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Response of ericoid mycorrhizal colonization and functioning to global change factors

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Summary

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- Here, we investigated effects of increased atmospheric CO₂ concentration, increased temperatures, and both factors in combination on ericoid mycorrhizal colonization, mycorrhizal functioning and below-ground carbon allocation in a subarctic forest understorey, to evaluate the hypothesis that photosynthesis is a primary driver for mycorrhizal colonization.
- Treatment effects on ecosystem processes were investigated using ¹⁴C-pulse labelling and photosynthesis measurements in combination with analysis of ergosterol content in roots. The effects on δ¹⁵N in leaves were also studied.
- Ergosterol content in hair roots was positively correlated with ecosystem photosynthesis and was higher in heat- and CO₂-treated plots. Leaves from CO₂ plots tended to be more depleted in ¹⁵N compared with controls both for *Vaccinium myrtillus* and *V. vitis-idaea*.
- Our results suggest that changes in ecosystem photosynthesis, plant carbon (C) allocation may give rise to changing mycorrhizal colonization under elevated CO₂ and temperature. The role of mycorrhizas in ecosystem N-cycling may change on a long-term basis as inorganic N availability declines with increasing levels of atmospheric CO₂.

Key words: ericoid mycorrhizas, photosynthesis, carbon allocation, climate change, enhanced CO₂, warming, ¹⁵N natural abundance, subarctic.

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Introduction

The ground vegetation of arctic tundra and boreal forests is often dominated by ericaceous plants colonized with ericoid mycorrhizas (ERM) (Michelsen *et al.*, 1998). This symbiosis is known to have a key role in the cycling of both carbon (C) and nitrogen (N) in arctic and boreal ecosystems (Read, 1991; Read & Perez-Moreno, 2003). The fungal partner gains its C from the atmosphere via the host plant photosynthesis (Stribley & Read, 1974). By exuding enzymes the fungus in turn mobilizes N from a wide range of organic sources often not available to plant roots alone (Bajwa & Read, 1985; Bajwa *et al.*, 1985; Leake & Read, 1990; Näsholm *et al.*, 1998). Since rates of organic matter mineralization are low in arctic tundra and boreal ecosystems, and almost all of the soil N is

organically bound, the direct involvement of ERM in N cycling and dynamics is of particular importance in these ecosystems. Ericaceous dwarf shrubs grown in controlled environments are known to utilize organic N taken up by their fungal symbionts (Sokolovski *et al.*, 2002), and as much as 90% of the N requirements of the ericoid dwarf shrub *Vaccinium myrtillus* may be met by amino acids (Näsholm *et al.*, 1998).

Field studies have shown that ectomycorrhizal (ECM) plants are depleted, whereas mycorrhizal fungus is enriched in ¹⁵N compared with the original soil (Gebauer & Taylor, 1999; Hobbie *et al.*, 1999). The plant δ¹⁵N has further been shown to decline as N availability decreases in the soil, which was found for both ECM and ERM plants (Hobbie *et al.*, 2000; Hobbie & Colpaert, 2003).

Climatic changes in association with rising atmospheric CO₂ are expected to be particularly marked at high latitudes (IPCC, 2001), where ericaceous dwarf shrub ecosystems are abundant. In view of the significance of ERM to the overall functioning of these ecosystems (Read, 1991), it is important to quantify changes in mycorrhizal functioning and colonization in response to rising temperatures and CO₂.

Ericoid mycorrhiza colonization is negatively correlated with altitude and positively with host vigour (Haselwandter, 1979). Plants at high altitudes are exposed to low growing season temperatures, which reduce total photosynthetic production and hence the supply of carbon to the mycobiont. In studies of arbuscular mycorrhizas (AM), colonization rates are positively correlated with the photoperiod (Johnson *et al.*, 1982). These findings indicate that the level of colonization may be determined by the photosynthetic rate of the host plant.

It has long been known that elevated CO₂ concentrations in the air surrounding plants can lead to enhanced photosynthesis rates. Results of whole-ecosystem CO₂ manipulations, such as FACE experiments (Field *et al.*, 1992; Pitelka, 1994), show consistently that the enhanced plant-level photosynthesis also leads to higher ecosystem production (NPP) and growth. Proportional allocation of assimilated C to below-ground structures, such as hair roots, may also increase under elevated CO₂ concentrations as soil resources replace light as the limiting plant resource (Bloom *et al.*, 1985; Pitelka, 1994). The present body of literature also indicates that increased CO₂ in the atmosphere might lead to increased sequestration of carbon into living hyphae in the soil for ECM and AM plants (Treseder & Allen, 2000).

Higher temperatures have been shown to increase ecosystem production, probably as a result of increased decomposition and turnover of soil organic matter pools and a subsequently enhanced N availability (Melillo *et al.*, 1993). However, additions of mineral N fertilizers containing NO₃ and NH₄ have been found to have little effect on the degree of ERM colonization in arctic ecosystems (Michelsen *et al.*, 1999; Johansson, 2000). Higher temperatures might also influence net photosynthesis directly through their effect on biochemical reaction rates.

Increases in atmospheric CO₂ over the coming century are a certainty, while warming is likely in most arctic and boreal regions (IPCC, 2001). Interactions between these two types of environmental change in their effects on ecosystems are currently regarded important, as it is uncertain whether interactions would be antagonistic or synergistic, for example with respect to ecosystem C storage and exchange (Norby & Luo, 2004).

The purpose of this study was to investigate how increased CO₂ concentration in the atmosphere, increased temperatures, and their combination influence the below-ground carbon allocation and the degree of ERM colonization in subarctic forest understorey. The effects on leaf $\delta^{15}\text{N}$ were also studied in two dominant ericaceous dwarf shrub species as a possible indicator of mycorrhizal role in ecosystem N-cycling. In addition, responses of mycorrhizal colonization rates to

the range of environmental conditions experienced during the growing season under ambient conditions was investigated, to address the hypothesis that photosynthetic rates are the primary driver for colonization.

Materials and Methods

Study sites

The study took place near Abisko in the subarctic region of Sweden (68°21' N, 19°00' E), 341 m above sea level. The mean annual temperature at the study site is -0.8°C while the warmest month average temperature is 11.0°C. Average annual accumulated precipitation (1913–99) is 300 mm. Two areas situated approximately 500 m apart and with some of the same dominant plant species, were studied during the growing seasons of 2001 and 2002. The seasonality study of ERM and ecosystem photosynthesis (study 1) was performed in the elevated ombrotrophic parts of Stordalen mire, which are dominated mainly by dwarf shrubs, in particular *Empetrum hermaphroditum*, *Andromeda polifolia*, *Vaccinium uliginosum*, and *Vaccinium vitis-idaea*. The response of mycorrhizal colonization to experimentally simulated global change (study 2) was studied within a subarctic birch forest understorey dominated by dwarf shrubs, especially *Vaccinium myrtillus*, *V. vitis-idaea*, *E. hermaphroditum* and *V. uliginosum*. The dominant plant species of both studies areas are colonized with ERM (Michelsen *et al.*, 1998).

Seasonality study (study 1)

Study setup A total of 18 plots were positioned at least 2 m apart in a stratified-random design over an overall area of 780 m². Each plot consisted of an aluminium base inserted 0.1–0.2 m into the ground, enclosing 0.22 × 0.22 m of vegetation representative for the study area. Since *Betula nana* was killed by the insertion of the frames, potential plots were rejected if this species constituted more than an estimated 5% of the vegetation cover. Similarly, plots were disqualified if vascular plants covered less than an estimated 70% of the plot area. Nine bases ('flux' plots) were used for CO₂ flux measurements, while the other nine ('core' plots) were used for repeated soil sampling.

Climatic parameters were logged, within the study area between 18 June and 24 September. Soil moisture was measured every 30 min by time domain reflectometry (TDR) using a permanently installed Theta probe connected to a data logger (EMS, Brno, Czech Republic). Photosynthetic photon fluence rate (PPFR) over the waveband 400–700 nm, soil temperature at soil depths 0–5 cm and 10–15 cm, and air temperature were logged every 10 min using PPFR sensors, thermistors and a CR10-X data logger (Campbell Scientific, Shepshed, UK). Air temperature, PPFR and relative air humidity (r.h.) were also logged in conjunction with measurement of CO₂

fluxes, which was carried out by an infrared gas analyser (IRGA), EGM-2 (PP-Systems, Hitchin, UK) coupled to an HTR-2 probe (PP-Systems) holding a quantum sensor. The probe was enclosed in an airtight Plexiglas chamber with an internal 12 V fan. Thaw depth and water table depth were measured once a week at three different locations using a metal rod.

CO₂ fluxes and multiple regression models The CO₂ flux measurements were performed weekly in the nine 'flux' plots, using a standard closed-chamber technique similar to that used by Christensen *et al.* (2000). At least once per month, a diurnal series of measurements was carried out at 3-h intervals. Ecosystem CO₂ fluxes were measured using an IRGA attached to a Plexiglas chamber (see above). The aluminium bases were equipped with a water-filled channel that ensured an airtight seal between chamber and base. Recordings of net ecosystem exchange (NEE), defined as total system CO₂ exchange under light conditions, were made by installing the chamber on the aluminium base and then continuously measuring the change in CO₂ concentration within the chamber over 2–3 min. Total system respiration was measured by the same procedure after darkening the chamber with a light-proof hood. Gross photosynthesis was calculated as the difference between NEE and measured respiration.

Two models were obtained (Olsrud & Christensen, 2003) by multiple regression analysis (SPSS 10.0, Chicago, IL, USA) describing photosynthesis at PPFR $\leq 400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Model 1); and photosynthesis at PPFR $> 400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Model 2). Both Models 1 and 2 were required since the linear relation between incoming radiation and photosynthesis showed a sharp discontinuity around the light saturation level of PPFR at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. Both models included, as independent variables, measured values of PPFR, r.h., air temperature, soil temperature at soil depths of 0–5 cm and 10–15 cm, soil moisture and thaw depth.

Soil sampling A soil core, 15 cm deep and 3.8 cm diameter, was taken weekly from each of the 'core' plots between 18 June and 24 September. After removing the uppermost 2 cm, which mostly consisted of cryptogam biomass, the next 5 cm of the core were sorted for hair roots ($< 200 \mu\text{m}$ diameter) on ice over 1–1.5 h in the laboratory. In general, only hair roots of $< 100 \mu\text{m}$ 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 c. 70% roots with $d < 80 \mu\text{m}$. A high incidence of colonization by ERM may therefore be assumed for this class. Living roots were distinguished from dead roots by their elasticity: dead roots fragmented easily when drawn with forceps (Aerts *et al.*, 1989). The roots were freeze-dried within 36 h after field sampling.

Determination of mycorrhizal colonization To obtain an estimate of ericoid mycorrhizal fungal biomass, hair roots were analysed for ergosterol by high-performance liquid chroma-

tography (HPLC) (Nylund & Wallander, 1992). The roots were first ground in a ball mill, then transferred to test tubes for ergosterol extraction. Approximately 5–20 mg roots were extracted with 1 ml cyclohexane and 4 ml 10% KOH dissolved in methanol (Nylund & Wallander, 1992). The chromatographic system consisted of a HPLC (model 2248; Pharmacia-LKB Biotechnology, Uppsala, Sweden), UV detector (model 2141; Pharmacia-LKB Biotechnology, Uppsala, Sweden) and a Nova-Pak 0.39 \times 7.5 cm C18 reverse-phase column (Waters, Milford, MA, USA) preceded by a C18 reverse-phase guard column (Waters). Extracts were eluted with methanol at a flow rate of 1 ml min^{-1} and monitored at 282 nm. Hair roots were visually examined for ERM colonization using the staining method of Phillips & Hayman (1970). The ERM colonization of roots was high and with some external mycelium present on the root surface, of which some could be nonERM.

Global change experiment (study 2)

Experimental setup The experiment includes a warming treatment and an elevated CO₂ treatment in a one-way factorial design with four treatments. At the time of the sampling in the present study (June–September 2002), the experiment had been run for two growing seasons and was in its third seasons. Each experimental plot is $0.45 \times 0.75 \text{ m}$, divided into $0.15 \times 0.15 \text{ m}$ subplots and surrounded by an open-top chamber (OTC). The chambers have a footprint of c. 1.5 m^2 . Four chambers in each of six experimental blocks are randomly assigned to one of following four treatments: (1) heated chambers in which soil and air temperature are elevated 5°C above ambient using buried heating cables and infrared heating lamps; (2) CO₂ chambers in which CO₂-enriched air is blown into the chamber to double internal atmospheric CO₂ concentrations; (3) combined heat and CO₂-enrichment; (4) ambient control chambers which are identical to heat and CO₂ treatments but receives no elevated CO₂ or heat.

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 thermistors, three in heated chambers and 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 air within the heated chambers.

In CO₂-enriched chambers, atmospheric concentrations are elevated to $730 \pm 25 \text{ p.p.m. CO}_2$. A LiCor 6262 Infrared Gas Analyser (Li-COR Biosciences, Lincoln, NE, USA) monitors CO₂ concentration in four of the six enriched chambers. A data logger coupled to flow sensors and a mass flow controller regulate the flow of CO₂ from the tanks to the chambers. Air enriched with CO₂ is blown into chambers from two sides in order to maintain an even concentration of CO₂ across each chamber. NonCO₂-treatment chambers have an identical design, but receive air without CO₂ enrichment. This ensures that all

chambers experience similar effects of blowing. Blowing also reduces convective heating effects within the chambers.

In each block two 0.15×0.15 m subplots from each treatment – heat, CO_2 , heat plus CO_2 and chambered control – were selected at random for isotope labelling and sampling.

Isotope labelling and soil sampling Twice during the growing season (i.e. 8–11 July and 20–22 August), the vegetation was pulse-labelled with 10–15 MBq $^{14}\text{CO}_2$. A different set of subplots, one in each treatment, was labelled on each occasion. Owing to time limitations each labelling occasion extended over 3 d, with two blocks being labelled each day. Labelling was performed within a $0.15 \times 0.15 \times 0.35$ m transparent Plexiglas chamber placed over the vegetation. The chamber was connected by 0.2 m plastic tubing to a glass vial containing $\text{NaH}^{14}\text{CO}_3$. Three millilitres of 1 M HCl were injected into the glass vial, causing $^{14}\text{CO}_2$ to be released. The glass vial was then flushed with 2×60 ml air, forcing the $^{14}\text{CO}_2$ into the chamber. After 2 h the Plexiglas chamber was removed and labelling was thereby terminated.

A soil core with a diameter of 2.1 cm and a depth of 5 cm was taken in the centre of each 0.15×0.15 m subplot 8 d following labelling. An incubation time of 8 d post-labelling has previously been shown to give the highest pulse of ^{14}C in hair roots regardless of when the labelling was performed over the season (Olsrud & Christensen, 2003). Soil cores were transported on ice to the laboratory where they were kept in a cooling room at 2°C . Each soil core was sorted for hair roots ($< 200 \mu\text{m}$) in the same way as described previously. Sorted hair roots were carefully rinsed in distilled water, freeze-dried within 12 h after field sampling and later analysed for ergosterol as an estimate of ERM fungal biomass. The remaining soil was shaken with 0.5 M K_2SO_4 for 1 h to extract the dissolved organic C (DOC; Jonasson *et al.*, 1996). Extracts were mixed with a scintillation liquid (Ultima Flo-AP; Packard, Groningen, The Netherlands) and the content of ^{14}C were determined on a liquid scintillation counter (Tri-Carb 2100TR; Packard).

In order to determine hair root density, a representative sample of 0.05 g dry wt soil was separated from the soil core for determination of total root biomass. The sum of all roots that could be manually extracted with forceps was considered as the total root biomass.

All roots were ground in a ball mill and oxidized on a sample oxidizer (Model 30710; Packard) and analysed for ^{14}C by liquid scintillation (as described earlier).

Soil moisture was measured in the OTC at four occasions over June–August 2003 using TDR. At each occasion 10 measurements were performed in each chamber, totalling 60 measurements for each treatment and occasion. Precipitation data was provided by Abisko scientific research station (www.ans.kiruna.se).

Determination of N concentration and $\delta^{15}\text{N}$ in leaves Three leaves from each species and plot were sampled directly after

the soil sampling. In order to obtain leaves developed during current year, leaves growing next under the uppermost leaf were sampled. Leaf samples were dried in 50°C for 24 h, ground in a ball mill and analysed for per cent N (%N) and $\delta^{15}\text{N}$ using a elemental analyser (EuroVector, Milan, Italy) coupled to a IsoPrime isotope ratio mass spectrometer (GV Instruments, Manchester, UK).

Statistics

Treatment effects on the degree of colonization, allocation of recent assimilates and content of %N and $\delta^{15}\text{N}$ in leaves, was tested in a one-way ANOVA and soil moisture in a repeated measures ANOVA, all followed by Dunnett's test (comparisons of treatments against controls). For two analyses (%N and $\delta^{15}\text{N}$) the significance of time was investigated in a two-way ANOVA before the data from July and August was merged. Before analysis data were tested for normality and homogeneity of variance followed by a transformation, if appropriate. A one-way ANOVA was used in all cases since different processes were responsible for responses in single factor and interacting factor treatments. Test for associations was done using a linear regression analysis. A model describing ecosystem photosynthesis was extracted from environmental variables using a multiple regression analysis. SPSS 10.0 was used for all analyses.

Results

Ecosystem photosynthesis rate and ergosterol content in hair roots

The ergosterol content in hair roots, over the period 18 June–24 September, was positively correlated with average ecosystem photosynthesis rates measured 17 ± 2 d before root sampling

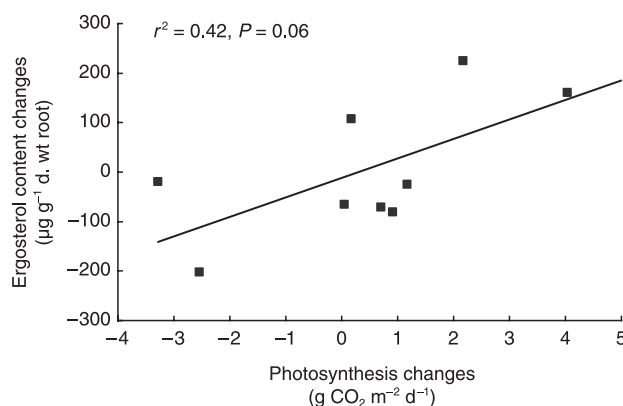


Fig. 1 The relationship between changes in ecosystem photosynthesis, and lagged changes in ergosterol content in hair roots over the period June–September 2001. Each of the nine data points are the difference between consecutive measurements 1 wk apart with no time overlap. Photosynthesis values used are means ($n = 5$) for 17 ± 2 d earlier than the ergosterol measurements (mean, $n = 3$) (Linear regression analysis; $n = 9$).

($r^2 = 0.42$, $P = 0.06$) (Fig. 1). The ergosterol content in hair roots over this period ranged from 230 to 950 μg ergosterol g^{-1} dry wt of root.

Effects of global change factors on ergosterol content in hair roots

There was a general trend towards higher content of ergosterol in hair roots (on a soil dry weight basis) in all treatments compared with control plots, both in July and August (Fig. 2). Heated plots had a higher ergosterol content in July compared with control ($P < 0.05$) (Fig. 2a), which might be explained by the higher ergosterol content per dry weight of hair roots in heated plots compared with control ($P < 0.05$). The CO_2 plots tended to have a higher ergosterol content in August compared with control plots ($P = 0.17$) (Fig. 2b), which are more likely to be associated with changes in root density (Table 1). No significant differences were found between the control and combined heat and CO_2 in either July or August.

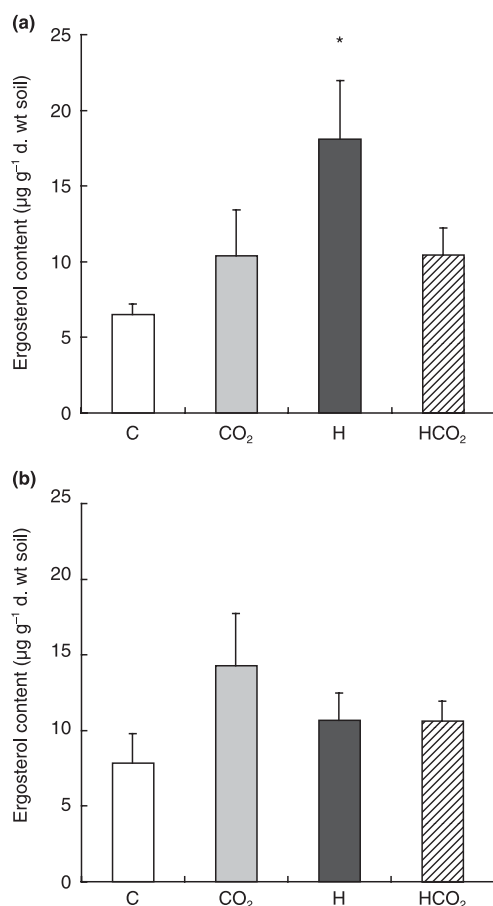


Fig. 2 Ergosterol content on a soil dry weight basis in hair roots (mean ± 1 SE; $n = 6$) in (a) July and (b) August, under increased temperature (H), increased CO_2 (CO_2), increased temperature and CO_2 (HCO_2), and untreated control plots (C) (Dunnett's test; $n = 6$; $*0.01 \leq P \leq 0.05$).

Table 1 Hair root density measured in treated and control plots on 8–11 July and 20–22 August 2002

Treatment	Month	Hair root density (g g^{-1} dry wt soil)
C	July	0.041 ± 0.003
CO_2	July	0.043 ± 0.01
H	July	0.060 ± 0.01
HCO_2	July	0.045 ± 0.01
C	Aug	0.032 ± 0.006
CO_2	Aug	$0.071 \pm 0.02^*$
H	Aug	0.052 ± 0.01
HCO_2	Aug	0.064 ± 0.01

H, increased temperature; CO_2 , increased CO_2 ; HCO_2 , increased temperature and CO_2 ; C, untreated control. Data are mean ± 1 SE ($n = 6$) $*0.01 \leq P \leq 0.05$ (Dunnett's test; $n = 6$).

Effects of global change factors on below-ground carbon dynamics and soil moisture

Allocation of recent assimilates to hair roots and DOC tended to be higher in treated plots compared with control in July–August (Fig. 3a,b). Heated plots had a higher allocation of recently assimilated carbon to DOC compared with control plots ($P < 0.05$) (Fig. 3b), and a similar trend was observed for hair roots ($P = 0.12$) (Fig. 3a). However, no differences were found in the relative carbon allocation to hair roots and DOC between CO_2 plots and plots with combined heat and CO_2 (Figs 3).

The hair root density in August was substantially higher in plots treated with additional CO_2 ($P < 0.05$). The same trend was also observed for CO_2 -treated plots with increased temperature ($P = 0.09$) (Table 1).

Soil moisture differed significantly between treatments. Heated plots were significantly drier compared with control (Fig. 4). The CO_2 -treated plots with increased temperature also tended to be drier than control plots ($P = 0.09$) (Fig. 4). The soil moisture in control plots could be estimated from average precipitation measured 10 d before the soil moisture ($r^2 = 0.95$, $P = 0.12$). Estimated from average precipitation, soil moisture in control plots were drier at the harvest in July ($0.09 \text{ m}^3 \text{ m}^{-3}$) compared with the harvest in August ($0.12 \text{ m}^3 \text{ m}^{-3}$).

Effects of global change factors on $\delta^{15}\text{N}$ and N content in leaves

Leaves sampled in July and August from plots treated with additional CO_2 tended to have lower $\delta^{15}\text{N}$ compared with control, both for *V. myrtillus* ($P = 0.16$) and for *V. vitis-idaea* ($P = 0.15$) (Fig. 5). Overall, the evergreen species *V. vitis-idaea* had lower $\delta^{15}\text{N}$ than the deciduous species *V. myrtillus* (Fig. 5).

The ergosterol content in the hair roots tended to be negatively correlated with the $\delta^{15}\text{N}$ in leaves from the dominant

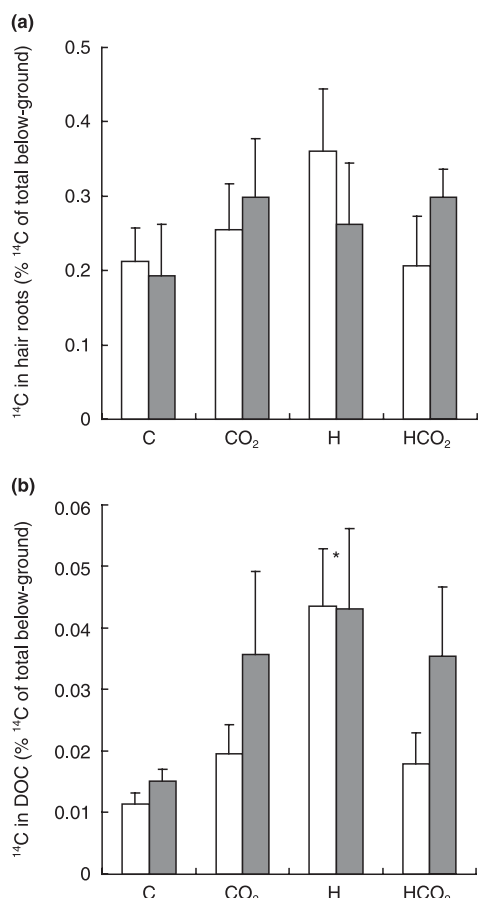


Fig. 3 Allocation of recently assimilated carbon to (a) hair roots and (b) dissolved organic carbon (DOC) in July (open columns) and August (tinted columns) as fraction of below-ground ¹⁴C allocation 8 d following labelling (mean \pm 1 SE; n = 6). Plot treatments: H, increased temperature; CO₂, increased CO₂; HCO₂, increased temperature; C, untreated control. Values were normalized by monthly means for statistical comparisons among treatments (Dunnett's test; n = 12; $0.01 \leq P \leq 0.05$).

species *V. myrtillus* in August (r^2 = 0.14, P = 0.07) whereas no such relationship was found for July (r^2 = 0.02, P = 0.559).

The N content in leaves was significantly lower in CO₂-treated plots in *V. myrtillus* and the same trend was observed for *V. vitis-idaea* (P = 0.10). In plots treated with heat or heat plus CO₂, the leaf N content tended to be lower than control for both *V. vitis-idaea* (P < 0.05; P = 0.18) and *V. myrtillus* (P = 0.09; P < 0.05) (Fig. 6).

Discussion

Ecosystem photosynthesis and ericoid mycorrhizal colonization

Results of the present study suggest that the degree of mycorrhizal colonization in hair roots is positively correlated with ecosystem

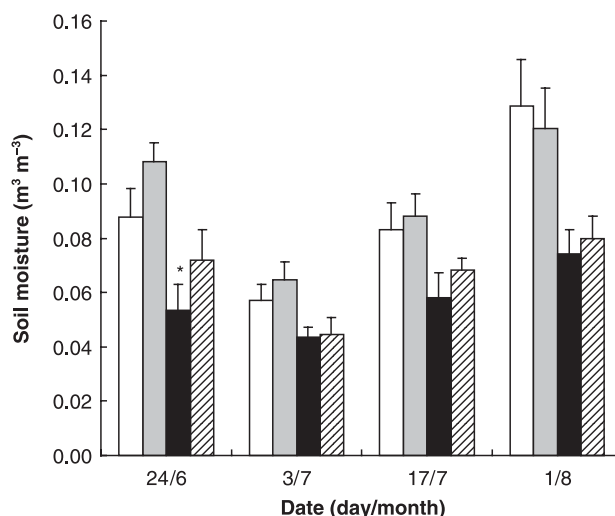


Fig. 4 Soil moisture in treated and control plots under increased temperature (filled bars); increased CO₂ (tinted bars); increased temperature and CO₂ (hatched bars); and untreated control plots (open bars) at 24 June, 3 July, 17 July and 1 August (mean \pm 1 SE; n = 6). Results from repeated measures ANOVA and *post hoc* test for differences between treatments and control (Dunnett's test; n = 6; $0.01 < P < 0.05$) are shown for 24 June but apply overall across sampling occasions.

photosynthesis (Fig. 1). This result, together with previous findings (Michelsen *et al.*, 1999; Johansson, 2000), suggests that a proportion of plant-assimilated carbon is consistently allocated to the symbiont regardless of the nutrient level in the soil. Studies of AM and ECM colonization have shown that the degree of mycorrhizal colonization is sensitive to nutrient levels in the soil (Smith & Read, 1997). In arctic and temperate heath ecosystems where ericaceous plants are colonized with ERM this relation has been sought but not confirmed (Michelsen *et al.*, 1999; Johansson, 2000). A positive relationship between ERM colonization and soil nutrient status has been found in long-term experiments, but the relation becomes weaker or disappears when normalized with above-ground or root biomass (Caporn *et al.*, 1995). A correlation between photosynthesis and ERM colonization point to a highly integrated relationship between the plant and fungal partner in these ecosystems, where the majority of the N is organically bound and therefore not available to plant roots alone. For this reason, plant C might be invested in the fungal partner without any primary soil nutrient control.

The level of ERM colonization in hair roots was estimated in this study by using ergosterol analysis. This method has been used previously for estimating ERM colonization, and the presence of vital ERM was found to increase the content of ergosterol in hair roots (Caporn *et al.*, 1995; Genney *et al.*, 2000). Ergosterol content in ERM fungi was determined to 2.3 mg/g by Padgett & Posey (1993), which is similar to the amount found in most ascomycetes and basidiomycetes (Weete & Gandhi, 1996). However, the relation

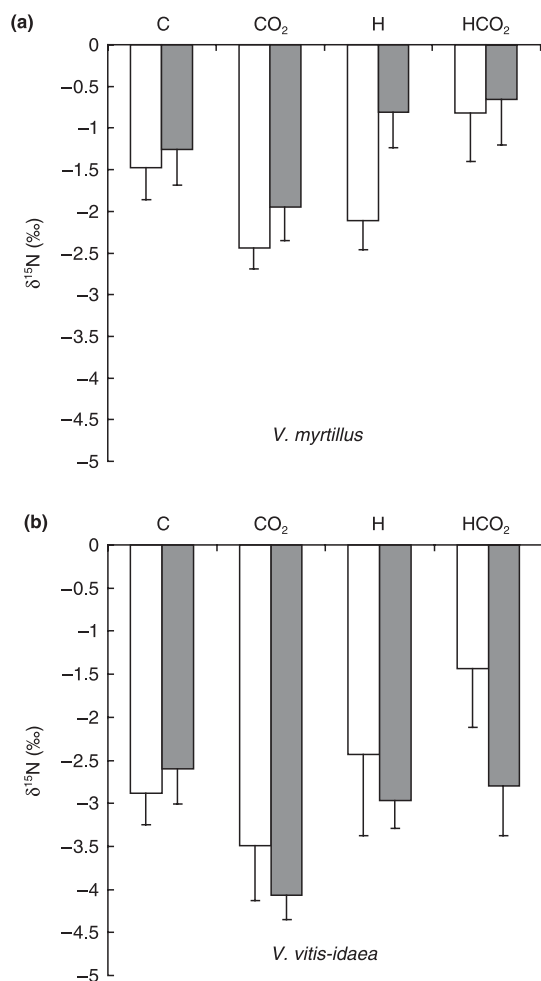


Fig. 5 Depletion of ^{15}N in leaves of (a) *Vaccinium myrtillus* and (b) *Vaccinium vitis-idaea* shown as $\delta^{15}\text{N}$ for treated and control plots 8–11 July (open columns) and 20–22 August (tinted columns) (mean \pm 1 SE; $n = 6$): H, increased temperature; CO_2 , increased CO_2 ; HCO_2 , increased temperature and CO_2 ; C, untreated control. Values were normalized by monthly means for comparisons between treatments and control (Dunnett's test; $n = 12$).

between ergosterol content in ERM-infected hair roots and the degree of ERM colonization has never been examined.

Effects of increased CO_2 and temperature on ericoid mycorrhizal colonization and below-ground C allocation

The observed responses of ERM colonization to elevated CO_2 and temperature (Fig. 2) can be interpreted with reference to the treatment effects on ecosystem photosynthesis and carbon allocation. Ecosystem photosynthesis is known to increase both in response to increased CO_2 and, under some conditions, temperature (Field *et al.*, 1992), and this could explain a tendency towards higher overall ERM colonization in all treatment plots compared with controls (Fig. 2).

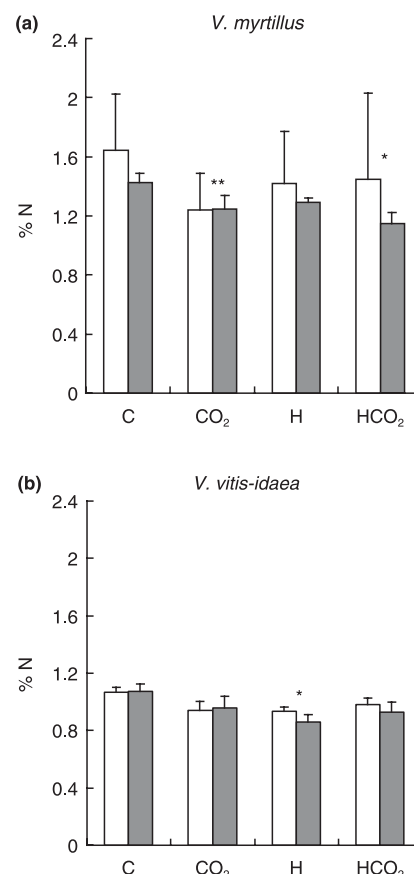


Fig. 6 Nitrogen content in leaves of (a) *Vaccinium myrtillus* and (b) *Vaccinium vitis-idaea* shown as percentage of leaf dry weight for treated and control plots 8–11 July (open columns) and 20–22 August (tinted columns) (mean \pm 1 SE; $n = 6$): H, increased temperature; CO_2 , increased CO_2 ; HCO_2 , increased temperature and CO_2 ; C, untreated control. Values were normalized by monthly means for comparisons between treatments and control (Dunnett's test; $n = 12$; * $0.05 \leq P \leq 0.05$; ** $0.001 \leq P \leq 0.01$).

The response of ecosystem photosynthesis alone does not appear to explain the superior colonization level in heated plots during July (Fig. 2a). Carbon allocation to hair roots and DOC tended to be higher in the heat treatment compared with controls in July according to ^{14}C measurements and observed root densities (Fig. 3; Table 1). Soil moisture levels were low during the July sampling period, owing to limited precipitation. It seems likely that the dry conditions were exacerbated by increased evapotranspiration in the heat-treated plots, resulting in significantly lower soil moisture than in untreated plots (Fig. 4). Plants exposed to reduced levels of soil resources are known to increase their proportional allocation of biomass to below-ground compartments in order to re-establish a balance in their overall resource budgets (Field *et al.*, 1992). An increased carbon allocation to hair roots and DOC (Fig. 3) might be a plant response that, in turn, enhances the level of mycorrhizal colonization. Both ecosystem photosynthesis and carbon allocation strategies

must be considered to explain the response of colonization level to CO₂ and temperature treatments.

The measurement period in August was preceded by a relatively large amount of precipitation, so that plants were exposed to little or no water stress during this period. Mycorrhizal colonization tended to be higher in the increased CO₂ treatment than in control (Fig. 2b), which could be explained by increased ecosystem photosynthesis in the treated plots (Field *et al.*, 1992). Both photosynthesis and leaf respiration are positively related to temperature. However, respiration may be more temperature-sensitive than CO₂ assimilation, suggesting that plants may have experienced lower net C uptake in the heated treatments owing to increased respiratory losses. Heat-treatment effects on ecosystem net capture are reflected in the tendency towards lower mycorrhizal colonization in roots from heated plots compared with CO₂-treated plots (Fig. 2b). In the absence of pronounced water stress, net photosynthesis alone may explain the degree of mycorrhizal colonization.

Effects of increased CO₂ and temperature on ecosystem N dynamics

The lower leaf N content and amount of $\delta^{15}\text{N}$ found in CO₂-treated plots than in control (Figs 5 and 6) suggest that an increased CO₂ concentration in the atmosphere might (1) reduce soil N availability and/or increase plant N demand, and (2) change the role of mycorrhizae in ecosystem N-cycling.

Leaves from all treatment plots had lower N content on a dry weight basis than controls (Fig. 6). However, in heated plots the total plant cover had increased significantly over the experimental period compared with control (B. Carlsson *et al.*, unpubl. data), which implies that N uptake on an ecosystem (ground area) basis was not necessarily lower as a result of heat treatment. By contrast, plant cover was not affected by CO₂ enrichment (B. Carlsson *et al.*, unpubl. data), suggesting that the lower leaf-tissue N concentrations in CO₂-treated plots (Fig. 6) might be indicative of N limitations (i.e. low soil N availability relative to plant demand).

Leaf N isotope composition might be affected by the N source in the soil (Michelsen *et al.*, 1996), physiological mechanisms in the plant (Evans, 2001) and type and degree of mycorrhizal association (Michelsen *et al.*, 1998; Hobbie *et al.*, 2003). In ERM plants the isotope signature of the N source is unlikely to be a major control of the plant ^{15}N content (Emmerton *et al.*, 2001). In ECM and NM plants there is little or no fractionation during N uptake under conditions of high plant N demand or low N availability, so that plant $\delta^{15}\text{N}$ is a good proxy for the $\delta^{15}\text{N}$ of the N source (Högberg *et al.*, 1999; Emmerton *et al.*, 2001). However, the opposite results were found for ERM plants, which showed increasing discrimination against ^{15}N at lower N supply (Emmerton *et al.*, 2001). This was found for various organic and inorganic N sources, which clearly shows that ERM significantly affect

^{15}N abundance in tissues of ericaceous plants and that the amount of $\delta^{15}\text{N}$ is not a reliable guide to the N source used.

The lower leaf $\delta^{15}\text{N}$ found in the CO₂-treated plots compared to control (Fig. 5) may reflect an N deficit in relation to plant demand (Hobbie *et al.*, 2000; Emmerton *et al.*, 2001) as also suggested by lower leaf N contents there. Several mechanisms might explain why the $\delta^{15}\text{N}$ in ERM plants is related to the plant N availability and demand: (1) different fungal enzymes of ammonium assimilation are active at low vs high supplies of ammonium (St John *et al.*, 1985); (2) mycorrhizal ^{15}N fractionation is more intensive at higher plant N uptake (Emmerton *et al.*, 2001); (3) a higher proportion of plant N uptake is mediated by the mycorrhizae under low N availability (Hobbie *et al.*, 2000).

Amount of mycorrhizal biomass (extraradical hyphae) has been shown to be negatively correlated with leaf $\delta^{15}\text{N}$ in ECM plants (Hobbie *et al.*, 2003). In the present study, a weak negative correlation was found between ERM colonization and leaf $\delta^{15}\text{N}$ in August but not in July.

Interactions between increased CO₂ and temperature

Interactive effects between increased CO₂ and temperature on ecosystems are important since simultaneous changes in both environmental drivers are likely according to climate scenarios for this century. However, the results of the present study illustrate the difficulty in interpreting such interactions; the interaction effects do not seem to be consistently antagonistic or synergistic when compared with the more frequently studied single-factor effects. As an example, the strong soil moisture-mediated effects on both below-ground carbon allocation and the degree of mycorrhizal colonization in heated plots are less likely to occur when warming is combined with elevated CO₂, since increased evapotranspiration associated with high temperatures may be counterbalanced by higher plant photosynthetic water-use efficiencies under elevated CO₂ (Table 2).

If water is assumed not to be a limiting factor, as seems reasonable in the case of the ecosystem studied, the combined effects of increased CO₂ and temperature might then be more readily inferred from a mechanistic understanding of the single-factor responses. The response matrix shown in Table 2 suggests that temperature effects may dominate ecosystem responses on a short-term basis, while CO₂ effects may become more important in the long term. The main reason for this is changes in N availability over time. Increased temperature enhances decomposition and N availability in the soil, while additional CO₂ suppresses this effect by decreasing tissue N content (Fig. 6) and litter quality (increased C : N ratio), with concomitant effects on decomposition and mineralization.

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Table 2 Response matrix for dwarf shrub dominated tundra showing how ecosystem parameters respond to increased temperature (Heat); increased CO₂ (CO₂); increased temperature and CO₂ (Heat + CO₂)

	Plant net C capture	Plant C allocation below-ground	Plant water balance	Plant phenology	Soil inorganic N availability	Soil organic N availability	Soil water relations	Mycorrhizal colonization
CO ₂	(+) Increased photosynthesis ^{3,11}	(+) Soil resource limitation ^{1,3,11}	(+) Increased water use efficiency ³	?	(–) In the long term increased C : N ratio of litter → lower N mineralization ³	(+) Increased mycorrhizal activity ^{7,10,12}	(+) Increased water use efficiency ³	(+) Increased photosynthesis and below-ground C allocation ^{5,10,13}
Heat	(+) Increased photosynthesis ^{3,14} (–) Increased respiration ¹⁴	(+) Water stress ^{1–3} (–) No water stress and increased mineralization ^{1,3}	(–) Increased transpiration ^{3,14}	(+) Speeded plant development ⁸	(+) Increased N mineralization ^{2–4,15}	(0) ^{6,9}	(–) Increased transpiration and evaporation ^{3,14,18}	(+) Increased photosynthesis ^{3,5,13} (+) Increased below-ground C allocation under water stress ^{1,3,5,10}
Heat + CO ₂	(+) Increased photosynthesis ³ (–) Increased respiration ¹⁴	(+) Water and/or nutrient stress ^{1–3,10} (–) No water and/or nutrient stress ^{1,3}	(0) Increased water use efficiency counterbalance increased transpiration ³	(+) Speeded plant development ⁸	(+) Increased N mineralization ^{2,3,4} (–) In the long term increased C : N ratio of litter → lower N mineralization ³	(0) ^{3,4,9,10} (+) In the long term, lower mineralization → increased mycorrhizal activity ^{7,12}	(0) Increased water use efficiency counterbalancing increased transpiration and evaporation ^{3,10,14}	(+) Increased photosynthesis ^{5,13}

The responses are shown as positive (+), negative (–) and no response (0), followed by a short description of the main responsible process involved.

Superscripts indicate the following references: 1, Bloom *et al.* (1985); 2, Shaver *et al.* (2000); 3, Field *et al.* (1992); 4, Hartley *et al.* (1999); 5, Haselwandter *et al.* (1979); 6, Michelsen *et al.* (1999); 7, Emmerton *et al.* (2001); 8, Menzel & Fabian (1999); 9, Johansson (2000); 10, this study; 11, Pitelka (1994); 12, Read & Perez-Moreno (2003); 13, Rillig *et al.* (2000); 14, Rustad *et al.* (2000); 15, Schmidt *et al.* (2002).

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