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TECHNICAL REPORT

Experimental design of multifactor climate change experiments with elevated CO_2, warming and drought: the CLIMAITE project

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Summary

1. Recent findings indicate that the interactions among CO_2, temperature and water can be substantial, and that the combined effects on the biological systems of several factors may not be predicted from experiments with one or a few factors. Therefore realistic multifactorial experiments involving a larger set of main factors are needed.

2. We describe a new Danish climate change-related field scale experiment, CLIMAITE, in a heath/grassland ecosystem. CLIMAITE is a full factorial combination of elevated CO_2, elevated temperature and prolonged summer drought. The manipulations are intended to mimic anticipated major environmental changes at the site by year 2075 as closely as possible. The impacts on ecosystem processes and functioning (at ecophysiological levels, through responses by individuals and communities to ecosystem-level responses) are investigated simultaneously.

3. The increase of [CO_2] closely corresponds with the scenarios for year 2075, while the warming treatment is at the lower end of the predictions and seems to be the most difficult treatment to increase without unwanted side effects on the other variables. The drought treatment follows predictions of increased frequency of drought periods in summer. The combination of the treatments does not create new unwanted side effects on the treatments relative to the treatments alone.

Key-words: ecosystem manipulation, FACE, grassland, heathland

Introduction

There is a growing consensus that 20th century human activities have induced dramatic and unprecedented changes in the global chemical and physical environment and current predictions indicate that, unless greenhouse gas emissions are significantly curtailed, atmospheric CO_2 concentrations will double during the present century (IPCC 2001). This will induce a 1·4–5·8 °C increase in mean global temperature, alterations in patterns of global air circulation and in the hydrologic cycle that will affect global and regional precipitation patterns, and increase the frequency and magnitude of severe weather events, including droughts and floods (Easterling *et al.* 2000; IPCC 2001). Such climatic and environmental changes will have strong effects on the terrestrial

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ecosystems and its organisms, create a complicated pattern of responses and induce a cascade of effects on ecosystem processes and ecosystem functioning that is extremely difficult to foresee.

In order to improve our understanding of the effects of climatic changes on ecosystems, many experimental manipulation studies have been conducted in terrestrial ecosystems around the world including elevated CO_2, increased temperature or changes in precipitation amounts or patterns (e.g. Wright 1998; Jonasson *et al.* 1999; Knapp *et al.* 2002; Beier *et al.* 2004). With very few exceptions, these manipulations have not combined CO_2 increase with changes in both temperature and precipitation. However, changes in CO_2, temperature and precipitation will interact, and the effects of multiple climatic and environmental stress factors may not be easily predicted from single factor studies as recently demonstrated by Shaw *et al.* (2002). Consequently, there is a clear need for studying effects of environmental multifactor changes on biological systems.

We here describe a new multifactor experiment, CLIMAITE – Climate change effects on biological processes in Terrestrial Ecosystem involving combined treatments manipulating the atmospheric CO_2 level, temperature and water supply. CLIMAITE aims to study the individual and combined effects of the climate change parameters on processes, structure and functioning of a semi-natural ecosystem. The paper describes documents and discusses the design and functionality of this multifactorial experimental project with particular focus on interactions and artefacts related to the combined treatments.

Methods

SITE DESCRIPTION

The experimental site is situated at Brandbjerg (55°53′ N, 11°58′ E) *c.* 50 km NW of Copenhagen, Denmark, on a hilly nutrient-poor sandy deposit. The site was chosen to be an unmanaged low vegetation ecosystem considered to be vulnerable to environmental pressures such as climate change and with a combination of annual and multiyear plant species for studies of plant species interaction and competition. The site is a dry heath/grassland ecosystem consisting of 30–40 cm tall vegetation cover dominated by a grass (*Deschampsia flexuosa*, *c.* 70% cover) and an evergreen dwarf shrub (*Calluna vulgaris*, *c.* 30% cover), a low cover of other herb and grass species (total 17 species) and an open moss cover beneath the canopy of vascular plants. The above-ground biomass is *c.* 720 g m^{-2} and the root biomass is *c.* 550 g m^{-2}. The yearly mean temperature is 8·0 °C, the yearly mean precipitation is 613 mm (www.DMI.dk), main wind directions (2005–2007) are westerly (31%) and easterly (27%), and average wind speed (2 m height) 2·5 m s^{-1} with the strongest winds from the West (average *c.* 4 m s^{-1}). Modelled N deposition in the region is *c.* 1·25 g N m^{-2} $year^{-1}$ and P deposition *c.* 0·003 g P m^{-2} $year^{-1}$ (Ellermann *et al.* 2005).

MANIPULATION DESIGN AND TREATMENTS

The CLIMAITE experiment was designed to, as closely as possible, match the climate scenario for Denmark year 2075 with elevated CO_2 at 510 ppm, elevated temperature of *c.* 2 °C and extended summer droughts, although with incidents of more heavy summer and winter rainfalls but with only small changes in annual rainfall (www.DMI.dk). The experiment includes plots with elevated CO_2 concentration (CO_2) elevated temperature (T), induced drought in late spring/summer (D) and untreated controls for reference (A). The treatments are combined in all combinations (A, T, D, CO_2, TD, TCO_2, DCO_2, $TDCO_2$). The eight treatments are placed in pairwise octagons of 6·8 m across receiving ambient and elevated CO_2, respectively. Each octagon is divided into four 'slices' (9·1 m^2 per plot) to provide all eight treatment combinations (Fig. 1). Each combination is replicated six times, (total of 48 plots) in a split-plot design with six octagons at ambient CO_2 and six receiving elevated CO_2. The distances between the octagons are at least 2·5 times the octagon widths to avoid CO_2 contamination from the elevated to the ambient CO_2 octagons. Boardwalks connect the octagons to avoid disturbance by trampling between the plots and a flexible boardwalk system in each octagon provides easy access and prevents trampling within the plots.

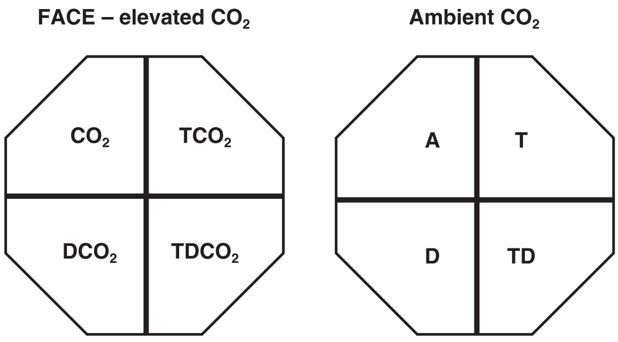

Fig. 1. Schematic presentation of a block with two separate octagons receiving elevated CO_2 and ambient CO_2, respectively, and together hosting all eight treatment combinations with CO_2 [CO_2], warming [T], drought [D] and untreated control [A].

FREE AIR CARBON ENRICHMENT (FACE)—CO_2 TREATMENT

The CO_2 is distributed by a FACE system (Miglietta *et al.* 2001). Pure CO_2 is distributed from a central CO_2 tank to the six CO_2-elevated octagons, each of which is equipped with a single-board computer controlling the CO_2 concentration at 510 ppm in the centre of the octagons at 7-s intervals. The CO_2 is supplied through eight PVC dosing pipes (2·5 m long, 20 mm internal diameter and 1·5 mm wall thickness) placed along each of the sides of the octagon *c.* 0·4 m above the soil having laser drilled holes (0·3 mm diameter) at every 54-mm facing away from the centre of the octagon. Each dosing pipe is connected to a pressure regulator connected in a manifold. The computer controls the degree of opening of each of these eight pressure regulators, thereby controlling the CO_2 dosing through each of the pipes. The CO_2 control is dynamic in relation to changes in [CO_2] monitored at the centre of the plot (30 cm height), wind speed and wind direction measured by a 2D-anemometer placed outside the octagon in 2 m height. The valve that controls the pipe on the upwind side of the octagon is 100% open while each of the two valves controlling the two neighbouring pipes are only 75% open in relation to the upwind pipe. All the other five valves/pipes are closed. The CO_2 fumigation starts 30 min after sunrise and ends 30 min before sunset all year round, except during periods with full snow cover of the vegetation. The amount of CO_2 injected is varied by increasing/decreasing the gas pressure in the pipes by means of automatic pressure regulators. A minimum pressure is maintained even at low wind speed to ensure that CO_2 jets stay near to sonic speed when fast air–CO_2 mixing is caused by a shockwave effect (Miglietta *et al.* 2001). The pressure variation in the pipes is adjusted on the basis of a Proportional–Integral–Differential (PID) control algorithm which is modified to take into account the effect of rapid changes in wind speed (Lewin, Hendrey & Kolber 1992). The dominant wind directions for all six octagons are W and E, and the average wind speed and hourly maximal wind speed varies between 1·0 and 2·3 m s^{-1}, and 8·3 and 13·3 m s^{-1}, respectively. In 2006, we used 3·0 kg CO_2 m^{-2} day^{-1}.

WARMING TREATMENT

The temperature enhancement is conducted as 'passive night-time warming' (Beier *et al.* 2004). The warming plots are covered by a light scaffolding ($h = 0{\cdot}5$ m) carrying a curtain-reflecting the infrared radiation. The scaffolding is a frame of steel tubes painted to avoid leaching of contaminants from the frame into the plots. The curtain is a white, water-proof woven acrylic cloth having 67% radiation reflection, 27% radiation transmission and 6% radiation absorption. The curtains are coiled on a beam and automatically operated by an electronic controller according to preset conditions during the whole year: Day/night – curtains are pulled over the vegetation at sunset and retracted at sunrise; Rain – curtains are retracted in case of rain during the night (sensitivity < 0·1 mm); Wind – the curtains are removed at high winds (> 7 m s^{-1}); and Dewfall – the curtains are removed in case of dewfall (max 30 min.). (All sensors specified in Table 1.)

DROUGHT TREATMENT

The drought treatment is constructed similar to the warming treatments, except that the curtains are controlled by a rain sensor activating the curtains to cover the plots whenever it rains and to remove the curtains when the rain stops. The water collected by the curtains is removed from the area by gutters. The curtains are removed automatically if the wind speed exceeds 7 m s^{-1}. In 2006, the drought treatment was started in late June and was continued for 5 weeks until early August when soil water content reached *c.* 5 vol% water content in the top 20 cm of the soil, which is slightly above the wilting point of the vegetation at the site.

TREATMENT CONTROL SYSTEM AND WEB INTERFACE

A web-based control and overview system is established to check the functioning of the treatments. Half hourly averages of CO_2 concentrations, air and soil temperatures, wind speed, soil moisture and dosing valve voltage are constantly shown graphically on a website. This allows identification of faulty sensors and provides continuous checks of the treatment functioning, for example, CO_2 dosing, allowing for rapid adjustments of the programme controlling the functioning, if needed.

Measurements

EXPERIMENTAL CONTROL MEASUREMENTS

Within each experimental octagon or plot, parameters are monitored to check the treatments and their effects on the physical and climatic conditions. Temperature is measured in the vegetation canopy (+20 cm), the top soil (–2 cm) and the soil (–5 cm) in each treatment plot every minute and averaged over 10-min intervals (for details on all measurements and sensors see Table 1). Soil moisture is measured by TDR at 0–60 and 0–20 cm soil depths providing half-hour averages. The CO_2 concentration is measured sequentially in all octagons and at two positions 30 m away from the nearest FACE octagon. The air is sampled continuously at the centre of each octagon through a 3-mm PVC tube, via a 1-µm Teflon filter, connected to a vacuum pump and a CO_2 monitor to provide measurements of [CO_2] of each octagon every 40 min.

The pattern of CO_2 distribution within one of the octagons was studied during a campaign by measuring the CO_2 concentration at 13 positions across the octagon under stable wind conditions and parallel to the wind direction. The [CO_2] measurements were repeated seven times across the octagon, and records were taken over 2 min in each position. The CO_2 distribution was further checked in one of the octagons by measuring ^{13}C content in current year leaves from *D. flexuosa* at 48 positions representing all positions (direction and distance from dosing pipes) within the octagon. The temperature distribution under the curtains and potential edge effects were studied in one octagon during 20 days in June 2006 by 23 thermocouples placed at 2 cm depth in the soil on two lines along and perpendicular to the curtain.

CLIMATIC CONDITIONS

Two independent weather stations situated within the experimental area and 75 m apart collect basic meteorological data at 2 m height, including: photosynthetic active radiation (PAR), relative humidity (RH), temperature and precipitation (Table 1). The potential for plant growth was calculated as the growing degree days (GDD) assuming a threshold for growth at 5 °C (Beier *et al.* 2004).

RESPONSE MEASUREMENTS

The impacts of the applied treatments are studied at the species, community and ecosystem level. A suite of measurements are employed to study and understand responses at the process level in the plants (e.g. leaf gas exchange, plant chemistry, root development, plant stress, litter production and phenology) and in the soil (e.g. decomposition, mineralization, soil water chemical composition, nutrient dynamics, soil organism physiology and activity). To reduce the risk of possible edge effects, the outer 20 cm of the plots are kept without any response measurements. These responses are integrated at the community (e.g. species composition, community changes) and the ecosystem level (e.g. carbon and nitrogen cycling, net ecosystem gas exchange, atmospheric feedback). The integration requires a highly interdisciplinary effort, and the results from the individual studies and the synthesis across the disciplines will be done by the use of dynamic process modelling.

Table 1. Basic sensors, sensor type, data logger hook-up, measurement frequencies and logging frequencies for climatic conditions and experimental control, and documentation parameters at the CLIMAITE site

Sensor	Sensor type	Make	Logger hook-up	Measurement–logging frequency
Temperature and soil moisture conditions				
Temperature, air (+20 cm), 1/plot	Pt100 1/3 DIN kl. B, TF-25 OD 6 mm, stainless steel IP67	Termokon, Mittenaar, Germany	AM16/32 multiplexer + data logger CR10×, Campbell Scientific Inc., Logan, UT, USA	1 min–1 h average
Temperature, soil (–2 cm), 1/plot	Pt100 1/3 DIN kl. B, TF-25 OD 6 mm, stainless steel IP67	Termokon	AM16/32 multiplexer + data logger CR10×, Campbell Scientific Inc.	1 min–1 h average
Temperature, soil (–5 cm), 1/plot	Pt100 1/3 DIN kl. B, TF-25 OD 6 mm, stainless steel IP67	Termokon	AM16/32 multiplexer + data logger CR10×, Campbell Scientific Inc.	1 min–1 h average
Soil moisture (0–20 and 0–60 cm), 1/plot/depth	TDR, TC-64 TDR-Controller	PRENART Equipment ApS, Frederiksberg, Denmark	PC	30 min–30 min average stored
CO_2 concentration				
CO_2 concentration (dosing control), 1/FACE ring	IRGA (WMA-4)	PP Systems, Amesbury, MA, USA	PK2600, Z-World, Inc., Davis, CA, USA	1 s–1 s
CO_2 concentration (documentation), 1/plot	LI-820	LI-COR, Lincoln, NE, USA	Data logger CR10×, Campbell Scientific Inc.	Sequential, 4 min average [CO_2] per 35 min
Climatic conditions				
Photosynthetic Active Radiation (PAR), 1/site	Cosine corrected quantum sensor, OL-4000q	Optisk Laboratorium, Hørsholm, Denmark	PK2600, Z-World, Inc.	1 s–1 s
Relative humidity (RH) + temperature, 1/site	HUMITTER® 50U/50Y	Vaisala, Helsinki, Finland	PK2600, Z-World, Inc.	1 s–1 s
Precipitation, 1/site	Rain-O-matic professional	Pronamic A/S, Silkeborg, Denmark	Data logger CR10×, Campbell Scientific Inc.	Event (0·2 mm)
CO_2 dosing control				
Pressure regulator, 1/FACE plot	ITV2050	SMC, Corporation, Tokyo, Japan	PK2600, Z-World, Inc.	1 s–1 s
Wind speed and wind direction, 1/FACE ring	2D-anemometer, WindSonic	GILL instruments LTD, Lamington, Hampshire, UK	PK2600, Z-World, Inc.	1 s–1 s
Curtain movement control and documentation				
Day/night, 1/site	Astro-switching, SC 28/172 4 × 3	Hugo Müller GmbH & Co. KG, VS-Schwenningen, Germany	Squirrel 453, Eltek Limited, Haslingfield, UK	Event (night/day)
Rain, 1/site	IR-light barrier (Thies Clima)	A. Thies GmbH & Co. KG, Goettingen, Germany	Squirrel 453, Eltek Limited	Event (rain/no rain)
Wind sensor, 1/site	Windwächter Plus 500	Vestamatic GmbH, Mönchengladbach, Germany	Squirrel 453, Eltek Limited	Event (threshold exceedance)
Dewfall sensor, 1/site	Regenwächter V2·0	Vestamatic GmbH, Mönchengladbach, Germany	Squirrel 453, Eltek Limited	Event (dew/no dew)

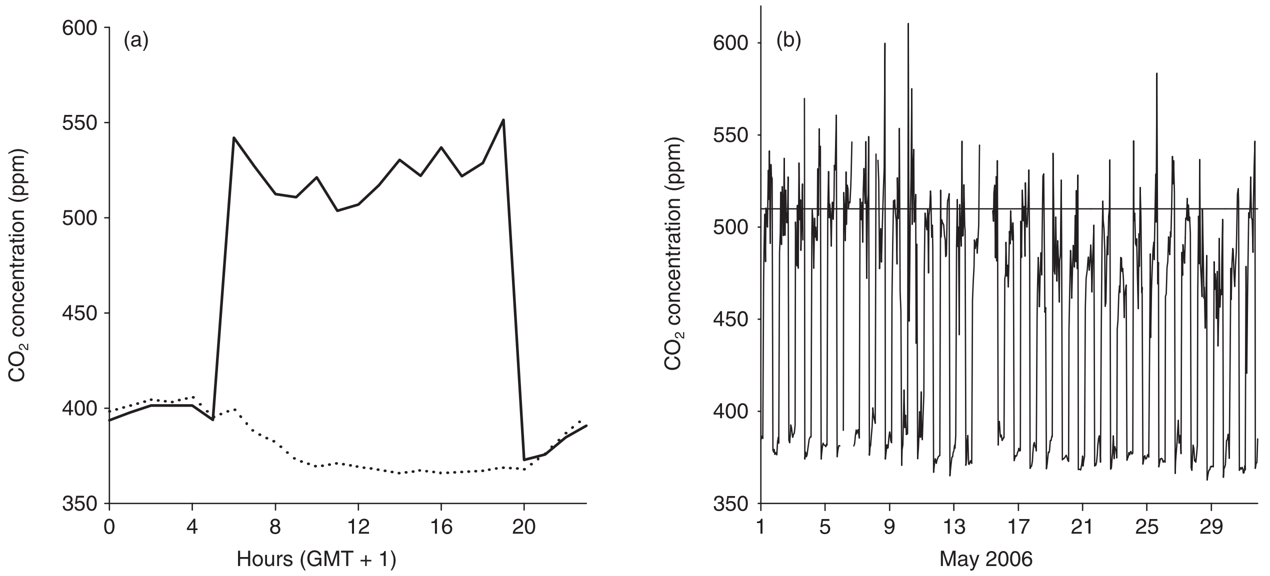

Fig. 2. CO_2 concentrations in the elevated CO_2 and ambient plots. (a) Diurnal and (b) daily average CO_2 concentrations for May 2006 in elevated FACE plots (bold line) and ambient plots (Fig. 2a dotted line) (hourly average across all six FACE and ambient octagons, respectively). The CO_2 concentration is sampled in the middle of the octagons in 20 cm and distributed to the CO_2-monitor within 7 s. The CO_2 addition is regulated every second.

Results

CO_2 TREATMENTS

The CO_2 addition to the FACE octagons was initiated on 3 October 2005 and has been running 90% of the time during the first year. Interruptions of the fumigation have been due to permanent snow cover with deliberate close down of fumigation in all FACE octagons for 30 days in January–March 2006, to interruptions during measurement campaigns or maintenance (< 4 h) and to postponed delivery of CO_2 (< 2 days).

The CO_2 concentrations were measured in both the FACE and the ambient octagons for 100 out of 330 days. The target of 510 ppm CO_2 is well met in all six FACE octagons with monthly average concentrations at 500–520 ppm CO_2. Also night-time [CO_2] in the control plots was slightly elevated relative to day-time, reflecting elevated concentrations due to respiration of soil and vegetation (Fig. 2). No CO_2 contamination from the FACE octagons into the controls has been observed (Fig. 2). Generally, the [CO_2] in the fumigated plots are higher and above the target in the morning and evening, and lower at midday. This is probably due to the generally low wind speeds in the early and late hours of the day making the [CO_2] control more difficult and the decline at midday probably linked with transport of CO_2 by vertical movement of warmed air columns. Because of local difference in wind exposure, the variation in [CO_2] differs slightly among the octagons (Fig. 3). The [CO_2] are within 10% of the target during 79·4% of the time in average (range 65·9%–86%) and within 20% during 96·2% of the time in average (range 89·4%–98·1%) for the six FACE octagons. The CO_2 distribution measurements show a decline in CO_2 concentration from the concentrated CO_2 at the outlet to 443 ppm at the centre and to 407 ppm at the far side of the octagon with the sharpest decline the first 20 cm from the exhaust pipe (Fig. 4). The [CO_2] at the octagon centre during these measurements was

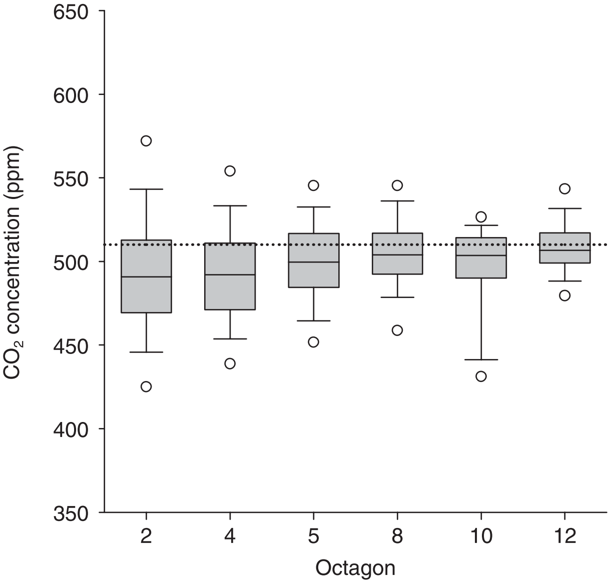

Fig. 3. Hourly [CO_2] in all six elevated FACE octagons during fumigation in May 2006. The boxes show the interquartile range with the median indicated as a thick horizontal line. Whiskers above and below the box indicate the 90th and 10th percentiles. Values outside of these bounds are 5th and 95th percentiles. The horizontal dotted line indicates the target value (510 ppm).

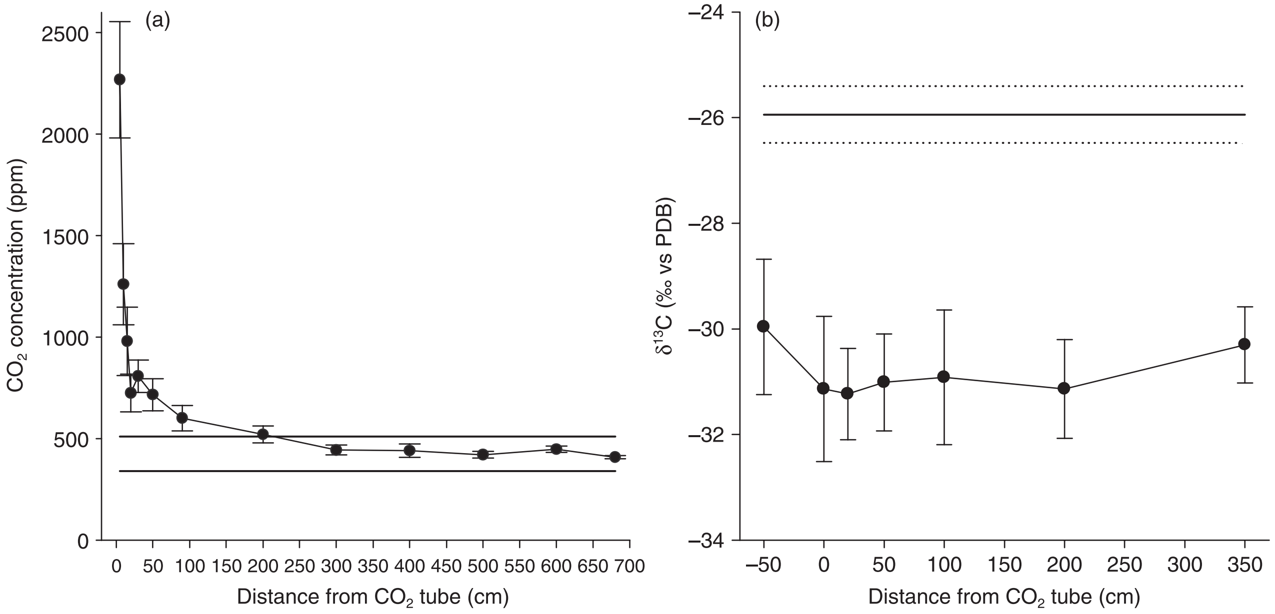

Fig. 4. CO_2 distribution in FACE plots. (a) CO_2 concentration gradient across FACE octagon 8 from the upwind distribution pipes (0 cm) to the downwind edge (700 cm). Solid lines indicate the target for the elevated CO_2 plots (510 ppm) and the ambient concentration (380 ppm). (b) ^{13}C signatures (± SD) in leaves of *Deschampsia* growing at varying distances from the distribution pipes in octagon 8. Samples were collected in June 2007 along eight equidistant radii. Horizontal line indicates $\delta^{13}C$ signatures (± SD) observed in adjacent non-FACE octagon 9.

67 ppm below the target due to sunny conditions and relatively low wind speeds causing significant thermal columns transporting the CO_2 enriched air upwards. The average ambient [CO_2] during the test was 339 ppm. The ^{13}C measurements show a uniform distribution with a significant depletion (–31·0 ± 0·73) relative to untreated plants (–25·9) and no systematic differences among directions or distance to the edge with a wind distribution pattern for the growing season equal to the overall annual pattern with prevailing winds from West and East.

WARMING

The warming treatment was initiated on 3 October 2005 and warming curtains were active 87% of the night-time. The 13% interruptions were due to rain (11%), dew (2%) and to wind exceeding the thresholds without simultaneous rain, occurring for only 0·7% of the nights. Additional interruptions in the warming treatment were due to deliberate turn-off of the treatments for 60 days in January–March because of permanent snow cover. Break down and short-term failures of single engines regulating the curtains resulted in short (days) interruption in single plots.

The warming treatment affected the temperature immediately after the start of the temperature enhancement treatment, and the full warming potential was achieved after four nights of treatment (Fig. 5). The slight decline in air temperature the following 5 days is the result of a slight increase in wind speed and a decline in incoming solar radiation. The air temperature difference is larger than the differences in soil temperature during the night, while the soil temperature difference is relatively larger during the day. The warming treatment affects the growth potential (GDD) significantly in the early spring with a 33% higher accumulated GDD during the period from 1 April to 15 May 2006 (data not shown) and an annual 7% increase in GDD in warmed plots (2630 GDD) relative to unwarmed plots (2468 GDD). Across the year the largest warming occurs in the autumn and early spring (Fig. 5). The average temperature elevation at the three heights (–5, –2 and 20 cm) are 0·6 °C, 0·8 °C and 1·4 °C, respectively. The warming effect shows a diurnal pattern with maximum warming in the late night/early morning in both the air and the soil, and a gradual reduced warming during the day (Fig. 6). In the air, the effect of the warming treatment disappears 3–5 h after sunrise and rapidly builds up again after sunset, while in the soil the warming effect is less dynamic and some warming is sustained throughout the day. The diurnal pattern is similar throughout the year.

The spot measurement of temperature distribution underneath a warming curtain showed an average temperature of 8·7 °C ± 0·53 (SD), ranging between a maximum of 9·3 °C and a minimum of 8·4 °C compared to ambient temperature 10–30 cm outside the curtain of 7·5 °C ± 0·11 (Fig. 7). This shows a minimal variation inside the warming treatment and that the adjacent non-warming treatments within the same octagon are largely unaffected by the adjacent warmed plot. Similar observations were also reported by Beier *et al.* (2004).

The temperature increase in the warmed plots was strongly dependent on wind speed with the strongest warming at low wind speed declining to very small or no warming at wind speeds exceeding 6 m s^{-1} (Fig. 8).

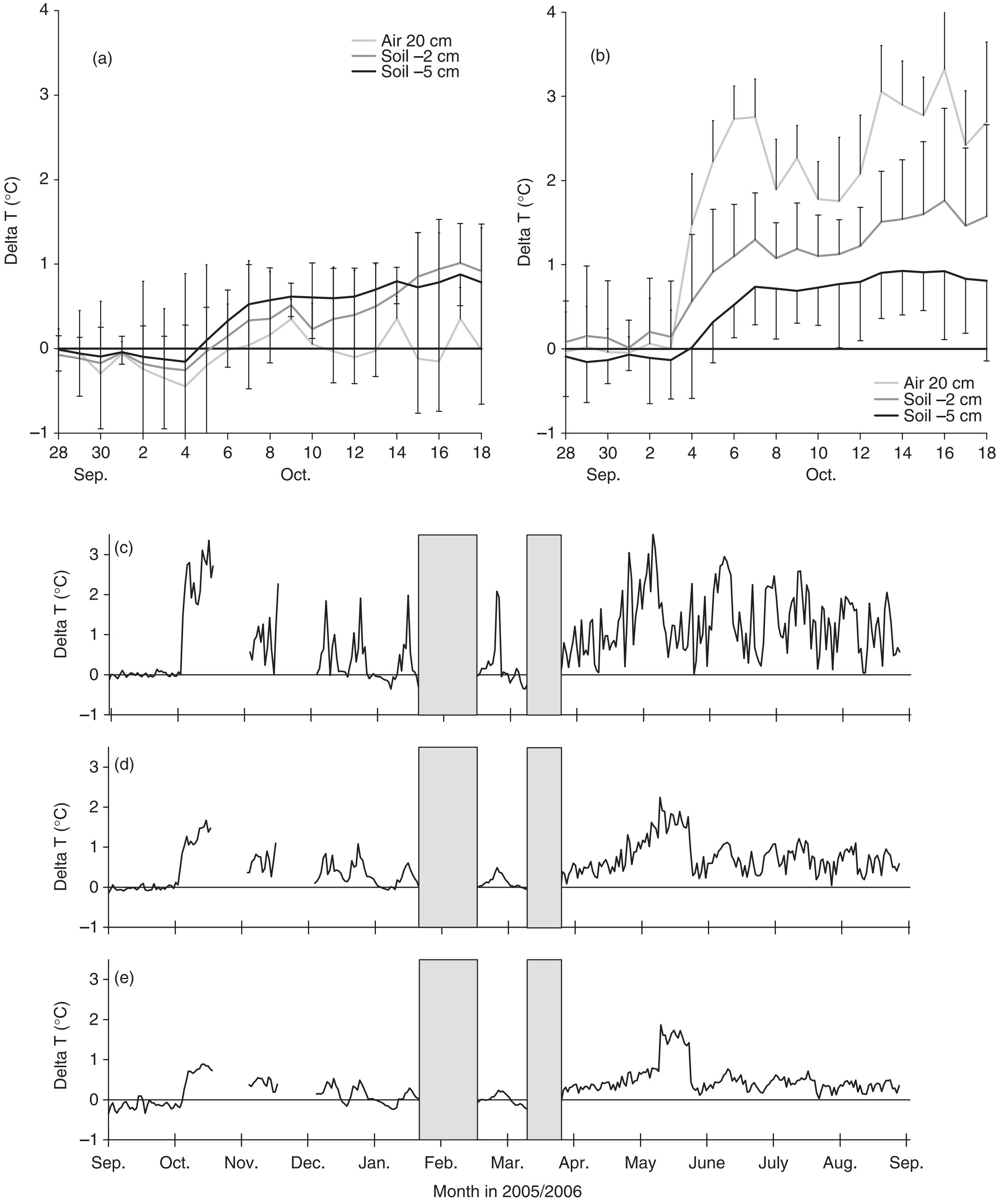

Fig. 5. Average temperature difference between warmed and non-warmed plots ($n = 24$) during (a) day and (b) night just before and after initiation of warming treatment on 3 October in three heights, and mean daily temperature differences during the first year of treatment (October 2005 to October 2006) (c) in the air (+20 cm), (d) in the top soil (–2 cm) and (e) in the soil (–5 cm). Grey areas between late January 2006 and late March 2006 indicate periods of deliberate interruption in warming treatment because of permanent snow cover.

DROUGHT

The first drought treatment was initiated in all octagons on 3 July 2006 and ended at 4 August 2006. During this period 95% of the precipitation (54 mm) was removed (Fig. 9). Delay time in curtain movement at the initiation of a precipitation event or removal of the curtains during very high winds during the rain events means that some rain may reach the

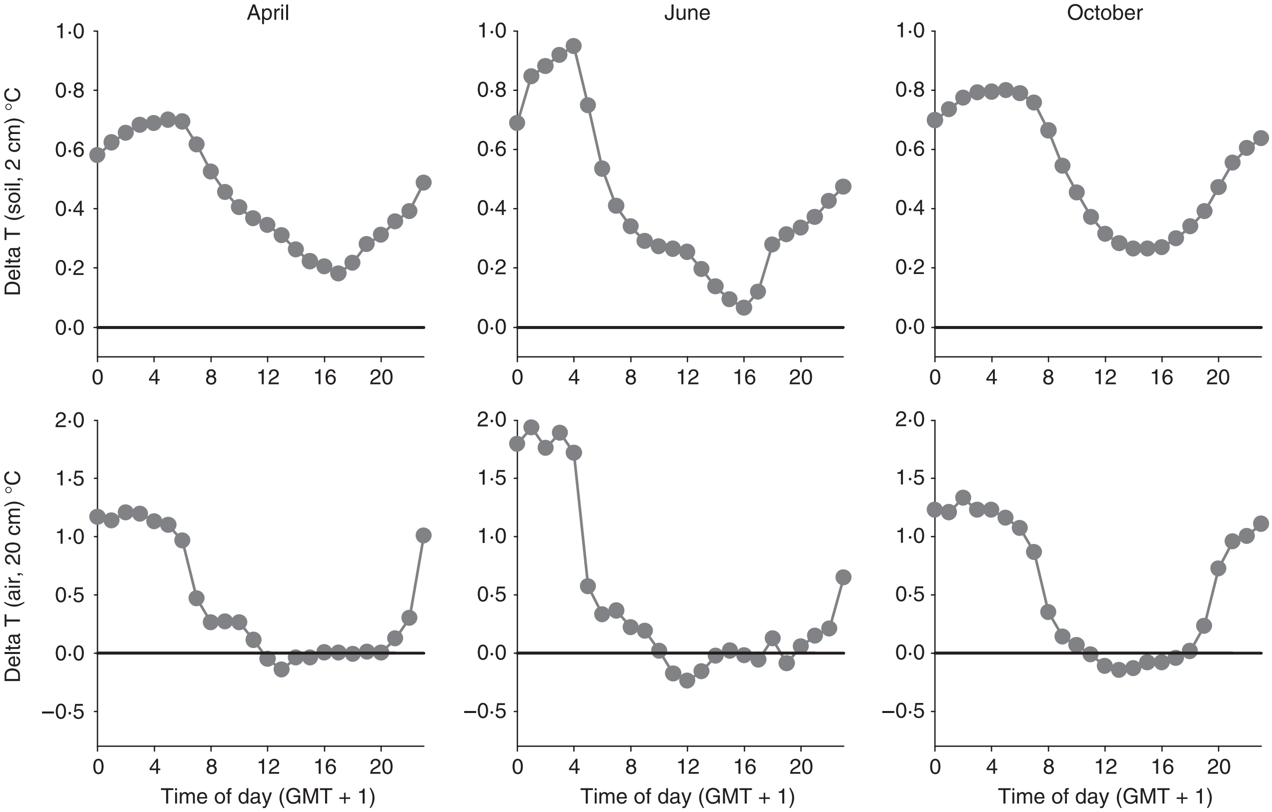

Fig. 6. Diurnal temperature enhancement by warming. Average hourly temperature difference per month between warmed and unwarmed plots, in the soil (–2 cm) and air (+20 cm) during April, June and October 2006.

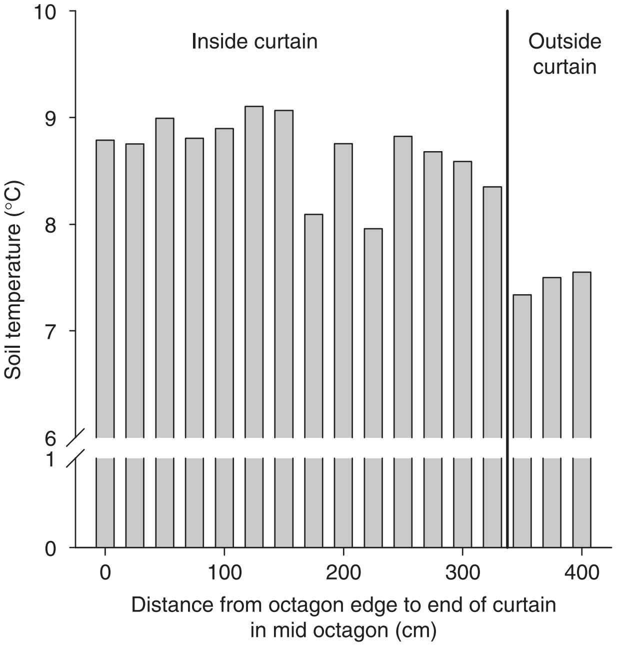

Fig. 7. Temperature distribution in warmed plots. Temperature measurements taken along a line underneath a reflective curtain used for passive warming ranging from the outer edge of the warmed plot (starting 70 cm from the edge) across the plot to 75 cm outside the curtain (three probes uncovered). The soil temperature is measured in 1 cm depth between 20 May and 9 June 2006, and data from 23.00 to 03.00 h are shown as an average for each sensor. Average temperature for all sensors under the curtain is 8·7 °C and outside the curtain edge the average temperature is 7·5 °C.

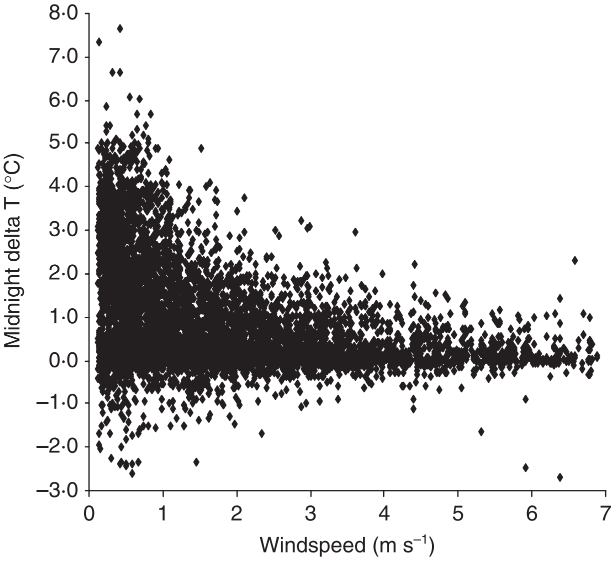

Fig. 8. Effect of wind speed on warming treatment. Increase in air temperature (+20 cm) at midnight in all warmed plots during the first treatment year (October 2005 to October 2006) relative to wind speed in 2 m height measured close to the plot (< 5 m distance) ($n = 12$ plots).

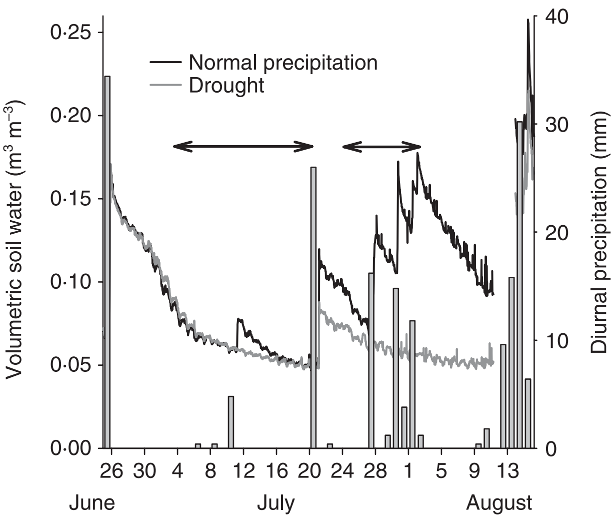

Fig. 9. Drought/precipitation and soil water content. Average volumetric soil water content in all plots exposed to drought (grey line) and normal precipitation (black line) during June–August 2006. Diurnal precipitation in the 'normal precipitation' plots are shown as grey bars. The arrows indicate periods for the experimental drought.

drought plots, but the amount of unintentional precipitation in the drought plots was small (< 1 mm per event). A natural drought occurred during the experimental period with little rain during the first 17 days, which reduced the difference between the drought and the non-drought treatments. By the end of the natural drought, the soil water content was as low as *c.* 0·05 $m^3\ m^{-3}$ in 0–20 cm soil depth in all treatments. On 21 July 2006, heavy rain showers damaged some of the curtains and the treatment was stopped for 4 days. During the last 2 weeks of treatment, the soil water content was again decreased to *c.* 0·05 $m^3\ m^{-3}$ in the drought treatment, while it increased to a maximum of 0·17 $m^3\ m^{-3}$ in the controls following precipitation events.

Discussion

Realistic studies of environmental impacts on ecosystem functioning in the field need to be designed and technically manufactured in a way so that the factors manipulated are relevant to scenarios, and so that all artefacts and unwanted side effects are avoided or, at least, minimised (Beier, Gundersen & Rasmussen 1998; Gundersen *et al.* 1998; Schulze *et al.* 1999; Beier *et al.* 2004). In CLIMAITE, we, as closely as possible, apply the predicted climate scenario for Denmark year 2075 (CO_2, temperature and precipitation) by combining methods that to our knowledge are the 'best available' in terms of applying the climate change factors in a realistic way and in causing minimal artefacts and disturbances on the key climatic variables light, wind, water and temperature.

The quality and the interpretation of the results depend on the technical quality of the treatments and the degree to which artefacts are avoided. All the three experimental approaches applied in CLIMAITE have been tested individually in previous projects: CO_2 fumigation by the FACE concept is widely used in many ecosystem types, for example, in forests (DeLucia *et al.* 1999), grasslands (Hovenden *et al.* 2006) and in agricultural fields (Miglietta, Giuntoli & Bindi 1996) Passive night-time warming have been tested and proven associated with minimal artefacts in similar ecosystems as in CLIMAITE (e.g. Beier *et al.* 2004) and automatic rain shelters have been widely used and tested in different ecosystems (e.g. Beier *et al.* 2004). However, the combination of all three manipulations in a multifactorial approach is novel, and poses new methodological challenges, considerations and opportunities.

CO_2 dosing by the FACE technique is widely used in many projects. CLIMAITE has adopted this technique because it is well developed, it uses a well-tested control system (Miglietta *et al.* 2001) and it avoids unwanted side effects on temperature, precipitation, light and wind as would be the case with any 'chamber' technique. The measurements show that the system controls the CO_2 dosing well near the target for all six CO_2 octagons despite the spatial variation in wind speed across the site and show no contamination from fumigated plots into ambient plots. The measured gradient of CO_2 near the edge at the upwind (dosing) side of an octagon during one event (Fig. 4a) might together with prevailing westerly and easterly winds lead to an uneven long-term distribution of CO_2 across the FACE plots. However, the ^{13}C measurements in the current year grass leaves show almost identical depletion at all positions indicating that the long-term dosing is equal across the plots. The FACE technique requires free air movement, and in the present set-up the combination with the retractable covers for warming and drought could potentially affect the wind movement and turbulence in the plots and thereby affect the CO_2 treatment. However, the measurements show that this is not the case, since the CO_2 concentration is well controlled near the 510 ppm target, and the [CO_2] distribution pattern is similar to what has been found in other studies (e.g. Hovenden *et al.* 2006), although in smaller FACE rings (diameter 1·5 m). In CLIMAITE, CO_2 dosing is restricted to day-time because dosing during night-time beneath the covers would likely cause problems with mixing, and because elevated CO_2 is assumed to have negligible effects during the night when photosynthesis stops. In the drought plots, the rain covers do affect the CO_2 distribution during rain events, but since rain events only lasted < 5% of the day-time during the drought experiment, we consider this to be of minor importance.

It should be noted that the web-based interface for checking the CO_2 dosing continuously during operation is not trivial but plays a significant role in the daily operation and documentation. Indeed, it was particularly important for the fine tuning of the control software during the start of the fumigation.

In CLIMAITE, warming by night-time passive covers was chosen because it reflects the already observed and predicted trends in future temperature increases with relatively higher elevation of minimum than maximum temperatures (e.g. Alward, Detling & Milchunas 1999), unwanted side effects on light, wind and hydrology are small (Beier *et al.* 2004) and the running costs are low (Beier *et al.* 2004). The most widely used alternative, IR heaters, have been criticized by ecophysiologists for creating unrealistic heating gradients and adverse conditions at the plant surface (Kimball 2005). The results from CLIMAITE showing a *c.* 1 °C warming match results found in another study with passive warming (Beier *et al.* 2004) and are similar to warming with IR-lamps applying 50–100 W m^{-2} energy (e.g. Shaw *et al.* 2002; Hovenden *et al.* 2006). The measurements of spatial distribution also agree with previous measurements showing very small edge effects (Beier *et al.* 2004) and in the present case shows that there is no significant transport of warm air from the warmed plots into the unwarmed plots. Since the predictions for future climatic conditions have 1 °C as an absolute minimum and temperature increases of 2–6 °C seem more likely, there is a need to develop existing or new techniques to increase the warming further. However, the disadvantage of all available systems for open air heating, which are needed if combined with the FACE technique, is that they allow lateral air flow in and out of the plots, thereby exchanging the warmer air in the plots with colder air from outside as shown by Kimball (2005) and also demonstrated in this study. Therefore, all reported projects using IR heaters or reflective covers have only heated the air and top soil by about 1 °C (e.g. Shaw *et al.* 2002; Beier *et al.* 2004). Increased heating could therefore be obtained by adding walls/sides on the study plots, which on the other hand would seriously affect the wind stress. Another alternative is soil heating cables (Hillier, Sutton & Grime 1994; Melillo *et al.* 2002) that can provide a specified amount of warming. This technique however involve disturbance effects from installation and require comparison to a cabled unheated control (McHale & Mitchell 1996). Cables may also create non-natural vertical temperature profiles and other unwanted experimental artefacts such as soil drying (Harte *et al.* 1995). The lack of complete experimental control on the warming treatment and the accompanying artefacts are tradeoffs inherent to *in situ* field studies. We have decided to keep a lower degree of warming in order to minimize the artefacts.

The methodology used to remove water and create drought works efficiently although very abrupt and heavy storm events may cause problems to cover the plots and remove the water fast enough. Therefore precipitation collectors or soil moisture measurements in each plot are important to check the efficiency of the covers. The covers are not transparent and therefore will affect the light conditions. However, as the drought covers are only operated for *c.* 6 weeks and only during rain events where light intensity is already low, we consider this problem of minor importance. Our drought treatment does not completely match the future precipitation scenarios for Denmark which, not only, expects longer dry spells but also more heavy rain showers and more rain in the winter. Technically we could have matched the future scenario more closely, but chose not to do so, because it would be difficult to assess to what extent any measured treatment effect would be related to the extended summer drought, the heavy rain showers or increased winter rain if these were applied simultaneously.

Major challenges in multifactor experiments are to manage the highly complex technical set-up, to balance the number of replicates with the number of treatments to make work feasible, and to restrict the area of the experimental site to a reasonable homogenous part of the terrain. The complex technical set-up with the combination of three different treatments requires frequent visits to the site in order to survey and maintain the system. Besides the scientific criteria for the site selection, we therefore included as an important criterion that the site should be close to the research institutes (< 1 h drive) in order to save resources (work hours for driving) and minimize delays and downtime when errors and faults occur. The 48 plots stemming from the three treatments plus the control in combination with six replicates make any set of measurements time-consuming. This, together with the high intensity of the field work with many different measurements and persons involved seriously increase the risk of rapid site deterioration by trampling and has required special attention by development of a flexible and easy-to-handle system for easy plot access. We therefore designed removable light weight aluminium boards at the octagons, which can be mounted by one person in less than a minute to provide access to all four plots. Such solutions have to be developed site specifically and depend on plot numbers, plot size, vegetation height, and so on. This may sound trivial, but the importance of this must not be underestimated. Finally, the split-plot design with combinations of four treatments in each octagon as opposed to a typical single-plot design was chosen in order to fit all plots within a reasonably homogeneous part of the terrain and still keep sufficient distance between the octagons to avoid contamination from elevated CO_2 to ambient plots.

With its combinations of CO_2, temperature and drought treatments, the CLIMAITE experiment is one of a few experiments in the world within a new generation of multifactor experiments related to global climate change. The multifactorial approach is essential as a tool to test potential nonlinear interactions among the applied individual factors, because these cannot be assessed from single factor experiments. Apart from the technical challenge in construction of the experiment, reported in this paper, the major challenge is to give research outputs that not only shows the environmental impact on single processes, but also, when combined, can be expected to give a holistic, integrated, view of how the system as a whole responds to a changed environment. For this reason, the researchers have been carefully selected across research groups with diverse specialties from ecophysiological to ecosystem and landscape levels, and covering processes in the main ecosystem components, for example, biogeochemistry, hydrology, microbial, plant and soil animal ecology. The combination of the multifactorial approach and a broad scientific coverage is crucial for development of realistic models on ecosystem functioning in a changing environment. The ultimate goal and expectation of the CLIMAITE experiment, therefore, is that it will provide unique opportunities for integration of responses by model testing through application of the models to the ambient conditions and subsequent validation by use of the data from the treatments (e.g. Beier 2004). We expect that ecosystem responses to the treatments will occur in cascades as is also known from effects of nitrogen (e.g. Galloway *et al.* 2003) with some processes reacting very rapidly (instantaneously, e.g. ecophysiology), some responses being transient (years, e.g. decomposition and carbon mineralization) and some reacting on a longer time-scale (decade, e.g. species composition). In order to provide overall and longer term assessments of ecosystem responses to combined climatic changes as well as developing robust mathematical models the CLIMAITE project and field site is, therefore, planned to run for at least 10 years.

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