

## Plant nutrient mobilization in temperate heathland responds to elevated CO<sub>2</sub>, temperature and drought

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**Abstract** Temperate terrestrial ecosystems are currently exposed to increased atmospheric CO<sub>2</sub> and progressive climatic changes with increased temperature and periodical drought. We here present results from a field experiment, where the effects of these three main climate change related factors are investigated solely

and in all combinations at a temperate heathland. Significant responses were found in the top soils below the two dominant species (*Calluna vulgaris* and *Deschampsia flexuosa*). During winter incubation, microbial immobilization of N and ammonification rate decreased in response to warming in *Deschampsia* soil, and microbial immobilization of N and P decreased in warmed *Calluna* soil. Warming tended to increase microbial N and P in *Calluna* but not in *Deschampsia* soil in fall, and more microbial C was accumulated under drought in *Calluna* soil. The effects of warming were often counteracted or erased when combined with CO<sub>2</sub> and drought. Below *Deschampsia*, the net nitrification rate decreased in response to drought and, while phosphorus availability and microbial P immobilization decreased, but nitrification increased in response to elevated CO<sub>2</sub>. Furthermore, leaf litter decomposition of both species decreased in response to drought. These complex changes in availability and release of nutrients from soil organic matter turnover and mineralization in response to elevated CO<sub>2</sub> and climate change may influence the future plant carbon sequestration and species composition at temperate heathlands.

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## Introduction

Natural ecosystems respond to changes in air and soil temperature, atmospheric carbon dioxide ( $\text{CO}_2$ ) concentration and drought, with changes in biological processes and ecosystem functioning. Model predictions forecast that the  $\text{CO}_2$  concentration in the atmosphere will increase, with an amount depending on the stabilization scenario and, as a consequence, the air temperature may increase by  $0.1^\circ\text{C}$  for each following decade (IPCC 2007). Furthermore, precipitation patterns will change in Denmark, leading to extended summer drought periods (Danish Meteorological Institute 2009; IPCC 2007). Investigations with experimental approaches of the combined effects of increased temperature, elevated atmospheric  $\text{CO}_2$  concentration and drought are scarce, but clearly needed, if we are to understand and forecast ecosystem responses to future climate scenarios (Beier et al. 2004a; Luo et al. 2008; Mikkelsen et al. 2008).

The relative strength of higher plants and soil microbes as carbon sinks and processors of soil organic matter is strongly controlled by the growth limiting factors, such as the availability of nitrogen (N) and phosphorus (P), labile carbon (C) or water (Beier et al. 2004a; Emmett et al. 2004; Finzi et al. 2006; Luo et al. 2006; Norby and Iversen 2006; Peñuelas et al. 2004; Rustad et al. 2001; Schmidt et al. 2004). Consequently, a progressive nitrogen or phosphorus limitation of the likely elevated  $\text{CO}_2$  induced increasing plant biomass (de Graaff et al. 2006b; Finzi et al. 2006; Hungate et al. 2006; Khan et al. 2008; Luo et al. 2004; Norby and Iversen 2006) may reduce C sequestration.

Microbial decomposition processes that release carbon by respiration are sensitive to temperature and moisture conditions and therefore respond to climatic changes, with ecosystem type and site specific directions of the responses (Rustad et al. 2001). Generally, net nitrification rates, N and P mineralization rates and leaching of inorganic nitrogen increase in response to warming (Jonasson et al. 2006; Rustad et al. 2001; Schmidt et al. 2002, 2004; van Meeteren et al. 2008). This is also the case for litter decomposition, at least in subarctic ecosystems (Cornelissen et al. 2007). Hence, mobilization of nutrients that are necessary for the increased primary production is likely to increase in response to elevated

temperature, altogether building a potential for increased carbon sequestration by terrestrial organisms.

In contrast, drought is expected to counteract the effects of elevated temperature. This was recently investigated in cross-European gradient investigations, on which extended drought periods and differences in moisture conditions strongly controlled N mineralization, mineral leaching and litter decomposition (Emmett et al. 2004; Schmidt et al. 2004; Sowerby et al. 2008; van Meeteren et al. 2007; van Meeteren et al. 2008). Responses to field manipulations are often small compared to the natural seasonal variation when investigated in temperate heath ecosystems (Anderson and Hetherington 1999; Emmett et al. 2004; Schmidt et al. 2004; Sowerby et al. 2005b), but model approaches across climatic zones have predicted, that absence of precipitation will decrease primary production, respiration and N mineralization in mesic ecosystems (Gerten et al. 2008; Knapp et al. 2008).

Acidification of soil exposed to elevated  $\text{CO}_2$  causes weathering of minerals (Andrews and Schlesinger 2001; Oh and Richter 2004) that may, depending on soil type, occlude phosphorus. This is caused by the increased  $\text{CO}_2$  concentration in the soil (Andrews and Schlesinger 2001), followed by formation of carbonic acid in the soil water, which reacts with soil minerals and may also alter the soil pH (Andrews and Schlesinger 2001; Oh et al. 2007; Oh and Richter 2004). A meta-analysis of mineralization studies concluded that N mineralization was unaffected while microbial immobilization of N and microbial N content increased under elevated  $\text{CO}_2$  (de Graaff et al. 2006b). Anyhow, a short term mineralization study at a calcareous grassland showed increased N mineralization and also increased enzyme activities (among those phosphatase) after 6 years of elevated  $\text{CO}_2$  (Ebersberger et al. 2003). In a study at a temperate grassland, soil N availability was reduced in response to elevated  $\text{CO}_2$  alone, but no effects on soil concentrations of nitrate, ammonium and DON were found in response to warming, or to warming combined with elevated  $\text{CO}_2$  (Hovenden et al. 2008).

Due to nutrient limitations, elevated  $\text{CO}_2$  could potentially lead to increases in C/N and C/P ratios in plant material (Finzi et al. 2004; Menge and Field 2007) and consequently alter the litter quality for decomposers, but a recent meta-analysis showed no

decreasing litter quality or decomposition of grass litter grown under elevated CO<sub>2</sub> (de Graaff et al. 2006a; Knops et al. 2007; Sowerby et al. 2005a). Hence, responses to elevated CO<sub>2</sub> in soil organic matter decomposition, chemical weathering and mineralization are in general soil type and vegetation type dependent.

The combined effects of warming, increased atmospheric CO<sub>2</sub> and summer drought on the soil processes of a temperate heathland ecosystem have not previously been investigated in the field (Ericc et al. 2006; Mikkelsen et al. 2008). Warming and elevated CO<sub>2</sub> combined with irrigation and N-addition (Barnard et al. 2006; Menge and Field 2007) have been studied in grasslands. Model studies combining warming, elevated CO<sub>2</sub> and drought (Sokolov et al. 2008) predict increased net primary production (NPP) and soil respiration (R) in response to warming and elevated CO<sub>2</sub>, and decreased NPP and R in response to drought (Luo et al. 2008). However, the potential controlling effects of the nutrients and the simultaneous nutrient responses to the climatic factors have not yet been accounted for in these models. The conclusion that nitrogen limits or even changes the direction of net carbon storage based on results from the Terrestrial Ecosystem Model (TEM) (Sokolov et al. 2008), strongly emphasizes the need for field investigations of nitrogen cycling e.g. of plant N demand, microbial N immobilization and mobilization of N from soil pools under multi factorial future climate conditions. The aim of the present study was to investigate soil nutrient dynamics under elevated CO<sub>2</sub>, imposed drought and elevated temperature in a multi factorial design and assess contingent effects of possible belowground sequestration of carbon by plants. As plants may influence the flow of labile carbon into soil and, hence, mineralization rates (Jonasson et al. 2006; Rinnan et al. 2007), the nutrient turnover in soil was studied both with and without the presence of plants.

It was hypothesized that, in the short term:

- Microbial processes in the soil would be stimulated by increased temperature leading to increased net rates of nitrification, mineralization and decomposition as well as increased microbial C, N and P.
- Moisture limitation in the drought treatment would lead to reduced mineralization, nitrification and decomposition.

- Presence of plants would lead to enhanced mineralization of N and P. Furthermore, both elevated temperature and CO<sub>2</sub> would increase the plant biomass due to increased availability and plant uptake of N and P.

## Methods

### The field site

The field site for the investigation covered an area of about two hectares at Brandbjerg (55°53'N 11°58'E), a hilly nutrient poor sandy deposit with pH 4.6, with a dry heath/grassland ecosystem dominated by *Deschampsia flexuosa* and *Calluna vulgaris* and with a low cover of other herbs and grass species, and an open moss cover beneath the canopy of vascular plants. The average precipitation per year is about 600 mm and the average temperature is 8°C (Danish Meteorological Institute 2009).

### The climate change manipulations

The climate manipulations started October 2005 and consisted of increased temperature (T), extended summer drought (D), increased atmospheric CO<sub>2</sub> (CO<sub>2</sub>) and all combinations of these treatments (TD, TCO<sub>2</sub>, DCO<sub>2</sub> and TDCO<sub>2</sub>), all in six replicates. Untreated controls were also included (A). The study plots consisted of 12 octagons each seven m in diameter, arranged in blocks of two, one with CO<sub>2</sub> fumigation and one without CO<sub>2</sub> fumigation. Each octagon comprised four plots with the treatments drought or elevated temperature solely or in combination, and a non-warmed, non-drought plot (Mikkelsen et al. 2008), hence each of the 48 plots were 9.2 m<sup>2</sup>. The temperature was increased by passive night-time warming by means of low automatic curtains that rolled over the vegetation during night (Beier et al. 2004a, b). To avoid changes in precipitation, the curtains were automatically removed during rain events. The precipitation in the drought plots was altered also with automatic curtains that automatically unfolded during rain events in early summer. The atmospheric CO<sub>2</sub> was increased with pipe fumigation as in a regular FACE experiment, and with a feed back control system linked to wind speed, wind direction, and CO<sub>2</sub> concentration in the centre of the octagon.

The temperature increase of the soil in 2 cm depth was c. 1°C, the increased CO<sub>2</sub> concentration in the air was maintained at 510 ppm during daylight hours. The drought period started in late June 2006 and continued for 5 weeks until early August when soil water reached c. 5 vol% in the top 20 cm of the soil, while soil water content was c. 17 vol % in the non-drought plots at this time. For further information about the experimental design of the multifactor set up and microclimate, see Mikkelsen et al. 2008.

#### Soil incubation in buried bag

Soil chemistry and mineralization was investigated both below *Calluna* and *Deschampsia* plants in all 48 plots. In November 2006, one year after treatments were initiated, two intact blocks of soil (20×20 cm), one from below *Calluna* plants and one from below *Deschampsia* plants, were cut from each plot from the top of the turf down to 5 cm, without removal of any litter or roots. One subsample was directly used for analysis of initial soil properties (see Table 1). Three other subsamples were carefully cut down to sizes of 4×4.5 cm. Each soil sample was carefully slipped into the incubation pot with no compression. A lid of Parafilm closed the pot but had a small slit to allow for plants in those incubated with plant and to allow for the same water vapor and dew exchange conditions (Eno 1960; Jonasson et al. 2006; Schmidt et al. 2002). Soil sampled below the two dominant plant species, viz. *Calluna* and *Deschampsia*, was incubated separately. For each plant type one sample was incubated with only soil and two identical (for sake of potential poor plant survival) incubations were made with soil including small *Calluna vulgaris* and *Deschampsia flexuosa* plants respectively. The plants had been pre-grown from seeds (*Deschampsia*) and cuttings (*Calluna*) for a period of 2 and 15 months respectively, in soil from the site prior to the incubations. Three *Deschampsia* seedlings (0.08 g fresh weight (FW) each) were planted in each pot with *Deschampsia* plant incubations, and two *Calluna* seedlings and one cutting (0.05 g FW each) were planted in each pot with *Calluna* plant incubation.

The incubation pots were placed in holes in the study plots in level with the surrounding soil. A 10 cm tall chicken net was tightened around the pots to exclude mice. After half a year of incubation

(6 months), in May 2007 after winter, the pots were sampled for analysis. The initial soil samples and the sampled buried bag incubations were kept cold until sorted within two weeks. The small plants were carefully removed, and roots and litter was sorted manually from the samples. Water content was measured after drying at 80°C and soil organic matter was measured as loss on ignition after 550°C for 6 h.

#### Organic acid in soil water

For analysis of organic acids, soil was sampled in each of the climate change treatments below grass in 0–5 cm depth in September 2006 and was kept cold until sorted after one day. Roots were removed manually and the soil was finally extracted with dematerialized water (1 soil : 5 water) for determination of the low molecular organic acids: acetic acid, formic acid, malic acid, tartaric acid, oxalic acid and citric acid with a Dionex HPLC system (Jonsson et al. 2007; Ström and Christensen 2007).

#### Leaf litter incubation in litter bags

Ambient leaf litter (standing dead biomass) from *Calluna* and *Deschampsia* was collected at the area of the field site in February 2006. The *Deschampsia* leaf litter was only current year leaf and inflorescence litter, still attached to the plant. It was dry at collection and kept at 5°C until use. The *Calluna* leaf litter was collected by picking the *Calluna* shrubs to collect only current year leaf litter and consisted of 27% (by dry weight) flowers, 36% leaves and small branches, 19% branches (>5 mm diameter) and 18% mixed, un-definable material. To ensure an even and natural chemical composition (e.g. phosphorus, phenolics and lignin) of the incubated litter, only current years litter was used (Anderson and Hetherington 1999; Frutos et al. 2002; van Vuuren et al. 1993).

The litter was cut down to lengths no longer than 3 cm and incubated in 4×4 cm litterbags with mesh size 1×1 mm (*Deschampsia*) or 0.05×0.05 mm (*Calluna*) to ensure that no small leaves would drop out. The *Deschampsia* litterbags each had 1.0 g FW litter and the *Calluna* litterbags each had 2.0 g FW litter. The litterbags were placed at the soil surface below the plant species of origin, and fixed with a small plastic pin and covered with the litter from the site.

**Table 1** Soil chemical and biological properties November 2006 for ambient plots (no treatment) below *Calluna* and below *Deschampsia* in 0–5 cm depth (no significant effect of soil type), and litter decomposition rates

Soil properties	<i>Calluna</i> soil		<i>Deschampsia</i> soil	
	mean	se	mean	se
% (wt dry wt <sup>-1</sup> )				
SOM	12.4	1.1	15.5	4.0
H <sub>2</sub> O	31.4	1.2	40.0	11.8
μg g SOM <sup>-1</sup>				
NO <sub>3</sub> -N	30.4	25.0	6.2	2.2
NH <sub>4</sub> -N	113	47.6	60.4	14.5
DON	148	104	113	78.8
Microbial N	1310	338	1320	92
DOC	725	87.6	905	110
Microbial C	9800	1390	7600	1780
PO <sub>4</sub> -P	34.7	28.2	5.00	1.41
Dissolved P	30.5	17.9	10.7	1.69
Microbial P	402	50.5	448	113
Microbial C:N	7.5		5.8	
Microbial N:P	3.5		2.9	
Leaf litter mass loss (%)	%			
6 months	25.8	1.31	33.4	1.09
12 months	34.3	1.42	45.1	4.28
14 months	.	.	46.1	4.67
Leaf litter decomposition	k (year <sup>-1</sup> )			
6 months	0.0014		0.0019	
12 months	0.0011		0.0017	
14 months	.		0.0014	

Litter mass loss and litter decomposition constant calculated for incubated *Calluna* or *Deschampsia* leaf litter after 6 months, 12 months and 14 months incubation in ambient plots. SOM is soil organic matter, DON is dissolved organic nitrogen, DOC is dissolved organic carbon

The litter incubation started March 20th 2006 and bags were collected after 6 months, 12 months and 14 months. The collected bags were frozen until cleaned for grown-in mosses and plant roots, freeze dried and weighed. The litter mass loss was calculated as:

$$\text{mass loss\%} = 100\% * (\text{DW}_{\text{initial}} - \text{DW}_{\text{sample}}) / \text{DW}_{\text{initial}}$$

(DW is dry weight).

and the litter decomposition constant k was calculated as:

$$k = -\text{Log}_e (\text{DW}_{\text{sample}} / \text{DW}_{\text{initial}}) / t$$

(t is the number of incubation days).

#### Chemical analysis and calculations

The fresh soil was extracted with 0.1 M K<sub>2</sub>SO<sub>4</sub> (1:5 of soil:water) for analysis of nitrate, ammonium, dissolved organic nitrogen (DON) and carbon (DOC) and dissolved phosphorus (P). Total dissolved nitrogen (TDN) was analyzed after digestion of the extract with potassium peroxide sulphate. A subset of samples were fumigated with chloroform and extracted with 0.1 M K<sub>2</sub>SO<sub>4</sub> for subsequent measurement of microbial carbon, phosphorus and, after digestion, also microbial nitrogen (Joergensen 1996; Joergensen and Mueller 1996; Schmidt et al. 2002, 2004). The incubation plants were washed and dried at 80°C for three days and weighed, and then digested

with  $\text{H}_2\text{O}_2$ ,  $\text{H}_2\text{SeO}_3$  and  $\text{H}_2\text{SO}_4$  for 1 h at 400°C (Jonasson et al. 2006).

N and P in extracted and digested samples were measured on Hitachi U 2010 Spectrophotometer. Carbon was measured on a Shimadzu TOC 5000A analyzer. The microbial C, N, and P fractions were calculated assuming extractability factors of 0.40, 0.45 and 0.40, respectively (Joergensen 1996; Joergensen and Mueller 1996; Schmidt et al. 2002, 2004), and were normalized relative to sample soil organic material content (SOM, loss on ignition).

Rates of net nitrification, mineralization and changes in microbial C, N and P and plant N and P were calculated as the difference between the concentration of the incubated sample (sample at the end) and the initial value (Beier et al. 2004b; Emmett et al. 2004). Hence for nitrate, ammonium, dissolved organic N and microbial N the net rate was calculated as:

$$\text{(incubated sample} (\mu\text{gN g}^{-1} \text{ SOM}) \\ - \text{initial} (\mu\text{gN g}^{-1} \text{ SOM})) / \text{days of incubation (187 days);}$$

For both positive and negative rates: net nitrate-N production is referred to as net nitrification, net ammonium-N production is referred to as net ammonification. Production of  $\text{PO}_4^{3-}$  and total dissolved P is referred to as net P mineralization. An increase of microbial N or P from initiation to end of incubation is termed microbial immobilization.

#### Statistical analysis

One-way analysis of variance (ANOVA) was used to test differences between plant specific soil types in ambient plots (*Calluna* or *Deschampsia* soil). Correlations of net nitrate, net ammonium and net P production rates were tested with Kendall and Pearson product moment correlation. A two factor analysis of variances was applied to test for additional effects of plant presence and soil type on microbial immobilization and net production of nutrients. Linear mixed models (proc mixed) were applied to analyze the data in SAS 8.0. Random effects terms were block, treatment, plot and octagons, respecting the nested structure of the design. Main effects terms were the treatment factors: CO<sub>2</sub>, T, and D. All

interaction terms between the factors were included. The models were gradually simplified, starting with the third order interaction, taking out non-significant terms until only significant ( $P < 0.05$ ) or close to significant ( $0.05 < P < 0.10$ ) terms remained. The variables ‘block’ and ‘water content’ of initial soil and incubated soil were included (SAS Institute Inc. 2003). Only data with significant ( $P < 0.05$ ) or close to significant ( $0.05 < P < 0.1$ ) effects are shown in Figs. 1, 2, 3 and 4.

## Results

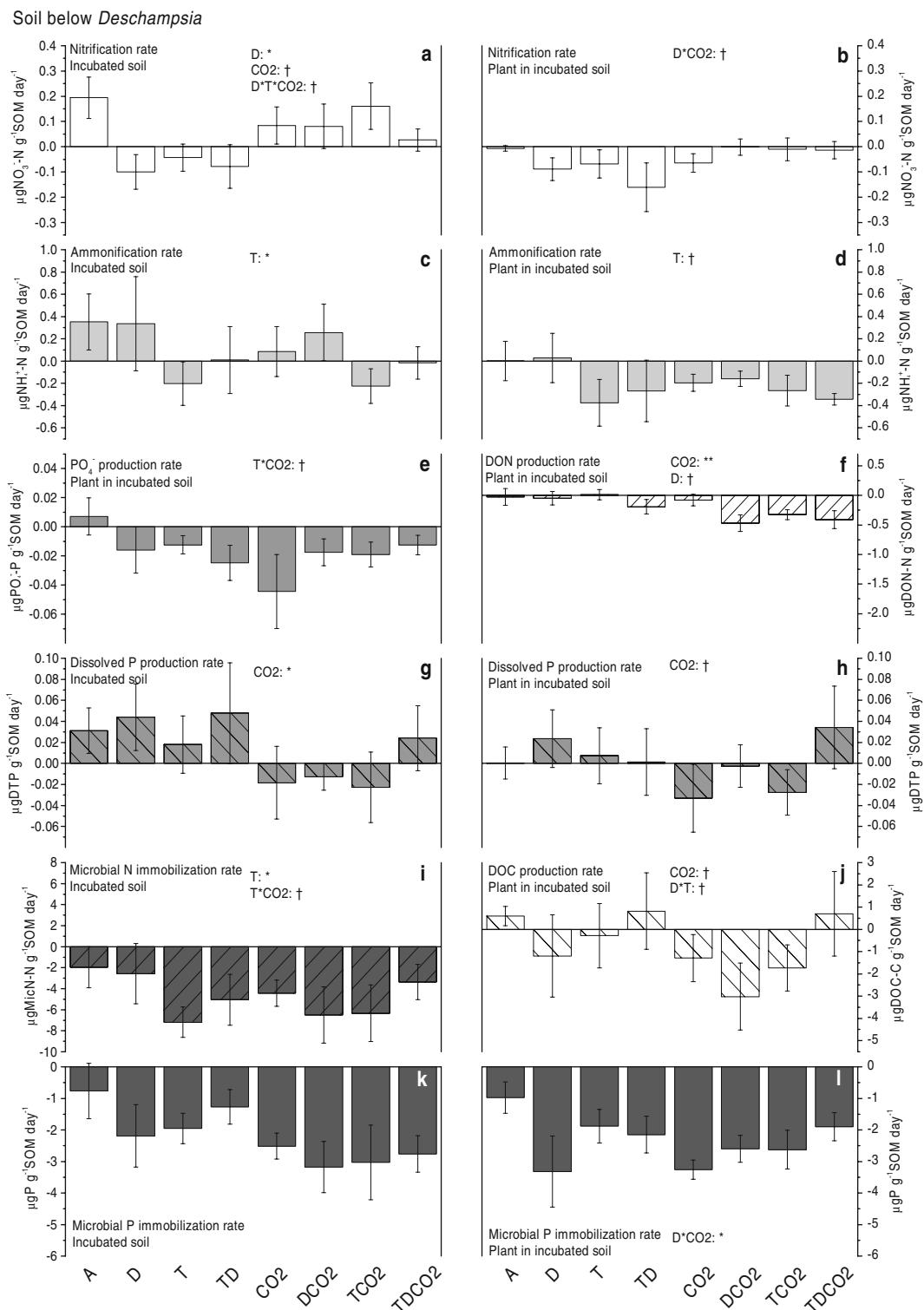
The soil properties of the ambient plots (Table 1) were not significantly different below the two species. However, after one full year of climate treatments, significant responses of soil properties to the main factors CO<sub>2</sub>, T and D and interactions were observed, and the responses differed for the two soil types. Consequently, the incubations with treated *Calluna* soil and *Deschampsia* soil were initially different, and incubations of the two soils responded differently to the climate change factors.

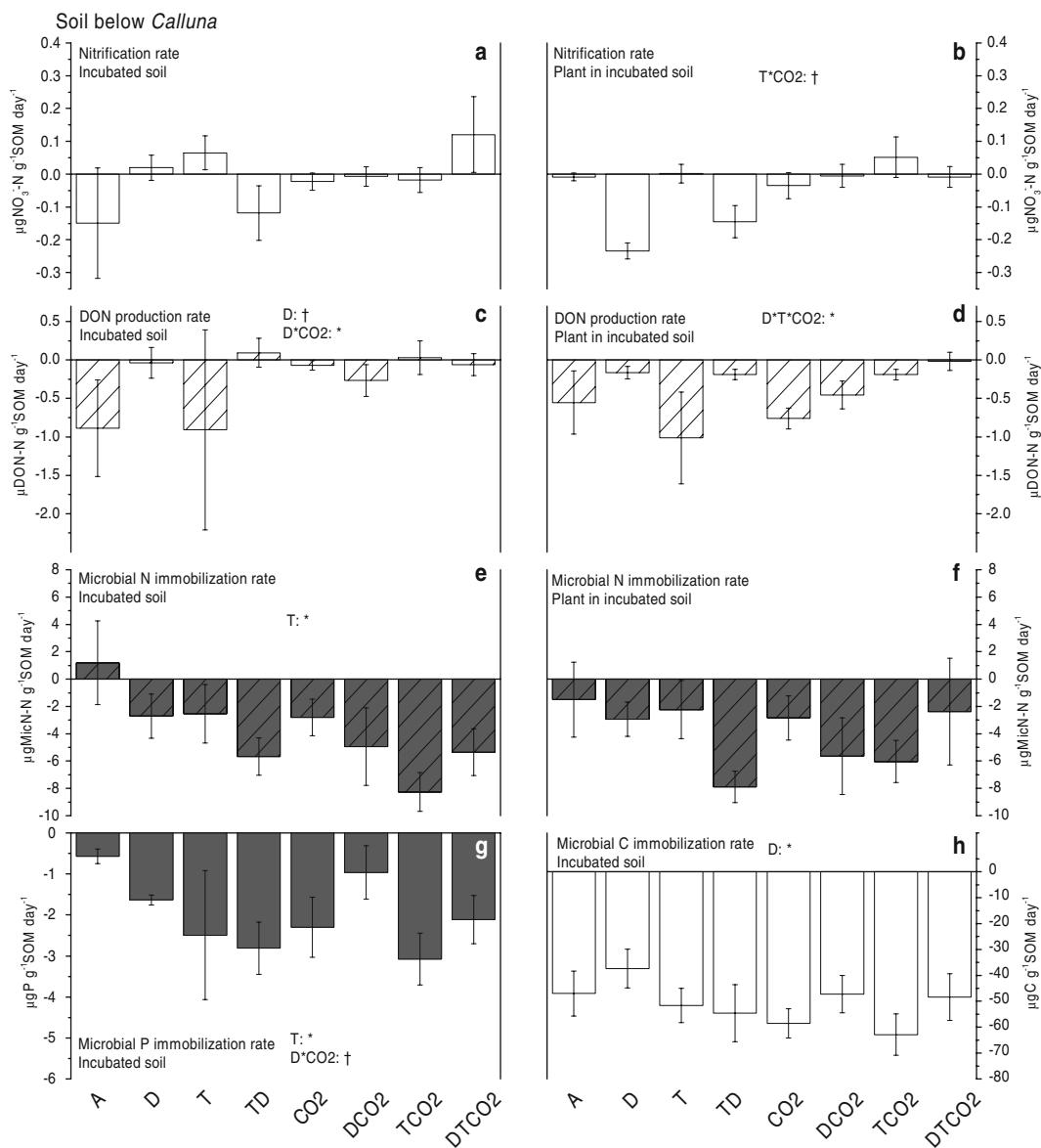
In general, the incubated soil had low or negative net N and P mineralization and microbial C, N and P immobilization. No significant correlations were found between net nitrification, net ammonification and P mineralization rates.

#### Effects of plants in the incubations

Plant survival in the buried bags was 98%. Overall, the *Deschampsia* plants in the incubations increased

**Fig. 1** Influence of plant presence in *Deschampsia* soil after ► incubation for 6 months, with no plant or with plant as indicated. **a** and **b**: nitrification rate, **c** and **d**: ammonification rate, **e**:  $\text{PO}_4$  production rate, **f**: dissolved organic N (DON) production rate, **g** and **h**: dissolved phosphorus production rate, **i**: microbial N immobilization rate and **j**: dissolved organic carbon (DOC) production rate, **k** and **l**: microbial P immobilization rate. Statistical significant effects from proc mixed model analyses of variance for the main effects: D, T and CO<sub>2</sub> and the interactions D\*T, D\*CO<sub>2</sub>, T\*CO<sub>2</sub> and D\*T\*CO<sub>2</sub> are indicated as follows: \*\*\* indicates  $P < 0.001$ ; \*\* indicates  $P < 0.01$ ; \*:  $P < 0.05$ ; †:  $P < 0.1$ . The treatments are: A is ambient (no treatment), D is drought, T is warming, CO<sub>2</sub> is elevated CO<sub>2</sub>. Only parameters with significant results or tendencies are shown



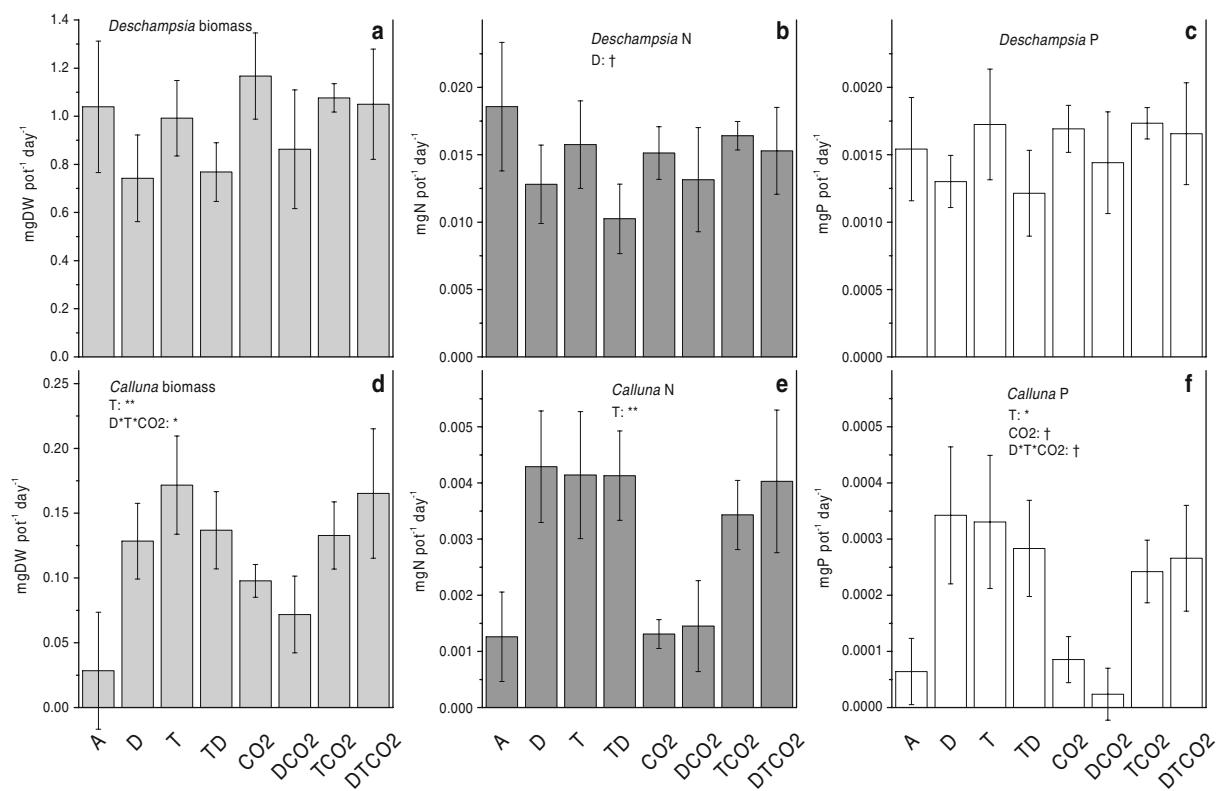


**Fig. 2** Influence of plant presence in *Calluna* soil after incubation for 6 months, with no plant or with plant as indicated. **a** and **b**: nitrification rate, **c** and **d**: dissolved organic N (DON) production rate, **e** and **f**: microbial N immobilization rate, **g**: microbial P immobilization rate and **h**: microbial C immobilization rate. Statistical significant effects from proc mixed model analysis of variances for the main effects: D, T

and CO<sub>2</sub> and the interactions D\*T, D\*CO<sub>2</sub>, T\*CO<sub>2</sub> and D\*T\*CO<sub>2</sub> are indicated as follows: \*\*\* indicates  $P < 0.001$ ; \*\* indicates  $P < 0.01$ ; \*:  $P < 0.05$ ; †:  $P < 0.1$ . The treatments are: A is ambient (no treatment), D is drought, T is warming, CO<sub>2</sub> is elevated CO<sub>2</sub>. Only parameters with significant results or tendencies are shown

their biomass from 0.013 g to 0.194 g, while *Calluna* plants did not gain much mass (from 0.051 g to 0.073 g). The presence of plants decreased the net nitrification rate ( $P$  values from two-factor ANOVA were for  $\pm$  plant: 0.0052 (*Deschampsia*), Fig. 1a and b;

for soil type: 0.0098, Figs. 1a, b and 2a, b), and decreased the net ammonification rate ( $\pm$  plant: 0.0140, Fig. 1c and d). In *Calluna* soil, the presence of plants decreased the net ammonification rate ( $\pm$  plant: 0.0399, data not shown).



**Fig. 3** a, b and c: *Deschampsia* plants incubated with soil. a: biomass, b: nitrogen pool, c: phosphorus pool. d, e and f: *Calluna* plants incubated with soil. d: biomass, e: nitrogen pool, f: phosphorus pool. Statistical significant effects from proc mixed model analysis of variances for the main effects: D:

T and CO<sub>2</sub> and the interactions D\*T, D\*CO<sub>2</sub>, T\*CO<sub>2</sub> and D\*T\*CO<sub>2</sub> are indicated as follows: \*\*\* indicates  $P < 0.001$ ; \*\* indicates  $P < 0.01$ ; \*:  $P < 0.05$ ; †:  $P < 0.1$ . The treatments are: A is ambient (no treatment), D is drought, T is warming, CO<sub>2</sub> is elevated CO<sub>2</sub>

### Responses to drought

In *Deschampsia* soil, the nitrification rate decreased in response to drought (incubated soil:  $P=0.0342$ ), and the DON production rate (soil incubated with plant:  $P=0.0532$ ) and plant immobilization of N ( $P=0.0949$ ) tended to decrease in response to drought (Fig. 1a, f and Fig. 3b). Furthermore, the DOC production rate decreased in non-warmed plots in response to drought (soil incubated with plant, T\*D:  $P=0.0760$ ; Fig. 1j).

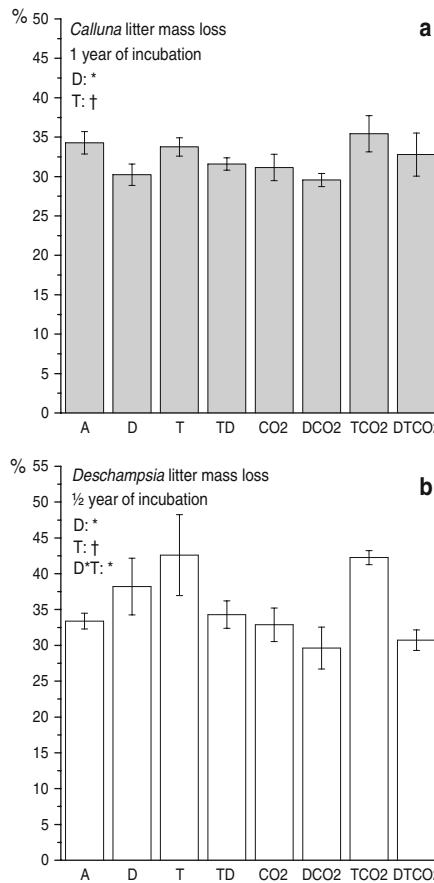
In *Calluna* soil the DON production rate (incubated soil:  $P=0.0646$ , Fig. 2c) and microbial biomass C accumulation (incubated soil:  $P=0.0189$ ) increased in response to drought (Fig. 2h).

Drought reduced the *Deschampsia* leaf decomposition in 6 months litterbag incubations (D: 0.0304, Fig. 4b) and *Calluna* leaf decomposition in 12 months litterbag incubations (D: 0.0212, Fig. 4a).

### Responses to warming

In *Deschampsia* soil the ammonification rate decreased (incubated soil:  $P=0.0294$ ) or tended to do so (soil incubated with plant:  $P=0.0794$ ) in response to warming (Fig. 1c and d). Microbial immobilization of N also decreased in response to warming in non-CO<sub>2</sub> plots (incubated soil:  $P=0.0249$ , Fig. 1i).

The microbes in initial *Calluna* soil for incubation had increased N content (T: 0.0929, T\*D\*CO<sub>2</sub>: 0.0401), and P content (T: 0.0558) in warmed plots (data not shown). However, during incubation of *Calluna* soil, warming decreased microbial immobilization of N (incubated soil:  $P=0.0156$ , Fig. 2e) and P (incubated soil:  $P=0.0122$ , Fig. 2g) while both *Calluna* plant biomass N ( $P=0.0039$ ) and plant P pools ( $P=0.0149$ ) increased in response to warming (Fig. 3e and f).



**Fig. 4** a: *Calluna* litter mass loss during 12 months of incubation of litter bags. b: *Deschampsia* litter mass loss during 6 months of incubation of litterbags. Statistical significant effects from proc mixed model analysis of variances for the main effects: D, T and CO<sub>2</sub> and the interactions D\*T, D\*CO<sub>2</sub>, T\*CO<sub>2</sub> and D\*T\*CO<sub>2</sub> are indicated as follows: \*\*\* indicates  $P<0.001$ ; \*\* indicates  $P<0.01$ ; \*:  $P<0.05$ ; †:  $P<0.1$ . The treatments are: A is ambient (no treatment), D is drought, T is warming, CO<sub>2</sub> is elevated CO<sub>2</sub>

Also, warming tended to increase leaf decomposition in *Calluna* ( $P=0.0605$ ) and *Deschampsia* ( $P=0.0884$ , Fig. 4a and b).

#### Responses to increased CO<sub>2</sub>

In *Deschampsia* soil the nitrification rate tended to increase in response to elevated CO<sub>2</sub> (incubated soil:  $P=0.0861$ ; soil incubated with plant: D\*CO<sub>2</sub>: 0.0558; Fig. 1a and b). The P mineralization rate (incubated soil:  $P=0.0335$ ; soil incubated with plant:  $P=0.0506$ ; Fig. 1g and h) and microbial P immobilization rate (soil incubated with plant, D\*CO<sub>2</sub>:  $P=0.0330$ , Fig. 1l)

also decreased in response to elevated CO<sub>2</sub> in non-drought plots. Furthermore, DON production rate decreased (soil incubated with plant:  $P=0.0063$ , Fig. 1f).

The soil concentration of citric acid below grass increased significantly from 0.055 (0.06s.e.) μM in response to elevated CO<sub>2</sub> (CO<sub>2</sub>\*D: 0.0165, T\*D\*CO<sub>2</sub>: 0.0438), mainly caused by an increase in elevated CO<sub>2</sub> alone from 0.06 μM (0.06s.e.) to 1.34 μM (0.61s.e.). The other organic acids did not respond to the treatments.

CO<sub>2</sub> tended to stimulate *Calluna* leaf decomposition after 6 months (CO<sub>2</sub>: 0.0744, data not shown).

## Discussion

#### Plants in incubations

All the incubations with plants, irrespective of treatment, showed reduced soil concentrations of nitrate and ammonium compared to the incubations with soil alone. This reduction was most likely a consequence of plant uptake of N during the incubation period. Hence, our hypothesis of plant presence increasing net mineralization could not be confirmed in this study. Previous studies have shown that both dominant plant species exhibit nitrate reductase activity, and hence have potential for nitrate acquisition (Högblom et al. 2002; Lee and Stewart 1978; Troelstra et al. 1995) and, furthermore, both species acquire ammonium (Andresen and Michelsen 2005). Alternatively, increased microbial N immobilization could explain the reduced soil N concentration. However, in contrast to similar studies at a subarctic heath (Jonasson et al. 2006; Rinnan et al. 2007), there were no indirect signs in the present incubations with *Deschampsia* and *Calluna* plants of root exudation which might have primed the decomposition and increased microbial immobilization of N and P, although we did not attempt to measure root exudation directly. Furthermore, the presence of plants generally did not change the direction of responses to the treatments. This implies that the frequently used, but also criticized, ‘buried bag’ (Eno 1960; Jonasson et al. 2006; Nielsen et al. 2009; Schmidt et al. 2002) method for studying nitrification and mineralization in some cases may adequately reflect net nutrient release.

## Drought suppresses nitrogen cycling in *Deschampsia* soil

In *Deschampsia* soil, the drought induced a smaller production of labile nitrogen, seen here as the decrease in net nitrification rate, DON production rate and litter decomposition. The lower N availability in response to drought was consistently reflected in the decreased uptake of N by the incubated plants. These responses to drought were in accordance with our hypothesis: that drought would work as a suppressor of nitrogen cycling. We also found that *Deschampsia* soil was more responsive to treatments than the *Calluna* soil. Note also that the soil incubations started immediately after ending the imposed summer drought. Consequently, any observed drought effects of the rates were caused by the differences that were developed during the pre-incubation history of the soil, though no effects on microbial biomass, as obtained from chloroform fumigation, was found to confirm other studies (Jensen et al. 2003; Sowerby et al. 2005b).

Below *Calluna*, drought reduced leaf litter decomposition, as was expected from other studies of *Calluna* litter decomposition, showing moisture limitation of *Calluna* leaf litter and soil organic matter decomposition, along a natural climatic gradient of several field sites in Europe (Emmett et al. 2004; van Meeteren et al. 2007; van Meeteren et al. 2008). Along the gradient in that study, moisture exerted a major control on the variability of the net N mineralization and nitrification rates.

## Effects of elevated temperature on N and P mobilization

The distinct responses for the soils below the two species suggest differential sensitivity to global change factors, with possible consequences for competition between the two plant species. Our hypothesis of the warming treatment promoting N cycling was confirmed. Warming increased leaf litter decomposition for both species, but not when combined with drought, confirming previous studies (van Meeteren et al. 2007, 2008). In *Calluna* soil, the microbial N (and P) pool in warmed plots was higher prior to incubation. This was driving the larger release of microbial N (and P)

in warmed plots, seen as the decreased immobilization during incubation. The increased *Calluna* plant uptake of both N and P was accompanied by increased plant biomass in warmed plots, which suggests that nutrient limitation of the *Calluna* plants was counteracted by warming, and that *Calluna* plants consequently will benefit from warmer climate.

In warmed *Deschampsia* soil, both ammonification rate and microbial N immobilization rate decreased. A decrease in net mineralization in response to warming has been found in other temperate heathlands (Emmett et al. 2004). This may be related to increased microbial immobilization in the bags, in contrast to the decreased microbial N immobilization (i.e. net release of N from the microbial pool) as seen in this study. But net mineralization and microbial immobilization of nutrients may also be regulated by soil fauna (Briones et al. 2009; Osler and Sommerkorn 2007) or gaseous losses through denitrification (Barnard et al. 2006) or by volatilization of ammonia (Chapin III et al. 2002). In studies of C, N and P mineralization of subarctic heath soil after a decade of summer warming, increased P mineralization (Jonasson et al. 2006), but decreased DOC and inorganic N production (Rinnan et al. 2007) was found, after similar incubations with and without plants. In our study, the promotion of nutrient release by warming was beneficial for *Calluna* when the two dominant plant species were studied in isolation. However, surveys of consequences of N deposition (Berendse 1990) for plant competition and heath landscapes generally suggest a long-term change in species composition towards more dominance of grasses (Aerts 1993; Bobbink and Heil 1993; Riis-Nielsen et al. 2005).

## Decreased phosphorus availability under elevated CO<sub>2</sub>

In response to elevated CO<sub>2</sub>, we found that phosphorus cycling was constrained or inhibited, seen as lower net P mineralization rates and microbial P immobilization. This may have been caused by increased CO<sub>2</sub> concentration in the soil (Andrews and Schlesinger 2001; Oh et al. 2007; Oh and Richter 2004), followed by formation of carbonic acid in the soil water that cause an

acidification of the soil, which, in this heathland soil, with an average pH of 4.6, would increase the amount of occluded phosphorus (Chapin III et al. 2002). A similar inhibition of the phosphorus cycle in the incubated soil, that we attribute to inorganic geochemistry, was also observed in a shrub oak forest, where decreased extractable P concentration was found after CO<sub>2</sub> fumigation for 5 years, although this was explained with increased plant nutrient demand (Johnson et al. 2003). These observations of decreased P availability are in contrast to effects of CO<sub>2</sub> fumiga-

tions at other soil types (Ebersberger et al. 2003; Khan et al. 2008). The sensitivity in soil P availability to enhanced CO<sub>2</sub> might, however, be soil specific and related to soil pH, contents of ion, aluminum and other adsorbing minerals important for soil P chemistry (Britton et al. 2003; Chapin III et al. 2002; Kooijman et al. 2009). In studies with short-term treatments, pulse releases of P after drying and rewetting of soils were observed but with no relation to microbial biomass C (Blackwell et al. 2009; Butterly et al. 2009).

**Table 2** Summary table of significant ( $P < 0.05$ ) and near-significant ( $0.05 < P < 0.1$ , in brackets) main effect results for: incubated soil, for incubated soil with plant, for the plant in the incubation and for leaf litter decomposition, separately for the two species

	Incubated soil			Incubated soil with plant		
	D	T	CO <sub>2</sub>	D	T	CO <sub>2</sub>
<i>Deschampsia</i>						
Nitrification rate	↓	.	(↑)	.	.	.
Ammonification rate	.	↓	.	.	(↓)	.
Dissolved organic N prod.	.	.	.	(↓)	.	↓
Dissolved organic C prod.	.	.	.	(↓) -T	.	(↓)
Microbial C accumulation	.	.	.	.	.	.
Microbial immobil. of N	.	↓ -CO <sub>2</sub>	.	.	.	.
Microbial immobil. of P	.	.	.	↓ -CO <sub>2</sub>	.	↓ -D
Phosphate prod.	.	.	(↓) -T -D	.	.	.
Dissolved P prod.	.	.	↓	.	.	(↓) -TD
Leaf litter decomp. 6 mo	↓+T+CO <sub>2</sub>	.	.	.	.	.
Inc. plant biomass	.	.	.	.	.	.
Inc. plant N pool	.	.	.	(↓)	.	.
Inc. plant P pool	.	.	.	.	.	.
<i>Calluna</i>						
Nitrification rate	.	.	.	.	.	(↑)+D+T
Ammonification rate	.	.	.	.	.	.
Dissolved organic N prod.	(↑) -CO <sub>2</sub>	.	.	↑	.	.
Dissolved organic C prod.	.	.	.	.	.	.
Microbial C accumulation	↑	.	.	.	.	.
Microbial immobil. of N	.	↓	.	.	.	.
Microbial immobil. of P	.	↓	.	.	.	.
Phosphate prod.	.	.	.	.	.	.
Dissolved P prod.	.	.	.	.	.	.
Leaf litter decomp. 12 mo	↓	.	.	.	.	.
Inc. plant biomass	.	.	.	↑	.	.
Inc. plant N pool	.	.	.	↑	.	.
Inc. plant P pool	.	.	.	↑	.	.

Arrow shows direction of response (increase or decrease), dot is no change. The arrow is followed by a specification if two-way interaction is found, see also Fig. 1, 2, 3 and 4

In our experiment we found that citric acid concentration in the soil more than doubled under elevated CO<sub>2</sub>. In rhizosphere soil, binding of phosphorus (P) to soil colloids is weaker when plant root exudation of citric acid is increased (Jones and Darrah 1994; Ström et al. 2005). Our results show, for the first time, that elevated CO<sub>2</sub> through one year may increase the soil water concentration of citric acid below *Deschampsia*, and this with possible consequences for phosphorus availability for the plants (Ström et al. 2005).

The altered mobilization of N and P from microbes appeared with no change in microbial biomass C. Hence, the microbial biomass was not limited by N or P at this heath, in consistence with a study of the consequences of inorganic N and P application during summer at the same field site (Nielsen et al. 2009). Altered mobilisation and availability of P may in the longer term have consequences for the plants, which due to N deposition may become progressively severely limited by P. This may affect plant species competition at the heath, in combination with the effects of future climate changes (Aerts 1993; Bobbink and Heil 1993; Riis-Nielsen et al. 2005).

A synthesis of belowground responses to combined elevated CO<sub>2</sub> and temperature suggested that soil organic matter cycling and decomposition would increase dramatically (Pendall et al. 2004). In the studies by Hovenden et al. (2008), Barnard et al. (2006) and Menge and Field (2007) in which warming and elevated CO<sub>2</sub> were combined, no synergistic responses in parameters related to decomposition processes were observed. However, nitrogen fertilization along with elevated CO<sub>2</sub> often triggers responses (Barnard et al. 2006; Bloor et al. 2009; Menge and Field 2007). In our study, microbial immobilization of N, P mobilization and leaf litter decomposition responded to warming with synergistic effects of elevated CO<sub>2</sub> and drought, in soil type specific patterns (Table 2). This suggests that biological and geochemical responses to the interactions of elevated CO<sub>2</sub> with other environmental factors are complex and still not fully understood.

#### Conclusions: climate change responses at the temperate heath

*Calluna* and *Deschampsia* soil showed different responses to elevated temperature, increased CO<sub>2</sub>

and summer drought. Treatments had significant effects on soil N and P net mineralization, microbial C, N and P immobilization, litter decomposition and plant growth in incubated soil (Table 2). Drought generally suppressed nitrogen cycling, in particular in *Deschampsia* soil where drought reduced net nitrification, litter decomposition and plant N uptake. Warming increased process rates of decomposition, net mineralization and microbial release of N and P, in soil type specific patterns beneficial for nutrient limited plants. The main effect of elevated CO<sub>2</sub> on P availability with consequences for soil microbes, may in the longer term have consequences for P limited plants, and thereby affect plant species competition at the field site.

In this short term study, the investigated ecosystem processes responded to drought, increased temperature and elevated CO<sub>2</sub>. However, the combined effects of these climate change related factors often counteracted the main effects. Thus, the study emphasizes the need to investigate interactions between the climate change factors, as these may be unpredictable based only on single factor studies.

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