009). Although Gaitnieks (2005) reported that *Heterobasidion* root-rot may affect fine root morphology of Norway spruce, this was not observed in the present study.

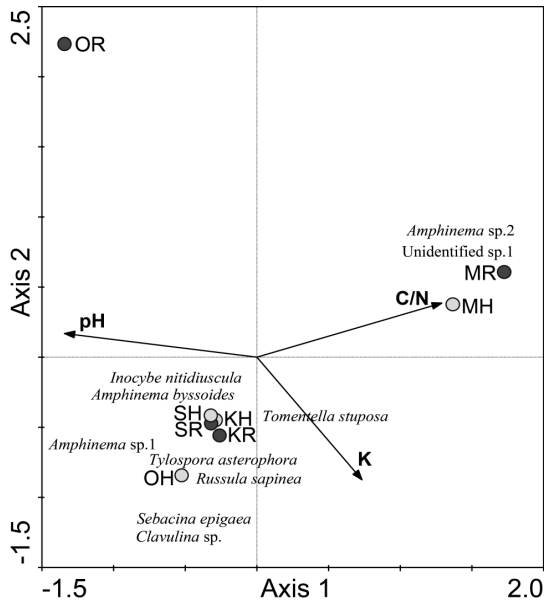

Figure 7. Ordination of fungal communities in roots of *Picea abies* from *Heterobasidion* root-rot infected (dark-grey circles) and uninfected (light-grey circles) stands, constrained on soil factors. Names of the plots (KR, KH, OR, OH, SR, SH, MR, MH) are as in Appendix 1. Arrows show environmental variables (pH, C/N and K) that were found to be of significant importance. Taxonomic names are shown in the ordination for the ten most common fungal species of in the study and correspond to position in the ordination.

4.3. Fertilization Study

4.3.1. Soil Parameters and Growth of Fine Roots

Soil pH was significantly higher in all wood ash fertilized plots in comparison to control plots (on average 5.1±0.1 and 4.5) (Table 5). Soil relative moisture varied only between sites; it was significantly higher in the *Myrtillosa turf. mel. / Caricoso-phragmitosa* forest type than in the other sites. Total N concentration was significantly higher and P, Ca and Mg concentrations were significantly lower in control plots than in wood ash fertilized plots (Table 5). Potassium concentration differed among forest types, with the lowest values in the *Myrtillosa turf. mel*. type, and in particular in the control plots.

Considerable increase in soil pH can be expected due to wood ash application (Augusto *et al*., 2008, Kuokkanen *et al*., 2009), as its neutralizing effect on the organic layer can have long duration (Bramryd & Fransman, 1995; Saarsalmi *et al*., 2001; Moilanen *et al*., 2002). This was also the case also in the experimental sites of the present study. The differences in soil pH after 12 years since treatment indicated that the used dosage of wood ash was high. Increased concentrations of Ca, Mg and P in fertilized plots also were likely due to the liming and fertilizing effect of wood ash. Lower K concentration in the *Myrtillosa turf. mel.* and

Caricoso-phragmitosa forest type than in the *Vacciniosa turf. mel.* type might be related with potassium deficiency observed in peatland forests, as described previously (Finer, 1989).

Table 5. Characteristics of soil and fine roots in samples from fertilized and control plots.

	Vacciniosa turf. mel.		***Myrtillosa turf. mel.***		***Myrtillosa turf. mel. / Caricoso-phragmitosa***		**Total**	
	Fertilized	**Control**	**Fertilized**	**Control**	**Fertilized**	**Control**	**Fertilized**	**Control**
pH	4.9±0.2a	4.3±0.1b	5.1±0.2a	4.3±0.1b	5.4±0.2a	4.8±0.1b	5.1±0.1 a	4.5 b
Relative moisture	9.8±0.5	9.9±0.5	9.7±0.5	9.1±0.3	11.6±0.4	11.6±0.4	10.2±0.3	10.4±0.3
N (Total, g/kg)	17.3±0.9	18.5±0.6	20.8±1.0	22.9±0.7	22.1±1.1	24.2±0.8	20.1±0.6 a	21.9±0.5 b
P (g/kg)	1.0±0.2	0.7	1.1±0.1	0.9±0.1	1.3 ±0.1a	1.0b	1.1±0.1 a	0.9 b
K (g/kg)	0.9±0.2	1.2±0.3	0.7±0.1	0.5±0.1	0.8±0.1	0.7±0.1	0.8±0.1	0.8±0.1
Ca (g/kg)	24.0±6.7a	9.9±0.8b	19.6±3.6a	10.2±0.9b	20.8±5.5	12.9±1.4	21.5±3.1 a	11.1±0.6 b
Mg (g/kg)	1.4±0.2a	1.0b	1.4±0.1a	0.8±0.1b	1.4±0.1	1.3±0.1	1.4 ±0.1a	1.0 b
Fine root biomass (kg/ha)	1498±214	1089±146	899±140	661±86	1284±315	998±166	1227±208a	916±144b
Abundance (%) of living fine roots**	41.6%a (4076)	55.3%b (4345)	54.6%a (3745)	41.7%b (4492)	50.9%a (3419)	58.6%b (4725)	49.2%a (10879)	51.9%b (13562)

* Different letters next to mean values indicate significant differences ($p < 0.05$) among treatments of the same forest type or in total.

** Number of root tips analyzed is given in parenthesis.

Fine root biomass was significantly higher in fertilized plots than in control plots, and lower in the *Myrtillosa turf. mel.* forest type in comparison to other sampled sites. Fine root viability was higher in control sites in the *Vacciniosa turf. mel.* and *Myrtillosa turf. mel. / Caricoso-phragmitosa* forest types, but in the *Myrtillosa turf. mel.* site type, fertilized sample plots had higher proportion of fine living roots than in control plots (54.6% and 41.7%, respectively).

Increased biomass of mycorrhized fine roots in the top humus layer has been reported as one of the effects of liming on fine roots (Nowotny *et al.*, 1998). Tree tissue lignification increases after wood ash treatment (Ferm *et al.*, 1992; Mandre, 2005; Mandre *et al.*, 2006; Lukjanova & Mandre, 2009), and therefore lignification of trees is thought to be promoted by an increase in the pH level and K concentration (Miidla, 1989; Mandre, 2005). Thus, active lignification might be one of the reasons for the higher fine root biomass in wood ash fertilized plots in the present study.

The observed differences in fine root production and vitality between wood ash fertilized and control plots might also be related to the fine root distribution pattern in the soil.

Norway spruce tends to have a shallower fine root system in plots treated with wood-ash than in control plots (Majdi & Viebke, 2004). Only the top 0–20 cm layer was sampled in this study, as more than 80% of all roots of pine and spruce are concentrated in this layer in drained forests (Ruseckas, 2000). However, rooting depth in peat soils can extend to a depth of 20–40 cm or more, which is much deeper than in mineral soils and drained peat soils (Ruseckas, 2000). Thus, it is possible that fine roots in control plots occurred in deeper layers of peat while in fertilized plots they were located in the top layer where growth conditions were optimal.

Reduced conifer fine root viability in wood ash fertilized *Vacciniosa turf. mel.* and Myrtillosa *turf. mel. / Caricoso-phragmitosa* forest types might be explained by increased proportion of dead fine roots as a result of an excessive amount of liming material (Persson, Ahlström, 1994). Since Scots pine was the dominant tree species in these sites, the observed decrease in root viability might be a specific response of Scots pine to wood ash fertilization. The *Vacciniosa turf. mel.* forest type typically has lower concentrations of nutrients (e. g. nitrogen concentration) than in other forest types, which might have restrained the fertilization effect of wood ash (Aronsson & Ekelund, 2004). As wood ash fertilization increases the activity of the microflora and modifies its species composition (Fritze *et al.*, 1994; Perkiömäki & Fritze, 2002), fine root turnover might be promoted, which in turn can explain the observed changes in ratio of living and dead fine roots.

In contrast, positive effect of wood ash fertilization on fine root vitality was observed in the *Myrtillosa turf. mel.* site dominated by Norway spruce, which is in accordance with results of other studies (Clemensson-Lindell & Persson, 1995; Majdi & Viebke, 2004). The lower K concentration in soil samples from the *Myrtillosa turf. mel.* site might indicate deficiency of this element for trees. Therefore, the increased fine root vitality in fertilized soil of this site might have been promoted by increased supply of potassium.

4.3.2. Fine Root Fungal Community

In total, 17 ECM morphotypes were observed. The most common were – dark brown with orange mycelium (*Amphinema*-type) and brown with swollen mantle (*Tylospora*-type). Abundance of these two morphotypes differed significantly between fertilized and control sample plots; the *Amphinema*-type was more abundant in wood ash fertilized plots, and the *Tylospora*-type in control samples. Black coloured mycorrhizas (*Cenococcum*-type) were more abundant in control samples.

A total of 131 sequences obtained from 216 root tips were identified by molecular methods to belong to 50 fungal species. Most of the fungal species were basidiomycetes (34

species). Ascomycetes were represented by 16 species. Identified fungal species are listed in Appendix 4 and partly in Table 6.

Table 6. ECM community richness, Shannon diversity indices and relative abundance of dominant fungal species (total occurrence in either control or fertilized plots greater than 5%) in fertilized and control samples. The first number refers to fertilized sites and the second – to control sites.

	Vacciniosa turf. mel.	***Myrtillosa turf. mel.***	***Myrtillosa turf. mel. / Caricoso-phragmitosa***	**Total**
Number of species (sequences)	15 (20) / 17 (27)	10 (22) / 10 (20)	15 (20) / 12 (22)	31 (62) / 29 (69)
Average number of species	6±0.6 / 6.5±0.3	5.7±0.3 / 4.5±0.6	5.7±0.7 / 4.5±0.3	5.8±0.3 / 5.2±0.4
Shannon diversity index	2.3±0.2 / 1.9±0.1	2.1±0.1 / 1.4±0.2*	2.1±0.2 / 1.6±0.2	2.2±0.1 / 1.7±0.1*
Number of ECM root tips analyzed	1198 / 1960	1966 / 1746	1376 / 2453	4540 / 6159
Ascomycota	33.4 / 25.0*	52.0 / 10.8*	24.7 / 29.4	38.8 / 22.7*
***Cenoccocum* sp.**	9.6 / 10.4	- / 3.6*	- / 2.2*	2.5 / 5.2*
***Pyronemataceae* sp.1**	-	8.4 / -*	11.2 / -*	7.0 / -*
Tuber anniae	-	27.0 / -*	1.6 / -*	12.2 / -*
***Tuber* sp.**	7.6 / -*	10.5 / -*	-	6.5 / -*
Basidiomycota	66.6 / 75.0*	48.0 / 89.2*	75.3 / 70.6	61.2 / 77.3*
Amphinema byssoides	14.1 / 1.3*	18.4 / 1.3*	20.3 / -*	17.8 / 0.8*
Inocybe nitidiuscula	-	20.4 / -*	-	8.9 / -*
Lactarius tabidus	2.8 / -*	- / 37.9*	- / 24.0*	0.7 / 20.3*
Russula sapinea	-	-	- / 13.0*	- / 5.2*
Tomentella coerulea	2.9 / -*	-	18.8 / -*	6.5 / -*
***Tomentella* sp. 1**	9.6 / 3.3*	-	8.6 / -*	5.2 / 1.1*
Tylospora asterophora	- / 15.8*	- / 26.6*	2.7 / 14.8*	0.8 / 18.5*

* Significant difference between fertilized and control samples ($p < 0.05$ for number of species and Shannon indices; $p < 0.001$ for species abundance).

Occurrence of almost all ECM species differed significantly between root samples from wood ash fertilized and control plots. The most common species in wood ash fertilized sample plots were *Amphinema byssoides* (17.8%) and *Tuber* cf. *anniae* (12.2%), and in control plots – *Tylospora asterophora* (18.5%) and *Lactarius tabidus* (20.3%). However, eight ECM species were present in all sample plots regardless of the treatment: *Amphinema byssoides*, *Cadophora finlandia*, *Cenococcum* sp., *Cortinarius casimiri*, *Lactarius tabidus*, *Otidea leporina*, *Tomentella stuposa* and *Tylospora asterophora*.

There were also variation at genus level between fungi inhabitating wood ash fertilized and control sample plots: *Tylospora*, *Lactarius* and *Russula* species were present in control plots and *Amphynema*, *Tuber* and *Inocybe* in wood ash fertilized plots.

The Sorensen similarity index among sample plots ranged from 0.0 to 0.9 (0.2 on average) indicating strong variation in community composition of ECMf. Community composition was more similar within control (0.3 on average) and wood ash fertilized sites (0.2 on average) than between these plot groups (0.1 on average). The largest differences in community composition between wood ash fertilized and control sites occurred in the *Myrtillosa turf. mel. / Caricoso-phragmitosa* forest type (Sorensen similarity index 0.1, on average), compared with the other forest types (0.2 and 0.2, on average).

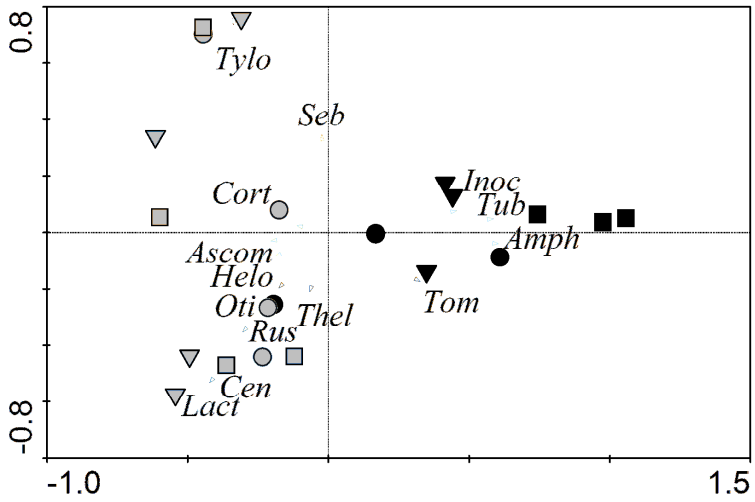

Figure 8. Principal component analysis (PCA) of fungal taxa. Rare taxa (observed only in one sample plot) were excluded from analysis. Only dominant taxa (observed in at least three sample plots) are shown. Symbols indicate plot treatment and site: black – wood ash fertilized sample plots; grey – control sample plots; circle – *Vacciniosa turf. mel.*; square – *Myrtillosa turf. mel.*; triangle – *Myrtillosa turf. mel. / Caricoso-phragmitosa*.

The first two principal components of a PCA ordination explained 41.3% of the variation in community composition of ECMf (Figure 8). Principal component 1, which explained 26.6% of data variation, clearly separated communities based on treatment. Communities of ECMf in control and fertilized plots overlapped slightly only in the pine dominated *Vacciniosa turf. mel.* forest type. The second component, which explained 14.7% of data variation, can be interpreted as a gradient from *Lactarius*-dominated communities closer to the drainage ditch (good soil aeration) to stronger *Tylospora* dominance in plots further from the drainage ditch (poor soil aeration).

Previous studies (Jonsson *et al*., 1999, Kjøller & Clemmensen, 2009) have shown changes in ECMf communities after forest liming. Observation of higher abundance of the fungal genera *Amphinema*, *Tuber* and *Inocybe* in wood ash fertilized sites than in control sites is in accordance with results of other studies (Qian *et al*., 1998; Erland & Taylor, 2002; Kjøller & Clemmensen, 2009). High abundance of these genera can be related to soil conditions. There are many *Inocybe* species that are associated with nutrient rich soils and calcareous soils (Ryberg *et al*., 2008) and also *Amphinema* (Jonsson *et al*., 1999) and *Tuber* species seem to prefer similar soil conditions. Increased abundance of *Amphimena byssoides* after liming was found not only on ECM root tips but also on dead wood substrates (Veerkamp *et al*., 1997). Dominance of mycorrhizas formed by *Tuber* spp. in wood ash fertilized sites might suggest practical importance, as some *Tuber* species form valuable edible fruitbodies.

The fungal genera *Tylospora*, *Lactarius* and *Russula* were associated with control plots in all sites and also in previous studies (LSFRI Silava, unpublished data) species of these genera were observed in unfertilized peat soils. Reduced occurrence of *Tylospora fibrillosa*, some *Russula* and *Lactarius* species and the entire Russulaceae clade has been reported in limed or wood ash fertilized sites (Qian *et al*., 1998; Jonsson *et al*., 1999; Taylor & Finlay, 2003; Kjøller & Clemmensen, 2009). Negative effect of liming on the abundance of *Tylospora fibrollosa* was reported from Sweden (Kjøller & Clemmensen, 2009). The present study showed reduced abundance of both *Tylospora* species, also *Tylospora asterophora*, in wood ash fertilized sites. Differences in site conditions, liming material and dosage might explain the various ecological outcomes from liming treatment. Typically, wood ash is used in dosages that are less than 1 kg per m^2 (Augusto *et al*., 2008), but in our experimental sites 5 kg per m^2 of wood ash was applied, which can represent extreme conditions for many forest species.

Potential saprotrophic ability due to ligninolytic enzyme production has been described for all of the dominant genera found in control plots (*Lactarius*, *Russula* and *Tylospora*) (Baldrian, 2008). The absence or reduction of those typically dominant fungal genera in ECM communities of the analyzed conifer forest types after fertilization might indicate changes in functioning and ecology of the ECM communities, and consequently altered mineral nutrition of forest trees. Overall patchy wood ash application in forest stands tends to increase total diversity of ECM species. However, soil chemical properties and the possible consequences need to be considered when applying wood ash fertilization, especially in high dosages or in a large territory.

4.4. Stump Removal Study

4.4.1. Seedling Growth

After the first growing season, seedling morphological parameters (shoot height, shoot biomass, root collar diameter, root biomass, total root length and number of root tips) in stump removal treatments were either significantly higher or did not differ significantly from those in corresponding trenching treatments in each of the surveyed forest types (Table 7). A single exception was seedling shoot height in the *Hylocomiosa* type (H-type), which was significantly higher in the trenching treatment than in the stump removal treatment (Table 7).

Chemical element concentrations in needles and soil of plots showed some differences between site preparation methods (Appendix 5). Concentrations of the main mineral elements (N, P and K) in needles were slightly higher in stump removal plots than in control plots. Soil parameters were quite variable among sites and did not show any general pattern related to site preparation method. Soil parameter variability reflects soil heterogeneity, which could be related also to recent disturbance and mixture of soil layers. As there was almost no replication of these analyses, the data sets obtained are descriptive and not allow statistical comparison of differences among treatments.

Table 7. Seedling morphological parameters after first growing season in different forest types where soil preparation was done using stump removal (Stump) and disc trenching (Control) methods.

	***Hylocomiosa* (H)**		***Myrtillosa-sphagnosa* (MS)**		***Myrtillosa mel.* (MM)**	
	Stump	**Control**	**Stump**	**Control**	**Stump**	**Control**
No. of seedlings analyzed	40	40	40	40	20	20
Shoot height, cm	26.3±0.9	29.1±0.8*	28.4±0.8	29.3±0.6	28.0±1.3	31.2±1.3
Shoot biomass, g	7.3±0.5	7.1±0.3	6.9±0.3	6.4±0.3	7.4±0.5	7.0±0.5
Root collar diameter, cm	0.7±0.01**	0.6±0.02	0.6±0.02	0.6±0.01	0.7±0.03	0.6±0.02
Old root biomass, g [a]	3.1±0.2	2.9±0.1	3.3±0.2**	2.6±0.1	3.1±0.3	2.6±0.2
New root biomass, g [b]	0.9±0.1	0.8±0.1	0.7±0.1*	0.5±0.1	0.7±0.1*	0.4±0.1
Total root biomass, g [a+b]	4.0±0.2	3.6±0.2	4.0±0.2**	2.9±0.2	3.8±0.3*	3.0±0.2
Root length, cm [b]	1108.8±89	1035.9±68	1036.5±75*	835.4±51	954.0±107	723.6±91
Number of root tips [b]	2877±231	3398±200	2612±156	2289±158	2349±295	1913±246
Mycorrhization, % [b]	84.0±2.1	87.1±1.4	81.7±1.7	76.9±2.0	81.1±3.6	80.4±3.4

Significantly greater at: * – $p < 0.05$; ** – $p < 0.01$.

[a] Roots produced during seedling cultivation in a forest nursery.

[b] Roots produced after seedling outplanting on a clear-cut (growing season 2012).

The short-term effects on seedling growth observed in this experiment might continue in the future, as better growth of the trees in stump removal sitee has also been observed 3–28 years after their outplanting (Kardell, 2008; Kardell & Eriksson, 2008; Menkis *et al*., 2010; Kataja-Aho *et al*., 2012). However, three years after planting in stump removal areas other authors (Page-Dumroese *et al*., 1998) observed smaller root collar diameter and lower total N content in needles in stump removal sites compared with control seedlings. Egnell and Leijon (1999) reported that after 15 years the height increment of Norway spruce was lower following whole-tree harvesting. The different results can probably be explained by natural variation imposed by specific conditions present in different forest types. However, observations from a longer period are necessary to determine if they are consistent with the short-term effects on seedling growth.

Greater height increment, but lower shoot biomass, of seedlings in disc trenching sites, compared with those in stump removal sites, is most likely related with greater spindling caused by understory vegetation in disc trenching sites. Kataja-aho *et al*. (2011) showed that the proportion of intact soil in stump removal sites was 32%, compared to 52% in mounding sites. The proportion of intact soil in disc trenching sites is likely even greater as this is a less extreme soil preparation method in comparison with mounding (Uotila *et al*., 2010).

There was significantly better root formation observed in stump removal sites, compared with that in disc trenching sites in the *Myrtillioso-sphagnosa* forest type (MS-type). This corresponds with findings of other authors who observed that soil preparation (mounding in that case) increased root growth significantly compared with untreated plots (Pennanen *et al*., 2005). Research from North America also indicates that soil compaction can result in decrease in root growth and ECM formation (Page-Dumroese *et al*., 1998).

4.4.2. Seedling Mycorrhization and ECM Community

The results of the present study highlight that stump removal, as compared with traditional trenching, has little or no effect on early mycorrhization of Norway spruce in forest types on podzolic soils differing in aeration and moisture ($p>0.05$). Mycorrhization in the H-type was 84.0±2.1% in the stump removal treatment and 87.1±1.4% in trenching treatment, 81.7±1.7% and 76.9±2.0% in the MS-type and 81.1±3.6% and 80.4±3.4%,in the *Myrtillosa mel.* forest type (MM-type), recpectively. Furthermore, richness of ECM morphotypes in each investigated forest type did not differ significantly between stump

removal and trenching treatments ($p>0.05$): 3.3±0.1 was in the stump removal treatment and 3.2±0.1 in the trenching treatment in the H-type, 3.3±0.1 and 3.5±0.1in the MS-type and 2.4±0.2 and 2.9±0.1 in the MM-type, respectively. Amplification and direct sequencing of fungal ITS rRNA from ECM morphotypes showed that the number of fungal taxa in the H-type was 13 in the stump removal treatment and 16 in the trenching treatment, 14 and 19 in the MS-type, and 7 and 8 in the MM-type, respectively (Table 8). Consequently, richness of fungal taxa in each investigated forest type did not differ significantly between stump removal and trenching treatments ($p > 0.05$). The total fungal community was comprised of 33 taxa, among which 15 (45.5%) were Ascomycetes and 18 (54.5%) were Basidiomycetes (Table 8, Appendix 4). The most common taxa were *Thelephora terrestris* (55.3%), *Wilcoxina* sp.1 (12.3%), *Acephala macrosclerotiorum* (4.7%), *Cenococcum geophilum* (4.0%) and *Amphinema byssoides* (3.6%) (Table 8). Previous results have shown that in different treatments the dominant ECM species are largely the same and the fungal community is similar as originated in the nursery (Table 8) (Menkis *et al*., 2005; Stenström *et al*., 2014). Such characteristic nursery fungi (e. g. *Thelephora terrestris*), which are known to be better adapted to the specific conditions of the nursery (frequent disturbance and higher amounts of mineral nutrients) (Marx *et al*., 1984), likely can benefited from alterations of soil properties as a result of site disturbance (Hope, 2007). However, such an effect is likely to be temporal, as in the later years these fungi are gradually replaced by indigenous ECMf present at a forest site (Menkis *et al*., 2007). In the present study, *T. terrestris* dominated in fungal communities of roots, with higher abundance in stump removal treatments than in corresponding trenching treatments, while the remaining taxa were less abundant than *T. terrestris* (Table 8).

Table 8. Occurrence of dominant fungal taxa (present in more than 2% of all sequenced root tips), shown as percentage of ECM roots of Norway spruce seedlings colonized in different forest types under stump removal (Stump) and trenching (Control) site preparation treatments.

	***Hylocomiosa* (H)**		***Myrtillosa-sphagnosa* (MS)**		***Myrtillosa mel.* (MM)**	
	Stump	**Control**	**Stump**	**Control**	**Stump**	**Control**
Number of ECM root tips analyzed	101	134	171	169	63	44
Ascomycetes						
Acephala macrosclerotiorum	3.0	4.2	7.1	4.1	-	6.9
Cenococcum geophilum	2.6	5.1	3.3	0.5	1.8	19.4
Meliniomyces bicolor	1.7	0.6	3.4	0.1	0.6	14.7
***Wilcoxina* sp.1**	24.7	34.8	1.6	4.1	-	20.9
All Ascomycetes	33.5	59.9	21.4	16.1	7.7	62.3
Basidiomycetes						
Amphinema byssoides	10.7	1.5	2.6	0.1	0.4	13.0
***Clavulina* sp.**	-	6.3	-	9.5	-	-
Thelephora terrestris	36.5	30.4	69.4	62.9	91.1	24.2
All Basidiomycetes	66.5	40.1	78.6	83.9	92.3	37.7
Total no. of taxa	13	16	14	19	7	8
Shannon diversity index	2.15	2.39	1.95	2.17	1.01	1.64

Among all taxa, eight were exclusively present in stump removal treatments; twelve in trenching treatments and thirteen were common to both treatments (Appendix 4). Shannon diversity indices of fungal communities in the treatments and study sites were between 1.0 and 2.4 (Table 8). The Sorensen similarity index between fungal communities in stump removal treatment and trenching treatment plots were 0.4 in the H-type, 0.6 in the MS-type and 0.7 in the MM-type. The PCA of fungal communities explained 76.1% of the variation on Axis 1 and 10.7% of the variation on Axis 2. The PCA ordination showed that stump removal and trenching treatments were in close proximity on Axis 1 (which explained most of the variation) for H-type and MS-type but slightly more distant for the MM-type (Figure 9). The forest types (H, MS and MM) were more or less well separated from each other in the ordination (Figure 9). In the present study, most of the dominant fungi were shared between different treatments of each forest type (Table 8, Appendix 4). Therefore, the treatment plots were close together within each MS- and H-types, showing that stump removal had little impact on fungal community structure (Figure 9). A slightly greater distance between plots of different treatments occurred for the MM-type was largely due to the abundance of *T. terrestris*, but composition of fungal communities was remained similar (Table 8). The

PCA also showed differences in the fungal communities among forest types (Figure 9), thereby indicating the relative importance of rare taxa (probably indigenous) present at each site. The Sorensen similarity index was moderate to high between treatments, indicating low effect of stump removal treatment. The Shannon diversity index of fungal communities was low (Table 8) and in the range of those reported from forest nurseries (Flykt *et al*., 2008; Stenström *et al*., 2014), indicating that diversity of fungal taxa also remained largely unchanged and unaffected by the stump removal treatment.

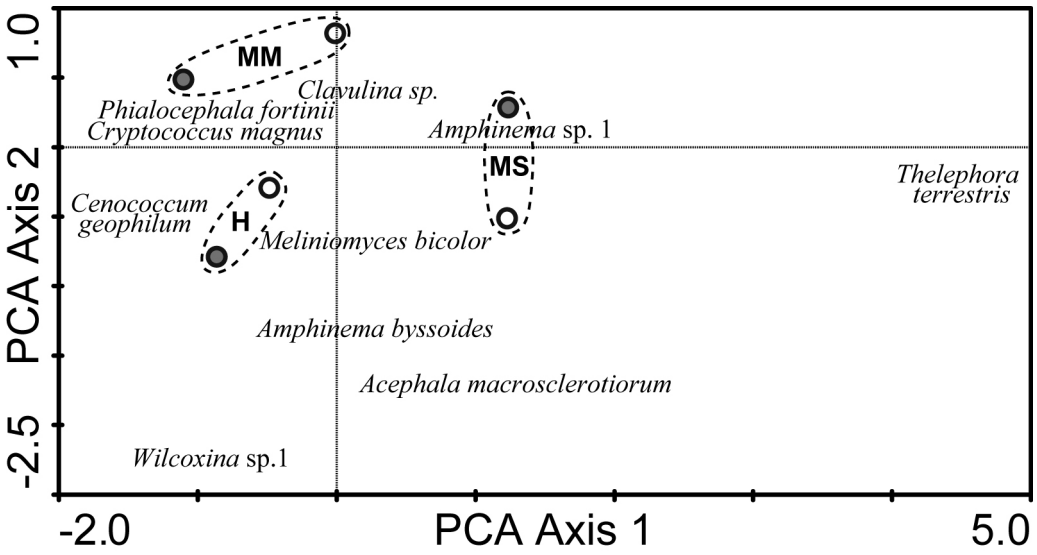

Figure 9. Ordination diagram based on a PCA of fungal communities in roots of Norway spruce seedlings outplanted on *Hylocomiosa* (H), *Myrtilloso-sphagnosa* (MS) and *Myrtillosa mel.* (MM) forest types where soil was prepared using stump removal (open circles) and disc trenching (filled circles). Taxonomic names correspond to position (centred) in the ordination and represent the ten most common taxa of the present study.

The study demonstrated that stump removal, in comparison to conventional trenching treatment, in each of investigated forest types had in general low but sometimes slightly positive impact on growth and mycorrhization of Norway spruce seedlings. This is in accordance with recent results of a study conducted in Finland (Huusko *et al*., 2015), which demonstrated high similarity of communities of ECMf on seedlings harvested three years after reforestation in clear-cuts with different wood harvesting intensity (removal of stump and logging residues; removal of logging residues; only mounding). However, their study indicated lower richness of ECMf in comparison with that on seedlings planted in uncut forest.

4.5. Seed Origin Study

After six years of cultivation in a forest nursery, significant differences in morphological parameters were observed among seedlings produced from seeds of different regions and provenances (Table 9). Shoot height was highest in seedlings of provenance C2, followed by E1, W2, W1 seedlings of, and lowest in C1 seedlings. Root collar diameter was highest in C2 and W2 seedlings, followed by W1 and E1seedlings and lowest in C1 seedlings (Table 9). Stem volume was highest in C2 seedlings, followed by W2, E1 and W1seedlings and lowest in C1 seedlings. The number of ECM morphotypes was highest in W2 seedlings, followed by E1, C2 and W1seedlings and lowest in C1 seedlings (Table 9). Consequently, seedlings of C2 and W2 showed significantly better growth and possessed significantly higher number of ECM morphotypes, while seedlings of W1, and in particular of C1, showed the poorest growth and possessed lowest number of ECM morphotypes (Table 9).

Table 9. Morphological parameters of seedlings from different seed regions (Western, Central, Eastern) and provenances (W1, W2, C1, C2, E1).

	Western		**Central**		**Eastern**
	W1*	**W2**	**C1**	**C2**	**E1**
Shoot height, cm	66.8±1.1ab**	70.5±1.1bc	63.2±1.0a	80.9±1.1d	71.7±1.1c
Root collar diameter, cm	1.42±0.03ab	1.45±0.02b	1.33±0.02a	1.45±0.03b	1.37±0.03ab
No. of ECM morphotypes	3.9±0.18ab	4.9±0.18c	3.6±0.16a	4.1±0.17ab	4.4±0.12bc

* 50 plants for each provenance were assessed.

** Within the same row, values followed by different letters are significantly different ($p < 0.05$).

The molecular analysis of 102 selected ECM root tips revealed the presence of nine distinct fungal taxa, including five basidiomycetes and four ascomycetes (Table 10). Despite the observed richness of taxa in each phylum, 60.5% of roots were colonized by ascomycetes and 39.5% were colonized by basidiomycetes (Table 10).

Table 10. Relative abundance of fungal taxa detected by morphotyping and Sanger sequencing of ECM root tips of Norway spruce seedlings representing different seed regions (Western, Central, Eastern) and provenances (W1, W2, C1, C2, E1).

Fungal taxa	**Western**		**Central**		**Eastern**	**All**
	W1 (15836/21)*	**W2 (16717/21)**	**C1 (15258/18)**	**C2 (14928/21)**	**E1 (15699/21)**	**(78438/102)**
Ascomycota						
Meliniomyces bicolor	3.8a**	3.6a	3.9a	4.5a	2.2a	3.6
***Tuber* sp.**	0.1a	0.4a	0.7a	3.9b	3.7b	1.7
***Wilcoxina* sp.**	49.0bc	46.5bc	63.4a	53.5ab	40.0c	50.3
Wilcoxina mikolae	5.1a	4.4a	5.0a	4.2a	5.6a	4.9
All Ascomycota	58.1	54.9	73.0	66.2	51.5	60.5
Basidiomycota						
Amphinema byssoides	37.0a	34.9a	24.5b	26.7b	41.5a	33.1
***Amphinema* sp.**	2.3a	4.0a	2.2a	6.6a	6.5a	4.3
Thelephora terrestris	0.2a	-	0.1a	-	0.5a	0.2
***Tomentella* sp.**	2.2a	4.7b	-	-	-	1.4
Tylospora asterophora	0.3a	1.5a	0.2a	0.5a	-	0.5
All Basidiomycota	41.9	45.1	27.0	33.8	48.5	39.5
Shannon diversity index	1.8	1.9	1.6	1.9	1.9	

* The number of ECM roots morphotyped / DGGE analyzed.

** Within the same row, values followed by different letters are significantly different ($p < 0.05$).

Basidiomycete *Amphinema byssoides* (33.1%) and ascomycete *Wilcoxina* sp. (50.3%) dominated in fungal communities in roots, but their abundances significantly varied among seedlings from different regions and provenances (Table 10). Abundance of *A. byssoides* was significantly higher in seedlings of provenances E1 (41.5%), W1 (37.0%) and W2 (34.9%) than in C1 (24.5) and C2 (26.7%). Abundance of *Wilcoxina* sp., in contrast, was highest on C1seedlings (63.4%), followed by C2 (53.5%), W1 (49.0%) and W2 (46.5%) seedlings and lowest in E1 seedlings (40.0%). The remaining fungal taxa were relatively rare (0.2%–4.9%). *Tomentella* sp. was observed exclusively on seedlings of Western provenances W1 (2.2%) and W2 (4.7%). *Tuber* sp. was more abundant on seedlings of C2 (3.9%) and E1 (3.7%). Abundances of other species were similar among seedlings from different regions and provenances (Table 10). *Thelephora terrestris* was observed exclusively on seedlings with lower growth rate (W1, C1 and E1), in comparison with those have higher growth rate (W2 and C2).

Shannon diversity index of fungal communities in seedlings of different regions and provenances was moderate and ranged between 1.6 and 1.9 (Table 10). Sorensen's similarity

index was between 0.9 and 1, showing that fungal communities in seedling roots of different provenances were very similar. Nevertheless, PCA showed that fungal communities in seedlings from Western, Central and Eastern seed regions were separated from each other on Axis 1, which explained most of the variation (84.6%) (Figure 10). Furthermore, provenances W1 and W2 of the Western region were in close proximity, thereby showing high similarity in their fungal community structure (Figure 10). By contrast, provenances C1 and C2 of Central region were placed more distantly, showing higher differences in their community structure (Figure 10). Spearman rank correlation analysis showed that there was no significant correlation between abundance of dominant ECM fungi and seedling morphological parameters.

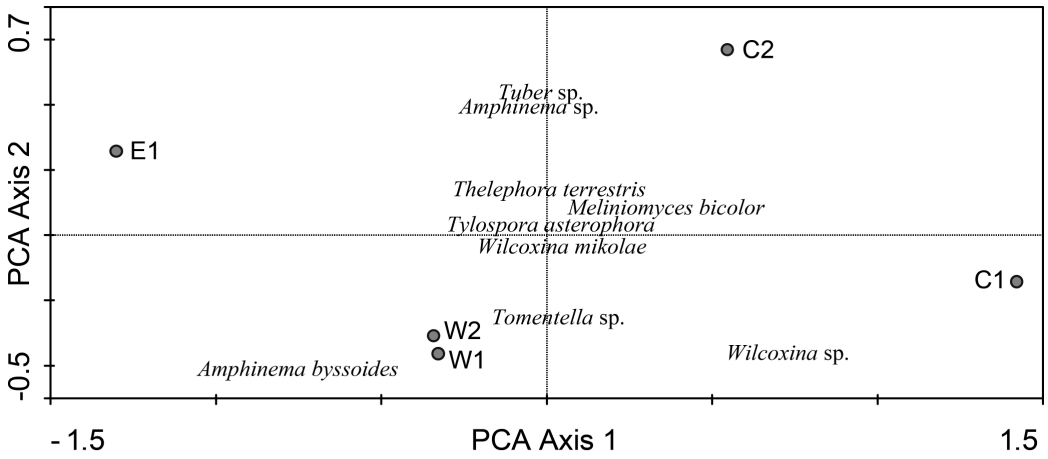

Figure 10. PCA ordination of fungal communities in roots of spruce seedlings from different seed regions: Western (W1 and W2 provenances), Central (C1 and C2 provenances) and Eastern (E1 provenance). In the diagram, 84.6% variation was explained by Axis 1 and 12.9% by Axis 2. Taxonomic names correspond to position in the ordination (centred).

The results demonstrated that, despite the fact that seedlings of different seed regions and provenances were exposed to the same growth conditions and natural ECM colonization, they differed in morphological parameters, and abundance and composition of ECM species (Tables 9 and 10, Figure 10). The results suggest that genetic background of the seedlings likely influenced both their growth and root colonization by ECMf, as these characteristics have previously been shown to vary within clonal families of Norway spruce (Korkama *et al*., 2006; Velmala *et al*., 2013). These traits may have practical importance for establishment and growth of seedlings in the nursery and after outplanting in the field (Menkis *et al*., 2007; Vaario *et al*., 2009).

Some differences were also observed between seedlings of different provenances within a particular region. In the Western region, compared with W1 seedlings, those of W2

showed higher shoot height, larger root collar diameter and higher number of ECM morphotypes. However, in both Western provenances the observed community of ECMf was similar (Tables 9 and 10, Figure 10). Previously, Norway spruce field trials showed that progeny of W2 were among most productive with high biomass yields (Gailis, 1993). Therefore this seed material has been widely used in tree breeding (Gailis, 2012) and for large-scale seedling production in forest nurseries. Even larger differences, both in morphological parameters and in the ECMf community, were observed between C2 and C1 seedlings of the Central region (Tables 9 and 10, Figure 10). Consequently, W2 and C2 seedlings not only showed the best growth parameters within each particular region but also supported significantly higher diversity of ECMf, compared with seedlings of other provenances. Therefore, the obtained data suggest that there is large variation in the growth performance of spruce seed provenances in Latvia, and that selection of the best Norway spruce seed provenances in different seed regions is needed to improve performance of future forest stands.

The ECMf observed in association with roots of spruce seedlings in the present study have been previously reported from forest nurseries and young forest plantations (Menkis *et al*., 2005; Flykt *et al*., 2008). The most dominant taxa, i.e. *A. byssoides* and *Wilcoxina* sp., were observed on all seedlings of different seed regions and provenances (Table 10) at variable abundances. Similarly, predominant occurrence of specific ECM species was also found in different Norway spruce clones (Korkama *et al*., 2006). *A. byssoides*, which is an efficient root colonizer of spruce seedlings (Menkis *et al*., 2007; Vaario *et al*., 2009; Menkis *et al*., 2011), was more dominant in Western and Eastern than in Central provenances. It occurs commonly in newly established forest plantations (Kranabetter, 2004; Menkis *et al*., 2007, Vaario *et al*., 2009), and, when abundant, it ensures better survival of seedlings after outplanting (Menkis *et al*., 2011). This may be of practical importance in terms of silviculture. *Wilcoxina* spp., the dominant ECMf in seedlings from Central provenances, belongs to a group of E-strain fungi that are early colonizers of seedling roots (Mikola, 1988). *Wilcoxina* spp. are commonly associated with tree seedlings in soils of disturbed sites (Yu *et al*., 2001; Menkis *et al*., 2010), and in soils with naturally high organic matter content or peat amendment (Rudawska *et al*., 2011). Therefore, high abundance of this genus in the analyzed seedlings in the present study could be related to the substrate. Velmala *et al*. (2014), demonstrated that *Wilcoxina* had high chitinase activity, which correlated with high N content of needles. The exploration type of ECM (Agerer, 2001) of *Amphinema* and *Wilcoxina* differs: *Amphinema* forms the *medium distance* and *Wilcoxina – contact type* mycorrhizas (Agerer, 2001; Rudawska *et al*., 2011). Those differences might have impact on seedling mineral

nutrition and further nutrient supply in the field, as the so-called ECM exploration types are believed to differ in their efficiency of carbon (C) storage, enzymatic activities, nutrient uptake and translocation (Hobbie & Agerer, 2010; Pritsch & Garbaye, 2011). In the present study, the source of ECM inoculum was not investigated, but likely included both inoculum of ECMf present in the soil and air-borne ECM spores from the surrounding forests. These studies on the ecology of both dominant taxa indicate that they might have some advantages for successful seedling growth and establishment after outplanting. However, there were no definite arguments provided that showed that they would be more beneficial taxa for seedling growth. In a study by Korkama *et al*. (2006), seedling provenances showing the best growth rate (W2 and C2) were characterized as provenances with diverse ECMf communities. Thus, it appears that the diversity of the fungal community and consequently their ecological plasticity are more essential for growth of the seedlings, compared to effect of a single dominant fungal species.

Thelephora terrestris was observed in provenances of seedlings that showed lower shoot growth rate. This ECM fungus, which is common in forest nurseries worldwide (Marx *et al*., 1984), often fails to support seedling establishment in the field (Ivory & Munga, 1983; Lee, 1992). Therefore, the presence of *Thelephora terrestris* in association with lower growth rate of seedlings suggests a disadvantage of these seedling provenances for successful establishment in the field.

In order to obtain a more comprehensive picture of the processes that determine patterns of ECM communities in tree roots, more studies are needed, encompassing a wide range of tree origin and environmental conditions.

4.6. Cultivation System Study

4.6.1. Seedling Growth

During four growing seasons (2006–2009) following seedling outplanting, a gradual decrease in seedling survival was observed each year in both cultivation systems, resulting in relatively low overall survival rates (Table 11). No significant differences in seedling survival were observed between containerized and bare-root seedlings after the 2006, 2007, and 2008 growing seasons, but after the 2009 season a greater dieback was observed in containerized seedlings, resulting in significantly lower survival than for bare-root seedlings (Table 11).

At the time of outplanting, height of containerized seedlings was significantly smaller as compared to bare-root seedlings ($p < 0.001$) (Table 11) and fluctuation in growth of the seedlings of different treatments was observed each year. Better growth of containerized

seedlings as compared to bare-root seedlings was observed during the two first seasons in the plantation, resulting in similar height of the seedlings in both treatments after the 2006 season and in significantly higher height of containerized seedlings after the 2007 season ($p < 0.001$). However, during the third and fourth seasons, containerized seedlings grew poorer when compared to bare-root seedlings, resulting in similar height of the seedlings in both treatments after the 2008 season and in significantly lower height of containerized seedlings after the 2009 season ($p < 0.05$) (Table 11).

Table 11. Survival and height of the Norway spruce seedlings during four growing seasons.

	Survival, %		**Height, cm**	
	Containerized	**Bare-rooted**	**Containerized**	**Bare-rooted**
May 2006	n.a.	n.a.	24.5±0.2	30.2±0.3 *
2006	83.2±6.2	81.5±5.1	34.2±0.4	33.6±0.4
2007	60.0±3.8	61.5±4.7	43.1±1.2 *	36.8±0.9
2008	39.8±3.2	45.2±4.8	59.5±1.4	60.3±1.3
2009	29.5±3.5	42.6±4.5*	75.6±2.3	82.5±1.9 *

* Significantly greater survival (in chi-squared tests) or height (in one-way ANOVA) within the same year.

The root collar diameter of the seedlings was measured once after the growing season 2009 and it did not differ significantly between the seedlings of different treatments (1.4 cm and 1.5 cm, respectively; $p > 0.05$). The chemical analysis of needles conducted after the 2009 season showed generally lower concentrations of all chemical elements in containerized seedlings as compared to bare-root seedlings (Table 12). An exception, Mo concentrations were several times higher in needles of containerized seedlings than in bare-rooted seedlings (Table 12).

Table 12. Chemical composition of needles (mg/g) of bare-rooted and containerized Norway spruce seedlings after four growing seasons (October 2009) in a plantation.

Chemical elements (%)	**Sample plots**		**Chemical elements (%)**	**Sample plots**	
	C2009	**B2009**		**C2009**	**B2009**
N	11.7	13.7	**Fe**	0.062	0.058
P	1.9	2.4	**Mn**	0.072	0.062
K	4	8	**Zn**	0.036	0.04
Ca	6	4.9	**Cu**	0.002	0.003
Mg	1.6	1	**Mo**	0.0007	0.0002
S	1.5	1.6	**B**	0.011	0.012

Several studies have previously reported that containerized seedlings exhibit faster growth and better survival than bare-root seedlings after their outplanting in the field or in the nursery (Leugner *et al*., 2009; Vaario *et al*., 2009; Menkis *et al*., 2011). In agreement with these studies, the present study also demonstrated that during the first three growing seasons following seedling outplanting, containerized seedlings showed generally similar or better survival and growth as compared to bare-root seedlings. Grossnickle (2005) reported that containerized seedlings can have greater root growth than bare-root seedlings during the first growing seasons. However, after the fourth growing season their survival and growth had decreased (Table 11). This may suggest that possibly a longer period of time is required to reveal the effects of different cultivation systems on seedling performance in the field and that this should be considered when performing similar studies in the future.

Differences in root architecture between seedlings may influence early survival and growth, since it affects the capacity of seedlings to produce new roots that extend outside the original root system into surrounding soil (Bernier *et al*. 1995). During cultivation in forest nurseries, roots of containerized seedlings are usually more compressed and compacted within growth containers, compared to naturally developing roots of bare-root seedlings. Despite the fact that roots of containerized seedlings usually have better primordia and larger nutrient reserves (Leugner *et al*., 2009) than for bare-root seedlings, containerized seedling roots to a large extent remain intact following seedling outplanting. In the present study, differences in age between containerized and bare-root seedlings (Appendix 2) might also have affected the development of roots and therefore growth and mineral nutrition of the seedlings of different treatments.

The results suggest that already after the fourth season in the plantation, in addition to survival and growth parameters, the nutritional status (as shown by the chemical composition of the needles) of bare-root seedlings was far better than for containerized seedlings (Table 12). This difference might be associated to water regime at the study site, as it has been reported that performance of containerized seedlings is better when planted on moist sites (Grossnickle, 2005). In the present study, however, the site corresponded to the *Oxalido-myrtillosa* forest type characterized by average fertility and temporarily excessive humidity in the soil.

The production of containerized seedlings is increasing in Northern Europe and production of bare-root seedlings is declining (Flykt *et al*., 2008). Data from forest nurseries of Seeds and Plants, Latvian State Forests JSC shows the same tendency – in last ten years, production of containerized spruce seedlings has increased aprox. six times, while production

of bareroot seedlings has decreased close to eight times. Therefore, since the results of the present study and related research (Leugner *et al*., 2009) suggest that containerized seedlings used for outplanting on dry and/or humid habitats may perform poorer than bare-root seedlings, caution should be taken when selecting seedlings for reforestation. On the other hand, seedling production using a Plug+1 system could be an alternative, as using this cultivation system seedling roots develop more naturally as compared to containerized seedlings and have better primordia as compared to bare-root seedlings. Also, their production is less expensive. However, in order to obtain a more comprehensive picture about the impact of cultivation system on seedling performance following their outplanting in the field, more related studies are needed in the region, encompassing different soil conditions, tree species and including Plug+1 system seedlings in experiments.

4.6.2. Seedling Mycorrhization and ECM Community

During four growing seasons (2006–2009) following plantation establishment, a total of 12 ECM morphotypes was found; ITS rRNA sequencing of representative root tips of each individual ECM morphotype revealed the presence of 17 fungal taxa (Table 13, Appendix 4).

Shannon diversity for bare-rooted seedling was quite similar at all study times. In contrast, species diversity for containerized seedlings varied strongly among sampling times (Table 13). The Sorensen similarity index of fungal communities between containerized and bare-rooted seedlings was moderate after the first, second and third growth season (0.4 to 0.6) and low after the fourth growth season (0.2). Furthermore, the PCA analysis showed that initially the fungal communities of both cultivation systems were largely different (Figure 11, Table 13). However, after the next seasons (2007, 2008), similar species composition was observed in both cultivation systems. The first component explained 34.7% of the species composition and can be interpreted as temporal succession from a *Thelephora terrestris* and *Amphinema byssoides* dominated community to a *Wilcoxina* sp. and *Amphinema* sp. dominated community (Figure 11). The second component explained 26.2% of the data variation and is associated with differences between cultivation systems.

Table 13. Occurrence and relative abundance (%) of dominant fungal taxa (represented on at least 2% of all analyzed root tips) directly sequenced from roots of bare-rooted (B) and containerized (C) Norway spruce seedlings before outplantation and after the first (2006), second (2007), third (2008) and fourth (2009) growing seasons in a plantation.

	2006		**2007**		**2008**		**2009**	
	B	**C**	**B**	**C**	**B**	**C**	**B**	**C**
Shannon diversity index	0.68	1.33	0.88	0.62	0.90	0.63	0.89	1.60
Amphinema byssoides	2.8	20.0	21.4	23.6	55.0	19.0	-	37.5
Amphinema sp.	-	-	-	-	1.3	1.0	39.5	28.1
Atheliaceae sp.	-	-	72.1	-	-	-	-	-
Leptodontidium elatius	-	50.5	-	-	-	-	-	-
Phlebiopsis gigantea	-	-	-	-	43.8	-	-	-
Thelephora terrestris	81.3	5.8	-	74.3	-	-	-	-
Wilcoxina rehmii	-	-	5.7	2.1	-	50.0	-	1.3
Wilcoxina sp.	-	-	-	-	-	-	50.9	-

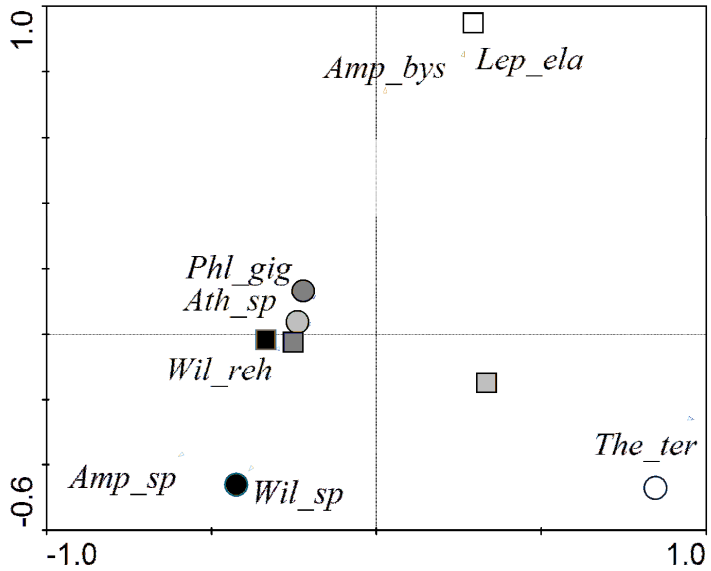

Figure 11. Ordination diagram based on PCA of fungal communities in roots of Norway spruce seedlings from different treatments after outplantation. Symbol colour – sampling year (white – 2006, light grey– 2007, dark grey – 2008, black – 2009); figure shape – cultivation system (circles – bare-root seedlings; square – containerized seedlings). Only dominant fungal species are shown.

In the present study, the communities of ECMf observed after the first growing season in a plantation were generally similar to the ones previously described from forest nurseries (Menkis *et al*., 2005; Flykt *et al*., 2008; Menkis & Vasaitis, 2011). The dynamic changes in the later seasons, i.e. from the predominance of *T. terrestris* to the dominance of *Wilcoxina* spp. and *Amphinema* spp., were likely driven by ECM inoculum availability, and

indicated certain adaptation of ECM communities to environmental conditions present at the site (Dahlberg & Stenström, 1991; Gagné *et al*., 2006; Menkis *et al*., 2007). On the other hand, sampling and analysis of a relatively small proportion of root-tips and plants in the plantation might be partially responsible for the observed rapid shift in composition and abundance of fungal taxa. Dominant taxa observed in the present study (genera *Thelephora*, *Wilcoxina* and *Amphinema*) were previously shown to be early stage root colonizers and widespread fungi (Horton & Bruns, 2001). Among these, the dominant forest nursery fungus *T. terrestris* (Marx *et al*., 1984), which despite its high ability to adapt to environmental conditions of the nursery (Perry *et al*., 1987), often fails to support seedling establishment in the field (Ivory & Munga, 1983; Lee, 1992). *Wilcoxina* species belong to a group of E-strain fungi that were found to be commonly associated with tree seedlings in soils following site disturbance and therefore could be important for seedlings in overcoming the replanting stress (Yu *et al*., 2001; Menkis *et al*., 2010). *A. byssoides* is known as an efficient root colonizer of Norway spruce seedlings and may play an important role in seedling survival and establishment following their outplanting (Menkis *et al*., 2007; Vaario *et al*., 2009; Menkis *et al*., 2011). In the present study, the observed predominant establishment of *A. byssoides* in roots of bare-root seedlings of Norway spruce after the second and third growing seasons might be associated with their better survival and growth in the following seasons (Table 11). Among other fungi, the presence of *Phlebiopsis gigantea* in ECM roots of spruce seedlings (Table 13) further supported the hypothesis about the multi-trophic nature of this wood-decay fungus (Vasiliauskas *et al*., 2007, Menkis *et al*., 2012).

4.7. Overall Results and Discussion on ECM Community and Species Ecology

In total, 148 fungal species were identified from the sequenced root tips. A large number of species represented basidiomycetes, 105 species, mainly from the ECM orders *Atheliales*, *Agaricales*, *Russulales* and *Thelephorales*. Ascomycetes were represented by 43 species, mainly from the *Helotiales* and *Pezizales* orders. The sequences of all studies are available from GenBank under accession numbers KP753291–KP753379, KR019832–KR019874, KR019781–KR019831, KF954060–KF954092, KP172303–KP172311 and JX907809–JX907827 (Appendix 4).

As no studies of Norway spruce ECM species from a belowground perspective was previously conducted in Latvia, there were many new species for Latvia recorded, mainly from ascomycetes and the basidiomycete orders *Atheliales* and *Thelephorales*. From basidiomycetes forming conspicuous fruitbodies, records of *Cortinarius casimiri*, *Russula*

sapinea, *Inocybe proximella*, *Inocybe reclina* and *Lactarius badiosanguineus* were new. Important records for Latvia were also for some hypogeous fungi, such as *Hydnotrya bailii*, *Hydnotrya cerebriformis*, *Pachyphloeus conglomeratus* and three *Tuber* species.

Some species, such as *Amphinema byssoides*, *Amphinema* sp. (SH197945.07FU), *Cadophora finlandica*, *Pyronemataceae* sp. (SH 194157.07FU), *Thelephora terrestris*, *Tomentella stuposa*, *Tylospora asterophora* and *Tylospora fibrillosa* were observed almost in all data sets.

High abundance of *Tylospora asterophora* in the studied sites indicates that this species is closely associated with nutrient rich spruce forests on peat soils. This might be related with the role of *Tylospora* spp. in degradation of remnants of decayed wood and soil humic polymers (Tedersoo *et al*., 2003). Also *Amphinema byssoides* was a very common species on fine roots of the analyzed trees. However, it was visually observed that vitality of colonized fine roots of this species in some cases was low and dense mycelium of *Amphinema byssoides* was forming around older fine roots. This suggests its saprotrophic activity, since mycorrhizal fungi can function both as endophytes and necrotrophs of host plants (Brundrett, 2004). As saprotrophic activity of this species is known, and since it is a frequent colonizer of dead wood substrates (Veerkamp *et al*., 1997), it would be valuable to test its saprotrophic activity in root tips as well, especially in nutrient rich peat substrate.

Some ECM species were exclusively found either in the root samples from mature stands or seedlings. Differences between young and mature spruce root samples were expected, since previous studies have explained such a pattern by soil disturbance v.s. continuity or tree maturity (Jones *et al*., 2003; Tedersoo *et al*., 2008; Twieg *et al*., 2009). Both *Wilcoxina* species (*W. mikolae* and *W. rehmii*) were present exclusively in young seedlings. Also, *T. terrestris* was much more abundant in young seedlings. Other authors have reported greater abundance of these species on seedlings in clear cuts, in comparison with forest sites with intact soil surface (Jones *et al*., 2003; Huusko *et al*., 2015). Species found exclusively in mature stands were *Acsomycota* sp. (SH214267.07FU), *Amanita* spp., *Cenococcum* sp. (SH214459.07FU), *Cortinarius* spp., *Elaphomyces* sp. (SH215736.07FU), *Hygrophorus* spp., *Inocybe flocculosa* (SH187316.07FU), *Inocybe proximella*, *Lactarius* spp., *Lactarius tabidus*, *Pseudotomentella* spp., *Russula sapinea* and *Thelephoraceae* sp. (SH184509.07FU) were determined. There were also some differences in occurrence of species from the same genera observed in mature and young stands: *Cenococcum* sp. (SH214459.07FU) and *Elaphomyces* sp. (SH215736.07FU) were observed in mature stands, while *Cenococcum* sp. (SH199613.07FU) and *Elaphomyces* sp. (SH215737.07FU) – in young plantings.

Soil acidity, aeration and nutrient content had effects on ECM communities. Some species, such as *Tomentolopsis echinospora*, *Tylopilus felleus* and members of the *Piloderma* genus were observed exclusively on sites with relatively lower soil pH. In contrast, other species, such as *Inocybe flocculosa*, *Amphinema* sp. (SH197945.07FU) were mostly observed in more calcifilous habitats. There were some fungal species forming ECM with conifer on sites with more neutral soil pH; these had previously been isolated mainly from broadleaved tree roots (e. g. *Thelephoraceae* sp. (SH177844.07FU)). Lower abundance of some typical taxa of boreal forests, such as *Cenococcum* and *Lactarius* in *Heterobasidion* root-rot infected trees and wood ash fertilized sites might also be primary related to soil pH or aeration.

There were some differences in occurrence of species from the same genus observed in sites with different pH values or soil fertility: *Tylospora asterophora* was more abundant in more fertile sites, and *Tylospora fibrillosa* in less fertile sites; *Amphinema byssoides* was present in almost all studied sites, while *Amphinema* sp. (SH197945.07FU) was more common in sites with higher soil pH. Among *Helotiales* fungi, there were also some soil preferences observed – *Phialocephala fortinii* was more common in nutrient poor soils with good tree health status; *Acsomycota* sp. (SH214267.07FU) preferred more nutrient rich soils with higher pH and higher tree decay incedence; *Cadophora / Meliniomyces* (SH214265.07FU / SH181080.07FU) was found often in young plantings and peat soils in general.

Analysis of fungal communities in relation with tree growth and vitality showed that in many cases there was no primary relation and that it is difficult to separate these interactions from the impact of soil factors. However, the study indicates the importance of ECM species composition and abundance on tree growth and nutrient cycling processes in Norway spruce forests. Therefore, this study should be continued in more detail to obtain more knowledge on the ecology of ECM species.

5. Conclusions

1. Decreased living fine root abundance on peat or gleyic soils was observed in severely damaged Norway spruce stands, which was correlated with soil parameters (peat layer thickness and groundwater level). Abundance of saprotrophic fungal species was greater in severely damaged Norway spruce stands.
2. *Heterobasidion* root-rot on peat soils had little or no effect on fine root morphology and on composition of fungal communities in roots. In general, peat soils in stands with *Heterobasidion* infection were either fertile with high pH and ECM species (*Amphinema* spp., *Inocybe* sp., *Tylospora asterophora*) or had extremely low pH and poor fine root mycorrhization in general.
3. Twelve years after wood ash fertilization, ECMf from genera *Amphinema*, *Tuber* and *Inocybe* were characteristic of wood ash fertilized plots, while *Tylospora*, *Lactarius* and *Russula* species – of unfertilized plots. Forest fertilization with high dosage (5 kg / m^2) of wood ash caused long-term effect on soil pH, chemical composition and communities of ECMf.
4. Stump removal in comparison to conventional trenching site preparation treatment showed positive effect on seedling growth and root development in the first growth season. However, high dominance of the typical nursery species *Thelephora terrestris* might indicate reduced mycorrhization inoculum of other species.
5. Seed origin affects growth of Norway spruce seedlings and their root colonization by ECMf. Abundance of dominant ECM species *Amphinema byssoides* and *Wilcoxina* sp. differed significantly on seedlings representing different seed origin in Latvia. *A. byssoides* was less abundant in seedlings representing Central seed origin of Latvia than in other provenances ($p < 0.05$). Seedlings with better growth rate had higher ECM species richness.
6. Higher growth parameters and better survival of spruce bare root seedling than containerized seedlings after the fourth growing seasons can be related to better adaptation to field conditions due to a more developed root system or to the mycorrhizal community. Ectomycorrhizal species *Amphinema* sp. and *Wilcoxina* sp. were more abundant on bare root seedlings.
7. In young plantings (up to eight years old), ECM species *Thelephora terrestris, Amphinema byssoides* and *Wilcoxina* species are the main root symbionts of Norway spruce seedlings. In mature Norway spruce stands and mainly on peat soils,

dominant ECM symbionts are species from genera *Tylospora*, *Amphinema, Lactarius* and *Tomentella*.

8. From the analyzed ECM root tips, several new species for Latvia were recorded, mainly from ascomycetes (also hypogeous fungi) and the basidiomycete orders *Atheliales* and *Thelephorales*. First time records in Latvia include the basidiomycetes *Cortinarius casimiri*, *Russula sapinea*, *Inocybe proximella*, *I. reclina* and *Lactarius badiosanguineus*.

Approbation of results

Publications

1. Klavina D., Menkis A., Gaitnieks T., Velmala S., Lazdins A., Rajala T., Pennanen T. 2015. Analysis of Norway spruce dieback phenomenon in Latvia – a belowground perspective. **Scandinavian Journal of Forest Research**, DOI 10.1080/02827581.2015.1069390.

2. Klavina D., Pennanen T., Gaitnieks T., Velmala S., Lazdins A., Lazdina D., Menkis A. 2015. The ectomycorrhizal community of conifer stands on peat soils 12 years after fertilisation with wood ash. **Mycorrhiza**, DOI 10.1007/s00572-015-0655-2.

3. Klavina D., Gaitnieks T., Menkis A. 2013. Growth and ectomycorrhizal community development of containerised and bare-root *Pinus sylvestris* and *Picea abies* seedlings outplanted on a forest clear-cut. **Baltic Forestry** 19: 39–49.

4. Klavina D., Zaluma A., Pennanen T., Velmala S., Gaitnieks T., Gailis A., Menkis A. 2015. Seed provenance impacts growth and ectomycorrhizal colonisation of *Picea abies* seedlings. **Baltic Forestry** 21(2), Accepted for publishing.

5. Klavina D., Menkis A., Gaitnieks T., Pennanen T., Lazdins A., Velmala S., Vasaitis R. Low impact of stump removal on mycorrhization and growth of replanted *Picea abies*: data from three types of hemi-boreal forest. Submitted to Baltic Forestry.

6. Gaitnieks T., Klavina D., Muiznieks I., Pennanen T., Velmala S., Vasaitis R., Menkis A. Impact of *Heterobasidion* root-rot on fine root morphology and associated fungi in *Picea abies* stands on peat soils. Submitted to Mycorrhiza.

Presentations in conferences

1. Klavina D., Pennanen T., Gaitnieks T., Lazdina D., Lazdins A., Velmala S., Menkis A. 2015. Ectomycorrhizal community in conifer stands on peat soils 12 years after wood ash treatment. COST action FP1305 „BioLink: Belowground biodiversity under changing environment" meeting, Krakow, Poland, March 17–20, 2015. Oral presentation.

2. Klavina D. 2014. Study of ECM community in conifer stands 10 years after wood ash treatment: preliminary results. NEFOM meeting, November 27–28, 2014, Riga. Oral presentation.

3. Klavina D., Pennanen T., Gaitnieks T., Lazdins A., Menkis A. 2014. Ectomycorrhizal and other root associated fungi on Norway spruce in declining Norway spruce stands in Latvia. XIX Symposium of mycologists and lichenologists of Baltic countries. 22–26 October, Talsi, Latvia. Poster presentation.

4. Klavina D. 2013. Impact of stump removal on mycorrhization and field growth of Norway spruce (*Picea abies*) seedlings in Latvia. NEFOM meeting, November 24–25, 2013, Uppsala. Oral presentation.

5. Klavina D., Velmala S., Zaluma A., Pennanen T., Gailis A., Gaitnieks T. 2012. Colonization by several ectomycorrhizal fungi might be related to reduced growth of *Heterobasidion annosum* s.l. mycelium in Norway spruce seedlings. „*Joint IUFRO 7.03.01 "Cone and seed insects" and 7.03.04 "Diseases and insects in forest nurseries" Working Party Meeting, Vilnius*, Lithuania. Oral presentation.

6. Klavina D., Pennanen T., Rajala T., Menkis A., Gaitnieks T. 2012. Mycorrhizae and fine root characterists of *Heterobasidion* infected and non –infected Norway spruce. „*Joint* IUFRO 7.03.10 – „Methodology of forest insect and disease survey" and IUFRO WP 7.03.06 – „Integrated management of forest defoliating insects" *Working Party Meeting, Palanga*, Lithuania. Oral presentation.

7. Klavina D., Lazdins A., Bardulis A., Gaitnieks T. 2011. Vitality of fine roots in spruce stands with different degree of foliage damage in Latvia. COST conference "Carbon balance after disturbances and drought". COST Action FP0803. Barcelona, 27–30 June, 2011. Poster presentation.

8. Klavina D., Gaitnieks T., Baumanis I., Vasaitis R. Menkis A. 2011. Field survival, growth and mycorrhization of containerised *Pinus sylvestris* and *Picea abies* seedlings of different provenances. Doctoral student conference „Next generation insights into geosciences and ecology" May 12–13, 2011, Tartu, Estonia. Oral presentation.

9. Kļaviņa D., Donis J., Gaitnieks J. 2011. Sakņu vitalitāte un stumbra koksnes pieaugums egļu audzēs ar dažādu vainaga bojājumu pakāpi. LU 69. Zinātniskā konference, Rīga, 1.februāris. Oral presentation (In Latvian).

10. Klavina D., Menkis A., Gaitnieks T. 2010. Field growth and mycorrhization of *Pinus sylvestris* and *Picea abies* seedlings produced under different nursery cultivation systems. IMC 9 „The biology of fungi", Edinburgh, UK, 1–6 August, 2010. Poster presentation.

11. Kļaviņa D., Gaitnieks T., Menkis A. 2010. Dažādas izcelsmes priežu un egļu stādmateriāla pieauguma dinamika. Latvijas Lauksaimniecības universitātes, Meža fakultātes zinātniski praktiskā konference – zinātne un prakse nozares attīstībai, Jelgava, 22. marts, 2010. gads. Oral presentation (In Latvian).

Acknowledgements

First of all, I would like to thank my supervisors Dr. Tālis Gaitnieks, Dr. Audrius Menkis and Dr. habil. prof. Indriķis Muižnieks. Thank you, Tālis, for accepting me back in Silava and trusting me this project, but most of all – for teaching, discussions and showing new insight to forest science. Many thanks to Dr. habil. prof. Indriķis Muižnieks for his support during this study period. I am deeply grateful to my supervisor Dr. Audrius Menkis from the Swedish University of Agricultural Sciences. Thanks for your generous availability to help and give advice. I have learnt a lot from your work and your way to organize it. I would like to thank here to Asoc. prof. Rimvydas Vasaitis from the same university for his support and ideas during this period.

I am grateful to Taina Pennanen, Sannakajsa Velmala and Tiina Rajala† from the Finnish Forest Research Institute Metla (now Natural Resources Institute Finland (Luke)) and all the colleagues who worked with me during my stay in Finland (Minna, Sipu, Sonja, Juha, Marja-Leena, Anna, Lu-Min, Michael, Kari, Jarkko and others). Thanks for everything you helped me with and taught me. It was a great experience to work at Metla and with you! I would also like to thank to our closest neighbours Estonian and Lithuanian colleagues for some valuable discussions and their work especially building the UNITE database.

All colleagues from LSFRI Silava involved in these investigations are gratefully thanked for their collaboration, advice and support during doctoral studies. I am deeply grateful to all colleagues from the Forest Environment laboratory for field work and chemical analysis and leading researcher of this lab Andis Lazdiņš for his support. Also colaboration of Genetic Resource Centre is acknowledged. My special thanks to my closest colleagues from the laboratory of Forest Phytopathology and Mycology – Natālija, Kristīne, Astra, Lauma, Agrita, Anete and people from this lab who worked there previously – Brigita, Dace, Guna, Alīna, Kristiāna, Ieva, Kristīne, Barbara and Undīne. I am grateful to Dr. Līvija Vulfa, who so kindly always takes care for all work and people of our laboratory. My friends who became involved in adventure of field or lab work, even as volunteers sometimes – Zane, Anna, Gundega, Guna, Kristaps, Līga T., Līga K., Rodika – are gratefully thanked.

My sincere is extended to reviewers of this doctoral thesis in different stages and all article reviewers before publishing.

Thanks to all the people from the University of Latvia, Faculty of Biology, who helped me during my studies there and work on this thesis. Especially, I would like to thank Doc. Edgars Vimba and Dr. Ligita Liepiņa for introducing me with fungi and mycorrhizae. I am

grateful to all personnel especially from the three departments I was more in contact with during this period – Department of Botany and Ecology, Department of Microbiology and Biotechnology and Department of Plant Physiology and Atonomy.

I would like to thank Dr. Roberts Matisons, Dr. Līga Strazdiņa, Dr. Didzis Elferts, Dace Čimoka and Anna Kļaviņa for consultations about data analysis and presentation and prof. Dr. Guntis Brūmelis and Gunta Kļaviņa for valuable comments and assistance with English revision.

Many thanks to colleagues from the Institute of Biology, Laboratory of Plant Mineral Nutrition for some chemical analysis and most of all to Dr. Anita Osvalde and Dr. Gunta Čekstere for sharing their knowledge in valuable comments and discussions. Special thanks to Laima Zvejniece, Indulis Brauners and other specialists from Latvia's State forests JSC for consultations, plant material and great examples of generous dedication to their work.

And finally many thanks to my family and friends, who encouraged me to start, continue and finish this work or just shared these moments with me, especially to my parents for so loving and scientific *background*! *Paldies*!

I dedicate this thesis to the memory of Tiina Rajala and my grandparents.

- Do you know from where apples come?

- From apple-tree.

- But from which part?

- From branches.

- No, everything comes from the root, if root is good, tree gives good fruits.

/St. Hosemaria and Rosalia/

The financial support:

a. The work was funded by the Latvia's State Forests, JSC project No. 5.5-5.1/000s/101/11/12; ERDF project (No. L-KC-11-0004) „Methods and technologies for increasing forest capital value", Riga Forests, LLC project No.: 5.5-5.1/001Y/110/08/8, ERDF project No. 2013/0065/2DP/2.1.1.1.0/13/APIA/VIAA/034, State research programme "Forest and earth entrails resources: research and sustainable utilization – new products and technologies" (ResProd) project "Even-age spruce stands cultivation potential in fertile forest ecosystem" and Grant project No.426/2012 of Latvian Council of Science.

b. Funding for attendance of some conferences and publishing was provided by ERDF project No 2DP/2.1.1.2.0/10/APIA/VIAA/021;

c. The lifelong learning program "ERASMUS", SNS project "NEFOM" and COST action FP1103 financially supported exchange visits to Finland for laboratory work.

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Appendix

Appendix 1. Coordinates and site description of forest sites studied

Study*, Site	Abbreviation of sample plots	Coordinates	Forest age, dominant tree species**	Forest type	Health status***	Soil type, drainage (^)
I, Olaine 1	1, 2	56º84.89´N, 24º03.21´E	50, S	*Myrtilliosa. turf. mel.*	1; 0	Raised bog soils ^
I, Olaine 2	3, 4	56º86.74´N, 23º99.35´E	35, Sbp	*Myrtilliosa. turf. mel.*	2; 0	Transitional mire soils ^
I, Olaine 3	5, 6	56º84.46´N, 24º02.65´E	50, Sbg	*Myrtilliosa. turf. mel.*	2; 0	Podzolic-gley soils ^
I, Olaine 4	7, 8	56º81.50´N 24º09.23´E	50, S	*Myrtilliosa. turf. mel.*	1; 0	Transitional mire soils ^
I, Olaine 5	35, 36	56º76.78´N, 23º80.62´E	50, Sb	*Mercurialiosa mel.*	1; 1	Gley soils ^
I, Kalnciems	11, 12	56º85.69´N, 23º68.16´E	50, Sb	*Myrtilliosa. turf. mel.*	2; 0	Gley soils ^
I, Ropaži 1	9, 10	56º99.55´N, 24º62.43´E	40, S	*Myrtilliosa mel.*	2; 0	Gley and Podzolic-gley soils ^
I, Ropaži 2	13, 14	56º98.95´N, 24º70.96´E	40, S	*Hylocomiosa*	1; 0	Podzolic gley soils
I, Ropaži 3	15, 16	56º99.14´N, 24º62.20´E	40, S	*Hylocomiosa*	2; 0	Podzolic soils
I, Ropaži 4	17, 18	56º99.08´N, 24º66.56´E	40, S	*Hylocomiosa*	1; 0	Podzolic gley soils
I, Ropaži 5	19, 20	56º98.99´N, 24º71.12´E	25, S	*Mercurialiosa mel.*	1; 0	Gley and Podzolic soils ^
I, Ropaži 6	21, 22	56º99.19´N, 24º71.19´E	25, S	*Mercurialiosa mel.*	2; 0	Podzolic gley and Gley soils ^
I, Dzimtmisa	23, 24	56º69.43´N, 24º17.06´E	70, Sba	*Oxalidosa turf. mel.*	2; 0	Transitional mire and Podzolic-gley soils ^
I, Nagļi 1	25, 26	56º64.63´N, 26º87.54´E	50, S	*Myrtilliosa. turf. mel.*	4; 4	Transitional mire soils ^
I, Nagļi 2	37, 38	56º64.81´N, 26º87.39´E	50, Sb	*Myrtilliosa. turf. mel.*	2; 2	Transitional mire soils ^
I, Viesīte 1	27, 28	56º40.18´N, 25º58.24´E	20, Sb	*Oxalidosa turf. mel.*	3; 1	Fen peat soils ^
I, Viesīte 2	29, 30	56º31.45´N, 25º41.94´E	45, S	*Myrtilliosa. turf. mel.*	3; 2	Transitional mire soils ^
I, Pēternieki	31, 32	56º68.24´N, 24º70.81´E	40, Sb	*Myrtilliosa mel.*	1; 0	Podzolic-gley soils ^
I, Iecava	33, 34	56º64.34´N, 24º34.11´E	40, S	*Myrtilliosa mel.*	1; 0	Gley soils ^

I, Varakļāni	39, 40	56º75.16´N, 26º62.83´E	60, S	*Mercurialiosa mel.*	3; 1	Transitional mire and Gley soils ^
I, Lubāna 1	41, 42	57º00.28´N, 26º79.83´E	40, Sb	*Oxalidosa turf. mel.*	2; 2	Fen peat soils ^
I, Lubāna 2	43, 44	57º00.06´N, 26º80.44´E	40, Sb	*Oxalidosa turf. mel.*	3; 2	Fen peat soils ^
I, Piebalga 1	45, 46	56º66.95´N, 24º33.70´E	40, Sp	*Myrtilliosa mel.*	1; 0	Podzolic-gley soils ^
I, Piebalga 2	47, 48	56º65.88´N, 24º33.46´E	45, S	*Myrtilliosa mel.*	1; 0	Podzolic-gley soils ^
II, Kalsnava	KR; KH	56º67.66´N 25º89.75´ E	41, Sb	*Myrtilliosa. turf. mel.*	Heterobasidon infected; Healty	^
II, Ogre	OR; OH	56º85.42´N 24º79.86´ E	44, Sb	*Oxalidosa turf. mel.*	Heterobasidon infected; Healty	^
II, Strautiņi	SR; SH	56º70.17´N 25º90.55´ E	67, Sb	*Oxalidosa turf. mel.*	Heterobasidon infected; Healty	^
II, Misa	MR; MH	56º77.47´N 24º13.36´ E	112, Sp	*Myrtilliosa turf. mel.*	Heterobasidon infected; Healty	^
III, A	AF, AC	56º71.30´ N; 25º86.00´ E	101, 76 Ps	*Vacciniosa turf. mel.*	n.a.	^
III, B	BF, BC	56º70.58´ N; 25º86.83´ E	76, 125, Sp	*Myrtillosa turf. mel.*	n.a.	^
III, C	CF, CC	56º70.69´ N; 25º87.02´ E	76, 119, SP	*Myrtillosa turf. mel. / Caricoso-phragmitosa*	n.a.	^
IV, MS	MSS, MSC	56°85.15' N; 25°65.70' E	100 (clear cut), Ps	*Myrtilloso-sphagnosa*	n.a.	
IV, H	HS, HC	56°89.29' N; 24°68.06' E	80 (clear cut), Spb	*Hylocomiosa*	n.a.	
IV, MM	MMS, MMC	56°88.18' N 24°66.85' E	80 (clear cut), Spb	*Myrtilliosa mel.*	n.a.	Mineral soil; ^
VI	n.a.	56˚84.19' N 23˚77.43' E	n.a. S	*Oxalido-myrtilliosa*	n.a.	Mineral soil; ^

* I – Foliar damage study; II – Root-rot study; III – Fertilization study; IV – Stump removal study; VI – Cultivation system study.

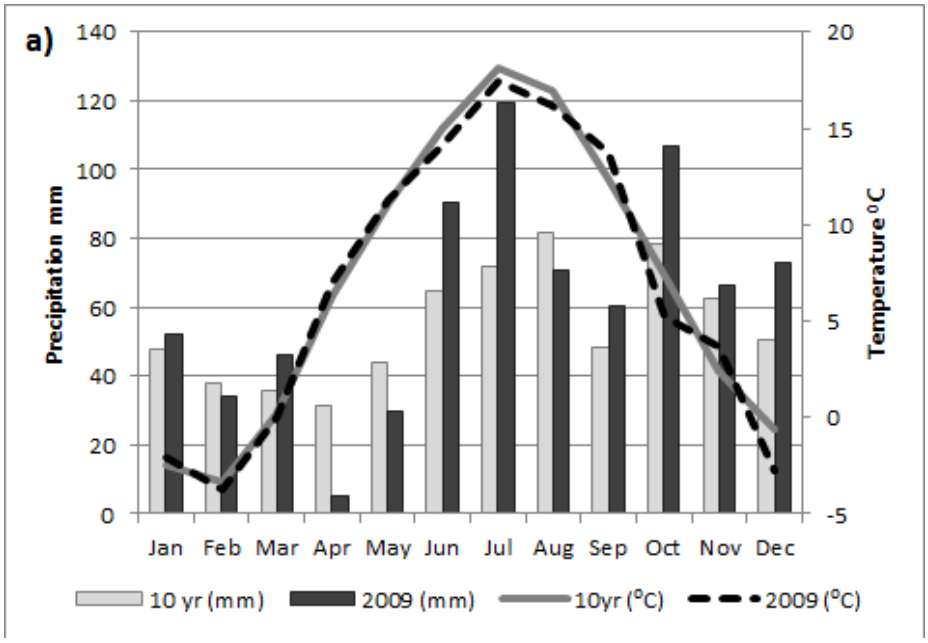
**Letters indicates tree species in stands: capital letter – dominant species; small letter – other trees (s – Norway spruce; p – Scots pine; b – silver birch; a – black alder; g – grey alder).

*** For Foliar damage study data of foliar damage class of each sample plot is given (0-healthy… 4 – all trees dead) in two sample plots (severely and minimally damaged).

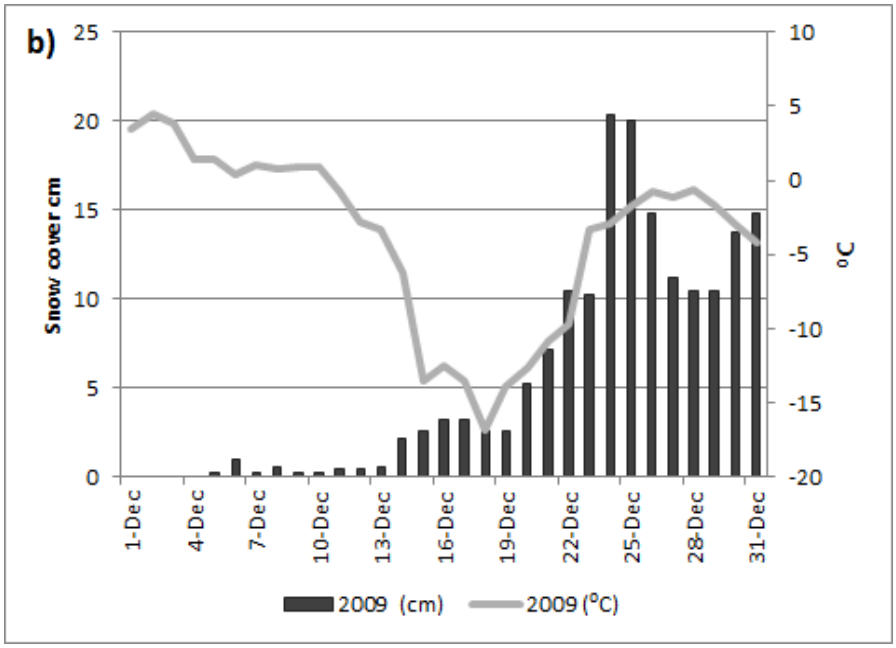
Appendix 2. Description of seedling material used in experiments

Study, Abbreviation	Nursery, cultivation system	Seed origin	Age at the time of root sampling
IV, n.a.	Norupe, conatinerized	n.d.	2.5
V, W1	Kalsnava, combined uncommercial method	Western (Saldus forest district, Sesile forestry)	6
V, W2	Kalsnava, combined uncommercial method	Western (Seed orchard Remte, Saldus forest district, Remte forestry)	6
V, C1	Kalsnava, combined uncommercial method	Central (Cesis forest district, Zaube forestry)	6
V, C2	Kalsnava, combined uncommercial method	Central (Jekabpils forest district, Briezi forestry)	6
V, E1	Kalsnava, combined uncommercial method	Eastern (Ludza forest district, Ludza forestry)	6
VI, SC2006, 2007, 2008, 2009	Strenči, containerized	Central (Seed orchard Suntazi, Ogre forest district)	2, 3, 4, 5
VI, SB2006, 2007, 2008, 2009	Strenči, bare-rooted	Eastern (Rezekne region)	4, 5, 6, 7

Appendix 3. Meteorological data for Foliar damage study



a) Monthly precipitation and temperature of Latvia in 2009 and 10-year mean values (mean data from seven meteorological stations).



b) Snow cover and soil surface temperature in December 2009 in Latvia (mean data from five meteorological stations).

Appendix 4. List of fungal species identified on Norway spruce root tips

	Taxa	**GenBank accession No.**	**Study ***	**Site**
	Ascomycetes			
Acephala macrosclerotiorum SH204989.07FU	*Acephala macrosclerotiorum*	KF954060	IV	All (SC)
Archaeorhizomycetales | SH004211.07FU	Unidentified S2	JX907826	VI	C2006
Ascomycota | SH198656.07FU	*Trichocladium opacum*	KP753370	I	6
Ascomycota | SH214267.07FU	*Helotiaceae* sp.	KP753310	I	3-4, 13, 24-27, 30, 35, 40-41, 46
	Unid. Ascomycete	KR019874	II	MR, OH, KH
	Ascomycota sp.	KR019781	III	AC (F)
	Rhizoscyphus ericae	KP753348	I	17, 48
Ascomycota | SH214904.07FU	*Paecilomyces carneus*	KP753337	I	20
Cadophora finlandica | SH214265.07FU	*Cadophora finlandica*	KP753341	I	5
	Cadophora finlandica	KR019782	III	AC (FC)
	Helotiales sp.	KR019786	III	B (C)
	Cadophora sp.	KF954063	IV	H, MS (SC)
	Meliniomyces sp.	KF954071	IV	H, MS (SC)
	Meliniomyces bicolor	KP172305	V	
Cenococcum | SH214459.07FU	*Cenococcum geophilum*	KP753297	I	19, 41, 47
	Cenococcum sp.	KR019868	II	M
	Cenoccocum sp.	KR019783 KR019784	III	ABC (Cf)
Cenococcum | SH199613.07FU	*Cenococcum geophilum*	KF954064	IV	All (SC)
Chalara holubovae | SH202699.07FU	*Cadophora* sp.	KP753296	I	7, 43, 48
Elaphomyces | SH215736.07FU	*Elaphomyces* sp.	KP753307	I	14, 16, 34
	Elaphomyces muricatus	KR019869	II	MR
	Elaphomyces sp.	KR019785	III	A (C)
Elaphomyces | SH215737.07FU	*Elaphomyces* sp.	KF954066	IV	H (S)
Helotiales | SH181532.07FU	*Helotiales* sp.	KP753311	I	10, 19, 25-26, 32, 34, 46
Helotiales | SH201700.07FU	Uncult. *Helotiales*	KP753377	I	16, 24
	Helotiales sp.	KR019871	II	M

	Unidentified sp. A	KF954077	IV	H (C)
Helotiales | SH211718.07FU	Uncult. Ascomycete	KP753376	I	18
Helvella elastica | SH005571.07FU	*Helvella elastica*	KR019787	III	B (F)
Helvella lacunosa | SH467563.07FU	*Helvella* sp.	KR019788	III	A (F)
Helvella | SH024288.07FU	*Pezizales* sp.	KP753339	I	16
Herpotrichiellaceae | SH217841.07FU	*Chaetothyriales* sp.	KP753375	I	44
Humaria hemisphaerica | SH179622.07FU	*Humaria hemisphaerica*	KP753312	I	38, 40, 43
	Humaria hemisphaerica	KR019872	II	MH
Hydnotrya cerebriformis (as Hydnobolites cerebriformis) | SH207912.07FU	*Hydnobolites cerebriformis*	KR019789	III	C (F)
Hydnotrya | SH218048.07FU	*Hydnotrya bailii*	KP753313	I	15
	Hydnotrya bailii	KF954069	IV	MS (C)
Hypocrea pilulifera | SH177682.07FU	*Hypocrea* sp.	KR019790	III	A (F)
Ilyonectria mors-panacis | SH202967.07FU	*Ilyonectria* sp.	KR019870	II	S
	Ilyonectria radicicola	KF954082	IV	H (C)
Leotia | SH196168.07FU	*Leotia lubrica*	KP753327	I	18
Leotiomycetes | SH181080.07FU	*Meliniomyces bicolor*	KP753332	I	8-9, 34, 39, 45, 47
Cadophora finlandica | SH214265.07FU	*Meliniomyces bicolor*	KR019791	III	C (C)
	Meliniomyces bicolor	KF954070	IV	All (SC)
	Cadophora finlandica	JX907811	VI	B2006
Leptodontidium | SH203226.07FU / SH205752.07FU	*Leptodontidium elatius*	KP753328	I	35
Meliniomyces variabilis | SH181078.07FU	*Meliniomyces variabilis*	KP753330	I	3-4, 24, 46-48
	Meliniomyces variabilis	KF954084	IV	MS (S)
Meliniomyces | SH181085.07FU	*Meliniomyces vraolstadiae*	KP753331	I	17, 23
Oidiodendron maius | SH216987.07FU	*Oidiodendron maius*	KP753335	I	1-2, 4-6, 9-12, 14, 20-22, 25-26, 34-36, 38, 41-42, 46
Otidea leporina | SH218038.07FU	*Otidea leporina*	KR019792	III	AC (Cf)
Otidea tuomikoskii | SH196383.07FU	*Otidea tuomikoskii*	KR019793	III	C (C)
Pezizaceae | SH193335.07FU	*Pachyphloeus conglomeratus*	KP753336	I	29-30
Phialocephala fortinii | SH204986.07FU	*Phialocephala fortinii*	KP753340	I	2, 9-12, 24, 26, 37, 44, 48

	Phialocephala fortinii	KF954086	IV	All (SC)
Pseudogymnoascus roseus | SH183329.07FU	*Geomyces* sp.	KP753309	I	2, 38
Pyronemataceae | SH194157.07FU	*Wilcoxina* sp.	KP753378	I	11-12, 34, 37, 39, 43
	Pyronemataceae sp.	KR019873	II	KH
	Pyronemataceae sp.	KR019795	III	BC (F)
	Wilcoxina sp.1	KF954079	IV	All (SC)
	Wilcoxina sp.2	KF954080	IV	All (SC)
	Wilcoxina sp.	KP172310	V	
Pyronemataceae | SH194158.07FU	*Pyronemataceae* sp.2	KR019794	III	C (F)
Rhizosphaera oudemansii | SH206397.07FU	*Rhizosphaera kalkhoffii*	KP753349	I	14, 31
Tuber anniae | SH202491.07FU	*Tuber* sp.1	KP753371	I	12
	Tuber anniae	KR019796	III	BC (F)
Tuber | SH185373.07FU	*Tuber* sp.2	KP753372	I	40
	Tuber sp.	KF954076	IV	MS (C)
Tuber | SH202492.07FU	*Tuber* sp.	KR019797	III	AB (F)
	Tuber sp.	KP172308	V	
Wilcoxina mikolae | SH194156.07FU	*Wilcoxina mikolae*	KF954078	IV	H (C)
	Wilcoxina mikolae	KP172311	V	
Wilcoxina rehmii | SH211927.07FU	*Wilcoxina rehmii*	JX907827	VI	B2007, C2007,2008
	Basidiomycetes			
Agaricales | SH179278.07FU	*Laccaria proxima*	JX907813	VI	B2006
Agaricales | SH223367.07FU	*Mycena* sp.	KP753333	I	16
Agaricomycetes | SH182081.07FU	*Amphinema* sp. 2	KR019836	II	M
Agaricomycetes | SH197944.07FU	*Amphinema* sp. 1	KF954062	IV	All (SC)
Amanita citrina | SH221026.07FU	*Amanita citrina*	KR019832	II	OH
Amanita muscaria | SH179028.07FU	*Amanita muscaria*	KP753291	I	1
Amanita porphyria | SH221019.07FU	*Amanita porphyria*	KR019833	II	MR
Amanita | SH221018.07FU	*Amanita spissa*	KP753292	I	5
Amphinema byssoides | SH197943.07FU	*Amphinema byssoides*	KP753293	I	20, 41, 43, 48
	Amphinema byssoides	KR019834	II	All sites
	Amphinema byssoides	KR019798	III	ABC (Fc)
	Amphinema byssoides	KF954061	IV	All (SC)
	Amphinema byssoides	KP172303	V	

	Amphinema sp.	KP172304	V	
	Amphinema byssoides	JX907809	VI	All (B,C); All time points
Amphinema | SH197945.07FU	*Amphinema* sp.	KP753294	I	11, 27, 30, 35
	Amphinema sp. 1	KR019835	II	S
	Amphinema sp. 2	KF954081	IV	MS (C)
	Amphinema sp.1	KR019800	III	BC (F)
	Amphinema sp.	JX907810	VI	B2008,C2008
Amphinema | SH199593.07FU	*Amphinema* sp. 2	KR019799	III	B (F)
Boletus badius (as Xerocomus badius) | SH216653.07FU	*Xerocomus badius*	KP753379	I	16, 47
Boletus edulis | SH190423.07FU	*Boletus edulis*	KP753295	I	22
Boletaceae | SH218065.07FU	*Xerocomus ferrugineus*	KR019867	II	OH
Byssocorticium | SH216107.07FU	*Byssocorticium* sp.	KR019837	II	OH
Clavulinaceae | SH184883.07FU	*Clavulina* sp.1	KP753298	I	30, 40
	Clavulina sp.	KR019838	II	OH
Clavulinaceae | SH220237.07FU	*Clavulina* sp.2	KP753299	I	10
Clavulina | SH220217.07FU	*Clavulina* sp.3	KP753300	I	32
	Clavulina sp.	KF954065	IV	H, MS (C)
Cortinarius camphoratus | SH222418.07FU	*Cortinarius camphoratus*	KP753301	I	17-18
Cortinarius casimiri | SH188471.07FU	*Cortinarius casimiri*	KR019802	III	AC (CF)
Cortinarius cinnamomeus | SH222334.07FU	*Cortinarius cinnamomeus*	KR019840	II	M
Cortinarius diasemospermus var. leptospermus | SH188470.07FU	*Cortinarius* sp.2	KR019805	III	A (Cf)
Cortinarius hemitrichus | SH188473.07FU	*Cortinarius sp.*	KR019839	II	M
	Cortinarius sp.4	KR019806	III	B (C)
Cortinarius sanguineus | SH212097.07FU	*Cortinarius sanguineus*	KR019803	III	A (C)
Cortinarius | SH188620.07FU	*Cortinarius praestigiosus*	KP753302	I	9-10
Cortinarius | SH188489.07FU	*Cortinarius* sp.	KP753303	I	16, 19
Cortinarius | SH188533.07FU	*Cortinarius* sp.1	KR019804	III	A (F)
Cortinarius | SH188480.07FU	*Cortinarius* sp.3	KR019801	III	A (C)
Cryptococcus terricola | SH190017.07FU	*Cryptococcus terricola*	KP753305	I	13, 37
Cryptococcus victoriae |	*Cryptococcus victoriae*	KP753306	I	12

SH181628.07FU				
Filobasidiales | SH197623.07FU	*Cryptococcus magnus*	KP753304	I	1-2, 6, 9-10, 12-15, 18, 21, 24-25, 27-31, 34, 36-43, 48
	Cryptococcus sp.	KR019841	II	SH, SR
	Cryptococcus magnus	KF954092	IV	H (S)
Flagelloscypha minutissima | SH182705.07FU	*Flagelloscypha minutissima*	KP753308	I	26
Hebeloma leucosarx (as Hebeloma velutipes) | SH215995.07FU	*Hebeloma leucosarx*	KR019807	III	A (F)
	Hebeloma sp.	KF954068	IV	H, MS (C)
Helvellosebacina helvelloides | SH214500.07FU	*Sebacina epigaea*	KP753358	I	28
	Sebacina epigaea	KR019857	II	OH
Hygrophorus olivaceoalbus | SH212614.07FU	*Hygrophorus olivaceoalbus*	KR019842	II	MH
Hygrophorus pustulatus | SH202868.07FU	*Hygrophorus pustulatus*	KP753314	I	5, 25, 33, 45
	Hygrophorus pustulatus	KR019843	II	OH, SH, SR
Inocybe flocculosa | SH187316.07FU	*Inocybe nitidiuscula*	KP753317	I	29-30, 35
	Inocybe nitidiuscula	KR019844	II	OR, SR, SH
	Inocybe nitidiuscula	KR019809	III	B (F)
Inocybe flocculosa | SH178660.07FU	*Inocybe flocculosa*	KR019808	III	A (F)
Inocybe lilacina | SH220309.07FU	*Inocybe geophylla var. lilacina*	KP753315	I	14
Inocybe napipes | SH196058.07FU	*Inocybe napipes*	KP753316	I	3, 23, 46
	Inocybe napipes	KF954083	IV	MS (S)
Inocybe ochroalba | SH220483.07FU	*Inocybe ochroalba*	KR019810	III	A (F)
Inocybe proximella | SH194466.07FU	*Inocybe proximella*	KP753318	I	27
	Inocybe proximella	KR019845	II	OR
	Inocybe proximella	KR019811	III	C (F)
Inocybe relicina | SH191846.07FU	*Inocybe relicina*	KP753319	I	24
	Inocybe relicina	KR019846	II	MR
Inocybe umbratica | SH191101.07FU	*Inocybe umbratica*	KP753320	I	7
Lactarius badiosanguineus | SH182388.07FU	*Lactarius badiosanguineus*	KR019812	III	B (C)
Lactarius camphoratus | SH220116.07FU	*Lactarius camphoratus*	KP753321	I	9, 21, 24
Lactarius helvus | SH214492.07FU	*Lactarius helvus*	KP753323	I	23
Lactarius necator | SH176406.07FU	*Lactarius necator*	KP753324	I	14-15, 17, 19, 23-24, 33, 47
	Lactarius necator	KR019847	II	S

Lactarius rufus | SH182377.07FU	*Lactarius rufus*	KP753325	I	17-18, 32
Lactarius torminosus (as Lactarius intermedius) | SH220109.07FU	*Lactarius* sp.	KR019848	II	OH
Lactarius tabidus | SH182379.07FU	*Lactarius tabidus*	KP753326	I	4, 6, 20, 25, 30, 37-38, 41, 43-44
	Lactarius tabidus	KR019849	II	MH, KR
	Lactarius tabidus	KR019813	III	ABC (Cf)
Lactarius trivialis | SH176405.07FU	*Lactarius trivialis*	KR019814	III	A (C)
Lactarius | SH220113.07FU	*Lactarius deterrimus*	KP753322	I	14, 21-22, 27
Leucosporidiella creatinivora | SH193763.07FU	*Leucosporidium* sp.	KP753329	I	30
Naucoria | SH188455.07FU	*Naucoria bohemica*	KP753334	I	10
Paxillus involutus SH210480.07FU	*Paxillus involutus*	KP753338	I	9, 33
	Paxillus involutus	KF954085	IV	MS (SC)
Phlebiopsis gigantea SH198804.07FU	*Phlebiopsis gigantea*	JX907814	VI	B2008
Piloderma fallax | SH203892.07FU	*Piloderma fallax*	KR019850	II	MH
Piloderma olivaceum | SH203891.07FU	*Piloderma olivaceum*	KP753343	I	14, 19
	Piloderma olivaceum	KR019851	II	MR
Piloderma sphaerosporum | SH196824.07FU	*Piloderma sphaerosporum*	KP753344	I	9, 17, 47
Piloderma | SH208787.07FU	*Piloderma byssinum*	KP753342	I	39-40
	Piloderma byssinum	KR019852	II	M
Piloderma | SH196704.07FU	*Piloderma* sp.	KP753345	I	7, 30, 38, 42
Piloderma SH196706.07FU	*Piloderma* sp.	JX907815	VI	B2007
Pseudotomentella mucidula | SH223399.07FU	*Pseudotomentella mucidula*	KP753346	I	3, 6, 9, 12-13, 17, 25, 45-46
	Pseudotomentella mucidula	KR019853	II	M
Pseudotomentella rhizopunctata | SH182252.07FU	*Pseudotomentella rhizopunctata*	KR019815	III	A (F)
Pucciniales | SH212621.07FU	*Pucciniales*	KR019816	III	B (C)
Russula aquosa | SH186210.07FU	*Russula aquosa*	KP753351	I	32
Russula emetica | SH218421.07FU	*Russula atrorubens*	KP753352	I	9, 40, 46
	Russula sp.	KR019817	III	A (C)
	Russula betularum	KF954087	IV	H (C)
	Russula densifolia	KF954088	IV	H (S)

Russula firmula SH190327.07FU	*Russula firmula*	KR019854	II	KH, OR
	Russula firmula	KF954089	IV	H (C)
Russula ochroleuca | SH190469.07FU	*Russula ochroleuca*	KP753353	I	34, 48
	Russula ochroleuca	KR019855	II	MH, MR
Russula puellaris | SH187352.07FU	*Russula puellaris*	KP753354	I	16
Russula sapinea | SH187353.07FU	*Russula sapinea*	KP753355	I	7, 38, 43-44
	Russula sapinea	KR019856	II	KH
	Russula sapinea	KR019818	III	C (C)
Russula | SH190322.07FU	*Russula velenovskyi*	KP753356	I	46, 48
	Russula velenovskyi	KR019819	III	A (C)
	Russula velenovskyi	KF954072	IV	MS (C)
Russula | SH219260.07FU	*Russula adusta*	KP753350	I	21
Sebacina | SH197124.07FU	*Sebacina dimitica*	KP753357	I	27
Sebacina | SH197126.07FU	*Sebacina* sp.	KR019820	III	BC (F)
Sebacina | SH214635.07FU	*Sebacina* sp.2	KR019821	III	C (C)
Sebacina | SH197129.07FU	*Sebacina* sp.3	KR019822	III	C (C)
SH180003.07FU	*Sebacina* sp.	KF954073	IV	H (S)
Thelephora palmata | SH209104.07FU	*Thelephora palmata*	KP753359	I	35, 37, 46
	Thelephora palmata	KR019858	II	SH, SR
Thelephoraceae | SH010097.07FU	*Thelephoraceae* sp.	KP753361	I	38
Thelephoraceae | SH184509.07FU	*Tomentella sublilacina*	KP753368	I	21, 38-39
	Tomentella sublilacina	KR019862	II	M
	Thelephoraceae sp.1	KR019823	III	A (C)
Thelephoraceae | SH184510.07FU	*Thelephora terrestris*	KP753360	I	24
	Thelephora terrestris	KP172306	V	
	Thelephora terrestris	KF954074	IV	All (SC)
	Thelephora terrestris	JX907820	VI	BC 2006, C2007
Thelephoraceae | SH189513.07FU	*Thelephoraceae* sp.2	KR019824	III	C (C)
Thelephoraceae | SH177844.07FU	*Thelephoraceae* sp.3	KR019825	III	C (F)
Thelephoraceae | SH189355.07FU	*Tomentella* sp.	KP172307	V	
Thelephoraceae | SH177802.07FU	*Tomentella lateritia*	KP753364	I	13, 24, 30
Tomentella bryophila | SH177792.07FU	*Tomentella bryophila*	KP753362	I	9, 20
Tomentella cinerascens | SH189368.07FU	*Tomentella cinerascens*	KR019859	II	OR

Tomentella coerulea | SH177784.07FU	*Tomentella coerulea*	KR019826	III	AC (F)
Tomentella stuposa | SH177785.07FU	*Tomentella bresadolae*	KP753365	I	28
Tomentella | SH177789.07FU Tomentella stuposa | SH177783.07FU	*Tomentella stuposa*	KP753366 KP753367	I	11, 14-16, 21, 29, 37, 40, 42
Tomentella stuposa | SH177783.07FU	*Tomentella stuposa*	KR019860	II	MH
Tomentella | SH177789.07FU	*Tomentella stuposa*	KR019861	II	O, S
	Tomentella stuposa	KR019829	III	AB (Cf)
	Tomentella stuposa	KF954075	IV	MS (S)
Tomentella atramentaria | SH177791.07FU	*Tomentella* sp.	KP753363	I	23, 26
Tomentella | SH189367.07FU	*Tomentella* sp.1	KR019827	III	AC (Fc)
Tomentella | SH177803.07FU	*Tomentella* sp.2	KR019828	III	C (F)
Tomentellopsis echinospora | SH184845.07FU	*Tomentellopsis echinospora*	KP753369	I	13-15, 20
Trichosporon dehoogii | SH196641.07FU	*Trichosporon* sp.	KR019863	II	OH
Tylopilus | SH217443.07FU	*Tylopilus felleus*	KR019864	II	MH, MR
Tylospora SH197766.07FU	*Tylospora asterophora*	KP753373	I	1, 4, 8, 10-12, 14-16, 22, 28, 31, 33-34, 36, 41-42, 44-46
	Tylospora asterophora	KR019865	II	S, K, OH
	Tylospora asterophora	KR019830	III	ABC (Cf)
	Tylospora asterophora	KF954090	IV	H (C)
	Tylospora asterophora	KP172309	V	
	Tylospora asterophora	JX907821	VI	B2006
Tylospora SH213927.07FU	*Tylospora fibrillosa*	KP753374	I	17, 32-33, 47-48
	Tylospora fibrillosa	KR019866	II	M, OH
	Tylospora fibrillosa	KR019831	III	AB (C)
	Tylospora fibrillosa	KF954091	IV	MS, MM (SC)
Tylospora | SH192266.07FU	Unidentified S1	JX907825	VI	C2008
Fungi SH009124.07FU	Unidentified sp. B	KF954067	IV	MS (S)

* Numbers refer different study (I – Foliar damage study; II – Root-rot study; III – Fertilization study; IV – Stump removal study; V – Seed origin study; VI – Cultivation system study).

Appendix 5. Amounts of different chemical elements in needles of Picea abies *and soil after the first growing season (2012) in* experimental sites of Study IV.

Chemical element	***Hylocomiosa* (H)**		***Myrtillioso-sphagnosa* (MS)**		***Myrtilliosa mel.*(MM)**	
	Stump removal	**Trenching**	**Stump removal**	**Trenching**	**Stump removal**	**Trenching**
Needles						
Total N, g/kg	22.5±0.5	21.5±0.5	23.0±1.0	21.0±3.0	25.0	23.0
Total C, g/kg	529.5±1.5	551.0±12.0	532.0±9.0	554.0±6.0	540.0	549.0
S, g/kg	1.15±0.1	0.9±0.1	1.05±0.1	0.95±0.1	1.0	0.9
Total P, g/kg	1.3±0.1	1.2±0.1	1.3±0.1	1.2±0.1	1.4	1.2
Ca, g/kg	4.4±0.5	3.7±0.1	3.4±0.2	3.4±0.7	3.4	3.4
K, g/kg	6.5±0.2	6.2±0.1	7.3±0.3	6.6±0.4	7.3	5.9
Mg, g/kg	1.8±0.1	1.8±0.1	1.7±0.1	1.6±0.1	1.5	1.6
Mn, g/kg	0.5±0.1	0.25±0.1	0.3±0.1	0.25±0.1	0.4	0.4
Fe, g/kg	0.4±0.2	0.35±0.1	0.3±0.1	0.3±0.1	0.3	0.4
Soil						
Absolute moisture, %	3.1±0.1	4.1±2.4	6.2±2.2	7.3±2.4	2.0	3.3
pH(KCl)	3.5±0.1	4.0±0.3	3.0±0.1	3.1±0.3	3.2	3.3
pH (H_2O)	4.0±0.1	4.3±0.2	3.6±0.3	3.8±0.2	4.0	3.8
EC, μS cm-1	288.5±36.5	262.0±74.0	331.0±11.0	365.0±104.0	173	380
Total N, g/kg	3.2±0.3	2.8±1.3	5.1±1.8	5.8±1.4	2.3	3.7
Total C, g/kg	84.5±12.5	72.0±20.0	147.0±84.0	147.5±55.5	156.0	337.0
Total S, mg/kg	210.0±12.0	155.5±55.5	346.0±220.0	314.0±106.0	357.0	744.0

P, mg/kg	101.5±57.5	127.5±33.5	49.5±4.5	44.5±15.5	21.0	39.0
K, mg/kg	67.0±11.0	66.0±6.0	133.5±56.5	122.5±3.5	139.0	196.0
Mn, mg/kg	29.4±9.8	54.8±28.0	7.3±1.2	29.0±23.9	15.9	13.2
Mg, g/kg	0.12±0.1	0.19±0.1	0.17±0.1	0.17±0.04	0.2	0.3
Ca, g/kg	0.48±0.2	1.29±1.2	1.0±0.7	1.0±0.7	0.7	0.8
Zn, mg/kg	6.6±0.2	4.5±0.6	9.3±2.8	4.7±1.8	14.1	15.2

Note: Numbers show mean ±SE. In MM, only one replicate was available.