Long-term climate change effects on dynamics of microorganisms and carbon in the root-zone

Reinsch, Sabine; Jakobsen, Iver; Ambus, Per

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

Long-term climate change effects on dynamics of microorganisms and carbon in the root-zone

PhD-Report

Sabine Reinsch

November 2012

DTU Kemiteknik
Institut for Kemiteknik

Ph.D. thesis

Supervised by: Prof. **Per Ambus** and Prof. **Iver Jakobsen**

Centre for Ecology and Environmental Sustainability

Department of Chemical and Biochemical Engineering

Technical University of Denmark (DTU)

4000 Roskilde, Denmark

Reference to this publication should be written as: Reinsch, Sabine (2012) "Long-term climate change effects on dynamics of microorganisms and carbon in the root-zone" Ph.D. Thesis.

THANKS TO

Jule and Heike and all friends for their faith in me.
Per Ambus for his great supervision, help and trust.
Iver Jakobsen for his friendly and open minded but also critical view.
Anja C. Nielsen for her patience, acceptance and help in the MS lab.
Marie P. Merrild for her great work establishing fatty acid analyses.
Mette Flodgaard for laboratory help and social events.
Nina W. Thomsen, Preben Jørgensen, Poul Sørensen for your help.

The whole ECO group for providing a very friendly, helpful and welcoming working environment. And thanks to Claus Beier who always keeps us together. Thanks to all ECO Ph.D. students: Kristine, Eike, Signe, Petra, Cathrine and Jian for being there.

The Climaite group for great cooperation and help with the labeling experiments. Thanks to the Climaite Ph.D. students Kristine, Marie M., Marie D., Merian, Pia, Jane, Marie A., Lasse and Johannes for good discussions and team spirit. Further thanks to the student helpers Nina B. Mikkelsen, Aslak K. Hansen and Matthias J. Justesen for bearing a big work load of the labeling experiment.

Eric Paterson for the welcoming cooperation, motivating discussions and for sharing his knowledge. Thanks for great laboratory help to Allan Sims, Noemí Martín and Barry Thornton. The exchange work at 'The James Hutton Institute' in Aberdeen was supported by NordSIR that covered the travel costs and the 'European Science Foundation (ESF)' that provided a grant for the external stay.

All co-authors: Per Ambus, Anders Michelsen, Zsuzsa Sárossy, Helge Egsgaard, Inger Kappel Schmidt, Iver Jakobsen, Eric Paterson and Barry Thornton for their individual contributions.

This Ph.D. project was financed by three instances to equal amounts: the Ph.D. grant of the Technical University of Denmark, the STAiR community and last but not least the CLIMAITE project.

Sabine Reinsch
November 2012

ABSTRACT

Climate change factors such as elevated CO_2 concentration, warming and changes in precipitation patterns have been shown to affect terrestrial carbon (C) cycling. The objective of this Ph.D. project is to track recently assimilated C into belowground compartments to investigate the effects of climate change on belowground C allocation. The impacts of climate change as single and combined treatments were applied to heath/grassland vegetation and the short-term terrestrial C turnover was investigated using *in-situ* $^{13}CO_2$ pulse-labeling. We developed a mobile and low-cost pulse-labeling setup applicable in remote natural environments. We present evidence that our new system works reliably and leads to results similar to former grassland pulse-labeling experiments. Allocation of recently assimilated C into roots and the microbial biomass was often similar among climate treatments, but C allocation patterns into microbial functional groups were treatment dependent. We observed a delayed C allocation into microbes under drought and a faster C flow through the microbial community under elevated CO_2 conditions. Especially the importance of actinomycetes in the utilization of recently assimilated C can have major impacts on the C balance under changing climatic conditions. A comparison of C allocation under ambient and simulated future climatic conditions showed that the terrestrial C balance might be changed by reducing soil organic matter mineralization. Our results suggest that the impact of future climatic conditions may change belowground processes involved in C cycling and that heath/grassland soils have the potential to serve as C sinks in the future. To confirm these results, a short-term C balance for the conducted study is needed to reveal if observed C allocation into different microbial communities affects the short-term C balance on the ecosystem scale.

SAMMENDRAG

Omsætningen af kulstof (C) i terrestriske økosystemer influeres af de faktorer, der medvirker til globale ændringer i klima og atmosfære, herunder stigende CO_2 koncentration og temperaturer samt ændrede nedbørsforhold. I dette studie blev lynghede udsat for de nævnte klima-faktorer, enten enkeltvis eller i kombination. Efterfølgende blev kort-tids omsætningen af kulstof analyseret ved brug af en *in-situ* indmærkning med C-13 mærket kuldioxid ($^{13}CO_2$). Formålet var at undersøge effekten af de simulerede klima-effekter på den underjordiske lagring af kulstof ved at spore den tilførte C-13 isotop til forskellige C-puljer i rødder og omgivende jord. For at gennemføre dette udviklede vi en forsøgsopstilling baseret på mobile og relativ billige komponenter, der muliggjorde $^{13}CO_2$ forsøg i vanskeligt tilgængeligt terræn. Vi dokumenterer, at metoden giver reproducerbare og troværdige resultater, bl.a. i sammenligning med tidligere studier i tilsvarende forsøgsterræn. De påførte klimafaktorer var uden betydning for fordelingen af nyligt fikseret C til rødder og den generelle pulje af mikroorganismer. Derimod havde klimabehandlingen betydning for fordelingen af C til forskellige funktionelle grupper af mikroorganismer. Generelt kunne det ses, at hastigheden hvormed nyligt fikseret C strømmede til jordens mikroorganismer blev nedsat under simuleret tørke, mens forholdene med øget CO_2 i atmosfæren gav en hurtigere C transport til mikroorganismerne. Resultaterne viser, at især gruppen af strålesvampe (aktinomyceter) kan have betydning for kulstofbalancen via deres udnyttelse og omsætning af nyligt fikseret C i fremtidens klima. En sammenligning af den underjordiske C lagring under nutidigt CO_2 niveau, med forventet C lagring under de fremtidige klimabetingelser, viser, at den fremtidige C balance i en lynghede vil ændres som følge af en øget oplagring via reduceret omsætning af nyligt fikseret C. Dermed kan det forventes, at den underjordiske C omsætning i lyngheder, og lignende græsklædte økosystemer, ændres i fremtidens klima. Ændringen vil indebære at nævnte type af økosystem vil bidrage til at optage C fra atmosfæren. For yderligere at understøtte disse resultater, bør der laves en kvantitativ opgørelse af kort-tids C balancen i et studie for at fastslå, hvorvidt den ændrede C allokering til forskellige funktionelle grupper af mikroorganismer har betydning for den overordnede C balance.

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LIST OF PAPERS

Papers of this thesis:

I: Reinsch S. and Ambus P. (2012) *In-situ* ^{13}C-CO_2 pulse-labeling in a temperate heath-land – development of a mobile multi-plot field setup. Submitted to *Rapid Communications in Mass Spectrometry*.

II: Reinsch S., Michelsen A., Sárossy Z., Schmidt I.K., Egsgaard H., Jakobsen I. and Ambus P. (2012) Short-term carbon utilization by the microbial community under changing climatic conditions in a temperate heath-land. To be submitted to *Global Change Biology*.

III: Reinsch S., Ambus P., Thornton B. and Patterson E. (2012) Impact of future climatic conditions on the potential for plant-mediated soil organic matter priming. Submitted to *Global Change Biology*.

1. TERRESTRIAL CARBON CYCLING IN FUTURE CLIMATE

Climate scenarios predict increasing temperatures, rising atmospheric carbon dioxide (CO_2) concentrations, changes in precipitation patterns and the occurrence of more frequent extreme weather events in the future (IPCC 2007). Terrestrial carbon (C) cycling is affected by e.g. warming, water availability and CO_2 concentration. Consequently, changing climatic conditions will alter processes involved in C cycling and can result into changed ecosystem C fluxes. Elevated atmospheric CO_2 concentration has been shown to accelerate the atmospheric in CO_2 concentrations even further (Hungate *et al.* 1997; Selsted *et al.* 2012), warming has a positive effect on the speed of C cycling but e.g. drought periods or heat waves can also diminish C turnover (Ciais *et al.* 2005; Luo 2007; Heimann & Reichstein 2008). In many ecosystems, limited water availability reduced C cycling processes (Ciais *et al.* 2005; Selsted *et al.* 2012). However, future climate will be a combination of all factors and it is important to understand climate factor interactions and their influence on ecosystem processes (IPCC 2007).

The global terrestrial soil organic matter (SOM) stock is with 1500 Pg C the biggest terrestrial C pool of which ca. 60 Pg C are turned over annually (Filley & Boutton 2006). Terrestrial C cycling starts with the autotrophic assimilation of atmospheric CO_2 by plants via photosynthesis (Figure 1). C is incorporated into plant components, stored in aboveground biomass or allocated belowground into roots (Bowling *et al.* 2008; Brüggemann *et al.* 2011). C compounds in roots are either used for root processes or can be released into the rooting zone (rhizosphere) to stimulate microbial activity (Kuzyakov *et al.* 2001; Paterson *et al.* 2007). Microbes use plant-derived C compounds for their own growth and invest it into exoenzymes for soil organic matter (SOM) and litter degradation, and as a result increase soil nutrient availability (Fontaine *et al.* 2011*)*. The soil microbial community can be (i) closely associated with plant roots (rhizoplane bacteria and mycorrhizal fungi), (ii) highly dependent on plant rhizodeposits (rhizosphere bacteria and saprophytic fungi) or (iii) specialised on SOM-mineralization in the soil matrix. Preferences in microbial substrate utilization can be assigned to ecological functionality (Cheng *et al.* 2005; Denef *et al.* 2009). Commonly, the soil microbial community is divided into five main functional groups namely gram-negative and gram-positive bacteria, actinomycetes,

mycorrhizal and saprophytic fungi. Bacterial groups are fast growing with short live cycles and thus able to rapidly respond to changing environmental conditions such as exudation of labile C compounds by plant roots (Fontaine *et al.* 2003). The actinomycetes group, classified as gram-positive bacteria, is an exception; this group has a more fungal-like life cycle and appearance and has the ability to mineralize recalcitrant soil C compounds (Lacey 1997). Mycorrhizal fungi are closely associated with plant roots in a symbiotic relationship (Talbot *et al.* 2008). In contrast, saprophytic fungi are the main decomposers of SOM in the soil matrix characterised by a long and steady but slow life cycle *(*Fontaine *et al.* 2003; Fontaine *et al.* 2011). Microbial and plant (leaves and roots) activity involve heterotrophic and autotrophic respiratory processes, respectively. C is eventually respired and diffuses back to the atmosphere closes the terrestrial C cycle (Schlesinger & Andrews 2000; Amundson 2001).

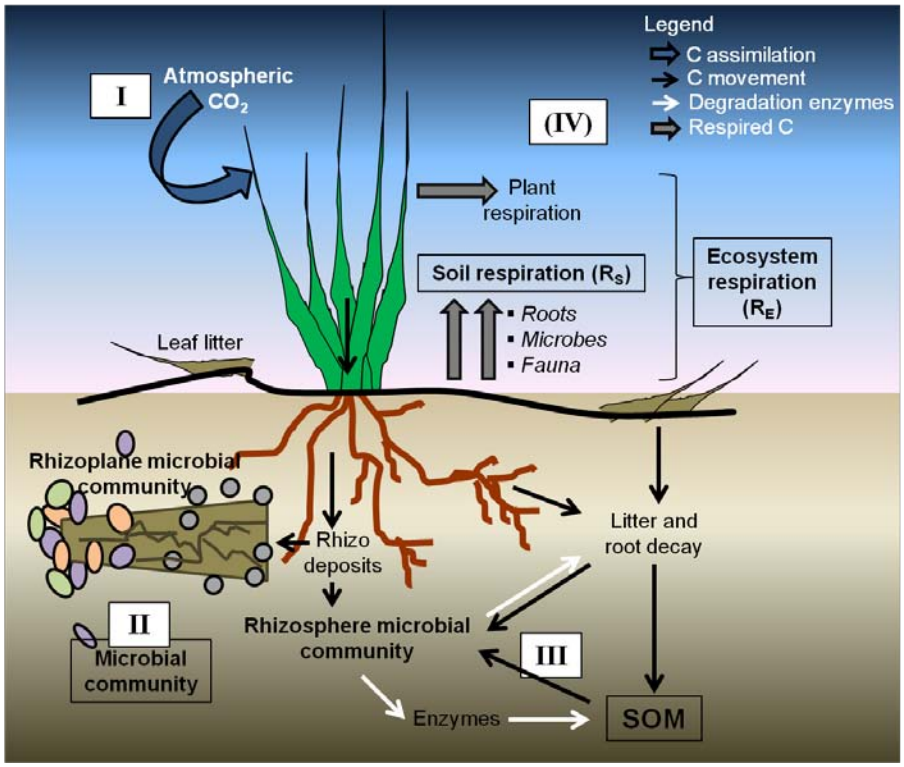

Figure 1: Terrestrial C cycle with the focus on the soil microbial community: atmospheric CO_2 is assimilated by plants, C is stored in aboveground plant biomass, respired or transported belowground into roots; roots can be directly colonized by the rhizoplane microbial community; plants can release rhizodeposits that are consumed by the microbial community; Microbes produce enzymes to mineralize litter and soil organic matter (SOM). Microbial and root activity involves respiration processes; respired C eventually leaves the soil (soil respiration). Boxes indicate studied ecosystem compartments in this thesis and the numbers I-IV show related papers: I: development of an *in-situ* ^{13}C-CO_2 pulse-labeling setup, II: trace of recently assimilated C into the soil microbial community, III: assessment of SOM priming and (IV): outlook of a short-term C balance.

Climate change factors that affect aboveground plant processes (e.g. increased atmospheric CO_2 concentration, warming) will also affect the closely linked belowground C cycling. Moreover, changes in precipitation patterns and thus soil water availability will influence belowground processes and aboveground plant performance. The aim of this thesis is to investigate the effects of future climatic condition on the soil microbial community composition and their importance for short-term belowground C turnover in a temperate heath/grassland.

2. CLIMATE CHANGE EXPERIMENTS

Free-**a**ir **c**arbon dioxide **e**nrichment (FACE) experiments have been established in grasslands (TasFACE, Swiss FACE, GiFACE), forests (Web-FACE, Bangor FACE, Duke FACE) and agricultural systems (AGFACE, FAL FACE, AZ FACE, ORNL FACE, SoyFACE, RiceFACE) to study the effects of elevated atmospheric CO_2 on ecosystems. FACE experiments have also been combined with ozone (SoyFACE), nutrient additions (Duke FACE, POP-EuroFACE, AZ FACE), warming (PHACE, TasFACE) and manipulation of precipitation (AZ FACE). In some cases, FACE experiments have been combined with more than one relevant climate factors such as OzFACE (three CO_2 concentrations, defoliation and nutrient additions) and the CLIMAITE experiment (drought and warming).

2.1 The CLIMAITE experimental site Brandbjerg

The CLIMAITE experiment is situated in temperate Northern Zealand (55˚53' N, 1158' E), Denmark in a hilly location covered by an unmanaged heath/grassland ecosystem. The yearly average temperature is 8 ˚C and the mean precipitation is ~610 mm. The soil is a sandy nutrient-poor deposit composed of ~70 % sand, ~20 % coarse sand, about 6 % silt and, ~2 % clay in 0-15 cm with low water holding capacity. The plant community is co-dominated by the grass *Deschampsia flexuosa* (~70 %) and the dwarf shrub *Calluna vulgaris* (~30 %), and minor occurrences of other grasses, herbs, mosses and lichens (Kongstad *et al.* 2012). The two species are distributed heterogeneously in patches throughout the study site. Total aboveground green biomass was about 700 g m^{-2} when the experimental site was established (Mikkelsen *et al.* 2008) and varied on a seasonal scale between ~300-730 g m^{-2} measured in campaigns between 2004 and 2008 (Kongstad *et al.* 2012). Total green biomass of *D. flexuosa* was ca. 30 g m^{-2} in March 2007 and was measured to be highest (ca. 230 g m^{-2}) in August 2004 in the same study. Plant heights ranged from 40 to 60 cm. Initial root biomass was ~550 g m^{-2} (Mikkelsen *et al.* 2008) with about 90 % of roots located in the top 10 cm soil.

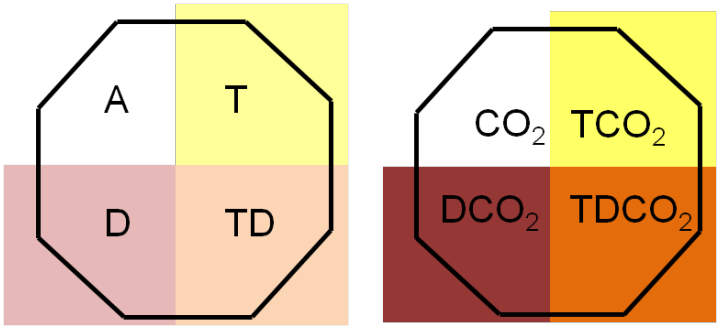

Figure 2: Experimental layout of the climate treatments at the CLIMAITE field site: one experimental block is composed of one non-CO_2 and one eleavated CO_2 octagon (FACE). One block contains a full-factorial combination of elevated CO_2 (CO2), prolonged spring/summer droughts (D) and temperature increase (T).

The experimental treatments were initiated in 2005. The experimental setup is a full-factorial split-plot design that consists of the following climate treatments: an un-treated control (A), elevated CO_2 concentration at 510 ppm during daytime hours, prolonged spring/summer droughts by horizontally moving curtains (D), increased temperature (1 ˚C) realized as passive night time warming by reflective curtains (T) and the treatment combinations TD, DCO2, TCO2, TDCO2. Each treatment is replicated 6 times (48 plots in total). Treatments are applied in 12 octagons (Ø 6.8 m) arranged pair-wise in six blocks (Figure 2). Each octagon is divided into four plots. Within each block, one octagon is exposed to elevated CO_2. Soil moisture (vol%), soil and air temperatures (˚C) and photosynthetic active radiation (PAR, µmol m^{-2} sec^{-1}) are constantly measured at the field site. For further information see Mikkelsen *et al.* (2008) and Selsted *et al.* (2012).

Within this Ph.D. project, two field experiments have been performed in subplots with at least 80 % grass cover to study the effect of changing climate on short-term carbon turnover. Experiment 1 (Exp1) took place in September/October 2010 and experiment 2 (Exp2) in May 2011 (Figure 3). During autumn (October), C allocation belowground is believed to be highest because plants prepare to outlast harsh winter conditions. In late spring (May), plants are in their growth phases and CO_2 uptake was expected to be maximum.

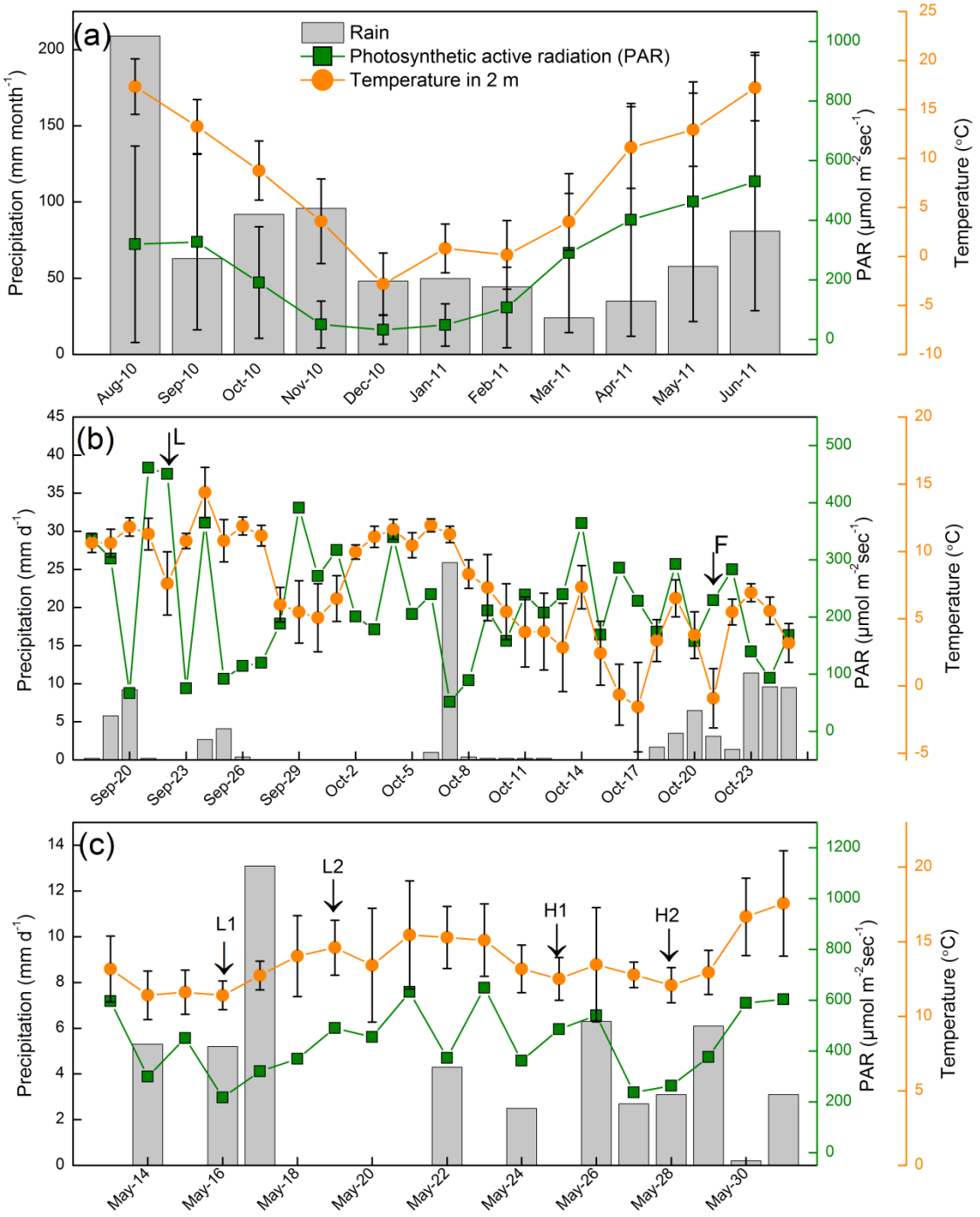

Figure 3: Climatic conditions (precipitation, photosynthetic active radiation (PAR), temperature) at the field site: (a) monthly averages from Aug 2010 to Jun 2011, (b) daily averages during Exp1 and (c) Exp2. 'L' indicates start of each labeling campaign, 'F': finish of Exp1, 'H1' and 'H2' indicate finish of Exp2 by aboveground biomass harvest. Values are means ± SE, only averages of PAR drawn in (b) and (c) due to high daily variability.

2.2 Effects of elevated CO_2, drought and temperature on terrestrial C-cycling

Elevated CO_2 was observed to increase C turnover by raising CO_2 assimilation efficiency, increasing aboveground and belowground plant biomass and by stimulating respiratory processes (King *et al.* 2004; Wan *et al.* 2007; Selsted *et al.* 2012). Carney *et al.* (2007) observed a decrease in soil C stocks after four years of elevated CO_2 exposure in a shrub-oak forest, which is in agreement with earlier modeling results (Amundson 2001). However, the effects of increased atmospheric CO_2 concentrations are still controversial, and elevated atmospheric CO_2 concentrations did not always result in soil C loss (Norby & Zak 2011).

Increased temperatures generally showed positive effects on C turnover by extending plant growth seasons due the close linkage with belowground C allocation processes, especially in cold ecosystems (Luo 2007; Heimann & Reichstein 2008) such as Alaska and Greenland. In already dry ecosystems, warming was observed to diminish physiological processes and thus C turnover. During the European heat-wave in 2003, a decrease of soil organic C stocks was observed as a result of decreased photosynthetic CO_2 assimilation but, soil processes including respiratory C losses remained unchanged (Ciais *et al.* 2005).

Future precipitation patterns are likely to be more extreme, leading to more heavy rain events alternated with longer drought periods (IPCC 2007). Extended drought periods decreased belowground C turnover (Selsted *et al.* 2012), soil properties and water availability. In contrast, heavy rain events can lead to flooding, resulting into anoxic soil conditions and thus, the dead of roots, soil microbes and animals going along with reduced belowground activity and diminished C utilization. Moderate increases in precipitation, however, facilitated soil CO_2 efflux significantly and it was suggested that soil C storage could be decreased (Wan *et al.* 2007).

Single climate factor effects on terrestrial C turnover have been studied fairly well and scientists got an idea about possible ecosystem responses. Effects of combined climate change factors on ecosystems are rarely studied, and can be difficult to predict. The combination of elevated CO_2, warming and extended drought periods has been shown not to result into additive ecosystem responses, but rather in antagonistic interactions (Larsen *et al.* 2011; Dieleman *et al.* 2012).

3. STUDYING CARBON CYCLING WITH PULSE-LABELING

C isotope tracers have become an important tool in studying C allocation patterns into terrestrial C pools (Meharg 1994; Leake *et al.* 2006). Continuous C-labeling (e.g. via long-term FACE treatments) is well suited to investigate C allocation to soil C pools due to continuous labeling of newly assimilated C, but it lacks resolution when studying environmental impacts on ecosystem C turnover. In contrast, pulse-labeling provides a higher spatial and temporal resolution, and allows the investigation of effects of changing climatic conditions on C cycling. But, low incorporation rates of recently assimilated C can limit the resolution (Meharg 1994).

3.1 Pulse-labeling setup (Paper I)

Field pulse-labeling studies using stable C isotopes have frequently been used to study short-term C-turnover in different ecosystems (Leake *et al.* 2006; Kaštovská & Šantrůčková 2007; Wang *et al.* 2007) and to investigate impacts of e.g. ecosystem management practices (Rangel-Castro *et al.* 2004; Denef *et al.* 2009; De Deyn *et al.* 2011) and climate change (Denef *et al.* 2007; Jin & Evans 2010). Although ^{13}C pulse-labeling is a very useful method to answer questions addressing terrestrial C-turnover, the conduction of a ^{13}C pulse study is challenging, especially under field conditions due to e.g. distances between plots of interest and weather conditions.

Two basic components are important for the deployment of a ^{13}C-pulse-labeling study under field conditions: (i) the experimental field plots should be confined and separated from the surroundings with the least disturbance. For the belowground part, this is often achieved by inserting soil collars to avoid exchange with the surroundings especially in terms of C exudation by roots that can mix into the soil matrix. For the aboveground part, air tight and transparent chambers or bags deployed isotopically modified CO_2 to the vegetation of interest. An important issue is to maintain ambient environmental conditions inside the chambers. ii) Supply of isotopically modified CO_2 either to the ambient air in chamber, or by a flow-through setup with forced air flow. Generally, flow-through systems are suitable to maintain stable chamber

concentrations without external adjustments and have an advantage over setups where chamber conditions have to be adjusted manually e.g. by giving CO_2 pulses (Kaštovská & Šantrůčková 2007; Wang *et al.* 2007; Hamilton *et al.* 2008; Mårtensson *et al.* 2012). A steady chamber CO_2 concentration is needed in order not to affect plant photosynthesis (Ainsworth *et al.* 2005), and is a prerequisite for assessing C-budgets that include plant assimilated C.

Within this Ph.D. project, we developed a mobile multi-plot field setup for *in-situ* ^{13}C-CO_2 pulse-labeling at relatively low costs (Paper I). Transparent acrylic chambers (Figure 4a) were mounted gas-tight in water-filled canals atop metal collars that were pushed into the soil. ^{13}C-CO_2 air was mixed with CO_2 free air and was stored in air reservoirs (3 m diameter advertisement balloons, Figure 4b). The air reservoirs contained air for a whole labeling event and was then transported to the place of interest (Figure 4c,d).

Pulse-labeling in Exp1 was carried out during one labeling campaign (7 h, sunny conditions, Figure 3b) in 1.5 x 1.5 m experimental plots located adjacent to the main climate treatment octagons. Exp1 was a two-factorial setup containing untreated control plots (ambient), frequent drought periods (drought) and increased precipitation (irrigation), all under ambient CO_2 concentrations (390 ppm). Drought periods were realized by covering the plots with transparent Plexiglas roofs raised ca. 1 m aboveground on metal racks. Precipitation was simulated by adding deionised water. Treatments were initiated in March 2007 and continued in 2008, paused in 2009, and resumed in 2010. Each treatment was replicated five times. In 2010, drought was induced at two two-week events in April and July. Artificial rain was applied every two weeks (n = 9) by adding ~28 litres of deionised water per plot equivalent to 12.44 mm precipitation between April and July 2010.

Pulse-labeling in Exp2 was performed in two campaigns (for 4 h) within the CLIMAITE setup (Figure 2) under ambient and elevated CO_2 concentrations. At the first labeling campaign, the weather conditions were dominated by grey skies and light showers, whereas the second campaign was performed under more sunny conditions (Figure 3c). For logistic reasons, the labeling plots were situated ca. 1.2 m outside the main octagons, and thus exposed to more randomly variable yet elevated CO_2 concentrations of about 500 ppm.

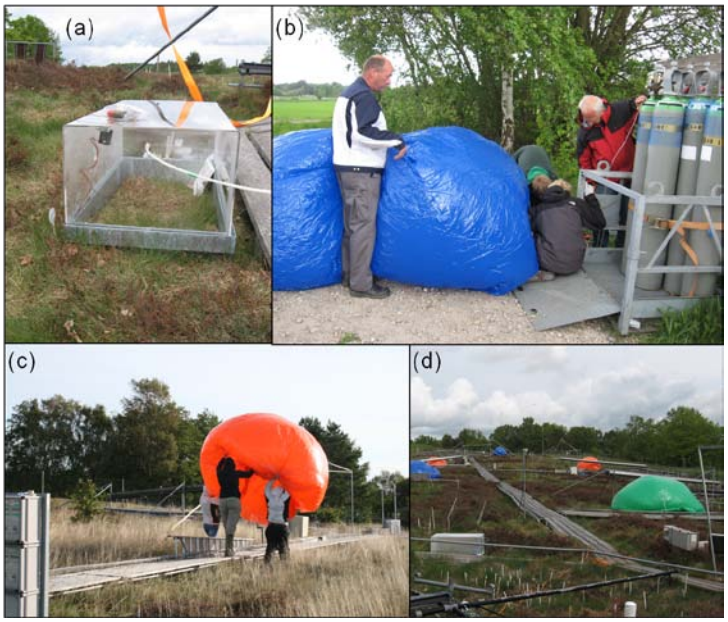

Figure 4:
In-situ ^{13}C-CO_2 pulse-labeling in practice: (a) pulse-labeling chamber mounted onto a soil frame, (b) filling of air-reservoirs with air, (c) transport of air-reservoirs to the location of interest and (d) field site during a labeling campaign.

3.2 Validation of the developed pulse-labeling setup (Paper I)

We successfully developed a mobile multi-plot *in-situ* ^{13}C-CO_2 pulse-labeling setup that was applied to low vegetation in a remote environment. A minimum of 15 plots were simultaneously pulse-labeled with ^{13}C-CO_2 for at least 4 hours. Conducting one pulse-labeling experiment with multiple plots instead of several successive smaller labeling campaigns ensured that environmental conditions (PAR, temperature, soil moisture, precipitation) were the same across replicates. The use of big (Ø ~3 m), robust (vinyl) and gas tight advertisement balloons as gas reservoirs provided sufficient air for at least four labeling chambers under the applied conditions. The robust balloon material allowed transportation of filled air reservoirs to the place of interest.

Observed stable CO_2 concentrations in air reservoirs were 390 ppm under ambient conditions and 510 ppm in plots treated with elevated CO_2 (Figure 5a). But, CO_2 concentrations in labeling chambers were always lower than 390 or 510 ppm (Figure 5b). This change in chamber CO_2 concentration compared to the supplied air was due to photosynthetic and respiratory processes in the soil and vegetation confined by the chamber.

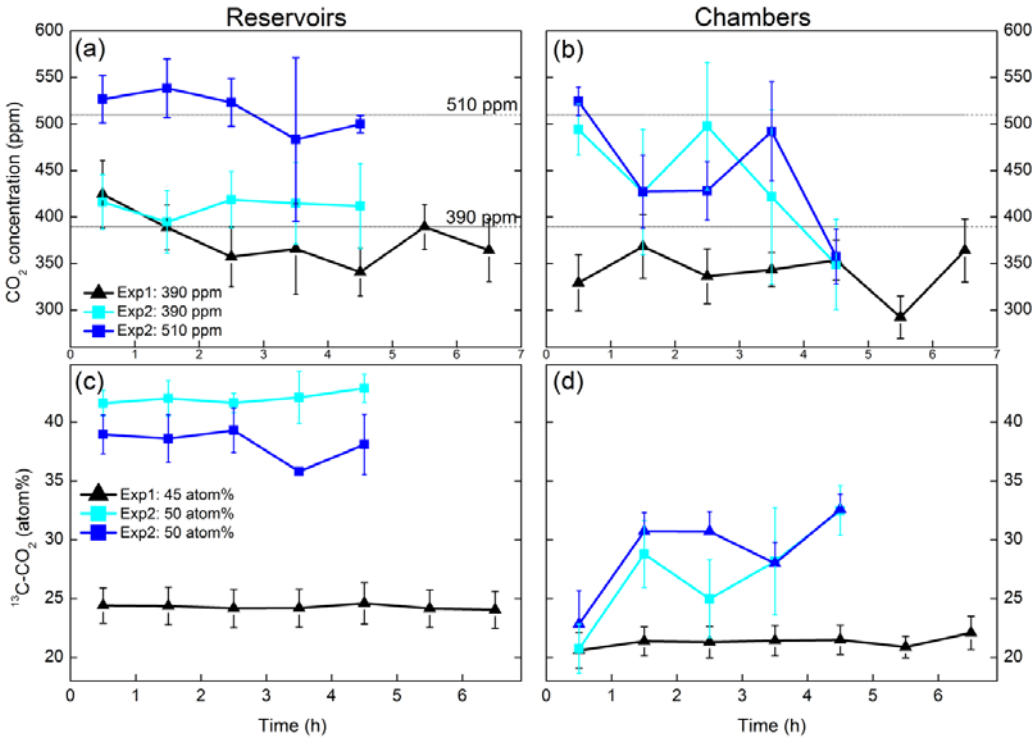

Figure 5: CO_2 concentrations and ^{13}C-CO_2 conditions in air reservoirs and labeling chambers during the course of the pulse-labeling campaigns in Exp1 and Exp2.

Observed isotopic enrichment of supplied CO_2 was stable over time, but always lower than the targeted concentration in reservoirs (Figure 5c) and chambers (Figure 5d) in both experiments. We have no obvious explanation for this unexpected observation. One reason might be an inaccurate correction for the intensity dependence in $^{13}C/^{12}C$ ratio determinations at enrichments >> natural abundance. Alternatively, the CO_2 isotopic composition may have changed significantly during the storage; however, this could not be observed in independent testing, and the CO_2 concentrations did not change during storage, confirming previous testing of the used gas vials (Joos *et al.* 2008). A test trial revealed that the apparent atom% ^{13}C-CO_2 values were strongly (linear) dependent on the CO_2 concentration in the sampled range of 500-2000 ppm. This empirical relationship was subsequently used to correct atom% ^{13}C-CO_2 in our analysis. Nevertheless, the corrected atom% ^{13}C-CO_2 values from our field samples never showed the expected values in gas reservoirs and labeling chambers. This suggests that either the equipment leaked and the enriched CO_2 was diluted with ambient air, or that the correction is wrong. Invasion of atmospheric air into the reservoirs is unlikely due to observed stable CO_2 concentrations. In case of mixed CO_2 sources we would also expect the CO_2 concentrations to be different from our target values. According to this, we have to assume that the measurement of gas samples highly enriched in ^{13}C-CO_2 has to be further tested and evaluated to achieve satisfactory results.

4. TRACING CARBON BELOWGROUND

Belowground C allocation of recently assimilated ^{13}C-carbon from the labeling pulse can be traced into belowground compartments such as roots, microbial biomass and the soil matrix (Stewart & Metherell 1999; Kaštovská & Šantrůčková 2007). C isotope analysis in roots, soil and soil microbial biomass can be used to study belowground C-sink dynamics (Leake *et al.* 2006), possibly in combination with the effect of climate change (Andresen *et al.* 2009). A more detailed insight into the soil microbial community can be achieved by extracting microbial biomarker fatty acids (Zelles 1997; Kirk *et al.* 2004; Frostegård *et al.* 2011). Microbial fatty acids are located in the outer membrane of microorganisms (except archaea) and can specifically be assigned to ecological functional groups (Table 1). Phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs) can be extracted from root and soil samples to quantify the soil microbial community (Bligh & Dyer 1959). Microbial fatty acid analysis can also be combined with pulse-labeling and recently assimilated C can be traced into microbial functional groups by using gas-chromatograph-combustion isotopic mass spectrometry (GC-c-IRMS) (Denef *et al.* 2007; Gray *et al.* 2011). C-13 enrichment in PLFAs can be very low, but is generally above the detection limits and provides a reliable tool to characterize the fate of C into microbial functional groups.

4.1 Belowground carbon pools and the soil microbial community under changing climatic conditions (Paper II)

With Exp2, we aimed to investigate the dynamics of short-term C turnover under different climatic conditions. It is known that the rate of C turnover can be influenced by elevated CO_2 concentration, drought and warming (Hungate *et al.* 1997; Ciais *et al.* 2005; Heimann & Reichstein 2008; Selsted *et al.* 2012). We sampled all treatments at similar short time intervals; however, with this sampling strategy we can only investigate temporal "snapshots" of the dynamic system, which has to be taken into account when interpreting the results in terms of a fast or delayed occurrence of recently assimilated C in different C. Different patterns of the fate of recently assimilated C can additionally be modified by microbial activity as well as water and nutrient availability in the soil.

Table 1: Microbial specific phospholipid fatty acids (PLFAs) used to characterize the soil microbial community in root and soil samples. PLFAs specific for arbuscular mycorrhiza were not detected in the present study. Neutral lipid fatty acids were not investigated.

Microbial functional group	PLFA	References
General	14:0	Zelles 1999
	15:0	Frostegård & Bååth 1996; Zelles 1999; Denef *et al.* 2009
	16:0	Zelles 1999; Denef *et al.* 2009
	18:0	Zelles 1999; Denef *et al.* 2009
Gram-negative bacteria	17:0cy	Frostegård & Bååth 1996; Denef *et al.* 2009
	19:0cy	Frostegård & Bååth 1996; Zelles 1999
	16:1ω7	Frostegård & Bååth 1996; Denef *et al.* 2009
	18:1ω7	Denef *et al.* 2009
Gram-positive bacteria	15:0a	Frostegård & Bååth 1996; Zelles 1999; Denef *et al.* 2009
	15:0i	Frostegård & Bååth 1996; Zelles 1999; Denef *et al.* 2009
	16:0i	Frostegård & Bååth 1996; Denef *et al.* 2009
	17:0a	Frostegård & Bååth 1996; Zelles 1999
	17:0i	Frostegård & Bååth 1996; Denef *et al.* 2009
Actinomycetes	10Me16:0	Kroppenstedt 1985; Denef *et al.* 2009
	10Me17:0	Kroppenstedt 1985
	10Me18:0	Kroppenstedt 1985; Denef *et al.* 2009
Fungi	18:1ω9	Stahl & Klug 1996; Zelles 1999; Denef *et al.* 2009
	18:2ω6	Stahl & Klug 1996; Denef *et al.* 2009
	18:3ω3	Stahl & Klug 1996; Denef *et al.* 2009

Recently assimilated C from the pulse-chase was traced into belowground C pools (roots, microbial biomass) as well as in different respiratory CO_2 effluxes (Figure 6). The atom% ^{13}C excess (APE) of each C pool was calculated as difference between natural abundance $^{13}C/^{12}C$ ratio and the $^{13}C/^{12}C$ ratio of belowground C pools 1, 2 and 8 days after the pulse-chase. APE measures are independent of pool sizes (e.g. root biomass, microbial community size), but APE measures are useful to reveal C flow dynamics of recently assimilated C and potential effects of climate factors on short-term C turnover. The pulse-labeling was successful as assessed by ^{13}C appearance in the tested C pools that were different from natural abundance $^{13}C/^{12}C$ ratios.

In all investigated compartments (below- and aboveground), APE was often highest two days after the pulse-chase and decreased thereafter. Enrichment peaks shortly after the pulse-labeling have been observed in other studies (Lu *et al.* 2004; Leake *et al.* 2006; Jin & Evans 2010) and illustrate the rapid turnover of plant assimilated C. In roots, APE was higher in the CO_2 treatment at one and two days after the pulse-chase, compared to ambient conditions (Figure 6, roots: brown). Even though we have no root biomass estimates yet, root biomass has been shown to be increased under elevated CO_2 conditions (Fitter *et al.* 1996), and we

can assume that a higher root ^{13}C enrichment indicates a facilitated belowground allocation of recently assimilated C (Jin & Evans 2010). Interactions of climate treatments on APE in roots were observed at days 1 and 8 in combination with increased temperatures (Figure 6, DT, CO2T, CO2TD).

Microbial biomass carbon (MBC) was equally enriched in ^{13}C across climate treatments (Figure 6, purple) going along with an unchanged microbial biomass size as revealed by total PLFA-C concentration (Paper II). Climate change factors have been observed not to affect MBC (Zhang *et al.* 2005; Jin & Evans 2010). However, a deeper insight into the microbial community at the level of microbial functional groups (MFG) showed different C allocation patterns dependent on climate treatments. The C-fate (%) into MFGs describes the relative allocation of recently assimilated C (from the pulse-chase) among the different microbial functional groups, e.g. if 100 % assimilated C was observed in the gram-negative group then, all the recently assimilated C that ended up in the microbial community was entirely used by that group. Thus, the measure of C-fate is an indirect measure of the importance of each functional group in the short-term C turnover.

We assessed the C-fate into MFGs in roots at days 1, 2 and 8 as well as into the soil at day 2 (Figure 7). Root samples showed an overall high C-fate into gram-negative bacteria (Figure 7, black bars) in terms of ^{13}C allocation into specific biomarker PLFAs. The temporal dynamic in the C-transfer pattern showed that gram-negative bacteria were generally the first group utilizing recently assimilated C followed by a delayed uptake of root exudates by gram-positive bacteria (Figure 7, green bars) since the sizes of the bars tend to increase over time. Gram-negative bacteria showed the most consistent C-fate patterns across climate treatments when taking into consideration that elevated CO_2 concentrations accelerate C turnover (Denef *et al.* 2007; Drake *et al.* 2011; Selsted *et al.* 2012) whereas dry conditions slowed down C cycling (Selsted *et al.* 2012). This observation offers the possibility to use C-fate patterns displayed in the gram-negative group to investigate the effects of climatic manipulations on the speed of C cycling.

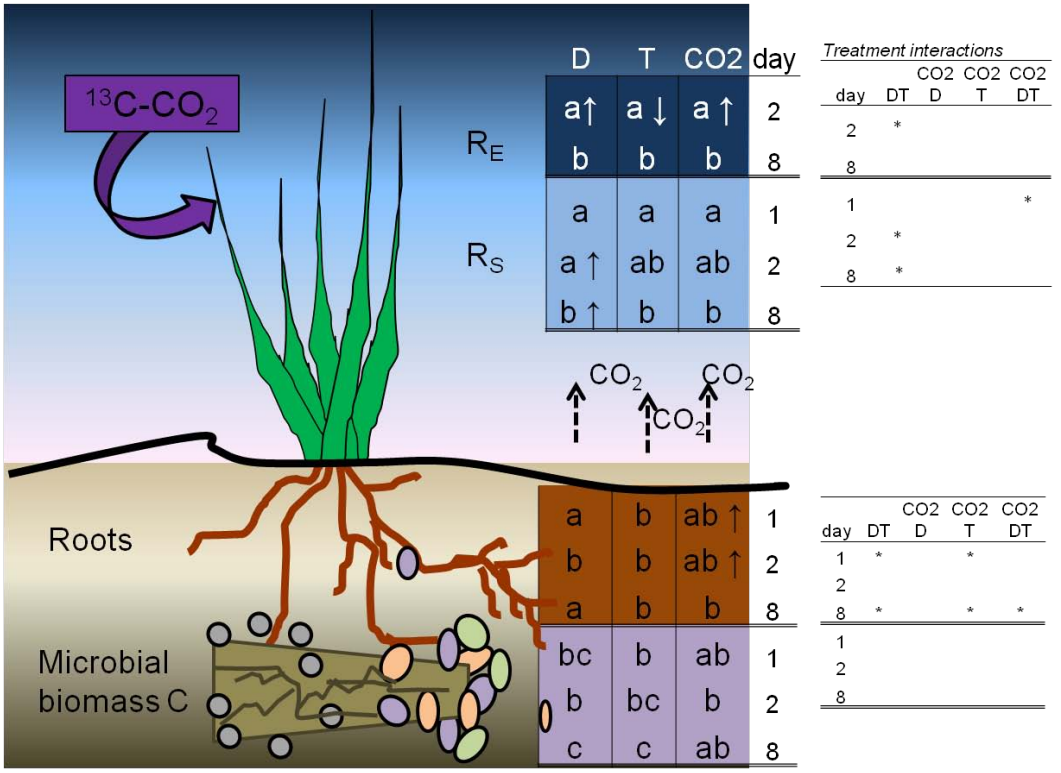

Figure 6: Atom% excess (APE) in different terrestrial C pools: Supplied ^{13}C-CO_2 is assimilated by plants and transported into belowground plant parts: roots (brown) and into the microbial biomass (purple). Re-allocated C can be respired and diffuses back to the atmosphere as soil respiration (R_S, light blue). Ecosystem respiration (dark blue) combines belowground and aboveground CO_2 respiration. APE was measured for the climate treatments 1, 2 and 8 days after the ^{13}C-CO_2 pulse-chase. APE dynamics within each climate treatment over time are indicated by letters; differences between treatments are indicated with arrows where the direction of the arrow indicates the APE development compared to the ambient treatment. Treatment interactions are indicated with * if $p \leq 0.05$.

Three out of four CO_2 treatments (CO2, DCO2 and TDCO2) showed a high C-fate into gram-negative bacteria one day after the pulse-label (Figure 7) suggesting an overall positive effect of elevated CO_2 on the speed of C transport and C allocation in the microbial community. In contrast, three out of four non-CO_2 plots (A, D and T), displayed a one-day delay in C allocation peak into gram-negative bacteria, confirming the hypothesis of a faster C turnover under elevated CO_2 concentrations. Drought and temperature treatments did not show consistent patterns of recent C allocation in gram-negative bacteria suggesting that the two treatments are not the main determinants of C turnover rates.

Actinomycetes (Figure 7, orange bars) and fungi (Figure 7, yellow bars) were generally less involved in the uptake of recently assimilated C in roots compared to soil. The uptake of recently assimilated C in

actinomycetes was delayed compared to the bacterial groups. The C-fate into actinomycetes in roots was very low one day after labeling (with exception of TCO2) but this changed over time. This can be a result of low actinomycetes activity in roots, or the utilization of an alternative C source. The actinomycetes community was more actively involved in the uptake of recently assimilated C in soil than in roots, with the exception of the TCO2 treatment. Fungi often took up a high proportion of recently assimilated C in roots at day one (average 10 %), but the C-fate was detected to be lower at day 2 (average 4 %), combined with a high fate of C into the fungal group in soil at day 2 (20 %). This pattern suggests a re-allocation of recently assimilated C from roots into the soil matrix due to fungal activity.

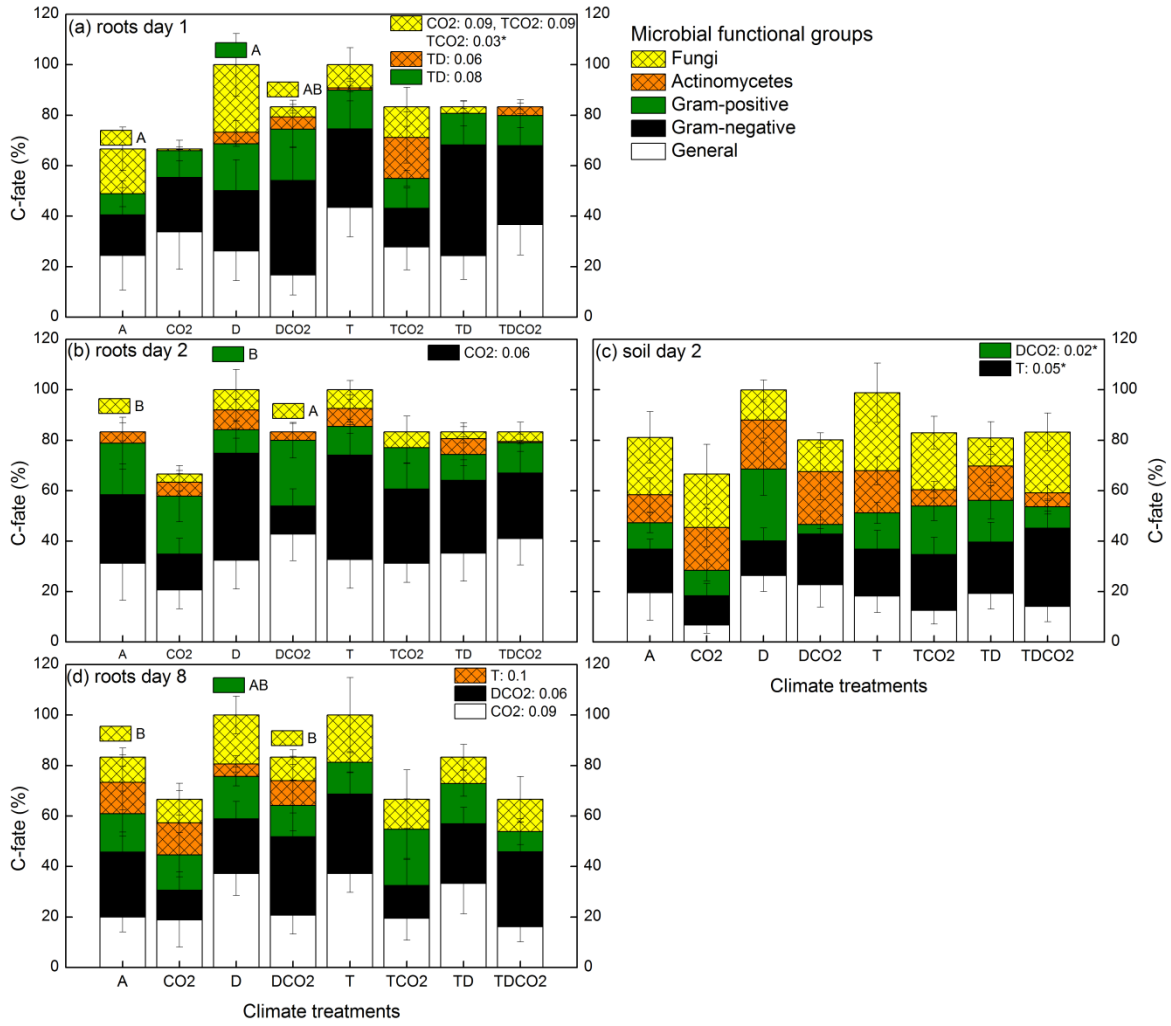

Figure 7: Fate of recently assimilated C (C-fate) into microbial functional groups in roots (a,b,d) and soil (c) over time. Legends within each plot show significant differences between treatments during that time point ($p \leq 0.1$). Boxes coloured as functional groups above treatments in combination with letters indicate within treatment differences in the soil microbial group over time.

Fungi, especially mycorrhizal fungi, are known to transport C through hyphae and provide easily available C to rhizosphere bacteria (Olsson & Johnson 2005). In the presented study, we did not detect mycorrhizal specific PLFAs, but we assume that the observed fungal biomarkers represent a mix of saprophytic and mycorrhizal fungi. Thus, low recovery of recently assimilated C in roots in e.g. drought plots at day 2 can be a result of C transport through hyphae away from roots and into different microbial groups such as actinomycetes (19 ± 7 %) and gram-positive bacteria (28 ± 10 %). C-fate patterns proposed that fungi generally transported recently assimilated C into the soil matrix, where especially actinomycetes profited from the labile C input (Figure 7, soil). Actinomycetes in our study might be dependent on C re-allocation by fungi. An active actinomycetes community in soil has further implication for mineralization of recalcitrant C compounds, since the availability of recalcitrant substances dominate in the soil matrix. Actinomycetes, like fungi, are decomposers of soil organic matter (Lacey 1997). Dependent on microbial community composition, size and activity in soils under changing climatic conditions, the soil C balance can be shifted towards the soil being either a C sink or a C source (Carney *et al.* 2007; Paterson *et al.* 2009; Garcia-Pausas & Paterson 2011).

A comparison of the ambient treatment vs. the treatment simulating future climatic conditions (TDCO2) revealed different roles of MFGs in short-term C utilization (Figure 7). Generally, under future climatic conditions the gram-negative bacteria persistently utilized 30 % of the recently assimilated C, emphasizing the quantitative importance of this functional group. C-fate into gram-positive bacteria was similar in ambient and future treatments. However, C-fate into actinomycetes in roots was notably lower under future conditions (average 2 %) compared to any other climate treatment, which can be explained by the fact that the enrichment peak was missed due to faster C re-allocation. The explanation, however, is rather unlikely because enrichment peaks in roots and MBC show the same patterns for all other treatments, and our observation rather suggest that the actinomycetes community was suppressed under future climatic conditions.

The re-allocation of C from roots into the soil via fungal hyphae was similar under ambient and future climatic conditions, but in the full-factorial treatment most of the recently assimilated C was observed in gram-negative bacteria. In contrast, the distribution of recently assimilated C in the soil was more even between the functional groups in ambient plots. The changed bacterial activity observed under future climatic conditions points towards a less active decomposer community

due to lower activity of actinomycetes under future climatic conditions, and thus possibly repressed mineralization of SOM from soil C stores.

Generally, responses of the microbial community to climatic changes are highly variable within and among ecosystems. Furthermore, timing (Butler *et al.* 2003) and scale of climatic changes affect microbial responses rather than the occurrence of the climate change itself (Hawkes *et al.* 2011; Sheik *et al.* 2011). In Exp2, C turnover was stimulated by elevated CO_2 resulting in different C allocation patterns in microbial functional groups over time. C allocation into MFGs could be changed under future climatic conditions, potentially leading to slower mineralization of SOM stocks.

4.2 Climate change as trigger of soil organic matter priming? (Paper III)

SOM is a complex mixture of plant and animal residues in different stages of decay. SOM includes particulate organic matter with a particle size between 0.053 and 2 mm, humus (< 0.053 mm) and recalcitrant organic C (mainly charcoal) (Six *et al.* 2000). SOM is a nutrient rich fraction in the soil (mainly N and K); it influences the soil water holding capacity and provides habitats for soil microorganisms (Fontaine *et al.* 2011). The SOM pool is balanced by the C input of dead plant material and SOM mineralization, and accounts for an exchange of about 60 Pg C yr^{-1} on a global scale (Filley & Boutton 2006). If climate factors favour SOM formation by e.g. increasing plant biomass under elevated CO_2 conditions (Fitter *et al.* 1996), soils can serve as C sinks. On the opposite, climate change has been shown to increase the magnitude of SOM mineralization under e.g. elevated atmospheric CO_2 conditions and consequently, soils can turn into C sources (Carney *et al.* 2007) and further accelerate atmospheric CO_2 increase (IPCC 2007).

SOM mineralization processes are still a matter of debate (Brookes *et al.* 2009; Kuzyakov *et al.* 2009; Paterson 2009). Kemmitt *et al.* (2008) suggested that SOM decomposition could be solely driven by soil physical processes and that the soil microbial community is not necessarily a key element in SOM mineralization dynamics. However, subsequent discussions pointed out that the soil microbial community is a crucial component of SOM mineralization processes (Fontaine *et al.* 2003; Blagodatskaya *et al.* 2008; Paterson *et al.* 2009; Garcia-Pausas & Paterson 2011).

Soil microbial communities are strongly controlled by plant-derived C released into the soil matrix by root exudation and litter inputs. Labile C input can induce increased SOM mineralization (the 'priming effect'); this effect is increasingly recognized as quantitatively significant in soil C dynamics (Carney *et al.* 2007; Fontaine *et al.* 2011). Labile C stimulates the soil microbial community and increases microbial activity coupled to increased SOM mineralization. The priming effect occurs after the microbial biomass has been triggered by labile plant C and microbes increase SOM utilization (Blagodatskaya & Kuzyakov 2008; Kuzyakov 2010). SOM mineralization leads to nutrient release into the soil matrix and nutrients become available for plants, microbes and other soil organisms (Fontaine *et al.* 2011).

We performed a laboratory incubation experiment with soil from five treatments from the CLIMAITE field experiment (A, CO2, DCO2, TCO2, TDCO2). The goal of this experiment was to investigate the impact of elevated CO_2 on the potential for plant-mediated SOM priming (paper III). Glucose was used as labile C substrate to induce priming (De Nobili *et al.* 2001; Mondini *et al.* 2006), in line with many previous studies, as it is utilized ubiquitously by soil microbes (Paterson *et al.* 2007; Blagodatskaya & Kuzyakov 2008; Fontaine *et al.* 2011). As a labile C compound that stimulates microbial activity, glucose is a relevant model substrate for root exudates under tightly controlled experimental conditions (Kuzyakov 2010). Homogenously labelled ^{13}C-glucose (3 APE) was added to soil microcosms (Figure 8a) and an identical set of microcosms was kept as controls (non-glucose). After glucose additions, soil respired CO_2 was accumulated in closed microcosms (Figure 8b) and the accumulated CO_2 concentration was measured (Figure 8c). The accumulated respired CO_2 was analyzed for $^{13}C/^{12}C$ ratios, and under the assumptions that microbes could either utilize glucose-derived C or SOM-derived C as C sources, a two-end member mixing model was used to separate glucose- and SOM-derived C in respired CO_2.

The total amount of SOM-derived CO_2 accumulated in non-glucose (NG) and glucose (G) microcosms during the experiment ($n = 9$) showed a significantly increased amount of respired SOM-C in all treatments, except for TDCO2 (Figure 9). A significant increase in SOM-respired CO_2 triggered by glucose (labile C) additions was a result of SOM-priming mediated by the soil microbial community.

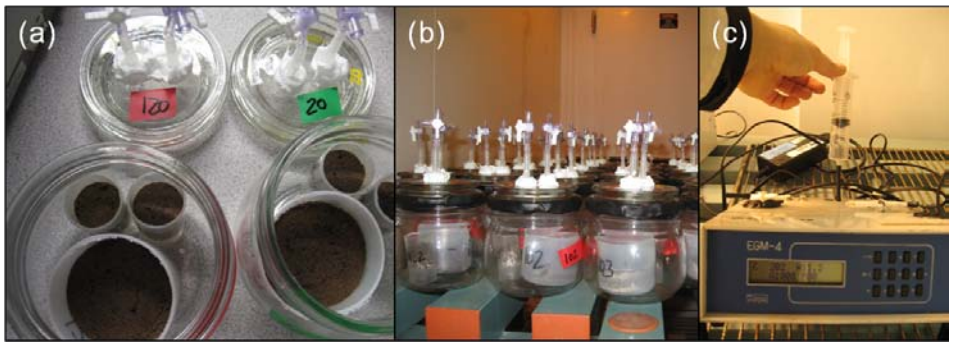

Figure 8: Laboratory experimental setup to investigate SOM priming under future climatic conditions: (a) paired microcosms for glucose (red) and non-glucose (green) treatments with three harvest compartments, (b) microcosm incubation (closed lids) at 8 °C and (c) injection of accumulated headspace CO_2 into an EGM-4 after incubation to measure the CO_2 concentration.

It has been shown that elevated CO_2 can induce SOM priming and thus lead to a reduction of soil C stocks (Carney *et al.* 2007). In our laboratory experiment, soils treated with elevated CO_2 in the field showed an increased proportion of SOM-derived C in soil CO_2 efflux. Remarkably, priming was also observed in the non-CO_2 treated soil, but not in the full factorial climate factor combination (TDCO2). The absence of SOM priming in TDCO2 soils can be due to C immobilization in the soil matrix or in the microbial biomass (Lagomarsino *et al.* 2006; Gude *et al.* 2012). SOM dynamics under elevated CO_2 has also been shown to be highly dependent on soil nutrient availability (van Groenigen *et al.* 2006; Phillips *et al.* 2011).

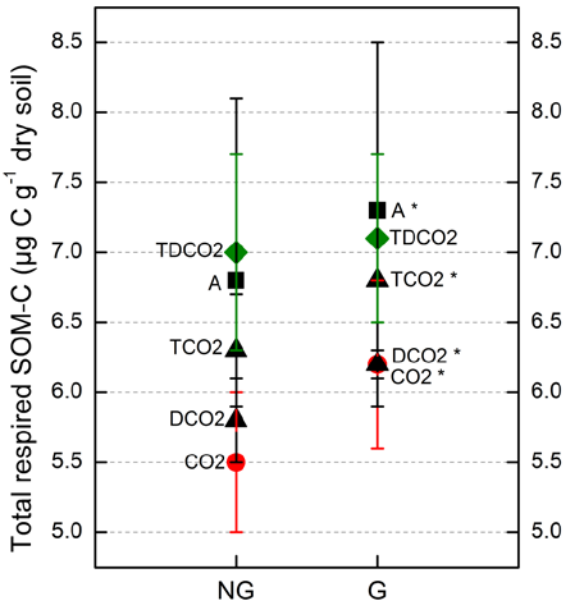

Figure 9: Total amounts of respired SOM-C in control (NG) and glucose-amended (G) microcosms over the course of the experiment (n = 9). Single factor CO_2 treatment is shown in red, and full treatment combination (TDCO2) in green. * indicates significant differences between NG and G treatments ($p \leq 0.05$). Values are means ± SE (n = 6).

C assimilation and belowground C allocation has been shown to be enhanced under elevated CO_2 conditions (Drake *et al.* 2011; Albert *et al.* 2011) suggesting an increase in soil C input. Meanwhile, Gude *et al.* (2012) observed a reduced C storage efficiency when C input into the soil was high. Their observations agree with our results showing that in our CO_2 fumigated soils (but not TDCO2) where the plant C input belowground is assumed to be high, SOM-C was 'wasted' and observed as SOM-priming. In contrast, Gude *et al.* (2012) also showed that soil C limitation reduced SOM priming in combination with the formation of new SOM-C. In our TDCO2 treated soil, the combination of elevated CO_2 with drought and warming could have reduced belowground C allocation (Albert *et al.* 2011) and consequently resulted into a C limited soil status going along with inhibition of SOM priming. The missing priming response under future climatic conditions (TDCO2) also confirms our conclusion presented in paper II and is in agreement with lower soil CO_2 effluxes in the TDCO2 treatment (Selsted *et al.* 2012) and suggests that the change in the microbial community can lead to a repressed mineralization of SOM (paper II).

5. OUTLOOK – CARBON BALANCE UNDER FUTURE CLIMATIC CONDITIONS

To what extend SOM priming will be facilitated or diminished in the future might have far-reaching consequences for the development of atmospheric CO_2 concentrations. CO_2 respired from the soil is the second largest flux between the soil and the atmosphere, and amounts to ca. 10-fold more than the annual fossil fuel emissions of ca. 6 Pg C yr^{-1} (Filley & Boutton 2006). Consequently, soil CO_2 effluxes increase under elevated atmospheric CO_2 conditions, as it has frequently been observed (Wan *et al.* 2007; Selsted *et al.* 2012), and CO_2 concentrations might accelerate considerably.

5.1 Soil and ecosystem CO_2 efflux as affected by climate change factors (outlook for paper IV)

In addition to the presented papers I-III, a fourth manuscript is planned to summarize C allocation patterns by means of C pool sizes to assess the short-term C turnover under anticipated future climatic conditions. Therefore, the following section presents results of measured soil and ecosystem fluxes and represents the last step in terrestrial C turnover (Figure 1) until the C cycle is closed.

Even though measured annual average soil CO_2 efflux rates are relatively small at the field site (1.7 μmol CO_2 m^{-2} sec^{-1}; Selsted *et al.* 2012), the contribution of soil respired CO_2 from heath/grasslands is not negligible as heath/grasslands cover about ~40 % of the global terrestrial surface (excluding Greenland and Antarctica; Reynolds 2005). Soil respiration rates measured in October 2010 (Exp1) showed a higher variability than soil CO_2 efflux rates in May 2011 (Exp2), probably as a result of seasonality (Figure A1). In May, drought tended to reduce soil respiration ($p = 0.07$) and drought, in combination with temperature and elevated CO_2, showed a significant interaction ($p = 0.02$). No positive effect of elevated CO_2 on soil respiration was observed which can be explained by high C allocation into the plant and limited belowground allocation at this time of the year. Ecosystem respiration showed the same flux patterns as soil respiration and resulted in a significantly reduced CO_2 efflux in drought plots ($p = 0.001$). The drought treatment in interaction with temperature ($p = 0.009$), and temperature*CO_2

(p = 0.079) revesed the positive effects of temperature and elevated CO_2 on soil respiration. Soil water availability can modulate climate effects (Wan *et al.* 2007), as suggested by lower CO_2 effluxes in our TDCO2 treatment. Reduced soil respiration rates were observed under drought conditions, possibly due to (i) lowered faunal activity, (ii) reduced root and microbial activity, (iii) reduced substrate availability and/or, (iv) limited solubility of substrates (Wan *et al.* 2007; Selsted *et al.* 2012).

The contribution of recently assimilated C to soil and ecosystem effluxes can be investigated by weighting the APE (^{13}C from the $^{13}CO_2$ pulse) in soil and ecosystem CO_2 effluxes (Figure 6) with the observed bulk fluxes (Figure 10). But beforehand, it is important to consider CO_2 uptake relative to plant biomass for the different climate treatments to evaluate these contributions (Figure 10a). The ^{13}C enrichment per g dry leaf per m^2 was not different between treatments, but the CO2 and TCO2 treatments showed exceptionally high variation. Both treatments showed outstandingly high contribution of recently assimilated C to ecosystem respiration (Figure 10b,c) suggesting that plant respired CO_2 contribution was highest in these treatments. This result is in agreement with the conclusion of Wan *et al.* (2007) that plant activity modulates effects of elevated CO_2 and can result in changed respiration patterns.

Treatments exposed to increased CO_2 concentrations always showed a higher amount of recently assimilated C per respired unit than their non-CO_2 counterparts, for both, soil and ecosystem respiration (Figure 10b,c). The only exception was found to be the TDCO2 treatment (Figure 10d) that showed the same contribution of recently assimilated C into soil and ecosystem respiration as the non-CO_2 treatments. Ecosystem CO_2 efflux of the future (TDCO2) treatment, compared to the ambient treatment, showed reduced contribution of recently assimilated C to ecosystem respiration.

Increased soil CO_2 efflux in CO_2 treatments are in agreement with our findings in the laboratory experiment, and we can draw the same conclusion that higher belowground C allocation may results in a higher utilization of C (Gude *et al.* 2012). Opposite to this, treatments including the summer drought resulted in lower soil and ecosystem respiration fluxes assuming C storage under the assumption that plant activity was the same among treatments. The TDCO2 treatment showed interactions between the three tested climate relevant factors and clearly displays the complexity of ecosystem responses to changing climatic conditions.

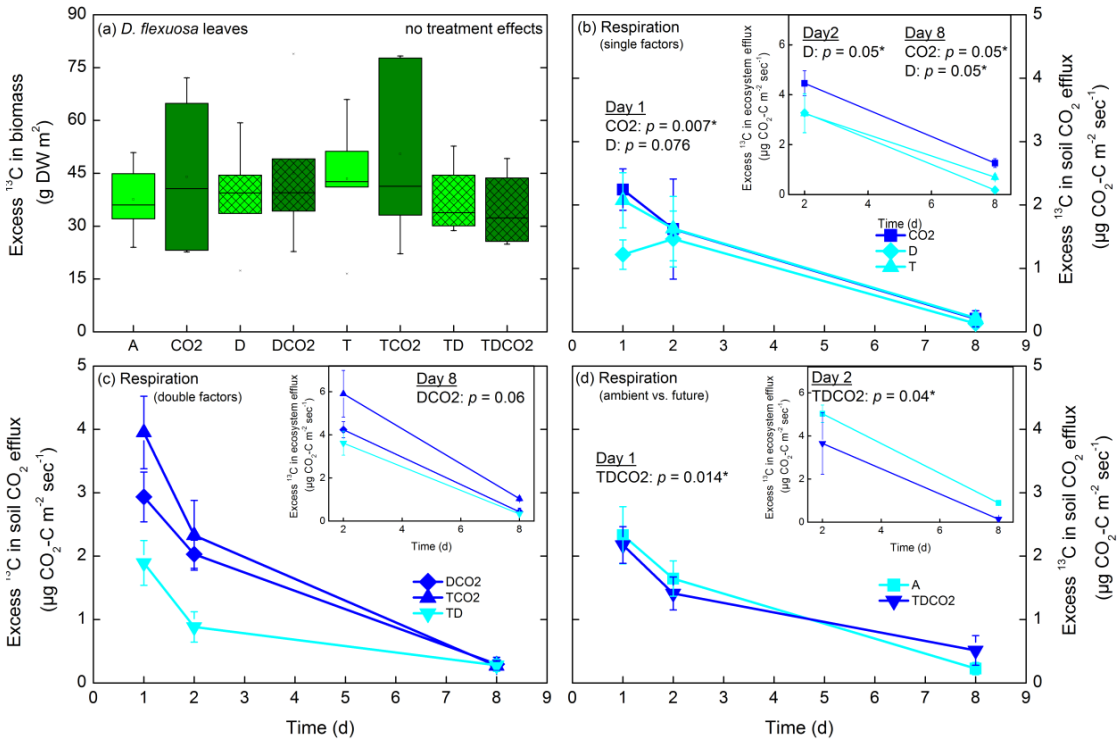

Figure 10: Excess ^{13}C in (a) *D. flexuosa* leaves (biomass * APE) (plant biomass estimates from pers. commun. with Inger Kappel Schmidt). Panals (b), (c) and (d) show ^{13}C excess in soil and ecosystem respiration over time under different climatic conditions (calculated as bulk flux * APE): (b) single factor treatments, (c) double factor interactions and (c) comparison of ambient and future (TDCO2) climatic conditions. Significance information with day and treatments in each panel.

5.1 Conclusions and perspectives

In-situ $^{13}CO_2$ pulse-labeling has been shown to be an excellent tool to track recently assimilated C into different terrestrial C pools to reveal terrestrial C turnover dynamics. In this study, a pulse-labeling setup was successfully developed and applied in a remote natural environment at relatively low costs. The pulse-chase provided enough label to track recently assimilated C into all below- and aboveground C pools of interest. The ^{13}C label appearance in the different pools was generally affected only to a minor extent by the applied climate treatments. But, on a finer resolution at the microbial functional group level, the allocation of recently assimilated C was altered under different climatic conditions. Our results suggest that the terrestrial C turnover is slowed down under drought conditions, and accelerated under elevated CO_2. Increased temperature was not observed to affect short-term C allocation. The simulation of future climatic conditions that combined the climate factors elevated CO_2, prolonged spring/summer droughts and increased temperature was found to induce a potential for increased

storage of recently assimilated C and to reduce soil C losses. To link our findings to the global C turnover, estimates of C pools in the studied heath/grassland are needed. However, the presented results clearly suggest that the combination of climate change factors change ecosystem responses, and the terrestrial C turnover cannot be derived from ecosystem responses of applied single factor treatments.

The current work does not include the analysis of NLFAs, but the identification of this group of cell membrane components can give a deeper insight into the microbial community composition, especially with respect to the importance of arbuscular mycorrhizal fungi (AMF). AMF are very important for the ecosystem function in terms of nutrient exchange with plants. To investigate the role of AMF in the terrestrial short-term C turnover in our nutrient poor heath/grassland would immensely improve our knowledge. Furthermore, research connected to the presented pulse-labeling experiment was carried out by Pia Lund to investigate the allocation of recently assimilated C into deeper soil horizons, which is particularly interesting under elevated CO_2 conditions when roots explore deeper down. Furthermore, C-budget calculations including photosynthetic capacity (CO_2 uptake) of *D. flexuosa* under different climatic conditions will be used to estimate how much ^{13}C was quantitatively taken up and distributed into different C pools, and respired back to the atmosphere respectively (planned Paper IV).

Future *in-situ* $^{13}CO_2$ pulse-labeling experiments should aim for a higher belowground labeling efficiency by e.g. applying the label at the end of the plant growth season when plants increase belowground C allocation, while plant aboveground biomass and photosynthesis are still high. This would offer the possibility to investigate the microbial community on a species level using RNA-SIP on field samples.

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7. APPENDIX

This appendix presents additional results collected during the time of the Ph.D. that are in addition to the synopsis and material that is not presented in either the synopsis or the papers.

Table A1 shows the atom% ^{13}C excess into individual functional groups. Tables A2 and A4 present the C-fate into in individual microbial PLFAs over time in Exp2 and the laboratory experiment, respectively. Table A3 displays relative abundances of each individual PLFA in Exp2.

Figure A1 shows average soil and ecosystem C fluxes for Exp1 (n = 7) and Exp2 (n = 3).

Table A1: Atom% ^{13}C excess in microbial functional groups over time in Exp2. Bold values indicate tendencies between treatments with $p \leq 0.1$ and * with $p \leq 0.05$. Small letters show differences within climate treatments over time. Values as mean ± SE.

	A	CO2	D	DCO2	T	TCO2	TD	TDCO2
Roots day 1								
Total	0.38 ± 0.14	0.24 ± 0.10	0.84 ± 0.27	1.04 ± 0.28 **a**	0.54 ± 0.13	1.17 ± 0.35 **a**	***0.71 ± 0.32*** *	***0.41 ± 0.20*** *
General	0.11 ± 0.06	0.13 ± 0.09	0.12 ± 0.05	0.23 ± 0.10	0.14 ± 0.02	0.40 ± 0.18	0.19 ± 0.12	0.17 ± 0.08
Gram-negative	0	0	0 **a**	0	0	0.03 ± 0.03	0.04 ± 0.03	0
Gram-positive	0.30 ± 0.12	0.12 ± 0.05	0.75 ± 0.24	0.87 ± 0.26 **a**	0.41 ± 0.13	0.74 ± 0.21 **a**	***0.44 ± 0.24*** *	0.24 ± 0.16
Actinomycetes	0	0	0	0	0	0	0	0
Fungi	0	0	0	0	0	0	0.04 ± 0.04	0
Roots day 2								
Total	0.74 ± 0.21	0.16 ± 0.17	0.96 ± 0.30	0.41 ± 0.19 **ab**	0.48 ± 0.21	***0.66 ± 0.19 *ab***	0.23 ± 0.17	0.43 ± 0.11
General	0.13 ± 0.07	0	0.35 ± 0.16	0.09 ± 0.12	0.20 ± 0.07	0.27 ± 0.07	0.21 ± 0.13	0.16 ± 0.08
Gram-negative	0.04 ± 0.06	***0*** *	0.20 ± 0.07 **b**	***0***	0.07 ± 0.04	***0.05 ± 0.02****	***0****	0
Gram-positive	0.56 ± 0.14	0.14 ± 0.18	0.42 ± 0.21	0.40 ± 0.11 **ab**	0.21 ± 0.12	0.34 ± 0.17 **ab**	***0.02 ± 0.09***	0.27 ± 0.07
Actinomycetes	0	0.06 ± 0.06	0	0	0	0	0	0.03 ± 0.03
Fungi	0	0	0	0	0	0	0	0
Roots day 8								
Total	0.63 ± 0.20	***0.33 ± 0.21*** *	0.55 ± 0.29	0.16 ± 0.09 **b**	0.58 ± 0.12	0.30 ± 0.18 **b**	0.45 ± 0.12	0.28 ± 0.21
General	0.20 ± 0.09	0.13 ± 0.10	0.36 ± 0.12	0.04 ± 0.08	0.13 ± 0.03	0.16 ± 0.015	0.24 ± 0.14	0.18 ± 0.10
Gram-negative	0.01 ± 0.05	0.02 ± 0.03	0.03 ± 0.03 **ab**	0	0.04 ± 0.04	0.01 ± 0.01	0.02 ± 0.04	0.01 ± 0.05
Gram-positive	0.29 ± 0.16	0.18 ± 0.11	0.14 ± 0.21	0.06 ± 0.13 **ab**	0.22 ± 0.12	0.14 ± 0.09 **ab**	0.18 ± 0.09	0.04 ± 0.17
Actinomycetes	0.02 ± 0.02	0	0	0	0	0	0	0
Fungi	0.11 ± 0.08	0	0.03 ± 0.02	***0.09 ± 0.06*** *	0.19 ± 0.12	0	0.01 ± 0.01	0.06 ± 0.05
Soil day 2								
Total	0.13 ± 0.15	0.08 ± 0.09	0.23 ± 0.22	0.11 ± 0.10	0.06 ± 0.06	0.16 ± 0.10	0.12 ± 0.08	0.11 ± 0.04
General	0.07 ± 0.07	0.05 ± 0.06	0.10 ± 0.12	0.07 ± 0.06	0	0.10 ± 0.07	0.05 ± 0.03	0.04 ± 0.04
Gram-negative	0.01 ± 0.02	0.01 ± 0.01	0	0	***0.01 ± 0.01***	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Gram-positive	0.05 ± 0.04	0	0	0	0.04 ± 0.04	0.03 ± 0.02	0.03 ± 0.04	0.02 ± 0.03
Actinomycetes	0	0	0.15 ± 0.13	0.02 ± 0.02	0	0	0	0
Fungi	0.01 ± 0.01	***0.02 ± 0.01***	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.03

Table A2: C-fate into microbial PLFAs (in %) over time in Exp2 for root and soil samples. Values as means ± SE.

Roots day 1		A	CO2	D	DCO2	T	TCO2	TD	TDCO2
General	14:0	0.4 ± 0.4	0.3 ± 0.3	1.6 ± 1.2	0	0	1.7 ± 1.3	0.6 ± 0.6	0.3 ± 0.3
	15:0	0.1 ± 0.1	10.5 ± 6.2	0.3 ± 0.1	1.9 ± 1.0	1.8 ± 0.9	2.1 ± 0.6	0.5 ± 0.4	5.6 ± 3.2
	16:0	22.4 ± 13.4	19.7 ± 12.5	20.1 ± 12.0	5.4 ± 5.1	35.2 ± 9.0	22.5 ± 7.8	20.7 ± 9.0	14.0 ± 8.1
	18:0	1.5 ± 1.5	3.3 ± 2.7	4.3 ± 2.2	9.4 ± 7.4	6.4 ± 3.2	1.4 ± 0.9	2.6 ± 0.8	16.8 ± 7.8
Gram-negative	17:0cy	12.4 ± 10.9	12.0 ± 12.0	12.0 ± 12.0	21.2 ± 13.4	22.0 ± 13.5	4.8 ± 4.8	34.7 ± 15.1	13.5 ± 13.4
	19:0cy	0	0	0	0	0	0	0	0
	16:1ω7	0.1 ± 0.1	4.8 ± 3.3	4.4 ± 3.3	5.5 ± 4.6	5.3 ± 4.0	0.8 ± 0.4	2.7 ± 2.6	3.3 ± 2.4
	18:1ω7	3.6 ± 1.8	4.8 ± 2.6	7.6 ± 3.6	10.7 ± 4.9	3.8 ± 2.0	9.8 ± 3.6	6.5 ± 2.4	14.4 ± 5.2
Gram-positive	15:0i	0.8 ± 04	0.3 ± 0.3	2.8 ± 0.2	10.2 ± 5.0	1.4 ± 0.6	1.6 ± 1.3	2.1 ± 0.8	1.0 ± 0.6
	15:0a	0.3 ± 0.2	1.3 ± 1.0	2.2 ± 0.7	1.6 ± 1.1	2.7 ± 2.0	1.9 ± 1.2	1.5 ± 1.4	0.5 ± 0.3
	16:0i	2.9 ± 2.3	2.3 ± 1.5	3.4 ± 0.8	3.4 ± 1.6	3.0 ± 1.9	1.7 ± 0.7	1.6 ± 1.1	3.6 ± 2.4
	17:0i	3.3 ± 1.9	5.2 ± 2.8	2.7 ± 1.1	2.6 ± 1.9	4.2 ± 2.7	2.1 ± 0.8	4.6 ± 2.2	4.3 ± 2.5
	17:0a	0.8 ± 0.6	0.3 ± 0.3	7.3 ± 1.6	2.1 ± 1.0	1.0 ± 0.7	2.5 ± 1.3	2.7 ± 2.1	1.4 ± 0.8
Actinomycetes	10Me16:0	0	0	0	0	0.8 ± 0.8	0.3 ± 0.3	0	0
	10Me17:0	0	0	0	4.9 ± 4.9	0	15.8 ± 10.3	0	0
	10Me18:0	0	0.0297	4.6 ± 4.6	0	0	0.2 ± 0.2	0	3.4 ± 2.8
Fungi	18:1ω9	0	0	0	0	0	0	0.3 ± 0.3	0
	18:2ω6	17.4 ± 8.4	0.7 ± 0.7	26.8 ± 12.3	4.0 ± 2.6	9.2 ± 6.7	12.2 ± 7.8	2.2 ± 2.2	0
	18:3ω3	0.3 ± 0.3	0	0	0	0	0	0	0

Table A2 *continued.*

Roots day 2		A	CO2	D	DCO2	T	TCO2	TD	TDCO2
General	14:0	0	1.4 ± 1.4	3.4 ± 3.3	1.3 ± 0.9	0	0.7 ± 0.5	0.7 ± 0.5	1.5 ± 0.9
	15:0	0.1 ± 0.1	1.2 ± 1.2	2.1 ± 1.2	1.5 ± 1.0	0.5 ± 0.3	0.6 ± 0.3	2.5 ± 1.6	0.5 ± 0.5
	16:0	16.6 ± 9.7	6.0 ± 3.7	11.7 ± 4.8	30.8 ± 8.6	20.6 ± 10.0	19.0 ± 6.6	28.0 ± 9.1	35.3 ± 10.0
	18:0	14.5 ± 9.6	11.9 ± 7.5	15.2 ± 11.2	9.2 ± 5.0	11.5 ± 5.3	10.8 ± 5.1	4.1 ± 2.2	3.8 ± 2.1
Gram-negative	17:0cy	7.2 ± 7.2	0	0	5.9 ± 5.9	14.9 ± 14.9	0.0	12.6 ± 9.1	0
	19:0cy	0	0	0	0	0	0	0	0
	16:1ω7	5.7 ± 4.0	3.7 ± 2.3	17.4 ± 7.5	2.2 ± 1.4	8.6 ± 4.1	16.1 ± 9.9	7.3 ± 4.6	13.2 ± 8.3
	18:1ω7	14.3 ± 11.2	10.6 ± 5.1	25.1 ± 10.4	3.0 ± 1.5	17.9 ± 7.6	13.4 ± 8.2	8.9 ± 3.1	12.7 ± 7.0
Gram-positive	15:0i	5.7 ± 3.9	7.6 ± 6.6	0.7 ± 0.6	1.5 ± 1.0	2.8 ± 0.8	1.7 ± 1.0	1.7 ± 1.0	4.3 ± 2.9
	15:0a	7.0 ± 6.2	5.8 ± 3.9	1.6 ± 1.2	7.0 ± 4.6	1.5 ± 0.6	2.8 ± 0.7	0.8 ± 0.6	1.7 ± 0.8
	16:0i	4.4 ± 1.7	1.3 ± 1.3	2.7 ± 1.1	3.0 ± 2.8	1.9 ± 1.3	2.8 ± 1.9	3.3 ± 2.6	2.3 ± 0.9
	17:0i	0.3 ± 0.3	1.8 ± 1.8	0	7.1 ± 7.1	0	0	0	0
	17:0a	2.0 ± 1.1	4.7 ± 3.1	2.5 ± 1.5	2.8 ± 2.1	3.6 ± 2.3	6.8 ± 5.2	0.8 ± 0.8	0.2 ± 0.2
Actinomycetes	10Me16:0	0	0	0.7 ± 0.7	0	0.2 ± 0.2	0	0.0	0.4 ± 0.4
	10Me17:0	3.7 ± 3.7	0	6.1 ± 4.7	0	5.5 ± 5.5	0	6.3 ± 6.3	0
	10Me18:0	0.8 ± 0.8	5.5 ± 3.5	1.0 ± 0.8	3.3 ± 3.3	1.5 ± 1.5	0	0	0
Fungi	18:1ω9	0	0	0	0	0	0	0	0
	18:2ω6,9	0	3.3 ± 3.3	8.0 ± 8.0	0	7.4 ± 3.7	6.2 ± 6.2	2.7 ± 2.0	3.8 ± 3.8
	18:3ω3	0	0	0	0	0	0	0	0

Table A2 *continued.*

Roots day 8		A	CO2	D	DCO2	T	TCO2	TD	TDCO2
General	14:0	0.3 ± 0.3	0.8 ± 0.5	0.7 ± 0.5	1.7 ± 1.0	0.9 ± 0.5	0.3 ± 0.3	1.0 ± 0.7	0.1 ± 0.1
	15:0	2.0 ± 1.0	1.8 ± 1.0	3.8 ± 1.1	0.2 ± 0.2	0.8 ± 0.4	3.6 ± 2.7	1.2 ± 1.0	1.7 ± 0.9
	16:0	11.2 ± 4.7	13.6 ± 8.5	28.8 ± 8.0	12.0 ± 5.1	26.6 ± 8.4	12.5 ± 4.9	26.3 ± 11.4	8.7 ± 4.5
	18:0	6.4 ± 3.1	2.7 ± 1.8	4.1 ± 4.0	6.8 ± 3.8	9.0 ± 4.2	3.2 ± 2.6	4.9 ± 1.7	5.6 ± 3.5
Gram-negative	17:0cy	11.4 ± 7.3	3.7 ± 3.2	0	0.9 ± 0.9	3.9 ± 3.4	1.0 ± 0.7	4.8 ± 2.3	4.5 ± 3.7
	19:0cy	0	0	1.8 ± 1.8	0	0	0	0	0
	16:1ω7	8.3 ± 3.9	3.2 ± 2.3	8.6 ± 6.4	7.7 ± 3.7	15.3 ± 8.8	4.7 ± 4.7	13.4 ± 6.2	10.7 ± 9.7
	18:1ω7	6.1 ± 5.9	4.9 ± 4.0	11.3 ± 7.2	22.5 ± 7.8	12.3 ± 5.4	7.2 ± 5.5	5.4 ± 3.3	14.5 ± 7.9
Gram-positive	15:0i	0.7 ± 0.5	2.9 ± 2.8	3.2 ± 1.4	0.0	4.5 ± 2.6	0.8 ± 0.8	0.6 ± 0.6	0.2 ± 0.1
	15:0a	1.2 ± 0.8	2.9 ± 2.0	0.5 ± 0.4	0.2 ± 0.2	1.9 ± 1.4	0.9 ± 0.5	0.7 ± 0.4	0.7 ± 0.5
	16:0i	1.9 ± 1.2	0.2 ± 0.2	4.3 ± 1.7	0.1 ± 0.1	2.4 ± 1.1	12.3 ± 9.2	5.4 ± 2.7	2.1 ± 1.3
	17:0i	9.3 ± 9.3	0	0	0	0	0	2.5 ± 2.5	0
	17:0a	2.1 ± 1.2	3.9 ± 3.4	2.9 ± 1.3	6.1 ± 4.8	1.9 ± 0.9	3.0 ± 1.9	3.8 ± 3.4	3.5 ± 3.5
Actinomycetes	10Me16:0	1.3 ± 1.3	0	0	0	0	0	0	0
	10Me17:0	11.0 ± 11.0	12.7 ± 12.7	5.0 ± 3.2	9.7 ± 9.7	0	0	0	0
	10Me18:0	0	0	0	0	0	0	0	0
Fungi	18:1ω9	3.7 ± 2.6	0	4.9 ± 3.8	1.8 ± 1.2	2.8 ± 2.4	0	0.1 ± 0.1	1.2 ± 1.2
	18:2ω6,9	0.9 ± 0.9	0	7.3 ± 7.3	4.0 ± 3.1	0	11.9 ± 11.6	0	0
	18:3ω3	5.4 ± 3.6	9.4 ± 6.3	7.1 ± 6.2	3.5 ± 2.2	15.9 ± 12.5	0	10.3 ± 5.0	11.6 ± 8.8

Table A2 *continued.*

Soil day 2		A	CO2	D	DCO2	T	TCO2	TD	TDCO2
General	14:0	0	0	0	0	0	0	0	0
	15:0	0.1 ± 0.1	1.0 ± 0.8	1.3 ± 0.8	0.1 ± 0.1	1.3 ± 0.9	1.4 ± 0.6	3.2 ± 2.0	2.4 ± 1.1
	16:0	17.7 ± 10.9	3.3 ± 1.4	16.9 ± 4.8	19.9 ± 9.0	8.7 ± 5.0	5.2 ± 3.2	11.6 ± 4.4	7.7 ± 3.5
	18:0	1.7 ± 1.7	2.5 ± 2.5	8.1 ± 3.6	2.7 ± 1.7	8.2 ± 3.7	5.9 ± 3.8	4.8 ± 3.1	4.0 ± 1.9
Gram-negative	17:0cy	1.8 ± .5	7.9 ± 4.7	3.3 ± 2.4	0.8 ± 0.8	4.7 ± 1.7	5.6 ± 4.9	2.2 ± 1.3	7.2 ± 3.1
	19:0cy	0	0	0	0	1.0 ± 1.0	0	0.5 ± 0.5	0
	16:1ω7	8.8 ± 4.4	0.2 ± 0.2	0	2.3 ± 2.3	6.3 ± 3.2	6.9 ± 3.4	3.0 ± 1.9	1.4 ± 1.1
	18:1ω7	6.8 ± 3.0	3.3 ± 2.8	10.5 ± 5.6	17.0 ± 8.5	6.6 ± 5.7	9.6 ± 5.0	14.7 ± 6.4	22.5 ± 5.0
Gram-positive	15:0i	2.0 ± 1.6	2.0 ± 1.2	7.8 ± 7.4	0.2 ± 0.2	2.3 ± 0.4	2.6 ± 1.5	2.5 ± 1.4	0.5 ± 0.3
	15:0a	0.7 ± 0.6	1.4 ± 1.0	0.2 ± 0.2	0.4 ± 0.4	0.7 ± 0.7	1.4 ± 0.8	1.5 ± 1.1	2.1 ± 0.9
	16:0i	1.4 ± 1.0	1.3 ± 0.8	1.2 ± 0.7	0.3 ± 0.2	0.6 ± 0.6	1.6 ± 0.6	2.5 ± 1.8	0.7 ± 0.5
	17:0i	3.3 ± 2.3	4.9 ± 2.7	15.0 ± 8.0	0.8 ± 0.8	7.8 ± 4.3	10.1 ± 6.1	6.7 ± 5.5	0.8 ± 0.5
	17:0a	2.8 ± 1.9	0.3 ± 0.3	3.1 ± 2.2	0.2 ± 0.2	1.6 ± 1.2	1.4 ± 0.6	1.7 ± 0.8	3.9 ± 1.7
Actinomycetes	10Me16:0	8.0 ± 6.7	13.7 ± 6.7	11.1 ± 7.1	18.2 ± 9.4	13.3 ± 5.9	4.5 ± 2.1	9.7 ± 6.0	2.4 ± 1.4
	10Me17:0	0	1.8 ± 1.1	3.4 ± 2.3	0.3 ± 0.2	0	0	0	2.0 ± 2.0
	10Me18:0	3.0 ± 3.0	1.5 ± 1.0	4.8 ± 4.6	2.5 ± 1.8	3.4 ± 2.4	2.0 ± 2.0	3.9 ± 2.5	1.1 ± 1.1
Fungi	18:1ω9	13.8 ± 5.1	7.2 ± 3.3	5.0 ± 2.2	7.8 ± 2.2	18.4 ± 5.2	11.7 ± 4.8	6.2 ± 3.4	14.8 ± 4.5
	18:2ω6,9	0	0	0	0	0	7.8 ± 7.8	0	0
	18:3ω3	9.0 ± 6.9	14.0 ± 11.9	7.0 ± 2.3	4.7 ± 2.3	12.4 ± 12.4	3.0 ± 2.1	5.0 ± 3.5	9.3 ± 5.2

Table A3: Relative abundance of each PLFA in microcosms with glucose additions in the laboratory experiment investigating soil organic matter priming. Values as means ± SE.

Day 0	PLFA	A	CO2	DCO2	TCO2	TDCO2
General	14:0	0.2 ± 0.1	0.4 ± 0.01	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
	15:0	0.4 ± 0.1	0.5 ± 0.04	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
	16:0	19.6 ± 0.5	19.2 ± 1.1	19.3 ± 0.6	18.7 ± 0.7	19.8 ± 0.5
	18:0	7.9 ± 0.5	7.2 ± 0.4	7.5 ± 0.5	7.0 ± 0.3	7.3 ± 0.5
Gram-negative	17:0cy	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
	16:1ω7	4.3 ± 0.7	4.9 ± 0.5	5.2 ± 0.3	5.3 ± 0.2	5.5 ± 0.2
	18:1ω7	16.7 ± 2.0	16.2 ± 1.9	14.9 ± 1.4	17.4 ± 1.9	13.6 ± 0.7
Gram-positive	15:0i	5.9 ± 0.2	6.2 ± 0.4	6.3 ± 0.2	6.6 ± 0.6	6.9 ± 0.3
	15:0a	2.4 ± 0.2	2.6 ± 0.2	2.4 ± 0.1	2.5 ± 0.2	2.8 ± 0.3
	16:0i	4.8 ± 0.3	4.7 ± 0.5	4.9 ± 0.3	4.9 ± 0.4	5.7 ± 0.3
	17:0i	1.4 ± 0.3	1.5 ± 0.2	1.3 ± 0.1	1.6 ± 0.2	1.3 ± 0.1
	17:0a	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.04	0.9 ± 0.04	0.9 ± 0.1
Actinomycetes	10Me16:0	2.5 ± 0.3	3.8 ± 1.6	2.0 ± 0.1	2.3 ± 0.2	2.2 ± 0.4
	10Me17:0	0.8 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.9 ± 0.2
	10Me18:0	1.9 ± 0.2	1.8 ± 0.3	1.8 ± 0.1	1.8 ± 0.2	1.9 ± 0.2
Fungi	18:1ω9	8.0 ± 0.6	7.2 ± 0.8	7.8 ± 0.6	7.7 ± 0.3	7.9 ± 0.6
	18:3ω3	19.1 ± 1.7	18.6 ± 1.0	20.7 ± 1.1	18.1 ± 1.2	18.7 ± 0.8

Day 6	PLFA	A	CO2	DCO2	TCO2	TDCO2
General	14:0	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.4 ± .1	0.4 ± .1
	15:0	0.4 ± 0.04	0.3 ± 0.1	0.5 ± 0.03	0.5 ± 0.04	0.4 ± 0.04
	16:0	18.7 ± 0.4	19.9 ± 1.1	19.1 ± 0.6	18.7 ± 0.7	19.1 ± 0.8
	18:0	7.2 ± 0.2	7.6 ± 0.5	7.3 ± 0.3	7.5 ± 0.5	7.3 ± 0.4
Gram-negative	17:0cy	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.2	1.6 ± .1	1.7 ± 0.1
	16:1ω7	6.1 ± 0.2	5.9 ± 0.3	6.3 ± 0.3	5.7 ± 0.3	5.8 ± 0.3
	18:1ω7	16.1 ± 0.9	16.1 ± 1.9	15.3 ± 1.0	16.5 ± 1.0	13.4 ± 0.5
Gram-positive	15:0i	5.5 ± 0.3	5.7 ± 0.4	5.9 ± 0.2	5.6 ± 0.3	6.0 ± 0.2
	15:0a	2.2 ± 0.2	2.3 ± 0.2	2.2 ± .1	2.1 ± 0.2	2.3 ± 0.1
	16:0i	4.5 ± 0.4	4.1 ± 0.5	4.5 ± 0.2	4.3 ± 0.5	5.1 ± 0.5
	17:0i	0.9 ± 0.1	1.3 ± 0.3	1.2 ± 0.1	1.3 ± 0.2	0.9 ± .2
	17:0a	0.8 ± 0.04	0.8 ± 0.05	0.8 ± 0.03	0.7 ± 0.1	0.8 ± 0.03
Actinomycetes	10Me16:0	1.5 ± 0.2	1.7 ± 0.2	1.7 ± 0.1	1.7 ± 0.4	1.5 ± 0.2
	10Me17:0	0.5 ± 0.2	0.8 ± 0.2	0.5 ± 0.1	1.3 ± 0.5	1.1 ± 0.2
	10Me18:0	1.5 ± 0.1	1.7 ± 0.2	1.6 ± 0.1	1.5 ± 0.1	1.7 ± 0.1
Fungi	18:1ω9	7.9 ± 0.7	6.7 ± 0.4	7.4 ± 0.3	7.0 ± 0.5	7.8 ± 0.3
	18:3ω3	17.7 ± 0.8	17.5 ± 1.0	19.2 ± 0.7	18.0 ± 0.8	18.7 ± 1.1

Table A3 *continued.*

Day 14	PLFA	A	CO2	DCO2	TCO2	TDCO2
General	14:0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
	15:0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
	16:0	18.8 ± 0.6	19.2 ± 1.2	18.8 ± 0.5	18.5 ± 0.7	19.0 ± 0.7
	18:0	7.6 ± 0.5	7.5 ± 0.6	7.3 ± 0.4	7.4 ± 0.5	7.4 ± 0.4
Gram-negative	17:0cy	2.0 ± 0.2	1.9 ± .2	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.2
	16:1ω7	6.0 ± 0.5	6.0 ± 0.6	6.5 ± 0.2	5.9 ± 0.6	6.1 ± 0.2
	18:1ω7	17.4 ± 0.8	17.6 ± 1.8	16.8 ± 0.9	18.3 ± 1.2	15.7 ± 0.6
Gram-positive	15:0i	5.3 ± 0.2	5.6 ± 0.3	5.6 ± 0.2	5.5 ± 0.3	5.7 ± 0.1
	15:0a	2.1 ± 0.2	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.2	2.1 ± 0.1
	16:0i	4.0 ± 0.2	3.9 ± 0.4	4.2 ± 0.1	3.9 ± 0.4	4.6 ± 0.3
	17:0i	1.0 ± 0.1	1.5 ± 0.2	1.3 ± 0.1	1.4 ± 0.2	1.1 ± 0.2
	17:0a	0.6 ± 0.1	0.8 ± 0.04	0.7 ± 0.04	0.7 ± 0.04	0.8 ± 0.04
Actinomycetes	10Me16:0	1.9 ± 0.4	1.9 ± 0.3	1.6 ± 0.2	1.8 ± 0.2	1.6 ± 0.2
	10Me17:0	0.4 ± 0.2	1.4 ± 0.5	0.3 ± 0.1	1.2 ± 0.5	0.8 ± 0.2
	10Me18:0	1.4 ± 0.2	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.7 ± 0.1
Fungi	18:1ω9	8.7 ± 0.7	6.9 ± 0.6	8.4 ± 0.5	7.9 ± 0.3	8.5 ± 0.3
	18:3ω3	16.3 ± .5	13.8 ± 2.8	17.4 ± 0.7	13.4 ± 2.7	17.1 ± 0.5

Table A4: C-fate into microbial PLFAs (in %) in microcosms with glucose additions in the laboratory experiment. Values as means ± SE.

Day 6		A	CO2	DCO2	TCO2	TDCO2
General	14:0	0.4 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
	15:0	0.1 ± 0.02	0.1 ± 0.04	0.2 ± 0.04	0.2 ± 0.03	0.2 ± 0.03
	16:0	24.0 ± 0.4	23.9 ± 1.0	24.1 ± 0.6	24.7 ± 0.9	24.3 ± 0.9
	18:0	3.4 ± 0.4	3.2 ± 0.1	3.1 ± 0.2	3.4 ± 0.3	3.6 ± 0.5
Gram-negative	17:0cy	3.1 ± 0.6	2.8 ± 0.3	3.5 ± 0.5	2.8 ± 0.2	2.9 ± 0.4
	16:1ω7	15.2 ± 1.4	15.3 ± 1.1	16.7 ± 0.8	14.8 ± 1.3	13.1 ± 1.4
	18:1ω7	25.8 ± 1.7	28.1 ± 1.6	27.9 ± 0.7	27.3 ± 1.3	25.6 ± 1.5
Gram-positive	15:0i	5.1 ± 0.5	6.2 ± 0.6	5.5 ± 0.5	5.7 ± 0.6	5.6 ± 0.6
	15:0a	1.9 ± 0.8	2.1 ± 0.3	1.8 ± 0.9	1.6 ± 0.3	1.8 ± 0.4
	16:0i	2.4 ± 0.2	2.9 ± 0.2	2.5 ± 0.2	2.5 ± 0.3	2.7 ± 0.3
	17:0i	1.7 ± 0.1	1.0 ± 0.2	0.7 ± 0.1	0.8 ± 0.1	0.5 ± 0.1
	17:0a	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.04
Actinomycetes	10Me16:0	0.01 ± 0.1	0	0.1 ± 0.1	0	0.1 ± 0.2
	10Me17:0	0	0	0	0	0.1 ± 0.03
	10Me18:0	0.4 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.04
Fungi	18:1ω9	14.1 ± 2.5	11.4 ± 1.4	11.2 ± 1.0	13.0 ± 1.6	16.1 ± 2.3
	18:3ω3	2.5 ± 0.3	2.2 ± 0.2	2.1 ± 0.2	2.5 ± 0.3	2.3 ± 0.1

Day 14		A	CO2	DCO2	TCO2	TDCO2
General	14:0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.04	0.1 ± .1	0.1 ± 0.1
	15:0	0.1 ± 0.03	0.1 ± 0.04	0.2 ± 0.04	0.1 ± 0.1	0.1 ± 0.04
	16:0	20.9 ± 0.6	20.6 ± 0.8	20.8 ± 0.2	21.0 ± 0.6	20.8 ± 0.3
	18:0	2.6 ± 0.1	2.6 ± 0.2	2.6 ± 0.1	2.7 ± 0.1	2.6 ± 0.2
Gram-negative	17:0cy	5.2 ± 0.8	4.7 ± 0.7	5.5 ± 0.5	5.4 ± 0.6	4.7 ± 0.6
	16:1ω7	12.1 ± 1.1	13.4 ± 1.0	12.9 ± 0.5	12.3 ± 0.8	11.7 ± 0.9
	18:1ω7	25.7 ± 0.7	27.5 ± 1.1	26.9 ± 0.6	27.3 ± 0.9	26.0 ± 0.8
Gram-positive	15:0i	4.8 ± 0.4	5.8 ± 0.3	5.0 ± 0.2	5.3 ± 0.6	5.3 ± 0.5
	15:0a	1.5 ± 0.3	1.8 ± 0.3	1.4 ± 0.5	1.5 ± 0.3	1.5 ± 0.2
	16:0i	2.6 ± 0.2	3.1 ± 0.3	2.9 ± 0.2	2.7 ± 0.3	2.9 ± 0.3
	17:0i	0.7 ± 0.1	1.0 ± 0.2	0.8 ± 0.04	0.9 ± 0.1	0.7 ± 0.1
	17:0a	0.3 ± 0.1	0.4 ± 0.04	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.04
Actinomycetes	10Me16:0	0	0	0	0	0.001 ± 0.1
	10Me17:0	0.03 ± 0.02	0	0.05 ± 0.02	0	0.1 ± 0.03
	10Me18:0	0.7 ± 0.3	0.4 ± 0.1	0.5 ± 0.03	0.4 ± 0.1	0.6 ± .1
Fungi	18:1ω9	18.4 ± 2.0	13.4 ± 1.5	16.2 ± 1.4	16.2 ± 1.4	18.4 ± 2.0
	18:3ω3	4.0 ± 0.4	3.1 ± 0.7	3.8 ± 0.1	3.5 ± 0.9	3.8 ± 0.3

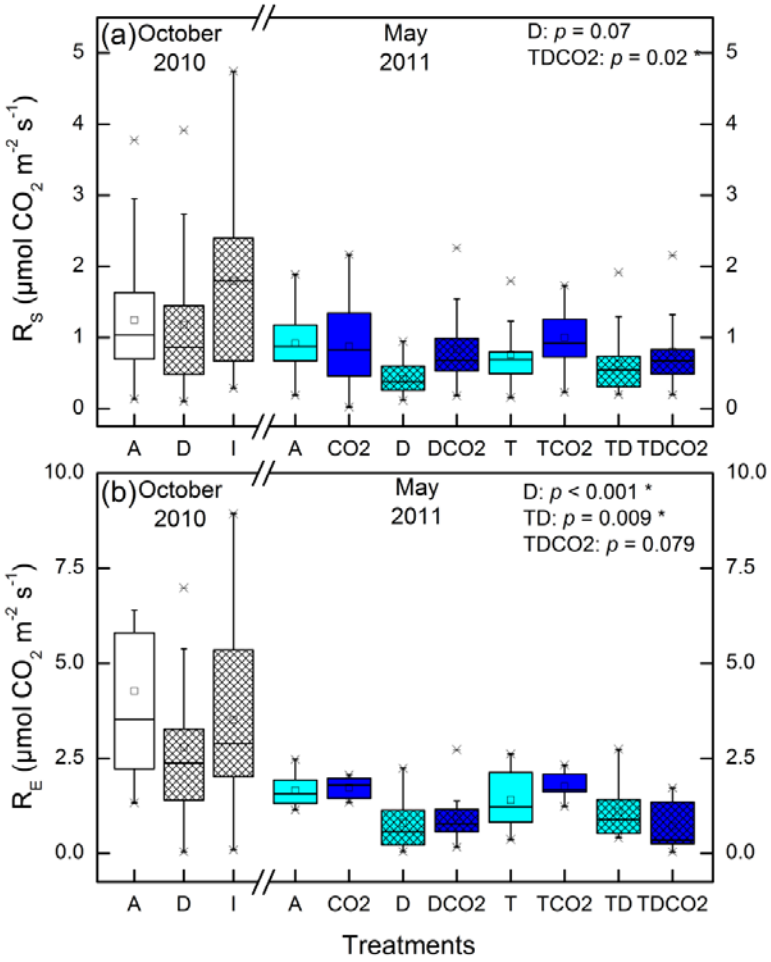

Figure A1: Night time soil (a) and ecosystem (b) respiration collected in October 2010 (Exp1, n = 7) and May 2011 (Exp2, n = 3). Shaded boxes are treatment with soil water manipulations, light blue are non-CO2 treatments and dark blue boxes are CO_2 treatments. Significance values (upper right corner) are for treatments in Exp2, no treatments effects on respiration were found in Exp1).

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8. PAPERS

8.1 Paper I

IN-SITU ^{13}C-CO_2 PULSE-LABELING IN A TEMPERATE HEAT-HLAND – DEVELOPMENT OF A MOBILE MULTI-PLOT FIELD SETUP

Sabine Reinsch and Per Ambus

Department of Chemical and Biochemical Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

Submitted to Rapid Communications in Mass Spectrometry

Abstract

RATIONALE: Isotopic manipulation experiments are useful tools to investigate biological and biochemical processes taking place in terrestrial nutrient cycling.

METHODS: A mobile field setup for *in-situ* ^{13}C-CO_2 pulse labeling was developed for grassland/low vegetation field experiments. Two pulse-labeling experiments were conducted in September 2010 (Exp1) and May 2011 (Exp2) in a Danish heath-land. A flow-through system was developed where labeling chambers were supplied with ^{13}C-CO_2 air from a gas reservoir. In Exp1, a total of 15 plots were pulse-labeled simultaneously over seven hours (390 ppm CO_2, 45 atom% ^{13}C-CO_2). Exp2 was conducted in two labeling campaigns operating a total of 41 plots within four hours (390 and 510 ppm CO_2, 50 atom% ^{13}C-CO_2). Reservoir and chamber air was sampled over the course of the experiments and CO_2 analyzed for concentration and isotopic composition. Soil CO_2 efflux and the atom% excess (APE) of soil respiration was measured after the pulse-chase to verify the performance of the setup.

RESULTS: Stable CO_2 concentrations and stable atom% ^{13}C-CO_2 values were maintained over the course of the experiments. CO_2 concentrations conformed to aimed values, whereas observed atom% ^{13}C-CO_2 values were lower than expected. We suggest corrects and sources of error for the deviation in observed atom% ^{13}C-CO_2 values, but more work has to be done. APE patterns in soil respiration agree with published observations of other grassland pulse-labeling studies, indicating satisfactory performance of the developed system.

CONCLUSIONS: We successfully developed a mobile flow-through system suitable for continuous *in-situ* ^{13}C-CO_2 pulse-labeling that is easily applicable in remote natural ecosystems.

Keywords

grassland, respiration, stable isotopes, carbon

Background

Carbon (C) is mainly stored in oceans, atmosphere and terrestrial ecosystems, but is mobile and exchangeable between these compartments. In terrestrial systems plants assimilate atmospheric CO_2, of which a part is stored temporary in biomass, and a part is transported rapidly into the plants rhizosphere, where it is consumed by soil microorganisms and animals. Eventually, the assimilated CO_2-C will be respired and diffuses back to the atmosphere and closes the cycle. Over decades, the biosphere-atmosphere C cycle has received more and more attention because of the rise in atmospheric CO_2 concentrations [(1)].

In parallel, methods have been developed to get deeper and more detailed insights into C cycling as well as processes involved. Rather than investigating C pools of ecosystems (roots, microbial biomass C, soil organic C etc.), studies on C turnover dynamics have drawn the attention of scientists. Carbon-14 tracing became a promising tool in e.g. ecosystem C decay studies and evaluations of human contributions to rising atmospheric CO_2 concentrations [(2)], but is applied randomly due to high resource demands. Chamber methods to investigate soil-atmosphere C exchange developed rapidly.[(3,4,5)] Methods investigating C dynamics in combination with the increasing capability for analysis of C-13 stable isotopes offers possibilities to study C turnover in great details.[(6,7,8,9)]

Forest ecosystems are of high interest when it comes to C cycling and especially soil C storage potentials. Trees are long-term accumulators of C and do reallocate comparatively large quantities of C into the soil. However, early succession stages such as grasslands, savannahs and heaths sum up to about 55 % (without Greenland and Antarctica[(10)]) of terrestrial cover and thus are of major concern too. Here, we focus on the application of C-13 isotope tracer to natural grassland ecosystems in order to evaluate their role in terrestrial C cycling.

Field experiments compared to laboratory studies are more challenging because natural environments cannot be controlled completely. Many studies on C cycling in soil-plant systems have used the application of a ^{13}C labeled CO_2 pulse to trace photosynthetically assimilated C.[(11,12,13)] The introduction of a ^{13}C-CO_2 pulse under *in-situ* conditions contains three basic elements and several challenges; i) Field plots have to be separated from the surroundings with the least disturbance, which is often achieved by inserting soil collars to avoid exchange with the surroundings especially in terms of C exudation by roots that can mix in the soil matrix. ii) Air tight and transparent chambers or bags should be deployed to apply isotopically

modified CO_2 to the vegetation of interest. An important issue is to maintain ambient environmental conditions inside the chambers. iii) Supply of isotopically modified CO_2 either to the ambient air in chamber, or by a flow-through setup with forced air flow. Isotopic manipulation has also been conducted in combination with free-air C enrichment (FACE) setups with long-term fumigated with elevated concentrations of ^{13}C depleted CO_2.[14,15]

Laboratory ^{13}C-CO_2 labeling experiments addressing C-cycling processes on a plant scale were reported in the early 1970th. For example ^{13}C-CO_2 labeling was used in connection with nuclear magnetic resonance spectroscopy (NMR) to reveal C processes in the plant metabolism.[16] However, it took about 30 years until *in-situ* ^{13}C-CO_2 pulse labeling became a relevant and applicable tool in the field. The main break through for *in-situ* grassland labeling studies was presented Ostle *et al.* published in 2000.[17] A stable isotope delivery (SID) system was introduced, which maintained stable conditions in terms of CO_2 concentration and air flow during the labeling period for up to 12 individual plots. This SID system was later used for grassland labeling studies variable in time (2.5 to 6 hours), ^{13}C-CO_2 enrichment (50 to 99 atom% ^{13}C-CO_2) and CO_2 concentration (350 to 400 ppm).[11,18,19,20] Another technically advanced setup was presented by Gamnitzer *et al.* in 2009 deploying flow meters, mass flow controllers and an outdoor continuous flow IRMS in the field and maintaining constant conditions in up to 20 labeling chambers for 15.5 hours.[21] Furthermore, tuneable diode lasers were shown to measure ^{12}C- and ^{13}C-CO_2 concentrations precisely under field conditions.[22,23]

In addition to these highly advanced *in-situ* ^{13}C-CO_2 labeling setups, grassland labeling was also performed with less technical equipment and with different approaches to maintain CO_2 concentrations within labeling chambers. For example instead of using compressed ^{13}C-CO_2, the tracer can also be released via acid reactions of $Na_2{}^{13}CO_3$ [9,24,25] or $Ba^{13}CO_3$ [26]. Furthermore, instead of using an air through-flow system, the CO_2 concentration within the labeling chambers can be manually maintained in a certain range by adding ^{13}C-CO_2 pulses through a septum.[26,27,28,29] With these more elaborative approaches the repeatability between replicates is limited and the number of plots operated simultaneously might become restricted.

In order to deploy a ^{13}C-CO_2 pulse to a large-scale, multi-factorial (eight treatments in n = 6 replicates) *in-situ* climate manipulation experiment in a temperate heath-land, we developed a low-cost and mobile ^{13}C-CO_2 flow-through system. Results of system performance and C-13 signal in soil respiration are presented from two labeling campaigns, *viz.* one campaign

including 15 plots labeled over 7 hours, and a second campaign including up to 22 plots operated at two different CO_2 concentrations exposed to ^{13}C-CO_2 over four hours. The basic and robust components enabled us to maintain an *in-situ* ^{13}C-CO_2 pulse simultaneously in 15-22 individual plots in a remote natural ecosystem.

Experimental

Experimental field site and setup

Two *in-situ* ^{13}C-CO_2 pulse-labeling experiments were conducted in a Danish heath-land [30] in September 2010 (Exp1) and May 2011 (Exp2). Dominating plant species were the grass *Deschampsia flexuosa* and the dwarf shrub *Calluna vulgaris*. Only plots with 80-100 % grass cover were chosen for pulse-labeling. Labeling was performed between 10:00-17:30 (Exp1) and 12:00-16:00 (Exp2). Exp1 was carried out at ambient CO_2 concentration (390 ppm) within 1.5 x 1.5 m experimental plots exposed to precipitation manipulations (described below). Exp2 was carried out at ambient and elevated CO_2 concentrations (510 ppm) and was deployed to experimental plots situated in close proximity to a series of octagon shaped (6.8 m diameter) main experimental plots exposed to multiple environmental treatments (increased temperature, extended spring/summer droughts and elevated atmospheric CO_2). Elevated CO_2 was maintained by the free-air CO_2 enrichment (FACE) approach taking advantage of wind-turbulence mixing of ambient air and concentrated CO_2 introduced along the up-wind sides of the octagons, and controlled by a central located CO_2 analyzer intake.[30] For logistical reasons, the experimental plots for the described labeling experiment were situated ca. 1.2 m outside the main octagons, and thus exposed to more randomly variable yet elevated CO_2 concentrations.

Therefore, CO_2 concentrations at different distances from the CO_2 release pipes were calculated on the basis of the $\delta^{13}C$ shift in green *D. flexuosa* leaves ($\delta^{13}C_{plant}$). This was possible because ambient air had a distinct higher C isotopic value ($\delta^{13}C_{amb\_air}$ = -8 ‰) than the concentrated CO_2 used for the FACE treatment ($\delta^{13}C_{FACE\_CO2}$ = -29 ‰, unpublished value). Plants assimilate atmospheric CO_2 and C discrimination ($\Delta^{13}C$) occurs during photosynthesis [31]. The C discrimination effect was calculated as:

$$\Delta^{13}C = \delta^{13}C_{amb\_plant} - \delta^{13}C_{amb\_air}$$

where $\delta^{13}C_{amb\_plant}$ was the C isotopic composition of plants growing under ambient CO_2 concentrations. Under the assumption that $\Delta^{13}C$ is constant at different CO_2 concentrations (= -20.5 ‰), the $\delta^{13}C$-value of FACE-air ($\delta^{13}C_{FACE\_air}$) was calculated as:

$$\delta^{13}C_{FACE\_air} = \delta^{13}C_{FACE\_plant} - \Delta^{13}C$$

where $\delta^{13}C_{FACE\_plant}$ was the $\delta^{13}C$ value of plants exposed to elevated CO_2 concentrations. A mass balance equation was then used to calculate the amount of CO_2 that was added due to the FACE treatment (x):

$$\delta^{13}C_{FACE\_air} * ([CO_2]_{amb} + x) = \delta^{13}C_{amb\_air} * [CO_2]_{amb} + (\delta^{13}C_{FACE\_CO2} * x)$$

and solved for x, with an ambient CO_2 concentration $[CO_2]_{amb}$ of 390 ppm. The actual, average CO_2 concentrations plants were exposed to was calculated as $[CO_2]_{amb}$ + x (Figure 2).

Green *D. flexuosa* leaves (3-5) were sampled inside the experimental octagons 0.6 m from the CO_2 release pipe, directly below the CO_2 release pipe as well as 0.6 and 1.2 m from the CO_2 in outer direction. Grass leaves were sampled at 5, 10 and 15 m distances from FACE treatments to assess natural ^{13}C abundances. Leaves were dried at 60 °C for 48 h, material was homogenized and analyzed for their C isotopic composition (see below).

Labeling setup

Metal soil collars (galvanized steel) were installed in July 2010 for Exp1, and early May 2011 for Exp2. The collars were installed at least two weeks prior to the labeling events, to allow vegetation recovery from root cutting. The labeling system was composed of a gas reservoir, gas pumps and labeling chambers (Figure 1). All units were connected via ~8 m Polyurethane tubes (TU0604, 4 mm, SMC Pneumatic A/S) and connectors (KQ2E06-00, SMC Pneumatic A/S).

Plexiglas labeling chambers were transparent and mounted gas tight on top of the collars by means of water filled funnels. Details about the components are given in Table 1. A flow-through system was realized by mounting the air inlet and outlet on opposing sides of the chambers. Incoming air from a gas reservoir was provided by an electric diaphragm pump (Vacuum pump, Model Thomas 107CCD20-164). A fan (670-OD401005HB, Orion) inside the chambers assured continuous mixing of chamber air. The transparent chambers were used for ecosystem respiration (R_E) measurements after labeling. Opaque soil respiration chambers were pressed ca. 2 cm into the ground in Exp1 and circular (10 cm diameter) collars for mounting opaque soil respiration (R_S) chambers were installed to ca. 2 cm depth inside the experimental plots shortly after the metal collars were inserted and vegetation was removed inside. Here we will only focus on R_S measurement for validation of pulse-labeling efficiency.

Gas tight vinyl balloons (Ø 3.048 m, different colours, Balloons Etc/Balloons Direct, Springfield, Virginia, USA) were used as gas reservoirs (Figure 1a). The reservoirs were inflated by pressurized CO_2 free air ($CO_2 \leq$ 1 ppm, Strandmøllen O_2, Klampenborg, Denmark), and a sample of pure, highly ^{13}C enriched CO_2 was added manually. The total amount of air was monitored with a gas meter (AL-800 Diaphragm Meter) in Exp1, and with a digital gas flow meter (MCF 250, Yamatake) in Exp2. A ^{13}C-CO_2 mixture of 45 atom% ^{13}C-CO_2 was achieved by mixing 5 L 99 atom% ^{13}C-CO_2 (ISOTEC^{TM}) with 6 L concentrated non-labeled CO_2 (Air Liquide, HEDE NIELSEN A/S, -4 ‰) in Exp1, and portions of 2.1 litres was added to each reservoir. Bottled 50 atom% ^{13}C-CO_2 (Cambridge Isotope Laboratories, Inc., France) was used in Exp2.

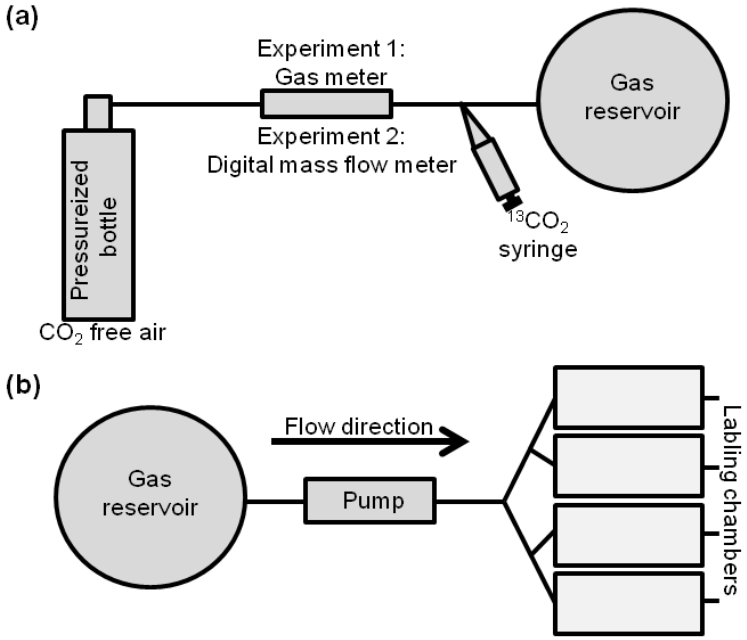

Figure 1: Experimental setup: gas transfer from pressurized bottle into the gas reservoir due to gas pressure. (a) and schematic overview of field equipment, (b) air was constantly pumped from the gas reservoir into labeling chambers.

Experimental units were adjusted to provide airflows of about 4.5 and 2.1 L chamber^{-1} min^{-1}, which correspond to theoretical gas residence times of 10 and 30 min per chamber^{-1} in Exp1 and Exp2, respectively. A residence time of 30 min under ambient CO_2 concentrations corresponds to a CO_2 supply equivalent to the maximal CO_2 consumption of the grass vegetation previously measured at the field.[32]

Table 1: Overview of experiment equipment and values. R_E = ecosystem respiration, R_S = soil respiration.

		Experiment 1	Experiment 2
General information			
Number of treatments		3	8
Replicates		5	4-6 [α]
Total number of plots		15	41
Number of labeling events		1	2 within one week
Weather conditions		Sunny	Rainy and sunny
Average PAR (mol m^{-2} d^{-1}) (± SE)		450 ± 57	217 ± 42 and 490 ± 80
Field setup			
Number of plots connected to one gas reservoir		3	2-4
Labeling duration (h)		7	4
Soil frame dimensions (m)[β]		0.5 × 0.2 × 0.2	0.8 × 0.4 × 0.1 + 0.2 corner spikes
Soil frame depth (m)		0.18	0.08
Chamber dimensions (m)	R_E[γ]	0.5 × 0.2 × 0.4	0.8 × 0.4 × 0.3
	R_S[Ø]	0.035 × 0.08	0.2 × 0.05
Labeling equipment			
Gas flow measurement device		gas meter	digital mass flow meter
^{13}C-CO_2 (atom%)		45 [δ]	50
CO_2 concentration (ppm)		390	390 and 510
Gas sample volume (ml)		5	20

[α] Plots ≤ 80 % grass coverage were excluded from the experiment
[β] length x width x height; height = belowground and aboveground, collars approx. 0.05 m aboveground
[γ] labeling chamber was used for R_E measurements
[Ø] diameter x height
[δ] 45 atom% ^{13}C-CO_2 was mixed from 99 atom% ^{13}C-CO_2 and non-labeled CO_2

Sample collection and analyses

Analysis of CO_2 concentration and C isotopic compositions in chambers and gas reservoirs were performed regularly during each experiment. Gas samples were taken with 5 and 20 ml syringes (Omnifix®) in Exp1 and Exp2 and transferred to 5.9 ml company evacuated vials (Exetainers®; Product code 819W, Labco Ltd, High Wycombe, UK). In Exp1 the 5 ml sample was injected into the Exetainer, whereas in Exp2 the 5.9 ml Exetainer was flushed with the 20 ml sample by a double-needle. Samples from gas reservoirs and labeling chambers were taken hourly. Chamber samples were taken from the chamber outlets in ambient (Exp1) and drought (Exp2) treatments, respectively.

R_S measurements were carried out one week before the ^{13}C-CO_2 pulse-labeling and 1, 2, 6, 12, 29 and 30 after the pulse-chase in Exp1 and 1, 2 and 8 in Exp2. Rates were achieved by analysis of increase in headspace CO_2 concentration during chamber enclosures of ca. 5 min (Exp1) and ca. 7 min (Exp2) obtained by sequential headspace sampling. The first sample was taken before closing the chamber, followed by 2 (Exp1) and 3 (Exp2)

samplings of accumulated headspace gas. All samples were stored at room temperature prior to analysis and processed as described. The CO_2 flux was calculated using the HMR-package in R.[33] Keeling plots were performed to calculate the isotopic characteristics of emitted CO_2, which is expressed as the atom% ^{13}C excess (APE) calculated as the difference between labeled and background samples.

Temperature measurements were performed inside and outside the chambers over the course of the experiments. All chambers were opened in the middle of each experiment to remove condensed water. In Exp2, two blue cooling packs were inserted into the chambers to minimize temperature increase. Photosynthetic active radiation (PAR) was measured at the experimental side at each labeling day (Cosine corrected quantum sensor, OL-4000q, Optisk Laboratorium, Hørsholm, Denmark).

Net CO_2 uptake was estimated as difference between average incoming and outgoing CO_2 concentration with the assumption that chamber air inflow and outflow were similar. The contribution of soil-derived C to the chamber CO_2 concentration (thus soil C 'loss') was calculated with a two-end-member mixing model where the source atom% ^{13}C was the measured atom% value of the reservoir, the mix atom% ^{13}C was the ^{13}C value determined in labeling chambers and the background ^{13}C value was assumed to be 1.1 atom% ^{13}C common to soils.

Gas samples from Exp1 were measured within one month, and gas samples from Exp2 within one week after sampling. Gas samples were analyzed for CO_2 concentration and ^{13}C-CO_2 on a GasBench-II coupled in continuous flow to a DeltaV Advantage isotope ratio mass spectrometer (IRMS; Thermo Fisher SCIENTIFIC). We used the default setting of the GasBench and deployed five analytical replicates on each sample. The second eluting sample peak was used to calculate CO_2 concentrations. An average of sample peaks 2-5 were used to calculate the ^{13}C isotopic value.

CO_2 concentrations were calculated from the measured peak areas of all masses [34]. Different CO_2 concentrations were prepared from concentrated CO_2 ($\delta^{13}C$ = -4 ‰) mixed with N_2 (Air Liquide, HEDE NIELSEN A/S) in gas tight Tedlar bags (1 litre, SKC). Certified isotopic mixtures of CO_2 in synthetic air were included, i.e. -2.7 ‰ at 362 ppm and -29.3 ‰ at 356 ppm (Messer Griesheim, Krefeld, Germany). Calibration lines for CO_2 concentrations were prepared in a range of 0-2000 ppm ($n \geq 3$) for 5 and 20 ml sample volumes (Figure 3). Each sample run was preceded by a triple analysis of pure CO_2 IRMS working reference gas, and peak areas were used to correct for changes in sensitivity of the system between runs, and to adjust the measurements to the calibration curves.

All gas samples were measured with the conventional GasBench-II method for natural abundance CO_2 samples, except samples taken from gas reservoirs and labeling chambers in Exp2. In the latter case, resistor values of mass 44 stayed unchanged ($3e^{+8}$ Ω), mass 45 resistance was increased from $3e^{+10}$ Ω to $3e^{+10} + 1e^{+9}$ Ω and mass 46 resistance increased from $1e^{+10}$ Ω to $1e^{+10} + 1e^{+11}$ Ω. This change in mass resistance for samples highly enriched in ^{13}C was applied to obtain higher measurement precision.

Exetainer vials were tested for storage of high atom% ^{13}C-CO_2. Company evacuated vials were used without further preparation; a set of four samples was prepared for each time point. Pure 50 atom% ^{13}C-CO_2 (as used in Exp2) was diluted with N_2 to 500 ppm CO_2 in Tedlar bags. A volume of 20 ml was flushed through each vial. The first measurement took place immediately after preparation; following measurements were carried out after 1, 2, 4 and 5 weeks storage in the lab. All samples were measured with the natural abundance method.

Analysis of the atom% ^{13}C in highly enriched CO_2 samples depended in a linear relationship on the total sample CO_2 concentration (Figure S1). Therefore, an atom% ^{13}C-CO_2 calibration curve was prepared from 50 atom% ^{13}C-CO_2 (Cambridge Isotope Laboratories, Inc., France) diluted with N_2 in a range of 500-2000 ppm. The measured atom% ^{13}C-CO_2 divergence vs. concentration was established (Figure 4) and this relationship ($Y_{divergence}$) was used to correct the observed ^{13}C atom% values:

$$Atom\,\%_{corrected} = Atom\,\%_{observed} + Y_{divergence}.$$

Leaf C isotopic ratios $^{13}C/^{12}C$ were measured from homogenized plant material (~2.5 mg) by Dumas combustion (1050 ºC) on an elemental analyzer (CE 1110, Thermo Electron, Milan, Italy) coupled in continuous flow mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). Acetanilide (Merck, Darmstadt, Germany) was used for elemental analyzer mass calibration. As working standard for isotope ratio analysis we used pure gases of CO_2 calibrated against certified reference materials of ^{13}C-sucrose (IAEA, Vienna, Austria). Performance of analysis (Qa/Qc) was assessed by the inclusion of reference samples of biological origin (Peach leaves (NIST 1547), National Institute of Standards and Technology, Gaithersburg, MD, USA). $^{13}C/^{12}C$ isotope ratios were expressed as the delta notation ($\delta^{13}C$ ‰) is the relative measurement against the $^{13}C/^{12}C$ ratio in an international standard (Vienna Pee Dee Belemnite):

$$\delta^{13}C = \frac{R_{sample} - R_{standard}}{R_{standard}} \cdot 1000\ ‰.$$

Field treatments

Exp1 was a two-factorial setup containing untreated control plots (ambient), frequent drought periods (drought) and increased precipitation (irrigation). Drought periods were realized with Plexiglas roofs for two weeks of rain exclusion. Precipitation was simulated by adding deionised water. Treatments were initiated in March 2007 and continued in 2008, paused in 2009, and resumed in 2010. Each treatment was replicated five times.

Drought periods took place two times in April and July 2010. Artificial rain was applied every two weeks (n = 9) by adding ~28 litres of deionised water per plot equivalent to 12.44 mm precipitation between April and July 2010. When the labeling was performed on September 22nd the plant biomass was not assessed, but *D. flexuosa* biomass has been shown to significantly be affected by prolonged drought summer drought resulting in a reduction of about 30 %. Biomass of *D. flexuosa* was about 100 g m^{-2} under ambient conditions in August 2008.[35]

Exp2 was a full-factorial combination of elevated CO_2, prolonged spring/summer droughts (four weeks) and passive night time warming (1 °C); each treatment was replicated six times resulting into 48 plots. For further details see Mikkelsen *et al.* (2008).[30] Vegetation in half the plots was exposed to elevated CO_2. Experimental climate treatments were established in 2004 and initiated in 2005. When the two labeling campaigns were performed in May (16th and 19th) the prolonged drought period was applied since two weeks. Grass biomass in non-CO_2 plots was 195 ± 16 g m^{-2} and in CO_2 plots 208 ± 25 g m^{-2} (means ± SE) (pers. communication with Inger Kappel Schmidt).

Statistics

Statistical analysis was carried out in R version 12.2.1. Data were tested for normality (Shapiro-Wilk Test of Normality) and homogeneity of variances (Levene Test) prior to analysis. A two-way ANOVA was performed on log-transformed data to test for differences in CO_2 concentrations at different distances from the CO_2 dosing pipe, and to test the maintenance of stable atom% ^{13}C contents in Exetainer vials. A one-way ANOVA for repeated measures was performed to test for the impact of time on the observed values. A one-sample t-test was used to test if the target values for CO_2

concentrations and ^{13}C-CO_2 label was achieved. If data were not normally distributed a "Wilcoxon Signed Rank Test" was performed. To test for differences between ambient and elevated CO_2 treatments (Exp2), a two-sample t-test, or in cases of missing normality, a "Wilcoxon Signed Rank Test" was used. Soil respiration data were tested for treatment differences over time using a linear mixed effect model ("lme" in the package "nlme") including a random statement, a weights structure (varIdent) and a correlation structure (corCAR1).

Results

A priori considerations

In Exp2, pulse-labeling plots were located outside the experimental octagons on the edge of treatment applications. Plant exposure to elevated CO_2 differed with distance from the CO_2 dosing pipes (Figure 2). Plants growing in a range of 0.6 m around the artificial CO_2 source were exposed to higher CO_2 concentrations, resulting in lower leaf $\delta^{13}C$ values compared to plants growing 1.2 m outside the octagons ($p = 0.002$). The $\delta^{13}C$ values of plants growing under ambient conditions showed significantly higher $^{13}C/^{12}C$ ratios compared to plants growing 1.2 m away from the FACE treatment ($p < 0.001$). Based on the isotopic analysis, we estimated that plants in the labeling-plots adjacent to the FACE octagons were exposed to an average of 500 ppm CO_2.

CO_2 calibration lines for different sample volumes stored in 5.9 ml Exetainer vials showed different intercepts and slopes (Figure 3). When the Exetainers were used with 5 ml sample volumes (Exp1), the measured CO_2 concentrations were generally more variable ($R^2 = 0.98$), and analysis of CO_2-free air (5 ml N_2) resulted in a significant peaks (4.3 ± 0.2 Peak Area; Figure 3). In contrast, flushing the vials with 20 ml N_2 completely showed no blank value and a reproducible CO_2 signal ($R^2 = 0.999$).

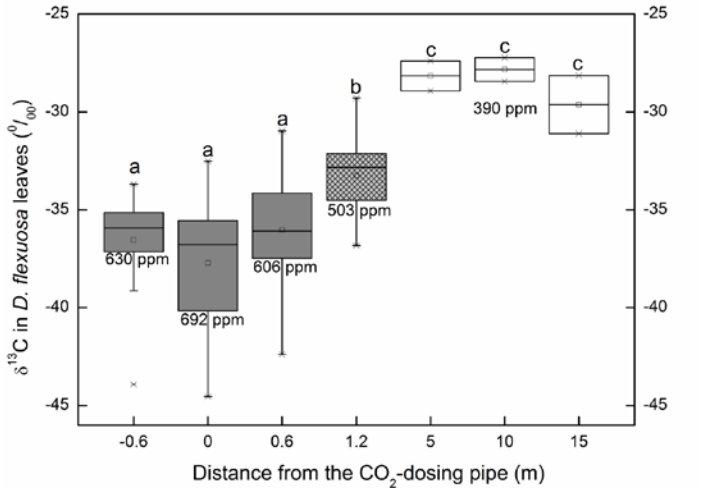

Figure 2: C isotopic values of green *D. flexuosa* leaves (‰) for different distances from the octagon CO_2 dosing pipes in Exp2. Sampling positions were located inside (negative values) and outside (positive values) the CO_2 octagons (n = 6). Plants exposed to elevated CO_2 treatment (grey boxes) and grown under atmospheric CO_2 conditions (white boxes), hatched box indicates pulse-labeling position. Calculated average CO_2 concentrations (see experimental part) are displayed below boxes. Differences between distances are indicated with letters.

Measured amounts of ^{13}C (atom%) were linearly dependent on the CO_2 concentration (Figure S1). Gas samples highly enriched in ^{13}C measured with the natural abundance method resulted into decreasing atom% ^{13}C measures with increasing CO_2 concentrations ($y = -0.012x + 47.9$, $R^2 = 0.966$). The same samples measured with the method suitable for highly enriched samples resulted in slightly increased atom% ^{13}C measures with increasing CO_2 concentrations ($y = 0.003x + 42.8$, $R^2 = 0.90$). Exetainer vials maintained a steady atom% ^{13}C concentration for at least two weeks ($p < 0.001$) of storage, after which a decline in ^{13}C was observed (Table S1).

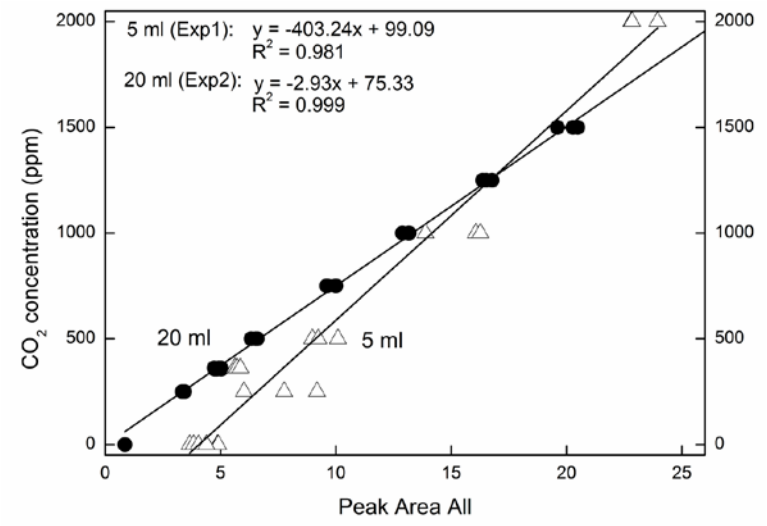

Figure 3:
Relationship between measured 'Peak Area All' and known CO_2 concentration for 5 ml (triangles) and 20 ml (circles) gas volume in 5.9 ml Exetainer vials.

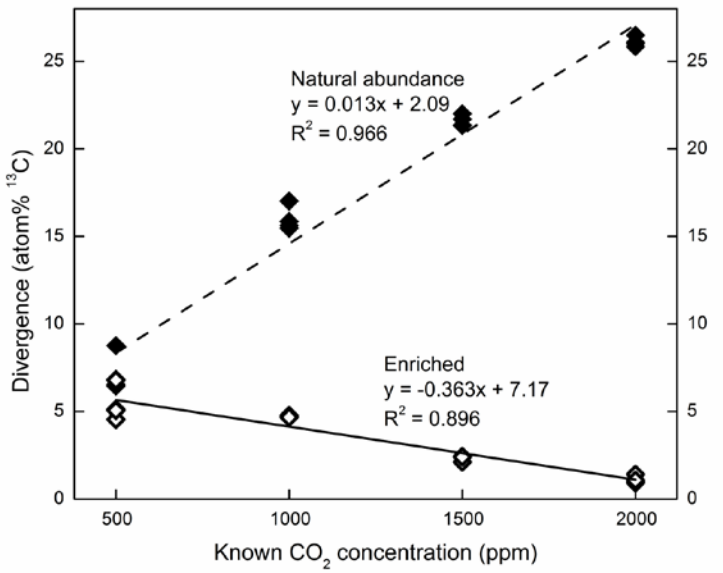

Figure 4:
Effect of CO_2 concentration on the divergence of measured atom% ^{13}C-CO_2 from known atom% ^{13}C-CO_2 values for the enriched method (open squares) and natural abundance method (solid squares). N = 3 for each concentration. Values are means ± SE.

Experiment 1

In Exp1 (Figure 5), observed CO_2 concentrations in gas reservoirs were stable over time ($F_{(1,4)}$ = 1.2; p = 0.335) and were similar to ambient (390 ppm) CO_2 conditions (p = 0.252). CO_2 concentrations in the chambers were constant over time ($F_{(1,4)}$ = 1.33; p = 0.313) but lower than 390 ppm (p < 0.001) and lower than reservoir CO_2 concentrations (p = 0.03). Measured atom% ^{13}C values in reservoirs and chambers were stable over time ($F_{(1,4)}$ = 2.82; p = 0.169; $F_{(1,4)}$ = 3.32; p = 0.142) but were always lower than 45 atom% (p < 0.001). Observed atom% ^{13}C values were higher in reservoirs than in chambers (p < 0.001). Overall, net CO_2 uptake was calculated as 5.8 ± 2.7 mg CO_2 m^{-2} h^{-1} and the net loss of soil C was observed in the same range (Table 3).

Chamber temperatures were increased compared to outside air temperatures (p = 0.009). Lowest chamber temperatures were measured at the beginning of the experiment (19.0 ± 0.5 °C) and peaked after 4.5 hours (30.0 ± 1.5 °C). Surrounding temperatures were on average 4 - 5 °C lower than chamber temperatures.

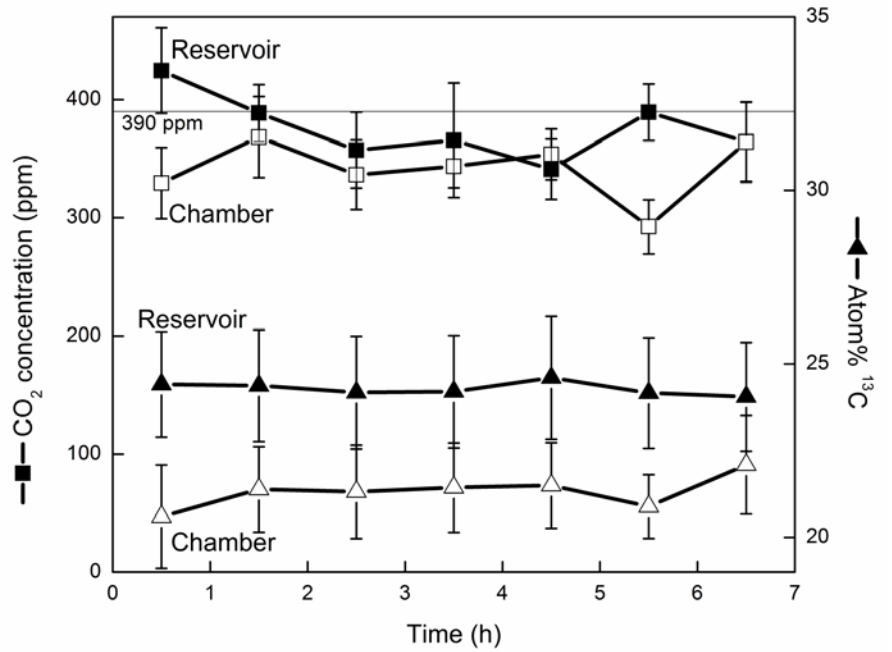

Figure 5: Exp1: CO_2 concentration (squares) and atom% ^{13}C-CO_2 values (triangles) in the gas reservoir (solid) and labeling chambers (open) over time. Means ± SE.

Experiment 2

In Exp2 (Figure 6), CO_2 concentrations in reservoirs were constant over time (Figure 6a) at both CO_2 levels ($F_{390\ ppm\ (1,5)} = -0.49$, $p = 1$; $F_{510\ ppm\ (1,5)} = 3.07$, $p = 0.140$). Ambient and elevated CO_2 concentrations were achieved in the gas reservoirs ($p_{390\ ppm} = 0.173$; $p_{510\ ppm} = 0.101$) and were significantly different from each other ($p < 0.001$). In labeling chambers (Table 2), CO_2 concentrations in CO_2 and non-CO_2 plots were constant over time ($F_{390\ ppm\ (1,5)} = -7.01$, $p = 1$, $F_{510\ ppm\ (1,5)} = 6.64$, $p = 0.05$). In non-CO_2 plots, CO_2 concentrations were similar to ambient conditions ($p = 0.066$) but, in CO_2 plots, CO_2 concentrations were lower than 510 ppm ($p < 0.001$). Chamber CO_2 concentrations were similar under ambient and elevated CO_2 conditions ($p = 0.848$).

Reservoir atom% ^{13}C-CO_2 values were constant over time (Figure 6b) at both CO_2 levels ($F_{390\ ppm\ (1,5)} = -12.45$, $p = 1$; $F_{510\ ppm\ (1,5)} = 3.41$, $p = 0.124$). Measured atom% ^{13}C-CO_2 values were lower than 50 atom% ($p < 0.001$). Reservoir and chamber atom% ^{13}C-CO_2 values were different in non-CO_2 and CO_2 treatments ($p = 0.001$) with higher atom% ^{13}C values in the reservoir in ambient plots and lower atom% ^{13}C measures in labeling chambers. Chamber atom% ^{13}C-CO_2 values (Table 2) in non-CO_2 plots were stable over time ($F_{390\ ppm\ (1,5)} = -1.94$, $p = 1$) but varied in CO_2 plots ($F_{510\ ppm\ (1,5)} = 10.48$; $p = 0.02$). Measured atom% ^{13}C-CO_2 values were lower than 50 atom% ($p < 0.001$) but were similar for both CO_2 levels ($p = 0.620$).

Table 2: Measured CO_2 concentrations and ^{13}C atom% values in the labeling chambers in ambient and elevated CO_2 plots (Exp2) over time. Values as mean ± SE. F-values from one-way repeated measure ANOVA with obtained *p*-values.

Time (h)	Ambient		Elevated CO_2	
	CO_2 concentration (ppm)	^{13}C atom%	CO_2 concentration (ppm)	^{13}C atom%
0.5	494 ± 27	21 ± 2	525 ± 15	23 ± 3
1.5	427 ± 67	29 ± 3	427 ± 39	31 ± 2
2.5	497 ± 69	25 ± 3	428 ± 31	31 ± 2
3.5	422 ± 94	28 ± 5	492 ± 53	28 ± 2
4.5	349 ± 49	33 ± 2	358 ± 29	33 ± 1
F(1,5)	-7.01	-1.94	6.64	10.48
p-value	1	1	0.05	0.02

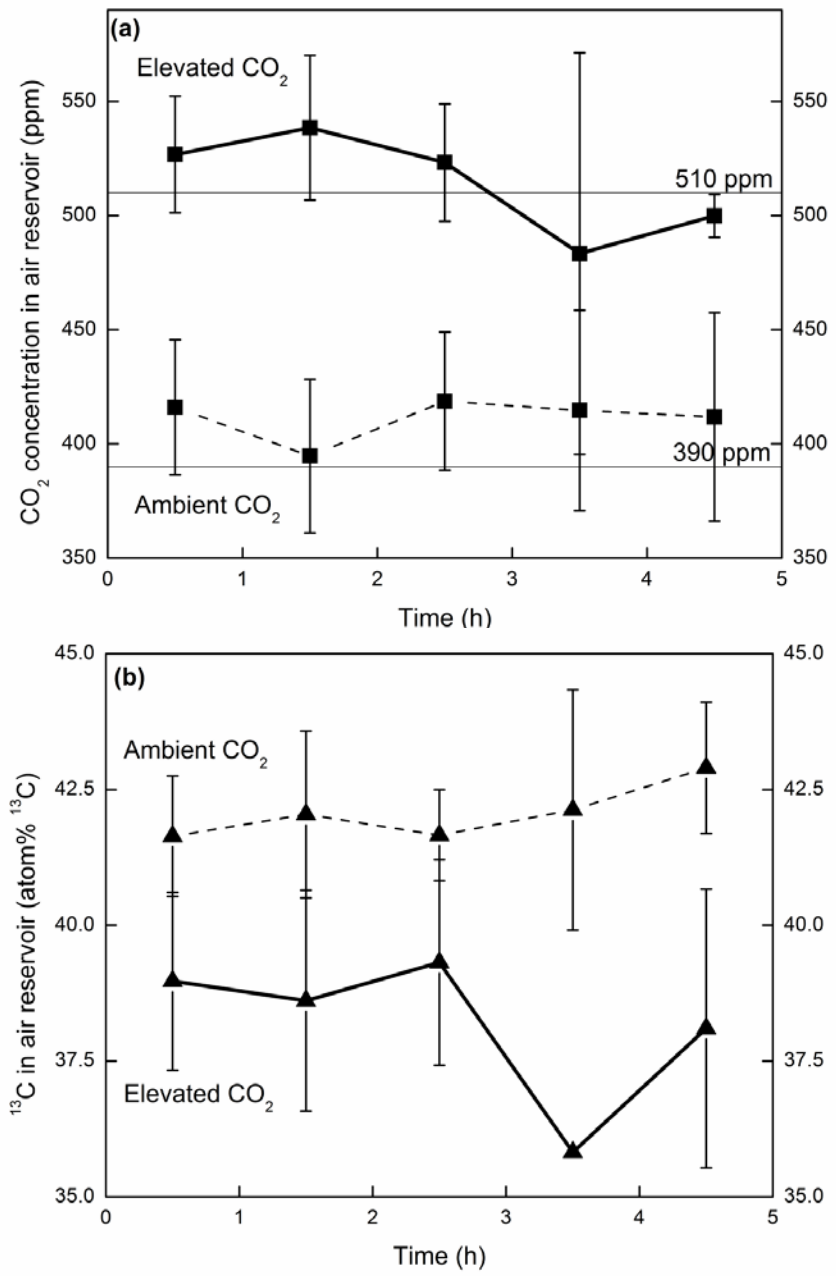

Figure 6: Exp2: CO_2 concentrations (a) and atom% ^{13}C values (b) in the air reservoirs under ambient (dashed) and elevated CO_2 (solid) concentrations over time. Straight lines indicate aimed CO_2 concentrations. Means ± SE.

In non-CO_2 plots, CO_2 concentrations in reservoirs and chambers were similar ($p = 0.557$), but atom% ^{13}C measurements were higher in reservoirs than in chambers ($p < 0.001$). In CO_2 plots, CO_2 concentrations and atom% ^{13}C values were higher in reservoirs than in chambers ($p_{CO2} < 0.001$, $p_{at\%} < 0.001$). Differences in CO_2 concentration between reservoirs and chambers resulted in an estimated negative net CO_2 uptake under ambient

CO_2 conditions in Exp1 (Table 3) and a five times higher net CO_2 uptake in elevated CO_2 plots in Exp2 (DCO2).
Chamber temperatures were higher than atmospheric temperatures ($p < 0.001$). Surrounding temperatures stayed constant during the day (13.6 °C ± 0.3 °C) whereas chamber temperature increased to 20.3 °C ± 1.9 °C after closing the labeling chambers, but stayed relatively constant thereafter.

Table 3: Net CO_2 uptake (Uptake, mg CO_2 m^{-2} h^{-1}) and soil CO_2 loss (Loss, mg CO_2 m^{-2} h^{-1}) in Exp1 and Exp2. Difference between incoming (reservoir) and outgoing (chamber) CO_2 concentration = ecosystem net CO_2 uptake. Soil CO_2 loss is the amount of CO_2 originating from the soil determined with a two end-member mixing model using incoming and outgoing atom% ^{13}C values. Average values for CO_2 concentrations over the whole experimental durations were used. Values are means ± SE.

Experiment	Treatment	Uptake	Loss
Exp1	Ambient	5.8 ± 2.7	7.3
	p –value	<0.001	0.03
Exp2	Drought	-1.6 ± 1.6	7.3
	p –value	0.557	<0.001
	Drought*CO_2	5.6 ± 1.5	6.1
	p -value	0.001	<0.001

Soil respiration

In Exp1, soil CO_2 efflux was fluctuating over time (Figure 7a-insert) with highest variation under ambient water conditions. Repeated measure analysis showed that soil respiration was similar under ambient and drought conditions ($p = 0.99$), but irrigation increased soil CO_2 efflux compared to soils exposed to ambient ($p = 0.072$) and drought treatments ($p = 0.062$).

The highest contribution of recently photosynthetically assimilated ^{13}C-CO_2 (^{13}C from the ^{13}C-CO_2 pulse-chase) was observed one day after pulse-labeling and decreased thereafter (Figure 7a). The amount of ^{13}C-CO_2 respired was treatment independent over time ($p = 0.680$). At day one, the APE in drought plots was ~25 % lower, and in irrigated plots ~12 % higher compared to ambient plots. Two days after the pulse-chase, this relative difference separated even further into ~31 % less ^{13}C-CO_2 in drought plots and ~51 % more ^{13}C-CO_2 in irrigated plots compared to ambient conditions. At day two, APE in soil CO_2 efflux was lower in drought than in irrigated plots ($p = 0.030$). After day two, APE decreased with time and was similar among water treatments.

In Exp2, soil CO_2 efflux was higher under elevated CO_2 (p = 0.1) than under ambient CO_2 concentrations (Figure 7b-insert). APE in soil respiration under ambient CO_2 concentration was highest one day after pulse-labeling and decreased thereafter. Under elevated CO_2 conditions, APE in soil CO_2 increased until day 2 and decreased thereafter. Chamber CO_2 concentration did not affect APE in R_S during the investigated time period in May (p = 0.375).

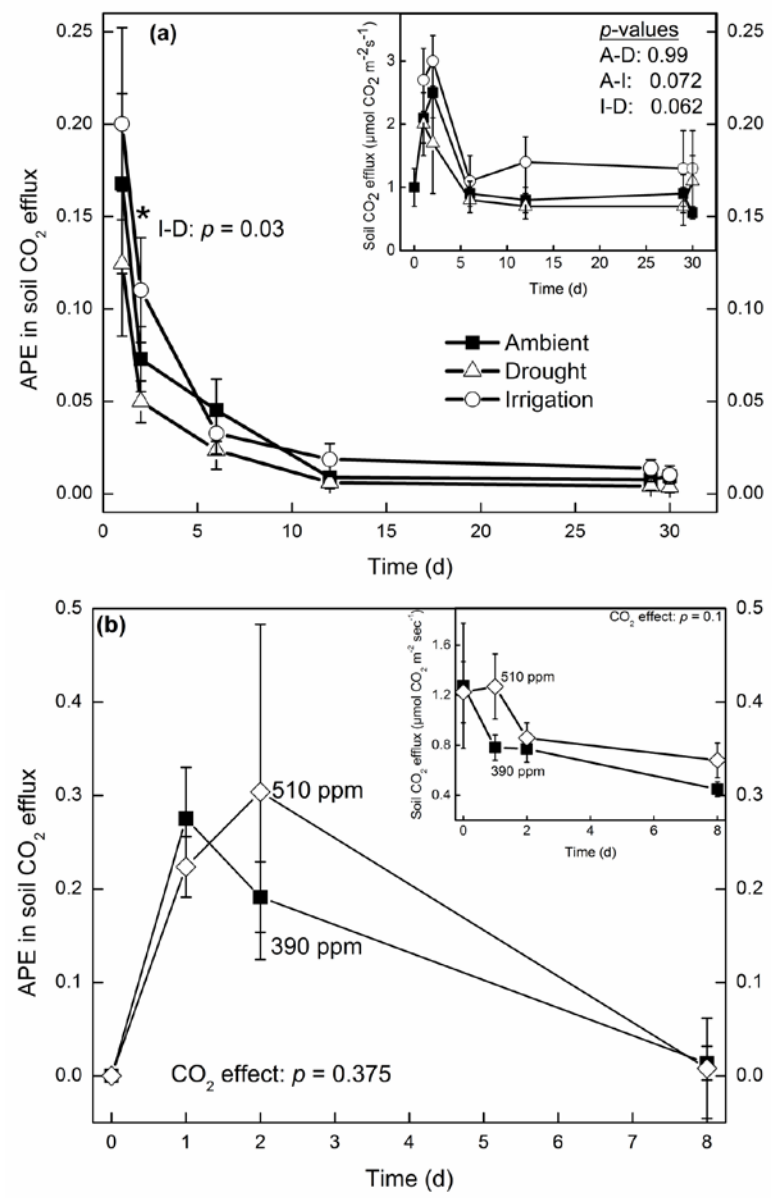

Figure 7: APE (atom% ^{13}C-CO_2 excess) in soil CO_2 efflux and soil CO_2 efflux rates (Figure insert) over time in (a) Exp1: under ambient (solid square), drought (open triangle) and irrigated (open circle) water regimes and, (b) Exp2: in ambient (solid square) and elevated CO_2 (open diamonds) treatments. Means ± SE.

Discussion

Equipment evaluation

We successfully developed a mobile multi-plot *in-situ* ^{13}C-CO_2 pulse-labeling setup that was applied to low vegetation in a remote environment at relatively low costs. A minimum of 15 plots were simultaneously pulse-labeled with ^{13}C-CO_2 in a temperate heath-land for at least 4 hours. Conducting one pulse-labeling experiment with multiple plots instead of several successive smaller labeling campaigns ensured that environmental conditions (PAR, temperature, soil moisture, precipitation) were the same across replicates. The use of big (Ø ~3 m), robust (vinyl) and gas tight advertisement balloons as gas reservoirs provided sufficient air for at least four labeling chambers under the applied conditions (Figure 1, Table 1). The robust balloon material allowed transportation of filled air reservoirs to the place of interest.

Pumps used to supply labeling chambers with air from the air reservoir supplied a minimum flow of 2.1 litre air $minute^{-1}$ $chamber^{-1}$ which was enough to account for the maximum rate of CO_2 uptake by the confined vegetation.[32] In general, higher flow rates are likely to stabilize chamber conditions in terms of temperature and moisture conditions. Due to distant plots it was necessary to use long tubes to connect all treatments per replicate to one gas reservoir, which limited the maximum air flow.

Equipment for filling gas reservoirs with air and CO_2 was modified between Exp1 and Exp2. The used gas meter was not sensitive enough to measure gas flows under high pressure as it was supplied from pressurized air bottles, and was replaced by a digital gas flow meter. Inflation of one reservoir (7 m^3 air) required about 15 min, and subsequent addition of 2.81 and 3.66 L ^{13}C-CO_2 (390 and 510 ppm) resulted in aimed CO_2 concentrations (Figure 6a).

Aimed vs. achieved reservoir and chamber values

CO_2 concentrations were stable in gas reservoirs and labeling chambers over time. Ambient and elevated CO_2 concentrations in the air reservoirs were as targeted (390 and 510 ppm), whereas target CO_2 concentrations in the labeling chambers were lower in Exp1 and in Exp2 in elevated CO_2 plots. This change in chamber CO_2 concentration compared to supply air was due to photosynthetic and respiratory processes in the soil and vegetation confined by the chamber. Net CO_2 uptake was of about the same magnitude as soil CO_2 loss in Exp1 and in Exp2 under elevated CO_2

conditions (Table 3). Under ambient CO_2 concentrations (combined with prolonged drought periods), the net CO_2 uptake was minimal whereas the soil CO_2 loss was similar to Exp1 and the CO_2 treatment in Exp2. A similar effect of drought was found when Europe was hit by a heat wave in 2003 and soils released unusual high amounts of soil C.[36] Uncertainty of CO_2 concentrations can be caused by air sample volumes (5 and 20 ml) and calculations of CO_2 concentrations [34] of samples highly enriched in atom% ^{13}C-CO_2 from IRMS chromatograms. Joos *et al.* (2008) demonstrated the potential to use 'Peak Areas' generated by the GasBench-IRMS to calculate CO_2 concentrations in natural abundance CO_2 samples.[34] Peak areas of samples highly enriched in ^{13}C-CO_2 were compared to natural abundance 'Peak Areas' of standards which resulted into inaccurate CO_2 concentration estimates. Therefore, it is highly recommended to use enriched gas standards close to the enrichment of the samples to be able to estimated CO_2 concentration accurate.

Measured atom% ^{13}C-CO_2 values were stable in gas reservoirs and labeling chambers over time. Generally, we were unable to confirm the target ^{13}C enrichments of 45 atom% in Exp1 and 50 atom% in Exp2 by GasBench-IRMS analysis of the reservoir air. We have no obvious explanation for this unexpected observation. One reason might be an inaccurate correction for the intensity dependence in $^{13}C/^{12}C$ ratio determinations at enrichments >> natural abundance. Alternatively, the CO_2 isotopic composition may have changed significantly during the storage in Exetainers; however, this could not be observed in independent testing (Table S1), and the CO_2 concentrations did not change during storage, confirming previous testing of Exetainer vials.[37]

Temperatures observed outside and inside the labeling chambers followed a daily pattern, but temperatures within the chambers were significantly higher than outside. In Exp2, we inserted two conventional blue ice packs into the chambers to maintain more stable temperatures without success; temperatures within the chambers increased compared to surrounding conditions. Solar irradiance was likely the main reason for the increased chamber temperatures. However, temperatures could have been increased further if the reservoir air that was supplied to the chambers was heated beyond chamber temperature. This was not tested in the current experiment. However, we believe that a decreased residence time of air within the chamber and a more powerful fan can help to maintain more temperatures within the labeling chambers.

Methodological evaluation

Gas sample volumes were 5 ml in Exp1 but were increased to 20 ml in Exp2. The sample volume in Exp1 was restricted due to the limited volume of the deployed soil respiration chambers. The Exetainers (5.9 ml) were pre-evacuated by the company without further preparation prior to use. Supposedly the "ready-to-use" Exetainers were of variable quality in terms of vacuum and potential residual gas contents as revealed by the variable determinations on CO_2 concentrations (Figure 3). In Exp2, soil respiration chambers were bigger and the sample volume was increased to 20 ml used for flushing the vials, which resulted in similar CO_2 concentration determinations between replicates.

Determination of atom% ^{13}C-CO_2 values in the highly enriched samples was not obvious. A test trial (Figure S1) revealed that the apparent atom% ^{13}C-CO_2 values were strongly (linear) dependent on the CO_2 concentration in the sampled range of 500-2000 ppm. This empirical relationship was subsequently used to correct atom% ^{13}C-CO_2 in our analysis (Figure 4). The correction needed was much stronger when the instrument was operated with cup-settings for analysis in the ^{13}C natural abundance range compared with the cup settings for enriched samples. We applied atom% corrections dependent on instrument settings. Nevertheless, the corrected atom% ^{13}C-CO_2 values from our field samples never showed the expected values in gas reservoirs and labeling chambers. This suggests that either the equipment leaked and the enriched CO_2 was diluted with ambient air, or that the correction is wrong. Invasion of atmospheric air into the reservoir is unlikely because the observed CO_2 concentrations are as expected. In case of mixed CO_2 sources we would also expect the CO_2 concentrations to be different from our target values. According to this, we have to assume that the measurement of gas samples highly enriched in ^{13}C-CO_2 has to be further tested and evaluated to achieve satisfactory results. In field experiments we suggest to take advantage of real-time measurements with calibrated CO_2 sensors to measure the actual ^{13}C-CO_2 concentration as an indication of the conditions (see e.g. [38]) and compare measurements to CO_2 concentrations obtained from IRMS chromatograms.

APE values in soil respiration and associated standard errors are within the range of previously observed values measured in grassland ^{13}C-CO_2 pulse labeling experiments. Therefore, we can assume that stable CO_2 concentrations and atom% ^{13}C-CO_2 values in labeling chambers assure (Figures 5, 6, 7) consistent and comparable results.[(11)]

Soil respired ^{13}C-CO_2

Respired CO_2 was enriched by less than one atom% ^{13}C-CO_2 illustrating the limited contribution of recently assimilated C to overall belowground respiratory activity. Similar dilution effects have been observed in other grassland labeling studies including studies performed with the technically advanced SID system.[17,19,28]

The assessment of atom% ^{13}C-CO_2 in soil CO_2 efflux of plots manipulated in soil water content (Exp1) revealed no overall effect of soil moisture on the respiration of recently assimilated C (Figure 7a), but irrigation increased soil CO_2 efflux (Figure 7a-insert). The APE was highest in irrigated plots and lowest under drought conditions. The greatest difference in respiration of recently assimilated CO_2 between drought and irrigated plots was significantly apparent two days after the ^{13}C-CO_2 pulse. A suppressing effect of drought on soil C turnover has been shown in several studies[32,39,40] and could be explained by i.e. changes in enzyme activities[41], diminished coupling of above- and belowground C processes[32,42] going along with negative effects on plant photosynthesis[43]. Our results obtained by isotopic manipulation of atmospheric CO_2 suggest that differences in water regimes will modulate the soil C turnover times.

Opposite to drought, elevated CO_2 concentrations stimulated soil CO_2 efflux (Figure 7b-insert) compared to ambient CO_2 conditions. However, no CO_2 effect was found on APE in soil respiration at the examined times (Figure 7b), but elevated CO_2 showed a tendency to contain more recently assimilated CO_2 in soil CO_2 efflux. Elevated CO_2 concentration has frequently be shown to increase soil CO_2 efflux in grasslands[32,44] and forests[45,46] and was considered a major driver in terrestrial C turnover that is important to investigate further.

Advantages and disadvantages

The mobility of the equipment was highly important for pulse labeling in our difficult to access experimental site, and worked out well. However, preparations before the experiment are time consuming (filling air reservoirs) and also subject to possible errors which can be avoided by constant flow controls and IRGA measurements.[9,17,21] Equipment composition was chosen to be least expensive but still suitable and efficient.

Generally, flow-through systems are suitable to maintain stable chamber concentrations without external adjustments and have an advantage over setups where chamber conditions have to be adjusted manually e.g. by

giving CO_2 pulses.[26,27,29,38] A steady chamber CO_2 concentration is needed in order not to affect plant photosynthesis[47], and a prerequisite for assessing C-budgets that include plant assimilated C.

The current setup was a pure flow-through system where the highly enriched ^{13}C-CO_2 was not reused, but released into the atmosphere. This, of course, can be a cost issue when labeling with 99 atom% ^{13}C-CO_2 in many plots for extended time periods. Emerged problems regarding the analysis of atom% ^{13}C-CO_2 values in highly enriched gas samples could not be solved fully. We suggest possible sources of error and demonstrate a first attempt to correct for measurement errors.

Acknowledgements

The CLIMAITE experiment is sponsored by the Villum Kann Raasmussen Foundation with cofounding from Air Liquide, DONG Energy and SMC Pneutmatic A/S. The authors would like to thank all colleagues within the CLIMAITE project for their help in conducting the experiments, helping with sampling and sample analysis as well as for general support. Furthermore, many thanks to Preben Jørgensen, Poul T. Sørensen and Nina W. Thomson for keeping the CLIMATE facilities running constantly and Anja C. Nielsen for laboratory assistance.

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Supplementary material

Table S1: Atom% ^{13}C-CO_2 loss over time (weeks), n=4. Letters indicate differences over time. Means ± SE.

Week	Atom%
1	-0.02 ± 0.22 a
2	-0.20 ± 0.23 a
4	-1.40 ± 0.33 b
5	-1.65 ± 0.17 b

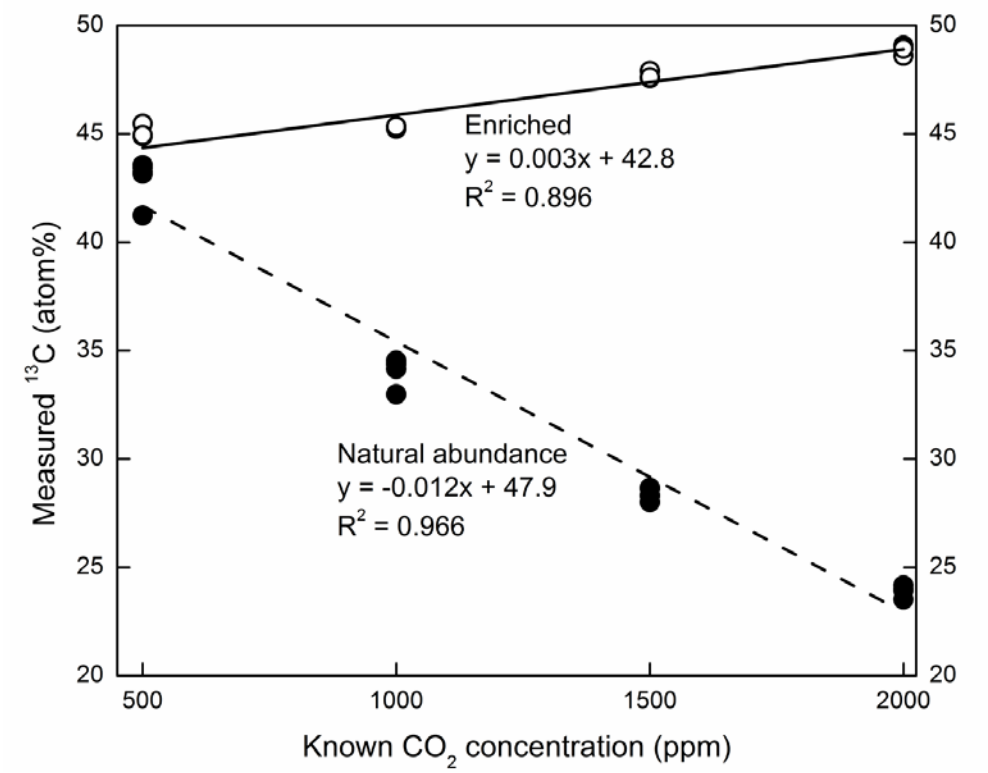


Figure S1: Known CO_2 concentration against measured atom% ^{13}C-CO_2 of 50 atom% ^{13}C-CO_2 samples. Open circles: samples measured with the GasBench methods for highly enriched samples and solid circles: measured with the conventionally applied method for natural abundance ^{13}C-CO_2 amounts. N = 3 for each concentration.

8.2 Paper II

SHORT-TERM CARBON UTILIZATION BY THE MICROBIAL COMMUNITY UNDER FUTURE CLIMATIC CONDITIONS IN A TEMPERATE HEATH-LAND

Sabine Reinsch[1*], Anders Michelsen[2], Zsuzsa Sárossy[1], Helge Egsgaard[1], Inger Kappel Schmidt[3], Iver Jakobsen[1] and Per Ambus[1]

[1] Department of Chemical and Biochemical Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

[2] Department of Biology, University of Copenhagen, 2100 Copenhagen, Denmark

[3] Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg, Demark

To be submitted to Global Change Biology

Abstract

An *in-situ* ^{13}C pulse-labeling experiment was carried out in a temperate heath/grassland to study the impact of elevated CO_2 (390 and 510 ppm), prolonged spring/summer droughts and increased temperature (1 °C) on belowground carbon (C) allocation. Recently assimilated C (^{13}C from the pulse-chase) was traced into leaves, roots, soil and microbial biomass 1, 2 and 8 days after pulse-labeling. The importance of the microbial community in C utilization was investigated using ^{13}C enrichment patterns in microbial functional groups on the basis of phospholipid fatty acids (PLFAs). Climate treatments did not affect total microbial abundance in soil and roots on the basis of PLFA-C concentration. Elevated CO_2 significantly reduced the abundance of gram-negative bacteria (17:0cy) but did not affect the abundance of decomposers (fungi and actinomycetes) in roots. Drought favoured the bacterial community in root samples whereas increased temperatures showed reduced gram-negative bacteria (19:0cy) and changed the actinomycetes community (10Me16:0, 10Me18:0). However, climate treatments had no effect on bacterial-to-total and fungal-to-total abundances, but bacteria were more abundant in root and fungi in soil samples, respectively. The fate of recently assimilated C into microbial functional groups showed a faster C allocation under elevated CO_2 conditions compared to ambient. C allocation belowground was different under ambient and anticipated future climatic conditions and indicated reduced utilization of soil organic matter in the future.

Keywords

Elevated CO_2, drought, increased temperature, phospholipid fatty acids, ^{13}C pulse-labeling

Introduction

Climate change reflects the change of several climatic variables such as carbon dioxide (CO_2) concentration, temperature and water availability. These factors affect the global carbon (C) cycle and are critical determinants of the terrestrial C turnover. Increasing atmospheric CO_2 concentrations may show a positive feedback on CO_2 concentrations and global warming (IPCC 2007). Warming often increases soil CO_2 efflux by e.g. extending plant growth periods (Luo 2007) and can reduce soil C residence time (Amundson 2001; Davidson & Janssens 2006; Heimann & Reichstein 2008). Temperature increase can turn low productive environments into physiologically active systems (Miller & Smith 2012), but can also negatively affect water availability leading to reduced gross primary production and may soil C loss (Ciais *et al.* 2005).
The C balance is determined by many processes starting with CO_2 assimilation (Körner 2006) followed by variable C allocation patterns within plants (Körner 2006), changes in soil nutrient availability (Poorter *et al.* 1995), water status (Albert *et al.* 2011), rhizodeposition (Hütsch *et al.* 2002) as well as the microbial community composition and activity (Carney *et al.* 2007).

Elevated CO_2 concentrations directly affects acclimatization of plant photosynthesis and increases water use efficiency (Leakey *et al.* 2009). In grasslands, increased CO_2 levels also increased C allocation into roots (Fitter *et al.* 1996; Hungate *et al.* 1997), yet after 10 years of CO_2 treatment in a temperate grassland no change in soil C stocks were observed (Theis *et al.* 2007). However, changing climatic conditions do not only influence processes directly but also indirectly. Soil CO_2 concentrations are several magnitudes higher than atmospheric CO_2 levels and thus elevated atmospheric CO_2 concentrations can only have indirect effects on C turnover processes belowground. Soil temperatures are also less responsive than air temperatures and less variable with increasing soil depth but are likely to increase on a longer time scale too. A change in soil water regimes, however, has the power to influence belowground processes involved in C turnover directly by changing water and nutrient availabilities (Jensen *et al.* 2003; Liu *et al.* 2009).

Effects of elevated CO_2 concentrations on the soil microbial community differ across ecosystems (Dunbar *et al.* 2012). In a scrub-oak system, increased CO_2 concentrations led to a fungal based community (Carney *et al.* 2007) and CO_2 fumigation in the Mojave desert revealed an increased fungal-to-bacterial ratio compared to ambient soils, but total PLFA-C concentrations were unaffected by treatments (Jin & Evans

2010). In contrast, the soil microbial community in a temperate grassland exposed to elevated CO_2 showed increased arbuscular mycorrhizal fungi abundance but decreased overall fungal activity (Denef *et al.* 2007), and in a calcareous grassland the microbial community composition was unaffected by increased atmospheric CO_2 concentrations after five years of CO_2 fumigation (Ebersberger *et al.* 2004).

Knowledge of longer-term effects of increased temperatures and water availability on the composition and activity of microbial communities are limited. Fungal-to-bacterial ratio increased under warmed-unclipped conditions in a tallgrass prairie (Zhang *et al.* 2005) and 12 years warming of a forest soil resulted into reduced microbial biomass and presence of fungal, but stimulated the gram-positive bacteria community (Frey *et al.* 2008). In contrast, warming did not affect the microbial community composition in a temperate mountain forest (Schindlbacher *et al.* 2011). The combination of temperature with elevated CO_2 and two levels of precipitation in an American old-field grassland revealed the potential of a 2 °C temperature to increase the abundance of gram-positive bacteria, whereas the abundance of gram-negative bacteria, arbuscular mycorrhizal fungi and saprophytic fungi were decreased. A changed composition of the soil microbial community can potentially change its functionality of the soil microbial community (Gray *et al.* 2011). In the same study, precipitation affected the soil microbial community only in combination with a temperature treatment. One year later, however, precipitation was the main predictor for changes in the microbial community (Castro et al., 2010).

In the present study, *in-situ* pulse-labeling with C-13 enriched CO_2 (^{13}C-CO_2) was performed in a temperate heath/grassland to investigate the utilization of recently assimilated C by the microbial community using microbial functional group specific biomarkers (phospholipid fatty acids) for distinction between groups. The experiment comprised the climate treatments: CO_2 (390 and 510 ppm), prolonged summer drought periods (exclusion of annual 7.6 ± 0.8 % precipitation (mean $\pm$ SE)) and increased temperature (1 °C) in a full factorial setup. The incorporation of ^{13}C-carbon into different C pools was analyzed, with emphasis on ^{13}C accumulation in different microbial functional groups to investigate their importance in short-term C cycling.

We hypothesize that (i) elevated CO_2 favours a fungus based community, (ii) increased temperature leads to a predominantly bacteria based community, (iii) prolonged drought has a negative effect on microbial biomass and increases the fungal component of the microbial community, (iv) C turnover is faster under elevated than ambient CO_2

concentrations and (v) allocation patterns of recently assimilated C are similar in the full factorial combination and the ambient conditions due to outbalancing effects of positive (elevated CO_2, temperature) and negative (drought) climate factor effects.

Materials and methods

Study site

The study site was a temperate unmanaged dry heath/grassland (55°53' N, 11°58' E) in North Zealand, Denmark. The annual average temperature is 8 °C and the mean precipitation is ~610 mm. The soil was a hilly and sandy nutrient-poor deposit composed of ~70 % sand, ~20 % coarse sand, ~6 % silt, and ~2 % clay in 0-15 cm. The plant community was co-dominated by the grass *Deschampsia flexuosa* (~70 %) and the dwarf shrub *Calluna vulgaris* (~30 %), and minor occurrences of other grasses, herbs, mosses and lichens (Kongstad *et al.* 2012). The two species were distributed heterogeneously in patches throughout the study site. Total aboveground green biomass was ca. 700 g m^{-2} when the experimental site was established (Mikkelsen *et al.* 2008) and varied on a seasonal scale between ~300-730 g m^{-2} measured in campaigns between 2004 and 2008 (Kongstad *et al.* 2012). Total green biomass of *D. flexuosa* was ca. 30 g m^{-2} in March (2007) and as ~230 g m^{-2} in August (2004). Plant heights ranged from 40 to 60 cm. Initial root biomass was ~550 g m^{-2} (Mikkelsen *et al.* 2008) with about 90 % of roots located in the top 10 cm soil.

Experimental climate treatments were established in 2004 and initiated in 2005. The experimental setup was a full-factorial split-plot design that consisted of the following climate treatments: an un-treated control (A), elevated CO_2 concentration at 510 ppm during daytime hours by the free-air C dioxide enrichment (FACE) approach (CO_2), prolonged spring/summer droughts by horizontally moving curtains (D), increased temperature (1 °C) realized as passive night time warming by reflective curtains (T) and the factorial combinations TD, DCO_2, TCO_2, $TDCO_2$. Each treatment was replicated 6 times (48 plots in total). Treatments were applied in 12 octagons (Ø 6.8 m) arranged pair-wise in six blocks. Each octagon was divided into four plots. Within each block, one octagon was exposed to elevated CO_2. Soil moisture (vol%), soil and air temperatures (°C) and photosynthetic active radiation (PAR, µmol m^{-2} sec^{-1}) were constantly measured at the field site. For further detailed information about the experimental site, see Mikkelsen *et al.* (2008) and Selsted *et al.* (2012).
Whereas the net area for the climate manipulations were defined by the area encompassed by the 6.8 m diameter octagons, the gross area actually extended into a 11 × 11 m^2 area confined by the dimensions of the drought- and heating curtains. Furthermore, elevated CO_2

concentrations (~500 ppm) could be observed up to 2 m outside the octagons, as opposed to the target value of 510 ppm in the octagon centre (Reinsch & Ambus 2012). Experimental plots for the current pulse-labeling were established 1.2 m outside the periphery of octagons in the corners of the gross area. The labeling plots were established two weeks prior to labeling to allow vegetation recovery, and were confined by galvanized steel collars (0.8×0.4×0.1 m) that were pushed into the soil to about 8 cm depth. The experiment was conducted in two campaigns in May 2011, which is a period of increasing plant photosynthetic activity and high CO_2 assimilation. During the first campaign on May 16th (Octagons 1-6) weather conditions were dominated by grey skies and light showers, whereas during the second campaign on May 19th (Octagons 7-12) sunny conditions prevailed (Figure 1). On both days, the pulse-labeling took place between 12:00-16:00. Only plots containing more than 70 % *D. flexuosa* were included (41 out of 48 plots in total). The drought treatment in 2011 was initiated two weeks prior to the pulse-labeling (2nd May). An amount of 11.2 mm and 29.5 mm of precipitation was excluded before labeling campaigns one and two respectively.

In-situ $^{13}CO_2$ pulse-labeling

The labeling setup has been described in detail in Reinsch & Ambus (2012). In brief, on the days of labeling, transparent flow-through Plexiglass chambers (0.8×0.4×0.3 m) were mounted gas tight on top of the soil collars by means of a water filled channel. A fan inside the chambers assured air mixing and two cooling packs per chamber were inserted to reduce heating of the chamber air. Incoming air (50 atom% ^{13}C-CO_2 at two CO_2 concentrations) from an air reservoir was provided by an electric diaphragm pump (Vacuum pump, Model Thomas 107CCD20-164) through Polyurethane tubing (TU0604, 4 mm, SMC Pneumatic A/S) at an air flow rate of 4.5 l air chamber^{-1} min^{-1}. The provided CO_2 accounted for the maximum measured CO_2 consumption of *D. flexuosa* (Selsted *et al.* 2012). Each octagon was equipped with one air reservoir (gas tight vinyl balloons, Ø ~3 m, Balloons Etc/Balloons Direct, Springfield, Virginia, USA) providing air to 4 chambers at 510 ppm CO_2 for the elevated CO_2 treatments, and at 390 ppm CO_2 for ambient treatments.

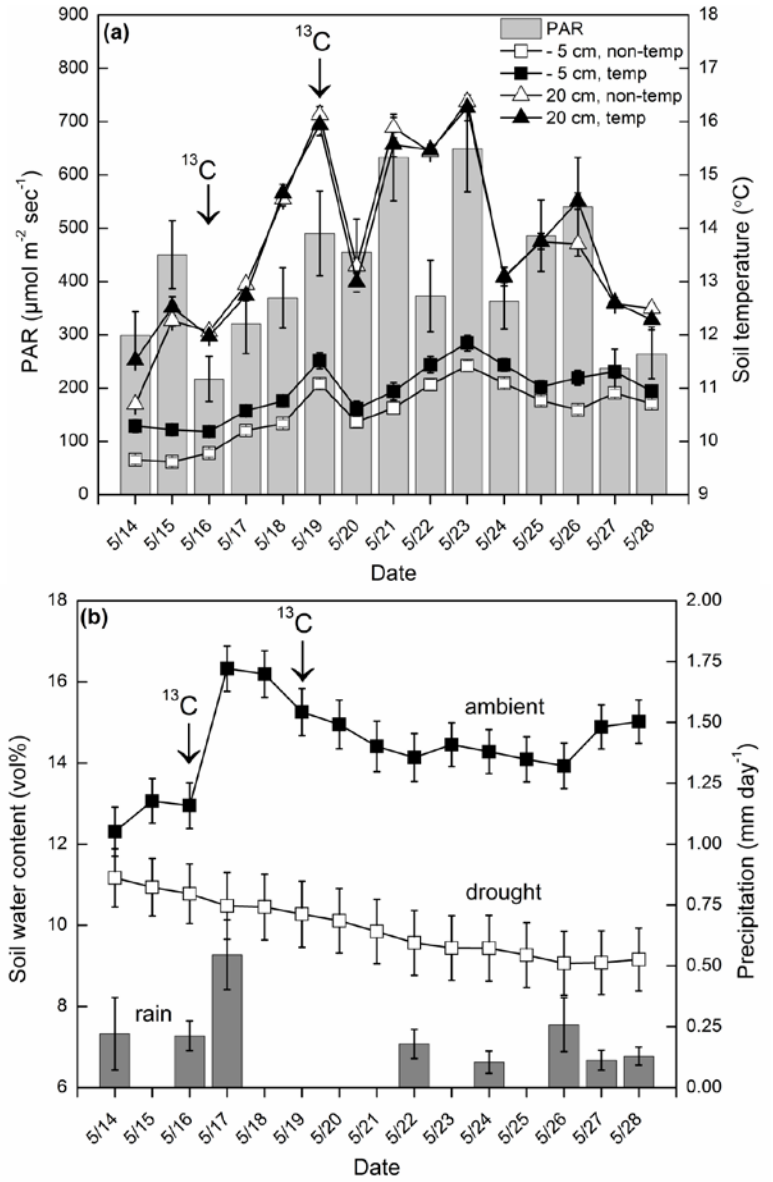


Figure 1: Soil and environmental conditions during the time of the experiment in May 2011. Arrows indicate the pulse-labeling events. (a) Averaged daily photosynthetic active radiation (PAR, bars) and soil temperature at 5 cm soil depth (squares) and 20 cm aboveground (triangles) for ambient (open) and temperature (solid) treatments; (b) soil water contents in ambient (open) and drought (solid) plots in connection with rain events (bars).

The pulse-chase started immediately when the chambers were mounted on the collars, and stopped when the chambers were removed after four hours of exposure. After two hours of labeling, all chambers were opened to remove inside condensed water on the chamber walls and to replace the cooling packs. At one-hour intervals during the pulse-chase, reservoir air and chamber air were sampled from all D plots. For this purpose, 20 ml (20 ml syringe, Omnifix®) air samples were taken from the air reservoirs and chamber outlets and used to flush a pre-evacuated 5.9 ml Exetainer gas

vial (Exetainer, 819W, Labco Ltd, High Wycombe, UK). Gas vials were stored at room temperature until analyses.

Tissue and soil sampling

Plant material (5-10 leaves) of the species present within the labeling plots was collected outside, but close to the soil frames the week before the pulse-labeling experiments, on May 12th. One day after labeling, 3-5 green leaves and litter of *D. flexuosa* were sampled randomly inside the collars. Nine days after the labeling all aboveground biomass was removed from a subplot of 0.2×0.2 m inside each collar. Plant material was immediately sorted into *D. flexuosa* green leaves and litter, shrubs, herbs, mosses and lichens. All plant material was oven dried at 60 °C for 48 hours, weighed and prepared for ^{13}C analysis. Total plant biomass (g m^{-2}) was calculated based on the day 9 harvest.

Pre-labeling soil samples were taken outside, but close to the soil frames on May 13th, and post-labeling soil samples were taken on 1, 2 and 8 days after the labeling, samples were taken inside the collars. Soil samples consisted of two bulked soil cores (Ø 5 cm, 10 cm deep). Soil samples were transported to the laboratory for further processing the same day. Samples were sieved (2 mm Ø) for a maximum of 5 min. Roots were sorted out, total root biomass (g) was recorded and unwashed roots were freeze dried thereafter. Soil subsamples were either processed freshly, air dried or freeze dried. Remaining soil was stored at -20 °C.

Analyses

Carbon contents and isotopic compositions in soil, leaf and root samples were measured on an Isoprime isotope ratio mass spectrometer (Isoprime Ltd, Cheadle, UK) coupled to a Eurovector CN elemental analyzer (Eurovector CN EA, Milan, Italy) using continuous flow. Samples were prepared by weighing 7-20 mg air dried soil and 2-5 mg air dried plant material (leaves, roots) into tin capsules.

Microbial biomass C (MBC) was assessed using chloroform fumigation. Paired fresh 10 g soil samples were either fumigated with chloroform for 24 hours or immediately extracted (1:5 w:vol) with re-demineralised water for 1 hour (Vance et al., 1987). Fumigated samples were treated the same way one day later. Gravimetric soil water content was determined from the weight loss in a 10 g soil sample after oven drying (105 °C, 24 hours). Total organic C of the extracts was determined (TOC-V_{CPH}, Shimadzu, Holm & Halby, Denmark). MBC was calculated

from the difference in C between fumigated and non-fumigated soils using a k_{EC} factor of 0.45 (Joergensen 1996). Non-fumigated (2-8 ml) and fumigated (0.5-5 ml) soil extracts were freeze dried on quartz filters (Quartz microfiber filter QMA Whatman) that were combusted for $^{13}C/^{12}C$ analysis on the EA-IRMS. The isotopic composition of chloroform labile C in unlabeled soil and in ^{13}C labeled soil plots was used to calculated atom% ^{13}C excess in MBC.

Phospholipid fatty acid extraction and analyses

Phospholipid fatty acids (PLFAs) were extracted from ~3 g freeze dried soil and 7-11 mg freeze dried roots. Extractions were carried out for all sampling days for roots, but only non-labeled soil samples and soil samples taken one day after the pulse-chase were processed. A one-phase mixture (CHCl3:MeOH:buffer) according to a modified Bligh and Dyer protocol was used (Bligh & Dyer 1959; White *et al.* 1979; Frostegård *et al.* 1991). PLFAs were eluted with methanol on pre-packed silica columns (Isolute SI-100 mg 10 ml-1, Biotage, Uppsala, Sweden). Internal standards C17:0 and C19:0 (Sigma-Aldrich, Munich, Germany) were added to the samples before mild alkaline-methanolysis and the separated fatty acid methyl esters (FAMEs of PLFAs) were solubilised in hexane.

FAMEs were analyzed via gas-chromatography-combustion-isotopic ratio mass spectrometry (GC-c-IRMS) using a GC HP6890 containing a fused silica column (Varian FactorFour WCOT, 0.25 mm id x 60 m coated with VF-23 ms at a thickness of 0.25 μm) with helium as carrier gas (1 ml min^{-1}). The GC was coupled via a GC combustion interface (Thermo Scientific, Bremen, Germany) in continuous flow mode with a Finnigan DeltaPLUS isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). The oxidation reactor on the interface was maintained at 940 °C, the reduction reactor at 650 °C.

Samples were injected (1 μl) at 240 °C using splitless mode. GC oven temperature program started at 50°C, held for 2 min, ramped at 15 °C min^{-1} to 100 °C, then at 2 °C min^{-1} to 220 °C and finally at 15 °C min^{-1} to 240 °C where the final temperature was held for 5 min. Separated compounds were measured against a CO_2 reference gas calibrated against Vienna PeeDee belemnite (VPDB) reference material. FAMEs were identified by relative retention time comparing samples with a FAME standard mix (Supelco 37 component FAME mix, 47885-U, Sigma Aldrich). All $\delta^{13}C$ values were corrected for the additional C during methanolysis:

$$\delta^{13}C_{CFA} = \frac{[(N_{FA} + 1)\delta^{13}C_{FAME} - \delta^{13}C_{MeOH}]}{N_{FA}}$$

where N_{FA} refered to the number (N) of C-atoms of the fatty acid (FA) component, $\delta^{13}C_{FAME}$ was the measured $\delta^{13}C$ value of the FAME after methylation and $\delta^{13}C_{MeOH}$ was the $\delta^{13}C$ value for the methanol used for methanolysis (-37.7 ‰ ± 3.2 ‰).

PLFA peak areas from GC-c-IRMS chromatograms were corrected for the number of C atoms in the fatty acid chain using the slope of individual PLFAs of the FAME standard (Denef *et al.* 2007) resulting into a PLFA concentration (mmol PLFA-C) that was used to calculate the mole fraction (%) of each individual PLFA. The quantitative amount of each PLFA (µg PLFA-C g^{-1} soil or mg^{-1} roots) was calculated using known internal standard concentrations.

The soil microbial community composition was investigated on the basis of microbial functional group specific PLFAs. General soil biomarkers are 14:0, 15:0, 16:0 and 18:0. Gram-negative specific PLFAs are the cyclic 17:0cy and 19:0cy as well as 16:1ω7c and 18:1ω7c. Gram-positive bacteria are presented by the PLFAs 15:0a, 15:0i, 16:0i, 17:0a, 17:0i. Actinomycetes (belonging to gram-positive bacteria) can specifically be distinguished on the basis of methylated PLFAs: 10Me16:0, 10Me17:0, 10Me18:0. The fungal biomarkers 18:1ω9c, 18:1ω9t and 18:3ω3 were used (Vestal & White 1989; Frostegård & Bååth 1996; Zak *et al.* 1996; Zelles 1999; Jin & Evans 2010).

Isotopic calculations and data analyses

Isotope ratios are expressed as the deviation (‰) from the standard Vienna Pee Dee Belemnite (VPDB) for C with $\delta^{13}C$ (‰) = $(R_{sample}/R_{standard} - 1) \times 1000$, where $R = {}^{13}C/{}^{12}C$. The standard deviation of isotope measurements of standard material (peach leaves, NIST) was ± 0.1 ‰ for $\delta^{13}C$. The isotopic excess in different C pools (leaves, roots, soil, MBC) was calculated as difference in atom% ^{13}C of pulse-labeled samples and natural abundance samples (non-labeled) and expressed as atom% excess (APE).

The distribution of recently assimilated C (^{13}C from the pulse-chase) in microbial specific biomarkers (C-fate) was calculated: firstly, the isotopic enrichment of PLFAs in each sample was related to the applied ^{13}C enriched air during the pulse-chase using a two end-member mixing model:

$$P_{assimilated} = \frac{\delta^{13}C_{PLFA} - \delta^{13}C_{controlPLFA}}{\delta^{13}C_{chamber} - \delta^{13}C_{controlPLFA}}$$

where $P_{assimilated}$ is the percentage of recently applied ^{13}C of pulse-chase air in each PLFA, $\delta^{13}C_{PLFA}$ is the isotopic value of each PLFA (post-labeling), $\delta^{13}C_{controlPLFA}$ is the isotopic value of PLFAs in non-labeled samples (pre-labeling) and $\delta^{13}C_{chamber}$ is the isotopic value measured in the labeling chambers (Reinsch & Ambus, 2012).

Secondly, the amount of recently assimilated ^{13}C into individual PLFAs was calculated by multiplying $P_{assimilated}$ with the amount of PLFA in the sample. Thirdly, the amounts of recently assimilated ^{13}C-carbon in each sample were converted to a percentage scale and grouped into microbial functional groups. Displaying isotopic values as fate of C into microbial functional groups facilitates an easy evaluation of the importance of each component of the microbial community in short-term C cycling.

Statistical analysis

Statistical analysis was carried out in R version 12.2.1. When necessary, data were tested for normality (Shapiro-Wilk test of normality) and were always tested for homogeneity of variances (Levene test of equality of variances, "car" package) prior to analysis. Differences within climate treatments over time were analyzed using an "ANOVA" using time as predictor. TukeyHSD test was used to extract differences between times. In case data showed no homogenous variances, the Kruskal-Wallis test was used instead. Bacterial-to-total and fungal-to-total ratios were tested with the Kruskal-Wallis test because the criteria of equal variances were not met.

Differences between climate treatments were analysed with a Fit Mixed-Effect Model (lmer) in the package "LMERConvenienceFunctions". This model has the advantage that the experimental design can be included in the random statement in its full complexity. The random statement "(1|Block) + (1|Block:CO2) + (1|Block:CO2:T) + (1|Block:CO2:D)" was used in all analyses and was extended by the factor (1|Time) when datasets were analysed across times. *p*-value outputs were generated using the MixMod-package that uses the SAS-algorithm. Differences between treatments were reported on two levels of significance with $p \leq 0.1$ and $p \leq 0.05$.

3. Results

Pulse-labeling conditions

Average photosynthetic active radiation (PAR) was variable over the experimental period (Figure 1a). Air temperatures 20 cm aboveground followed that pattern closely, whereas soil temperatures at 5 cm depth were less influenced by PAR but rose continuously from 9.6 ± 0.1 to 10.7 ± 0.1 °C in ambient (A, CO2, D, DCO2) and from 10.3 ± 0.1 to 11 ± 0.1 °C in temperature plots (T, TCO2, TD, TDCO2). PAR was 217 ± 42 μmol m^{-2} sec^{-1} and 490 ± 80 μmol m^{-2} sec^{-1} on the first and second labeling days, respectively. The higher PAR at the second day was also reflected in increased air and soil temperatures.

Soil water content in D plots (D, DCO2, TD, TDCO2) decreased continuously during the experimental period from 11.7 ± 0.7 to 9.1 ± 0.8 vol% (Figure 1b, Table 2). In contrast, non-drought plots (A, T, CO2, TCO2) received precipitation during the experimental period and soil water contents varied. Due to a rain event between the two labeling campaigns, the soil water content in non-drought plots increased from 12.9 ± 0.6 to 15.3 ± 0.6 vol% but drought plots stayed unaffected. Total plant biomass ranged between 207 ± 32 g m^{-2} in DCO2 plots and 420 ± 79 g m^{-2} in A plots with a significant negative effect ($p < 0.05$) of the applied drought treatment (Table 1). The single factor drought treatment affected the total grass biomass (*D. flexuosa*) positively.

Soil microbial community and its structure

Soil C and nitrogen (N) contents varied between 1.8-3.1 % and 0.12-0.17 %, respectively (Table 2). C and nitrogen contents should be constant over time but varied highly between individual samples. Soil C content was constant over time for all treatments but the single factor T treatment. Single factors CO2, D and T showed significantly increased C contents compared to A at sampling day 8. Soil N in D and T treatments was patchily distributed and variable over time. The single factor treatments CO2 and T increased soil N content compared to A at day 8. The climate treatment interactions TD and TDCO2 significantly affected soil N contents before the pulse-chase.

Table 1: Characteristics of *D. flexuosa* aboveground biomass in the different climate treatments. Values as means ± SE (n=4-6). Significant differences between treatments are marked as bold: $p \leq 0.1$, and * $p \leq 0.05$.

Measure		Unit	A	CO2	D	DCO2	T	TCO2	TD	TDCO2
Total plant biomass		g m^{-2}	420 ± 79	372 ± 65	***300 ± 48*** *	207 ± 32	364 ± 64	344 ± 13	225 ± 32	275 ± 38
Ratio Grass:Total			0.6 ± 0.1	0.5 ± 0.1	***0.8 ± 0.1*** *	0.9 ± 0.04	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Carbon content	Green leaves	%	47 ± 0.2	***47 ± 0.1***	***47 ± 0.2***	47 ± 0.3	48 ± 0.2	47 ± 0.1	48 ± 0.1	47 ± 0.1
	Litter		47 ± 0.2	47 ± 0.5	48 ± 0.3	47 ± 0.2	48 ± 0.2	47 ± 0.2	47 ± 0.6	48 ± 0.2
Nitrogen content	Green leaves	%	**1.7 ± 0.1**	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.5 ± .1	1.8 ± 0.1	1.6 ± 0.1
	Litter		1.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.1 ± 0.1

Microbial biomass C (MBC) concentrations were harmonized against soil C content and showed high variability dependent on the individual sample at different sampling days (Table 2). MBC varied over time in all climate treatments with the highest MBC two days after pulse-labeling and lowest 8 days after the pulse-chase. MBC was significantly reduced in D plots and also TD showed reduced MBC one day after labeling whereas TDCO2 showed decreased MBC 8 days after labeling.

In contrast to MBC, microbial biomass in terms of total PLFA-C (general PLFAs and functional group specific biomarkers) extracted from root and soil samples suggested a stable microbial community size that was unaffected by climate variables and time (Table 2). One exception to the persistent abundance was observed one day after the pulse-chase where the full climate combination showed significantly reduced total root PLFA-C content (Table 2).

Patterns of individual PLFA-C concentrations in roots showed a dominant appearance of gram-negative bacteria especially due to 17:0cy and 16:1ω7c (Figure 2). The actinomycetes specific PLFA 10Me17:0 and the fungi specific PLFA 18:3ω3 were highly abundant under all climatic conditions. Elevated CO_2 (CO2, DCO2, TCO2, TDCO2) tended to reduce PLFA-C concentrations (Figure 2a) and had a significantly negative effect on the occurrence of the general biomarker 16:0, the gram-negative biomarker 17:0cy, the gram-positive specific PLFA 16:0i and the fungal biomarker 18:2ω6,9. In contrast, prolonged spring/summer droughts (D, DCO2, TD, TDCO2) tended to increase biomarker abundances and had a

significantly positive effect on the actinomycetes specific 10Me18:0 PLFA (Figure 2b). Elevated temperature (T, TCO2, T, TDCO2) affected the gram-negative biomarker 19:0cy negatively and had a positive effect on the actinomycetes biomarker 10Me16:0 and decreased the 10Me18:0 abundance (Figure 2c). Individual PLFA-C concentration in bulk soil samples were not affected by climate treatments (Table S1).

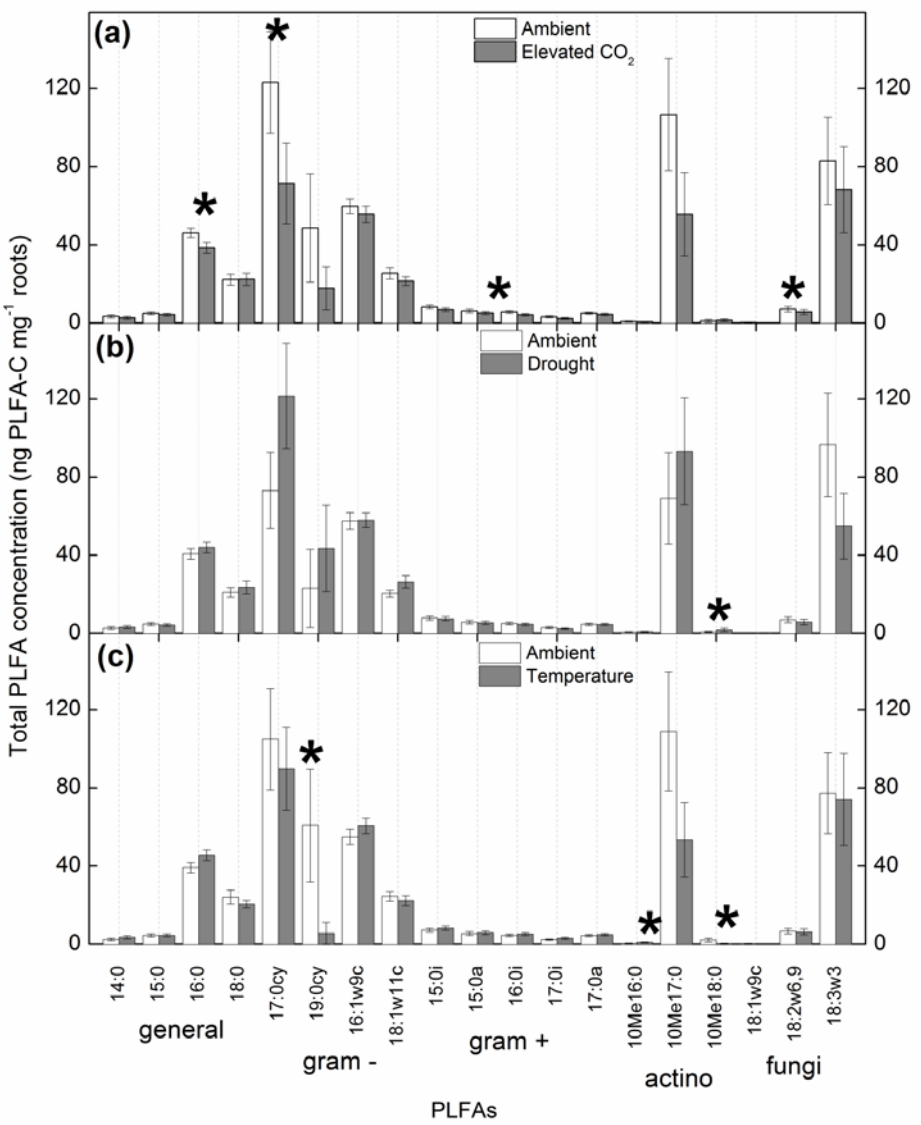


Figure 2: Concentrations of PLFA-C (ng PLFA-C mg^{-1} root) for individual PLFA biomarkers. (a) ambient CO_2 concentration vs. elevated CO_2 concentration, (b) ambient (non-drought) vs. drought plots and (c) ambient (non-temperature) vs. increased temperature plots. Letter "a" refers to background atom% ^{13}C excess (natural abundance ^{13}C). * indicate differences between ambient and the climate treatment with $p \leq 0.1$.

Table 2: Soil and microbial properties for each climate treatment: Day 0 implies the day before pulse-labeling. Values are means ± SE (n=4-6). Bold values are significant treatment effects $p \leq 0.1$, * $p \leq 0.05$, letters indicate differences within climate treatments over time.

	Day	A	CO2	D	DCO2	T	TCO2	TD	TDCO2
Soil moisture (vol%)	0	15.4 ± 2.3	14.4 ± 1.1	11.6 ± 1.9	12.7 ± 1.8	13.5 ± 1.0	13.6 ± 1.0	8.4 ± 2.1	9.8 ± 0.9
Soil total C content (%)	0	2.4 ± 0.4	1.9 ± 0.3	2.8 ± 0.2	2.6 ± 0.1	2.1 ± 0.1 ab	2.3 ± 0.2	***2.2 ± 0.2***	2.0 ± 0.5
	1	2.5 ± 0.2	1.8 ± 0.4	2.6 ± 0.4	2.7 ± 0.2	2.5 ± 0.2 a	2.8 ± 0.6	2.4 ± 0.3	2.3 ± 0.7
	2	2.3 ± 0.3	2.4 ± 0.2	3.0 ± 0.2	2.8 ± 0.4	2.0 ± 0.1 b	2.1 ± 0.2	2.2 ± 0.3	2.5 ± 0.5
	8	1.8 ± 0.2	***1.9 ± 0.3***	***2.1 ± 0.3*** *	2.4 ± 0.2	***2.1 ± 0.1 ab***	2.2 ± 0.2	2.2 ± 0.2	3.1 ± 0.4
Soil total N content (%)	0	0.14 ± 0.02	0.12 ± 0.01	0.15 ± 0.01 **a**	0.15 ± 0	0.13 ± 0.003 **ab**	0.15 ± 0.02	***0.14 ± 0.01***	***0.13 ± 0.01*** *
	1	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01 **ab**	0.16 ± 0.01	0.16 ± 0.01 **a**	0.18 ± 0.05	0.15 ± 0.02	0.15 ± 0.03
	2	0.14 ± 0.01	0.16 ± 0.01	0.17 ± 0.01 **a**	0.16 ± 0.02	***0.13 ± 0.01 *b***	0.13 ± 0.01	0.13 ± 0.01	0.15 ± 0.03
	8	0.12 ± 0.01	***0.13 ± 0.02*** *	0.12 ± 0.01 **b**	0.14 ± 0.01	***0.13 ± 0.01 b***	0.15 ± 0.02	0.13 ± 0.01	0.17 ± 0.02
MBC/Soil C content	0	198 ± 23 **ab**	303 ± 116 **ab**	***151 ± 21 a***	192 ± 22 **ab**	189 ± 18 **ab**	195 ± 24 **ab**	169 ± 48 **ab**	164 ± 31 **ab**
(µg C g^{-1} soil)	1	238 ± 59 **ab**	416 ± 162 **a**	***196 ± 21 a***	149 ± 40 **a**	169 ± 42 **ac**	263 ± 86 **ab**	***147 ± 22*** **ab**	219 ± 47 **a**
	2	359 ± 59 **a**	249 ± 38 **ab**	***210 ± 15 a****	246 ± 28 **b**	302 ± 48 **b**	419 ± 80 **a**	272 ± 59 **a**	***270 ± 41*** **a***
	8	101 ± 34 **b**	45 ± 2 **b**	***55 ± 11b***	50 ± 7 **c**	55 ± 11 **c**	82 ± 21 **b**	71 ± 17 **b**	***50 ± 19*** **b***
Root PLFA-C concentration †	0	0.9 ± 0.4	0.5 ± 0.3	0.9 ± 0.3	0.7 ± 0.3	0.9 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.2
(µg C mg^{-1} dry roots)	1	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	**0.2 ± 0.1**
	2	0.3 ± 0.2	0.2 ± 0.1	0.6 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.1 ± 0.04	0.5 ± 0.2	0.2 ± 0.1
	8	0.4 ± 0.2	0.6 ± 0.2	0.8 ± 0.4	0.4 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
Soil PLFA-C concentration †	0	10.4 ± 2.8	8.6 ± 2.9	8.9 ± 1.4	10.5 ± 2.2	12.3 ± 2.3	10.9 ± 2.4	8.8 ± 2.5	6.7 ± 1.6
(µg C g^{-1} dry soil)	2	11.1 ± 2.3	9.3 ± 3.5	15.0 ± 1.3	13.0 ± 3.1	11.8 ± 2.0	9.5 ± 2.2	9.8 ± 2.4	10.7 ± 2.8

† PLFA concentrations as sum of all PLFAs (general, gram-negative, gram-positive, actinomycetes and fungi biomarkers).

Ratios of bacteria-specific PLFAs vs. total PLFA abundance, and fungi-specific PLFAs vs. total PLFA concentrations were not affected by climate treatments, but were different in soil and root samples (Figure 3). The bacterial-to-total ratio was lower in soil ($p < 0.05$) than in roots, fungal-to-total ratio showed the opposite pattern and was lower in roots than in soil ($p < 0.05$).

APE aboveground and belowground

Green leaves of *D. flexuosa* showed an APE between 180 ± 20 and 260 ± 40 (values presented as measured atom% ^{13}C excess * 10^3) where the lowest enrichment was observed in A, and the highest in the CO2 treatment (Table 3, leaves). APE in leaves tended to be higher in the single factor CO2 treatment compared to all other climate treatments and the full factorial treatment combination (TDCO2) showed a reduced isotopic enrichment and significant interaction effect ($p \leq 0.05$).

Root APE showed a maximum enrichment after two days of labeling for all treatments except the single factor CO2 treatment, which showed the highest enrichment after 8 days (Table 3, roots). One day after labeling, root APE was significantly higher in CO2 compared to A and, the two factor treatments TCO2 and TD showed significant climate factor interactions on root APE. The CO2 treatment persisted at significantly higher isotopic enrichment throughout the 8 day period. At day 8, root APE was significantly influenced by the interactions of CO2, D and T (TDCO2). This was also true for the two-factor treatments TCO2 and TD. A transfer of assimilated C-13 into the soil matrix was barely detectable as displayed by the very low APE values in soil (Table 3, soil). The observed peak of ^{13}C enrichment in the soil was similar to the peak in root enrichment.

Average APE in soil MBC often peaked two days after labeling, which goes along with isotopic peak enrichments in roots and soil (Table 3, MBC). Isotopic enrichment of MBC was generally not affected by climate treatments, which is in accordance with the finding of unchanged MBC and unchanged total PLFA-C concentrations under different climatic conditions. In general, APE of MBC one and two days after labeling were twice as high as enrichments observed in root samples. Temperature related treatments (except the TDCO2) tended to have lower APE in MBC than the non-temperature treatments.

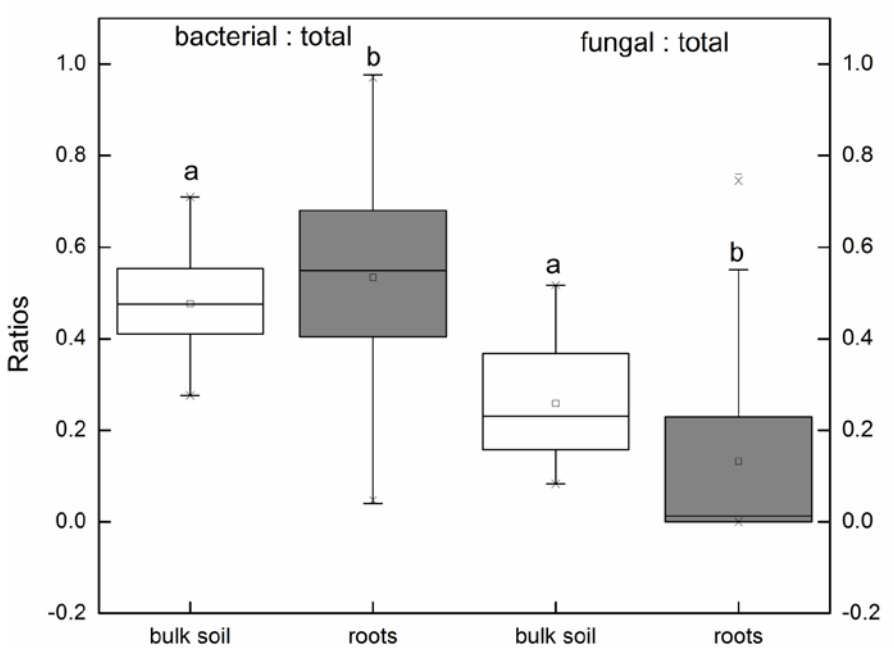


Figure 3: Ratios of bacterial-to-total and fungal-to-total PLFA biomarkers in bulk soil and root samples. Bacteria are the sum of PLFA-C concentrations (µg C g^{-1} soil or mg^{-1} root) of 17:0cy, 19:0cy, 16:1ω9c, 18:1 ω11c, 15:0i and a, 16:0i and a, 17:0:I and a. Fungal abundance is the sum of the PLFAs 18:1 ω9c, 18:2 ω6,9 and 18:3 ω3,6,9. Boxes show 95 % of the values and the line indicates median values. Letters indicate significant differences ($p < 0.05$) between bulk soil and roots for each bacterial-to-total or fungal-to-total ratios.

C allocation into PLFAs

The C-fate (%) describes how much recently assimilated C (from the pulse-chase) was allocated to each microbial functional group, e.g. if 100 % assimilated C was observed in the gram-negative group then all the recently assimilated C that ended up in the microbial community was exclusively utilized by that group. Thus, the measure of C-fate is an indirect measure of the importance of each microbial functional group in the short-term C turnover.

Root samples showed an overall high C-fate into gram-negative bacteria (Table 3, Table S3) in terms of C-13 allocation to the specific biomarkers. The sum of general biomarkers on average showed an allocation of recently assimilated C of 20-40 % into general PLFAs (Table 3). The temporal dynamic in the C-transfer pattern, as indicated by the shading in Table 3, shows that gram-negative bacteria were generally the first group utilizing recently assimilated C followed by a delayed uptake of root exudates by gram-positive bacteria. Actinomycetes and fungi were generally less active in roots compared to soil. The uptake of recently assimilated C in actinomycetes was delayed compared to gram-negative and gram-positive bacteria. The C-fate into actinomycetes in roots was

very low one day after labeling (with exception of TCO2) but increased over time. The actinomycetes community was more active in soil than in roots with exception of the TCO2 treatment. Fungi often took up a high proportion of recently assimilated C in roots at day one (average 10 %), but the C allocation into fungi was lower at day 2 (average 4 %), combined with a high proportion of recently assimilate C in the fungal group in soil at day 2 (20 %). This pattern suggests a re-allocation of recently assimilated C from roots into the soil matrix due to fungal activity.

Detailed data for C incorporation into microbial functional groups are first presented for root samples followed by soil samples (Table 3, S3). If possible, climate effects on C allocation patterns were described along microbial functional groups starting with gram-negative bacteria, followed by gram-positive bacteria, actinomycetes and fungi.

In the CO2 treatment, allocation of recently assimilated CO_2 into gram-negative bacteria was significantly decreased at day 2 ($p < 0.05$). The climate factor combination DCO2 (also in TDCO2) displayed a significantly higher C-fate into gram-negative bacteria in roots at day 8 (31 ± 9 %, $p < 0.05$) compared to the single factor treatments CO2 and D (average 17 %). The combination TD showed a significantly reduced C-fate into gram-positive bacteria in roots at day 1 (13 ± 5 %, $p < 0.05$) than it was observed for single factor D and T treatments (average 17 %). C-fate into actinomycetes was not affected by climate treatments and no recently assimilated C was detected in roots in A (day1), T, (day 8), TCO2 (days 2 and 8), TD (days 1 and 8) and TDCO2 (day 8). C-fate into fungi in root samples was negatively affected by elevated CO_2 (CO2, DCO2, TDCO2) exposure and showed less than 5 % C allocation, with exception of the TCO2 treatment. The combination of T and CO2 (average 5 %) showed a positive interaction on the fate of C into fungi at day 1 (12 ± 8 %, $p < 0.05$) and the combination of T and D (average 18 %) reduced the C allocation into fungi at day 1 (3 ± 2 %, $p < 0.05$).

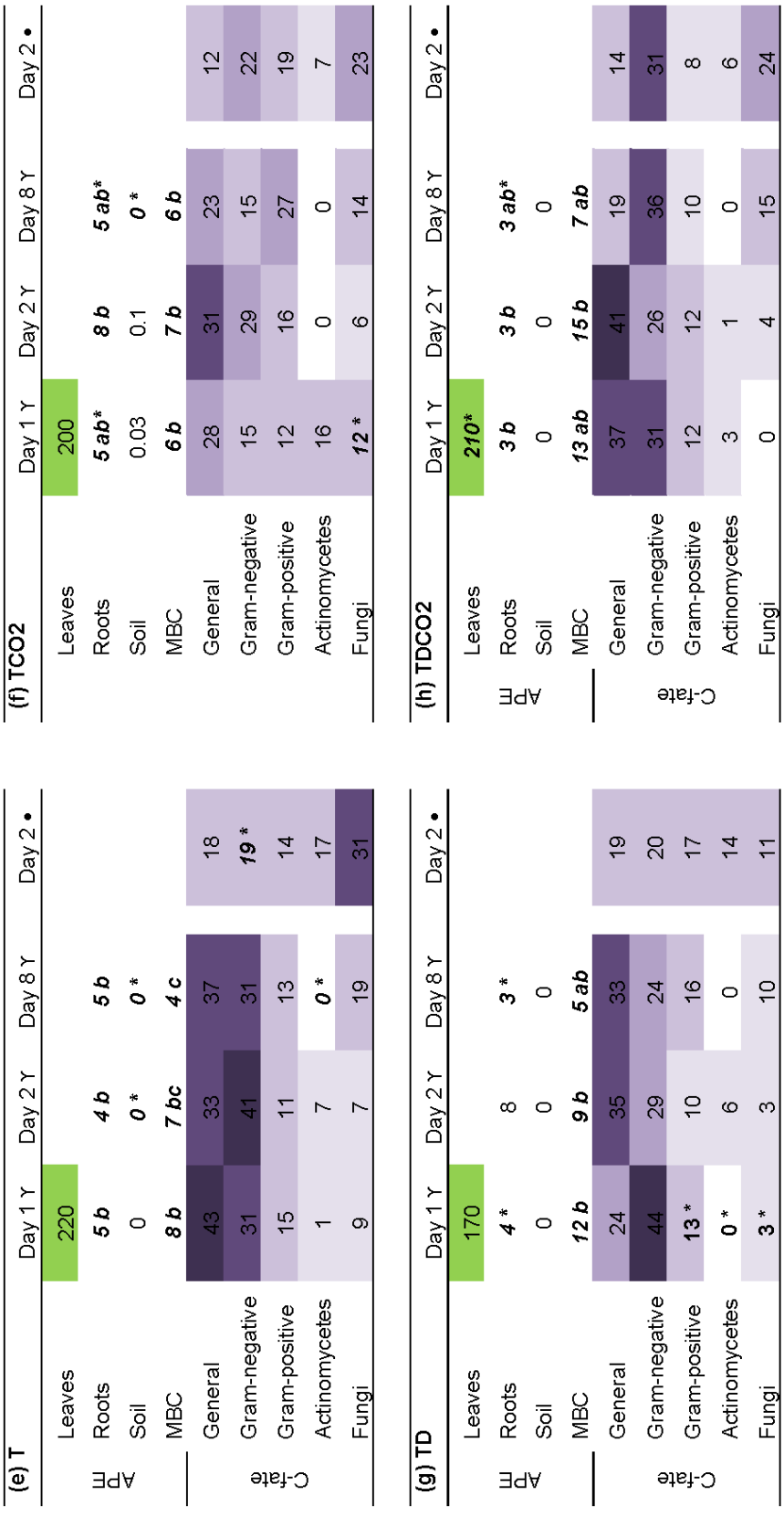
Table 3: APE (mean values of atom% ^{13}C excess × 10^3) for green *D. flexuosa* leaves, roots, soil and microbial biomass C (MBC), and recently assimilated C (C-fate in %) in different microbial functional groups (general, gram-negative, gram-positive, actinomycetes, fungi) in root samples (ϒ: days 1, 2 and 8) and in soil (•: day 2). Bold: differences among climate treatments $p \leq 0.1$, letters indicate differences between days; letter "a" refers to background atom% ^{13}C excess (natural abundance ^{13}C) for APE data. * indicates differences between treatments ($p \leq 0.05$). C-fate values (%) grouped into 0, 1-10, 11-20, 21-30, 31-40 and 41-50 regimes and marked with increasing purple shading intensity. Standard errors are avoided for clearness, but full dataset is available in supplementary tables S1 and S2.

(a) Ambient		Day 1 ϒ	Day 2 ϒ	Day 8 ϒ	Day 2 •
APE	Leaves	180			
	Roots	***3 ab***	***5 b***	***4 b***	
	Soil	0	0.4	0.2	
	MBC	***10 b***	***19 c***	***4 ab***	
C-fate	General	29	31	20	19
	Gram-negative	19	27	26	17
	Gram-positive	10	20	15	10
	Actinomycetes	0	4	12	11
	Fungi	***21 a***	***0 b***	***10 ab***	23

(b) CO2		Day 1 ϒ	Day 2 ϒ	Day 8 ϒ	Day 2 •
APE	Leaves	260			
	Roots	***7 ab****	***5 ab****	***9 b***	
	Soil	0.2	***0.4 ****	***0.3****	
	MBC	***12 ab***	***17 b***	***5 ab***	
C-fate	General	34	21	***20 ****	7
	Gram-negative	22	***14 ****	12	11
	Gram-positive	11	23	14	10
	Actinomycetes	0.03	5	13	17
	Fungi	***1 ****	3	9	21

(c) D		Day 1 ϒ	Day 2 ϒ	Day 8 ϒ	Day 2 •
APE	Leaves	210			
	Roots	***0.1 a***	***5 b***	***2 a***	
	Soil	0	0.2	0	
	MBC	***7 bc***	***10 b***	***4 c***	
C-fate	General	26	32	37	26
	Gram-negative	24	42	22	14
	Gram-positive	***19 a***	***9 b***	***17 ab***	28
	Actinomycetes	5	8	5	19
	Fungi	27	8	19	12

(d) DCO2		Day 1 ϒ	Day 2 ϒ	Day 8 ϒ	Day 2 •
APE	Leaves	240			
	Roots	***2 ab***	***4 b***	***3.2 b***	
	Soil	0.2	0.1	0.2	
	MBC	***15 b***	***14 b***	***2 b***	
C-fate	General	17	43	21	23
	Gram-negative	37	11	***31 ****	20
	Gram-positive	20	26	12	***4 ****
	Actinomycetes	5	3	10	21
	Fungi	***4 ab ****	***0 a***	***9 b***	13


(e) T
Day 1 Y
Day 2 Y
Day 8 Y
Day 2 •
APE
Leaves 220
Roots 5 b 4 b 5 b
Soil 0 0 * 0 *
C-fate
MBC 8 b 7 bc 4 c
General 43 33 37 18
Gram-negative 31 41 31 19 *
Gram-positive 15 11 13 14
Actinomycetes 1 7 0 * 17
Fungi 9 7 19 31
(f) TCO2
Day 1 Y
Day 2 Y
Day 8 Y
Day 2 •
APE
Leaves 200
Roots 5 ab* 8 b 5 ab*
Soil 0.03 0.1 0 *
C-fate
MBC 6 b 7 b 6 b
General 28 31 23 12
Gram-negative 15 29 15 22
Gram-positive 12 16 27 19
Actinomycetes 16 0 0 7
Fungi 12 * 6 14 23
(g) TD
Day 1 Y
Day 2 Y
Day 8 Y
Day 2 •
APE
Leaves 170
Roots 4 * 8 3 *
Soil 0 0 0
C-fate
MBC 12 b 9 b 5 ab
General 24 35 33 19
Gram-negative 44 29 24 20
Gram-positive 13 * 10 16 17
Actinomycetes 0 * 6 0 14
Fungi 3 * 3 10 11
(h) TDCO2
Day 1 Y
Day 2 Y
Day 8 Y
Day 2 •
APE
Leaves 210*
Roots 3 b 3 b 3 ab*
Soil 0 0 0
C-fate
MBC 13 ab 15 b 7 ab
General 37 41 19 14
Gram-negative 31 26 36 31
Gram-positive 12 12 10 8
Actinomycetes 3 1 0 6
Fungi 0 4 15 24

In soil samples, C allocation into fungi in CO_2 plots was approximately twice as high as in roots suggesting that the peak of fungal activity in roots was not detected. As illustration: C-fate into fungal biomarkers in roots under CO2 and T conditions at day 1 was low (average 5 %) compared to C allocation in soil at day 2 (average 26 %). In contrast, in the two factor treatments TD and DCO2 the C-fate into roots (days 1-8) was low (average 5 %) without an increased C-fate into the soil (average 12 %). TDCO2 showed a different fungal allocation pattern of recently assimilated C than any other treatment since in the full combination there was no allocation of CO_2-derived C into fungal PLFAs at day 1.

C-fate into gram-negative and gram-positive bacteria in soil was little affected by climate treatments or interactions. However, T had a significant positive effect on the C allocation into gram negative bacteria (19 ± 7 %) compared to other single factor treatments (average 14 %). Besides, C-fate into gram-positive bacteria was significantly negative affected by the two factor combination D and CO2 (average 19 %) to 4 ± 2 ($p < 0.05$).

Discussion

A priori considerations

Variable environmental conditions between the two pulse-chase campaigns and increased soil water contents (~1.5 vol%) in plots without drought treatments (Figure 1) likely resulted into high spatial variability and, probably contributed to the high variability observed in the presented data. Spatial variability in vegetation cover (Kongstad *et al.* 2012) and soil nutrient patchiness (Table 2) across the experimental site are further variables that can result into high data variability. Therefore, in view of the expected high influence of variable field conditions, we reported *p*-values not only at the generally accepted level of significance, $p \leq 0.05$, but also for trends in the data, $p \leq 0.1$.

To investigate C allocation patterns belowground, we identified the microbial community composition and activity in soil and root samples. Soil cores were taken from 0-10 cm soil depth containing up to 90 % root biomass. Roots were separated from soil samples, but were not washed. Independent analysis of washed roots resulted in undetectable low PLFA concentrations (not shown) and we thus assume that our results reported as samples from roots represent the 'rhizoplane' microbial community. Results referring to soil samples might be considered as a measure of the 'rhizosphere' microbial community due to very high root density and similar PLFA-C concentration patterns observed in root and soil samples (Figure 2, Figure S1) suggests an intimate linkage between the two sample types.

Climate effects on carbon allocation patterns

The uptake of ^{13}C-carbon during pulse-labeling was successful and green leaves of *D. flexuosa* were significantly labeled (Table 3, Table S2) and provided a good basis for studying further C re-allocation patterns. The month May was chosen for the tracer study because *D. flexuosa* at the current site is in a rapid growth stage and shows increased C assimilation (personal comm., K. Boesgaard). Generally, about 5-25 % of photosynthetically assimilated C is released by roots into the rhizosphere (Helal & Sauerbeck 1984). In our study, APE observed in roots was only 0.05 % of the APE observed in leaves in the D treatment but it was 2.7 % in roots compared to leaf APE in CO2. These low values for C re-allocation from aboveground plant parts into belowground plant structures can have different reasons: firstly, plants invested more C into

aboveground structures for growth and reduced belowground C allocation. Secondly, the dilution effect due to mixing of non-enriched ^{13}C root C compounds with recently assimilated C from the pulse-chase results into lower APE values. Thirdly, C allocated to the roots may have been released as rhizodeposits and taken up by the microbial community, this is supported by the observations that MBC is the major sink for recently assimilated C (Kaštovská & Šantrůčková 2007). The fast appearance of recently assimilated C into belowground compartments shows the high dynamic and linkage between aboveground and belowground C allocation (Wu *et al.* 2009; Brüggemann *et al.* 2011).

Elevated CO_2 treatments showed a higher excess of assimilated ^{13}C-CO_2 in *D. flexuosa* leaves without any change in grass biomass (except for the single factor D treatment, Table 1) supporting observations that elevated CO_2 concentrations increase CO_2 uptake (Leakey *et al.* 2009; Albert *et al.* 2011). The full factorial combination (TDCO2) showed a significantly decreased leaf APE compared to the single factor CO_2 treatment, suggesting that the multi-factorial setup, simulating future climatic conditions, outbalanced the positive influence of elevated CO_2 on C assimilation (Albert *et al.* 2011; Dieleman *et al.* 2012). APE patterns in belowground compartments showed the general trend of peak enrichment two days after labeling in roots, soil and MBC (Table 3). This pattern might also depend on the speed of C re-allocation under different climatic conditions and on pool sizes and activities of plants, roots and MBC. This finding is consistent with other studies showing observed peak ^{13}C enrichments in different plant compartments after one or two days of pulse labeling (Lu *et al.* 2004; Leake *et al.* 2006; Jin & Evans 2010). In general, observed APE patterns were often similar across climate treatments. This suggests either small effects of climatic conditions on general C allocation patterns or a large effect of pool dilution would mask climatic impacts.

Climate effects on soil microbial community composition

Within this study, APE in MBC was relatively similar between treatments (Table 3, Table S2). This is in accordance with stable microbial biomass abundances observed in soil and roots on the basis of PLFA-C abundances (Table 2) and with previous studies on different climate change effects on the soil microbial community (Zhang *et al.* 2005; Jin & Evans 2010). The observed microbial community is dominated by gram-negative bacteria (Figure 2, Table S1), but fungi (arbuscular mycorrhizal and saprophytic fungi) were underrepresented compared to results from other grassland studies (Butler *et al.* 2003; Denef *et al.* 2007). Comparably, high abundance of actinomycetes (Figure 2, 10Me17:0) could have been

antagonistic to fungi as observed by Jayasinghe & Parkinson (2008) where the abundance of actinomycetes inhibited the abundance of the fungal decomposer community.

A broader picture of treatment effects on microbial abundances can be obtained by grouping treatments into ambient vs. elevated CO_2, drought and temperature responses. This approach might result into higher variation within a group due to grouping of single and combined treatments, but displays more clearly effects of the single climate treatments. First, application of this analysis to PLFA-C concentrations (Figure 2, Table S1) shows that elevated CO_2 tended to reduce bacterial PLFA-C abundances, especially in gram-negative bacteria (17:0cy), decrease gram-positive bacteria abundance (16:0i), and slightly reduce in fungal PLFA-C concentrations (18:3ω3). This trend towards a reduction in the bacterial community combined with a more stable fungal community is in accordance with hypothesis (i) suggesting that elevated CO_2 favours the fungal community. This finding is furthermore in accordance to other studies simulating increased atmospheric CO_2 concentrations. In the Mojave Desert and in a shrub-oak ecosystem, the soil microbial community shifted towards a fungal dominance after several years of exposure to elevated CO_2 (Carney *et al* 2007; Jin & Evans 2010).

Second, increased temperature in our study had a similar, but dampened effect as observed under CO_2 fumigation. This does not agree with our hypothesis (ii) and is also in conflict with results from a 12 year warming experiment in the Harvard Forest showing a reduced fungal community but increased abundance of gram-positive bacteria (Frey *et al.* 2008). However, in a warming and clipping experiment in a tallgrass prairie, the soil fungal community was favoured under warming conditions probably due to an indirect positive effect of warming on plant growth (Zhang *et al.* 2005). Moreover, Yuste *et al.* (2011) observed a drought-resistant fungal community in response to warming in a shrubland and holm-oak forest, confirming our results. Finally, we observed that extended drought periods tended to favour the bacterial community by increasing the abundance of the gram-negative PLFAs 17:0cy and 19:0cy (Figure 2b) and decreasing the fungal abundance (18:3ω3) compared to ambient (non drought) plots. A high abundance of cyclic PLFAs (17:0cy, 19:0cy) can result from drought stress on gram-negative bacteria that produce stronger membrane lipids to better tolerate drought periods (Bossio & Scow 1998). Our results suggest that we reject hypothesis iii that a fungal based community is established under drought conditions to meet minimal nutrient demands that can be achieved by the exploration of fungal hyphae into the soil matrix. Hawkes *et al.* (2011) reported fungal

community responses to different precipitation treatments (droughts and increase precipitation), and also showed that fungal communities are more stable under drought conditions. Furthermore, gram-negative bacteria are mainly present in biofilms because they are highly vulnerable to changing environmental conditions due to their thin cell membranes. The free- living species *Azotobacter sp.* is very important in nitrogen cycling due to its ability to fix atmospheric nitrogen (Strandberg & Wilson 1968) and *Nitrosomonas sp.* is an important component of the nitrogen cycle (Hofman & Lees 1952). Therefore, the maintenance of gram-negative bacteria community under drought conditions can also fulfil nutrient demands.

Climate effects on carbon distribution and turnover

C cycling is faster at elevated CO_2 than under ambient or drought conditions (Selsted *et al.* 2012). Prolonged drought periods at the current study site have negatively affected plant photosynthesis (Albert *et al.* 2011), plant biomass (Kongstad *et al.* 2012) and the microbial community biomass size (Andresen *et al.* 2010). Therefore, we assume that C cycling under ongoing drought conditions was slower in the single factor D treatment than in the other single factor treatments, whereas C turnover is assumed to be fastest in single factor CO2 plots. Furthermore, we could assume that the combination of D and T amplifies the drought effect by further decreasing soil water availability or, that the combination of T and CO2 amplifies the positive CO2 effect on C cycling. However, as it was shown that factor interactions are rarely additive (Larsen *et al.* 2011; Dieleman *et al.* 2012) the climate factor interactions should be experimentally tested to evaluate potential effects on (short-term) C turnover.

With respect to C allocation patterns in the D treatment (Table 3c), utilization of recently assimilated C in gram-negative bacteria was high on day 1 (24 ± 12 %, mean ± SE). C allocation was further increased until day 2 (42 ± 10 %), after which it decreased to 22 ± 7 % on day 8, very likely due to the dilution of the ^{13}C-pulse. In CO2 plots (Table 3b), gram-negative bacteria also utilized a high proportion of recent C from the labeling-pulse at day 1 (22 ± 11 %), but two days after the pulse, the percentage of recently assimilated C was significantly lower than in D plots (14 ± 6 %) and stayed low until day 8 (12 ± 7 %). Taking our assumptions on C turnover times in D and CO2 plots into account, this observed C utilization patterns suggests our sampling window was too short to pick the enrichment peak in gram-negative bacteria in the CO2 treatment.

The gram-positive group in the D treatment utilized on average 19 ± 1% of recently assimilated C, but another C source might be co-utilized leading to a significantly lower C-fate into gram-positive bacteria at day 2 (9 ± 3 %) maybe due to competition for C with gram-negative bacteria. At day 8, the amount of recently assimilated C allocated to gram-positive bacteria had increased again to 17 ± 4 %. The C-fate into gram-positive bacteria under elevated CO_2 conditions showed the opposite pattern with a high allocation of recent C at day 2 (23 ± 10 %), suggesting that drought and elevated CO_2 affected the group of gram-positive bacteria differently.

Only small amounts of recently assimilated C were utilized by actinomycetes in roots across treatments. This can result from low activity in roots or from the use of an alternative C source. However, the fungal community e.g. in D treatments had utilized about 27 ± 12 % of assimilated C, but the C-fate into fungal PLFAs at day two was only 8 ± 8 %. This suggests that another microbial functional group (gram-negative bacteria in D treatments) received more recently assimilated C via. e.g. plant exudates or that the enriched C was transported via fungal hyphae.

In CO2 soils measured at day 2, fungi received a high amount of recently assimilated C (21 ± 12 %) indicating a transport of C from roots into the soil. Due to discrete temporal sampling, the transformation of C through the ecosystem, intermediate stages and utilization patterns might have been missed. Generally, fungi are known to transport C through hyphae and provides easily available C to rhizosphere bacteria (Olsson & Johnson 2005). Thus, low recovery of recently assimilated C in roots in D plots at day 2 can be a result of C transport through hyphae away from roots and into different microbial groups such as actinomycetes (19 ± 7 %) and gram-positive bacteria (28 ± 10 %). C-fate patterns (Table 3) display that fungi generally transport recently assimilated C into the soil matrix where especially actinomycetes profited from the labile C input (Table 3c,d,e,g).

Microbial community composition and functioning

Bacteria and fungi are the two main groups in ecosystem functioning in terms of spatial distribution and as shown above, soil C utilization and allocation. Climate treatments did not affect bacterial-to-total and fungal-to-total ratios in roots and soil. However, the bacterial-to-total PLFA-C ratio in roots was higher than in soil, whereas the fungal abundance was highest in the soil (Figure 3). This agrees with previous findings that bacteria are closely related to roots and associated rhizo-depositions,

whereas fungi can expand into the soil matrix via their hyphal system (Olsson & Johnson 2005; Leake *et al.* 2006). Furthermore, C transport through hyphae and C exudation as well as hyphal debris can be substrates for bacterial growth outside the rhizoplane zone around the roots. Actinomycetes in our study might depend on C re-allocation by fungi as shown by C-fate patterns in soil samples two days after pulse-labeling (Table 3c,d,e,f,g). An active actinomycetes community in soil has further implication for mineralization of recalcitrant C compounds, since the availability of recalcitrant substances dominates in the soil matrix. Actinomycetes, like fungi, are decomposers of soil organic matter (Lacey 1997). Dependent on the composition, size and activity of the microbial community, soils can be shifted towards a soil C sink or source under changing climatic (Carney *et al.* 2007; Paterson *et al.* 2009; Garcia-Pausas & Paterson, 2011).

Gram-negative bacteria as indicator of carbon turnover rates

The microbial functional group of gram-negative bacteria was the first to utilize recently assimilated C (Figure 3) and this is in accordance with previous observations (Butler *et al.* 2003; Treonis *et al.* 2004). Furthermore, gram-negative bacteria showed the most consistent patterns of utilization of recently assimilated C across climate treatments if delays in C transport due to climatic conditions are allowed for (discussed above). This observation suggests that C-fate patterns displayed in the gram-negative group can be used to investigate the effects of climatic manipulations on the rate of C cycling.

Three out of four CO2 treatments, CO2, DCO2 and TDCO2, showed a high C allocation into gram-negative bacteria one day after the pulse-label (Table 3b,d,h) suggesting an overall positive effect of elevated CO_2 on the speed of C transport and C allocation in the microbial community. In contrast, three out of four non-CO2 plots, A, D and T, showed a one-day delay in C allocation peak into gram-negative bacteria (Table 3a,c,e) and this supports our hypothesis iv of a faster C turnover under elevated CO_2 concentrations and is supported by other studies (Denef *et al.* 2007; Drake *et al.* 2011; Selsted *et al.* 2012). The two-factor treatments TCO2 and TD were outlier treatments (Table 3f,g) where climate factor interactions seemed to affect the fate pattern of C cycling differently. Drought and temperature treatments did not show consistent patterns of allocation of recent C into gram-negative bacteria suggesting that the two treatments are not the main determinants of C turnover rates. Warming and drought are known to affect plant photosynthesis and thus C uptake indirectly and can have reducing and accelerating effects on C turnover

depending on plant acclimatization (Ciais *et al.* 2005; Luo 2007; Albert *et al.* 2011).

Consequences of future climatic conditions on short-term carbon cycling

Applied climate treatments were chosen to simulate climatic conditions for Denmark in 2075 (Mikkelsen *et al.* 2008) as realized in the full factorial combination TDCO2 (future). Ambient vs. future climatic conditions showed only minor differences in the assimilation of APE into different compartments (grass, roots, soil, MBC; Table 3a,h), which is in accordance with our hypothesis (v). This implies that the positive effect of elevated CO_2 on ^{13}C assimilation in green leaves was counterbalanced by the climatic factors T and D emphasizing that climatic effects are not necessarily additive but sometimes act antagonistic in their induced responses as also suggested by others (Larsen *et al.* 2011; Dieleman *et al.* 2012).

Even though the overall APE patterns were not different between ambient and future treatments, C turnover did vary as revealed by C allocation patterns into microbial functional groups (Table 3a,h); this disagrees with our hypothesis (v). Evaluating C turnover on the basis of the gram-negative group in roots (as described above), C turnover was faster under future climatic conditions as also observed for all treatments with increased CO_2 concentrations. Generally, under future climatic conditions the gram-negative bacteria persistently utilized 30 % recently assimilated C, emphasizing the quantitative importance of this functional group (Table 3h). C-fate into gram-positive bacteria was similar in ambient and future treatments. However, C-fate into actinomycetes in roots was always lower under future conditions (average 2 %) compared to any other climate treatment. One explanation is that the time resolution of sampling was too low to pick up an enrichment peak in the fast C re-allocation. Still, the is rather unlikely because enrichment peaks in roots and MBC show the same patterns for all other treatments and therefore our observation rather suggests that the actinomycetes community was suppressed under future climatic conditions.

The re-allocation of C from roots into the soil via hyphae was similar under ambient and future climatic conditions but the utilization of re-distributed C by other microbial functional groups changed. In the full-factorial treatment most of the recently assimilated C was allocated into gram-negative bacteria. In contrast, recently assimilated C was evenly distributed between the functional groups in ambient plots. The changed

bacterial activity (gram- negative and positive) observed under future climatic conditions points towards a less active decomposer community (especially actinomycetes) under future climatic conditions with possibly repressed mineralization of soil organic matter from soil C stores.

Our results suggest that changing climatic conditions affect the soil microbial community and C allocation patterns within microbial functional groups. The same conclusion was drawn on the basis of single factor treatment manipulations (Carney *et al.* 2007; Frey *et al.* 2008; Jin & Evans 2010; Kim *et al.* 2012) as well as from a climate change experiment manipulating CO_2 concentration, precipitation and temperature in an old-field ecosystem (Gray *et al.* 2011). More precisely, we can conclude that the utilization of recently assimilated C into different microbial functional groups can potentially turn heath/grassland soils into a C sink under anticipated future climatic conditions.

Considerations for future studies

With the current experiment, we aimed to investigate the dynamics of short-term C turnover under different climatic conditions. It is known that the speed of C turnover is influenced by the tested climate change factors (Hungate *et al.* 1997; Ciais *et al.* 2005; Heimann & Reichstein 2008; Selsted *et al.* 2012), but we sampled all plots at similar time intervals for comparison. The sampling strategy, however, only revealed temporal "snapshots" of the dynamic system, which has to be taken into account when interpreting the results in terms of a fast or delayed occurrence of recently assimilated C into different C pools and microbial functional groups. Different patterns of the fate of recently assimilated C can additionally be modified by microbial activity as well as water and nutrient availability in the soil. Generally, responses of the microbial community to climatic changes are highly variable within and among ecosystems and are also dependent on plant community composition (De Deyn *et al.* 2008). Furthermore, timing (Butler *et al.* 2003) and scale of climatic changes affect soil microbial responses rather than the occurrence of climate change itself (Hawkes *et al.* 2011; Sheik *et al.* 2011). However, in our study, C turnover was stimulated by elevated CO_2 resulting in different C allocation patterns into microbial functional groups over time. C allocation into microbial functional groups could be changed under future climatic conditions potentially leading to slower mineralization of soil organic matter stocks. This study presented a qualitative assessment on C allocation pattern and the role of microbial functional groups but, future work is needed to investigate the pulse-chase ^{13}C balance to assess whether observed changes in the speed of C turnover and changes in C allocation patters into microbial functional

groups are important on an ecosystem scale. Mycorrhizal lipid biomarkers (e.g. neutral lipid fatty acid 16:1ω5, Olsson & Johnson 2005) can give further information about C allocation into the microbial community and should be included in further studies if they can be detected.

Acknowledgements

The experiment was carried out within the CLIMAITE project that is financially supported by the Villum Kann Rasmussen Foundation, Air Liquide DenmarkA/S and Dong Energy. In particular, we thank Preben Jørgensen, Nina W. Thomson, Svend Danbæk and Poul T. Sørensen for keeping the experiment running constantly. The authors would like to thank everybody who has helped to carry out the pulse-labeling with special thanks to Nina Bonke Mikkelsen, Aslak Kappel Hansen and Matthias Just Justesen for their great effort during the whole experimental time, all involved Climaite Ph.D. students, student helpers and technicians. Special thanks go to Marie P. Merrild for her contribution to the fatty acid analysis.

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Supplementary material

Table S1: Concentrations of soil PLFA-C (µg PLFA-C g^{-1} soil) for individual PLFA biomarkers. For ambient CO_2 concentration (non-CO2) vs. elevated CO_2 concentration (CO2), ambient (non-D) vs. drought plots (D) and ambient (non-T) vs. increased temperature plots (T).

Functional group	PLFA	non-CO2	CO2	non-D	D	non-T	T
General	14:0	9.4 ± 2.9	13 ± 4	10 ± 3	13 ± 4	13 ± 4	9.8 ± 2.7
	15:0	46 ± 6	51 ± 7	46 ± 6	51 ± 7	53 ± 7	44 ± 6
	16:0	1364 ± 92	1310 ± 119	1352 ± 113	1322 ± 100	1356 ± 106	1318 ± 106
	18:0	275 ± 45	196 ± 26	247 ± 44	224 ± 28	210 ± 27	261 ± 44
Gram-negative	17:0cy	298 ± 33	324 ± 42	291 ± 34	331 ± 41	284 ± 32	338 ± 42
	19:0cy	187 ± 51	70 ± 37	172 ± 51	86 ± 38	95 ± 39	163 ± 51
	16:1w7c	619 ± 70	668 ± 77	710 ± 73	577 ± 72	615 ± 75	671 ± 72
	18:1w7c	1920 ± 166	1861 ± 197	1819 ± 162	1962 ± 199	1943 ± 188	1838 ± 175
Gram-positive	15:0i	618 ± 50	667 ± 67	639 ± 58	646 ± 61	670 ± 65	615 ± 53
	15:0a	227 ± 18	252 ± 27	236 ± 23	243 ± 23	248 ± 25	231 ± 21
	16:0i	568 ± 43	546 ± 51	562 ± 48	552 ± 46	596 ± 52	518 ± 41
	17:0i	386 ± 75	361 ± 66	347 ± 69	400 ± 71	373 ± 77	375 ± 63
	17:0a	147 ± 15	124 ± 17	149 ± 18	122 ± 14	131 ± 14	139 ± 18
Actinomycetes	10Me16:0	718 ± 80	605 ± 78	621 ± 77	701 ± 81	746 ± 89	577 ± 66
	10Me17:0	251 ± 34	243 ± 43	283 ± 44	212 ± 31	272 ± 43	223 ± 33
	10Me18:0	114 ± 25	80 ± 16	114 ± 24	80 ± 17	110 ± 25	84 ± 15
Fungi	18:1w9c	1067 ± 106	969 ± 115	1056 ± 117	980 ± 104	1135 ± 118	901 ± 100
	18:2w6,9	190 ± 66	346 ± 119	311 ± 109	226 ± 82	252 ± 83	284 ± 109
	18:3w3	1947 ± 280	1151 ± 246	1464 ± 278	1634 ± 261	1691 ± 281	1407 ± 257

Table S2: Natural abundance $\delta^{13}C$ (‰) (Day 0) and atom% ^{13}C excess (Days 1–8) in different C pools. Atom% ^{13}C excess indicated as 1×10^{-3}. Values are means ± SE (n=4-6). Significant differences between treatments in bold with $p \leq 0.1$ and * with $p \leq 0.05$. Letters indicate within treatment differences over time.

Measure	Day	A	CO2	D	DCO2	T	TCO2	TD	TDCO2
δ13C (‰)									
Grass	0	-27.1 ±0.4	***-32.0 ±0.4 ****	***-26.9 ±0.3***	***-31.1 ±1.2***	-27.0 ±0.3	-32.9 ±1.2	-27.4 ±0.5	-30.7 ±0.9
Root	0	-27.8 ± 0.1 a	***-30.1 ± 0.7 ****	***-27.8 ± 0.2 ****	***-29.7 ± 0.3***	-27.7 ± 0.1	***-32.4 ± 1.0 ****	-27.4 ± 0.1	-30.0 ± 0.4
Soil	0	-27.3 ± 0.1	***-27.6 ± 0.2 ****	-27.3 ± 0.1	-27.6 ± 0.1	-27.2 ± 0.03	***-27.9 ± 0.2***	-27.2 ± 0.1	-27.7 ± 0.1
MBC	0	-25.9 ± 0.1	***-27.3 ± 0.3 ****	-25.9 ± 0.1	-27.3 ± 0.3	***-26.0 ± 0.2 ****	***-28.1 ± 0.3 ****	-25.7 ± 0.2	-27.6 ± 0.2
Atom% 13C excess									
Grass	1	0.18 ± 0.02	***0.26 ± 0.04 ****	0.21 ± 0.05	0.24 ± 0.05	0.22 ± 0.03	0.20 ± 0.02	0.17 ± 0.02	***0.21 ± 0.03***
Roots	1	***2.5 ± 0.2 ab***	***5.6 ± 0.3 ab****	***0.1 ± 0.1 a***	***1.9 ± 0.2 ab***	**4.6 ± 0.2 b**	***4.8 ± 0.3 ab****	***4.1 ± 0.4 ****	***2.7 ± 0.2 b***
	2	***5.0 ± 0.3 b***	***5.2 ± 0.3 ab****	***5.2 ± 0.3 b***	***3.9 ± 0.1 b***	***3.9 ± 0.2 b***	***7.8 ± 0.2 b***	8.3 ± 0.9	***3.1 ± 0.2 b***
	8	***4.2 ± 0.1 b***	***8.6 ± 0.3 b***	***2.1 ± 0.1 a****	***3.2 ± 0.2 b***	***5.4 ± 0.2 b***	***5.3 ± 0.3 ab****	***2.6 ± 0.2***	***2.6 ± 0.2 ab****
Soil	1	0	0.2 ± 0.04	0	0.2 ± 0.03	0	0.03 ± 0.05	0	0
	2	0.4 ± 0.03	***0.4 ± 0.05 ****	0.2 ± 0.05	0.1 ± 0.02	***0 ****	0.1 ± 0.03	0	0
	8	0.2 ± 0.04	***0.3 ± 0.03***	0	0.2 ± 0.03	***0 ****	***0***	0	0
MBC	1	0.010 ± 0.003 **b**	0.012 ± 0.004 **ab**	0.007 ± 0.001 **bc**	0.015 ± 0.004 **b**	0.008 ± 0.001 **b**	***0.006 ± 0.006 b***	0.012 ± 0.003 **b**	***0.013 ± 0.006 ab***
	2	0.019 ± 0.003 **c**	0.017 ± 0.004 **b**	0.010 ± 0.002 **b**	0.014 ± 0.002 **b**	***0.007 ± 0.001 *bc***	0.007 ± 0.001 **b**	***0.009 ± 0.002 *b***	0.015 ± 0.004 **b**
	8	0.004 ± 0.004 **ab**	0.005 ± 0.001 **ab**	0.004 ± 0.001 **c**	0.009 ± 0.002 **b**	0.004 ± 0.001 **c**	0.006 ± 0.001 **b**	0.005 ± 0.003 **ab**	0.007 ± 0.002 **ab**

Table S3: C-fate (%) into different microbial functional groups in root samples and soil for each climate treatment, and over time. Values are means ± SE (n=4-6). Bold numbers indicate significant differences between treatments with $p \leq 0.1$.

	day	A	CO2	D	DCO2	T	TCO2	TD	TDCO2
General									
roots	1	29 ± 14	34 ± 15	26 ± 12	17 ± 8	43 ± 12	28 ± 9	24 ± 9	37 ± 12
	2	31 ± 15	21 ± 7	32 ± 11	43 ± 11	33 ± 11	31 ± 8	35 ± 11	41 ± 11
	8	20 ± 6	**20 ± 11**	37 ± 9	21 ± 8	37 ± 8	23 ± 9	33 ± 12	19 ± 6
soil	2	19 ± 11	7 ± 3	26 ± 6	23 ± 9	18 ± 7	12 ± 5	19 ± 6	14 ± 6
Gram-negative									
roots	1	19 ± 12	22 ± 11	24 ± 12	37 ± 13	31 ± 15	15 ± 8	44 ± 15	31 ± 15
	2	27 ± 12	**14 ± 6**	42 ± 10	11 ± 7	41 ± 12	29 ± 10	29 ± 8	26 ± 11
	8	26 ± 8	12 ± 7	22 ± 7	**31 ± 9**	31 ± 9	15 ± 12	24 ± 7	36 ± 11
soil	2	17 ± 4	11 ± 5	14 ± 5	20 ± 9	**19 ± 7**	22 ± 7	20 ± 8	31 ± 7
Gram-positive									
roots	1	10 ± 5	11 ± 4	19 ± 1	20 ± 7	15 ± 4	12 ± 3	**13 ± 5**	12 ± 5
	2	20 ± 10	23 ± 10	9 ± 3	26 ± 7	11 ± 3	16 ± 6	10 ± 4	12 ± 3
	8	15 ± 9	14 ± 9	17 ± 4	12 ± 10	13 ± 4	27 ± 12	16 ± 5	10 ± 5
soil	2	10 ± 4	10 ± 4	28 ± 10	**4 ± 2**	14 ± 4	19 ± 6	17 ± 7	8 ± 3
Actinomycetes									
roots	1	0	0.03 ± 0.03	5 ± 5	5 ± 5	1 ± 1	16 ± 10	**0**	3 ± 3
	2	4 ± 4	5 ± 3	8 ± 4	3 ± 3	7 ± 5	0	6 ± 6	0.5 ± 0.5
	8	12 ± 11	13 ± 13	5 ± 3	10 ± 10	**0**	0	0	0
soil	2	11 ± 7	17 ± 7	19 ± 7	21 ± 11	17 ± 6	7 ± 3	14 ± 8	6 ± 3
Fungi									
roots	1	21 ± 9	**1 ± 1**	27 ± 12	4 ± 3	9 ± 7	**12 ± 8**	**3 ± 2**	0
	2	0	3 ± 3	8 ± 8	0	7 ± 4	6 ± 6	3 ± 2	4 ± 4
	8	10 ± 4	9 ± 6	19 ± 8	9 ± 3	19 ± 15	14 ± 13	10 ± 5	15 ± 10
soil	2	23 ± 10	21 ± 12	12 ± 4	13 ± 3	31 ± 12	23 ± 7	11 ± 6	24 ± 8

8.3 Paper III

IMPACT OF FUTURE CLIMATIC CONDITIONS ON THE POTENTIAL FOR PLANT-MEDIATED SOIL ORGANIC MATTER PRIMING

Sabine Reinsch[1], Per Ambus[1], Barry Thornton[2] and Eric Paterson[2]

[1] Department of Chemical and Biochemical Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

[2] The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH, Scotland

Submitted to Global Change Biology

Abstract

Terrestrial carbon (C) storage and turnover are of major interest under changing climatic conditions. We present a laboratory microcosm study investigating the effects of anticipated climatic conditions on the soil microbial community and related changes in soil organic matter (SOM) decomposition. Soil samples were taken from a heath-land after six years of exposure to elevated carbon dioxide (eCO_2) in full combination with summer drought and increased temperature. Soil C-dynamics were investigated in soils from: (i) ambient, (ii) eCO2, and (iii) plots exposed to the full combination of factors simulating future climatic conditions. C-13 enriched glucose (3 APE) was added to soil microcosms, soil CO_2 efflux was measured and separated into glucose- and SOM-derived C. Microbial biomass, community composition and activity were assessed with microbial functional group specific biomarkers (PLFAs). We observed that glucose additions induced SOM priming in ambient and eCO_2 treated soils, but not in soil exposed to future climatic conditions. Climate treatments and glucose additions did not affect relative abundances of microbial functional groups but the fate of labile C through the microbial community was changed by climate treatments as revealed by the incorporation of C-13 in PLFAs. Soil treated with eCO_2 showed a high flow of labile C through gram-positive bacteria whereas in ambient and future soils utilisation of glucose by actinomycetes and fungi (putative SOM-decomposers) was greater. Our results suggest that individual climate change factors may influence pathways of C-flux through microbial communities and therefore affect soil processes; these factors may cause opposing effects and act to maintain ecosystem stability. This highlights the importance of studying climate change factors in combination to fully assess consequences of environmental change on plant-soil systems.

Key words:

Priming effects, climate change, microbial community, PLFA, rhizodeposition, glucose, C-13

Introduction

Although the turnover of terrestrial soil carbon (C) stocks is relatively slow, the large size of these stocks (~2200 Pg C) means that the gross flux of CO_2 to the atmosphere is very large (~60 Pg C yr^{-1}). For soils in equilibrium, these losses are balanced by the inputs of plants, but under expected climate change scenarios this balance may be disrupted and soils may turn into net CO_2 sources (Carney *et al.* 2007). If this does occur, depletion of terrestrial C stocks would accelerate climate change.

During recent years, impacts of elevated carbon dioxide (eCO_2) on ecosystem processes have been investigated *in-situ* with Free-Air Carbon Enrichment (FACE) experiments (Leakey *et al.* 2009; Larsen *et al.* 2011). eCO_2 has been found to accelerate C turnover in terrestrial ecosystems (Martin-Olmedo *et al.* 2002; Carney *et al.* 2007; Carrillo *et al.* 2011), i.e. through increased plant CO_2 uptake (Leakey *et al.* 2009), plant belowground C exudation (Hagedorn *et al.* 2008; Phillips *et al.* 2011) and soil C effluxes (Martin-Olmedo *et al.* 2002; Hagedorn *et al.* 2008). To date it is of major debate if eCO_2 will lead to soil C sequestration (Lagomarsino *et al.* 2006; Iversen *et al.* 2012) or net soil C mineralization (Martin-Olmedo *et al.* 2002; Carney *et al.* 2007; Hagedorn *et al.* 2008; Phillips *et al.* 2011).

Soil C turnover is not only influenced by atmospheric CO_2 concentration. Soil temperature (Fierer *et al.* 2006), nutrient availability (Martin-Olmedo *et al.* 2002; Cheng & Kuzyakov 2005; Paterson *et al.* 2008; Phillips *et al.* 2011), hydrology (Xiang *et al.* 2008) and soil characteristics (Kemmitt *et al.* 2008) are also important internal and external drivers for soil C decomposition. Kemmitt *et al.* (2008) attempted to describe SOM dynamics solely by physical and chemical processes considering the soil microbial community as a constant soil parameter. However, subsequent discussions illustrate that soil C dynamics are intensely modulated by the soil microbial community (Kuzyakov *et al.* 2009; Garcia-Pausas & Paterson 2011).

Rhizosphere microbes are consumers of plant-derived C released into the soil matrix by root exudation and litter inputs. Between 5 to 25 % of photosynthetically fixed CO_2 is exuded as rhizodeposits (Helal & Sauerbeck 1984) and strongly impacts the microbial community structure, activity and SOM mineralization (Phillips *et al.* 2011; Fontaine *et al.* 2011). Increased SOM mineralization induced by labile C input to the soil (the "priming effect") is increasingly recognized as quantitatively significant in soil C-dynamics (Fontaine *et al.* 2007; Kuzyakov *et al.* 2009).

The mechanisms and controls of SOM priming are still a matter of debate (Kemmitt *et al.* 2008; Paterson 2009) and impacts of eCO_2 are difficult to anticipate (Billings *et al.* 2010). eCO2 has been reported to increase microbial biomass size (Martin-Olmedo *et al.* 2002; Paterson *et al.* 2008) and to support SOM priming (Martin-Olmedo *et al.* 2002; Lagomarsino *et al.* 2006). However, eCO_2 and facilitated C turnover do not necessarily lead to net soil C loss because plant-derived C can also be stabilised within the soil matrix and immobilized within the microbial community (Lagomarsino *et al.* 2006; Gude *et al.* 2012). Microbial community composition is a determinant of the magnitude of priming (Garcia-Pausas & Paterson 2011), and activities of fungi and actinomycetes have been implicated in increased rates of priming compared to those of the bacterial community (Carney *et al.* 2007; Garcia-Pausas & Paterson 2011).

Here we present a laboratory incubation experiment to investigate long term impacts of eCO_2 in combination with drought and temperature on the soil microbial community and connected SOM mineralization. Potential priming was assessed by adding C-13 labelled glucose to soil samples from a temperate heath that has been exposed to climatic manipulations for six years. We hypothesize that (H1) microbial C turnover in soils treated with eCO_2 show an increased utilization of SOM-derived C in the presence of labile C (priming) and (H2) that eCO_2 induces a change in activity of different microbial functional groups associated with higher SOM mineralization rates.

Materials and methods

Soil collection and processing

Soil was collected in March 2012 from a temperate heath-land exposed to climatic manipulation for six consecutive years including elevated CO2 (eCO2 at 510 ppm) by Free-air carbon enrichment (FACE), extended summer drought (D, 4 weeks) and increased night time temperature (T, 1 °C) (Mikkelsen et al. 2008). The full factorial combination simulates the climatic conditions predicted for Denmark in 2075 (Mikkelsen et al. 2008). Each treatment is replicated six times. Samples were taken from five different treatments (Table 1). Four soil cores (Ø 1.5 cm, 10 cm deep) were taken below the grass *Deschampsia flexuosa* L. and bulked together. Soils were transported to the UK (The James Hutton Institute, Aberdeen) within 24 hours and stored at 4 ℃ until further processing within one week. Soil was sieved (2 mm) and the soil gravimetric water content (SWC) was determined by oven drying.

Microcosm incubation and treatments

In each microcosm (0.5 litre glass jar) the soil was divided into 3 separate compartments (2 x 15 g, 1 x 70 g soil) to facilitate sequential sampling during the experiment, without causing soil disturbance by sampling one compartment per harvest. Two sets of microcosms (treatment and control) were prepared from each field treatment sampled. All microcosm units were packed to a bulk density of 1 g dry soil cm-3 which is close to field bulk density. Samples were adjusted to the same SWC (16.4 % on dry soil basis), which was maintained throughout the experiment by addition of deionised water (dH_2O). Microcosms were incubated at 8 ℃ within a controlled-environment room; all experimental manipulations and sampling were conducted at this temperature.

An acclimatisation period (addition of water only) of 18 d was imposed, during which soil CO2 efflux rates stabilised following the disturbance of microcosm preparation. Following the acclimatisation period, glucose was applied to half of the microcosms (glucose treatment) at a rate of 200 μg C g-1 dry soil per day (3 atom% excess: APE, uniformly labelled C-13-glucose dissolved in dH2O, Sigma Aldrich) for 6 consecutive days where day 1 was the first day of glucose addition. Control microcosms received dH_2O only. Soil harvests took place at days 0, 6 and 14.

Soil CO_2 efflux

Measurements of soil CO_2 efflux rates were conducted at days 1, 2, 3, 4, 6, 7, 9, 11 and 14. For CO_2 accumulation, microcosms were tightly sealed (plastic sealing strip, Terostat VII) and microcosm headspace was flushed with CO_2 free air for 4 min, reducing CO_2 concentrations to ≤ 10 ppm. Then, after 6 hours incubation, CO_2 concentrations were immediately measured (EGM-4, PP-Systems, Amesbury, USA) and $\delta^{13}C$ isotopic values were determined from microcosm head space samples stored in 12 ml N_2 flush-filled Exertainer® vials (GasBenchII, Delta^{PLUS} Advantage IRMS, Thermo Finnigan, Bremen, Germany). The EGM-4 was not calibrated for ^{13}C but the bias on CO_2 concentration was assumed to be negligible.

Analyses

Soil microbial biomass C (MBC) was assessed with chloroform fumigation. Paired 5 g soil samples were either fumigated with chloroform over night or immediately extracted (1:5 w:vol) with 0.5 M K2SO4 (Vance et al. 1987). Fumigated samples were treated the same way one day later. Total organic C of the extracts was determined (TOC Analyser, model 700, Corporation College Station, XT). MBC was calculated from the difference in C between fumigated and non-fumigated soils using a k_{EC} factor of 0.45 (Joergensen 1996).

Isotopic values of the MBC were determined from K2SO4 extracts as described in Garcia-Pausas & Paterson (2011) using wet oxidation. Headspace CO2 was transferred to N2 flush-filled 12 ml Exertainer® vials in a closed loop via a peristaltic pump (Midwood *et al.* 2006). Samples were measured on the same GasBenchII system as described above.

Table 1: Overview of climate treatments: soil temperature (5 cm soil depth), soil water content (SWC) in the field and before the experiment and soil C content. Field data are from the day of soil sampling. Values are means ± SE.

Treatments	Abbreviations	Soil temp (° C)	SWC (vol %)		Soil C content (%)
		Field	Field	Experiment	Experiment
Ambient	ambient	4.4 ± 0.1	19.1 ± 1.6	11.2 ± 0.4	1.3 ± 0.1
Elevated CO_2	eCO_2	4.5 ± 0.1	18.4 ± 1.7	11.7 ± 0.9	1.3 ± 0.1
Summer droughts * eCO_2	DCO_2	4.6 ± 0.1	20.4 ± 1.5	12.5 ± 0.5	1.4 ± 0.1
Increased temperature * eCO_2	TCO_2	4.7 ± 0.1	17.7 ± 0.4	12.3 ± 0.6	1.4 ± 0.1
Temperature*drought*eCO_2	future	4.7 ± 0.1	18.8 ± 1.7	12.3 ± 1.0	1.4 ± 0.1

Glucose- and SOM derived C

The proportions of glucose- and SOM-derived C in gas samples were calculated using a two end-member-mixing model:

$$P_{glucose} = \frac{\delta^{13}C_{sample} - \delta^{13}C_{control}}{\delta^{13}C_{glucose} - \delta^{13}C_{control}}$$

where $P_{glucose}$ describes the proportion of glucose-derived CO_2 of the microcosm headspace, $\delta^{13}C_{sample}$ is the measured isotopic value of the head space gas of microcosms with glucose addition and $\delta^{13}C_{control}$ without glucose addition. $\delta^{13}C_{glucose}$ is the isotopic value of the added glucose solution, assuming that no isotopic fractionation takes place during glucose mineralization. Glucose- and SOM-derived CO_2 effluxes were calculated from the proportional contribution of each source, multiplied by the total soil CO_2 efflux.
Proportions of C sources (glucose or SOM) in MBC were calculated using the isotopic values of the MBC. Glucose-derived C in the microbial biomass was calculated as:

$$MBC_{glucose} = \frac{(P_{glucose} * C)_F - (P_{glucose} * C)_{NF}}{k_{EC}}$$

where $MBC_{glucose}$ is the amount of glucose that was incorporated into the microbial biomass, C is the total MBC of fumigated (F) and non-fumigated (NF) soils with corresponding $P_{glucose}$ values for fumigated and non-fumigated soils. The amount of primed SOM was calculated by subtraction of SOM-derived CO_2 in the control treatments from the SOM-derived CO_2 in glucose treated soil. The amount of primed SOM in MBC was calculated the same way.

Phospholipid fatty acid extraction and analysis

Phospholipid fatty acids (PLFAs) were extracted from ~3 g freeze dried soil of glucose treatments at days 0, 6 and 14. A one-phase mixture ($CHCl_3$:MeOH:buffer) according to a modified Bligh and Dyer protocol was used (Bligh & Dyer, 1959; Frostegård *et al.* 1991). PLFAs were eluted with methanol on pre-packed silica columns (Isolute SI-100 mg 10 ml^{-1}, Biotage, Uppsala, Sweden). Internal standard C17:0 (Sigma-Aldrich, Munich, Germany) was added to the samples before mild

alkaline-methanolysis and the separated fatty acid methyl esters (FAMEs of PLFAs) were solubilised in hexane.

FAMEs were analyzed via gas-chromatography-combustion-isotopic ratio mass spectrometry (GC-c-IRMS) using a GC HP6890 containing a fused silica column (Varian FactorFour WCOT, 0.25 mm id x 60 m coated with VF-23 ms at a thickness of 0.25 μm) with helium as carrier gas (1 ml min^{-1}). The GC was coupled via a GC combustion interface (Thermo Scientific, Bremen, Germany) in continuous flow mode with a Finnigan Delta^{PLUS} isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). The oxidation reactor on the interface was maintained at 940 °C, the reduction reactor at 650 °C.

Samples were injected (1 μl) at 240 °C using splitless mode. GC oven temperature program started at 50°C hold for 2 min, ramped at 15 °C min^{-1} to 100 °C, then at 2 °C min^{-1} to 220 °C and finally at 15 °C min^{-1} to 240 °C where the final temperature was hold for 5 min. Separated compounds were measured against a CO_2 reference gas calibrated against certified reference material, and reported versus Vienna PeeDee belemnite (VPDB) reference material. FAMEs were identified by relative retention time comparing samples with a FAME standard mix (Supelco 37 component FAME mix, 47885-U, Sigma Aldrich). PLFA-C concentrations were calculated relative to the concentration of the internal standard C17:0. All $\delta^{13}C$ values were corrected for the additional C during methanolysis:

$$\delta^{13}C_{CFA} = \frac{[(N_{FA}+1)\delta^{13}C_{FAME} - \delta^{13}C_{MeOH}]}{N_{FA}}$$

where N_{FA} refers to the number (N) of C-atoms of the fatty acid (FA) component, $\delta^{13}C_{FAME}$ is the measured $\delta^{13}C$ value of the FAME after methylation and $\delta^{13}C_{MeOH}$ is the $\delta^{13}C$ value for the methanol used for methanolysis (-37.7 ‰ ± 3.23 ‰).

Soil microbial community composition was investigated using relative PLFA abundance (mol%) and relative glucose-derived ^{13}C incorporation into each individual PLFA (%). The following PLFA designations were used: gram-positive bacteria 15:0a, 15:0i, 16:0i, 17:0a, 17:0i, gram-negative bacteria 17:0cy, 19:0cy, 16:1ω7, 18:1ω7, actinomycetes 10Me16:0, 10Me17:0, 10Me18:0 and saprotrophic fungi 18:1ω9c, 18:2ω6 and 18:3ω3 (Kroppenstedt 1985; Frostegård & Bååth 1996; Zelles 1999). No PLFAs specific for mycorrhizal fungi were observed with the described GC-c-IRMS setup. Total amounts of extracted specific PLFAs

plus the unspecific PLFAs 16:0 and 18:0 were a second measure of total microbial biomass C (μg PLFA-C g^{-1} dry soil).

Statistical analyses

Statistical analysis was carried out in R version 12.2.1. Data were tested for normality (Shapiro-Wilk test of normality) and homogeneity of variances (Levene test of equality of variances) prior to analysis. If data showed no homogeneity of variances models were expanded with a 'weights=varIdent' structure. A linear mixed-effects model 'lme' was used for all repeated measure analyses ('correlation=corCAR1' structure) including random effects (Mikkelsen *et al.* 2008) and climate treatments as fixed effects (Table 1). Tukey HD test (as part of the general linear hypotheses 'glht') was used to test for differences among means.

The impact of glucose addition on SOM-derived soil CO_2 efflux was tested separately for each climate treatment using a paired t-test. Effects of different field climate treatments on total SOM-derived CO_2 in control plots and glucose amended plots, glucose-derived soil CO_2 efflux, primed SOM carbon, the ratio of primed SOM carbon to glucose-derived C, SOM-derived and glucose-derived MBC, total and priming-derived MBC were tested using the linear mixed-effects model structure.

Principal component analyses (PCA) was performed with the R-package 'prcomp' that uses a covariance matrix. Standardized PLFA data on relative abundance and relative community activity were tested. Scores (representing the variation within treatments) of each PCA were extracted and used in the 'lme' model as described above. Scores of the principle components 1 (PC1) and 2 (PC2) were tested individually. Analyses were performed with all treatments and measurements.

Results

All measurements were conducted for all the five treatments (Table 1) to evaluate the impact of imposed future climatic condition in the field on potential SOM priming under controlled laboratory conditions. Results from the two-factor treatments DCO_2 and TCO_2 have been included in the statistical analysis to explain possible drought and temperature interactions within the eCO_2 environment. However, for a clear presentation, we will focus only on the results for the three soil treatments ambient, eCO_2 and future and results of the two factor treatments DCO2 and TCO2 are available as supplementary material.

Microbial biomass C

Total microbial biomass C (MBC) increased from day 0 to day 6 in response to the glucose treatment (Table 2, Table S2), and entirely in the form of glucose-derived C (see equation for MBC glucose). Glucose addition had no effect on SOM-derived MBC. Glucose-derived MBC at the end of the glucose amendment (day 6) were not significantly different between treatments, but showed a tendency to be highest in ambient soil (169 ± 69 μg C) and lowest in future soil (83 ± 27 μg C).

MBC decreased after stopping glucose additions ($p < 0.001$) (Table 2): SOM-derived MBC stayed constant whereas glucose-derived MBC decreased. Total PLFA abundances suggest that there was no change in the microbial biomass size over time (Table 2, Table S2).

Soil CO_2 efflux rates and SOM priming

Glucose amendment increased total CO_2 efflux from all treatments (Table 3, Table S1). Glucose-derived CO_2 showed a steady increase during the period of glucose addition (Fig. 1). The amount of glucose-derived CO_2 decreased gradually after glucose amendment ceased. There were no differences in glucose-derived CO_2 between climate treatments ($p = 0.98$). Total CO_2 production was on average lower in eCO_2 soil than in ambient and future soils for both, control and glucose amended microcosms (Table 3).

Table 2: Soil organic matter (SOM)-derived and glucose-derived microbial biomass carbon, total PLFA-C concentrations and relative PLFA abundances of microbial functional groups as affected by ambient, eCO_2 and future treated soils over time. Results are means ± standard error of 6 field replicates. Different letters indicate differences of SOM-derived or glucose-derived MBC development for treatments over time. Values are means ± SE.

		Day 0			Day 6			Day 14		
		Ambient	eCO_2	Future	Ambient	eCO_2	Future	Ambient	eCO_2	Future
*Microbial biomass C (µg C * g^{-1} dry soil)*										
Control	SOM	236 ± 18 **a**	235 ± 38 **a**	253 ± 33 **a**	222 ± 22 **a**	213 ± 41 **a**	247 ± 34 **a**	42 ± 20 **b**	36 ± 30 **b**	53 ± 17 **b**
Glc add	SOM	255 ± 21 **a**	246 ± 35 **a**	283 ± 33 **a**	234 ± 26 **a**	223 ± 39 **a**	231 ± 33 **a**	22 ± 9 **b**	58 ± 24 **b**	65 ± 19 **b**
	Glucose	-	-	-	169 ± 54 **a**	138 ± 69 **a**	83 ± 27 **a**	31 ± 13 **b**	59 ± 13 **b**	64 ± 14 **b**
	Total	255 ± 21 **a**	246 ± 35 **a**	283 ± 33 **a**	572 ± 118 **b**	498 ± 147 **b**	397 ± 73 **b**	85 ± 31 **c**	177 ± 35 **c**	192 ± 39 **c**
Total PLFA content (µg PLFA-C g^{-1} dry soil)										
Glc add	Total	9.3 ± 0.4 **a**	10.3 ± 0.7 **a**	10.0 ± 0.7 **a**	11.9 ± 1.0 **b**	12.3 ± 1.3 **b**	11.7 ± 1.0 **b**	12.6 ± 0.8 **b**	11.7 ± 1.0 **b**	11.4 ± 0.6 **b**
Relative PLFA abundance for microbial functional groups (mol%)										
Gram-negative		22.6 ± 2.3 **a**	22.7 ± 1.7 **a**	20.8 ± 0.8 **a**	23.9 ± 0.8 **a**	23.7 ± 1.5 **a**	21.0 ± 0.6 **a**	25.7 ± 0.7 **a**	24.0 ± 0.8 **a**	26.6 ± 1.1 **a**
Gram-positive		15.7 ± 0.5 **b**	16.2 ± 1.2 **b**	18.1 ± 0.9 **b**	14.3 ± 0.7 **b**	14.4 ± 1.2 **b**	15.5 ± 0.6 **b**	12.7 ± 0.5 **b**	12.9 ± 0.3 **b**	14.4 ± 0.8 **b**
Actinomycetes		5.2 ± 0.4 **c**	6.3 ± 1.7 **c**	5.1 ± 0.5 **c**	3.4 ± 0.2 **c**	4.2 ± 0.2 **c**	4.3 ± 0.2 **c**	4.2 ± 0.6 **c**	4.2 ± 0.6 **c**	4.2 ± 0.3 **c**
Fungi		27.1 ± 1.3 **a**	25.9 ± 0.8 **a**	26.7 ± 0.3 **a**	26.3 ± 0.7 **a**	24.8 ± 0.7 **a**	27.1 ± 0.8 **a**	24.1 ± 0.6 **a**	22.6 ± 3.1 **a**	21.8 ± 2.5 **a**

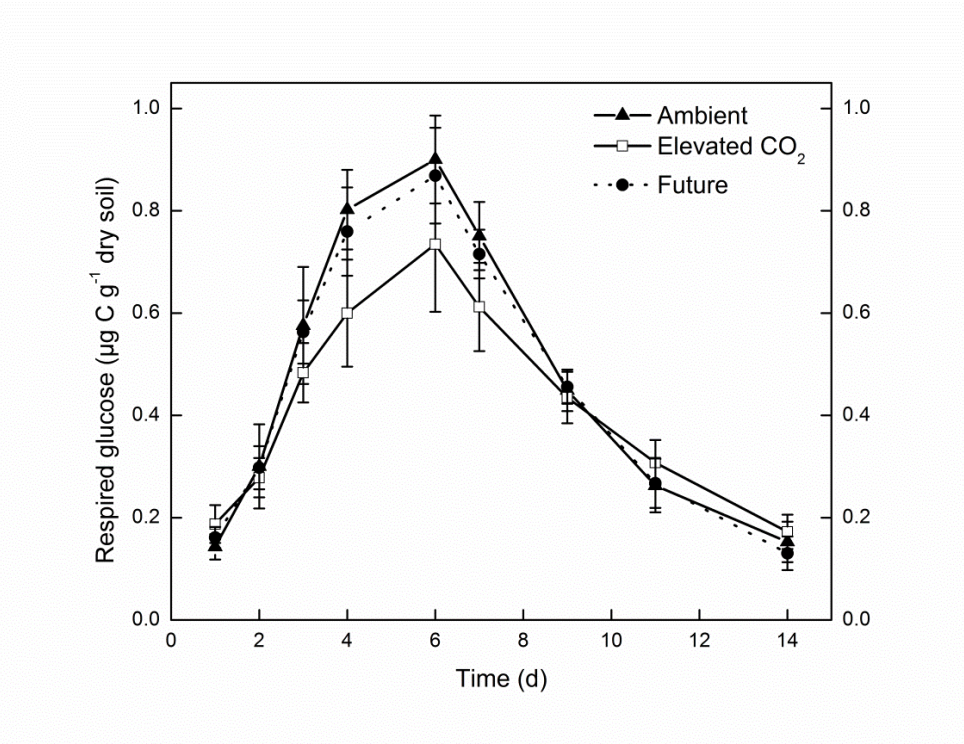

Figure 1: Glucose-derived CO_2 over time.

Total amounts of SOM-derived CO_2 efflux over the experimental period were significantly increased in ambient (p = 0.042), eCO_2 (p = 0.005), DCO2 (p = 0.024) and TCO2 (p = 0.046) soils when glucose was added, but not for the future soil (p = 0.61) (Table 3, Table S1). This release of additional SOM-derived CO_2 indicates SOM priming due to labile C addition and constituted up to 0.02 µg CO_2-C g^{-1} h^{-1} (Fig. 2). Negative values for primed SOM-derived CO_2 indicate that labile C addition reduced mineralization of SOM. The ratios of SOM- to glucose-derived CO_2 effluxes showed a sharp decrease from day 1 to day 2 for all treatments (Fig. 3, Table S1) followed by a close to zero constant ratio until day 9, when the ratio increased to 0.05 or more during the remaining part of the incubation.

Table 3: Total amounts of accumulated soil organic matter (SOM)-derived and glucose-derived CO_2 throughout the experimental period (µg C g^{-1} dry soil) for ambient, eCO_2 and future treated soils. P-values (paired t-test) indicate differences in SOM-derived CO_2 accumulation between controls and glucose treated soils. (Values as means ± SE).

	SOM-derived			Glucose-derived
	Control	Glc add	p-value$_{priming}$	
Ambient	6.8 ± 1.3 **a**	7.3 ± 1.2 **b**	0.043	26.0 ± 1.5 **c**
eCO_2	5.5 ± 0.5 **a**	6.2 ± 0.6 **b**	0.005	22.9 ± 2.4 **c**
Future	7.0 ± 0.7 **a**	7.1 ± 0.6 **a**	0.61	25.3 ± 1.3 **c**

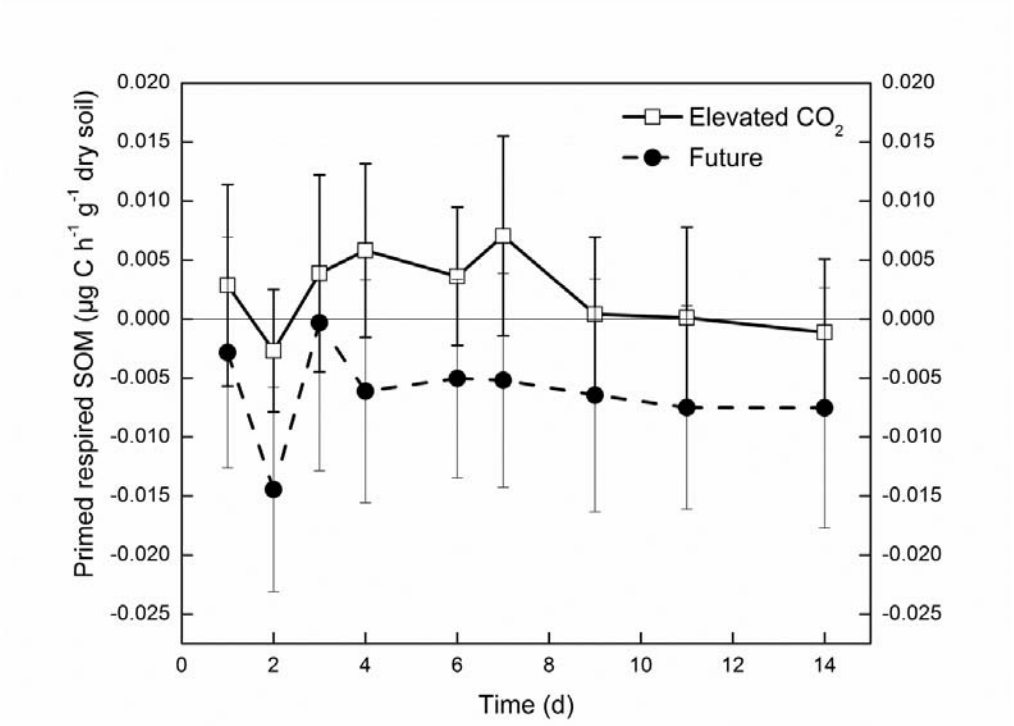

Figure 2: Primed SOM-derived CO_2 (daily measures) for eCO_2 (open squares) and future (solid circles) soils compared to ambient soils (thin continuous line) over time. Comparison to the ambient treatment using error propagation.

Microbial community structure and C utilization pathways

Total PLFA concentrations increased with glucose additions ($p < 0.001$) independent of climate treatments ($p = 0.570$) (Table 2, Table S2). The mol% fractions of specific functional groups were not affected by glucose additions, treatments or time (Table 2). Gram-negative specific PLFAs accounted for 23.4 ± 0.6 mol% whereas gram-positive bacteria were only half as abundant (15.1 ± 0.3 mol%). The relative abundance of actinomycetes was lowest (4.4 ± 0.4 mol%) and fungi specific PLFAs accounted for 25.6 ± 0.8 mol%.

Glucose-derived 13C distribution in the PLFA profile showed significantly different C allocation patterns for the eCO2 soil compared to ambient and future soils at days 6 ($p = 0.03$) and 14 ($p < 0.0001$). PCA (Fig. 4, Table S3) shows a separation of gram-positive bacteria (16:0i, 15:0a, 15:0i and 17:0i) and decomposers (actinomycetes: 10Me17:0, 10Me18:0; fungi: 18:1ω9, 18:3ω3) on PC1 that were assigned to eCO2 soil on one side and ambient and future soils on the other side. Activity of gram-negative bacteria (17:0cy, 16:1ω7) separate from gram-positive bacteria and decomposers on PC2

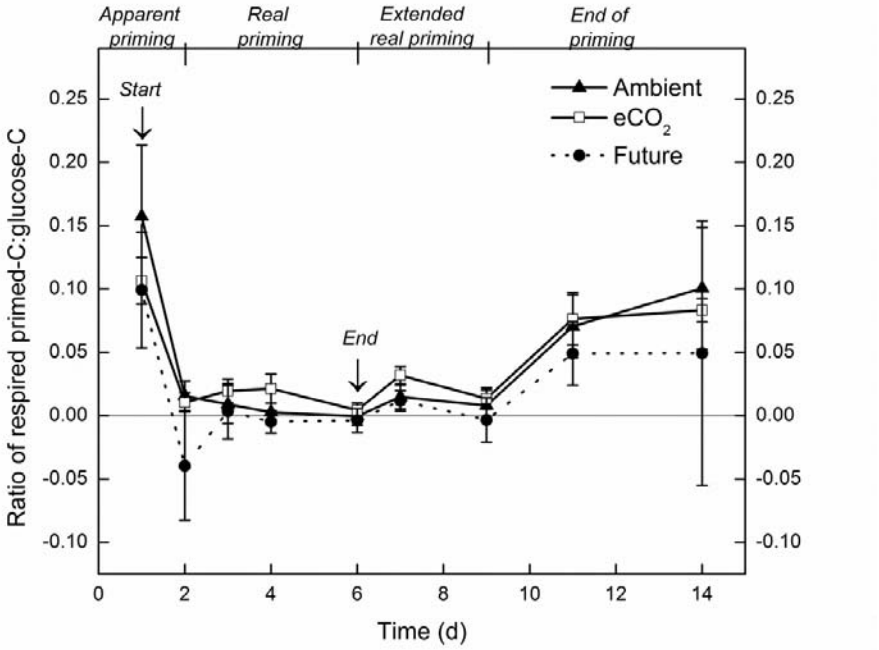

Figure 3: Ratio of primed SOM-derived and glucose-derived carbon over time. Arrows indicate start and end of daily glucose additions. Phases of priming above the graph are related to specific time frames.

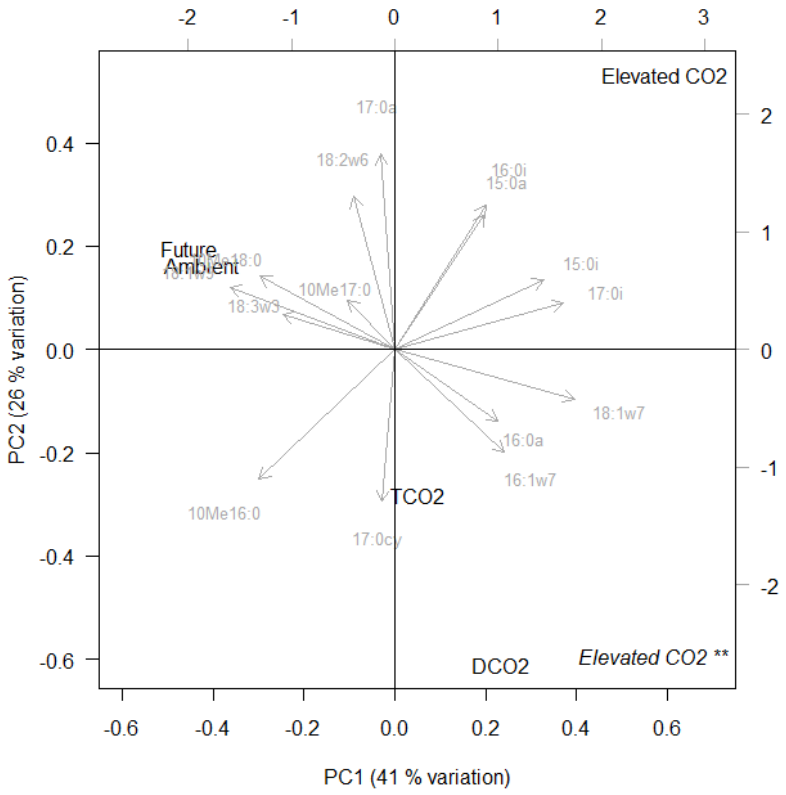

Figure 4: Principle component analysis of ^{13}C incorporation into individual PLFAs as affected by climate treatments at day 6. Results are plotted means for relative glucose-derived ^{13}C distribution (%).

Discussion

Glucose-induced priming

We investigated how 6 years exposure of a temperate heath-land soil to predicted future environmental conditions affected the extent to which priming processes affect rates of SOM mineralization. Glucose was used as the C-substrate to induce priming (De Nobili *et al.* 2001; Mondini *et al.* 2006), consistent with many previous studies, as it is utilized ubiquitously by soil microbes (Paterson *et al.* 2007; Blagodatskaya & Kuzyakov 2008; Fontaine *et al.* 2011). As a labile C compound that stimulates microbial activity, it is a relevant model substrate for root exudates and to simulate plant-mediated impacts on SOM mineralization, under tightly controlled experimental conditions (Kuzyakov 2010). Here, using C13-glucose as a model substrate facilitated quantification of impacts on SOM mineralization and the determination of C-flux pathways through components of the microbial community, as functions of previous exposure of the heath-land soil to future climate conditions.

Basal CO_2 efflux

Rates of SOM-derived mineralization, over the course of the experiment (Table 3), were less than in many previous studies (e.g. Garcia-Pausas & Paterson 2011) suggesting low activity of the microbial community, but was consistent over treatments (Table 3). Low microbial activity was likely a result of the low soil temperatures in this study. The lack of significant treatment effects on basal SOM mineralization are supported by identical microbial biomass sizes (Table 2, day 0) and community composition (Table 2, day 0).

Glucose utilization

Respiration of glucose-C increased during the period of glucose additions and declined after glucose amendment was stopped (Fig. 1). The response shows activation of the microbial community across all treatments as a response to labile C addition (Table 2). Glucose amendment affected rates of SOM mineralization in all treatments (Fig. 2). The magnitude of priming was small (< 15 % increase in SOM mineralization) relative to many previous studies, some of which have reported priming effects greater than 300 % (Zhu & Cheng 2011).

Microbial activity and related substrate mineralization are influenced by temperature (Kirschbaum 2006; Conant *et al.* 2011). SOM priming processes are often studied under temperatures higher than is common for temperate soils (Mondini *et al.* 2006; Fontaine *et al.* 2011; Garcia-Pausas & Paterson, 2011), potentially increasing microbial activity and sensitivity to labile C input, accelerating SOM decomposition. The observed lower rates of priming in the current study may be a result of suppression of glucose mineralization due to low, but more realistic soil temperature (Table 1).

Priming effect

SOM priming was observed in ambient soil and soil previously exposed to eCO_2 (Table 3, Fig. 3) as displayed in increased cumulative SOM-derived CO_2 in glucose amended soil (Table 2). The within-treatment variability of glucose induced priming was reduced when the magnitude of priming was expressed relative to the rate of glucose mineralization (Fig. 3). This strongly supports that there is a direct interaction between the utilisation of labile substrate and the process of microbial mineralization of SOM. This presentation of results also allows clearer identification of specific states of SOM mineralization as response to added glucose. Initially (day1), a priming response to glucose addition was seen in all treatments (Fig. 3). Such rapid responses of SOM-derived CO_2 efflux are characteristic of 'apparent priming' where pool-substitution of unlabelled C in the microbial biomass with labelled substrate is quantitatively significant (Blagodatskaya & Kuzyakov 2008). Apparent priming is followed by a phase of real priming during glucose additions presumably as a result of increased utilization of the added labile glucose. After glucose additions were stopped, an extended period of priming was observed. The last stage shows SOM respiration approaching initial ratios and the end of glucose-mediated priming.

In previous studies, priming of SOM was discussed to be driven by eCO_2 (Carney *et al.* 2007; Briones *et al.* 2009) and the change of soil C storage was explained by a shift of the microbial community from bacteria to fungi domination in a scrub-oak ecosystem (Carney *et al.* 2007). In contrast, the current study shows a high fungal activity in ambient and future soils (Fig. 4) while the latter doesn't show SOM priming. However, the overall tendency of ambient and future soils to respire more SOM-derived (Table 3) suggests that increased fungal dominance may be mirrored by increased soil CO_2 efflux. Dependent on soil dynamics, a decomposer dominating microbial community can be responsible for decreased SOM storage. Gude *et al.* (2012) suggested

fungal dominance in soils as a general important driver for decreasing SOM mean residence times.

Pathways of glucose utilisation

Glucose amendments induced a doubling of the microbial biomass (Table 2, day 6) where 20 – 30 % of the MBC was glucose-derived. No change in relative abundance within the microbial groups was observed (Table 2), but principle component analysis of the fate of C revealed that eCO_2 soil separated from ambient and future soils by the activity of gram-positive bacteria vs. the decomposer groups: actinomycetes and fungi (Fig. 4). eCO_2 treated soil had a tendency towards smaller soil CO_2 efflux rates, probably due to relatively low activity of decomposer organisms (fungi and actinomycetes). Potentially, increased belowground C allocation under eCO_2 (Drake *et al.* 2011) may not necessarily lead to a higher SOM mineralization activity, but can also lead to C sequestration (Lagomarsino *et al.* 2006).

Increased activity of gram-positive bacteria in the eCO_2 soil could have caused less soil CO_2 efflux i.e. gram-positive bacteria outcompeted decomposers for labile whereas active fungi and actinomycetes observed in ambient and future soils potentially resulted into higher soil respiration rates (Fig. 4; Carney *et al.* 2007). This soil CO_2 efflux pattern is in agreement with the observed C turnover in a grassland labelling study manipulating CO_2 concentration and nitrogen availability where they showed increased belowground C allocation but a decreased C decomposition leading to an overall decrease of C turnover (Loiseau & Soussana 1999). Additionally, the same study showed a reduced belowground C allocation but increased SOM mineralization (potentially SOM priming) when eCO_2 was combined with increased temperature. Effects of eCO_2 on soil microbial activity seem to be changed when combined with other climate change factors as seen in our future soil.

Different activities of microbial functional groups between climate treatments can be a result of water limitation. Adequate soil moisture conditions allow the microbial community to be abundant and active (eCO_2), but low soil moisture conditions discriminate against the bacterial part of the community and facilitate fungal activity (ambient and future). Soil moisture content also modulates nutrient availability, as high soil moisture results in good nutrient distribution (eCO_2), whereas low soil moisture contents require local nutrient mineralization processes to maintain the required nutrient supply (ambient and future). Even though water content was kept constant during the incubations,

we assume that our climatically treated field soils show a long term memory (Selsted *et al.* 2012). In the presented study, future soil treatment included long-term manipulations of soil water content (summer droughts) and is the treatment without observed SOM priming indicating that the 'soil-memory' drives SOM mineralization processes under labile C input. As shown by Marhan *et al.* (2010), soil C loss rather than C sequestration was predicted in eCO_2 treatments when soil moisture was included into their model.

Considering our hypothesis (H1), the eCO_2 only soil showed a significant increased respiration of SOM-C (priming), but in combination with summer drought and increased temperature this increase could not be shown for the future climate scenario. However, this result can be misleading. Observed basic SOM-derived respiration rates tend to be slightly (although not significantly) lower in the eCO_2 soil probably due to ecosystem acclimatization leading towards a soil C-storage potential as in agreement with the study of Marhan *et al.* (2010). Under ambient conditions, plants modulate C allocation as a response to current climatic conditions but soils showed priming of SOM, too. The future climate simulation didn't show priming of SOM presumably because positive effects of eCO_2 and negative effects of drought and increased temperature are counteracting presumably as a function of water availability.

Observed SOM-priming in ambient and eCO_2 soils can partly be assigned to fungal abundance in soil (Table 2) which is in accordance with previous studies (H2) (Carney *et al.* 2007; Gude *et al.* 2012) but, fungal activity in eCO_2 soil was different than under ambient conditions. Future soil exhibited an active fungal community but didn't show SOM-priming possibly due to climate treatment interactions and feedback mechanisms affecting rates of SOM-priming not displayed in the microbial community composition on the basis of PLFA data. Our data suggest that climatic changes (i.e. elevated CO_2 concentration, precipitation, warming) affect the C-flux through the soil microbial community and therewith soil processes. SOM-mineralization might be influenced by single climatic factors mediated by soil microbial community composition and activity but interactive effects of climate factors on ecosystem processes might have opposing effects on ecosystem C-turnover (Larsen *et al.* 2011; Dieleman *et al.* 2012).

Acknowledgements

We thank Noemí Martín and Allan Sims for their assistance with the laboratory work. The CLIMAITE experiment is sponsored by the Villum Kann Raasmussen Foundation with co-funding from Air Liquide, DONG Energy and SMC Pneumatic A/S. The project was supported with an Exchange Grant from the European Science Foundation (ESF) within the framework of the ESF activity 'Climatic Change - Manipulation Experiments in Terrestrial Ecosystems' and a travel grant from the "Nordic Network for Stable Isotope Research" (NordSIR). The James Hutton Institute receives funding from the Scottish Government.

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Supplementary material

Table S1: Soil CO_2 efflux partitioned into glucose- and SOM-derived carbon of climatically treated soils in the field over time. Supplementary data of respired glucose-C to Figure 1 and supplementary data of primed-C:glucose-C to Figure 3. Respired SOM-C in controls and glucose treatments are qualitative measures to Figure 2. "Total" amounts are the sum of collected respired CO_2 throughout the experiment as addition to table 3; letters indicate significant differences between total SOM-derived C in control and glucose amended soils. Values as means ± SE.

	1	2	3	4	6	7	9	11	14	Total
Respired glucose-C (µg C g^{-1} dry soil)										sums
DCO2	0.13 ± 0.02	0.24 ± 0.02	0.53 ± 0.05	0.79 ± 0.09	0.87 ± 0.08	0.72 ± 0.06	0.49 ± 0.02	0.32 ± 0.05	0.16 ± 0.03	25.5 ± 1.3
TCO2	0.15 ± 0.02	0.28 ± .03	0.54 ± 0.07	0.70 ± 0.09	0.82 ± 0.09	0.71 ± 0.06	0.48 ± 0.04	0.31 ± 0.04	0.17 ± 0.02	25.1 ± 1.4
Respired SOM-C in controls (µg C g^{-1} dry soil)										
Ambient	0.14 ± 0.04	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.12 ± 0.02	0.13 ± 0.02	0.12 ± .02	0.11 ± 0.02	0.11 ± 0.02	6.8 ± 1.3 **a**
eCO2	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	5.5 ± 0.5 **a**
DCO2	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.004	0.11 ± 0.01	0.10 ± 0.01	0.09 ± .01	5.8 ± 0.3 **a**
TCO2	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	6.3 ± 0.4 **a**
Future	0.14 ± 0.02	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	7.0 ± 0.7 **a**
Respired SOM-C in glucose treatment (µg C g^{-1} dry soil)										
Ambient	0.16 ± 0.03	0.14 ± 0.04	0.13 ± 0.03	0.13 ± 0.02	0.12 ± 0.02	0.14 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	7.3 ± 1.2 **b**
eCO2	0.13 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	6.2 ± 0.6 **b**
DCO2	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.005	0.14 ± 0.01	0.11 ± 0.005	0.12 ± 0.01	0.11 ± 0.002	6.2 ± 0.3 **b**
TCO2	0.14 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	6.8 ± 0.5 **b**
Future	0.15 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	7.1 ± 0.6 **a**
Ratio primed-C : glucose-C										
DCO2	0.20 ± 0.12	-0.03 ± 0.03	-0.01 ± 0.01	-0.002 ± 0.01	-0.01 ± 0.002	0.04 ± 0.01	0.01 ± 0.01	0.07 ± 0.02	0.10 ± 0.05	-
TCO2	0.07 ± 0.05	-0.01 ± 0.02	0.005 ± 0.02	0.01 ± 0.01	0.001 ± 0.004	0.02 ± 0.01	0.01 ± 0.005	0.08 ± 0.04	0.13 ± 0.04	-

Table S2: Soil organic matter (SOM)-derived and glucose-derived microbial biomass carbon in control and glucose treated (Glc add) soils at different time points for DCO2 and TCO2 treated soils. Relative PLFA abundance (in mol%) of microbial functional groups as affected by DCO2 and TCO2 over time. Results are means ± standard error of 6 field replicates (supplemental data to Table 2).

		Day 0		Day 6		Day 14	
		DCO2	TCO2	DCO2	TCO2	DCO2	TCO2
Microbial biomass C (µg C g-1 dry soil) (mean ± SE)							
Control	SOM	255 ± 19	250 ± 11	224 ± 20	219 ± 20	-	-
Glc add	SOM	256 ± 16	280 ± 18	233 ± 19	254 ± 16	248 ± 33	248 ± 24
	Glucose	-	-	-19 ± 37	135 ± 24	21 ± 7	36 ± 7
	Total	256 ± 16	280 ± 18	215 ± 52	289 ± 24	369 ± 38	284 ± 31
PLFA content (µg PLFA-C g-1 dry soil) (mean ± SE)							
		10.1 ± 0.3	10.2 ± 0.4	11.8 ± 0.6	12.2 ± 0.6	11.5 ± 0.6	11.5 ± 0.4
Relative PLFA abundance for microbial functional groups (mol%) (mean ± SE)							
Gram-negative		21.8 ± 1.4	24.3 ± 1.8	23.3 ± 1.0	23.7 ± 0.8	23.3 ± 1.0	23.7 ± 0.8
Gram-positive		16.1 ± 0.7	16.9 ± 1.1	14.7 ± 0.2	14.4 ± 0.9	14.7 ± 0.2	14.4 ± 0.9
Actinomycetes		4.4 ± 0.3	4.7 ± 0.4	3.7 ± 0.3	4.6 ± 0.4	3.7 ± 0.3	4.6 ± 0.4
Fungi		28.5 ± 0.6	25.8 ± 1.2	27.0 ± 0.5	25.3 ± 0.4	27.0 ± 0.5	25.3 ± 0.4

Table S3: Loadings from the principle component analysis (PCA) of the glucose-derived C distribution at day 6. Supplement to Figure 4.

Functional groups	PLFAs	PC1	PC2
Gram-negative	17:0cy	0.276	-0.328
	16:1w7	0.416	-0.100
	18:1w7	0.300	0.299
Gram-positive	15:0i	0.189	0.391
	15:0a	0.239	-0.459
	16:0i	0.194	0.216
	17:0i	0.328	0.195
	17:0a	0.138	-0.486
Actinomycetes	10Me16:0	-0.242	0.176
	10Me17:0	-0.085	-0.036
	10Me18:0	-0.273	-0.127
Fungi	18:1w9	-0.460	-0.101
	18:2w6	-0.015	-0.168
	18:3w3	-0.185	-0.066

ECO's research is aimed at assessing various energy technologies effects on the climate and the environment, including ways of improving nature and ecosystems adapt to new climatic conditions and promote a negative feedback between the atmosphere and the planet through land use.

DTU-KT

Centre for ecosystem and environmental sustainability (ECO)

Department of Chemical and Biochemical Engineering

Technical University of Denmark

Kongens Lyngby,

DK-2800, Copenhagen

Denmark

Phone +45 4677 4100

Fax +45 4677 4100

http://www.kt.dtu.dk/