Masters Thesis

The role of symbiotic arbuscular mycorrhizal fungi (Glomeromycota) in roots of the host plant *Deschampsia flexuosa* in vegetation succession of inland sand dunes in Finnish Lapland

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ABSTRACT

Most of all land plant species and families are mycorrhizal, and the majority of them house the symbiont *arbuscular mycorrhizal* (AM) fungi in their roots. The attachment between the host plant and the mycorrhizal fungus is called a mutualistic symbiosis. The heterotrophic AM fungi rely on organic carbon provided by their plant hosts and in return the fungi can improve their host plants nutrients acquisition, provide protection from pathogens and herbivores, and tolerance of water deficit. By affecting the plant's resource allocation and growth, AM fungi may affect the interactions between plants and thus alter the composition of plant and soil microbial communities. I studied the role of AM fungi in vegetation succession of inland sand dunes in Finnish Lapland. I assessed the mycorrhizal status of a host plant as percent root length colonized (%). The study plant, *Deschampsia flexuosa*, grew both in the deflation basins (the beginning of succession) and in the vegetated dunes (the climax) in the study area. Mycorrhizal colonization is generally common in old and stabilized ecosystems and absent or low in early successional phases of ecosystems, or in ecosystem suffering from severe disturbances. Colonization tends to be high when the amount of inorganic nutrients in soil is low and competition for sparse resources is intense. In this study I found that the colonization of roots was greater in the disturbed deflation basins than in the stabilized vegetated dunes, which was contrary to what was expected. For one reason or the other it seems that the host plants in the deflation basins are more dependent on AM fungi - but to find out for what, we need to study further.

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TIIVISTELMÄ

Suurin osa maalla elävistä putkilokasvilajeista ja -heimoista elää symbioosissa mykorritsasienten kanssa. Mykorritsatyyppejä on useita, ja näistä yleisin ja vanhin on arbuskelimykorritsasymbioosi, jossa Glomeromycota-sienet muodostavat arbuskelimykorritsaa (AM) kasvien juurien sisällä. Tämä kasvin ja sienen välinen tiivis suhde on molempia osapuolia hyödyttävä symbioosi. Toisenvarainen AM sieni on riippuvainen kasvin tuottamasta orgaanisesta hiilestä, ja vastapalveluksena sieni voi parantaa kasvin ravinteiden- ja vedenottokykyä, sekä suojata tätä taudinaiheuttajilta, tuhohyönteisiltä ja laiduntajilta, sekä parantaa kasvin kuivuudensietokykyä. Vaikuttamalla isäntäkasvien kasvuun ja resurssien kohdentamiseen AM sienet vaikuttavat myös kasvien välisiin suhteisiin, sekä edelleen kasvien ja maaperän pieneliöstön eliöyhteisöiden rakenteisiin ja monimuotoisuuteen. Tutkin AM sienten roolia hietatievojen sukkessiossa Suomen Lapissa. Mittasin sienten merkitystä isäntäkasville juurten kolonisaatioasteen (%) avulla. Tutkimuskasvilajina oli metsälauha, *Deschampsia flexuosa*, joka kasvoi sekä hietatievojen deflaatioaltaissa (sukkession alkuvaihe) että jo metsittyneillä dyyneillä (kliimaksi). AM symbioosin merkityksen on aikaisemmissa tutkimuksissa todettu nousevan ekosysteemin ikääntyessä ja vakaantuessa. Kolonisaatio on tyypillisesti suurempaa ekosysteemeissä, joissa epäorgaanisten ravinteiden saatavuus on heikko ja kilpailu resursseista on kovaa. Tässä tutkimuksessa kolonisaation määrä isäntäkasvien juurissa oli kuitenkin suurempaa deflaatioaltaissa kuin metsittyneillä dyyneillä, mikä oli vastoin ennakko-oletuksia. Näyttää siis siltä, että metsälauhakasvit deflaatioaltaissa ovat riippuvaisempia AM sienistä kuin metsittyneiden dyynien metsälauhat - jatkotutkimus on tarpeen selvittämään miksi.

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1. INTRODUCTION

1.1. Arbuscular mycorrhizal fungi

The majority of plants growing in natural conditions are dual organisms: their chief organs of nutrient and water uptake are not their own roots but "fungus-roots", *mycorrhizas* (Wang & Qiu 2006, Smith & Read 2008). Arbuscular mycorrhizas (AM), which were previously also known as *endomycorrhizas*, are the most common mycorrhizal type (Smith & Read 2008), and ancestral to all other mycorrhizal types (Wang & Qiu 2006). AM symbiosis is so ubiquitous that it is easier to list the plant families in which it is not known to occur than to compile a list of families in which it has been found (Gerdemann 1968, Wang & Qiu 2006, Smith & Read 2008). It is also evolutionarily old: the existence of arbuscules in the early Devonian (400 million years ago) indicates a role for these fungi in very early plant colonization of land (Simon et al. 1993, Remy et al. 1994, Taylor et al 1995, Brundrett 2002, Wang & Qiu 2006) via symbiosis with cyanobacteria (Gehrig et al. 1996, Schüßler 2002). Also the occurence of AM symbiosis in vast majority of extant land plants and in all early-diverging lineages of major clades strongly supports this claim (Wang & Qiu 2006). AM symbiosis is thus far from being an oddity in ecosystems studied - although its ecological importance is not so widely appreciated (Allen 1991, Smith & Read 2008, Redecker 2008).

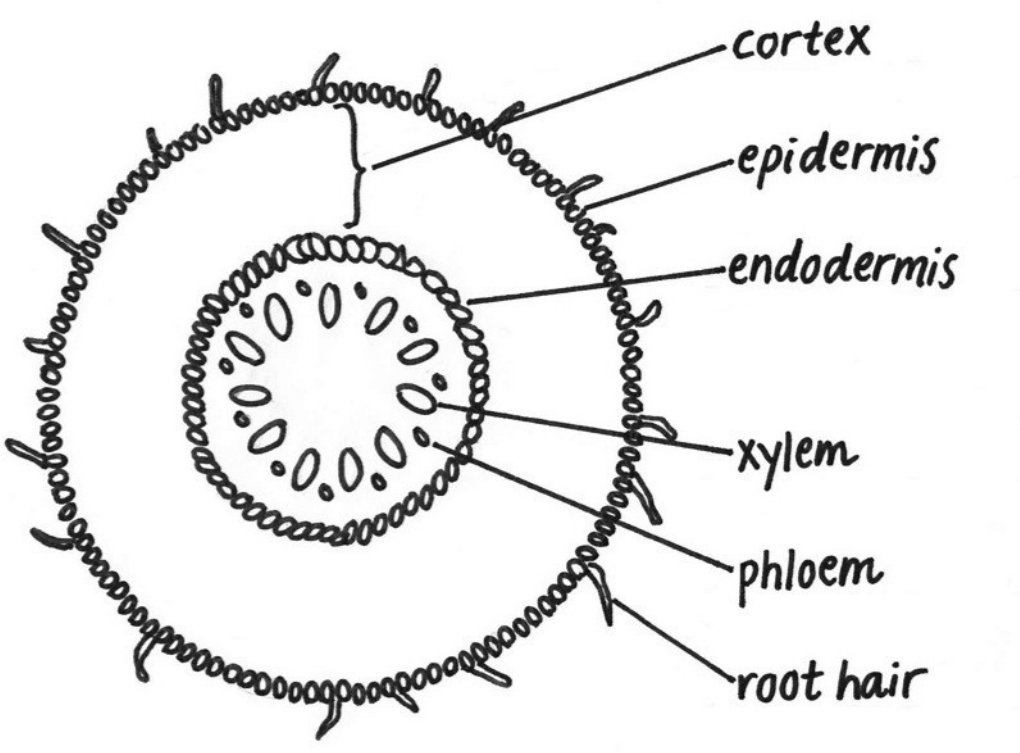

Figure 1. Anatomy of a typical monocotyledon root. Endophytic fungal colonization typically occurs in the cortex, epidermis and root hairs of the plant.

The life of an AM fungus typically begins from the soil as a spore (~80-500µm) germinates. From the spore, hypha (~5-10µm wide) elongates to the surrounding soil in

search for plant roots. When suitable host plant root is found, hypha enters the root through *appressoria*, a flattened hyphal pressing organ, and continues to grow inside the cortex of the root (Figure 1). Inside the root, the AM fungi form three different structures: the constantly elongating and moderately branching inter- or intracellular hyphae, *vesicles* (~50-100µm in diameter), enlarged portions of hyphae, that are considered to function as storage units containing lipids, and *arbuscules* (~50-100µm in diameter) made of extremely finely dichotomously branched hypha, that are considered to be the organs through which the exchange of nutrients and carbon between the plant and the fungus mainly takes place (Smith & Read 2008). These structures are called *intraradical* for they are inside the cortex. A large proportion (estimates up to 95%) of fungal hyphae is nevertheless *extraradical* - it extends beyond the root and ramifies into the surrounding soil to uptake nutrients, and the fairly large spores of AM are also formed mostly in the extraradical mycelium.

The basis of the mutualistic AM symbiosis is bidirectional transfer of nutrients with the result that fitness of both organisms increases. AM fungi provide plants with soil-derived mineral nutrients (Marschner & Dell 1994), and in exhange, up to 20% of plants net photosynthate, organic C, is transferred to the fungus (Jakobsen & Rosendahl 1990). The most important feature of the associations seems to be the ability of the extraradical hyphae of the fungus to take up and transport resources to the plant from the soil outside depletion zones created by the root itself (Allen 1991). For plants, colonization can lead to increased vegetative growth, especially in soils of low nutrient status, particularly if nutrient P is in short supply (Hayman & Mosse 1971, 1972, Mosse & Hayman 1971). Increased fitness does not however always lead to increase in vegetative growth (which is widely used because it is an easy and straightforward parameter to measure in laboratory), and there is increasing evidence that AM colonization in the field may amongst other things increase resistance to pathogens and insect herbivores, and tolerance of water deficit (Augé 2001, Smith & Read 2008). However, the responses of plants are diverse and range from positive to negative, depending at least on the nutrient availability of the soil, amount of irradiation, identity of fungal symbionts and community interactions (Grime et al. 1987, Smith & Read 2008). Though the line between parasitism and mutualism is fine, and negative interactions between plants and mycorrhizal fungi can and do occur, the mutualistic nature of the interaction is nevertheless a critical character that differentiates a mycorrhiza from other plant-fungus associations (Allen 1991).

All AM species (~150) are obligate symbionts and belong to one monophyletic phyla, the Glomeromycota (Schüßler et al. 2001). In contrast, although majority of AM host plant species in the field are normally colonized by AM fungi, some grow satisfactorily in the absense of colonization as long as mineral nutrient supplies are adequate (Wang & Qiu 2006, Smith & Read 2008). Species which are sometimes, but not always, colonized by mycorrhizas are referred to as "facultatively mycorrhizal", to distinguish them from those "obligately mycorrhizal" species that are consistently colonized. There are also plant families that usually are not colonized by any type of mycorrhiza, and these are referred to as "nonmycorrhizal", however, even in these colonization of roots is sometimes observed (Smith & Read 2008). The intensity of the interaction is most commonly measured as percent root length colonized, whose magnitude is species-specific, and varies both seasonally (Ruotsalainen et al. 2002) and geographically (Read & Haselwandter 1981).

Other types of mycorrhiza differ from AM in their taxonomy, structure and host plant species range (Smith & Read 2008). Together there are seven types of mycorrhizas, and the ones found from the study area of this thesis besides AM are: ectomycorrhizas, ectendomycorrhizas, arbutoid mycorrhizas and ericoid mycorrhizas. In general,

ectomycorrhizal symbiosis is a common association e.g. in boreal and subarctic trees (such as *Betula, Picea* and *Pinus*) and ericoid mycorrhizal symbiosis in ericaceous dwarf shrubs (such as *Arctostaphylos, Calluna* and *Vaccinium*). Different types of mycorrhiza might enable plants to tap different sources of nutrients in the soil (Read 1993 cit. Smith & Read 2008, Michelsen et al. 1996), on the other hand, an individual plant may simultaneously be colonized by several types of mycorrhizas (Wang & Qiu 2006). The individual hyphae of mycorrhizas growing from root to root in soil form belowground linkages between plant root systems, called *common mycorrhizal networks*, connecting plants of same and different species, potentially altering nutrient and carbon transfers between them (Simard et al. 1997, Robinson & Fitter 1999, Simard & Durall 2004, Meding & Zasoski 2008). Common mycorrhizal networks are an important means by which seedlings become mycorrhizal when establishing in the neighbourhood of colonized plants (Simard & Durall 2004, van der Heijden 2004, Smith & Read 2008). By regulating belowground competition between plants, AM fungi have been shown affect plant community structure and diversity (Grime et al. 1987, van der Heijden et al. 1998a, van der Heijden et al. 1998b). In conclusion, the interactions in the soil are labyrinthine, and it might sometimes be difficult to tell apart competition from coexistence and facilitation.

1.2. Root associated fungi in cold climate

Rhizodeposition, the release of C compounds from living plant roots into the surrounding soil, results in different chemical, physical and biological characteristics in the rhizosphere compared with those in the bulk soil (Lambers et al. 2009). In the vicinity of roots, a great variety of fungi exist, and plants live in association with a rich diversity of microorganisms during their entire development (Peterson et al. 2008, Lambers et al. 2009). The loss of C from root epidermal and cortical cells leads to a proliferation of microorganisms inside (*endophytes*), on the surface, and outside the roots (Lambers et al. 2009).

In general the percentage of mycorrhizal infection decreases towards poles and higher altitudes (Gardes & Dahlberg 1996). The frequency of plants not colonized by mycorrhizas increases at higher latitudes, largely owing to an increase in nonmycorrhizal and a decrease in obligately mycorrhizal plant families (Gardes & Dahlberg 1996, Newsham et al. 2009). In general terms, AM are characteristically found in species-rich ecosystems (e.g. in tropical forests) in contrast to ecto- and ericoid mycorrhizas which predominate in boreal and arctic forests and heaths in which levels of organic nitrogen are typically high in soil, and only a few host species are present or dominate (Read 1991, Smith & Read 2008). In addition, it also seems that AM may be rare in the arctic even when potential host plants are present (Olsson et al. 2004). Alternatively AM associations are found frequently in low arctic tundra and subarctic, albeit the level of colonization is highly variable (Read & Haselwandter 1981). It also seems intuitive that mycorrhizal, saprotrophic and parasitic soil fungi must play an essential role in enhancing nutrient availability for plants and other living organisms in the arctic and subarctic, where decaying is slow due to low temperatures (Kankaanpää 2001).

Dark septate endophytes (DSE) are a taxonomically ambiguous group of globally ubiquitous root-associated fungi that frequently colonize plant roots especially in cold-stressed alpine, arctic and sub-antarctic habitats (Read & Haselwandter 1981, Currah & van Dyk 1986, Stoyke & Currah 1991, Väre et al. 1992, Laursen et al. 1997). They form melanised structures such as extensive wefts of hyphae on the root surface and intraradical tensely packed *microsclerotia* (Stoyke & Currah 1991, Jumpponen & Trappe 1998). DSE colonization has been found from approximately 600 plant species including species usually considered AM, ecto-, ericoid- and nonmycorrhizal (Jumpponen & Trappe 1998). There seems to be little or no host specificity, and simultaneous colonization with other

types of mycorrhiza has been observed (Jumpponen & Trappe 1998). At least under some conditions, DSE are capable of forming mutualistic associations with plants functionally similar to mycorrhizas (Jumpponen 2001), but as they are capable of living on organic nutrient sources, they may also grow as freeliving saprobes in soil. DSE are apparently more frequent than mycorrhizal fungi in polar regions (Newsham et al. 2009). Understanding the species composition and ecological significance of this group is however still in its infancy (Mandyam & Jumpponen 2005).

Fine endophytes (formerly classified as *Glomus tenue*) are species of Glomeromycotan AM fungi with identifiably narrow hypha, and based on this and other features in their morphology they form a separated group from the AM. The function and taxonomical status of the group remains to be largely enigmatic, but they are often found from truly harsh cold (Olsson et al. 2004, Smith & Read 2008) and dry (Rabatin et al. 1993) environments, were few other mycorrhizas exist and typically nonmycorrhizal plant species thrive (Olsson et al. 2004). They are also the dominant form of AM in roots at higher latitudes (Newsham et al. 2009).

Yeasts are a systematically artificial group of fungi designated by presence of a unicellular stage in their life cycle, and they are found in soils worldwide (Botha 2011). Due to rhizodeposition, the number of yeasts and diversity in species composition tends to be higher in the vicinity of plant roots in rhizophere than further away in the bulk soil (El-Tarabily & Sivasithamparam 2006, Cloete et al. 2009). Over the years evidence has accumulated that soil yeasts may exert a positive effect on soil structure, nutrient recycling and plant growth (Botha 2011). There is also evidence from symbiotic relationship between yeasts and plants (Cloete et al. 2009).

1.3. Mycorrhizas in vegetation succession

Succession refers to the changes observed in an ecological community following a major environmental perturbation (Connell & Slatyer 1977). It is a complicated set of processes associated with this vegetative recovery, and it includes alterations in soils, nutrient cycling, and composition of organisms that occur (Allen 1991). Important phases in primary succession are 1) *nudation*, the exposure of a bare area, 2) *migration*, the reaching of seeds, spores, and propagules to the area, 3) *ecesis*, the establishment of new species, 4) *competition*, the development of intra- and interspecies competition among the members of the pioneer community as resources become limited, 5) *reaction,* the modification of the environment influenced by living organisms, where existing community is replaced by next seral community and the process is repeated, until 6) *stabilization,* where the final terminal climax community becomes stabilized and can maintain itself in equilibrium with climate of the area.

Mycorrhizal symbioses are major factors in the successional processes (Janos 1980), and both types and species of mycorrhizal associations change with succession and alter processes such as organic material development, nutrient cycling and plant species composition (Allen 1991). Odum (1969) proposed that entropy decreases with succession, as symbioses become more apparent and nutrients become associated with living or cycling biomass. As a result, according to this tradional view, succession culminates in a stabilized ecosystem in which maximum biomass (or high information content) and high symbiotic function between organisms are maintained per unit of available energy flow (Odum 1969). Therefore, according to Odum, we should expect symbioses to be ubiquitous in old ecosystems and absent in young soils of primary succession. Is this really the case in nature?

Many observations certainly support this claim. Many invading pioneer plant species, typically weedy annuals, are nonmycorrhizal (e.g. members of the nonmycorrhizal

plant families such as Brassicaceae and Chenopodiaceae) (Allen 1991). On the other hand, climax communities tend to be composed of plant species that are obligately mycorrhizal (Janos 1980). In e.g. coastal sand dunes plants nearest the sea are often nonmycorrhizal, but as one proceeds inland AM fungal infection and spore densities tend to increase and the species become more diverse as the habitat becomes more stabilized (Nicolson 1960, Nicolson & Johnston 1979, Read 1989, Harner et al. 2011, Oehl et al. 2012). In the study of Reeves et al. (1979) 99% of the plant cover in a natural, undisturbed habitat was mycorrhizal - whereas next to it on a narrow disturbed area only 1% of the plant cover was mycorrhizal. Furthermore it seems that mycorrhizas and organic matter accumulation during succession are tighly coupled: as soil development proceeds and organic matter increases, also mycorrhizal activity increases (Anderson et al. 1984, Allen 1991, Gould et al. 1996).

The initial reason for low mycorrhizal activity in pioneer communities can be the lack of fungal inoculum, and a prerequisite for any fungal impact is that propagules (or any other form of AM functioning as a source of inoculum, e.g. soil hyphae or root pieces) are transported to the newly exposed substrates by wind, animals or soil erosion (Smith & Read 2008). A heavy disturbance itself might cause the inoculum potential of the soil to be lost (Cazares et al. 2005), and as a result mycorrhizal inoculum is typically extremely low in drastically disturbed land (Gould et al. 1996). As it takes time for the migration of propagules to occur, and because succesful colonization requires also a suitable host plant growing in the area, nonmycorrhizal plants generally establish faster to a newly exposed site than the obligately mycorrhizal plants. Recovery of an ecosystem in part is thus dependent on either the rate of migration of propagules of mycorrhizal fungi which are viable, or roots supporting root associated fungi (Reeves et al. 1979). Some plants are able to form associations with several types of mycorrhiza, and their establishment to a bare area might be a prerequisite for other plant species to establish.

Yet, even when the mycorrhizal inoculum potential does exist in the soil, nonmycorrhizal plants are be better competitors in situations were easily accessible mineral nutrients and water resources are unlimited. In a pioneer community with low density vegetation, mycorrhizal infection might only be a cost for a plant - afterall up to 20% of plants net photosynthate is transferred to the fungus (Jakobsen & Rosendahl 1990), regardless of the magnitude of achieved benefit. An ecosystem in the ecesis-phase is hence likely to favor nonmycorrhizal plants. As individuals, nonmycorrhizal plants compete poorly with obligately mycorrhizal plants (Allen & Allen 1984, Ruotsalainen & Aikio 2004), which explains the ubiquity of mycorrhizal symbioses in climax ecosystems. As an ecosystem proceeds from the ecesis-phase towards competition, reaction and stabilization, the readily accessible mineral nutrients are depleted from the soil and organic matter starts to accrue, and mycorrhizal strategy is favoured, for it gives an increased competitive advantage for the later successional species compared with the earlier colonizing nonmycorrhizal species (Allen 1991). On the other hand, an intensive nonmycorrhizal vegetation might prevent an ecosystem to recover to its climax (Gould et al. 1996).

The pioneer community of a subarctic heath is commonly composed of nonmycorrhizal or facultatively AM graminoids. Due to low temperatures during the summer, nutrient cycling in the subarctic is slow, leading to the accruement of organic material. Those organisms that can take up and use nutrients in their organic forms are favoured in the ecosystems approaching stabilization-phase and climax, which include ericoid mycorrhizal fungi in the soil, and their host plants aboveground, Ericaceae and Empetraceae. But is the role of mycorrhizal fungi passive in succession, or are these fungi able to direct the course of succession? Could the established vegetation in part be the

result of the composition of the *fungal pioneer community* of the habitat, and not vice versa?

Mycorrhizal symbiosis is believed to contribute to the survival of host plants in stressful conditions, and it has also been suggested that the recovery of disturbed ecosystems may depend upon the reestablishment of mycorrhizal fungi (Reeves et al 1979, Janos 1980, Allen & Allen 1980, Perry et al. 1989). Common mycorrhizal networks are known to aid in the establishment of vegetation by providing inoculum potential and additional support for seedlings of same and other plant species. In primary succession, plants species that support mycorrhizal fungi in their roots can facilitate the establishment of other mycorrhizal species, and thus direct the natural succession of the habitat (van der Heijden 2001, van der Heijden & Vosatka 1999 , Nara & Hogetsu 2004, Nara et al. 2003a, Nara et al. 2003b). It might also be that nonmycorrhizal pioneer plants facilitate the establishment of later mycorrhizal plants by hosting a small inoculum potential of AM fungi in their roots (Allen & Allen 1988). In addition, in a sand dune ecosystem, the extensive mycelial network of AM hyphae not only facilitates the capture of the critical element N, but also provides the aggregation of sand grains necessary for dune stabilization (Read 1989).

Whether mycorrhizal fungi are of benefit during the early development of disturbed areas is not known (Gould et al. 1996). It seems likely that mycorrhizal fungi would play a role in facilitating the succession but until recently, there was little direct evidence for such a role in nature (Smith & Read 2008). The accumulating evidence makes one doubt the thoughts of Odum: although it might seem like mycorrhizal activity and the progress of succession correlate and are interdependent, this view might be nothing but an oversimplification. Rather, it seems intuitive that the types and species of mycorrhizal fungi play a much more complicated and diverse role in vegetation succession and in the formation of major biomes, that can only be understood after precise step by step study of the phases of succession, and the species and communities of endophytic mycorrhizal fungi and host plants that occur.

1.4. Aims of the study

Aim of this study is to cast more light on the role that symbiotic AM fungi play in vegetation succession of inland sand dunes. The inland sand dunes of the study consist of deflation basins and vegetated dunes (Seppälä 1995) - ecosystems in ecesis-phase and respectively in the stabilization-phase of succession. The specific aim of the study is to test whether the successional phase (deflation basin vs. vegetated dune) will have an effect on the intensity of AM colonization in roots of a host plant and on host plant performance. Based on previous studies it can be expected that mycorrhizal infection tends to be lower in disturbed habitats of early succession. Opposite results would indicate that AM fungi play a role in the establishment of pioneer community in the deflation basins, possibly facilitating the establishment. The intensity of mycorrhizal colonization was measured as percent root length colonised. Performance of the host plant was measured as plant abundance, mass allocation and percentage of N in plant leaves. Isotope N fractioning in plant leaves ($\delta^{15}N$) was measured to trace the differences in plant nutrient N supply.

2. MATERIALS AND METHODS

2.1. The study area

The study area, "Hietatievat", is located in northern Finland in the municipality of Enontekiö (68°25' N, 15°43' E). Climate at these latitudes is quite harsh: the average temperature of the year falls between -3 and -2ºC, and the growing season is short, it typically begins in late May and ends in late September (Finnish Meteorological Institute 2010). In the vegetated patches of the dunes birch (*Betula pubescens* ssp. *czerepanovii*) grows sparsely, in company of smaller bushes of juniper (*Juniperus communis*) and willows (*Salix* ssp.), upper understory layer is dominated by shrubby heathers (Ericaceae) and the ground layer by Cladonia lichens (*Cladonia* ssp.) (Figure 3). Vegetation of the deflation basins consists only of fairly large separated graminoid tussocks (Figure 2). The species composition is described in more detail in Table 6 in the appendices on page 34.

Figure 2. Deflation basin.

Figure 3. Vegetated sand dune.

The sand of the dune is extremely fine, it was refined by the melting waters of the last glacial period and wind (Johansson & Kujansuu 2005). After the ice cover withdraw approximately 10 000 years ago, strong winds under periglacial conditions drifted the fine sands, forming parabolic sand dunes that are widespread in many parts of Finnish Lapland (Seppälä 1995, Heinonen et al. 1996). During preboreal time, soil formation began and the sand dunes were gradually stabilized by vegetation (Seppälä 1995). At present, erosion is causing the deflation of these sand dunes and it has been so pronounced that only small remnants of sand dunes remain (Seppälä 1995). Deflation basins can be several hectares in area and over 10m in depth (Seppälä 1995). Human impact and overgrazing by reindeers seem to increase deflation (Seppälä 1995, Heinonen et al 1996).

2.2. Vegetation succession in the study area

Intense summer grazing by reindeers has been reported to promote a shift of drier vegetation communities towards early successional phases with considerably reduced total plant cover (Nadelhoffer et al. 1992). The lack of plant cover in turn exposes the sandy soil to other disturbances preventing the rapid succession of vegetation. The fine sand is easily blown by the wind, making the deflation basins hollow in their shape. The lack of insulation provided by the vegetation cover causes the soil to freeze more rapidly and deeper in autumn, and to thaw much later in spring (Nadelhoffer et al. 1992). The temperature of the topsoil fluctuates strongly daily and depending on the weather. Precipitation is absorbed by vegetation and rhizophere micro-organisms in the vegetated dunes but percolates in the deflation basins making them arid. The lack of vegetation cover together with strong wind might also lead into thinner snow cover at winter, which in turn protects poorly the topsoil from freezing. These abiotic and biotic factors together make the vegetated dunes a considerably different habitat compared to the deflation basins, and an ideal place to study the role of AM fungi, whose absense in early successional phases and in habitats suffering from high disturbances has long troubled scientists working with mycorrhizal symbiosis (Smith & Read 2008). It must be kept in mind, however, that the "climax" of the vegetated dunes is actually far from undisturbed and stabilized - it is as exposed to overgrazing by reindeers as are the deflation basins.

Succession of an ecosystem is typically classified as being either primary or secondary, but in Hietatievat it´s easy to find features of both. Primary succession, in theory, occurs on a substratum devoid of life, e.g. on a bare rock, sand dunes on a new coast line, or on a new island exposed out of the sea (Tirri et al. 2001). In addition, primary succession is typically a slow event taking place in large unvegetated areas, and its phases are coverned by the initial lack of nutrients, seeds, spores and propagules (Tirri et al. 2001). Secondary succession, on the other hand, occurs on a shorter timescale in an area which has had an existing biotic community, but that has come bare due to destruction by e.g. fire, landslide or earthquake (Tirri et al. 2001). The deflation basins of the study consist of fine sand with little or no organic soil, but they are circled by the climax community of the vegetated dunes, that provide them with nutrients, seeds, spores and propagules.

Based on my observations, I considered succession to progress from pure sand to just grasses growing sparsely as individual tussocks of *Deschampsia flexuosa* and *Festuca ovina*, from there to the addition of *Polytrichum* mosses around them, then to the appearance of woody dwarf shrubs, *Empetrum nigrum* ssp. *hermaphroditum* and *Arctostaphylos uva-ursi*, and finally at the climax many species of herbaceous plants such as *Antennaria dioica*, *Hieracium alpina* (coll.) and *Solidago virgaurea*, feather mosses (*Pleurozium schreberi)*, Cladonia lichens (*Cladonia* ssp.) and birch trees (*Betula pubescens* ssp. *czerepanovii)*. The phases classified for the study therefore are: "Deflation basin": *Deschampsia flexuosa* or *Festuca ovina,* and "Vegetated dune": *Pleurozium shreberii* and *Cladonia* ssp. and *Betula pubescens* ssp. *czerepanovii*.

2.3. Sampling design

Data was collected from 10 deflation basins and 10 vegetated dunes. The size of the study area is approximately 4,5km x 2km, and most of it is vegetated. The deflation basins are scattered, and their shape, direction and size (1-200m in diameter) varies greatly, and the distance from one basin studied to another was typically 100-500m. *Deschampsia flexuosa* was chosen as study plant species because it is an abundant species in both phases. It is an abundant perennial grass (Hämet-Ahti & Hackman 1998) with holarctic distribution, and it

is also known to be an AM host plant (Vosatka & Dodd 1998, Wang & Qiu 2006). Data was collected 12.- 24.7.2010.

2.4. Intensity of mycorrhizal colonization

2.4.1. Percent root length colonized

In determining the extent of colonization of roots, the percentage of the root length colonized by AM fungi following staining remains the most widely used method of assessment (Smith & Read 2008). It is a relative measure which is recognized as having limitations, such as its inability to take into account the rate of root growth and variations in it, and the ignorance of extraradical fungal mycelium altogether. To detect and observe the intensity of intraradical colonization, roots were stained with trypan blue (Phillips & Hayman 1970, Vierheilig et al 2005). This method was calibrated for *D. flexuosa* roots prior to the actual study to assure fungal structures to be easily distinguished in stained samples. Percent root length colonized by fungal structures was assessed under a light compound microscope using the magnified intersection method (McGonigle et el. 1990, Giovannetti & Mosse 1980).

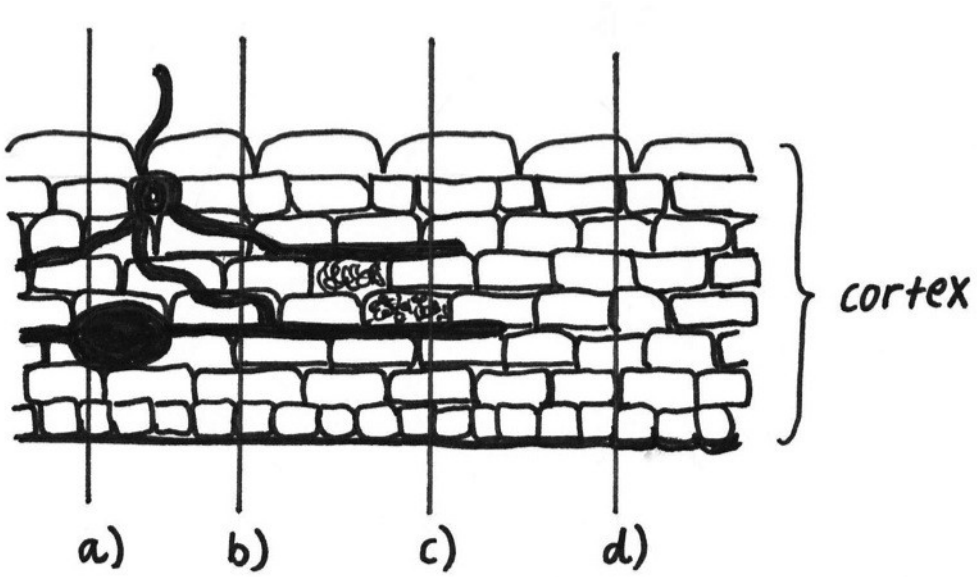

Figure 4. The assesment of percent root length colonized by arbuscular mycorrhizal fungi under a light compound microscope using the magnified intersection method (McConigle et al. 1990). In every randomly chosen field-of-view an eye-piece cross-hair was set perpendicular to the root, and the presence or absense of fungal structures were recorded where the structures intersected the cross-hair. Four possible field-of-views are presented in the picture: a) hyphae and vesicle, b) hyphae, c) hyphae and arbuscule and d) root only.

In field roots were dug up, cleansed mechanically from soil and sand, cut to pieces of ~2cm and preserved in 50% ethanol. In laboratory roots were kept overnight (16h) in 10% KOH, to make them soft and translucent. Roots were then rinsed several times with water, and then acidified with diluted HCl (1,5%) for 2 x 1,5h (the acid was refreshed after the first 1,5h). After this, the samples were stained with 0,02% trypan blue (lactic acid, glycerol, water and trypan blue) and kept at +80°C for two hours. After staining, samples were preserved in glycerol before mounting them on microscope slides in polyvinyl-lacto-glycerol. Slides were covered with cover glasses, and before polyvinyl-lacto-glyserol

dried, roots were gently squashed against the slide with the aid of a fingernail, along the root, to enable better visibility inside the cortex. Between 2 to 4 slides were used for each subsample.

In every subsample 100 randomly selected intersections were studied. 5-10 intersections were studied per one root (depending on their length), and hence 10-20 roots were studied per subsample. In every randomly chosen field-of-view along the root, an eye-piece cross-hair was set perpendicular to the root, and the presence or absence of fungal structures was recorded (Figure 4). The magnification used was x140, but if structures were unclear they were rechecked with x280. Percent root length colonized was defined as PRLC= ("Number of observations" * "Number of intersects"$^{-1}$) * 100%.

Fungal colonization was observed from young roots and preferably at a short distance from the growing apex of the root. Reason for this is that mycorrhizal structures in roots are not permanent but have an initial process of development, a mature phase and a senescent phase - the meristamatic apex of the root typically is free of fungal colonization, because the root is growing, and fungal colonization occurs typically behind the growing apex (Harley & Harley 1987). Because there were plenty of roots in our subsamples, the studied root pieces were chosen so that the variation in their size and degree of branching was small, and this was done visually before viewing them under the microscope, to avoid any personal preferences on the extent of colonization.

2.5. Performance of the host plant

Determining the enhanced fitness or survival value of the AM association over a long time period is difficult or even impossible (Allen 1991), especially in field studies were plants live longer than studies last and control plants or situations are difficult to find or establish. In this study the performance of the host plant was measured as abundance, mass allocation and percentage of N in plant leaves. Abundance of the plant was measured both with point frequency analysis and as aboveground dry biomass per squaremeter. As a part of plant performance, also the origin of nutrient N was traced via isotope N fractioning in plant leaves ($\delta^{15}N$). In previous studies the $\delta^{15}N$ values of plant tissues are found to be useful markers of the mycorrhizal role in nutrient N supply (Michelsen et al. 1996, Michelsen et al 1998, Hobbie & Colpaert 2002). While all these parameters are insufficient in describing the enhanced performance due to co-existence with AM, and their values might differ because of many factors not controlled in this study - such as the prevailing conditions of the previous years, the time of year and growing season, the intensity of grazing and variations in it - they are widely used variables comparable with other studies, and thus give us an insight of the performance of the study plant.

2.5.1. Point frequency analysis

Assessing the point intercept method to find out the total number of hits of different species (Goodall 1952, Jonasson 1983, Jonasson 1988) was done with the aid of a square frame (approximately the size of 60cm x 60cm). Two set of fishing lines were attached to the frame, dividing the square into smaller 5,5cm x 5,5cm squares, one just a few centimeters below the other (Figure 5). This frame was first balanced to be horizontal with the aid of two spirit levels and adjustable legs. When estimated visually from above, the lines on top of each other create 100 precise intercepts (covering an area of 49,5cm * 49,5cm = 2450,25cm² = ~0,25m²). All species of plants and lichens, and hits of bare ground, rock and biotic crust were registered from all intercepts. In an intercept many observations were possible, even of the same species. The stratified vegetation was recorded from up to bottom and plants were moved out of the way with the aid of a stick. All hits of a species were summed up to get total number of hits.

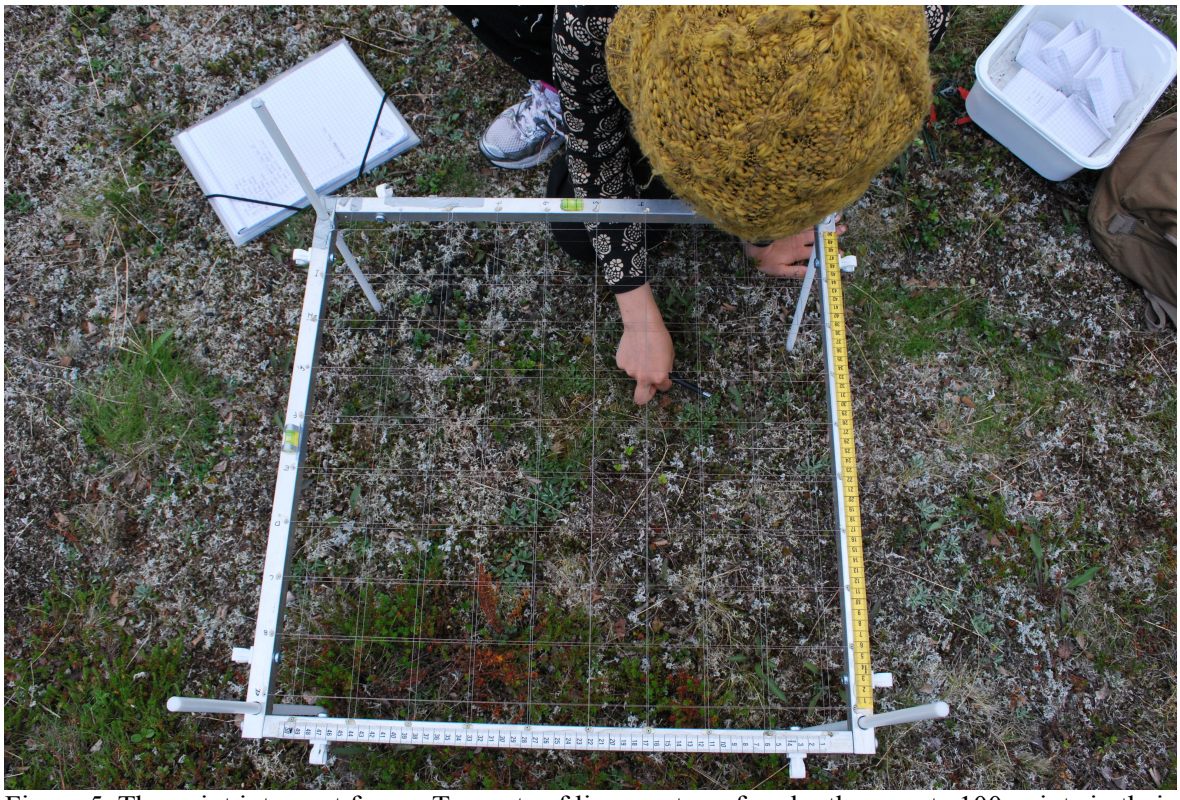

Figure 5. The point intercept frame. Two sets of lines on top of each other create 100 points in their intercepts - from these points all species of plants and lichens, and hits of bare ground, rock and biotic crust are registered. Hits are summed to get the total number of hits.

In the vegetated dunes, due to continuity of vegetation, the disposition of the frame was random, it was chosen by tossing a stick over the shoulder. Nevertheless to sustain the comparability of observations, the frame was to contain the following abundant species: *Antennaria dioica, Arctostaphylos uva-ursi, Deschampsia flexuosa, Empetrum nigrum* ssp. *hermaphroditum* and *Solidago virgaurea*. In the deflation basins *D. flexuosa* grew in separated tussocks typically with >1m distance to others (of *D. flexuosa* and *F. ovina)* (Figure 2). Hence, the disposition of the frame was done by choosing a tussock of typical size and placing it in the centre of the frame.

2.5.2. Biomass and mass allocation

To find out the aboveground dry biomass of different species per 1m², in the vegetated dunes a sample the size of 22cm x 22cm was taken inside the point intercept frame from one corner of the frame (Figure 6). The data from the weighing was multiplied with $0{,}0484^{-1}$ to express the biomass per 1m². In one sample there was by mistake no *D. flexuosa* at all, and this sample was not included in the analysis comparing the mean values. This is because the idea is to find out the mean values of biomass in the two phases of succession when *D. flexuosa* is present, or its abundance when it is present, and hence a better estimate of the mean was achieved by leaving the one sample with $0 g/m^2$ out.

In deflation basins a biomass sample was taken from the whole point intercept frame (49,5cm x 49,5cm), because it always contained only one tussock of *D. flexuosa.* Due to sparsity of vegetation, the number of graminoid tussocks was counted from an area the size of 3m x 3m ($9m^2$) around the sample plant. There were only two species of plants in this area, and these were *D. flexuosa* and *F. ovina*. The appearance of the two grasses is quite similar, and I therefore expected that the weight of a *F. ovina* tussock is quite the same as

the weight of a *D. flexuosa* tussock, if they are of the same size. To express the biomass of each species per $1m^2$, the weight of the sample plant (inside the point intercept frame) was multiplied with the number of tussocks of each species inside the area the size of $9m^2$ surrounding the sample plant, and then divided with 9.

From samples all the species of plants and lichens were separated, and from *D. flexuosa* also rhizomes, leaves, flowers and litter, to study the differences in mass allocation (Figure 7). Samples were stored in room temperature, and later in laboratory they were kept overnight in +50º celsius before measuring their weight with a scale on the precision of 0,001mg.

Figure 6. Biomass sample (22cm x 22cm) from a vegetated dune.

Figure 7. Mass allocation of the study plant, *Deschampsia flexuosa*. In the left is the litter, in the centre are the leaves and flower, and in the right are the rhizomes. In the top of the figure are the roots - their biomass was not weighed, but the samples were used to measure the intensity of mycorrhizal colonization.

2.5.3. Percentage of N and $\delta^{15}N$ in plant leaves

Growth in the species poor subarctic forest is limited due to shortage of nutrients. Nitrogen is the nutrient element which most often limits growth of plants in tundra and boreal forest, at the same time as the total N is often high in these soils (Nadelhoffer et al. 1992). Cold, wet soil environments and short summers of the arctic slow organic matter decomposition and nutrient mineralization, and hence severely restrict nutrient availability to plants (Nadelhoffer et al. 1992). Estimates of net nitrogen mineralization by the buried bag technique have shown that the annual release of N in tundra is often only 10% of the annual uptake by the plants, and the net mineralization may often even be negative during the period of growth (Nadelhoffer et al. 1992). Therefore the percentage of N in plant leaves can be considered as a quite direct indicator of performance.

Nitrogen exists as two naturally occuring stable isotopes, ^{15}N and ^{14}N. The ratio of the two isotopes varies in the biosphere as a result of isotope fractionation in physical, chemical and biological processes (Högberg 1997). Variation in the absolute abundance of ^{15}N is small, therefore nitrogen isotope composition is expressed as: $\delta^{15}N$ (‰) = 1000 * (R_{SAMPLE} - $R_{STANDARD}$) * ($R_{STANDARD}$)$^{-1}$, where R = mass 28 /mass 29, and the standard is calibrated against atmospheric N_2, which is 0‰ by definition. $\delta^{15}N$ can provide information on sources and transformations of N in ecosystems (Evans 2001, Robinson 2001). It is especially practical in field studies where it is difficult otherwise to follow the cycling of nutrients. The $\delta^{15}N$ of a system (e.g. a plant, an animal or an ecosystem) reflects the $\delta^{15}N$ of the source, but it is affected also by isotope fractionings during absorbtion, N gains and losses and N pool mixing - therefore the provided information is often indicative rather than definitive (Robinson 2001). In general, discrimination is positive in most biological

systems, therefore the product (e.g. a plant) should have a lower $\delta^{15}N$ value than the substrate (Evans 2001). However under conditions of strong N limitation plants should take up virtually all of the N supplied, which would leave no possibility of a potential discrimination (Nadelhoffer & Fry 1994, Högberg et al. 1999).

Under natural conditions most plants are mycorrhizal, and hence N from the soil is often taken up - not by the plant roots - but through mycorrhizal fungi. Several studies have shown that host plants and mycorrhizal associates differ in their $\delta^{15}N$ values as much as 8‰ (Högberg 1997), and sporocarps of ectomycorrhizal fungi are often enriched in ^{15}N by 5-10‰ relative to their alleged host plants (Taylor et al. 1997, Högberg et al. 1999). This is believed to result either from a) that ectomycorrhizal fungi use sources of N with a high $\delta^{15}N$ that are not used by or transferred to the host plants or b) that ectomycorrhizal fungi become enriched in $\delta^{15}N$, whereas the N passed to the hosts become depleted in ^{15}N relative to source N (Högberg et al. 1999). At all events, $\delta^{15}N$ values of plant tissues are useful markers of the mycorrhizal role in plant nitrogen supply (Michelsen et al. 1996, Michelsen et al 1998, Hobbie & Colpaert 2002). $\delta^{15}N$ of plant tissues is closely correlated with the presence and type of mycorrhizal association, as the $\delta^{15}N$ of ectomycorrhizal or ericoid mycorrhizal plants is 3,5-7.7‰ lower than that of nonmycorrhizal and AM species (Högberg 1990, Michelsen et al. 1998). It has also been found that AM plants have 2‰ lower $\delta^{15}N$ values than nonmycorrhizal plants (Hobbie & Högberg 2012).

The percentage of N and stable isotope analysis was carried out using a Flash EA1112 elemental analyzer (Carbo Erba) connected to a Finnigan $Delta_{plus}$ Advantage (Thermo Electron Corp., Waltham, USA) continuous flow isotope ratio mass spectrometer (CFIRMS).

2.6. Statistical analysis

To seek for meaningful statistical differences between the successional phases, data from percent root length colonized by AM arbuscules, hyphae, vesicles, DSE, fine endophytes and yeasts, total number of hits, biomass, mass allocation, N% in leaves and $\delta^{15}N$ in leaves was explored with PAWS Statistics using Independent samples t-test. To fulfil the prerequisite of normal distribution the data was first tested with Kolmogorov-Smirnov -test. Variables that were not normally distributed were tested with non-parametric Mann-Whitney U-test. Levene´s test was used to test the homogeneity of variances. Linear regression was used to seek for dependency between colonization intensity and $\delta^{15}N$ in plant leaves.

3. RESULTS

3.1. Intensity of mycorrhizal colonization and nutrient N supply

Arbuscular mycorrhizal hyphal colonization was greater in roots of *D. flexuosa* host plants growing in the deflation basins compared to those in the vegetated dunes (Table 1, Figure 8). On the other hand fungal colonization of roots by DSE was greater in the vegetated dunes (Table 1, Figure 8). In addition, $\delta^{15}N$ in *D. flexuosa* leaves was greater in plants growing in vegetated dunes (Table 1, Figure 9). There was no relationship found in between percent root length colonized by AM structures and the $\delta^{15}N$ in leaves, but positive correlation was found to be statistically significant between percent root length colonized by DSE and $\delta^{15}N$ (Table 2). The percent root length colonized by AM arbuscules, AM vesicles, fine endophytes and yeasts did not differ between the two successional phases (Table 1).

Table 1. Results from the comparison of mean values of percent root length colonized (PRLC) by fungal structures (AM= arbuscular mycorrhiza, DSE= dark septate endophytes) and $\delta^{15}N$ in leaves of *Deschampsia flexuosa* host plants growing in the deflation basins versus in the vegetated dunes. The degrees of freedom are 18.

Variable	Test used	t	P
PRLC by AM arbuscules	Mann-Whitney U test	-	0,095
PRLC by AM hyphae	Independent samples t-test	2,757	0,021
PRLC by AM vesicles	Independent samples t-test	2,139	0,054
PRLC by DSE	Independent samples t-test	-5,689	<0,001
PRLC by fine endophytes	Mann-Whitney U test	-	0,133
PRLC by yeasts	Mann-Whitney U test	-	0,655
$\delta^{15}N$ of leaves	Independent samples t-test	-3,537	0,005

Table 2. Results from linear regression analysis between percent root length colonized (PRLC) by fungal structures (AM= arbuscular mycorrhiza, DSE= dark septate endophytes) and $\delta^{15}N$ in leaves of *Deschampsia flexuosa* host plants growing in the deflation basins versus in the vegetated dunes (n=20).

Independent variables	t	Sig.
PRLC by AM arbuscules	0,758	0,460
PRLC by AM hyphae	0,755	0,462
PRLC by AM vesicles	0,443	0,664
PRLC by DSE	2,147	0,049
Dependent variable: $\delta^{15}N$ in leaves		

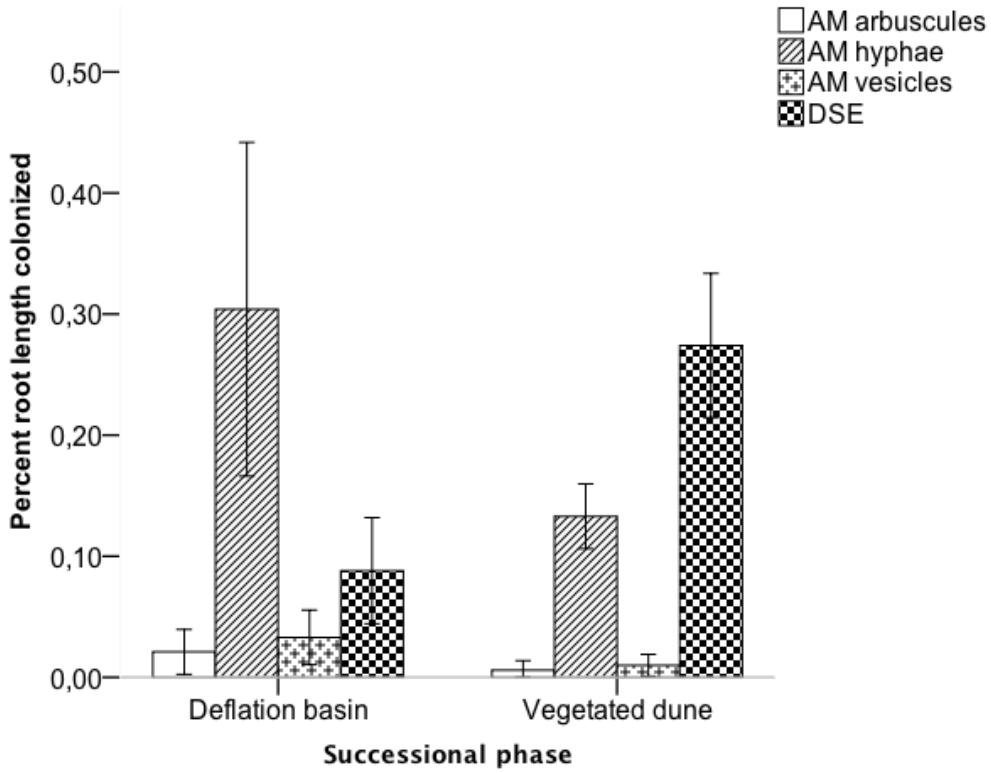

Figure 8. Mean values of percent root length colonized by fungal structures (AM=arbuscular mycorrhiza, DSE= dark septate endophytes) in *Deschampsia flexuosa* host plants growing in the deflation basins and in the vegetated dunes (n=10). Error bars represent 95% confidence interval.

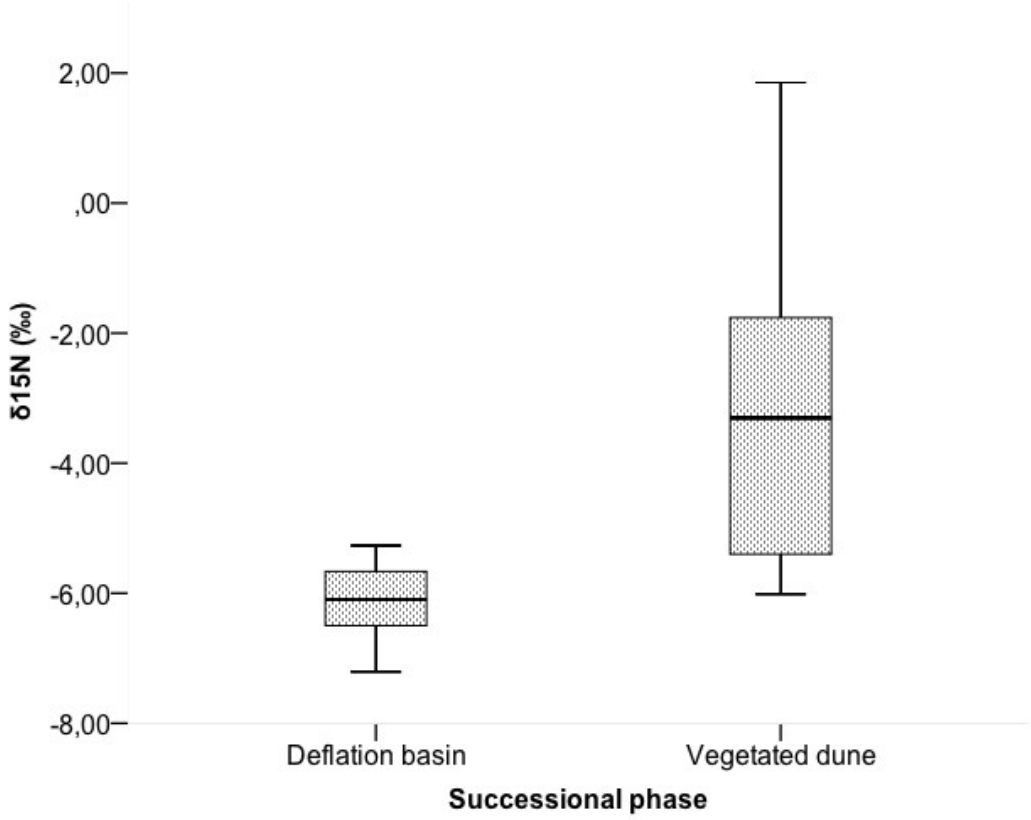

Figure 9. Boxplot showing the median values of $\delta^{15}N$ in leaves of *Deschampsia flexuosa* plants growing in the deflation basins and in the vegetated dunes (n=10).

3.2. Performance of the plant

The total number of hits from the point frequency analysis, and the dry biomass (g/m^2) of *D. flexuosa* were both greater in the vegetated dunes (Table 3, Figure 10, Figure 11). The mass of rhizomes of *D. flexuosa* was greater in the vegetated dunes (Table 3) but there were no statistically significant differences in the masses of leaves and flowers, and litter. *D. flexuosa* plants in the vegetated dunes allocated more in rhizomes than plants in deflation basins, where plants allocated more in flowers and leaves and litter than in the vegetated dunes (Table 3, Figure 12). The percentage of N in the leaves of *D. flexuosa* did not differ between the successional phases (Table 3).

The communities of plants and lichens in the deflation basins and vegetated dunes were dramatically different. The total number of hits, biomass (g/m^2) and number of species of vascular plants, mosses, lichens and litter are presented in the appendices in Table 4 and Table 5 on pages 32 and 33.

Table 3. Results from the comparison of mean values (Independent samples t-test) of total number of hits, dry biomass (g/m^2), dry biomass of leaves and flowers, rhizomes and litter (g/m^2), mass allocation (the percentage of leaves and flowers, rhizomes and litter from the whole biomass of the plant per m^2) and the N% in leaves of *Deschampsia flexuosa* host plants growing in the deflation basins versus in the vegetated dunes. The degrees of freedom are 17-18.

Variable	**t**	**P**
Total number of hits	-6,72	<0,001
Biomass (g/m^2)	-2,364	0,045
Mass of leaves and flowers (g/m^2)	-1,905	0,093
Mass of rhizomes (g/m^2)	-2,440	0,040
Mass of litter (g/m^2)	-2,197	0,059
% of leaves and flowers	3,562	0,002
% of rhizomes	-4,371	<0,001
% of litter	2,994	0,008
N% in leaves	1,97	0,075

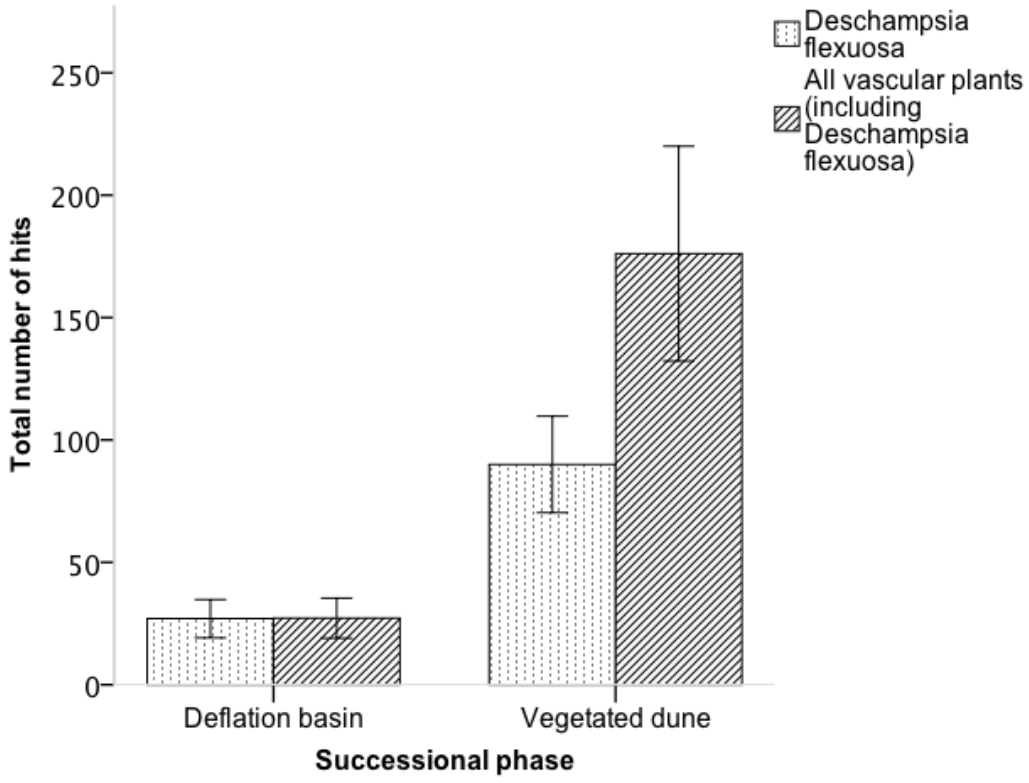

Figure 10. Mean values of total number of hits (from the point frecuency analysis) of *Deschampsia flexuosa* and of all vascular plant species growing in the deflation basins and in the vegetated dunes (n=10). Error bars represent 95% confidence interval.

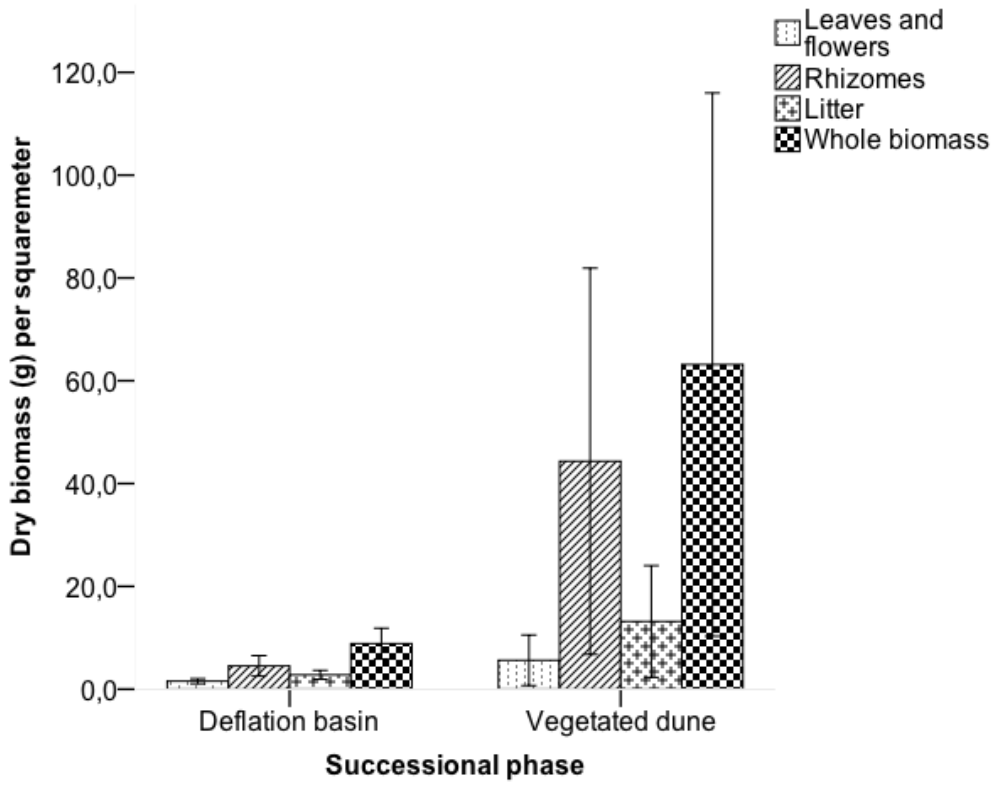

Figure 11. Mean values of dry biomass (g/m^2) of *Deschampsia flexuosa* in the deflation basins and in the vegetated dunes (n=9-10). Error bars represent 95% confidence interval.

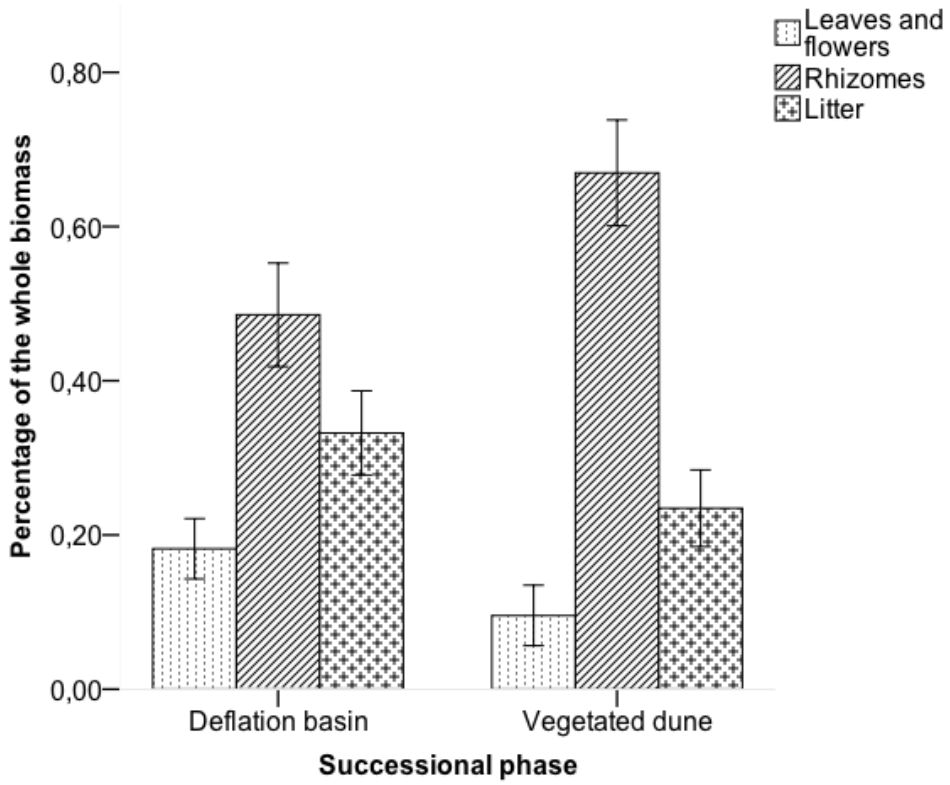

Figure 12. Mass allocation of *Deschampsia flexuosa*: mean values of percentages of flowers and leaves, rhizomes and litter from the whole biomass of the study plant (g/m^2) in the deflation basins and in the vegetated dunes (n=9-10). Error bars represent the 95% confidence interval.

4. DISCUSSION

Percent root length colonized by AM hyphae was greater in roots of *D. flexuosa* host plants growing in the deflation basins (~30%) compared to those in the vegetated dunes (~13%). This is problematic, because from the amount of hyphae it is difficult to know how intense the relationship between the host plant and the mycorrhiza really is. Dead and alive hyphae cannot faithfully be distinguished under a microscope, and in addition fungal hyphae is actually a structure that is most likely not interacting with the host plant. The amount of arbuscules in the roots would be better sign of intense co-operation between the host plant and the AM fungus. The mean values of percent root length colonized by arbuscules and vesicles were both higher in the deflation basins (~2% arbuscules, ~3% vesicles) than in the vegetated dunes (~0,6% arbuscules, ~1% vesicles), but the differences are not statistically significant, albeit they are quite close. On top of this the $\delta^{15}N$ in leaves of *D. flexuosa* was significantly lower in the deflation basins (~-6‰) than in the vegetated dunes (~-3‰), and AM and mycorrhizal plants tend to have lower values of $\delta^{15}N$ than the nonmycorrhizal ones (Högberg 1990, Michelsen et al. 1998, Hobbie & Högberg 2012). All things considered, I conclude that AM infection is more intense in the deflation basins.

Host plants are able to control the rate of infection of their roots by mycorrhizal fungi, and therefore it can be stated that *D. flexuosa* plants benefit from AM fungi in the deflation basins. However, it must be kept in mind that the percent root length colonized of

D. flexuosa roots by AM structures in the field is generally something in between 60 and 80% (Vosatka & Dodd 1998, Zijlstra et al. 2005, Ruotsalainen et al. 2007) and in this respect the intensity of colonization in this study is all in all quite low. At the very least it seems clear that *D. flexuosa* plants benefit more from AM fungi in the deflation basins than in the vegetated dunes, and this is contrary to what was expected. In fact in previous studies, when *D. flexuosa* individuals and their roots have been compared between disturbed and undisturbed environments, the percent root length colonized is found to be lower in disturbed sites (Vosatka & Dodd 1998, Ruotsalainen et al. 2007), and the same trend is also found from other plant species (Chaudry et al. 2009).

What is the benefit of housing AM fungi? The benefit in the deflation basin cannot result from aid in intense competition (because competition is low) in a situation where nutrients are mainly in their organic forms (because, although nutrients must be sparse, they are mainly in their inorganic, easily accessible forms). I have two hypotheses to present. The first one is that AM fungi enable or facilitate the establishment of seedlings, by integrating the emerging seedlings into extensive hyphal networks and by supplying nutrients to them. It is probable, that the establishment is actually the most difficult action for a plant that grows in the deflation basin, and a seedling growing on its own in the fine sand with low water retention capacity must have low initial chances of survival. In a study of Gange et al. (1990) a fungicide was applied in attemp to reduce AM infection of plants during early stages of secondary succession. As a result, total cover of the vegetation (73% of the community were annual forbs) was significantly reduced, and fewer plants species recruited into communities were fungicide was applied (Gange et al. 1990). Furthermore in a microcosm study of van der Heijden (2004) seedlings grew larger and obtained more phosphorus when AM fungi were present. On the other hand it has also been found in some studies that the extraradical mycelium radiating from large plants depresses the growth of nearby seedlings in a nutrient deficient substrate, indicating intra-species competition (Janoušková et al. 2011).

The second hypothesis is that AM fungi increase the capacity of host plants for nutrient acquisition. In a study of Zangaro et al. (2012), in several successional tropical ecosystems in Brazil, it was found that in all ecosystems, soil fertility, fine-root mass and root diameter increased with the succession, while root length, root-hair length, AM colonization and AM spore density decreased. These results suggest that plant species from early phases of tropical succession, with inherent rapid growth, invest in fine roots and maintain a high degree of AM colonization in order to increase the capacity for nutrient acquisition (Zangaro et al. 2012). Conversely, fine root morphological characteristics and low degree of AM colonization exhibited by plants of the later phases of succession lead towards a low nutrient uptake capacity that combine with their typical low growth rates (Zangaro et al. 2012). In the subarctic such a rapid initial growth is unlikely, but it could be that the adult plants growing in the sand with low water retention capacity benefit from their mycorrhiza as the fungal extraradical hyphae effectively catch nutrients and water when they are occasionally available. When it rains, or when reindeers defecate, the effective exploitation of the pulses might be essential for survival. The fluctuation of nutrients and water overall must be greater in the deflation basins compared to the more stabilized vegetated dunes.

AM colonization of *D. flexuosa* roots was higher in the deflation basins, but it is likely that the fungal species composition of the phases differ. Sikes et al. (2012) compared the fungal inoculum of early, intermediate and late phases of sand dune ecosystem, and found that AM fungal communities were phylogenetically different among dune successional phases. AM fungi were phylogenetically diverse in early succession compared to intermediate and late successional phases, but late successional fungi consistently

produced more soil hyphae and arbuscules (Sikes et al. 2012). Despite these differences, inoculum from different successional phases had similar effects on the growth of all plant species, indicating small role in determining plant succession (Sikes et al. 2012). It would be interesting to be able to compare more specifically the communities of AM fungi of the deflation basins to those in the vegetated dunes - in all probability they are very different from each other.

Percent root length colonized by DSE was greater in roots of *D. flexuosa* host plants growing in the vegetated dunes (~27%) than in the deflation basins (~9%). The result is logical, for DSE are commonly described as saprobes, meaning that they are capable of living on organic nutrient sources, and the amount of organic material in the deflation basins is extremely low compared to the vegetated dunes. Moreover, the percent root length colonized by DSE has also been found to be lower at disturbed sites compared to undisturbed sites (Chaudhry et al. 2009). This, however, raises a question that cannot be solved in this thesis, but which is actually quite interesting: How can a saprobe fungus accustomed to organic soil live in fine sand at all? Could it be that in the deflation basin the strategy favoured by the DSE fungus is actually a mutualistic symbiosis with our study grass? It is known that at least under some conditions, DSE are capable of forming mutualistic associations with plants functionally similar to mycorrhizas (Jumpponen 2001). It would be interesting to know if the species composition of DSE are the same in the deflation basins and in the vegetated dunes.

There was a slight statistically significant correlation between DSE colonization in roots and $\delta^{15}N$ in leaves of *D. flexuosa*. In all likelihood this is a result from two separate correlations of these factors with the successional phase. The high $\delta^{15}N$ in leaves in the vegetated dunes is probably the result of low mycorrhizal dependency in nutrient N supply, and DSE colonization in turn is most likely higher in the vegetated dunes because of the high amount of organic material in the soil. However, if there actually exists a correlation between these two, it is quite interesting. Then the "host plant" would not acquire nutrients throught the DSE fungus, or any fungus at all, when DSE colonization is intense in its roots. Instead when DSE are present, the plant takes up its own nutrients from the soil with its own roots. It seems logical that it can do so - since as saprobes DSE fungi break down organic material in the rhizophere.

Was the performance of the host plant better in the deflation basins or in the vegetated dunes? According to the total number of hits and the dry biomass (g/m^2), the performance of *D. flexuosa* was clearly better in the vegetated dunes (~90 hits, ~63g) than in the deflation basins (~27 hits, ~9g). There was, however, no statistically significant difference in the percentage of N in leaves, but the mean was a bit higher in the deflation basins (~1,5%) than in the vegetated dunes (~1,3%).

There were differences in mass allocation in the successional phases: in the vegetated dunes *D. flexuosa* plants allocated ~10% of their mass in leaves and flowers, ~67% in rhizomes and ~23% of their mass was litter. In the deflation basins *D. flexuosa* plants allocated ~18% of their mass in leaves and flowers, ~49% in rhizomes and ~33% of their mass was litter. All three differences were statistically significant. Since *D. flexuosa* is a perennial plant, it can be expected that the amount of flowers and green leaves that are able to photosynthetise and produce seeds are a sign of performance during this growth season. So, in this respect, it seems that the host plants in the deflation basins are performing better - they allocate less on storage structures (rhizomes) than their neighbours in the vegetated dunes. This correlates with field observations, for the grasses in the deflation basins did seem strikingly green, and their leaves were tall and thick, compared to those in the vegetated dunes. Reason for this might be the lack of competition in the deflation basins, but it must be kept in mind that in the subarctic plants do not usually compete for light or

space: they compete for nutrients in the soil. If AM fungi in deflation basins aid in catching the occasional fluxes of nutrients (rain, urine and faeces of reindeers), it would help to explain the relatively good performance of the study plants in the deflation basins compared to the vegetated dunes. To conclude, the performance of the host plant was not indisputably better in either of the phases of succession, which is altogether surprising, as the deflation basins are an extremely hostile environment for a plant to grow in.

Unfortunately it is impossible to conclude anything about the allocation of biomass in the roots - it was fairly easy to collect almost the entire root system of the plant in the deflation basins, because there were no other plants and the fine sand was easy to dig, but impossible to do the same in the vegetated dunes, because of the organic soil and all the other roots of other plants. It might be safe to state, however, based on a visual estimate, that the amount of roots in relation to dry biomass per squaremeter seemed to be higher in the deflation basins. This is intuitive, because in the sand plants need a large amount of roots to effectively scavenge for nutrients, that are drastically sparse in the fine sand. In the vegetated dunes, in contrast, competition for nutrients is intense everywhere in the rhizosphere, and therefore the advantages of a large root system are more difficult to think of.

To conclude, the colonization of roots by AM fungi was more intense in the disturbed deflation basins of early succession than in the more stabilized vegetated dunes, and therefore, the results of this thesis are opposite to many previous studies. The performance of the study plant was not indisputably better in either of the phases of succession. The results of this thesis force us to widen our conception of the ecological role that mycorrhiza play in ecosystems and in their formation - their role is not simple but intricate.

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APPENDICES

Table 4. Mean values (and the standard error of the mean) of total number of hits (from the point frequency analysis), aboveground dry biomass (g/m²) and the number of species of vascular plants, mosses, lichens and litter in the deflation basins and in the vegetated dunes (n=10).

	Vascular plants	**Mosses**	**Lichens**	**Litter**
Total number of hits				
Deflation basin	27,2 ± 3,63	0	0	1,4 ± 1,0
Vegetated dune	176,1 ± 19,41	41,6 ± 6,82	100,9 ± 9,27	68,4 ± 6,82
Biomass g/m²				
Deflation basin	10,52 ± 1,35	0	0	0
Vegetated dune	131,99 ± 31,04	ND	ND	95,54 ± 45,34
Number of species				
Deflation basin	1,1 ± 0,1	0	0	-
Vegetated dune	7,00 ± 0,33	2,4 ± 0,22	8,90 ± 0,82	-

Table 5. The mean values (and the standard error of the mean) of total number of hits (from the point frequency analysis), aboveground dry biomass (g/m^2) of vascular plant species and groups of species in the deflation basins and in the vegetated dunes (n=10).

Vascular plant species and groups	**Total number of hits**		**Biomass g/m²**	
	Deflation basin	Vegetated dune	Deflation basin	Vegetated dune
Forbs	0	16,4 ± 2,86	0	5,65 ± 2,3
Antennaria dioica	0	9,2 ± 2,31	0	3,34 ± 2,32
Hieracium alpina (coll.)	0	0,10 ± 0,10	0	0
Solidago virgaurea	0	7,1 ± 1,28	0	2,31 ± 0,49
Graminoids	27,2 ± 3,63	115 ± 13,53	10,58 ± 1,11	71,14 ± 21,68
Deschampsia flexuosa	27 ± 3,46	90 ± 8,71	8,99 ± 1,04	56,84 ± 21,45
Festuca ovina	0,2 ± 0,2	22,2 ± 6,13	1,6 ± 0,81	14,3 ± 3,14
Juncus trifidus	0	2,8 ± 2,7	0	0
Dwarf shrubs	0	43,3 ± 7,09	0	53,78 ± 13,41
Arctostaphylos uva-ursi	0	1,3 ± 0,94	0	0
Empetrum nigrum ssp. *hermaphroditum*	0	25,4 ± 6,5	0	33,04 ± 12,81
Linnaea borealis	0	4,5 ± 2,37	0	2,93 ± 2,45
Vaccinium vitis-idaea	0	12,1 ± 3,26	0	17,81 ± 6,11
Club-mosses	0	1,4 ± 1,4	0	1,42 ± 1,42
Diphasiastrum complanatum ssp. *montellii*	0	1,4 ± 1,4	0	1,42 ± 1,42

Table 6. The common plant and lichen species of the inland sand dune ecosystem. Nomenclature follows Hämet-Ahti & Hackman (1998) for vascular plants, Hallinbäck & Holmåsen (1985) for mosses and Stenroos et al. (2011) for lichens. Mycorrhizal status of vascular plants from Wang & Qiu (2006). Mycorrhizal types according to widely used system of Smith & Read (2008): AM arbuscular mycorrhiza, ABM arbutoid mycorrhiza, ECM ectomycorrhiza, EEM ectendomycorrhiza, ERM ericoid mycorrhiza, NM nonmycorrhizal. *Species has not been studied, mycorrhizal status taken from closely related species.

	Common plant and lichen species	
	Vegetated dune	**Deflation basin**
Trees and bushes	*Betula pubescens* (ECM, EEM) ssp. *czerepanovii* *Juniperus communis* AM, ECM *Salix* ssp. AM, ECM, EEM, NM	-
Upper understory	*Antennaria dioica* AM *Arctostaphylos alpina* ABM,ERM *Arctostaphylos uva-ursi* ABM, ECM, EEM, ERM *Calluna vulgaris* ERM *Deschampsia flexuosa* AM *Diphasiastrum complanatum* (NM) ssp. *montellii* *Empetrum nigrum* (ERM) ssp. *hermaphroditum* *Festuca ovina* AM,NM *Hieracium alpina* (coll.) (AM,NM)* *Juncus trifidus* AM *Linnaea borealis* AM *Lychnis alpina* (AM,NM)* *Lycopodium annotinum* (AM) ssp. *alpestre* *Solidago virgaurea* AM *Vaccinium uliginosum* ERM *Vaccinium vitis-idaea* ERM	*Deschampsia flexuosa* AM *Equisetum arvense* AM, NM *Festuca ovina* AM, NM *Leymus arenarius* AM, NM *Luzula spicata* AM
Ground layer, mosses:	*Dicranum* ssp., *Pleurozium schreberi, Pohlia nutans, Polytrichum juniperinum, Polytrichum piliferum, Ptilidium ciliare*	*Polytrichum* ssp.
Lichens:	*Alectoria ochroleuca, Cetraria islandica* subsp. *islandica, Cetraria muricata, Cladonia amaurocreae, Cladonia arbuscula* subsp. *squarrosa, Cladonia carneola, Cladonia coccifera, Cladonia cornuta* subsp. *cornuta, Cladonia crispata* var. *crispata, Cladonia deformis, Cladonia fimbriata, Cladonia furcata, Cladonia gracilis* subsp. *elongata, Cladonia maxima, Cladonia mitis, Cladonia pleurota, Cladonia rangiferina, Cladonia stellaris, Cladonia stygia, Cladonia uliginosa, Cladonia uncialis* subsp. *biuncialis, Cladonia uncialis* subsp. *uncialis, Flavocetraria nivalis, Flavocetraria cucullata, Nephroma arcticum, Peltigera aphthosa, Peltigera malacea, Solorina crocea, Stereocaulon alpinum* var. *alpinum, Stereocaulon paschale*	