JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
200

Anne Pietikäinen

# Arbuscular Mycorrhiza, Resource Availability and Belowground Interactions between Plants and Soil Microbes

JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Villa Ranan Paulaharjun salissa
huhtikuun 24. päivänä 2009 kello 12.

Academic dissertation to be publicly discussed, by permission of
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2009

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Publishing Unit, University Library of Jyväskylä

Jyväskylä Studies in Biological and Environmental Science


Jehkas meadow and experimental plots in June 2002. Photo by Mikko Kursula

URN:ISBN:978-951-39-3555-9
ISBN 978-951-39-3555-9 (PDF)

ISBN 978-951-39-3514-6 (nid.)
ISSN 1456-9701



Jyväskylä University Printing House, Jyväskylä 2009

# ABSTRACT

Pietikäinen, Anne
Arbuscular mycorrhiza, resource availability and belowground interactions between plants and soil microbes
Jyväskylä: University of Jyväskylä, 2009, 38 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 200)
ISBN 978-951-39-3555-9 (PDF), 978-951-39-3514-6 (nid.)
Yhteenveto: Arbuskelimykorritsa, resurssien saatavuus ja maanalaiset kasvien ja mikrobien väliset vuorovaikutukset
Diss.

Most vascular plants house the Glomeromycotan fungi that form arbuscular mycorrhizal (AM) symbioses in their roots. This symbiosis is usually considered mutualistic. The AM fungus relies on the carbon provided by its plant host and in return the AM fungus can improve plant nutrients and water acquisition and provide protection from pathogens. By affecting the plant growth and resource allocation AM fungi may affect the interactions between plants, the plant community composition and the soil microbial community dependent on the plant derived carbon. I studied the interactions between plants and soil microbial community focusing on the symbiotic relationship between plants and arbuscular mycorrhizal fungi in low arctic meadow ecosystem. I found that changes in resource availability caused by defoliation and fertilization did not have significant effect on functioning of AM symbiosis on the basis of the AM fungal colonization rate in plant roots in low arctic meadow. It seems that other factors than resource availability are more important determining the AM fungal colonization rate in natural conditions. I found no evidence of mycorrhiza mediated facilitative interactions between seedlings and adult plants and the results of this thesis emphasize the importance of below-ground competition in seedling establishment. Furthermore, I showed that defoliation of the neighboring adult plant may improve seedling establishment by decreasing the competition for mycorrhiza mediated resources. In the low arctic meadow, the AM fungal diversity was not affected by short term changes in plant community composition. Functional AM fungal and saprophytic microbial community necessary for successful seedling establishment persisted in the soil for two years without vegetation cover. In general, the functioning AM symbiosis in low arctic meadow seems not to be markedly different from what is known about AM symbiosis in other ecosystems.

Keywords: Arbuscular mycorrhiza; competition; low-arctic meadow; plant community; seedlings; soil microbial community.

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## LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-V.

I am the first author in papers I-IV and I have done large part of the experimental work, analyses and writing in these papers. I have contributed significantly to the field work and laboratory analyses and participated as a co-author to the preparation of the manuscript in paper V.

I Pietikäinen, A., Kytöviita, M.-M. & Vuoti, U. 2005. Mycorrhiza and seedling establishment in a subarctic meadow: Effects of fertilization and defoliation. Journal of Vegetation Science 16: 175-182.

II Pietikäinen, A. & Kytöviita, M.-M. 2007. Defoliation changes mycorrhizal benefit and competitive interactions between seedlings and adult plants. Journal of Ecology 95: 639–647.

III Pietikäinen, A., Kytöviita M.-M., Husband, R. & Young, P. 2007. Diversity and persistence of arbuscular mycorrhizas in a low-Arctic meadow habitat. New Phytologist 176: 691-698.

IV Pietikäinen, A., Mikola, J., Vestberg, M. & Setälä, H. Defoliation effects on arbuscular mycorrhizal symbiosis and nematodes in soils with different nutrient availability. Manuscript.

V Kytöviita M.-M., Pietikäinen, A. & Fritze, H. Plant and soil responses to absence of plant cover and monoculturing in low arctic meadows. Manuscript.

# 1 INTRODUCTION

## 1.1 Symbiotic relationship between plant and arbuscular mycorrhizal fungi

Mycorrhiza can be defined as a symbiotic association between a fungus and plant root. In arbuscular mycorrhizal (AM) symbiosis the extramatrical hyphae grow in soil and from root to root. The hyphae enter into the plant cell producing dichotomously-branching invaginations called arbuscules and often, but not always, balloon-like structures called vesicles. Arbuscular mycorrhizal symbioses are globally distributed and most vascular plants house the Glomeromycotan fungi that form AM symbioses in their roots (Brundrett 2002). Globally, c. 150 species of AM fungi have been recognized on the basis of spore morphology (Morton & Benny 1990).

AM fungi are obligate symbionts that depend solely on plant carbon and they may consume up to 20% of total net photosynthetic carbon (Jakobsen & Rosendahl 1990). Thus, maintenance of AM fungi may pose a considerable carbon cost to the plant. In return, the fungi can improve plant nutrient and water acquisition (Marschner & Dell 1994, Augé 2001) and defend plant roots against pathogens (Newsham et al. 1995). AM fungi are considered particularly important in acquisition of P, but it has been reported to supply also N, K and some micronutrients (Marschner & Dell 1994).

The exact physiological mechanisms regulating resource exchange in AM symbioses are currently unknown (Fitter 2006). Experiments have shown that when production of photosynthates is reduced by defoliation or shading the root colonization rate by AM fungus is decreased (Tester et al. 1986, Gehring & Whitham 1994, Heinemeyer et al. 2003). Also it has been shown using axenic carrot root cultures that transfer of P by an AM fungus was affected by the C availability to the root (Bücking & Shachar-Hill 2005). These findings give indications that the nutrient acquisition by the plant is coupled with the carbon allocation to the AM fungus.

## 1.2 Quantifying mycorrhizal symbiosis: mycorrhizal benefit and colonization rate

Mycorrhizal symbiosis can be considered beneficial (mutualistic) to the plant when the net costs are less than the net benefits and parasitic when the net costs exceeds the net benefits (Johnson et al. 1997). The current methods for measuring the actual net costs and net benefits and the functioning of AM symbiosis are limited. The mycorrhizal effects on the plant host are often measured by comparing the biomass (or growth) of mycorrhizal and non-mycorrhizal plants growing in identical conditions. The biomass gain summarizes the nutritional benefits as well as the carbon costs of supporting the AM fungus over a period of time. However, with this approach, it is not possible to specify the gross costs and gross benefits of AM symbiosis to the plant host. Also, this method has limited use in nature where comparable non-mycorrhizal conditions are difficult to establish as the fungicides used for eliminating AM fungi can have non-target effects on other organisms (West et al. 1993) and fungicide effects can vary between AM fungal species (Schreiner & Bethlenfalvay 1997).

Another commonly used approach for measuring the functioning of AM symbiosis is percent root length colonized by AM fungal structures. Specific AM structures are associated with nutrient and C exchange between plant host and fungus. Arbuscules are believed to be the main sites of nutrient transfer to the host plant, whereas carbon may be absorbed also by the intraradical hyphae (Smith & Smith 1996). Thus, the frequency of the AM structures in the plant roots can be considered as indirect indicator of costs and benefits (Rillig & Allen 1998). However, the interpretation of percentage root length colonized is not straight forward. The observed AM fungal colonization rate depends on both the nutritional needs and root growth rate of the plant as well as the carbon and nutrients available to the AM fungal growth (Smith & Walker 1981, Allen 2001).

The mycorrhizal benefit and functioning of AM symbiosis is assessed in this thesis mainly based on two approaches: the root length colonized by AM fungi and the biomass benefit to the host plant. By this approach I focus on the benefits of improved nutrient acquisition to the host plant. However, the protection from root pathogens and improved water economy can be equally important and may be particularly vital in harsh natural conditions overriding the possible high carbon costs and low nutritional benefits.

## 1.3 AM fungi as mediators of competitive and facilitative interactions

Competition is one of the main biotic factors shaping plant communities. Much of the competition among plants takes place underground and below-ground

competition can reduce plant performance more than above-ground competition (Wilson 1988, Wilson & Tilman 1993). AM fungi have been shown to mediate belowground competition between plants (Hart et al. 2003, van der Heijden et al. 2003) and thus can potentially affect the plant community composition and diversity.

By increasing nutrient acquisition the AM fungi can improve the growth and fecundity of its host plant. However, these benefits may not be equal for all members of the plant community. Firstly, not all plants form mycorrhizal symbiosis. Such non-host plant species are common in families Brassicaceae, Caryophyllaceae, Chenopodiaceae and Polygonaceae. Experiments have shown that when non-host plant species compete with mycorrhizal plants the competitive balance is usually altered in favor of the host species by the AM fungus (Titus & del Moral 1998, van der Heijden et al. 1998b). Secondly, even when all members of the plant community are mycorrhizal, the benefits may not be equal for all host plants as there is increasing evidence of specificity in AM symbiosis. The mycorrhizal benefit to the plant host can depend on the particular combination of host plant species and AM fungal species or strain (van der Heijden et al. 1998a, Helgason et al. 2002, Munkvold et al. 2004). AM fungi can also affect the competition between conspecific plants. There are several studies showing that intraspecific competition is amplified by AM fungi (Allsopp & Stock 1992, Hartnett et al. 1993, Moora & Zobel 1996, Watkinson & Freckleton 1997, Facelli et al. 1999, but see Eissenstat & Newman 1990). Late-successional plant communities are typically intensively mycorrhizal (Allen & Allen 1990) and the effects of AM fungi on competitive interactions between plants may be particularly important in these ecosystems. There is increasing evidence from temperate and tropical ecosystems that AM fungal community may affect the plant community production and diversity (Gange et al. 1993, van der Heijden et al. 1998a, Vogelsang et al. 2006) and that the AM fungal community composition depends on the identity of the plant host (Husband et al. 2002a, Helgason et al. 2002, Vandenkoornhuyse et al. 2002).

AM fungi may also mediate facilitative interactions. The AM fungi can grow in the soil from root to root (Hirrel & Gerdemann 1979) and can also connect plants of different species (Heap & Newman 1980, Chiariello et al. 1982, Francis & Read 1984) thus forming a common mycorrhizal network (CMN). In contrast to the general view that plants compete for resources, it has been proposed that plants may provide support to neighboring plants connected by CMN. This CMN mediated net benefit is suggested to be generated by direct carbon or nutrient transfer from one plant to the other (Whittingham & Read 1982, Grime et al. 1987), but experiments have not provided strong evidence that significant net transfer of carbon or nutrients occurs from plant to plant (Francis & Read 1984, Watkins et al. 1996, Graves et al. 1997, Fitter et al. 1998, Yao et al. 2003, Pfeffer et al. 2004). However, facilitative interactions may also take place without net transfer of resources in CMN. Simard & Durall (2004) postulated that seedlings growing in the vicinity of an established plant are colonized faster by AM fungi than seedlings growing alone. Furthermore, seedlings growing among the established plants may access nutrients using the

pre-existing fungal hyphal network and gain the nutritional benefits from AM symbiosis earlier.

## 1.4 Interactions between AM fungi and other soil microbes

The interactions between AM fungi and other soil microbes range from positive to negative. There are several mechanisms by which the soil saprophytic microbial community can be affected by AM fungi. Firstly, the AM fungi may affect the quality and quantity of carbon entering the soil food-web. As the AM fungus colonizes the plant roots, it improves the plant's nutrient uptake and changes the allocation patterns in plant. Thus, AM colonization is likely to alter the quality and quantity of plant litter (Langley & Hungate 2003) as well as the amount of root exudates entering the soil (Jones et al. 2004). Both root exudation and plant litter are significant sources of C for soil microbes (Tiessen et al. 1994, Kramer & Gleixner 2006). In experimental systems it has been shown that AM fungal colonization affects the soil bacterial community structure (Wamberg et al. 2003) and this effect may be at least partly plant mediated (Marschner & Baumann 2003).

The AM fungal hyphae constitute a considerable source of carbon for the soil microbes and fungal feeding fauna. The lengths of extraradical mycorrhizal mycelium of AM fungi typically range from 3-30 m g soil$^{-1}$ (Leake et al. 2004) and it has been estimated that AM fungi constitute over 50% of the fungal length in some soils (Rillig et al. 2002). In grasslands as much as 15% of the soil organic C pool may be contributed by AM fungi (Miller & Kling 2000). The results from experimental systems studying the effects of AM fungi on soil bacterial and fungal biomass are inconclusive. In an experiment by Olsson et al. (1998), the soil saprophytic fungi were negatively affected by AM mycelium and no effect was found on soil bacterial biomass, whereas Cheng & Baumgartner (2006) showed that AM fungal hyphae may be important in supporting other microbes within the mycorrhizosphere.

Soil microbes compete with plants for inorganic nutrients (Kaye & Hart 1997) and it has been suggested that AM fungal colonization may affect the soil microbial community by increasing the competitive ability of plants against the saprophytic rhizosphere microflora for common nutrients (Christensen & Jakobsen 1993). On the other hand, there is evidence of possibly mutualistic relationship between AM fungi and bacteria as the uptake of sparingly soluble P by external AM mycelium may be facilitated by P solubilizing bacteria (Villegas & Fortin 2001). These complex direct and plant mediated interactions make it difficult to predict the effect AM fungi on soil microbial community.

## 1.5 AM fungi in Arctic ecosystems

The Arctic can be defined as area around the north pole beyond the tree line. The Artic areas are characterized by low solar radiation, long cold winters and short cool summers. The decomposition and mineralization of organic matter is slow at low temperatures (Schmidt et al. 1999) and the primary production in Arctic ecosystems is typically nutrient limited (Haag 1974, Shaver & Chapin 1986, Jonasson et al. 1999). There is a general trend of declining species richness with increasing latitude (Hillebrand 2004) and the Arctic flora is generally quite species poor. The typical plant life form of arctic environments is an herbaceous perennial with relatively large root and/or rhizome system (Billings 1974). Shortness and severity of growing season sets limits to regeneration by seeds as seedlings have only few weeks to develop a root system and to produce enough carbohydrates to allow survival through winter. In some arctic areas, a considerable proportion of vegetation cover consists of plant species that do not reproduce vegetatively (Welling & Laine 2000) and therefore factors affecting seedling establishment have significant impact on plant community composition in these areas. The plant community composition and biomass production is often significantly affected by mammalian herbivores such as semi-domesticated reindeer and microtine rodents (Virtanen et al. 1997, Moen and Oksanen 1998, Grellmann 2002).

The AM associations are rare or non-existent in the high Arctic, but are commonly found in low-arctic and alpine ecosystems (Haselwandter & Read 1980, Read & Haselwandter 1981, Bledsoe et al. 1990, Blaschke 1991, Treu et al. 1996, Väre et al. 1997). There is evidence that the effects of AM on plant growth and nutrient acquisition depend on temperature resulting in a lower mycorrhizal benefit for the host plant in low temperatures (Ruotsalainen & Kytöviita 2004, Gavito et al. 2005, Kytöviita & Ruotsalainen 2007). This may have implications for the functioning of arbuscular mycorrhizal symbiosis in arctic conditions. Besides the AM fungi, a phylogenetically diverse fungal group belonging to Ascomycota referred to as dark septate (DS) fungi is found frequently colonizing plant roots in the Arctic (Ruotsalainen et al. 2002, Treu et al. 1996). However, their ecological role is still largely unknown (Jumpponen 2001).

## 1.6 Aims of the study

In this thesis, I studied the interactions between plants and soil microbial community focusing on the symbiotic relationship between plants and arbuscular mycorrhizal fungi in low arctic meadow ecosystem.

The first aim was to study how altering resource availability (fertilization and defoliation) affects the AM symbiosis and AM fungal colonization rate in plant roots. I manipulated plants' photosynthetic capacity and the nutritional

benefits of symbiosis by defoliation and fertilization in simplified systems in greenhouse and growth chamber conditions (II, IV) and in a field experiment (I).

The second aim was to study the role of AM fungus in plant-plant below-ground interactions - particularly between seedlings and adult plants. In a greenhouse experiment seedlings were grown alone and with non-defoliated or defoliated adult plant as a neighbor (II). In this experiment I tested i) does the AM affect the competitive balance between conspecific seedlings and adult plants and ii) does defoliation affect the competitive balance in mycorrhizal and non-mycorrhizal systems. Furthermore, the role of AM fungus in competitive and facilitative interactions between seedlings and adult plants were studied in field experiments where neighboring vegetation was subjected to defoliation (I) and where the composition and density of vegetation was experimentally manipulated (V).

The third aim was to study the effect of established plant community on the symbiotic AM fungal community found in seedling roots and the soil saprophytic microbial community. In a field experiment, plots without vegetation cover, plots with monoculture of single plant species and plots with natural vegetation were established (III, V). The manipulations were expected to affect the amount and quality of resources available to the soil symbiotic and saprophytic microbial community and thus result in changes in soil microbial community diversity and composition.

All the field experiments were carried out in a low-arctic meadow ecosystem. The knowledge of this ecosystem is quite limited and my fourth aim was to gain general information about seedling establishment and soil symbiotic and saprophytic microbial communities in low-arctic meadow ecosystem.

# 2 MATERIALS AND METHODS

## 2.1 Field experiments (I, III, V)

Two field experiments were carried out in low-Arctic meadow ecosystems at about 600 m above sea level in Kilpisjärvi area in north-western Finland. The growing season in these areas is about 90 days. The meadow vegetation consists mainly of perennial herbs and grasses. This area is grazed by reindeer in summers and the history of reindeer herding in this area can be dated back at least a few centuries. During the experiments the reindeer were excluded from the experimental plots by fences. The plant material (seeds and adult plants) used in the field experiments originated from the area surrounding the experimental plots.

The effect of plant community diversity and composition on soil symbiotic and saprophytic microbial community and seedling establishment was studied in two south facing meadows with similar vegetation located about 2.5 km apart (69°03′N, 20°50′E and 69°05′N, 20°47′E) (III, V). In 1999, nine experimental plots 3.5 m in diameter were established at each site and allocated to three treatments (control, monoculture and no-vegetation). In monoculture and no-vegetation treatments the vegetation cover was removed from the plots. The monoculture plots were revegetated with the perennial herb *Solidago virgaurea*. In the control plots the vegetation was left untouched. The plots were protected from natural seed rain from mid-August to early June every year by covering the plots with fine transparent mesh. In spring 2001, stratified seeds of three perennial herb species (*Solidago virgaurea*, *Alchemilla glomerulans* and *Potentilla crantzii*) were sown to the plots. The seedlings germinated from these seeds were sampled in 2001 (*S. virgaurea* only) and 2002 (all three species). Their shoots were collected for biomass analysis (V) and roots were sampled to determine AM fungal colonization rate (all three species) (V) and AM fungal community composition and diversity (*S. virgaurea* 2002 only) (III). Soil samples were collected in June and August in 2001 and 2002 from the experimental plots

and used for analyzing soil microbial community and soil nutrient content (V). In August 2005, a vegetation analysis was conducted in the control plots (III).

To explore which factors affect AM fungal colonization and seedling establishment a defoliation and fertilization experiment was performed in summer 2001 in a low arctic meadow (69°03′N, 20°50′E) close to the site described above (I). I used randomized complete block design with defoliation and fertilization as treatments. In total there were four treatment combinations: control, defoliated, fertilized and defoliated + fertilized. In June 2001, five blocks with similar vegetation were marked and in each block four plots 1.5 m in diameter were established and allocated to treatments according to the experimental design. Stratified seeds of two perennial herb species *Solidago virgaurea* and *Gnaphalium norvegicum* were sown on the plots. Plots were defoliated by clipping and fertilized with NPK-fertilizer twice during the experiment. In total, the fertilized plots received 8 g N, 2 g P and 8.6 g K per $m^2$ and these fertilization levels were selected based on other arctic fertilization experiments (eg. Press et al. 1998). Six weeks after sowing the seeds the number of seedlings was recorded and seedling shoots were collected for biomass and N concentration analyses. The natural vegetation in the experimental plots was sampled. The shoot N concentrations were analyzed from the seedlings and natural established vegetation. Root samples of the germinated seedlings and of mature individuals from three plant species (*Solidago virgaurea*, *Trollius europaeus*, *Deschampsia flexuosa*) growing in the plots were collected for analysis of AM fungal colonization rate.

## 2.2 Greenhouse experiment (II)

The role of AM fungus in below-ground interactions between adult plant and seedlings was studied in a greenhouse experiment (II). In this experiment seedlings of the perennial herb *Gnaphalium norvegicum* were grown with and without an arbuscular mycorrhizal fungus (G*lomus claroideum*), in the presence and in the absence of a conspecific adult plant. Each adult plant was assigned to one of the three defoliation treatments: no defoliation, 50% leaf area removed or 75% leaf area removed. In total there were eight different treatment combinations with 14 replicates in each. The adult plants were defoliated by clipping twice during the experiment: just after sowing the seeds and 3 weeks later. The final harvest was conducted 5 weeks after sowing the seeds when the seedlings were approximately 4 weeks old. The total biomass, shoot to root ratio, AM fungal colonization in the roots and the shoot nitrogen (N) concentration were measured in the seedlings and the adult plants. Shoot phosphorus (P) concentration was measured in the shoots of the adult. Ratio of mycorrhizal to non-mycorrhizal in terms of biomass and N content was used as a measure of mycorrhizal benefit. The index for below-ground competitive intensity (BCI; Cahill & Casper 2000) was calculated separately for the mycorrhizal and non-mycorrhizal seedlings.

## 2.3 Growth chamber experiment (IV)

In the growth chamber experiment, the effects of defoliation and fertilization on AM fungal colonization as well as plant biomass allocation patterns were studied in microcosms containing simplified decomposer community (IV). First 80 microcosms were established with sterilized soil. Natural soil microbes and nematodes were re-introduced to soil and one non-mycorrhizal seedling of the perennial herb *Plantago lanceolata* was planted in each microcosm. Plants were subjected to defoliation, fertilization and mycorrhizal inoculation treatments in a fully factorial experimental design. Mycorrhizal microcosms were inoculated with arbuscular mycorrhizal fungus *Glomus claroideum*. Defoliation treatment was performed twice and plants in fertilization treatment received on each watering occasion low concentration of full-nutrient-solution. At the final harvest, roots and shoots and inflorescence (if present) were harvested, dried and weighed. N concentration was measured in plant tissues. Nematodes were extracted from soil samples and AM fungal colonization rate was estimated in the plant root samples.

## 2.4 Laboratory analyses

Plant biomass was dried and measured as dry weight (I, II, IV, V). Nitrogen concentration was analyzed in dried and ground plant material using the dynamic flash combustion technique (CE Instruments EA 1110 Elemental Analyzers) (I, II, IV, V). Dried and milled plant shoots were used in analysis of P concentration (II). The P analysis was modified from the procedure described by John (1970) and phosphorus concentration was measured as absorbance at 882 nm (UV-160A; Shimadzu).

Root samples for analysis of AM fungal colonization rate were stored in ethanol and later stained with trypan blue (Phillips & Hayman 1970). Mycorrhizal root colonization rate was assessed under a light transmission microscope using the gridline intersection method (McGonigle et al. 1990) (I, II, V) or estimated visually (IV).

AM fungal community in the seedling roots (III) was analyzed using the terminal restriction fragment length polymorphism (T-RFLP) method (Liu et al. 1997). With this method AM fungal taxa are identified by the differences in their small sub-unit (SSU) rRNA (Helgason et al. 1999).

Soil microbial community composition (V) was analyzed using PLFA-method in which phospholipids were extracted from soil, subjected to mild alkaline methanolysis, and the fatty acid methyl esters were separated by gas chromatography (Hewlett Packard 5890) (Frostegård et al. 1993, modified by Pennanen et al. 1999).

Extractable ions (P, Ca, Mg, Mn, S, Fe, Al, Na) were analyzed by ICP-IRIS and dissolved organic carbon (DOC) and nitrogen (DON) with an Analytic

Jenan MULTI-NC analyzer from the soil samples (V). C, N, and C/N ratio were analyzed according to descriptions given in Tamminen and Starr (1990) except that total C and N were determined with a LECO CHN-1000 analyzer. Organic matter content was analyzed by loss-on-ignition at 550 $^{o}$C for 4 h and pH soil was measured in water.

Nematodes (IV) were extracted from soil using a wet funnel device (Sohlenius 1979). The number of nematodes was counted and 50 individuals of each sample were identified to genus and allocated into trophic groups according to Yeates et al. (1993).

## 2.5 Data analyses

Treatment effects on dependent variables were tested with factorial ANOVA. When a significant interaction between treatments was found, a one-factor ANOVA followed by a multiple comparisons test (Student-Newman-Keuls or Tukey's) was performed to compare the means in different treatment combinations. The homogeneity of variances was tested with Levene's test prior conducting ANOVA. The variables were log, square root or arcsin square-root transformed if necessary to meet the assumptions of ANOVA (normal distribution and homogeneity of variance). These statistical analyses were performed using SPSS for Windows (SPSS Inc.).

The significance of treatment and site effects and sampling time on the total amount of PLFA extracted, and the mole % of single or pooled signature PLFAs and bacterial to fungal ratios were analyzed with repeated measures ANOVA with time as within subject factor. The relative abundances of the 40 identified PLFAs were reduced to two principal components and the soil nutrient contents were reduced to one principal component and these were also subjected to repeated measures ANOVA.

Jaccard similarity coefficients were calculated for the T-RFLP patterns of the root samples, which were clustered by the unweighted pair-group average (UPGMA) algorithm, with 1000 bootstrap replicates to obtain confidence estimates. These calculations were performed using FREE TREE (Hampl et al. 2001).

# 3 RESULTS AND DISCUSSION

## 3.1 Resource availability and AM symbiosis

### 3.1.1 Effects of soil nutrient availability

I found that fertilization affected the functioning of AM symbiosis only in simplified conditions in the growth chamber, but not in field experiment in low arctic meadow on the basis of the AM fungal colonization rate. In the growth chamber experiment, fertilization increased the N concentration and total biomass production in *Plantago lanceolata* and AM colonization rate was decreased (IV). This result is well in line with several studies reporting a decrease in AM fungal colonization rate in response to fertilization (eg. Hetrick et al. 1990, Braunberger et al. 1991, Egerton-Warburton & Allen 2000). However, in the field experiment in the low arctic meadow fertilization did not decrease the AM colonization rate although the nutrient status in plants was improved on the basis of higher N content in shoots (I). I found also similar results in the two meadows with naturally different soil nutrient availability: the AM fungal colonization rate in seedling roots did not differ between the two low arctic meadows (V). The AM fungal colonization rate response to N and P fertilization can depend among other things on the amount of nutrients given as well as the initial soil nutrient status (Abbott et al. 1984, Sylvia & Neal 1990, Corkidi et al. 2002). Similar results with no effect of fertilization on AM fungal colonization rate has also been found in other ecosystems such as grass dominated prairie (Jumpponen et al. 2005), annual serpentine plant community (Koide et al. 1988) and in rainforest (Treseder & Allen 2002).

It is unclear if plant can eliminate the existing AM fungal colonization inside the roots. However, there is some new evidence of mechanisms by which the plant host may control the AM fungal colonization rate of new roots. Strigolactones released by the plant root induce hyphal branching and metabolic activity in AM fungus (Akiyama et al. 2005) and encourage formation of AM symbiosis. Recently it was shown that the synthesis of strigolactones is promoted by phosphate starvation (López-Ráez et al. 2008). Thus, plant may

control the AM fungal colonization depending on its nutrient status. On the basis of these findings it seems that in the low-arctic meadow ecosystem small and/or short-term changes in nutrient availability do not affect the plants or their AM fungal symbionts to such a degree that the formation of AM symbiosis is affected and colonization rate decreased.

At the community level, nutrient enrichment has been reported to change the AM fungal community composition and decrease the number of AM fungal species (Johnson 1993, Egerton-Warburton & Allen 2000, Jumpponen et al. 2005). However, the naturally higher soil fertility in one of the two low artic meadows seems not to have similar effects on AM fungal community. The AM fungal diversity was higher in the meadow with higher soil fertility (III). This higher AM fungal diversity coincided with higher plant species richness. Therefore, it was not possible to deduce if the higher soil fertility was behind the differences in diversity of AM fungi or if plant and AM fungal diversity were depended on each other.

### 3.1.2 Effects of defoliation

When leaf biomass is removed by defoliation plant photosynthetic capacity decreases. This is likely to have implications on the functioning of AM symbiosis. I found that in the short-term defoliation experiments performed with low arctic herbs defoliation had no effect (I, II) on the AM colonization rate. Furthermore I found that in the roots of grassland species *P. lanceolata* the colonization rate was increased by defoliation (IV). These results contrast the traditional view that defoliation decreases mycorrhizal colonization rate. In their review Gehring and Whitham (2002) found that mycorrhizal colonization rate was reduced by defoliation or herbivory in 64.3% of the 42 plant species examined, including 6 ectomycorrhizal species, rest being AM. However, since their review several new articles have been published reporting that the AM colonization rate was not affected (Lugo et al. 2003, Busso et al. 2001) or it was increased (Eom et al. 2001, Techau et al. 2004, Kula et al. 2005, Wearn & Gange 2007) by defoliation or herbivory. Thus, it can be concluded that defoliation does not automatically result in decreased AM colonization rate, but rather the response is variable.

Variable responses to defoliation may be due to the AM fungal species and their tolerance to herbivory and reductions in carbon supply. In the present greenhouse and growth chamber experiments (II, IV) different strains of the same AM fungal species *Glomus claroideum* was used. For this fungal species, an increase in colonization rate following defoliation has been also reported by Hokka et al. (2004) and it is thus plausible that this species is particularly tolerant of low carbon supply.

The intensity of defoliation in different experiments can also be a factor causing this variance in AM colonization response (Gehring and Whitham 1994). Some experiments have been able to link the colonization rate to the intensity of defoliation (Allsopp 1998). In low the arctic meadow (I) defoliation treatments removed about one third of annual above ground vascular plant

production and in the greenhouse (II) and growth chamber (IV) experiments the defoliation treatment decreased the final standing biomass as much as by 60%. In temperate grassland removal of nearly 90% of the aboveground foliage was required to cause such C limitation that the AM colonization rate was decreased (Wearn & Gange 2007). Thus, it seems that a relatively large proportion of plant photosynthetic tissue can be removed without any marked changes in AM colonization rate.

The AM fungal colonization response to defoliation can also depend on soil nutrient availability and plant nutritional needs. Resembling my results from growth chamber experiment (IV), Hartley and Amos (1999) found an increase in AM colonization rate following defoliation in nutrient limited plants and a decrease with nutrient addition. Also, on the basis of the AM fungal colonization response to defoliation it seems that herbivory depletes more strongly plants' nutrient than carbohydrate reserves in the low arctic meadow ecosystem. Chapin (1980) suggested that in nutrient poor tundra soils selection favors plants capable of maintaining or increasing nutrient absorption rates and root activity following defoliation. In this thesis I did not compare the regrowth of plants in mycorrhizal and non-mycorrhizal conditions in the low arctic meadow following defoliation and there is no direct evidence of mycorrhizal benefit in this ecosystem. However, it is possible that mycorrhizal symbiosis may increase tolerance of herbivory through increasing the availability of P and other growth limiting soil resources following defoliation.

## 3.2 AM symbiosis and plant-plant interactions

### 3.2.1 AM benefit in systems with single host plant

In the present work, the effects of AM fungus *G. claroideum* on the host plant growth were not uniform. In *P. lanceolata* the mycorrhizal colonization was not found beneficial to the host plant as the mycorrhizal plants had lower biomass production than their non mycorrhizal controls although AM symbiosis tended to increase the reproductive biomass (IV). For the adult *G. norvegicum* plants AM colonization was very beneficial in terms of biomass and nutrient acquisition: The AM plants had higher N and P concentrations in their shoots and their biomass was higher than that of the non-mycorrhizal plants (II). The effects of AM fungi on host plant are known to depend among other things on the host plant species (Helgason et al. 2002) and the AM fungal strain (Munkvold et al. 2004). Also the environmental conditions such as light, soil nutrient availability, pH, moisture and temperature (Hayman 1974, Schenck & Smith 1982, Graham et al. 1997, Clark et al. 1999, Augé 2001) affect the AM symbiosis and all these factors are possible explanations for the contrasting responses in these two experiments. In simplified experimental systems with single plant host and one or more AM fungal partners a positive effect of AM

symbiosis on growth has been frequently observed and based on these findings the AM symbiosis is generally considered beneficial to plant hosts (Fitter 1985).

### 3.2.2 Effects of neighboring plants on mycorrhizal benefit and mycorrhizal symbiosis in seedlings

My results emphasize the importance of below-ground competition during seedling establishment and show that competition for mycorrhiza-mediated resources may be an important factor affecting seedling establishment. In the greenhouse experiment with seedlings and adult *G. norvegicum* plants, the mycorrhizal benefit to the seedlings was low in the vicinity of non-defoliated adult plants (II). Also, in the low arctic meadow, the biomass gain in seedlings was highest in absence of adult plants (V). These results are in line with previous findings from greenhouse (Moora & Zobel 1998, Kytöviita et al. 2003) and field experiments (Scherff et al. 1994, Gehring & Connell 2006) showing that seedlings do not receive significant benefits when associated with adult plants, and that the mycorrhizal benefit is greatest in the absence of neighboring vegetation. These findings raise the question if adult plants and seedlings host a different AM fungal community in nature in order to avoid competition for mycorrhiza mediated resources. In tropical trees the AM fungal community composition has been reported to change with age (Husband et al. 2002b). However, to my knowledge, there are no similar studies done with herbaceous plant species and I found no information on the AM fungal community hosted by seedlings vs. adult plants.

I also showed that defoliation of neighboring conspecific adult plant can increase the mycorrhizal benefit in the *G. norvegicum* seedlings in comparison to the seedlings growing with non-defoliated adult plant in greenhouse conditions (II). On the basis of this result it seems that defoliation of vegetation by mowing or herbivory may improve seedling establishment by decreasing the competition for mycorrhiza mediated resources. In the low arctic meadow the defoliation of neighboring vegetation did not improve the seedling growth (I) illustrating that in natural conditions with complex biotic and abiotic interactions other factors may override the effect of defoliation on below ground competition between adult plants and seedlings. However, there are several reports by others showing increased seedling establishment following defoliation in temperate and also in low-arctic meadows (Kotorová & Lepš 1999, Lepš 1999, Eskelinen & Virtanen 2005) and the effects of defoliation on competition for mycorrhiza mediated resources may have been one underlying factor in these experiments.

### 3.2.3 Effect of neighboring plants on AM fungal colonization rate

I found no evidence that neighboring adult plants facilitated colonization in seedlings. The AM fungal colonization rate was high in the seedlings and it was not affected by the presence or absence of neighboring conspecific adult plants or defoliation of neighboring plants either in greenhouse or in the meadow (I,

II, V). These results indicate that if facilitation by faster colonization did take place, the effect was short-term and had no significant long-term effect on seedling colonization rate.

I found that the colonization rate by AM fungal structures did differ between seedlings growing among natural vegetation in control plots and seedlings growing in plots where vegetation was manipulated (monoculture and no-vegetation) (V). Plants may potentially affect the AM fungal colonization rate in the roots of their neighboring plant by (i) allocating resources to shared AM fungus and thus affecting fungal growth or (ii) by affecting the resource availability to the neighboring plant and thus its resource allocation to the AM fungus and/or to the roots. The vegetation was more dense in the control plots than in the monoculture plots and as the effects of neighboring vegetation on resource availability are likely to be density dependent the differences in colonization rate may at least in parts be explained by the density of neighboring vegetation. The neighboring *S. virgaurea* plants did not have any clear effect on AM fungal colonization rate in conspecific and heterospecific (*P. crantzii* and *A. glomerulans*) seedlings (V). Others have reported that neighboring plants of different species can have a significant impact on AM fungal colonization rate in plant roots (Miller et al. 1983, Chen et al. 2005). In the field experiment by Chen et al. (2005), the highly mycorrhizal neighboring plant species tended to increase the colonization rate in the roots of poorly mycorrhizal plant species. They suggested that the highly mycorrhizal plant species provided higher diversity and abundance of AM fungal spores thus enhancing the colonization of less mycorrhizal species. In the low arctic meadow the AM fungal community diversity in seedlings was not affected by the vegetation manipulations (III) suggesting that other factors were more important in determining the AM colonization rate. In *P. crantzii* seedlings there was a significant relationship between mycorrhizal colonization rate and soil microbial community profile (V) indicating that biotic interactions might be important in determining the AM colonization rate.

### 3.2.4 Effects of neighboring plants on AM fungal community in seedling roots

The AM fungal community in the low arctic meadow ecosystem seems to be resilient to short-term disturbances in plant community diversity and composition. My results from field experiment indicated that a high inoculum potential and diversity of AM fungi remained in the soil for at least two years after the vegetation supporting the AM fungal community was removed. The AM fungal diversity in the *S. virgaurea* seedling roots was as high in the no-vegetation plots as in the seedling roots growing among natural vegetation in control plots (III). This result suggests that AM fungi were able to survive at least two years without host plants. This ability buffers the AM fungal community against short-term changes in plant community diversity and composition. Arctic sites are characterized by large fluctuations in soil

temperature (Coulson et al. 1995) and in the length of the growing season (Forland et al. 2004), which may have selected for traits associated with the ability to remain dormant over unfavourable periods.

It seems that neighboring vegetation can affect the AM fungal community composition in seedling roots. Although the vegetation manipulations did not affect the diversity of AM fungi, the composition of AM fungal community in seedling roots differed between control and the vegetation manipulation treatment (no-vegetation, monoculture) (III). This result is in line with findings of Mummey et al. (2005) who showed a shift in AM fungal community composition in roots of the grass *Dactylis glomerata* in when it was grown within 10 cm distance of forb *Centaurea maculosa.* The same mechanisms which I mentioned in Chapter 3.3.3 in respect to neighbor effects on colonization rate may also operate on AM fungal community composition. In case of *D. glomerata* and *C. maculosa* plants, Mummey et al. (2005) suggested as possible explanations for the shift in AM fungal community the direct phytotoxic mechanisms, alteration of fungal/bacterial relationships, selection of catechin-resistant AM fungal species [catechin is phytotoxic and antimicrobial substance produced by *C. maculosa* root] and modified environmental conditions (pH, nutrient availability).

My results indicate that the adult *S. virgaurea* plants did not have significant effect on the AM fungal community composition in the conspecific seedlings. I assumed that adult *S. virgaurea* plants which were planted as monoculture would have supported a specific AM fungal community (III) as there is evidence from temperate and tropical ecosystems that the AM fungal community composition depends on the identity of the host plant (Husband et al. 2002a, Helgason et al. 2002, Vandenkoornhuyse et al. 2002). I expected this "*S. virgaurea* specific" fungal community to be reflected in the AM fungal community in the roots of *S. virgaurea* seedlings establishing in the vicinity of their conspecific adult plants. However, I found no significant effect of *S. virgaurea* monoculture on AM fungal community diversity and composition in roots of *S. virgaurea* seedlings (III). Considering the resilience of diverse AM fungal community in the no-vegetation plots, the outcome could have been different if the period of monoculture had been longer. It is also possible that *S. virgaurea* does not host a particularly specific fungal community. In Swedish pasture Santos et al. (2006) did not detect any plant host specificity when the AM fungal community composition of *Festuca pratensis* and *Achillea millefolium* was compared.

In greenhouse experiments the AM fungal community diversity and composition has been shown to affect host plant performance (Edathil & Udaiyan 1996, van der Heijden et al. 1998a). However, neither the diversity nor the composition of AM fungal community explained the variance in seedling size within the monoculture and no-vegetation plots (III). This result demonstrates that in natural conditions other factors, such as patchiness of soil nutrient availability or possibly soil saprophytic microbial community composition (V), may be more important in determining the seedling growth.

## 3.3 Plants, AM fungi and soil microbial community

Plants are the main source of organic carbon to the soil food-web and therefore changes in plant community, plant physiology and resource allocation can have significant impacts on the soil saprophytic microbial community. In simplified system in a microcosm experiment defoliation and fertilization of grassland species *P. lanceolata* affected the resource allocation to the roots and these effects were reflected in the numbers of bacterial-feeding, but not those of fungal-feeding nematodes (IV). Considering that microbial populations are mostly bottom-up regulated in the soil (Mikola & Setälä 1998) and that predators responsible for the nematode turnover rate were not abundant in this system, these results suggest that bacteria respond more readily to changes in resource availability than fungi. The AM fungus seemed to have a weak positive effect on the fungal feeders. However, I found no evidence of a plant-mediated effect of the AM fungus on the bacterial feeders despite the fact that AM had significant effects on plant biomass allocation to roots indicating that effects of AM fungi on soil bacterial biomass production may be small. There are other experiments showing no effect of AM fungi on soil bacterial biomass (Olsson et al. 1998) and bacterial-feeding nematode abundance (Hokka et al. 2004), but the effects may vary between bacterial taxa (Vestergård et al. 2008).

Removal of vegetation in low arctic meadow resulted in decreased soil organic matter and nutrient content and changes in microbial community composition based on the PLFA profiles (V). Total microbial biomass as well as the abundance of fungi and gram negative bacteria was lower and gram positive bacteria higher in no-vegetation plots in comparison to control plots with natural vegetation. The changes in soil microbial community were expected as the absence of plants should result changes in resource availability to the soil microbial community i.e. shortage of easily assimilable carbon in form of rhizodeposition as well as in reduced amounts of more complex C source in form of root litter. However, some of the changes in microbial community were different from what had been found in temperate disturbed soils. Generally, gram negative bacteria are considered respond strongly to easily assimilable C inputs such as rhizodeposition (Griffiths et al. 1999) and gram positive bacteria seem to use more soil organic matter derived carbon sources (Kramer and Gleixner 2008). In temperate ecosystems the abundance of gram positive bacteria has been reported decrease in soil in response to disturbance (McKinley et al. 2005, Peacock et al. 2001).

I expected that the monoculture *S. virgaurea* plants would have provided resources to the soil microbial community not available on the no-vegetation plots thus resulting in differences in the composition of soil microbial community in the low arctic meadow. Surprisingly, the microbial community in *S. virgaurea* monoculture plots did not differ from that of no-vegetation plots (V). Thus, it seems that in order to affect soil microbial community high plant cover and root biomass is necessary.

Dark septate fungi is a taxonomically unclear group of fungi characterized by the formation of dark, pigmented and septate fungal hyphae inside plant roots. These fungi are frequently observed in roots of arctic and alpine plants (Read & Haselwandter 1981, Treu et al. 1996). DS fungal colonization was also found in the low arctic meadows in Kilpisjärvi (I, V). I found no correlation between AM fungal and DS fungal colonization rate (I). There were large species-specific differences in DS fungal colonization rate suggesting that the host plants differ in their susceptibility to be colonized by DS fungi (V, I). A relatively high DS fungal colonization rate was observed already in young (about one month old) seedlings implying that DS colonization is not a sign of senescence in roots (I). The DS fungal colonization was found to be highest in experimental plots with natural unmanipulated vegetation cover (V) and responded negatively to defoliation and fertilization (I). The ecology of DS fungi is still largely unknown, and the ecological interpretation of these results is not clear. There is evidence that DS fungi have good saprophytic capacity (Caldwell et al. 2000) and the root colonization pattern might be partly explained by soil organic matter content (V).

# 4 CONCLUSIONS

Although AM fungal colonization rate can be affected by manipulating plants' resource availability by defoliation and fertilization as was demonstrated in the microcosm experiment, the results from field experiments showed that the AM fungal colonization rate was not affected by short-term changes in resource availability. The low arctic meadow ecosystems are naturally subjected to herbivory and other small scale disturbances and my results suggest that AM symbiosis in the low arctic meadow ecosystem is not sensitive to these kinds of disturbances affecting resource availability, at least in the short-term. Other factors, such as interactions with soil saprophytic microbial community may be more important in determining the AM fungal colonization rate. The short growing season and low temperatures are characteristic for the low arctic meadow ecosystem, but the functioning of AM symbiosis seems not to be markedly different from what is known about AM symbiosis in other ecosystems.

I found no evidence of mycorrhiza mediated facilitative interactions between seedlings and adult plants and the results of this thesis emphasize the importance of below-ground competition in seedling establishment. I showed in a simplified system in greenhouse conditions that defoliation of the neighboring adult plant may improve seedling establishment by decreasing the competition for mycorrhiza mediated resources. It is known that mowing and herbivory can improve seedling establishment in meadow ecosystems and I propose that the decrease in below ground competition may be one underlying factor increasing seedling success.

I showed that in the low arctic meadow a functional soil AM fungal and saprophytic microbial community necessary for successful seedling establishment can persist in the soil at least for two years without vegetation cover. Thus, the soil microbial community seems to be quite well buffered against short-term changes in plant community composition. Planting *Solidago virgaurea* as monoculture to the devegetated experimental plots did not have significant effect on soil symbiotic and saprophytic microbial community composition nor did it improve seedling establishment indicating that higher plant cover and root biomass may be necessary in order to affect soil microbial community.

*Acknowledgements*

This study was conducted at the Department of Biology in the University of Oulu and at the Department of Biological and Environmental Science in the University of Jyväskylä. The field experiments were carried out in Kilpisjärvi Research Station. Financing for this thesis was provided by the Kone Foundation, Finnish Cultural Foundation, Maj and Tor Nessling Foundation and Oskar Öflund Foundation, which all are greatly appreciated.

My utmost thanks go to my supervisor Dr. Minna-Maarit Kytöviita for guidance and encouragement in this work. I am especially grateful that she always had time for stimulating discussions about science. I also owe much gratitude towards my collaborators - I could have not done this study without their expertise. Professor Peter Young kindly welcomed me into his laboratory and Dr. Rebecca Husband guided me in the T-RFLP analysis of AM fungi in the University of York. Dr. Hannu Fritze provided his expertise in the PLFA analysis at Metla research station, Vantaa. I also want to express my thanks to Dr. Juha Mikola who introduced me to the below-ground ecosystems and to my co-authors Dr. Heikki Setälä and Dr. Mauritz Vestberg. MSc Ulla Vuoti assisted me in the field experiments in the summer 2001 - I could not imagine having a nicer person to work with – Thanks! During my PhD work I have also had many good advice and comments in regards to science and practical matters from my fellow students, scientists and staff members of the above mentioned institutes and universities to whom I am very grateful.

Last but not least I thank those people "behind the scenes" that have provided me love, support and friendship. So my warmest thanks go to my parents Matti and Kaija , my brother Antti and sister Sanna, to my dear friends, and most of all, to my fiancé Mikko.

# YHTEENVETO (RÉSUMÉ IN FINNISH)

## Arbuskelimykorritsa, resurssien saatavuus ja maanalaiset kasvien ja mikrobien väliset vuorovaikutukset

Arbuskelimykorritsa (AM) on kasvin ja Glomeromycota-pääjaksoon kuuluvan sienen muodostama symbioottinen suhde. Arbuskelimykorritsaa tavataan yleisesti ympäri maailman ja se on tavallinen myös ala-arktisissa ekosysteemeissä. Arktiset olosuhteet, lyhyt kasvukausi ja kylmä ilmasto, asettavat rajoitteita kasvien kasvulle ja lisääntymiselle, ja mahdollisesti myös AM-symbioosin toiminnalle. AM-symbioosia muodostavat sienet ovat täysin riippuvaisia isäntäkasvin fotosynteesituotteista ja voivat kuluttaa niistä merkittävän osan. Vastineeksi kasvin juurissa elävä AM-sieni voi parantaa isäntäkasvin ravinteiden saantia ja vesitaloutta sekä suojata patogeeneiltä. Yleensä AM-symbioosin katsotaan olevan mutualistinen, mutta hiili- ja ravinneresurssien saatavuus voi vaikuttaa isäntäkasvin symbioosista saamaan hyötyyn. AM-symbioosin vaikutuksien isäntäkasvin kasvuun ja menestykseen tiedetään olevan jossain määrin lajispesifisiä riippuen sekä AM-sienilajista että isäntä-kasvilajista. Parantamalla isäntäkasviensa kasvua AM-sienet voivat vaikuttaa kasvien väliseen kilpailuun sekä sitä kautta kasviyhteisön koostumukseen. Paljon keskustelua on herättänyt AM-sienten mahdollinen rooli fasilitatiivisten vuorovaikutusten välittäjänä taimien ja aikuisten kasvien välillä. Lisäksi AM-sienet voivat vaikuttaa suoraan tai välillisesti isäntäkasvinsa kautta myös maan mikrobiyhteisöön, jolla on tärkeä rooli ravinteidenkierroissa.

Väitöskirjatyössäni tutkin kasvien ja maan mikrobien välisiä vuorovaikutuksia keskittyen erityisesti AM-symbioosiin ala-arktisilla niityillä. Työni tavoitteina oli selvittää i) miten resurssien saatavuus vaikuttaa AM-symbioosin toimintaan ja AM-sienikolonisaatioon kasvin juurissa, ii) mikä on AM-sienen rooli aikuisten kasvien ja taimien välisissä maanalaisissa vuorovaikutuksissa sekä iii) millaisia vaikutuksia kasvinyhteisöllä on maan saprofyyttiseen mikrobiyhteisöön ja AM sieniyhteisöön taimien juurissa. Lisäksi tavoitteena oli lisätä ekologista tietämystä ala-arkisesta niittyekosysteemistä liittyen erityisesti taimien menestykseen ja maan symbioottiseen ja saprofyyttiseen mikrobiyhteisöön.

Tutkimukseni koostuivat ala-arktisilla niityillä Kilpisjärvellä tehdyistä maastokokeista sekä yksinkertaistetuissa systeemeissä toteutetuista kasvihuone- ja kasvukammiokokeista. Resurssien saatavuuden vaikutusta AM-symbioosin toimintaan tutkin vähentämällä isäntäkasvin yhteytyskapasiteettia leikkauskäsittelyin ja lisäämällä ravinteiden saatavuutta lannoituskäsittelyin sekä maastossa että kasvihuoneolosuhteissa. AM-sienen roolia aikuisen kasvin ja taimen välisissä maanalaisissa vuorovaikutuksissa selvitin kasvihuonekokeella, jossa taimia kasvatettiin yksin ja aikuisen kasvin kera, ilman mykorritsasientä ja mykorritsasienen kanssa. Kasviyhteisön vaikutuksia maan mikrobistoon ja taimien menestymiseen tutkin maastokokeella, jossa kasvillisuuden koostumusta ja monimuotoisuutta muokattiin, ja koealoille kylvettiin niityillä

luonnostaan esiintyvän kasvilajin siemeniä. AM-symbioosin toiminnan mittareina käytin AM-sienikolonisaatiota kasvin juurissa sekä kasvin biomassaa ja ravinnepitoisuutta. AM-sieniyhteisön koostumusta ja monimuotoisuutta analysoin terminaalinen restriktiofragmenttipituuspolymorfia (T-RFLP) -menetelmällä, ja maan mikrobiyhteisön analyysi perustui fosfolipidirasvahappo (PLFA) -menetelmään.

Kokeissani havaitsin, että leikkauskäsittely ei merkittävästi vaikuttanut AM-sienikolonisaatioon kasvin juurissa. Vain kasvukammiokokeessa leikkaus nosti AM-sienikolonisaatiota. Samoin lannoituskäsittely laski kolonisaatiota vain kasvukammiokokeessa, muttei ala-arktisella niityllä. Tulosten perusteella vaikuttaa siltä, että ravinteiden saatavuus tai lehtipinta-alan vähentämisen aiheuttamat lyhytaikaiset vaikutukset kasvin hiilen assimilaatioon eivät ole AM-sienikolonisaatiota määrääviä tekijöitä ala-arktisilla niityillä. Maastokokeet antoivat viitteitä, että vuorovaikutuksilla maan mikrobiston kanssa voisi olla merkitystä AM-kolonisaatioon ja symbioosin toimintaan vaikuttavana tekijänä.

En löytänyt viitteitä siitä, että aikuiset kasvit edesauttaisivat taimien menestymistä AM-sienen välityksellä. Tutkimukseni vahvistivat käsitystä, että symbioosi AM-sienen kanssa lisää kasveilla lajinsisäisen kilpailun intensiteettiä, ja osoitti, että kilpailu maanalaisista resursseista aikuisten kasvien kanssa voi olla tärkeä taimien menestystä rajoittava tekijä myös luonnossa. Lisäksi kasvihuonekokeessa havaitsin, että naapurina kasvavan aikuisen kasvin leikkaus voi vähentää taimien kokemaa kilpailua mykorritsasienen tarjoamista resursseista. Tämä voikin olla yksi mekanismi, jolla niittykasvillisuuden laidunnus tai niitto edesauttaa taimien rekrytoitumista. Maastokokeet osoittivat myös, että symbioottinen AM-sieniyhteisö sekä saprofyyttinen mikrobiyhteisö säilyivät taimien rekrytoitumisen kannalta riittävänä myös koealoilla, joilta kasvillisuus poistettiin kokonaan kahdeksi vuodeksi.

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