Carbon Dynamics in Heathlands in Response to a Changing Climate
Pia Lund Nielsen

Carbon Dynamics in Heathlands in Response to a Changing Climate
ABSTRACT

Climate is changing, and more adverse changes are expected in the future. Changes are caused by continuously rising atmospheric concentrations of greenhouse gases as CO₂. These changes will affect ecosystem processes and functions in the future and hence the cycling of carbon.

Many studies have been conducted observing effects of climate change on aboveground biomass, less have been conducted observing changes in belowground biomass, and even fewer studies exist that comprise both above- and belowground biomass and take interactions of climate change factors into account. This thesis tried to do that. First two studies were carried out in a Danish heathland (CLIMAITE) dominated by the grass *Deschampsia flexuosa*, while last study was carried out in three heathlands (INCREASE) along a precipitation gradient from a dry heathland (Mols) in Denmark, a mesic heathland (Oldebroek) in Netherlands and a wet heathland (Clocaenog) in Wales. The vegetation at the sites was dominated by the evergreen dwarfshrub *Calluna vulgaris* and *Deschampsia flexuosa*. First part focus on how climate changes affected carbon assimilation and partitioning with in plant and soil and represent the short term effects of climate changes on carbon cycling. Second part investigated the effects of climate change on biomasses of shoots, roots, dissolved organic carbon and microbes after six years of treatment. Last study investigated effects of climate change of decomposition and chemistry of fine roots from the three heathland in INCREASE.

To follow the fate of carbon in the ecosystem we used the stabile isotope ¹³C. We found elevated CO₂ to increase enrichment of ¹³C in leaves and deep roots of *Deschampsia flexuosa*, and to speed up carbon cycling, while drought reduced ¹³C enrichment in top roots and slowed down carbon cycling. Both elevated CO₂ and drought led to more carbon being allocated in the deepest roots and effects added up, while often the two factors eliminated each other. Long term effects showed that root biomass increased due to elevated CO₂, and the effects seemed to increase over time. However it was also evident that the effect size was dependent on sampling month. Aboveground biomass varied substantially over season, but showed no persistent changes over the years. Responses of aboveground and belowground biomass were coupled, and *Deschampsia flexuosa* showed high ability to adapt to treatments. Only fine roots from Clocaenog responded to climate change resulting in slower decomposition in roots from drought plots, likely due to longer drought periods and hence more adverse effects on soil conditions. Lignin, and to some extend suberin, seemed to protect against unfavourable conditions in the soil. However no coupling between treatments induced changes in fine root decomposition and chemistry were found.
In contrast to the slower cycling of new carbon in drought plots in the first study, we found a higher rate of decomposition of fine roots of *Deschampsia flexuosa* grown in drought plots. Further fine roots of *Deschampsia flexuosa* from deep layers showed much slower decomposition than fine root from top layer. Higher roots biomass and deeper allocation of carbon in response to elevated CO2 combined with the slower decomposition of deep roots could affect future carbon cycling. Decisive for consequences of climate changes on cycling of C and potential for soils to sequester carbon depend on the further fate of carbon in the rhizosphere.
RESUMÉ

Vores klima forandrer sig, og kraftigere ændringer er forventet i fremtiden. Forandringerne er forårsaget af den kontinuerte stigende atmosfæriske koncentration af drivhusgasser som CO₂. Disse forandringer vil påvirke økosystem processer og funktioner i fremtiden og dermed også kulstofkrede løbet.

Der er udført mange studier der observerer effekter af klimaforandringerne på overjordisk plantebiomasse, mens langt færre har studeret ændringer i underjordisk biomasse. Endnu færre studier eksisterer der både inkluderer over- og underjordisk biomasse og tager interaktioner af forskellige klimabehandlinger med. Denne PhD afhandling forsøger netop dette. De første to studier blev udført på en dansk hede (CLIMAITIE) domineret af græsset *Deschampsia flexuosa*, mens det sidste studie blev udført i tre forskellige heder (INCREASE) bevokset med både den stedsegrønne dværgbusk *Calluna vulgaris* og *Deschampsia flexuosa*. Hederne er lokaliseret langs en nedbørsgradient fra en tør hede (Mols) i Danmark, en semi-tør hede (Oldebroek) i Holland til en våd hede (Clocaenog) i Wales. Første del fokuserer på hvordan klimaforandringerne påvirker kulstof assimilering og allokering i plante og jord, og repræsenter korttidseffekter af klimabehandlingerne på kulstofomsætningen. Anden del undersøger effekter af klimaforandringerne på biomasser af skud, rødder, opløst organisk kulstof og mikrober efter seks års behandling. Det sidste studie undersøger nedbrydning og kemi af finrødder fra de tre heder i INCREASE.

For at følge kulstoffet i økosystemet anvendte vi den stabile isotop ¹³C. Forhøjede CO₂ koncentrationer øgede berigelsen af ¹³C i blade og dybe rødder i *Deschampsia flexuosa*, og øgede hastigheden af kulstofomsætningen, mens tørke reducerede ¹³C berigelsen in top-rødder og sænkede hastigheden af kulstofomsætningen. Både forhøjede CO₂ koncentrationer og tørke førte til at mere kulstof blev allokert i de dybe rødder, og i felter med combinerede behandlinger blev effekterne lagt sammen. Studiet af langtidseffekter viste at rodbiomassen blev øget som respons på forhøjede CO₂ koncentrationer, og effekten lod til at øges over årene. Det var dog også tydeligt at størrelsen på effekten af forhøjede CO₂ koncentrationer afhæng af hvilken måned røddrøverne blev taget. Overjordisk plantebiomasse varierede kraftigt over sæsonen, men ingen blivende effekter af klimapåvirkningerne blev fundet. De fundne responser på over- og underjordisk plantebiomasse var koblede, og *Deschampsia flexuosa* udviste stor tilpasningsdygtighed til klimabehandlingerne. Klimabehandlingerne resulterede i lavere nedbrydningshastighed for finrødder fra Clocaenog men ikke for finrødder fra de andre lokaliteter, sandsynligvis pga. en længere tørkeperiode i Clocaenog og en derfor større påvirkning på jorden. Lignin, og i nogen grad suberin, så ud til at beskytte rødderne mod ugunstige forhold i jorden. Vi kunne dog ikke finde nogen kobling mellem ændringer i nedbrydningsraten og rodkemi forårsaget af klimabehandlingerne.
Som kontrast til den langsomme kulstofsætning vi fandt i tørkefelterne i det første studie, fandt vi en højere nedbrydningsrate af finrødder fra *Deschampsia flexuosa*, der havde vokset i tørkefelterne. Desuden fandt vi at finrødder af *Deschampsia flexuosa* fra de dybe lag blev nedbrudt langsommere end finrødder fra det øverste lag. Øgede rodbiomasser og dybere allokering af kulstof som respons på forhøjede CO₂ koncentrationer kombineret med en langsommere nedbrydning af dybe rødder kan påvirke den fremtidige kulstofsætning. Afgørende for konsekvenserne af klimaforandringerne på kulstofbalancen og potentielle for jordens evne til at binde kulstof bliver den videre skæbne af kulstof i rhizosfæren.
This PhD thesis is submitted to the Faculty of Science, University of Copenhagen, Denmark. The study was conducted at the Department of Geosciences and Natural Resource Management, University of Copenhagen, and was partly funded by CHANCE and the Danish VKR Centre of Excellence CLIMAITE (Funded by Villum Kann Rasmussen Foundation). The PhD was part of the Danish CLIMAITE experiment and the European INCREASE experiment and was conducted in the period of 2011-2015, including one maternity leave in 2013/14 and visits to the INCREASE sites in The Netherlands (University of Amsterdam) in September + December 2012 and to Wales (University of Bangor) in September 2013.

I wish to thank several people who helped me within the period I conducted this thesis. First of all thanks to Inger Kappel Schmidt, my boss and supervisor for believing enough in me to give me this chance of doing a PhD, for continuous support through the years, for remembering life with small kids and hence giving me the opportunity to balance my time for family and work. Thanks to Albert Tietema for hospitality and use of the Respicond at UvA, to Louise C. Andresen for hosting me in Amsterdam and for help with planning, setting up and running the experiment in the Respicond conducted at UvA. Preben Frederiksen for several times dragging me out in the beautiful field of Mols to do practical work after sitting too long periods in my office. Anders Michelsen for advice and reviewing of articles. Søren Barsberg for teaching me how to perform and analyse FT-IR spectra. Helle Sørensen for help with statistics. Thanks to the CLIMAITE group for great meetings, discussions and days at Kullen. Likewise thanks to the INCREASE group for great meetings and discussions at different places in Europe. To the biogeochemistry group and all the people in the hall way for making everyday life more cheerful. My first office mate Johannes for interesting ideas and for making statistic seem so simple and my second office mate Marie for good conversations and friendship.

Thanks to friends for being there when needed. Special thanks to my mother for taking extraordinary good care of our girls in the last time of finishing this thesis, my farther for fixing practical issues and hence making life easier and my brother for still wishing to compete with me and thus keeping me to the fire.

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>1</td>
</tr>
<tr>
<td>RESUMÉ</td>
<td>3</td>
</tr>
<tr>
<td>PREFACE AND ACKNOWLEDGEMENT</td>
<td>5</td>
</tr>
<tr>
<td>LIST OF PAPERS</td>
<td>7</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>8</td>
</tr>
<tr>
<td>AIMS OF THE PROJECT</td>
<td>14</td>
</tr>
<tr>
<td>METHODOLOGY</td>
<td>15</td>
</tr>
<tr>
<td>RESULTS</td>
<td>24</td>
</tr>
<tr>
<td>SUMMARY DISCUSSION</td>
<td>30</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>36</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>38</td>
</tr>
<tr>
<td>PAPERS</td>
<td>47</td>
</tr>
</tbody>
</table>
INTRODUCTION

Climate change
Ongoing and future increases in atmospheric CO₂ concentration will affect ecosystems directly and indirectly via changes in temperature and precipitation. Climate changes could lead to changes in carbon assimilation, biomass production, carbon partitioning and (re)allocation, carbon release as root exudates, microbial biomass, composition and activity, litter quantity and quality, decomposition rates and hence changes in carbon cycling.

Greenhouse gasses in the atmosphere have now reached an unprecedented level in at least 800,000 years (IPPC, 2014). Simultaneous we have seen increased warming since the mid-20th century, which is linked to the rising greenhouse gasses (IPPC, 2014). The surface temperature of earth has increased by 0.8 degrees Celsius since 1880 and temperature is expected to rise 0.2 degrees Celsius per decade within this and next decade (IPPC, 2007). Along with temperature changes, changes in precipitation are also predicted.

Climate scenarios for Denmark/the northern hemisphere include an increase in temperature of 2-3 degrees Celsius in 2100 compared to 1990. Night temperatures are expected to increase more than day temperatures. Precipitation is expected to increase in winter time and decrease in summer time with increased risk of drought periods (www.dmi.dk). These three factors, atmospheric CO₂, temperature and precipitation, are all drivers for important ecosystem processes, and changes in these are therefore expected to lead to changes in ecosystem functions.

In order to study how present and future changes in climate will affect ecosystem processes and functions two different set-ups of treatments simulating expected climate change was established and used in this thesis. The first set-up is the CLIMAITE experiment and comprises manipulations of precipitation (drought), temperature (warming) and elevated CO₂ and all combinations, while the latter set-up is the INCREASE experiment and comprises drought and warming separately.
Figure 2. Predicted changes in temperature (°C) in summer in the period of 2071-2100 compared to the period of 1961-1990 in Denmark (www.dmi.dk)

Figure 3. Predicted changes (%) in precipitation in winter (a) and summer (b) for the period 2071-2100 compared to the period 1961-1990 (www.dmi.dk)

Plant responses to climate change – current knowledge
Climate is one of the factors distributing plants, so when climate is changing, distribution might also change. The change in climate we have seen so far is only minor compared with what is expected by the end of the century, but nonetheless climate change is already changing living systems as seen in several studies (Walther et al. 2002, Parmesan and Yohe 2003, Rosenzweig 2007).
Plant response to elevated CO₂
Elevated CO₂ have been found to increase carbon assimilation into plants (Albert et al. 2011, Ge et al. 2012) and hereby increase the biomass of plants both aboveground and belowground (de Graaff et al. 2006). In natural ecosystems as grasslands increases in aboveground biomass have also been found as a result of elevated CO₂ (Barnard et al. 2005, Hovenden and Williams 2010), but more often belowground biomass is found to increase in grasslands as a consequence of elevated CO₂ (Higgins et al. 2002, Adair et al. 2009, Anderson et al. 2010). Higgins et al. (2002) found an increase in carbon fixation of 30-70% in elevated CO₂ in grassland, but this only let to a 30% increase in above and belowground biomass. Morgan et al. (2004) likewise concluded in a review that photosynthetic responses to elevated CO₂ were often unreliable predictors of plant growth responses. Biomass increases as a response to elevated CO₂ in natural ecosystems may be moderate due to nutrient constraint of N (Gill et al. 2006) or P (Menge and Field 2007). In nutrient limited ecosystems carbon have often been found allocated belowground for acquisition of nutrients (Saggar et al. 1997). Elevated CO₂ can also change litter quality and quantity (Cotrufo et al. 1998, Knops et al. 2007) and increase rhizodeposition of labile carbon into the soil (Eisenhauer et al. 2012), which again can lead to increased microbial biomass or activity (Blagodatskaya et al. 2010, Drigo et al. 2010).

Plant responses to drought
Drought tends to induce the opposite response than elevated CO₂, since drought increases the stomatal closure (Chaves et al. 2003), which reduces photosynthetic activity of plants and decrease the amount of CO₂ fixed by plants (Ruehr et al. 2009) and hence the amount of carbon available for biomass production. A review by Wu et al. (2011) reported suppressed aboveground biomass production in response to decreased precipitation, and Brunner et al. (2015) reported decreased fine root biomass in a review of tree root response to drought. Microbial activity and/or biomass is expected to decrease caused by both lower carbon availability and as a direct effect of drought (Jensen et al. 2003, Kardol et al. 2009), possibly resulting in a lower turnover of organic matter (Schmidt et al. 2004, Allison and Treseder 2008) or a longer residence time of carbon as found in Ruehr et al. (2009).

Plant responses to warming
The response to warming is depended on the soil moisture status. Warming can increase carbon assimilation in grass (Ge et al. 2012), but if water is scarce warming induced drought offsets the higher assimilation (Wang et al. 2014). If water is plenty warming may increase microbial activity and autotrophic respiration, and result in higher mineralization and hence nutrient availability (Rustad et al. 2001, Andresen et al. 2010). Warming has been found to increase number of Growing Degree Days (GDD) and Growing Season Days (GSD) (Beier et al. 2004), but warming can also induce earlier plant senescence (Zavaleta et al.
2003), which shortens time for photosynthesis and thereby reducing the stimulating effect of elevated CO₂ (Cheng et al. 2009, Cheng et al. 2010).

**Interactions of climate factors**
Larsen et al. (2011) found that interactions between climate change factors (elevated CO₂, drought and warming) were very common but also complex, and studies including several factors and interactions are therefore important. Interactions are especially expected between drought and elevated CO₂ since these induce the opposite effect on carbon assimilation and as the latter is leading to e.g. increased water use efficiency (WUE) diminishing the effect of drought. Warming can have a drought effect in warm periods, where higher temperature dries out the soil. The combination of elevated CO₂ and warming may increase biomass production due to combination of enhanced nutrient cycling due to warming (Ruess et al. 1999, Rustad et al. 2001) and increased photosynthesis. Warming and drought may counteract each other if water is available, or if water is scares they may exhibit an even stronger drought (Wang et al. 2014).

**Interface between aboveground and belowground plant responses**
Many studies have been conducted observing effects of climate change on aboveground biomass, less have been conducted observing changes in belowground biomass, and even fewer studies exist that comprise both above- and belowground biomass and take interactions of climate change factors into account. Therefore not much knowledge is available considering how above and belowground interact as a result of climate change, and if this is further complicated by interaction of climate change factors. Whether biomass responds more above- or belowground depends on several factors e.g. the nutrient state of the soil. In nutrient limited ecosystems higher carbon allocation was observed belowground for acquisition of nutrients (Saggar et al. 1997) and higher carbon allocation belowground has been found in soils under drought stress (Sanaullah et al. 2012), indicating that both drought and elevated CO₂ can lead to carbon being allocated belowground in a nutrient limited system. The higher allocation of carbon belowground could result in changes in the root:shoot ratio (Niklaus et al. 2001) and a higher root C:N ratio with possible effects on ecosystem carbon balance.

**Decomposition of plants - responses to Climate Change**
Decomposition of plant litter involves the physical and chemical processes that reduce litter to CO₂, water and mineral nutrients, and by doing so decomposition is a key process in carbon and nutrient cycling (Aerts 1997). The amount of carbon returned to the atmosphere by decomposition of dead organic matter is an important component of the global carbon budget (Vitousek 1982, Vitousek et al. 1994). Decomposition of plant litter is controlled by three types of factors; the physical environment (temperature, soil moisture etc.), the quantity and quality of substrate available to decomposers, and the characteristics of the
microbial community (Swift et al. 1979, Santonja et al. 2015). Much focus has been on decomposition of leaf litter, while less effort has been made to investigate root litter, although the picture has been changing the last decade. Freschet et al. (2013) estimated that root litter represented approximately 33% and 48% of annual litter input in grassland and forest respectively, underlining the importance of incorporating this pool in the carbon cycle. Climate affects decomposition directly (Jenny et al. 1949), which have been the most common focus in decompositions studies looking at how climate affects decomposition (Schmidt et al. 1999, Rustad et al. 2001, Schmidt et al. 2002). But climate also affects decomposition indirectly via changes in litter quantity and quality either due to changes in species composition or changes in leaf and root litter chemistry, see figure 4.

Litter quality have been found to describe the greatest proportion of variability in root decomposition rates, in contrast to previously established leaf litter decomposition models (Silver and Miya 2001). A recent review by Zhang and Wang (2015) showed that fine and coarse roots differ in which factors are main control of decomposition rate. Most important predictor of fine root decomposition was initial lignin content, while coarse roots also depended on lignin to nitrogen, mean annual temperature and precipitation (Zhang and Wang 2015).

**Figure 4.** How climate change affects decomposition directly and indirectly. Focus for this thesis is the indirect effects of climate change on decomposition via changes in litter chemistry (red arrows).

**Root chemistry: cellulose, lignin and suberin concentrations**

Where cellulose is a simple polymer of glucose, and therefore easily digestible, lignin and suberin are both complex compounds and hence considered recalcitrant (Soler et al. 2007, Prescott 2010). Both lignin and
suberin protect the roots from the surrounding environment (Schreiber et al. 1999, Watanabe et al. 2013), and both have been found to increase due to different stressors as drought, salinity, waterlogged conditions and e.g. heavy metals (Enstone et al. 2002, Moura et al. 2010), further suberin protect against pathogens (Enstone et al. 2002). Cellulose have been found both to decrease in response to drought (Jiang et al. 2012), to be unaffected (Brunner et al. 2015) and to increase (Gall et al. 2015). Lignin is, after cellulose, the second most abundant terrestrial biopolymer, accounting for approximately 30% of the organic carbon in the biosphere (Boerjan et al. 2003). Therefore the decomposition of lignin is a major potential source of C to the atmosphere. Conventionally, cellulose has been seen as positively correlated with decomposition of litter, while lignin was inversely related to decay rates especially in later stages of decomposition (Berg 1984). However this concept has been challenged by Klotzbucher et al. (2011) who found decomposition of lignin to depend on presence of labile carbon sources.

Ecosystems investigated

Heathlands

Heathlands are wide open landscapes and semi natural ecosystems on sandy soils low in pH and nutrient content and dominated by dwarf shrubs as Calluna vulgaris (L). A unique association of plants and animals adapted to withstand such inhospitable conditions has evolved to form the distinctive heathland community (Gimmingham 1972). Heathland have declined dramatically in past centuries due to agricultural conversion (Thompson et al. 1995, Price and Price 2003), human activities resulting in increased N deposition, fragmentation, lack of management, and climate change (Aerts and Heil 1993). Increasing N deposition and lack of management leads to rising availability of the former so sparse nitrogen in heathland soils, which again leads to changes in the vegetation towards more nutrient demanding species as the grass Deschampsia flexuosa or Molinia coerulea (Riis-Nielsen et al. 1998). The heathlands of Europe are cultural landscapes created by grazing, fires, mowing or cutting of vegetation, and today most heathlands are protected be law (in Europe e.g. by the European Union’s Habitat Directive (92/43/EEC)), and therefore no longer at risk of agricultural conversion. Degradation however continues. One of the treats is present and future climatic change.

This Ph.D. comprises investigations of climate manipulation experiments in four different heathlands in Denmark (Mols and CLIMAITE), Netherlands (Oldebroek) and in Wales (Clocaenog). Sites are described in methods.
Aims of the project

The objectives of this thesis are divided into two major questions. (1) How does climate change affect carbon partitioning and biomass production in a Danish heathland? And (2) how does drought and warming affect root decomposition and root chemistry at three different heathlands in Europe along a precipitation gradient, and does the change in decomposition and chemistry correlates?

The specific research questions raised were:

1. How climate change affects partitioning and turn-over of new carbon?
2. How climate change affects biomass allocation?
3. How climate change induced responses in biomass allocation developed over time?
4. How will drought and warming indirectly affect the decomposition of roots (and leaves) from three different European heathlands?
5. How does drought and warming affect cellulose, lignin and suberin concentration in roots from three different European heathlands and can this response be coupled to changes in decomposition rates?

Answer to the first question comes from a $^{13}$C labelling study carried out in 2011. Here plots subjected to enhanced CO$_2$, warming and precipitation and all treatment combinations were labelled with $^{13}$C (new carbon) by exposing them to $^{13}$C-CO$_2$ for four hours and then following the new carbon in the plant soil system within the first 8 days. Turn-over were studied by re-measuring after four months. To answer the second question above- and belowground biomasses from the labelling study were used in combination with pin-point analysis for the aboveground biomass, to give a detailed picture of climate change induced changes over the season and coupled responses. Question 3 were addressed by comparing result on biomasses after six years of exposure to climate change (question 2) to earlier results on biomasses responses after 2-3 year of exposure at the same site. To answer question 4 a decomposition study were carried out. Roots from three different European heathlands were decomposed for two months in a Respicond that automatically measures CO$_2$ released from the roots hourly. Later leaves from one of the sites were decomposed in a similar way. Question 5 on climate change responses in cellulose, lignin and suberin concentration were analysed by Fourier transform infrared spectroscopy (FT-IR). Afterwards data from the decomposition study and the FT-IR analysis were related to see if responses were comparable.

Paper I address the question on carbon partitioning and turn-over. Paper II address changes in biomass production/allocation and response to climate change over time. Paper III addresses the question on root decomposition and root chemistry.
METHODOLOGY

All methods applied in this thesis are described in the different papers, but in the following section I will outline some considerations of the chosen methods. Not all methods are discussed here, but merely it presents the principal methods ($^{13}$C labelling, soil coring, plant harvest, pin-point method, decomposition in Respicond and FT-IR) and thoughts behind these choices, while other more specific methods in the lab is explained in the articles. All methods used in the thesis are well known, but the methods used in article 3 (Respicond and FT-IR analysis) are not usually applied in this manner. This has the disadvantage that it is very hard to find references comprising similar experiments, but also the potential to gain new knowledge.

Study sites

The CLIMAITE experiment

The CLIMAITE experiment started in October 2005, with the purpose of studying climate change effects on biological processes in terrestrial ecosystems. The experiment was a cooperation of Danish universities: University of Copenhagen, University of Aarhus and the Technical University of Denmark (former Risø). The climate manipulations at CLIMAITE were designed accordingly to Danish Meteorological Institutes climate predictions for Denmark 2075. Predictions included an atmospheric CO$_2$ level on 510 ppm (CO2); prolonged spring/summer drought (D) and increased temperature (warming) at night of 1-2 degrees (T). The predictions forecasted no change in annual amounts of precipitation, but with a different annual distribution leading to droughts in spring/summer and increased winter precipitation. Climate manipulations for the experiment were however simplified, so only spring/summer drought is included, since responses would be difficult to interpret in a combined summer drought and winter addition scenario.

Manipulations are done by use of FACE (Free-Air Carbon dioxide Enrichment) techniques and roofs covering the plots during nights and rain events, respectively. A PAR sensor triggers the alu-roof at sunset increasing the minimum night-time temperature by about 1 °C and a rain sensor set off the drought roofs during rain events for 4-6 weeks in spring/summer (prolonged spring/summer drought) removing 95 % of the precipitation during the drought and 5.1-11.3% of the annual precipitation (data from 2006-2013). The roofs are released from the warming plots during rain event to minimize the disturbance of the hydrological cycle. The experiment includes a control (A) which received no treatments and the site contains full factorial combinations of the three climate factors adding up to 8 treatments: A, D, T, DT, CO2, DCO2, TCO2, DTCO2.
Figure 5. CLIMAITE Site from above

The eight treatments are placed in pair wise octagons with a diameter of 6.8 m receiving ambient (380 ppm) and elevated CO2 (510 ppm), respectively (figure 6). Each octagon is divided into four plots (9.1 m² per plot) to provide all eight treatment combinations in a split-plot design. Each combination is replicated six times (total of 48 plots). The distances between the octagons are at least 2.5 times the octagon widths to avoid CO₂ contamination from the elevated to the ambient CO2 octagons. For further details see Mikkelsen et al. (2008) and Larsen et al. (2011).

Figure 6. Octagons with treatments at CLIMAITE. Treatments are ambient (A), drought (D), warming (T) plus drought and warming (DT) in the first octagon. All plots in second octagon are treated with elevated CO₂ alone (CO2), and in combination with drought (DCO2), warming (TCO2) and last with both drought and warming (DTCO2).
Figure 8. Precipitation, soil moisture and temperature at the CLIMAITE site in 2011
The INCREASE experiment

The INCREASE experiment includes sites at heathlands in Hungary, Italy, Netherlands, Denmark and Wales. The five sites differ in annual temperature and precipitation. All sites have been manipulated with the same climate factors by same methods since 1999. Climate treatments are drought, temperature and control. The experiment was laid out as a randomized block design at each site including three replicates of the three treatments. Each plot was 4*5 m (20 m²). The warming treatment is achieved using a passive nighttime warming method designed to mimic an increased minimum night temperature of 1-2 degrees (W). Exceptions are at rain falls during night, where curtains are retracted to make sure these plots receive same amounts of rain water as control plots. Drought plots are based on same methods, but curtains are here made of rain proof material. For a period of one to two months during spring the drought (D) is applied to drought plots at all sites. At Clocaenog, which is the site receiving far most rain fall, the drought is extended and last from May to September. The drought system is connected to a rain censor that extends the curtain over the drought plots during rain event over 0.2 mm. Curtains are retracted immediately after rain events to reduce artefacts from changed conditions of light, wind and temperature. Over control plot scaffolding (similar to the scaffolding holding the curtains at drought and temperature plots) is established to ensure same impacts of light and wind to the controls as the other treatments. Soil temperature and moisture is measured using in-situ thermocouple and time domain reflectometry sensors installed 5 cm below the soil surface, sensor data is recorded on an hourly basis using CR1000 data loggers (Campbell Scientific Ltd, UK).

Figure 9. Heathland sites within INCREASE network.
The three heathlands used in this study are (presented from east) located in the eastern part of Jutland in Denmark (Mols), east of Amsterdam in Netherlands (Oldebroek) and in Clocaenog Forest in NW Wales (Clocaenog). At the Mols site *Deschampsia flexuosa* is dominant with patches of *Calluna vulgaris*, while at Oldebroek and Clocaenog, *Calluna vulgaris* is dominant. Soil at Oldebroek and Mols are both sandy podzols with a pH of 3.9 while Clocaenog is an organic-rich humo-ferric podzol, also with a pH of about 3.9. Further details of the locations and description of the sites are given in Beier *et al.* (2004) and Sowerby *et al.* (2008).

**Table 1.** Drought period 2010, mean precipitation (1999-2011), mean soil moisture (May 2010- June 2011) and mean temperature (1999-2011) for Mols, Oldebroek and Clocaenog

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<thead>
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<th>Oldebroek</th>
<th>Clocaenog</th>
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</thead>
<tbody>
<tr>
<td>Drought period 2010</td>
<td>11&lt;sup&gt;th&lt;/sup&gt; of June – 14&lt;sup&gt;th&lt;/sup&gt; of July</td>
<td>22&lt;sup&gt;nd&lt;/sup&gt; April – 30&lt;sup&gt;th&lt;/sup&gt; May</td>
<td>7&lt;sup&gt;th&lt;/sup&gt; of May – 17&lt;sup&gt;th&lt;/sup&gt; of September</td>
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<tr>
<td>Mean annual precipitation (mm)</td>
<td>669</td>
<td>962</td>
<td>1289</td>
</tr>
<tr>
<td>Mean soil moisture (%)</td>
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<td>20.4</td>
<td>41.0</td>
</tr>
<tr>
<td>Daily mean temperature (°C)</td>
<td>8.7</td>
<td>10.1</td>
<td>6.9</td>
</tr>
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Pictures of Mols (upper left), Oldebroek (upper right) & Clocaenog (lower)
**13C labelling**

*In situ* 13CO2 pulse-labelling have been used widely to assess partitioning of new carbon in grasslands since the millennium (Ostle *et al.* 2000, Leake *et al.* 2006, Denef *et al.* 2009, De Deyn *et al.* 2011, Hafner *et al.* 2012, Bahn *et al.* 2013). When using 13CO2 pulse-labelling under *in-situ* conditions three basic issues must be handled (Reinsch and Ambus 2013) (i) field plots have to be separated from each other to avoid disturbances of particular root exudates. This was done by soil collars, which are placed in the soil two weeks in advance, (ii) keep the 13CO2 labelled air at the plot without changing other environmental conditions. This was done by using air-tight and transparent chambers placed on the soil collars and a relative low labelling time (4 hours), and (iii) 13CO2 must either be added to the air in the chamber or employed using a flow-through setup with forced air flow. The latter was chosen here to keep ambient and elevated CO2 concentrations equal over time.

The 13CO2 Pulse labelling was done in May 2011 on two days (the 16th and 19th of May), since it was a very time consuming procedure. Between the 16th and the 19th a rain event took place, changing the soil moisture of the non-drought plots. This may have had some effect on partitioning of new carbon, but no pattern was found in the data.

*Figure 10.* 13CO2 pulse-labelling at CLIMAITE in May 2010

*Figure 11.* 13CO2 passes from the balloon through a hose and enters the Plexiglas frame forced by a constant flow
Samples of natural abundance in leaves, roots and soil 0-10 cm were taken a few days before labelling, while natural abundance in 10-30 cm were taken outside the labelling area but inside the treatments in the autumn. This happened as a consequence of changing the depth of which we took soil samples two days after labelling (days 2 were soil samples to 30 cm depth, while day 1 and 8 were soil samples to 10 cm depth). We did not expect this to have any influence on the natural abundance in the soil samples. Enrichment of $^{13}$C measured in leaves and roots will be referred to as $^{13}$C enrichment or simply as new carbon, to illustrate that this fraction is considered a ‘cohorte’ of carbon that we follow through its way in the plant/soil system. To avoid disturbances to other studies at the site, the labelling was done within treatment areas, but outside the defined plots. This was possible since the experimental design consisted of octagons, leaving corners to studies outside the normal frame.

**Belowground biomass – sequential soil coring**

Plant sampling is notoriously much easier aboveground than belowground. Therefore other methods, than the common sequential soil coring, have been developed for studying root dynamics. Especially the relationship of climate change and effects on plant growth and the role of carbon sequestration have encouraged the refinement of estimates of root biomass (Vogt et al. 1998). However when doing an experiment that is comprised of both aboveground and belowground responses to climate change, a method combining aboveground harvest and belowground soil coring and root sorting is considered appropriate. The methods are relatively similar and therefore direct comparison of responses above and below responses are more straightforward. Sequential soil coring can be used for studying how roots are distributed in depth and how biomass changes over time. Disadvantages are that this is a destructive method, and therefore can disturb plants in the proximity. Also experiments often have a limited space and number of sequential soil coring may therefore also be limited to avoid the swizz cheese syndrome. In our study of partitioning of new carbon by $^{13}$CO$_2$ pulse-labelling, several soil cores short after each other were necessary (day 1, 2 and 8).

**Aboveground biomass - plant harvest and pin-point analysis**

Aboveground biomass harvest was chosen as a direct and easy method of biomass measures. Because of limited space for harvesting this method was only applied twice in 2011 and combined with pin-point analysis in permanent plots, to have a more detailed picture of biomass changes over the year from early spring (April) to late autumn (November) and from year to year. Plant harvest is simple and biomass of grass leaves can be used directly (dried and weighted). Pin-point analysis, described by Jonasson (1988), are non-destructive and can be converted into biomass estimates. A pin-point analysis provides estimates of
plant cover, defined as the relative number of pins in the grid that touch the species, and the compactness of the species measured as the average number of hits per pin (Damgaard et al. 2009). This measure of compactness has shown to be correlated to plant biomass (Jonasson and Skold 1983, Jonasson 1988), a relation that has been used in the conversion. The relationship was tested by Kongstad et al. (2012), where further details can be found. Since we use two methods for analysing changes in biomass over time, and since biomass showed to respond differently to treatments over time, we have chosen to do statistical tests of treatments effect on one sampling at the time.

Respicond – measuring decomposition

Decomposition measured as CO₂ efflux from litter was monitored in a conductivity measuring respirometer, Respicond VI (from here referred to as Respicond), which is a simply way of continuously measuring release of CO₂ from many samples simultaneously (Nordgren 1988). Besides it is a more controlled way of measuring decomposition compared to litterbags. Fine roots from all three sites and leaves from one site (Clocaenog) were inoculated with microbes from soil from the site they stem from and placed in moist sand to keep the roots moist during the period of decomposition. The Respicond was set to measure the conductivity every half hour, which was automatically converted to accumulated CO₂ and CO₂/hour. Decomposition of fine roots was compared across sites according to the moisture gradient, and within sites according to climate treatments. Further decomposition of leaves and fine roots of Calluna vulgaris from Clocaenog were compared. Using a Respicond instead of the more common litter bag method have the advantage of giving much more precise values of decomposition (CO₂ efflux per hour), and hence smaller differences due to treatments could be measured. Disadvantages are that preparations and running of instruments is more time consuming than leaving litter bags to decompose in the field, and that we could find no other studies to compare our result directly with.
Measurement of cellulose, lignin and suberin concentration by FT-IR analysis

Intact roots were analysed for relative content of surface (1 mm) lignin, suberin (waxy substances) and cellulose using a FT-IR spectrometer. Afterwards roots were grinded and the analysis was repeated on the bulk samples, to be able to distinguish between concentration of the substances on surface and in bulk roots. Further analysis was made on Deschampsia roots from Mols after decomposition to be able to compare changes in the relative concentration of cellulose, lignin and suberin in non-decomposed and decomposed roots. Advantages of the FT-IR methods was that differentiation of concentrations of the three substances on surface and bulk were possible. Disadvantages were that concentrations were always relative to concentration of other substances, and hence changes in e.g. lignin and suberin concentrations could result in changes in cellulose concentration, without the concentration of cellulose in absolute terms were changed. Unfortunately only samples from Mols included enough root material for both decomposition and FT-IR analysis. However for the Clocaenog site we were able to get older root material, while no extra material were available for Oldebroek site. Hence FT-IR analyses were only done on roots from Mols and Clocaenog.
RESULTS

Paper I and II were carried out at the CLIMATE site. Paper I studied partitioning of new carbon \(^{13}\text{C}\) between different ecosystem compartments; leaves, roots and soil, and how climate change (elevated \(\text{CO}_2\), drought and warming) affected this partitioning. The paper follows the fate of a \(^{13}\text{C}\) pulse in the ecosystem within a short-term perspective from days to a growing season. Paper II studied biomass changes in the ecosystem as a result of six years of climate treatment (elevated \(\text{CO}_2\), drought and warming), and hereby present the long-term perspective. Paper III was carried out in three of the INCREASE sites (Wales, Holland and Denmark) and investigates how climate change (warming and drought) affects root carbon chemistry and decomposition and if changes in decomposition are linked to changes in root chemistry. Paper III represents a more method oriented experimental approach, where known methods are used in new ways. Results are presented in paper I-III, so here I will only show the most important results of each paper.

Uptake and allocation of new carbon

Elevated \(\text{CO}_2\) resulted in higher \(^{13}\text{C}\) enrichment in leaves at day 8, but the effect was diminished by drought. We also found higher allocation of new carbon to deep roots (20-30 cm). Here \(^{13}\text{C}\) enrichment was 3 times higher in elevated \(\text{CO}_2\) plots than in non-\(\text{CO}_2\) plots and 5 times higher when comparing \(^{13}\text{C}\) enrichment in roots under future climate (combined treatment with elevated \(\text{CO}_2\), warming and drought) to ambient climate. Drought also resulted in relatively more new carbon \(^{13}\text{C}\) allocated to deeper roots, although total amount of new carbon \(^{13}\text{C}\) in roots did not change.

![Graph](image-url)

**Figure 12.** Enrichment of \(^{13}\text{C}\) in roots at day 2. Elevated \(\text{CO}_2\) versus ambient \(\text{CO}_2\).
**Turnover of new carbon**

We found that elevated CO$_2$ tended to reduce retention of $^{13}$C in shoots ($P=0.0741$), roots ($P=0.0678$) and significantly so in total plant pool ($P=0.0191$). Drought tended to increase the $^{13}$C roots pool by a factor of 2.6 compared to a factor of 1.3 in non-drought plots between day 8 and day 120 ($P=0.0615$) (figure 6).

**Figure 13.** $^{13}$C retention at day 120 (percentage of $^{13}$C label at day 8 still present at day 120) for $^{13}$C pools for shoots (a), roots (b) and total plant (c) Treatments are: non-CO$_2$ vs CO$_2$ and non-Drought vs Drought. Level of significance is show using: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ and †$p < 0.1$. 
Changes in biomasses above- and belowground due to climate treatments

Effects of climate change differed on aboveground and belowground biomass. Aboveground we found that treatments had no general effect on biomass, but effects varied over season. Drought was the treatment with most effects on aboveground biomass. Drought seemed to strengthen the seasonal variation in biomass, with increased green biomass in early spring and autumn, and decreased green biomass during drought. Elevated CO₂ primarily affected green biomass by increasing Water Use Efficiency (WUE) and hereby reduced the effect of drought. Belowground elevated CO₂ was the treatment that affected root biomass the most. Elevated CO₂ increased root biomass by 21.5% across sample months and depths. Impact, however, varied with depth and season, with higher percentage increase in root biomass below 10 cm and higher percentage increase in spring and late autumn. We only found few effects on DOC, MBC and on CN ratios in plants.

![Figure 14](image)

**Figure 14.** Total root biomass in May, September and November 2011. Treatments are non-CO₂ versus elevated CO₂. Statistics were done across months and depths, but only statistical result for month and CO₂ are shown here.
Figure 15. Biomass of green grass (grey) and litter (white) in March, May, June, August and September. Treatments are A (ambient), D (drought), T (temperature), CO2 (elevated CO2) and combinations (DT, DCO2, TCO2, DTCO2). Note different scale on y-axis. Statistics were done months wise for green grass and litter separately. P values of significant results are shown in the graph. P values of <0.05 are considered significant and p < 0.1 are considered trends.
Changes in root decomposition due to indirect effects of drought and warming

Roots grown in drought plots at the Clocaenog site showed lower amount of accumulated CO$_2$ after 50 days (figure 9), reflecting a lower decomposition rate, while roots grown in drought plots from the two other sites did not differ from roots grown in control plots. We saw a much higher amount of accumulated CO$_2$ from Calluna leaves compared to Calluna roots from the Clocaenog site (figure 9), reflecting faster turnover of leaves compared to roots.

![Graph showing accumulated CO$_2$ from 1g of sample (roots or leaves) at day 50 released from decomposition of Mols roots (MR), Oldebroek roots (OR), Clocaenog roots (CR) and Clocaenog leaves (CL). Treatments are control (C), drought (D) and warming (W).]

Site: $p<0.0001$
MR = CL > OR > CR

Treatment (separate analysis):
MR: Treat: n.s.
OR: Treat: n.s.
CR: Treat: 0.0732
DLSM C vs D $P=0.0282$
CL: Treat: n.s.

Changes in root chemistry due to drought and warming and coupling to decomposition

Due to low root biomass in samples from Oldebroek and Clocaenog, only roots sample from Mols site was analysed by FT-IR. Additional root samples from Clocaenog were supplied, but none could be supplied from Oldebroek. Both Deschampsia roots from Mols and Calluna roots from Clocaenog showed increased relative concentration of lignin in roots grown in warmed plots, and Calluna roots from Clocaenog also showed increased suberin concentration. Roots from neither site showed changes in cellulose due to drought or warming. Comparison of root chemistry in decomposed versus non-decomposed roots from...
Mols, showed that lignin and suberin decrease equally within initial stage (here 50 days) of decomposition. Responses in root decomposition could not be coupled directly to changes seen in root chemistry, and vice versa.

**Figure 17.** Lignin concentration at the Mols and Clocaenog sites for control plots (C), drought plots (D) and warming plots (W). Black bars are surface and grey bars are bulk samples.

Decomposition of roots from CLIMAITE was affected by drought, leading to a higher amount of accumulated CO₂ after 50 days. Amount of accumulated CO₂ from decomposition of roots grown in drought was higher than from roots grown in elevated CO₂.
SUMMARY DISCUSSION

This thesis investigated how climate change affected different aspects of carbon cycling in heathland/grassland ecosystems. The CLIMAITE site included plots exposed to elevated CO₂, night-time warming and drought in spring/summer and all combinations, while the INCREASE sites included plots subjected to drought and warming separately within sites, but also included a gradient of precipitation and hence contrasting soil moisture across sites with decreasing soil moisture from west to east. This gradient made it possible to investigate how local conditions as precipitation and soil moisture affected the response to climate change. Disadvantages of combining the two experimental approaches at CLIMAITE and INCREASE are of course that they differ in experimental set-up (primarily regarding lack of elevated CO₂ and interactions at the INCREASE sites), but it also gives some opportunities. Since the CLIMAITE site and Mols site in INCREASE were located on same soil type and both dominated by *Deschampsia flexuosa*, we can to some degree combine the results from these two sites up to form a more detailed picture of how this grass common to dry heathlands responds to expected climate change, however with the notion of INCREASE not included the response to elevated CO₂. Different species were dominant at the three sites within INCREASE, which gave us the opportunity to compare the response of *Deschampsia flexuosa* to the response of *Calluna vulgaris*, two species with very different life form and strategy (Kongstad et al. 2012, Ransijn et al. 2015). To some degree we were also able to compare responses of *Calluna vulgaris* at a wet site (Clocaenog) with a mesic site (Oldebroek).

Effects of elevated CO₂ on carbon uptake and allocation in *Deschampsia flexuosa*

In a short term perspective elevated CO₂ led to a higher assimilation of CO₂ in plants as reflected in the higher enrichment of ¹³C in leaves of *Deschampsia flexuosa* in plots with elevated CO₂ eight days after labelling (paper I). This is consistent with the higher assimilation of CO₂-C in the same species in elevated CO₂ plots as measured by Albert et al. (2011). Also elevated CO₂ resulted in a 3 times higher enrichment in the deep roots (20-30 cm depth) measured two days after labelling. Despite the increased enrichment of new carbon (¹³C), there was no effect in the long term perspective (after 6 years of treatment) in aboveground biomass, which was not different in elevated CO₂ plots compared to ambient CO₂ plots (figure 15), similar to the result found after 3 years (Kongstad et al. 2012). Roots on the other hand showed higher biomass after 6 years (figure 14), a response that was relatively stronger in the deeper roots compared to the roots in the top 10 cm (despite a higher increase in the top 10 cm in absolute terms; paper II). Our results confirmed result of higher root biomasses in elevated CO₂ plots after 3 years (Arndal et al. 2013, Arndal et al. 2014). This strongly indicates that the ecosystem is more affected by elevated CO₂.
belowground than aboveground, which has also been found in other studies (Pritchard and Rogers 2000, Madhu and Hatfield 2013) probably related to nutrient limitation (Nielsen et al. 2009).

**Effects of drought on carbon uptake and allocation in Deschampsia flexuosa**

In the short term perspective drought did not directly change enrichment of $^{13}$C in leaves of *Deschampsia flexuosa*, however it eliminated the increasing effect of elevated CO$_2$ on leaf $^{13}$C enrichment, resulting in an unchanged $^{13}$C enrichment in plots treated with both elevated CO$_2$ and drought compared to plots only treated with elevated CO$_2$ (paper I). Root $^{13}$C enrichment was decreased in the top 10 cm by drought (paper II). However, we found that drought significantly increased the relative ratios of $^{13}$C enrichment in the two deeper layers compared to the top layer (paper I), indicating that the plants merely distributed their carbon deeper in roots. When effects of drought were measured as biomass responses after 6 years of treatment, we found a large variation in response due to seasonality (figure 14 and 15). Drought decreased the aboveground biomass during the drought treatment in June. However this was compensated for in late summer with higher aboveground biomass, but also by simultaneously lower belowground biomass in September in drought plots (figure 14+15). Ransijn et al. (2014) found a similar response in green fraction of *Deschampsia flexuosa* with lower values during drought and higher values in autumn. After September root biomass increased more in drought plots than non-drought plots resulting in equal root biomass in November. All in all *Deschampsia flexuosa* is demonstrating high flexibility towards drought inflicted changes in demand and limitations. Similarly, Kongstad et al. (2012) found fast and fully recovery in the grass after drought indicating high resilience in this species.

**Effects of warming on carbon uptake and allocation in Deschampsia flexuosa**

Warming only affected distribution of new carbon in *Deschampsia flexuosa* wagely (as tendencies; paper I) although measurements of photosynthesis showed increased photosynthesis capacity in the warmed plots (Albert et al. 2011) and increased NDVI (Ransijn 2014). Biomasses of *Deschampsia flexuosa* after six years of treatment were marginally affected by warming, with an increasing effect on biomass of green grass in September and an increasing effect on biomass of litter in March (figure 15), while it only tended to affect root biomass (paper II). Main reason for this is likely the low effect of warming on the temperature at the CLIMAITE site (figure 8). However warming did affect soil moisture in all months from June to December, making warming treatment a wage but continuous drought through summer and autumn suggesting that a positive effect of warming could be counterbalanced by decreased soil moisture in the warmed plots.
Effects of climate change on turnover and differences on leaves and roots

Lower retention of new carbon in shoots, root and total plant of *Deschampsia flexuosa* in plots exposed to elevated CO₂ at the CLIMAITE site was found (figure 13) suggesting a higher carbon cycling in these plots, supporting earlier findings of Hungate *et al.* (1997) and Reinsch *et al.* (2014). In contrast, drought led to a higher retention of ¹³C in roots of *Deschampsia flexuosa* at the CLIMAITE site suggesting slower carbon cycling in roots in drought plots (figure 13), also supporting earlier findings (Gorissen *et al.* 2004, Ruehr *et al.* 2009). However, in contrast to this, roots of *Deschampsia flexuosa* from the CLIMAITE site decomposed faster when roots had grown in drought compared to ambient, pointing in the opposite direction with regards to turn-over.

Roots of *Calluna vulgaris* from Clocaenog showed a slower decomposition rate, when plant originated from the drought plots, compared to the lack of response to drought in decomposition of roots of *Deschampsia flexuosa* from Mols and root of *Calluna vulgaris* from Oldebroek, which was likely due to the length of the drought period. This period was more than 16 weeks in 2010 at Clocaenog, while only 5 weeks at Oldebroek and Mols in 2010 (paper III). Further, the fact that the soil at Clocaenog showed decreased soil moisture year round possibly caused by drought induced hydrophobicity in these plots (Sowerby *et al.* 2008).

Comparing our results with earlier finding of Emmett *et al.* (2004) who studied leaf response to climate change at the same sites highlighted two important differences between roots and leaves. Primarily we found fine roots of *Deschampsia flexuosa* to decompose much faster than fine roots of *Calluna vulgaris* roots, while Emmett *et al.* (2004) found no difference in decomposition of leaves between the two species. This was interpreted as roots varying more than leaves between the two species. Secondly we found that leaves and roots responded differently on climate treatments e.g. decomposition of fine roots of Calluna vulgaris was slowed by drought, while leaves were not affected, and opposite leaves of *Calluna vulgaris* and *Deschampsia flexuosa* from the drier sites of Oldebroek and Mols, respectively, showed slower rates of initial decomposition, while roots from these sites (and species) were not affected by treatments. Some of the discrepancy between the studies could be due to sampling in different seasons (autumn versus spring). Potential differences in response between leaves and roots could be important as root litter form the main source of soil organic matter (Balesdent and Balabane 1996, Rasse *et al.* 2005) and hence has a relatively higher effect on the ability of the ecosystem to sequester carbon.

Even though we found lower retention of new carbon due to elevated CO₂, decomposition of litter is not necessarily faster in elevated CO₂ plots. Van Groenigen *et al.* (2005) showed decomposition in an elevated-
CO₂ world to be slower than in an ambient-CO₂ world. Their finding was in contrast to Sowerby et al. (2000) and suggested that the higher amount of labile carbon could instead have been leached or exudated (van der Krift et al. 2002). Our data from Deschampsia roots from the CLIMAITE site showed no difference between decomposition of roots grown in ambient compared to elevated CO₂, but where roots grown in drought decomposed faster, this effects were not seen in roots grown in plots with combined drought and elevated CO₂, indicating that elevated CO₂ diminished the increasing effect of drought on decomposition rate. Hence these results point to same conclusion as van Groenigen et al. (2005).

**Effects of climate change on root litter quality**

Increased lignin concentration in fine roots of *Deschampsia flexuosa* at the Mols site as a response to lower soil moisture, was related to the ability of lignin to enable the plant to resist a higher water pressure due to stronger tissue (Lee et al. 2007). In dry soil with lower water potentials than inside the roots, water can be lost to the rhizosphere by diffusion (Enstone et al. 2002). Increased amounts of lignin could prevent this by forming a strong barrier (Hose et al 2001). Increased lignin and suberin concentrations in fine roots of *Calluna vulgaris* at the Clocaenog site as response to warming was related their role in rendering roots more resistant to belowground herbivory simply due to increased mechanical strength (Johnson et al. 2010). Earlier studies have shown that warming increased the species diversity of enchytraeids (Holmstrup et al. 2012) and the density of root-zone dwelling Collembola (Holmstrup et al. 2013) which possibly feed directly on fine roots (Ruess et al. 1999, Endlweber et al. 2009, Scheunemann et al. 2015). Increased activity of pathogens due to warming could possibly also have induced increased concentrations of lignin and suberin (Enstone et al. 2002).

**Methodology advantage and limitations**

Discussion of methods used in the thesis was partly done in Method section, however I will here add a few considerations of the methods. The CO₂ pulse labelling was successful with both aboveground and belowground plant parts enriched with ¹³C. Unfortunately dissolved organic carbon (DOC) and microbial biomass carbon (MBC) in the soil layers below 10 cm were too weakly enriched to follow the fate of new carbon in these fractions. Information of new carbon in these fractions would have given us information on whether the extra amounts of new carbon to the deep roots were exudated to the rhizosphere as a labile carbon source for microbes, and hence given us information about whether to expect higher break down of old carbon bound in the soil due to priming. However stronger dose of ¹³C-CO₂ could lead to unrealistic partial pressure just after the ¹³C-CO₂ application and increase the risk of contamination of the ecosystem.
with the tracer, which could possibly compromise future measurements of natural abundances of $^{13}$C (Schmidt et al. 2013).

Our analysis of aboveground biomasses consisted of a mix of two different methods, harvest and estimates of biomasses from pin point analysis. This had the great advantage that we were able to get data from five different months in 2011 plus data from autumn in 2010, and not just the two months where we harvested. Due to limited space and many different investigations carried out within the sites, numbers of harvests had to be very limited. Same restriction applied for soil coring for belowground biomasses, unfortunately no belowground equivalent of pin point analysis exist.

To my knowledge there have so far been no attempts to use FT-IR for chemistry of roots or other plant parts in decomposition studies in Respicond – or in the field. Unfortunately we were not able to correlate the results of the two methods, which likely was due to the fact that we analysed initial decomposition. Prolonging the experiment to last at least a half or a whole year might have given some correlation between decomposition and chemistry of the roots. However both methods separately gave interesting results and new insight.
Figure 20. Response of Deschampsia flexuosa to expected future climate as found in this thesis (simplified). Elevated CO₂ is illustrated with green, drought with red, and warming with blue. Black illustrates ambient condition (present climate). Thicker arrows illustrate faster processes and smaller arrows a slower process. Likewise arrows up and down besides boxes illustrate respectively higher enrichment (new carbon), concentration (lignin) or biomass, and lower enrichment/concentration/biomass. * Cycling of new carbon is decreased by drought, but decomposition rate of fine roots grown in drought were higher, while decomposition of fine roots from elevated CO₂ plots were unchanged.
CONCLUSION

The expected future climate with elevated CO$_2$, warming and prolonged drought is complex and included many interactions, primarily between elevated CO$_2$ and drought. Generally elevated CO$_2$ leads to faster processes and higher enrichment plus allocation of new carbon in roots deeper in the soil profile. Drought slows down processes and leads to lower enrichment, however the allocation of new carbon is also enhanced in roots in deeper soil layers. Response of these two climatic factors often eliminates each other; except the deeper allocation of new carbon in roots where their responses added up. Long term effects showed that root biomass increased due to elevated CO$_2$, and the effects seemed to increase over time. However it was also evident that the effect size was dependent on sampling month. Aboveground biomass varied substantially over season, but showed no persistent changes over the years.

Only fine roots from Clocaenog responded to climate change resulting in slower decomposition in roots from drought plots, likely due to longer drought periods and hence more adverse effects on soil conditions. This changes could however not be explained by changes in fine root chemistry, and likewise the higher lignin concentrations in fine roots from respectively drought and warmed plots did not affect fine root decomposition within the first 50 days. Lignin concentrations seemed to protect against unfavourable conditions in the soil. In contrast to the slower cycling of new carbon in drought plots, we found a higher rate of decomposition of fine roots from drought plots. When combined the two processes could eliminate each other to some degree. Roots decomposed slower than leaves of Calluna vulgaris as expected, and fine roots of Deschampsia flexuosa from deep layers showed much slower decomposition than fine root from top layer. Data on shoot:root ratios and rooting depth illustrate the importance to take these parameters into account in predictive models for the contribution of decomposition in ecosystem carbon balances. Decisive for consequences of climate changes on cycling of C and potential for soils to sequester carbon depend on the further fate of carbon in the rhizosphere.

Future research perspectives

Some research topics were identified that could add knowledge to our understanding on ecosystem response to climate change. We were not able to follow the newly labelled $^{13}$C in dissolved organic carbon and in microbial biomass in the deep soil layers. Since results have shown that increasing concentration of CO$_2$ in the atmosphere will lead to increased root biomass and increased concentration of new carbon in deeper root layers, it is important to investigate the further fate of this carbon, and to what extent it will be
exudates by roots or respired, taken up by microbes, built into soil or possibly induce priming in these
deeper soil layers, resulting in positive feedback of the CO₂ levels in the atmosphere.

We found biomass responses to climate change to be very influenced by sampling month. This had mayor
effects on the possible outcomes when analysing effects of climate change over time, and we were able to
both find increasing and decreasing effects of elevated CO₂ over six years. Further knowledge of how
sampling month affect root biomasses response to climate change are needed in order to make standards
for which types of data can be compared over the years.
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LIST OF PAPERS

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**Paper 2:** Pia Lund Nielsen, Inger Kappel Schmidt, Klaus Steenberg Larsen and Anders Michelsen. Climate change affects seasonal allocation of biomass above- and below-ground. Results from six years of elevated CO2, drought and warming in a grass dominated heathland. To be submitted to Functional Plant Biology

**Paper 3:** Pia Lund Nielsen, Louise Andresen, Søren Barsberg, Helle Sørensen, Andy Smith, Albert Tietema and Inger Kappel Schmidt. Impact of recurrent summer drought and warming on fine root decomposition and chemistry of Shrubland plant communities. To be submitted to Plant and soil.

The papers are not included in this version of the theses due to copyright.
## FORMER ISSUES

<table>
<thead>
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<th>Title</th>
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<td>Landskabsbyens æstetik. En undersøgelse af fikmmediet som redskab til belysning af forstadens omgivelseskarakter</td>
<td>Mads Farsø</td>
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<td>August 2013</td>
<td>Translating Harbourscape. Site-specific Design Approaches in Contemporary European Harbour Transformation</td>
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<td>Johannes Ransijn</td>
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<td>April 2014</td>
<td>Deriving harmonised forest information in Europe using remote sensing methods. Potentials and limitations for further applications</td>
<td>Lucia Maria Seebach</td>
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<td>May 2014</td>
<td>Health Promoting Pocket Parks in a Landscape Architectural Perspective</td>
<td>Karin Kragsig Peschardt</td>
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<td>October 2014</td>
<td>Cemeteries–Organisation, management and innovation. Diffusion of maintenance specifications in Danish national church cemetery administrations</td>
<td>Christian Philip Kjøller</td>
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<td>November 2014</td>
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