



The VKR center of excellence

Climate

November 2006



Midterm report



CLIMAITE was initiated 1st April 2004 with the aim of establishing a strong national interdisciplinary collaboration between the best Danish research groups in studies of climate change effects on biological processes in terrestrial ecosystems. More specifically the aim was to establish a high quality experimental climate change research facility, conduct 4 years of climatic manipulations with CO₂, temperature and water and study the effects on the ecosystems organisms, structure and functioning. CLIMAITE has been running for almost 3 years now and is more than half way through. In the following report we will provide an overview and assessment of the status today relative to the original goals and provide some perspectives for the final 2½ years and further.

Status of the establishment and organisational functioning of CLIMAITE

Establishment and organization

The CLIMAITE centre is established as a collaboration between Risø, KU, KVL and DMU and involve 15 permanent researchers (professors, senior researchers and researchers) and 10 technicians from these institutes. CLIMAITE has initiated 6 PhD-projects and has announced 2 further PhD projects and a 2 year post doc to be evaluated and hopefully initiated at the start of 2007. The centre is organised in a number of work areas (workpackages) which are responsible for the specific scientific activities and each one led by a senior researcher. The work package leaders form the steering group which is responsible for the overall steering of the centre. The daily management of the centre is carried out by the center leader Claus Beier, Risø and the administration at Risø. The activities are further evaluated by an international scientific advisory board consisting of three internationally acknowledged researchers within the field. Strong emphasis has been put on creating a constructive and dependent collaboration among the involved institutes and scientists. This is among other things obtained through bi-annual science meetings involving all researchers and technicians to discuss the activities, results, and new ideas, and frequent meetings with all involved Ph.D students.

Scientific activities

WP1 – site building and experimental manipulation

The field site was designed and build during the first year of the project and the manipulations were initiated in October 2005. The experimental setup consists of automatic FACE system to fumigate the plants with CO₂ at a level of 510 ppm and automatically operated curtains to conduct warming (passive night time warming) and extended summer drought. Installations include instruments to measure general climatic conditions (wind speed, wind direction, humidity and precipitation) and sensors to check the treatments (CO₂-concentrations in fumigated and non fumigated plots, temperature in the air and soil, soil water in various depths) and a web-based control and surveillance system to check the functioning of the system. The field site provides housing for equipment and personal, power supply and all infrastructure needed for the people involved with the experiment. The results from the first year of treatment show that CO₂ is well controlled around the target with a variation from 570 at 20 cm from the dosing pipes to 480 ppm at the down wind site of the ring. The temperature increase during the night ranges from 0.5 to 4 °C, diurnal average

increase of 0.8 °C and average night time increase of 1.1 °C. In agreement with findings in other projects with night time warming, there is no edge effect observed. The drought treatment effectively removes all rain water during the drought period. These results have been presented at the Ecological Society of America (ESA) conference in Memphis in August and have been submitted for scientific publication. **Deviations from plans:** the start of the treatments was postponed by ½ year in accordance with recommendations from the scientific advisory panel in order to allow for more pre treatment measurements. This will reduce the number of growing seasons from 4 to 3. There have been some technical problems with the warming covers and the rain covers, which have reduced the efficiency of the covers slightly, but these problems seem to be under control now and the exact treatment has been recorded anyway.

WP2 – species responses

Activities so far: WP2 is divided into three activities:

- 2.1 *Plant physiological responses.* Intensive field campaign from May to November including monthly measurements of photosynthesis (CO₂ and light response curves, daily variation in simple CO₂ response curves). In parallel, measures of plant stress were detected by conducting chlorophyll fluorescence measurements monthly to weekly during and after the drought period. Harvested material fractioned for area determination (*D. flexuosa* leaf area – *C. vulgaris* projected shoot area), fresh and dry weight, planned to be analyzed for C/N and Carbon isotope ratios. Further, the fresh material was weighed and homogenized for chlorophyll analysis. New activities were measurements of water potential in *C. vulgaris* initiated from June 2006.
- 2.2 *Physiological responses of the soil fauna.* Laboratory investigations combined with field trials including manipulated drought exposure at other field sites than Brandbjerg have been used to assess soil fauna responses to realistic drought levels. In two studies we have investigated the effects of drought on enchytraeids and microarthropods in the field, and investigated how soil pollution interact with low soil moisture. Using a “space for time” approach we have investigated clinal variation in desiccation tolerance of the collembolan, *O. cincta*, to elucidate the questions of climatic stress adaptations.
- 2.3 *Responses in trophic interactions.* Work on foliar herbivory as a factor affecting below-ground organisms in the rhizosphere have been slightly reduced because the natural plant community at the Brandbjerg site is composed of grasses where herbivores easy to manipulate are not readily available. Therefore the herbivore study will be on the plant *Teesdalia nudicaulis*, a crucifer present at Brandbjerg. Instead, more focus will be on functional differences in the decomposer community in the rhizosphere of different plants. Investigations performed during the pre-treatment period at Brandbjerg has shown that Heather (*C. vulgaris*) and the grasses *D. flexuosa* and *Festuca ovina* have different densities of microbes in the rhizosphere and moreover that these microbes have different physiology.

Deviations from plans: PhD student Kristine Maraldo is on maternal leave November 2006 – August 2007. This will delay some of the planned activities in 2007. Otherwise have been no major deviations from original plans. The PhD student Karen Stevnbak has been on maternal leave from early summer 2005-2006. For this reason her project is delayed with a year at the moment.

WP3 – community responses

Activities so far: WP 3 is subdivided into four activities:

- 3.1 *Treatment effects on plant community composition and dynamics.* Pre-treatment analysis including aboveground plant biomass and structure, root biomass by depth and species and phenological patterns during the growing season. Calibration between pin-point analysis and plant biomass has successfully been obtained (Riis-Nielsen and Schmidt, in prep) and a two species plant competition model obtained from pin-point data of the dominant species, the grass *D. flexuosa* and the evergreen dwarf shrub *C. vulgaris* has been developed (see under scientific results below). The responses to the first year treatments are described under scientific results.
- 3.2. *Treatment effects on community structure of the soil fauna.* Soil fauna sampling was made for characterisation of the site and to reveal any pre-treatment differences between the subplots and sampled again during the first treatment year. The pre-treatment measurements showed that the structure of the soil fauna community is typical for temperate heathland and both micro- and mesofauna are most abundant in the organic rich upper soil layers (0-3 cm) while rapidly decreasing in deeper layers. Across the site there seem to be no big differences in mean abundance between the different plots. Preliminary results show that drought during the first treatment in summer 2006 negatively affected nematodes while rewetting subsequently showed a short-lasting stimulation. A long-lasting experiment (VULCAN, Mols) on the same vegetation type showed no effect on soil nematodes to drought treatment whereas a heating treatment resulted in an increase.
- 3.3 *Population structure of soil bacteria and microbial and fungal biomass.* The microbial biomass, structure and metabolic profile of soil bacterial and soil fungal communities has been analysed on pre-treatment samples as well as on samples obtained from treatment year one. A specific effort to investigate bacterial and fungal community structure in the rhizosphere of the three dominant plant species at the study site, *i.e.* *D. flexuosa*, *C. vulgaris*, and *Festuca ovina* has been conducted.
- 3.4 *Basic site characteristics and their variability:* These have been characterised for each plot before the treatments started (e.g. carbon and nutrient pool sizes in plants and soil by depth, soil texture, density and water holding capacity and wilting point) and nutrient and microbial dynamics have been measured by depth throughout the year outside the plots. The results show some differences in soil carbon fractions and nutrient cycling (see WP4) among the plots and under the two main plant species. These results will be crucial for subsequent response assessment (see Site characteristics and Nutrient limitation under Scientific results below).

Deviation from plans: Overall, the activities in WP3 have followed the plans except that we postponed collection of litter and calibration between NIR spectroscopy and wet chemistry to 2007 due to lack of man power in the initial phase.

WP4 – Effects on ecosystem functioning

The main WP4 activities have been:

- 4.1 *Ecosystem-Atmosphere gaseous exchange.* Ecosystem respiration and fluxes of N₂O and CH₄ (GHGs) were obtained at three occasions under pre-treatment conditions in 2005. GHG fluxes measured regularly since June 2006. Regular measurements for obtaining seasonal variations of CO₂ exchange initiated in March 2006. Data of Net ecosystem exchange (NEE), ecosystem respiration (ER) and soil respiration (SR) are obtained; also the aim of getting the diurnal variation is implemented. Apparently SR is increased by enhanced CO₂. The same trend is observed for ER. Mostly the effect is pronounced at the SR observations. It remains to be thoroughly verified if the impact of enhanced CO₂ on SR is a

biological effect only, and not influenced by “physical” mechanisms induced by increased soil CO₂.

4.2 *Effects on belowground C-transfer.* In April 2006 four cylinders (height 15 cm, Ø 15 cm) were pushed down in vegetation dominated by *D. flexuosa*. In October 2006 one cylinder was removed from each plot and subject to a laboratory based ¹³CO₂ -pulse tracer experiment. The experiment is completed pending analysis of plant and soil materials. Plant-, root- and soil samples have been collected *in situ* to assess the incorporation of CO₂ in FACE plots based on ¹³C/¹²C ratio analysis. The average ¹³C isotope signal in CO₂ supplied for fumigation is around -28 ‰ (PDB). A change in vegetation and belowground ¹³C/¹²C ratio, however, may not be seen upon several years of fumigation.

4.3 *Internal nutrient transformations.* Pre-treatment analysis of soil chemistry: i) soil mineralization through a half and one year, respectively, below *C. vulgaris* and *D. flexuosa*. ii) seasonal changes in soil chemistry through a year (Single amino acids, organic acids, microbial C, N and P, NO₃⁻, NH₄⁺, DON, DOC, dissolved P, fine root biomass of *C. vulgaris* and graminoids). iii) determination of mycorrhizal colonization, and ergosterol of soil and roots in progress. Start of mineralization (buried bag +/- plant) was initiated in September/October one year after treatment start.

4.4 *Gross rates and potentials of N cycling processes.* Potential denitrification rates (with and without addition of acetylene), potential nitrous oxide reductase rates, and potential nitrification rates have been obtained from the twelve octagons before and after treatments were initiated. Analysis of soil samples for nitrous oxide reductase enzyme activity has been included as new activity. The enzyme activity proved to be very small. No apparent treatment effects within half a year of initiating warming and carbon dioxide.

4.5 *Mechanisms and pathways of C and N assimilation.* Labelling experiment with ¹⁵N¹³C Glycine in the treatments was initiated in September/October one year after treatment start.

4.6 *Leaching losses of N and C from the system.* Soil water content of C, N and P has been collected and measured monthly/bimonthly since the spring 2004.

Deviations from plans: Overall, the activities in WP4 have not been affected by major delays, methodological problems or other. Few minor activities, however, have been cancelled and a couple of new activities have been initiated. Regular measurements of CO₂ exchange was delayed until spring 2006 due to a combination of technical difficulties and a white long winter. Application of closed chamber technique to FACE-rings raised some problems caused by high initial chamber CO₂ concentrations giving rise to artificial chamber effects. Upon several weeks of testing it was decided to use the closed chamber only under ambient CO₂ concentrations. Measurements of nitric oxide (NO) fluxes have been cancelled because NO fluxes at the site are neither relevant (low activity) nor feasible (expensive to apply dynamic chambers in numerous separate plots). A new activity was included in WP4.2 focusing on the evolution of soil ¹³C-CO₂ concentrations in association with the ¹⁵N¹³C Glycine labelling in WP4.5 to assess dynamics of Glycine decomposition. A litter bag experiment was initiated in April 2006 (WP4.3) as a new initiative to study above-ground litter decomposition; the first sampling taking place October 2006. Measurements of phosphatase activity have been cancelled, however, P-availability and dynamics is assessed in WP3. In WP4.6 mice have caused some problems gnawing through the tubes connecting suction cells with soil water reservoirs. Tubes are being reinforced. Soil water measurements of C have been delayed, but will be on track from spring 2007.

WP5 – Syntheses

The synthesis work has been an integrated part of the work in the three main scientific work packages, with the main aim to ensure that all results are evaluated with respect to their relation to the overall ecosystem responses. The work has so far primarily consisted in collaboration with the technical University in Lund, with the aim of investigating the possibilities of using the FORSAFE model complex as a tool to model the output from CLIMAITE. This work will be intensified in the final two year period by employing a modelling PostDoc.

Scientific results

The field experiment

An important part of the activities until now has been aimed to establish the field scale manipulation facility at Brandbjerg. The facility has been established in a part of the military practise area near Jægerspris, which has been transformed from a remote natural area into a modern and technically advanced research facility. It has been a huge practical, scientific and technical challenge, which has only been possible because the centre has strong expertise in designing and running manipulation experiments from previous projects, and because we have developed fruitful collaboration with research groups and private companies to help and advice on matters not covered within the centre. The research facility is now up and running and we have just finished the first year with elevated CO₂, elevated temperature and extended drought. According to judgements from international experts, who have visited Brandbjerg through the last year, the CLIMAITE research facility is a world leading state of the art facility. The technical design and setup has already been presented at the Ecological Society of America's annual congress in Memphis, US in August 2006, and a scientific paper based on the presentation has been submitted.

Pre-treatment measurements and treatment efficiency

The manipulations and the scientific results are the core of the activities within CLIMAITE. An important part of the scientific work in parallel with the design and construction of the field site has focused on measurements of site characteristics and pre treatment conditions. These measurements are essential for the subsequent evaluation and assessment of the results and will be important to focus the measurements on the most important processes in the ecosystem. The most important results from these measurements are:

Site characteristics - Brandbjerg is situated on a hilly sandy moraine from the Weichsel glaciation. The experimental area is a 7.5 ha dry heath/grassland dominated by the graminoid *D. flexuosa* and the evergreen dwarfshrubs *C. vulgaris*. A number of herb and grass species characteristic to acid grassland occur with low cover, e.g. *F. ovina* and *S. decumbens*. The aboveground biomass is approximately 700 g/m², where mosses account for 25 %. Root biomass is approximately 500 g/m². The soil consists of sand (70 %) and coarse sand (20 %) with low clay content of about 2 % and 6 % silt. Except for the 5 cm thick mor-layer, the soil has low C content and low base saturation. Field capacity and wilting point are about 12 and 2.5 vol%, which is in accordance with thresholds in the TDR-measurements.

Nutrient status and limitations (N&P) - A fertilizer experiment with factorial addition of N or P or combined N and P addition was established at Brandbjerg. The results indicate that nitrogen addition stimulated the biomass production of both *C. vulgaris*

and *D. flexuosa*. Nitrogen addition also affected the phenology of *D. flexuosa* with an increased production of daughter shoots in the end of the growth season which makes this species a superior competitor relative to *C. Vulgaris*. If nutrient availability is increased by any of the treatments, this may in the long run increase grass abundance and grass litter production, which will decrease the nutrient turnover time. On the other hand **nitrogen is unlikely to be a proximate factor limiting net primary production** at the site because the sum of N deposition and annual net N mineralization seem to balance the plant N uptake and losses from the system. The first indications of “non-growing season” biological activity has already been shown. During this period net mineralization were higher below *D. flexuosa* than below *C. vulgaris*, while there were no significant effects of plant species in mineralization, release of DON and microbial N after one year of incubation.

Rhizosphere microorganisms and rhizodeposition - Pretreatment measurements have shown that microbivorous fauna controls the bacterial biomass in the rhizosphere and directly indicate differences in rhizodeposition from living plants (Christensen, Bjørnlund, and Vestergård 2006). The functional physiology of rhizosphere microorganisms differ among the dominant plant species at Brandbjerg with rhizosphere organisms associated with *D. flexuosa* and *F. ovina* having a more vigorous growth potential relative to *C. vulgaris* organisms. Moreover, indigenous microbial activity was higher in the rhizosphere of *F. ovina* compared to *D. flexuosa*. **The differences in types and biomasses of microorganisms associated to the roots of the different plant species at Brandbjerg are believed to be important factors determining the success of these plants in situ.** A manuscript on these effects on functional physiology as well as the genetic diversity of rhizosphere microorganisms is in preparation (Christensen, Priemé, Stevnbak).

N-leaching - Monthly/bimonthly measurements of soil water concentrations of nitrogen in the main rooting depth and in the leaching water have shown that N concentrations are reduced by a factor of 10 from the main rooting depth to the leaching and **leaching of nitrogen is small and amounts to ca. 0.05 g/m²/yr.** As expected, the NH₄ and NO₃ concentrations are about equal in the top soil and dominated by NO₃ in the leaching water. No treatment effects have been detected over the first 8 months of treatment.

Plant growth and plant species competition estimates - Net photosynthesis levels were 3-4 times higher in *D. flexuosa* than in *C. vulgaris* across all treatments and across the two levels of CO₂ and in correspondence the transpiration was 2-4 times higher in *D. flexuosa* vs. *C. vulgaris*. Thus, **the potential carbon sequestration appear to be much higher in *D. flexuosa* vs. *C. vulgaris*. In parallel, *D. flexuosa* may have a stronger impact on the hydrological cycle through water consumption relative to *C. vulgaris*.**

A method for estimating plant competition and predicting the dynamics of plant communities from non-destructive pin-point measurements was developed and tested on measurements from the 8-year long climatic treatments at the VULCAN site at Mols. Significant competitive interactions between *C. vulgaris* and *D. flexuosa* have been observed. *D. flexuosa* seem to affect both the cover and compactness of *C. vulgaris* individuals as a function of the compactness the previous year, whereas *C. vulgaris* significantly affect only the compactness and not the cover of *D. flexuosa*. When both species are found at relatively high plant covers the two species are predicted to coexist for a long time period relatively to the time scale of the ageing process of *C. vulgaris*. **The non-destructive measurements combined with model estimates show a strong potential for analyses of the competitive interactions in natural plant communities** and can provide important new insight into the processes that determine the composition of plant communities.

Treatment effects after one growing season

Photosyntheses - The maximal photosynthetic and transpiration rates (P_{\max} and Tr_{\max}) differed across treatments in the order $A > T > CO_2 > TCO_2$ (P_{\max}) and $A > CO_2 > T \approx TCO_2$ (Tr_{\max}). This means that **the photosynthetic activity was downregulated by CO_2** , which is in accordance with other FACE studies. On the other hand **an expected improvement of water use efficiency (WUE) was not significant at this level**. Despite of the downregulation of the CO_2 uptake the numeric **photosynthetic rate was 1.5-2 times higher in both *D. flexuosa* and *C. vulgaris* plants acclimated to the elevated CO_2 level** relative to ambient CO_2 . In addition these rates were in parallel with an improved WUE. **Night warming affected the photosynthesis negatively** and pointed to *D. flexuosa* as being more sensitive to the temperature treatment compared to *C. vulgaris*. The direction of responses to the combination of passive night warming and elevated CO_2 (TCO₂) were the same as for the single factors. However, the TCO₂ treatment also indicated a species dependent impact since the photosynthesis and transpiration rates for both *C. vulgaris* and *D. flexuosa* were numerically lowest in the TCO₂ treatment. **Plant water potential was negatively affected by both the drought and temperature treatments, whereas CO_2 had the opposite effect**. The effect of drought was relatively stronger than other factors leading to negative effects in the combination treatments.

Plant community - After the first experimental drought in August 2006, we observed a significantly negative effect on *D. flexuosa* green leaves in the plots where drought was applied indicating decreased compactness of the grass. There was no main effect of enhanced CO_2 while temperature had a close to significant negative effect on *D. flexuosa* green leaves in ambient CO_2 plots but a positive effect in high CO_2 plots ($p=0.08$). We observed both inter annual variation and seasonal variation in the percentage of dead *D. flexuosa* leaves. These variations between and within years may be caused by climatic factors such as the long dry summer period experienced in 2006. The drought treatment affected the compactness of both *C. vulgaris* and *D. flexuosa* individuals negatively and increased the mortality of *D. flexuosa* leading to an increased amount of dead leaves, whereas night warming had no significant effects on either species. **This potentially negative effect of the drought appeared to be slightly reduced in plots with combinations of drought and CO_2 . This may be due to the better water use efficiency following increased CO_2** . The fact that this response was only seen in *D. flexuosa* and not in the other abundant species *C. vulgaris* may be explained by the different life forms and strategy of the two species with *C. vulgaris* responding slower due to the lower growth rate and its adaptations to nutrient poor habitats.

Physiology of drought tolerance in soil fauna - Studies on soil fauna adaptations and responses to increased drought stress employed a combination of field trials with manipulated drought exposure at other field sites in order to reduce destructive field sampling at Brandbjerg and laboratory investigations to supplement this research. The effects of drought on enchytraeids and microarthropods in the field showed that surprisingly, the severe drought levels applied (around the permanent wilting point of plants) did not have long-lasting effects on enchytraeids or microarthropods, and combinations with copper pollution did not enhance effect of drought. We consider these studies to be of considerable value as “training examples”, which will aid in the design of future field and laboratory experiments within CLIMAITE. In another study using a “space for time” approach clinal variation in desiccation tolerance of the collembolan, *O. cincta*, showed genetically based differences in resistance to high and low temperature, desiccation and water loss rate. **This provides evidence for geographical variation in stress resistance of springtails related to climatic**

conditions, and thus, that sufficient genetic variation is present in this species to make adaptation to climatic changes possible. On the other hand, a similar study on the enchytraeid, *C. spagnetorum*, sampled from the plots in the VULCAN experiment, suggested that this species did not have the potential to adapt to increased occurrence of drought.

Soil respiration - Within all treatments soil respiration (SR) shows the expected seasonal pattern with higher SR during the summer relative to winter. The yearly average SR in the control plots was $1.68 \pm 0.62 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$, equivalent to $53 \pm 20 \text{ mol CO}_2 \text{ m}^{-2} \text{ year}^{-1}$. The highest SR was recorded in plots with the combined temperature and CO₂ treatment ($2.11 \pm 0.71 \mu\text{mol m}^{-2} \text{ sec}^{-1} \approx 66 \pm 23 \text{ mol m}^{-2} \text{ year}^{-1}$), while the lowest flux was found in the temperature-drought treated plots ($1.26 \pm 0.49 \mu\text{mol m}^{-2} \text{ sec}^{-1} \approx 39 \pm 16 \text{ mol m}^{-2} \text{ year}^{-1}$). **These observations do support the hypothesis that enhanced CO₂ and temperature increases belowground activity due to stimulated plant growth.** It is interesting, however, that **SR in all treatment combinations including CO₂ were consistently higher than control plot SR.** This was not the case for temperature treatments suggesting that CO₂ is more important for SR than temperature and that CO₂ may even reverse depressed activities induced by temperature and drought.

Other important results

Initiation of PhD studies

Over the past 1½ year CLIMAITE has initiated 6 PhD studies, which is one more than originally planned. The initiated PhD studies cover the basic scientific areas within the project. By 6 PhD studies it has been possible for the PhD students to create a strong and lively PhD environment, where the PhD students arrange their own meetings to discuss practical and scientific ideas, problems and results. The steering group of the centre view this PhD collaboration as very fruitful and unique. The CLIMAITE PhD projects contributes directly to the activities within 3 of the PhD schools at KU and KVL. These are:

- COGCI – Copenhagen Global Change Initiative: Louise Andresen
- REFOLANA – Jane Kongstad
- PhD school in Biology – Kristian Albert, Karen Andersen, Merete Selsted, Kristine Maraldo

Additionally 2 PhD projects have been announced and are awaiting the evaluation of applications. The two PhDs are:

- Root studies: examination of below-ground root production, input of root litter to the soil and root litter decay rate.
- Fungi: Studies of responses of the overall fungal communities and meso-fauna fungal diet to the different treatments.

Scientific networks and collaboration

In CLIMAITE we have specifically aimed to involve the centre in national and international networks. This is because we strongly believe that the results from CLIMAITE will be many times more valuable and useful if they are combined with results from other projects under different conditions around the world. Furthermore we believe that CLIMAITE has a lot to offer in terms of scientific results and technical experience, which will be useful for other scientists. Development and involvement in networks, distribution of information and involvement in international projects has had high priority in parallel with the scientific activities. CLIMAITE has been involved in the following networks and collaborations:

Lund University – Collaboration with Harald Sverdrups group to set use the FORSAFE model framework to model Brandbjerg (hydrology as well as ecological responses. Collaboration with Pål Axel Olsson, Plant Ecology and Systematics, on isotopic analysis in fatty acids. Collaboration with Lena Ström, Department of Physical Geography and Ecosystem Analysis, GeoBiosphere Science Centre.

Helsinki University – Collaboration with Eeva-Stiina Tuittila, Dept. Forest Ecology, on CO₂ flux measurement techniques.

KVL - statistics – In order to assure a thorough and consistent statistical treatment of the responses, a collaboration has been set up with the statistical department at KVL who has developed standard SAS models for data treatment of in the split-plot design we are using.

TERACC – Terrestrial Ecosystem Research and Climate Change. A US based network aiming to coordinate terrestrial ecosystem research projects and synthesise results. CLIMAITE is connected to this network.

PrecipNet – Precipitation change Network. An international scientific network of research projects and manipulation studies focusing on effects of precipitation change. CLIMAITE participates in this network.

IBIMET. University of Firenze. Franco Miglietta. Experts in designing FACE-experiments. Has been responsible for the design and technical control system for the FACE experiment.

Publicity

Publication of the CLIMAITE activities to the public and the press has been done through launching of a website (www.climaite.dk) describing the activities and the results in both Danish and English, press releases in relation to important activities (project opening and international workshop), and direct contacts to the press and publication of popular science papers. Furthermore, we have continuously filmed the activities aimed at later TV broadcasts.

Publications

The building of the field site was finished in late 2005, and just recently the first years of treatments was finalized. Consequently, the number of scientific papers based on the responses has been limited. However, several papers have been published based on the considerations behind the experiments in CLIMAITE and conceptual and modeling considerations.

Organisation of international workshop - EPRECOT

Climate change will lead to changes in amounts and distribution of the precipitation. This aspect has so far received limited attention despite the fact that effects of precipitation changes in many cases are as big as or even bigger than effects of elevated CO₂ and temperature. Therefore CLIMAITE participated in the organization of the EPRECOT workshop aiming to provide an overview of the present knowledge on effects of precipitation change on terrestrial ecosystems and advising future research. The workshop was held in Helsingør in May 2006 and was organized in collaboration with the US networks TERACC and PrecipNet and economically supported by EU. A field excursion to the experimental field site at Brandbjerg was arranged for all participants during the workshop. The results from the workshop will be published in a number of scientific papers over the next year and was summarized in a "brochure" for policy makers and laymen.

European research project - NitroEurope

The experimental facility at Brandbjerg has provided a unique possibility for involvement in international networks and EU projects. In February 2006 a new EU project was initiated focusing on carbon and nutrient interactions and emissions of green house gasses, where CLIMAITE participates. Brandbjerg is a core site within the "Experimental manipulation" component in the project where intensive measurements of the green house gasses N_2O and CH_4 will be conducted to highlight the relationship to elevated CO_2 , temperature and drought. The project runs until the beginning of 2011.

Education

In addition to the PhD projects, CLIMAITE has participated in several educational activities, and with the experiment running, a large number of new projects have been planned. A list of student projects is provided in the appendix.

New planned activities include:

- **New student projects** – A catalogue of student projects have been collected and students are presently being recruited for the next growing season.
- **Involvement in future courses**
Four new student projects have been designed for at total of 12-20 students during three weeks annually in the course Experimental Ecology Projects, KU:
 - Photosynthesis during winter
 - Plant respiration during winter
 - The effect of simulated root exudation on microbial C and N
 - Anthropogenic N deposition on heaths: Effects on plants, soil and microbes

Plan for next 2-year period

The activities for the full 5 year project were largely outlined already in the project description when the project started. This 5 year plan still holds, and no major changes are needed. The overall project activities are therefore:

WP1

The final 2 years of the project period will be devoted to keep the treatments running and documenting the system and the treatments

WP2

Activities during the next 2 year include plant photosynthetic measures at a reduced frequency and with a particular aim to generate response functions of ecophysiological processes to temperature and soil-moisture. Seasonal dynamics of enchytraeids and microarthropods will be mapped and their vertical distribution in relation to soil water potential changes as well as physiological capacities in tolerance of drought conditions and the potential of climate stress adaptation will be measured. Additional lab. experiments will be conducted to estimate excretion rates of N (ammonium, urea, allantoin and uric acid) in soil invertebrates and microfauna. Rhizosphere populations will be followed on identified plants supplemented with introduction of plants from warmer and cooler climates (F, D, DK, S) and subjected to a herbivory treatment with aphids in the field. If possible the impact of precipitation events and additional stress i.e. ozone or heat will be investigated.

WP3

Basically, the scheduled activities will be continued according to the plans. In 3.1 repeated measurements of the abundance and phenology of plants will be performed to follow the resistant and resilience in the species to climatic stress. Reflectance measurements of plants to reveal changes in timing of phenological events will be

initiated in 2007. In 3.2, the scheduled activities will proceed as planned. In 3.3, the scheduled activities will continue according to the original plans except that soil sampling frequency will decrease to twice a year and metabolic profiling on BIOLOG plates will be out-phased. On the other hand, new activities will be introduced (see below). In 3.4 litter quantity and quality will be measured by NIR and wet chemistry to reveal resource allocation to growth or defence compounds. Decomposability of the litter is followed by litter bag studies.

WP4

Basically the scheduled activities will be continued according to the planning. In WP4.1 frequent measurements of CO₂ fluxes will go on at least until April 2007. Thereafter shorter intensive campaigns will take over. Pulse-labelling (WP4.2) will be repeated in spring and autumn 2007 to detect whether the system is adapting. In WP4.3 sampling of litter bags and buried bags will occur April 2007 and finally in October 2007. Nitrogen-15 isotope pool dilution experiments will be initiated spring 2007, and currently efforts are made to attract a master student to perform the work. Measurements of potential nitrification rates and potential denitrification rates are continued as are also the monthly soil water collections.

WP5

The synthesis work will increase its importance as the results start to become available. In order to have a frame for organisation of the synthesis we will continue the efforts to develop a modelling concept in collaboration with Lund University. A crucial point in this work will be the employment of a modelling post doc from the start of 2007. Also, half year meetings involving all researchers have been agreed where the results will be presented and discussed.

WP6

The management and coordination of the project is generally running very efficiently with the main scientific decisions made at the work package level and with the centre manager and steering group making the overall decisions. Therefore no major changes are needed. The efforts to publish the results scientifically as well as popular will continue. Publication plans have been sketched for each activity and popular writings have been agreed with Jord & Vand for early 2007 and 2008. An international conference will be organised in 2008, either as a CLIMAITE conference or more likely as a session within an existing international conference series.

Account 2004-2006 and Budget 2007-2009

	VKR-funding						External funding				Total	
	2004-2006		2007-2009		2004-2009		2004-2006		2007-2009		2004-2009	
	mmth	kkr	mmth	kkr	mmth	kkr	mmth	kkr	mmth	kkr	mmth	kkr
WP1	28	3592	15	1186	43	4778	36	3256	21	2279	100	10513
WP2	79	3262	55	2269	134	5531	16	972	14	822	164	7325
WP3	39	1852	44	2195	83	4047	11	711	9	561	103	5319
WP4	55	2484	82	3158	137	5642	20	1257	20	1242	177	8141
WP5	15	802	34	1477	49	2279	15	1125	10	750	74	4154
WP6	13	1168	8	1555	21	2723	0	150	0	0	21	2873

	VKR-funding		External funding			
	2004-2006	2007-2009	2004-2006	2007-2009		
	Kkr	Kkr	Kkr	Kkr		
Total	229 13160	238 11840	467 25000	98 7571	74 5654	639 38225
Investments and consumables						
Site	2622	852	500	0		0
Travel	259	437	0	0		0
Meetings & communication	224	225	150	0		0
Management	35	65	0	0		0
Investments	1174	0	0	0		0
Consumables	1405	1938	600	1000		
Salary						
VIP	2563	1818	3150	1800		
Tek	2460	1836	1356	904		
PhD	2123	3673	1680	1680		
PostDoc	296	996	135	270		
Total	13160	11840	7571	5654		

Account (2004-2006) for VKR funding and external funding separated into work packages (Table 1) and Main cost categories. External funding includes inputs from participating institutes, DONG Energy, Air Liquide Denmark and EU.

Perspectives and recommendations for the future

The experiments at Brandbjerg have been in operation for one full year and will continue for another 2 years under the framework of CLIMAITE based on the present funding. Within the full extent of the project we are confident that the project will lead to new and exciting findings highlighting interactions between the main climatic factors CO₂, temperature and water. These will mainly be the "first stage" responses and will be useful for model development and short term evaluations of climate change effects.

As climate change will act over decades it would be very relevant to extend the CLIMAITE experiments and the research for a longer time in order to obtain information on the long term effects and the more stable changes in the ecosystem. Long term responses to climatic perturbations are hypothesized based on experience from previous short term studies, but it is not until recently documented in the scientific literature (e.g. Reich et al., 2006). This new information emphasises the need to maintain and extend ongoing activities in studies on climatic effects on terrestrial ecosystems. Furthermore, since the effort and resources put into the development of the field site has been quite substantial and the site is considered to be of a high quality, it would be very appropriate to keep it running past the originally planned 5 year period and to take advantage of the facility in order to attract European funding under the 7th framework programme and to develop the networks already established. It is therefore our wish to continue the experiment for another 5 year period and we hope that VKR will consider an extension.

We therefore suggest that the VKR foundation considers a 5-year extension of the current project, beginning in April 2009 when the current 5 year period expires. It is proposed that a continuation is initiated with a 3 year commitment, and that a potential final 2 year extension will be decided by the end of 2009.

The reason to bring the question up at this moment only half way into the first 5 year period is that it will provide an opportunity for more long term and continuous planning of the activities. If for example we want to start new PhD projects or engage ourselves in long term EU contracts and international networks, we need some security that the basic activities will still be running throughout the commitment period. Furthermore, if we know the funding situation after year 5 already now, we can keep a high momentum and start activities to continue into the next phase rather than winding down towards the end.

To provide a solid basis for such a decision within VKR we have tried in the following to sketch a work plan for CLIMAITE beyond 2009.

Basic idea - adaptation

The present organisational and scientific structure of CLIMAITE has proven very strong and logic and we see no pressing reasons to change that. This means that a scientific division of the activities into species responses, community responses and ecosystem functioning responses will still be the framework for the activities and that a specific activity to focus on syntheses is needed.

We plan to change the scientific focus slightly. While much of the activities in the first phase has been devoted to relatively short term responses we want to look at more long term responses and adaptations to the changed climatic conditions. Therefore

the overall focus of an extension will be placed on "adaptation" trying to answer the question: "*What determines the success of organisms under Global Change?*".

The altered climate will result in changes in the abundance of the different plants, animals and microorganisms in the area and/or establishment of new species. Which properties determine the success of these species relative to those species being reduced or disappearing? Central properties could be species physiology, life history patterns, and/or species interactions such as the relations between rhizosphere organisms and plant roots.

Under permanently changed climatic conditions the ecosystem is expected to adapt to the new conditions. Such ecosystem adaptations and changes can happen through a number of different processes:

- Genetic adaptation: Microbial and plant genetic changes within species
- Community changes: structural changes of the ecosystem (due to extinction of some species and increased abundance of other, or invasion of new species)
- Physical ecosystem structure: soil structural changes, canopy structure
- Chemical changes: soil carbon, leaf chemistry and litter quality

The present CLIMAITE experiment offers a unique setting for answering such questions that attempts to approach an understanding of the mechanisms underlying the threats to natural ecosystems due to Global Change.

This means that new scientific activities and potentially new partners will be involved, while the present monitoring programme will remain the same. Thus, most of the measurements being done now will continue in order to get a long term and development picture of the responses, while other activities can be reduced in intensity, frequency or even be stopped. In the plan sketched below we will mark these differences.

WP1 - experimental manipulation

The site at Brandbjerg is fully developed and provides a high quality site for experimental manipulations. At present we do not foresee specific needs for change in the setup of the experiment. The main activities during a second phase will be:

- **Maintenance** – the high technical level of the experiment require a strong effort in maintaining and developing the field site and the technical installations
- **Intensified warming** – the warming experiment is in the lower end of the expected future temperature increase. Attempts to intensify the degree of warming without compromising the present setup will be explored.
- **Additional experiments** – in addition to the present experiments smaller studies outside the plots may be initiated to explore specific questions and processes, e.g. the effect of water on the biological processes. Also, small flexible units to apply other treatments within the present plots may be considered. (e.g. UV radiation, ozone, nutrients, additional water manipulations).

WP2 - Species responses

Under changing environmental conditions, the survival of a population depends on the ability of the individuals to either tolerate or avoid stress. The development of tolerance can either be due to phenotypic plasticity (acclimation), e.g. physiological/biochemical acclimatisation and activation of alternative genes, or be an inherited tolerance that is favoured by selection (adaptation). Genetic adaptation to increased climatic stress may occur, if sufficient genetic variation for increased resistance is present in the population under consideration. Therefore, we will particularly address the important question whether a species is able to adapt to changing environmental conditions in its habitat. Will some species be able to compensate for increased stress (e.g. from drought), or will populations suffer from stress and decline in numbers over a longer period and eventually become extinct? Will increased CO₂ cause adaptational changes in some plant species making them more stress tolerant or providing them with competitive advantages?

Continued activities

Plant physiology:

Responses of primary plant physiological processes will be continued, although with a reduced intensity and with a gradual shift towards physiological response functions. Such response functions will also be of central importance to the development of plant ecophysiological modelling.

Potential new activities

Genetic variation and adaptation

Investigation of genetic variation for increased stress resistance by comparison of iso-genetic lines derived from field populations in the differently treated plots at Brandbjerg indicating the potential for climatic stress adaptation in keystone plant and soil animal species, and the speed with which this process proceeds. Combinations with a “space for time” approach will further elucidate the questions of adaptation. Climate changes such as increased severity of droughts could reduce the contribution of soil fauna on the turnover of live fungal hyphae in the soil. Such complex interactions between fauna and fungi in a changing climate will be investigated through studies of the consumption of fungi by meso-fauna by modern molecular and genetic marker techniques will reveal the fungal diet of the individual animal species and will open for investigations of seasonal and climate-related changes in diet.

WP3 - Community responses

Climate change affects the performance of species in different ways and may potentially change community composition. The rate of climate change may exceed the rate of adaptation within both species and community which may result in poor performance or loss of existing species and may not be compensated by recruitment of new species if the dominant species form a dense vegetation cover. At Brandbjerg *C. vulgaris* being a specialist plant with a narrow niche may be expected to be more sensitive to the climatic changes as a compared to the other dominant species, *D. flexuosa*, which has a higher potential for adaptation to the new conditions. However, *C. vulgaris* is very resistant to environmental changes as a mature plant, whereas it is highly sensitive to climate change during its regeneration phase. Therefore, species changes may most likely be related to the phase where the *C. vulgaris* plants die due to age and have to regenerate from seeds.

Species composition change will have consequences for changes in litter quality and quantity and subsequently changes in the turn over rate of soil organic matter and carbon sequestration. Furthermore, aboveground and belowground litter may have different turn over rate and a shift in root:shoot ratio and rooting depth in response to climate change may also be important for soil carbon sequestration.

Continued activities

Community composition of plants, bacteria, fungi and ammonium-oxidizing bacteria: Plant performance will be studied continuously in the permanent vegetation plots to follow changes in plant performance e.g. growth, cover, flowering and seed production and potential loss of species. A simple plant competition model developed within CLIMAITE will be applied to the longer time series and more intensive plant competition studies e.g. asymmetric plant competition, and testing of more complex plant competition model. The timing of key biological processes (e.g. flowering, bud burst and senescence) are key parameters describing the changes in seasonality caused by climate change factors. These will be followed continuously.

New activities

Quantification of bacteria, fungi, and plants using molecular techniques. Studies of functional and genetic properties of rhizosphere microbes of adapted and invading plants will be important to understand the success of these invading organisms under global change. Molecular techniques are becoming more and more powerful and can be applied, e.g. Q-PCR, T-RFLP and PLFA, to identify community

and functional changes in plants and microorganism and evaluate changes in the potential for decomposition of complex organic compounds.

Regrowth and recruitment

Plant recruitment and plant competition and adaptation during the regeneration phase can be studied in areas within the experimental plots where plant biomass has been harvested due to other activities. This will mimic the conditions in the heather life cycle where *C. vulgaris* plants die due to age and have to regenerate.

Plant chemical composition and secondary metabolites in plants

The effect of treatments on plant litter quality can be followed with spectral analysis and/or NMR. The changes in litter quality within specific plant species as well as changes in litter quality due to changes in plant abundance will be a determining factor for long term adaptation of mineralization processes and nutrient cycling.

Soil structure and changes in soil profile distributions

Changes in soil structural and vertical distribution and functioning of roots and associated organisms in response to the treatments with significant effects on the availability of nutrients, soil carbon and water availability will be important long term changes to study. Root density and root distribution by depth can be determined by the use of rhizotrons supplemented with estimation of root biomass in soil cores used for other purposes as well as estimation of soil enzymes and root phosphatase activity by depth. Further, uptake experiments can be designed to investigate uptake capacity of roots by depth. Functional rooting depth and the plant water uptake potential at various depths may be studied in the manipulated plots by stable isotopes of oxygen and/or hydrogen. In addition, hydraulic lift caused by the plants may benefit microbes and enhance mineralization and organic matter turnover. This role of plants in facilitating microbial acquisition of water from deeper soil layers can be studied as well.

WP4 - Functional responses

Ecosystem adaptation may be manifest through permanently changed cycling and interaction of the main elements (C, N and P) due to species and structural changes. For example, prolonged exposure to enhanced CO₂ may change the relative importance and availability of e.g. N and P. Such changes may affect the exchange of carbon and greenhouse gases (GHGs) between the biosphere and the atmosphere, the leaching of elements to the ground water, and the C pools in soil and plants. An important question to be addressed in an extended project therefore is to what extent the system is capable of allocating additional N/P resources and how this affects the permanent storage and exchange of carbon and nutrients with the environment.

Continued activities

CO₂-sequestration, GHG exchange and feedback to the atmosphere

The long term integrated responses of the overall carbon and nitrogen budget due to the altered climate change factors and consequent altered size and stability of the SOM-pool will also be continuously studied by use of chambers, lysimeters and stable isotope labelling. This will focus on potential feedback to the atmosphere through C and N sequestration and GHG emissions, and contamination of the groundwater through leaching of C, N and P compounds.

New activities

Isotopic tracing

Combined ^{15}N tracer experiment and an *in-situ* $^{13}\text{CO}_2$ pulse tracer experiment will provide a basis for studying the temporal dynamic of C-sequestration and –allocation over prolonged periods and under natural conditions with respect to species interactions and climatic perturbations. Application of the $^{13}\text{CO}_2$ at this scale will also provide access for other groups (fauna, microbiology, soil chemistry, soil gases) to study detailed C-cycling in the site.

C-mineralization kinetics.

The CLIMAITE fumigation with ^{13}C -depleted CO_2 serves as an isotopic label of new plant tissues and soil organic matter. This can be used to better characterize soil heterotrophic activity and soil organic matter decomposition under various environmental conditions. Previous work, for example, has shown that the microbial community structure changes with temperature causing a shift in mineralizable carbon pools. Studies of the soil organic matter mineralization at contrasting temperatures can be used to examine if the mineralizable carbon pools are affected by long-term climatic perturbations.

Mycorrhizal function and diversity

Mycorrhizal fungi are an important, integrated part of the plant-soil system, and long-term climatic changes affect the diversity and functioning of mycorrhizal fungi and thereby the ecosystem functioning. Therefore studies of mycorrhizal colonization of the dominant plants *D. flexuosa* (arbuscular mycorrhiza) and *C. vulgaris* (ericoid mycorrhiza), and the mycorrhizal fungal function, community composition and diversity by in-growth bags previously used for fungi in sandy soils and application of molecular techniques and isotopic labeling will be important.

Litterfall and litter decomposition

Decomposition of leaf litter from *D. flexuosa* and *C. vulgaris* is expected to be altered by the global change factors and an investigation of decomposition of initial leaf litter is ongoing. Leaf litter quality (e.g. C/N ratio) may change due to altered carbon sequestration and production of secondary metabolites. Comparison of decomposition of plant litter collected inside and outside of the treatment plots can reveal the impact of litter quality changes and leaf litter from the manipulated plots (including ^{15}N labeled litter) may be used as a tool to reveal long term effects changes in litter quality on nutrient cycling. Turn over rates of leaf and root litter, different growth forms and knowledge on the changes in their abundance can be used to access the impact of the different climate manipulations on soil carbon balance.

WP5 – Synthesis

Process, species and ecosystem adaptation to climatic changes may work at different time scales and be linked in a highly non linear fashion (e.g. the process from individual species becoming stressed by the climatic changes translating into changes in community composition and litter quality and finally into changed structure and functioning of the ecosystem). Therefore, synthesizing the adaptation responses across the different work packages is crucial to understand how climatic change will affect the ecosystem on the long term. Furthermore, if our knowledge on the adaptive changes should be translated into assessment of future consequences these have to be included and tested in mathematical models. Therefore, the model

work which will be initiated during the first CLIMAITE phase will be continued and slightly changed into a more predictive and assessment mode.

Continued activities

Development and application of the model concept started in phase 1. This may include 3 major advancements:

- Development of individual model compartments such as plant carbon and water exchange, plant competition, plant-microbe interaction and soil fauna-microbial interactions.
- Extrapolation of the results into the future and to other geographical and biological conditions.

WP6 – Networking and outreach

The majority of the activities under WP6 will be continued and strengthened under an extended project period.

Management: The management of CLIMAITE has worked very well with much of the scientific work managed by the work package leaders and coordinated through the steering committee. Consequently, no major changes in management structure will be needed.

Scientific advisory board: The scientific advisory board has been very useful and inspiring for the project participants and the management. They have provided excellent external feedbacks on the project and its progress and we therefore expect to engage the advisory board also during an extension.

Networking: During the first 3 years of the project CLIMAITE has already engaged with researchers and research groups from all over the world. Since multifactor experiments is a relatively new and challenging area, the interaction with other groups and networks is important in order to share experiences and develop a better understanding of the complex results. CLIMAITE will continue to engage with networks already formed and develop new networks wherever needed.

Publication and publicity: With only one year of treatment until now, the results from the experiments are still limited. However, a number of publications have already been produced or have been submitted based on the initial considerations and results or previous experiences. In the next 2 years and in an extended period we will have a strong focus on scientific publications in high quality international journals. Also, publications in more popular scientific journals and other media will be followed as has been the case until now.

Education: We are strongly focused on using the CLIMAITE facility in the education of master and PhD students at the universities. A series of potential PhD projects are described in the appendix as well as a catalogue of student projects for the universities. Also, international PhD summer courses may be offered.

Budget 2009-2011

Total budget	Requested from VKR						Own contribution	
	Sci+P hD mmnths	Tech	Salary kDKK	Invest kDKK	Cons kDKK	Sub-Total kDKK	mths	kDKK
WP1 – Experimental treatments								
Running costs (CO ₂ , Power etc.)					1000			1000
Staff	18	18	1400				18	650
<i>Total</i>	18	18	1400		1000	2400	18	1650
WP2 – Species responses								
WP2 – Science	14	14	1100	200	300		6	300
<i>Total</i>	14	14	1100	200	300	1600	6	300
WP3 – Effects on Ecosystem Structure								
WP 3 – Science	14	14	1100	100	300		6	300
<i>Total</i>	14	14	1100	200	300	1600	6	300
WP4 – Effects on Ecosystem Function								
WP 4 – Science	14	14	1100	200	500		18	800
<i>Total</i>	14	14	1100	200	500	1800	18	800
WP5 – Syntheses								
Scientific syntheses	12		600				9	400
PostDoc	36		1200					
Workshops					250			
3 PhD studies	108		3600				54	1800
<i>Total</i>	156		5400	0	250	5650	63	2200
WP6 – SENDOUT								
Management & information	6	6	450		200		6	300
Travel costs					400			
Reserve					1000			
<i>Total</i>	6	6	450	0	1600	2050	6	300
External funding – EU projects etc.								
NitroEurope							10	400
FP7								1000
Forskningsråd								1000
Abisko ??								500
Total	222	66	10550	500	3950	15000	127	8650

Total budget (mmths (Scientific+PhD and Technician) and kDKK) for CLIMAITE showing requested budget from VKR (Salary, investments, consumables) and resources provided from participating institutes and external sources separated into work packages