

Root growth and N dynamics in response to multi-year experimental warming, summer drought and elevated CO₂ in a mixed heathland-grass ecosystem

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Abstract. Ecosystems exposed to elevated CO₂ are often found to sequester more atmospheric carbon due to increased plant growth. We exposed a Danish heath ecosystem to elevated CO₂, elevated temperature and extended summer drought alone and in all combinations in order to study whether the expected increased growth would be matched by an increase in root nutrient uptake of NH₄⁺-N and NO₃⁻-N. Root growth was significantly increased by elevated CO₂. The roots, however, did not fully compensate for the higher growth with a similar increase in nitrogen uptake per unit of root mass. Hence the nitrogen concentration in roots was decreased in elevated CO₂, whereas the biomass N pool was unchanged or even increased. The higher net root production in elevated CO₂ might be a strategy for the plants to cope with increased nutrient demand leading to a long-term increase in N uptake on a whole-plant basis. Drought reduced grass root biomass and N uptake, especially when combined with warming, but CO₂ was the most pronounced main factor effect. Several significant interactions of the treatments were found, which indicates that the responses were nonadditive and that changes to multiple environmental changes cannot be predicted from single-factor responses alone.

Additional keywords: *Calluna vulgaris*, CLIMAITE, *Deschampsia flexuosa*, excised roots, ingrowth core, ¹⁵N-assay.

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Introduction

Most climate change scenarios project that greenhouse gas concentrations will increase over the next decades with a continued increase in average global temperatures (Solomon *et al.* 2007). Along with increasing CO₂ concentrations and a projected warming of ~0.2°C per decade, changes in precipitation patterns are also expected. These three factors are drivers for many important ecosystem processes, and changes in ecosystem function are therefore expected.

A substantial amount of carbon assimilated by plants is transported underground and transferred to the soil; in temperate grasslands, the largest fraction of total ecosystem carbon is stored below the ground (Mokany *et al.* 2006). The calculated root surface area and fine root length is often greater than leaf area, and in grasslands, it is more than an order of magnitude larger (Jackson *et al.* 1997). Hence soils are often the critical C sink in grasslands, in contrast to the large aboveground biomass sink in forests (Gill *et al.* 2006).

Regulation of the ability to take up nutrients such as nitrogen might have a great influence on carbon sequestration (Bassirirad 2000), and nutrient uptake kinetics are expected to be stimulated under high CO₂ (Jackson and Reynolds 1996). Both nutrient uptake capacity and root growth generally increase in response to increasing temperature, but not always (Bassirirad 2000). This can be due to increased photosynthesis at higher temperatures, where the extra fixed carbon is allocated belowground to sustain new root growth (Pregitzer and King 2005). However, high temperatures often increase evapotranspiration and lead to drought, and low soil moisture reduces nutrient availability and nutrient uptake in the rooting zone, thus decreasing nutrient uptake rates (Gutschick and Pushnik 2005).

Even though elevated CO₂ is expected to increase plant growth, this increase may not be sustained in the long term if nutrients or water are limited. The Progressive Nitrogen Limitation hypothesis suggests that without additional N input or reduced N loss, the N availability decreases over time at elevated CO₂ (Luo *et al.* 2004), leading to reduced C uptake in

the long term. The direct effects of warming and CO₂ on plant growth in natural ecosystems can be limited if nutrient stocks are gradually depleted (Arnone *et al.* 2000; Gill *et al.* 2006). However, several examples exist that show no evidence of progressive nitrogen limitation even after several years of treatment with elevated CO₂ in forests (Barnard *et al.* 2006; Norby and Iversen 2006; Phillips *et al.* 2006).

Plant responses to elevated CO₂ are very dependent on plant N acquisition; however, studies of potential changes in root uptake capacity in response to the projected climate changes are few and inconsistent. It is critical to know, if the plants can positively adjust to the expected increased demand for nitrogen under elevated CO₂ by increasing root growth or physiological properties. There are, to our knowledge, no longer term investigations of the effects of combined temperature, drought and elevated CO₂ on root dynamics and root–plant–nutrient interactions.

Plants adapted to acid soils, like heathlands, have a preference for ammonium (Marschner 1995), but NO₃[−] and organic N is also taken up by the heathland plants. Plant uptake of organic nitrogen (glycine) was studied at our experimental site 1 year after treatments started (Andresen *et al.* 2009). Here, we report the response of root growth and uptake of ammonium (NH₄⁺-N) and nitrate (NO₃[−]-N) to changes in atmospheric CO₂, temperature and prolonged summer drought in all combinations. The objectives of this study were to study the inorganic N uptake and root production in a future climate, and determine whether an expected higher root growth would be followed by increased nutrient acquisition.

We hypothesised that root growth would be stimulated in response to elevated CO₂ due to increased C uptake (Albert *et al.* 2011a; Albert *et al.* 2011b), and that therefore there would be greater C allocation to the roots. Drought was expected to slow down root growth, whereas the temperature treatment was expected to increase root growth, depending on soil moisture content. We hypothesised that nitrogen uptake would increase in response to elevated CO₂, demonstrating a larger nutrient deficiency, but it would decrease under warming due to higher mineralisation and nutrient availability (Andresen *et al.* 2010b; Larsen *et al.* 2011). Drought, by contrast, was expected to decrease N acquisition. The single-factor effects were expected to be simply additive, when looking at the full combination of warming, drought and elevated CO₂.

Materials and methods

Site description

The experimental site is situated in a dry mixture of heathland and grassland ~50 km north-west of Copenhagen (55°53'N, 11°58'E), Denmark, on a hilly nutrient-poor acid sandy deposit. The soil consists of 71.5% sand, 20.5% coarse sand, 5.8% silt and 2.2% clay (Nielsen *et al.* 2009). The soil is well drained with a pH_{CaCl2} in the topsoil of 3.3 increasing to 4.5 in the B-horizon and an organic top layer of 2–5 cm (O-horizon).

The yearly mean temperature is 8°C with 613 mm of precipitation (Danish Meteorological Institute, 2009, <http://www.dmi.dk>). The site had a relatively low atmospheric N bulk deposition of $1.35 \pm 0.04 \text{ g N m}^{-2} \text{ year}^{-1}$ in 2007 (Larsen *et al.* 2011).

The dominant and studied plant species are an evergreen dwarf shrub *Calluna vulgaris* Hull. (~30% cover) and a perennial grass *Deschampsia flexuosa* Trin. (~70% cover). A few other grasses, herbs and mosses that are common in acidic grasslands are also present at the site (Kongstad *et al.* 2012).

Experimental manipulations

The manipulations in the CLIMAITE (CLIMate change effects on biological processes In Terrestrial Ecosystems) experiment were designed according to the climate predictions for Denmark in year 2075. The climate scenario was an elevated atmospheric CO₂ concentration at 510 parts per million (ppm), elevated temperatures of 1–2°C above current mean air temperatures and a prolonged summer drought period (4–6 weeks).

The experiment consisted of 12 octagons (7 m in diameter) laid out pairwise in six blocks. Each block consisted of two octagons with one octagon receiving elevated CO₂ (CO₂, 510 ppm) using the free air CO₂ enrichment technique and the other receiving ambient CO₂. The CO₂ concentration is highest at the edge, where the CO₂ is released (the position of this depends upon the wind direction); the concentration sharply declines within the first 20 cm and towards the middle of the octagon where the mean target value of 510 ppm is reached. Within each octagon, there were four subplots with the following treatments: summer drought (D, exclusion of rain by automatic shelters), elevated temperature (T, passive night-time warming by reflective curtains, which covered the experimental plots 50 cm above the ground during the night, reducing the loss of heat and thereby increasing the temperature), a combination of drought and elevated temperature (TD) and an untreated control for reference (A, i.e. ambient CO₂ and neither temperature- nor drought-treated) in a split-plot design.

The experiment provided a full factorial design replicated 6 times with the treatments and combinations: A, T, D, CO₂, TD, TCO₂, DCO₂ and TDCO₂, giving a total of 48 plots. The treatments were initiated in October 2005. See Mikkelsen *et al.* (2008) and Larsen *et al.* (2011) for further details of the experimental design and set-up. Fig. 1 shows the climatic data from the site and the change in soil water content due to treatments and temperature in some of the treatments from 2007 to 2008.

The imposed drought periods were in 2008: 21 May to 22 June and again from 16 September to 2 October. The experimental drought treatment in 2008 was weak due to an extremely dry summer.

Root biomass

Soil samples were taken in October 2008 to give an estimate of root biomass in undisturbed areas under both *Calluna* and *Deschampsia* vegetation. One core was taken under each species per experimental plot. The soil auger was 4.5 cm in diameter and the soil core was divided into horizons: O-horizon, 0–5 cm deep and 5–10 cm deep. Fine roots (<1 mm in diameter) were separated from the soil in the soil laboratory by use of sieves and hand picking the roots with forceps, and then washed carefully. The roots were dried in the oven at ~60°C and weighed.

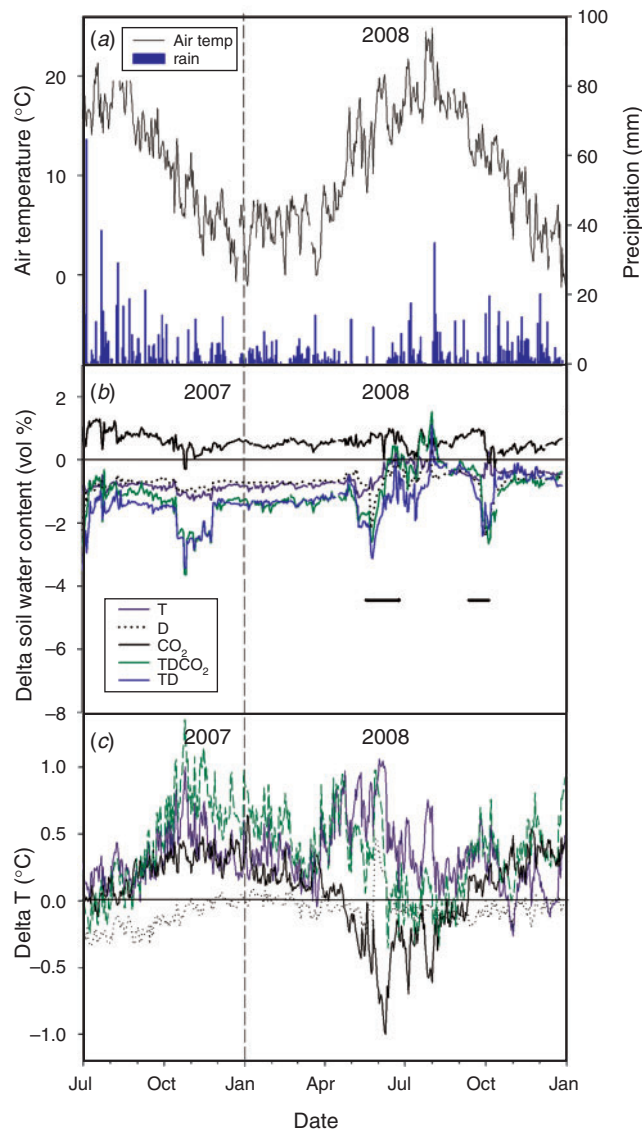


Fig. 1. (a) Average precipitation (mm) and air temperature (°C) at the experimental site. (b) Delta soil water content in soil 0–20 cm deep, showing the difference in SWC 0–20 cm depth between warmed and nonwarmed treatments (T), between elevated CO_2 and ambient CO_2 treatments (CO_2), between drought and nondrought treatments (D), between the control treatment and TD and, and between the control and TDCO_2 . In (b), the experimental drought periods are shown as black horizontal lines. (c) Mean temperature difference (Delta T) in soil 5 cm deep for the T, D, CO_2 and TDCO_2 treatments.

Root ingrowth cores and N pool size estimation

Annual net root productivity was estimated by in-growth cores, covering a full year of root growth from October 2007 to November 2008. In each treatment plot, two soil cores were sampled under patches of *Calluna* and *Deschampsia*, respectively. Roots were separated from the soil by the use of sieves and hand picking the roots with forceps. A tube of plastic mesh (mesh size: ~2 mm) lined the soil pit to mark the original hole (6 cm in diameter) and this was then filled up with root-free

soil in the same original order of depth, divided into O-horizon, 0–5 cm deep and 5–10 cm deep, separated from each other by a small cloth inside the bag. The majority of roots in this ecosystem are found in the upper 10 cm of soil. After 13 months (November 2008), a soil auger of 4.5 cm was used to take a sample inside the original hole. The 96 samples were brought back to the laboratory and put into a fridge until the time of root sorting. Roots were not separated into species, but we assumed that the majority of the roots would belong to the species under which the in-growth cores were placed due to the patchy distribution of species. In an earlier in-growth core study, we found that some roots from *Deschampsia* grew into the mesh bags of *Calluna* (especially in the O-horizon), whereas the opposite was rarely seen. A mean estimate of *Deschampsia* roots growing into *Calluna* mesh bags indicated ~20% grass roots in the O-horizon, 12% in soil 0–5 cm deep and 15% in soil 5–10 cm deep under the dwarf shrubs, but little or no ingrowth of *Calluna* roots into the mesh bags under *Deschampsia* (unpublished results). In-growth bag samples are therefore referred to as ‘under’ *Calluna* or *Deschampsia*, due to the assumption that the majority of the root samples taken belonged to the aboveground plant species directly above.

The standing pool of belowground plant nitrogen was calculated as the root biomass from the in-growth core multiplied by the nitrogen concentrations (see next section).

Nitrogen uptake

The study of nitrogen uptake was done by ^{15}N assay (Jones *et al.* 1991) and used roots from the in-growth cores. Hence, all the roots had been exposed to the experimental treatments and had a maximum age of 13 months.

Due to the large number of samples and the time-consuming root sorting procedure, sampling was done sequentially in three campaigns in autumn 2008 (four octagons per campaign: 24 November, 30 November and 7 December). The soil samples were separated into O-horizon, 0–5 cm deep and 5–10 cm deep. Each horizon was sorted by hand and fine roots were washed in demineralised water. The roots were put in a plastic bag with a moist hand towel and kept in the dark at 5°C until the assay was conducted.

The N bioassay was performed using the method described by Jones *et al.* (1991). Fresh roots from each species and soil layer were divided into two subsamples for analysis of absorption of $^{15}\text{NH}_4^+$ -N and $^{15}\text{NO}_3^-$ -N, respectively. Root samples from the O-horizon were not sufficient for the two assays and were therefore used for $^{15}\text{NH}_4^+$ -N uptake assay only. All roots of both *Deschampsia* and *Calluna* were approximately of same diameter (<1 mm in diameter) and were not separated into different size classes. Hence all roots were considered active and able to take up nutrients.

The fresh root bundles were marked with a name tag and presoaked for 30 min (5×10^{-4} M CaCl_2 solution) to maintain root cell membrane integrity and to remove ammonium, nitrate and nitrite ions from the cell-free space (Rosengren *et al.* 2003). After the presoaking treatment, roots were transferred to the uptake solution (5×10^{-4} M CaCl_2 labelled with 2 mL ^{15}N - NH_4Cl or - KNO_3 and 8 mL of ^{14}N - NH_4Cl or - KNO_3 , to get a 20% enrichment of ^{15}N) and soaked for 2 h, washed in running demineralised water for 15 min, put in paper bags, dried in the

oven at 80°C, ground and weighed and finally analysed for ^{15}N , %N and %C (Eurovector elemental analyser, (Milan, Italy) coupled to an Isoprime mass spectrometer (Cheadle Hulme, UK)). Excess ^{15}N (atom %) was converted to absorption rate of N (in $\mu\text{g N g}^{-1}$ root DW 2 h^{-1}). The total N concentration was also calculated, subtracting the N that had been taken up during the bioassay (Michelsen *et al.* 1999).

The nitrogen deficiency test was developed as a tool to relate root nitrogen levels to the fertiliser regime of trees in excised roots (Jones *et al.* 1991). High ^{15}N uptake demonstrates nitrogen limitation (Jones *et al.* 1991; Rosengren *et al.* 2003). The results obtained with excised roots represent a relative measurement of the root net uptake capacity at different soil depths and not the actual uptake rate, as would have been found with the roots still attached to the plant (Göransson *et al.* 2007).

Statistical analysis

Statistical analyses were conducted by using the PROC MIXED procedure of SAS (SAS Institute, Cary, NC, USA). The same model was used for all variables, with all main factor effects and their interactions. The main effects (factors) of the statistical model were drought (D), warming (T) and elevated CO_2 (CO_2), and their interactions ($T \times D$, $T \times \text{CO}_2$, $D \times \text{CO}_2$, $T \times D \times \text{CO}_2$). This means, for instance, that with a significant main effect for CO_2 , all treatments with CO_2 (CO_2 , TCO_2 , DCO_2 , TDCO_2) will be different overall from all treatments with ambient CO_2 (A, T, D, TD). The statistical model chosen included a random statement that accounted for the experimental design (random block octagon, octagon \times D, octagon \times T). The same model was used for all variables, with all main factor effects and their interactions, except when the differences between soil depths were studied. Soil sample identification was also included in the random statement to account for possible correlations between biomasses at different depths in the same soil sample.

Data were transformed in case requirements for the normality and homogeneity of variances were not met. P -values < 0.05 were considered significant, but trends at $P < 0.1$ are also reported. Model reduction was done to obtain the best model by sequential

removal of the terms in the model with the highest P -value. Differences of least-squares means were used to interpret significant interactions.

Results

Fine root biomass and annual net root productivity

The total root biomass of *Deschampsia* sampled in October was significantly lower in the drought treatment (Fig. 2). Prolonged summer drought reduced *Deschampsia* root biomass in the O-horizon ($P = 0.031$), and at 0–5 cm deep, the combined warming and drought treatment reduced the biomass compared with the combination with elevated CO_2 ($T \times D \times \text{CO}_2$, $P = 0.039$). Elevated CO_2 tended to increase the root biomass across all temperature and drought treatments by an average of 43%. Elevated CO_2 increased the root biomass of *Deschampsia* significantly in the upper 5 cm (0–5 cm, $P = 0.044$); at 5–10 cm deep, there was only a tendency ($P < 0.1$) towards increased biomass in both *Calluna* and *Deschampsia*. We observed no significant effects of any treatment on the standing root biomass of *Calluna* (Fig. 2).

Elevated CO_2 stimulated the total root production of both plant species in the in-growth cores by 28% in *Calluna* and by 55% in *Deschampsia* compared with plots under ambient CO_2 (Fig. 3). The response to elevated CO_2 was significant or tended to be in both species and in all three soil depths. Furthermore, there was an interaction where drought in combination with either T or TCO_2 decreased the biomass in *Deschampsia*, mainly in the organic layer (D, $P = 0.046$ and $T \times D$, $P = 0.019$). These results indicate that the *Deschampsia* roots are more affected by the drought treatment compared with *Calluna* roots.

Root nitrogen concentration and N pool sizes

Elevated CO_2 significantly reduced fine root N concentration under both *Calluna* (0–5 cm, $P = 0.004$; 5–10 cm $P = 0.004$) and *Deschampsia* (0–5 cm, $P = 0.011$; 5–10 cm, $P = 0.016$) by almost 10% when viewed across all depths (Fig. 4). The root N

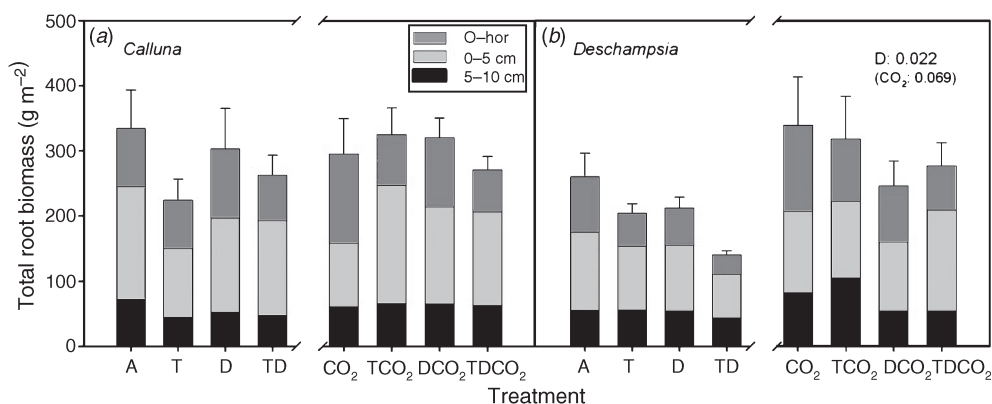


Fig. 2. Total root biomass (g m^{-2}) in October 2008 after 3 years of treatment, in (a) *Calluna* and (b) *Deschampsia* (mean \pm 1 SE). The treatments are: A (ambient), CO_2 (elevated CO_2), D (drought), T (elevated temperature) and all treatment combinations (TD, TCO_2 , DCO_2 , TDCO_2). All plot types are included in the evaluation of the main factor effects and their interactions. P -values of the treatment effects are shown in the graph. Only significant ($P < 0.05$) or near-significant ($P < 0.1$) effects are shown.

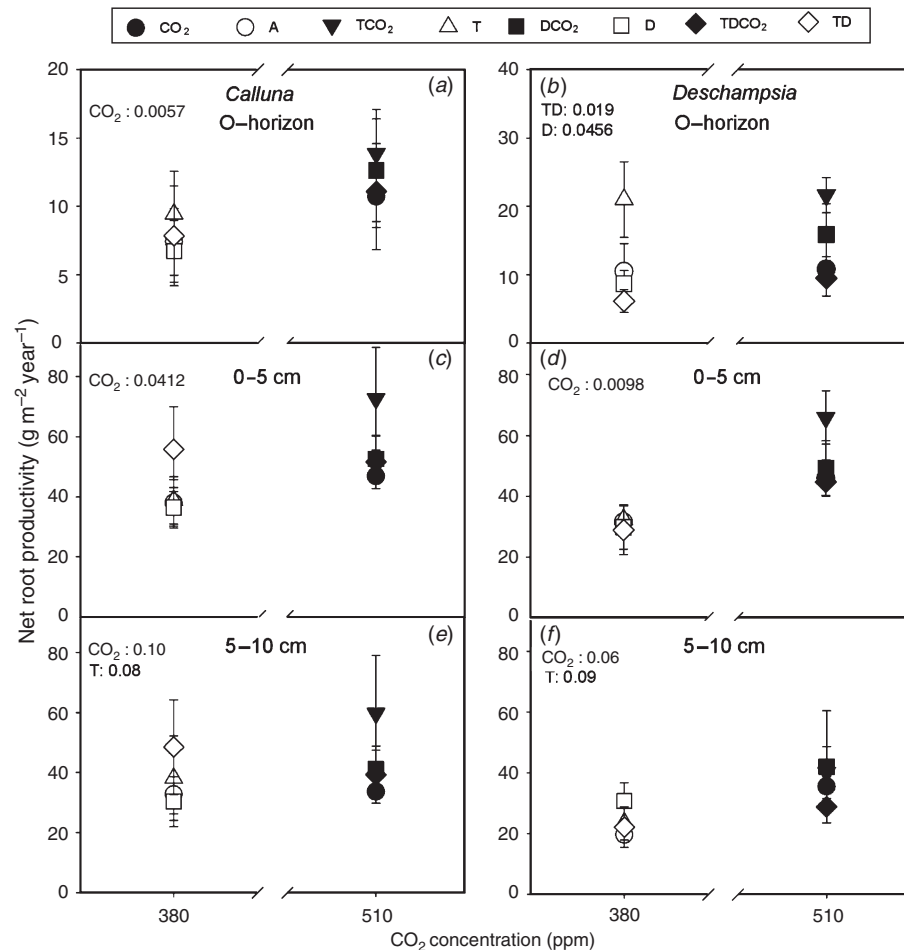


Fig. 3. Annual net root productivity (biomass g m^{-2}) from in-growth cores in (a, c, e) *Calluna* and (b, d, f) *Deschampsia* (mean \pm 1 s.e.) grown under ambient CO_2 (380 parts per million (ppm)) or elevated CO_2 (510 ppm) at (a, b) the O-horizon, (c, d) at 0–5 cm deep and (e, f) at 5–10 cm deep. Note the different scales on y-axis for the O-horizons and for the two the species. The treatments are: A (ambient), CO_2 (elevated CO_2), D (drought), T (elevated temperature) and all treatment combinations (TD, TCO_2 , DCO_2 , TDCO_2). *P*-values of treatment effects are shown in the graph. Only significant ($P < 0.05$) or near-significant ($P < 0.1$) effects are shown.

concentration also decreased with increasing soil depth. Reductions in N concentration were associated with corresponding increases in the C : N ratio, significantly in *Calluna* roots (0–5 cm deep and 5–10 cm deep, $P = 0.003$ for both depths; data not shown).

The root N pool size was increased in elevated temperature in the *Deschampsia* O-horizon, but when combined with drought, the N pool decreased ($T \times D$ interaction) (Table 1). Elevated CO_2 stimulated the root N pool size in *Deschampsia* at 0–5 cm deep and tended to do so at 5–10 cm deep, and in *Calluna* in the organic horizon. Hence, overall root N pool size was increased per unit of area under elevated CO_2 , due to the increased root growth that compensated for decreases in root N concentration.

Nitrogen uptake

The highest uptake rate of NH_4^+ -N in *Calluna* roots (per unit of root biomass) was found at 0–5 cm and 5–10 cm deep (Fig. 5).

In *Deschampsia* roots, uptake in soil 0–5 cm deep was the highest. The uptake of NH_4^+ -N was similar in the two species, with only a few treatment effects. Drought reduced the uptake of NH_4^+ -N in *Deschampsia* roots especially in the deeper layers (5–10 cm, $P = 0.014$), except in the full treatment combination ($T \times D \times \text{CO}_2$, $P = 0.031$). Elevated CO_2 interacted with both T and TD, and increased the uptake of NH_4^+ -N ($T \times \text{CO}_2$, $P = 0.047$). The uptake of NH_4^+ -N in *Deschampsia* roots in the TD treatment was generally low at all three depths (not significant).

The uptake rate of NH_4^+ -N was three to four times larger than the uptake rate of NO_3^- -N for both species. The NO_3^- -N uptake showed a similar response pattern to that observed for NH_4^+ -N uptake with respect to treatment effects in *Deschampsia* (Fig. 6). In *Deschampsia*, drought reduced or tended to reduce the uptake, and the TD treatment generally decreased the NH_4^+ -N uptake in all depths, but not significantly.

$\text{NH}_4^+\text{-N}$ was the preferred nitrogen form, reflecting the differential abundance of the two inorganic N forms (Andresen et al. 2010a).

Discussion

Net root growth and standing root biomass

Increased root growth and biomass under elevated CO_2 was observed, similar to other grassland studies (Jackson and Reynolds 1996; Sindhøj et al. 2004). In *Calluna*, a clear CO_2 response was only seen in the in-growth cores, and a response in undisturbed areas might become pronounced only after a disturbance or after a longer time period.

In the CLIMAITE experiment, Albert et al. (2011a, 2011b) found increased net photosynthesis and increased leaf C : N ratios in response to elevated CO_2 . This is comparable to our higher root C : N ratio, which supports our hypothesis of the improved carbon status of the plant and a higher C allocation to roots grown under elevated CO_2 . Hence our increased net root production under elevated CO_2 would result in more C-enriched root litter to the soil when the roots die. Due to the slower decomposition rate of roots compared with leaves (Franck et al. 1997; Gorissen and Cotrufo 2000), this might have a

positive effect on soil carbon storage, especially in soils under *Calluna* due the recalcitrant compounds of the litter.

The few drought effects seen were found mostly in the O-horizon, where the drought effect on soil moisture is also most pronounced and the combined treatment of drought and elevated temperature seemed to reduce soil moisture enough to reduce root biomass under *Deschampsia* but not under *Calluna* (Fig. 2). The standing root biomass from *Deschampsia* was negatively affected in the TD treatment, but the opposite was true for *Calluna*. The explanation for this could be the leaf dieback that *Deschampsia* experiences during drought (Kongstad et al. 2012); the same adjustment in root biomass stocks has been reported. Many grass species cope with dry soil by rapidly shedding the fine lateral roots (Eissenstat and Yanai 1997), a strategy *Deschampsia* might also use. This may have given *Calluna* a competitive advantage, with its roots being able to grow without competition from *Deschampsia*'s roots (the drought effect was apparently sustained until autumn and winter).

An earlier study at our experimental site showed no persistent changes in the aboveground biomass under any of the treatments (Kongstad et al. 2012). This could indicate that the higher root growth in the CO_2 -treated plots is a response to limited nitrogen supply, as larger root systems relative to shoots are common when N is more limited than C (Poorter and Nagel 2000).

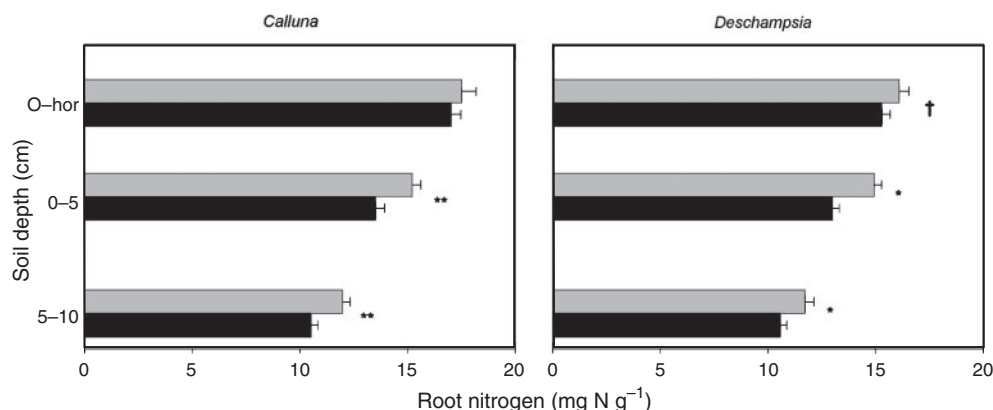


Fig. 4. Nitrogen concentration (mg g^{-1}) in (a) *Calluna* and (b) *Deschampsia* roots at three soil depths (mean \pm 1 s.e.). Grey bars represent treatments with ambient CO_2 and black bars represent treatments with elevated CO_2 ($n=24$). Significant differences between ambient and elevated CO_2 treatments are indicated with asterisks (*). The statistics are derived using the full dataset with $n=6$, but elevated CO_2 was the only significant treatment effect.

Table 1. Fine root plant nitrogen pool (mg N m^{-2}) under *Calluna* and *Deschampsia* plants at three depths

All values are mean \pm 1 s.e. P -values of the treatment effects are shown in the last column. The treatments are: A (ambient), CO_2 (elevated CO_2), D (drought) T (elevated temperature) and all treatment combinations. Statistics shown are the main effects D, T and CO_2 , and their interactions ($T \times D$, $T \times \text{CO}_2$, $D \times \text{CO}_2$, $T \times D \times \text{CO}_2$). All plot types are included in the evaluation of main factor effects and interactions. Only significant ($P < 0.05$) or near-significant ($P < 0.1$) effects are shown.

Species and soil depth	A	T	D	TD	CO_2	TCO_2	DCO_2	TDCO_2	P -value
	mg N m^{-2}								
<i>Calluna</i> O-horizon	116 \pm 32	156 \pm 52	117 \pm 39	149 \pm 72	181 \pm 63	213 \pm 50	195 \pm 52	202 \pm 46	CO_2 : 0.0040
<i>Deschampsia</i> O-horizon	171 \pm 67	332 \pm 98	138 \pm 30	106 \pm 29	170 \pm 30	314 \pm 40	241 \pm 65	172 \pm 23	$T \times D$: 0.020
<i>Calluna</i> 0–5 cm	538 \pm 88	535 \pm 95	567 \pm 100	834 \pm 168	687 \pm 27	903 \pm 199	685 \pm 100	656 \pm 106	
<i>Deschampsia</i> 0–5 cm	474 \pm 49	443 \pm 67	433 \pm 95	451 \pm 120	637 \pm 75	837 \pm 137	636 \pm 123	567 \pm 57	CO_2 : 0.051
<i>Calluna</i> 5–10 cm	372 \pm 70	381 \pm 117	382 \pm 104	572 \pm 160	392 \pm 38	567 \pm 152	436 \pm 64	387 \pm 87	
<i>Deschampsia</i> 5–10 cm	222 \pm 36	256 \pm 53	365 \pm 76	285 \pm 88	352 \pm 72	435 \pm 94	400 \pm 170	328 \pm 49	

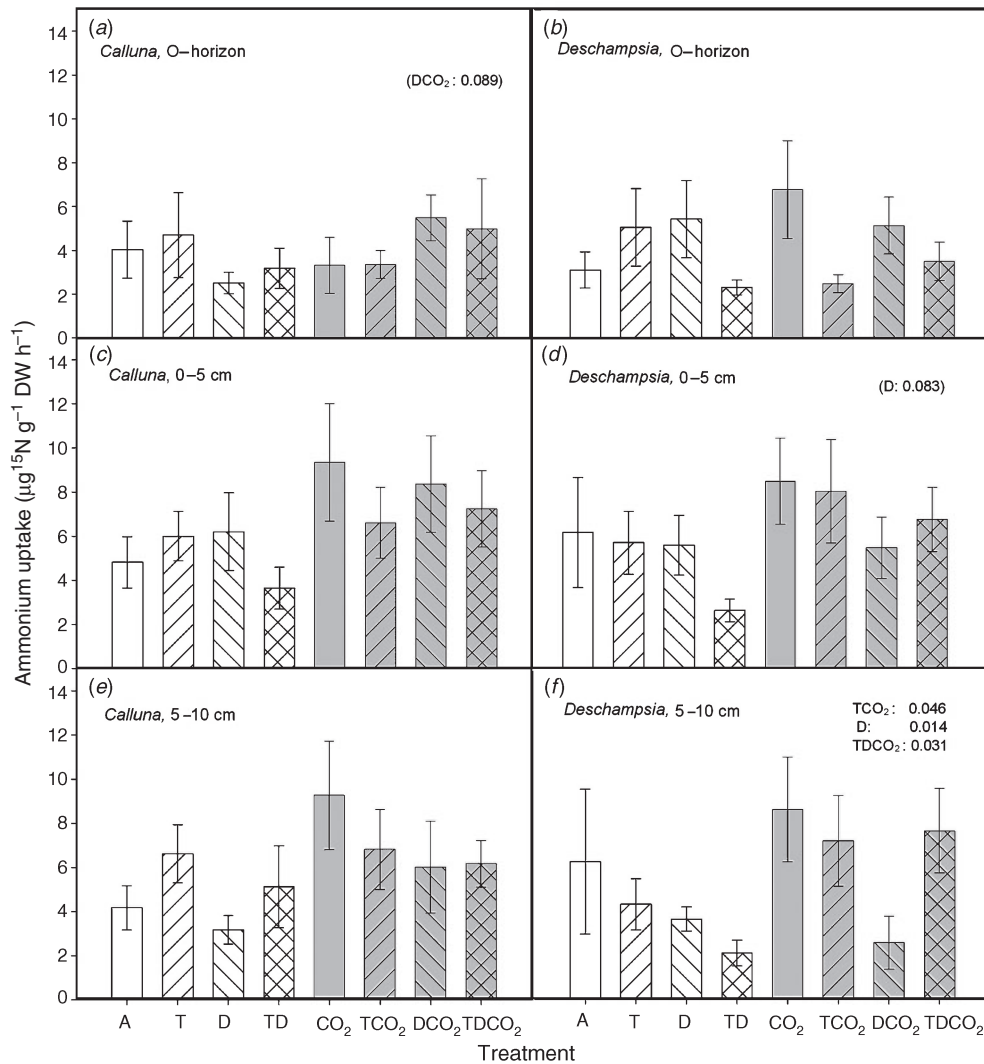


Fig. 5. Root NH_4^+ -N uptake ($\mu\text{g}^{15}\text{N g}^{-1} \text{DW h}^{-1}$) in (a, c, e) *Calluna* and (b, d, f) *Deschampsia* roots at three depths, from the in-growth cores (mean \pm 1 s.e.). (a, b) O-horizon; (c, d) 0–5 cm; (e, f) 5–10 cm. The treatments are: A (ambient), CO_2 (elevated CO_2), D (drought), T (elevated temperature) and all treatment combinations (TD, TCO_2 , DCO_2 , TDCO_2). Statistics shown in the graph are the main effects D, T and CO_2 , and their interactions ($\text{T} \times \text{D}$, $\text{T} \times \text{CO}_2$, $\text{D} \times \text{CO}_2$, $\text{T} \times \text{D} \times \text{CO}_2$). All plot types are included in the evaluation of main factor effects and interactions. Only significant ($P < 0.05$) or near-significant ($P < 0.1$) effects are shown.

Root N concentration and N uptake

Our results support the hypothesis that N concentration decreases in plants grown under elevated CO_2 , as has been found by many others (for example Cotrufo *et al.* 1998). Elevated CO_2 commonly results in lower tissue N concentration, for reasons that are not fully understood but include the dilution effect due to the accumulation of nonstructural carbohydrates (Bassirirad *et al.* 1997; Taub and Wang 2008). The stimulated root growth results in more roots that can exploit soil nutrients better than root systems in plots maintained at ambient CO_2 concentrations.

Our results from the ^{15}N -assay indicate that nitrogen uptake rate per unit of root mass would not increase under elevated CO_2 . This means that if our results are consistent with no increases in nutrient uptake per unit of root mass, other compensatory adjustments by the plants may take place. If a

greater proportion of the additional carbon resulting from growth under elevated CO_2 is allocated to root growth, then a high CO_2 concentration can lead to an increased N uptake on a whole-plant basis, although the uptake rate per unit of root mass remains unchanged (Bassirirad *et al.* 1999). We also observed that the belowground N pool increased under elevated CO_2 , indicating an increased N allocation below the ground.

Different studies have provided highly variable results with increasing, decreasing or unchanged uptake under elevated CO_2 (Rogers *et al.* 1994; Newbery *et al.* 1995; Jackson and Reynolds 1996; Bassirirad 2000; Gavito *et al.* 2001; Bielenberg and Bassirirad 2005; Taub and Wang 2008). Dijkstra *et al.* (2010) found that an increase in plant biomass with CO_2 enrichment was mostly a result of increased nitrogen use efficiency, whereas

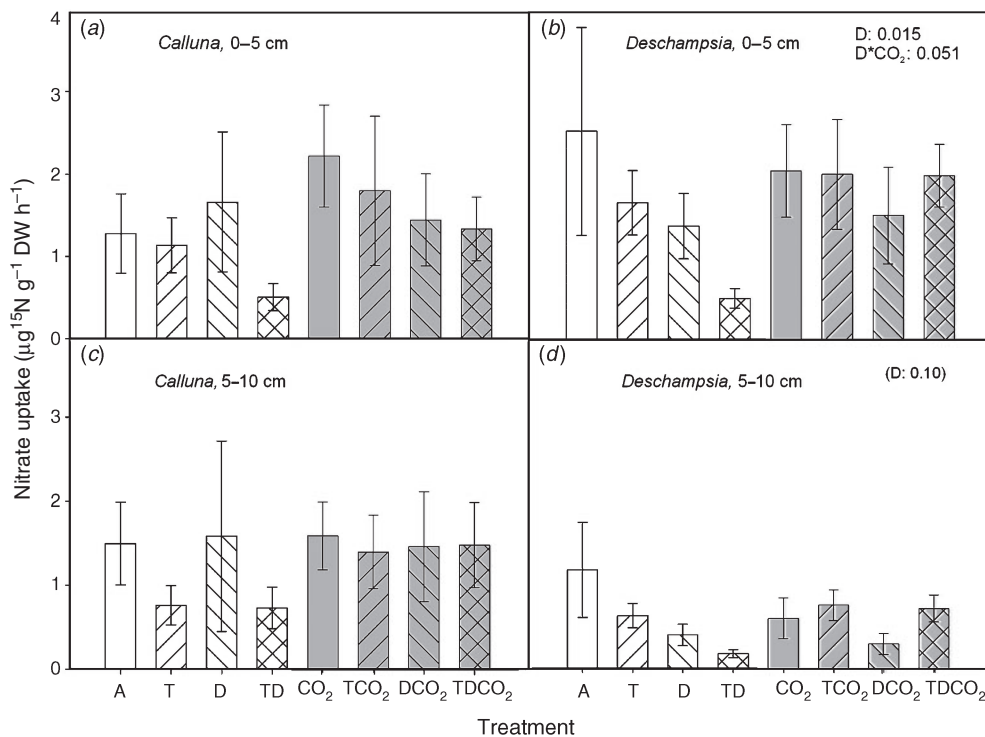


Fig. 6. Root NO_3^- -N uptake ($\mu\text{g } ^{15}\text{N g}^{-1} \text{DW h}^{-1}$) in (a, c) *Calluna* and (b, d) *Deschampsia* roots at two depths, from the in-growth cores (mean \pm 1 s.e.). (a, b) 0–5 cm; (c, d) 5–10 cm. There were no data available for the organic horizon. The treatments are: A (ambient), CO_2 (elevated CO_2), D (drought), T (elevated temperature) and all treatment combinations (TD, TCO_2 , DCO_2 , TDCO_2). Statistics shown in the graph are the main effects D, T and CO_2 , and their interactions ($\text{T} \times \text{D}$, $\text{T} \times \text{CO}_2$, $\text{D} \times \text{CO}_2$, $\text{T} \times \text{D} \times \text{CO}_2$). All plot types are included in the evaluation of main factor effects and interactions. Only significant ($P < 0.05$) or near-significant ($P < 0.1$) effects are shown.

increased plant N uptake contributed to the increase in biomass with increased soil moisture. Furthermore, both root age and diameter of individual roots have a large effect on the N uptake capacity (Volder *et al.* 2009), which also results in large variability.

Since the availability of NH_4^+ -N is most often highest at the top of the soil where mineralisation is greatest, we expected a decrease in NH_4^+ -N and NO_3^- -N uptake with depth. This was, however, not the case and corresponds to the results of Göransson *et al.* (2006), who did not find any significant differences in the uptake rate of NH_4^+ -N at different soil depths in a beech (*Fagus sylvatica* L.) and a spruce (*Picea abies* (L.) Karst.) stand in Denmark.

The highest N uptake was found at a depth of 0–5 cm due to the large root biomass here. The N concentration in the roots decreased with increasing soil depth but no clear correlation was found between the uptake rate of NH_4^+ -N or NO_3^- -N, and the root nitrogen concentration, as also seen in the study of Göransson *et al.* (2007).

Although *Deschampsia* and *Calluna* acquire some form of organic N, as amino acids (Andresen *et al.* 2010a, 2011), NH_4^+ -N and NO_3^- -N are probably the most important sources of nitrogen at the site, and many grasses and ericoid species from heathlands prefer NH_4^+ -N as their main N source (de Graaf *et al.* 1998; Falkengren-Grerup and SchötteIndreier 2004).

The roots of both species and at all depths were able to absorb both forms of N, but NH_4^+ -N was always taken up at a greater rate: at a 4-fold higher rate, on average. The uptake ratio of NH_4^+ -N to NO_3^- -N corresponds well with that of inorganic N in the soil, with NH_4^+ -N roughly 4 and 10 times higher than NO_3^- -N in soil under *Calluna* and *Deschampsia*, respectively (Andresen *et al.* 2010a). Jin and Evans (2007) found increased NH_4^+ -N and NO_3^- -N uptake under elevated CO_2 in arid shrub lands, with NO_3^- -derived N taken up at the greatest rate. However, our results did not indicate that elevated CO_2 increased the relative preference of NO_3^- -N to NH_4^+ -N, similar to Jackson and Reynolds (1996).

In the combined treatment TD, NO_3^- -N uptake was low (although not significantly) in both species and at both depths, whereas the respective nitrogen concentration in TD was not lower than in the other treatments. As a low uptake of ^{15}N indicates lack of nutrient limitation in the plant, the explanation for low uptake in the TD treatment could be due to higher nutrient availability, caused by delayed mineralisation after an intensive drought during summer combined with lysis of microbial nitrogen. In most plant roots, the kinetics of N uptake are regulated by demand, which is likely to exhibit a seasonal pattern (Gessler *et al.* 1998). The seasonal activities of soil organisms produce substantial changes in the availability of required nutrients (Glass 2005). This winter study may,

therefore, not fully describe the overall CO₂ effects on N uptake in this mixed heathland and grassland.

In summary, the CO₂ response in the measured root parameters was not significantly moderated by the full treatment combination (TDCO₂) and we did not observe an additive effect of the manipulations, as the effects of T and D were moderate. The response to TDCO₂, which mimics the future climate, was not straightforward to interpret from single-factor effects. The root growth and nitrogen concentration in this study showed a clear effect of CO₂, with no antagonistic effects of temperature and drought. *Calluna* root production seems to be less affected by drought, which could be an advantage when competing for nutrients in a future climate with longer drought events. The increased root growth in elevated CO₂ will add more belowground litter to the soil, and C could be accumulated in a pool with relatively slow turnover, at least for the non-woody *Deschampsia*.

The increased root growth in elevated CO₂ plots might be a strategy for the plants to cope with low nutrient supply, leading to an increased N pool size on a whole-plant basis, although the N concentration in the roots decreased.

In conclusion, root growth was stimulated by elevated CO₂, leading to a higher biomass, but with a lower N concentration. However, the overall N content per unit of area was increased demonstrating an increased N allocation belowground. The nutrient uptake was not much affected by the treatments after 3 years of experimental manipulations, which suggest that both plant species are not strongly N-limited at present.

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