

**MYCORRHIZAL COLONIZATION
AND PLANT PERFORMANCE
IN ARCTO-ALPINE CONDITIONS**

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Abstract

Mycorrhizal symbiosis is generally advantageous for plants in nutrient-poor soils. Arcto-alpine areas are relatively nutrient-poor, but abundantly inhabited by non-mycorrhizal species. Possibly, mycorrhizal symbiosis is not favoured due to the harsh climatic conditions and the short growing season, which constrain the photosynthetic gain and growth of the arcto-alpine plants. This hypothesis was theoretically evaluated by assuming that optimal mycorrhizal colonization maximizes the net carbon gain of the host plant. In addition, the prevalence of arbuscular mycorrhizal (AM) and dark-septate endophytic (DSE) fungi along an altitudinal gradient was studied in the field, and their effects on the plant performance were tested in the laboratory.

In the model, the photosynthetic nutrient use efficiency (PNUE) had a key role in determining whether mycorrhizal strategy would be optimal for the plant net carbon gain. The model generated several colonization patterns depending on possible changes in PNUE and soil nutrient concentrations along altitudinal gradients. Field studies indicated that species-level colonizations do not yield a consistent pattern along the altitude except for fine endophyte, which increased along an altitudinal gradient. In a high-alpine field site root fungal colonizations were rare. Seasonal shifts in colonizations in low-alpine conditions were not found. DSE fungi were common root-associates in the field. In the laboratory, AM had a positive impact on the performance of *Gnaphalium norvegicum* at 15°C, but not at 8°C. DSE-inoculation did not colonize the roots, but it had a positive impact on seedling performance, which may be due to the saprophytic activity of the fungus in the substrate. Additionally, mycorrhizal inoculum was found to decrease the performance of a non-mycorrhizal plant in a competition experiment.

Species-level mycorrhizal colonization patterns may differ from community-level pattern along altitudinal gradients and the relative abundance of different fungal symbionts may change along with the altitude. The performance of mycorrhizal plants in high-alpine conditions may be decreased due to several factors e.g. low temperature constraints on plant and fungal physiology and allocation, soil disturbances and low availability of inoculum. Climatic constraints for plant photosynthesis may thus affect the mycorrhizal colonization patterns in arcto-alpine conditions, but are not necessarily the primary cause for lower performance of mycorrhizal plants at higher altitudes.

Keywords: alpine ecology, arbuscular mycorrhiza, benefit, cost, dark-septate endophytes

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Oulu, March 2003

Annu Ruotsalainen

Abbreviations

AM	arbuscular mycorrhiza
DSE	dark-septate endophytes
FE	fine endophyte
HSE	hyaline septate endophytes
PNUE	photosynthetic nutrient-use efficiency (CO_2 nutrient $\text{unit}^{-1} \text{time}^{-1}$)
SLA	specific leaf area ($\text{cm}^2 \text{mg}^{-1}$)

List of original papers

This thesis is based on the following publications, which are referred to in the text by their roman numerals.

- I Ruotsalainen AL, Tuomi J & Väre H (2002) A model for optimal mycorrhizal colonization along altitudinal gradients. *Silva Fennica* 36: 681–694.
- II Ruotsalainen AL, Väre H, Oksanen J & Tuomi J (2003) Root fungal colonizations along an altitudinal gradient. Submitted manuscript.
- III Ruotsalainen AL, Väre H & Vestberg M (2002) Seasonality of root fungal colonization in low-alpine herbs. *Mycorrhiza* 12: 29–36.
- IV Ruotsalainen AL & Aikio S (2003) Competition between mycorrhiza-forming *Trientalis europaea* and non-mycorrhizal *Carex bigelowii*. Manuscript.
- V Ruotsalainen AL & Kytöviita M-M (2003) Ecophysiology of *Gnaphalium norvegicum*: impact of fungal inoculation at two temperatures. Manuscript.

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1 Introduction

1.1 Mycorrhizal symbiosis

Most plants in natural ecosystems form mycorrhiza with soil fungi. This form of symbiosis, which is considered to be beneficial to both partners, has co-evolved in the course of several hundred million years since the first land plants appeared (Simon *et al.* 1993, Remy *et al.* 1994, Taylor *et al.* 1995, Selosse & Le Tacon 1998). In mycorrhizal symbiosis the host plant translocates photosynthates to its fungal symbiont and the fungus translocates nutrients and water to the plant. Thus, the nutritional benefit due to mycorrhizal associations for the host plants simultaneously implies a carbon cost (e.g. Jakobsen & Rosendahl 1990, Fitter 1991, Jones *et al.* 1991). The majority of the plant kingdom have mycorrhiza, but certain taxonomic units, families such as Brassicaceae, Caryophyllaceae, Cyperaceae and Juncaceae are largely non-mycorrhizal (Newman & Reddell 1987, Trappe 1987, Peat & Fitter 1993). These families are very common in arcto-alpine habitats. Mycorrhizas can be categorized into seven main groups according to their morphology and on the basis of the fungal and plant taxa forming the symbiosis. In general, herbs and grasses are colonized by arbuscular mycorrhizal (AM) fungi from the phylum Glomeromycota (Schüssler *et al.* 2001), certain tree families such as Betulaceae, Fagaceae, Pinaceae and Salicaceae by ectomycorrhizal fungi (mainly Agaricales, Basidiomycetes) and ericaceous shrubs by ericoid mycorrhizal fungi (Ascomycetes and Fungi imperfecti). Other mycorrhizal types are arbutoid, orchid, monotropoid and pyrolid mycorrhizas, where fungal partners are Basidiomycetes (Smith & Read 1997). In addition to mycorrhizal fungi, roots are commonly associated with other endophytic, parasitic and in old roots also saprophytic fungi. One group, dark-septate endophytes (DSE) has been found to elicit also mutualistic responses in the host plants in a few experiments (Haselwandter & Read 1982, Fernando & Currah 1996, Jumpponen *et al.* 1998). The DSE-complex is highly heterogeneous including several taxa which probably belong to the Ascomycetes (Carlsen 2002, Vrålstad *et al.* 2002).

The primary importance of mycorrhiza for vascular plants is the enlarged nutrient absorption surface provided by fungal hyphae which enhances the nutrient absorption capacity of the roots (Koide 1991), but mycorrhiza can also considerably enhance the

nutrient mobilisation ability from organic and inorganic sources (Mitchell & Read 1981, Abuzinadah & Read 1986a,b, 1988, Northup *et al.* 1995, Michelsen *et al.* 1996, Näsholm *et al.* 1998). Generally, vascular plants absorb inorganic nutrients from soil solution, but mycorrhizal symbiosis may provide access to additional nutrient sources through hyphal enzyme activity or by other physical or chemical modification of the rhizosphere (Marschner 1995). Mycorrhizal types have considerable differences in their ability for nutrient uptake from organic sources and the capacity of mycorrhizal fungi to maintain their energy requirements without the connection to host plant is also different among the mycorrhizal types (Smith & Read 1997). Ericoid mycorrhizal fungi are known to possess e.g. cellulases, ligninases, proteinases and phenol oxidases (Bending & Read 1997, Burke & Cairney 2002), which gives them a relatively good ability to break down complex molecules in the soil and satisfy their carbon and nutritional requirements this way. In the case of ectomycorrhizal fungi, the saprotrophic activity is present, but lower than in ericoid mycorrhiza (Bending & Read 1997). Arbuscular mycorrhizal fungi are obligate biotrophs, which means that the growth and reproduction of the fungus is not possible without a connection to the host plant (Smith & Read 1997). It has recently been shown that also AM fungi have at least some ability to utilize organic nutrients (Koide & Kabir 2000, Hodge *et al.* 2001).

Maintenance of mycorrhizal fungi implies a considerable carbon cost for the plant in terms of the relative investment of photosynthates to the maintenance of the symbionts. Arbuscular mycorrhizal plants have been estimated to allocate 4–20% more photosynthates to mycorrhizal roots compared to non-mycorrhizal roots (Jakobsen & Rosendahl 1990, Smith & Read 1997), and in ectomycorrhizal plants the mycorrhizal roots have been found to consume as much as 2.5 times more carbon than the non-mycorrhizal roots (Jones *et al.* 1991).

Mycorrhizal symbiosis can be either obligatory or facultative for the plants (Trappe 1987). Functionally all other types except AM are obligate, i.e. plant reproduction cannot be completed without a fungal partner at some stage of the life cycle. Facultatively mycorrhizal vascular plants provide a tool to study conditions, which favour mycorrhizal and non-mycorrhizal strategy. For example, plants of alpine habitats are often facultatively mycorrhizal (Trappe 1988). Due to high nutrient uptake capacity, it could be expected that mycorrhiza would be favoured in nutrient poor soils. In general this is the case (Smith & Read 1997), but certain deviations exist. Non-mycorrhizal species have successfully colonized extreme habitats, such as primary soils (e.g. land upheaval areas, deglaciated terrain), rocks and saline soils (Allen & Cunningham 1983, Allen *et al.* 1989, Peat & Fitter 1993), ruderate landscapes (Allen 1989, Peat & Fitter 1993) and arcto-alpine areas (e.g. Trappe 1988, Väre *et al.* 1992, 1997).

1.2 Arcto-alpine conditions

Alpine vegetation exists above the natural high altitude treeline. Even though the word originates from the Alps, it is applied worldwide. Arctic and alpine vegetation have many common characteristics, and these terms are often combined (Euroala 1974, Gardes & Dahlberg 1996). In the higher latitudes, e.g. in northern Fennoscandia, alpine areas merge with the arctic zone (Ahti *et al.* 1968, Euroala 1974, Oksanen & Virtanen 1995). The

amount of solar radiation decreases towards higher latitudes and simultaneously the annual fluctuations become stronger. Consequently, the altitudinal lower limit of the alpine belt differs between latitudes. The treeline is at about 600 m a.s.l. in northern Scandinavia and in above 2000 m a.s.l. in the Alps (Körner 1999). In the literature, the term alpine is sometimes replaced by the term oroarctic when northern Fennoscandian mountain vegetation is discussed, which emphasizes the arctic characteristics of these environments (e.g. Ahti *et al.* 1968, Euroala 1974, Haapasaari 1988, Oksanen & Virtanen 1995, Väre *et al.* 1997).

The alpine zone can be divided in low-, middle-, and high-alpine belts based on the prevailing vegetation (e.g. Kalliola 1939, Hedberg 1952, Euroala 1974, Haapasaari 1988, Oksanen & Virtanen 1995). In the low-alpine belt the vegetation cover is continuous, in the middle-alpine belt more patchy and in high-alpine belt all vegetation occurs scattered among snow patches, stone and polygon fields (Hedberg 1952). The plant species composition differs between the alpine belts. Within these relatively roughly described belts vegetation is heterogeneous for example due to exposition and the steepness of the slope.

‘Alpine gradient’ refers to changes that can be observed along with increasing altitudes. Globally, along the alpine gradients a consistent ecological factor is the decrease of temperature, which is 0.6°C per 100 m of altitude (adiabatic lapse rate) (Lamb 1972, Körner 1999). This is mostly due to the decrease in energy flow with altitude. Energy is carried with convection and radiation from the Earth surface (Lamb 1972). In addition to the direct impacts on the alpine plants and their physiology, temperature affects the rain, wind and soil conditions (e.g. freeze-thaw cycles) at high altitudes. The exposition, macro- and microtopography significantly modify these large-scale climatic effects (Körner 1999). Another global characteristic is the decline in atmospheric pressure with increasing altitudes (Lamb 1972). Solar radiation is stronger at higher altitudes, but this effect is counteracted by more cloudy conditions. Precipitation tends to increase with increasing altitude especially in extratropical mountains (Körner 1999).

1.2.1 Adaptations to low temperature and air pressure

The effects of temperature on the plant growth can be divided into both direct (e.g. effects on enzymatic processes) and indirect ones (impacts via soil microbial activity and nutrient availability, rain and wind conditions). Temperature conditions crucially affect all enzymatic processes, like photosynthesis, nutrient uptake and growth in plants (Larcher 1995). Along altitudinal and latitudinal gradients the decreasing mean temperatures slow down these processes, but the arctic and alpine plants are also highly adapted to live in low temperatures (Bliss 1971, Morecroft & Woodward 1990, 1996, Körner 1999, Weih & Karlsson 1999). These adaptations may include morphological changes such as specific growth forms (cushion and tussock plants) and shifts in leaf morphology and physiology (Körner *et al.* 1989). Alpine plants, for example, have lower photosynthesis temperature optima and higher photosynthetic capacity per leaf area than low-altitude plants (Berry & Björkman 1980, Körner & Diemer 1987). In the laboratory, low temperature has been found to increase leaf thickness and nitrogen concentration (Woodward 1979, Morecroft

& Woodward 1996). Decreasing atmospheric pressure implies decreasing CO₂ partial pressure along the altitude (Lamb 1972). Plants also cope with this decrease by enhancing their photosynthetic systems by increasing the thickness of leaf mesophyll and maintaining higher nitrogen concentration in the leaves, which increase carbon fixation (Körner & Diemer 1987, Morecroft & Woodward 1990, 1996).

High tissue nutrient concentration, 'luxury consumption', is typical of arcto-alpine plants (Chapin 1980). It is probably related to slow growth rates and temporally variable availability of nutrients in these conditions (Chapin 1980, Weih & Karlsson 1999). The plant tissue nutrient concentrations have also been found to increase along with increasing altitude (worldwide review by Körner 1989). Due to high nutrient and also high non-structural carbon levels in the arcto-alpine plant tissues it has been proposed that plant growth in these environments would not be primarily limited by these resources, but direct low temperature effects on the growth processes at cellular level (Körner 1999, Hoch *et al.* 2002). By contrast, Weih & Karlsson (1999, 2001) have emphasized that high tissue nutrient levels imply an active physiological and possibly genetical adaptation to cold climates and, despite the unfavourable conditions for nutrient uptake, arcto-alpine plants maintain relatively high nutrient uptake rates.

1.2.2 Soil nutrient availability in arcto-alpine conditions

Low nutrient availability is typical of alpine and arctic soils (Chapin 1980, Kielland & Chapin 1992, Körner 1999). This is due to slow decomposition and mineralization of organic matter at low temperatures (e.g. Rehder & Schäfer 1978, Schmidt *et al.* 1999), which also results in a relative increase of organic matter along with increasing altitude (Väre *et al.* 1997, Körner 1999). Spatial heterogeneity is typical for soil nutrient concentrations (Marschner 1995), but decreasing nutrient gradients along with increasing altitude may exist (Kyllönen 1988, Väre *et al.* 1997). Nitrogen availability may especially decline along altitude because it is highly correlated with the amount of organic matter and mineralization rate, which both decrease along with the altitude (Väre *et al.* 1997, Körner 1999, Kernaghan & Harper 2001). However, at highest altitudes soil physical disturbances may be intense, which may locally create relatively high nutrient patches (Jonasson & Sköld 1983).

Due to the slow mineralization, the ability of direct utilization of the organic matter could be an useful trait. Some arctic sedges are able to this (Kroehler & Linkins 1991, Chapin *et al.* 1993, Kielland 1994). Additionally, such mycorrhizal symbionts that could short-circuit nutrients this way could be of high value for their host plants. As presented above (Chapter 1.1) especially ericoid mycorrhizal plants have this capacity, but ectomycorrhizal plants and also arbuscular mycorrhizal plants only to a limited extent. DSE-fungi possess a wide range of enzymes needed for relatively efficient utilization of organic matter (Currah & Tsuneda 1993, Caldwell *et al.* 2000). Therefore, if these fungi would behave mutualistically (Haselwandter & Read 1982, Jumpponen *et al.* 1998) this could be advantageous for the associated plants.

1.3 Mycorrhizas and DSE in arctic and alpine areas

All the main mycorrhizal types can be found in arctic and alpine areas (Väre *et al.* 1992, Gardes & Dahlberg 1996, Körner 1999). Below the treeline ectomycorrhizal symbiosis is common in trees and shrubs, and above the treeline ectomycorrhizal fungi are hosted by *Dryas octopetala*, *Betula nana*, *Salix* spp and by herbaceous plants such as *Kobresia* spp. and *Polygonum viviparum* (Massicotte *et al.* 1998). Ericaceous shrubs are also obligatorily mycorrhizal, and they are commonly found in low- and middle-alpine zones (like *Cassiope* spp., *Empetrum* spp., *Loiseleuria procumbens*, *Phyllodoce* spp., *Vaccinium* spp.). Arbutoid mycorrhiza is regionally common depending on the abundance of *Arctostaphylos alpina*. Arbuscular mycorrhiza is also common, but it appears that the species or genera capable of forming AM are often found to be less colonized or even non-mycorrhizal at higher altitudes and latitudes when compared to lower ones (Haselwandter & Read 1980, Read & Haselwandter 1981, Christie & Nicolson 1983, Bledsoe *et al.* 1990, Kohn & Stasovski 1990, Väre *et al.* 1992, Gardes & Dahlberg 1996). However, it must be emphasized that AM propagules and root colonizations can also be found in high-alpine zone (Haselwandter & Read 1980, Mullen & Schmidt 1993) and from the high arctic (e.g. Dalpé & Aiken 1998) even when they may be rare and possibly the colonization is formed by specific fine endophyte-type (Haselwandter & Read 1980). The relative coverage of non-mycorrhizal plants has been found to increase along with increasing altitude (data of Oksanen & Virtanen 1995 in Väre *et al.* 1997). This is mainly due to the relative increase of families such as Brassicaceae, Caryophyllaceae, Cyperaceae and Juncaceae (Trappe 1987, Trappe 1988, Gardes & Dahlberg 1996, Väre *et al.* 1997), which are generally found to be non-mycorrhizal (Smith & Read 1997, but see e.g. Miller *et al.* 1999). Orchids are not rare in arctic and alpine floras and thus, orchid mycorrhizas probably exist, because the germination and seedling development of orchids is dependent on mycorrhizal partners (Smith & Read 1997).

There are partly conflicting results about the shifts in mycorrhizal colonizations along the altitudinal gradient (Haselwandter 1979, Haselwandter & Read 1980, Read & Haselwandter 1981, Väre *et al.* 1997). Ecto- and ericoid mycorrhiza are obligatory for their host plants, which means that plants forming these symbioses are always mycorrhizal in natural conditions. However, Haselwandter (1979) and Haselwandter and Read (1980) have detected that mycorrhizal colonizations clearly decrease along with increasing altitude in ericoid mycorrhizal *Vacciniums* in the Alps. Opposite to that, Väre *et al.* (1997) have found increasing colonization patterns among some ericoid mycorrhizal plants in Finnish Lapland. Ectomycorrhizal *Salix herbacea* showed a constant colonization along with increasing altitude (Väre *et al.* 1997). Decreasing, constant and intermediate colonization patterns have been reported between arbuscular mycorrhizal plant communities (Haselwandter & Read 1980, Read & Haselwandter 1981, Väre *et al.* 1997), even though a general shift towards a non-mycorrhizal community seems to be the rule when the highest altitudes for vascular plant life are considered (Haselwandter & Read 1980, Read & Haselwandter 1981, Trappe 1987). However, shifts of mycorrhizal colonizations along with altitude at the species-level are rarely studied, which is probably due to the limited altitudinal distributions of the plant species.

The possible seasonal shifts in mycorrhizal colonizations may also influence the interpretation of the root colonization studies carried out in the field. Mullen and Schmidt

(1993) and Mullen *et al.* (1998) found seasonal changes in root colonization by AM and DSE-fungi in high-alpine conditions. Also Baikalova and Onipchenko (1988) found seasonal trends in AM in alpine conditions. These changes could follow intensive periods of the plant phosphate and nitrogen uptake during the peaks of their availability in the soil (Chapin & Bloom 1976, Chapin *et al.* 1978, Jaeger & Monson 1992, Kielland & Chapin 1992, Mullen & Schmidt 1993, Mullen *et al.* 1998). In the arbuscular mycorrhiza the root fungal structures are thought to have relatively short life-spans (Smith & Read 1997). Thus, the maintenance of active AM-colonization could be connected to the nutrient uptake (especially phosphate) and possible phosphate availability peak in the soil in alpine conditions (Mullen & Schmidt 1993).

Dark-septate endophytes are ubiquitous in arctic and alpine environments (Haselwandter & Read 1980, Currah & Van Dyk 1986, Bledsoe *et al.* 1990, Väre *et al.* 1992, Carlsen 2002). They may also become more common with increasing altitude (Haselwandter & Read 1980, Read & Haselwandter 1981). The real nature of DSE is obscure so far. DSE colonizations in roots possibly include both mutualistic fungal species, but also weak parasites (reviewed by Väre *et al.* 1992, Jumpponen & Trappe 1998). Recent studies show that taxa belonging to the DSE-complex may include the same taxa that form ericoid — and ectomycorrhiza (Vrålstad *et al.* 2002). Increased growth response and phosphorus uptake when colonized by DSE has been detected by Haselwandter and Read (1982), Fernando and Currah (1996) and Jumpponen *et al.* (1998). Haselwandter and Read (1982) and Mullen *et al.* (1998) have posed the question that DSE could somehow adopt the mycorrhizal function in the alpine conditions.

A specific fine-endophyte-type AM colonization (FE) may be especially common in high altitude conditions (Crush 1973b, Haselwandter & Read 1980). This rarely mentioned colonization type has been originally described from New Zealand (Hall 1977), and reported from several places worldwide (e.g. Haselwandter & Read 1980, Christie & Nicolson 1983, Bledsoe *et al.* 1990, Mullen & Schmidt 1993). It is unclear if this colonization type represents one fungal species *Glomus tenuis* or several species (Abbott 1982, Brundrett *et al.* 1996). Fine endophyte has been, however, found to be beneficial for its host plants in especially low nutrient conditions (Crush 1973b, Rabatin 1979), which could fit with its possible abundance in generally nutrient poor arctic and alpine areas. It may be that there are shifts in colonization types from coarse AM types to FE along with the altitude (Haselwandter & Read 1980).

1.4 Aims of this study

The aims of this study were (i) to propose and critically evaluate hypotheses for explaining performance of mycorrhizal and non-mycorrhizal plants along altitudinal gradients, (ii) to analyse theoretically environmental factors that may constrain optimal mycorrhizal colonization in plant roots and (iii) to empirically study the colonization patterns in arcto-alpine conditions and test the factors affecting mycorrhizal colonization and mycorrhizal benefit for plant performance, both in the laboratory and in the field.

On the basis of these aims the following specific questions were addressed:

1. Do root fungal colonizations (AM and DSE) have plant species-level changes along an altitudinal gradient? (II)
2. In which way could climatic, nutrient and competitive factors interact with the mycorrhizal status of plants and what kind of role DSE-fungi play? (I, IV, V)
3. Does fungal (AM and DSE) colonization change through the growing season in low-alpine conditions? (III)

Two basic factors that were considered at the start of this project were soil nutrient concentration and photosynthesis, because mycorrhizal symbiosis: (i) enhances plant nutrient uptake by e.g. providing larger nutrient absorption surface and (ii) causes an additional carbon cost due to hyphal respiration and the construction costs of the hyphal network.

Consequently, my first working hypothesis was that climatic factors would determine the success of mycorrhizal plants mainly by affecting the photosynthetic yield of plants. As plants invest considerable amounts of energy on mycorrhizal fungi, it was predicted that the energy allocation to fungi would become too expensive for a plant beyond a certain climatic point. Therefore, it was predicted that the performance (growth, survival or reproductive output) of mycorrhizal plants would decrease relative to non-mycorrhizal plants along altitudinal gradients, as described in Fig. 1.

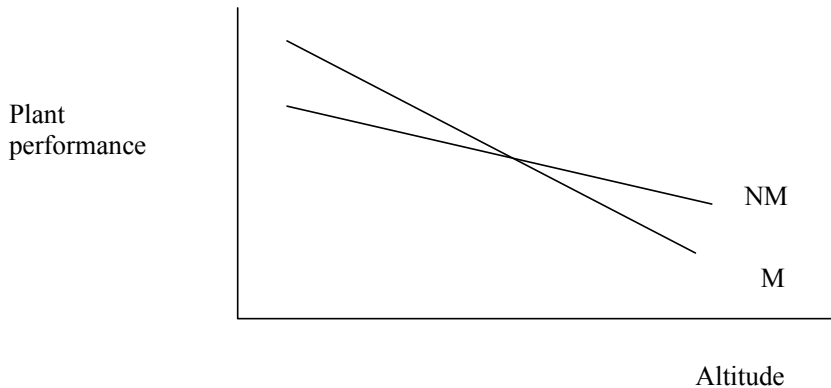


Fig. 1. A schematic picture of the hypothesis that the performance of the mycorrhizal plants decreases more steeply towards higher altitude compared to non-mycorrhizal plants. M = mycorrhizal, NM = non-mycorrhizal.

The hypothesis was studied by modelling and by carrying out experiments in controlled and field conditions. First, a model of optimal mycorrhizal colonization along with increasing altitude was constructed (I). The aim of this theoretical work was to analyze how the colonization pattern might change from a mycorrhizal to non-mycorrhizal condition or vice versa when PNUE and soil nutrient levels change along with environmental gradients. Second, a field study along a long altitudinal gradient was conducted to see if AM and DSE colonizations of herbaceous plants would have plant species level shifts along with increasing altitude (II). The possible seasonal shifts in AM

and DSE colonizations and the influence of bud removal on them was studied in low-alpine herbs, to evaluate the importance of sampling time and plant nutrient sinks on the root fungal colonizations (III). The role of competitive interactions between mycorrhiza-forming *Triantalis europaea* and non-mycorrhizal *Carex bigelowii* with or without AM inoculum was studied in a climate chamber experiment (IV), and also the effects of temperature and fungal inoculation on the ecophysiology and germination of *Gnaphalium norvegicum* were studied (V).

2 Materials and methods

2.1 The model

A root system with R roots was assumed, of which fraction p were mycorrhizal and $1 - p$ non-mycorrhizal. The average nutrient gain per unit time by a non-mycorrhizal root was N_{nm} and by a mycorrhizal one N_m , where $N_m > N_{nm}$, and C_m was the average carbon demand of a mycorrhizal root per unit time and C_{nm} that of a non-mycorrhizal root ($C_m > C_{nm}$). The net carbon gain of a plant with a fraction of p mycorrhizal roots was defined as

$$C(p) = \alpha R(p N_m + (1 - p) N_{nm}) - R(p C_m + (1 - p) C_{nm}) \quad (1)$$

where α was the nutrient use efficiency of photosynthesis (PNUE) (Larcher 1995), $N(p) = R(p N_m + (1 - p) N_{nm})$ was the total nutrient gain of the whole plant, and the last term $R(p C_m + (1 - p) C_{nm})$ represented the carbon loss to the root system (Tuomi *et al.* 2001).

The two main terms on the right-hand side of Eq. (1), i.e. gross carbon gain and carbon cost of the root system, were linear functions of p . If mycorrhizal roots were more effective in nutrient absorption, i.e. $N_m > N_{nm}$, but had a greater carbon demand, i.e. $C_m > C_{nm}$, the net carbon gain, C , would increase with p for

$$\alpha > (C_m - C_{nm}) / (N_m - N_{nm}) \quad (2)$$

If α assumed smaller values, C would decrease as a function of p . In other words, in terms of the net carbon gain, the nutrient use efficiency of photosynthesis determined whether a mycorrhizal condition ($p = 1$) or a completely non-mycorrhizal condition ($p = 0$) would be most economical for the carbon balance of the plant (see also Fitter 1991, Tuomi *et al.* 2001).

The kinetics of nutrient uptake at different soil nutrient concentrations was used to determine the nutritional benefits, i.e. $N_m > N_{nm}$, that the plant would obtain from mycorrhizal associations. Nutrient uptake as a function of the external concentrations of mineral nutrients is often presented in terms of Michaelis-Menten kinetics. The difference in nutrient uptake was assumed to be in one of the parameters defining the equation:

maximum uptake capacity of a root (V_{\max}), minimum value of x leading to zero net uptake (x_{\min}), or half-saturation constant (K_m) (e.g. Marschner 1995). When these possibilities were studied in the present model in the condition (2), three cases were obtained indicating how the advantage of mycorrhizal symbiosis may change along a gradient of the external availability of mineral nutrients in the soil (Fig. 2). These theoretical results were used to deduce how the mycorrhizal status of plants may change along environmental gradients when the nutrient use efficiency and the soil nutrient concentration change along the gradient (Fig. 2) (Table 1 and Fig. 2 in paper I).

2.2 Field studies (II, III)

The field studies were carried out at Mts Jeahkkas and Saana, Finland (69°01' N, 20°50' E) (III) and at Mt Paras, Norway (69°06' N, 20°07' E) (II), both sites representing subarctic Fennoscandia. In the study II root samples of *Ranunculus glacialis*, *Saxifraga aizoides*, *Sibbaldia procumbens*, *Solidago virgaurea*, *Trientalis europaea* and *Viola biflora* were collected, mainly on the basis of their wide altitudinal distribution. Samples were collected between sea level and 1400 m a.s.l. at Mt Paras.

The seasonality and bud removal study was conducted at three sites in the low-alpine belt between Mts Saana and Jeahkkas. The sites were situated just above the tree-line, at 630 m a.s.l., at 1 km distance from each other (III). Root samples of *Alchemilla glomerulans*, *Carex vaginata*, *Ranunculus acris* ssp. *borealis* and *Trollius europaeus* were collected four times and from *R. acris* three times during the growing season. In addition, a bud removal experiment was conducted to see the effect of sink removal to root fungal colonizations. For this purpose about 60 plants per species and site were marked and flower buds were removed from these plants once. *T. europaeus* was excluded from the bud removal study, because it did not flower during the study year.

In all field studies the plants and their roots were collected with one litre of soil. The replicate number was 5 per site (and in case of bud removal in the study III also per treatment). Samples were brought to the laboratory and kept in a refrigerator until roots were cleaned and preserved in 50% alcohol, which was done either immediately or 1–2 days after sampling. Roots were stained by the method of Phillips and Hayman (1970) with some modifications, and fungal colonizations were determined by the magnified intersections method (McGonigle *et al.* 1990). Colonizations of arbuscules, vesicles, hyaline hyphae, DSE and hyaline-septate hyphae (HSE) were calculated separately. 50 intersects/root sample were studied. In study II it was also found possible to separate fine endophyte-type colonizations from common coarse AM. The colonization percentages were then calculated as colonized intersects/total number of intersects \times 100.

In the seasonality study (III), soil inorganic phosphorus availability throughout the season was determined. Soil was separated from the root samples in the laboratory, and the soil samples were then frozen until the analyses ($n = 35/\text{date}$). Soil soluble inorganic P was extracted to ammonium acetate as described in Väre *et al.* (1997) and phosphorus was determined using a standard method described in Halonen *et al.* (1983).

2.3 Studies in climate chamber (IV, V)

In the competition study (IV), intra- and interspecific competition between mycorrhiza-forming *Trientalis europaea* and non-mycorrhizal *Carex bigelowii* was examined. The micropropagated specimens of low-alpine origin were grown either singly or together with a conspecific or heterospecific individual with AM inoculum (*Glomus mosseae*-spores with adhering root pieces in sand) present or absent for 90 days. Shoot and root dry weights and relative growth rates (RGR) were determined, and root AM colonizations assessed as in the field studies (Chapter 2.2).

In the study V, the impact of two temperatures (8° or 15°C) and DSE (*Phialocephala fortinii*) and AM (*Glomus claroideum*) inoculations were evaluated on the ecophysiology and germination of an arcto-alpine plant *Gnaphalium norvegicum*. Shoot biomass, shoot nitrogen concentrations (N %) and nitrogen contents (mg plant⁻¹) were determined. The root fungal colonization percentages were assessed as in the field studies (Chapter 2.2). The root biomass was not included because sampling for root fungal colonization assessment made the data inconsistent. At 15°C, the photosynthesis rate (μmol CO₂ m⁻²s⁻¹) and specific leaf area (SLA cm² mg⁻¹) were measured and, finally, the photosynthetic nutrient use efficiency (PNUE, nmol CO₂ mmol N⁻¹ s⁻¹) was determined (Larcher 1995). Photosynthesis measurements were not conducted at 8°C because the seedlings were too small for this purpose.

2.4 Statistical analyses

In most cases the statistical analyses were made using ANOVA models with the SPSS software (versions 8–10, SPSS Inc.) (III, IV, V). Percentage data (root colonization %, nitrogen %) were arcsin-transformed before the tests and for some other variables logarithm [LOG ($x + 1$)], square root [SQRT ($x + 1$)] and rank transformations were used when the normality and variance assumptions for ANOVA were not met. In study II, a logistic regression model was applied to the root colonization data using the R-program (version 1.1.1, Ihaka & Gentleman 1996).

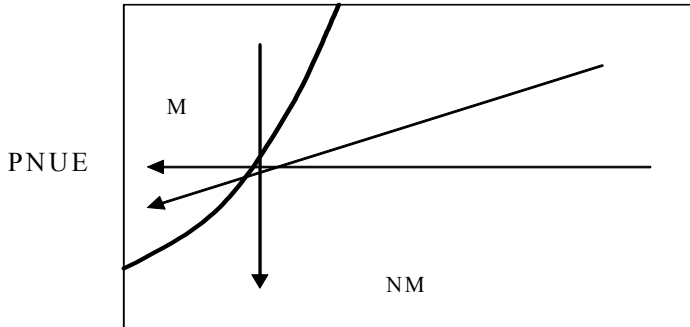
3 Results

3.1 Optimal mycorrhizal colonization along altitudinal gradients

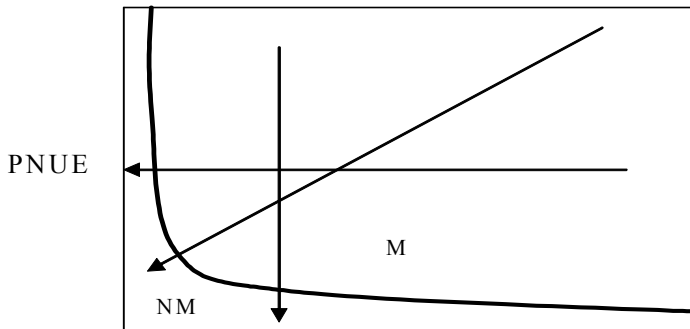
According to the model (I), mycorrhizal symbiosis can be favoured at low nutrient concentrations if mycorrhizal roots had a lower threshold value of nutrient uptake (lower x_{\min}), but only with relatively high nutrient use efficiency of photosynthesis (PNUE) values (Fig. 2a). At higher soil nutrient concentrations only the non-mycorrhizal strategy prevailed. If mycorrhizal roots had higher maximum capacity of nutrient uptake (higher V_{\max}) then non-mycorrhizal strategy was favoured at the lowest PNUE and soil nutrient concentration values (Fig. 2b). When soil nutrient concentration increased, the advantage of mycorrhizas increased steeply and they were favoured with all other, but not at the lowest PNUE-values. If mycorrhizal roots had higher affinity of uptake (lower half-saturation constant, K_m) then mycorrhizal symbiosis was favoured at intermediate nutrient concentrations, but only at relatively high PNUE values (Fig. 2c).

From these results the possible patterns along altitudinal gradients were deduced (arrows in Fig. 2). First, I considered the situation when only PNUE would decrease and soil nutrient concentration remained constant. Second, I considered the situation where PNUE would stay constant or decrease, and soil nutrient levels would decrease. It appeared that, if soil nutrient concentration is constant and PNUE decreases, the only emerging pattern was from mycorrhizal (M) to non-mycorrhizal (NM) condition, independent on the kinetics of nutrient uptake (Fig. 2a–c). If both the soil nutrient concentration and PNUE decrease, several patterns may emerge depending on the nutrient uptake kinetics of the roots. Qualitatively similar results were obtained when PNUE was assumed to stay constant with decreasing nutrient levels. Nevertheless, in all cases PNUE should be relatively high to favour the mycorrhizal condition (I).

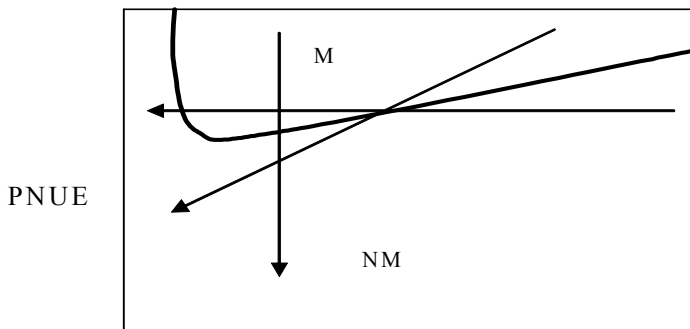
a.



b.



c.



Soil nutrient concentration

Fig. 2. A schematic picture of the main results of the model (I). The arrows show the possible directions of the altitudinal gradients when the photosynthesis nutrient use efficiency (PNUE) and soil nutrient concentration either stay constant or decrease. M = parameter area where mycorrhizal strategy is favoured and NM = area where non-mycorrhizal strategy is favoured. Three cases are shown: a. Mycorrhizal roots have lower threshold concentration (x_{\min}) of nutrient uptake, b. Mycorrhizal roots have higher maximum capacity (V_{\max}) of nutrient uptake and c. Mycorrhizal roots have lower half saturation constant (K_m) of nutrient uptake.

3.2 Root fungal colonizations along an altitudinal gradient

In *Ranunculus glacialis*, *Sibbaldia procumbens*, *Solidago virgaurea*, *Trientalis europaea* and *Viola biflora* clear AM structures (= arbuscules, vesicles and typical non-septate hyphae) were detected (II). Hyaline hyphae resembling AM and occasionally vesicles were detected in *Saxifraga aizoides* but no arbuscules. So, these were not considered as AM. DSE colonizations were common in all studied species and fine endophyte (FE) was also present in all AM plant species. FE-colonizations were found to have a positive relationship with altitude, but in other colonization parameters no consistent changes along with increasing altitude were found. The results suggest that the root fungal colonizations at the species-level were rather constant along with altitude, but the mean colonization rates between species were different. In *Ranunculus glacialis* all fungal colonizations were low in the high-alpine belt (1400 m a.s.l.). DSE had no clear relationship to altitude, but at 1400 m (*R. glacialis*) it was absent.

3.3 Seasonality of root fungal colonizations

Fungal colonization patterns during a growing season were found to be species-specific in the low-alpine meadows. A statistically significant decline towards the end of the the season was detected only in *Trollius europaeus* (III). Also the hyphal and DSE colonizations in *Alchemilla glomerulans* decreased towards the autumn. *Ranunculus acris* maintained relatively high colonizations through the summer. *Carex vaginata* did not form arbuscular structures, but it was often colonized by DSE and HSE, which both had no seasonal shifts. Soil soluble phosphorus concentration had seasonal variation, but the concentration patterns were also highly variable between study sites. Flower bud removal decreased the arbuscular colonization of *R. acris*, but in the other species studied no effect was detected. The root colonizations did not correlate with soil phosphorus to any considerable extent and also the correlations between DSE and AM did not show any consistent trend.

3.4 Competition between a mycorrhizal and a non-mycorrhizal plant

Inoculated *Trientalis europaea* formed abundant AM structures, but mycorrhiza did not increase its shoot biomass or the whole plant's relative growth rate (RGR) (IV). *Carex bigelowii* did not form mycorrhiza, but its shoot biomass and RGR were lower in the inoculated pots. The presence of a competitor had no statistically significant effect on the shoot biomasses or RGR in either plant species. Mycorrhizal inoculum increased the root/shoot ratio of *C. bigelowii* in all competitive situations, while the root/shoot ratio decreased in *T. europaea* in the presence of *C. bigelowii* in both mycorrhizal and non-mycorrhizal state.

3.5 Temperature and fungal inoculation effects on seedlings

AM-fungus *Glomus claroideum* colonized the roots at both temperatures, but the colonization at 8°C was distinctly low and often totally absent (V). DSE-fungus *Phialocephala fortinii* did not colonize the roots at any temperature. At 15°C, *G. claroideum* had a positive impact on the shoot biomass of *Gnaphalium norvegicum*, but not at 8°C. Shoot N % was lower in *G. claroideum*-inoculated seedlings at both temperatures, but N content (mg N plant⁻¹) was higher only at higher temperature, where also the seedling size was considerably larger. At 15°C, photosynthesis rate, specific leaf area (SLA) and photosynthesis nutrient use efficiency (PNUE) were positively affected by *G. claroideum*. *P. fortinii* had a positive effect on all mean values of the above mentioned parameters especially at the higher temperature, but these effects generally lacked statistical significance. Germination of *G. norvegicum* seeds was considerably slower at 8°C compared to 15°C. *P. fortinii*-inoculation had a positive impact on germination at both temperatures whereas *G. claroideum*-inoculation had no effect.

4 Discussion

4.1 Mycorrhizal benefit for the host plant

Increased nutrient acquisition is the crucial benefit that a host plant gets from mycorrhizal associations. When acquired nutrients are effectively allocated to growth, improved nutrient economy leads to positive mycorrhizal growth response, which has been observed in several experiments (Smith & Read 1997). The growth response, however, is affected by several factors that constrain the balance of symbiosis, for example temperature (Furlan & Fortin 1973, Hayman 1974), light (Hayman 1974, Son & Smith 1988) and substrate nutrient availability (Koide & Li 1990, Koide 1991). Additionally, plant species differ in their responsiveness to mycorrhizal symbiosis (Koide 1991).

The shoot biomass of *Gnaphalium norvegicum* was considerably higher at 15°C than at 8°C (V). Colonization by the AM fungus *Glomus claroideum* further resulted in increased nitrogen acquisition and growth at 15°C but not at 8°C, indicating positive mycorrhizal response at higher temperature, but not at lower temperature. At 15°C, photosynthesis rate and specific leaf area were increased in the AM plants. These results agree with the earlier studies of positive growth responses in mycorrhiza-inoculated plants (Smith & Read 1997). The increased photosynthesis rate detected in mycorrhizal plants implies that carbon translocated to the fungal symbiont may relax the sink limitation and enhance photosynthesis (Herold 1980). Higher specific leaf area suggests improved water economy due to symbiosis (Snellgrove *et al.* 1982, Al Karaki 1998). Our study also showed that AM may have an important role also in plant nitrogen uptake, which has been reported only rarely before (Francis *et al.* 1986, Barea *et al.* 1987, Cliquet *et al.* 1997).

Mycorrhizal colonization was lower at 8°C than at 15°C (V). Low mycorrhizal colonization rates at low temperatures are a common observation in several earlier studies where they are also often associated with low growth responses (Furlan & Fortin 1973, Hayman 1974, Chilvers & Daft 1982, Graham *et al.* 1982, Smith & Roncadori 1986, Baon *et al.* 1994). This seemed to be the case also in the present study. Seedling nitrogen concentrations were lower at 8°C than at 15°C, both in mycorrhizal and non-mycorrhizal plants, which suggests that the seedling growth may have been nutrient limited in the cold (V). This was either due to lower microbial activity in the substrate (Rehder & Schäfer

1978) or decreased root nutrient uptake capacity (Marschner 1995). At 8°C, the functioning of the mycorrhizal hyphae was probably decreased in the AM plants (Wang *et al.* 2002).

At 15°C, AM plants had an increased photosynthetic nutrient use efficiency (PNUE) (V). It was probably related to the high growth rates of the seedlings, where nutrients acquired via mycorrhizal symbiosis were effectively allocated ('diluted') into the new growth resulting in lower N % in the mycorrhizal plants. Mycorrhizal plants have been found to have higher PNUE than non-mycorrhizal plants in a few studies (Brown & Bethlenfalway 1988, data in Peng *et al.* 1993, data in Nielsen *et al.* 1998). As the higher photosynthesis (see above), the higher PNUE in mycorrhizal plants indicates that AM acted as a carbon sink. The model (I) suggested that the mycorrhizal condition would be favoured only at relatively high PNUE values. If mycorrhizal plants had higher PNUE than non-mycorrhizal plants, this would increase the range of ecological conditions where mycorrhizal symbiosis is favoured (see also Tuomi *et al.* 2001).

In addition to the direct benefits, mycorrhiza may change plant performance in competition with other plants (IV, Marler *et al.* 1999). In the present study, mycorrhizal inoculation could have enhanced the competitive ability of the mycorrhizal plant *Trientalis europaea* by having adverse effects on its non-mycorrhizal competitor *Carex bigelowii* (IV). The inoculated mycorrhizal fungus did not colonize the *C. bigelowii* roots at all, suggesting that the presence of mycorrhizal inoculum itself elicited a negative response. Francis & Read (1994) have found that soil water extract from a mycorrhizal system inhibited root growth of a non-mycorrhizal host. It is impossible to state if their observation and the present result are due to direct antagonistic actions between organisms or, for example, other soil biota in the inoculum, but both studies suggest that antagonism between mycorrhizal and non-mycorrhizal plants is possible. The negative response of *C. bigelowii* when grown alone with AM rather suggests some kind of antagonism due to mycorrhizal inoculum other than resource competition between the plant and the fungus, because AM fungal inoculum does not develop hyphae to any substantial extent in the absence of a compatible host (Schreiner & Koide 1993, Logi *et al.* 1998). Resource competition between the AM fungus *Glomus mosseae* and the non-mycorrhizal plant is an alternative explanation in such treatments where *T. europaea* was grown in the same pot with *C. bigelowii*. However, *G. mosseae* did not either improve the growth of *T. europaea*, which suggests that if *G. mosseae* and *C. bigelowii* competed for nutrients, there was either an imbalance between the fungus and *T. europaea* in the carbon-nutrient exchange or that *T. europaea* was not able to allocate the acquired nutrients to growth.

Though laboratory studies are problematic to interpret into field conditions, the present observation (IV) could still indicate that there may exist antagonism between mycorrhizal inoculum and those non-mycotrophic plants that do not belong to the mycorrhizal community (see also Peat & Fitter 1993). Mycorrhizal fungi have been demonstrated to have an important role in determining plant population and community level patterns (e.g. Grime *et al.* 1987, Van der Heijden *et al.* 1998). Our study suggests that relationships between mycorrhizal and non-mycorrhizal plants may be one aspect.

4.2 Significance of different root colonizing fungi

In the present thesis, the focus was on arbuscular mycorrhiza (AM) due to its facultative nature for the host plants (Trappe 1987) and therefore, possible altitudinal shifts in its colonization rates. In addition to the direct effects of AM to the host plant performance and species interactions (above chapter I, IV,V), the AM colonization patterns were studied in the field (II, III). In the study II, the AM colonizations were not found to have any consistent changes along an altitudinal gradient. By contrast, colonization rates and patterns along the altitude tended to be species-specific. In the high-alpine belt AM was nearly absent. The seasonal changes in AM in a low-alpine environment were found to be species-specific (III). Flower bud removal decreased the arbuscular colonization in *Ranunculus acris*, suggesting that the nutrient demand for flowers and the rate of AM colonization may be related to each other (III). The lack of seasonal changes in AM colonization may also indicate that AM structures in the roots have longer life spans in indigenous nature (Brundrett & Kendrick 1990), than in more widely studied agricultural environments (Alexander *et al.* 1989, Smith & Read 1997).

The present results suggested that fine endophyte (FE) is present in subarctic mountaineous areas, and it becomes more common towards higher altitudes, but is rare in the high-alpine belt (II). Colonization morphology, in general, does not enable species-level identification of AM fungi (Smith & Read 1997). FE may be an exception, because it has relatively distinctive morphology (Abbott 1982, Brundrett *et al.* 1996, Thippayarugs *et al.* 1999). It has been proposed to be formed by *Glomus tenuis* (Hall 1977), but the relationship between colonization type and the fungal species may be questionable (Abbott 1982, Brundrett *et al.* 1996). The studies on FE are quite rare, but it has been reported to inhabit grasslands (Crush 1973a, Rabatin 1979), southern hemisphere mixed forest (Johnson 1977), coal wastes (Daft & Nicolson 1974), arctic and antarctic areas (Christie & Nicolson 1983, Bledsoe *et al.* 1990) and mountains at high altitudes (Haselwandter & Read 1980, Read & Haselwandter 1981, Lesica & Antibus 1986, Blaschke 1991, Mullen & Schmidt 1993).

FE has been found to have a relatively fast colonization capacity (Rabatin 1979), positive response to phosphate availability (Crush 1973b, Powell & Daniel 1978, Rabatin 1979) and it often appears as a pioneer endophyte (Daft & Nicolson 1974). These kinds of traits could be useful also at high altitudes, where for example soil physical disturbances may locally create phosphate-rich patches (Jonasson & Sköld 1983). Furthermore, soil disturbances *per se* may have negative impacts on the AM hyphal network (Stahl *et al.* 1988, Jasper *et al.* 1989, McLellan *et al.* 1995). Faster recovery rate after disturbances, higher colonization capacity and ability to effectively utilize phosphorus peaks could favour FE at high altitudes. High surface to volume ratio compared to that of the coarse AM may also imply that FE is more economical for the host plant in terms of carbon/nutrient exchange. This kind of trait could also, hypothetically, favour FE in the high altitude conditions.

The significance and function of DSE fungi in plant roots is reported to be variable, from mutualistic to parasitic responses (Haselwandter & Read 1982, Fernando & Currah 1996, Jumpponen & Trappe 1998, Jumpponen *et al.* 1998). This is at least partly due to the heterogeneity of the group. In the field studies DSE-type colonization was found to be common both in mycorrhizal and non-mycorrhizal plant roots in alpine areas (II, III), but it was also found below the treeline (II). However, in the present laboratory study a DSE

fungus did not colonize the roots (V). DSE are commonly found in diverse habitats, but in arctic, antarctic and alpine areas they are especially frequent (Haselwandter & Read 1980, Read & Haselwandter 1981, Christie & Nicolson 1983, Currah & Van Dyk 1986, Bledsoe *et al.* 1990, Blaschke 1991, Väre *et al.* 1992, Mullen *et al.* 1998). In this study (II), DSE colonizations were not found to be common in the high-alpine belt, which differs from the observations by Haselwandter and Read (1980) and Read and Haselwandter (1981). DSE colonization was neither found to have any consistent seasonal variation in the low-alpine environment, and flower bud removal did not alter its colonizations (III).

Several DSE fungal strains have been shown to be capable to utilize organic molecules in their nutrition (Currah & Tsuneda 1993, Caldwell *et al.* 2000). If DSE colonized plant roots and behave mutualistically (Haselwandter & Read 1982) their importance for the host plant in the alpine conditions would probably be the enhanced nutrient uptake from organic sources (Jumpponen *et al.* 1998, Caldwell *et al.* 2000). Additionally, Mullen *et al.* (1998) suggested that DSE could be important for early season nitrogen uptake during nutrient flushes. In the present laboratory study the DSE fungus *Phialocephala fortinii* did not colonize the roots of *Gnaphalium norvegicum* (V). However, *P. fortinii*-inoculation tended to increase the growth and nitrogen acquisition of *G. norvegicum*, which suggests that *P. fortinii* may have performed active mineralization in the substrate. Consequently, positive effects on photosynthesis rate and PNUE were also probably due to somewhat enhanced nitrogen acquisition in *P. fortinii*-inoculated plants. *P. fortinii*-inoculation enhanced the seed germination, which may also be related to its possible mineralizing activity (V, Baskin & Baskin 1998).

In the seasonality study (III) a colonization of hyaline septate endophytes (HSE) was reported from *Carex vaginata*, but not from the other species due to their irregular occurrence (Kohn & Stasovski 1990). HSE are relatively difficult to detect if AM colonization is abundant. According to Barrow and Aaltonen (2001) it is possible that HSE are formed by DSE fungi which become less dark and even hyaline when they colonize roots inter- and intracellularly. On the basis of the hyphal structure, both are formed by Ascomycetes. HSE probably form another heterogeneous fungal group in the plant roots, and their relationship to host plants may be as diverse as DSE fungi are assumed to have.

4.3 Altitudinal gradients

4.3.1 Plant performance and mycorrhizal colonization along altitudinal gradients

The model indicated that PNUE would play a key role in determining if mycorrhizal symbiosis would be beneficial for the plant net carbon gain or not (I). Decreasing PNUE along environmental gradients would generally suggest that the mycorrhizal benefit for the net carbon gain would decrease. However, this prediction is somewhat complicated to be evaluated with precision along altitudinal gradients on the basis of published studies because the photosynthetic capacity per leaf area, tissue nutrient concentration and leaf

biomass per area all increase along with the altitude (Friend *et al.* 1989, Körner & Diemer 1989, Körner 1999). The increase in the photosynthetic capacity per leaf area would imply an increasing trend in PNUE along with the altitude, but simultaneously an increase in tissue nutrient concentrations and leaf biomass per area would imply a reverse change in PNUE. The few published studies of PNUE at different altitudes have resulted in variable patterns (Friend *et al.* 1989, Westbeek *et al.* 1999). The overall changes may thus differ between species and higher taxonomic groups and it is difficult to predict any consistent pattern along altitudinal gradients. Nevertheless, if PNUE decreases along altitudinal gradients, the present model suggests that it would generally imply decreasing mycorrhizal colonization patterns.

Possible shifts in the soil nutrient concentrations along with altitude also affect the optimal mycorrhizal colonization (I). Soil nutrient concentrations may decrease along with increasing altitude (Kyllönen 1988, Väre *et al.* 1997, Körner 1999) even though nutrient-rich patches created by cryoperturbation exist at high altitudes (Jonasson & Sköld 1983). The shifts in mycorrhizal colonization were different when soil nutrient concentration was constant, compared to the situation when it decreased (with declining PNUE, I). In that case the nutrient uptake kinetics became crucial. It is also possible that fine endophyte could differ from coarse arbuscular mycorrhiza in the nutrient uptake kinetics for example due to thinner hyphae and higher surface to volume ratio (Chapter 4.2). The general validity of the model can only be evaluated to a limited extent on the basis of the present empirical studies. The model includes a number of simplifying assumptions and neglects species-specific complexity of plant-fungal interactions. In spite of this, the model generated various colonization patterns along environmental gradients and, hence, my original hypothesis of declining colonization toward higher altitudes seems in fact be only a possibility among others. The observations from the alpine gradients (II, Haselwandter 1979, Haselwandter & Read 1980, Read & Haselwandter 1981, Väre *et al.* 1997) have also yielded variable colonization patterns, especially at species-level.

The availability of arbuscular mycorrhizal propagules may be low at high altitudes for example because AM spores are soil- and animal-dispersed (Allen 1987, Trappe 1988). Additionally, the number of fungal species in soil generally decreases along the alpine gradient (Bissett & Parkinson 1979, Kernaghan & Harper 2001) and the physical conditions may not be favourable for extensive fungal networks (see below). Plants invest relatively more biomass below ground, especially on fine roots at higher altitudes (Körner & Renhardt 1987). This has been assumed to be a functional substitute for the decreasing availability of mycorrhizal inoculum (Körner & Renhardt 1987). Plants also tend to reserve energy to be usable for reproduction during a favourable season in alpine conditions (Bliss 1971). Thus, it could also be that it would be more favourable for high altitude plants to translocate carbon to roots where it constitutes a reserve, rather than to fungal symbionts. Non-structural carbon is abundant in alpine plants (Körner 1999) indicating that plant carbon availability as such may not limit the mycorrhizal symbiosis. However, if the most favourable carbon allocation would change along the altitude, it could also affect the advantage of mycorrhizal symbiosis.

Towards higher altitudes, both the intensity and frequency of soil physical disturbances increase, which limit the amount of favourable patches for vascular plant growth in high-alpine conditions (Oksanen & Virtanen 1995). Low mycorrhizal colonization levels found in patchy high-alpine vegetation (Haselwandter & Read 1980) suggest that the

availability of mycorrhizal inoculum may be low in these patches and, from the fungal viewpoint, the sparse vegetation may not be able to maintain effective mycorrhizal hyphal networks in the soil (Johnston & Ryan 2000, Genney *et al.* 2001). Soil disturbances *per se* have also been found to have a negative effect on the hyphal network and the nutrient transfer for the host plants (Stahl *et al.* 1988, Jasper *et al.* 1989, McLellan *et al.* 1995). The availability of mycorrhizal inoculum or the effect of soil disturbances were not evaluated in this work, but it is probable that these factors may be of crucial importance for determining the performance of mycorrhizal plants and the plant community-level shift towards decreasing mycotrophy at high altitudes (Trappe 1987, 1988).

4.3.2 Is there any generalized pattern of mycorrhizal colonization along altitudinal gradients?

Species-level differences in mycorrhizal colonization along alpine gradients may differ from community-level patterns. According to the present study and literature, the relative coverage of mycorrhizal species decreases with altitude, but plant species seem to differ in their specific colonization patterns (II, Haselwandter 1979, Haselwandter & Read 1980, Read & Haselwandter 1981, Väre *et al.* 1997). Allison & Goldberg (2002) proposed that the community-level responses to mycorrhizal colonizations along environmental gradients are not necessarily the same as the responses found at species-level. This is due to changes in species composition along the gradients and their different responses to mycorrhiza. Modifying this hypothesis, the mycorrhizal colonizations along altitudinal gradients could have different species-level and community-level patterns (Fig. 3).

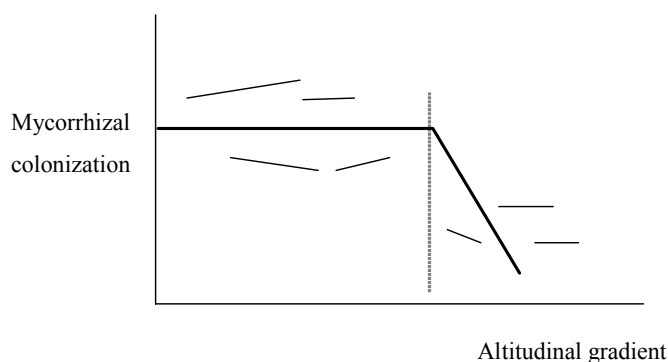


Fig. 3. A schematic hypothesis of the mycorrhizal colonization patterns along altitudinal gradients. The dashed line denotes the transition zone from middle- to high-alpine belt. The mycorrhizal colonization has species-specific patterns with altitude, which average a straight line at lower altitudes, but decrease, on average, in high-alpine belt.

Along the altitudinal gradients several abiotic and biotic factors affect the performance of mycorrhizal plant species and their colonization rates. Below the high-alpine conditions the average colonization rates would result in a relatively constant line (Fig. 3)

(II, Väre *et al.* 1997). Different colonization patterns found at the species-level may be due, for example, to the microtopography and different mycorrhizal dependency of the species. By contrast, in the high-alpine belt the average colonizations would decline (II, Haselwandter & Read 1980, Read & Haselwandter 1981). In addition to the low colonization rates of the mycorrhizal taxa in the high-alpine belt presented in the figure 3, the increasing relative abundance of non-mycorrhizal taxa towards higher altitudes emphasizes the non-mycorrhizal nature of high-alpine plant communities (Trappe 1987, Gardes & Dahlberg 1996). It may also be that there are shifts towards possibly more favourable mycorrhizal symbionts, such as fine endophyte (II, Haselwandter & Read 1980). Previous chapters discussed possible factors affecting the advantage of mycorrhizal symbiosis and the amount of colonization. It appears that in high-alpine conditions several of these may not favour mycorrhizal condition: (i) low temperature constraints on mycorrhizal fungi, (ii) low temperature and short growing season constraints on plant physiology and carbon allocation, (iii) soil disturbances, and (iv) low availability of inoculum.

5 Conclusions

Contrary to expectations, I did not detect species-level shifts in arbuscular mycorrhizal and dark-septate endophytic root colonizations along with altitude, except in the case of fine endophyte-type arbuscular mycorrhiza, which increased along with the altitude. Low temperature decreased the mycorrhizal growth benefit in a laboratory experiment and also the mycorrhizal colonization was found to be lower at lower temperature. A theoretical model suggested soil nutrient concentration to affect the mycorrhizal colonization, depending on photosynthetic nutrient use efficiency and differences in nutrient uptake kinetics between mycorrhizal and non-mycorrhizal roots. Mycorrhizal inoculum was found to have adverse effects on a non-mycorrhizal plant without direct nutritional benefit to the host plant. Dark-septate endophytes were common root associates at the field sites. A dark-septate endophyte *Phialocephala fortinii* was found to increase seedling growth and seed germination, possibly via increased nutrient availability due to the saprophytic activity in the substrate, but no signs of root-colonization or mutualistic relationships were detected. Plant species did not have any consistent root fungal colonization cycles and colonization rates were quite stable during growing season. Plant species may have their own colonization patterns which are possibly related to their own phenological rhythms.

Species-level mycorrhizal colonization patterns along with increasing altitude may differ from the community-level change from a mycorrhizal to a non-mycorrhizal plant community. This is probably related to different altitudinal distributions of the plant species, microtopography and the species-specific dependency on mycorrhizal symbiosis. The fungal symbionts and their relative abundance in the roots may also change with the increasing altitude.

Several factors may not favour mycorrhizal symbiosis in high-alpine conditions. This suggests that the performance of mycorrhizal plants would decline along the alpine gradient relative to non-mycorrhizal plants, which was hypothesized. However, it appears that the rate of the decline may not be constant along with the altitude and mycorrhizal plants may perform, in average, relatively well at lower altitudes but in the transition to high-alpine conditions the advantage of mycorrhizal symbiosis may drop. Consequently, declining mycorrhizal colonizations and lower performance of mycorrhizal plants compared to non-mycorrhizal plants may not be primarily due to changes in individual plant ecophysiology and photosynthesis but are also affected by plant and fungal

community-level phenomena and direct environmental impacts. Further studies will be required to evaluate the relative importance of the factors affecting the mycorrhizal symbiosis in the arcto-alpine conditions. For example, laboratory experiments on PNUE of mycorrhizal and non-mycorrhizal plants at different temperature and nutrient conditions would be required for precise model testing. Use of indigenous AM fungal species and native soils as a substrate would increase the predictive power of experiments. Additionally, identification of AM and DSE fungal communities from different altitudes would be useful, especially when looking for the role and identity of the fine endophyte. Manipulative field experiments where both the structure and function of fungal communities would be monitored in the high-alpine conditions would considerably increase our understanding of the role of mycorrhizal symbiosis in these environments.

6 References

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