Ecosystem-atmosphere exchange of carbon in a heathland under future climatic conditions

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Risø-PhD-Report

Merete Bang Selsted
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Risø DTU
National Laboratory for Sustainable Energy

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Abstract (max. 2000 char.):
Global change is a reality. Atmospheric CO_2 levels are rising as well as mean global temperature and precipitation patterns are changing. These three environmental factors have separately and in combination effect on ecosystem processes. Terrestrial ecosystems hold large amounts of carbon, why understanding plant and soil responses to such changes are necessary, as ecosystems potentially can ameliorate or accelerate global change. To predict the feedback of ecosystems to the atmospheric CO_2 concentrations experiments imitating global change effects are therefore an important tool.
This work on ecosystem-atmosphere exchange of carbon in a heathland under future climatic conditions, shows that extended summer drought in combination with elevated temperature will ensure permanent dryer soil conditions, which decreases carbon turnover, while elevated atmospheric CO_2 concentrations will increase carbon turnover. In the full future climate scenario, carbon turnover is over all expected to increase and the heathland to become a source of atmospheric CO_2.

The methodology of static chamber CO_2 flux measurements and applying the technology in a FACE (free air CO_2 enrichment) facility is a challenge. Fluxes of CO_2 from soil to atmosphere depend on a physical equilibrium between those two medias, why it is important to keep the CO_2 gradient between soil and atmosphere unchanged during measurement. Uptake to plants via photosynthesis depends on a physiological process, which depends strongly on the atmospheric CO_2 concentration. Photosynthesis and respiration run in parallel during measurements of net ecosystem exchange, and these measurements should therefore be performed with care to both the atmospheric CO_2 concentration and the CO_2 soil-atmosphere gradient.

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Preface

This thesis was written for the CLIMAITE project, supported by the Villum Kann Rasmussen Foundation. The work has been carried out at the Ecosystem Programme, Biosystems Division at Risø National Laboratory for Sustainable Energy, Danish Technical University, DTU by supervision of professor Per Ambus. As a PhD-student I have been enrolled at the Department of Biology, University of Copenhagen with supervision from associate professor Anders Michelsen.

My CLIMAITE adventure started in autumn 2005 and concludes now, summer 2010, by present thesis.
The CLIMAITE project is, I believe, the best possible setting for a PhD-student. From the beginning we have been a group of PhD-students, which it has been fantastic to be a part of, both socially; we have spend much time together including good food and fun, but also it has been invaluable to discuss and collaborate with peers. Kristine, Jane, Marie, Louise, Karen and Kristian thanks for being the best PhD-network one could ask for. The project involves several Danish research groups with highly skilled scientist. During meetings it has been extremely inspiring at moments when the synergistic effect of bringing researchers together has evolved. Claus Beier, our project leader has been running the project strictly – which I have learned is needed as scientists have no limits for bringing up suggestions and new ideas. However, the atmosphere has always been pleasant and one feels welcome and respected.

Risø has been the daily safe base for my work and could not have been situated anywhere better, arriving here in the morning lights up one's mind. During the day, the good mood is held up by staff at ECO: Technicians, researchers and students always make the Ecosystem Programme such a friendly and inspirational place to work. It would be nice to be able to bring this special Risø spirit with me as I move on. A special thanks goes to Poul Sørensen, Liselotte Meltofte, Anja Nielsen, Bente Andersen, and Nina Wiese Thomsen for invaluable help at Brandbjerg and in the lab, and to Klaus Steenberg Larsen for sharing your enthusiasm for chamber measurements with me. Andreas Ibrom has invested much time for my benefit, playing around with my data and creating models. It has at times been frustrating trying to follow your thoughts, but it has also been exciting and fun work, which has broadened my horizon.

During my study I have had two supervisors, Anders Michelsen and Per Ambus. As my internal supervisor far away in Copenhagen, Anders has been most helpful when I have needed it. I want to thank you for your qualified help and for making me feel welcome at your office in the Botanical Garden. A very big thank you goes to Per. I do not know how you succeeded, but I believe you have managed through the whole process to guide me in the right directions and to make me focus on what was important. Thanks, you are a good supervisor.

I am grateful that Claus Beier and Per Ambus agreed on giving me the PhD-scholarship.

Merete Bang Selsted
July 2010

Contents

1. Introduction

Global change started by elevation in atmospheric CO_2 levels, which have increased from 280 ppm before the industrialisation in the 18th century to present 380 ppm. The development is not linear and models predict that the concentration will rise up to 700 ppm within this century (IPPC 2007). Rising mean global temperature is a consequence and has already increased by ~0.8 ^{o}C during the past century and is modeled to further increase 1.4-5.8 ^{o}C by the end of this century (IPCC 2001). Higher temperatures are projected to cause a change in precipitation patterns besides leading to more unpredictable extreme weather events such as flooding and drought (IPCC 2001). The Danish scenario for the future climate as predicted by the Danish Meteorological Institute is a rise in mean temperature comparable with the mean global temperature rise. Furthermore, precipitation will change towards longer drought periods during the growth season, more heavy rain during autumn and increased amounts of rain during winter (DMI, 2010).

Environmental changes, such as accumulation of atmospheric CO_2 and climate change, are expected to affect the structure and function of terrestrial ecosystems (IPCC 2007). Understanding plant and soil response to such changes is necessary, because ecosystems are invaluable to human and can potentially ameliorate or accelerate the global change (Foley et al. 2003).

1.1 Elevated temperature

Temperature naturally changes by season and thereby differentially stimulates several ecosystem processes such as photosynthesis, root and microbial activity (Wan et al., 2007), and start of growing season (Cleland et al., 2006). Elevated temperatures will directly impact and promote soil processes, primary production and early flowering, as well as extend the period of active plant growth, but will also as an indirect effect further raise respiration due to higher carbon turnover (Wan et al., 2007). Since both the primary production and respiration processes are stimulated by higher temperatures, it is difficult to predict the overall effect on carbon balance, and presumably the accumulated effect differs between ecosystems.

1.2 Changed water regimes

Changes in precipitation go in the direction of extremes; drought and heavy rain falls. For the Danish climate, the summer drought will have a higher impact on ecosystems than the increased rain in the colder months. In shrublands, droughts will be responsible for restriction

of growth and plant survival (Llorens et al., 2004) and soil water contents will permanently decrease (Sowerby et al., 2008). Also factors of lower root activity (Borken *et al.,* 2006) followed by reduced microbial activity (Jensen et al., 2003) and soil fauna activity (Maraldo *et al.,* 2009) will be of importance for ecosystem functioning. Ecosystem C-balances may be more susceptible to changes in primary productivity compared to respiratory losses. During the 2003 hot and dry climatic extreme in Europe, the persistent respiratory CO_2 losses outbalanced at least four years of net uptake due to decreased productivity (Ciais et al., 2005; Arnone III et al., 2008).

While elevated temperature alone promotes ecosystem processes, extended summer drought in combination with elevated temperature have the potential to lead to even dryer soil conditions than the drought treatment alone (Wan et al., 2007). This synergistic effect will result in further depressing the ecosystem processes.

1.3 Elevated atmospheric CO_2

Carbon dioxide is the substrate for photosynthesis and elevated atmospheric levels are therefore expected to increase carbon uptake of plants. Experiments at the ecosystem level (Fredden et al., 1995) and at leaf level (Jackson et al., 1995) have shown that elevated atmospheric CO_2 immediately increases short-term photosynthetic rates in grasslands, but after acclimatisation to the elevated environment, photosynthesis down regulates to a lower level, though still with higher photosynthetic rates than at ambient CO_2. The extra amount of assimilated carbon does, however, not necessarily result in enhanced biomass (Luo et al., 1997). Measurements have shown that carbon turnover increases when grasslands are exposed to higher amounts of atmospheric CO_2 (Ross et al., 1996; Niklaus et al., 2004 Xi et al., 2005; Baronti et al., 2008).

Due to easy access of CO_2, plant leaves may reduce their stomatal conductance in elevated CO_2 environments (Ainsworth et al., 2004; Long et al., 2004) resulting in an enhanced water use efficiency, again leading to higher soil water contents (Garten et al., 2007; Leuzinger and Körner, 2010). As an example, Lou et al. (2008) report that prolonged summer drought treatments decreased both photosynthesis and ecosystem respiration in a Danish heathland, while in combination with elevated CO_2, photosynthesis increased and the drought effect on respiration was mitigated. Improved water use efficiency will be of significant importance to ecosystem processes, especially during drought events (Morgan et al., 2001; Pendall et al., 2003).

In order to predict the fate of ecosystems in future climates, it is thus important to gain insight into the combined effects of key climate change factors.

1.4 Aims and outline of PhD-work

The overall aim of this PhD-work was to examine the effect of global change (elevated temperature, extended summer drought and elevated atmospheric CO_2 concentrations) on carbon balance in a Danish heathland with focus on annual and seasonal patterns. This was expected to be performed by two different approaches: 1) Direct measurements of CO_2 exchange between atmosphere and ecosystem and 2) a survey based on ^{13}C pulse labeling for assessing where to newly assimilated carbon were allocated in the ecosystem.

During the work period, the focus was, however, shifted. Ecosystem atmosphere CO_2 fluxes proved to be very difficult to measure under elevated CO_2 due to the method (FACE) of elevating the atmospheric concentration. Besides evaluating global changes effects on carbon balance, lots of work was therefore put into developing and analysing the methodology of static chamber flux measurements in an elevated CO_2 atmosphere.

2. Background

2.1 CLIMAITE

CLIMAte change effects in biological processes in Terrestrial Ecosystems. The aim of the CLIMAITE project is to investigate how elevated temperature, changes in precipitation and elevated atmospheric CO_2 concentrations in combination affect biological processes and the functioning of natural ecosystems.

Figure 1. Patch of heater surrounded by grasses (photo by Poul T Sørensen)

2.2 Brandbjerg, the experimental site

Brandbjeg is the experimental site, which provides the basis for research conducted within the CLIMAITE project. Brandbjerg is a hilly nutrient poor sandy deposit in a heathland situated in northern Zealand, Denmark (55°53´ N, 11°58´ E). Vegetation on the site is dominated by the dwarf shrub *Calluna Vulgaris* and the annual grass *Deschampsia flexuosa*, each covering about 30 and 70% of the surface (fig. 1). The annual average precipitation is about 600 mm and the annual average temperature is 10 °C.

2.3 Experimental setup

The experiment is a full factorial design of three manipulative factors; elevated temperature (T), extended summer drought (D) and elevated atmospheric CO_2 (CO2). The untreated control is labeled A. The experiment thus holds eight treatment combinations (A, T, D, TD, CO2, TCO2, DCO2 and TDCO2). The treatments are arranged in blocks, where each block is split into two octagons, one of which is exposed to elevated CO_2. As each octagon is split in four

plots, all eight treatment combinations exist within every block (see fig. 2). Each block is 6.8 m in diameter, leaving 9.1 m^2 per plot. The experiment holds six replicates, resulting in a total of 48 plots distributed in six blocks.

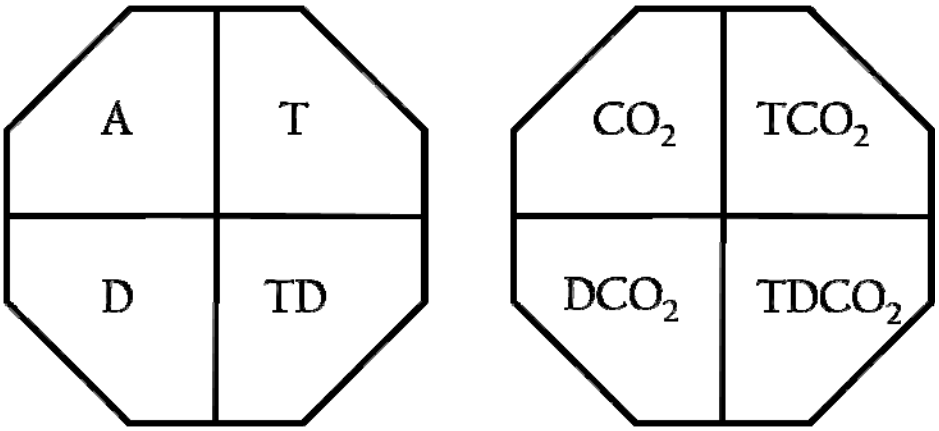

Figure 2. Schematic overview of one block. Two octagons holds all eight treatments, one of the octagons is exposed to elevated CO_2.

2.3.1 Elevated temperature

To elevate temperature, an approach for hindering emission of infrared radiation during the night was applied. The treatment is referred to as passive night time warming and is implemented by white reflective curtains 50 centimeters above ground that cover the ecosystem from sunset until sunrise. In case of dewfall, rain and strong wind, the curtains are programmed to retract.

2.3.2 Extended summer drought

During summer an extended drought is applied for about one month. Rain is prevented to access the drought treated plots by curtains that run over the plots, when precipitation sensors detect rain. The curtains are mounted on a slant, and the collected water is drained out of the experimental area.

2.3.3 Elevated CO_2

The aim of the CO_2 treatment is to elevate current level of atmospheric CO_2 to 510 ppm. This is done by a FACE (Free Air Carbon dioxide Enrichment) system, where CO_2 is injected along the perimeter of the octagonas via injection tubes situated 40 cm above ground. Enrichment with CO_2 is only activated during daylight hours, i.e. from 30 minutes after sunrise until 30

minutes before sunset. Moreover, CO_2 is only released on the upwind site of the octagon. The target CO_2 concentration is monitored in the centre of the seven m diameter experimental plots, and along with current wind conditions, this value controls the FACE CO_2 dosing system.

2.3.4 Automated meteorological monitoring

To continuously follow treatment effects of physical conditions, temperature - and TDR probes are installed in all experimental plots to continuously measure soil and air temperature as well as soil moisture. Temperature is measured in 2 and 5 cm soil depths and 20 cm above ground every hour. TDR probes are situated at 0–20 cm and 0–60 cm depths and collect soil moisture values every 30 minutes. Photosynthetic radiation (PAR) and temperature in 2 m height is measured every second at two stations at the experimental site. Likewise, two rain sensors, two dewfall sensors and two wind speed sensors are installed.

In other words, the experimental site at Brandbjerg is high-tech and ready for field work. Furthermore, it displays itself beautifully (see fig. 3).

Figure 3. The Climaite experimental site displaying both heat and drought curtains (photo by Kim Pilegaard)

3. Ecosystem-atmosphere CO_2 flux measurements

3.1 Methodology of flux measurements

The closed chamber technique is in theory an easy-to-apply method for measuring gas fluxes – and of interest here, of cause fluxes of CO_2. However, it is not possible to do chamber measurements without considering how to obtain the true flux. Paper I briefly discusses the actual measurement, how to apply the chamber and the importance of recording the CO_2 chamber concentration right after enclosure.

CO_2 flux measurements in combination with FACE are an additional challenge. From measurements we know that CO_2 concentrations within the FACE plots vary significantly. The target concentration, 510 ppm, is maintained in the centre of the CLIMAITE octagons. But as described earlier, the CO_2 is only dosed in the upwind direction, implying that the CO_2 concentration is higher in upwind plots compared to the opposite plots in the downwind direction (fig. 4).

Figure 4. Measurements of atmospheric CO_2 concentration above the chamber flux bases in two octagons(FACE and non-FACE), morning June 5, 2006. Measurements were made in 0-60 cm height above ground.

According to Ficks law of diffusion:

$$J = -D\frac{\partial[CO2]}{\partial z} \quad (1)$$

The diffusion flux (*J*) is described by a diffusion coefficient (*D*), the soil depth ∂z and the difference in CO_2 concentration between to compartments, e.g., soil and atmosphere. It is therefore of importance that the starting concentrations resemble the system we want to describe, or at least that the CO_2 concentration difference between the compartments is the same as during treatment. Our first assumption was that a good approach for making starting atmospheric CO_2 levels stable and uniform was to turn off the FACE system, let the soil and atmosphere equilibrate by degassing to ambient level and then measure the flux, expecting that the soil - as the atmosphere - was elevated by 130 ppm (510ppm - 380 ppm). Investigating the soil CO_2 concentrations we concluded, however, that the CO_2 treatment gave rise to higher soil activity, as we did not only measure a soil CO_2 increase of only 130 ppm, but up to 500 ppm (paper I, fig. 4). This observed higher soil activity in CO2 plots could be a result of three factors: Higher root biomass, higher microbial activity and/or more allocation of substrate from roots to soil. We know that root biomass in the CLIMAITE CO2 plots are approximately 10 % higher than plots not treated with elevated CO_2 (Arndal 2010, personal communication). A higher root biomass also indicates higher allocation of substrate from roots to soil and thereby higher levels of microbial biomass. However, from analysis of microbial C, we found that microbial biomass does not depend on treatment, see fig. 5. This finding is supported by Andresen et al. (2009), who also performed her experiments at the CLIMAITE study site. The higher soil activity must therefore first of all be a result of higher root biomass. We show in paper I that soil respiration in CO2 plots drops to ambient level after exposure to ambient CO_2 levels for several hours, indicating a down regulation of root activity in CO2 plots (argued for below).

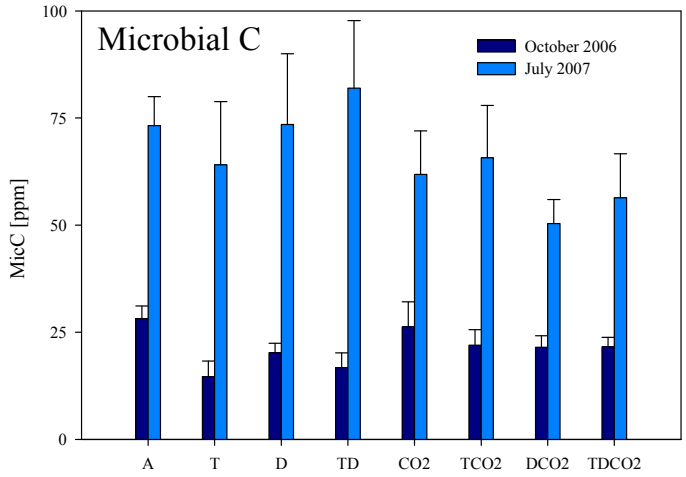

Figure 5. Microbial C measured in October 2006 and July 2007, no significant treatment effects were found.

Concerning soil respiration measurements in FACE plots, we conclude in paper I that measurement after a degassing period of 10 minutes results in valid fluxes, as the higher biological activity caused by the elevated CO_2 concentration is kept for up to 18 hours.

The measurements are further complicated by bringing in aboveground vegetation into the system. The flux between soil and atmosphere is a physical diffusion of CO_2 from a high soil CO_2 concentration to a lower atmospheric concentration, whereas photosynthesis is an active physiological process. It is well documented that plants grown in an elevated CO_2 atmosphere down regulate their photosynthetic activity (Fredden et al., 1995; Jackson et al., 1995; Moore et al., 1999), which means photosynthesis measurements in FACE plots at ambient CO_2 most likely result in fluxes lower than those obtained from the corresponding non-FACE plots. To get a correction factor for this, detailed studies on photosynthetic response to initial CO_2 concentration on the leaf level could be performed at each of the treatments including elevated CO_2 (CO2, TCO2, DCO2 and TDCO2), optimally with seasonal variation included.
The above mentioned issues are further discussed in paper I. We did not find a perfect solution for performing closed chamber CO_2 flux measurements, but we concluded that the consequences of performing the measurements at ambient CO_2 in FACE plots are many, and that they can be divided in two - biological and physical. We believe that this is a very important issue, which needs attention as FACE experiments becomes more frequent.

3.2 Calculating CO_2 fluxes obtained from closed chambers

When chamber measurements are performed and data collected in a satisfactory way, focus shifts towards calculating the flux rate. Much literature discuss and argue for the use of non-linear regression, when determining CO_2 fluxes obtained from closed chambers (Hutchinson et al., 2000; Kutzbach et al., 2007). The argument for application of non-linear regression to linear regression is the immediate disturbance of the CO_2 gradients that were in effect prior to chamber deployment. In case of net uptake of CO_2 (when photosynthesis exceeds ecosystem respiration), the chamber CO_2 concentration will decrease and the ecosystem-atmosphere CO_2 gradient increase. Consequently this instantly lead to recordings of reduced CO_2 uptake as soil respiration will increase according to Ficks law (eq. 1). The initial flux rate after chamber deployment is therefore important to capture, in attemp to calculate the true flux. Net ecosystem exchange was measured in a control plot midday 14th of April 2008. By use of linear regression, the flux was -5.20 µmol m^{-2} s^{-1}, compared to -6.80 µmol m^{-2} s^{-1} using the initial rate when applying non-linear regression (fig. 6). In this case, linear regression underestimates the flux by 24 % compared to the -6.80 µmol m^{-2} s^{-1}, which is considered the more correct flux.

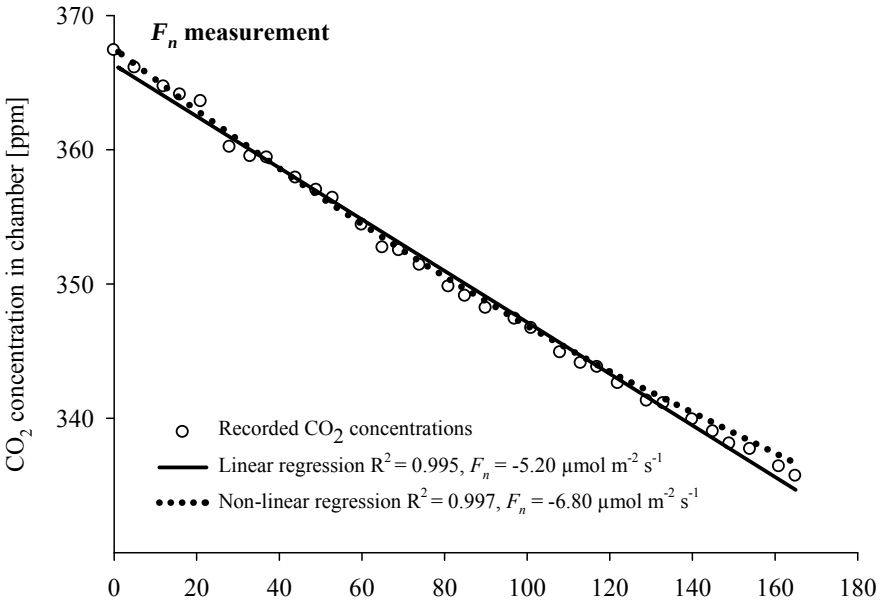

Figure 6. Chamber recordings of CO_2. Net ecosystem exchange, F_n, Text in graphs shows regression coefficients of the regression curves pictured, and the corresponding calculated flux.

Ecosystem respiration rates are obtained by shutting off any photosynthetic activity by applying an opaque chamber (PAR = 0). Opposite to photosynthesis measurements, respiration will - as

discussed in paper I - be correctly measured as long as the gradient between ecosystem and atmosphere is the same, and therefore we accept a linear development of CO_2 in the flux chamber during measurement. However, when darkening the ecosystem by the chamber, the photosynthetic activity is not turned off instantly, as the plant uses already produced ATP and NADPH to fixate CO_2 (Peracy., 1990). It therefore takes a while, dependent on the ATP/NADPH productivity rate in light, before the CO_2 exchange is pure respiration. Midday measurements the 14th of April 2008 (fig. 7) show a typical course of chamber CO_2 development in the darkened chamber. Initially the photosynthesis activity is visible active, as the CO_2 concentration is decreasing. After c. 20 seconds the chamber CO_2 concentration starts to increase (R_E = 0.45 µmol m^{-2} s^{-1}), however, photosynthesis might still be active for at least another 70 seconds, as the highest respiration rate is calculated after that time (R_E = 2.36 µmol m^{-2} s^{-1}).

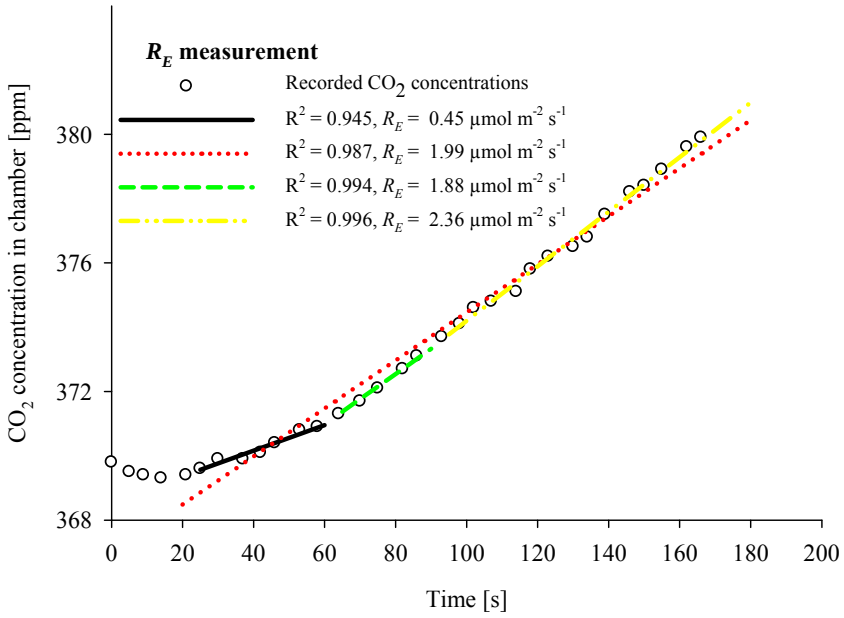

Figure 7. Chamber recordings of CO_2. Ecosystem respiration, R_E, Text in graphs shows regression coefficients of the regression curves pictured and the corresponding calculated flux.

Kutzbach et al. (2007) argues that the regression coefficient is not a valid value for examining whether the calculated flux is correct. This observation is also demonstrated here and visible from fig. 6 and 7 comparing the R^2 values. Analysing the net ecosystem fluxes obtained for present carbon balance study was met with non-linear regression, while ecosystem respiration fluxes were calculated by use of linear regression.

4. Ecosystem-atmosphere exchange of carbon in a heathland under future climatic conditions

4.1 Results from the ecosystem atmosphere carbon flux studies

The aim of the CO_2 flux measurements was to obtain detailed knowledge on seasonal variation and to determine to which extent the climatic and atmospheric manipulations affect the carbon balance of the system. Three main parameters were measured: Net ecosystem exchange of CO_2 (F_n), ecosystem respiration (R_E) and soil respiration (R_S). From these parameters photosynthesis (P_g) were calculated (R_E- F_n). For extrapolating a multiple regression model describing soil respiration were developed (paper II), and simple extrapolations of ecosystem fluxes were made (paper III), both to get a picture of the yearly budgets of C-balance and the seasonal variation.

Methods for gas flux measurements, data analysis and model development are described in detail in paper II and III. Fig 8, 9 and 10 displays the setup for soil respiration and net ecosystem exchange measurements, respectively.

Figure 8. Bases for flux measurements. The large frame for ecosystem fluxes, small collar without aboveground vegetation for soil respiration fluxes.

Figure 9. Soil respiration was recorded by the Li-Cor 6400.

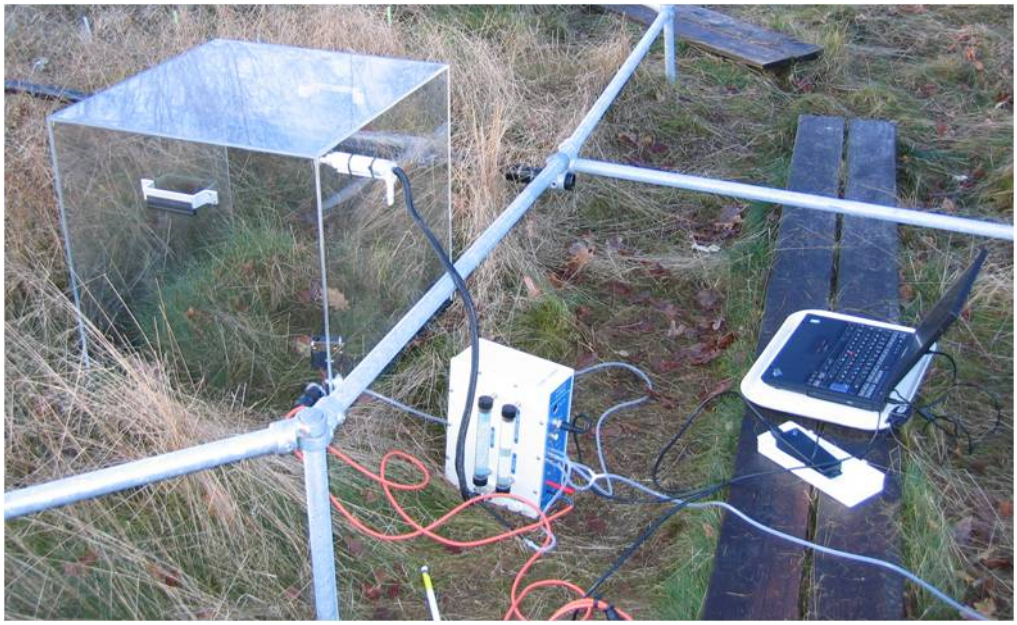

Figure 10. The ecosystem flux chamber connected to a CIRAS DC 10 measuring net ecosystem exchange of CO_2.

4.1.1 Soil respiration in response to global change

Main conclusion from paper II, is an anticipated increase in annual soil respiration by 15 % as a consequence of changes in atmospheric and climatic conditions. Though with the reservation that long-term changes of soil conditions may occur due to repeated prolonged droughts which potentially would reduce the 15 % increase in soil respiration. More over we developed an empirical soil respiration model that describes current soil respiration with soil temperature, soil moisture and substrate supply as important drivers, underlining that soil respiration depends on several climatic factors.

Elevated atmospheric CO_2 did have the highest impact on soil respiration, namely a rise of to up to 40 % increase. In itself elevated temperature did not have any effect on soil respiration, but in combination with drought a synergistic effect showed 3 timers further decrease in soil respiration than drought caused alone. Fig. 11 shows the average of all field measurements, visualising the significant effects of elevated CO_2, drought and the interaction between elevated temperature and drought.

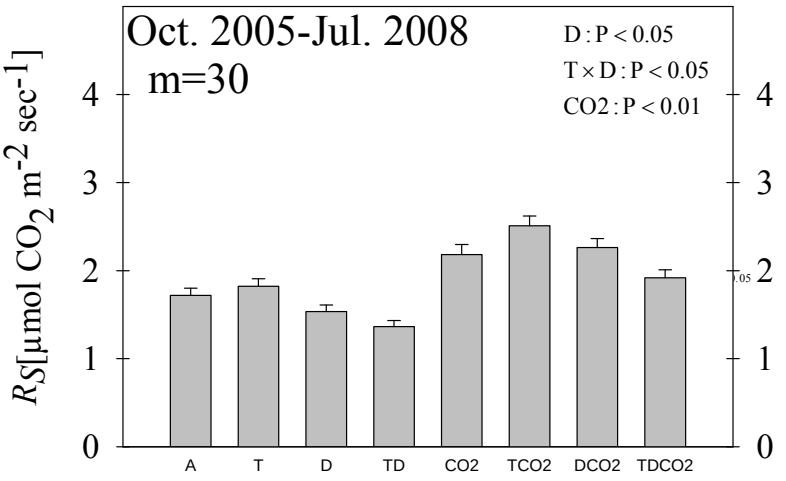

Figure 11. Average of all soil respiration measurements.

By the synergistic effect of elevated temperature and drought on soil respiration this study additionally confirms the importance of performing multifactor experiments when aiming at predicting global change effects on ecosystem processes.

4.1.2 Ecosystem carbon exchange in response to global change

By linear extrapolation of measured fluxes to yearly sums the ambient control ecosystems showed a net loss of carbon during 2006, and while elevated temperature caused a further loss, the drought treatment resulted in a net uptake of carbon. The interaction of elevated temperature and drought showed additive effect on net carbon balance, ecosystem respiration and photosynthesis (paper III, table 1), opposite to what was found in the soil respiration study, where the temperature increased the drought effect.

Elevated CO_2 generally increased ecosystem respiration by 10 %, while the effect on net carbon balance ends up as with a net loss of carbon from the system. Presumably measurements of photosynthesis were underestimated by 10 %, however this underestimation in not enough to counterbalance the increased respiration. In conclusion we anticipate a minor net loss of carbon from Danish heathlands in the projected future climatic conditions.

The effect of elevated CO_2 on respiration is significant ($P<0.05$), while the CO_2 effect on photosynthesis only becomes significant ($P<0.05$) if the underestimation by method of measuring is included. Fig. 12 shows the average midday measurements (photosynthesis is calculated form net ecosystem exchange and respiration measurements), the underestimation of photosynthesis is not included in the figure.

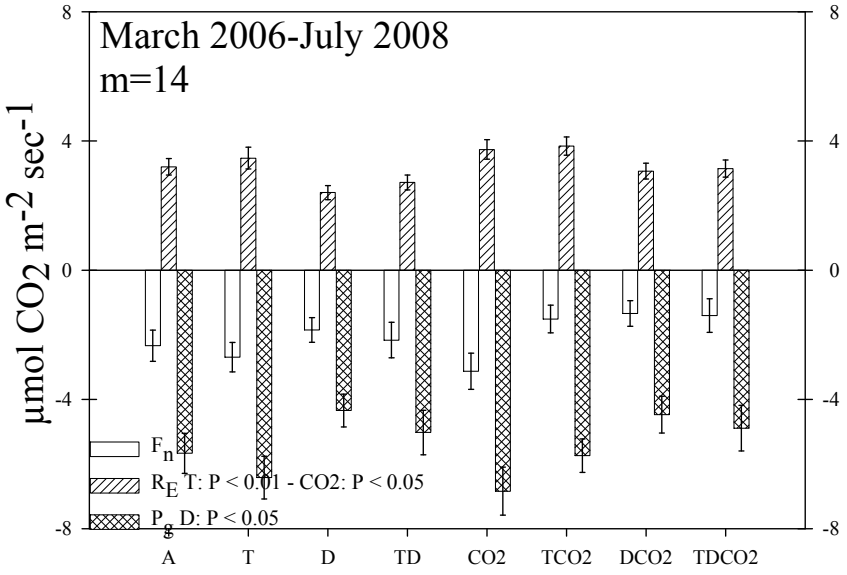

Figure 12 . Average of daytime ecosystem flux measurements. F_n = net ecosystem exchange, R_E = ecosystem respiration, P_g = photosynthesis.

4.2 Results from ^{13}C labeling study

At the end of this thesis a $^{13}CO_2$ pulse labeling study is presented. Mesocosms from the CLIMAITE study site were exposed to $^{13}CO_2$ during 6 hours, with the aim of examining where the ^{13}C taken up via photosynthesis were allocated during the next seven days particularly with respect to treatment. The experiment was performed two times; October 2006 and July 2007.

The two experiments showed different results. In October plants from the CO_2 treatment assimilated 33 % more carbon pr m^2 via photosynthesis than the non CO_2 treated plants and total respiration were in the CO_2 treatment increased by 27 %. However, relatively less newly assimilated carbon was lost via respiration by CO_2 treated plants compared to the ambient controls, indicating that elevated atmospheric CO_2 do increase carbon turnover of older soil carbon. In July respiration was decreased by 22 % as a consequence of the CO_2 treatment, while allocation of carbon though the ecosystem did not reveal any treatment effects.

5. Conclusions and perspectives

The results from the work that comprise this thesis end up in the following main conclusions for a Danish heathland year 2075 compared to current levels:

- Extended summer drought in combination with elevated temperature will ensure permanent dryer soil conditions, which decreases soil carbon turnover
- Elevated atmospheric CO_2 concentrations will increase ecosystem carbon turnover
- In the full climate scenario, carbon turnover is over all expected to increase and the heathland to become a source of atmospheric CO_2

These conclusions are based on short term effects on carbon fluxes of the projected future Danish climate. Differences in response time of different ecosystem processes could influence the carbon balance and therefore long term studies are needed to confirm or reformulate the above mentioned conclusions. Flux measurements do continue at the CLIMAITE study site, and they will contribute to a extended description of the carbon balance and budget at the site.

Along with the attempt to describe the carbon balance, emphasis should also be on the methodology. Static chamber CO_2 flux measurements in combination with the FACE facility should be further examined and developed in order to obtain reliable fluxes either by direct measurements or by subsequent correction. To further discuss is also the reasonability of bringing e.g. mesocosms from a FACE facility to the lab expecting the treatment effects to persist: Rates of photosynthesis and soil respiration strongly depend on current atmospheric CO_2 levels and thereby other ecosystem processes.

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Paper I

Measurement of carbon dioxide fluxes in a free-air carbon dioxide enrichment experiment using the closed flux chamber technique

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Measurement of carbon dioxide fluxes in a free−air carbon dioxide enrichment experiment using the closed flux chamber technique

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Abstract

Carbon dioxide (CO_2) fluxes, composing net ecosystem exchange (NEE), ecosystem respiration (ER), and soil respiration (SR) were measured in a temperate heathland exposed to elevated CO_2 by the FACE (free air carbon enrichment) technique, raising the atmospheric CO_2 concentration from c. 380 µmol mol^{-1} to 510 µmol mol^{-1}. All CO_2 fluxes were measured by the static chamber methodology. Although the FACE technique enriches the atmosphere with CO_2 to a fixed level, the above ground CO_2 concentrations may nevertheless locally vary strongly (from about ambient to ~1000 µmol mol^{-1}). Deployment of static chambers to FACE experiments should therefore be performed with great care in order to ensure reproducible conditions with respect to chamber headspace CO_2 concentration. We demonstrate that that the fluxes measured by closed chambers relate linearly to the initial headspace CO_2 concentration. When changing the initial headspace CO_2 concentration from 380 to 510 µmol mol^{-1} the net CO_2 assimilation expressed by NEE increased instantaneously 1.51 times in control plots and 1.71 times in FACE plots. By contrast, ER in control plots decreased, being 0.87 times that measured at the low CO_2 concentration, and the flux also decreased in FACE plots, to 0.79 times that at low concentration. Similar SR in control plots was decreased 0.94 times in control plots and 0.88 times in FACE plots. We found that a useful method to achieve stable and reproducible chamber headspace and soil CO_2 concentration prior to commencement of flux measurements was to turn off the FACE system at least 10 minutes in advance. Within 10 minutes a new equilibrium was established between the soil and atmosphere, apparently due to CO_2 degassing from the top soil. The observed increase in SR in response to increased CO_2 persisted for up to 18 hrs during which measurements should be performed. Soil CO_2 concentrations were increased by up to 500 µmol mol^{-1} by the FACE treatment, substantially more than the 130 µmol mol^{-1} enrichment achieved in the atmosphere suggesting that the increased SR flux was caused by increased belowground respiration.

Keywords: FACE, CO_2, net ecosystem exchange, ecosystem respiration, soil respiration.

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1. Introduction

The increasing atmospheric carbon dioxide (CO_2) concentration has the potential to alter plant photosynthetic activity, with significant consequences for ecosystem turnover and storage of carbon. A growing number of investigations have, consequently, addressed the impacts of atmospheric CO_2 concentrations on biosphere−atmosphere exchange of CO_2 in combination with manipulative experiments at the square−meter scale by application of open−top chambers (Zak et al., 1993) or FACE (free air CO_2 enrichment) technique (Miglietta, 1997). Under these experimental scales, the closed chamber methodology is commonly applied to measure the CO_2 exchange and determine soil respiration (SR), ecosystem respiration (ER) and net ecosystem exchange (NEE).

Many challenges are faced when applying closed chambers for flux measurements and a considerable amount of literature is available on that particular subject (Hutchinson and Livingston, 1993; Healy et al., 1996; Conen et al., 2000; Hutchinson and Livingston, 2001; Davidson et al., 2002; Pumpanen et al., 2004). In the context of soil CO_2 effluxes, the concentration gradient across the soil−atmosphere boundary is an important driver for the CO_2 diffusion into the atmosphere. In order to achieve an accurate flux rate it is of crucial importance that the concentration gradient remains undisturbed during application of chamber enclosures. Davidson et al. (2002) reviewed several studies in which closed non−steady state chambers of a height of 10−20 cm underestimate soil CO_2 fluxes by up to 15%. A simplified estimate of soil CO_2 emissions can be given by Fick's first law of diffusion:

$$J = -D\frac{d[CO_2]}{dz} \qquad (1)$$

Where J is the flux given in μmol m^{-2} s^{-1}, D is the diffusion coefficient, in m^{-2} s^{-1}, dependent on physical soil conditions, z [m] is the soil depth, and $[CO_2]$ is the CO_2 concentration in μmol m^{-3}.

Equation (1) can be expressed as:

$$J = -K([\mathrm{CO2}]\mathrm{atm} - [\mathrm{CO2}]\mathrm{soil}) \qquad (2)$$

Here K is a constant containing information on the diffusion coefficient and soil depth, and $[CO_2]_{atm}$ and $[CO_2]_{soil}$ denote the concentrations in atmosphere and soil gas, respectively. From eq (2) it follows that a change in $[CO_2]_{atm}$ will affect the flux rate (J) of CO_2 across the air−soil boundary. Hence, if the CO_2 concentration in the flux chamber during measurement increases due to net

respiration and the soil concentration is unchanged because the production in the soil is unaffected, the flux measured will be lower than the true flux outside the chamber since the diffusion of CO_2 will slow off in order to compensate the increased chamber CO_2 concentration (Conen and Smith, 2000; Davidson et al., 2002). This suggests that short measuring times that maintain ambient or near−ambient CO_2 concentrations inside flux chambers should avoid biased results.

In analogy, it is of great importance that the initial chamber headspace concentration resembles the outside concentrations when the chamber is deployed. Closed chamber measurements in experiments with elevated atmospheric CO_2 treatments should thus be deployed with particular care and attention to experimental CO_2 concentration dynamics. Specifically, under conditions where the CO_2 fumigation is applied discontinuously, e.g. when CO_2 is not applied during night time, diurnal alterations in the soil and atmosphere CO_2 concentrations occur that may bias chamber derived flux measurements. Although numerous studies report on CO_2 fluxes in elevated CO_2 environments (e.g. Pendall et al., 2001; King et al., 2004; Xu et al., 2006; Bernhardt et al., 2006; Wan et al., 2007), to our knowledge only one study reports on the methodological concerns associated with the use of closed chambers. Nakayama et al. (1994) addressed the issue of initial chamber CO_2 concentrations when performing flux measurements. They found that increased initial chamber CO_2 concentrations resulted in significantly lower flux rates than rates obtained under ambient CO_2.

The objective of this study was to investigate and clarify the experimental conditions needed to achieve unbiased measurements of SR, ER and NEE by application of the closed chamber technique to a FACE experiment in a low vegetation heathland.

2. Material and methods

2.1. Experimental site

The experiment was conducted at the CLIMAITE study site situated at 55°53'N 11°58'E, northern Zealand, Denmark (Mikkelsen et al., 2008). The ecosystem is a temperate heathland on a hilly, nutrient poor, sandy deposit, with a 5 cm organic layer with a pH of c. 5. Vegetation height is 40 – 60 cm and is dominated by the perennial shrub (*Calluna vulgaris*) and annual grass (*Deschampsia flexuosa*). Exposure to elevated CO_2 is achieved by the FACE technique where CO_2 is injected along the perimeter of octagonal plots via injection tubes situated c. 50 cm above ground. The CO_2 target concentration is 510 μmol mol^{-1}, which is monitored in the centre of the 7 meter diameter experimental plots (Mikkelsen et al., 2008). The FACE system has been in operation since October 2005. Enrichment with CO_2 is activated only during daylight hours, i.e. from 30 mins after sunrise

until 30 mins before sunset. Measurements have shown that monthly average CO_2 concentrations in FACE plots are relatively uniform, 500 – 520 μmol mol^{-1}. However, the CO_2 dosing is characterised by a high degree of short−term variability driven by wind speed and direction that may lead to marked concentration fluctuations, in particular within 50 – 70 cm distance from the injection tubes (Mikkelsen et al., 2008). Moreover, CO_2 is only released on the upwind site of the plot, and depending on the distance of the chamber collar relative to the CO_2 release tubes and current wind direction, fluctuations in CO_2 concentrations above the collars may occur. This will lead to differences in initial chamber CO_2 concentrations among chambers and measurements, and make the measurements non−replicable. Therefore precautions need to be taken before mounting the chambers.

2.2. Measurement techniques

For measurements of net ecosystem CO_2 exchange (NEE) and ecosystem respiration (ER) we used a cubic 60·60·60 cm Plexiglas chamber that could be mounted gas tight on 60·60·10 cm stainless steel collars placed permanently in the experimental plots. Proper mixing of air inside the chamber was ensured by a fan mounted to the chamber wall. Concentrations of CO_2 inside the flux chamber were recorded by an infrared gas analyser (IRGA) (CIRAS DC 10, PP Systems, Amesbury, Massachusetts, USA). Temperature and light intensities inside the chamber was measured by a TRP−1 Temperature/Light (PAR) Probe (PP Systems). For ER measurements the chamber was covered by opaque black Beaver Nylon in order to exclude all sunlight. Between each measurement the chamber was vented thoroughly to replace the chamber air and avoid heating of the chamber during measurement series. Typically, the rate of chamber CO_2 concentration change was achieved by linear regression analysis based on c. 40 observations at 5 seconds intervals. Soil respiration (SR) measurements were performed by a Portable Gas Exchange and Fluorescence System combined with a soil CO_2 flux chamber (LI−6400, LICOR Biosciences, Lincoln, Nebraska, USA). Each measurement was applied to a 10 cm diameter vegetation free area confined by a PVC collar permanently placed inside the stainless steel collar.

2.3. Spatial variation within FACE octagons

Preliminary measurements indicated that CO_2 concentrations at 0−60 cm above ground may vary considerably within the FACE octagons. On one occasion the CO_2 concentration averaged 851 ± 93 μmol mol^{-1} (mean ± SE, n=4) (measured in 20 cm increments) above a collar situated in the upwind

direction, which was almost twice as much as the concentration observed above the opposite downwind collar (462 ± 51 μmol mol^{-1}). Therefore it was decided to turn off the CO_2 dosing system at least 30 minutes prior to measurements were initiated, unless otherwise stated, in order to achieve consistent and reproducible CO_2 concentrations between the different plots.

2.4. Impact of initial chamber CO_2 concentrations on CO_2 fluxes

The effect of initial chamber CO_2 concentration on the CO_2 flux were investigated by recording the NEE, ER, and SR fluxes at different initial chamber CO_2 concentrations ranging from ambient to about 1000 μmol mol^{-1}. NEE and ER fluxes at different initial chamber CO_2 concentrations were studied by injecting varying amounts of concentrated CO_2 gas into the 216 L cubic chamber immediately upon mounting the chamber on the collar. The LI−COR 6400 was set to measure SR at different initial chamber CO_2 concentrations. The system either scrubs out CO_2 from the chamber or waits for the selected concentration to build up via ongoing respiration from the enclosed soil before the flux is recorded (www.licor.com). However, instead of waiting for the CO_2 concentration to build up only via soil respiration, CO_2 was injected into the respiration chamber when initial concentrations above 400 μmol mol^{-1} were required. Measurements were carried out at two campaigns in May 2006. For all flux measurements, the chamber was applied successively to the same collar with careful venting prior to each enclosure event. Measurements were made in one control plot and one FACE plot. Average soil water content down to 20 cm depth was c. 0.12 m^3 m^{-3} and differed by less than 0.02 m^3 m^{-3} between the two plots, and soil temperature in 5 cm depth was c. 12°C and differed less than 0.1 °C. Soil texture and vegetation cover was judged to be similar in the two plots.

Fluxes are reported in μmol m^{-2} s^{-1}, a positive sign indicating effluxes of CO_2 and negative sign assimilation of CO_2 by the ecosystem.

2.5. Changes in soil respiration CO_2 fluxes in response to discontinued CO_2 fumigation

Short−term SR dynamics in response to discontinued CO_2 fumigation was investigated at two different temporal resolutions. Intense, short time campaigns of c. 1 hr duration were performed on May 23, 2008 and again on December 1, 2009. SR was recorded every 5 minutes, alternating between a control plot and a FACE plot. In the May 2008 campaign one plot of each treatment was included; in the December 2009 campaign three replicate plots were included. Secondly, a whole−day campaign was initiated at 8:00 (4 hours after sunrise) on May 22, 2007, lasting for 23

hours. SR was recorded every three hours in three replicate FACE plots and three control plots. The FACE system was turned off immediately before the SR measurements were initiated. On all dates differences in soil moisture and soil temperature between control and FACE plots were negligible: May 22 2007: Soil water content down to 20 cm depth was 0.14 $m^3 m^{-3}$ ±0.02 in control plots and 0.12 $m^3 m^{-3}$ ±0.03 in FACE plots, and soil temperature at 5 cm depth was 11.9 °C ±0.6 in control plots and 12.0 °C ±0.4 in FACE plots. May 23 2008: Soil water content down to 20 cm depths was 0.07 $m^3 m^{-3}$ ±0.01 in control plots and 0.06 $m^3 m^{-3}$ ±0.02 in FACE plots, soil temperature at 5 cm depth was 12.5 °C ±1.3 in control plots and 12.2 °C ±1.3 in FACE plots. December 1 2009: Soil water content down to 20 cm depths was 19.2 $m^3 m^{-3}$ ±2.2 in control plots and 18.2 $m^3 m^{-3}$ ±2.1 in FACE plots, soil temperature at 5 cm depth was 6.7 °C ±0.4 in control plots and 6.8 °C ±0.4 in FACE plots.

2.6. Soil CO_2 and $\delta^{13}C-CO_2$ profiles

Sets of five soil gas probes were installed in three control plots and three FACE plots in February 2007. The probes were made of 6.4 mm outer diameter brass tubes varying in lengths from 18.5 to 63.5 cm. The 0−5 cm tip of the probe was perforated by side holes to facilitate gas exchange with soil gas. An extractable rod was inserted into the tube during installation to prevent clogging of the probe lumen with soil. Upon installation the top of the probe was sealed gas tight with at silicone membrane. For sampling a 20 ml sample was extracted from each probe and flushed through a crimp sealed 1.8 ml vial. The vials were left over−pressurised with c. 1 ml sample until analysis of CO_2 concentrations and $\delta^{13}CO_2$ by GC−TCD (Hewlett−Packard 6890) in continuous flow mode with a Preparation Concentration unit (PreCon, Thermo Scientific, Germany) and stable isotope ratio mass spectrometer (Finnigan Delta PLUS, Thermo Scientific, Germany). The soil CO_2 profiles were examined in early season (March) and mid season (June) in 2007.

2.7. Statistical analyses

Statistical analyses were carried out using SAS 9.1 (SAS Institute Inc., 2003). The procedure was to first analyse the overall effect of initial chamber CO_2 concentration for each treatment, control and FACE by a one−way repeated measurements ANOVA, the repeated proc glm procedure (SAS Institute Inc., 2003). Secondly, effect of treatment or period of time by treatment were analysed by a one−way ANOVA, the proc glm procedure (SAS Institute Inc., 2003).

3. Results

3.1. Impact of initial chamber CO_2 concentrations on CO_2 fluxes

All measured fluxes responded linear to initial chamber CO_2 concentrations (Fig. 1). The net CO_2 assimilation, NEE (Fig. 1(NEE)) increased linearly with the initial CO_2 concentration in control (R^2= 93%, $P < 0.001$), as well as in FACE plots (R^2 = 81%, $P < 0.05$). There was no significant difference between NEE flux responses to initial chamber CO_2 concentrations between the FACE plot and the control plot, $P > 0.1$ (Fig. 1(NEE)). The CO_2 efflux by ER was significantly depressed by increasing the initial chamber CO_2 concentration (Fig. 1(ER)) both in control (R^2= 54%; $P < 0.01$) and FACE plots (R^2 = 81%, $P < 0.01$). A suppressing effect of CO_2 treatment on ER was found, $P < 0.05$ (Fig. 1(ER)). Gross ecosystem photosynthesis (GEP), as calculated from NEE and ER regressions by subtracting the two values (NEE−ER), showed that control plots assimilated more carbon than FACE plots at same initial chamber CO_2 concentrations (Fig. 1(GEP)). However, the latter were more sensitive to the initial CO_2 concentration as the regression line had a 10% steeper slope than the control. The response of SR to increasing initial CO_2 (Fig. 1(SR)) was significant for both the control (R^2= 37%, $P < 0.01$) and the FACE plots (R^2 = 96%, $P < 0.001$). A strong effect of CO_2 treatment on SR was found, $P < 0.001$. In contrast to ER, SR is influenced positively by the CO_2 treatment as SR flux rates were lower in ambient plots compared to FACE plots. The response to initial CO_2 concentrations also differed between the two treatments, indicating a respiration rate up to 4 times higher in the FACE plot compared to the control plot. Table 1 shows relative and absolute changes (calculated from regression parametres) of the individual fluxes when initial chamber CO_2 concentrations are altered from 380 μmol mol^{-1} to 510 μmol mol^{-1}. The highest impact on change in initial concentration was found for NEE measurements in FACE plots (1.7), whereas SR in control plots was only changed by a factor of 0.94 when CO_2 concentration changes from ambient to 510 μmol mol^{-1}. In absolute values, the CO_2 losses from ER and SR in control plots were reduced by 1.07 and 0.14 μmol m^{-2} s^{-1} respectively compared with a change in NEE that gives rise to an increased CO_2 assimilation of 3.51 μmol m^{-2} s^{-1}.

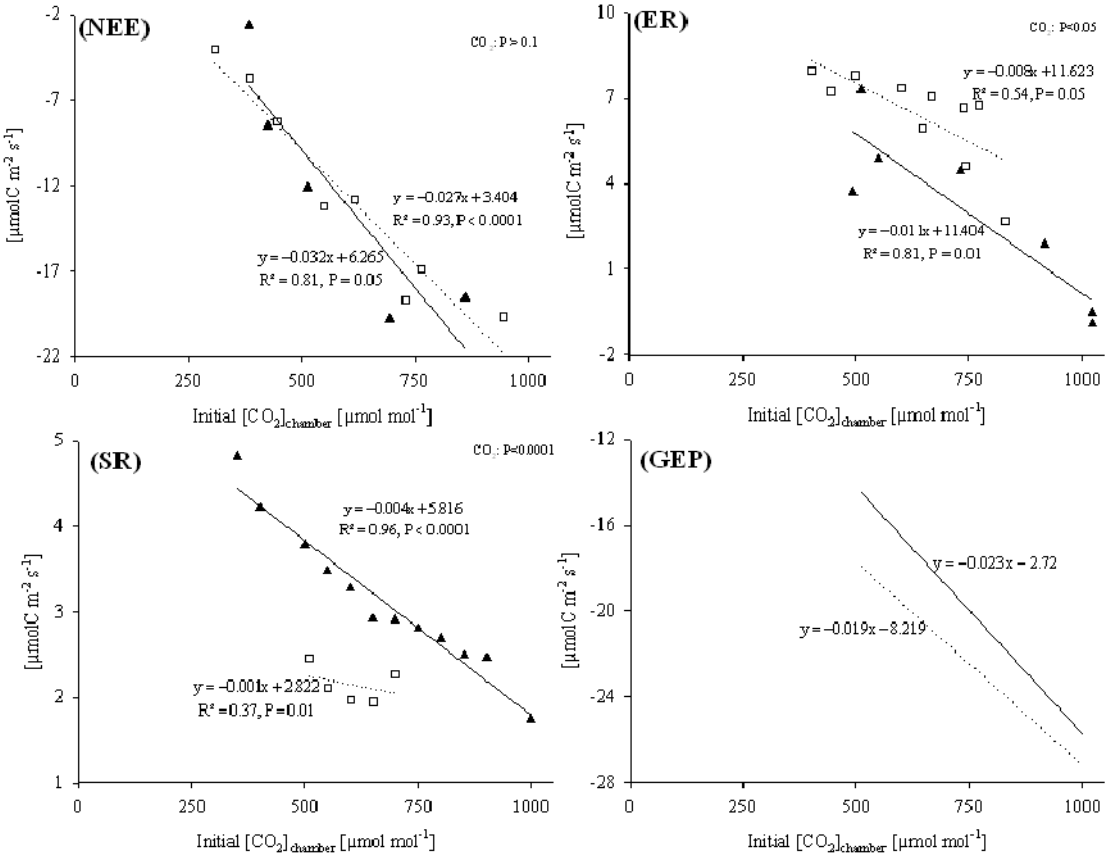

Figure 1. NEE, ER, and SR measured with different initial chamber CO_2 concentrations. Open squares indicate measurements from a control plot and closed triangles indicate measurements from FACE plot. FACE system was turned off prior to measurements. GEP is calculated on basis of NEE and ER regressions, dotted line is the control, straight line the FACE experiment.

Table 1. Relative and absolute changes in measured net ecosystem exchange (NEE), ecosystem respiration (ER), and soil respiration (SR) and calculated gross ecosystem photosynthesis (GEP) fluxes in response to increasing the initial chamber CO_2 concentration from 380 ppm (ambient) to 510 ppm. Changes are given for both control and FACE plots. Please note that NEE has negative sign when the ecosystem gains carbon and correspondingly that respiration rates are positive.

Treatment	NEE	ER	GEP	SR
		Relative change		
Control	1.51	0.87	1.16	0.94
FACE	1.71	0.79	1.26	0.88
		Absolute change [μmol CO_2 m^{-2} s^{-1}]		
Control	-3.51	-1.07	-2.47	-0.14
FACE	-4.16	-1.47	-2.99	-0.52

3.2. Changes in soil respiration CO_2 fluxes in response to discontinued CO_2 fumigation

SR fluxes decreased immediately upon turning off the CO_2 fumigation and appeared to decrease asymptotically for up to 10 min, after which the decline in activity levelled out (Fig. 2), while in contrast, SR was relatively stable in the control plot during the 60 minutes campaign. The measuring period was analysed by dividing it into three time intervals, 0−10 min, 11−40 min, and 41−60 min after onset of measurements, and normalised flux means were grouped by Tukey's test. In the control plot no difference in normalised SR was found between the three intervals, whereas in the FACE plot SR in the initial 0−10 min period exceeded that in the subsequent periods ($P < 0.05$), confirming the asymptotical course.

Over the course of 23 hrs the SR showed a distinct diurnal pattern with peak activity during the afternoon both in control and FACE plots (Fig. 3). SR in FACE plots tended to exceed SR in the control plots from the start of the campaign at 8 am and the following 18 hrs (>01:00 hr) after which SR in the FACE plots and the control plots converged. The first 18 hours of measurements tends to differ with respect to treatment (Repeated Measures ANOVA, $P = 0.056$) while at the last period no treatment effect were found ($P = 0.99$).

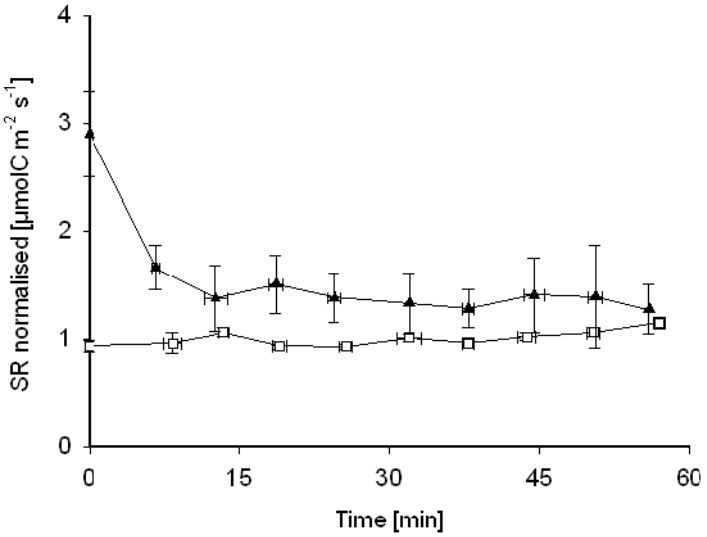

Figure 2. Soil respiration (SR) measured at 5 min intervals for 60 min in May 2008 and December 2009. Open squares indicate measurements from control plots (n=4) and closed triangles indicate measurements from FACE plots (n=4). Measurements are normalised against the average SR flux observed in the control plot for each pair of FACE−control plots. CO_2 fumigation was turned off at time 0 in the FACE plots. Initial concentration of CO_2 in SR chamber reflected present ambient level at the site (~380 µmol mol^{-1}).

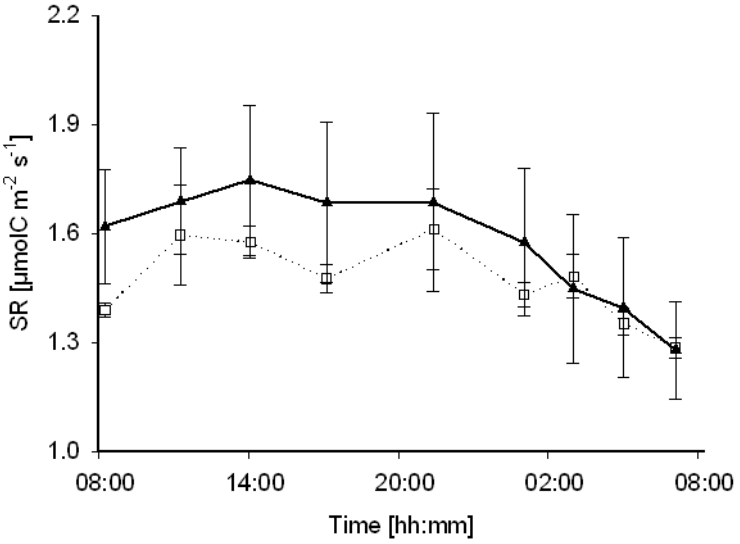

Figure 3. Soil respiration (SR) measured at three hours intervals for 23 hrs in May 2007. Open squares indicate measurements from control plots and closed triangles indicate measurements from FACE plots, mean ± SE, n=3. CO_2 fumigation was turned off at time 0 in the elevated CO_2 plots. Initial concentration of CO_2 in SR chamber reflected present ambient level at the site (~380 µmol mol^{-1}).

3.3. Soil CO_2 and $\delta^{13}C-CO_2$ profiles

On March 27, soil CO_2 concentrations in control and FACE plots, respectively, ranged between 1100 to 1187 μmol mol^{-1}, and 1398 to 1624 μmol mol^{-1} (Fig. 4). On June 20, soil CO_2 concentrations were substantially higher ranging in control plots between 2150 to 2657 μmol mol^{-1}, and in FACE plots between 2622 to 3816 μmol mol^{-1}. On both dates a significant effect of the CO_2 treatment on average soil CO_2 concentration was detected ($P<0.05$ and $P<0.001$ for March 27 and June 20, respectively). The CO_2 concentrations in March were uniform throughout the profile, while in June there was a tendency towards higher concentrations at the deepest samplings points.

The $\delta^{13}C$ values measured in March ranged between -18.4 to -19.7 ‰ vs VPDB (Vienna pee dee belemnite) in control plots and between -20.7 to -22.6 ‰ vs VPDB in FACE plots. In the control plots in June, values were -19.8 to -22.9 ‰ vs VPDB and -21.4 to -25.9 ‰ vs VPDB in FACE plots. Profiles from March were consistent down through the profile, while measurements in June at the top soil might be higher due to better mixing with ambient air as the soil were drier in June, ~0.13 and ~0.16 m^3 m^{-3} in June and March respectively. In both control plots and FACE plots we found a significant difference between measurements in March and June ($P<0.001$). The difference in $\delta^{13}C$ signals between control and FACE plots is due to the ^{13}C depletion (c. -25‰) of the CO_2 used for fumigation, which when mixed with the ambient air (δ ^{13}C -8‰) provides a source signal of c.-13‰ (unpublished data).

SR measured six times (three replicates) from mid March to late June 2007 by the LI−COR 6400 showed a relationship in CO_2 flux between FACE and control plots: $J(_{CO2})/J(_{Control})$ ± SE = 1.07 ± 0.03, while soil profile measurements from March and June gave a flux: $J(_{CO2})/J(_{Control})$ ± SE = 1.44 ± 0.11. The latter relationship was calculated from measured soil concentrations from ~18 cm depth via Ficks law (eq. 2).

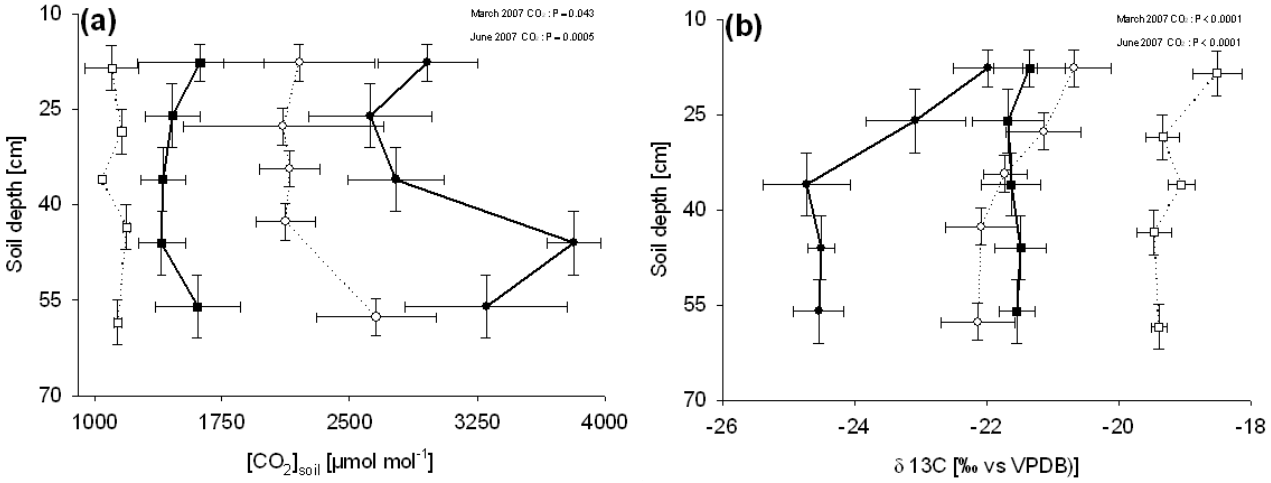

Figure 4. (a) Soil profile CO_2 concentrations measured at 16 to 61 cm soil depths in March and June 2007. Open and closed squares indicate measurements from control plots and FACE plots in March, respectively. Open and closed circles indicate measurements in June. All data are mean of n=3 replicates ± SE. (b) $^{13}C-CO_2$ characteristics measured at 16 to 61 cm soil depths March and June. Open and closed squares, respectively, indicate measurements from control plots and FACE plots in March. Open and closed circles indicate measurements in June. All numbers are average of n=3 replicates ± SE

4. Discussion

4.1. Initial chamber CO_2 concentrations

The application of the closed chamber technique in FACE experiments to measure exchange of CO_2 between the atmosphere and ecosystems faces several challenges. First, experimentally elevated CO_2 concentrations may fluctuate significantly over very short time scales making it difficult to obtain initial chamber CO_2 concentrations similar to target concentration. We have demonstrated that chamber based observations on NEE, ER, and SR are very sensitive to fluctuations in initial chamber headspace concentrations within the range of CO_2 concentrations frequently deployed in FACE experiments. Nevertheless, this relationship is only rarely taken into consideration in the literature. In a FACE experiment in Arizona, Nakayama et al. (1994) reported for SR measurements in the control plots, that if the initial concentration of chamber CO_2 concentration was high compared to the average the flux was unusually low, in agreement with the observations in our work. Nakayama et al. (1994) concluded, that if the initial CO_2 concentrations differed by more than 30 µmol mol^{-1} from the average initial CO_2 concentration, then the measurement should be discarded. However, the authors did not describe upon which criteria the measurements in the FACE plots were met. Among others, Bernhardt et al. (2006), King et al. (2004) and Pendall et al.

(2001) describe the measurement of soil respiration in several FACE experiments, without mentioning to what extent chamber CO_2 concentrations were considered important, and we have not been able to identify other papers which address this issue.

To overcome problems with fluctuations in chamber headspace CO_2 concentrations it may be necessary to adjust the concentrations to match the experimental target values, respectively, and ambient conditions. Automated SR measurement systems (e.g. LI−COR LI−6400) can automatically adjust CO_2 in the soil chamber by scrubbing excess CO_2 chemically or allowing SR to build up CO_2 to preset levels in order to avoid biased measurements. A similar solution could theoretically be applied to whole ecosystem flux chambers to overcome the inherent CO_2 fluctuations in FACE experiments. Though, to our experience adjusting the CO_2 headspace concentration to a certain level in a 216 L chamber is not immediate due to the delays associated with fluxes which proceed upon the in situ photosynthetic and respiration rates dependent on present environmental conditions. If the chamber has to be closed for a substantial amount of time in order to reach the desired headspace CO_2 concentration, the ecosystem might have been disturbed substantially by the enclosure, e.g. chamber H_2O concentration and temperature can change rapidly affecting the fluxes. More over if the wind has been in the same direction for a long period soil in the upwind plot is equilibrated to a high atmospheric CO_2 concentration. An alternative to adjusting chamber CO_2 concentration is to turn off the FACE system prior to measurements. However, both methods raises concern, as by changing the atmospheric CO_2 concentration we instantly alter the soil−atmosphere CO_2 gradient. The challenge is how to quantify a flux that reflects the true picture of CO_2 release in an elevated CO_2 environment.

4.2 FACE and chamber CO_2 flux measurements

When performing repeated measurements at short (5 min) intervals (Fig. 2) it appeared that the soil CO_2 efflux in FACE plots stabilised c. 10 min after shutting off the CO_2 fumigation. Fig. 3 shows that the enhanced soil respiration caused by CO_2 fumigation may last for at least 10 hours. This indicates that during night time, when the FACE system is off, the soil respiration is stabilised all night only in the periods when night length is maximum 9 hours, as the FACE system switches off half an hour after sunset and on half an hour before sunrise (at the CLIMAITE study site: late April – mid August). According to Fick's first law of diffusion, (eq 2), it is evident that if both soil and atmosphere are changed by e.g., 130 µmol mol^{-1} CO_2 (from an elevated atmosphere of 510 µmol mol^{-1} to ambient 380 µmol mol^{-1}) the measured flux will remain the same given the same rate of

production in the soil. We have, though, indications from our soil profile measurements that the soil CO_2 concentration does not only increase by 130 μmol mol^{-1}, but rather in the order of 500 μmol mol^{-1} when comparing control and FACE plots (Fig. 4(a)). An increase of this magnitude can not only be assigned to the increase in atmospheric CO_2 *per se*, but also implies a biological response in soil processes, as either, or both, increased autotrophic activity or heterotrophic activity. The increase in soil CO_2 with season (Fig. 4(a)) is very likely also driven by altered biological activity; in support of this theory, Fig. 4(b) shows a decrease in the $\delta^{13}C$ signal of about 2‰ as we move from spring (March) into summer (June). This signature shift implies that more plant derived carbon is found in the soil gas CO_2; plant biomass has a $\delta^{13}C$ of c. -31‰ and -26‰ biomass in FACE and non−fumigated plots respectively (Mikkelsen et al., 2008). The direction would have been the opposite, namely towards the atmospheric $\delta^{13}C$ of c. -8‰, if a passive flux of CO_2 from the atmosphere was the dominant process.

The application of Fick's law to compare the CO_2 flux relationship $J_{(CO2)}/J_{(Control)}$ from SR measurements made by the LI−COR 6400 and soil profile CO_2 measurements, showed that the method employing the LI−COR 6400 might underestimate the true flux in FACE plots. This is contrasting our finding that SR and ER rates increased when initial chamber CO_2 concentrations decreased, meaning that we would expect an overestimated flux rate in FACE plots when measured under ambient conditions. The immediate decline in SR in FACE plots when CO_2 is shut off is supposedly due to a degassing across the soil−atmosphere boundary rather than a biological response, and should be disregarded. It should also be emphasised that our soil profile data do not reflect the CO_2 gradient across the very top few cm soil where most biological activity is expected to occur, which may bias the comparison of calculated vs. observed SR rates. We conclude that after closing the CO_2 fumigation, the soil looses CO_2 because of a simple physical equilibration of the soil air. Soil respiration measured in spring and winter (Fig. 2) both show the same response to a sudden decrease in atmospheric CO_2 when closing off the FACE system. Such a fast response is very unlikely to be due to shifts in biological activity, especially during winter. The equilibrium lasts for up to 18 hrs, after which the soil biologically adapts to the changed atmospheric CO_2 concentrations, and respiration in FACE and control plots converge.

4.3. Effect of elevated CO_2 concentrations on photosynthesis and net ecosystem CO_2 exchange

Specific changes in vegetation CO_2 uptake by photosynthetic activity need to be emphasised. From various studies, among others Jackson et al. (1995) and Nowak et al. (2004), we know that plant net

photosynthesis increases with increased atmospheric CO_2, and in the study by Jackson et al. (1995) it was shown that a C3 grass, *Avena barbata* down regulates the photosynthetic activity when exposed to high concentration of atmospheric CO_2 for longer periods. Hence, grass grown in a 380 μmol mol^{-1} CO_2 atmosphere has a higher photosynthetic rate when exposed to an elevated CO_2 environment compared to specimens that are fully grown in the enriched atmosphere. This down regulation of results are in full agreement with the experiments performed in this study (Fig. 1(GEP)) supported by measurements made on the leaf level at same study site (unpublished data). The ecosystem carbon net balance depends on the sum of two factors, uptake by photosynthesis and the ecosystem respiration. To increase the net carbon uptake the photosynthesis rate should either increase, respiration rate decrease, or both. In May 2006 photosynthesis surpassed respiration and net assimilation was occurring, in both control and in FACE plots. The stimulation of GEP by increasing initial chamber CO_2 concentration was in FACE plots higher than in control plots (slope in regression line, Fig. 1(GEP)) and correspondingly the decrease of ER by increasing initial chamber CO_2 was also described by a steeper slope in FACE plots compared to control plots. In total this results in a relative change of 1.7 on NEE in FACE plots in measurements collected at 510 μmol mol^{-1} compared to measurements in 380 μmol mol^{-1}.

5. Conclusion

We demonstrate that soil−plant−atmosphere CO_2 fluxes obtained by closed flux chambers are highly sensitive to initial chamber CO_2 concentrations in the range from ambient 380 μmol mol^{-1} up to c. three−fold ambient concentrations. In our current FACE experiment, the CO_2 concentration is increased to 510 μmol mol^{-1}, which instantaneously decreases CO_2 effluxes from soil (SR) and ecosystem (ER) by ~20 %. The decrease in ER partly explains a ~70 % increase in net assimilation (NEE). Extreme care should be employed when applying static chambers in FACE experiments where atmospheric CO_2 concentrations and thereby also soil concentrations inevitably may fluctuate significantly. We did not find any easy solution for measuring with the aim of collecting the true flux. Well knowing that vegetation is sensitive to atmospheric CO_2 concentration we suggest turning off the CO_2 fumigation at least 10 minutes before measurements are commenced to allow degassing of the soil atmosphere and achieve reproducible chamber and soil conditions. Supplementary CO_2 fluxes made at the leaf level, where it is easy to adjust headspace CO_2

concentrations can support ecosystem CO_2 fluxes by estimating a true correction factor for GEP and plant respiration for measurements made at ambient instead of elevated CO_2.

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Paper II

Soil respiration in a temperate heathland responds strongly to elevated temperature, extended summer drought and elevated CO_2

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Soil respiration in a temperate heathland responds strongly to elevated temperature, extended summer drought and elevated CO_2

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Abstract

This study was conducted to investigate the impact of the predicted future Danish climate and atmospheric conditions, including elevated atmospheric CO_2 concentrations, elevated temperature and changes in water regimes, on soil respiration in a *Calluna-Deschampsia* heathland. A full factorial experiment with treatments of elevated CO_2 (+130 ppm), elevated soil temperature (+0.4 ^{o}C) and extended summer drought was established in autumn 2005. Soil respiration was measured in campaigns over three years. An empirical soil respiration model was developed that describes current soil respiration with soil temperature, soil moisture and substrate supply via plant photosynthesis as important drivers. Data analyses and model extrapolations showed that elevated temperature alone did not influence soil respiration. Extended summer drought decreased soil respiration by 7 %, whereas the combination of elevated temperature and extended drought, decreased soil respiration by 23 %. As main effect, elevated CO_2 was by far the most important driver for soil respiration and increased the soil respiration CO_2 losses by up to 40 %, irrespective of the combination with the other treatments. The multi-factorial model described soil respiration within -12 % to 18 % of the observed values, and we suggest that model performance could be improved by more temporal resolution of substrate inputs. We conclude that soil respiration rates are likely to increase at ca. 15 % in Danish heathlands in the projected future climatic conditions, in particular as a consequence of increased levels of CO_2.

Keywords:

Soil respiration*, Deschampsia-Calluna heath,* Climate change, CO_2 flux*,,* FACE, Modelling soil respiration, CLIMAITE.

Introduction

Soil respiration (R_S) is a key parameter for the global biospheric and atmospheric carbon (C) budgets, and, in terms of carbon quantities, together with net primary production, the most important flux between the atmosphere and ecosystems (Schimel 1995). Extensive research has focused on temperature and soil moisture sensitivity of R_S in various soil types and ecosystems shrub steppes over temperate forests to Mediterranean ecosystems (Wildung *et al.* 1975; Orchard and Cook 1983; Fang and Moncrieff (2001). Almagro *et al.* (2009) found in accordance with others, e.g. Davidson *et al.* (1998), that R_S is largely controlled by soil moisture at low soil water contents. However, above a certain soil water threshold, which is determined by the specific soil type, temperature becomes the primary controller of R_S. Increased temperatures in humid environments may stimulate soil activity leading to more respiration, whereas in dry soils increased temperatures may contribute to even dryer conditions, resulting in depressed R_S response (Wan *et al.* 2007; Ciais *et al.* 2003). In addition to water availability and temperature, R_S depends on photosynthesis, which is a possible indicator of substrate availability and supply (Larsen *et al.* 2007). Some research suggests that this relationship, accompanied by seasonal variation, is even more important than immediate water accessibility and temperature (Högberg *et al.* 2001; Mencuccini and Höltta 2010). In accordance with this, Tang *et al.* (2005) found that R_S correlated about 100% to soil temperature in vegetation free soils, while in tree covered soils R_S was 86-93 % correlated to the photosynthesis measured 7 hours earlier in the tree canopy. Increasing atmospheric CO_2 concentrations are likely to increase ecosystem root biomass and litter production (Johnson *et al.* 1994). Consequently, as R_S is intimately linked to biomass and litter fall, CO_2 fertilised ecosystems may exhibit elevated soil respiration (Raich and Nadelhoffer 1989; Raich and Schlesinger 1992).

Given that ecosystem responses are sensitive to the above mentioned climatic factors, R_S is an important issue in global change research. Human activities in the form of burning of fossil fuels and land-use changes are causing present atmospheric CO_2 level to rise by ~2 ppm per year, and global climate models (GCMs) consequently predict increased temperatures as well as changes in precipitation patterns (IPCC 2007). Overall, this calls for a detailed knowledge of the sensitivity and changes of the main fluxes and pools in the terrestrial carbon cycle. Concerning soils and the assessments of R_S, an important task is to examine whether a given soil will release more or less carbon to the atmosphere as a result of climate changes. In order to examine the consequences of the changing environment on ecosystem processes, field-scale global change experiments are valuable tools, as demonstrated in numerous field studies (e.g. Pendall *et al.* 2001; Emmett *et al.* 2004; Wan *et al.* 2007; Garten *et* al. 2009, de Dato *et al.* 2010). Single factor manipulation experiments are by far the most common, while two or more factors applied in concert are less

frequent. Shaw *et al.* (2002) showed that ecosystem responses to single factor treatments of several climatic and environmental parameters are not necessarily additive in relation to the carbon balance of an ecosystem. For instance, Shaw *et al.* (2002) found that elevated atmospheric CO_2 and increased precipitation as single factor treatments increased net primary production, while in combination the two treatments decreased net primary production. Multi-factorial experiments investigating ecosystem responses to global change are therefore needed to understand and predict consequences for the terrestrial C-balance.

The objective of this study was to examine the impact of elevated soil temperature, elevated atmospheric CO_2, and extended summer drought on R_S in a low vegetation heathland. Treatments were applied in a fully factorial design in order to identify the treatment interactions. We hypothesised that (1) increased atmospheric CO_2 concentration both as single factor and in combination with increased temperature and extended summer drought would lead to higher R_S as a result of the CO_2 fertilisation effect on plants, (2) R_S would increase in response to higher temperature as single factor, while (3) extended summer drought as a single factor would decrease R_S. We also hypothesised that (4) extended summer drought in combination with increased temperature would lead to even dryer soil conditions during drought treatment, reducing R_S even more.

Material and methods

Study site

The experiment was conducted in the period from October 2005 to August 2008 at the CLIMAITE study site Brandbjerg, situated at 55°53'N 11°58'E, Northern Zealand, Denmark (Mikkelsen *et al.* 2008). The ecosystem is a temperate heathland on a hilly nutrient poor sandy deposit, with a 5 cm organic layer with a pH of c. 5. The vegetation is dominated by two perennial species, heather (*Calluna vulgari*s L.) and wavy hair-grass (*Deschampsia flexuosa* L.). Plant height ranges from 40 – 60 cm and the two species are distributed heterogeneously in patches. The annual precipitation sum was 630, 850 and 640 mm in 2006, 2007 and 2008, respectively and average annual air temperature in 2 m height was 10 C°

Climate change manipulations and meteorological observations

The heathland was exposed to climatic and atmospheric conditions expected for the Danish region in year 2075 (Mikkelsen *et al.* 2008). Treatments include daytime atmospheric CO_2 concentrations

elevated to 510 ppm (CO2), soil temperature in 5 cm depth raised by ~0.4^{o}C (T), and extended summer drought (D). The experiment includes an untreated control (A). All factors and their combinations (A, T, D, TD, CO2, TCO2, DCO2, and TDCO2) were replicated 6 times. The experimental plots (48 in total) were distributed in 12 octagons of 7 m in diameter, which were arranged pair-wise in six blocks, one octagon of each pair being exposed to elevated CO_2. Each octagon was split into 4 plots, of which one was exposed to extended summer drought, one to raised temperature, one to both drought and temperature, and the fourth plot was either a control (non CO_2 fumigated octagons) or a CO2-plot (elevated CO_2 octagons) Temperature was elevated by passive night time warming (Beier *et* al., 2004) by means of curtains 0.5 m above ground covering all the 24 plots designated for warming. The curtains reflect emitted infrared radiation back to the soil and vegetation. They were automatically removed in case of dew fall or rain. During selected periods, in each summer, the drought treatment (D) was applied. This was carried out by automated rain exclusion curtains, controlled by a rain sensor, programmed to cover all 24 plots excluding rain during precipitation. Exposure to daytime elevated CO_2 was achieved by the FACE technique where CO_2 was injected along the perimeter of octagonal plots via injection tubes situated c. 40 cm above ground. The target concentration, 510 ppm_{vol} was measured in the centre of the 7 meter diameter experimental octagons. In all plots temperature and TDR probes were installed to measure temperature and moisture with mean values derived for every hour. Temperature probes were situated at 20 cm aboveground, at the soil surface and in 5 cm depths, TDR probes at 0–20 cm and 0–60 cm depths. Photosynthetically active radiation (PAR) was measured continuously with cosine corrected quantum sensors (OL-4000q, Optisk Laboratorium, Hørsholm, Denmark), precipitation (Rain-O-matic professional, Pronamic A/S, Silkeborg, Denmark) and air temperature in 2 meters height was measured at two stations at the site. The manipulations started in October 2005. Extended summer drought was carried out from July 3 – August 4 2006, May 21 – June 22 2007, and May 5 – May 27 2008. In 2006 8% and 2007 11 % of the annual rain fall was removed, while in 2008, 6 % was excluded. For further information about the experimental design, see Mikkelsen *et al.* (2008).

Soil respiration measurements

Soil respiration, R_S measurements were performed using the Portable Gas Exchange and Fluorescence System (LI-6400, LICOR Biosciences, Lincoln, Nebraska, USA) combined with a soil CO_2 flux chamber (LI-6400-09, LICOR Biosciences). Measurements were applied to 78.5 cm^2 plots confined by 10 cm diameter permanent PVC collars inserted 10 cm into the soil one year before measurements started. The aboveground vegetation in the plots was removed at installation;

any re-growth was removed subsequently. It is, however, assumed that the roots from the neighbouring plants reoccupied the soil under the collars before measurements were initiated. Measurements in FACE plots required special precautions; the CO_2 fumigation was switched off 30 minutes prior to measuring. Previous measurements have shown that the average CO_2 concentrations were relatively uniform across the CO_2 treated plots; however, the CO_2 dosing was characterised by a high degree of temporary variability driven by wind speed and direction, which might lead to marked concentration fluctuations, in particular within 50 – 70 cm distance from the injection tubes (Mikkelsen *et al*. 2008). Moreover, CO_2 was only released from the upwind side of the plot and depending on the distance of the R_S chamber collar relative to the CO_2 release tubes and current wind direction, significant fluctuations in CO_2 concentrations above the collars occurred. Measurements showed that CO_2 concentration near the soil collars could vary with several hundred ppm making it difficult to maintain stable and uniform starting conditions. The methodology is discussed in detail Selsted *et al.* (submitted 2010). Soil respiration, $R_{S,}$ was monitored 30 times, in all treatments and replicates, between October 2005 and August 2008, in total 1374 valid measurements. Particular attention was given to R_S during the drought treatments, where intensive measurement campaigns took place up to one month before the drought treatment was initiated, during the treatment, and following the first rewetting of the ecosystem.

Plant biomass estimates

Total aboveground biomass in an area of 60 x 60 cm^2 surrounding the 10 cm diameter collars were estimated in October 2006. We used the non-destructive pin-point analysis method (Jonasson & Skold 1983, Jonasson 1988). A frame with a 10 x 10 cm^2 fixed grid pattern was placed above the vegetation, and a 2 mm diameter pin was lowered vertically into the vegetation at each of the 25 grid points. Each hit on plant parts by the pin was registered by species and height until the tip of the pin was no longer visible. Further it was recorded if the pin hit newly dead or alive plant parts. The pin-point analysis was converted into estimates of above-ground biomass by correlations with vegetation height and number of hits per pin on a plant species as outlined for the site by Riis-Nielsen & Schmidt, unpublished.

Data analysis and statistics

Treatment responses were analysed by the proc mixed procedure (SAS 9.1, SAS institute Inc. 2003) using the repeated design. Main effects were the treatment factors T, D, and CO2 and all their interaction terms. Random factors included block and octagon. Biomass and air temperature were included as covariates if significant at $p < 0.05$. Air temperature in 2 m and PAR might explain part

of the same variation, which may relate to photosynthesis rate. Air temperature was preferred to PAR as the first is a more stable parameter during the day, whereas PAR oscillates in case of clouds. Only air temperature was included in the ANOVA.

Soil temperature, CO_2 concentrations and soil water content were not included as covariates since they are manipulated by the experimental design and part of the fixed effects. Homogeneity of variance was investigated with residual plots and data were log transformed when necessary. As covariance structure we used Heterogeneous Compound Symmetry (CSH) on R_S data. When testing treatment effects on environmental parameters Compound Symmetry (CS) was used as covariance structure. Tukey adjusted least square means were used to compare interaction terms and treatment effects.

Model parameters of the soil respiration model were fitted by multiple, nonlinear regression (Proc Model, SAS 9.1, SAS institute Inc. 2003).

Our starting point of the modelling exercise was the modified van't Hoff equation (e.g. Janssens and Pilegaard, 2003; Davidson *et al*. 2006):

$$R_{0,j} = \mathrm{Q}_{10}^{\frac{\mathrm{T_S}}{10}} \qquad (1)$$

Where R_0 is the base respiration at 0°C, Q_{10} is the factor by which respiration is changed when temperature increases by 10 °C, T_S is the soil temperature [°C] in 5 cm depth. In applying Eq. 1, we assumed that the base respiration was treatment specific, whereas Q_{10} was the same across treatments, i.e. the temperature sensitivity of R_s was assumed to be independent from the treatments. To further improve the model predictions we included soil water content and above ground biomass surrounding the measurement plots, which are proxies for differences in root biomass and litter production. Similar to temperature sensitivity, the relation of soil respiration to soil water content and plant biomass was considered independent of treatments. A good description of observed R_S was obtained by the following multiplicative model:

$$R_S = R_{0,j} Q_{10}^{\frac{T}{10}} * \mathrm{f}(\Delta_S) * \mathrm{g(B)} \qquad (2)$$

Where $R_{0,j}$ is the base respiration at 0°C at treatment j, Δ_S is soil water saturation deficit expressed by:

$$\Delta_S = 1 - \frac{\theta}{\theta_{fc,j}} \quad (3)$$

Where θ is the soil water content in percent measured in 0-20 cm depth in the experimental plots and $\theta_{fc,j}$ the soil water content at field capacity of each treatment. Field capacity is calculated as the average soil water content in periods after heavy rain, corrected for a short-term overshoot in θ. The sensitivity of R_S on soil moisture is described by a continuous function including two constants, a and b:

$$f(\Delta_S) = 1 - e^{a - \frac{b}{\Delta_S^2}} \quad (4)$$

Values of $f(\Delta_S)$ will always be equal to or less than 1 (Fig. 1a; Fig. 1b), and the function predicts that soil moisture has a reducing effect on R_S in A plots when lower than ~20 %.

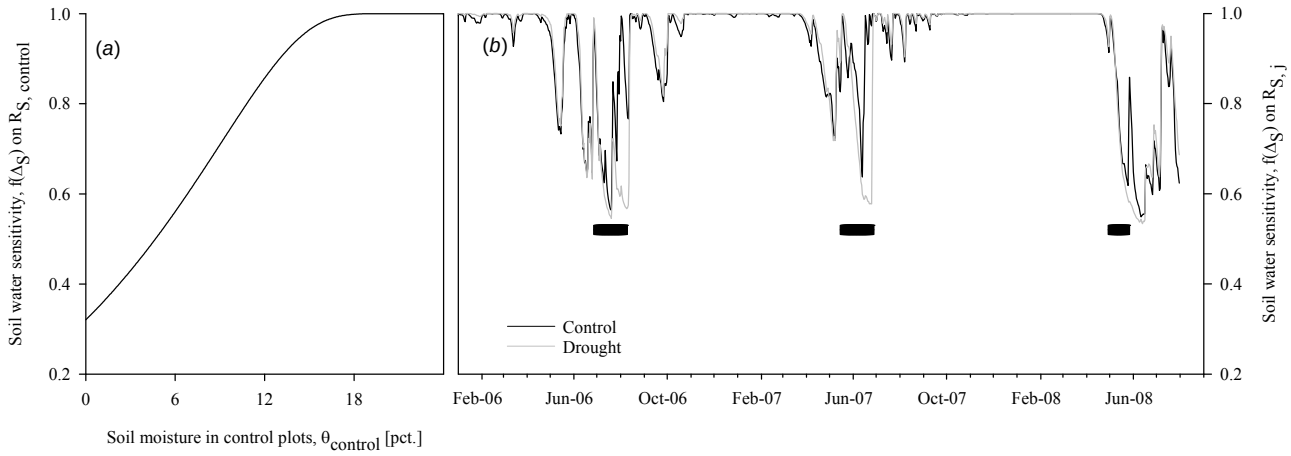

Figure 1. (a) Relative soil water sensitivity function, $f(\Delta_S)$, on R_S in control plots. (b) Seasonal course of $f(\Delta_S)$ in control plots (black line) and drought treated plots (gray line). Horizontal black bars indicate exclusion of precipitation in drought treatments.

The third parameter of the model is a linear relationship with surrounding biomass as a proxy for heterotrophic and autotrophic soil activity at the investigated plots:

$$g(B) = \frac{B}{B_{max}} + c \quad (5)$$

Biomass, B in each plot is normalised with regard to the plot with the highest amount of biomass, B_{max}, based on the measurements from October 2006 .

We fitted the model with 12 parameters ($R_{0,A}$, $R_{0,T}$, $R_{0,D}$, $R_{0,TD}$, $R_{0,CO2}$, $R_{0,TCO2}$, $R_{0,DCO2}$, $R_{0,TDCO2}$, Q_{10}, a, b and c) in accordance to eq 2. The model was used to extrapolate measured R_S to continuous estimates using the continuous environmental data collected at the study site.
To investigate whether the treatment effects were captured fully by the model, we performed an analysis of variance (the proc mixed procedure, SAS 9.1, SAS institute Inc. 2003) on model residuals. If model residuals ($R_{S\_field\_measurement}$ - $R_{S\_modelestimate}$) were independent of treatments, any treatment effects were considered to be satisfactorily described by the model.

Results

Plant biomass estimates

Biomass estimates from 2006 did not reveal any difference among treatments in, *Deschampsia, Calluna* or total, biomass. The *Deschampsia/Calluna* ratio differs among plots from 0.07 to 325, but no trends towards differences among treatments were detectable because of initial patchiness of vegetation. Large differences were seen between single plot observations, resulting in large standard errors at each treatment (Fig.2).

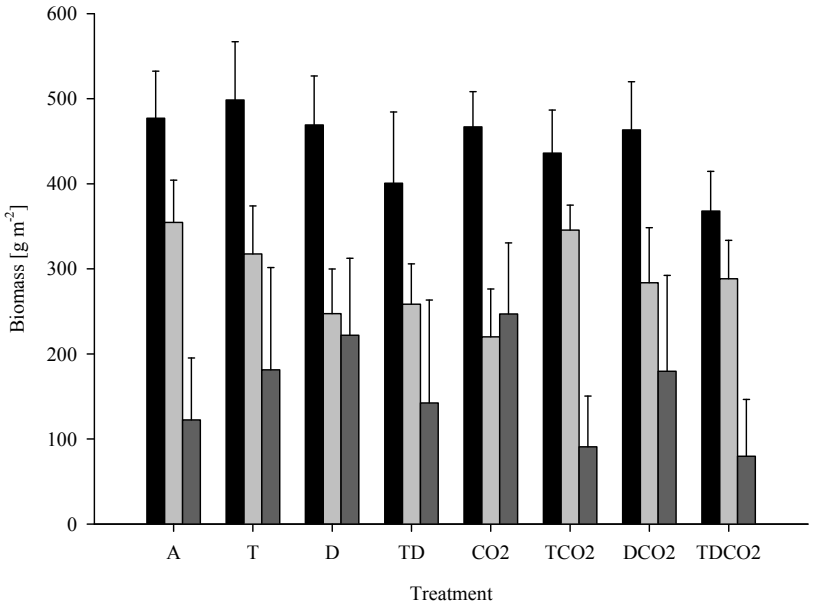

Figure 2. Biomass estimations ± SE from October 2006. Black bars indicate total biomass, light gray the *Deschampsia* grass biomas and the dark gray bars indicate the *Calluna* heather biomass at each treatment.

Treatment effects on ecosystem conditions

During January 2006 – July 2008 the main effects T and D reduced the soil water content in 0-20 cm depth ($P < 0.001$) with significant interactions D×CO2 and T×D×CO2. Soil water in D and TD treatments were on average 2.1 vol% ±0.3 and 3.3 vol% ±0.3 lower than the control, which on average over the whole period was 12.7 vol% ±0.3 (Fig. 3a). As expected, the drought treatment showed the highest impact on soil water content during drought treatment campaigns. Soil water content in drought treatments recovered up to a level of 0.5 vol% less than non-drought treated plots during the first month after ended treatment. This level was maintained until next year's drought treatment. Soil water showed a different pattern in T and CO2 treatments, where moisture levels were close to ambient until May 2007, hereafter the T treatment showed a decrease in soil water content compared to control plots (Fig. 3b and c).

The only significant main effect on soil temperature in 5 cm depth was T ($P < 0.01$), which enhanced the soil temperature by 0.36 C^o ±0.12 on average during 2006-2008. Separated into seasons the temperature treatment raised soil temperature during spring (March, April and May) by 0.43 C^o ±0.15, during summer (June, July and August) by 0.36 C^o ±0.07, during autumn (September, October and November) by 0.43 C^o ±0.17 and during winter (December, January and February) by 0.23 C^o ±0.09.

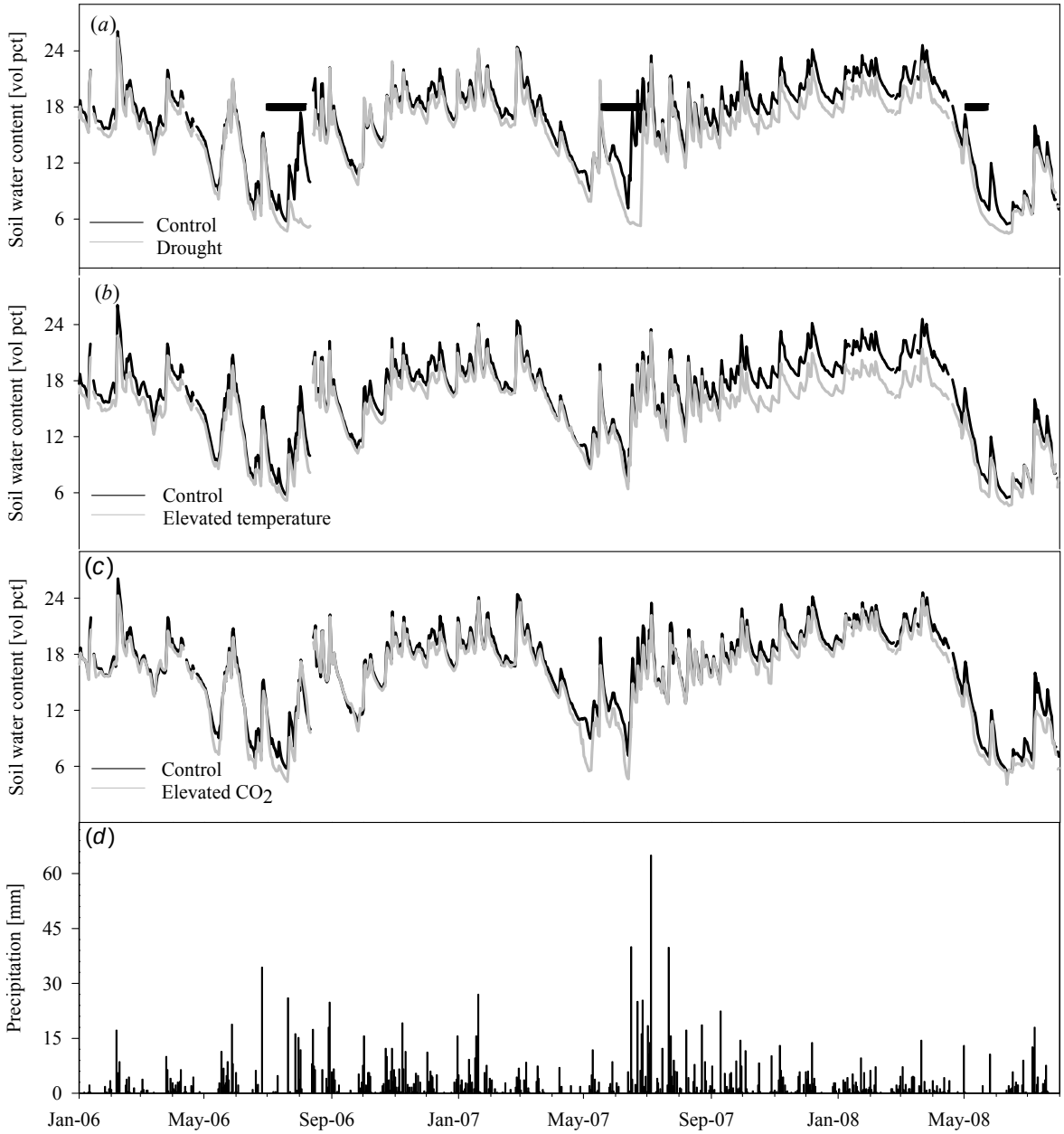

Figure 3. (a) Soil water content in control (black line) and drought plots (gray line), horizontal black bars indicate exclusion of precipitation in D-plots. (b) Soil water content in control (black line) and elevated temperature plots (gray line). (c) Soil water content in control (black line) and elevated CO_2 plots (gray line). (d) Precipitation at the site.

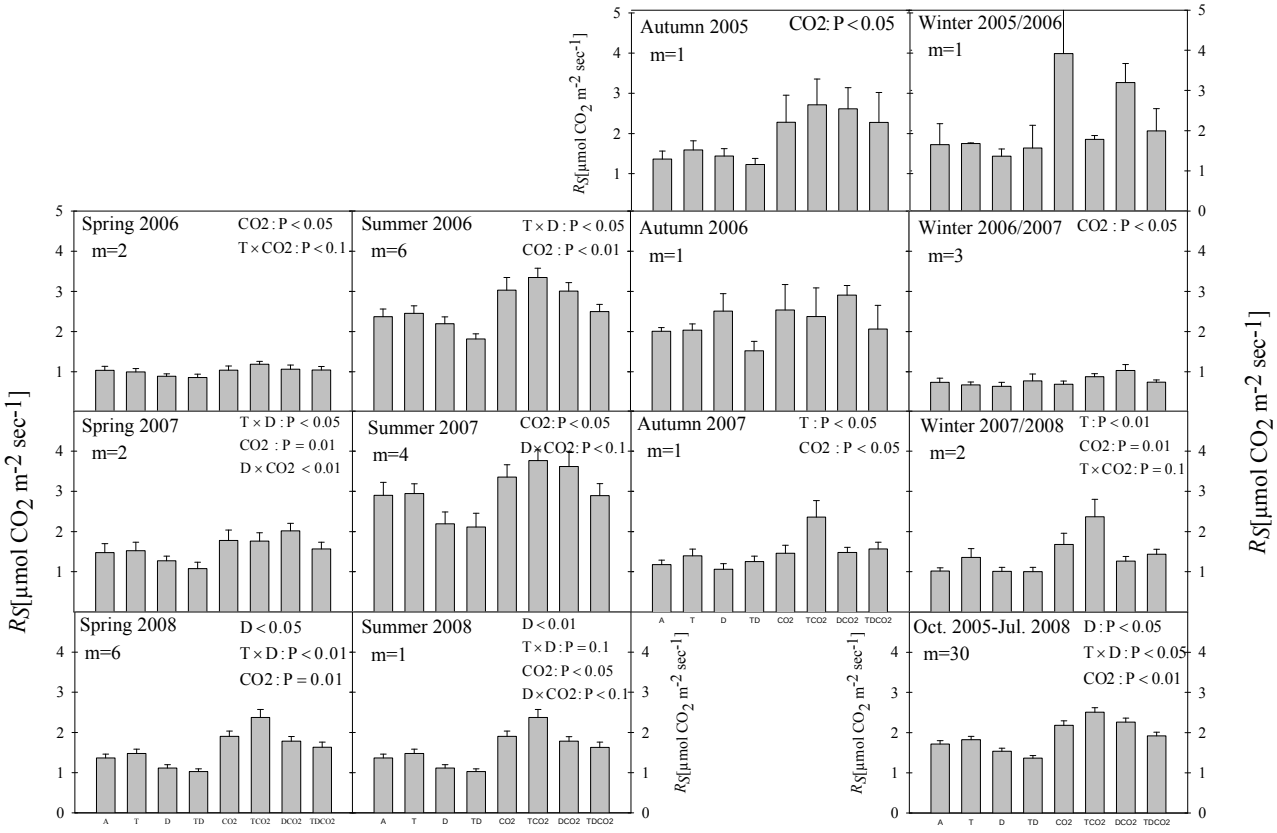

Figure 4. Measurements of soil respiration R_S, grouped by season and overall average, ranging from October 2005 to July 2008. Significant treatment effects are indicated in each plot. The number of measurement campaigns is indicated by m; each campaign includes 6 replicates per treatment. The overall average R_S of Oct 2005 to July 2008 is indicated at bottom right, which is the average of 30 rounds of measurement on each of the 48 plots.

Observed soil CO_2 fluxes

Analysis of variance on all field observed R_S (Fig. 4, October 2005 to August 2008) showed significant responses to the main effects D ($P < 0.05$), CO2 ($P < 0.01$) and also a significant interaction between temperature and drought, T×D ($P < 0.05$). Drought had a negative effect on R_S, ($P < 0.05$), T and D interacted to further intensify the drought effect, while CO2 had a strong positive effect, on average increasing R_S by a factor of 1.35 ±1.04 regardless of interactions with other treatments. Air temperature was as covariate highly significant ($P < 0.0001$), with a positive effect on R_S. The possible effect of elevated CO_2 on R_S was apparent throughout most seasons, except winter 2005/2006 and autumn 2006, during which periods only one measurement was made (Fig. 4). Temperature treatment had a positive effect on R_S during autumn 2007 and winter 2007/2008, but not earlier during the measurement period. The positive effect of warming was maintained in spring and summer 2008, but not when warming was combined with drought, as shown by the significant T×D effect. Highest fluxes up to 4 µmol CO_2 m^{-2} sec^{-1} were measured in

TCO2 plots during summer 2007. Lowest fluxes ~0.7 µmol CO_2 m^{-2} sec^{-1} were measured in winter 2007 in non CO2 plots.

Model performance and estimates

All observed fluxes (October 2005 to August 2008) were fitted by the model (eq. 2-5), which described 53.5 % of the variation in data. Q_{10} was estimated to 2.44 ± 0.07, *a* and *b* to 0.21 ± 0.15 and 0.63 ± 0.09, respectively, and *c* to 1.10 ± 0.20. All fitted parameters were highly significant ($P < 0.0001$) except *a* ($P = 0.17$), indicating that the model sensitivity to *a* is small compared to the other parameters. The model estimates a base respiration, R_0 at 0^oC for each treatment. A clear effect of CO2 both as single treatment and in combination with T and/or D was found on R_0, raising R_0 by a factor of 1.39 ±0.06 (Fig. 5). Single factors T and D did not show any treatment effects. The combination TD tended to show a reduced R_0, also in combination with CO2 (Fig. 5).

Analysis of variance was performed on model residuals showing no difference in residuals between treatments concluding that the model described all 8 treatments without treatment specific bias and similar performance.

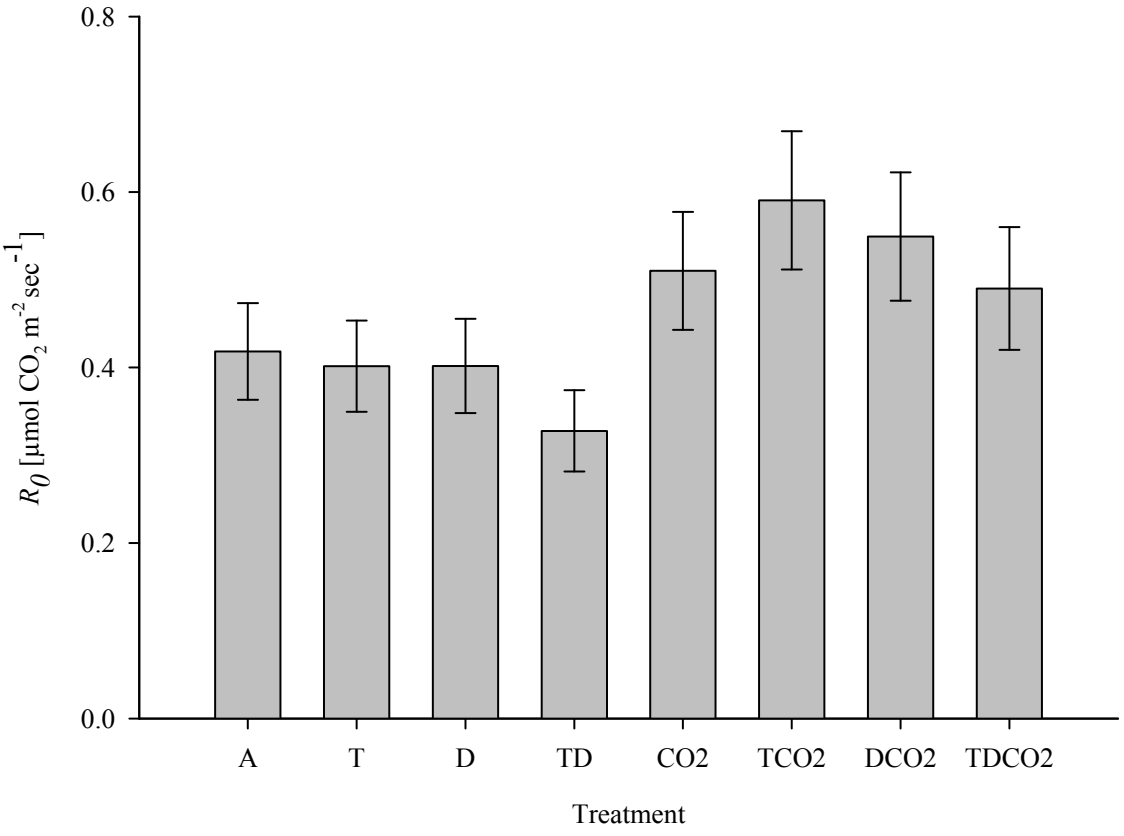

Figure 5. Base respiration R_0 for each treatment compared to control ± SE as fitted by the model.

Seasonal patterns and annual sums of soil respiration

Model extrapolations of $R_{S,j}$ generally followed the seasonal pattern of soil temperature, low during winter with minimum in February, and then increasing until peak values in August. All treatments followed the same overall pattern, see Fig. 6 for ambient soil respiration.

Annual CO_2 loss predicted by model extrapolations was 5% higher in 2007 compared to 2006 across all treatments (Table 1). Treatment effects compared to control were nearly identical in both 2006 and 2007. T and D as single factor treatments did not change R_S significantly, whereas in combination they showed 23% lower respiration than control. In all treatments which included CO2, the annual sum of R_S was raised, by up to 45% when in combination with increased temperature.

Table 1. Upscaled, predicted total soil respiration year 2006 and 2007.

Treat	R_S 2006 [g m^{-2} $year^{-1}$]	R_S 2007 [g m^{-2} $year^{-1}$]	2006 relative change to control [pct]	2007 relative change to control [pct]
A	722	761	0	0
T	717	761	0	0
D	669	709	-7	-7
TD	556	589	-23	-23
CO2	881	937	22	23
TCO2	1041	1118	45	45
DCO2	906	962	26	26
TDCO2	824	877	15	15

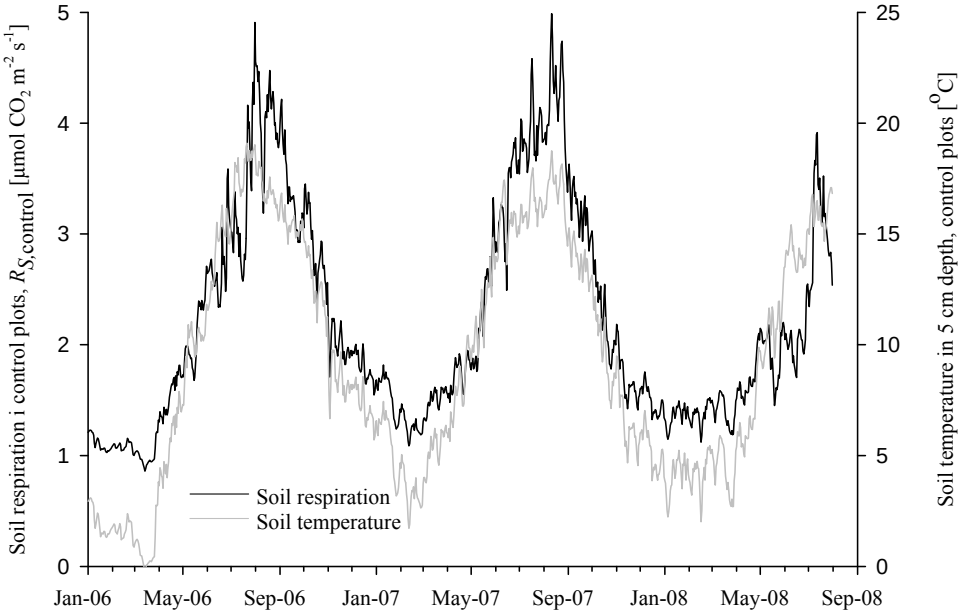

Figure 6. Soil respiration in control plots predicted by the model (black line) and soil temperatures (gray line) for the period between January 2006 and August 2008.

Table 2. Treatment effects before, during and after first rewetting after the experimental summer drought. Number of measurement campaigns is indicated by m, each observation includes 6 replicates, date ranges indicate interval where data were collected. Effects of the factor D were always negative compared to the corresponding interaction not including D. Level of significance is labeled by: P < 0.1: ‡, P < 0.05: *, P < 0.01: ** and P < 0.001: ***

	Before drought	During drought	After drought	Before drought	During drought	After drought	Before drought	During drought	After drought
Interaction term	n=2	n=2	n=2	n=2	n=1	n=3	n=2	n=2	n=1
		2006			2007			2008	
	Jun7-Jul5	Jul14-Jul25	Aug17-Aug24	Apr26-May14	Jun13-Jun21	Jun25-Jul19	May6-May14	May21-May27	Jul1-July1
D		*			**			***	
T×D	‡		**	‡				*	
D×CO2				‡					
T×D×CO2								*	

Response to soil moisture and recovery after experimental summer drought

Observations showed that R_S was significantly reduced by the drought treatment during the extended summer drought in all three years (Table 2). With the first rewetting after the drought treatment (Fig. 3a) activity returned to levels similar to ambient (Table 2). However, despite similar water contents between control and drought treated plots, the drought effect reappeared both, in

spring 2007 and 2008 with negative influence on R_S, in 2007 as the interaction T×D and in 2008 both as main effect D and interaction T×D, (Fig. 4). The persistence of the drought effect is not explicitly included in the model estimations; only the small persistent differences in soil water content in the drought plots could contribute to modeling this phenomenon (Fig.3). Therefore, modelled R_S in D plots is only different from A plots when the sensitivity to soil water content is different (Fig. 1), as the model applied does not explicitly include substrate pools, which would impart memory-like properties, as observed as persistent effects in the ecosystem.

Discussion

Extended summer drought

Soil respiration was significantly reduced by drought, both as main effect (D) and in interaction with temperature (T×D). Jensen *et al.* (2003) investigated R_S in a Danish heathland, similar to the CLIMAITE study site, and found that summer (May-September) flux rates in control plots were similar to those in the current study, about 3 μmol m^{-2} s^{-1}. After a two-month experimental drought, Jensen *et al.* (2003) noticed that soil respiration had decreased by 27% during summer (May-September 2000). For comparison, a one month drought period in the current study decreased soil respiration by 12 and 10 %, respectively, for the May-September periods in 2006 and 2007. We found that soil respiration generally was constrained under reduced soil water conditions, probably due to lower root activity (Borken *et al.,* 2006) followed by reduced microbial activity (Jensen *et al.*, 2003), and soil fauna activity (Maraldo *et al.,* 2009). The effect of extended summer drought was significant when considering all fluxes observed throughout the year, which indicates a strong impact of the short term drought treatment. Respiration in drought treated plots did initially recover after the first rewetting, however suppression of R_S in drought treated plots reappeared in the spring of the following year, both in 2007 and 2008. This could be ascribed to a drought-induced temporary water repellency of the soil, creating water flow patterns unfavourable for vegetation access (Muhr and Borken, 2009). Alternatively, the delayed suppression of R_S could be a consequence of reduced plant growth (Penuelas *et al*., 2007) during the drought period leading to reduced litter production and subsequent lower respiration rates. The reappearance of the drought effect is not predicted by the model, as the model is only dependent on instantaneous soil water content. However, our model is based on data from the three first years of drought treatment. Over time one could expect a changed soil texture or decreased root biomass as a result of repeated summer droughts leading to permanent reductions in R_S rates as soil water holding capacity will become smaller, and plant available soil water will become reduced (Muhr and Borken, 2009).

Elevated temperature

The temperature treatment led to reduced soil water content, which could possibly be assigned to two main reasons: (1) higher evapotranspiration from soil and vegetation due to higher temperatures (Liu *et al.*, 2009) and (2) an unintended exclusion of precipitation by the heat reflecting curtains. The warming curtains covered the ecosystem during night time and were programmed to withdraw during rainfall and dew formation. However, dew formation is difficult to detect and the responsiveness of the precipitation sensors is not immediate and a minor fraction of precipitation might be excluded before the curtains are fully withdrawn.

The main effect T had no overall significant effect on R_S, assumingly because the treatment effect on soil temperature was limited, with an increase of c. 0.4 ^oC on average. We hypothesised elevated temperature and extended summer drought to interact and cause further drying of the ecosystem, and hence further reduced R_S as compared to drought as a single factor. This was confirmed by a significant interaction between temperature and drought when analysing observed data (Fig. 4), and from the trend towards reduced R_0 in model prediction for TD (Fig. 5). In agreement, Wan *et al.* (2007) found that the temperature dependence of R_S in a grassland changes with season, whereas an overall increase of 2.7 ^oC in air temperature did not change R_S. In dry periods, Wan *et al*. (2007) found that elevated temperature reduced R_S, whereas temperature had the opposite effect in wet periods. In the current *Calluna-Deschampsia* heathland, there were no periods of negative temperature effects on R_S, except when T was combined with D. Ecosystem C-balances, however, may be more susceptible to changes in primary productivity compared to respiratory losses. During the 2003 hot and dry climatic extreme of Europe the persistent respiratory CO_2 losses outbalanced at least four years of net uptake due to decreased productivity (Ciais *et al*., 2005; Arnone III *et al.*, 2008).

Elevated atmospheric CO_2

Elevated atmospheric CO_2 is reported to increase R_S, likely due to higher photosynthetic activity (Nowak *et al.,* 2004) promoting higher above and below ground litter production (Luo *et al*., 1996; Craine *et al*., 2001, Wan *et al*,. 2007; Pregitzer *et al*., 2008). Our investigation confirms these findings and demonstrated at least 35 % increased R_S under elevated CO_2, regardless of interactions with other environmental parameters. The analysis of experimental data and the model predictions revealed comparable results suggesting an increase in R_S by 35 % (data) to 39 % (model predictions). In an elevated CO_2 environment, increased stomatal CO_2 concentration gradients and influx rates reduces stomatal conductance, often leading to enhanced plant water use efficiencies

and consequently higher soil water contents (Craine *et al.* 2001; Dermody *et al*. 2007; Garten *et al*. 2009*;* Leuzinger and Körner 2010). This interaction between elevated CO_2 and soil moisture could particularly become important for R_S in dry periods where even small amounts of extra available water can be critical. Pendall *et al.* (2003) found that elevated CO_2 over a grassland increased R_S by 25% during a moist season, while increases up to 85% were reported in a dry season. In our experiment we also observed a positive interaction between elevated CO_2 and extended drought, resulting in higher soil moisture contents, thus the effect on R_S was only significant in spring 2007.

Model performance

Analysis of variance on model residuals revealed no differences between residuals across treatments, and we concluded that the model describes all 8 treatments with similar accuracy. Regression analysis of R_s measured on R_s predicted, however, suggest a tendency that the model overestimates R_s at low activity levels and underestimates R_S at high activity levels, as indicated by the regression line slopes exceeding 1 in five of eight treatments (Fig. 7). An explanation for this bias in model performance could be that the R_S dependency on substrate input is inadequately described in the model. As mentioned, R_S depends on substrate input which is strongly correlated to plant photosynthesis and seasonality (Tang *et al.,* 2005). The current model does not directly consider substrate input as a driving parameter, whereas above ground living biomass is used as a proxy for both root activity and microbial activity. However, seasonal variations of these activities are not taken into account by the model. Living biomass might be a good proxy for substrate input, but only to a certain degree, as photosynthesis rates vary strongly with weather and environmental conditions. We observed a significant co-variance of air temperature with R_S ($P < 0.0001$), which suggests air temperature as a proxy for photosynthesis. Larsen *et al.* (2007) modelled ecosystem respiration in a temperate heathland and found a similar bias as in our example. When photosynthesis rate was incorporated in the model, the model performance was improved and the over- and underestimation of fluxes, respectively for high and low $R_{S,}$ was minimised. Likewise, our model could probably be improved by including photosynthesis as an input, which would allow for a seasonal variation of the base respiration (Larsen *et al*., 2007).

The model was parameterised with a ubiquitous and treatment independent Q_{10}, assuming that temperature sensitivity of R_S does not change with season, and that Q_{10} remains independent of treatment. Janssens and Pilegaard (2003) concluded that application of one Q_{10} is sufficient to estimate total annual carbon loss, while seasonal specific Q_{10} values are needed to capture seasonality. Davidson *et al.* (2006) suggested a more sophisticated proposition, that soil respiration

is controlled by several processes that should be considered separately, and applying only one Q_{10} to describe temperature sensitivity. This view is also supported by Schindlbacher *et al.* (2008) who concluded that only one model parameter is needed to describe temperature sensitivity of R_S at larger temporal and spatial scales. These authors also stated that seasonal variation in Q_{10}-values does not reflect varying temperature sensitivity, but rather the influence of other parameters. Our attempts to parameterise the model with different Q_{10} values for different treatments did not improve model performance, justifying that treatment effects are not described by Q_{10} but lie within R_0 and present environmental conditions.

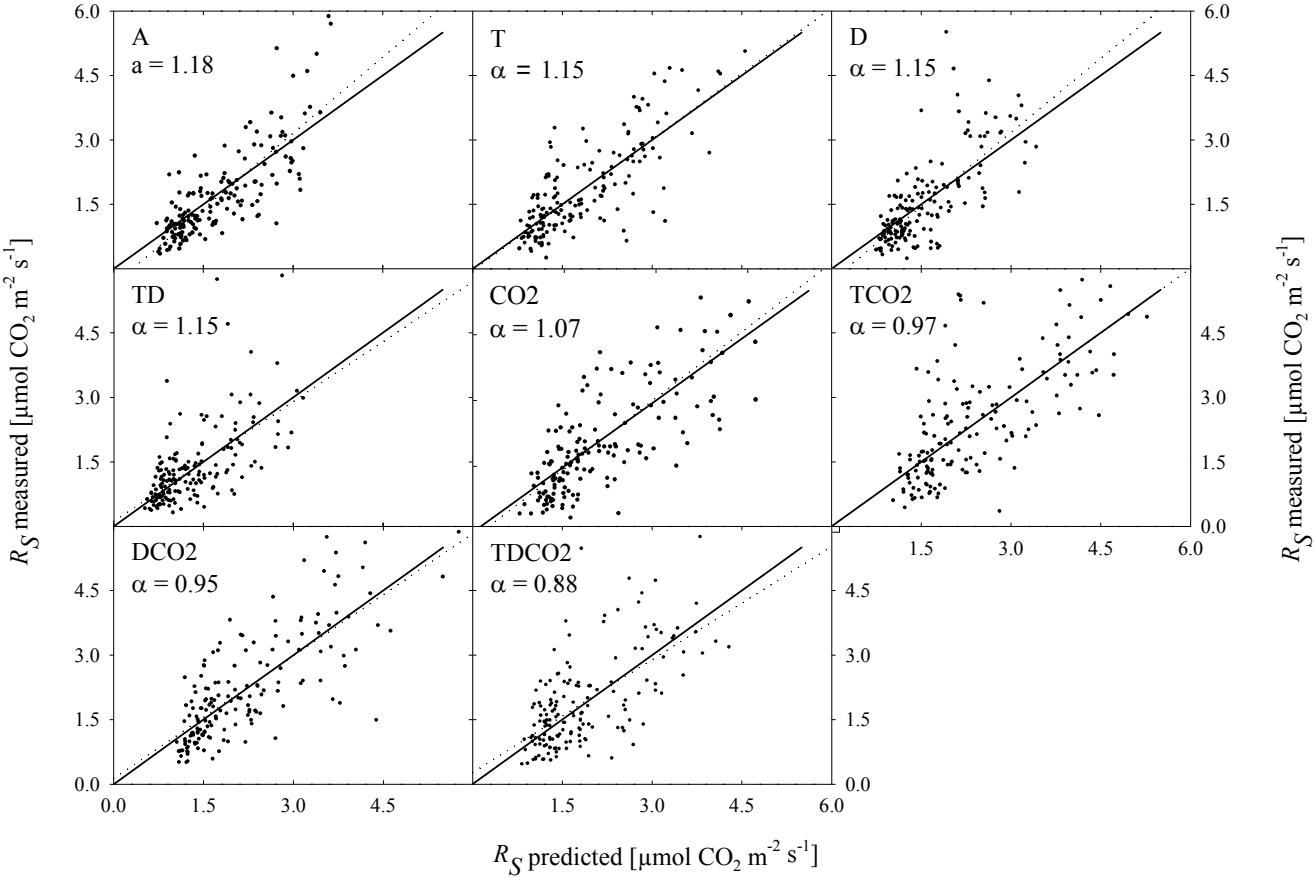

Figure 7. Observed vs. predicted R_S for the individual treatments. The solid line indicates the line x = y and dotted lines indicate the position of the regression lines R_S observed vs R_S predicted. α indicate the slope of the regression line.

Soil respiration in response to global change

We investigated soil respiration responses to elevated temperature, extended summer drought and elevated CO_2 in a low vegetation heathland. A strong increase in soil respiration, up to 40 % was observed under elevated CO_2, both as single factor treatment and in concert with temperature and drought. The elevated temperature treatment as single factor treatment had no effect on soil respiration. Extended early summer drought decreased annual soil respiration by 7 %; however,

immediate drought effects were transient with a rapid system recovery upon rewetting, whereas long-term and delayed decreases in soil respiration appeared at the onset of the following season. Our hypothesis, that increased temperature would strengthen the reducing effect of drought on soil respiration was supported by the findings showing a 23 % reduction in R_S. Soil CO_2 losses could be described within -12 % to 18 % of observed fluxes by a multiplicative model taking into consideration temperature, soil moisture and plant biomass as drivers. By model extrapolations, we estimate that the future climate (combining elevated temperature, CO_2 and drought) will increase annual soil respiration by 15 %, compared to current levels. Long-term changes, however, were observed in response to episodic summer drought, and it can be speculated that further changes may appear during persistent changes in environmental and climatic conditions.

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Paper III

Ecosystem CO_2 fluxes in respond to elevated temperature, extended summer drought and elevated CO_2

Paper in preparation for submission to Plant and Soil

Ecosystem CO_2 fluxes in respond to elevated temperature, extended summer drought and elevated CO_2

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Abstract

This study was conducted to investigate the impact of the predicted future Danish climate and atmospheric conditions, including elevated atmospheric CO_2 concentrations, elevated temperature and changes in water regimes on ecosystem CO_2 fluxes (net ecosystem exchange, ecosystem respiration and photosynthesis) in a *Calluna-Deschampsia* heathland. A full factorial experiment with treatments of elevated CO_2 (+130 ppm), elevated soil temperature (+0.4 ^oC) and extended summer drought was established in autumn 2005. Fluxes were measured in two long term campaigns (spring 2006 throughout winter 2006/2007 and winter 2007/2008 throughout summer 2008). Data analysis and extrapolations of fluxes measured in 2006 to yearly sums showed that elevated temperature alone did increase respiration in the autumn and winter, while photosynthesis were not influenced. On the yearly scale the temperature treatment decreased net carbon uptake to 54 gC m^{-2} $year^{-1}$ compared to ambient 17 gC m^{-2} $year^{-1}$. Extended summer drought decreased both respiration and photosynthesis making the drought treatment the sole treatment that assimilated carbon during 2006, 140 gC m^{-2} $year^{-1}$. When elevated temperature was in combination with drought the drought effect were at the yearly scale reduced (a net uptake of 3 gC m^{-2} $year^{-1}$). Elevated CO_2 generally increased ecosystem respiration and photosynthesis, while the effect on net carbon balance tends of a net loss of carbon from the system. In conclusion we anticipate a minor net loss of carbon from Danish heathlands in the projected future climatic conditions.

Keywords FACE, *Calluna, Deschampsia,* modelling ecosystem fluxes, CO_2 flux, climate change

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Introduction

The carbon balance of terrestrial ecosystems is expected to change due to the continued increase in atmospheric CO_2. Ecosystems assimilate and loose carbon in the form of CO_2 by mainly two opposed processes, that is gross uptake by photosynthesis (P_g) and losses through respiration from soil and vegetation, the ecosystem respiration (R_E). Of particularly interest is the net ecosystem exchange (F_n), which is the balance of P_g and R_E that reveals if ecosystems are a sink or a source of CO_2, and hence mitigate or adds to the atmospheric CO_2 pool. A stimulation of P_g may not necessarily result in increased biomass production, as the net uptake of carbon also depends on the concurrent stimulation of R_E (Verburg et al. 2004).

Experiments with elevated atmospheric CO_2 in grasslands have shown that enhanced CO_2 generally stimulates both photosynthesis (P_g) and ecosystem respiration (R_E) (Fredeen et al. 1995; Ellsworth et al. 2004; Bachman et al. 2010). The outcome of such experiments is often an increased net production (F_n) proposing that photosynthetic activity (P_g) is stimulated more than respiratory processes. The change in atmospheric CO_2 concentrations is pushing not only ecosystem processes, but is also pushing the climate systems towards a rise in mean temperature and changed precipitation patterns (IPCC 2007). These climatic changes may accelerate effects of increased CO_2 on ecosystem processes, or work in opposite directions. Recent studies have shown that impacts of several climatic factors on ecosystems are not straight forward additive as interactions occur (Shaw et al. 2002). When elevated CO_2 is considered in combination with altered water regimes, F_n might both be influenced negatively or positively by the elevated CO_2. Due to easy access of CO_2 plant leaves may reduce their stomatal conductance in elevated CO_2 environments (Ainsworth et al. 2004; Long et al. 2004) resulting in an enhanced water use efficiency, again leading to higher soil water contents (Craine *et al.* 2001; Zavaleta et al. 2003; Dermody *et al*. 2007; Garten *et al*. 2007*;* Leuzinger and Körner 2010). E.g. Lou et al. (2008) report that prolonged summer drought treatments decreased both P_g and R_E in a Californian heathland, while in combination with elevated CO_2 P_g increased and the drought effect on R_E was mitigated. Improved water use efficiency will be of significant importance to ecosystem processes, especially during drought events (Morgan et al. 2001; Pendall et al. 2003). In contrast, productivity in ecosystems exposed to higher precipitation might not benefit from the elevated CO_2 as the gain from improved water use efficiency will be neglected, and growth may even be constrained due to nutrient limitations (Shaw et al. 2002; Zavaleta et al. 2003). In a study on soil respiration in a Danish heathland (Selsted el al. 2010) it was found that elevated soil temperature and extended summer drought in combination intensified the negative drought effect, whereas the temperature alone had no effect on soil respiration. In order to

predict net carbon balance of ecosystems in a future climate, it is thus important to gain insight into the combined effects of key climate change factors on ecosystem processes regulating carbon balance.

The current study forms part of the CLIMAITE project, a multifactor experiment investigating ecosystem responses of a semi-natural heathland to the climatic and atmospheric conditions predicted for 2075 in Denmark (Mikkelsen et al. 2008). The objective of this study was (1) to identify impacts of elevated temperature, extended summer drought, and elevated atmospheric CO_2 on ecosystem CO_2 fluxes (F_n, R_E, and P_g). By this we aimed at testing four main hypotheses: (a) elevated temperature enhances both P_g and R_E, (b) extended summer drought constrains P_g and R_E, (c) elevated atmospheric CO_2 increases P_g as well as R_E, and (d) elevated atmospheric CO_2 in combination with extended summer drought will result in higher P_g and R_E rates compared to the sum of the two treatments alone, both with and without warming.

Material and methods

Study site

The experiment was conducted in the period October 2005 to August 2008 at the CLIMAITE study site Brandbjerg, situated at 55°53'N 11°58'E, northern Zealand, Denmark (Mikkelsen *et al.* 2008). The ecosystem is a temperate heathland on a hilly nutrient poor sandy deposit, with a 5 cm organic layer with a pH of c. 5. The vegetation is dominated by two perennial species, heather (*Calluna vulgaris*) and wavy hair-grass (*Deschampsia flexuosa*). Aboveground biomass height is 40 – 60 cm and distributed heterogeneously in patches. The annual average precipitation was 630, 850 and 640 mm in 2006, 2007 and 2008, respectively and average annual air temperature 10 °C (unpublished data).

Climate change manipulations and meteorological observations

The heathland was exposed to climatic and atmospheric conditions expected for the Danish region in year 2075 (Mikkelsen *et al.* 2008). Treatments include daytime atmospheric CO_2 concentrations elevated to 510 ppm (CO2), soil temperature raised by ~0.5°C (T), and extended summer drought (D). The experiment includes an untreated control (A). All factors and their combinations (A, T, D, TD, CO2, TCO2, DCO2, and TDCO2) were replicated 6 times. The experimental plots (48 in total) were distributed in 12 octagons of 7 m in diameter, which were arranged pair-wise in six blocks, one octagon of each pair being exposed to elevated CO_2. Each octagon was split into 4 plots, of which one was exposed to extended summer drought, one to raised temperature, one to both drought and temperature, and the fourth plot was either a control (non CO_2 fumigated octagons) or a

CO2-plot (elevated CO_2 octagons). Temperature was elevated by passive night time warming (Beier *et* al., 2004) by means of curtains 0.5 m above ground covering all the 24 plots designated for warming. The curtains reflect emitted infrared radiation back to the soil and vegetation. They were automatically removed in case of dew fall or rain. During selected periods, in each summer, the drought treatment (D) was applied. This was carried out by automated rain exclusion curtains, controlled by a rain sensor, programmed to cover all 24 plots excluding rain during precipitation. Exposure to daytime elevated CO_2 was achieved by the FACE technique where CO_2 was injected along the perimeter of octagonal plots via injection tubes situated c. 40 cm above ground. The target concentration, 510 ppm_{vol} was measured in the centre of the 7 meter diameter experimental octagons. In all plots temperature and TDR probes were installed to measure temperature and moisture with mean values derived for every hour. Temperature probes were situated at 20 cm aboveground, at the soil surface and in 5 cm depths, TDR probes at 0–20 cm depth. Photosynthetic active radiation (PAR) was measured continuously with cosine corrected quantum sensors (OL-4000q, Optisk Laboratorium, Hørsholm, Denmark), precipitation (Rain-O-matic professional, Pronamic A/S, Silkeborg, Denmark) and air temperature in 2 meters height was measured at two stations at the site. The manipulations started in October 2005. Extended summer drought was carried out from July 3 – August 4 2006, May 21 – June 22 2007, and May 5 – May 27 2008. In 2006 8% and 2007 11 % of the annual rain fall was removed, while in 2008, 6 % was excluded. For further information about the experimental design, see Mikkelsen *et al.* (2008).

CO_2 flux measurements

For measurements of net ecosystem CO_2 exchange (F_n) and ecosystem respiration (R_E) we used a cubic 60*60*60 cm Plexiglas chamber that could be mounted gas tight on 60*60*10 cm stainless steel collars placed permanently in the experimental plots. Proper mixing of air inside the chamber was ensured by a fan mounted to the chamber wall. Concentrations of CO_2 inside the flux chamber were recorded every five seconds by an infrared gas analyser (IRGA) (CIRAS DC 10, PP Systems, Amesbury, Massachusetts, USA). Temperature and light intensities inside the chamber was obtained by a TRP-1 Temperature/Light (PAR) Probe (PP Systems). To obtain F_n at different light intensities, the chamber was after the first measurement in full light shaded by two different types of fabric. The first shade was thin white satin that excluded 30% of incoming PAR, the second shade coarse jute that excluded 70% of PAR. For R_E measurements the chamber was covered by opaque black Beaver Nylon in order to exclude all sunlight. For each measurement the chamber was in position for three minutes. Estimates of F_n were based on the initial 20 data points fitted by a

second order polynomium, and using the initial slope as the flux rate. The R_E fluxes were achieved by linear regression analysis based on the initial 40 data points. Flux chamber measurements in the FACE plots had to be conducted with special attention to the initial chamber CO_2 concentrations. Previous measurements have shown that air CO_2 concentrations in FACE plots is characterised by a significant short-term temporary variability, driven by wind speed and direction that may lead to marked concentration fluctuations. Under such conditions, chamber measurements can be substantially biased, depending on initial conditions (Selsted *et al*. 2010). In order to achieve uniform and reproducible conditions for chamber measurements, the CO_2 fumigation was consequently interrupted 30 minutes prior to commencement. Fluxes were monitored 14 times, in all treatments and replicates, between April 2006 and February 2007 and between December 2007 and July 2008, in total 583 and 654 observations of F_n and R_E, respectively. Moreover, at sunny days F_n was also measured excluding 30% and 70% of incoming PAR, adding 628 observations for modelling light response. Primary production by photosynthesis, P_g were calculated as $F_n - R_E$.

Fluxes are reported in μmol m^{-2} s^{-1}. A positive sign indicates effluxes of CO_2 and negative sign assimilation of CO_2 by the ecosystem.

Plant biomass estimates

Total aboveground biomass inside the 60*60 cm collars was estimated October 2006 by the non-destructive pin-point analysis method (Jonasson and Skold 1983, Jonasson 1988). A frame with a 10 x 10 cm fixed grid pattern was placed above the vegetation, and a 2 mm diameter pin was lowered vertically into the vegetation at each of the 25 grid points. Each hit on plant parts by the pin was registered by species and height until the tip of the pin was no longer visible. Further, it was recorded if the pin hit newly dead or alive plant parts. The pin-point analysis was converted into estimates of above-ground biomass by correlations with vegetation height and number of hit per pin on a plant species as outlined for the site by Riis-Nielsen and Schmidt (pers com).

Data analysis and statistics

Treatment responses were analysed by the repeated proc mixed procedure (SAS 9.1, SAS institute Inc. 2003). Main effects were the treatment factors T, D, and CO2 and all their interactions. Random factors included block and octagon. Biomass, PAR and air temperature were included as co-variates if significant at $P < 0.05$. Co-variates as soil temperature, CO_2 concentrations and soil water content were never included since they are manipulated by the experimental design and part

of the fixed effects. Homogeneity of variance was investigated with residual plots and data were log transformed when necessary. As covariance structure we used Heterogeneous Compound Symmetry (CSH) on ecosystem fluxes, and when testing treatment effects on environmental parameters Compound Symmetry (CS) was used as the most appropriate covariance structure. Tukey adjusted least square means were used to pairwise comparison of main effects and interaction terms.

Linear extrapolations

For the purpose of simple, linear extrapolations of F_n, light response functions of P_g were established for each season in 2006 (Fig. 1). Treatments specific functions were also established for the growing season when adequate data were available. Average P_g for each season was then calculated from the fitted curves by using the average PAR at light hours during the season. R_E was estimated for each treatment by simple arithmetic means and F_n calculated as P_g *(fraction of light hours per 24 hours) + R_E.

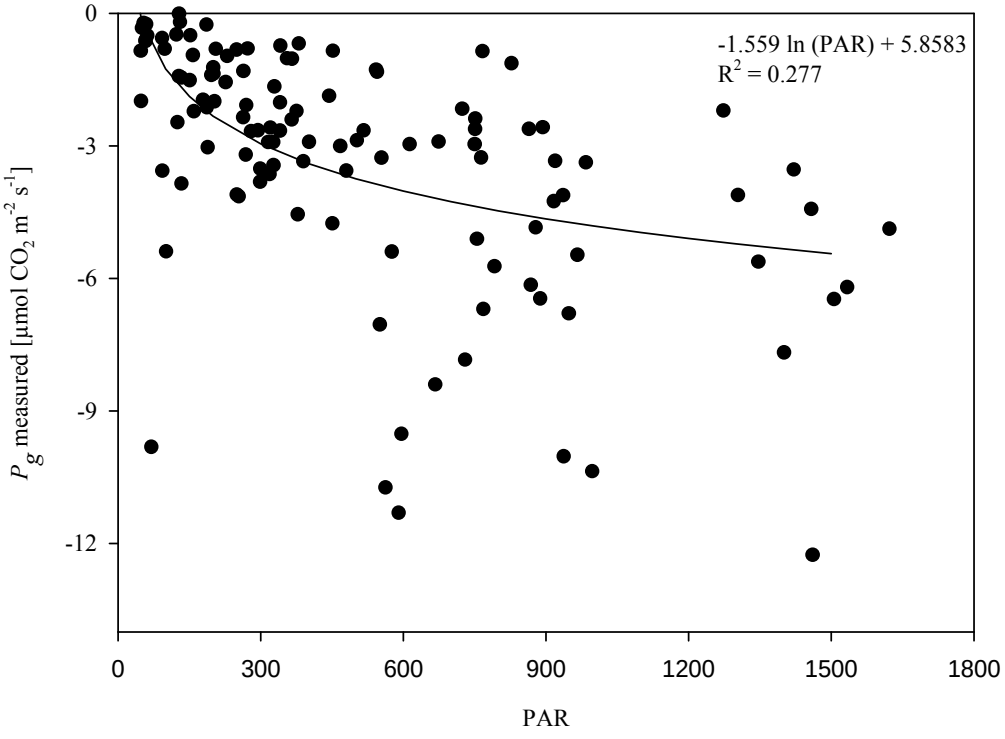

Figure 1. The relationship between photosynthesis and photosynthetic active radiation (PAR) measured in spring 2006. The line indicates the fitted light response curve.

Results

Plant biomass

Biomass estimates from 2006 did not reveal any treatment differences in biomass of *Deschampsia, Calluna* or total plant cover. The *Deschampsia/Calluna* ratio differed among plots, from 0.07 to 325, but no trends towards differences among treatments appeared, presumably because of initial patchiness of vegetation (Fig. 2).

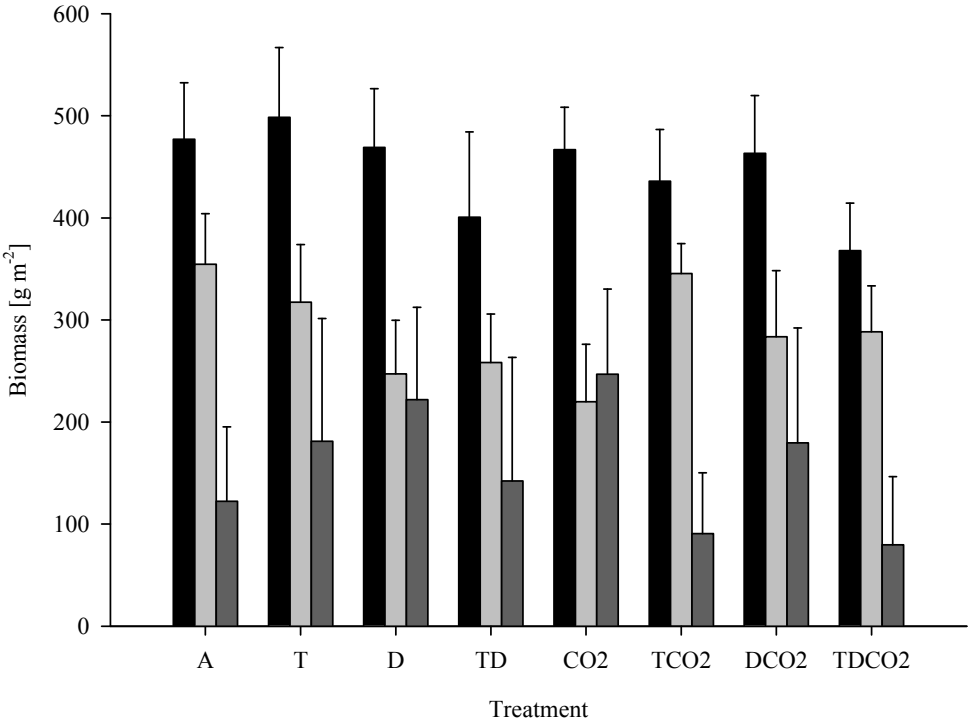

Figure 2. Plant aboveground biomass estimations from October 2006. Black bars indicate total biomass light gray bars the Deschampsia biomass, and dark gray bars the Calluna biomass in each treatment. Mean ± SE, n=6.

Treatment effects on ecosystem conditions

During January 2006 – July 2008 the main effects of T and D significantly ($P<0.0001$) reduced the soil water content in 0-20 cm depth (Fig. 3), with significant interactions D×CO2 ($P < 0.0001$) and T×D×CO2 ($P < 0.001$). Soil water in the D and TD treatments were on average 2.1 vol% ±0.3 and 3.3 vol% ±0.3 lower than the control, which on average over the whole period was 12.7 vol% ±0.3 (Fig. 3a). As expected, the drought treatment showed the highest impact on soil water content during drought treatment campaigns (Fig. 3a). Soil water content in drought treatments rapidly increased, up to a level of 0.5 vol% less than non-drought treated plots, during the first days after ended treatment. This level was maintained until the drought treatment the following year. Soil

water contents showed a different pattern in the T treatment, where moisture levels were close to ambient until May 2007, after which soil moisture decreased in the T treatment compared to control plots (Fig. 3b).

The only significant main effect on soil temperature in 5 cm depth was T ($P < 0.01$), which enhanced the soil temperature by 0.36 °C ±0.12 on average during 2006-2008. Separated into seasons, the temperature treatment raised soil temperature during spring (March, April and May) by 0.43 °C ±0.15, during summer (June, July and August) by 0.36 °C ±0.07, during autumn (September, October and November) by 0.43 °C ±0.17 and during winter (December, January and February) by 0.23 °C ±0.09.

Chamber based measurements of ecosystem CO_2 fluxes

Ecosystem respiration, R_E

The R_E fluxes ranged between 0.5 and 8 µmol CO_2 m^{-2} s^{-1}, and were in mere cases changed by the experimental treatments (Fig. 4). During the period April 2006 to July 2008 main effects T and CO2 significantly ($P < 0.01$ and $P < 0.05$ respectively) increased R_E, on average from 3.1 ±0.2 to 3.3 ±0.2 µmol CO_2 m^{-2} s^{-1} and from 3.0 ±0.2 to 3.5 ±0.2 µmol CO_2 m^{-2} s^{-1}. Variations in R_E were significantly related to the total plant biomass, ($P<0.01$), PAR ($P < 0.01$) and air temperature ($P < 0.05$). The drought treatment during summer 2006 and spring 2008 resulted in significant ($P < 0.001$ and $P < 0.05$) reductions in R_E of 24 % (2006) and 27 % (2008). Main effect T significantly increased ($P <0.05$) R_E in autumn 2006 (39 %), winter 2006/07 (42 %) and winter 2007/08 (44 %). The main effect CO2 increased ($P < 0.05$) R_E in autumn 2006 (90%) and spring 2008 (43 %), and in summer 2006 CO2 tended ($P < 0.1$) to increase R_E.

Primary production by photosynthesis, P_g

The P_g fluxes ranged between -0.7 and -14 µmol CO_2 m^{-2} s^{-1}. Analysis of variance on the dataset covering April 2006 – July 2008 showed significant ($P<0.05$) reductions in P_g of main effect D, on average from -5.9 ±0.4 to -4,9 ±0.4 µmol CO_2 m^{-2} s^{-1} (Fig. 4). As for ecosystem respiration, the primary production was significantly related to the covariates ait temperature, PAR and total plant biomass ($P<0.0001$). Main effect D was significant ($P<0.05$) in summer 2006 and reappeared in winters 2006-07 and 2007-08 ($P <0.05$ and $P<0.01$) reducing the photosynthesis by 20, 62 and 38 %, respectively (Fig. 4). Elevated temperature only became significant ($P < 0.01$) in winter 2007/08 by reducing P_g 41 % from -1.75 to -1.0 µmol CO_2 m^{-2} s^{-1}.

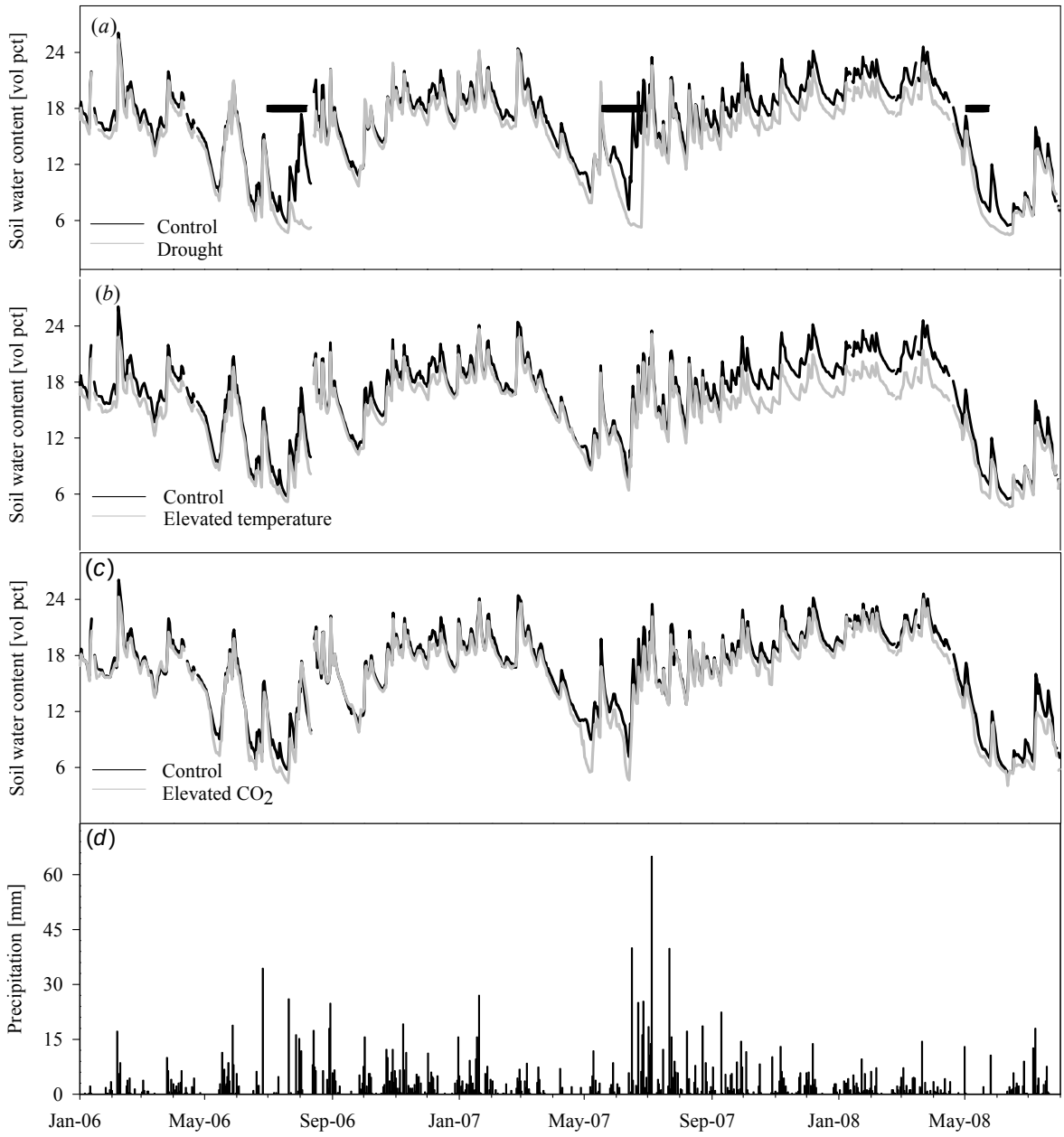

Figure 3. (a) soil water content in control (black line) and drought plots (gray line), horizontal black bars indicate exclusion of precipitation in D-plots. (b) soil water content in control (black line) and elevated temperature plots (gray line). (c) soil water content in control (black line) and elevated CO_2 plots (gray line). (d) pricipitation at the site.

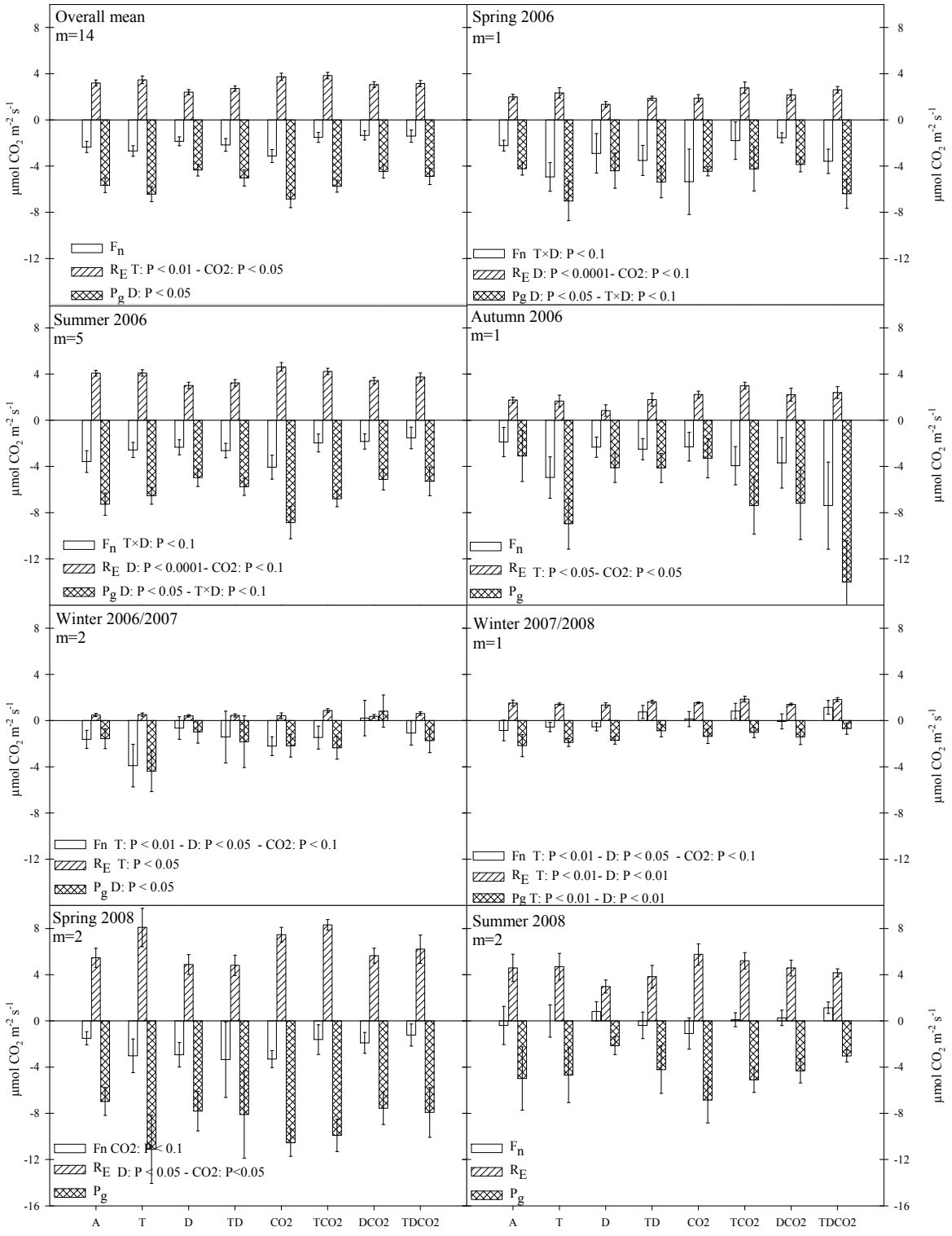

Figure 4. Measurements of net ecosystem exchange, F_n ecosystem respiration, R_E and claculated net photosynthesis, P_g. Fluxes are gruped by season and as overall mean ranging from spring 2006 to summer 2008. Statistical effects of factors and interactions are indicated in each subplot. Mean ± SE, n=6. Number of observations in each period is indicated by m, where each m includes 6 replicates.

Net ecosystem exchange, F_n

Analysis of variance on all field observed F_n (April 2006 to July 2008) did not show any significant difference in main effects or treatment interactions. Significant covariates were total biomass, air temperature and PAR ($P<0.001$). Looking more detailed into seasons drought reduced F_n ($P<0.05$) in winters 2006/2007 and 2007/2008 by 74 and 300 % respectively but not while treatment was applied in summer 2006 and spring 2008. Notably, warming promoted F_n in the winter 2006/07 by 40 % but had the opposite effect the following winter ($P<0.01$), where a reduction of 25 % was recorded. Enhanced CO_2 tended ($P<0.01$) to reduce the size of F_n both during winter 2006/07 and 2007/08 and during spring 2008 (Fig. 4).

Linear extrapolations

The linear extrapolations derived from seasonal PAR response functions and total light hours per season show that net ecosystem exchange, F_n in the control plots almost were in a steady state, with equal rates of respiration and photosynthesis (Table 1). The net carbon balance seems to be dependent on the atmospheric CO_2 conditions, with a trend towards a net loss of carbon in all CO_2 treated plots, and a neutral to accumulating balance for non-CO_2 treatments. Most carbon accumulation was found for the D-plots (140 ± 36 gC m^{-2} $year^{-1}$), while TCO2 plots lost most carbon (313 ± 426 gC m^{-2} $year^{-1}$). On the yearly scale ecosystem respiration, R_E was limited by the drought treatment, promoted by the elevated CO_2 and not influenced by the temperature treatment as single factor treatment. The D plots respired 32 % less than the control plots, respiration in CO2-plots were 10 % enhanced, while D and CO2 in combination neutralised each other. The temperature treatment increased respiration when in combination with both D and CO2, resulting in 13 % respiration in the three factor combination TDCO2 compared to the ambient control. Photosynthetic activity was calculated to be very similar in all combinations, with a trend of a reducing effect of the drought treatment.

Table 1. Linear extrapolations of measurements, year 2006, total net carbon uptake, F_n total ecosystem respiration, R_E and total photosynthesis, P_g (standard errors in brackets) and the treatment change relative to the ambient control. Negative fluxes indicate a carbon uptake to the ecosystem, positive fluxes a loss.

2006 - linear extrapolations						
	F_n		R_E		P_g	
	[gC m-2 year-1]	[pct] change to A	[gC m-2 year-1]	[pct] change to A	[gC m-2 year-1]	[pct] change to A
A	17 (98)	0	774 (81)	0	-758	0
T	54 (183)	228	803 (129)	4	-749	-1
D	-140 (36)	-942	524 (104)	-32	-664	-12
TD	-3 (106)	-116	684 (108)	-12	-687	-9
CO2	66 (180)	300	855 (114)	10	-788	4
TCO2	313 (426)	1783	1014 (113)	31	-702	-7
DCO2	109 (246)	558	763 (136)	-1	-654	-14
TDCO2	218 (339)	1213	874 (121)	13	-656	-13

Discussion

Methodology

As mentioned in the methods section, we measured all fluxes under ambient CO_2 concentrations, regardless of treatment, due to the restrictions of the chamber methodology. Fredden *et al.* (1995) showed that a C3 grass grown in an elevated CO_2 atmosphere had about 100 % higher F_n when measured in elevated CO_2 compared to measurements at ambient CO_2, as the Rubisco activity is reduced in plants grown in elevated CO_2 (Fredden *et al.,* 1995; Moore *et al.*, 1999). At the CLIMAITE site we found that F_n in elevated CO_2 plots, and measured under ambient conditions might be underestimated by as much as 70% (Selsted et al, in revision). Consequently, comparison of chamber based CO_2 fluxes between non-CO_2 vs CO_2 treated plots should be done under careful attention to possible biases in data. In the current work we have considered our observations among all treatments as an attempt to strengthen the analysis of main effects of elevated soil temperature and summer droughts. Based on leaf photosynthesis response to CO_2 concentration (A/c_i) measured throughout 2007 at the CLIMAITE site a correction factor of P_g of 1.1 was obtained (per comm Ibrom A, 2010), however measurements were not corrected by that factor, but the discussion should of cause include a possible underestimation of measured P_g in CO2 plots.

Ecosystem respiration, R_E

It is well documented that ecosystem respiration depends on soil water content, seasonal temperature fluctuations and substrate input (e.g. Davidson et al., 2006; Larsen et al. 2007; Aires et al., 2008). This is in line with our findings and, as hypothesised, R_E was generally constrained under reduced soil water conditions, probably due to lower microbial activity (Skopp *et al.* 1990) and root activity (Borken *et al.* 2006). Moreover, that strong relationship between R_E and co-variates PAR and plant biomass also confirms the general conception that the ecosystem respiration is controlled by substrate inputs from photosynthesis (Larsen et al. 2007). The suppression of R_E by the drought observed in summer 2006 and spring 2008 was only transient and not apparent at subsequent measurements in autumn 2006 and summer 2008, respectively. In contrast, the suppression in R_E implied by the drought treatment in summer 2007, with a strength similar to the 2006 drought, persisted throughout the following winter 2007. However, as in contrast to the 2006 and 2008 campaigns, soil moisture in 2007 did not fully recover to the pre-treatment level (Fig. 3a), which further emphasises the importance of soil moisture for overall ecosystem respiration.

Our experiment confirms the general perception that elevated CO_2 increases R_E . The increase in R_E can be ascribed to higher photosynthetic activity in CO2 plots (Nowak *et al.,* 2004) promoting more growth of biomass above and below ground, which will lead to higher autotrophic and heterotrophic respiration (Luo *et al*., 1996; Craine *et al*., 2001, Wan *et al*,. 2007; Pregitzer *et al*., 2008). However, change in R_E could not be observed in the winters 2006/07 and 2007/08, which could be explained by low heterotrophic and autotrophic activity in the cold period.

We hypothesised that elevated temperature would have a positive effect on R_E. This was partly confirmed by the significant positive temperature effects observed in the colder periods, i.e. autumn-winter 2006-2007 and in winter 2007/08. This seasonality in temperature response is very likely due to the fact, that the relative experimental temperature increase was relatively higher during winter months compared to the summer months, i.e. 0.23 ^{o}C increase from 4.4 ^{o}C versus 0.36 ^{o}C increase from an average of 16 ^{o}C.

By comparison to another study in a similar Danish heathland (Larsen et al., 2007), the current R_E during summer control plots were rather low, 4.59 ± 1.18 μmol m^{-2} sec^{-1} , while Larsen et al. (2007) reported rates of 12.2 ±1.0 μmol m^{-2} sec^{-1}. Winter recordings from the two sites were similar, 0.48 ± 1.14 μmol m^{-2} sec^{-1} in winter 2006/07 and 0.59-0.99 μmol m^{-2} sec^{-1}in the heathland investigated by Larsen et al. (2007). The differences between the two sites can probably be explained by different hydrological conditions, where soil water contents in the current heathland approaches 7 vol% during the summer, while the other heathland maintained soil water above 15 vol% during summer Larsen et al. (2007), again showing that water is an important control on R_E.

Primary production by photosynthesis, P_g

As for R_E, we hypothesised that elevated temperature would enhance the overall P_g due to an earlier onset of growing season (Penuelas, 2007), and that biological activity generally is enhanced from higher temperatures. The temperature treatment did, however, not influence P_g, besides in winter 2007/08 where it reduced P_g. This reduction was probably an indirect effect of the elevated temperature, namely a result of reduced soil water content in warmed plots (Fig. 3), which is also confirmed by the general 17% reduced P_g in response to the drought treatments. Aires et al. (2008) found for a Mediterranean grassland that both on the seasonal and inter-annual scales P_g correlates positive with water use efficiency and light use efficiency, which magnitudes were correlated to water availability. If this applies to the current heathland, reduced P_g in winters could be explained by a general lower biomass in drought treated plots. Even when soil water contents in drought plots re-established to control level during autumn and winter, we would as a consequence of less growth expect reduced photosynthetic activity. Low water availability might be caused by a drought-induced temporary water repellency of the soil creating water flow patterns unfavourable for vegetation to access. Over time, one could expect a changed soil texture as a result of repeated summer droughts leading to permanent all year changes in biomass production as soil water holding capacity will become smaller, and plant water availability will become less (Muhr and Borken 2009).

Based on several studies (e.g. Fredden et al. 1995; Leakey et al. 2009; Bachman et al., 2010) we hypothesised that P_g would increase when exposed to elevated CO_2. Due to easy access to CO_2, plant leaves may reduce their stomatal conductance in elevated CO_2 environments resulting in enhanced water use efficiency, again leading to higher soil water content (Craine *et al.* 2001; Dermody *et al*. 2007; Garten *et al*. 2009*;* Leuzinger and Körner 2010). Our results did, however, not reveal any effect of elevated CO_2 on P_g. When taking a underestimation of P_g of 10 % into account the effect of elevated CO_2 does significantly (P = 0.05) increase P_g in CO2 plots.

Net ecosystem exchange, F_n

Generally, none of the treatments affected the magnitude of the net ecosystem exchange. Transient effects of the modest elevation of soil temperature was observed in winter 2006/07 when net carbon uptake was increased by 40 % by the elevated temperature, and in winter 2007/08 when F_n was reduced 25%, the latter probably due to reduced soil water content. Transient effects of the drought treatments were also observed in the winters 2006/2007 and 2007/2008, when the net carbon uptake

was reduced by 74% and 300%, respectively. The net ecosystem exchange was vaguely reduced under elevated CO_2 conditions during three periods, i.e. winter 2006/07, winter 2007/08 and spring 2008, but effects were not statistically significant ($P < 0.1$; Fig. 4). This trend is in agreement with the conclusions by Shaw *et al,.*(2002), who found that elevated CO_2 in combination with e.g. elevated temperature suppressed F_n in a California grassland, however taking the expected underestimation of photosynthesis into account the reducing tendency disappears.

Linear extrapolations

The extrapolation of the measured fluxes to the entire year of 2006 showed that ambient plots approximately had balanced P_g and R_E, and hence a F_n close to 0. The ecosystem has, however, shown increased and not decreased plant cover since the beginning of the experiment (Kongstad et al., unpublished), This in combination with a balanced carbon budget indicates that the increased plant cover has promoted soil activity and by that decomposition of soil organic matter, hence increased soil respiration. The highest net uptake of carbon was surprisingly found in the drought treatment (140 gC m^{-2} $year^{-1}$), giving that ecosystem respiration was more restricted than production, which is in contrast to Arnone III *et al.*(2008) who during the 2003 hot and dry climatic extreme in Europe found that carbon loss through respiration outbalanced at least four years of net uptake due to decreased productivity. On the yearly scale measurements of net carbon balance decreased as a consequence of the elevated CO_2, respiration increased relatively much more than photosynthesis (table 1). Taking an underestimation of 10 % on photosynthesis into account the net balance tends of a system in a steady state, although still with a net loss of carbon in the combined treatments. Comparing with others (Aeschlimann et al., 2005 and Li et al., 2004) reports no or very little effect of elevated atmospheric CO_2 in two grasslands.

Conclusion

Ecosystem carbon exchange in response to global change

We investigated ecosystem CO_2 fluxes responses to elevated temperature, extended summer drought and elevated CO_2 in a low vegetation heathland. From field measurements we saw an enhancing effect of elevated CO_2 on ecosystem respiration. The extended summer drought decreased photosynthesis and ecosystem respiration during treatment and also during winter, an effect that we ascribe to a lower production in the growth season resulting in reduced activity in winter. We hypothesised that elevated temperature would enhance fluxes, and for ecosystem respiration that applied. On net ecosystem exchange we did, however, not see this effect. When

combining our findings with others, we conclude that elevated CO_2 does enhance both respiration and photosynthesis, whereas the effect on net carbon balance is very limited and might even suppress ecosystem carbon net uptake.

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Presentation of results of ^{13}C labeling experiment

Carbon allocation of newly assimilated carbon in a Danish heathland under future climatic and atmospheric conditions – a ^{13}C labeling study

The strongest conclusions from the measurements presented in paper II and III are the increase in soil respiration and ecosystem exchange due to elevated CO_2. Moreover, we found a reducing effect of extended summer drought as well as interactions between elevated temperature and extended summer drought, which decreased the fluxes even further. Effects on net ecosystem exchange were not as strong as effects on respiration. Measurements showed that the control plots were either in a steady state or in fact were a net source of carbon and overall, the conclusion of the projected Danish climate year 2075 on the heathland ecosystem is emissions of additional carbon. It is a well established theory that the carbon turnover is higher in ecosystems treated with elevated CO_2 (Ross et al., 1996; Niklaus et al., 2004), which is also supported by our flux measurements. To strengthen findings from flux measurements and to reveal where newly assimilated carbon were allocated a $^{13}CO_2$-pulse labeling study were performed. The aim was to fractionate between loss by respiration, carbon used for plant growth, and carbon sequestered to the root-soil environment and eventually incorporated into microbial biomass.
In the following, the experiment is outlined and the results presented.

Material and Methods
In April 2006, four PVC cylinders (height 15 cm, Ø 15 cm) were pushed down in every plot (in total 48) at the study site around already established *Deschampsia flexuosa* patches. In October 21 2006 and June 30 2007 one cylinder (mesocosm) was collected from each plot for ^{13}C -pulse labeling. In 2007, the mesocosms were collected eight days after the first rain event following termination of the experimental drought treatment. Five days before collection of mesocosms, background samples of 5 cm diameter soil cores intact with roots and grass were collected next to each cylinder. Background samples were not wetted, incubated or labeled, but with regard to analysis treated as the mesocosms. Arriving at the laboratory, the mesocosms were given water equal to 15 mm rain, reflecting field capacity. The Mesocosms were placed on a table to drain and left for 3 days in a growth chamber. In October 2006, the temperature in the chamber was 10^oC with 14 hours of light and 10 hours of darkness per 24 hours. In July 2007, the temperature was 15^oC with 18 hours of light and 6 hours of darkness per 24 hours. The ^{13}C-pulse labeling was carried out in a gastight Plexiglas chamber by exposing the mesocosms to an

atmosphere enriched with $^{13}CO_2$, see fig 1. Lights were dosed from above and from the sides to ensure that all mesocosms were exposed to the same intensity of light.

Figure 1 . $^{13}CO_2$ labeling experiment

The background level of CO_2 in the climatic chamber was very high, about 600 ppm. During the six hours of labeling, the aim was to reach a CO_2 level of 1000 ppm, which should ensure a high fraction of ^{13}C, see table 1 for further details.

	Adding of $^{13}CO_2$	Labeling time [hours]	Avg [CO2] during labeling [ppm]	Avg $\delta^{13}C$ during labeling [‰ vs VPDB] and [AT%]	Avg temp during labelling [oC]
October 2006	0.5 l ^{13}C02 was added in the beginning of the experiment and again after 3 hours.	6	870	12,061/12.74	15
July 2007	0.5 l ^{13}C02 was added in the beginning of the experiment.	6	1600	8,485/9.59	18

Table 1. Details on labeling of mesocosms in 2006 and 2007

Gas samples for $^{13}CO_2$ detection by GC-IRMS were collected every hour simultaneously with total CO_2 measurements by a connected IRGA, CIRAS DC 10, PP-System. After labeling, the mesocosms were incubated for one week in the climatic chamber. During incubation, respiration and ^{13}C content in respiration air were measured on each mesocosms at day 1, 2, 4 and 6. This was done by a cylindrical respiration chamber (Ø=23.5 cm, H=29 cm) placed gas tight over one mesocosm at a time. Via a rubber stopper in the lid, 20 ml sample air was taken by syringe at time 0, 4 and 8 minutes after chamber closure (see fig. 2). A 1.8 ml vial was flushed by approximately 17 ml air before filled with 1 ml pressure above the atmosphere. Gas samples were analysed by GC-IRMS for both total CO_2 concentration and ^{13}C. Respiration rates were calculated by linear regression with the unit μmol m^{-2} s^{-1}. The content of ^{13}C in respiration air was found by use of Keeling plots: 1/[CO2] vs $\delta^{13}C$, where the intercept expresses the value of respired $\delta^{13}C$. Respiration rates were averaged over the period with the assumption that the rate did not change during incubation. The $\delta^{13}C$ signal in respiration air was expected to follow a decreasing course during the period of incubation. Due to large standard errors, a simplification was, however, taken and an average was calculated. The accumulated loss of carbon during the seven days of incubation related to carbon taken up via photosynthesis during the six hours of labeling were calculated as follows:

$$\frac{Average\ respiration\ rate * 13C\ enriched\ fraction\ of\ respiration * 7\ days}{fraction\ of\ 13CO2\ in\ air\ during\ labeling} \qquad (2)$$

Where an assumption of background respiration of $\delta\ ^{13}C$ = -26 ‰ in control plots and -32.5 ‰ in FACE plots were taken, as it was not measured. The value of -26 ‰ is an estimate from literature (Yakir and Sternberg, 2000), while the offset of -6.5 ‰ is the difference between aboveground biomass ^{13}C content in control plots and FACE plots.

Figure 2. Respiration measurements of mesocosms, open chambers to the right, closed chambers at left.

The seventh day after labeling, the experiment was stopped by separating the mesocosms. The top 10 cm soil was sieved, thereby separating soil from roots. Soil was analysed for water content and dried at room temperature for further analysis of total C and ^{13}C contents on EA-IRMS. Plant tissue (above green, and roots) were separated and dried at 80^{o}C, also for analysis of total C and ^{13}C on EA–IRMS. Enrichment of ^{13}C was calculated ($^{13}C_{\text{labeled tissue}} - {}^{13}C_{\text{background tissue}}$). Enrichments of ^{13}C were converted to total C uptake per area by:

$$\frac{Total\ weight\ of\ tissue * fraction\ of\ C\ content\ in\ tissue * 13C\ enriched\ fraction}{fraction\ of\ 13CCO2\ in\ air\ during\ labeling * area\ of\ mesocosm} \qquad (3)$$

Microbial biomass C was obtained by the chloroform fumigation–extraction procedure (Vance et al., 1987). Organic C extracted from fumigated and non-fumigated samples of 10 g soil with 40 ml of 0.5M K_2SO_4 was measured on an organic C analyser. The biomass C was calculated from the relationship $C_{biomass}$=2.22*EC, where EC is [$C_{fumigated}$]-[$C_{non\text{-}fumigated}$] (Wu et al., 1990). Extracts were analysed for ^{13}C content on EA-IRMS, and total C uptake to microbial biomass was measured and calculated as described above for plant tissue and soil.

Data analysis and statistics

Treatment responses were analysed by the proc mixed procedure (SAS 9.1, SAS institute Inc. 2003) using the repeated design when repeated measures were collected (e.g. respiration of mesocosms). Main effects were the treatment factors T, D, and CO2 and all their interaction terms. Random factors included block and octagon. Covariates as biomass were included if

significant at p<0.05. Homogeneity of variance was investigated with residual plots and data were log transformed when necessary. As covariance structure in the repeated design, Compound Symmetry (CS) was used. Tukey adjusted least square means were used to compare interaction terms and treatment effects.

Results and discussion

Respiration

The two labeling experiments were conducted in October 2006 and July 2007. In 2006, biomasses (roots and above ground green) in mesocosms were not significantly different between treatments, while in 2007, the above ground biomass were significantly (P<0.05) increased by main effect CO2, while root biomasses were reduced (P<0.01) in T plots (fig. 3). In the following, these effects are taken into account by using biomass as covariate when performing the statistical tests. The CO2 and T effects could be argued as a consequence of the applied treatments, and should in any case be discussed.

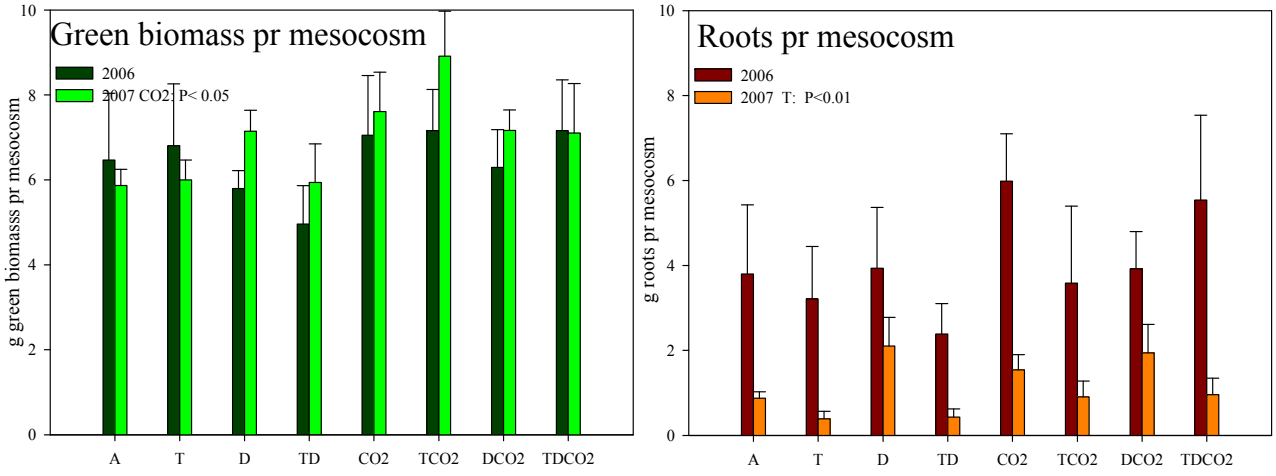

Figure 3. Biomasses in mesocosms collected for $^{13}CO_2$ labelling study.

The average content of $\delta^{13}C$ in respiration air during the four measuring days in autumn 2006 indicated no treatment effects on back respiration of newly assimilated carbon. In summer 2007, when conditions were dry and even dryer in drought and temperature treated plots (fig. 3 in paper II), the content of $\delta^{13}C$ in respiration air was significantly reduced in temperature and drought treated plots, P<0.05 and P<0.01, respectively (fig. 4). This effect could either indicate lower photosynthetic rates in T and D plots resulting in less respiration or a higher assimilation of carbon. With reference to flux measurements of soil respiration (paper II) and ecosystem respiration (paper III), higher respiration rates in CO_2 treated plots were expected, but as shown

in paper I, the high respiration only lasted for a limited amount of time (18 hours) at ambient levels and roots activity decreases (paper I). CO_2 levels in the growth chamber, were, however, very high and the opposite might be the case, namely an up regulation of plant activity in ambient CO_2 treatments (Fredden et al. 1995). Measurements from present study show 27% higher respiration rates from CO2 plots (P<0.01) in October 2006 and reduced rates (22 %) in same treatment July 2007 (P<0.05). That respiration were higher in the CO2 treatment (in 2006) and no effect were found in ^{13}C respired, indicates that the CO2 treatment stimulates soil activity and decomposition of soil organic matter. This theory that is also discussed and applies to results in paper III.

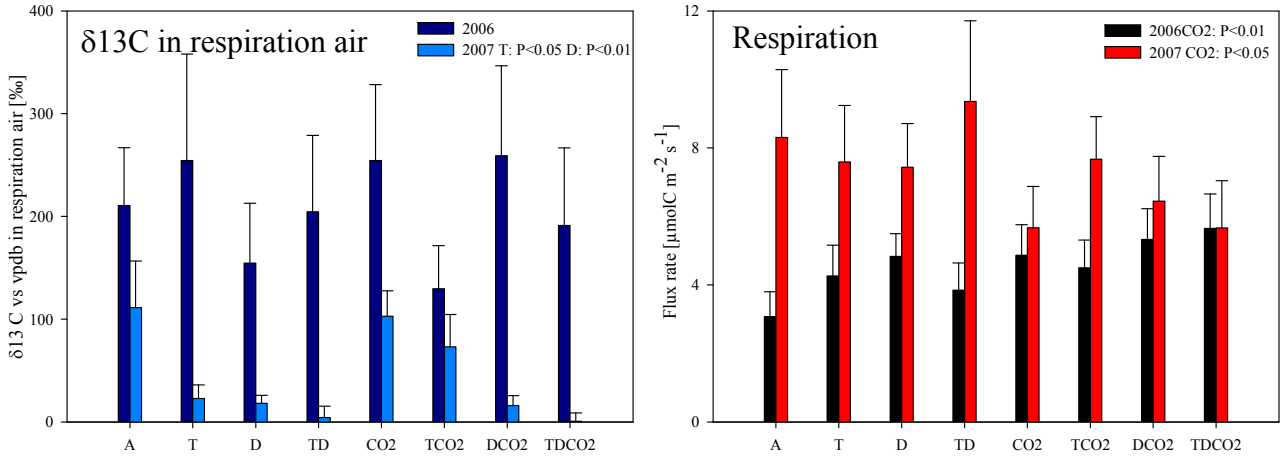

Figure 4. Left: Average δ13 C in respiration air during seven days of incubation. Right: Average respiration rate during 7 days of incubation.

Compared with field ecosystem respiration measurements (paper III), the fluxes from mesocosms were up to twice as high as field measurements. In autumn 2006, field observations ranged between 1 and 3 µmol m^{-2} s^{-1}, while rates from mesocosms were between 3 and 6.5 µmol m^{-2} s^{-1}. No field observations were collected during summer 2007, but summer field measurements in 2006 yielded rates between 3 and 4.5 µmol m^{-2} s^{-1} compared to mesocosms rates in July of 5.5 to 9.5 µmol m^{-2} s^{-1}. Elevated CO_2 generally enhanced field ecosystem respiration (P at least <0.1); by 11 % in the summer of 2006, 26 % in the summer of 2008 and 63 % in the autumn of 2008 (paper III). In autumn 2006, elevated temperature as main effect increased soil respiration by 20 % (P<0.05) and in the summer, extended drought treatment resulted in 15 % reduced respiration, though only in summer (2006) where the treatment was actually applied during the summer months. In 2008, the drought treatment was applied in spring and the effect was present in spring and absent in summer. Unfortunately there are no

data from summer 2007, but following the pattern of 2006 and 2008 a drought and CO_2 effect was expected.

These labeling experiments show how difficult it is to bring the eight CLIMAITE treatments to the lab with the idea of performing realistic experiments. The increased effect of elevated CO_2 was both seen in field observations and in the lab in autumn 2006, while the reduced CO_2 effect in summer 2007 was opposite in the lab experiment as compared to the general trend of field observations. Background concentrations of CO_2 were high in the growth chamber, where respiration measurements took place ~600 ppm. As discussed in paper I and III, high levels of atmospheric CO_2 results in an up regulation of photosynthesis in plants grown at the site at ambient CO_2 and consequently leading to increased respiration rates. However, it is difficult to compare treatment effect from field observations with the response of the mesocosms. Fluxes were calculated per square meter by linear regression, knowing that in the field root biomasses presumably were 10 % higher in FACE plots compared to ambient plots. This relationship was not found in mesocosms, but fluxes were nevertheless calculated per square meter and not per biomass. Moreover, the vegetation in the field flux-frames were covered by both *Calluna* and *Deschampsia* (fig. 2, paper II), while the mesocosms were only occupied by *Deschampsia.* The first speaks for measurements of relative lower fluxes in FACE mesocosms compared to field observations, and the latter for general higher respiration rates measured in mesocosms compared to field observations as the grass at the CLIMAITE site is more productive than the heather during optimal conditions (Albert *et al.,* 2010 unpublished)
The effect of elevated temperature on respiration were limited and almost absent in field flux measurements (paper II and III). Though in winter elevated temperature tends of a positive effect enhancing ecosystem fluxes as the relative temperature enhancement were larger in winter compared to summer (paper II). The consequences of bringing the mesocosms to the lab with regard to the temperature treatment might therefore not be critical to flux measurements.

Tissue enrichments

After exposure to six hours of an enriched $^{13}CO_2$ atmosphere, the mesocosms incubated for seven days followed by measurements of ^{13}C enrichment in carbon contents of green biomass, roots, soil and microbes (see fig. 5).

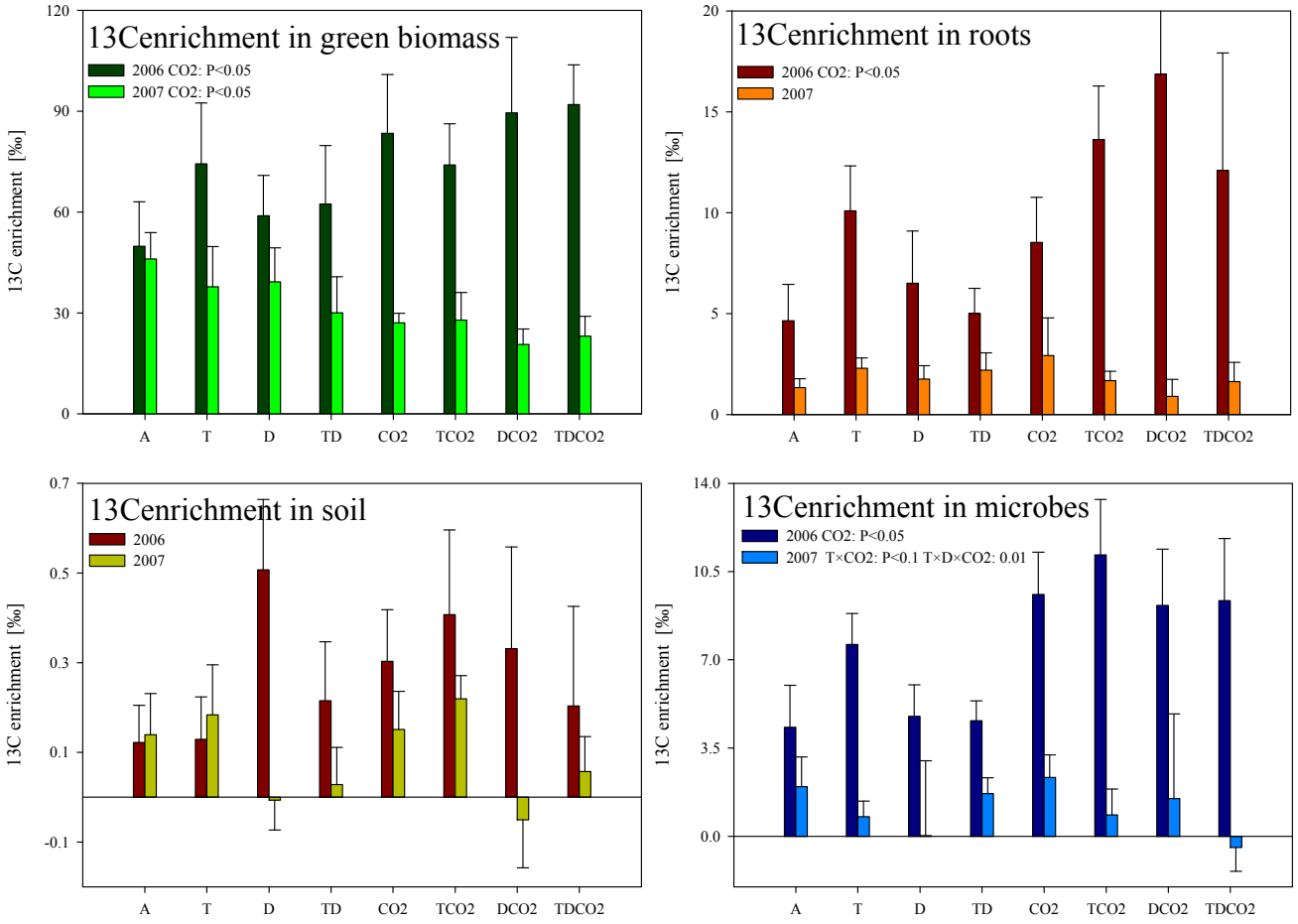

Figure 5. ^{13}C enrichment in green biomass, roots, soil and microbes 7 days after labeling.

In 2006 the ^{13}C content of green biomass was on average enriched by 73 ‰, roots 10 ‰, soil 0.3 ‰ and microbial biomass 8 ‰. In 2007 the corresponding values were: 31 ‰, 2 ‰, 0.1 ‰ and 1 ‰. In 2007 the $\delta^{13}C$ content of labeling air was 41 % lower compared to the 2006 experiment (Table 1). The enrichments were therefore lower, regardless of photosynthetic activity.

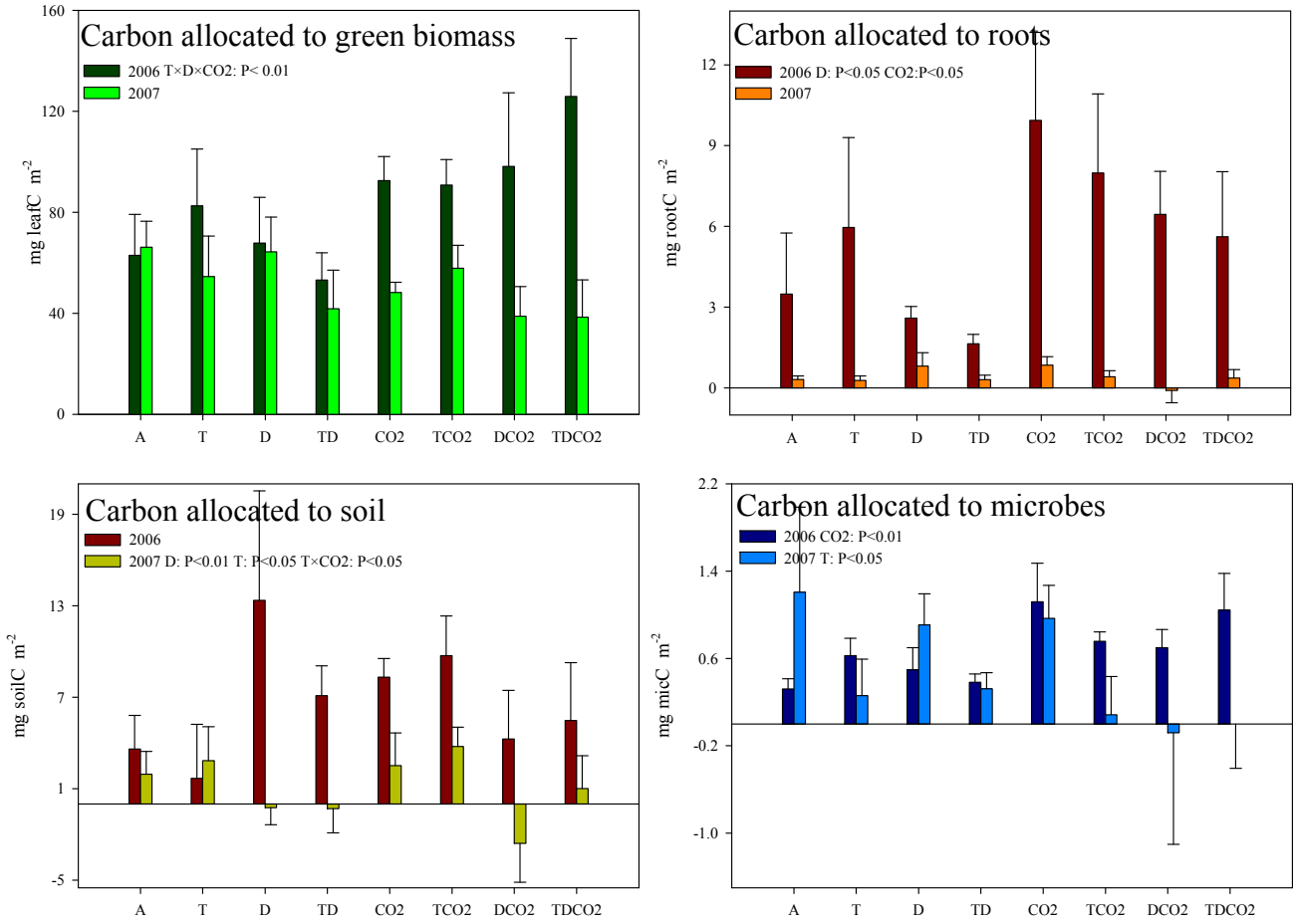

Figure 6. Total carbon incorporated in green biomass, roots, soil and microbes from uptake during 6 hours of labeling 7 days earlier.

^{13}C enrichments in 2006 points towards an effect of elevated CO_2: Both green biomass, roots and microbial biomass were more enriched if treated with elevated CO_2 ($P<0.05$). In 2007 respiration and the ^{13}C enrichment in green biomass were reduced by the FACE treatment. A simple explanation could be up regulation of photosynthetic activity in treatments grown in ambient CO_2, when exposed to the high level of CO_2 during labeling (paper I and III). If this effect was present in October 2006, it was not as pronounced. Calculating the total uptake of carbon per square meter, the pattern is still the same as the ^{13}C enrichments (see fig. 6). The reduced CO_2 effect on green biomass July 2007 does, however, disappear as the mesocosms treated with elevated CO_2 had increased green biomass (fig. II).

Seven days carbon budget

Carbon budgets of each treatment in October and July were calculated. Averages were calculated separately for ambient CO_2 treatments and elevated CO_2 treatments for October data,

but for July data, total averages were calculated as no consistent effect of treatment were found (see fig 7). The budgets represent the accumulated respiration of carbon that has been assimilated during labeling the seven following days and the distribution of carbon in plant and soil also after seven days. Their sum represents the carbon that has been assimilated by photosynthesis during six hours of labeling.

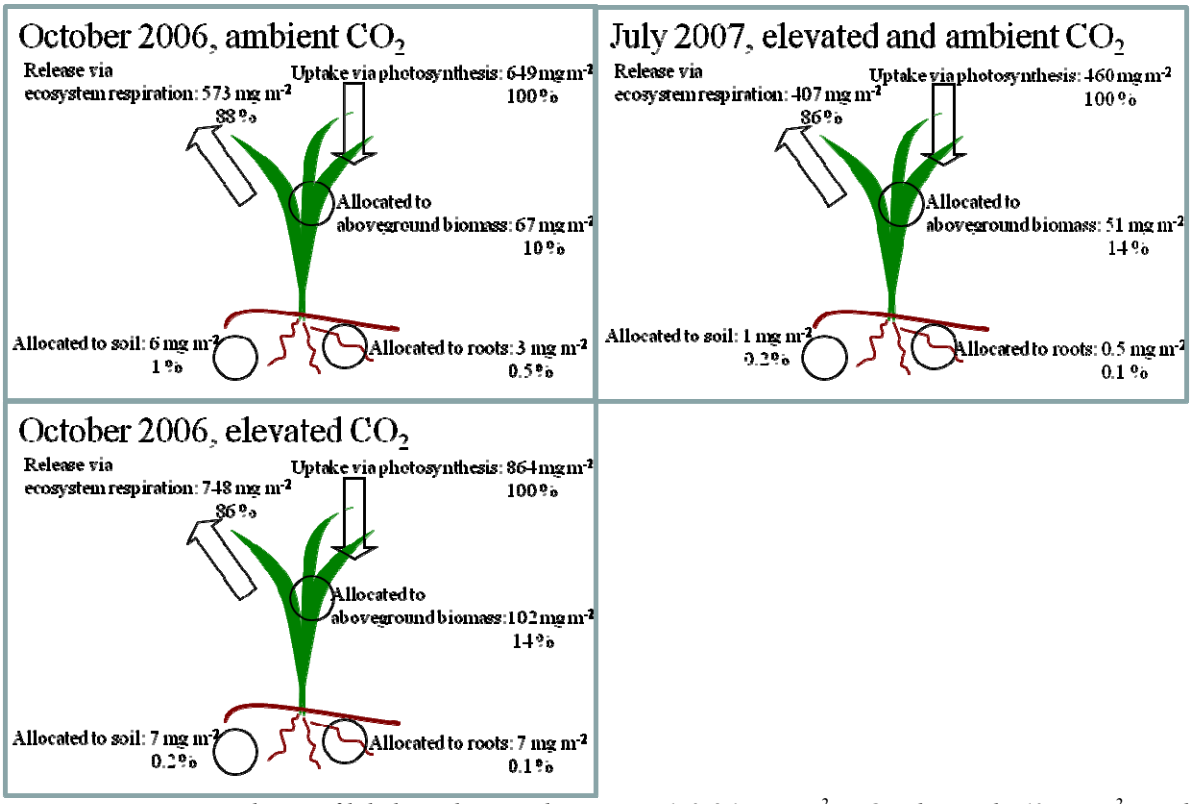

Figure 7. During six hours of labeling photosynthesis were 649-864 mg m^{-2} in October and 460 mg m^{-2} in July. During the next seven days, 86-88 % of assimilated carbon was lost via respiration, 10-14 % allocated to the aboveground biomass, 0.1-0.5 % to roots and 0.2-1 % ended up in the soil.

In October 2006 photosynthetic activity per square meter was 33 % higher in plants grown in elevated CO_2 compared to plants grown in ambient CO_2. Ambient plants lost 2 % more of the assimilated carbon via respiration, while plants grown in elevated CO_2 kept 4 % more in green tissue and did not allocate as high a fraction of carbon to roots and soil. Ambient grown plants allocated 1.5 % of assimilated carbon to roots and soil during the seven days of incubation. This number was only 0.3 % for plants from FACE plots. In July 2007, the distribution of assimilated carbon was the same as for October 2006, elevated CO_2. Though only 460 mg C m^{-2} were taken up by photosynthesis, which were half of what the plants grown in elevated CO_2 assimilated in October 2006 (see fig. 7). As the biomasses in 2006 and 2007 per ground area were very similar (see fig II), the plants were less active in July. The growth pattern of

Deschampsia at the CLIMAITE site is divided in two periods. Growth starts in spring with biomass peak late June, hereafter the mortality exceeds growth. Late July the plants start to grow again until late October, where the biomass decreases (Kongstad, 2010). Growth pattern of *Dechampsia* at the CLIMAITE site confirms the findings from this labeling experiment. In July, the measurements were made on an ecosystem that was in a state of decreasing its biomass, while in October, the biomass was more in a steady state.

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