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Effects of twice-ambient carbon dioxide and nitrogen amendment on biomass, nutrient contents and carbon costs of Norway spruce seedlings as influenced by mycorrhization with *Piloderma croceum* and *Tomentellopsis submollis*

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Abstract Elevated tropospheric CO_2 concentrations may increase plant carbon fixation. In ectomycorrhizal trees, a considerable portion of the synthesized carbohydrates can be used to support the mutualistic fungal root partner which in turn can benefit the tree by increased nutrient supply. In this study, Norway spruce seedlings were inoculated with either *Piloderma croceum* (medium distance "fringe" exploration type) or *Tomentellopsis submollis* (medium distance "smooth" exploration type). We studied the impact of either species regarding fungal biomass production, seedling biomass, nutrient status and nutrient use efficiency in rhizotrons under ambient and twice-ambient CO_2 concentrations. A subset was amended with ammonium nitrate to prevent nitrogen imbalances expected under growth promotion by elevated CO_2. The two fungal species exhibited considerably different influences on growth, biomass allocation as well as nutrient uptake of spruce seedlings. *P. croceum* increased nutrient supply and promoted plant growth more strongly than *T. submollis* despite considerably higher carbon costs. In contrast, seedlings with *T. submollis* showed higher nutrient use efficiency, i.e. produced plant biomass per received unit of nutrient, particularly for P, K and Mg, thereby promoting shoot growth and reducing the root/shoot ratio. Under the given low soil nutrient availability, *P. croceum* proved to be a more favourable fungal partner for seedling development than *T. submollis*. Additionally, plant internal allocation of nutrients was differently influenced by the two ECM fungal species, particularly evident for P in shoots and for Ca in roots. Despite slightly increased ECM length and biomass production, neither of the two species had increased its capacity of nutrient uptake in proportion to the rise of CO_2. This lead to imbalances in nutritional status with reduced nutrient concentrations, particularly in seedlings with *P. croceum*. The beneficial effect of *P. croceum* thus diminished, although the nutrient status of its host plants was still above that of plants with *T. submollis*. We conclude that the imbalances of nutrient status in response to elevated CO_2 at early stages of plant development are likely to prove particularly severe at nutrient-poor soils as the increased growth of ECM cannot cover the enhanced nutrient demand. Hyphal length and biomass per unit of ectomycorrhizal length as determined for the first time for *P. croceum* amounted to 6.9 m cm^{-1} and 6.0 μg cm^{-1}, respectively, across all treatments.

Keywords Elevated CO_2 · Ectomycorrhiza · Mycelium · Fungal biomass · Nitrogen · Phosphate · Potassium · Magnesium · Calcium · Macronutrients · Micronutrients · Nutrient use efficiency · Rhizotrons · *Piloderma croceum* · *Tomentellopsis submollis* · *Picea abies*

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Introduction

The global change scenario with an expected increase of CO_2 concentrations (IPCC 2007) raises the question as to

what extent trees may contribute to CO_2 sequestration. The stimulation of photosynthesis by elevated tropospheric CO_2 furthers primary production and plant growth (e.g. Rey and Jarvis 1997; Wang et al. 1998; Iversen 2010; McCarthy et al. 2010). A resulting surplus of carbohydrates can be allocated belowground and used for root growth and the formation of mycorrhizae (Rogers et al. 1992; Janssens et al. 1998; Pritchard et al. 2001; Norby et al. 2004). As ectomycorrhizae (ECM) have a higher carbon (C) demand than other mycorrhizal associations (Leake et al. 2004), this mutualistic relationship of trees in temperate and boreal forests is currently discussed as means for sequestration of excessive CO_2 (Alberton et al. 2005). The participating fungi represent a strong sink of carbohydrates, particularly through mantle formation (Bidartondo et al. 2001; Högberg and Högberg 2002; Simard et al. 2002) and growth of extramatrical mycelium (Rygiewicz and Andersen 1994; Rygiewicz et al. 1997).

Many qualitative and quantitative studies on ECM under elevated CO_2 showed positive effects on both the formation and quantity of ECM, indicating an increased availability of photosynthates to the mycorrhizal fungi (e.g. Norby et al. 1987; O'Neill et al. 1987; Rygiewicz and Andersen 1994; Gorissen and Kuyper 2000; Alberton et al. 2005; McCarthy et al. 2010). Most of the studies report changes in mycorrhizal formation (e.g. Segmüller and Rennenberg 1994; Rey and Jarvis 1997; Tingey et al. 2000), community composition or morphotype assemblages (Godbold and Berntson 1997; Runion et al. 1997; Parrent et al. 2006; Parrent and Vilgalys 2007). Only a limited number of investigations refer to biomass data and the development of the extramatrical mycelium (Alberton et al. 2007; Alberton and Kuyper 2009).

In comparison to ambient CO_2 (aCO_2), elevated CO_2 concentrations increase ECM abundance and the amount of extramatrical mycelium (Tingey et al. 2000; Fransson et al. 2005; Garcia et al. 2008; Pritchard et al. 2008), although in some studies (Godbold et al. 2006; Parrent and Vilgalys 2007) a significant contribution of extramatrical mycelium to soil organic matter could not be found. As ECM differ in their amount of extramatrical mycelium (Raidl 1997; Agerer 2001; Agerer and Raidl 2004), the ECM community is likely to change in response to continuously increasing tropospheric CO_2 concentrations (Godbold and Berntson 1997; Parrent et al. 2006; Parrent and Vilgalys 2007), possibly promoting species with higher amounts of mycelium.

Studies on ECM regarding impacts of elevated CO_2 refer to either forest soils (O'Neill et al. 1987; Godbold and Berntson 1997; Godbold et al. 1997) or artificial substrates (Segmüller and Rennenberg 1994; Ineichen et al. 1995; Gorissen and Kuyper 2000). Only a few focus on reactions of ECM associations (Godbold and Berntson 1997; Godbold et al. 1997). Rey and Jarvis (1997) observed a stronger growth promotion of *Leccinum* ECM under elevated CO_2 in comparison to ECM of *Hebeloma*, *Laccaria* or *Thelephora*, with *Leccinum* seemingly having a higher demand for carbohydrates than the latter three species (Agerer 2001, 2007). Godbold and Berntson (1997) and Godbold et al. (1997) found an evidence for a shift towards ECM with higher amounts of extramatrical mycelium, whereas according to Runion et al. (1997) all morphotypes responded similarly in their abundance to elevated CO_2. Increased biomass production under eCO_2 was also reported from *Pisolithus tinctorius* (Mont.) E. Fisch. (Ineichen et al. 1995) and *Hebeloma crustuliniforme* (Bull.) Quél. (Fransson et al. 2005). Alberton and Kuyper (2009) found an increased hyphal length of the fungal species *Hebeloma cylindrosporum* Romagn., *Laccaria bicolor* (Maire) P.D. Orton and *Suillus bovinus* (Pers.) Roussel in response to elevated CO_2. Most studies do not refer to the absolute biomass data of both the mycorrhizal mantle and the extramatrical mycelium (Anderson and Cairney 2007), even though Colpaert and van Tichelen (1996) already pointed out that 'probably one of the best ways of studying the effect of environmental stress factors on mycorrhizas is to focus on the growth of the external mycelium'.

Apart from being carbohydrate sinks, ECM are generally accepted as the primary nutrient- and water-absorbing organs of trees (Smith and Read 2008). Depending on the amount, the distribution and organization of their extramatrical mycelia, so-called exploration types (Agerer 2001, 2007) of ECM, have been distinguished. In most species of ECM fungi, nutrient uptake is performed by the extramatrical mycelium (Duddridge et al. 1980; Kammerbauer et al. 1989; Allen 1991; Read 1992) comprising a single hyphae or strands of bundled hyphae, i.e. rhizomorphs (Agerer 1987–2008; Cairney et al. 1991).

Piloderma croceum Erikss. & Hjortst. (= *Piloderma fallax* (Libert) Stalpers) and *Tomentellopsis submollis* (Svrček) Hjortstam — the target organisms of the present study — both belong to the so-called medium distance exploration type (Agerer 2001; Agerer and Rambold 2004–2009) but differ in the amount and organization of their extramatrical mycelium (Agerer 1998; Agerer and Rambold 2004–2009; Brand 1991b; Haug and Pritsch 1992). *P. croceum,* with dense mats of hairy rhizomorphs and repeatedly dividing and unifying individual filaments of uniform-loose construction (Agerer 1999), is affiliated to the "fringe" subtype, whereas *T. submollis*, with less extramatrical mycelium and rather smooth, uniform-compact rhizomorphs (Agerer 1999), belongs to the "smooth" subtype. It was therefore of interest how the fungi and the mycorrhizal tree seedlings perform under elevated CO_2 with regards the biomass of both seedlings and fungal partners and the plant nutritional status.

At community level, soil nutrients can influence the fungal species composition (Agerer and Göttlein 2003), particularly under a deficiency or oversupply of nitrogen (N) (Alexander and Fairley 1983; Nilsson 2004; Parrent et al. 2006; Parrent and Vilgalys 2007). Nutrient supply can also represent a limiting factor for plant growth, being crucial when photosynthesis and carbohydrate formation increase due to elevated CO_2 concentrations (Alberton et al. 2007; Millard et al. 2007; Alberton and Kuyper 2009). Especially, nitrogen should be available and well balanced (Walker et al. 1995; Rygiewicz et al. 1997; Treseder 2004), but species seem to react differently to supply (Alberton and Kuyper 2009).

In this study, we investigated the combined effect of elevated CO_2 (eCO_2) and nitrogen availability on (a) the production of ectomycorrhizal biomass and aimed to assess the absolute biomass for the mycorrhizal mantle of *T. submollis* and *P. croceum*. The biomass of extramatrical mycelium was analysed only for the latter species. Further aims were to (b) focus on potential relations between biomass of extramatrical mycelium and amount of *P. croceum* ECM and to (c) determine whether the two ECM species vary in their effects on growth and nutrient supply of spruce seedlings under aCO_2 and eCO_2 treatments modified by N availability.

Materials and methods

Fungal isolates and culture conditions

T. submollis (Svrček) Hjortstam Germany, Bayern, Oberpfalz, district Regensburg, Bayerischer Wald, Rabenzipfel south of Forstenmühle and Ziegelhaus (circa 7 km north east of Donaustauf), 510–530 m asl, 14.03.1999, leg. et det. S. Raidl, SR 806, ectomycorrhizae, in M (Holmgren et al. 1990).

P. croceum J. Erikss. & Hjortstam: stock culture of SR 430: (= *Piloderma bicolor* (Peck) Jülich 1969, = *Piloderma fallax* (Libert) Stalpers (Stalpers 1993)), Germany, Bayern, Oberbayern, district Kelheim, Siegenburg, in the Dürnbucher Forst near the Fuchsberg, approximately 1 km west of Siegenburg, pine forest on sandy soil (Leucobryo-Pinetum Matuszkiewicz 1962), stand of *Picea abies* mixed with *Pinus sylvestris*, approximately 400 m asl, 08.10.1997, SR 430, *Picea* ectomycorrhizae, vouchers in M (Holmgren et al. 1990). The stock culture was kept at room temperature in the dark and subcultured regularly.

Ectomycorrhiza synthesis

Seedlings of Norway spruce (*P. abies* (L.) Karst.) were germinated according to Schubert et al. (2003). At approximately 4 weeks after germination, the seedlings were planted into square petri dishes (size 12×12×1 cm, VWR, Darmstadt, Germany) used as rhizotrons. Rhizotrons were filled to approximately 8 mm in height with 9 g (dry matter) pure peat substrate (specification H_3–H_5; pH in $CaCl_2$, 2.5–3.5; Kölle, Munich, Germany), which had been grinded in a knife blender and homogenized with a sieve of 2 mm mesh width (comp. Raidl 1997), and three seedlings were planted into each dish. Following 2 months of root formation, the seedlings were inoculated with freshly collected ECM of *T. submollis*, which were carefully placed nearby actively growing short root tips (comp. Raidl 1997). For inoculation with *P. croceum*, sterile mycelium was precultured for 5 weeks on square agar petri dishes (12×12×1 cm) on MMN medium (Marx 1969) supplemented with 1% (*w*/*v*) tetracycline. As described by Schubert et al. (2003), a sterile nylon grid (80 µm mesh width, Draht Center, Stuttgart, Germany) placed on the agar surface allowed the removal of inoculum from agar plates without any damage and containing only minimal agar residues. The nylon net bearing the mycelium was placed top down onto the roots of seedlings. The rhizotrons were covered with aluminium foil to keep light off the roots and were exposed upright to daylight at a north-facing window. After successful mycorrhization (circa 4–6 weeks), the inoculum net was removed and 40 rhizotrons (ten replicates for each treatment) with a similar degree of mycorrhization and almost equally sized seedlings were selected and randomly distributed over the different treatments. Ten rhizotrons with substrate but without seedlings and inoculum were used as controls for the growth of fungal saprotrophs.

Peat was used as substrate, as both ECM species are able to grow on spruce roots in organic layer (Brand 1991a [sub nomine *Fagirhiza rosea*], Haug and Pritsch 1992 [sub nomine *Piceirhiza rosea*]).

CO_2/N treatments

The rhizotrons were transferred to the greenhouse of the German Research Center for Environmental Health (http://www.helmholtz-muenchen.de/eus/neu/green_en.php) with standardized conditions (15/10°C day/night temperatures, relative humidity 75%, additional irradiance for 12 h/day by Natrium high-pressure lamps, Phillips; photosynthetic photon flux density approx. 130 µmol m^{-2} s^{-1}). 20 rhizotrons, 10 of each mycorrhizal species, as well as the control rhizotrons were exposed to aCO_2 (charcoal-filtered ambient air at an annual mean of ~400 ppm CO_2) for 13 weeks and the other 20 rhizotrons to eCO_2 (= aCO_2+ 300 ppm, i.e. aCO_2 mixed with 300 ppm CO_2). Out of each CO_2 treatment, ten rhizotrons were moistened weekly with circa 10 ml deionised water, while ten others received a weekly nitrogen amendment of 5 ml of ammonium nitrate

solution (concentration, 9 mg/l) plus circa 5 ml deionised water (aCO_2+N and eCO_2+N). The parallel treatment with moderate nitrogen fertilization served to keep the nutritional equilibrium between N and carbohydrates which had been expected to be synthesized in higher amounts under eCO_2 (Iversen 2010; Lewis et al. 1994; McCarthy et al. 2010).

Harvest procedure

First, the surrounding peat substrate including the extramatrical mycelium was removed from each rhizotron by carefully stripping off the extramatrical mycelium from the ECM mantle using fine forceps and a stereoscope. The complete, 7-month-old plants were removed from the rhizotron and each plantlet was separated into three fractions, with the fractions of the three seedlings of each rhizotron combined to one sample: (1) aboveground parts of the plant, i.e. needles and shoot—combined for receiving sufficient material for element analysis, (2) mycorrhized roots, recognizable as a typical yellow mantle cover in *P. croceum* (Brand 1991b) and pinkish in *T. submollis* (Agerer 1998) and (3) roots. Needles/shoot and roots were dried (2 days/60 ° C) and weighed, while ECM were stored in FEA (formol–ethanol (70%)–acetic acid = 5:90:5, *v*/*v*/*v*). The substrate including the extramatrical mycelium was gently mixed in a mortar with a pestle, and a small substrate aliquot (approximately 10%) was removed and dried for the calculation of total substrate dry matter. The remaining substrate was mixed further while adding water to a total volume of 150 or 200 ml (depending on the mycelium density), resulting in a homogeneous suspension. A 10-ml aliquot was separated and mixed strongly in a small mortar to break the rhizomorphs and longer hyphae into shorter fragments. The time and intensity of this final procedure had to be calibrated individually for every rhizotron while the result was checked step by step by light microscopy to test whether the hyphae had been separated well enough.

Measurement of mantle and mycelial length and calculation of fungal biomass

The total length of ECM was measured via image analysis using WinRhizo (Version 3.09, Regent Instruments Inc., Quebec, Canada). The conversion of mycorrhizal length into volume of mycorrhizal mantle was achieved by assuming ECM to be perfect cylinders, with a mean diameter of spruce ECM of 0.387±0.072 mm and an ECM mantle thickness of 0.028±0.012 mm (based on the data compiled by Agerer and Rambold 1998). The mantle cross-section area comprises, according to geometrical formulas, 26.9% of the total cross-section area of the mycorrhizal root and therefore on average 0.0316 mm^2 for spruce ECM. Multiplication with mycorrhizal length resulted in the total volume of the mycorrhizal mantle and the multiplication with dry weight (see below) in biomass.

The length of extramatrical hyphae was determined by the agar film method (after Bååth and Söderström 1979; Kunzweiler and Kottke 1986; modified by adding 90% lactic acid instead of agar solution and without staining with phenol aniline blue), producing four replicate agar films for each rhizotron. Hyphae were detected with a Nomarski interference contrast microscope (Zeiss, Germany, magnification ×400) and sketched on paper with the aid of a camera lucida. After measuring the length of the drawn hyphae with a map measuring instrument (Eschenbach, Germany) and calibrating these values to real hyphal lengths and total substrate aliquots, total hyphal length per gramme substrate (dry matter) could be calculated for each rhizotron. In order to determine the length of saprotroph hyphae, the controls were processed accordingly. Assuming that the amount of saprotroph hyphae was the same in both controls and mycorrhizal rhizotrons, the mean length of saprotroph hyphae was subtracted from the total hyphal length to obtain the length of the extramatrical hyphae only for each treatment. These corrected values were used for further calculations.

Mycelial biomass was calculated using the formula $B = r^2\pi \times L \times D \times M$ (Frankland et al. 1978), with B= mycelial biomass [g dry mass], r=hyphal radius [mm], L= hyphal length [m], D=relative hyphal density [g cm^{-3}] and M = % dry mass = (100 − mycelial moisture content as % of fresh weight)/100. $r^2\pi \times L$ = hyphal biovolume (assuming hyphae to be perfect cylinders), with r based on the species-specific hyphal diameter of 2.2 µm for the extramatrical mycelium of *P. croceum* (deduced from Brand 1991a and Raidl 1997). L was measured via WinRhizo (see above). D=1.09 g cm^{-3} and M=21%, following Bakken and Olson's (1983) suggestion for the conversion of hyphal biovolume into biomass with $D \times M = 0.2289$ g dry mass cm^{-3}.

Nutrient content of seedlings

Dried plant fractions were powdered with a mortar mill (Retsch, Germany), digested with HNO_3 and analysed by ICP-AES for macro- and micro-nutrients (according to BMVEL 2005). The total N content of plant samples was measured by combustion using an elemental analyzer (LECO, USA).

Data analysis and statistical treatment

The biomass and nutrient status of seedlings as well as the length and biomass of ECM and extramatrical mycelium

were calculated for each rhizotron. As seedlings within rhizotrons were connected via their mycorrhizae and in order to get a sufficient material for analysis, all three seedlings per rhizotron were regarded as one entity, with the rhizotron as the replication unit. The differences between CO_2/N treatments (aCO_2, aCO_2+N, eCO_2 and eCO_2+N) and between ECM species were compared by two-way ANOVA (n=10 rhizotrons) with the factors 'fungal species', 'treatment' and the interaction 'fungal species×treatment', in the case of mycelium (*P. croceum* only) by one-way ANOVA with the factor 'treatment'. For multiple comparisons between species and treatments, the Tukey's HSD test was applied (p<0.05). Statistical analyses were performed using JMP INTRO, Version 5.0.1a (SAS Institute Inc., Cary, NC, USA). The data on the nutrient contents of the spruce seedlings were compared accordingly.

The initially intended comparison of extramatrical mycelia of both species could not be performed as the method used for hyphal length measurements was only applicable for *P. croceum*. The hyphae of *T. submollis* could not be separated thoroughly enough and could not be reliably discerned in the hemocytometer cuvettes.

Results

Seedling biomass and C/N ratios

Seedling biomass was differently influenced by the two fungal species. Across all CO_2/N treatments, root/shoot ratio was significantly higher with *P. croceum* than with *T. submollis* as ECM partner (Table 1), and the same trend was found for total plant biomass (p=0.0515). Root mass was particularly pronounced in seedlings with *P. croceum*. Within CO_2/N treatments, however, these species-specific differences were significant only in roots under eCO_2. In general, elevated CO_2 did not affect seedling biomass or biomass allocation within the timeframe of the experiment, although seedlings with *P. croceum* tended to increase biomass in response to elevated CO_2 (eCO_2 and eCO_2+N). However, the C/N ratio in the shoot differed between treatments, showing an increase under both eCO_2 and eCO_2+N, particularly with *P. croceum* as fungal partner.

Ectomycorrhizae and extramatrical mycelia

The ECM length and the mantle biomass of *P. croceum* were considerably higher than those of *T. submollis* (Tables 2 and 3). Assuming identical thickness of the mantles in both species, the mantle biomass was four to five times higher in *P. croceum* than in *T. submollis*, ranging between 2.3–3.1% and 0.6–0.9% of total seedling dry mass, respectively (Table 3). In comparison to root biomass, the mantle biomass of *P. croceum* and *T. submollis* represented 4.2–5.8% and 1.4–2.0% per rhizotron, respectively.

There was no significant effect of elevated CO_2. However, ECM length and mantle biomass of *P. croceum* tended to be increased by about 25% under the combination of eCO_2+N, whereas in *T. submollis* these traits tended to be increased under both eCO_2 and eCO_2+N by up to 61%.

The hyphal length and biomass of *P. croceum* extramatrical mycelium, for the first time determined in relation to ECM length, amounted to an average of 6.9 m cm^{-1} ECM^{-1} and 6.02 μg cm^{-1} ECM^{-1}, respectively. As for ECM length and

Table 1 Dry matter (DM), root–shoot ratio and C/N ratio of total plant, shoot and root in seedlings colonized with *P. croceum* and *T. submollis* under the treatments aCO_2, aCO_2+N, eCO_2 (aCO_2+300 ppm) and eCO_2+N (aCO_2+300 ppm +N). Data represent means ± standard deviation, n=10. *Asterisks* within a column indicate significant differences between species (p<0.05); only the highest value was marked

	aCO_2	aCO_2 + N	eCO_2	eCO_2 + N
Piloderma croceum				
DM shoot [mg]	93.5±34.5	88.7±26.2	101.9±36.8	108.6±22.2
DM root [mg]	109.4±40.0	103.6±32.6	120.3±32.9*	120.8±29.6
DM total plant [mg]	202.9±74.0	192.3±56.2	222.2±68.7	229.4±49.1
Root/shoot ratio	1.2*	1.2*	1.2*	1.1*
C/N shoot	49.2±9.8	47.9±11.7	55.0±11.2	59.2±6.9
C/N root	50.4±4.9	50.9±3.8	46.8±5.3	53.0±3.1
C/N total plant	50.5±5.9	49.2±7.3	50.7±6.1	55.6±4.1
Tomentellopsis submollis				
DM shoot [mg]	117±14.5	108.7±16.5	104.4±16.8	109.3±16.2
DM root [mg]	80.4±12.4	80.8±17.6	75.4±12.5	84.6±12.5
DM total plant [mg]	197.4±24.8	189.5±33.1	179.8±27.3	193.9±27.1
Root/shoot ratio	0.7	0.7	0.7	0.8
C/N shoot	54.2±6.6	47.1±4.9	47.8±8.0	52.5±4.5
C/N root	56.0±6.9	53.3±5.5	51.5±4.6	53.3±4.9
C/N total plant	55.0±6.7	50.1±4.0	49.4±6.9	52.7±3.7

Table 2 Length of ectomycorrhizae (ECM) of *P. croceum* and *T. submollis*, as well as the length of extramatrical mycelium and mycelial biomass of *P. croceum* per unit of ECM length and per unit of soil dry mass (DM) and their percentage in comparison to the treatment with ambient CO_2 alone (=100%). Hyphal length adjusted by subtraction of saprotroph hyphae (13.12 m g^{-1} for aCO_2 and eCO_2 and 14.83 m g^{-1} for aCO_2+N and eCO_2+N, respectively). Data represent means ± standard deviation, n=10. *Asterisks* within column indicate significant differences between species ($p<0.05$); only the highest value was marked

	aCO_2	aCO_2 + N	eCO_2	eCO_2 + N
Piloderma croceum				
ECM length [mm g^{-1} soil DM]	9.5±3.3*	7.9±3.7*	8.2±4.0*	11.9±4.8*
In percent of aCO_2 [%]	100	83	86	125
Hyphal length [m g^{-1} soil DM]	61.8±40.3	58.0±36.4	63.0±56.1	86.8±42.7
In percent of aCO_2 [%]	100	94	102	140
Hyphal length [m cm^{-1} ECM]	6.4±3.3	6.7±2.9	6.9±4.0	7.6±3.6
Mean [m cm^{-1} ECM]	*6.9±3.4*			
Hyphal biomass [μg g^{-1} soil DM]	53.8±35.1	50.8±33.6	54.79±48.8	75.51±37.1
Mean [μg g^{-1} soil DM]	*58.9±39.0*			
Mantle biomass [μg g^{-1} soil DM]	690.3±237.4*	574.3±265.5*	594.1±286.6*	860.1±349.1*
Hyphal biomass [μg cm^{-1} ECM]	5.57±2.83	5.81±2.52	6.04±3.44	6.62±3.17
Mean [μg cm^{-1} ECM]	*6.02±2.93*			
Tomentellopsis submollis				
ECM length [mm g^{-1} soil DM]	1.8±1.1	2.1±1.3	2.2±1.7	2.9±1.9
In percent of aCO_2 [%]	100	116	123	161
Mantle biomass [μg g-1 soil DM]	130.1±80.2	150.3±91.7	160±126.5	209.8±134.7

mantle biomass, there was no effect of elevated CO_2 apart from a slight trend of increased hyphal length and biomass under eCO_2 and eCO_2+N.

Nutrient concentrations in seedlings in relation to fungal associates and CO_2/N treatments

The concentrations of P, K, Ca, Mg, Cu and Zn were significantly higher in the shoot and/or total plants of seedlings inoculated with *P. croceum* as compared to those with *T. submollis* (Fig. 1). The most striking fungal species effect was observed for P concentrations which were more than twice as high in seedlings inoculated with *P. croceum*. A different pattern of plant nutrient allocation was found for Ca, showing significantly lower concentrations in roots with *P. croceum* as compared to those with *T. submollis*.

Significant treatment effects were only found in seedlings with *P. croceum* (exception roots in seedlings with *T. submollis* for Ca), in most cases with lower nutrient concentrations in the shoot and/or total plant in response to eCO_2 and eCO_2+N, although variation was high within treatments (Fig. 1).

Table 3 ECM mantle biomass per unit of soil dry mass (DM), per rhizotron and in percentage of total tree biomass and root biomass (dry mass, DM); mycelial biomass and total ECM biomass including the mycelium of *P. croceum* per rhizotron and in percentage of total tree biomass and root biomass; proportion of mycelial biomass on total ECM biomass of *P. croceum*. Data represent means ± standard deviation, n=10. *Asterisks* within column indicate significant differences between species ($p<0.05$); only the highest value was marked

	aCO_2	aCO_2 + N	eCO_2	eCO_2 + N
Piloderma croceum				
ECM mantle per rhizotron [mg]	5.745±2.069*	4.917±2.485*	4.851±2.099*	6.957±2.884*
In percent of seedling DM [%]	2.9±0.5*	2.6±1.1*	2.3±1.0*	3.1±1.2*
In percent of root DM [%]	5.4±1.0*	5.0±2.3*	4.2±1.9*	5.8±2.0*
Mycelium per rhizotron [mg]	0.448±0.294	0.424±0.260	0.438±0.363	0.603±0.291
In percent of seedling DM [%]	0.22±0.11	0.23±0.15	0.21±0.20	0.27±0.13
ECM mantle + mycelium per rhizotron [mg]	6.193±2.258	5.298±2.662	5.289±2.368	7.561±3.100
In percent of seedling DM [%]	3.1±0.5	2.8±1.2	2.5±1.2	3.3±1.3
In percent of root DM [%]	5.8±1.0	5.4±2.6	4.6±2.2	6.3±2.1
Proportion of mycelium on total ECM biomass [%]	7.0±3.3	7.4±2.9	7.5±4.0	8.3±3.6
Tomentellopsis submollis				
ECM mantle per rhizotron [mg]	1.118±0.731	1.341±0.867	1.388±1.130	1.762±1.100
In percent of seedling DM [%]	0.6±0.3	0.7±0.4	0.8±0.6	0.9±0.5
In percent of root DM [%]	1.4±0.8	1.6±0.8	1.8±1.4	2.0±1.1

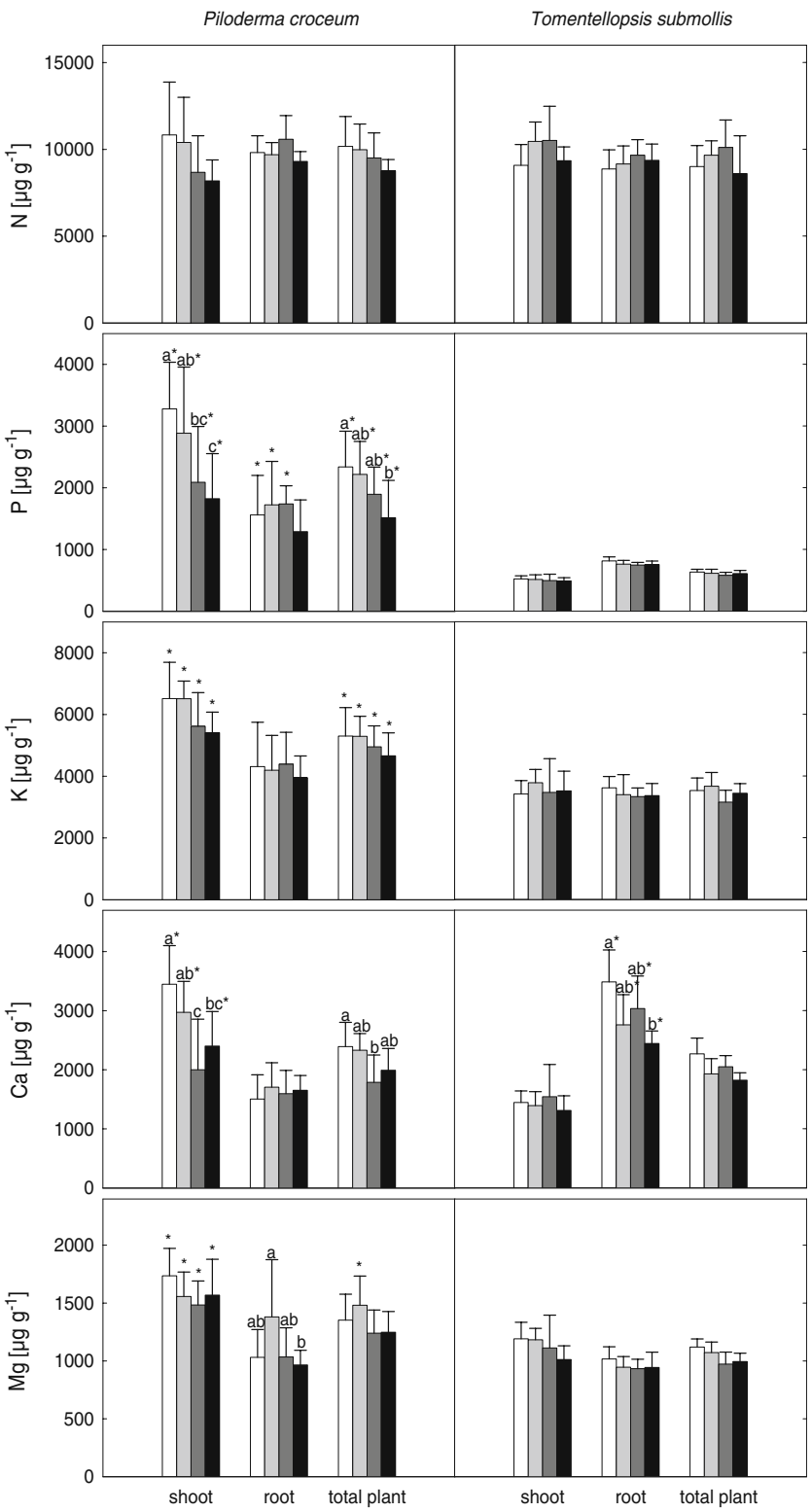
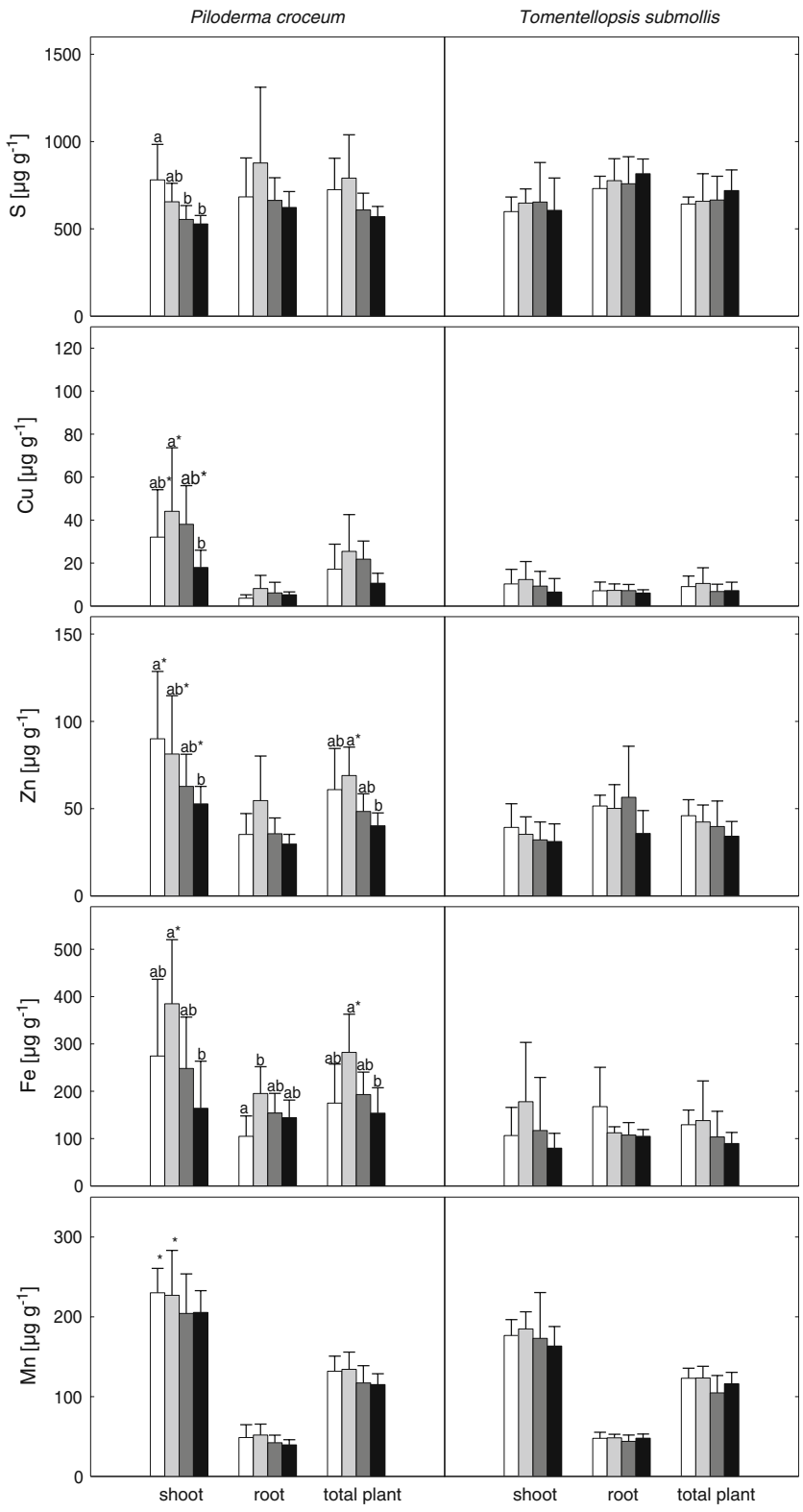


Fig. 1 Nutrient concentrations in shoot, root and total plant of spruce seedlings, mycorrhizal with *P. croceum* (*left*) and *T. submollis* (*right*). Data represent means ± standard deviation (n= 10) per treatment: aCO_2 (*white bars*), aCO_2+N (*light grey bars*), eCO_2 (*dark grey bars*), eCO_2+N (*black bars*). Different *letters* within grouped *bars* indicate significant differences between treatments ($p<0.05$). *Asterisks* indicate significant differences between species in the same treatment ($p<0.05$); only the highest value was marked

Nutrient contents in seedlings and nutrient use efficiency in relation to fungal partners and CO_2/N treatments

As nutrient concentrations only mirror the nutrient status of plants, total nutrient contents express the capability of plants for total uptake in relation to biomass production. When including seedling biomass, total nutrient accumulation per three seedlings (rhizotron) was significantly higher for all nutrients when seedlings were inoculated with *P. croceum* compared to seedlings inoculated with *T. submollis* (Table 4).

Fig. 1 (continued)



Significant CO_2/N treatment effects on total nutrient content were found only for Zn as reduced amounts under eCO_2+N compared to aCO_2+N in seedlings with *P. croceum*.

Regarding nutrient use efficiency, i.e. biomass produced per unit of invested nutrient, seedlings with *P. croceum* were less efficient in biomass production per invested unit of the macro-nutrients P, K and Mg (Fig. 2). This was most

Table 4 Nutrient content per total seedling biomass of rhizotron (three seedlings per rhizotron), as means ± standard deviation, $n=10$. Different *letters* within column and fungal species indicate significant differences between CO_2/N treatments ($p<0.05$).

Treatment	Seedling biomass per rhizotron [g]	N [µg seedlings^{-1}]	P [µg seedlings^{-1}]	K [µg seedlings^{-1}]	Ca [µg seedlings^{-1}]	Mg [µg seedlings^{-1}]	S [µg seedlings^{-1}]	Cu [µg seedlings^{-1}]	Zn [µg seedlings^{-1}]	Fe [µg seedlings^{-1}]	Mn [µg seedlings^{-1}]
P. croceum											
aCO_2	0.203±0.074	2,168±531	491±112*	1,130*±242	513±132	288±62	151±29	3.5±2.3	12.7±5.2 ab	42.5±21.5	28.4±7.2
aCO_2 + N	0.192±0.056	1,994±479	506±169*	1,192*±216	551±148	336±92*	175±43	5.8±3.9	15.6±4.3 a*	61.4±9.8*	31.5±7.4
$eCO2$	0.222±0.069	2,349±420	442±113*	1,173*±296	424±131	291±64*	145±39	5.6*±2.6	11.9±4.8 ab	50.1±22.1	27.0±3.9
eCO_2 + N	0.229±0.049	2,066±341	363±146*	1,109*±168	474±91	302±75*	145±24	2.5±1.0	9.6±1.7 b	38.0±10.1	27.6±4.7
T. submollis											
aCO_2	0.197±0.025	1,822±133	127±19	707±94	458±96	226±40	129±17	1.9 ± 1.1	9.2±2.1	25.9±6.8	24.8±4.3
aCO_2 + N	0.190±0.033	1,900±260	121±18	732±177	379±66	212±41	128±30	2.1±1.7	8.4±2.6	27.5±16.8	24.4±5.2
eCO_2	0.180±0.027	1,826±239	109±17	595±120	388±76	183±30	124±23	1.2±0.5	7.5±2.8	19.9±12.2	19.7±4.8
eCO_2 + N	0.194±0.027	1,749±504	111±31	676±146	348±75	195±39	143±43	1.4±0.6	6.6±1.7	18.5±5.2	23.0±6.3

*$p<0.05$ (significant differences between fungal species; only the highest value was marked)

striking for P, as seedlings with *T. submollis* showed a threefold higher efficiency than seedlings with *P. croceum*.

Nutrient use efficiency was not affected by any of the CO_2/N treatments, except for a significantly higher efficiency in P uptake by seedlings with *P. croceum* under eCO_2+N as compared to aCO_2.

Discussion

Growth parameters and biomass of seedlings and ectomycorrhiza

Influence of fungal partner

The considerably higher root and ECM mantle biomasses of the seedlings inoculated with *P. croceum* as compared to those of seedlings inoculated with *T. submollis* resulted in a higher root/shoot ratio of seedlings with *P. croceum* (cf. Tables 1 and 2). Whereas total seedling biomass with *P. croceum* was on average 1.1 times higher than with *T. submollis*, the proportion of ECM mantle biomass was about four times higher with *P. croceum*. This suggested that seedlings with *P. croceum* invested much more carbohydrates into the root system and the fungal partner than those with *T. submollis*. The difference in biomass production and carbon allocation thus highlight a strong species-specific fungal impact.

Similar fungal species-specific influences on plant biomass were reported from other ECM species (Fransson et al. 2005; Alberton et al. 2007; Alberton and Kuyper 2009). Fransson et al. (2005) reported on a higher biomass of Scots pine seedlings inoculated with *Paxillus involutus* (Batsch) Fr. compared to seedlings inoculated with *H. crustuliniforme* (Bull.) Quél, while at the same time the fungal biomass of *P. involutus* was lower than that of *H. crustuliniforme*. This corresponds to our results with respect to shoot mass but not with root and total seedling mass. *T. submollis*, with considerably less ECM length, furthered shoot biomass production more than *P. croceum*. Both studies suggest a species-specific impact on seedling growth and biomass allocation. Colpaert et al. (1992) showed a negative correlation between the extent of fungal development of several ECM fungi and the growth of host plants under low substrate nutrient concentrations that were similar to our peat substrate. Species with large amounts of hyphae apparently consumed more carbohydrates than species with sparse mycelial development, as the former species caused a smaller plant growth (Colpaert et al. 1992). Although the mycelial biomass of *T. submollis* could not be measured in the present study, visual differences indicated a higher investment of the seedlings into the mycelium of *P. croceum* than into that of *T. submollis*.

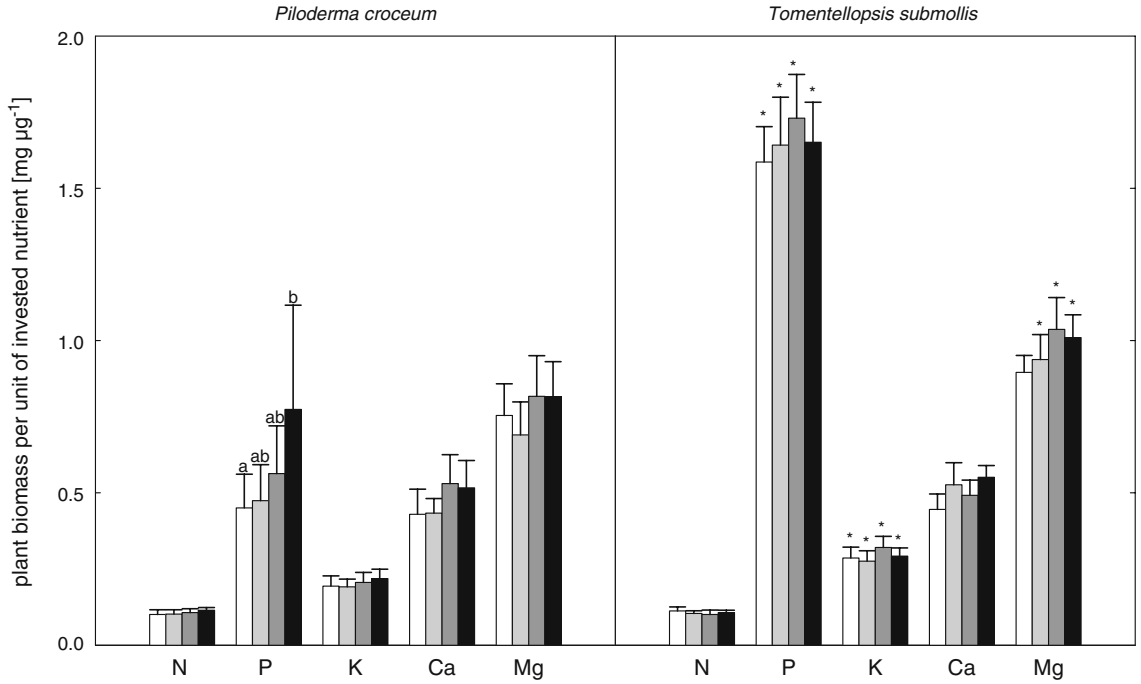


Fig. 2 Nutrient use efficiency of spruce seedlings colonized with *P. croceum* (*left*) and *T. submollis* (*right*) expressed as plant biomass per unit of invested nutrient of the macro-nutrients N, P, K, Ca and Mg. Data represent means ± standard deviation (n=10) per treatment: aCO_2 (*white bars*), aCO_2+N (*light grey bars*), eCO_2 (*dark grey bars*), eCO_2+N (*black bars*). Different *letters* within grouped *bars* indicate significant differences between treatments ($p<0.05$). *Asterisks* indicate significant differences between species in the same treatment ($p<0.05$); only the highest value was marked

Species-specific mantle thickness likewise was not considered here. Yet it is apparent that *T. submollis* forms denser mantles and less extramatrical mycelium than *P. croceum* (Agerer 1998; Brand 1991b). This contrasts with the findings of Colpaert et al. (1992) as seedling biomass was still higher with *P. croceum* than with *T. submollis*.

The mycelium of *P. croceum* represented about 7.5–11.5% of the mycorrhizal mantle biomass. This fits well to the extrapolation made by Smith and Read (2008) for *Lactarius rufus* (Scop.) Fr., likewise a medium-distance exploration type, with an estimated production of mycorrhizal mantles of 730 kg ha^{-1} $year^{-1}$ and an extramatrical mycelium amounting to 70 kg ha^{-1} $year^{-1}$ or 9.6% of the mantle biomass. However, *P. croceum* obviously owns much more extramatrical mycelium and its mantle is less thick than that of *L. rufus* (Agerer and Rambold 2004–2009; Agerer 2001). The figures given by Smith and Read (2008) are therefore possibly overestimations, which is supported by the fact that *L. rufus* belongs to the medium distance "smooth" exploration type.

Hyphal length and dry mass relative to the ECM length as evaluated for the first time with the described method may be a useful tool for estimating mycelial length and biomass produced by mycorrhizal systems. Averaged across all CO_2/N treatments, 6.9 m, equivalent to 6.02 µg hyphae per centimetre ECM have been produced. Rousseau et al. (1994) reported for the long-distance exploration type *P. tinctorius* on 5 m cm^{-1} ECM^{-1} and Jones et al. (1990) for the medium-distance exploration types *Laccaria proxima* and *Thelephora terrestris* 1.93–3.13 m hyphae per cm mycorrhizal root and 0.29–0.53 m, respectively, depending on phosphorus content of the substrate. The figures obtained for *L. proxima* and *T. terrestris* are possibly similar to those that could be expected for *T. submollis*, as all three species belong to the "smooth" subgroup within the medium-distance exploration type, whereas *P. croceum* is affiliated to the "fringe" subgroup (Agerer and Rambold 2004–2009).

However, mycelial biomass is not sufficient to judge the carbohydrate sink on, as carbohydrates could have been differently stored as glycogen granula within the hyphae. Preliminary comparisons of both species have shown a higher density of glycogen granula in the hyphae of *T. submollis* in comparison to those of *P. croceum* (unpubl. data, Franz 1994).

Effects of elevated CO_2 and nitrogen availability

Elevated CO_2 had no significant effect on biomass but tended to promote seedling growth with *P. croceum* as well

as ECM growth of both fungal species, particularly in combination as eCO_2+N. Similar tendencies of growth promotion of *P. croceum* hyphae and biomass of *P. sylvestris* seedlings in response to elevated CO_2 were reported by Alberton et al. (2007). Both eCO_2 and eCO_2+N indicated stronger impacts on mantle biomass and ECM length of *T. submollis* than of *P. croceum* (cf. Table 2). These findings correspond to those reported by Godbold et al. (1997) in that respect such that mycorrhizal morphotypes forming thicker mantles are favoured under elevated CO_2.

Elevated CO_2 is generally known to increase root growth, particularly in nutrient-deficient substrates, often leading to an increasing root/shoot ratio (e.g. Janssens et al. 1998; Thomas et al. 1999; Tingey et al. 2000; Alberton et al. 2007). Yet the contrary has been found, too (Kasurinen et al. 1999; Rouhier and Read 1999; Johnson et al. 2006; Handa et al. 2008), and nitrogen availability can influence root growth under elevated CO_2 (Pregitzer et al. 2000; Wiemken et al. 2001). However, in our experiments, neither CO_2 nor N addition significantly influenced the root growth of seedlings with *P. croceum* or *T. submollis* apart from an indicated trend of increased root mass under elevated CO_2 with *P. croceum*. Comparing different ECM species, Alberton et al. (2007) found that increased root biomass and root/shoot ratio under elevated CO_2 was most prominent with species exhibiting the largest external mycelium.

Godbold et al. (1997) observed a predominant increase of mycorrhizal morphotypes forming a greater amount of extramatrical mycelia and rhizomorphs under elevated CO_2 concentrations, whereas morphotypes with less mycelium were reduced. However, the hyphal length measured (438–1,216 mm g^{-1} soil) was about 50–200 times lower than that found in *P. croceum* in the present study (cf. Table 2). The mycelial biomass in a *Pinus taeda* stand showed no change in response to CO_2 concentrations of 200 ppm above aCO_2 (Parrent and Vilgalys 2007). Ineichen et al. (1995) found, for *P. sylvestris* seedlings with *P. tinctorius* (Mich.: Pers.) Coker & Couch, a threefold increase in the number of mycorrhizal systems and a doubling of mycelial biomass following 3 months of elevated CO_2 (600 ppm) exposure. The number of ECM and extent of mycelial systems of *S. bovinus* and *P. involutus* contemporarily increased considerably in response to twice-ambient CO_2 concentrations, being remarkably larger in *P. involutus* than in *S. bovinus* rhizotrons (Rouhier and Read 1999). Tingey et al. (2000) concluded that elevated CO_2 causes increases in ECM colonization on conifer roots and in the amount of produced extramatrical mycelium. Based on PLFA quantification, an increased amount of ECM soil hyphae under elevated CO_2 was also found on deciduous *Fagus sylvatica* (Wiemken et al. 2001). Conversely, Kasurinen et al. (1999) found no evidence of increased carbon allocation to ECM under elevated CO_2 in young Scots pines at nutrient-poor forest sites. In a study similar to the one presented here, mycelial production and spread of *H. crustuliniforme* were either increased or unchanged in response to twice-ambient CO_2, depending on the experimental approach, whereas *P. involutus* showed no clear effect (Fransson et al. 2005). The differences in mycelial spread between *H. crustuliniforme* and *P. involutus*, particularly in greater distances from the inoculated root system, are possibly due to the different exploration types of the two fungal species (Agerer 2001; Agerer and Rambold 2004–2009). The rhizomorph-forming long-distance exploration type *P. involutus* usually exhibits a less uniformly distributed mycelium than the short-distance or medium-distance exploration type *H. crustuliniforme*.

Apart from growth enhancement, the carbohydrate storage in hyphae can also be increased in response to elevated CO_2, as shown for glycogen in the mantle of *Amanita muscaria* (L.: Fr.) Hooker (Turnau et al. 2001).

When assessing the total annual carbon allocation to ectomycorrhizae and the influence of elevated CO_2, the turnover and respiration of the ECM should also be considered. It is known that ECM respiration, as considerable consumer of carbohydrates (Ek 1997; Koch et al. 2007; Rygiewicz and Andersen 1994), can amount to 30% of soil respiration (Söderström and Read 1987). Thus, increasing soil respiration under elevated CO_2 (e.g. Nakayama et al 1994; Schlesinger and Andrews 2000; Zak et al. 2000; King et al. 2004) may be attributed to a considerable part to ECM respiration. The respiration of ECM and of their extramatrical mycelia are suggested to amount to 60% of the carbon allocated to the fungus or 4.3% of total carbon assimilated (Rygiewicz and Andersen 1994). Based on Rygiewicz' and Andersen's respiration percentage of 60%, when calculating for *P. croceum* extraradical mycelial biomass, between 5.9% and 8.3% of seedling dry matter was transferred to the fungal partner. As seedling biomass prior to inoculation was not measured, an estimation of carbon allocation to the fungal partner relative to the carbon assimilation was not possible. Assuming a mycelial carbon content of 40% to 50% (Zhu and Miller 2003, Smith and Read 2008), *P. croceum* should have sequestered between 6.6 and 9.4 mg and between 5.3 and 7.6 mg carbon, respectively.

Moreover, ECM turnover plays a role when assessing C allocation. Rygiewicz et al. (1997) calculated an average median ECM lifetime of 139 days and Sittig (1999) of 76 days for *Xerocomus chrysenteron* (Bull.: St. Amans) Quél, 83 days for *Lactarius subdulcis* Bull.: Fr. and 94 days for *Cenococcum geophilum* Fr. Due to the relatively short duration of our experiment (approximately 90 days), ECM turnover might not have influenced the conclusions regarding carbohydrate allocation to the fungal partner. As

fine roots have lifetimes of years (Smith and Read 2008), their turnover rates can be neglected in the present studies.

Elevated CO_2 concentrations increase the trees' demand for N (O'Neill 1994; Walker et al. 1995; Runion et al. 1997; McCarthy et al. 2010) to be covered for regular growth, whereas the moderate addition of nitrogen can equalize a nitrogen imbalance caused by the increased availability of CO_2 (Pregitzer et al. 2000; Turnau et al. 2001). The lacking increase in ECM length and biomass of *P. croceum* under aCO_2+N and eCO_2 and, conversely, a trend of higher production under eCO_2+N indicate that fertilization balanced the increased nitrogen demand of the seedlings under eCO_2. The same trend was evident for the extramatrical mycelium. The reaction to nitrogen may be a consequence of the different levels of sensitivity of both species to the availability of this nutrient (Alberton and Kuyper 2009). Therefore, a refined approach with nitrogen amendment adjusted to the special demands of either species would be necessary. Such slightly controversial reactions as found for *P. croceum* and *T. submollis* are little known for ectomycorrhizae, although a shift in ectomycorrhizal community structure with reference to N availability has been observed (Fransson et al. 2000, Parrent et al. 2006).

A synergistic effect of N amendment and eCO_2 on fungal growth was apparent only as a trend in *P. croceum* as well as in *T. submollis* with an increase in length of 25% and 61%, respectively. This may hint at the differing capabilities for nitrogen uptake or nitrogen use efficiencies. However, no difference in N use efficiency was observed between the two fungal species (see below). According to Arnebrant and Söderström (1992), N fertilization with ammonium nitrate inhibited the growth of *P. croceum* mycelium and, conversely, promoted the growth of another ECM morphotype (now identified as *T. submollis* according to Agerer 1998; Köljalg et al. 2001). This species-specific N uptake capacity under different N availability indicates a different optimum range of N between these two fungal species, although only weakly pronounced in the present study.

Nutrient concentrations of seedlings

Influence of fungal partner

The higher concentrations of most nutrients in seedlings with *P. croceum* compared to those with *T. submollis* indicated an improved nutrient status for spruce with *P. croceum*. Additionally, a different plant internal nutrient allocation under the influence of these two fungal species was observed.

The striking differences in almost all element concentrations, particularly in phosphate accumulation, were most likely due to the fact that *Piloderma* produced 3.7–5.3 times greater ECM lengths, respectively mantle biomass (cf. Tables 2 and 3), than *Tomentellopsis*, leaving differences in extramatrical mycelium unconsidered. Therefore, with respect to the low nutrient availability of the peat substrate, seedlings with *P. croceum* had better access to nutrients. A prerequisite for this was to enhance carbon investment into the roots, ECM and extramatrical mycelia for an appropriate nutrient uptake, with the consequence of a reduced shoot growth. In contrast, seedlings were less effective in nutrient uptake when growing with *T. submollis*. This resulted in a slight, though not significant, reduction in total seedling biomass in comparison to seedlings with *P. croceum*. Low nutrient availability, particularly N and P, usually promotes root growth (e.g. Chapin 1980; Ericsson 1995; Marschner et al 1996). Instead, the increased biomass allocation to the shoot in seedlings with *T. submollis* may also be interpreted as investment into assimilation organs for increment in photosynthesis products and following compensation of restricted root growth under the influence of this fungal species.

The contrasting Ca concentration in roots might be explained by differences in crystal formation. *P. croceum* forms high amounts of calcium oxalate crystals (Arocena et al. 2001) on its hyphal surfaces, both of mantle surface as well as of the extramatrical mycelium (Brand 1991a, b), which is lacking in *T. submollis* (Agerer 1998; Köljalg et al. 2001). As the extramatrical mycelium was stripped off and the ECM were removed from the roots, most of the Ca taken up by *P. croceum* is deposited on hyphae in the peat or on the ECM separated for length measurements and could apparently not be transferred to the roots to be measured. As shoot Ca concentrations of seedlings with *P. croceum* are significantly higher than those of *T. submollis*, in spite of the possibly massive use for crystal formation by *P. croceum*, a great deal of Ca has likely been sequestered in the ECM-bearing roots of seedlings with *T. submollis* and not delivered to the shoots.

Effects of elevated CO_2 and nitrogen availability

Elevated CO_2 and particularly the combination eCO_2+N resulted in reduced concentrations of most nutrients in seedlings with *P. croceum*. This is consistent with findings on *P. croceum* and other ECM species as reported by Alberton et al. (2007) for nitrogen and phosphorus. As simultaneously the biomass of seedlings and ECM was rather promoted (see above), elevated CO_2 seemed to affect the nutrient balance of seedlings with *P. croceum*. In contrast, treatment effects on nutrient concentrations were lacking in seedlings with *T. submollis*, with the exception of Ca in the roots. While ECM biomass of *T. submollis* also

tended to be increased in response to elevated CO_2, Ca concentration in roots decreased. Thus, for both ECM species, the capacity in nutrient acquisition obviously could not be increased by elevated CO_2, possibly due to the insufficient availability of nutrients in the peat substrate.

The lacking difference between the treatments with and without nitrogen addition might indicate that, in our system, the N content of the substrate was, in spite of nitrogen amendment in eCO_2+N, still too low to reach an equilibrium between C and N. Seedlings with *T. submollis* apparently acquired enough nitrogen, keeping the C/N ratio rather constant throughout all treatments. The low P content and P/N ratios (approx. 0.05–0.06) in the shoot of spruce seedlings synthesized with *T. submollis* indicate a very poor P supply. This suggests that these seedlings probably could not respond to eCO_2 due to their deficiency in phosphate (see Conroy et al. 1990; Johnson et al. 1995). Elevated CO_2 caused *P. taeda* seedlings with *P. tinctorius* to increase P uptake, at least under P limitation (Lewis and Strain 1996). This is possibly dependent on increased phosphatase activities (Moorhead and Linkins 1997). Our studies, however, showed a decrease in shoot P concentrations under eCO_2. This might be a dilution effect as discussed for N, too, with less P available than would be necessary for an enhanced seedling growth under eCO_2. This is also evident for other macronutrient concentrations (S, K, Ca, Mg; cf. Fig. 1). The applied substrate might influence the outcomes as substrate type and nutrient availability play an important role for the growth of ectomycorrhizal plants under elevated CO_2 concentrations (Mousseau and Saugier 1992), although there seem to be exceptions (Johnson et al. 2006).

Norby et al. (1986) is, to our knowledge, the only publication that studied the uptake of a diversity of macro- and micronutrients by ECM under the influence of elevated CO_2. In *Quercus alba* seedlings grown in nutrient-deficient natural soil, they found no increase in total uptake of N, S and B under elevated CO_2, resulting in lower tissue concentrations. In contrast, P and K uptake increased in proportion to growth, therefore leaving tissue concentrations unchanged. The authors explained this by the proliferation of fine roots and associated ECM and phosphate dissolution stimulating rhizosphere bacteria.

Impact of ECM fungi on nutrient use efficiency

Influence of fungal partners

Conversely to the growth benefits and higher nutrient supply with *P. croceum*, when considering the biomass production per unit of acquired nutrient, seedlings with *P. croceum* proved to be less efficient than those with *T. submollis*. This was most pronounced for P, for which the produced biomass per unit P was about three times higher with *T. submollis* than with *P. croceum*. This clearly shows a species-specific influence of the fungus not only on plant nutrient supply but also on the usage of the nutrients for biomass production. Jones et al. (1991) found higher nutrient use efficiency for P of *Salix* cuttings with *T. terrestris* Ehrh. in comparison with that of non-mycorrhizal cuttings.

K is important for the transport of short-chained polyphosphate (Bücking and Heyser 1999). Therefore, it is not surprising that the significantly higher P use efficiency in *T. submollis*-inoculated seedlings is associated also with significantly higher K and Mg use efficiency (both have relatively high plant internal mobility), although the concentrations of these elements (P, Ca, K, Mg) were all significantly higher in seedlings with *P. croceum*. This is in accordance with the findings of Jentschke et al. (2001) on the interdependence of K, Mg and N fluxes with long-distance P translocation in mycorrhizal mycelium of *P. involutus* of pine seedlings. Different impacts on K fluxes in roots synthesized with the short/medium-distance exploration type *H. cylindrosporum* and the long-distance exploration type *Rhizopogon roseolus* (Corda) Th. Fr. in comparison to non-mycorrhizal roots have been found by Plassard et al. (2002). *H. cylindrosporum* did not positively influence the K flux, whereas *R. roseolus* increased it considerably.

Effects of elevated CO_2 and nitrogen availability

CO_2/N treatment effects were observed only in P use efficiency of seedlings with *P. croceum* (cf. Fig. 2). The increase under eCO_2+N as compared to aCO_2 suggested an increased energy supply for uptake under elevated CO_2. The weak response of seedlings with *T. submollis* is reflected by the unchanged seedling biomass. Under both eCO_2 treatments, the increased ECM growth of this fungal species did not promote a better nutrient supply. In contrast, Alberton et al. (2007) reported on significantly reduced N uptake per unit of root mass in response to elevated CO_2 for *P. croceum* and other ECM species indicating N immobilization by the mycorrhizal fungi.

High nutrient use efficiency may be due to the fact that the plant can afford higher biomass production per unit of nutrient. On the other hand, nutrient-deficient plants are forced to enhance their efficiency simply to survive. So, a high nutrient use efficiency may indicate either beneficial or detrimental conditions As shown by the very low P concentrations for seedlings with *T. submollis* in combination with their lower biomass, in our case, the higher P use efficiency seems to be an adaptation to cope with conditions of deficiency. In contrast, the higher P use efficiency for seedlings with *P. croceum* under elevated CO_2 seems to be a beneficial effect, indicated by the slightly higher biomass production under these conditions.

Conclusions

The two fungal species used in the present study revealed considerably different influences on growth, biomass allocation as well as nutrient uptake of spruce seedlings. Under the low soil nutrient availability of the peat substrate used here, *P. croceum* promoted seedlings better than *T. submollis* did by increased nutrient uptake. At the expense of higher carbon investment into roots and the fungal partner, this resulted in an increased root/shoot ratio and a higher total biomass of seedlings with *P. croceum*. In contrast, the seedlings profited less from the association with *T. submollis* in terms of plant growth and nutrient status. However, shoot growth was favoured in this species which may be of advantage in above-ground competition. At considerably lower carbon costs for roots and ECM, nutrient use efficiency was much higher with *T. submollis* than with *P. croceum*, particularly for P but also for K and Mg. As soil nutrient availability was low, the increased biomass allocation to the shoot in seedlings with *T. submollis* in combination with higher nutrient use efficiency may indicate a response to the low nutrient transfer from the fungi to the plant.

Under elevated CO_2 treatments, particularly in the combination eCO_2+N, seedling biomass was slightly increased in association with *P. croceum* but not with *T. submollis.* Although ECM biomass and length tended to be increased in both species in response to eCO_2 and particularly to eCO_2+N, nutrient uptake by ECM was relatively decreased. The beneficial effect of *P. croceum* diminished under these treatments, although nutrient status was still above that of plants with *T. submollis.* Thus, in the long term, increased plant growth under elevated CO_2 can be accompanied by imbalances in nutritional status as the capacity in nutrient uptake by ECM is not proportionally increased. These effects may likely be more severe in nutrient-poor soils.

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