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Belowground heathland responses after 2 years of combined warming, elevated CO_2 and summer drought

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Abstract Terrestrial ecosystems are exposed to atmospheric and climatic changes including increases in atmospheric CO_2 concentration, temperature and alterations of precipitation patterns, which are predicted to continue with consequences for ecosystem services and functioning in the future. In a field scale experiment on temperate heathland, manipulation of precipitation and temperature was performed with retractable curtains, and atmospheric CO_2 concentration was increased by FACE. The combination of elevated CO_2 and warming was expected to affect belowground processes additively, through increased belowground sequestration of labile carbohydrates due to elevated CO_2 in combination with temperature increased process rates. Together, these changes might increase microbial activity and availability of plant nutrients. Two years after the start of the experiment, belowground processes responded significantly to the treatments. In the combined temperature and CO_2 treatment the dissolved organic nitrogen concentration decreased and the ammonium concentration increased, but this release of nutrients was not mirrored by plant parameters. Microbial biomass carbon and microbial enrichment with ^{13}C and ^{15}N (1 year after $^{13}C_2^{15}N$-glycine was injected into the soil) increased in warmed plots and in elevated CO_2 plots, but not when these treatments were combined. Furthermore, drought led to an increase in *Calluna* biomass and total plant nitrogen pool. The full combination of warming, elevated CO_2 and periodic drought did not unambiguously express the ecosystem responses of single factors additively, which complicates predictions of ecosystem responses to multifactor climate change.

Keywords ^{13}C · Climate change · Microbial carbon · Microbial turnover · ^{15}N · Plant nutrients · Temperate heath

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Introduction

Terrestrial ecosystems are exposed to atmospheric and climatic changes including increases in atmospheric CO_2 concentration, temperature and alterations of precipitation patterns, which are predicted to

continue with consequences for ecosystem services and functioning in the future (Luo et al. 2006; IPCC 2007). Biotic responses to climate forcing may appear as suppression or increase of the abundance and functioning of specific organisms, and the direction of the response depends e.g. upon whether the ecological networks are in dynamic steady state and balanced regarding demographic flow and competition (Ims et al. 2007; Ings et al. 2009). Other factors which influence the outcome are the potential limitations on primary production and its consumers caused by low supply of nutrient element or water (Finzi et al. 2006; Hungate et al. 2006; Sokolov et al. 2008), and the initial position of key species along their ecological amplitude regarding optimal climatic conditions (Sætersdal and Birks 1997; Gornall et al. 2007; Willis et al. 2007). A forced change in any of these conditions may affect the magnitude and time scale of the responses at ecosystem level.

On a short-term time scale, an increased primary production caused by CO_2 may, together with warming lead to increased acquisition of nitrogen (N) and phosphorus (P) by plants. On a longer time scale, this may result in a progressive limitation of primary production (Finzi et al. 2006; Hungate et al. 2006; Sokolov et al. 2008) and the associated consumers and symbionts (Olsrud et al. 2004; Briones et al. 2009). Stimulated primary production, root longevity (Eissenstat et al. 2000) and root biomass (Runion et al. 1994; Iversen et al. 2008) under elevated CO_2, have been found in a number of field studies (Mauney et al. 1994; Niklaus and Körner 2004; Dijkstra et al. 2008; Liberloo et al. 2009). Depending on site and vegetation type, this was controlled and limited by nutrient availability (e.g. nitrogen or phosphorus) (Niklaus and Körner 2004; Luo et al. 2006). The resulting effect with lower nutrient concentration in the plant, when carbon assimilation is faster than nitrogen uptake and transport (Taub and Wang 2008), may regulate the quality of the food source for herbivores (Osler and Sommerkorn 2007). Consequently, it is suggested that cascade effects of elevated CO_2 through the rhizosphere food-web may be controlled by plant root exudation (Giesler et al. 2007) and plant litter deposition, though this has not been consistently observed (Zak et al. 2000; Arnone et al. 2000; Woodward and Osborne 2000; Ostle et al. 2007; Handa et al. 2008).

Increased allocation of carbon (C) and nitrogen (N) into root biomass in response to warming and to elevated CO_2 is found in temperate forests and grasslands (Volder et al. 2007; Pregitzer et al. 2008). Warming may consequently initiate a belowground shift in the primary producer controlled food-chain, based on plant litter amount and chemical composition (Graglia et al. 2001; Hansen et al. 2006; Briones et al. 2009; Niklaus et al. 2007). Due to changes in these substrates, shifts in soil microbial communities may occur (Hamer and Marschner 2002; Chung et al. 2007). For instance, 12 years of chronic soil warming reduced microbial biomass, the utilization of a suite of carbohydrate substrates and the abundance of fungal biomarkers (FAMEs) in a temperate deciduous forest (Frey et al. 2008). The possibility that soils become depleted of readily decomposable substrates has been suggested as a confounding factor of the microbial responses to long-term warming of heathlands (Biasi et al. 2005; Hartley et al. 2008). Hence, availability of labile carbohydrates may be limiting factors for soil microorganisms.

Droughts influence carbon sequestration in terrestrial ecosystems through inhibited plant growth (Peñuelas et al. 2004; Zavalloni et al. 2009), and altered decomposition of soil organic matter. For instance, terrestrial ecosystems in North America shifted from being a weak to a normal carbon sink with the return of rains after 5 years of heavy droughts, as inferred from remote observations of the normalized differential vegetation index and CO_2 concentrations in the air (Buermann et al. 2007). Likewise, the 2003 climate anomaly in Europe with increased temperature (+6°C) and reduced precipitation (−50%) was predicted to induce an anomalous net loss of CO_2, roughly undoing 4 years of net ecosystem C sequestration (Ciais et al. 2005). The normal moisture status of an ecosystem is critical for the impact of drought on belowground carbon storage and sequestration. By induced repeated summer drought treatments at three temperate heathland sites, a sustained reduction in soil moisture was found during winter due to hydrophobicity of soil organic matter and cracking of the soil (Beier et al. 2004; Sowerby et al. 2008). As response, site specific mineralization and litter decomposition correlated with soil water content (Emmett et al. 2004; Schmidt et al. 2004), and decreased enzyme activity under dry conditions was suggested as a possible controlling

factor (Sardans et al. 2008). Furthermore, soil microorganisms were inhibited by droughts that constrain microbial immobilization of nutrients and carbon dioxide release (Jensen et al. 2003). For the grasslands of North America, lignin content and mean annual precipitation were the best predictors of belowground litter decomposition, while temperature did not correlate with decomposition (Bontti et al. 2009), hence, it is suggested from various investigations that elevated temperature only increase decomposition when soil moisture is not limiting.

Although effects of enhanced CO_2, elevated temperature and incidents of drought clearly interact on ecosystem processes, field studies combining all these factors are scarce. In the present study, a temperate heathland was exposed to manipulations of CO_2, temperature and precipitation in order to study how belowground ecosystem processes respond to single- and multifactorial climate change treatments.

We expected that after 2 years, the treatments would lead to:

- Higher turnover and mineralization of substrates in response to warming.
- Enhanced nutrient demand by plants in warmed and elevated CO_2 treatments.
- Inhibited microbial nutrient cycling in response to drought.
- Possible additive responses by combined warming, CO_2 and drought.

Methods

The field site

The experiment took place at the site of the CLIMAITE experiment (Mikkelsen et al. 2008) at Brandbjerg (55°53′N 11°58′E) approximately 50 km NW of Copenhagen, Denmark. The site was a dry, temperate heath on a hilly nutrient-poor sandy deposit, with an organic layer of approximately 5 cm depth and a pH of around five. The vegetation was dominated by *Calluna vulgaris* (an evergreen dwarf shrub of the *Ericaceae*) and *Deschampsia flexuosa* (a grass) and also included small proportions of the grass *Festuca ovina* and other heathland herbs and mosses. The average precipitation per year was approximately 600 mm and the average annual temperature was 8°C (Danish Meteorological Institute 2008).

The climate change treatments

The climate manipulations started in October 2005 (Mikkelsen et al. 2008) and consisted of eight treatments: plots with elevated temperature (T), extended summer drought (D), elevated atmospheric CO_2 concentration (CO_2), all combinations of these treatments (TD, TCO_2, DCO_2 and $TDCO_2$) and untreated reference plots (A). All treatments were replicated six times. The field site covered an area of about two hectares and the experimental plots were distributed in 12 seven meter diameter octagons arranged pair-wise in six blocks, one octagon exposed to elevated CO_2 and one octagon at ambient CO_2 in each of the six blocks. Each octagon comprised four plots with the treatments D or T solely or in combination, and a non-warmed, non-drought plot, giving a total of 48 plots. The temperature was increased by passive night-time warming, by means of low curtains (blinds) which were automatically removed during rain events. This gave rise to an average 1°C temperature increase in two cm soil depth. The drought was imposed also with curtains that automatically unfold during rain events. The atmospheric CO_2 was increased to 510 ppm by a FACE technique including automated feed-back control on CO_2 concentrations driven by wind speed and wind direction (Mikkelsen et al. 2008). The drought treatment period was initiated in late June 2006 and was continued for 5 weeks until early August when soil water reached approximately 0.05 m^3m^{-3} in the top 20 cm of the soil. In 2007, the drought treatment period started in late May and was ended after 5 weeks in late June.

All of the 48 experimental plots had temperature probes installed at 5 cm depth in the soil, at the soil surface, and in the vegetation canopy at 20 cm height, recording temperature on an hourly basis. TDR probes were installed at 0–20 cm depth and 0–60 cm depth for registration of soil water content on an hourly basis. In addition, the gravimetric water content of the soil samples from 0 to 5, 5 to 10 and 10 to 15 cm was measured after soil sampling in August 2007 (80°C for 48 h). Cups for collection of precipitation water were installed on two masts at the field site.

$^{15}N^{13}C_2$-glycine labelling of the soil

In each of the 48 plots an area of 80 × 80 cm was chosen prior to the start of the climate treatments to contain an approximately equal amount of *Calluna vulgaris* and grasses (mainly *Deschampsia flexuosa*). Within each of these areas, a subplot of 20 × 20 cm was amended with $^{15}N^{13}C_2$-labelled glycine on 26th September 2006 (Andresen et al. 2009). Each subplot received 100 ml of re-demineralised water with 0.027 g glycine (U–$^{13}C_2$, 98%; ^{15}N 98%; H_2NCH_2COOH), corresponding to 130 mg N m^{-2}. The glycine solution was injected into the soil just below the soil surface with a syringe at 20 evenly distributed points within the 20 × 20 cm plots. The glycine concentration in the soil prior to labelling was 0.197 μgN g^{-1} SOM ± 0.052. Consequently, addition of glycine enhanced the soil concentration of glycine 100-fold, which is unavoidable if uptake of label in plants and microbes is to be determined with sufficient precision.

Sampling of plant biomass and soil

In late August 2007, 1 year after labelling with $^{15}N^{13}C_2$-glycine and 2 years after the start of the climate change treatments, aboveground vegetation of the undisturbed 20 × 20 cm subplots was sampled down to soil surface. The vegetation was separated into fractions: *Calluna*, grasses (mostly *Deschampsia* but also *Festuca ovina*; including leaf meristem) and mosses (mostly: *Hypnum cupressiforme*, *Pleurozium schreberi* and *Dicranum scoparium*). Additionally, soil cores were sampled in the subplots from the soil surface (including the litter layer) down to 15 cm depth. Three soil cores (4.5 cm diam.) were taken from each plot and divided into three soil depths: 0–5, 5–10 and 10–15 cm. These were mixed depth wise into a composite sample from each depth and immediately sorted by hand into soil and roots. The *Calluna* material was divided into: green shoots with green leaves attached, flowers, coarse (non-green) branches, coarse roots (>0.5 mm in diameter) and fine roots (<0.5 mm). The grasses were sorted into leaves, coarse roots (>1 mm), and fine roots (<1 mm). Mosses and aboveground litter (mainly of grasses, but also of *Calluna*) constituted additional fractions. All plant material (roots and shoots) was washed with 0.5 mM $CaCl_2$, frozen and freeze dried.

The soil samples were kept on ice until further processing. Within 48 h, a subsample of the fresh soil from each plot was extracted with re-demineralised water (1:5) on a shaker for 1 h. Another set of subsamples was vacuum-incubated with chloroform for 24 h to release microbial carbon and nitrogen (Joergensen and Mueller 1996; Brookes et al. 1985) before extraction with water as above. A third subsample of the sorted soil was freeze dried and weight loss was used for estimating soil water content. Additional soil samples and plant shoot samples from non-labelled plants, microbes and soil within the climate treated plots, were required for $\delta^{15}N$ and $\delta^{13}C$ natural abundance from all the investigated fractions. The same procedures as for the labelled samples were followed, with caution not to cross-contaminate with ^{13}C and ^{15}N labelled samples.

Pot incubations for net mineralization rates

Seven weeks after the isotope label was injected into the soil (November 2006, 1 year after treatments were initiated) separate blocks of soil (20 × 20 cm, adjacent to the isotope labelled area) from below *Calluna* plants and below *Deschampsia* plants respectively, were cut down to 5 cm depth without removal of any litter or roots. One subsample from each block was directly used for analysis of initial soil properties. Two other subsamples were carefully cut down to sizes of 4 × 4.5 cm and cut in two vertically. A small plant of *Calluna* or *Deschampsia* was sandwiched in, and the whole sample was slipped into PVC incubation pots with no compression. A lid of Parafilm sealed the pot, but a small slit allowed for plant growth and additionally allowed for water vapour exchange (Andresen et al. 2010). The pot incubations were placed in holes at the field site in level with the surrounding soil. A 10 cm tall wire mesh was tightened around the pots to exclude mice. During summer (June, July and August), all pots were kept humid with de-mineralized water by adding one ml of water four times to all pots. Furthermore, germinating plants were weeded from the pots. After 1 year (November 2007) the pots were sampled for analysis. The small plants were carefully removed, and roots and litter was sorted manually from the samples. The fresh soil from the pots (kept cold for max. 2 weeks) was extracted with

0.1 M K_2SO_4 (1:5 soil:water) for analysis of nitrate (NO_3^-), ammonium (NH_4^+), dissolved organic carbon (DOC) and phosphate (PO_4^+). The same procedure was conducted for the initial subsamples. The incubation plants were washed and dried at 80°C for 3 days and weighed, and then digested with H_2O_2, H_2SeO_3 and H_2SO_4 for 1 h at 400°C (Nielsen et al. 2009).

Chemical and isotope ratio analysis

The soil water extracts were analyzed for NH_4^+ and total dissolved nitrogen (TDN) with a Hitachi U 2010 spectrophotometer, and for NO_3^- with a Tecator FIAstar analyzer. Total mineral N was subtracted from TDN to determine DON. Microbial nitrogen (MicN) was calculated as the subtraction of TDN in the non-fumigated samples from TDN in the fumigated samples, and 0.4 was used as extractability factor. Dissolved organic carbon (DOC) was measured with a Shimadzu TOC 5000A analyzer. Microbial carbon (MicC) was calculated as DOC in the fumigated samples subtracted DOC in the non-fumigated samples, with 0.45 as extractability factor. N and P in extracted and digested plant samples were measured with Hitachi U 2010 Spectrophotometer (Jensen et al. 2003; Nielsen et al. 2009).

For the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotope ratio analysis of the fumigated and non-fumigated soil extracts, the extracts were freeze dried in a small vial containing a quartz filter (Quartz microfiber filters QMA Whatman) with a parafilm lid with a small hole. Filters, dried ground soil and plant material were analyzed with a Eurovector CN analyzer coupled to an Isoprime isotope ratio mass spectrometer. Plant material calibrated against certified IAEA standards was used as working standards.

Calculations and statistics

The atomic percentage was calculated from $\delta^{15}N$ values: $\text{atom\%} = 100\% * 0.003676 * ((\delta^{15}N/1000) + 1)/(1 + 0.003676 * (\delta^{15}N/1000 + 1))$ or $\delta^{13}C$ values: $\text{atom\%} = 100\% * 0.011237 * ((\delta^{13}C/1000) + 1)/(1 + 0.011237 * (\delta^{13}C/1000 + 1))$.

The measured ^{15}N or ^{13}C natural abundance atomic percentage of the material was then subtracted. In particular, the plots with elevated CO_2 exhibited a change in initial ^{13}C natural abundance due to different isotopic composition of CO_2 in air and in FACE fumigation CO_2. Thus, for all treatment combinations and each plant or soil fraction, the measured ^{15}N or ^{13}C contents were adjusted using plot specific values for isotope natural abundance for each sample component (Fry 2006). ^{15}N and ^{13}C enrichments of the microbial biomass are reported as millimole per m^2 in excess of natural abundance ^{15}N and ^{13}C. The linear regression of these excesses ($^{13}C = b + a \times {}^{15}N$), expressed the relative content of each stable isotope from the original glycine. The maximum potential intactness of acquired glycine molecules, assuming that all ^{15}N and ^{13}C enrichment was potentially still glycine in the microbes, was calculated based on '2' as the C:N ratio of glycine, and based on the slope of the linear regression: $a/2 \times 100\% = \text{intact\%}$.

The ^{15}N recovery in the subplots was calculated as the percentage of total added ^{15}N label per m^2 units, which was recovered in the TDN, MicN and total soil N pools and in the total plant biomass per m^2 units:

$$^{15}\text{N recovery} = \text{mg}^{15}\text{N m}^{-2}\,(\text{measured}) / \text{mg}^{15}\text{N m}^{-2}\,(\text{added}) \times 100\%$$

Net nitrification and mineralization rates and dissolved phosphate (P) and carbon (C) production rates were calculated as the difference between the concentration of the incubated sample (sampled at the end) and the initial value (Eno 1960; Emmett et al. 2004). Hence, for nitrate, ammonium, DOC and phosphate the net element (E) production rate was calculated as:

$$\left(\text{incubated sample}\left(\mu\text{gE g}^{-1}\ \text{SOM}\right) - \text{initial}\left(\mu\text{gE g}^{-1}\text{SOM}\right)\right)/\text{days of incubation.}$$

Linear mixed models were applied to analyse the responses using SAS 9.1. Random effect terms were block, treatment plot and octagons, respecting the nested structure of the design. Main effects terms were the treatment factors: CO_2, T, and D. All interaction terms between the factors CO_2, D and T were included. Soil water content was included as a covariate in all proc mixed analysis of soil variables. 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

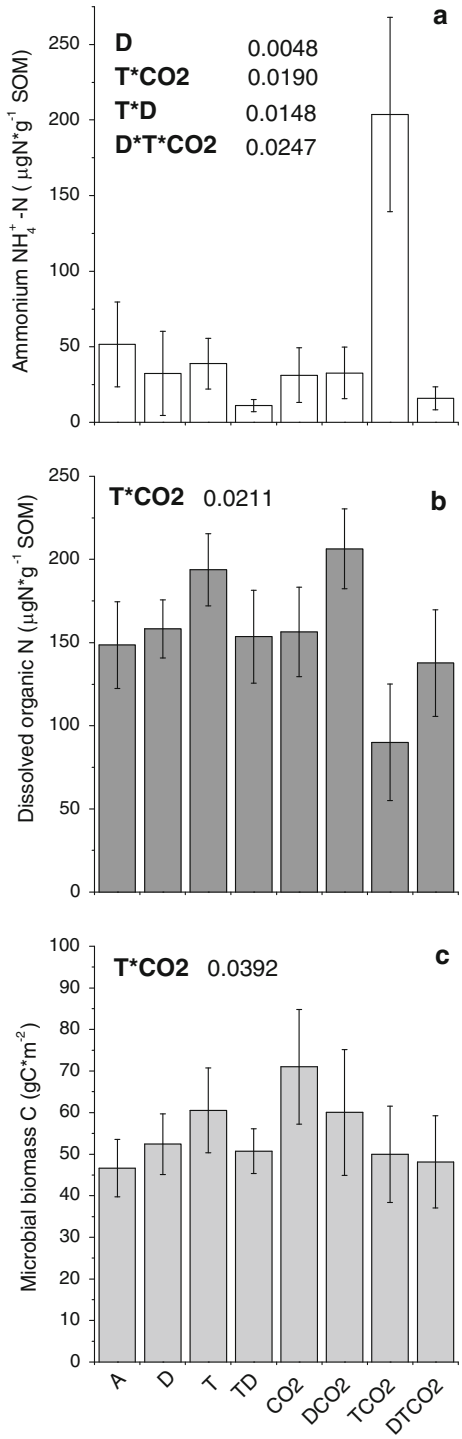

Fig. 1 **a** Mean soil ammonium concentration (NH_4^+–N in µgN g^{-1} soil organic matter (SOM)) and **b** mean soil dissolved organic nitrogen concentration (DON in µgN g^{-1}SOM) in the top 5 cm soil and **c** mean microbial biomass carbon (C) (µgC m^{-2}) in the top 15 cm soil. Observations were made in late August 2007, after 2 years of treatment with the climate change treatments: warming (T), elevated atmospheric CO_2 concentration (CO_2) and summer drought (D) and those treatments in all combinations. A is ambient conditions (control). *Error bars* represent standard error ($n = 6$). *P* values of significant main effects and the interactions are presented

significant ($0.05 < P < 0.10$) terms remained. Homogeneity of variances was investigated with residual plots and appropriate transformations done if necessary (SAS Institute Inc. 2003).

Results

The soil NH_4^+ concentration decreased in response to drought and strongly increased in the combined temperature and CO_2 treatment (TCO_2 treatment) (Fig. 1a), while DON correspondingly decreased in the TCO_2 treatment (Fig. 1b). Microbial biomass carbon pool summed over 0–15 cm depth, increased in response to warming and elevated CO_2 alone but not when these treatments were combined (Fig. 1c).

The microbial turnover during the year of incubation was assessed by the remaining enrichment of the microbial biomass with ^{15}N and ^{13}C, which was added as dual labelled $^{15}N^{13}C_2$-glycine 1 year earlier. There were significant treatment effects on microbial ^{13}C enrichment and ^{15}N enrichment, with higher ^{13}C and ^{15}N enrichment in warmed and elevated CO_2 plots, but not when these treatments were combined, leading to significant T * CO_2 interactions (Fig. 2). ^{13}C and ^{15}N enrichment in the microbial biomass were significantly correlated ($^{13}C = 0.1366 + 1.299 * {}^{15}N$, $P < 0.0001$, $R^2 = 0.548$) (Fig. 2), indicating a potential 65% intactness of acquired glycine molecules 1 year after addition, compared to a potential 79% intactness of microbial acquired glycine 1 day after addition (Andresen et al. 2009).

The recovery of ^{15}N in the total *Calluna* plant increased significantly in response to drought (Fig. 3a), mainly as a consequence of the increased *Calluna* biomass. All main factors interacted on the recovery of ^{15}N in the *Deschampsia* total plant biomass (Fig. 3b). In the microbes the recovery of ^{15}N from the glycine label almost doubled in T, CO_2 and DCO_2 treatments (Table 1; Fig. 3c). Summed for 0–15 cm depth there were significant effects of treatment on the microbial ^{15}N recovery

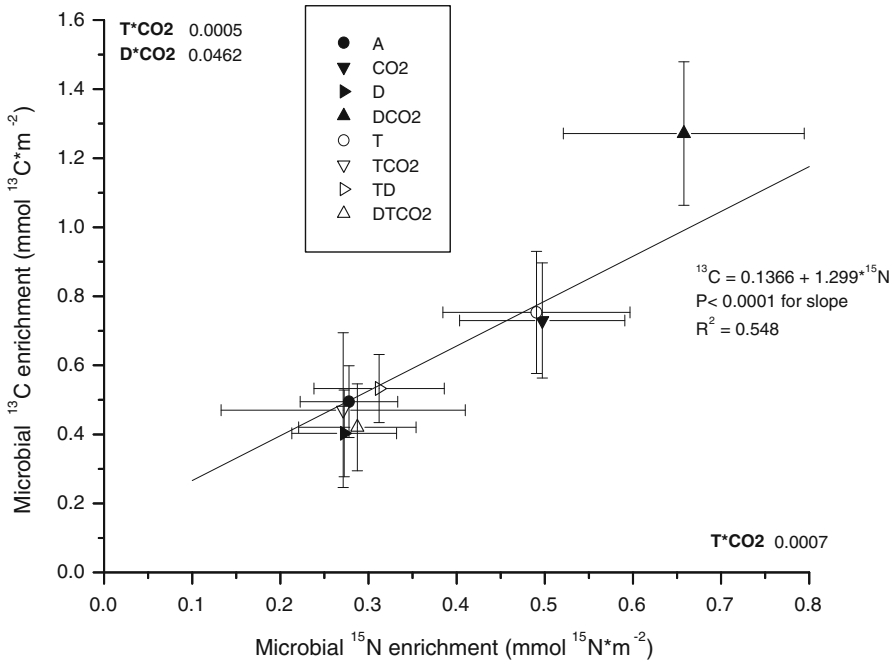

Fig. 2 Microbial biomass enrichment with ^{13}C (second axis in mmol ^{13}C m^{-2}) versus microbial biomass enrichment with ^{15}N (first axis in mmol ^{15}N m^{-2}) in the top 5 cm soil. Observations were made in late August 2007, 1 year after in situ labelling with $^{15}N^{13}C_2$-glycine, and after 2 years of treatment with the climate change factors (see Fig. 1 for legend)

(T * CO_2: 0.0004 (*P*-value)), the ^{15}N recovery in the dissolved nitrogen pool (T * CO_2: 0.0157 and T * D: 0.0113) and ^{15}N recovery in the soil (T * CO_2: 0.0091). A third order interaction was also found for recovery of ^{15}N in mosses (D * T * CO_2: 0.0492; Table 1).

Total above- plus belowground plant biomass of *Calluna* increased in response to drought, while *Deschampsia* total plant biomass was unaffected (Fig. 4a, d). The green biomass, flower biomass and fine root fractions of *Calluna* all increased under drought (Fig. 4c, e, f). The green biomass fraction of *Deschampsia* (Fig. 4b) and moss biomass responded with no main effect but with interactions between main factors (mosses; T * D * CO_2: 0.0216; data not shown). *Deschampsia* fine root biomass tended to decrease in response to warming (T: 0.0689; data not shown), while *Calluna* fine root biomass increased in response to warming in non-CO_2 plots (Fig. 4c). The total *Calluna* nitrogen pool increased in response to drought (Fig. 5a), while the *Deschampsia* N pool remained unchanged (Fig. 5b).

The *Calluna* flower N concentration decreased in response to elevated CO_2 (Fig. 6a). The *Deschampsia* green leaf fraction had a tendency to a lower N concentration in response to elevated CO_2, except when CO_2 was combined with warming (T * CO_2: 0.0717, Fig. 6f). In the top soil a third order interaction was found for *Deschampsia* and *Calluna* fine root N concentration (Fig. 6b, c).

In the soil incubated with plants, warming reversed the direction of NO_3^- turnover from an average net production of: 0.008 μgNO_3–N g^{-1} SOM day^{-1}, without warming, to a net consumption of: −0.024 μgNO_3–N g^{-1} SOM day^{-1}, with warming (Table 2). Furthermore, elevated CO_2 significantly increased the net DOC production rates from a mean of 7.61 $\mu gDOC$ g^{-1} SOM day^{-1} with ambient CO_2 to 9.76 $\mu gDOC$ g^{-1} SOM day^{-1} with elevated CO_2. Drought treatments decreased NO_3^- consumption from −0.049 μgNO_3–N g^{-1} SOM day^{-1} without drought, to −0.027 μgNO_3–N g^{-1} SOM day^{-1} with drought, and increased the plant N to P ratio from 8.5 without drought to 9.2 with drought (Table 2). There was no effect of treatments on ammonification rate (mean: −0.073 μgNH_4–N g^{-1} SOM day^{-1}) and phosphate production rate (mean: −0.022 μgPO_4–P g^{-1} SOM day^{-1}).

Discussion

Two years of treatment with warming, elevated atmospheric CO_2 and summer drought affected the belowground processes at the temperate *Calluna*–*Deschampsia* heathland. The concentrations of

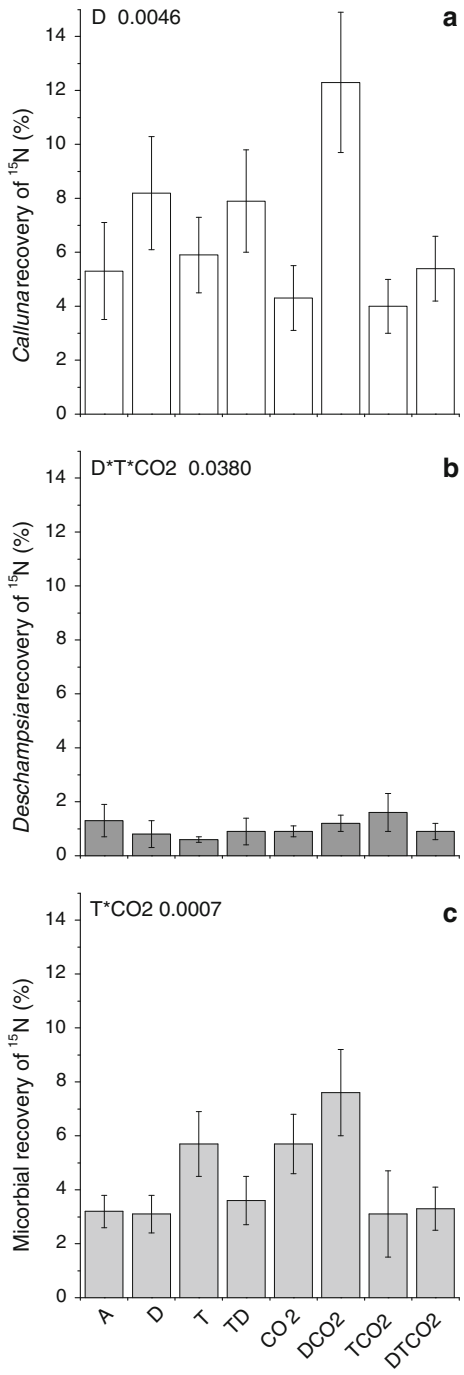

Fig. 3 Recovery of ^{15}N (%) in whole plants of **a** *Calluna vulgaris* and **b** *Deschampsia flexuosa* and **c** soil microbes in the top 5 cm soil. Observations were made in late August 2007, 1 year after in situ labelling with $^{15}N^{13}C_2$-glycine, and after 2 years of treatment with the climate change factors (see legend Fig. 1)

ammonium and DON (Fig. 1a, b) responded strongly and in opposite directions in the combined warming and elevated CO_2 treatment. This may be a consequence of a rapid microbial mineralization, supporting our hypothesis of higher turnover and mineralization in warmed plots (Emmett et al. 2004; Andresen et al. 2010). In combination with an altered process rate, we suggest that a change in the composition of the microbial community (Habekost et al. 2008; Frey et al. 2008), e.g. via a seasonal dieback of microorganisms, may have released ammonium. When additionally the drought treatment was combined with warming plus elevated CO_2, no increase in ammonium concentration occurred, which may be because the drought treatment inhibited the microbial ammonification processes, as hypothesized based on earlier studies (Emmett et al. 2004; van Meeteren et al. 2008; Andresen et al. 2010). Furthermore, the decreased nitrification rate in response to warming (Table 2) suggests that less ammonium was lost through this pathway in warmed plots. In a mesocosm study of nitrogen availability at a grassland treated with warming, there were no effects on soil ammonium and nitrate concentrations, N leaching or plant N, and this was attributed to a concurrent drought (Verburg et al. 2009). Contrastingly, in a study at a Mediterranean shrubland, nitrate concentration increased and ammonium concentration decreased in response to warming in seasonal patterns (Sardans et al. 2008). Hence, the imposed environmental stress factors may affect soil inorganic nitrogen concentration through controls at many different steps in the cascade of processes during microbial decomposition, mineralization, nitrification and denitrification, and the rates are limited by water and substrate availability and by the timing with the growing season. At a temperate grassland, Hovenden et al. (2008a, b) showed reduced soil concentrations of inorganic nitrogen in elevated CO_2 plots, but this effect disappeared when combined with warming (Hovenden et al. 2008a). These findings contrast with results from our study, which however confirms the general idea that warming counteracts the effects of elevated CO_2 on nutrient availability.

The recovery of ^{15}N in soil microbes was one order of magnitude smaller than 1 year earlier in similar labelled adjacent plots (Andresen et al. 2009), yet the ratio of ^{15}N to ^{13}C enrichment in microbes

Table 1 Percentage (%) recovery of ^{15}N from glycine label added 1 year earlier, in mosses and in 0–15 cm soil depth for: dried soil, soil microbes and dissolved nitrogen

	A	D	T	TD	CO_2	DCO_2	TCO_2	$DTCO_2$	Proc mixed ANOVA
Mosses	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.4 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	D * T * CO_2
Soil	26.2 ± 3.1	29.0 ± 5.7	48.1 ± 6.8	31.2 ± 4.8	67.9 ± 28.6	68.4 ± 11.1	33.5 ± 7.9	42.7 ± 8.6	T * CO_2
Microbes	3.6 ± 0.6	3.7 ± 0.6	7.1 ± 1.1	4.4 ± 0.6	6.4 ± 1.3	9.8 ± 2.8	3.7 ± 1.6	3.9 ± 0.8	T * CO_2
Dissolv. N	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.8 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	T * CO_2 T * D

Significant effects of 2 years of climate change treatments for mean values over six replicates and standard error. The treatments are: no treatment (A), drought (D), warming (T), elevated CO_2 (CO_2) and their combinations TD, DCO_2, TCO_2 and $DTCO_2$. Significant ($P < 0.05$) effects by proc mixed ANOVA of main treatments are indicated with D, T and CO_2 and the interactions indicated as T * D, T * CO_2, D * CO_2 and T * D * CO_2

(Fig. 2) suggest that 65% of the glycine acquired 1 year earlier possibly remained as intact compounds in the microbes, however since this was not confirmed by compound specific analysis, other molecules could contain the acquired ^{15}N and ^{13}C (Jones et al. 2005). The microbial pool of carbon (Fig. 1c), the microbial enrichment with ^{13}C and ^{15}N, the recovery of ^{15}N label in microbes and the total soil ^{15}N were all larger in response to warming and CO_2, but non-additively (Figs. 2, 3c; Table 1). This suggests that treatments with warming and elevated CO_2 removed one or several limiting factors for soil microbial activity and growth. We suggest, that an important limiting factor for the microbes was ready available labile carbohydrates (Hartley et al. 2008), and when elevated CO_2 and warming were combined, a new limiting factor was introduced, possibly low availability of water. In parallel, Hu et al. (2001) observed increased microbial biomass (N and C) in response to elevated CO_2 over grassland but with changes by seasons (Hu et al. 2001). Furthermore, Ross et al. (2006) found more microbial biomass in response to 6 years of elevated CO_2 over a (fertilized) *Pinus*/*Nothofagus* plantation (Ross et al. 2006). The increased microbial carbon pool we found as short-term response to warming and elevated CO_2, may indicate an increased amount of ericoid mycorrhiza associated with *Calluna vulgaris* (Read and Perez-Moreno 2003; Treseder 2004). In parallel, in subarctic heaths, fungal biomarkers (ergosterol) increased in response to warming and to elevated CO_2 (Olsrud et al. 2004; Clemmensen et al. 2006), suggesting that increased photosynthesis may stimulate soil microbial biomass, mycorrhization and belowground carbon sequestration. Furthermore, assessed from molecular biomarkers, fungal abundance increased in a perennial C3–C4 grassland in response to elevated CO_2 (Chung et al. 2007). It has been suggested, that readily decomposable substrates (labile carbohydrates) are gradually depleted from soil through long-term warming, which consequently reduces the microbial responses to warming as the low molecular carbohydrates becomes a limiting factor (Hartley et al. 2008). The increased DOC production under elevated CO_2 found in this study (Table 2) and elsewhere (Lagomarsino et al. 2009), may suggest that the combination of warming with elevated CO_2 could counterbalance a DOC depletion of the soil. However, if warming and elevated CO_2 remove a carbohydrate limitation of soil microbes only when these treatments are applied alone but not in combination, the soil moisture regulated by a higher evapotranspiration (Bontti et al. 2009) becomes limiting for the soil microbes, when elevated temperature and CO_2 are combined.

The large release of NH_4^+ in the combined warming and CO_2 was a response pattern with additive effects of warming and elevated CO_2 were like we hypothesized, though no effect was observed of the factor alone. The combined treatment did not, however, lead to increased plant biomass or N-uptake which was not a supporting evidence of our hypothesis of enhanced nutrient demand by plants in warmed and elevated CO_2 treatments. This could be the consequence of a shift in timing of plant phenology in this treatment, as shown by Shaw et al. (2002), who found decreased grass NPP in response to elevated CO_2 (Shaw et al. 2002). However, as the N

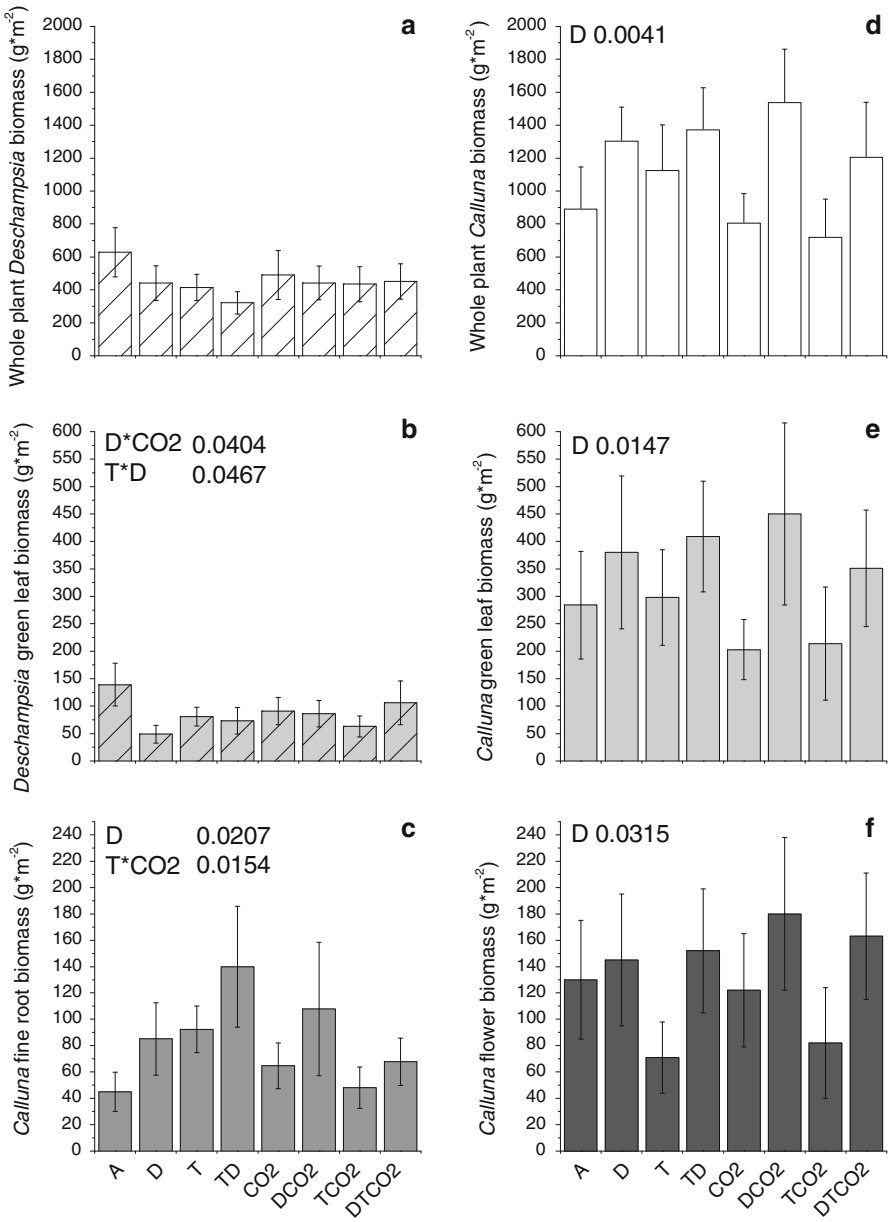

Fig. 4 Biomass (g m^{-2}) of **a** whole plant *Deschampsia* biomass, **b** *Deschampsia* leaf, **c** *Calluna* fine roots, **d** *Calluna* whole plant, **e** *Calluna* green leaf and **f** *Calluna* flowers from 20 × 20 cm plots. For further details, see Fig. 1

concentration decreased in fine roots of both *Deschampsia* and *Calluna* and in *Calluna* flowers (Fig. 6) in response to elevated CO_2, a growth dilution of nitrogen in the plant tissue was caused by a relatively larger carbon assimilation, as hypothesized (Taub and Wang 2008; Hyvönen et al. 2009).

Drought led to a strong increase in *Calluna* biomass and nitrogen pool (Figs. 4, 5a). The increased flower biomass in *Calluna* in response to drought (Fig. 4f) contrasts with previous observations of flowering responses in plants belonging to the *Ericaceae* family. In a Mediterranean shrubland, *Erica multiflora* was unaffected by drought (Prieto et al. 2008), and in cultivated *Vaccinium corymbosum*, drought reduced the number of flowers (Mingeau et al. 2001). Flowering of grasses in a temperate C4–C3 grassland was not affected by elevated CO_2, but was accelerated by warming, also when combined with elevated CO_2 (Hovenden et al. 2008b). The drought-increased *Calluna* biomass in our experiment was accompanied by a larger uptake of nitrogen in the last year, as shown by the enhanced recovery of

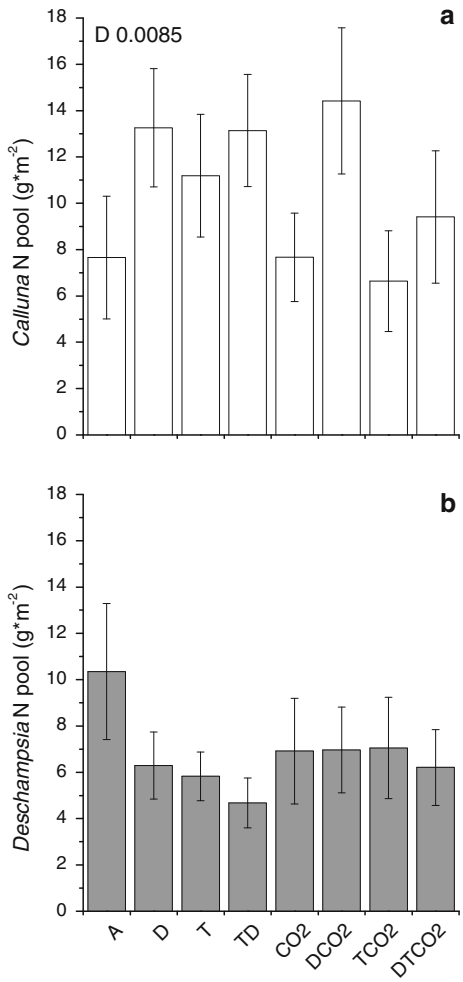

Fig. 5 Plant N pool (gN m^{-2}) in whole plants of **a** *Calluna* and **b** *Deschampsia.* For further details, see Fig. 1

added ^{15}N in *Calluna* (Fig. 3a). This ^{15}N recovery was six- to tenfold larger than 1 year earlier with respect to *Calluna*, but was similar or smaller for *Deschampsia* in other labelled adjacent plots (Andresen et al. 2009). Especially, in the treatment with combined drought and CO_2, *Calluna* biomass and ^{15}N recovery was increased (Figs. 3a, 4d). This suggests that *Calluna* benefits from both elevated CO_2, which increased growth, and from a drought inhibition of the production of exoenzymes by saprotrophs (Sardans et al. 2008; Ebersberger et al. 2003), easing the competition for nutrients in favour of *Calluna*. Furthermore, the drought treated plants from the incubations had a larger N to P ratio (Table 2), as was also found in situ for several drought and warming treated heathland species across Europe (Peñuelas et al. 2004). This indicates a larger plant uptake of nitrogen in drought plots, possibly combined with a lower mobility of phosphates (Blackwell et al. 2009; Butterly et al. 2009; Andresen et al. 2010). Due to the association with ericoid mycorrhizal fungi, *Calluna* is able to access nitrogen on organic form (Read and Perez-Moreno 2003; Andresen and Michelsen 2005), however, the smaller concentration of ammonium in drought treated plots (Fig. 1) supported that a sink, such as plant uptake of ammonium, was increased.

Previous investigations have shown that plant biomass and growth of dwarf shrubs (*Calluna* and *Erica*) was reduced (Gordon et al. 1999; Peñuelas et al. 2004) or non-responsive (Britton et al. 2003) in field scale drought manipulations, depending on the position of the ecosystem along an aridity gradient and on stand age (Berendse 1990). The diversity of the responses in different ecosystem types with feed-back control of primary production and release of nutrients suggest that the controlling parameters are site specific and not general across soil types and vegetation types, but may indeed be related to the dynamics and limiting factors of the organisms (Niklaus et al. 2007; Briones et al. 2009). We suggest that drought was beneficial for *Calluna* by inhibiting the competition from microbes for nutrients and when subject to elevated CO_2, *Calluna* was a strong competitor for the available nitrogen. However, this is a short-term response after only 2 years with removal of precipitation during summer. A further prolongation of this environmental stressor may reveal inter-annual variability and that water at some point becomes limiting for *Calluna* biomass production.

Conclusions

The concentrations of DON and ammonium responded strongly and in opposite directions in the combined warming and elevated CO_2 treatment, as a possible consequence of a rapid microbial mineralization, supporting our hypothesis of higher turnover and mineralization in warmed plots. The large, additive release of NH_4^+ in the combined warming and CO_2 corresponded to our hypothesis, although no effect was observed of the factors alone. The combined treatment did not, however, lead to

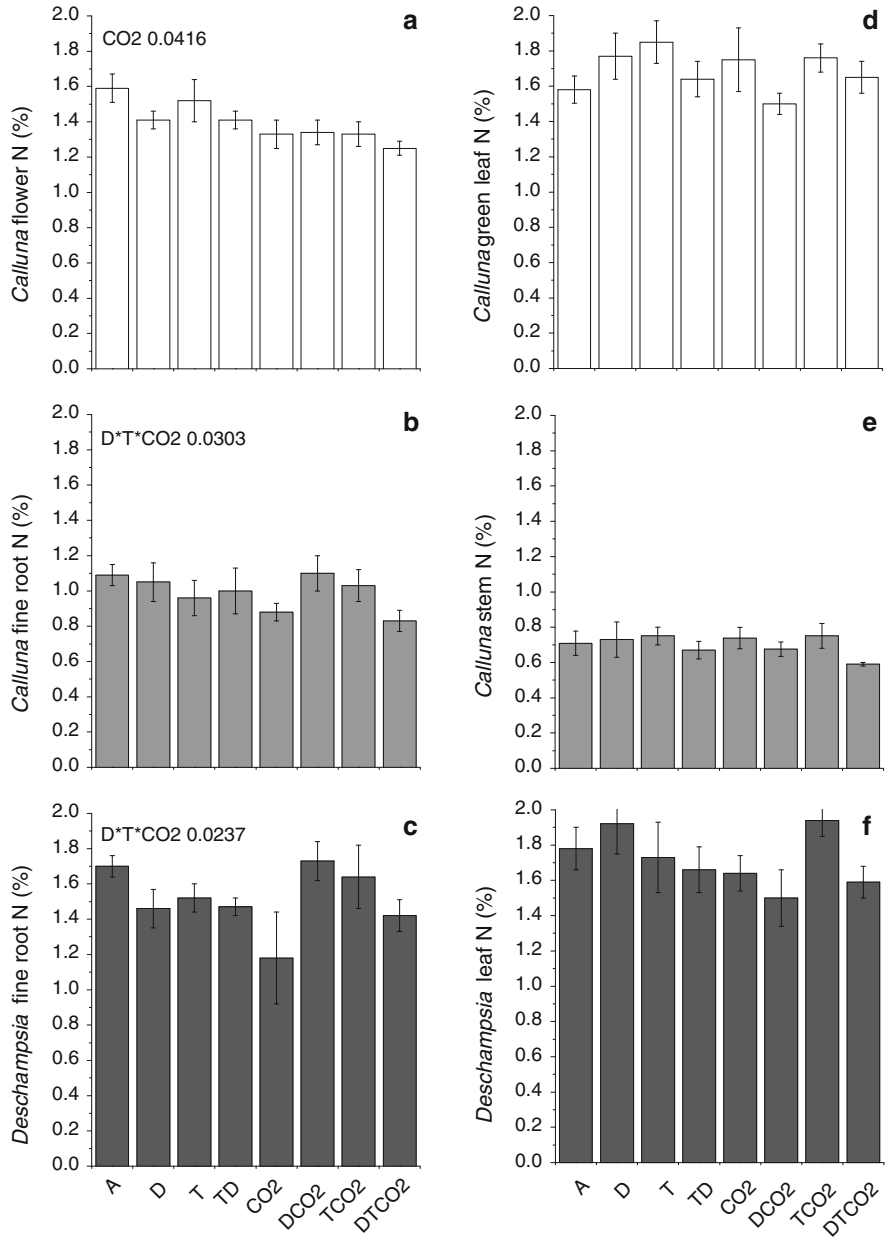

Fig. 6 Nitrogen concentration (%) in **a** *Calluna* flowers, **b** *Calluna* fine roots in 0–5 cm depth, **c** *Deschampsia* fine roots in 0–5 cm depth, **d** *Calluna* green leaf, **e** *Calluna* stem and **f** *Deschampsia* leaf. For further details, see Fig. 1

increased plant biomass or N-uptake, which did not support our hypothesis of enhanced nutrient demand by plants in warmed and elevated CO_2 treatments.

A smaller concentration of ammonium in drought treated plots suggested that a sink, such as plant uptake of ammonium, was increased. We suggest that drought promoted *Calluna* by periodically reducing competition from saprotrophic microbes for nutrients. When subject to drought and elevated CO_2, *Calluna* was a strong competitor for the available nitrogen. The recovery of ^{15}N in soil microbes was tenfold smaller than 1 year earlier in similar labelled adjacent plots, yet the ratio of ^{15}N to ^{13}C enrichment in microbes suggest that most of the carbon acquired as glycine 1 year earlier remained in the microbes. The microbial pool of carbon, the microbial enrichment with ^{13}C and ^{15}N, the recovery of ^{15}N label in microbes and the total

Table 2 Significant effects ($P < 0.05$, proc mixed) of treatments, soil type (species) and the interactions on: net nitrification rate (NO_3–N), net ammonification rate (NH_4–N), net production rate of DOC and phosphate net production rate (PO_4–P) in soil incubated with plants, through 1 year (November 2006 to November 2007)

Nitrification rate	T ↓	D ↑
Ammonification rate	ns	
DOC production rate	CO_2 ↑	*Deschampsia* < *Calluna*
PO_4–P production rate	ns	
Plant N:P	D ↑	*Deschampsia* < *Calluna*

soil ^{15}N were all larger in response to warming and CO_2, but non-additively.

The future climate change scenario with the full combination of warming, elevated CO_2 and drought periods did not lead to additive responses of single factors, or more factors. The ecosystem seemed to counteract the changes with an inbuilt capacity for buffering environmental stress factors, although the effects of individual treatments indicate that all treatments were stressors for the ecosystem.

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References

Andresen LC, Michelsen A (2005) Off-season uptake of nitrogen in temperate heath vegetation. Oecologia 144:585–597

Andresen LC, Michelsen A, Jonasson S, Beier C, Ambus P (2009) Glycine acquisition in temperate heath vegetation and soil microorganisms in influenced by elevated temperature, CO_2 and drought. Acta Ecol 313:283–295

Andresen LC, Michelsen A, Jonasson S, Schmidt IK, Mikkelsen TN, Ambus P, Beier C (2010) Plant nutrient mobilization in temperate heathland responds to elevated CO_2, temperature and drought. Plant Soil 328:381–396

Arnone J A III, Zaller JG, Spehn EM, Niklaus PA, Wells CE, Körner C (2000) Dynamics in root systems in native grasslands: effects of elevated atmospheric CO_2. New Phytol 147:73–85

Beier C, Emmett B, Gundersen P, Tietema A, Penuelas J, Estiare M, Gordon C, Gorissen A, Llorens L, Roda F, Williams D (2004) Novel approaches to study climate change effects on terrestrial ecosystems in the field: drought and passive night-time warming. Ecosystems 7:583–597

Berendse F (1990) Organic matter accumulation and nitrogen mineralization during secondary succession in heathland ecosystems. J Ecol 78:413–427

Biasi C, Rusalimova O, Meyer H, Kaiser C, Wanek W, Barsukov P, Junger H, Richter A (2005) Temperature dependent shift from labile to recalcitrant carbon sources of arctic heterotrophs. Rapid Commun Mass Spectrom 19:1401–1408

Blackwell MSA, Brookes PC, de la Fuente-Martinez N, Murray PJ, Snars KE, Williams JK, Haygarth PM (2009) Effects of soil drying and rate of re-wetting on concentrations and forms of phosphorus in leachate. Biol Fertil Soils 45:635–643

Bontti EE, Decant JP, Munson SM, Gathany MA, Przeslowska A, Haddix ML, Owens S, Burke IC, Parton WJ, Harmon ME (2009) Litter decomposition in grasslands of Central North America (US Great Plains). Glob Chang Biol 15:1356–1363

Briones MJI, Ostle NJ, McNamara NP, Poskitt J (2009) Functional shifts of grassland soil communities in response to soil warming. Soil Biol Biochem 41:315–322

Britton A, Marrs R, Pakeman R, Carey P (2003) The influence of soil-type, drought and nitrogen addition on interactions between *Calluna vulgaris* and *Deschampsia flexuosa*: implications for heathland regeneration. Plant Ecol 166:93–105

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:827–842

Buermann W, Lintner BR, Koven CD, Angert A, Pinzon JE, Tucker CJ, Fung IY (2007) The changing carbon cycle at Mauna Loa Observatory. PNAS Environ Sci 104: 4249–4254

Butterly CR, Bünemann EK, McNeil AM, Baldock JA, Marschner P (2009) Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biol Biochem 41:1406–1416

Chung H, Zak DR, Reich PB, Ellsworth DS (2007) Plant species richness, elevated CO_2, and atmospheric nitrogen deposition alter soil microbial community composition and function. Glob Chang Biol 13:1–10

Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, Aubinet M, Buchman N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grünwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437:529–533

Clemmensen KE, Michelsen A, Jonasson S, Shaver GR (2006) Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems. New Phytol 171:391–404

Danish Meteorological Institute (2008) Danish Meteorological Institute (Internet Communication)

Dijkstra FA, Pendall E, Mosier AR, King JY, Milchunas DG, Morgan JA (2008) Long-term enhancement of N availability and plant growth under elevated CO_2 in a semi-arid grassland. Funct Ecol 22:975–982

Ebersberger D, Niklaus PA, Kandeler E (2003) Long term CO_2 enrichment stimulates N-mineralization and enzyme

activities in calcareous grassland. Soil Biol Biochem 35:965–972

Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL (2000) Building roots in a changing environment: implications for root longevity. New Phytol 147:33–42

Emmett BA, Beier C, Estiarte M, Tietema A, Kristensen HL, Williams D, Peñuelas J, Schmidt I, Sowerby A (2004) The response of soil processes to climate change: results from manipulation studies of shrublands across an environmental gradient. Ecosystems 7:625–637

Eno CF (1960) Nitrate production in the field by incubating the soil in polyethylene bags. Soil Sci Soc Am J 24:277–279

Finzi AC, Moore CJP, DeLucia EH, Lichter J, Hofmockel KS, Jackson RB, Kim H-S, Matamala R, McCarthy HR, Oren R, Pippen JS, Schlesinger WH (2006) Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest. Ecology 87:15–25

Frey SD, Drijber R, Smith H, Melillo J (2008) Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biol Biochem 40: 2904–2907

Fry B (2006) Stable isotope ecology, 1st edn. Springer, New York

Giesler R, Högberg MN, Strobel BW, Richter A, Nordgren A, Högberg P (2007) Production of dissolved organic carbon and low-molecular weight organic acids in soil solution driven by recent tree photosynthate. Biogeochemistry 84:1–12

Gordon C, Woodin JS, Alexander IJ, Mullins CE (1999) Effects of increased temperature, drought and nitrogen supd temperature, drought and nitrogen supply in two upland perennials of contrasting functional type: *Calluna vulgaris* and *Pteridium aquilinum*. New Phytol 142:243–258

Gornall JL, Jónsdottir IS, Woodin JS, van der Wal R (2007) Arctic mosses govern below-ground environment and ecosystem processes. Oecologia 153:931–941

Graglia E, Julkunen-Titto R, Shaver GR, Schmidt IK, Jonasson S, Michelsen A (2001) Environmental control and intersite variations of phenolics in *Betula nana* in tundra ecosystems. New Phytol 151:227–236

Habekost M, Eisenhauer N, Scheu S, Steinbeiss S, Weigelt A, Gleixner G (2008) Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment. Soil Biol Biochem 40: 2588–2595

Hamer U, Marschner B (2002) Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. J Plant Nutr Soil Sci 165: 261–268

Handa T, Hagedorn F, Hättenschwiler S (2008) No stimulation in root production in response to 4 years of in situ CO_2 enrichment at the Swiss treeline. Funct Ecol 22:348–358

Hansen AH, Jonasson S, Michelsen A, Julkunun-Tiitto R (2006) Long-term experimental warming, shading and nutrient addition affect the concentration of phenolic compounds in deciduous and evergreen dwarf shrubs. Oecologia 147:1–11

Hartley IP, Hopkins DW, Garnett MH, Sommerkorn M, Wookey PA (2008) Soil microbial respiration in arctic soil does not acclimate to temperature. Ecol Lett 11: 1092–1100

Hovenden MJ, Newton PCD, Carran RA, Theobald P, Wills KE, Vander Schoor JK, Williams AL, Osanai Y (2008a) Warming prevents the elevated CO_2-induced reduction in available soil nitrogen in a temperate, perennial grassland. Glob Chang Biol 14:1018–1024

Hovenden MJ, Wills KE, Schoor JKV, Williams AL, Newton PCD (2008b) Flowering phenology in a species-rich temperate grassland is sensitive to warming but not to elevated CO_2. New Phytol 178:815–822

Hu S, Chapin F S III, Firestone MK, Field CB, Chiariello NR (2001) Nitrogen limitation of decomposition in a grassland under elevated CO_2. Nature 409:188–191

Hungate BA, Johnson DW, Dijkstra P, Hymus G, Stiling P, Megonigal JP, Pagel AL, Moan JL, Day F, Li J, Hinkle CR, Drake BG (2006) Nitrogen cycling during seven years of atmospheric CO_2 enrichment in a shrub oak woodland. Ecology 87:26–40

Hyvönen R, Ågren GI, Linder S, Persson T, Cotrufo F, Ekblad A, Freeman M, Grelle A, Janssens IA, Jarvis PG, Kellomäki S, Lindroth A, Loustau D, Lundmark T, Norby RJ, Oren R, Pilegaard K, Ryan MG, Sigurdsson BD, Strömgren M, van Oijen M, Wallin G (2009) The likely impact of elevated [CO_2], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. New Phytol 173:463–480

Ims RA, Henden J, Killengreen ST (2007) Collapsing population cycles. Trends Ecol Evol 23:79–86

Ings TC, Montoya JM, Bascompte J, Blüthgen N, Brown L, Dorman CF, Edwards F, Figueroa D, Jacob U, Jones JI, Lauridsen RB, Ledger ME, Lewis HM, Olesen JM, van Veen FJF, Warren PH, Woodward G (2009) Ecological networks—beyond food webs. J Anim Ecol 78:253–269

IPCC CWT (2007) IPCC, 2007: Climate change 2007: synthesis report. Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change. IPCC, Geneva, Switzerland, 104 pp

Iversen CM, Ledford J, Norby RJ (2008) CO_2 enrichment increases carbon and nitrogen input from fine roots in a deciduous forest. New Phytol 179:837–847

Jensen KD, Beier C, Michelsen A, Emmett BA (2003) Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions. Appl Soil Ecol 24:165–176

Joergensen RG, Mueller T (1996) The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{en} value. Soil Biol Biochem 28:33–37

Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A (2005) Dissolved organic nitrogen uptake by plants—an important N uptake pathway? Soil Biol Biochem 37:413–423

Lagomarsino A, De Angelis P, Moscatelli MC, Grego S (2009) The influence of temperature and labile C substrates on heterotrophic respiration in response to elevated CO_2 and nitrogen fertilization. Plant Soil 317:223–234

Liberloo M, Lukac M, Calfapietra C, Hoosbeek MR, Gielen B, Miglietta F, Scarascia-Mugnozza GE, Ceulemans R (2009) Coppicing shifts CO_2 stimulation of poplar productivity to above-ground pools: a synthesis of leaf to stand level results from the POP/EUROFACE experiment. New Phytol 182:331–346

Luo Y, Hui D, Zhang D (2006) Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems. A meta-analysis. Ecology 87:53–63

Mauney JR, Kimball BA, Pinter P J Jr, LaMorte RL, Lewin KF, Nagy J, Hendrey GR (1994) Growth and yield of cotton in response to a free-air carbon dioxide enrichment (FACE) environment. Agric For Meteorol 70:49–67

Mikkelsen TN, Beier C, Jonasson S, Holmstrup M, Schmidt IK, Ambus P, Pilegaard K, Michelsen A, Albert K, Andresen LC, Arndal MF, Bruun N, Christensen S, Danbæk S, Gundersen P, Jørgensen P, Linden LG, Kongstad J, Maraldo K, Priemé A, Riis-Nielsen T, Ro-Poulsen H, Stevnbak K, Selsted MB, Sørensen P, Larsen KS, Carter MS, Ibrom A, Martinussen T, Miglietta F, Sverdrup H (2008) Experimental design of multifactor climate change experiments with elevated CO_2, warming and drought: the CLIMAITE project. Funct Ecol 22:185–195

Mingeau M, Perrier C, Améglio T (2001) Evidence of drought-sensitive periods from flowering to maturity on highbush blueberry. Sci Hortic 89:23–40

Nielsen PL, Andresen LC, Michelsen A, Schmidt IK, Kongstad J (2009) Seasonal variations and effects of nutrient applications on N and P and microbial biomass under two temperate heathland plants. Appl Soil Ecol 42:279–287

Niklaus PA, Körner C (2004) Synthesis of a six-year study of calcareous grassland responses to in situ CO_2 enrichment. Ecol Monogr 74:491–511

Niklaus PA, Alphei J, Kampichler C, Kandeler E, Körner C, Tscherko D, Wohlfender M (2007) Interactive effects of plant species diversity and elevated CO_2 on soil biota and nutrient cycling. Ecology 88:3153–3163

Olsrud M, Melillo JM, Christensen TR, Michelsen A, Wallander H, Olsson PA (2004) Response of ericoid mycorrhizal colonization and functioning to global change factors. New Phytol 162:459–469

Osler GHR, Sommerkorn M (2007) Toward a complete soil C and N: incorporating the soil fauna. Ecology 88: 1611–1621

Ostle N, Briones MJI, Ineson P, Cole L, Staddon P, Sleep D (2007) Isotopic detection of recent photosynthate carbon flow into grassland rhizosphere fauna. Soil Biol Biochem 39:768–777

Peñuelas J, Gordon C, Llorens L, Nielsen T, Tietema A, Beier C, Bruna P, Emmett B, Estiarte M, Gorissen T, Williams D (2004) Non-intrusive field experiments show different plant responses to warming and drought among sites, seasons and species in a North-South European gradient. Ecosystems 7:598–612

Pregitzer KS, Burton AJ, King JS, Zak DR (2008) Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3. New Phytol 180:153–161

Prieto P, Peñuelas J, Ogaya R, Estiarte M (2008) Precipitation-dependent flowering of *Globularia alypum* and *Erica multiflora* in Mediterranean shrubland under experimental drought and warming, and its inter-annual variability. Ann Bot 102:275–285

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance? New Phytol 157:457–492

Ross DJ, Grayston SJ, Whitehead D (2006) Changes in soil carbon and nitrogen properties and microbial communities in relation to growth of *Pinus radiata* and *Nothofagus fusca* trees after 6 years at ambient and elevated atmospheric CO_2. Glob Chang Biol 12:1690–1706

Runion GB, Curl EA, Rogers HH, Backman PA, Rodríguez-Kábana R, Helms BE (1994) Effects of free-air CO_2 enrichment on microbial populations in the rhizosphere and phyllosphere of cotton. Agric For Meteorol 70: 117–130

Sætersdal M, Birks HJB (1997) A comparative ecological study of Norwegian mountain plants in relation to possible future climatic change. J Biogeogr 24:127–152

Sardans J, Peñuelas J, Estiare M (2008) Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Appl Soil Ecol 39:223–235

SAS Institute Inc. (2003) The SAS system for windows (Computer Program)

Schmidt IK, Tietema A, Williams D, Gundersen P, Beier C, Emmett BA, Estiarte M (2004) Soil solution chemistry and element fluxes in three European heathlands and their responses to warming and drought. Ecosystems 7: 638–649

Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, Field CB (2002) Grassland responses to global environmental changes suppressed by elevated CO_2. Science 298:1987–1990

Sokolov AP, Kicklighter DW, Melillo JM, Felzer BS, Schlosser AC, Cronin TW (2008) Consequences of considering carbon-nitrogen interactions on the feedbacks between climate and the terrestrial carbon cycle. J Clim 21:3776–3796

Sowerby A, Emmett BA, Tietema A, Beier C (2008) Contrasting effects of repeated summer drought on soil carbon efflux in hydric and mesic heathland soils. Glob Chang Biol 14:2388–2404

Taub DR, Wang X (2008) Why are nitrogen concentrations in plant tissues lower under elevated CO_2? A critical examination of the hypotheses. J Integr Plant Biol 50: 1365–1374

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorous, and atmospheric CO_2 in field studies. New Phytol 164:347–355

van Meeteren MJM, Tietema A, van Loon EE, Verstraten JM (2008) Microbial dynamics and litter decomposition under a changed climate in a Dutch heathland. Appl Soil Ecol 38:119–127

Verburg PS, Johnson DW, Schorran DE, Wallace LL, Luo Y, Arnone J A III (2009) Impacts of an anomalously warm year on soil nitrogen availability in experimentally manipulated intact tallgrass prairie ecosystems. Glob Chang Biol 15:888–900

Volder A, Gifford RM, Evans JR (2007) Effects of elevated atmospheric CO_2, cutting frequency, and differential day/night atmospheric warming on root growth and turnover of *Phalaris* swards. Glob Chang Biol 13:1040–1052

Willis KJ, Kleczkowski A, New M, Wittaker RJ (2007) Testing the impact of climate variability on European plant diversity: 320000 years of water-energy dynamics and its

long-term influence on plant taxonomic richness. Ecol Lett 10:673–679

Woodward FI, Osborne CP (2000) The representation of root processes in models addressing the responses of vegetation to global change. New Phytol 147:223–232

Zak DR, Pregitzer KS, King JS, Holmes WE (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytol 147:201–222

Zavalloni C, Gielen B, De Boeck HJ, Lemmens MHM, Ceulemans R, Nijs I (2009) Greater impact of extreme drought on photosynthesis of grasslands exposed to a warmer climate in spite of acclimation. Physiol Plant 136:57–72