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Responses of fungal root colonization, plant cover and leaf nutrients to long-term exposure to elevated atmospheric CO_2 and warming in a subarctic birch forest understory

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Abstract

Responses of the mycorrhizal fungal community in terrestrial ecosystems to global change factors are not well understood. However, virtually all land plants form symbiotic associations with mycorrhizal fungi, with approximately 20% of the plants' net primary production transported down to the fungal symbionts. In this study, we investigated how ericoid mycorrhiza (ErM), fine endophytes (FE) and dark septate endophytes (DSE) in roots responded to elevated atmospheric CO_2 concentrations and warming in the dwarf shrub understory of a birch forest in the subarctic region of northern Sweden. To place the belowground results into an ecosystem context we also investigated how plant cover and nutrient concentrations in leaves responded to elevated atmospheric CO_2 concentrations and warming. The ErM colonization in ericaceous dwarf shrubs increased under elevated atmospheric CO_2 concentrations, but did not respond to warming following 6 years of treatment. This suggests that the higher ErM colonization under elevated CO_2 might be due to increased transport of carbon belowground to acquire limiting resources such as N, which was diluted in leaves of ericaceous plants under enhanced CO_2. The elevated CO_2 did not affect total plant cover but the plant cover was increased under warming, which might be due to increased N availability in soil. FE colonization in grass roots decreased under enhanced CO_2 and under warming, which might be due to increased root growth, to which the FE fungi could not keep up, resulting in proportionally lower colonization. However, no responses in aboveground cover of *Deschampsia flexuosa* were seen. DSE hyphal colonization in grass roots significantly increased under warmer conditions, but did not respond to elevated CO_2. This complex set of responses by mycorrhizal and other root-associated fungi to global change factors of all the fungal types studied could have broad implications for plant community structure and biogeochemistry of subarctic ecosystems.

Keywords: Dark septate endophyte, ericoid mycorrhiza, fine endophyte, global change, leaf nitrogen and phosphorus, open top chamber, subarctic

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Introduction

Climatic changes in association with rising atmospheric CO_2 are expected to be particularly marked at high latitudes (ACIA, 2005; IPCC, 2007), where birch forests and ericaceous dwarf shrub dominated understorys are abundant. Research on ecosystem effects of rising atmospheric CO_2 concentrations and temperature has focused on photosynthesis and aboveground plant growth (van Wijk *et al*., 2004; Ainsworth & Long, 2005). However, relatively little attention has been given to the belowground part of the terrestrial ecosystem (Pendall *et al*., 2004, 2008).

The mycorrhizal fungal community is a key belowground component. Mycorrhizal fungi form symbiotic associations with plant roots and are important parts of the terrestrial carbon cycle, with approximately 20% of the net primary production transported down to the fungal symbionts (Jakobsen & Rosendahl, 1990). Both arbuscular mycorrhiza (AM) and ectomycorrhiza (EM), which are the most commonly studied types of mycorrhizas, have been shown to respond significantly to global change factors. Elevated atmospheric CO_2 has

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often been found to increase colonization levels of AM in roots of herbaceous plants, and of EM in roots of woody plants (Rillig *et al*., 2002; Treseder, 2004). It has been suggested that fungal responses to elevated CO_2 are controlled by host–plant responses; that is, when plant growth increases, the investment of carbon in mycorrhizal fungi should increase (Rillig *et al*., 2002; Staddon *et al*., 2002). Consequently, the root fungal responses to elevated CO_2 often disappear when expressed in relation to plant biomass (Gebauer *et al*., 1996; Alberton *et al*., 2005).

Warming, on the other hand, has been shown to increase AM colonization, but to reduce EM colonization independently of plant growth (Rillig *et al*., 2002). However, some studies on total AM and EM colonization did not show any response to warming (Rillig *et al*., 2002). Effects of warming could be mediated directly by warming effects on the plants and fungi, but also indirectly by changes in soil mineralization rates and hence N availability or changes in relative abundance of EM species (Clemmensen & Michelsen, 2006).

Research on how global-change factors affect mycorrhizae, including effects of elevated CO_2 concentrations and warming, is often laboratory based and short-term (Rillig & Field, 2003; Staddon *et al*., 2004). The extrapolation of laboratory results to field conditions is not always appropriate because laboratory conditions cannot mimic all the variables represented in the field. Furthermore, short-term studies do not often reflect what is happening over a longer time scale in the ecosystems.

In this long-term field study, we focus on less often studied root fungal types: ericoid mycorrhizal (ErM) fungi, fine endophytic (FE) fungi and dark septate endophytic (DSE) fungi. The responses of ErM fungi and DSE hyphal colonization to enhanced CO_2 levels and warming have never been studied before, and studies of the effects of changes in CO_2 levels and warming on FE fungi are rare (Rillig *et al*., 1999; Rillig & Field, 2003; Staddon *et al*., 2004).

ErM are common in boreal and arctic ecosystems, where ericaceous dwarf shrubs growing in raw humus soils dominate (Read & Perez-Moreno, 2003; Read *et al*., 2004). In these ecosystems, essential nutrients such as N and P are almost exclusively bound in organic compounds (Read & Kerley, 1995). ErM produce enzymes to release amino acids and amino sugars from detrital material, which can then be taken up by the plants (Näsholm *et al*., 1998; Olsrud & Michelsen, 2009). In this way, ErM fungi both improve host plant growth and stimulate soil organic matter decomposition. ErM fungi produce more extracellular enzymes than AM and therefore ErM fungi will contribute more to soil organic matter decomposition in boreal and arctic ecosystems than AM fungi (Read & Perez-Moreno, 2003). ErM fungi can grow on, and decompose protein, chitin, cellulose, hemicellulose, starch, and more recalcitrant compounds such as polyphenols (Read *et al*., 2004). The ability to decompose such a broad range of organic compounds in soil and plant debris makes ErM a possible important controller of soil organic matter decay in arctic and boreal ecosystems. Therefore, global change effects on fungal symbionts are interesting since they can control loss of large soil C stocks at northern latitudes (Talbot *et al*., 2008).

The AM group includes two main types of fungi, FE fungi and coarse endophytic fungi (Thippayarugs *et al*., 1999). The FE fungi have a hyphal diameter that does not exceed 2 μm, while the coarse endophytic fungi have hyphal diameters of 3–10 μm (Gianinazzi-Pearson *et al*., 1981). In harsh environments with low pH, such as boreal and arctic ecosystems, FE fungi are more common than the coarse endophytic fungi (Olsson *et al*., 2004; Postma *et al*., 2007; Newsham *et al*., 2009). It has been suggested that FE fungi can improve host nutrient uptake (Powell & Daniel, 1978), although their ecological function remains uncertain. AM fungi have been shown to produce glomalin, a glycoprotein that is resistant to decomposition in soil and may represent up to 3–8% of soil organic C (Rillig *et al*., 2001; Treseder *et al*., 2007). As glomalin concentrations in soil are related to AM fungi abundance, an increased colonization of AM of roots may contribute to an increase in long-term C storage in soil (Treseder, 2004).

The DSE often co-occur with both ErM and FE fungi and are common in cold and nutrient-stressed environments (Olsrud *et al*., 2004, 2007; Postma *et al*., 2007; Newsham *et al*., 2009). They are ascomycetous fungi, and may function as pathogens or saprophytes as well as developing mutualistic associations like mycorrhiza (Jumpponen, 2001; Addy *et al*., 2005). Shoot P concentration has been shown to increase by inoculation with DSE in alpine host plants, which suggests that DSE have mycorrhizal functions in harsh environments. DSE are also able to improve plants' ability to withstand drought and to reduce infection by pathogens (Addy *et al*., 2005).

The aim of this study was to investigate how long-term enhanced atmospheric CO_2 concentrations and warming affect ErM colonization, FE colonization and DSE hyphal colonization in hair roots collected in a birch forest dwarf-shrub understory in the subarctic region of northern Sweden. To place our belowground results into an ecosystem context, we also investigated how total plant cover and leaf C, N and P concentrations responded to enhanced atmospheric CO_2 concentrations and warming.

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Materials and methods

Study site

The study took place in a birch forest near Abisko in the subarctic region of Sweden (68°21′N, 19°00′E), 341 m a.s.l. The mean annual temperature at the study site is −0.8 °C while the warmest month average temperature is 11.0 °C. Average annual precipitation (1913–2006) is 307 mm. The subarctic birch forest understory was dominated by four dwarf shrubs; *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, *Vaccinium uliginosum* and *Empetrum hermaphroditum*. The grass *Deschampsia flexuosa* was also frequently found (Table 1).

Experimental setup

The experiment was established in the year 2000 and includes a warming and an elevated CO_2 treatment in a two-way factorial design. At the time of the sampling for the present study (August 2006), the experiment had been run for six growing seasons and was into the seventh season. Each experimental plot for soil sampling is 0.45 m × 0.75 m, divided into 15 different 0.15 m × 0.15 m subplots and is surrounded by an open-top chamber (OTC) with a footprint of ca. 1.5 m^2. The experimental plot for point-frequency analyses is 0.105 m × 0.75 m and included in the OTC. Four chambers in each of six experimental blocks are randomly assigned to one of the following four treatments: (1) heated chambers in which soil and air temperatures are elevated 5 °C above ambient, using buried heating cables and infrared heating lamps, (2) CO_2 chambers in which CO_2-enriched air is blown into the chamber to double ambient atmospheric CO_2 concentrations, (3) combined heat and CO_2 enrichment, (4) ambient control chambers which are identical to heat and CO_2 treatments, but which receive no elevated CO_2 or heat.

Table 1 Total plant biomass in control plots with the most dominant plants showed first in the table and the following in descending order

Species	Biomass (g m^{-2})	Type of symbiont
Vaccinium myrtillus	175.2 ± 43.7	ErM fungi*
Vaccinium vitis-idaea	88.3 ± 24.4	ErM fungi*
Deschampsia flexuosa	66.7 ± 25.4	AM fungi*
Empetrum hermaphroditum	34.3 ± 27.8	ErM fungi*
Linnaea borealis	5.9 ± 3.6	AM fungi*
Cornus suecica	0.43 ± 0.43	AM fungi†

Values are presented as means ± SE, $n = 6$. Type of symbiont is also shown.
*Harley & Harley (1987).
†Taylor (1999).

The soil is warmed by resistance cables, which have been threaded through the organic upper layer of the soil. Heating cables are controlled by a data logger coupled to a set of thermistors – three in heated chambers, two in ambient chambers. Cables are switched on and off automatically on a 2-min cycle to maintain a temperature difference of 5 °C between heated and ambient chambers. Infrared lamps, suspended 1.2 m above the soil surface, warm the plants within the heated chambers.

In CO_2-enriched chambers, atmospheric concentrations are elevated to 730 ± 25 ppm CO_2. A LiCor 6262 (Li-COR Biosciences, Lincoln, NE, USA) Infrared Gas Analyser monitors CO_2 concentration in four of the six enriched chambers. A data logger coupled to flow sensors and a mass flow controller regulate the flow of CO_2 from the tanks to the chambers. CO_2-enriched air is blown into chambers from two sides in order to maintain an even concentration of CO_2 across each chamber. Non-CO_2-treatment chambers have an identical design, but receive air without CO_2 enrichment. This ensures that all chambers experience similar effects of blowing. Blowing also reduces convective heating effects within the chambers. Both warming and CO_2-enrichment is applied during the growing season each year (late May to early September).

In each block one 0.15 m × 0.15 m subplot from each of the four treatments – heat, CO_2, heat plus CO_2 and chambered control – were selected at random for soil sampling and initial survey of aboveground biomass.

Plant cover

Plant cover was measured using point-frequency analysis. The occurrence of all species of vascular plants, bryophytes and lichens, as well as litter and bare soil, was recorded at 5-cm intervals using a 10-mm-thick sheet of transparent Plexiglas. The sheet had 97 holes and was standing on three adjustable metal legs enabling us to position it in the same place every year, with the help of small markers on the soil surface and a spirit level. The holes had been precision-drilled using a milling cutter to enable a 5-mm-diameter brass rod to be inserted with a minimum of angular error. All hits on a 20-mm-long zone on the sharpened tip of the rod were recorded as it was moved downward through the vegetation canopy.

Initial survey of aboveground biomass

The aboveground biomass was harvested in each 0.15 m × 0.15 m subplot over 17–22 August 2006 by clipping of the vegetation at ground surface, that is just below the green part of cryptogams. The harvested biomass was sorted in the laboratory into separate

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vascular plant species, moss and litter. For all vascular plant species leaves were separated from stems. The biomass samples were dried at 70 °C over 24 h where after leaves, stems, moss and litter were weighed.

Analyses of plant C, N and P concentration

Samples of *D. flexuosa*, *V. myrtillus*, *V.vitis-idaea* and ericaceous coarse roots were dried at 70 °C over 24 h and finely ground. Leaves of *D. flexuosa*, *V. myrtillus* and *V. vitis-idaea* and ericaceous coarse roots were analysed for total N and P. About 0.1 g leaf and root material was digested in 5 mL of concentrated H_2SO_4 with 20 mg H_2SeO_3 and 1 mL 30% H_2O_2 added. The digest was analysed for N and P with the indophenol method and the molybdenum blue method, respectively (Allen, 1989), using a Hitachi U-2000 (Hitachi, Tokyo, Japan) spectrophotometer. The total C and N concentration in leaves and roots of ericaceous plant species were analysed using an elemental analyser (EuroVector, Milano, Italy) coupled to an IsoPrime isotope ratio mass spectrometer (GV Instruments, Manchester, UK). Total C concentration in leaves of *D. flexuosa* were analysed on LECO Truespec CN-analyzer.

Soil sampling

Three soil cores with a diameter of 2.1 cm and a depth of 7 cm was taken around the centre of each 0.15 m × 0.15 m subplot over 17–22 August 2006. Soil cores were transported on ice to the laboratory where they were kept in a cooling room at 2 °C. After removing the uppermost 2 cm, which mostly consisted of cryptogam biomass, the next 5 cm of the core were sorted for hair roots (< 200 μm diameter) on ice during 1–1.5 h in the laboratory. In general, only hair roots of < 100 μm diameter are colonized by ErM (Read, 1996). A representative sample of roots in the class 'hair roots' from the study site was found to consist of ca. 70% roots with $d < 80$ μm. A high incidence of colonization by ErM may therefore be assumed for this class. Living roots were distinguished from dead roots by their elasticity, with dead roots fragmenting easily when drawn with forceps (Aerts *et al*., 1989). The roots were rinsed in water and put in lactoglycerol within 3 days after field sampling.

Determination of mycorrhizal colonization

Roots were visually examined for ErM colonization, FE colonization and DSE hyphal colonization using the staining method of Phillips & Hayman (1970), followed by visual examination according to the magnified intersections method described by McGonigle *et al*. (1990). Grass roots from *D. flexuosa* and ericaceous dwarf shrub roots were distinguished from each other by colour and separated before aligned on slides. Grass roots were intensively blue coloured while dwarf shrub roots were dark brown. Between one and six slides, that is on average 81 cm roots were used for each sample. Roots were aligned in five rows parallel to the long axis of the slides and observed at × 400 magnification. Eight passes across each slide perpendicular to its long axis were made with 0.5 cm intervals. All intersections between roots and the vertical eyepiece crosshair, that is, on average 160 intersections per sample, were considered, except when epidermal cells were missing. The plane of focus was moved completely through the root sample.

For ericaceous hair roots, it was noted whether the vertical crosshair cut any ErM hyphal coils (Massicotte *et al*., 2005) or DSE hyphae (Yu *et al*., 2001). Only scores on hyphal coils that filled up the entire plant cell were counted in order to minimize the subjectivity of the visual determination. Colonizations with a loose net of thick melanized intercellular and intracellular hyphae as well as microsclerotia were rated as DSE fungi in the roots (Jumpponen & Trappe, 1998; Yu *et al*., 2001). Difficult-to-detect hyaline hyphae belonging to DSE complex were not counted and therefore the DSE hyphal colonization might be underestimated. Grass roots were examined for FE colonization, coarse AM colonization and DSE hyphal colonization. All sharp blue coloured hyphae with no septa or irregular-shaped ones and < 1.5 μm in diameter were classified as FE, while blue-stained hyphae > 1.5 μm in diameter were counted as AM. Vesicles, of various sizes and shapes, including both terminal and internal vesicles were also classified as FE if attached to FE hyphae of < 1.5 μm in diameter (Thippayarugs *et al*., 1999). Many of these FE fungi formed fan-shaped appressorium similar to those illustrated by Gianinazzi-Pearson *et al*. (1981). Some of the FE formed appressorium-like swelling before entering into roots (Daft & Nicolson, 1974). DSE hyphal colonization was examined as described above. All slides were examined with the identity hidden from the observer.

Statistical analysis

The effects of warming, CO_2 and their interaction on fungal root colonization were analysed with two-way ANOVAs with the factors warming and CO_2, each with two levels, and their interaction, plus block. The effects of warming, CO_2 and their interaction on vegetation cover were analysed using univariate general linear model ANOVA. The data for the initial season (2000) was used as a covariate. SPSS 14 were used for all analyses.

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Results

Plant cover

Based on the method used a significant increase in the total plant cover was found for warming ($P<0.05$) but not for elevated CO_2 ($P=0.78$) (Fig. 1a). This was also true for ericaceous dwarf shrubs alone for which a significant effect of warming was found ($P<0.05$), while there was no effect for elevated CO_2 ($P=0.72$) (Fig. 1b). There were no treatment effects on the cover for graminoids ($P=0.29$, $P=0.59$, warming and elevated CO_2, respectively) (Fig. 1c). Also, there were no interaction effects between warming and elevated CO_2 in any of the plant groups.

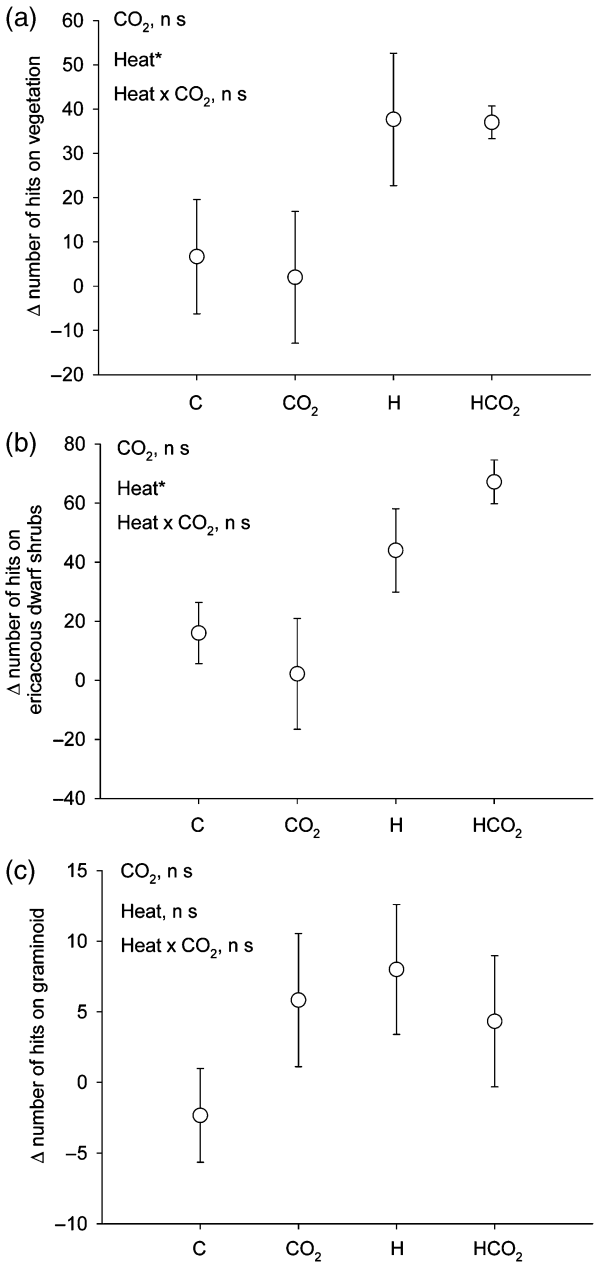

Fig. 1 The number of hits, using point-frequency analysis, on (a) vegetation, (b) ericaceous dwarf shrubs, and (c) graminoids in year 2006 minus the number of hits in year 2000 from four different treatments: untreated control plots (C); increased CO_2 (CO_2); increased temperature (H); increased temperature and CO_2 (HCO_2). Values are means ± SE. Statistical significance in ANOVAs are *$P<0.05$; ns, nonsignificant.

C, N and P concentration in plants

A significant lower N concentration was found in leaves of ericaceous plants under elevated CO_2 treatments ($P<0.05$) (Fig. 2a). The interaction effect between elevated CO_2 concentrations and warming was also significant in ericaceous plant species ($P<0.05$) (Fig. 2a). *D. flexuosa* showed a tendency to higher N concentration in leaves under warming ($P=0.16$) (Fig. 2b). The C/N ratio in leaves tended to be higher in ericaceous plants under elevated CO_2 ($P=0.05$), while the C/N ratio in leaves of *D. flexuosa* tended to decrease under warming ($P=0.16$) (Fig. 2e and f). No significant treatment effects were seen for P concentration in leaves. The N/P ratio for all plant species were low ranging between 6 and 10 in control plots.

ErM colonization

ErM colonization in ericaceous hair roots was significantly increased in CO_2-treated plots ($P<0.001$) (Fig. 3a). However, no responses of ErM colonization to warming could be seen ($P=0.69$), and there was no interaction. A trend towards a block effect was also found ($P=0.06$). There was no significant correlation between ErM colonization and DSE hyphal colonization ($P=0.90$).

FE colonization

FE colonization in grass roots was significantly lower in CO_2-treated plots ($P<0.05$) and tended to be lower in heat-treated plots ($P=0.06$), with no interaction between the warming and CO_2 treatments. (Fig. 3b). A significant block effect was also seen ($P<0.05$). No coarse AM fungi were found in association with grass roots.

DSE hyphal colonization

The DSE hyphal colonization did not differ between treatments in ericaceous hair roots ($P>0.05$) (Fig. 3c). However, in grass roots, heat significantly increased the DSE hyphal colonization while CO_2 had no effect on the DSE colonization ($P<0.05$) (Fig. 3d). The correlation

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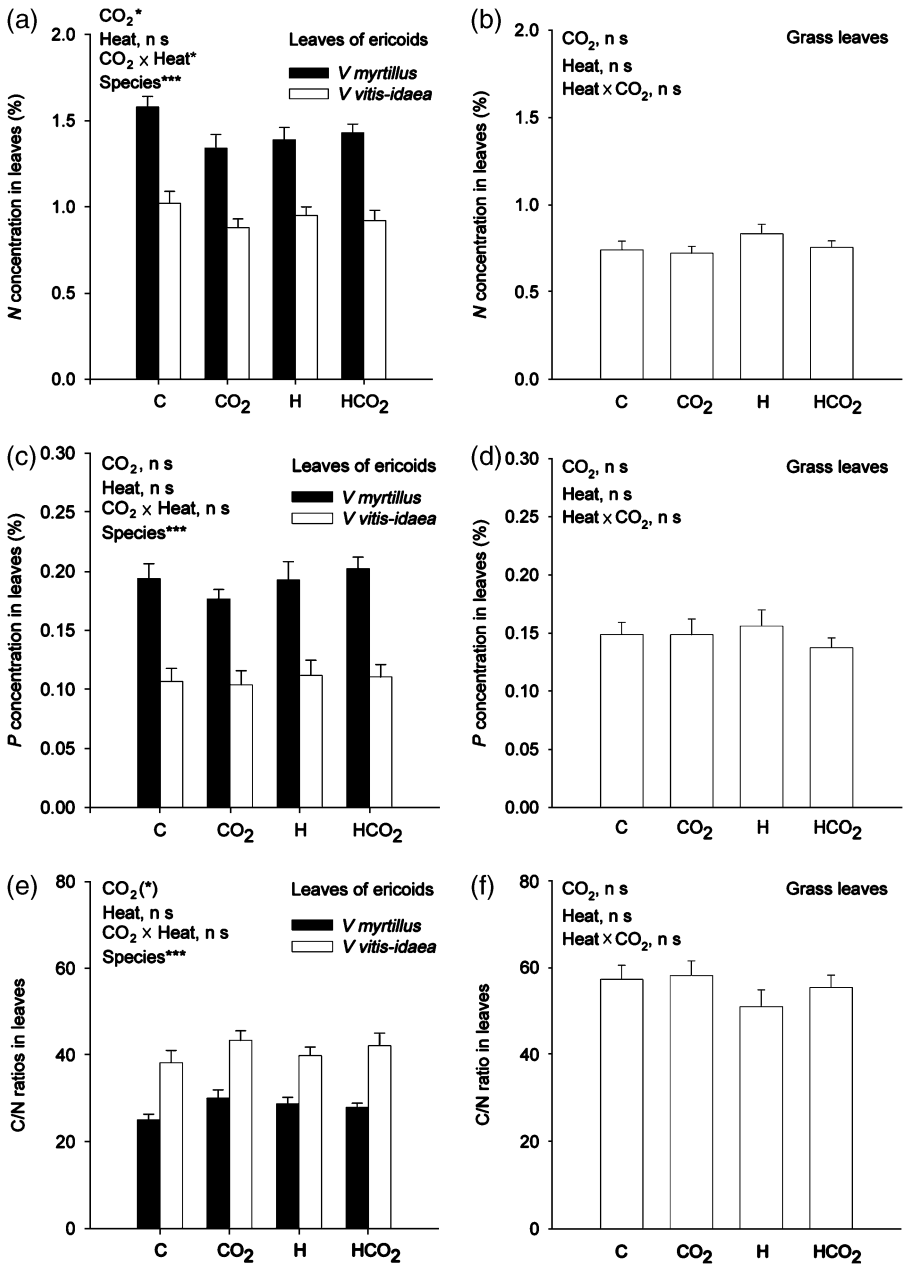

Fig. 2 Nutrient concentration (a–d) and C/N ratio (e–f) in leaves of ericoid dwarf shrubs, and *Deschampsia flexuosa* under different treatments: untreated control plots (C); increased CO_2 (CO_2); increased temperature (H); increased temperature and CO_2 (HCO_2). Values are means ± SE. Statistical significance in ANOVAs are ***$P<0.001$; *$P<0.05$; (*) $P<0.1$; ns, nonsignificant.

between FE colonization and DSE hyphal colonization in grass roots was significantly negative ($R^2 = 0.35$, $P<0.01$).

Discussion

Plant cover

The ericaceous dwarf shrub community responded positively to warming, that is, plant cover and assumingly plant biomass increased under warming. It has been shown previously in a similar OTC warming experiment in the subarctic that net N mineralization rates doubled in warmed soil (Hartley *et al.*, 1999). This increase in plant N availability likely contributes to the increased plant growth in warmed plots. The absence of a synergistic effect (i.e., no interaction effect) between warming and elevated CO_2 concentrations is likely due to plant N deficiency despite the increased N mineralization rate. A N/P ratio <10 indicates N deficiency in terrestrial plants

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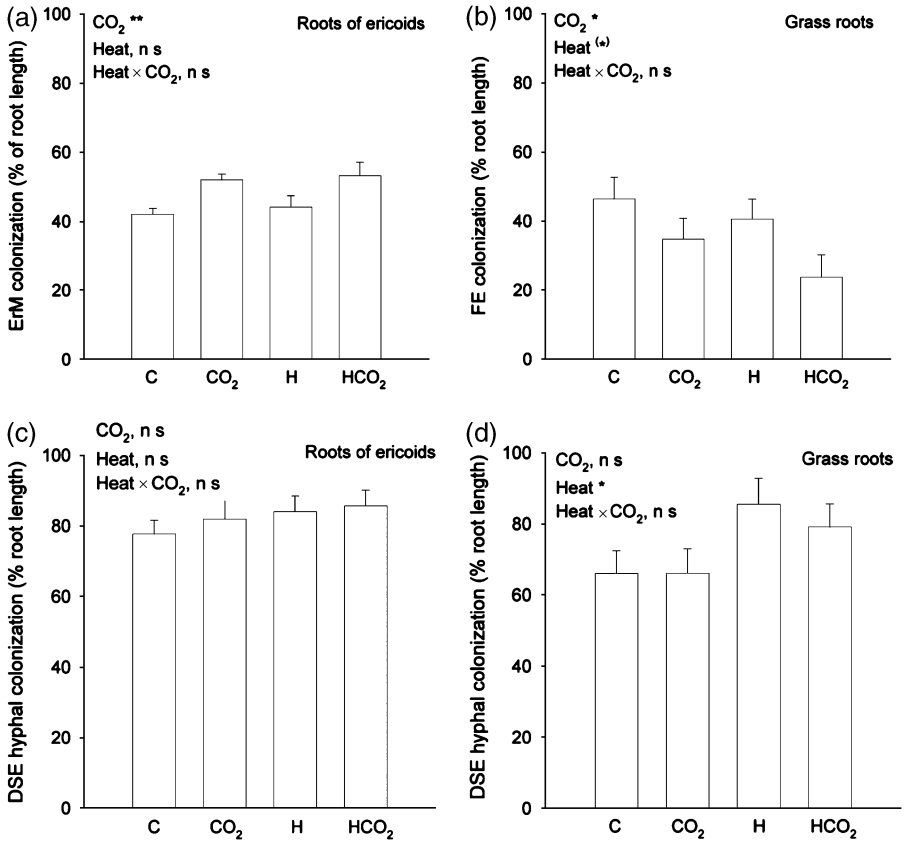

Fig. 3 Colonization by (a) ericoid mycorrhizae (ErM) in ericaceous hair roots, (b) fine endophytes (FE) in roots of the grass *Deschampsia flexuosa*, (c) dark septate endophytes (DSE) in ericaceous hair roots, and (d) dark septate endophytes (DSE) in roots of *D. flexuosa*, collected from four different treatments: untreated control plots (C); increased CO_2 (CO_2); increased temperature (H); increased temperature and CO_2 (HCO_2). Values are means ± SE. Statistical significance in ANOVAs are $^{**}P<0.01$; $^{*}P<0.05$; (*) $P<0.1$; ns, nonsignificant.

(Lambers *et al*., 1998), and in this study the plant N/P ratio ranged between 6 and 10 showing that plants were N limited, irrespective of treatment. This means that the extra CO_2 could not be used for additional plant growth. If N was sufficient we would probably have seen a positive growth response also to enhanced CO_2 concentrations in the atmosphere. The plant N deficiency prevents the plants from increasing their aboveground biomass as a response to increased CO_2 concentrations in the atmosphere. The increased root density (Olsrud *et al*., 2004) and increased ErM fungal colonization found under elevated CO_2 does not necessarily contribute to an increased N transfer to the plant shoots since the fungus and roots can also become a large sink for nutrients (Fransson *et al*., 2005). This might explain why we do not see a synergistic interaction effect between elevated CO_2 and warming on plant cover.

D. flexuosa did not respond to either warming or enhanced CO_2 concentrations in the atmosphere. The extra N available in soil in warmed plots did not benefit growth of *D. flexuosa*. Strengbom *et al*. (2004) showed that light and not N limits the growth of *D. flexuosa* in boreal forests. This might be one reason to why a growth response to warming and, hence, increased N availability in soil is absent for the grass in the birch forest. However, past studies in the open Arctic have reported an increased biomass production of graminoids to fertilization (Shaver & Chapin, 1980) and warming (Parsons *et al*., 1995).

ErM colonization

This is the first study to show that ErM colonization in hair roots is increased when host plants are exposed to enhanced atmospheric CO_2 concentrations. The results suggest that future increases in atmospheric CO_2 concentrations will lead to increased mycorrhizal colonization in dwarf shrub species dominating the understory of boreal and arctic ecosystems. The higher ErM colonization found in the current study might be due to increased transport of carbon belowground in an enhanced CO_2 environment. A tendency towards increased carbon allocation to hair roots in this dwarf-shrub plant community under enhanced CO_2 concentrations was observed previously (Olsrud *et al*.,

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2004). Some of this carbon is most likely transported to the ErM fungi and will be beneficial for the fungal symbiont.

The concentration of N in leaves of ericaceous plants was significantly lower in CO_2-treated plots compared with controls. In order to compensate for this lower N concentration the plant may allocate more resources to the roots and the fungal symbiont in an attempt to take up more N that can be allocated to aboveground tissue. Plants grown under elevated CO_2 tend to allocate more carbon to belowground relative to aboveground tissues (Rogers *et al*., 1996). This could result in more carbon being available to the root symbiont belowground resulting in strongly increased colonization per unit root length, as found in this study. Not only did the ErM colonization level increase, but also the hair-root density was significantly increased under elevated CO_2 concentrations (Olsrud *et al*., 2004). However, we found no effect of CO_2 enrichment on total plant cover. It therefore seems that the significant belowground responses to elevated CO_2 may take place irrespective of lack of changes in aboveground plant growth.

The abundance of ErM in roots might have broad implications on ecosystem C storage (Read & Perez-Moreno, 2003). The ErM have been shown to facilitate decomposition of recalcitrant carbon compounds (Bajwa & Read, 1985). An increased ErM colonization might therefore potentially have implications for the long-term storage of carbon in arctic and boreal soils. Talbot *et al*. (2008) suggested in the 'Priming Effect' hypothesis that mycorrhizal fungi decompose soil organic matter when the allocation of photosynthates to mycorrhizal roots are high. According to this hypothesis, enhanced atmospheric CO_2 concentrations may lead to increased decomposition, since more C was allocated to the roots under this treatment (Olsrud *et al*., 2004). Dorodnikov *et al*. (2009) found a significantly higher chitinase activity in soil under elevated CO_2 concentrations in the atmosphere. This reveals a mechanism on how ErM fungi potentially could increase their contribution to C turnover processes in soil under global change.

In this study, we also show, for the first time, that ErM colonization does not respond to warming. The difference between the responses to CO_2 and warming could be due to differential effects of the two treatments on soil nutrient availability, with warming leading to enhanced mineralization and nutrient release (Hartley *et al*., 1999). In a parallel study from the same experimental site, the ammonium concentration in the soil increased three to four times with warming, but remained unaffected in the elevated CO_2 treatment (R. Giesler *et al*.; unpublished data). Hence, when no N deficiency is present in plants the allocation of C to the ErM fungi is not changed and, consequently, the ErM colonization is also unchanged. The increased amount of N required for increased biomass production aboveground was met via increased N mineralization rate in the soil and not via an increased ErM colonization level that could potentially increase the N uptake in plants.

FE colonization

In this study, the grass *D. flexuosa* was the third most abundant plant species in terms of biomass, and hence an important component of the understory ecosystem. Both enhanced atmospheric CO_2 and warming caused decreased root colonization of FE fungi in roots of *D. flexuosa*. A decreased colonization level under elevated atmospheric CO_2 concentrations has previously been reported for FE fungi in a mediterranean-type climate field site (Rillig *et al*., 1999). However, this response was plant-species-dependent, as some host species did not show any responses to elevated CO_2 while others did. In laboratory studies, no changes in root colonization of FE were found in CO_2-treated plants (Rillig & Field, 2003; Staddon *et al*., 1998, 2004).

Soil warming has previously been shown to have no effect on FE fungal colonization (Staddon *et al*., 2004). On the other hand, drought has been shown to decrease FE fungal colonization (Braunberger *et al*., 1994; Staddon *et al*., 2004). This should be noted since increased temperature may have indirect effects via decreased soil moisture, as was found previously for this experiment (Olsrud *et al*., 2004). This might be a reason for the lower colonization level of FE fungi found in heated plots (Braunberger *et al*., 1994; Staddon *et al*., 2004). Another possible reason for the decreased FE fungal colonization level in grass roots from CO_2-treated plots and heated plots might be the increased proportion of newly produced grass roots in these two treatments compared with control. The faster root growth at elevated atmospheric CO_2 concentrations and heated conditions could have meant that FE fungi could not keep up, resulting in lower root colonization (Staddon *et al*., 1998, 2004). Olsrud *et al*. (2004) showed that hair root density measured after 2 years of treatment at the present site was increased in both CO_2-treated plots and heated plots. A tendency towards higher N concentration in *D. flexuosa* under warmed conditions could also be a reason to a lower FE colonization in heated plots.

D. flexuosa was colonized with FE fungi, but not with coarse AM fungi. This provides further evidence that FE fungi are more abundant than coarse AM fungi in northern ecosystems. Olsson *et al*. (2004) showed that arctic soil collected under *Deschampsia cespitosa*, a close relative to *D. flexuosa*, contained FE fungi, but not coarse AM fungi. The occurrence of FE fungi has also been shown to be related to soil pH (Wang *et al*., 1984; Postma *et al*., 2007). In acid soils, roots were found to be

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exclusively colonized with FE fungi but not with coarse endophyte fungi (Wang *et al*., 1984). This study was performed in the organic layer of an acidic podsol which might be another reason to why only FE fungi, but not coarse endophyte fungi, were present in roots.

DSE hyphal colonization

DSE hyphal colonization ranged between 80% and 90% in all treatments and was higher than co-occurring ErM colonization (40–50%) and FE fungi colonization (20–50%). This seems to be a general pattern, especially in cold regions, where nutrient-poor conditions prevail (Routsalainen *et al*., 2002; Olsrud *et al*., 2007). In dwarf shrub roots, no response to either warming or enhanced CO_2 levels was seen, while in grass roots, warming increased DSE hyphal colonization levels. The FE fungal colonization decreased under warmed conditions while DSE hyphal colonization were found to increase. Competition between the two fungal symbionts probably affects the level of colonization in cases where DSE hyphal colonization is favored during warmer conditions. It is possible that these results point towards a fungal community shift when the climate warms. The question then is whether such changes in the mycorrhizal fungal community structure may decrease or enhance plant responses. This is hard to speculate around since the function of the different fungal complexes is yet relatively unknown (Jumpponen, 2001; Addy *et al*., 2005). Some studies suggest that DSE associations can stimulate host plant growth by improving the nutritional status of plants or the ability of plants to withstand drought, while other claim that DSE fungi are exclusively parasitic (Addy *et al*., 2005). However, the cover of *D. flexuosa* did not increase under warmer conditions in this study, despite the enhanced DSE colonization level. Further studies are needed in order to elucidate the functional basis of these fungi.

Concluding remarks

This study shows that ErM fungi, FE fungi and DSE fungi respond significantly to six years of warming and enhanced CO_2 in a subarctic birch forest understory. Increased colonization of ErM in hair roots of ericoid dwarf shrubs due to elevated atmospheric CO_2 concentrations might have implications for ecosystem carbon and nitrogen cycling. Hence, an increased enzyme production in soil following enhanced ErM colonization might increase decomposition of organic matter in soil. However, changes in ErM colonization level did not affect total plant cover. The lower FE colonization due to global change factors might have effects on the nutritional status of host plant if FE acts as mycorrhizal fungi do, but the ecological function of FE fungi is still uncertain. Furthermore, the increased DSE colonization in grass roots increased under warming could have positive effects on plant performance but the function of DSE are still little understood and such changes in colonization level are therefore hard to interpret.

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