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The effect of elevated atmospheric CO_2 on soil C and N dynamics and its feedback on CO_2 and N_2O emissions from a temperate grassland ecosystem

Results from a long-term Free Air CO_2 Enrichment (FACE) experiment

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ANPP	Aboveground NPP
C	Carbon
C_{def}	C saturation deficit
C_{sat}	C saturation
C_{new}	C input into SOC that has been fixed since the change in in $\delta^{13}C$ signature under eCO_2 in July 2004
CO_2	Carbon dioxide
DOC	Dissolved organic carbon
eCO_2	Elevated atmospheric CO_2 concentrations
FACE	Free Air CO_2 Enrichment
GHG	Greenhouse gases
Gi-FACE	Giessen Free Air CO_2 Enrichment
GPP	Gross primary production
LM	Large macroaggregates (>2000 µm)
MIC	Microaggregates (53-250)
MRT	Mean residence time
N	Nitrogen
N_2O	Nitrous oxide
NH_4^+	Ammonium

NH_3	Ammonia
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NPP	Net primary production
NUE	Nitrogen use efficiency
P	Phosphorus
PNL	Progressive nitrogen limitation
POM	Particulate organic matter
SC	Silt and clay (<53 μm)
SM	Small macroaggregates (250-2000 μm)
SOC	Soil organic carbon
SOM	Soil organic matter
SSOC	Stable soil organic carbon

Summary

Rising atmospheric CO_2 concentrations are affecting the cycling of carbon (C) and nitrogen (N) in ecosystems, which has the potential to alter the emissions of the stable greenhouse gases CO_2 and N_2O to the atmosphere. Despite the relevance of these processes to affect global warming current knowledge is fragmentary and relies mostly on short-term studies.

At the Giessen Free Air CO_2 Enrichment Experiment (Gi-FACE) the effect of +20% above ambient CO_2 concentration (corresponds to conditions reached 2035-2045) in a temperate grassland has been investigated since 1998. Consequently, observations from this site allow to investigate long-term effects of elevated CO_2 (eCO_2).

The main objective of the present work was to contribute to a better understanding of soil C and N dynamics under long-term eCO_2, which are governing the formation and emission of CO_2 and N_2O from a temperate grassland ecosystem. Towards this objective we assessed the seasonal effects of long-term eCO_2 on soil respiration (study I). We further elucidated the distribution of soil aggregate-size classes at different soil depths under eCO_2 (within 13.5 years) by physical fractionation, estimated the associated mean residence time (MRT) under eCO_2 by applying an isotope mixing model and measured the resulting soil organic carbon (SOC) content (study II). Moreover, we quantified N transformations via ^{15}N labelling and by applying a ^{15}N tracing model and measured the resulting N_2O emissions (study III).

The results of weekly soil respiration measurements for a period of three years (2008-2010) revealed a pronounced and repeated increase of soil respiration under eCO_2 during late autumn and winter dormancy. Increased CO_2 losses during the autumn season (September–October) were 15.7% higher and during the winter season (November–March) were 17.4% higher compared to respiration from ambient CO_2 plots. However, during spring time and summer, which are characterized by strong above- and below-ground plant growth, no significant change in soil respiration was observed at the Gi-FACE site under eCO_2. Further, a depth-dependent response of macroaggregation to eCO_2 was observed: While in subsoil (15–45cm depth) macroaggregation increased under eCO_2, no CO_2-induced change in macroaggregation was detected in topsoil (0–15 cm). MRT of SOC in aggregate-size classes were not different among each other under eCO_2. However, macroaggregates and bulk soil differed in their MRT between soil depths under eCO_2. Despite increased macroaggregation and an estimated high SOC sequestration potential in subsoil, we could not observe an increase in SOC content of bulk soil within 13.5 years of eCO_2.

Results from the ^{15}N study showed that the major source for twofold increases of N_2O emissions under eCO_2 was the oxidation of organic N followed by incomplete NO_2^- reduction. From these results we suggest that a CO_2-induced priming effect resulted in stimulated mineralization of soil organic matter (SOM) and fostered the activity of bacterial nitrite reductase, which was responsible for increased N_2O emissions.

To sum up, the present work showed a positive feedback of long-term eCO_2 in a temperate grassland on N_2O and soil CO_2 emissions which further accelerate global warming. This indicates that temperate European grasslands may gradually turn into greenhouse gas (GHG) sources with rising atmospheric CO_2 due to enhanced CO_2 losses during autumn and winter and increased N_2O emissions.

Zusammenfassung

Der zunehmende Anstieg atmosphärischer CO_2 Konzentrationen beeinflusst die Umsetzungsprozesse von Kohlenstoff (C) und Stickstoff (N) in unseren Ökosystemen, welches zu Rückkoppelungseffekten hinsichtlich atmosphärischer CO_2 und N_2O Konzentrationen führen kann. Trotz der Relevanz dieser Zusammenhänge und der beteiligten Prozesse hinsichtlich der Beeinflussung globaler Erwärmung, ist der aktuelle Wissensstand noch lückenhaft und beruht größtenteils auf Kurzzeitstudien.

Im Rahmen des Giessener Freiland-CO_2 Anreicherungsexperiments (Free Air CO_2 Enrichment; Gi-FACE) werden seit 1998 die Auswirkungen von +20% erhöhten CO_2 Konzentrationen (entspricht den Bedingungen, die 2035-2045 erwartet werden) in einem gemäßigten Grünlandökosystem untersucht. Somit bietet das Gi-FACE die Möglichkeit Langzeitstudien zu den Auswirkungen von erhöhten atmosphärischen CO_2 Konzentrationen zu untersuchen.

Das Ziel der vorliegenden Arbeit war es, zu einem besseren Verständnis von C- und N-Umsetzungsprozessen im Boden unter langzeitig erhöhtem CO_2 beizutragen, die für die Entstehung von CO_2- und N_2O-Emissionen aus einem gemäßigten Grünlandökosystem verantwortlich sind. Dazu wurde der jahreszeitliche Effekt von langzeitig erhöhtem CO_2 auf die Bodenatmung untersucht (Studie I). Weiterhin wurden die Effekte von erhöhtem CO_2 auf die Aggregatstruktur des Bodens in verschiedenen Bodentiefen über einen Zeitraum von 13,5 Jahren, anhand physikalischer Fraktionierung, untersucht, sowie der C-Umsatz mit Hilfe eines Isotopenmischungsmodells ermittelt und der organische C-Gehalt des Gesamtbodens sowie der Aggregatsklassen analysiert (Studie II). Darüber hinaus wurden in der ^{15}N-Markierungsstudie (Studie III), anhand eines angewandten Markierungsmodells, die N-Transformationen im Boden quantifiziert und die aus den verschiedenen Boden-N-Umsetzungsprozessen resultierenden N_2O-Emissionen gemessen (Studie III).

Über einen Zeitraum von 3 Jahren (2008-2010) mit wöchentlichen Messungen zeigten die Ergebnisse einen ausgeprägten und wiederholten Anstieg der Bodenatmung unter erhöhtem CO_2 im spätem Herbst und in der Vegetationsruhe an. Im Herbst war die Bodenatmung um 15.7% angestiegen, über die Vegetationsruheperiode um 17.4% im Vergleich zur Bodenatmung auf den Kontrollflächen. In den Frühlings- und Sommerperioden, die durch ein starkes Pflanzenwachstum charakterisiert sind, wurde hingegen keine signifikante Änderung der Bodenatmungsrate unter erhöhtem CO_2 festgestellt. Weiterhin wurde eine von der Bodentiefe abhängige verstärkte Makro-Aggregation unter erhöhtem CO_2 festgestellt: Während im

Unterboden (15-45 cm Tiefe) die Makro-Aggregation unter erhöhtem CO_2 zunahm, wurde keine CO_2-abhängige Veränderung der Makro-Aggregation im Oberboden (0-15 cm Tiefe) beobachtet. Der C-Umsatz unterschied sich nicht zwischen den verschiedenen Bodenaggregatsklassen unter erhöhtem CO_2. Allerdings unterschied sich der C-Umsatz beim Gesamtboden und bei den Makro-Aggregaten zwischen den Bodentiefen unter erhöhten CO_2. Trotz zunehmender Makro-Aggregation und eines ermittelten hohen C-Bindungspotentials des Unterbodens konnte keine Zunahme des organischen Kohlenstoffgehaltes des Gesamtbodens innerhalb 13.5 Jahren CO_2 Anreicherung festgestellt werden.

Ergebnisse der ^{15}N-Markierungsstudie zeigten, dass die Oxidation von organischen N gefolgt von unvollständiger NO_2^--Reduktion die hauptsächlichen Prozesse für die Verdoppelung der N_2O-Emissionen unter erhöhtem CO_2 im untersuchten Grünlandökosystem darstellen. Anhand der Ergebnisse schließen wir, dass, bedingt durch die CO_2-Anreicherung, eine angeregte Mineralisierung der organischen Bodensubstanz erfolgt, welches die Aktivität von bakterieller Nitritreduktase fördert und für die zusätzlichen N_2O-Emissionen verantwortlich ist.

Insgesamt zeigt die vorliegende Arbeit eine positive Rückkoppelung von langzeitig erhöhtem CO_2 auf N_2O- und CO_2-Emissionen eines gemäßigten Grünlandökosystems auf, die zu einer weiteren Erderwärmung beiträgt. Folglich deuten die Ergebnisse darauf hin, dass europäische gemäßigte Grünlandökosysteme sich eher zu Treibhausgasquellen entwickeln können aufgrund von erhöhten CO_2 Verlusten während Herbst und Winter und höheren N_2O-Emissionen mit zunehmenden atmosphärischen CO_2 Konzentrationen.

1 Introduction

Carbon dioxide (CO_2) concentrations in the atmosphere are steadily rising (Raynaud and Barnola, 1985; Moss et al., 2010; Monastersky, 2013) and CO_2, as the largest radiative forcing component (IPCC, 2013), causes global warming.

Moreover, elevated atmospheric CO_2 (eCO_2) is affecting cycling of carbon (C) and nitrogen (N) in ecosystems. This may impose feedback effects to climate change through altered rates of greenhouse gas (GHG) emissions from ecosystems and through changes in C storage within ecosystems.

Within the terrestrial biosphere, soil organic carbon (SOC) represents the largest pool of C and stores about 1500 Gt of C down to a depth of 1 m (Amundson, 2001). The potential of increasing the SOC pool is widely discussed in the scientific literature as a contribution to offset the rise in global atmospheric CO_2 concentrations (Stockmann et al., 2013; Minasny et al., 2017). On the other hand, limitations to soil C sequestration are debated i.e. nutrient constraints, particularly by N (Coskun et al., 2016) and that the SOC concentration can become saturated with respect to C input (Stewart et al., 2007). Further, for effective C sequestration it is relevant that additional C is allocated to pools that are stable over long-term scales (Paustian et al., 1997).

In contrast, soils have also received increasing attention as a potentially large and uncertain source of GHGs to the atmosphere in the future in response to eCO_2 (Wieder et al., 2015; Mystakidis et al., 2016). The release of GHGs from soil and indirect effects may offset climate mitigation effects of soil carbon sequestration. Besides carbon-based GHGs (CO_2, CH_4), nitrous oxide (N_2O) is another GHG, which derives largely from agricultural soils. Given its role as a climate-relevant gas with a global warming potential over a 100-year period of 298 and a steady increase in atmospheric concentration (IPCC, 2007), it is important to understand the processes and factors that control its production in particular under future CO_2-enriched atmospheres.

Because of its wide ranging appearance and high SOC content, grassland ecosystems were suggested to play an important role in the global C cycle. Further, grasslands under eCO_2 may provide mitigation services by increased C sequestration in soil thereby counteracting atmospheric CO_2 increases and therefore climate change (O'Mara, 2012).

In order to hold climate change below a warming threshold of 2°C according to the Paris agreement (UNFCCC, 2015), SOC sequestration and adequate management of soils have been discussed as a possible contribution to reduce GHG emissions (Minasny et al., 2017). However, due to complex interactions of biogeochemical cycles it is difficult to provide reliable estimates of how much C sequestration can realistically be achieved in soils and of possible feedback effects to eCO_2 affecting the GHG balance of soils. These uncertainties have implications for science and policy.

Elucidating the soil processes that control whether a soil will be a sink or source of GHGs to eCO_2 is therefore essential for developing effective soil management practices in climate mitigation plans and policies. Due to the interconnectedness of the C- and N- soil processes involved in their diverse GHG emissions it is important to include both C- and N-cycling in soil CO_2 budgets.

Especially long-term experiments that represent natural conditions and integrate potential feedback effects (i.e. shifts in the species composition) and nutrient interactions to eCO_2 are required to provide data for reliable process-based models and to verify existing models. Further, a better process understanding would improve current estimates in the National greenhouse gases Inventory.

To sum up, leveraging adequate management of soils for climate change mitigation will require a better understanding of the multiple processes under eCO_2 including C and N cycling relating to the release and storage of GHGs.

2 Literature overview on C and N dynamics and interactions under eCO_2

The following chapter presents an overview on the C and N dynamics at the soil-atmosphere interface of ecosystems under eCO_2. The state of knowledge on those key processes that are relevant for evaluating ecosystems in terms of their GHG balance is addressed.

Whenever available, results are presented from grassland studies. Chapter 2.1 describes the C dynamics under eCO_2 within ecosystems, while chapter 2.2 presents the interaction of C and N under eCO_2 which includes N_2O production pathways under eCO_2.

2.1 C dynamics under eCO_2

Rising levels of atmospheric CO_2 were shown to affect numerous processes within terrestrial ecosystems at various scales. The C cycle of an ecosystem is defined by several C pools and C exchanges between system components. The grassland C cycle involves three major pools: the atmosphere, soil and biomass. Soil and biomass pools are often separated into further pools with different characteristics. Due to the complexity of ecosystem C cycling not all relevant processes can be described in detail. Instead, the present work focusses on soil C dynamics, pools and the fluxes of CO_2 between the grassland soil and the atmosphere.

Soil organic carbon (SOC)

SOC is considered as the largest pool of C in the terrestrial biosphere (Jobbagy and Jackson, 2000). Especially grassland ecosystems were found to have a high belowground C allocation (Hungate et al., 1997). According to Lal et al. (2015) soil C sequestration is defined as the process of transferring CO_2 from the atmosphere into the soil through plants, plant residues and other organic solids, which are stored and retained as part of the soil organic matter (SOM) with differing mean residence times (MRT). The capacity of a soil to sequester C is determined by the net balance between soil C inputs and C losses through decomposition (Schlesinger, 1997; Amundson, 2001).

Further, research of the last decades has proposed that the SOC concentration has an upper limit (Stewart et al., 2007) which is referred to as SOC saturation and depends on the clay and silt content (Hassink, 1997; Six et al., 2002). Consequently, soil C sequestration may not be linear in response to soil C input (Gill et al., 2002; Kool et al., 2007). Taking this into account, it may be of interest to assess the respective C sequestration potential of a soil based on its specific C

saturation deficit (C_{def}) which is defined as the difference between the theoretical maximum SOC content (C_{sat}) of the mineral fraction and its current stable SOC (SSOC) content (Angers et al., 2011). Moreover, subsoils have been discussed as potential C sinks due to their unsaturated mineral surfaces (Schrumpf et al., 2013; Poirier et al., 2014). However, the C saturation deficit of subsoil horizons has rarely been estimated (Chen et al., 2018, Castellano et al., 2017; Reis et al., 2014).

2.1.1 Inputs to soil organic carbon under eCO_2

In a review of previous grassland studies Jones and Donnelly (2004) showed that eCO_2 influenced C input rates to the soil. In the following sections above- and below-ground processes of C input to ecosystems are described and the effects of eCO_2 on these processes.

Gross primary production

C is derived naturally by vegetation from the atmosphere through photosynthesis, also known at the ecosystem level as gross primary production (GPP) (Lorenz and Lal, 2018). Grassland GPP is controlled by atmospheric CO_2 concentration among many other factors (Chapin et al., 2002).

It is well established that eCO_2 can stimulate photosynthesis (Drake et al., 1997) and aboveground biomass growth with differing magnitudes of increases.

In a meta-analysis of FACE (Free Air CO_2 Enrichment) studies, Leakey et al. (2009) reported biomass increases of about 19–46% under eCO_2 and that in the longer term acclimation responses to eCO_2 were taking place. In various FACE grassland studies a positive biomass response trend was found across different climatic conditions, concentrations of eCO_2, nutrient fertilization intensities and management practices (Feng et al., 2015). Seasonal rainfall balance affected the biomass responses to eCO_2 in a Southern Hemisphere grassland (Tasmania, "TasFACE") (Hovenden et al., 2014).

For the Gi-FACE study site, Andresen et al. (2017) reported an increase in total aboveground biomass in response to eCO_2 by about 15%. A more modest increase of about 10% was found in a study with 13 grassland species (Lee et al., 2011).

In contrast, no biomass gains were found within 5 years of eCO_2 for a grassland in California (Jasper Ridge Global Change Experiment), irrespective of N supply. It was suggested that

phosphorus (P) limitations were responsible for this observation, since eCO_2 reduced total plant P uptake (Dukes et al., 2005).

Belowground carbon input

The Aboveground NPP (ANPP) of grasslands is a source for the inputs of belowground C. Moreover, grasslands translocate a large proportion of assimilates (30–50%) belowground through their roots (Kuzyakov and Domanski, 2000) and additional photosynthetic C under eCO_2 was shown to stimulate belowground biomass growth in a sward of *Lolium perenne* (Casella and Soussana, 1997) and at the Swiss FACE with *Trifolim repens* where soil C input was greater under eCO_2 (Nitschelm et al., 1997). In a grazed grassland (New Zealand pasture FACE) root production and turnover were greatly stimulated after 4 years exposure to eCO_2 (Allard et al., 2005).

Root biomass production has often been stimulated by eCO_2, especially in grasslands (Fitter et al., 1997; Jastrow et al., 2000; Higgins et al., 2002). However, some studies showed no effect or even reduced root biomass under eCO_2 (Kandeler et al., 1998; Arnone et al., 2000). Other studies found that eCO_2 resulted in more fine and secondary roots (Pregitzer et al., 1995; Treseder and Allen, 2000; Treseder, 2004; Arndal et al., 2018). A meta-analysis of Sillen and Dieleman (2012) reported that root biomass of grasses increased only when eCO_2 was combined with N fertilization.

Plant roots contribute to soil C not only through their decomposition, but also by rhizodeposition which consists of soluble root exudates, sloughed cells and tissue root fragments from root turnover (Jones et al., 2009; Nguyen, 2009). Allard et al. (2006) reported increased rhizopedosition after 4 years for isolated plants of *Lolium perenne* grown under eCO_2. They suggested that eCO_2 stimulated soil microbial growth and acted as a priming effect which increased SOM decomposition (Shahzad et al., 2015). Increased rhizodeposition was also observed in a semiarid C3-C4 grassland ecosystem growing 5 years in open-top chambers with eCO_2 (Pendall et al., 2004).

In contrast, greater rhizodeposition resulted in a suppressive effect on decomposition of older SOC when nutrients were abundant in a grassland exposed to eCO_2 for 2 years (Cardon et al., 2001).

However, any changes in rhizodeposition may have a large impact on C cyling in grassland ecosystems due to the high fraction of below-ground C translocation (Milchunas and Lauenroth, 2001) and through the interaction with microbial processes (Zak et al., 1993).

2.1.2 Storage, stabilization and turnover of soil organic carbon

The amount of SOC stored in a particular soil is dependent on the quantity and chemical quality of organic matter returned to the soil, the soil's ability to retain SOC and abiotic influences (Cardon, 1996; Grace et al., 2006).

The SOC pool is characterized by a wide range of turnover rates (Jenkinson and Rayner, 1977) depending on microbial ecology and of the resource availability within the soil environment (Kleber et al., 2011). According to Six and Jastrow (2002) the turnover of an element (e.g. C) is quantified as the element´s mean residence time (MRT), which is defined as the average time required to completely renew the content of the element in the pool at steady state.

In order to separate and characterize SOC pools, researchers have used various methods, including particle size and density fractionation (Cambardella and Elliott, 1992; Jastrow et al., 1996) as well as separation with models into two or three conceptual pools with short, medium and long residence times (Stockmann et al., 2013). The application of isotopic techniques to determine the MRTs of separated SOC fractions demonstrated the existence of various turnover rates for different pools (Six and Jastrow, 2002).

According to Lützow et al. (2006) two types of processes are relevant for stabilization of SOC: (i) physical protection within soil aggregates, reducing spatial accessibility of SOC to decomposers and their enzymes, substrates, water, and oxygen and (ii) organo-mineral complexes and organo-metal interactions, i.e., interactions of organic matter with minerals, metal ions, and other organic substances.

Soil aggregates of different sizes and stability are formed by the association of mineral particles with organic matter (Tisdall and Oades, 1982). Differences in physical protection of the various soil aggregates-size classes are widely used to gain insight into the changes in soil C and N dynamics and turnover (Christensen, 2001; Accoe et al., 2002; Liao et al., 2006). Physical fractionation may provide a more sensitive measure than detecting changes in total SOC of bulk soil due to the large pool size of total SOC in comparison to small changes and the spatially great variation (Hungate et al., 1996).

Effect of eCO_2 on soil organic carbon storage, stabilization and turnover

Net C sequestration is sustained only under eCO_2 if additional C input is allocated to pools that are stable over long-term scales and have a slow turnover. This implies that soil C decomposition lags behind the increase in soil C input (Friedlingstein et al., 1995). Otherwise, increased C losses via enhanced soil respiration and dissolved organic carbon (DOC) losses could counterbalance the input of extra C under eCO_2. Moreover, according to the C saturation concept (Stewart et al., 2007), the potential of soil to sequester additional C may be limited. This was demonstrated in two grassland studies in which plants were exposed to a CO_2 concentration gradient (Gill et al., 2002; Kool et al., 2007).

Studies from a grassland ecosystem under eCO_2 (Swiss FACE) showed that increased photosynthesis did not lead to a higher C storage of bulk soil (van Kessel et al., 2000; Van Kessel et al., 2006). Several meta-analyses reported only marginal SOM changes, especially due to nutrient limitations (De Graaff et al., 2006; Luo et al., 2006; Hungate et al., 2009; Norby and Zak, 2011; Liu et al., 2018). However, this may also be related to the difficulty of detecting changes in total SOC content of bulk soil (Hungate et al., 1996), especially in short-term studies, as mentioned before.

eCO_2 may alter many factors known to influence the distribution of soil aggregate-size classes (Díaz, 1995; Eviner and Chapin, 2002) through changes in quantity and quality of residue input and microbial activity. After six and eight years of eCO_2 at the Swiss FACE experimental site soil aggregation increased in the grassland ecosystem (Six et al., 2001; van Groenigen et al., 2002) but without any increase in total SOC content in topsoil (0-10 cm).

Any assessment of eCO_2 effects on C sequestration should consider the stability of the C pools i.e. their turnover. Accelerated SOM decomposition was frequently reported under eCO_2 (Groenigen et al., 2017; Thaysen et al., 2017). These processes may be caused by priming, that is, the effect of increased substrate availability on microbial decomposition of SOM, and may explain the absence of any SOC increase (Phillips et al., 2012).

Among major uncertainties is the response of the subsoil SOC stock, turnover and distribution of soil aggregate-size classes to eCO_2. It has been suggested that subsoils may play an important role in the global C cycle due to their reduced turnover and greater C saturation deficit relative to topsoil (Rumpel and Kögel-Knabner, 2011). Consequently, results from long-term studies

such as the Gi-FACE study are required which are investigating the response of subsoil soil aggregate-size classes and their SOC dynamics to eCO_2.

2.1.3 C losses from soil under eCO_2

A large proportion of the C that enters the soil is lost by soil respiration which was estimated to account for two-thirds of the total C loss from terrestrial ecosystems (Bitzer et al., 2010). Besides soil respiration, further losses of C occur through erosion, leaching (Kalbitz et al., 2000), fire and removal of biomass by grazing animals or through biomass harvesting (Jones and Donnelly, 2004; Lorenz and Lal, 2018).

Soil respiration is considered as the sum of autotrophic root respiration and heterotrophic respiration associated with the decomposition of litter, roots and SOM through microorganisms and soil meso- and macrofauna (Bernhardt et al., 2006).

The annual flux of soil respiration was estimated to account 77 Gt C $year^{-1}$ and represents the second-largest terrestrial C flux (Raich and Potter, 1995).

Soil respiration under eCO_2

The large contribution of soil respiration in the terrestrial C cycle points out that even small changes in soil respiration in response to eCO_2 can have large effects on atmospheric CO_2 concentrations.

Increased delivery of C substrate to the soil due to greater photosynthetic C fixation and plant biomass under eCO_2 may provide additional C substrate to decomposers (Zak et al., 2000) which may affect rates of soil respiration.

If under eCO_2 losses of SOC through soil respiration (outputs) are greater than the uptake through photosynthesis and sequestration of C in soil (inputs) it provides a positive feedback to global warming by exacerbating rising atmospheric CO_2 levels.

The majority of studies, to date, observed that soil respiration rates increased under eCO_2 (Janssens and Ceulemans, 2000; De Graaff et al., 2006, Zak et al., 2000, Liu et al., 2018); mostly based on short-term exposure (less than 5 years) with eCO_2 and measurements during growing season, neglecting the dormant season. However, short- and long-term responses of soil respiration to eCO_2 may be quite different since it is a product of several processes from various pools with different turnover times (Luo et al., 2001) and due to the CO_2 step increase effect

(Klironomos et al., 2005) at the beginning of any CO_2 enrichment (Luo, 2001; Newton et al., 2001).

Moreover, soil respiration during vegetation dormancy may represent a significant component of the annual C budget and contributes to the observed winter CO_2 maximum in the atmosphere (Raich and Potter, 1995). A study from a temperate heathland showed that soil respiration was increased under eCO_2 during winter season (Selsted et al., 2012).

2.2 Linked C and N cycle under eCO_2

Due to the coupled cycling of C and N, eCO_2 was found to affect soil N processes (Reich et al., 2006b) and consequently the production processes of N_2O (van Groenigen et al., 2011), a potent greenhouse gas with a global warming potential of 298 on a 100-year basis (Myhre et al., 2013).

Further, it was suggested that N in ecosystems controls C sequestration in plants and soil (Gill et al., 2006). Consequently, understanding N feedbacks under eCO_2 is relevant for evaluating ecosystems in terms of their GHG balance.

N_2O emissions from soils are dependent on the availability of C and N substrates that influence the involved microbial processes. The concentrations of the major N sources available to plants, i.e. NO_3^-, NH_4^+, and organic N (e.g. amino acids), have the potential to vary under eCO_2 which may also constrain the CO_2 responses of the ecosystem.

Additionally, soil N availability for plant growth may limit the degree to which eCO_2 enhances plant and soil C sequestration (Hungate et al., 2003; Luo et al., 2004; De Graaff et al., 2006; Reich et al., 2006a; van Groenigen et al., 2006).

The size of the soil mineral N pools are controlled by simultaneous processes such as production and consumption of NH_4^+ and NO_3^-, which may be changed under eCO_2.

Results on soil N availability for plant growth under eCO_2 have been highly variable, having been observed to decrease (Gill et al., 2002; Reich et al., 2006a), remain constant (Finzi and Schlesinger, 2003; Zak et al., 2003) or increase (Dijkstra et al., 2008; Langley et al., 2009) under eCO_2.

Reduced soil N availability was often related to a hypothesis referred to as progressive N limitation (PNL). PNL proposes that soil N availability becomes increasingly limited under

long-term exposure to eCO_2 as N is sequestered into long-lived plant biomass and SOM (Luo et al., 2004).

However, the large variations in the response of soil N availability to eCO_2 may reflect mechanisms that can alleviate resource limitation through (i) increased N use efficiency (NUE) (Rastetter et al., 1997; Gill et al., 2006), (ii) within-ecosystem redistribution of N from fractions with low C:N ratios to those with higher ratios (Gill et al., 2006), (iii) increased growth of deep roots (Hofmockel et al., 2011; Iversen et al., 2012), (iv) a shift in mycorrhizal fungal distribution towards deeper soil (Pritchard et al., 2008), (v) increased biological N_2 fixation (Hungate et al., 2004; Rütting and Andresen, 2015) or (vi) soil microbial processes i.e. accelerated decomposition and N mineralization (Dijkstra et al., 2008; Rütting et al., 2010) that may sustain ecosystem N availability under eCO_2.

Rütting and Andresen (2015) concluded in their meta-analyses that gross mineralization was only stimulated in N limited ecosystems, but not in P limited ecosystems under eCO_2.

Feng et al. (2015) suggested that CO_2-induced decreases in mineral N were related to suppressions of plant N acquisition under eCO_2 rather than to growth dilution of plant N (Luo et al., 1994; Gifford et al., 2000; Ellsworth et al., 2004; Taub and Wang, 2008).

Cheng et al. (2012) suggested that the form, rather than the total amount, of soil N is controlling belowground C turnover and plant N acquisition under eCO_2. In line with this finding, several studies demonstrated that eCO_2 inhibited NO_3^- assimilation in C3 plants (Bloom et al., 2014; Wu et al., 2017) thereby potentially increasing N_2O emissions from soil.

Production of N_2O in soil

On a global scale, vegetated soils are the main natural terrestrial sources of N_2O. Natural soils and fertilized fields were identified as important sources of N_2O (Bouwman et al., 2002a, b) and agriculture as the main anthropogenic source and the main driver of increasing atmosphere N_2O concentrations (Syakila and Kroeze, 2011). However, feedback effects of eCO_2 on N_2O emissions have not yet been included in climate change models and projections.

The emission of N_2O from soils results from microbe-mediated processes of which autotrophic nitrification and heterotrophic denitrification are considered to be the predominant processes (Barnard et al., 2005; Syakila and Kroeze, 2011).

Nitrification is the biological oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) through nitrite (NO_2^-). Denitrification is a process by which NO_3^- is stepwise reduced via NO_2^- and nitric oxide (NO) to the gaseous compounds N_2O or dinitrogen (N_2), which then diffuse into the atmosphere. The factors controlling denitrification rates are the amount of C and of NO_3^- supply and anoxic soil conditions. Oxic conditions are needed for NO_3^- production by nitrification (Whitehead, 2000). With limited supply of O_2 nitrifying bacteria may use NO_2^- as an electron acceptor and reduce it to NO and N_2O (Bollmann and Conrad, 1998).

Moreover, a variety of microbial species were shown to produce N_2O through further pathways. This includes the production of N_2O by fungi, which was demonstrated in grassland soils (Laughlin and Stevens, 2002) and many other ecosystems (Chen et al., 2014). Codenitrification is considered as a possible process for N_2O production by fungi among other microorganisms where one N atom originates from NO_2^- and the other from organic or reduced inorganic N (Spott et al., 2011). Further N_2O producing processes include heterotrophic nitrification which is considered as the oxidation of organic N to NO_3^- and was found to play a significant role in acidic forest soils with high C/N ratio (Zhang et al., 2015) and even in soils near neutral pH such as the old grassland study site (Müller et al, 2014). N_2O was also found to be produced by nitrifier denitrification which is a pathway of nitrification and describes the oxidation of ammonia (NH_3) to NO_2^- followed by the reduction of NO_2^- to NO, N_2O and N_2 and carried out within one group of microorganisms (ammonia oxidizing bacteria) (Wrage et al., 2001) which contrasts coupled nitrification-denitrification, which is carried out by distinct groups of microorganisms (Butterbach-Bahl et al., 2013). Dissimilatory NO_3^- reduction to NH_4^+ (DNRA) is another N transformation which was found to be relevant for the production of N_2O from soils (Smith, 1982).

Each N_2O production pathway is dependent on specific soil conditions (pH, oxygen content, availabilty of C and N substrates) and the presence of specific soil organisms (Butterbach-Bahl et al., 2013). These conditions are highly heterogeneous in soils at a small scale, with microsites i.e. within soil aggregates that may provide suitable conditions for the respective microbial community and may result in “hot spots” with high activity of N_2O production (Kuzyakov and Blagodatskaya, 2015; Ley et al., 2018).

Effect of eCO_2 on N_2O emissions

In a meta-analysis Van Groenigen et al. (2011) found that eCO_2 stimulated emissions of N_2O by 18.8%. Increasing amounts of N_2O was also observed for the temperate grassland study site

of the GiFACE experiment (Kammann et al., 2008; Regan et al., 2011) as well as for other grassland sites under eCO_2 (Baggs et al., 2003; Cantarel et al., 2012).

eCO_2 may indirectly alter microbial processes and the microbial community structure by (i) increasing soil moisture (Rice et al., 1994; Niklaus et al., 1998; Körner, 2000), (ii) altering nutrient concentrations of plant litter, (iii) changing C and N input into the soil via rhizodeposition (Norby et al., 1987; Rogers et al., 1994; De Graaff et al., 2007) and (iv) changing soil aggregation (Rillig et al., 1999; van Groenigen et al., 2002) which regulates oxygen (O_2) content via microhabitat formation (Kuzyakov and Blagodatskaya, 2015) . As a result, CO_2-induced changes to microbial processes could potentially impact N transformations and N_2O emissions. Further, eCO_2 may modify the amount or form of N in soil through complex interactions between the C and N cycle, which also control N_2O emissions.

3 Study site and general objectives

3.1 Study site

FACE experiments and requirements for conducting long-term studies

The majority of studies, analyzing changes in C and N cycling under eCO_2 have been based on short-term exposure (less than 5 years) with eCO_2, often using open-top chamber or greenhouse experiments (Zak et al., 2000). Results from these experiments should be analyzed with appropriate caution because of the known "chamber effect" on the microclimate (Leadley and Drake, 1993), size constraints of the chambers, limited growing periods and their relevance to natural ecosystems in which longer-term biogeochemical feedbacks operate (Rastetter et al., 1991).

In the last decades, FACE facilities have become a premier approach for conducting CO_2 experiments on intact ecosystems (Hendrey et al., 1999; Miglietta et al., 2001; Okada et al., 2001). They have been implemented in numerous ecosystems, including grassland ecosystems (van Kessel et al., 2000; Edwards et al., 2001; Reich et al., 2001) such as the Gi-FACE study site (Jäger et al., 2003). FACE experiments proofed to be a powerful approach to examine C and N cycles under eCO_2 (Ainsworth and Long, 2005) without enclosure.

However, it has been reported that the sudden increase in atmospheric CO_2 (CO_2 step increase) at the beginning of a CO_2-enrichment, may cause certain short-term responses of the ecosystem that differ from long-term responses (Luo, 2001; Newton et al., 2001, Klironomos et al., 2005). Accordingly, Kammann et al. (2005) showed that yield responses to eCO_2, in the Giessen Free Air Carbon Enrichment Experiment were different in the initial compared to the subsequent years. Moreover, plants may undergo micro-evolutionary changes in response to eCO_2, which may also be reflected in belowground processes (Klironomos et al., 2005). Consequently, to avoid misinterpretations due to insufficient experimental duration, results from long-term exposure studies are required.

Gi-FACE study site

The study site of the following three studies (I-III) is the Giessen Free Air Carbon Enrichment (Gi-FACE) experiment, which is located on permanent semi-natural grassland. It is situated near Giessen, Germany (50°32′N and 8°41.3′E) at an elevation of 172m above sea level. The set-up and performance of the Gi-FACE system has been described in detail by Jäger et al.

(2003) and Andresen et al. (2018). In brief, from May 1998 until present, atmospheric CO_2 concentrations were enriched by 20% above ambient, all-year-round during daylight hours. The CO_2 enrichment was applied in three rings, each eight meter in diameter (E plots). Three equally sized control plots were maintained at aCO_2 levels (A plots). The experimental design was a randomized block design. A block consisted of two plots to which ambient and eCO_2 treatments were randomly assigned. A characteristic attribute of the study site is a soil moisture gradient, resulting from a gradual terrain slope (2–3°) and varying depths of a subsoil clay layer. Within each of the three blocks, soil moisture conditions were relatively homogeneous (Jäger et al., 2003). The soil of the study site is classified as a Fluvic Gleysol (FAO classification).

The vegetation is an Arrhenatheretum elatioris Br.Bl. Filipendula ulmaria subcommunity, dominated by *Arrhenaterum elatium, Galium album* and *Geranium pratense*. At least 12 grass species, 15 non-leguminous herbs and 2 legumes are present within a single ring. For at least 100 years, the grassland has not been ploughed. Since at least 60 years, it was managed as a hay meadow with two cuts per year, and fertilized at the rate of 50–100 kgN ha^{-1} yr^{-1}. From 1996, fertilizer was applied in mid-April with granular mineral calcium-ammonium-nitrate fertilizer at the rate of 40 kgN ha^{-1} yr^{-1} (Kammann et al., 2008).

Figure 1: Gi-FACE experiment (Hessisches Landesamt für Bodenmanagement und Geoinformation 2010)

3.2 General objectives and hypotheses

Despite a great variety of studies that have been conducted since several decades on C and N related processes in ecosystems under eCO_2 there is still uncertainty on evaluating whether a certain soil will act as a net sink or source of GHGs to eCO_2. Moreover, the majority of studies to date have assessed short-term responses to eCO_2, which may differ from long-term responses (Luo, 2001; Newton et al., 2001). It has been proposed that short-term CO_2-enrichment experiments tended to overestimate the potential for grasslands to sequester C in the long term (Hungate et al., 1997). Further, the number of studies on natural conditions, where intact ecosystems are exposed to eCO_2 - as in FACE experiments - are limited.

Further, the role of subsoils as potential C sinks due to their unsaturated mineral surfaces and high mean residence times of organic C have been increasingly reported (Rumpel and Kögel-Knabner, 2011) but information on subsoil C processes under eCO_2 are very scarce (Schortemeyer et al., 2000; Pendall and King, 2007).

Moreover, soil respiration during vegetation dormancy may represent a significant component of the annual C budget and contributes to the observed winter CO_2 concentration maximum in the atmosphere (Raich and Potter, 1995; Keeling et al., 1996), which shows the necessity to integrate year-round measurements of soil respiration into ecosystem C balances.

Consequently, the main objective of the present work was to contribute to a better understanding of soil C and N processes under long-term eCO_2 governing the formation and emission of CO_2 and N_2O from a temperate grassland soil.

Towards this objective, we

(1) assessed the seasonal effects of long-term eCO_2 on soil respiration as a potential feedback effect (study I),

(2) elucidated the distribution of soil aggregate-size classes at different soil depths, the associated MRT and the resulting SOC content under long-term eCO_2 (study II) and

(3) quatified N transformations and the resulting N_2O emissions under long-term eCO_2 (study III).

We hypothesized that

(i) long-term (> 10 years) moderate CO_2 enrichment causes increased soil respiration (study I)

(ii) soil respiration is more enhanced in the growing season than during vegetation dormancy (winter) (study I)

(iii) soil respiration is significantly enhanced in winter under eCO_2 in the Gi-FACE where the CO_2 enrichment is continuing during winter (study I)

(iv) topsoil will be close to C saturation and will show small increases in SOC content under long-term eCO_2 (study II) and

(v) subsoil will have a higher C saturation deficit and will therefore increase to a higher extent in SOC relative to topsoil under eCO_2 (study II).

(vi) eCO_2 will result in enhanced N_2O emissions due to increased plant growth stimulating root exudation and thus denitrification, which would be reflected in altered soil NO_3^- dynamics (study III).

4 Major results

Main results obtained from the Gi-FACE study (study I-III) are summarized in this chapter. The methods of sampling and analysis are provided in the single chapter of each study (chapter 6-8).

C saturation deficit (C_{def}) and soil organic carbon content under eCO_2

C_{def} was estimated for different soil depths at the Gi-FACE study site (study II, chapter 7). Results showed that in topsoil C_{def} was close to C saturation, while C_{def} was increasing with soil depth (Figure 2 and 3), which confirmed part of our hypotheses (iv and v) (chapter 3.2).

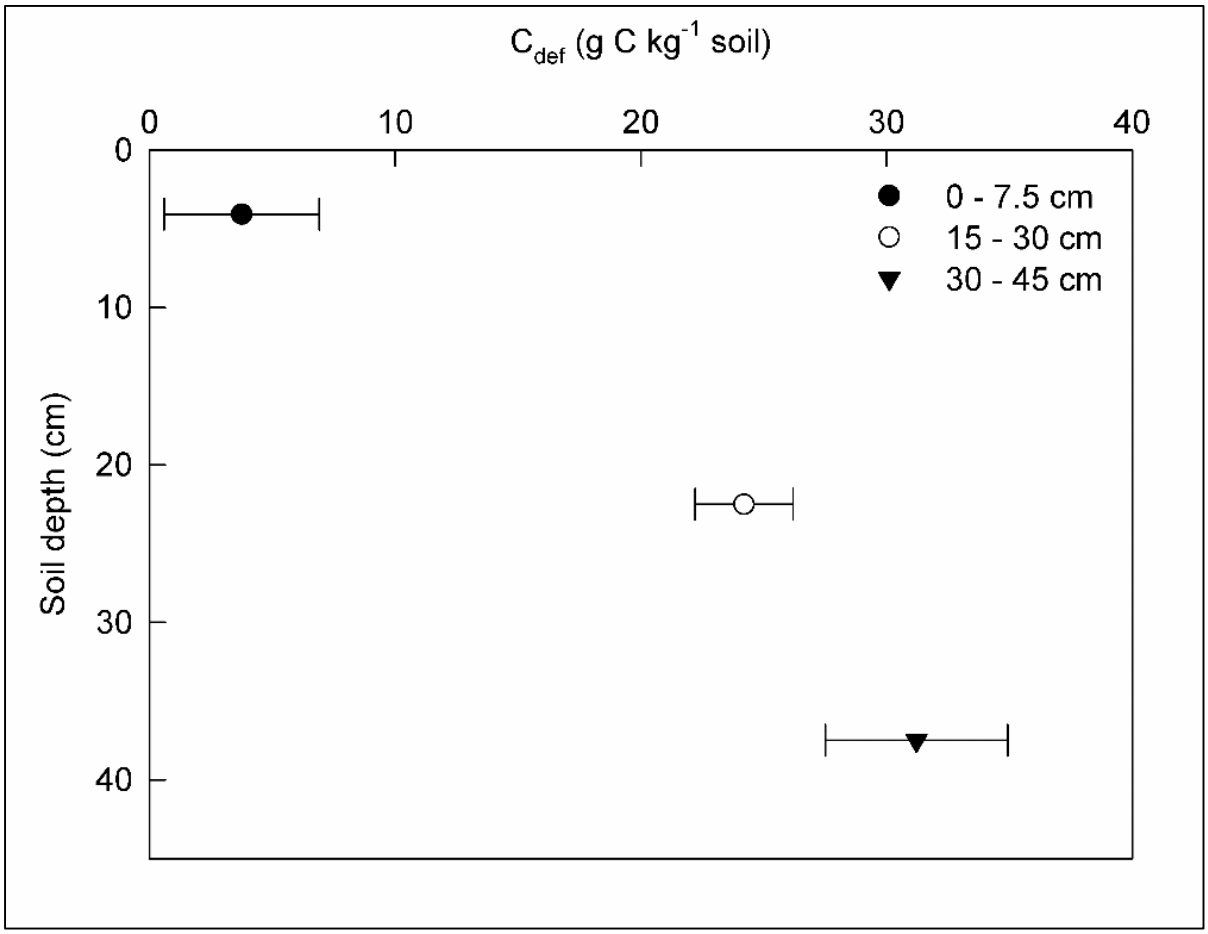

Figure 2: C saturation deficit (C_{def}) estimated for the grassland study site at different soil depths after 6 years of the FACE experiment. Values are presented as means, based on ring pairs (n=3).

However, our hypothesis (v) (chapter 3.2) that subsoil will increase to a higher extent in SOC relative to topsoil under eCO_2 due to its higher C_{def} could not be confirmed. Within 13.5 years of CO_2 enrichment no change in SOC content of bulk soil was observed in any soil depth at the Gi-FACE (Table 1, study II). Internal aggregate-SOC content increased only in silt and clay

aggregate-size classes (SC) in lower soil depths (below 7.5 cm) and in small macroaggregates (SM) in 7.5 – 15 cm but not in deeper soil layers under eCO_2 (study II and Figure 3). Further, no increases in internal aggregate-SOC content were observed in any other soil aggregate-size classes under eCO_2 which contradicted part of hypothesis (v) (chapter 3.2).

Table 1:ANOVA table of effects of eCO_2 on SOC content of bulk soil at different soil depths.

Depth	*df*	*P*
0-7.5 cm	1	0.866
7.5-15 cm	1	0.367
15-30 cm	1	0.471
30-45 cm	1	0.129

Belowground C input under eCO_2

At the Gi-FACE experiment the proportions of C input (C_{new}) under eCO_2 that have been fixed since the change in in $\delta^{13}C$ signature in July 2004 (within 7 years) were calculated for bulk soil and different soil aggregate-size classes (study II). Results showed that, within 7 years since the switch in $\delta^{13}C$ signature, C_{new} was allocated within 30 cm soil depth and that C_{new} in the top 7.5 cm soil depth differed from lower soil depths in bulk soil, SM and microaggregates (MIC) under eCO_2 (Table 2 and Figure 3). Highest amounts of C_{new} in bulk soil in the top 7.5 cm of soil were explained by a relative high fraction of C_{new} in free particulate organic matter (POM) that was not occluded within soil aggregates in the top soil.

Storage, stabilization and turnover of soil organic carbon under eCO_2

Various turnover rates for different pools could not be confirmed at the Gi-FACE study site, where MRT of SOC in different soil aggregate-size classes did not differ significantly among each other under eCO_2 (study II and Table 2). However, different MRTs of SOC were observed in macroaggregates (LM and SM) and bulk soil between different soil depths under eCO_2 (Table 2 and Figure 3).

Table 2:Relative and absolute amounts of C_{new}, k-value and MRT of SOC in soil aggregate-size classes and bulk soil after 13.5 years of eCO_2. Values are presented as means ± standard error, n=3. Results of a Tukey´s HSD post-hoc test show significant differences among aggregate-size classes and among soil depths for C_{new}. Different uppercase letters indicate significant differences among aggregate-size classes within same depth for MRT. Different lowercase letters indicate significant differences of aggregate-size classes among depths for MRT.

Depth	**aggregate-size class**	**Cnew**				**Tukey´s HSD comparisons**								***k***		**MRT**		
(cm)		**(g 100 g^{-1} SOC)**		**(g kg^{-1} soil)**		**LM**	**SM**	**MIC**	**SC**	**bulk soil**	**0-7.5**	**7.5-15**	**15-30**			**(yr)**		
0 - 7.5	**LM**	24.42	± 0.01	3.07	± 0.06				0.044	< 0.01				0.038	± 0.00	27	± 2.05	Aa
	SM	26.44	± 0.02	4.04	± 0.03			0.022	< 0.01	< 0.01		< 0.01	< 0.01	0.041	± 0.00	25	± 2.08	Aa
	MIC	19.17	± 0.01	0.63	± 0.15		0.022			< 0.01		0.043	0.041	0.029	± 0.01	41	± 9.70	Aa
	SC	20.09	± 0.03	0.07	± 0.01	0.044	< 0.01			< 0.01				0.030	± 0.01	35	± 4.70	Aa
	Bulk soil	30.57	± 0.03	11.85	± 1.25	< 0.01	< 0.01	< 0.01	< 0.01			0.007	0.002	0.049	± 0.01	21	± 2.90	Aa
7.5 - 15	**LM**	16.99	± 0.02	2.73	± 1.02									0.025	± 0.00	42	± 5.62	Aa
	SM	17.65	± 0.02	1.23	± 0.15						< 0.01			0.026	± 0.00	39	± 3.59	Ab
	MIC	9.51	± 0.02	0.18	± 0.06						0.043			0.013	± 0.00	81	± 15.66	Aa
	SC	19.30	± 0.05	0.13	± 0.06									0.029	± 0.01	40	± 9.23	Aa
	Bulk soil	14.56	± 0.05	4.03	± 1.50			0.042	0.040		0.007			0.021	± 0.01	68	± 29.28	Aa
15 -30	**LM**	15.26	± 0.02	2.13	± 1.02				0.084					0.022	± 0.00	47	± 7.23	Ab
	SM	11.50	± 0.01	0.60	± 0.02						< 0.01			0.016	± 0.00	62	± 4.93	Ac
	MIC	11.66	± 0.04	0.18	± 0.02					0.094	0.041			0.017	± 0.01	79	± 30.88	Aa
	SC	18.10	± 0.04	0.07	± 0.02	0.084				0.074				0.027	± 0.01	41	± 9.21	Aa
	Bulk soil	10.35	± 0.02	2.18	± 0.41			0.094	0.074		0.002			0.015	± 0.00	76	± 19.00	Ab

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay. No δ13C- data was available for soil aggregate size classes in 30-45 cm soil depth after 13.5 years.

At the Gi-FACE experiment a depth-dependent response of macroaggregation to eCO_2 was observed (study II and Figure 3). While the abundance of large macroaggregates (LM) increased in subsoil (15-45 cm depth) with a concomitant decrease in the abundance of smaller aggregate-size classes, no CO_2-induced increase in macroaggregation was observed in topsoil (0-15 cm). However, eCO_2 decreased the abundance of MIC and SC within the top 7.5 cm (study II and Figure 3).

Despite increased macroaggregation and the calculated C_{def} in subsoil no indication of SOC sequestration in bulk soil was detected at the Gi-FACE experiment within 13.5 years of CO_2 enrichment. This is in line with the observation that MRT of different soil aggregate-size classes did not differ among each other under eCO_2 (Table 2).

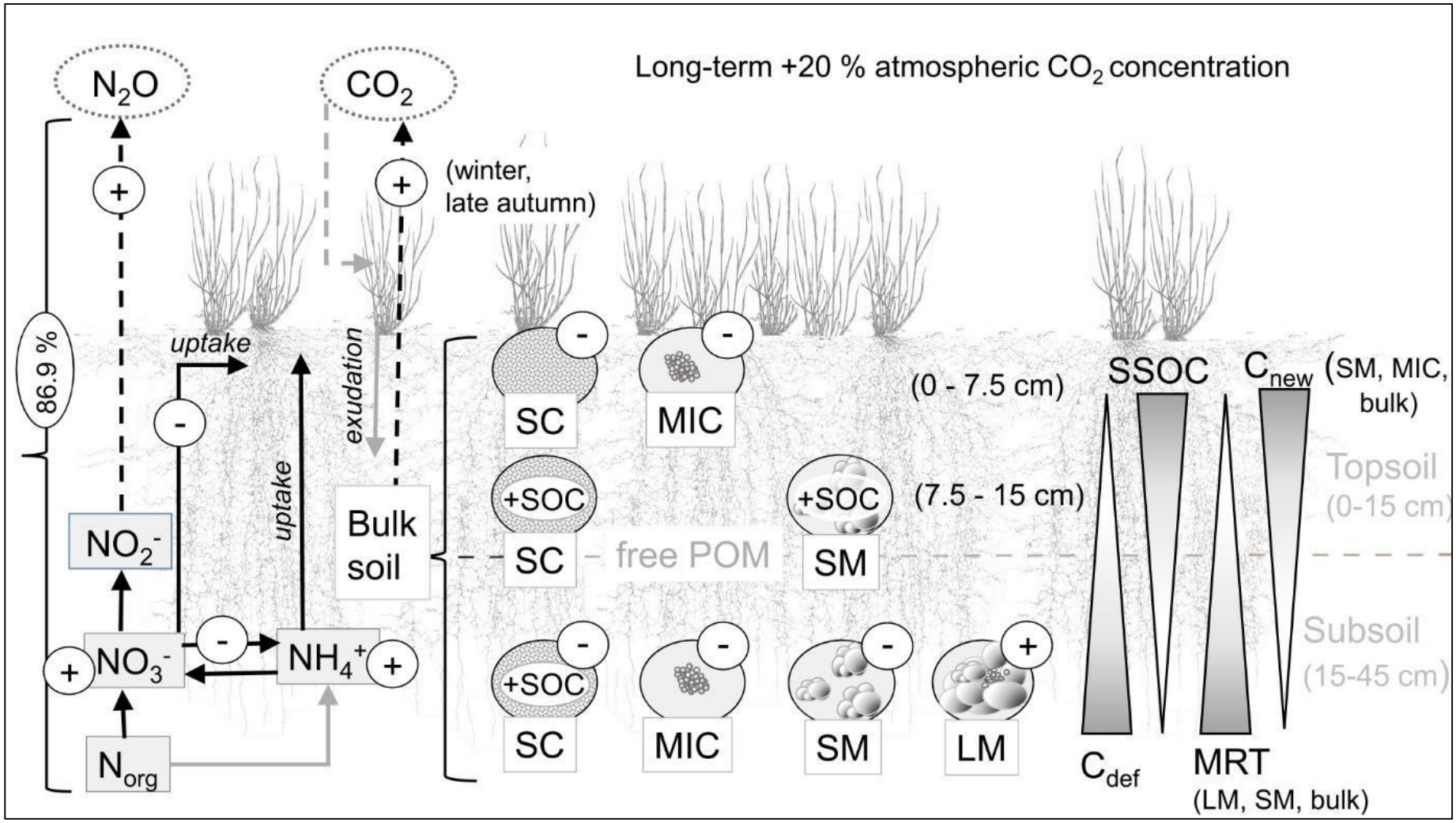

Figure 3: Significant changes of C- and N- soil dynamics and between top- and subsoil under long-term elevated CO_2 (eCO_2) at the Gi-FACE study site. "**+**" mark increases and "**-**" mark decreases under eCO_2. C_{def}: C saturation deficit; MRT: mean residence time; SSOC: stable soil organic carbon, LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay.

Soil respiration under eCO_2

Study I (chapter 6) showed that at the Gi-FACE experiment soil respiration rates under eCO_2 were significantly higher during autumn (15.7 %) and winter (17.4 %) compared to rates under ambient CO_2 (Figure 3 and 4). During all other seasons, covering most of the vegetation period, no significant CO_2 effect was observed (Figure 4). These results contradicted the majority of FACE studies (Pendall et al., 2001; Pregitzer et al., 2008; Jackson et al., 2009; Adair et al., 2011; Dawes et al., 2013) but confirmed hypothesis (iii). Since annual sums of soil respiration did not differ significantly between the CO_2 treatments this contradicted hypothesis (i). However, increased soil respiration during winter and autumn may play an important role concerning the global C balance by increasing the observed winter CO_2 maximum concentration in the atmosphere (Raich and Potter, 1995; Keeling et al., 1996) when respiration exceeds photosynthesis. Consequently, the results from the Gi-FACE study emphasize the relevance of conducting year-round measurements of soil respiration.

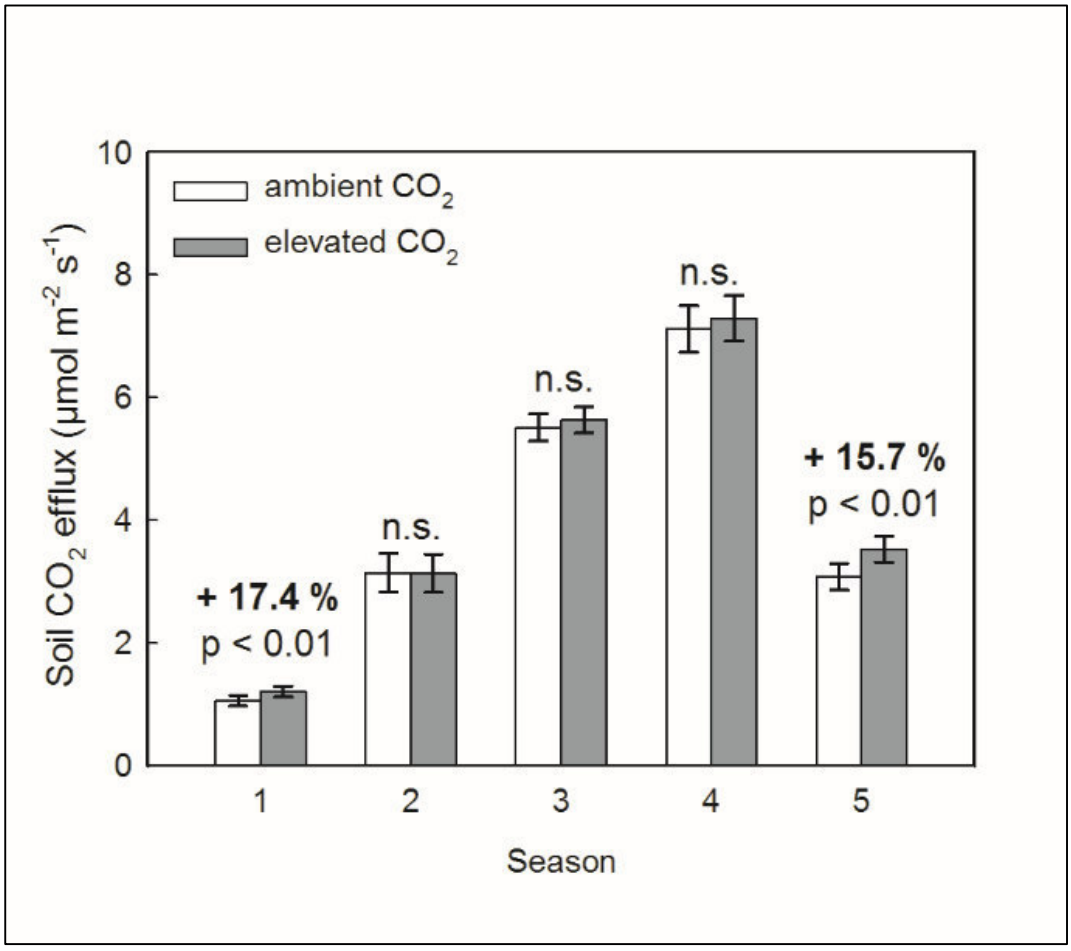

Figure 4: Mean soil respiration rates during the five defined seasons under ambient and elevated CO_2 averaged over three years from 2008 – 2010 (a); (1) = winter dormancy; (2) = start of vegetation period; (3) = spring; (4) = summer; (5) = autumn.

Effect of eCO_2 on N_2O emissions and N transformations

Study III (chapter 8) confirmed earlier results from the Gi-FACE study site by showing that after 15 years of eCO_2 N_2O emissions under eCO_2 were still more than twofold higher than under ambient CO_2. As the major source for additional emissions the oxidation of organic N followed by incomplete NO_2^- reduction to N_2O was identified (Figure 3) which contradicted parts of hypothesis (vi) (chapter 3.2). Decreased NO_3^- uptake rates under eCO_2 were observed at the Gi-FACE (Figure 3) and are in line with other studies (Bloom et al., 2014; Wu et al., 2017) but did not completely explain the increase in N_2O emissions under eCO_2.The sources of additional N_2O emissions under eCO_2 were associated with NO_3^- (+2.0 %), NH_4^+ (+11.1 %) and organic N (+86.9 %) (study III).

5 General conclusions and implications

Contrary to our hypotheses (i) annual estimates of soil respiration were not different between the CO_2 treatments and soil respiration was not significantly affected during the growing season to moderate long-term CO_2 enrichment (ii). However, in line with our hypotheses (iii), the results revealed that 10 years of moderate CO_2 enrichment increased soil respiration during winter and autumn (study I). These results highlight the importance of including winter soil CO_2 fluxes in ecosystem C budgets. Otherwise, soil-respiratory C losses may be underestimated in C balances that are based on measurements exclusively from the growing season.

In contrast to our hypotheses (iv and v), long-term eCO_2 did not change the SOC content of bulk soil in any soil depth (study II), neither in topsoil, for which we estimated a small C saturation deficit, nor in subsoil for which we estimated a higher C saturation deficit than in topsoil. However, increased macroaggregation in subsoil and higher MRT in subsoil compared to topsoil under eCO_2 indicate that C stabilization processes are taking place in subsoil under eCO_2. However, we suggest that CO_2-induced soil processes are taking place that are resulting in C losses that outbalance the increases in soil C under eCO_2. This is in line with our finding of increased soil respiration under eCO_2 during late autumn and winter, which indicates that microbial decomposition is accelerated under eCO_2 in this seasons.

Results from the ^{15}N tracing study (study III) confirm part of our hypothesis (vi) that the 20% increase in the atmospheric CO_2 concentration triggered changes in soil N transformations that resulted in long-term higher N_2O emissions. However, our hypothesis (vi) that stimulated denitrification is mainly responsible for increased N_2O emissions was not confirmed since our results revealed that the major source for additional emissions was the oxidation of organic N followed by incomplete NO_2^- reduction. We suggest from our results that increased root exudation under eCO_2 provided an additional source of bioavailable supply of energy that triggered as a priming effect the stimulation of microbial SOM mineralization and increased activity of bacterial nitrite reductase, which caused a shift in N_2O:N_2 ratio via incomplete denitrification. Accordingly, our studies indicate that any potential N limitation was likely alleviated by an CO_2-induced priming effect. We suggest that such an effect had a negative consequence on C sequestration through SOM decomposition and also explains increased oxidation of organic N that allowed sustained N availability. While N_2O emissions were very similar between aCO_2 and eCO_2 treatments during autumn and winter months (study III), soil CO_2 emissions were significantly different between CO_2 treatments in these seasons (study I).

However, measurements in study I and III were carried out in different years and due to differing abiotic factors (soil moisture conditions, soil temperature, freeze-thawing effects) care should be taken in comparing results from the different studies. This aspect is further supported by an earlier study at the Gi-FACE, which showed different seasonal effects of N_2O emissions under eCO_2 (Kammann et al., 2008), making generalizations difficult.

To sum up, the present thesis leads to the conclusion that temperate European grasslands which were characterized by a greenhouse gas balance near zero (Soussana et al., 2007) may gradually turn into greenhouse gas sources with rising atmospheric CO_2 due to enhanced CO_2 losses during autumn and winter and increased N_2O emissions. No bulk soil C sequestration could be observed in any soil depth within 13.5 years of CO_2 enrichment. This was in contrast to increased macroaggregation under eCO_2 in subsoil, which was expected to provide a greater protection from microbial decomposition and also did not confirm the estimated higher C sequestration potential in subsoil based on the applied $C_{sat\text{-}def}$ concept. Increased CO_2 efflux from soil indicate faster C cycling in soil under eCO_2, at least during late autumn and winter, which may explain that no C sequestration occurred in bulk soil or large macroaggregates. Only SC increased in their internal SOC content in deeper soil depths and received a high fraction of C_{new}. However, this did not have any effects on the SOC content of bulk soil or any larger soil aggregate-size class to date. However, it is possible that sequestration of C in subsoil will require longer periods than the observed 13.5 years since only small fractions of C_{new} is allocated to these depths where it is protected for longer periods than in topsoil. Nevertheless, results from studies I-III do not support any climate mitigation strategies which define temperate grasslands per se as a sink to eCO_2 without any adequate management which may promote C sequestration, but was beyond the scope of this thesis. In contrast, our results showed a positive feedback of eCO_2 on N_2O and soil CO_2 emissions which further accelerate global warming and call out for a holistic perspective of GHG emissions in current models and climate change mitigation strategies.

Outlook: Need for future research work and open questions

Although our studies I-III gave further insight into relevant C stabilization processes, C losses and N transformations under long-term eCO_2 of a temperate grassland soil, further studies are required. These studies are necessary as a basis for defining adequate mitigation policies, accurate estimates in the National greenhouse gases Inventory and to support process-based models. Further studies should address the following points or questions:

- Comparison of soil C turnover between aCO_2 and long-term eCO_2 taking top- and subsoil into account
- Long-term and multi-factor (warming, eCO_2, drought) studies of climate change on soil C and N processes, which also take subsoil as well as seasonal effects into account
- Is the observed macroaggregation in subsoil under eCO_2 related to mycorrhizal fungal distribution towards deeper soil as observed by (Pritchard et al., 2008)?
- How does subsoil respond to eCO_2 in terms of N_2O production and N transformation processes? Which effects does CO_2-induced soil aggregation have on N_2O production?
- Which effects would an increased supply of nutrients have at the Gi-FACE on the suggested priming effect and the resulting GHG balance of the grassland ecosystem?
- What are the effects of the increasing level of N deposition that is projected to rise (Galloway et al., 2004; Galloway et al., 2008) on the GHG balance of grassland ecosystems under eCO_2?
- Identification of soil management practices that create a net C sink of atmospheric CO_2

The urgency of understanding the underlying processes of ecosystem feedbacks to eCO_2 and integration of potential mitigation options into policy emphasizes the need for interdisciplinary work incorporating input from different disciplines.

6 Study I:

Positive feedback of elevated CO_2 on soil respiration in late autumn and winter.

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Positive feedback of elevated CO_2 on soil respiration in late autumn and winter

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Abstract. Soil respiration of terrestrial ecosystems, a major component in the global carbon cycle is affected by elevated atmospheric CO_2 concentrations. However, seasonal differences of feedback effects of elevated CO_2 have rarely been studied. At the Gießen Free-Air CO_2 Enrichment (GiFACE) site, the effects of +20 % above ambient CO_2 concentration have been investigated since 1998 in a temperate grassland ecosystem. We defined five distinct annual seasons, with respect to management practices and phenological cycles. For a period of 3 years (2008–2010), weekly measurements of soil respiration were carried out with a survey chamber on vegetation-free subplots. The results revealed a pronounced and repeated increase of soil respiration under elevated CO_2 during late autumn and winter dormancy. Increased CO_2 losses during the autumn season (September–October) were 15.7 % higher and during the winter season (November–March) were 17.4 % higher compared to respiration from ambient CO_2 plots.

However, during spring time and summer, which are characterized by strong above- and below-ground plant growth, no significant change in soil respiration was observed at the GiFACE site under elevated CO_2. This suggests (1) that soil respiration measurements, carried out only during the growing season under elevated CO_2 may underestimate the true soil-respiratory CO_2 loss (i.e. overestimate the C sequestered), and (2) that additional C assimilated by plants during the growing season and transferred below-ground will quickly be lost via enhanced heterotrophic respiration outside the main growing season.

1 Introduction

The atmospheric concentration of CO_2 has increased from pre-industrial values of 275–285 ppm (Raynaud and Barnola, 1985) to 400 ppm in 2013 (Monastersky, 2013). Projections of future atmospheric CO_2 concentration in the year 2100 range between 490 and 1370 ppm depending on representative concentration pathways (Moss et al., 2010). As the major radiative forcing component (IPCC, 2013), atmospheric CO_2 is positively correlated with air temperature and is therefore an important component for global warming. Additionally, indirect effects of elevated atmospheric CO_2 (eCO_2), which are altering carbon (C) fluxes in ecosystems, may impose a feedback to climate change. About half of photosynthetically assimilated C returns immediately to the atmosphere as plant-respired CO_2 (autotrophic respiration) (Chapin et al., 2002). Portions of the net carbon gain (net primary production) are transferred to the soil via root exudates, fine root growth and turnover or other litter, providing the substrate for soil organic carbon (SOC) buildup (Kirschbaum, 2000).

Soil functions as an important C reservoir within the global carbon cycle and stores about 1500 Gt of C (Amundson, 2001; Lal, 2004; Batjes, 1996), which is about twice the amount of C in the atmosphere (Schils et al., 2008).

Soil respiration, the sum of autotrophic root respiration and heterotrophic respiration from microorganisms and soil meso- and macrofauna, accounts for two-thirds of the total C loss from terrestrial ecosystems (Luo, 2006). Enhanced net C losses under eCO_2 cause a positive feedback.

Many past studies focused on soil–atmosphere CO_2 exchange during the growing season. However, soil respiration during vegetation dormancy may represent a significant component of the annual C budget and contributes to the ob-

served winter CO_2 maximum in the atmosphere (Raich and Potter, 1995). Accordingly, analysis of CO_2 data from an air sampling network identified seasonal oscillation with highest concentrations occurring each winter when respiration exceeds photosynthesis (Keeling et al., 1996). This emphasizes the necessity to study seasonal dynamics of soil respiration under future CO_2 conditions to gain a better understanding of how soil respiration responds to changing atmospheric CO_2 concentrations.

A meta-analysis of Zak et al. (2000) revealed a 51 % increase of soil respiration as a mean response in a grassland ecosystem under elevated CO_2, Janssens and Ceulemans (2000) provided evidence for consistent stimulation of soil respiration under a variety of tree species. However, the majority of studies, to date, are based on short-term exposure (less than 5 years) with eCO_2, often using open-top chamber experiments (Zak et al., 2000). Results from these experiments should be analysed with appropriate caution because of the known "chamber effect" on the microclimate (Leadley and Drake, 1993) and their relevance to natural ecosystems in which longer-term biogeochemical feedbacks operate (Rastetter et al., 1991). Since soil respiration is a product of several rhizospheric processes i.e. root exudation, root respiration, and root turnover, as well as decomposition of litter and bulk soil organic matter from various pools with different characteristic turnover times, short- and long-term responses to eCO_2 may be quite different (Luo et al., 2001).

The most suitable approach for conducting ecosystem CO_2 experiments under natural conditions are Free Air CO_2 enrichment (FACE) experiments, where intact ecosystems are exposed in situ to a higher atmospheric CO_2 concentration. However, it has been reported that the sudden increase in atmospheric CO_2 (CO_2 step increase) at the beginning of a CO_2-enrichment, may cause certain short-term responses of the ecosystem that differ from long-term responses (Luo, 2001; Newton et al., 2001). Accordingly, Kammann et al. (2005) showed that yield responses to eCO_2, in the Gießen Free-Air CO_2 Enrichment (GiFACE), were different in the initial compared to the subsequent years. Moreover, plants may undergo micro-evolutionary changes in response to eCO_2 (Ward and Kelly, 2004), which may also be reflected in belowground processes (Klironomos et al., 2005). Consequently, to avoid misinterpretations due to insufficient experimental duration, results from long-term exposure studies are required. In the GiFACE this was after approximately 5–6 years (Kammann et al., 2005). In the following we use the expression "short-term" for CO_2 enrichment durations < 5 years and "long-term" for durations > 5 years.

Based on a literature overview, we found 13 other FACE studies, from a wide variety of ecosystems, where in-situ soil respiration under eCO_2 has been investigated. All of these FACE studies operated at higher CO_2 enrichment concentrations than the GiFACE experiment (with +20 % CO_2 above ambient), i.e. they imposed larger initial step increases (Klironomos et al., 2005). Klironomos et al. (2005) have demonstrated that ecosystem responses to eCO_2 may differ between using a sudden step increase and a gradual rise in the CO_2 concentration. However, in any CO_2 enrichment study a step increase – also if lower than usual – cannot be avoided. Thus, experimental FACE results are more indicative for future predictions. However, experimental studies with durations of > 10 years are scarce (Carol Adair et al., 2011; Jackson et al., 2009). To our knowledge, 10 of the 16 investigations on soil respiration across these 13 FACE studies were carried out within the first 5 years of exposure, thus reporting short-term responses (Craine et al., 2001; King et al., 2001; Allen et al., 2000; Andrews and Schlesinger, 2001; Selsted et al., 2012; Masyagina and Koike, 2012; Soe et al., 2004; Lagomarsino et al., 2013; Liu et al., 2006; Nakayama et al., 1994). All short-term study results pointed towards a consistent stimulatory effect of eCO_2 on soil respiration. The average increase ranged from 12 % under a sweet gum plantation (King et al., 2004) to 70 % under a mixed plantation of *Populus* species (Lagomarsino et al., 2013). In two of the short-term studies, significant effects were only observed on days with high photosynthetic activity (Masyagina and Koike, 2012; Soe et al., 2004); measurements during dormancy were not carried out.

Three of the short-term studies conducted measurements during winter dormancy with contrasting results (Allen et al., 2000; Andrews and Schlesinger, 2001; Selsted et al., 2012; Lagomarsino et al., 2013). In a temperate heathland (CLIMAITE study), soil respiration was significantly increased under eCO_2 during three consecutive winter seasons (Selsted et al., 2012). Allen et al. (2000) detected a significant effect of eCO_2 on soil respiration during December 1997 in the Duke Forest FACE study but not during the previous growing season beneath the loblolly pine forest. Andrews and Schlesinger (2001) reported from the same site greater increases of soil respiration during fumigation periods (26–59 %) than during non-fumigated periods (8–15 %). Fumigation was stopped when ambient air temperature dropped below 5 °C for more than 1 hr. In line with these results, much larger percentage enhancements of the soil CO_2 efflux were observed during the growing season (up to 111 %) than during dormant season (40 %) from a mixed plantation of *Populus* species exposed to eCO_2 (EUROFACE) (Lagomarsino et al., 2013). CO_2 enrichment was provided from bud burst to leaf fall at this site.

Out of six long-term studies on soil respiration (Carol Adair et al., 2011; Pregitzer et al., 2008; Jackson et al., 2009; Pendall et al., 2001; Bader and Körner, 2010; Dawes et al., 2013), only one study reported measurements throughout the dormant season, showing that after 10 years of eCO_2 during the growing season at a loblolly pine forest (Duke FACE) soil respiration was consistently higher in midsummer to early fall and diminished or disappeared in winter (Jackson et al., 2009). This was explained by a reduction in assimilation and hence available root exudate during dormancy. If the fumigation may continue during the dormant season in an ecosys-

tem with a green canopy e.g. in a permanent grassland, the stimulation may theoretically continue on a higher level.

Reports from other long-term FACE studies in temperate ecosystems (disregarding the dormant season) were consistent by reporting an increase in soil respiration under eCO_2, with the exception of the Swiss Canopy Crane experiment in an old-growth, mixed deciduous forest. Bader and Körner (2010) reported that soil respiration from the site was only stimulated when volumetric water content was $\leq 40\,\%$ at soil temperatures above 15 °C.

In summary, only fragmented information is available on how soil respiration responds to eCO_2 during vegetation as well as dormant periods after long-term eCO_2. To our knowledge, no long-term FACE study in a grassland ecosystem exists which has investigated soil CO_2 fluxes across several years. Consequently, it is difficult to generalize temporal patterns of soil respiration under eCO_2, and thus the soil respiratory response to eCO_2 at all.

Based on the available studies and earlier observations at our site, where whole-ecosystem respiration including the green canopy was increased under eCO_2, mainly during non-growing season (Lenhart, 2008), we hypothesized that (1) long-term (> 10 years) moderate CO_2 enrichment causes increased soil respiration, (2) soil respiration is more enhanced in the growing season than during vegetation dormancy (winter), and (3) soil respiration is significantly enhanced in winter under eCO_2 in the GiFACE where the CO_2 enrichment is continuing during winter.

2 Materials and methods

2.1 Study site and design

The Gießen Free Air Carbon Enrichment (GiFACE) experiment is located on permanent semi-natural grassland. It is situated near Gießen, Germany (50°32′ N and 8°41.3′ E) at an elevation of 172 m above sea level.

The set-up and performance of the GiFACE system has been described in detail by Jäger et al. (2003). In brief, from May 1998 until present, atmospheric CO_2 concentrations were enriched by 20 % above ambient, all-year-round during daylight hours. At present the GiFACE experiment is still ongoing.

The CO_2 enrichment was applied in three rings, each 8 m in diameter (E plots). Three equally-sized control plots were maintained at ambient atmospheric CO_2 levels (A plots). The experimental design was a randomized block design. A block consisted of two plots to which ambient and eCO_2 treatments were randomly assigned. A characteristic attribute of the study site is a soil moisture gradient, resulting from a gradual terrain slope (2–3°) and varying depths of a subsoil clay layer. Within each of the three blocks, soil moisture conditions were relatively homogeneous (Jäger et al., 2003).

The vegetation is an *Arrhenatheretum elatioris* Br.Bl. *Filipendula ulmaria* subcommunity, dominated by *Arrhenaterum elatium, Galium mollugo* and *Geranium pratense*. At least 12 grass species, 15 non-leguminous herbs and 2 legumes are present within a single ring. For at least 100 years, the grassland has not been ploughed. For several decades, it was managed as a hay meadow with two cuts per year, and fertilized in mid-April with granular mineral calcium-ammonium-nitrate fertilizer at the rate of $40\,kg\,N\,ha^{-1}\,yr^{-1}$. Before 1996, fertilizer was applied at a rate of $50–100\,kg\,N\,ha^{-1}\,yr^{-1}$ (Kammann et al., 2008).

The soil of the study site is classified as a Fluvic Gleysol (FAO classification) with a texture of sandy clay loam over a clay layer (Jäger et al., 2003).

Observations in this study were carried out from January 2008–December 2010 (i.e. more than 9 years after the onset of CO_2 enrichment). During the observation period the mean annual temperature was 9.2 °C and mean annual precipitation was 562 mm, which was identical to the average rainfall since the beginning of recording in 1995. Rainfall was recorded at the site in 30 min intervals with 20 randomly distributed "Hellmann" samplers. Air temperature was recorded continuously at two locations at the site at 2 m height and averaged 9.5 °C since 1995.

2.2 Measurement of soil CO_2 fluxes at the field site

In each of the six FACE plots, soil respiration rates were measured using an automated closed dynamic chamber system with an infrared gas analyzer (LI-COR 8100, LI-COR, Inc., Lincoln, Nebraska, USA) with a patented vent for pressure equilibration between the closed chamber and the atmosphere (McDermitt et al., 2005). Carbon dioxide fluxes were reported in $\mu mol\,CO_2\,m^{-2}\,s^{-1}$. The measurements were performed at four permanently installed PVC soil collars per FACE ring, to cover the spatial heterogeneity within each ring. The soil collars had a diameter of 20.3 cm (8 inch) and were about 11 cm high. A bevelled edge at one end facilitated the insertion into the soil, which took place on 9 May 2006 and the vegetation cover, including surficial rhizomes, was removed manually. Subsequently, the surface was held vegetation-free by removing germinated seedlings weekly. Due to uneven soil conditions, soil collars varied ±1 cm in their insertion depth. Generally, the insertion was chosen to be as shallow as possible, minimizing the trenching effect (Heinemeyer et al., 2011) while maintaining an airtight connection between soil and chamber. A foam gasket and rubber seal between the bottom of the chamber and the top of the soil collar minimized leaks between the collar and the chamber. Before each measurement, the distance between the soil surface and the top of each soil collar (i.e. chamber offset) was measured and entered into the LI-COR software to enable correct flux calculations (= total chamber volume). After installation in May 2006, soil CO_2 efflux measurements were carried out over a period of 1 month to record the insertion

and disturbance effects (Fig. S1 in the Supplement). The investigation period spanned over 3 years (January 2008 until December 2010), after the collars were well established and held vegetation free for 1.5 years, allowing a die-back and decomposition of trenched roots, and in-growth of new roots from the outside vegetation. This ensured that soil respiration measurements in a dense, closed grassland canopy were taken as unbiased as possible. Measurements of soil respiration were carried out weekly in the evening, except in July 2009. From May to July 2010 and from October to December 2010, measurements were carried out every second week. No measurements were carried out in November and December 2008.

During the measurement, a pump provided circulating air flow from the closed chamber on its collar to the infrared gas analyzer for thorough mixing of the systems' inner volume. Chamber closure time was between 1 and 3 min, depending on the season (i.e. the strength of the CO_2 efflux and thus the detection limit). CO_2 and H_2O concentrations were measured simultaneously. The software calculated soil respiration rates by using the changes in CO_2 concentration over a period of time, taking the dilution of water vapour into account. Rates were calculated either by linear regression (lin_flux) or as the efflux rate at time t_0 at chamber closure using an exponential CO_2 efflux function (exp_flux) (LI-COR, 2007). The latter takes the diminishing CO_2 concentration gradient between the soil and the chamber headspace into account (Hutchinson and Mosier, 1981) and is implemented by LI-COR in the LI-8100 to avoid underestimations of the CO_2 efflux. We used the following algorithm to choose between these two types of flux calculation for the subsequent processing of all obtained flux data. The use of the exp_flux calculation was only allowed when (1) the R^2 of the exp_flux calculation was better than that of the lin_flux calculation, and (2) when the number of iterations necessary for the exp_flux calculation was lower than five. By applying these comparatively strict criteria (stricter than those that are inbuilt by the manufacturer) we minimized miscalculations caused either by large initial CO_2 concentration fluctuations at chamber closure (when the exp_flux calculation is used) or underestimations of the true soil CO_2 efflux (when only the lin_flux calculation is used). The algorithm was applied to each measurement with the same settings. In general, CO_2 flux rates with an R^2 below 0.90 were excluded. This was the case in 0.6 % of all measurements taken in this study throughout the 3-year investigation period.

Soil moisture was measured in each FACE plot as the volumetric water content (VWC) with time-domain-reflectometry (TDR) probes (Imko, Ettlingen, Germany, type P2G). The probes were permanently installed (in March 1998) within the top 15 cm. The probes were monitored manually once a day, except on weekends or holidays. Soil temperature was logged in every plot at 10 cm depth as 15 min means (Imko, Ettlingen, Germany, Pt-100 sensors).

2.3 Data analyses

In order to describe changes in soil respiration during different seasons and to test for differences in soil respiration between ambient and elevated CO_2, we performed a linear mixed-effect model analysis with SPSS version 18. We used all measured data of 3 years for the linear mixed-effect model analysis to obtain seasonal estimates of soil respiration. CO_2 treatment was considered as a fixed effect in the model. Coding variables were introduced to indicate the hierarchical order of the data. The six mean fluxes taken in one measurement cycle received the same numerical code; this variable ("measurement cycle") was considered as a random effect in the linear mixed effect model. A further variable ("ringreplicate") was introduced to define the ring where the measurement was taken (1–6). "Ringreplicate" was selected as a repeated measure in the SPSS software using linear mixed effect model analysis. Maximum likelihood was used as the estimation method for the parameters in the model. The total observational data set was split by season to analyse seasonal CO_2-response patterns. Therefore, we distinguished the following five seasons (1–5), depending on major dates of phenology and management practices at the grassland study site (Fig. 1): 1 is winter (November–March); 2 is the start of vegetation period up to the date of spring fertilizer application (March–middle of April); 3 is spring until first biomass harvest (middle of April–end of May); 4 is regrowth and summer growing season (end of May–beginning of September); 5 is regrowth and autumn growing season (beginning of September–end of October).

The start of the vegetation period for the grassland ecosystem was identified according to the calculations defined by Wasshausen (1987). The date of leaf discoloration of *Quercus robur* in the nearby phenological garden was used to identify the beginning of winter dormancy. All other dates were chosen according to the management practices at the study site (Fig. 1); the exact dates varied by a few days between the years.

2.4 Soil respiration model

We applied a temperature response model to fill gaps in the measured data set. Therefore a function was fitted according to Lloyd and Taylor (1994) (Eq. 1) to 20 % of the data that were randomly selected. We defined values for coefficients $E0(= 62.16)$, $T0(= 262.47)$ and $R10(= 2.85)$ for the first run of the model. Subsequently, $E0$, $T0$ and $R10$ were fitted for each treatment (ambient and eCO_2) by using the dynamic fit function in the SigmaPlot 11.0 software package (Systat Software, San Jose, CA, 2008). Mean soil temperature values were converted from °C to K.

$$f = R10e^{E0\left(\frac{1}{(283.15-T0)}-\frac{1}{(x-T0)}\right)}, \quad (1)$$

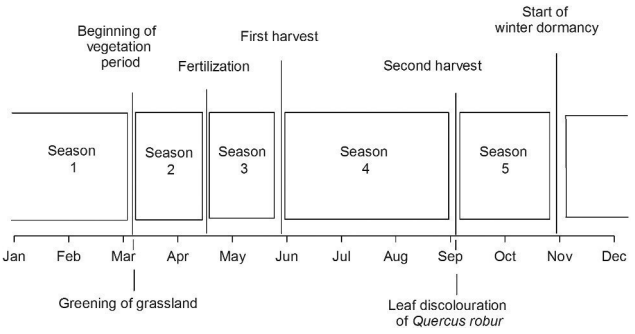

Figure 1. Seasonal patterns and the five defined seasons at the GiFACE grassland study site.

with $E0$ = activation-energy-type empirical coefficient, $T0$ = lower temperature limit for soil respiration in K, $R10$ = respiration rate at 10 °C.

Consequently, the quality of the soil respiration model was evaluated by plotting modelled soil respiration rates against the remaining 80 % of the observed respiration values to test if the linear trend line meets the requested slope of 1 (Fig. 5).

2.5 Annual estimates of soil respiration

To obtain annual sums of soil respiration, measured data was used whenever available, and modelled data for data gaps. Modelled soil respiration rates were calculated, based on the almost continuous data set of soil temperature in 10 cm depth measured at 2–3 positions per ring. We received modelled fluxes for every 15 min over the 3-year period for all gaps where no observational data were available. Estimates of annual sums were then calculated with the observational data and the modelled data per ring and averaged between treatments as true steps ($n = 3$). Differences in annual soil respiration between the CO_2 treatments were tested by using a paired t test. Further, the absolute difference and relative change of monthly mean soil respiration rates under eCO_2 were calculated in comparison to soil respiration under ambient CO_2, based on observational and modelled data. For calculating the relative change ambient soil respiration was set to 0 %.

3 Results

3.1 Annual variability of soil respiration

From 2008 to 2010, soil respiration rates at the GiFACE experiment showed distinct annual dynamics, following the seasonal temperature cycle with lowest soil respiration effluxes during winter months and highest effluxes during midsummer (Fig. 2c, g). Thus, soil respiration rates responded to abiotic factors in particular temperature and moisture. This is exemplified by the high CO_2 efflux rates in June 2009 which occurred shortly after a period of high precipitation while soil temperatures were > 20 °C (Fig. 2g).

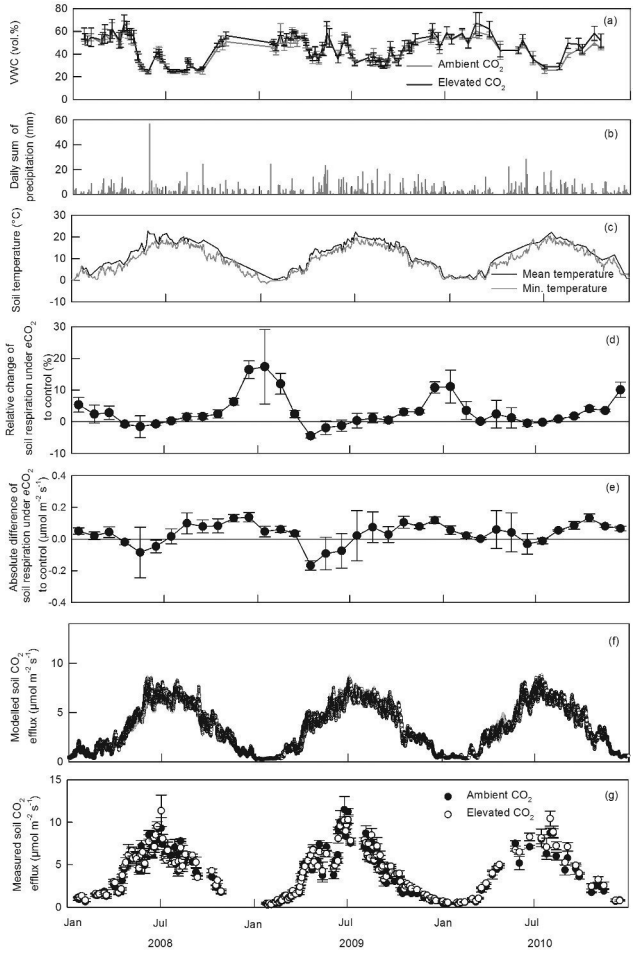

Figure 2. Volumetric water content under ambient and elevated CO_2 **(a)**, daily sums of precipitation at the GiFACE **(b)**, mean soil temperature during soil respiration measurements and minimum daily soil temperature at 10 cm depth **(c)**, the relative mean monthly change of soil respiration under elevated CO_2 based on measured and modelled data **(d)**, the absolute mean monthly difference in soil respiration under elevated CO_2 based on measured and modelled data **(e)**, modelled soil respiration under ambient and elevated CO_2 from 2008 to 2010 **(f)** and measured soil respiration under ambient and elevated CO_2 from 2008 to 2010 **(g)**. Data are presented as averages ($n = 3$) ±1 SE.

The relative and absolute change of soil respiration under eCO_2 (Fig. 2d, e) followed a seasonal pattern with greatest increases under eCO_2 during autumn and winter. During midsummer, when the largest absolute soil respiration rates occurred, the relative increase due to the CO_2 enrichment was lowest or non-existent. A linear mixed effect model analysis confirmed that soil respiration rates under eCO_2 were significantly higher compared to rates under ambient CO_2 during autumn (15.7 %) and winter (17.4 %) (Fig. 3). During all other seasons (beginning of vegetation period (season

Table 1. Results of fitting the temperature-dependence model after Lloyd and Taylor (1994) to 20 % of our observation data under ambient and elevated CO_2.

CO_2 treatment	R	R^2	Adjusted R^2	Standard error of estimate
Ambient CO_2	0.87	0.75	0.75	1.35
Elevated CO_2	0.91	0.82	0.82	1.19

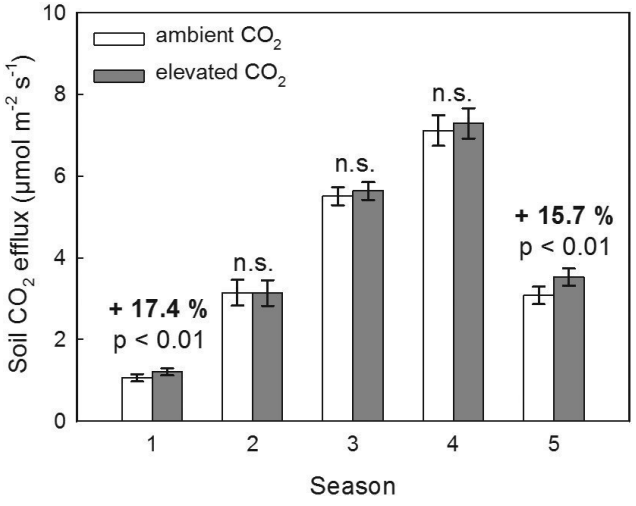

Figure 3. Mean soil respiration rates during the five defined seasons under ambient and elevated CO_2 averaged over 3 years from 2008–2010. Error bars show ±1 SE associated by averaging across the three replicates per treatment ($n = 3$) (1) is winter dormancy; (2) is the start of vegetation period; (3) is spring; (4) is summer; (5) is autumn (for details see methods). P values indicate the difference between treatments obtained by a linear mixed-effect model analysis.

2), spring (season 3) and summer (season 4)), covering most of the vegetation period, a trend towards higher soil respiration, but no significant CO_2 effect was observed with eCO_2 (Fig. 3).

3.2 Model performance and parameter estimation

By comparing modelled soil respiration with observed soil respiration for all observation dates from 2008–2010 a significant linear relationship was observed with a slope of 1.02 (Fig. 5).

Based on the temperature-respiration function by Taylor and Lloyd (1994), soil respiration was significantly correlated to soil temperature under ambient as well as eCO_2 ($p = <0.0001$). From 2008 to 2010, 75 % of the variability of soil respiration rates was explained by soil temperature under ambient CO_2 and 82 % under eCO_2 (Fig. 4, Table 1). Soil respiration rates did not differ in their relationship to soil temperature between the treatments (Fig. 4).

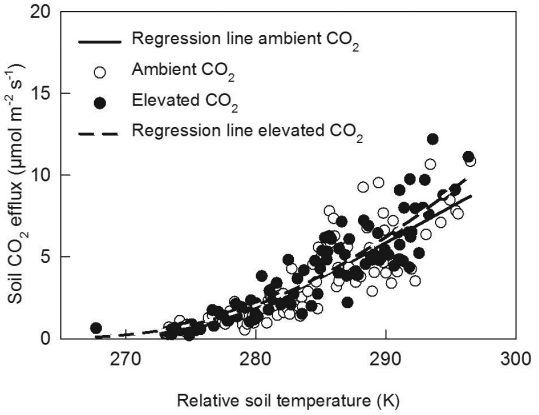

Figure 4. Relationship between soil respiration rate and soil temperature under ambient and elevated CO_2. Equation of dynamic fit (Lloyd and Taylor, 1994): $f = R10e^{E0\left(\frac{1}{(283.15-T0)}-\frac{1}{(x-T0)}\right)}$.

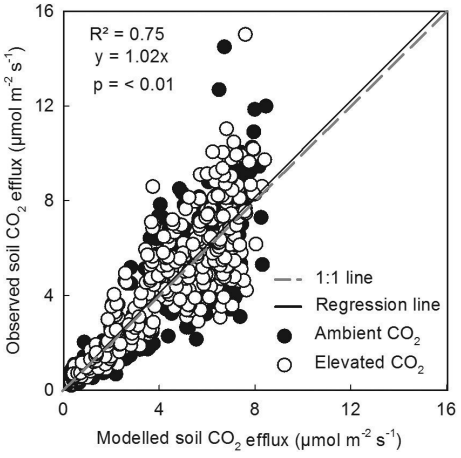

Figure 5. Observed versus modelled soil respiration rates under ambient and elevated CO_2.

3.3 Annual sums of soil respiration

Comparing annual sums of soil respiration, no mean treatment effect of elevated CO_2 (over all seasons) was observed in any of the observation years (Table 2). Mean annual estimates of soil respiration under ambient CO_2 ranged from 1283 to 1344 and under eCO_2 from 1300 to 1352 g C [CO_2] m^{-2} yr^{-1} (Table 2).

4 Discussion

4.1 Annual sums of soil respiration

In contrast to our initial hypotheses, annual estimates of soil respiration were not different between the CO_2 treatments (Table 2). Mean annual sums of soil respiration were 1317 ± 18 g C m^{-2} yr^{-1} under ambient CO_2 and

Table 2. Annual sums of soil respiration under ambient and eCO_2 from 2008–2010. Data are presented as averages ($n = 3$) ± standard error (SE). P values indicate the difference between treatments per year obtained by a paired t test.

Year	CO_2 treatment	Mean annual sum of soil respiration ($g\,CO_2\,m^{-2}\,yr^{-1}$)	Mean annual sum of soil respiration ($g\,C[CO_2]\,m^{-2}\,yr^{-1}$)	Relative change to control (%)	P value
2008	Ambient CO_2	4854 ± 34	1324 ± 9	1.22	0.17
	Elevated CO_2	4913 ± 14	1340 ± 4		
2009	Ambient CO_2	4928 ± 48	1344 ± 13	0.56	0.64
	Elevated CO_2	4956 ± 39	1352 ± 11		
2010	Ambient CO_2	4702 ± 37	1283 ± 10	1.38	0.23
	Elevated CO_2	4767 ± 12	1300 ± 3		

$1331 \pm 16\,g\,C\,m^{-2}\,yr^{-1}$ under elevated CO_2. Raich and Schlesinger (1992) estimated much lower rates of annual soil respiration, reporting 400 to $500\,g\,C\,m^{-2}\,yr^{-1}$ for temperate grasslands. Annual soil respiration sums from a sandstone and serpentine grassland were 485 and $346\,g\,C\,m^{-2}\,yr^{-1}$ (Luo et al., 1996). These soil respiration rates were lower than those from the wet grassland site investigated here due to the larger net primary productivity of the wet temperate grassland with a year-round more or less moist climate, compared e.g. to a seasonally dry Mediterranean-type grassland. A lower net ecosystem productivity (NEP) will automatically result in lower overall soil respiratory C losses. Methodological differences may have been to a lesser extent responsible, because the studies of Luo et al. (1996) and Raich and Schlesinger (1992) may have overestimated rather than underestimated the annual soil respiration. Their measurements did not exceed 2 years in duration and soil respiration was less frequently measured for a portion of the year. Other recent studies reported higher rates of annual soil respiration which are closer to our estimates; however climatic factors are different from our site: in a tallgrass prairie in Oklahoma annual soil respiration rates were 1131 and $877\,g\,C\,m^{-2}\,yr^{-1}$ in 2002 and 2003 respectively (Zhou et al., 2006). In a Texas grassland annual soil respiration rates increased with annual precipitation and were 1600, 1300, 1200, 1000, 2100 and $1500\,g\,C\,m^{-2}\,yr^{-1}$ in 1993 through 1998 respectively (Mielnick and Dugas, 2000). At the Texas grassland site measurements were conducted year-round with a high time resolution. Consequently annual rates could be estimated by more measured (than gap-filled) data compared to other studies. However the most important factors were likely the annual precipitation, its distribution over the year, and the annual mean temperature: High annual rainfall, a long growing season and large soil organic C contents explained the higher soil respiration rates (as a consequence of a higher NEP) at the Texas study site. Mean annual precipitation at the GiFACE study site (562 mm) was close to the mean precipitation reached in 1995 at the Texas grassland with 657 mm, when annual soil respiration averaged $1200\,g\,C\,m^{-2}\,yr^{-1}$ at the Texas grassland.

4.2 Seasonality of soil respiration

Also, contrary to our initial hypotheses is the observation that soil respiration was not significantly affected during the growing season (start of vegetation period, spring and summer) by moderate long-term CO_2 enrichment. This indicates that any increase in the ecosystem respiration (Lenhart, 2008) during this season will not have been due to enhanced soil (root-derived) respiration but rather to increases in the respiration of the green canopy.

The majority of long-term FACE studies reported significantly increased soil respiration under eCO_2 during the growing season (Pregitzer et al., 2008; Jackson et al., 2009; Pendall et al., 2001; Dawes et al., 2013; Carol Adair et al., 2011), whereas Bader and Körner (2010) reported that 7 years of eCO_2 failed to stimulate cumulative soil respiration significantly during the growing season. Among the mentioned long-term FACE experiments, the GiFACE operates at the lowest CO_2 enrichment step increase (20 % above ambient CO_2), which may have contributed to this result.

However, in line with our hypotheses, the results revealed that 10 years of moderate CO_2 enrichment increased soil respiration during winter and autumn (Fig. 3). These seasonal stimulations of soil respiration under eCO_2 were not observed by comparing the annual sums of soil respiration (Table 2). This may be because soil respiration fluxes were lower in winter and autumn compared to fluxes from the other seasons where no differences in soil respiration between the CO_2 treatments were observed. However, within the winter and autumn season differences in soil respiration may play an important role concerning the global C balance. Increased rates of winter soil respiration under eCO_2 may increase the observed winter CO_2 maximum in the atmosphere (Raich and Potter, 1995; Keeling et al., 1996) when respiration exceeds photosynthesis. Another reason why annual sums of soil respiration were not different between the CO_2 treatments may be that our model underestimated high soil

respiration fluxes ($> 10\,\mu mol\,m^{-2}\,s^{-1}$). However these fluxes occurred only in 1.72 % of all observations. Our model did not take soil moisture into account. The high variability of observed soil respiration during summer may be partly due to differing soil moisture conditions, which were not significantly different between ambient and eCO_2 plots (Kammann et al., 2005, 2008).

In most FACE studies which reported the effect of eCO_2 on soil respiration, the winter was excluded since fumigation during this period was mostly switched off (often in response to sub-zero freezing temperatures or deciduous forest ecosystems). This was the case in the Swiss FACE study, where seeded grassland was exposed to 600 ppm CO_2 (de Graaff et al., 2004), the BioCON FACE, also a grassland study (Craine et al., 2001; Carol Adair et al., 2011), the Aspen FACE, an aspen forest enriched with eCO_2 (Pregitzer et al., 2008; King et al., 2001), a Japanese model forest ecosystem exposed to 550 ppm CO_2 (Masyagina and Koike, 2012) and in a 9-year FACE study of an alpine tree line ecosystem (Dawes et al., 2013). In the Swiss Canopy Crane study soil respiration was measured during the beginning of the dormant season but not over the complete dormant season while fumigation was switched off (Bader and Körner, 2010). In the Maricopa FACE, where a wheat field was exposed to eCO_2, no winter measurements were carried out because this season was a fallow season (Pendall et al., 2001). Outside the cultivation period no soil respiration measurements were made on a cotton plantation exposed to eCO_2 (Nakayama et al., 1994).

Increased winter soil CO_2 fluxes are in line with results from Selsted et al. (2012), who reported stimulated rates during three consecutive winter periods in a Danish N-limited *Calluna-Deschampsia*-heathland exposed to FACE at 510 ppm (CLIMAITE study). Fumigation was carried out all year round except during periods with full snow cover. Contrary to our results, in the CLIMAITE study, the stimulatory effect of eCO_2 on soil respiration persisted throughout most of the year, i.e. also in summer and not only during winter. However, in the CLIMAITE study, monthly soil respiration measurements were carried out within the first 3 years after the experimental start and may therefore reflect short-term responses, driven by the initial CO_2 step increase (Klironomos et al., 2005). Thus the results are not completely comparable to this study where measurements were carried out in the eleventh to thirteenth year of CO_2 enrichment.

To our knowledge, the Duke Forest FACE is the only other FACE experiment where soil respiration was measured in an evergreen ecosystem year-round for several years and after long-term fumigation with eCO_2 (+200 ppm). On average, soil respiration was significantly higher by 23 % under eCO_2. Jackson et al. (2009) summarized, after 10 years of CO_2 enrichment, that the greatest stimulation of soil respiration under eCO_2 occurred from midsummer to early fall, in contrast to our observations, during winter the CO_2 response of soil respiration was weakest. However, fumigation was stopped at the Duke Forest FACE when ambient air temperature dropped below 5 °C for more than 1 hr.

After short-term enrichment with eCO_2 (550 ppm) on a mixed plantation of *Populus* species (EUROFACE; in the fourth and fifth year of enrichment), Lagomarsino et al. (2013) recorded much larger stimulation of soil respiration during the vegetation (up to 111 % enhancement) than dormant season (40 % enhancement), when fumigation was stopped, which is also contrary to our results. However, experimental setup and climate differed from our site. While minimum soil temperatures reached −1.7 °C in the GiFACE experiment during winter (Fig. 2b), comparably warm and mild winters without sub-zero temperatures were typical at the EUROFACE site located in Italy. Moreover, the *Populus* plantation was a fertilized agro-ecosystem, where coppicing was carried out every 3 years, while the GiFACE was an old established, species-rich ecosystem where N-supply was limited.

In line with results from the EUROFACE but in contrast to our findings, Volk and Niklaus (2002) did not observe any wintertime increase in the ecosystem CO_2 efflux from a calcareous grassland in response to 3 years of CO_2 enrichment (600 ppm) with a screen-aided CO_2 enrichment facility.

Investigations from the GiFACE experiment showed that N_2O emissions also exhibited a "seasonality response", with the greatest stimulation of N_2O emission under eCO_2 being observed in late-summer and autumn (Kammann et al., 2008). These findings support the hypothesis that the driving mechanism of the eCO_2 seasonality responses of enhanced microbial activity may have been related to the mineralization of previously accumulated organic matter, fuelling denitrification (Kammann et al., 2008).

4.3 Root-derived soil respiration

Increased root biomass was frequently recorded under eCO_2 (Rogers et al., 1994; Jastrow et al., 2000; Lukac et al., 2009), potentially affecting soil respiration rates (Zak et al., 2000). However, at the GiFACE, root biomass, picked with forceps (for set time intervals per sample, $n = 3$ per FACE ring), was only different in December 2005 between the CO_2 treatments but not at other dates during 2004–2007 (Lenhart, 2008) or in November 2011 (unpublished results). Lenhart (2008) observed in the GiFACE eCO_2 plots, using Keeling plots and two-component mixing models that the fraction of root-derived CO_2 (root- and root-exudate respiration and fine root decay), as part of the total soil CO_2 efflux was lower in winter than during the growing season. Accordingly, during winter, the soil CO_2 efflux originated mainly from microbial soil respiration.

Higher fine root turnover under eCO_2, resulting in higher C input via root necromass could explain increased autumn soil respiration but unlikely the winter increase in soil CO_2 efflux at the GiFACE since root necromass was not changed under eCO_2 in November 2011 (unpublished results). Al-

ternatively, differences in the root necromass could already have been decomposed at this time of sampling or may be observed later in the year, so that "enhanced fine root decomposition" as a cause of the autumn and winter soil respiration increase under eCO_2 cannot be ruled out.

4.4 N availability

Since soil microorganisms require C as well as N for maintenance and growth (De Graaff et al., 2006; Zak et al., 1993), N availability plays an important role in determining soil CO_2 efflux. Root respiration rates were observed to correlate with tissue nitrogen concentration (Burton et al., 1996, 1998). In the GiFACE, eCO_2 caused reduced tissue N concentrations and higher C : N-ratios of aboveground plant biomass (Kammann et al., 2008). Through freezing effects in winter, mineral N, which was immobilized into the microbial biomass shortly after fertilizer application in spring, became partly available again (Müller et al., 2003). It is possible that N, as a limiting factor in the temperate grassland, may partly be responsible for the increase in soil C loss during the autumn and winter season under eCO_2.

4.5 Microbial community

Multiple observations from the GiFACE indicated that increases in winter soil respiration under eCO_2 were largely associated with microbial respiration (including rhizosphere microbiota). Recent studies from other FACE sites detected differences between microbial communities at eCO_2 compared to ambient CO_2 (Drigo et al., 2008, 2009). At the GiFACE, stimulated rhizosphere-C utilization by arbuscular mycorrhizal fungi were found under eCO_2 by a ^{13}C-PLFA study (Denef et al., 2007), which may have contributed to altered soil respiration. Recent measurements in 2013 did not indicate any differences in the abundance of bacteria and archaea between the ambient and eCO_2 plots (K. Brenzinger, personal communication, 2014) so that this can be ruled out as a cause for differed soil respiration between the CO_2 treatments if this observation persists throughout autumn and winter.

4.6 Soil moisture

Several studies showed that eCO_2 can affect soil moisture (Niklaus et al., 1998; Field et al., 1995; Hungate et al., 1997), which in turn regulates soil respiration. However, large effects are only expected and were detected at the dry end of the spectrum (Moyano et al., 2012; Guntinas et al., 2013; Rodrigo et al., 1997). During the investigation period, the volumetric water content ranged from 20 to 80 vol. % at the GiFACE site, with an average of 44 % during 2008–2010, and 39 % over the vegetation periods of these years. Thus, the soil moisture effect is likely not to be large. Moreover, no significant effect of eCO_2 on the soil water content was observed either during the first 5 years of enrichment (Kammann et al., 2005) or after 13 years of enrichment (Meine, 2013). Consequently, a CO_2-induced soil moisture effect is unlikely governing increased soil respiration rates.

However, it can be assumed that annual dynamics of soil moisture with wettest conditions in winter, i.e. close to saturation, and driest conditions in summer (Fig. 2a) contributed to the seasonal dynamics of soil respiration under eCO_2 due to diffusion limitations. Previous results from the GiFACE site show that in periods when soil moisture in the main rooting zone was low ($0.3\,m^3\,m^{-3}$), soil continued to produce N_2O from deeper soil layers (20–50 cm), where soil moisture remained high (ca. $0.6\,m^3\,m^{-3}$) (Müller et al., 2004). The production of N_2O at deep soil layers seemed to coincide with the production of CO_2 during summer, which was also characterized by a homogenous $\delta^{13}CO_2$ profile during vegetation period at our study site (Lenhart, 2008). However, a detailed investigation on layer-specific CO_2 production was beyond the scope of this study. At times of high soil moisture CO_2 diffusion was slowed down, coinciding with limited oxygen supply (Skopp et al., 1990). At these times, soil respiration was likely to be originating mainly from the topsoil. However, increased autumn soil respiration under eCO_2 cannot be attributed to this phenomenon since soil water content is relatively low at this season (Fig. 2a). We suggest that increased substrate supply under eCO_2 from end-of-season dieback of roots and enhanced root-associated microbiome activity may explain stimulated soil respiration rates in autumn.

4.7 Plant community

Another aspect which may have contributed to altered soil respiration rates under eCO_2 is a shift in the plant community composition. Grüters et al. (2006) observed that summergreens decreased, whereas evergreens increased under eCO_2 in the GiFACE experiment. Since soil respiration is controlled by substrate supply via rhizodeposition (Verburg et al., 2004; Wan and Luo, 2003; Craine et al., 1999), higher photosynthetic activity in eCO_2 plots during mild winter may have contributed to the observed increase in soil respiration. In addition, since the vegetative aboveground growth is dormant and does not provide an assimilate sink, the relative proportion of assimilate partitioned below-ground towards the root-associated microbiota may increase, contributing to the relative increase under eCO_2 during winter. The higher abundance of evergreens at eCO_2 also underlines the importance of a year-round CO_2 enrichment strategy in such ecosystems with the respective climatic conditions. To date, increased winter soil respiration at eCO_2 was only found in FACE experiments with year-round fumigation and a photosynthesizing at least partly green canopy, i.e. in the CLIMAITE study (Selsted et al., 2012) and in this study.

5 Conclusions

In conclusion, our results demonstrate the importance of winter soil respiration measurements, by showing that soil respiration was increased during autumn and winter after moderate long-term eCO_2. Measurements and year-round CO_2 enrichment should not be neglected, at least in winter-green temperate ecosystems. Studies in such ecosystems excluding measurements during the dormant season may thus underestimate the effect of eCO_2 on annual soil-respiratory CO_2 losses (i.e. leading to an overestimation of C sequestered). Consequently, winter soil CO_2 fluxes may play a crucial role in determining the carbon balance and dynamics of temperate grassland ecosystems. Our results indicate that temperate European grasslands which are characterized by a greenhouse gas balance near zero (Soussana et al., 2007) may gradually turn into greenhouse gas sources with rising atmospheric CO_2 due to enhanced CO_2 losses during autumn and winter, in particular if N_2O emissions are significantly increased as well as observed in the GiFACE (Kammann et al., 2008; Regan et al., 2011).

To generalize and explain the variation in the temporal dynamics of soil respiration under eCO_2 more studies of winter C dynamics under long-term eCO_2 are required. For such future studies it is advisable to include frequent samplings of root biomass, including the fine root fraction and necromass, in particular during the autumn/winter period under eCO_2. Another beneficial research strategy may be combined (pulse) labelling of ^{15}N and ^{13}C to elucidate gross C and N turnover processes after long-term (> 10 years) of CO_2 enrichment to study the C-N gross dynamics and associated carbonaceous gas losses.

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References

Allen, A. S., Andrews, J. A., Finzi, A. C., Matamala, R., Richter, D. D., and Schlesinger, W. H.: Effects of free-air CO_2 enrichment (FACE) on belowground processes in a Pinus taeda forest, Ecol. Appl., 10, 437–448, doi:10.2307/2641105, 2000.

Amundson, R.: The carbon budget in soils, Annu. Rev. Earth Pl. Sc., 29, 535–562, 2001.

Andrews, J. A. and Schlesinger, W. H.: Soil CO_2 dynamics, acidification, and chemical weathering in a temperate forest with experimental CO_2 enrichment, Global Biogeochem. Cy., 15, 149–162, doi:10.1029/2000gb001278, 2001.

Bader, M. K. F. and Körner, C.: No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment, Global Change Biol., 16, 2830–2843, doi:10.1111/j.1365-2486.2010.02159.x, 2010.

Batjes, N. H.: Total carbon and nitrogen in the soils of the world, Eur. J. Soil Sci., 47, 151–163, doi:10.1111/j.1365-2389.1996.tb01386.x, 1996.

Burton, A. J., Pregitzer, K. S., Zogg, G. P., and Zak, D. R.: Latitudinal variation in sugar maple fine root respiration, Can. J. For. Res., 26, 1761–1768, doi:10.1139/x26-200, 1996.

Burton, A. J., Pregitzer, K. S., Zogg, G. P., and Zak, D. R.: Drought reduces root respiration in sugar maple forests, Ecol. Appl., 8, 771–778, doi:10.1890/1051-0761(1998)008[0771:drrris]2.0.co;2, 1998.

Carol Adair, E., Reich, P. B., Trost, J. J., and Hobbie, S. E.: Elevated CO_2 stimulates grassland soil respiration by increasing carbon inputs rather than by enhancing soil moisture, Global Change Biol., 17, 3546–3563, doi:10.1111/j.1365-2486.2011.02484.x, 2011.

Chapin III, F. S., Matson, P. A., and Mooney, H. A.: Principles of terrestrial ecosystem ecology, Springer, New York, 436 pp., 2002.

Craine, J. M., Wedin, D. A., and Chapin, F. S.: Predominance of ecophysiological controls on soil CO_2 flux in a Minnesota grassland, Plant Soil, 207, 77–86, 1999.

Craine, J. M., Wedin, D. A., and Reich, P. B.: The resonse of soil CO_2 flux to changes in atmospheric CO_2, nitrogen supply and plant diversity, Global Change Biol., 7, 947–953, 2001.

Dawes, M. A., Hagedorn, F., Handa, I. T., Streit, K., Ekblad, A., Rixen, C., Korner, C., and Hattenschwiler, S.: An alpine treeline in a carbon dioxide-rich world: synthesis of a nine-year free-air carbon dioxide enrichment study, Oecologia, 171, 623–637, doi:10.1007/s00442-012-2576-5, 2013.

de Graaff, M.-A., Six, J., Harris, D., Blum, H., and van Kessel, C.: Decomposition of soil and plant carbon from pasture systems after 9 years of exposure to elevated CO_2: impact on C cycling and modeling, Global Change Biol., 10, 1922–1935, doi:10.1111/j.1365-2486.2004.00862.x, 2004.

de Graaff, M.-A., Van Groenigen, K.-J., Six, J., Hungate, B. A., and Van Kessel, C.: Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis, Global Change Biol., 12, 2077–2091, 2006.

Denef, K., Bubenheim, H., Lenhart, K., Vermeulen, J., Van Cleemput, O., Boeckx, P., and Müller, C.: Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO_2, Biogeosciences, 4, 769–779, doi:10.5194/bg-4-769-2007, 2007.

Drigo, B., Kowalchuk, G. A., and van Veen, J. A.: Climate change goes underground: effects of elevated atmospheric CO_2 on microbial community structure and activities in the rhizosphere, Biol. Fertil. Soils, 44, 667–679, doi:10.1007/s00374-008-0277-3, 2008.

Drigo, B., Van Veen, J. A., and Kowalchuk, G. A.: Specific rhizosphere bacterial and fungal groups respond differently to elevated atmospheric $CO_2$2, Isme J., 3, 1204–1217, doi:10.1038/ismej.2009.65, 2009.

Field, C. B., Jackson, R. B., and Mooney, H. A.: Stomatal response to increased CO_2: implications from the plant to the global scale, Plant Cell Environ., 18, 1214–1225, 1995.

Grüters, U., Janze, S., Kammann, C., and Jäger, H.-J.: Plant functional types and elevated CO_2: a method of scanning for causes of community alteration, J. Appl. Bot. Food Qual., 80, 116–128, 2006.

Guntinas, M. E., Gil-Sotres, F., Leiros, M. C., and Trasar-Cepeda, C.: Sensitivity of soil respiration to moisture and temperature, J. Soil Sci. Plant Nutr., 13, 445–461, doi:10.4067/s0718-95162013005000035, 2013.

Heinemeyer, A., Di Bene, C., Lloyd, A. R., Tortorella, D., Baxter, R., Huntley, B., Gelsomino, A., and Ineson, P.: Soil respiration: implications of the plant-soil continuum and respiration chamber collar-insertion depth on measurement and modelling of soil CO_2 efflux rates in three ecosystems, Eur. J. Soil Sci., 62, 82–94, doi:10.1111/j.1365-2389.2010.01331.x, 2011.

Hungate, B. A., Chapin III, F. S., Zhong, H., Holland, E. A., and Field, C. B.: Stimulation of grassland nitrogen cycling under carbon dioxide enrichment, Oecologia, 109, 149–153, 1997.

Hutchinson, G. L. and Mosier, A. R.: Improved soil cover method for field measurement of nitrous oxide fluxes, Soil Sci. Soc. Am. J., 45, 311–316, 1981.

Jackson, R. B., Cook, C. W., Pippen, J. S., and Palmer, S. M.: Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest, Ecology, 90, 3352–3366, doi:10.1890/08-1609.1, 2009.

Jäger, H.-J., Schmidt, S. W., Kammann, C., Grünhage, L., Müller, C., and Hanewald, K.: The University of Gießen Free-Air Carbon Dioxide Enrichment Study: Description of the experimental site and of a new enrichment system, J. Appl. Bot., 77, 117–127, 2003.

Janssens, I. A. and Ceulemans, R.: The response of soil CO_2 efflux under trees grown in elevated atmospheric CO_2: A literature review, Phyton-Ann. REI Bot., 40, 97–101, 2000.

Jastrow, J. D., Miller, R. M., and Owensby, C. E.: Long-term effects of elevated atmospheric CO_2 on below-ground biomass and transformation to soil organic matter in grassland, Plant Soil, 224, 85–97, 2000.

Kammann, C., Grünhage, L., Grüters, U., Janze, S., and Jäger, H.-J.: Response of aboveground grassland biomass and soil moisture to moderate long-term CO_2 enrichment, Basic Appl. Ecol., 6, 351–365, 2005.

Kammann, C., Müller, C., Grünhage, L., and Jäger, H.-J.: Elevated CO_2 stimulates N_2O emissions in permanent grassland, Soil Biol. Biochem., 40, 2194–2205, 2008.

Keeling, C. D., Chin, J. F. S., and Whorf, T. P.: Increased activity of northern vegetation inferred from atmospheric CO_2 measurements, Nature, 382, 146–149, 1996.

King, J. S., Pregitzer, K. S., Zak, D. R., Sober, J., Isebrands, J. G., Dickson, R. E., Hendrey, G. R., and Karnosky, D. F.: Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric Co-2 and tropospheric O-3, Oecologia, 128, 237–250, 2001.

King, J. S., Hanson, P. J., Bernhardt, E., DeAngelis, P., Norby, R. J., and Pregitzer, K. S.: A multiyear synthesis of soil respiration responses to elevated atmospheric CO_2 from four forest FACE experiments, Global Change Biol., 10, 1027–1042, doi:10.1111/j.1529-8817.2003.00789.x, 2004.

Kirschbaum, M. U. F.: Will changes in soil organic carbon act as a positive or negative feedback on global warming?, Biogeochem., 48, 21–51, doi:10.1023/a:1006238902976, 2000.

Klironomos, J. N., Allen, M. F., Rillig, M. C., Piotrowski, J., Makvandi-Nejad, S., Wolfe, B. E., and Powell, J. R.: Abrupt rise in atmospheric CO_2 overestimates communitiy response in a model-plant soil system, Nature, 433, 621–624, 2005.

Lagomarsino, A., Lukac, M., Godbold, D. L., Marinari, S., and De Angelis, P.: Drivers of increased soil respiration in a poplar coppice exposed to elevated CO_2, Plant Soil, 362, 93–106, doi:10.1007/s11104-012-1261-0, 2013.

Lal, R.: Soil carbon sequestration impacts on global climate change and food security, Science, 304, 1623–1627, 2004.

Leadley, P. W. and Drake, B. G.: Open top chambers for exposing plant canopies to elevated CO_2 concentration and for measuring net gas-exchange, Vegetatio, 104, 3–15, doi:10.1007/bf00048141, 1993.

Lenhart, K.: The effects of long-term Free Air CO_2 Enrichment (FACE) on soil aggregation, soil carbon input, and ecosystem CO_2 dynamics in a temperate grassland ecosystem, Department of Plant Ecology, Justus-Liebig University, Gießen, 134 pp., 2008.

LI-COR: LI-8100 Instruction Manual, LI-8100 automated soil CO_2 flux system., Li-COR, Inc, Lincoln, NE, USA 68504, 2007.

Liu, Q., Edwards, N. T., Post, W. M., Gu, L., Ledford, J., and Lenhart, S.: Temperature-independent diel variation in soil respiration observed from a temperate deciduous forest, Global Change Biol., 12, 2136–2145, 2006.

Lloyd, J. and Taylor, J. A.: On the temperature-dependence of soil respiration, Funct. Ecol., 8, 315–323, doi:10.2307/2389824, 1994.

Lukac, M., Lagomarsino, A., Moscatelli, M. C., De Angelis, P., Cotrufo, M. F., and Godbold, D. L.: Forest soil carbon cycle under elevated CO_2 – a case of increased throughput?, Forestry, 82, 75–86, doi:10.1093/forestry/cpn041, 2009.

Luo, Y., Jackson, R. B., Field, C. B., and Mooney, H. A.: Elevated CO_2 increases belowground respiration in California grasslands, Oecologia, 108, 130–137, doi:10.1007/bf00333224, 1996.

Luo, Y.: Transient ecosystem responses to free-air CO_2 enrichment (FACE): experimental evidence and methods of analysis, New Phytol., 152, 3–8, 2001.

Luo, Y., Wu, L., Andrews, J. A., White, L., Matamala, R., Schäfer, K. V. R., and Schlesinger, W. H.: Elevated CO_2 differentiates ecosystem carbon processes: deconvolution analysis of Duke forest data, Ecol. Monogr., 71, 357–376, 2001.

Luo, Y. and Zhou, X.: Soil Respiration and the Environment, Academic/Elsevier, San Diego, 328 pp., 2006.

Masyagina, O. V. and Koike, T.: Soil Respiration in Model Plantations under Conditions of Elevated CO_2 in the Atmo-

sphere (Hokkaido Island, Japan), Russ. J. Ecol., 43, 24–28, doi:10.1134/s1067413611060099, 2012.

McDermitt, D., Xu, L., Gracia, R., Madsen, R., and Anderson, D.: On equalizing pressure in a soil respiration chamber with pressure in the ambient air under windy conditions, Geophysical Research Abstracts, 7, 05841, 2005.

Meine, M.: Charakterisierung und Quantifizierung der mikrobiellen Bodenrespiration eines Grünlandbodens unter erhöhten atmosphärischen CO_2-Konzentrationen, diploma, Geography, Phillipps-Universität Marburg, Marburg, 101 pp., 2013.

Mielnick, P. C. and Dugas, W. A.: Soil CO_2 flux in a tallgrass prairie, Soil Biol. Biochem., 32, 221–228, doi:10.1016/S0038-0717(99)00150-9, 2000.

Monastersky, R.: Global carbon dioxide levels near worrisome milestone, Nature, 497, 13–14, 2013.

Moss, R. H., Edmonds, J. A., Hibbard, K. A., Manning, M. R., Rose, S. K., van Vuuren, D. P., Carter, T. R., Emori, S., Kainuma, M., Kram, T., Meehl, G. A., Mitchell, J. F. B., Nakicenovic, N., Riahi, K., Smith, S. J., Stouffer, R. J., Thomson, A. M., Weyant, J. P., and Wilbanks, T. J.: The next generation of scenarios for climate change research and assessment, Nature, 463, 747–756, 2010.

Moyano, F. E., Vasilyeva, N., Bouckaert, L., Cook, F., Craine, J., Curiel Yuste, J., Don, A., Epron, D., Formanek, P., Franzluebbers, A., Ilstedt, U., Kätterer, T., Orchard, V., Reichstein, M., Rey, A., Ruamps, L., Subke, J.-A., Thomsen, I. K., and Chenu, C.: The moisture response of soil heterotrophic respiration: interaction with soil properties, Biogeosciences, 9, 1173–1182, doi:10.5194/bg-9-1173-2012, 2012.

Müller, C., Kammann, C., Ottow, J. C. G., and Jäger, H.-J.: Nitrous oxide emission from frozen grassland soil and during thawing periods, Z. Pflanzenern. Bodenk., 166, 46–53, 2003.

Müller, C., Stevens, R. J., Laughlin, R. J., and Jäger, H.-J.: Microbial processes and the site of N_2O production in a temperate grassland soil, Soil Biol. Biochem., 36, 453–461, 2004.

Nakayama, F. S., Huluka, G., Kimball, B. A., Lewin, K. F., Nagy, J., and Hendrey, G. R.: Soil carbon dioxide fluxes in natural and CO_2-enriched systems, Agric. For. Met., 70, 131–140, doi:10.1016/0168-1923(94)90052-3, 1994.

Newton, P. C. D., Clark, H., Edwards, G. R., and Ross, D. J.: Experimental confirmation of ecosystem model predictions comparing transient and equilibrium plant responses to elevated atmospheric CO_2, Ecol. Lett., 4, 344–347, 2001.

Niklaus, P. A., Spinnler, D., and Korner, C.: Soil moisture dynamics of calcareous grassland under elevated CO_2, Oecologia, 117, 201–208, doi:10.1007/s004420050649, 1998.

Pendall, E., Leavitt, S. W., Brookes, T., Kimball, B. A., Pinter, P. J., Jr, Wall, G. W., LaMorte, R. L., Wechsung, G., Wechsung, F., Adamsen, F., Matthias, A. D., and Thompson, T. L.: Elevated CO_2 stimulates soil respiration in a FACE wheat field, Bas. App. Ecol., 2, 193–201, 2001.

Pregitzer, K. S., Burton, A. J., King, J. S., and Zak, D. R.: Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3, New Phytol., 180, 153–161, doi:10.1111/j.1469-8137.2008.02564.x, 2008.

Raich, J. W. and Potter, C. S.: Global patterns of carbon dioxide emissions from soils, Global Biogeochem. Cy., 9, 23–36, 1995.

Raich, J. W. and Schlesinger, W. H.: The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate, Tellus, 44B, 81–99, 1992.

Rastetter, E. B., Ryan, M. G., Shaver, G. R., Melillo, J. M., Nadelhoffer, K. J., Hobbie, J. E., and Aber, J. D.: A general biogeochemical model describing the response of the C and N cycles in terrestrial ecosystems to changes in CO_2, climate, and N deposition, Tree Phys., 9, 101–126, 1991.

Raynaud, D. and Barnola, J. M.: An Antarctic ice core reveals atmospheric CO_2 variations over the past few centuries, Nature, 315, 309–311, 1985.

Regan, K., Kammann, C., Hartung, K., Lenhart, K., Muller, C., Philippot, L., Kandeler, E., and Marhan, S.: Can differences in microbial abundances help explain enhanced N(2)O emissions in a permanent grassland under elevated atmospheric CO(2)?, Global Change Biol., 17, 3176–3186, doi:10.1111/j.1365-2486.2011.02470.x, 2011.

Rodrigo, A., Recous, S., Neel, C., and Mary, B.: Modelling temperature and moisture effects on C-N transformations in soils: comparison of nine models, Ecol. Mod., 102, 325–339, 1997.

Rogers, H. H., Runion, G. B., and Krupa, S. V.: Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere, Environ. Pollut., 83, 155–189, 1994.

Schils, R. L. M., Kuikman, P., Liski, J., van Oijen, M., Smith, P., Webb, J., Alm, J., Somogyi, Z., van den Akker, J., Billett, M., Emmett, B. A., Evans, C. D., Lindner, M., Palosuo, T., Bellamy, P. H., Jandl, R., and Hiederer, R.: Review of existing information on the interrelations between soil and climate change, Alterra, Wageningen, 208, p. 23, available at: http://ec.europa.eu/environment/soil/review_en.htm, 2008.

Selsted, M. B., van der Linden, L., Ibrom, A., Michelsen, A., Larsen, K. S., Pedersen, J. K., Mikkelsen, T. N., Pilegaard, K., Beier, C., and Ambus, P.: Soil respiration is stimulated by elevated CO_2 and reduced by summer drought: three years of measurements in a multifactor ecosystem manipulation experiment in a temperate heathland (CLIMAITE), Glob. Change Biol., 18, 1216–1230, doi:10.1111/j.1365-2486.2011.02634.x, 2012.

Skopp, J., Jawson, M. D., and Doran, J. W.: Steady-State Aerobic Microbial Activity as a Function of Soil Water Content, Soil Sci. Soc. Am. J., 54, 1619–1625, doi:10.2136/sssaj1990.03615995005400060018x, 1990.

Soe, A. R. B., Giesemann, A., Anderson, T. H., Weigel, H. J., and Buchmann, N.: Soil respiration under elevated CO_2 and its partitioning into recently assimilated and older carbon sources, Plant Soil, 262, 85–94, doi:10.1023/B:PLSO.0000037025.78016.9b, 2004.

Soussana, J. F., Fuhrer, J., Jones, M. B., and Van Amstel, A. R.: The greenhouse gas balance of grasslands in Europe, Agric. Ecosyst. Environ., 121, 1–4, 2007.

Verburg, P. J., Arnone III, J. A., Obrist, D., Schorran, D. E., Evans, R. D., Leroux-Swarthout, D., Johnson, D. W., Luo, Y., and Coleman, J. S.: Net ecosystem carbon exchange in two experimental grassland ecosystems, Glob. Change Biol., 10, 498–508, 2004.

Volk, M. and Niklaus, P. A.: Respiratory carbon loss of calcareous grasslands in winter shows no effects of 4 years' CO_2 enrichment, Funct. Ecol., 16, 162–166, 2002.

Wan, S. Q. and Luo, Y. Q.: Substrate regulation of soil respiration in a tallgrass prairie: Results of a clipping and shading experiment,

Global Biogeochem. Cy., 17, 1054, doi:10.1029/2002gb001971, 2003.

Ward, J. K. and Kelly, J. K.: Scaling up evolutionary responses to elevated CO_2: lessons from *Arabidopsis*, Ecol. Lett., 7, 427–440, 2004.

Wasshausen, W.: Frühjahrspflege auf dem Grünland: Zehn Punkte beachten, Landwirtschaftsblatt Weser-Ems, 8, 6–8, 1987.

Zak, D. R., Pregitzer, K. S., Curtis, P. S., Teeri, J. A., Fogel, R., and Randlett, D. L.: Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles, Plant Soil, 151, 105–117, 1993.

Zak, D. R., Pregitzer, K. S., King, J. S., and Holmes, W. E.: Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis, New Phytol., 147, 201–222, 2000.

Zhou, X., Sherry, R. A., An, Y., Wallace, L. L., and Luo, Y.: Main and interactive effects of warming, clipping, and doubled precipitation on soil CO_2 efflux in a grassland ecosystem, Global Biogeochem. Cy., 20, GB1003, doi:1010.1029/2005GB002526, 2006.

Supplement of Biogeosciences, 12, 1257–1269, 2015
http://www.biogeosciences.net/12/1257/2015/
doi:10.5194/bg-12-1257-2015-supplement

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Supplement of

Positive feedback of elevated CO_2 on soil respiration in late autumn and winter

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Supporting Information

Fig. S1

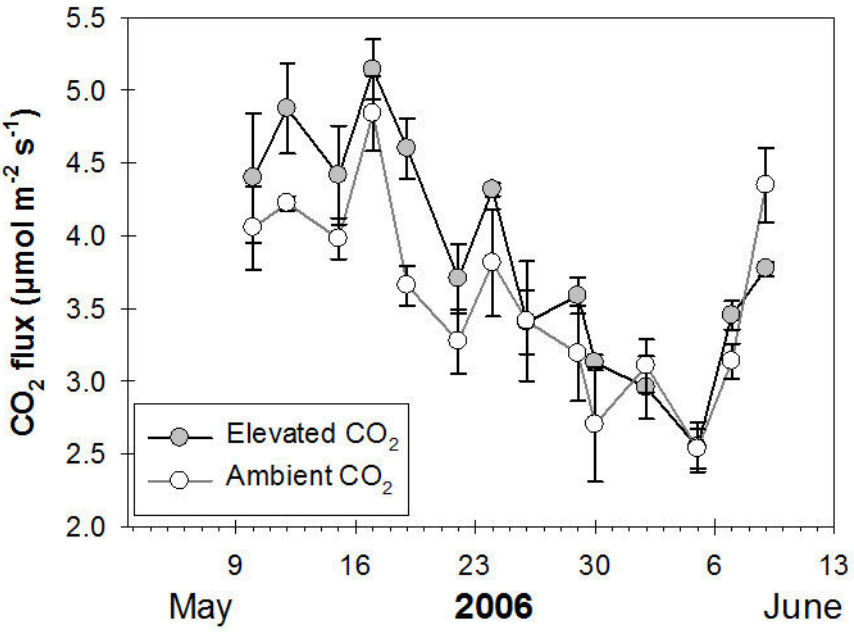

Fig S1: Mean CO_2 efflux +/- standard error (n=3) after installation of the frames and removal of the aboveground biomass on 9th May 2006.
On 11 out of 14 measurement occasions all three E-plot fluxes where higher than those of their corresponding A-plot partner. A mixed Model analysis (SPSS version 18) with the factors CO_2-treatment and time revealed that the soil CO_2 efflux was significantly increased by CO_2 enrichment.

Table S1

Parameter estimates of the temperature-dependence model after Lloyd and Taylor (1994)

CO_2 treatment	Model parameter	Coefficient	P value
Ambient CO_2	E0	61.92 ± 33.59	0.07
	R10	3.00 ± 0.19	< 0.001
	T0	261.18 ± 6.53	< 0.001
Elevated CO_2	E0	143.68 ± 103.57	0.17
	R10	3.11 ± 0.17	< 0.001
	T0	248.72 ± 13.35	< 0.001

7 Study II:

Depth-dependent response of soil aggregates and soil organic carbon content to long-term elevated CO_2 in a temperate grassland soil.

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Depth-dependent response of soil aggregates and soil organic carbon content to long-term elevated CO_2 in a temperate grassland soil

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ABSTRACT

Facing rising atmospheric CO_2 concentrations, subsoils may play an important role in the global carbon (C) cycle due to the presence of unsaturated mineral surfaces. Further, macroaggregation is considered a crucial process influencing C sequestration. However, analyses on subsoil aggregation and C retention processes under long-term elevated CO_2 (eCO_2) are lacking. In this study we investigated the long-term effect of +20% above ambient CO_2 concentration (corresponds to conditions reached 2035–2045) in a temperate grassland ecosystem at the Giessen Free Air CO_2 Enrichment (Gi-FACE), Germany. A depth-dependent response of macroaggregation to eCO_2 was observed: While in subsoil (15–45 cm depth) macroaggregation increased under eCO_2, no CO_2 induced change in macroaggregation was detected in topsoil (0–15 cm). Increased macroaggregation in subsoil coincided with higher SOC content of large macroaggregates (LM). Mean residence time (MRT) of SOC in aggregate-size classes were not different among each other under eCO_2. However, macroaggregates and bulk soil differed in their MRT between soil depths. Despite increased macroaggregation and an estimated high SOC sequestration potential in subsoil we could not observe an increase in SOC content of bulk soil.

1. Introduction

Since soil organic carbon (SOC) presents the largest terrestrial pool of C (Amundson, 2001), its potential to store additional C from the atmosphere has been widely discussed in the scientific literature (Stockmann et al., 2013). Accordingly, the 4 per mille initiative considers SOC sequestration as a contribution to mitigate climate change (Minasny et al., 2017) and calls out for accounting the rate of SOC sequestration and to identify mechanisms increasing SOC stocks.

It is widely accepted that SOC sequestration depends on the distribution of soil organic matter (SOM) in soil aggregates. The potential to physically protect certain SOM fractions from decomposition varies with aggregate-size class, which governs their residence time in soil (Tisdall and Oades, 1982; Van Veen and Kuikman, 1990; Jastrow et al., 1996). Further, subsoils may play an important role in the global C cycle due to their high mean residence times (MRT) relative to topsoil (Rumpel and Kögel-Knabner, 2011) and the presence of unsaturated mineral surfaces which was shown to be related to the formation of macroaggregates and C accrual (Kaiser and Guggenberger, 2003; Poirier et al., 2014).

However, in view of rising atmospheric CO_2 concentrations, it remains unclear how elevated CO_2 (eCO_2) affects the distribution of SOC to soil aggregate-size classes in different soil depths, the associated MRT and the resulting SOC content. For effective C sequestration, it is relevant that additional C is allocated to pools with long-term stabilization and not fast cycling pools.

It has been reported that eCO_2 may alter many factors known to influence the distribution of soil aggregate-size classes (Díaz, 1995; Eviner and Chapin, 2002). For example, eCO_2 can alter the vegetation community composition and related fungal biomass which was shown to affect aggregate stability (Rillig et al., 2002). Six et al. (2001) showed that eCO_2 changed the quality of residue inputs and enhanced the proportion of recently photosynthesized C with increasing aggregate size. They concluded that the quantity and quality of residues, which was altered by eCO_2, determined the turnover time of macroaggregates. Furthermore, it was reported that eCO_2 enhanced rhizodeposition which may stimulate fungal biomass (Phillips et al., 2006) that may serve as a binding-agent for macroaggregates (Tisdall and Oades, 1982).

Free-Air CO_2 Enrichment (FACE) experiments proofed to be a powerful approach to examine ecosystem responses to eCO_2 (Ainsworth and Long, 2005). FACE experiments allow the investigation of intact ecosystems which are exposed in-situ to eCO_2 concentration without enclosure. Nine FACE studies that investigated the effect of eCO_2 on the

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distribution of soil aggregate-size classes across a variety of ecosystems showed contrasting results (Table S1). Eight out of nine FACE studies reported results after short-term enrichments (< 10 years of CO_2 enrichment) which may not be representative of long-term dynamics. Not all of the studies incorporated measurements of SOC-content and some focused on microbial responses within aggregates (Dorodnikov et al., 2009; Nie et al., 2014) or the influence of arbuscular mycorrhizal fungi to aggregation changes (Rillig et al., 2001). In five of the FACE studies, assessment of aggregate-size class distribution was limited to the topsoil, while two studies analyzed pooled samples of top- and subsoil, consequently losing any depth-dependent information. As a result, only very limited information is available on how the distribution of soil aggregate-size classes responds to soil depth under long-term eCO_2.

To our knowledge only one other FACE study (Hofmockel et al., 2011) exists to date that investigated long-term effects (> 10 years) of eCO_2 on the distribution of soil aggregate-size classes and SOC-content. Hofmockel et al. (2011) demonstrated that eCO_2 changed C turnover of different particle-size classes in a forest soil suggesting a eCO_2 induced priming of older, relatively stable SOC.

Thus our main objective was to quantify long-term and depth-dependent effects of eCO_2 on the abundance of soil aggregate-size classes and soil C dynamics in a FACE-experiment which, to our knowledge, has not been investigated in detail so far. Since the Gi-FACE is located on temperate managed grassland our study complements the results from the long-term forest FACE study (Hofmockel et al., 2011).

In this study we investigated if eCO_2 (1) affected the distribution of soil aggregate-size classes at different soil depths; (2) induced a change in aggregate and bulk SOC content at different soil depths and (3) affected the mean residence time (MRT) and distribution of newly sequestered C (C_{new}) in soil aggregates and bulk soil at different depths.

Based on studies reporting higher C sequestration potential in subthan topsoil (Kaiser and Guggenberger, 2003; Poirier et al., 2014) we hypothesized that (i) topsoil will be close to C saturation and will show small increases in SOC content under long-term eCO_2 and (ii) subsoil will have a higher C saturation deficit and will therefore increase to a higher extent in SOC relative to topsoil under eCO_2.

2. Materials and methods

2.1. Study site and design

The Giessen Free Air Carbon Enrichment (Gi-FACE) experiment, is located on permanent semi-natural grassland. It is situated near Giessen, Germany (50°32′N and 8°41.3′E) at an elevation of 172 m above sea level.

The set-up and performance of the Gi-FACE system has been described in detail by Jäger et al. (2003) and Andresen et al. (2017). In brief, from May 1998 until present, atmospheric CO_2 concentrations were enriched by 20% above ambient, all-year-round during daylight hours. From May 1998 to June 2004 the $\delta^{13}C$ signature of the CO_2 used for enrichment was −25‰ (compared to ambient atmospheric CO_2 (aCO_2): −8‰). From July 2004 onwards the $\delta^{13}C$ signature of the CO_2 was changed to −48‰ without altering the CO_2 concentration. The CO_2 enrichment was applied in three rings, each eight meter in diameter (E plots). Three equally sized control plots were maintained at aCO_2 levels (A plots). The experimental design was a randomized block design. A block consisted of two plots to which ambient and eCO_2 treatments were randomly assigned. A characteristic attribute of the study site is a soil moisture gradient, resulting from a gradual terrain slope (2–3°) and varying depths of a subsoil clay layer. Within each of the three blocks, soil moisture conditions were relatively homogeneous (Jäger et al., 2003). The soil of the study site is classified as a Fluvic Gleysol (FAO classification). The soil texture and the depth of the clay layer is presented in Table 1.

The vegetation is an Arrhenatheretum elatioris Br.Bl. Filipendula ulmaria subcommunity, dominated by *Arrhenaterum elatium, Galium album* and *Geranium pratense*. At least 12 grass species, 15 non-leguminous herbs and 2 legumes are present within a single ring. For at least 100 years, the grassland has not been ploughed. Since at least 60 years, it was managed as a hay meadow with two cuts per year, and fertilized at the rate of 50–100 kg N ha^{-1} yr^{-1}. From 1996, fertilizer was applied in mid-April with granular mineral calcium-ammonium-nitrate fertilizer at the rate of 40 kg N ha^{-1} yr^{-1} (Kammann et al., 2008).

Table 1
Soil texture in the soil profile of each ring pair at the Gi-FACE study site according to Lenhart (2008).

Horizon	Lower horizon boundary	Sampling depth	Depth of clay layer	Sand	Silt	Clay	Silt and clay
	(cm)			(%)			
Ring pair 1							
Ah	10	2–7	128–155	43.25	39.00	17.75	56.75
M	32	12–17		40.89	42.13	16.97	59.10
SwM	78	40–45		48.10	51.90	nd	51.90
Ring pair 2							
Ah	12	2–7	48–110	59.26	20.89	19.85	40.74
MSw	42	15–20		34.52	40.50	24.98	65.48
GoSw	65	50–55		35.34	52.33	12.33	64.66
Ring pair 3							
Ah	12	2–7	65–135	9.98	58.13	31.89	90.02
M	20	15–20		9.78	55.56	34.66	90.22
MSw	50	40–45		14.94	50.56	34.50	85.06

nd: not determined.

2.2. Soil sampling

Soil samples were taken at nine sampling dates (April 1998, June 2004, December 2004, July 2005, December 2005, June 2006, June 2007, November 2011 and December 2015) in 0–7.5 cm depth. After six (June 2004), nine (June 2007) and 13 years (November 2011) of CO_2 enrichment soil samples were taken in 0–7.5, 7.5–15, 15–30 and 30–45 cm depth (soil sampler: Ejkelkamp, Giesbeek, The Netherlands) with three sub-samples per plot in each depth. Soils were passed through an 8 mm sieve and air-dried. Subsequently, roots were picked out with tweezers until all visible roots were removed. The soil samples were split partly for bulk soil analysis and 80 g of the samples were used for the wet sieving procedure to separate soil aggregate-size classes.

2.3. Soil aggregate fractionation

Soil samples were separated into four aggregate-size classes by wet sieving of 80 g of soil according to a method adapted from Cambardella and Elliott (1993). Soil samples were submerged for 2 min in deionized water on top of the 2000 µm sieve and subsequently a series of three sieves (2000 µm, 250 µm and 53 µm) was used to obtain the four aggregate-size classes: > 2000 µm (large macroaggregates (LM)), 250–2000 µm (small macroaggregates (SM)), 53–250 µm (microaggregates (MIC)) and < 53 µm (silt and clay (SC)). The separation of water-stable aggregates was achieved by manually moving the sieve up and down with 50 repetitions during a 2 min period. Each aggregate-size class was transferred into aluminum pans and dried at 60 °C until a constant weight was reached.

2.4. Carbon analysis

All solid samples were ground with a ball mill (Retsch, type MM). 15–20 mg of bulk soil and of isolated soil aggregates were placed into tin capsules to determine stable carbon ($\delta^{13}C$) isotope composition, as well as C and N contents. The same procedure was applied with two milligrams of roots for each depth on composite samples. Stable carbon

($\delta^{13}C$) isotope composition was determined for bulk soil for each soil depth (down to 45 cm). For soil aggregates no $\delta^{13}C$- values were determined for 30–45 cm soil depth in November 2011. Consequently, C-content and MRT of aggregates are shown down to depths of 30 cm, while of bulk soil down to 45 cm. Samples collected between 1997 and December 2005 were measured using a continuous flow, isotope ratio mass spectrometer (CF-IRMS, PDZ-Europa Scientific, Sandbach UK) interfaced with a CN analyzer (Carlo Erba). Samples collected from June 2006 till June 2007 were measured on a combined elemental analyzer and gas purification module (SerCon-GSL). Samples from November 2011 were analyzed on an isotope mass spectrometer (IRMS, DeltaXP Plus, Thermo Finnigan, Waltham, USA) and for December 2015 on a IRMS (GV Isoprime combined with an Elemental analyzer, Eurovector EA).

2.5. Estimation of C saturation and C saturation deficit

We determined C saturation (C_{sat}) of our study site for different soil depths by applying a model where C_{sat} is related to the silt and clay content in grassland (Six et al., 2002) (1).

$$C_{sat} = 16.33 + 0.32\,(Clay + Silt) \quad (1)$$

where C_{sat} is the C saturation (g C kg^{-1} soil) expressed as the C content of the Clay + Silt fraction on a whole-soil basis and Clay + Silt is the clay and silt (0–50 μm particles) contents (%). We used the soil texture data as presented in Table 1 and allocated the soil horizons to the increments of soil sampling. We did not present any results of C_{sat} for the depth 7.5–15 cm since we could not allocate a specific soil horizon to this depth (Table 1).

We then estimated C saturation deficit (C_{def}) according to Angers et al. (2011) (2), where the deficit is determined by the difference between the theoretical saturation and the actual stable SOC (SSOC) content.

$$C_{def} = C_{sat} - SSOC \quad (2)$$

where SSOC is stable SOC which is bound to minerals. SSOC was estimated to account for 78.63 ± 6.15% of SOC content in 0–7.5 cm, 94.15 ± 2.21% in 7.5–15 cm, 95.74 ± 1.77% in 15–30 cm and 96.38 ± 1.78% in 30–45 cm soil depth. According to Schrumpf et al. (2013) we determined the contribution of the free light fraction to the SOC content for different soil depths of three grassland sites. We applied these values as estimates of the unbound part of SOC to our grassland study site. Our estimate of 21.37 ± 6.15% for the fraction of SSOC in topsoil is in agreement with an average value of 20.8 ± 10.9% for the unbound part of SOC from 22 grassland sites (review by Gregorich et al. (2006)).

2.6. Assessment of aggregate-SOC content

We reported aggregate-SOC content in two ways. Mostly, we presented aggregate-SOC content on a whole soil basis (g C kg^{-1} soil) as this unit integrates the C concentration of the aggregate-size class (g C kg^{-1} aggregate) as well as the distribution of aggregate-size classes (g aggregate kg^{-1} soil). Additionally, we presented aggregate-SOC content in the unit g C kg^{-1} aggregate to elucidate if eCO_2 caused a change in the proportion of SOC within a given soil aggregate-size class (internal aggregate-SOC content).

2.7. Calculation of C input (C_{new}) and mean residence times (MRT)

The depleted $\delta^{13}C$ signature in the eCO_2 treatments enabled the application of an isotope mixing model to calculate the proportions of C_{new} that has been fixed since the change in $\delta^{13}C$ signature in July 2004 according to Equation (3) (Balesdent and Mariotti, 1996):

$$fC_{new} = \frac{\delta(t_1) - \delta(t_0)}{\delta_B - \delta(t_0)} \quad (3)$$

where fC_{new} is the fraction of new C in the SOC pool, $\delta(t_1)$ is the $\delta^{13}C$ signature of SOC in the elevated plots at t_1, $\delta(t_0)$ is the $\delta^{13}C$ signature of SOC in the elevated plots at t_0 and δ_B is the corresponding $\delta^{13}C$ signature of root biomass at t_1. We chose the $\delta^{13}C$ of root material because root material is the main input at the grassland study site as above ground biomass is harvested from the study plots (mimicking silage production).

Equation (3) was applied for soil aggregate-size classes and bulk soil at different soil depths. To calculate the absolute C_{new} content (g C_{new} kg^{-1} soil) we multiplied the relative fraction of C_{new} (g C_{new} $100\,g^{-1}$ SOC), which we derived from equation (3) with the SOC content of the corresponding aggregate-size class.

MRT of SOC in soil aggregate-size classes in different soil depths were estimated based on changes in their $\delta^{13}C$ over time after the switch in the signature of $^{13}CO_2$ in 2004. MRT of C in a pool (bulk soil or soil aggregate-size class) was defined as the average time required to completely renew the content of C in the pool at steady state (Six and Jastrow, 2002).

To describe changes in $\delta^{13}C$ vs. time, non-linear regressions of the form of $C_t = C_0 \cdot e^{-kt}$ were fitted to the data using SigmaPlot (ver 12.5, Systat Software Inc.). The equation was fitted to the C_{old} data vs. time, where $C_{old} = 1 - C_{new}$. C_{old} was forced to be equal to 1.0 at time zero (June 2004). The coefficient k is the first order decay constant for the organic matter pool and was derived from fitting the model to the data. C_t is the amount of C_{old} at the respective time t, t is the elapsed time since the signature switch of $\delta^{13}C$ in July 2004 and C_0 is the initial C content before the switch of the ^{13}C signature. MRT was then calculated as: $MRT = \frac{1}{k}[years]$. For estimation of MRT we included the earliest data from June 2007, as from this date on the ^{13}C signature was significantly different between aCO_2 and eCO_2 in all aggregate-size classes in the top 30 cm depth. Lower soil depths did not show sufficient change in their ^{13}C signature at this time and therefore no MRT could be estimated.

2.8. Data analysis

A General Linear Model (SPSS, version 24) was used to calculate univariate analysis of variance (ANOVA) and to evaluate CO_2 effects on soil aggregate-size classes in 0–7.5 cm depth at the full time series (1998–2015) and for the soil profile data which incorporated measurements from 6, 9 and 13.5 years of the experiment. No transformation of data was required as results of a Shapiro-Wilk-Test verified normal distribution of residuals. We split the data by aggregate-size class and by depth and applied separate ANOVAs to evaluate CO_2 effects in different depths and within soil aggregate-size classes. According to the experimental design the ANOVA model included the factors CO_2, block and time and their interactions.

To identify significant differences of MRT among aggregate-size classes we split the MRT data by depth and applied an ANOVA with the factor aggregate-size class. Significant differences of MRT within aggregate-size classes and between depths were performed by splitting the data by aggregate-size class and performing an ANOVA with the factor depth. Tukey's HSD was used as a post-hoc test to determine significant differences between groups. All effects and comparisons were considered significant at $p \leq 0.05$ and marginally significant at a p-value between 0.05 and 0.10.

3. Results

3.1. Distribution of aggregate-size classes in 0–7.5 cm depth within 17 years of eCO_2

Within the top 7.5 cm soil depth, a single observation showed an

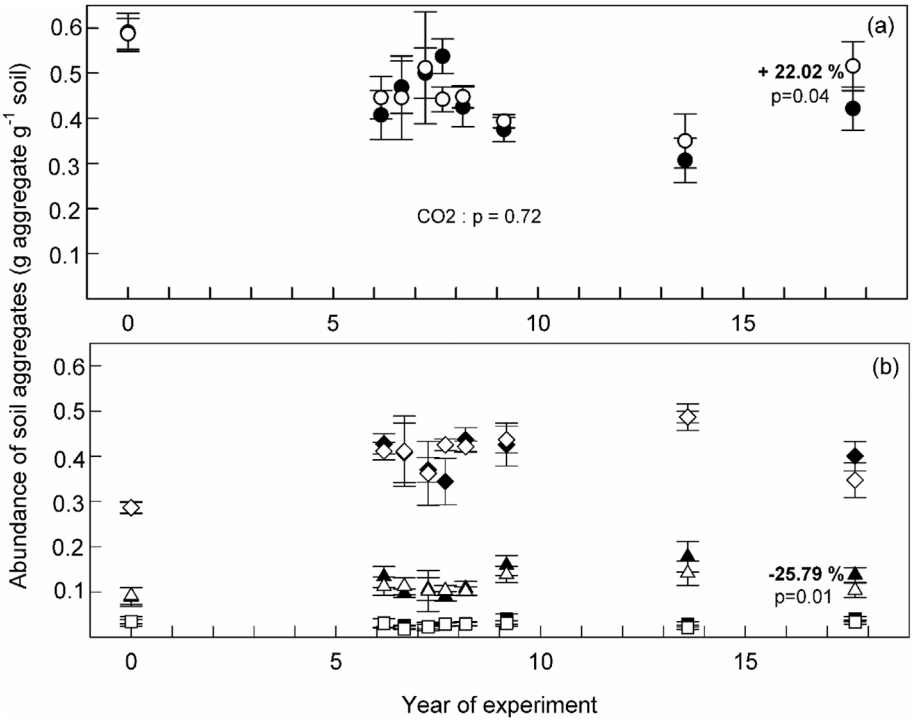

Fig. 1. Distribution of soil aggregate-size classes under aCO_2 (solid symbols) and eCO_2 (open symbols) in 0–7.5 cm soil depth during 17 years at the Gi-FACE experiment. Abundance of large macroaggregates (circles) **(a)**, small macroaggregates (diamonds), microaggregates (triangles) and silt and clay aggregates (squares) under aCO_2 (solid symbols) and eCO_2 (open symbols) in 0–7.5 cm soil depth **(b)**. Values are presented as means ± standard error, n = 3. Reported P values are for CO_2 effects.

Table 2
ANOVA table of effects of eCO_2 (CO_2), time and their interactions on the abundance of soil aggregate-size classes at the full time series (17 years of eCO_2) in 0–7.5 cm depth. Significant values are bolded.

Source	df	LM	SM	MIC	SC
		P	*P*	*P*	*P*
CO_2	1	0.724	0.525	**0.042**	**0.050**
Time	8	**0.000**	**0.000**	**0.000**	**0.001**
CO_2 x Time	8	0.519	0.449	0.450	0.742

LM: large macroaggregates, SM: small macroaggregates, MIC. microaggregates, SC: silt and clay.

eCO_2-induced increase in the abundance of LM by 22.02 ± 3.59% (p = 0.04) relative to aCO_2 after 17 years (Fig. 1a). However, this single observation of increased macroaggregation under eCO_2 did not impose a significant CO_2 effect on the whole investigation period in topsoil (Table 2). Increased macroaggregation after 17 years of eCO_2 was concomitant with a decreased abundance of MIC by 25.79% (p = 0.01) relative to MIC in aCO_2 plots (Fig. 1b).

Over the whole investigation period eCO_2 had no effect on the fraction of SM (p = 0.525) but decreased the fraction of MIC (p = 0.042) and SC (p = 0.050) in the top 7.5 cm soil depth (Table 2, Fig. 1b).

3.2. Soil aggregation effects in the soil profile within 13 years of eCO_2

Within the soil profile (0–45 cm depth) we observed CO_2-induced differences in soil aggregate-size distribution among depths (Table 3). While the abundance of LM increased in subsoil (15–45 cm depth) with a concomitant decrease in the abundance of SM (Fig. 2c + d), eCO_2 did not change the abundance of LM and SM in topsoil (0–15 cm depth) (Table 3, Fig. 2a + b). However, eCO_2 decreased the abundance of MIC and SC within the top 7.5 cm and in 15–45 cm soil depth (Table 3a – d).

Table 3a
Mass balance of aggregate-size classes and of aggregate-SOC content under aCO_2 and eCO_2 after 6, 9 and 13.5 years of the FACE experiment in 0-7.5 cm soil depth. Values are presented as means, n = 3 and reported P values show significant CO_2 effects. Significant values are bolded.

			0-7.5 cm depth			
Property	Year of experiment	Aggregate-size class	aCO2	eCO2	*df*	*P*
C content (g C kg-1 soil)	6	LM	15.74	17.47		
		SM	17.33	16.54		
		MIC	5.15	3.99		
		SC	0.81	0.96		
		total	**39.03**	**38.95**		
	9	LM	15.58	16.81		
		SM	18.52	19.64		
		MIC	5.41	5.07		
		SC	1.07	0.83		
		total	40.59	42.35		
	13.5	LM	11.69	12.91	1	0.270
		SM	14.78	15.29	1	0.773
		MIC	4.40	3.33	1	**0.079**
		SC	0.45	0.35	1	0.635
		total	**31.32**	**31.87**		
Abundance (g aggregate g-1 soil)	6	LM	0.41	0.45		
		SM	0.43	0.41		
		MIC	0.13	0.11		
		SC	0.03	0.03		
		total	**1.00**	**1.00**		
	9	LM	0.38	0.39		
		SM	0.43	0.44		
		MIC	0.16	0.14		
		SC	0.04	0.03		
		total	1.00	1.00		
	13.5	LM	0.31	0.35	1	0.165
		SM	0.49	0.49	1	0.937
		MIC	0.18	0.14	1	**0.035**
		SC	0.03	0.02	1	**0.087**
		total	**1.00**	**1.00**		

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay.

Table 3b
Mass balance of aggregate-size classes and of aggregate-SOC content under aCO_2 and eCO_2 after 6, 9 and 13.5 years of the FACE experiment in 7.5 - 15 cm soil depth. Values are presented as means, n = 3 and reported P values show significant CO_2 effects. Significant values are bolded.

			7.5-15 cm depth			
Property	Year of experiment	Aggregate-size class	aCO_2	eCO_2	*df*	*P*
C content (g C kg^{-1} soil)	6	LM	17.47	17.51		
		SM	12.02	10.36		
		MIC	2.98	1.82		
		SC	0.73	0.75		
		total	33.20	30.44		
	9	LM	15.40	20.40		
		SM	12.64	12.07		
		MIC	3.03	2.77		
		SC	0.56	0.48		
		total	31.63	35.73		
	13.5	LM	10.90	15.16	1	0.109
		SM	7.29	6.97	1	0.438
		MIC	3.66	2.08	1	**0.022**
		SC	0.47	0.63	1	0.748
		total	22.32	24.83		
Abundance (g aggregate g^{-1} soil)	6	LM	0.48	0.56		
		SM	0.36	0.33		
		MIC	0.12	0.08		
		SC	0.03	0.03		
		total	1.00	1.00		
	9	LM	0.46	0.52		
		SM	0.38	0.35		
		MIC	0.13	0.11		
		SC	0.03	0.02		
		total	1.00	1.00		
	13.5	LM	0.43	0.53	1	0.167
		SM	0.36	0.31	1	0.260
		MIC	0.18	0.13	1	0.111
		SC	0.03	0.03	1	0.172
		total	1.00	1.00		

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay.

Table 3c
Mass balance of aggregate-size classes and of aggregate-SOC content under aCO_2 and eCO_2 after 6, 9 and 13.5 years of the FACE experiment in 15-30 cm soil depth. Values are presented as means, n = 3 and reported P values show significant CO_2 effects. Significant values are bolded.

			15-30 cm depth			
Property	Year of experiment	Aggregate-size class	aCO_2	eCO_2	*df*	*P*
C content (g C kg^{-1} soil)	6	LM	8.39	10.72		
		SM	4.02	2.36		
		MIC	0.97	0.63		
		SC	0.41	0.26		
		total	13.79	13.98		
	9	LM	7.07	10.29		
		SM	6.91	5.58		
		MIC	1.76	1.16		
		SC	0.49	0.41		
		total	16.23	17.45		
	13.5	LM	9.32	12.89	1	**0.015**
		SM	5.81	5.18	1	0.100
		MIC	2.62	1.52	1	**0.005**
		SC	0.48	0.40	1	**0.016**
		total	18.23	19.99		
Abundance (g aggregate g^{-1} soil)	6	LM	0.54	0.76		
		SM	0.30	0.16		
		MIC	0.12	0.06		
		SC	0.04	0.02		
		total	1.00	1.00		
	9	LM	0.41	0.58		
		SM	0.40	0.31		
		MIC	0.15	0.09		
		SC	0.04	0.03		
		total	1.00	1.00		
	13.5	LM	0.40	0.55	1	**0.000**
		SM	0.38	0.30	1	**0.002**
		MIC	0.19	0.12	1	**0.000**
		SC	0.04	0.03	1	**0.005**
		total	1.00	1.00		

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay.

3.3. Aggregate-SOC content on a whole soil basis (g C kg^{-1} soil)

Elevated CO_2 increased the SOC content of LM in 15–30 cm soil depth (p = 0.015) (Table 3c, Fig. 3c) but not in the top 15 cm of soil (Table 3a + b, Fig. 3a + b) and significantly decreased the SOC content of MIC in all soil depths (Table 3a–c, Fig. 3a–c), while SOC in SC was decreased in 15–30 cm soil depth (Table 3a – c, Fig. 3a–c).

3.4. Internal aggregate-SOC content (g C kg^{-1} aggregate)

Internal aggregate-SOC content increased in SC in 7.5–30 cm but not in the top 7.5 cm soil depth under eCO_2 (Table 4). Internal SM-SOC increased under eCO_2 in 7.5–15 cm depth (Table 4). No change in internal LM-SOC was observed under eCO_2 (Table 4).

3.5. SOC content of bulk soil in the soil profile

Over the whole investigation period no change in SOC content of bulk soil was observed in any soil depth (Table 5, Fig. 4).

3.6. SOC saturation and saturation deficit in the soil profile

Our estimates of C_{sat} were similar for top- and subsoil, while SSOC and C_{def} differed among soil depths (Table 6). SSOC decreased with soil depth. In the top 7.5 cm of soil C_{def} was close to C_{sat} with a mean value of 4.07 ± 3.16 g C kg^{-1} soil for all plots. In subsoil C_{def} was 24.20 ± 1.99 g C kg^{-1} soil in 15–30 cm and 31.22 ± 3.71 g C kg^{-1}

Table 3d
Mass balance of aggregate-size classes and of aggregate-SOC content under aCO_2 and eCO_2 after 6, 9 and 13.5 years of the FACE experiment in 30-45 cm soil depth. Values are presented as means, n = 3 and reported P values show significant CO_2 effects. Significant values are bolded.

			30-45 cm depth			
Property	Year of experiment	Aggregate-size class	aCO_2	eCO_2	*df*	*P*
Abundance (g aggregate g^{-1} soil)	6	LM	0.22	0.34		
		SM	0.47	0.44		
		MIC	0.23	0.17		
		SC	0.08	0.05		
		total	1.00	1.00		
	9	LM	0.22	0.30		
		SM	0.43	0.41		
		MIC	0.27	0.22		
		SC	0.08	0.07		
		total	1.00	1.00		
	13.5	LM	0.22	0.38	1	**0.003**
		SM	0.43	0.38	1	**0.080**
		MIC	0.29	0.20	1	**0.005**
		SC	0.06	0.04	1	**0.059**
		total	1.00	1.00		

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay; C content is not presented in 30-45 cm since no $\delta^{13}C$- values were determined at this soil depth.

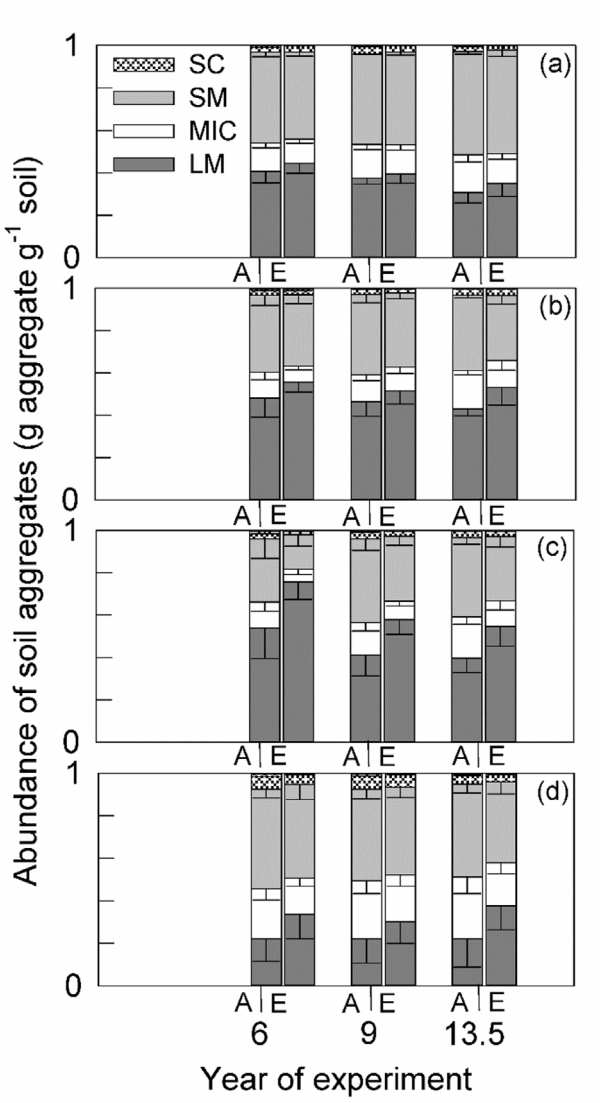

Fig. 2. Distribution of aggregate-size classes under aCO_2 (A) and eCO_2 (E) in 0–7.5 cm (a), 7.5–15 cm (b), 15–30 cm (c) and 30–45 cm (d) soil depth. Values are presented as means ± standard error, n = 3. LM: large macroaggregates, SM: small macroaggregates, MIC:microaggregates, SC: silt and clay aggregates.

soil in 30–45 cm (Table 6).

3.7. Soil C input in the CO_2 enriched plots and MRT

Highest absolute amounts of C_{new} (g C_{new} kg^{-1} soil) were found in SOC of bulk soil in the top 7.5 cm of soil (Table 7). C_{new} of bulk soil was significantly higher than in any soil aggregate-size class at this soil depth (Table 7). Among soil aggregate-size classes absolute amounts of C_{new} differed between macroaggregates and SC and between SM and MIC (Table 7). In lower soil depths bulk soil and macroaggregates showed the highest absolute amounts of C_{new} (Table 7). C_{new} in bulk soil was significantly higher than in MIC and SC in 7.5–30 cm soil depth (Table 7). C_{new} in SM, MIC and bulk soil was significantly lower in 7.5–15 and 15–30 cm soil depth than in the top 7.5 cm soil depth, while LM and SC did not differ in their C_{new} content among soil depths (Table 7).

MRT of SOC in soil aggregate-size classes were not different among aggregate-size classes at the same depth (Table 7). However, MRT of SOC in macroaggregates and bulk soil was significantly different among top- and subsoil (Table 7). We did not observe any significant differences of the MRT among depths for MIC and SC (Table 7).

4. Discussion

4.1. Changes in SOC content and distribution of aggregate-size classes

In contrast to our initial hypotheses, long-term eCO_2 did not change the SOC content of bulk soil in any depth (Fig. 4, Table 5). Despite our estimations of high SOC sequestration potential (C_{def}) in subsoil of the grassland ecosystem (Table 6), we did not observe an increased SOC content in subsoil within 13 years of eCO_2. In topsoil, for which we estimated a small SOC sequestration potential (C_{def}), we also did not observe an increase in SOC content under eCO_2.

There have been recent discussions on the suitability of the applied $C_{sat\text{-}def}$ concept for assessing the bulk soil SOC sequestration potential (Barré et al., 2017). It was criticized that C_{sat} based on the fine fraction does not account for C of coarse fractions such as particulate organic matter or sand-sized particles. We are aware that these aspects may limit the accuracy of the estimated C_{sat} and following C_{def} values. However, we took account of the fraction of unbound POM-C and incorporated the SOC bound to minerals (SSOC) into our equation (2). Despite the known limitations, our results of higher C_{def} in sub- than topsoil would arguably also persist with more detailed modelling approaches as they are in line with other studies (Kaiser and Guggenberger, 2003).

Despite no changes in bulk SOC content between CO_2 treatments we found a depth-dependent response in macroaggregation. We observed CO_2 induced macroaggregation in 15–45 cm depth but not in topsoil. Consequently, increased macroaggregation in subsoil did not result in C sequestration at the study site.

Even though we did not detect an increased SOC content in subsoil we found that LM-SOC content increased concomitantly with a decreased SOC content in MIC and SC. Consequently, increased LM-SOC content on a whole soil basis may have been counterbalanced by decreases in MIC and SC fractions. The analysis of internal aggregate-SOC content provided a different picture of SOC dynamics: Despite CO_2 induced increases of LM-SOC on a whole soil basis we did not observe any difference in internal LM-SOC content between CO_2 treatments. This may also explain why we did not detect any increased SOC content in bulk soil under eCO_2.

SC actually increased in their internal SOC content in 7.5–30 cm soil depth under eCO_2. However, the observed decrease of the SC fraction probably outbalanced the increase in SOC content, as seen on a whole soil basis. The increase in internal SC-SOC content are in line with our findings that SC-SOC contained a high fraction of C_{new} in 7.5–30 cm soil depth relative to the other aggregate-size classes (Table 7). These findings support the concept that subsoils possess a higher fraction of unsaturated mineral surfaces than topsoil where organic substances can be absorbed to (Poirier et al., 2014). However, this could not be confirmed for other aggregate-size classes or bulk soil as we did not observe any concomitant increase in internal SOC content. Decreased abundance of SC fractions under eCO_2 may be explained by absorption of organic substances to these particles and subsequent formation of macroaggregates (Blanco-Canqui and Lal, 2004).

However, no changes in bulk SOC under eCO_2 are in line with observations from other FACE experiments (Table S1) (Six et al., 2001; van Groenigen et al., 2002; del Galdo et al., 2006; Lichter et al., 2008) but contrast observations by Hoosbeek et al. (2006) and Hofmockel et al. (2011).

As the SOC content at a given time represents the balance between C inputs und losses we argue that the increase of C_{new} under eCO_2 may be counterbalanced by the rate of microbial decomposition resulting in no net C increase in SOC. This is in accordance with earlier findings from the Gi-FACE reporting increased soil respiration rates under eCO_2 in late autumn and winter (Keidel et al., 2015).

Macroaggregation has been related to temporary binding agents

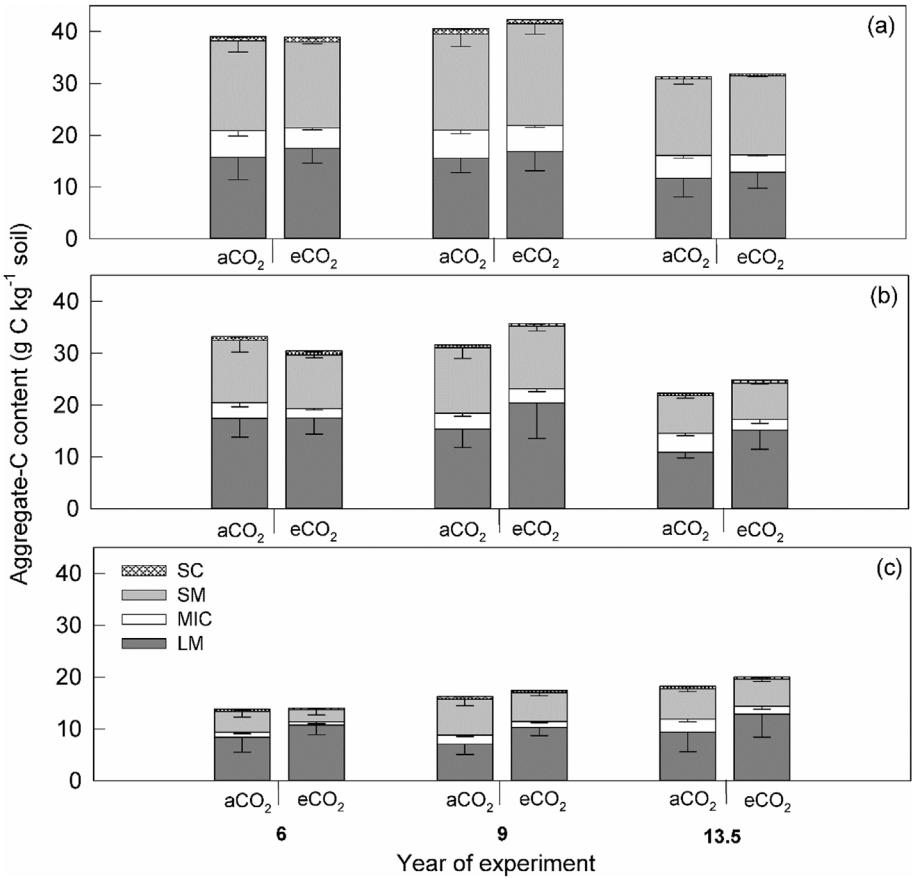

Fig. 3. Aggregate-C content under aCO_2 and eCO_2 in 0–7.5 cm (a), 7.5–15 cm (b), 15–30 cm (c) soil depth after six, nine and 13.5 years. Values are presented as means ± standard error, n = 3. C content is not presented in 30–45 cm since no $\delta^{13}C$- values were determined at this soil depth after 13.5 years. LM: large macroaggregates, SM: small macroaggregates, MIC:microaggregates, SC: silt and clay aggregates.

Table 4
ANOVA table of effects of eCO_2 on internal aggregate-SOC content (g C kg^{-1} aggregate) after six, nine and 13.5 years of CO_2 enrichment in different soil depths. Significant values are bolded.

Depth	*df*	LM	SM	MIC	SC
		P	*P*	*P*	*P*
0–7.5 cm	1	0.723	0.544	0.938	0.155
7.5–15 cm	1	0.307	**0.051**	0.689	**0.041**
15–30 cm	1	0.802	0.452	0.175	**0.062**

LM: large macroaggregates, SM: small macroaggregates, MIC. microaggregates, SC: silt and clay. No $\delta^{13}C$- data was available for soil aggregate size classes in 30–45 cm soil depth after 13.5 years.

Table 5
ANOVA table of effects of eCO_2 on SOC content of bulk soil at different soil depths.

Depth	*df*	bulk soil
		P
0–7.5 cm	1	0.866
7.5–15 cm	1	0.367
15–30 cm	1	0.471
30–45 cm	1	0.129

such as roots and fungal hyphae (Tisdall and Oades, 1982). However, more recent studies reported that higher root length densities increased the proportions of smaller aggregates (Materechera et al., 1992). Increased root biomass was often observed in response to eCO_2 (Jastrow et al., 2000; Eviner and Chapin, 2002), however, at Gi-FACE there is no such evidence because even after 13 years of eCO_2 no CO_2 effect on root biomass was observed over the soil profile (0–45 cm depth) (Fig. S2).

Still, fungal-derived binding agents cannot be ruled out to be responsible for the observed increase in macroaggregation (Rillig et al., 1999). Glomalin has been linked to aggregate stability (Wright and Upadhyaya, 1998). Rillig et al. (1999) reported an increased glomalin content and macroaggregate abundance under eCO_2, and concluded that arbuscular mycorrhizal fungi (AMF) mediated the CO_2-induced increase in soil aggregation. However, recent studies question that glomalin originates from AMF and refer to it as glomalin-related soil protein (Gillespie et al., 2011). In a different study, Rillig and Field, 2003 reported that AMF responses to plants exposed to eCO_2 followed a soil-depth dependent pattern. About 5-fold increases of AM fungal root colonization were observed in the subsoil in response to eCO_2, but no significant changes in the corresponding topsoil of *Bromus hordeaceus* L. This is in line with observations from a forest FACE study, where CO_2 enrichment increased mycorrhizal root tip production in deep soil (15–30 cm) but did not influence mycorrhizal production in shallow soil (0–15 cm) (Pritchard et al., 2008).

To date studies of AMF at the Gi-FACE were limited to the topsoil layer showing no CO_2 induced increases in abundance of AMF (Gerstner, 2014) after 15 years of eCO_2. Our results point out that studies on AMF should also include subsoil layers in CO_2 enrichment experiments to test if a CO_2-induced increase in AMF colonization can explain increases in soil aggregation in the subsoil.

4.2. Soil C input in the CO_2 enriched plots and MRT

We suggest that highest amounts of C_{new} in bulk soil in the top 7.5 cm of soil may be explained by a relative high fraction of C_{new} in free particulate organic matter (POM) that was not occluded within soil aggregates at this soil depth.

The relative high fraction of C_{new} in SC may partly result from wet

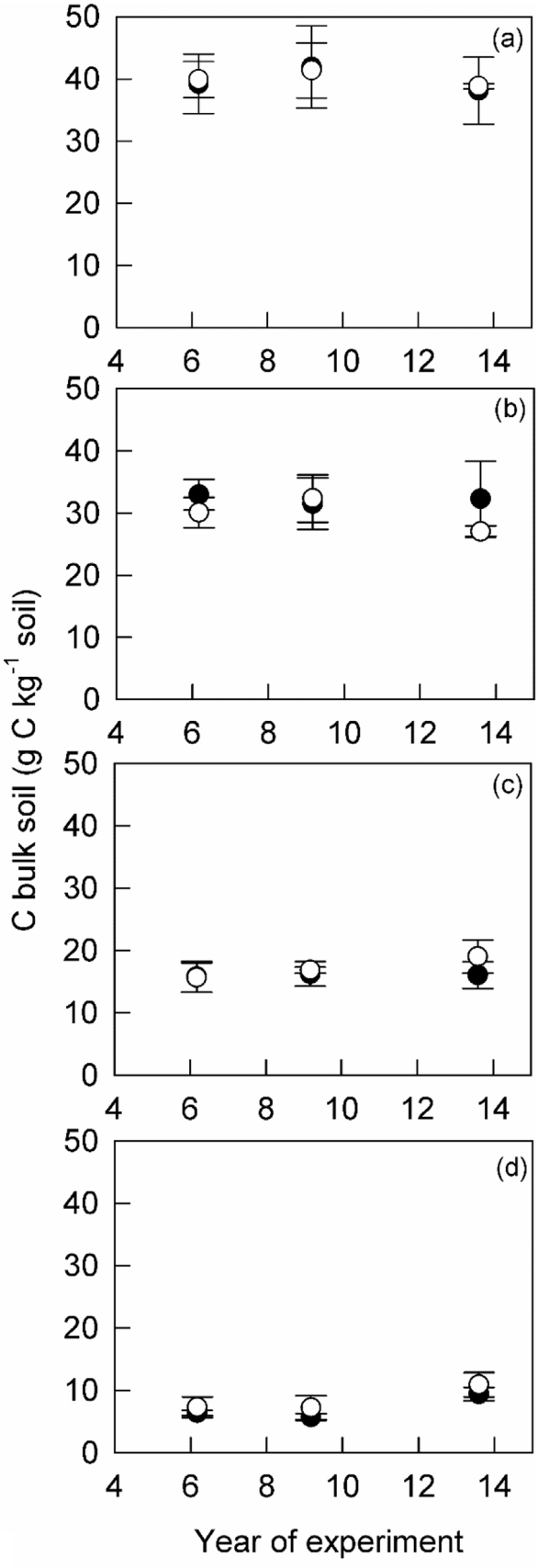

Fig. 4. SOC content of bulk soil under aCO_2 (solid circles) and eCO_2 (open circles) in 0–7.5 cm (a), 7.5–15 cm (b), 15–30 cm (c) and 30–45 cm (d) soil depth after six, nine and 13.5 years. Values are presented as means ± standard error, n = 3.

Table 6
C saturation (C_{sat}), stable soil organic carbon content (SSOC) and C saturation deficit (C_{def}) estimated for the grassland study site at different soil depths after 6 years of the FACE experiment. Values are presented as means of all rings, based on ring pairs (n = 3). Different letters represent significant differences among soil depths ($p > 0.1$).

Soil depth	C_{sat}		SSOC		C_{def}	
	(g C kg^{-1} soil)					
0–7.5 cm	36.33 a	± 4.64	32.26 a	± 4.05	4.07 a	± 3.16
15–30 cm	39.24 a	± 3.04	15.04 b	± 1.17	24.20 b	± 1.99
30–45 cm	37.84 a	± 3.09	6.61 c	± 0.95	31.22 c	± 3.71

Soil depth 7.5–15 cm is not presented since it could not be assigned to a particular soil horizon (Table 1).

sieving where soluble C associated with micro- and macroaggregates may have entered the SC fraction which are known to absorb organic substances to its surfaces (Blanco-Canqui and Lal, 2004). However, due to the small pool size of this aggregate-size class, high relative values had only a negligible influence on the absolute amount of C_{new} (Table 7). The high fraction of C_{new} in SC resulted in relatively fast MRT of SOC within this aggregate-size class (Table 7).

Our study showed that the MRT of SOC in different aggregate-size classes did not differ significantly among each other. However, macroaggregates and bulk soil differed in their MRT between soil depths. These results are in contrast to other experiments where MRT of SOC increased with aggregate size (Six et al., 2001). Our observations are also in contrast to results from a review of Von Lützow et al. (2007) reporting MRT of about 15–50 years for SOC in macroaggregates and 100–300 years for SOC in microaggregates. On the other hand, van Groenigen et al. (2002) found no significant differences in C_{new} between aggregate-size classes under eCO_2 and suggested that this was due to the high level of aggregation and the incorporation of MIC into macroaggregates. In line with these results we suggest that similar values of C_{new} in subsoil and consequently similar MRT at the Gi-FACE study may be caused by aggregation dynamics under eCO_2.

5. Conclusions

The study of 17 years of moderate CO_2 enrichment showed that despite an estimated high SOC sequestration potential of the grassland subsoil and an increased macroaggregation under eCO_2 no increase in total SOC content under eCO_2 could be observed. However, we found a CO_2 induced increase in LM-SOC on a whole soil basis but no internal LM-SOC increase in subsoil. SC aggregates also showed a depth-dependent pattern with internal SOC increases in lower soil depths. Since the MRT of macroaggregates and the bulk soil was higher in subsoil than in topsoil, C_{new} allocated to these depths at the grassland study site will be sequestered for longer periods than in topsoil. We conclude from our study that approaches estimating the SOC sequestration potential, based on the fraction of silt and clay particles, may not reflect appropriately the actual SOC sequestration under eCO_2. The investigation of soil aggregates provided insight into the C protection dynamics and C allocation patterns under eCO_2.

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Table 7
Relative and absolute amounts of C_{new}, k-value and MRT of SOC in soil aggregate-size classes and bulk soil after 13.5 years of eCO_2. Values are presented as means ± standard error, n = 3. Results of a Tukey's HSD post-hoc test show significant differences among aggregate-size classes and among soil depths for C_{new}. Different uppercase letters indicate significant differences among aggregate-size classes within same depth for MRT. Different lowercase letters indicate significant differences of aggregate-size classes among depths for MRT.

Depth	aggregate-size class	Cnew			Tukey's HSD comparisons						k		MRT	
(cm)		(g 100 g^{-1} SOC)		(g kg^{-1} soil)	LM	SM	MIC	SC	bulk soil	0–7.5	7.5–15	15–30	(yr)	
0–7.5	LM	24.42	± 0.01	3.07 ± 0.06				0.044	< 0.01				0.038	± 0.00	27	± 2.05	Aa
	SM	26.44	± 0.02	4.04 ± 0.03			0.022	< 0.01	< 0.01		< 0.01	< 0.01	0.041	± 0.00	25	± 2.08	Aa
	MIC	19.17	± 0.01	0.63 ± 0.15		0.022			< 0.01		0.043	0.041	0.029	± 0.01	41	± 9.70	Aa
	SC	20.09	± 0.03	0.07 ± 0.01	0.044	< 0.01			< 0.01				0.030	± 0.01	35	± 4.70	Aa
	Bulk soil	30.57	± 0.03	11.85 ± 1.25	< 0.01	< 0.01	< 0.01	< 0.01			0.007	0.002	0.049	± 0.01	21	± 2.90	Aa
7.5–15	LM	16.99	± 0.02	2.73 ± 1.02									0.025	± 0.00	42	± 5.62	Aa
	SM	17.65	± 0.02	1.23 ± 0.15						< 0.01			0.026	± 0.00	39	± 3.59	Ab
	MIC	9.51	± 0.02	0.18 ± 0.06						0.043			0.013	± 0.00	81	± 15.66	Aa
	SC	19.30	± 0.05	0.13 ± 0.06									0.029	± 0.01	40	± 9.23	Aa
	Bulk soil	14.56	± 0.05	4.03 ± 1.50				0.042	0.040	0.007			0.021	± 0.01	68	± 29.28	Aa
15–30	LM	15.26	± 0.02	2.13 ± 1.02					0.084				0.022	± 0.00	47	± 7.23	Ab
	SM	11.50	± 0.01	0.60 ± 0.02						< 0.01			0.016	± 0.00	62	± 4.93	Ac
	MIC	11.66	± 0.04	0.18 ± 0.02					0.094	0.041			0.017	± 0.01	79	± 30.88	Aa
	SC	18.10	± 0.04	0.07 ± 0.02	0.084				0.074				0.027	± 0.01	41	± 9.21	Aa
	Bulk soil	10.35	± 0.02	2.18 ± 0.41			0.094	0.074		0.002			0.015	± 0.00	76	± 19.00	Ab

LM: large macroaggregates, SM: small macroaggregates, MIC: microaggregates, SC: silt and clay. No $\delta^{13}C$- data was available for soil aggregate size classes in 30–45 cm soil depth after 13.5 years.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2018.05.005.

References

Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytologist 165, 351–372.

Angers, D.A., Arrouays, D., Saby, N.P.A., Walter, C., 2011. Estimating and mapping the carbon saturation deficit of French agricultural topsoils. Soil Use & Management 27, 448–452.

Amundson, R., 2001. The carbon budget in soils. Annual Review of Earth and Planetary Sciences 29, 535–562.

Andresen, L.C., Yuan, N., Seibert, R., Moser, G., Kammann, C.I., Luterbacher, J., Erbs, M., Müller, C., 2017. Biomass responses in a temperate European grassland through 17 years of elevated CO_2. Global Change Biology. http://dx.doi.org/10.1111/gcb.13705.

Balesdent, J., Mariotti, A., 1996. Measurement of soil organic matter turnover using ^{13}C natural abundance. In: Boutton, T.W., Yamasaki, S.-i. (Eds.), Mass Spectrometry of Soils. Marcel Dekker, Inc, New York, pp. 83–111.

Barré, P., Angers, D.A., Basile-Doelsch, I., Bispo, A., Cécillon, L., Chenu, C., Chevallier, T., Derrien, D., Eglin, T.K., Pellerin, S., 2017. Ideas and perspectives: can we use the soil carbon saturation deficit to quantitatively assess the soil carbon storage potential, or should we explore other strategies? Biogeosciences Discussions 2017, 1–12.

Blanco-Canqui, H., Lal, R., 2004. Mechanisms of carbon sequestration in soil aggregates. Critical Reviews in Plant Sciences 23, 481–504.

Cambardella, C.A., Elliott, E.T., 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science Society of America Journal 57, 1071–1076.

del Galdo, I., Oechel, W.C., Cotrufo, M.F., 2006. Effects of past, present and future atmospheric CO_2 concentrations on soil organic matter dynamics in a chaparral ecosystem. Soil Biology and Biochemistry 38, 3235–3244.

Díaz, S., 1995. Effects of elevated [CO2] at the community level mediated by root symbionts. Plant and Soil 187, 309–320.

Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Fangmeier, A., Kuzyakov, Y., 2009. Stimulation of r- vs. K-selected microorganisms by elevated atmospheric CO2 depends on soil aggregate size. FEMS Microbiology Ecology 69, 43–52.

Eviner, V.T., Chapin III, F.S., 2002. The influence of plant species, fertilization and elevated CO_2 on soil aggregate stability. Plant and Soil 246, 211–219.

Gerstner, J., 2014. Influence of Abiotic Factors like Carbon Dioxide, Soil Water and Nitrogen Content on the Abundance of Arbuscular Mycorrhiza Fungi (AMF) in the GiFACE Study in Leihgestern. Faculty 08 – Biology and Chemistry. Justus-Liebig-Universität Giessen, Giessen, pp. 55.

Gillespie, A.W., Farrell, R.E., Walley, F.L., Ross, A.R.S., Leinweber, P., Eckhardt, K.-U., Regier, T.Z., Blyth, R.I.R., 2011. Glomalin-related soil protein contains non-mycorrhizal-related heat-stable proteins, lipids and humic materials. Soil Biology and Biochemistry 43, 766–777.

Gregorich, E.G., Beare, M.H., McKim, U.F., Skjemstad, J.O., 2006. Chemical and biological characteristics of physically uncomplexed organic matter. Soil Science Society of America Journal 70, 975–985.

Hofmockel, K.S., Zak, D.R., Moran, K.K., Jastrow, J.D., 2011. Changes in forest soil organic matter pools after a decade of elevated CO_2 and O_3. Soil Biology and Biochemistry 43, 1518–1527.

Hoosbeek, M.R., Li, Y., Scarascia-Mugnozza, G., 2006. Free atmospheric CO_2 enrichment (FACE) increased labile and total carbon in the mineral soil of a short rotation Poplar plantation. Plant and Soil 281, 247–254.

Jastrow, J.D., Boutton, T.W., Miller, R.M., 1996. Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. Soil Science Society of America Journal 60, 801–807.

Jäger, H.-J., Schmidt, S.W., Kammann, C., Grünhage, L., Müller, C., Hanewald, K., 2003. The university of Giessen free-air carbon dioxide enrichment study: description of the experimental site and of a new enrichment system. Journal of Applied Botany 77, 117–127.

Jastrow, J.D., Miller, R.M., Owensby, C.E., 2000. Long-term effects of elevated atmospheric CO_2 on below-ground biomass and transformation to soil organic matter in grassland. Plant and Soil 224, 85–97.

Kaiser, K., Guggenberger, G., 2003. Mineral surfaces and soil organic matter. European Journal of Soil Science 54, 219–236.

Kammann, C., Müller, C., Grünhage, L., Jäger, H.-J., 2008. Elevated CO_2 stimulates N_2O emissions in permanent grassland. Soil Biology and Biochemistry 40, 2194–2205.

Keidel, L., Kammann, C., Grünhage, L., Moser, G., Müller, C., 2015. Positive feedback of elevated CO_2 on soil respiration in late autumn and winter. Biogeosciences 12, 1257–1269.

Lenhart, K., 2008. The Effects of Long-term Free Air CO_2 Enrichment (FACE) on Soil Aggregation, Soil Carbon Input, and Ecosystem CO_2 Dynamics in a Temperate Grassland Ecosystem. Department of Plant Ecology. Justus-Liebig University, Giessen, pp. 134.

Lichter, J., Billings, S.A., Ziegler, S.E., Gaindh, D., Ryals, R., Finzi, A.C., Jackson, R.B., Stemmler, E.A., Schlesinger, W.H., 2008. Soil carbon sequestration in a pine forest after 9 years of atmospheric CO_2 enrichment. Global Change Biology 14, 2910–2922.

Materechera, S.A., Dexter, A.R., Alston, A.M., 1992. Formation of aggregates by plant roots in homogenised soils. Plant and Soil 142, 69–79.

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.-C., Vågen, T.-G., van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. Geoderma 292, 59–86.

Nie, M., Pendall, E., Bell, C., Wallenstein, M.D., 2014. Soil aggregate size distribution mediates microbial climate change feedbacks. Soil Biology and Biochemistry 68, 357–365.

Phillips, D.A., Fox, T.C., Six, J., 2006. Root exudation (net efflux of amino acids) may increase rhizodeposition under elevated CO_2. Global Change Biology 12, 561–567.

Poirier, V., Angers, D.A., Whalen, J.K., 2014. Formation of millimetric-scale aggregates and associated retention of 13C–15N-labelled residues are greater in subsoil than topsoil. Soil Biology and Biochemistry 75, 45–53.

Pritchard, S.G., Strand, A.E., McCormack, M.L., Davis, M.A., Oren, R., 2008. Mycorrhizal and rhizomorph dynamics in a loblolly pine forest during 5 years of free-air-CO_2-enrichment. Global Change Biology 14, 1252–1264.

Rillig, M.C., Field, C.B., 2003. Arbuscular mycorrhizae respond to plants exposed to elevated atmospheric CO2 as a function of soil depth. Plant and Soil 254, 383–391.

Rillig, M.C., Wright, S.F., Eviner, V.T., 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant and Soil 238, 325–333.

Rillig, M.C., Wright, S.F., Kimball, B.A., Pinter, P.J., Wall, G.W., Ottman, M.J., 2001. Elevated carbon dioxide and irrigation effects on water stable aggregates in a *Sorghum* field: a possible role for arbuscular mycorrhizal fungi. Global Change Biology 7, 333–337.

Rillig, M.C., Wright, S.F., Allen, M.F., Field, C.B., 1999. Rise in carbon dioxide changes soil structure. Nature 400, 628.

Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. Plant and Soil 338, 143–158.

Schrumpf, M., Kaiser, K., Guggenberger, G., Persson, T., Kögel-Knabner, I., Schulze, E.D., 2013. Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. Biogeosciences 10, 1675–1691.

Six, J., Carpentier, A., van Kessel, C., Merckx, R., Harris, D., Horwath, W.R., Lüscher, A., 2001. Impact on elevated CO_2 on soil organic matter dynamics as related to changes in aggregate turnover and residue quality. Plant and Soil 234, 27–36.

Six, J., Jastrow, J.D., 2002. Organic Matter Turnover, Encyclopedia of Soil Science. Marcel Dekker, New York, pp. 936–942.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil 241, 155–176.

Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchia, N., Jenkins, M., Minasny, B., McBratney, A.B., de Remy de Courcelles, V., Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, B.C., Chenug, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell, A.G., Parton, W.J., Whitehead, D., Zimmermann, M., 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agriculture, Ecosystems & Environment 164, 80–99.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science 33.

van Groenigen, K.-J., Harris, D., Horwath, W.R., Hartwig, U.A., van Kessel, C., 2002. Linking sequestration of ^{13}C and ^{15}N in aggregates in a pasture soil following 8 years of elevated atmospheric CO_2. Global Change Biology 8, 1094–1108.

Van Veen, J.A., Kuikman, P.J., 1990. Soil structural aspects of decomposition of organic matter by micro-organisms. Biogeochemistry 11, 213–233.

Von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanism. Soil Biology and Biochemistry 39, 2183–2207.

Wright, S.F., Upadhyaya, A., 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil 198, 97–107.

Supporting Information

Table S 1 Review of FACE experiments studying responses of soil aggregate distribution under eCO_2.

Name	**Location**	**Ecosystem**	**N** (kg ha^{-1} y^{-1})	**CO_2 treatment** (µL L^{-1})	**Duration** (years)	**Depth** (cm)	**Fractionation method**	**Aggregate size distribution changes**	**C content**	**Reference**
	Maricopa, AZ, USA	Sorghum field	279	ambient + 200 , 24 hours	from emerge to plant maturity	**0-30**	wet sieving	soil aggregate (SM) water stability increased		Rillig et al., 2001
Swiss FACE	Eschikon, 20 km NE of Zurich, Switzerland	Lolium perenne and trifolium repens pastures	560	600 during daytime	6	0-10	wet sieving	L. perenne: increase in LM	no effect	Six et al., 2001
Swiss FACE	Eschikon, 20 km NE of Zurich, Switzerland	Lolium perenne pasture	140, 560	600 during daytime	8	0-10	pysical fractionation by wet sieving	increase in LM; decrease in SM, decrease in MIC only under high N and eCO_2	no effect	Van Groenigen et al., 2002
	northwestern Switzerland	alpine calcareous grassland		600, 24 hours, except during mid-winter	6	0-10	chemical and physical fractionation	shift towards smaller aggregate sizes at macro- and microaggregate scales	no increase in DOC	Niklaus et al., 2003
Sky Oaks CO_2 enrichment	near Warner Springs, California	chaparral ecosystem (shrubland)	N limited	gradient: 250 - 750	6	0-10	pysical fractionation by wet sieving	decrease in LM / SM	bulk soil C did not change; C content of MIC decreased with rising levels of CO_2	Del Galdo et al., 2006
PHACE experiment	Wyoming, USA	northern mixed grass prairie		600 ppm	6	0-15	dry sieving	no changes		Nie et al., 2013

FACE-Hohenheim	Stuttgart, Germany	oilseed rape (Brassica napus)	140	540	5	0-10	"optimal moist" sieving	no changes		Dorodnikov et al., 2009
Rhinelander Free Air CO_2-O3 Enrichment (FACE)	Rhinelander, Wis-consin, USA	mixed forest		ambient + 200	10	**0-20**	chemical and physical fractionation		C increased in cPOM> 250 µm and decreased in MAOM < 53 µm	Hofmockel et al., 2011
PopFACE	Viterbo,Italy	Poplar plantation	212 in 2002 and 290 in 2003 and 2004	560 µmol mol^{-1}	5	**0-10 ; 10-20**	chemical fractionation (acid hydrolysis)	labile C fraction increased	bulk soil C increased; refractory and stable C pools were not afffected	Hoosbeek et al., 2006
Duke Forest free-air CO_2 enrichment (FACE)	near Chapel Hill, North Carolina, USA	loblolly pine forest (Pinus taeda L.)		ambient + 200	6	**0-15; 15-30**	wet sieving		no effect	Lichter et al., 2005

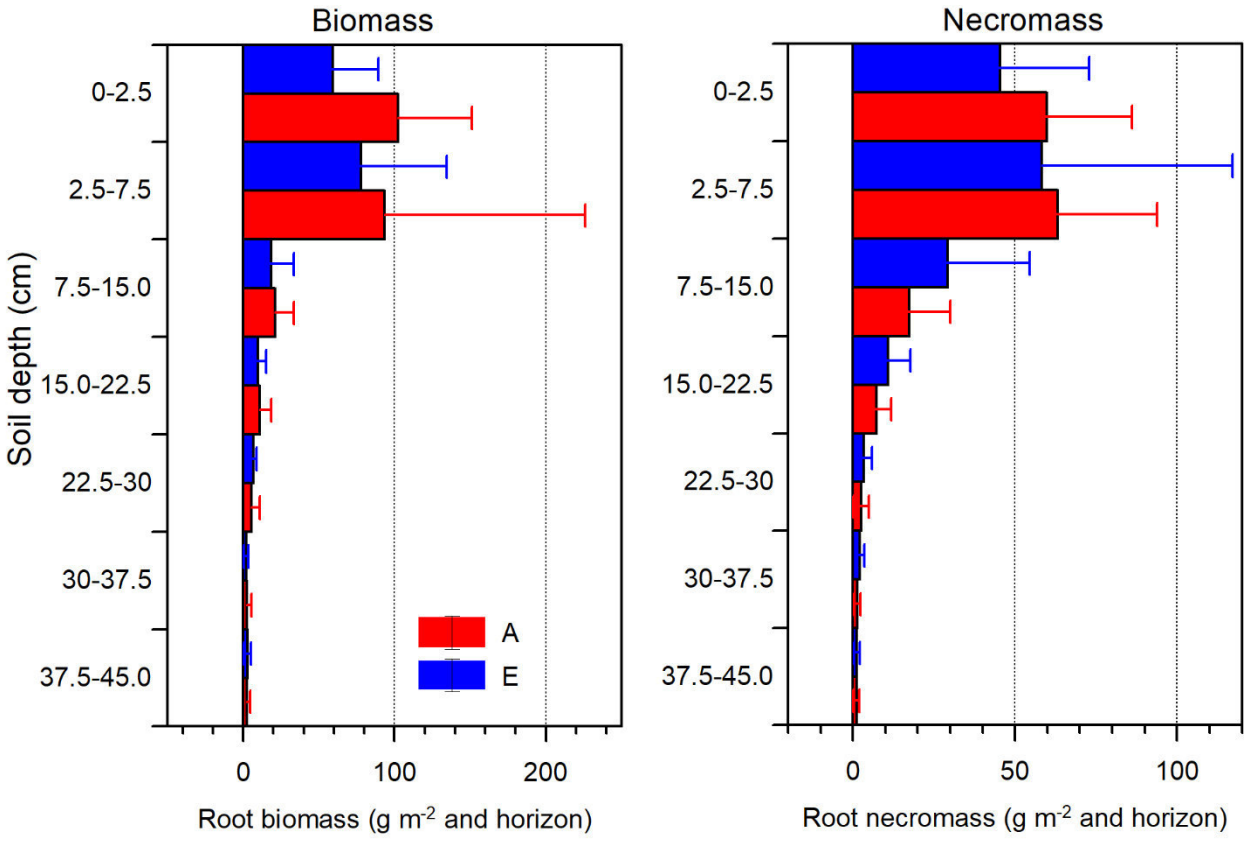

Fig. S1. Root biomass and necromass under aCO_2 (A) and after 13 years of eCO_2 (E)

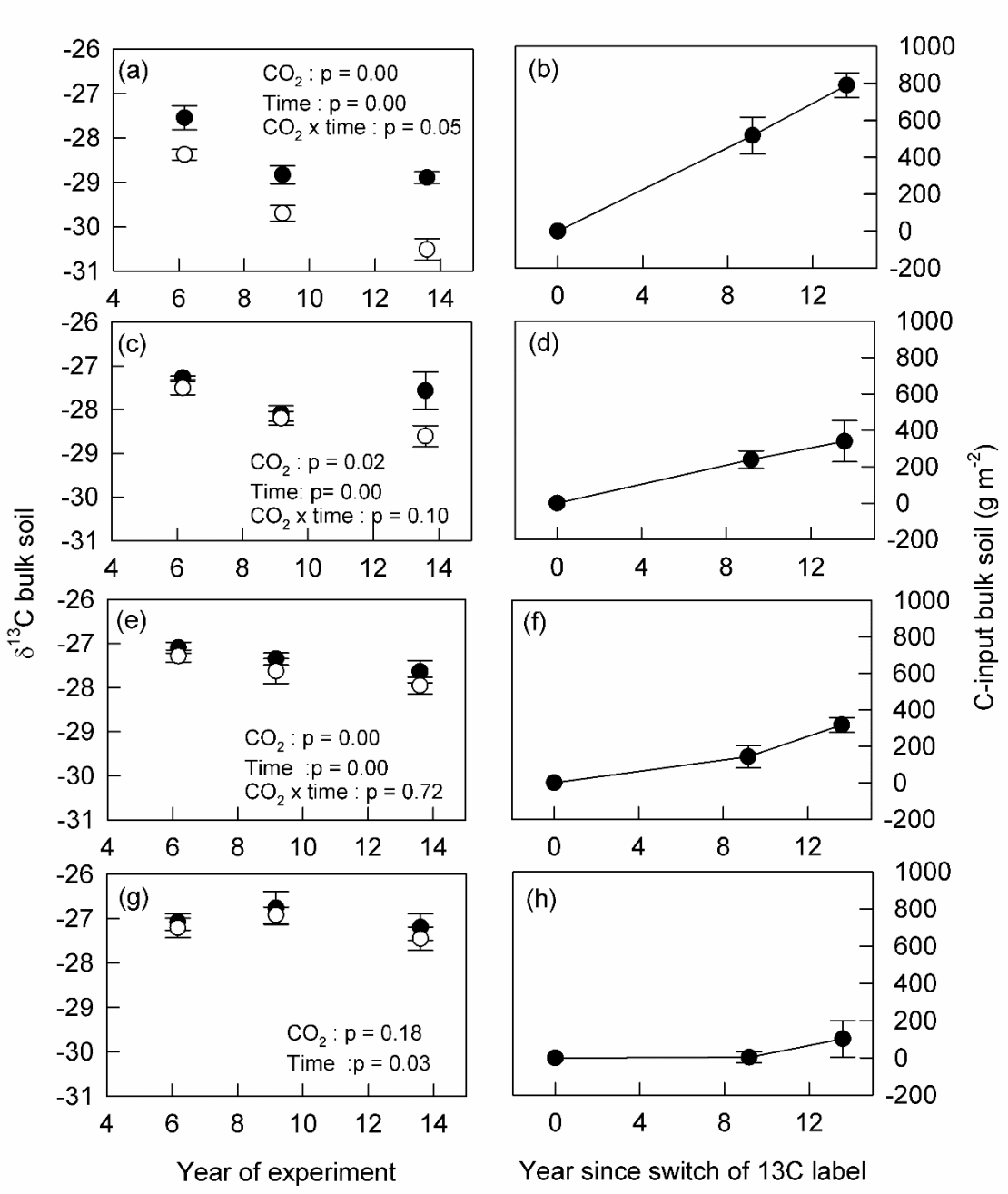

Fig. S2. $\delta^{13}C$ of bulk soil and C input in bulk soil under aCO_2 (solid circles) and eCO_2 (open circles) in 0-7.5 cm (a & b), 7.5-15 cm (c & d), 15-30 cm (e & f) and 30 – 45 cm (g & h) soil depth. Values are presented as means ± standard error, n=3.

8 Study III:

Explaining the doubling of N_2O emissions under elevated CO_2 in the Giessen FACE via in-field ^{15}N tracing.

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PRIMARY RESEARCH ARTICLE

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Explaining the doubling of N_2O emissions under elevated CO_2 in the Giessen FACE via in-field ^{15}N tracing

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Abstract

Rising atmospheric CO_2 concentrations are expected to increase nitrous oxide (N_2O) emissions from soils via changes in microbial nitrogen (N) transformations. Several studies have shown that N_2O emission increases under elevated atmospheric CO_2 (eCO_2), but the underlying processes are not yet fully understood. Here, we present results showing changes in soil N transformation dynamics from the Giessen Free Air CO_2 Enrichment (GiFACE): a permanent grassland that has been exposed to eCO_2, +20% relative to ambient concentrations (aCO_2), for 15 years. We applied in the field an ammonium-nitrate fertilizer solution, in which either ammonium (NH_4^+) or nitrate (NO_3^-) was labelled with ^{15}N. The simultaneous gross N transformation rates were analysed with a ^{15}N tracing model and a solver method. The results confirmed that after 15 years of eCO_2 the N_2O emissions under eCO_2 were still more than twofold higher than under aCO_2. The tracing model results indicated that plant uptake of NH_4^+ did not differ between treatments, but uptake of NO_3^- was significantly reduced under eCO_2. However, the NH_4^+ and NO_3^- availability increased slightly under eCO_2. The N_2O isotopic signature indicated that under eCO_2 the sources of the additional emissions, 8,407 μg N_2O–N/m^2 during the first 58 days after labelling, were associated with NO_3^- reduction (+2.0%), NH_4^+ oxidation (+11.1%) and organic N oxidation (+86.9%). We presume that increased plant growth and root exudation under eCO_2 provided an additional source of bioavailable supply of energy that triggered as a priming effect the stimulation of microbial soil organic matter (SOM) mineralization and fostered the activity of the bacterial nitrite reductase. The resulting increase in incomplete denitrification and therefore an increased N_2O:N_2 emission ratio, explains the doubling of N_2O emissions. If this occurs over a wide area of grasslands in the future, this positive feedback reaction may significantly accelerate climate change.

KEYWORDS
climate change, elevated CO_2, free air CO_2 enrichment, grassland, long-term response, N transformation, N_2O emission, positive climate change feedback

1 | INTRODUCTION

The rising atmospheric CO_2 concentration, which has recently reached 400 ppm (Dlugokencky & Tans, 2017), is unprecedented in the last 800,000 years (IPCC, 2013). This increase in CO_2 concentration stimulates plant growth (Andresen et al., 2017; Obermeier et al., 2017) and is expected to affect soil nitrogen (N) cycling and the production pathways of nitrous oxide (N_2O; van Groenigen,

Osenberg, & Hungate, 2011). Microbial N transformations via nitrification and denitrification contribute about 70% of the annual N_2O emissions worldwide (IPCC, 2007; Mosier, Delgado, & Keller, 1998) and anthropogenic contributions to N_2O emissions are triggered by N fertilizer application in agriculture (Singh, Bardgett, Smith, & Reay, 2010). Nitrification and denitrification are the most prominent N transformation processes that produce N_2O, but in agricultural soils denitrification often dominates (Wrage, Velthof, Van Beusichem, & Oenema, 2001) as was the case for the grassland soil in this study (Müller et al., 2002). However, in old grassland soils such as this study, the production of nitrite (NO_2^-) via heterotrophic nitrification and its subsequent reduction to N_2O may also be an important pathway for N_2O production (Müller, Laughlin, Spott, & Rütting, 2014). Both, single case studies (Baggs, Richter, Hartwig, & Cadisch, 2003; Kammann, Müller, Grünhage, & Jäger, 2008; Kettunen, Saarnio, Martikainen, & Silvola, 2006, 2007) and review articles (van Groenigen et al., 2011) have reported increased N_2O emissions under elevated atmospheric CO_2 (eCO_2), with a mean increase of 19%. In the case of the Giessen Free Air CO_2 Enrichment (GiFACE) experiment, situated in a temperate grassland, a doubling of N_2O emissions has been observed after 8 years (Kammann et al., 2008).

The global warming potential of N_2O over a 100-year period is 298 (Myhre et al., 2013), and thus a positive feedback of eCO_2 on N_2O emissions (Knohl & Veldkamp, 2011) could accelerate global warming, which is not yet included in climate change models and scenarios. It is therefore crucial to understand the soil processes behind increased N_2O emissions under climate change conditions.

It has often been reported, and discussed, that the CO_2 fertilization effect on plant growth is not proportional to the N uptake under eCO_2, resulting in a lower N concentration in plant biomass (Ainsworth & Long, 2005; Luo, Hui, & Zhang, 2006) either by dilution or because of reduced N availability (Luo et al., 2004). Feng et al. (2015) suggested that eCO_2 may reduce the strength of the plant N sink and thus constrain plant N utilization. Other studies have shown that eCO_2 reduced nitrate (NO_3^-) assimilation in C3 plants (Asensio, Rachmilevitch, & Bloom, 2015; Bloom, Burger, Rubio-Asensio, & Cousins, 2010; Bloom, Smart, Nguyen, & Searles, 2002) which could leave more NO_3^- substrate available for denitrification. In their meta-analysis, van Groenigen et al. (2011) attributed increased N_2O emissions under eCO_2 to enhanced denitrification resulting from both higher soil labile carbon (C) and soil moisture under eCO_2. The increased C assimilation rate of plants, under eCO_2, may trigger increased root exudation (Phillips, Bernhardt, & Schlesinger, 2009) of labile, energy-rich, C compounds such as sugars or amino acids. The recognized increased water use efficiency of plants (Leakey et al., 2009; Morgan et al., 2004), under eCO_2, and the higher shading caused by increased aboveground biomass may result in higher soil moisture (Leakey et al., 2009). Such an effect may also be counterbalanced if more biomass results in more evapotranspiration (Tricker et al., 2009). At the GiFACE site Regan et al. (2011) found that increased soil moisture and eCO_2 increased N_2O emissions as a result of a decreased proportion of N_2O reducers within the denitrifier community in the wettest plots, in which higher N_2O emissions were observed in response to CO_2 enrichment.

Nearly all published studies, with the aim to improve the process understanding of changes in N cycling and N_2O emissions under eCO_2, have been either microcosm and greenhouse experiments or laboratory incubations of bare soil from free air CO_2 enrichment (FACE) experiments, typically with rather short observation periods. For the first time, this study traces in a long-term field experiment, soil N transformations, using ^{15}N tracing, under ambient concentrations (aCO_2) and eCO_2 in situ. The study includes plant growth and its subsequent effects on soil moisture and N dynamics, under FACE conditions to enlighten the processes responsible for the observed doubling of N_2O emissions under eCO_2. We hypothesized that eCO_2 would result in enhanced N_2O emissions due to increased plant growth stimulating root exudation and thus denitrification, which would be reflected in altered soil NO_3^- dynamics.

2 | MATERIALS AND METHODS

2.1 | Study site and design

The GiFACE field experiment is situated on permanent temperate grassland. It is located near Giessen, Germany (50°32′N and 8°41.3′E) at an elevation of 172 m above sea level. The set-up and performance of the GiFACE system has been described in detail by Jäger et al. (2003). In brief, from May 1998 until present, atmospheric CO_2 concentrations were enriched by 20% above ambient, all-year-round during daylight hours.

The CO_2 enrichment was applied to three circular plots, each 8 m in diameter (eCO_2). Three equally sized control plots were maintained at ambient atmospheric CO_2 levels (aCO_2). The soil of the study site is classified as a Fluvic Gleysol (FAO classification) with a sandy clay loam texture overlying a subsoil clay layer (Jäger et al., 2003). The experimental design was a randomized block design. A block consisted of two plots to which either aCO_2 or eCO_2 treatments were randomly assigned. A characteristic attribute of the study site is a soil moisture gradient, resulting from a gradual terrain slope (2–3°) and varying depths of the subsoil clay layer. Within each of the three blocks, soil moisture conditions were relatively homogeneous, small moisture differences between blocks may occur during summer, while over the rest of the year the water table is close to the soil surface. Volumetric soil water content of the 0–15 cm soil depth was measured daily with four permanently installed TDR probes (Imko, Germany, type P2G) per plot.

The vegetation is an Arrhenatheretum elatioris Br.Bl. Filipendula ulmaria subcommunity, dominated by *Arrhenaterum elatius*, *Galium album* and *Geranium pratense*. At least 12 grass species, 15 nonleguminous herbs and up to 5 legumes with small biomass contributions (<5%) are present within a single plot. The grassland has not been ploughed for at least 100 years, being managed as a hay meadow with two cuts per year, with granular mineral calcium-ammonium-nitrate fertilizer applied at the rate of 40 kg N ha^{-1} $year^{-1}$ in mid-April. Before 1996, fertilizer was applied at a rate of 50–100 kg

N ha^{-1} $year^{-1}$ (Andresen et al., 2017; Kammann et al., 2008). Meteorological data were available from meteorological stations at the field site.

In March 2013 two subplots for a ^{15}N labelling experiment were installed in all plots (Figure 1). No fertilizer was applied to these subplots in April 2013. Each 60 × 90 cm big subplot contained a plant and soil sampling area (for 10 different time steps) and a metal frame (38 × 38 cm) inserted 8 cm into the ground with a manually determined mean offset of 1–3 cm aboveground for static chamber (40 × 40 × 20 cm) gas flux measurements (Figure 1). One day before the ^{15}N tracer application (on 7th May 2013), gas samples were taken manually with 60 ml syringes at time 0, 15 and 30 min after closure using the static dark chamber (mean headspace 35,000 cm^3) to determine the in situ N_2O fluxes before fertilization. The samples were directly analysed at the field site using a gas chromatograph (see below). At the same time samples of soil and plants were taken within the respective subplots to determine the natural ^{15}N signature in plants and soil.

2.2 | ^{15}N labelling in the GiFACE and sampling

On 7th of May 2013, during the maximum growth stage of the grassland plants, the ^{15}N labelling experiment commenced with ammonium-nitrate (NH_4NO_3) application at a rate equal to the annual fertilization of 40 kg N ha^{-2} $year^{-1}$. Both, of the ^{15}N experiment subplots, situated within the main plots, were labelled simultaneously by dispensing an NH_4NO_3 solution. We did not wash the solution into the soil by additional watering, but during application, care was taken to ensure that the labelled fertilizer solution was only applied at a height of 0–10 cm aboveground, so that no ^{15}N was deposited onto plant leaves positioned higher than 10 cm above the soil surface. The first subplot was labelled with $NH_4{}^{15}NO_3$ and the second with $^{15}NH_4NO_3$ solution (5 L per subplot equivalent to 9.3 mm of precipitation) enriched at 60 atom% excess (Figure 1).

2.3 | Determination of N concentration and ^{15}N enrichment

After application of ^{15}N fertilizer, the first samples of soil, plants and gas fluxes were taken for each subplot (Day 0). Additional soil and plant samples for ^{15}N analyses were taken on days 1, 3, 8, 20, 57, 145 and 305 after ^{15}N application (the remaining two sample location were spared to be able to quantify the ^{15}N contamination for future experiments). Gas sampling for N_2O fluxes also started immediately after ^{15}N application and sampling was repeated again on the same day. Additional gas samples were taken daily until Day 9 after application, afterwards at least weekly sampling was continued until January 2014.

During plant sampling, all plant parts higher than 15 cm above the soil surface, and within the 10 × 10 cm sampling area, were harvested first. Then the rest of the aboveground plant biomass, which had been in contact with the fertilizer solution, was harvested. Plant samples were dried at 60°C for 48 hr, weighed and milled. The isotopic signatures of the upper plant parts were measured using an elemental analyser (EA, Euro EA 3000, Euro Vector, Milan, Italy) coupled with an isotope ratio mass spectrometer (IRMS, DeltaXP Plus, Thermo Finnigan, Waltham, USA).

After plant sampling, an 8 cm diameter soil auger (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) was used to take samples to 7.5 cm soil depth within the 10 × 10 cm square. The soil core was divided in the field into a 2.5 cm top depth and a 5 cm lower depth; both were transferred to the laboratory, the latter within the metal rings used with the corer.

In the laboratory, half of each soil core (2.5–7.5 cm soil depth) was extracted with 2M KCl to determine the concentrations of NH_4^+ and NO_3^- by an auto analyser 3 (Seal Analytical GmbH, Norderstedt, Germany). The ^{15}N enrichments of the NO_3^- and NH_4^+ in the extracts were determined using methods based on the conversion of the inorganic salts to N_2O (Laughlin, Stevens, & Zhuo, 1997; Stevens & Laughlin, 1994).

The other half of the soil core, including half of the densely rooted 0–2.5 cm soil depth sample (0–7.5 cm soil depth), was used for root washing and soil organic matter fractionation. The soil was washed with 50 L H_2O_{dest} through a 2 mm sieve and the remaining roots were sorted into live and dead roots and dried at 60°C, weighed, milled and analysed. A fresh subsample of living roots was transferred into alcohol and the rates of mycorrhiza infection were quantified (Gerstner, 2014). A further dry root subsample was used for molecular analysis of the mycorrhizal community (Macek et al., *unpublished data*).

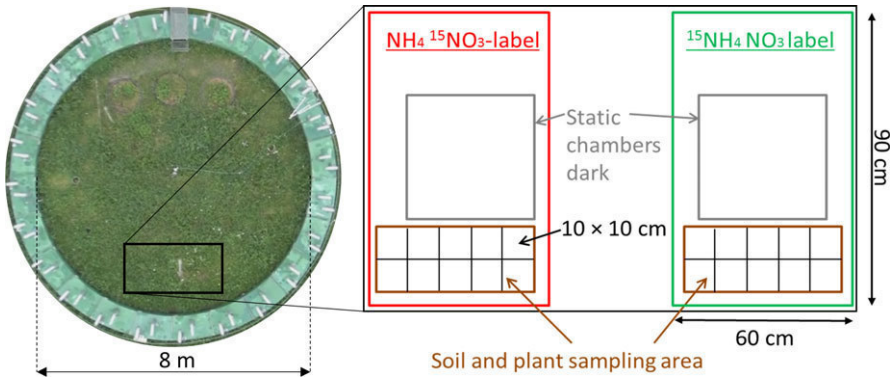

FIGURE 1 One of six GiFACE plots (left) with expanded subplot layout and dimensions showing the labelling and sampling scheme (right). The ^{15}N-labelled fertilizer treatments were simultaneously applied as a liquid solution. Thereafter, the gas sampling with closed static chambers for N_2O flux measurement and the first plant and soil samples were taken

The rinse-water from the 50 L of H_2O_{dest} used to wash the roots, containing the organic and mineral soil compounds, was passed through a 250 μm sieve and then transferred into a 500 ml beaker, where it was rinsed and decanted 25 times (10 s after filling) to separate labile from recalcitrant soil organic matter (SOM). This technique was developed in advance of the labelling experiment with the aim to create a method that allows a quick separation of organic and mineral soil compounds and a fast sample preparation to minimize changes and time shifts to sampling of N pools and their ^{15}N signature due to the continuation of N transformations during processing. Both SOM fractions were dried (60°C), weighed, milled and analysed for isotopic signatures of C and N as reported for plant biomass samples.

At the same time as plant and soil was sampled, the static chambers were closed for gas sampling. One set of gas samples were taken from the static chambers with 60 ml syringes for direct analysis on a gas chromatograph (HP6890, Hewlett Packard, Palo Alto, USA) linked to an automated sampling unit to which the 60 ml syringes have been connected (Loftfield, Flessa, Augustin, & Beese, 1997). A second set of gas samples was transferred to 12 ml Exetainers® vials (Labco Ltd, High Wycombe, Buckinghamshire, UK) for $\delta^{15}N$–N_2O analyses using an automated isotope ratio mass spectrometry (Sercon Ltd 20-20), as described by Stevens, Laughlin, Atkins, & Prosser (1993), interfaced to a TGII cryfocusing unit (Sercon Ltd 20-20).

2.4 | ^{15}N tracing model

To quantify the simultaneously occurring gross N transformations in soil, a ^{15}N tracing model Ntrace, based on Müller et al. (2009) and Inselsbacher, Wanek, Strauss, Zechmeister-Boltenstern, and Müller (2013), was applied (Figure 2). The model considered seven N pools and 14 gross N transformations: M_{Nrec}, mineralization of recalcitrant organic N to NH_4^+; M_{Nlab}, mineralization of labile organic N to NH_4^+; I_{NH_4-Nlab} and $I_{NH_4-Nrec,}$ immobilization of NH_4^+ to N_{lab} and to N_{rec}, respectively; I_{NO_3}, immobilization of NO_3^-; O_{NH_4} and O_{Nrec} oxidation of NH_4^+ to NO_3^- and of N_{rec} to NO_3^-; D_{NO_3}, dissimilatory NO_3^- reduction to NH_4^+; A_{NH_4} and A_{NO_3}, adsorption of NH_4^+ and NO_3^-, respectively; R_{NH_4} and R_{NO_3}, release of adsorbed NH_4^+ and NO_3^-, respectively; U_{NH_4} and U_{NO_3}, plant uptake of NH_4^+ and NO_3^-, respectively.

The transformation rates were calculated either by zero- or first-order kinetics (Table 1). The model calculated gross N transformation rates by simultaneously optimizing the kinetic parameters for the various N transformations by minimizing the misfit between modelled and observed (mean ± standard deviations) NH_4^+ and NO_3^- concentrations and their respective ^{15}N enrichments via a Markov chain Monte Carlo method. A unique parameter set was optimized for the entire duration of the study and the performance of different model runs was evaluated by the AIC criterion. The uniqueness of the parameter set was evaluated by least three parallel sequences and evaluated by the Gelman reduction factor ($R < 1.3$) (Gelman, Carlin, Stern, & Rubin, 2003). The Ntrace model was programmed in the software MatLab (Version 7.2, The MathWorks Inc.) and Simulink (Version 6.4, The MathWorks Inc.). A description of all model parameters, the kinetic settings and the parameter values after optimization are presented in Table 1.

2.5 | Calculation procedures and statistics

To calculate the cumulative N_2O fluxes of the treatments over the observation period, we used linear interpolation between sampling dates, that is, similar to the procedure applied by Kammann et al. (2008). The solver method (Microsoft Excel 2007) was used to calculate the N_2O fractions associated with NH_4^+ (n – nitrification) and NO_3^- (d – denitrification) and organic N (h – heterotrophic nitrification of organic N followed by reduction to N_2O) by minimization of the absolute difference between observed and calculated ^{15}N enrichments of N_2O according to the equation (Müller et al., 2014):

$$a_{N_2O} = d \times a_d + n \times a_n + (1 - d - n) \times a_h$$

where n and d are the fractions related to the NH_4^+ and NO_3^- pools, respectively, and a_d, a_n and a_h represent the ^{15}N abundance of the NO_3^-, NH_4^+ and N_{org} (assumed to be at natural abundance) respectively.

For N transformations following first-order kinetics, average gross N rates were calculated by integrating the gross N rates over the experimental period, divided by the total time (Rütting & Müller, 2007). To determine cumulative NH_4^+ production, the results of the rates for M_{Nrec}, M_{Nlab}, D_{NO_3} and R_{NH_4} were summed up, while for cumulative NH_4^+ consumption the sum of I_{NH_4-Nrec}, I_{NH_4-Nlab}, O_{NH_4}, A_{NH_4} and U_{NH_4} was calculated. The sum of the rates of O_{Nrec}, O_{NH_4} and R_{NO_3} was calculated to determine cumulative NO_3^- production, and the sum of the rates of I_{NO_3}, D_{NO_3}, A_{NO_3} and U_{NO_3} was used to calculate cumulative NO_3^- consumption.

We analysed parameter results based on the comparisons of standard deviations and ANOVA, using Fisher's LSD as post hoc test with a 5% probability level of significance. Due to the high number of iterations of the ^{15}N tracing model, further statistical tests are inappropriate for the comparison of parameter results (Yoccoz, 1991). Statistical calculations (ANOVA) were carried out with SigmaPlot-SigmaStat 12.

3 | RESULTS

We found no significant differences in above- or belowground biomass, or N pools, in the small (10 × 10 cm) subplots. Conversely, Andresen et al. (2017) reported, for the year 2013, that total aboveground biomass yields in the plots were significantly greater for the eCO_2 plots than the aCO_2 plots (i.e. spring: 485.9 ± 9.0 g/m^2 eCO_2, 450.4 ± 4.7 g/m^2 aCO_2; summer: 296.8 ± 30.0 g/m^2 eCO_2, 226.5 ± 19.5 g/m^2 aCO_2; $p < .05$). Volumetric soil moisture data from permanently installed TDR probes within the 0–15 cm soil depth during the study period showed no significant difference between the treatments (Figure 3b).

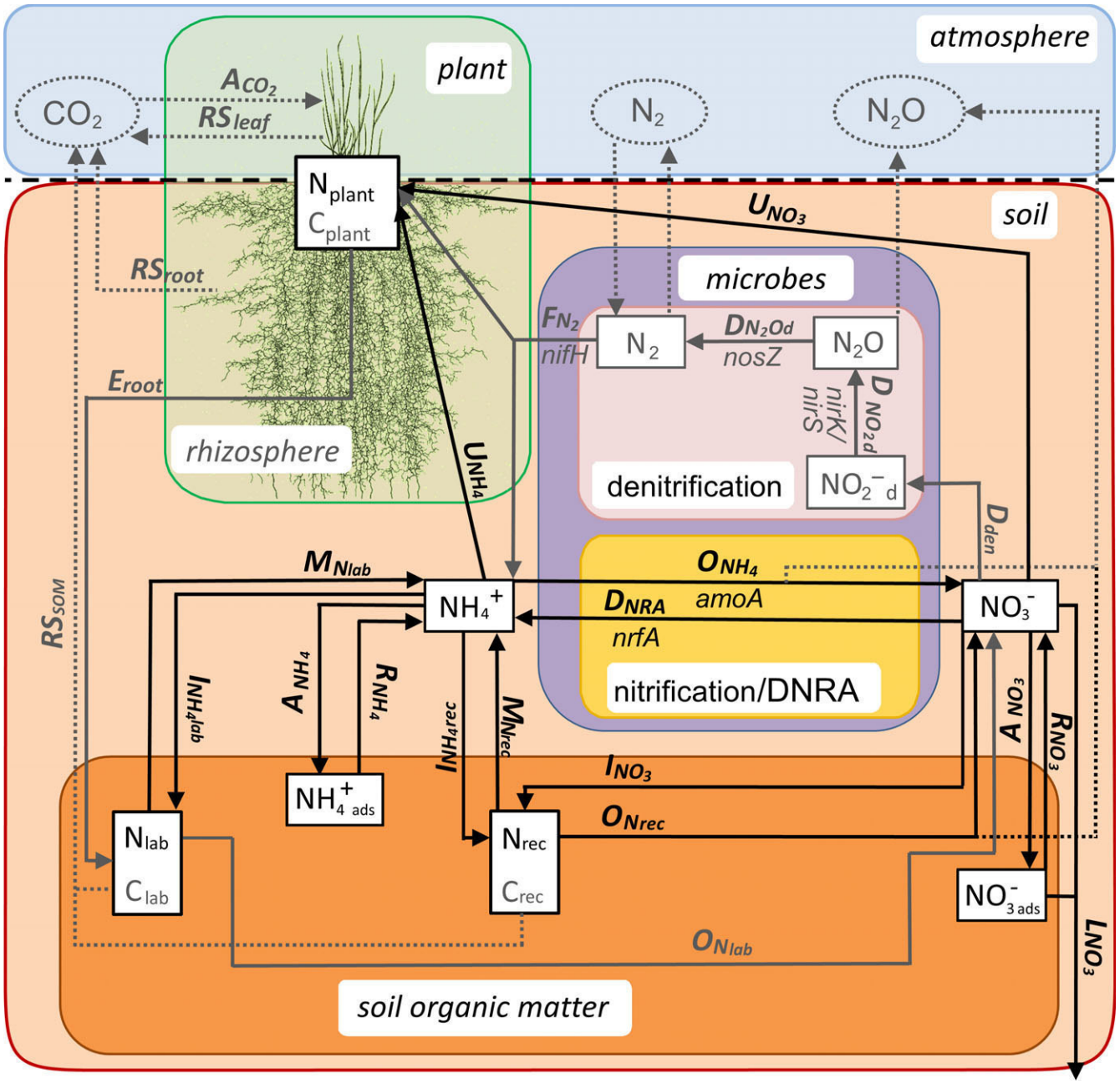

FIGURE 2 Scheme of C and N transformations in the GiFACE grassland. Dotted ellipses mark gases, dotted arrows indicate transformation to gaseous state or gas diffusion. Rectangular boxes mark soil and plant pools, solid line arrows indicate transformations within microorganisms and/or liquid phase. In the applied ^{15}N tracing model only transformations and soil and plant pools marked in black were included, the solver method considered NH_4^+, NO_3^- and SOM (N_{lab} + N_{rec}) as sources for N_2O. The abbreviation above each arrow indicates the respective N transformation, while below the arrows the respective microbial functional marker genes are displayed: A_{CO_2} – assimilation, *A* – adsorption of NH_4^+ or NO_3^-, *C* – carbon pool, *D* – dissimilatory reduction, *d/den* – denitrification, *E* – exudation, *F* – fixation of N_2, *I* – immobilization, *L* – leaching, *lab* – labile, *M* – mineralization, *N* – nitrogen pool, *NRA* – Nitrogen reduction to NH_4^+, *O* – oxidation, *R* – release of adsorbed NH_4^+ or NO_3^-, *RS* – respiration, *rec* – recalcitrant, *SOM* – soil organic matter, *U* – uptake by plants

3.1 | N_2O fluxes and ^{15}N enrichment under elevated CO_2

In the study period, May 2013 to January 2014, the N_2O emissions from eCO_2 plots were, on average, 2.25-fold higher (the median was 1.48-fold higher) than from aCO_2 plots (Figure 3a) and the ratio ln (E/A) showed that on 75% of the sampling days the N_2O emissions were higher from eCO_2 compared to aCO_2 (Figure 3a). The cumulative fluxes of N_2O, calculated with linear interpolation within the observation period from May 2013 to January 2014, resulted in a 2.88-fold increase in average N_2O emissions from eCO_2 compared to aCO_2 plots (i.e. eCO_2: 37.1 ± 2.5 *SE* g N_2O–N/m^2 during 266 days; aCO_2: 12.9 ± 0.2 *SE* g N_2O–N/m^2 during 266 days; the median was 1.36-fold higher).

TABLE 1 Description of model parameters and optimized values (mean and standard deviations) of the temperate GiFACE grassland under ambient and after 15 years of elevated atmospheric CO_2

			Parameter values			
Parameters	**Description**	**Kinetics[a]**	**Ambient mean**	**Ambient *SD***	**Elevated mean**	**Elevated *SD***
M_{Nrec}	Mineralization of N_{rec} to NH_4^+	0	87.6195	10.8860	80.5763	6.9571
M_{Nlab}	Mineralization of N_{lab} to NH_4^+	1	1.1×10^{-5}	5.81×10^{-6}	1.27×10^{-5}	9.74×10^{-6}
I_{NH_4-Nrec}	Immobilization of NH_4^+ to N_{rec}	1	0.0102	0.0067	0.0084	0.0073
I_{NH_4-Nlab}	Immobilization of NH_4^+ to N_{lab}	1	0.0179	0.0039	0.0224	0.0188
I_{NO_3}	Immobilization of NO_3^- to N_{rec}	1	0.2596	0.0303	0.3505	0.0264
O_{Nrec}	Oxidation of N_{rec} to NO_3^-	0	0.0026	0.0013	0.0008	0.0005
O_{NH_4}	Oxidation of NH_4^+ to NO_3^-	1	0.1771	0.0271	0.1689	0.0341
D_{NO_3}	Dissimilatory NO_3^- to NH_4^+	0	15.3343	1.9821	8.3756	1.0143
A_{NH_4}	Adsorption of NH_4^+	1	0.0432	0.0200	0.0294	0.0240
A_{NO_3}	Adsorption of NO_3^-	1	6.66×10^{-5}	5.43×10^{-5}	7.18×10^{-5}	2.92×10^{-5}
R_{NH_4}	Release of adsorbed NH_4^+	1	0.0030	0.0004	0.0037	0.0005
R_{NO_3}	Release of adsorbed NO_3^-	1	0.0041	0.0009	0.0081	0.0019
U_{NH_4}	Plant uptake of NH_4^+	1	0.8005	0.1019	0.8843	0.0832
U_{NO_3}	Plant uptake of NO_3^-	1	0.2459	0.0102	0.2339	0.0125

[a]Kinetics: 0 = zero order (mg N m^{-2} day^{-1}), 1 = first order (day^{-1}).

The highest emission peaks occurred 2 days after the application of the labelled N fertilizer and reached 2,047 and 1,744 μg N_2O–N m^{-2} day^{-1} for eCO_2 and aCO_2 plots, respectively. Emission events >100 μg N_2O–N m^{-2} day^{-1} occurred under eCO_2 plots up to day 115. High precipitation at the end of May caused similar high volumetric water content in both aCO_2 and eCO_2 (Figure 3b,c), but triggered higher N_2O emissions under eCO_2 than aCO_2 (Figure 3a). During autumn and winter months, when the soil moisture difference between treatments was constant but not significant, N_2O emission differences were smaller or nonexistent.

The observed ^{15}N enrichment of emitted N_2O increased in both treatments, aCO_2 and eCO_2 plots, and for both ^{15}N-labelled moieties immediately after the labelling occurred, peaking within the first 23 hr (Figure 4c,d). The average peaks of the ^{15}N enrichment from the plots labelled with ^{15}N–NO_3^- were 17.6 and 19.5 atom% excess for aCO_2 and eCO_2, respectively, and much lower for the plots labelled with ^{15}N–NH_4^+, with 1.9 and 1.8 atom% excess for aCO_2 and eCO_2, respectively. Ten days after the labelling occurred, the ^{15}N enrichments of all the N_2O emissions were <0.5 atom% excess.

The observed ^{15}N enrichment of N_2O, NH_4^+ and NO_3^-, and the analysis of the N_2O emission pathways, revealed that the highest relative contribution of denitrification to the N_2O emissions of 41.4 and 51.0% occurred 23 hr (0.95 days) after labelling for the aCO_2 and eCO_2 plots, respectively, which then fell below 1% contribution after 8 days (Table 2 and Figure 4a,b). The portion of N_2O emissions from nitrification peaked at 2.75 days after labelling (19.2 and 19.1% for aCO_2 and eCO_2, respectively), which corresponded to the largest total N_2O emission peak (Table 2 and Figure 4a,b). The largest contributor to the total N_2O emissions was heterotrophic nitrification of organic N followed by reduction to N_2O and ranged between 77.8 and 93.0% (Table 2).

The absolute contributions of denitrification and nitrification, were continuously higher under eCO_2 compared to aCO_2 plots (Figure 4a,b): nitrification contributed 1409.8 and 2340.6 μg N_2O–N m^{-2} over the first 58 days after labelling to the cumulative N_2O emissions of aCO_2 and eCO_2 plots, respectively, while denitrification contributed 157.8 and 329.9 μg N_2O–N m^{-2} over the first 58 days after labelling to the cumulative N_2O emissions of aCO_2 and eCO_2 plots, respectively (Table 2). N_2O emissions from heterotrophic nitrification of organic N followed by reduction to N_2O were 7304.5 6 μg N_2O–N m^{-2} higher under eCO_2 plots than under aCO_2 plots.

These linear interpolations of the results of the solver method showed a 2.09-fold increase in N_2O emissions from denitrification (which equals an additional 172 μg N_2O–N m^{-2} over 58 days) and a 1.64-fold increase from nitrification (which equals additional 931 μg N_2O–N m^{-2} over 58 days) and a 1.66-fold increase in N_2O emissions from heterotrophic nitrification under eCO_2 compared to aCO_2 plots (Table 2).

3.2 | Plant N uptake, soil NH_4^+ and NO_3^- concentrations and ^{15}N enrichment

The observed and modelled changes in soil NH_4^+ and NO_3^- concentrations after the application of the ^{15}N labelled NH_4NO_3 were very similar and no significant differences between the observed concentrations occurred between aCO_2 and eCO_2 plots (data not shown). The detectable NH_4^+ concentrations (i.e. aCO_2: 779 mg N m^{-2}, eCO_2: 618 mg N m^{-2}) were only half that of NO_3^- (i.e. aCO_2: 1,358 mg N m^{-2}, eCO_2: 1,233 mg N m^{-2}) at the first sampling date a few hours after the application, despite all plots receiving the same rate of NH_4NO_3. The soil NH_4^+ concentration had decreased within 5 days to background concentrations in both CO_2 treatments. The

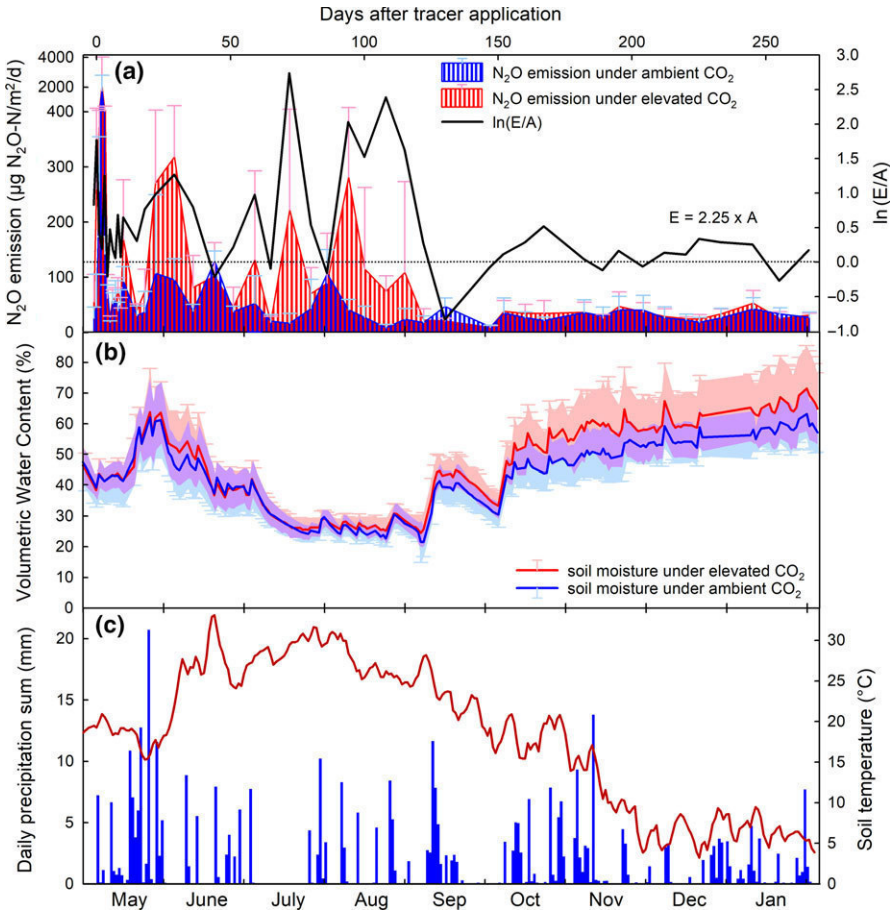

FIGURE 3 N_2O emissions and abiotic factors. (a) N_2O emissions (mean ± *SD*) and the ln(E/A) ratio of N_2O emissions for ambient and elevated CO_2 plots, (b) volumetric soil water content (mean ± *SD*) in 0–15 cm under ambient and elevated CO_2 and (c) daily rainfall (bars) and soil temperature at 10 cm (line). If the ln(E/A) ratio is above zero, the emissions were higher under eCO_2

soil NO_3^- concentration took 10 days to decrease to the background concentration, with no significant differences due to CO_2 treatment (data not shown).

The observed ^{15}N enrichments of aboveground biomass showed no significant differences due to CO_2 treatment (Figure 5a,b). The modelled total gross uptake of NH_4–N by plants (U_{NH_4}) did not differ with CO_2 treatment, but the modelled total gross uptake of NO_3^- (U_{NO_3}) decreased under eCO_2 (Table 3). When NH_4–^{15}N was applied the ^{15}N enrichment of the NH_4^+ pool declined rapidly regardless of CO_2 treatment (Figure 5c,d).

When ^{15}N–NO_3^- was applied, both the observed and modelled ^{15}N enrichment of the NH_4^+ pool became more enhanced under aCO_2 conditions at days 3 to 8 when compared with eCO_2 (Figure 5c,d). After applying NH_4–^{15}N the^{15}N enrichment of the NO_3^- pool initially increased to ca. 10 atom% excess before declining with no effect of CO_2 treatment (Figure 5e,f). When ^{15}N–NO_3^- was applied the ^{15}N enrichment of the NO_3^- pool decreased over time with significantly higher ^{15}N–NO_3^- atom% excess in the eCO_2 treatment only at Day 22 (Figure 5e,f).

The DNRA (D_{NO_3}) was significantly reduced under eCO_2 (Table 3). The gross release rates of adsorbed NH_4^+ and NO_3^- ($R_{NH_4} + R_{NO_3}$) were significantly higher under eCO_2 than aCO_2. The rates of gross NH_4^+ immobilization to recalcitrant soil organic N (I_{NH_4-Nrec}) and the adsorption of NH_4^+ (A_{NH_4}) tended to be greater under aCO_2 conditions, but because of relatively large standard deviations the rates did not differ from those under elevated CO_2. Significant treatment differences were not observed for any of the other modelled N transformation rates measured under aCO_2 and eCO_2 (Table 3). Cumulative NH_4^+ production, as the sum of processes that produce NH_4^+ calculated by the model, decreased by 10.8 mg N m^{-2} day^{-1} under eCO_2, while cumulative NH_4^+ consumption decreased by 15.0 mg N m^{-2} day^{-1}, but their ratio remained the same in both treatments (Table 3). The sum of NO_3^- production under eCO_2 increased by 2.2 mg N m^{-2} day^{-1}, which was the result of a 3.8-fold increase in R_{NO_3} and a 21% decrease in the sum of autotrophic and heterotrophic nitrification. The NO_3^- consumption under eCO_2 decreased by 5.7 mg N m^{-2} day^{-1} (Table 3). The ratio of NO_3^- consumption to production was slightly lower under eCO_2.

4 | DISCUSSION

This ^{15}N tracing study in the GiFACE grassland field confirms earlier results obtained after only 8 years of eCO_2 (Kammann et al., 2008) that eCO_2 increased N_2O emissions by at least twofold, as our data indicate that cumulated N_2O emissions after 15 years under eCO_2 were 2.88-fold higher than under the control. This confirms our hypothesis that the 20% increase in the atmospheric CO_2 concentration triggered changes in soil N transformations that were responsible for the long-term higher N_2O emissions.

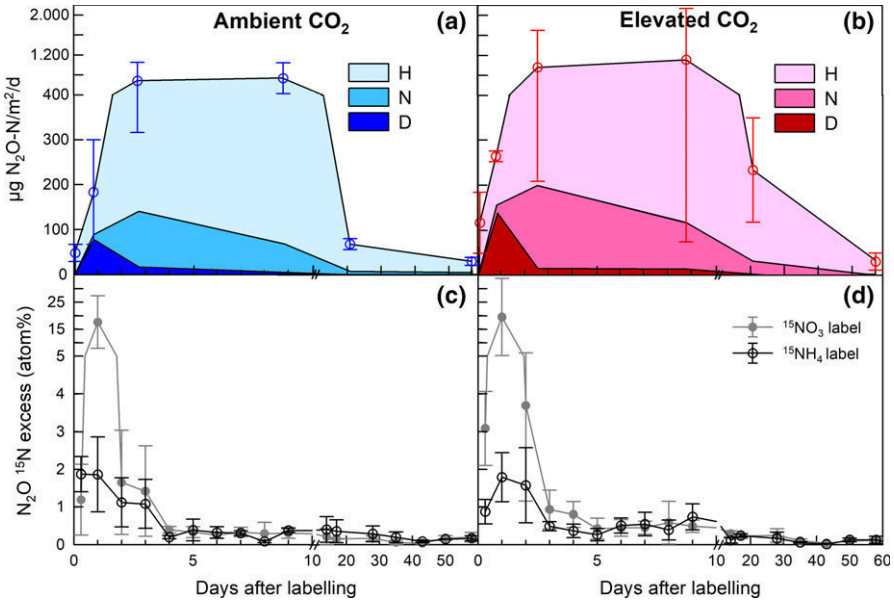

FIGURE 4 Contribution to N_2O emissions and ^{15}N enrichment in the GiFACE grassland after the application of labelled NH_4NO_3 solution. Total N_2O emission (means ± *SD*) and the relative contribution of H – heterotrophic nitrification of organic N followed by reduction to N_2O, N – nitrification and D – denitrification per treatment as results of the solver method for (a) ambient CO_2 and (b) elevated CO_2. Measured ^{15}N enrichment of emitted N_2O (c) under ambient and (d) after 15 years of elevated atmospheric CO_2 concentration; given are means ± *SD* (n = 3) of ^{15}N enrichment in N_2O for the two different subplots where either ^{15}N–NH_4^+ or ^{15}N–NO_3^- was applied. The scaling of x and y axis were adjusted for a better visualization of the data during the first 10 days after labelling

TABLE 2 Results of the solver method on N_2O emissions related to denitrification, using NO_3^- as source of N_2O emissions, nitrification, using NH_4^+ as source of N_2O emissions, and heterotrophic nitrification, using organic N as a source of N_2O emission for the sampling times (days) after ^{15}N tracer application under aCO_2 and eCO_2 treatments, and the cumulated N_2O emissions calculated by linear interpolation over the observation period of 58 days after labelling

	Ambient CO_2			Elevated CO_2		
Days after labelling	Denitrification %	Nitrification %	Heterotrophic Nitrification %	Denitrification %	Nitrification %	Heterotrophic Nitrification %
0.17	2.2	20.0	77.8	6.0	1.0	93.0
0.95	41.4	2.4	56.2	51.0	3.3	45.8
2.75	1.8	19.2	79.1	1.3	19.1	79.6
8.73	0.1	8.6	91.2	0.9	8.7	90.4
20.85	0.0	12.3	87.7	0.2	12.4	87.4
57.77	0.4	17.6	82.0	0.0	9.4	90.6
Cum. N_2O emission over 58 days (µg N_2O–N/m^2)	157.8	1409.8	10347.6	329.9	2340.6	17652.1

4.1 | NH_4^+ dynamics under elevated CO_2

In an earlier plant-free laboratory incubation study with soil from the GiFACE grassland (Müller et al., 2009), the NH_4^+ concentration directly after the tracer application was higher than the NO_3^- concentration. Conversely, in the field, we observed that the NH_4^+ concentration at the first sampling after tracer application was only half of the NO_3^- concentration for both treatments. This can be explained by the much higher rate of NH_4^+ uptake by the plants compared to NO_3^- uptake (Table 3).

The observed and modelled steep decline in the portion of ^{15}N–NH_4^+ from the labelled NH_4^+ subplots under aCO_2 and eCO_2 showed a very similar pattern, while the different peaks after 5 days from labelled NO_3^- subplots indicated a reduced DNRA under eCO_2 (Figure 5) that contradicts the former incubation study (Müller et al., 2009).

Under eCO_2 the decreased sum of NH_4^+ production rates was lower than the reduced consumption of NH_4^+, but its ratio was the same between treatments. Overall, plant uptake of NH_4^+ (U_{NH_4}) was the dominant transformation process (Table 3), accounting for 76% and 79% of total consumption under ambient and elevated CO_2 conditions, respectively.

This field tracing study after 15 years of CO_2 enrichment revealed that M_{Nrec} accounted for 83 and 85% of total NH_4^+

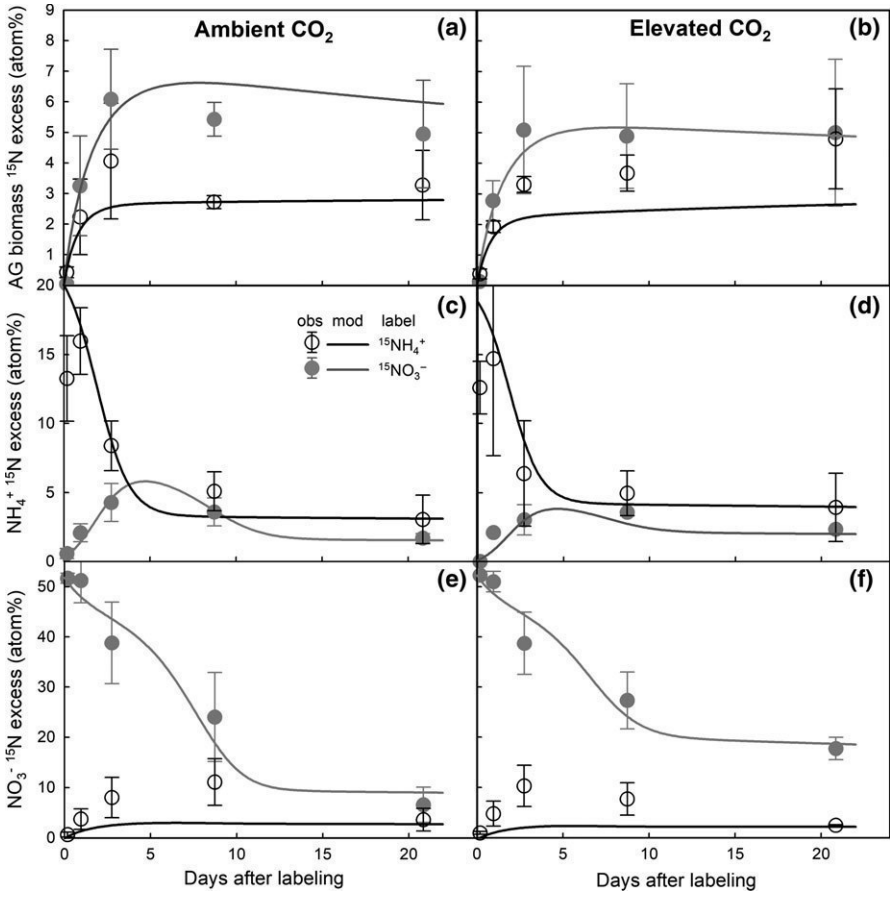

FIGURE 5 Measured and modelled ^{15}N enrichment of aboveground biomass (a–b), NH_4^+–N (c–d) and NO_3^-–N (e–f) in the GiFACE grassland under ambient and after 15 years of elevated atmospheric CO_2 concentration

production under aCO_2 and eCO_2 conditions, respectively. There was a tendency for the absolute M_{Nrec} rates to be lower under eCO_2, but the difference was not significant. This is in contrast with Hungate, Chapin, Zhong, Holland, and Field (1997) who found that within the first 24 hr after labelling the gross rate of NH_4^+ mineralization increased significantly in a grassland soil under eCO_2, while in a Florida scrub oak under eCO_2 the rate of gross N mineralization was reduced (Hungate, Dijkstra, Johnson, Hinkle, & Drake, 1999). Other previous observations from CO_2 experiments, including microcosm studies (Hungate, Lund, Pearson, & Chapin, 1997), soil incubations without plants (Niklaus et al., 2003; Richter, Hartwig, Frossard, Nösberger, & Cadisch, 2003), a modelling study excluding plant dynamics (Pepper, Del Grosso, McMurtrie, & Parton, 2005), and meta-analyses and reviews (van Groenigen et al., 2006; Zak, Pregitzer, King, & Holmes, 2000) failed to account for the subsequent CO_2 effect of increased plant assimilation and root exudation on mineralization. An incubation study in the laboratory with soil from the GiFACE (Müller et al., 2009) did detect a higher gross N mineralization rate from labile organic N but no difference in mineralization from recalcitrant organic N. Potential explanations for the different outcomes of the laboratory and the field studies are that sieved soil without plants was used for the incubation study, and the direct influences of plants via N uptake and rhizodeposition of energy-rich labile C compounds were absent. In addition, the different duration of the CO_2 enrichment could play a role.

Current studies indicate that under climate change conditions increased root exudation, as a source of bioavailable supply of energy triggers a stimulation of microbial SOM mineralization called priming (Phillips, Finzi, & Bernhardt, 2011), which may explain the observed increase in organic N as a source for N_2O emissions. Some root exudates, such as oxalic acid, promotes SOM loss by liberating organic compounds from protective association with minerals (Keiluweit et al., 2015).

4.2 | NO_3^- dynamics under elevated CO_2

The observed and modelled ^{15}N enrichment of NH_4^+ when ^{15}N–NO_3^- was applied, reached slightly higher values under aCO_2 than eCO_2 (Figure 5c,d). This difference in the model was caused by a nearly twofold higher gross rate of DNRA (D_{NO_3}) reducing more NO_3^- to NH_4^+ under aCO_2 conditions. The observed ^{15}N enrichment of NO_3^- was significantly higher under eCO_2 only at 22 days after labelling ($p < .01$; Figure 5e,f). This may be related to the lower dilution rate via nitrification (O_{NH_4}) under eCO_2, the lower sum of gross mineralization rates ($M_{Nrec} + M_{Nlab}$) and the reduced gross rate of NO_3^- uptake by plants (U_{NO_3}) under eCO_2.

Bloom, Burger, Kimball, and Pinter (2014) showed that NO_3^- assimilation was slower under elevated than ambient CO_2 in field-grown wheat (*Triticum aestivum L.*), similar to our findings. There is evidence that C3 plants under eCO_2 preferentially take up NH_4^+ over

TABLE 3 Gross N transformation rates in the permanent GiFACE grassland under ambient CO_2 concentration and after 15 years of elevated atmospheric CO_2 concentration

	N-species		Production/Consumption rate (mg N m^{-2} day^{-1})		
Process	Produced	Consumed	aCO_2	eCO_2	Difference
M_{Nrec}	NH_4^+		87.6060 a	80.5763 a	
M_{Nlab}	NH_4^+		0.3772 a	0.3845 a	
I_{NH_4-Nrec}		NH_4^+	1.3635 a	0.9403 a	
I_{NH_4-Nlab}		NH_4^+	2.3840 a	2.5106 a	
I_{NO_3}		NO_3^-	40.6380 a	48.2308 a	
O_{Nrec}	NO_3^-		0.0026 a	0.0007 a	
O_{NH_4}	NO_3^-	NH_4^+	23.6017 a	18.9277 a	
D_{NO_3}	NH_4^+	NO_3^-	15.3343 a	8.3756 b	
A_{NH_4}		NH_4^+	5.7590 a	3.2929 a	
A_{NO_3}		NO_3^-	0.0104 a	0.0099 a	
R_{NH_4}	NH_4^+		1.8630 b	5.0848 a	
R_{NO_3}	NO_3^-		2.7298 b	9.5821 a	
U_{NH_4}		NH_4^+	106.7154 a	99.1194 a	
U_{NO_3}		NO_3^-	38.4984 a	32.1916 b	
Cum NH_4^+	Production		105.1805	94.4212	−10.8
	Consumption		139.8236	124.7909	−15.0
	Ratio		1.33	1.33	
Cum NO_3^-	Production		26.3341	28.5105	2.2
	Consumption		94.4811	88.8079	−5.7
	Ratio		3.59	3.11	

The gross N transformation rates are outputs from the Ntrace model. For abbreviation, see Table 1. Within rows, means followed by the same letter are not significantly different (Fisher's LSD, $p < .05$).

NO_3^- from soil, because of physiological mechanisms (Bloom et al., 2002, 2010, 2012), for example, the dependence of NO_3^- assimilation on eCO_2 affected photorespiration (Rachmilevitch, Cousins, & Bloom, 2004). Wu et al. (2017) found that eCO_2 effects on the plant preference of different N forms may alter plant and microbial N acquisition and N_2O emissions. These authors suggested that eCO_2 inhibition of plant NO_3^- uptake and/or increased soil labile C under eCO_2 enhances the N and/or C availability for denitrifiers and increased the intensity and/or duration of N_2O emissions. However, in this study, we found no significant changes in the absolute rate of NH_4^+ uptake (U_{NH_4}), but a decreased NO_3^- uptake rate (U_{NO_3}) and therefore a relative shift to a preferred uptake of NH_4^+ under eCO_2.

Similar to the GiFACE incubation study by Müller et al. (2009), we observed a tendency of declining rates of oxidation of NH_4^+ (O_{NH_4}) and organic N (O_{Nrec}), as NO_3^- sources under eCO_2 in the field experiment. Together with an increased release of adsorbed NO_3^- (R_{NO_3}), this caused a total net increase in NO_3^- production of 7.8% under eCO_2. At the same time, total NO_3^- consumption under eCO_2 (as the sum of NO_3^- immobilization (I_{NO_3}), dissimilatory NO_3^- reduction to NH_4^+ (D_{NO_3}), adsorption of NO_3^- (A_{NO_3}) and plant uptake of NO_3^- (U_{NO_3})) decreased by 5.8%. Cheng et al. (2012) documented increased soil NO_3^- (26.7%), but decreased soil NH_4^+ (7.9%) under eCO_2, explainable either via increased soil available N and/or reduced plant N uptake. An increased NO_3^- availability for the denitrification process under eCO_2 may cause higher N_2O emissions (Wu et al., 2017). Our data indicate that it was a change in the other N transformation rates and not, as previously suggested, only decreased NO_3^- uptake by plants that increased NO_3^- availability (Bloom et al., 2012; Wu et al., 2017). In total, the changes in NO_3^- transformation rates were only small, and in contrast to our hypothesis could not fully explain the increase in N_2O emissions under eCO_2.

4.3 | N_2O emissions under elevated CO_2

The gas flux measurements confirmed our hypothesis of increased N_2O emissions under eCO_2 and showed that the formerly reported doubling of N_2O emissions under eCO_2 during the first 8 years (Kammann et al., 2008) still prevailed after 15 years as the cumulative N_2O emissions over the study period were 2.88-fold higher under eCO_2 than under aCO_2. While the highest N_2O emissions in the first 8 years under eCO_2 occurred during the summer months and not directly after the fertilization in April (Kammann et al., 2008), the new results documented highest emissions within a few weeks after fertilization. The difference is likely related to the application of liquid fertilizer solution during the labelling experiment,

which made NH_4^+ and NO_3^- directly available for microbial N transformations, while usually the GiFACE plots receive solid fertilizer, which is not immediately available to soil microorganisms.

An increase in soil moisture under eCO_2 has been suggested to stimulate denitrification (van Groenigen et al., 2011) caused by a change in the microbial community (Brenzinger et al., 2017), for example, a reduced abundance of N_2O reducers (Guenet et al., 2012; Regan et al., 2011). We could not detect significant soil moisture differences in this study, but slightly higher soil moisture under eCO_2 occurred only during the autumn and winter months, when N_2O emissions were low and very similar under the aCO_2 and eCO_2 treatments.

The ^{15}N tracing model includes denitrification only as part of the I_{NO_3} rate. However, our results clearly showed that under field conditions, including plant uptake, there was a greater availability of NO_3^- for denitrification. This resulted from changed N transformation rates under eCO_2 (Table 3). Furthermore, the results from the independent solver method used in this study showed that most N for the additional N_2O emissions under eCO_2 was associated with the organic N pathway (Zhang, Müller, & Cai, 2015). The N_2O isotopic signature indicated that under eCO_2 the sources of the additional emissions of 8407.2 μg N_2O–N/m^2 during the first 58 days after labelling were associated with NO_3^- (+2.0%), NH_4^+ (+11.1%) and organic N (+86.9%) (Table 2). These results are in line with the documented importance of the heterotrophic contribution to N_2O emissions at the GiFACE site (Müller, Stevens, & Laughlin, 2006) and its increase under eCO_2 in the New Zealand grassland FACE (Rütting, Clough, Müller, Lieffering, & Newton, 2010; Zhong, Bowatte, Newton, Hoogendoorn, & Luo, 2018). It also confirms results from an earlier study that mainly reduction processes are responsible for the N_2O emissions, because the N_2O associated with the oxidation of organic N to nitrite (NO_2^-) and subsequent reduction to N_2O was found to be the predominant reduction process in this ecosystem (Müller, Stevens, Laughlin, & Jäger, 2004).

Therefore, our results provide evidence that the increased N_2O emissions under eCO_2 result from incomplete reduction in NO_2^-, which is an intermediate from the oxidation of organic N, as well as from the reduction in NO_3^-, which is in line with earlier findings (Müller et al., 2014). In our case, it seems that an increase in the activity of nitrite reductase encoded by *nirS*, rather than a decrease in the activity of the nitrous oxide reductase encoded by *nosZ* (Figure 2), was crucial for increased N_2O emissions during the first day of this study (Brenzinger et al., *unpublished data*).

We were not able to measure root exudation rates to quantify higher belowground allocation, which was documented in the GiFACE by a former study (Denef et al., 2007). The root biomass data did not show significant differences between treatments, but the increased soil respiration rates particularly during the autumn and winter months indicated higher belowground allocation (Keidel, Kammann, Grünhage, Moser, & Müller, 2015) that hints that our hypothesis of plant-induced stimulation and alteration of the microbial activity is true. Our hypothesis that stimulated denitrification is mainly responsible for the doubling of N_2O emissions was not confirmed by our results, because the solver method revealed that the major source for additional emissions was the oxidation of organic N followed by incomplete NO_2^- reduction.

It is difficult to evaluate, whether the documented increase in N_2O emissions under eCO_2 from this or other FACE studies and experiments (Baggs et al., 2003; van Groenigen et al., 2011; Kammann et al., 2008; Kettunen et al., 2006, 2007; Wu et al., 2017) provide a realistic picture of ecosystem reactions under progressive global warming. That is, most of these climate change experiments manipulated only one factor, the atmospheric CO_2 concentration, and not air and soil temperatures or precipitation patterns as predicted by climate change models.

Changes in soil temperature and moisture may also have significant effects on the microbial soil communities and their activity. Increased soil temperature may result in lower soil moisture and less N_2O production as shown for a grassland warming experiment at the GiFACE field site (Jansen-Willems, Lanigan, Clough, Andresen, & Müller, 2016), which may counterbalance eCO_2 effects. In contrast, Griffis et al. (2017) found a positive correlation between N_2O emissions and temperature in a 6-year data series from the US corn belt.

Combined CO_2 enrichment and warming experiments in a paddy field showed only minor and counteracting effects of these factors on soil N dynamics (Chen, Zhang, Xiong, Pan, & Müller, 2016). Brown et al. (2012) found in a review of studies that soil N_2O efflux from combined environmental changes ranged from a −1.1-fold decrease to a 1.8-fold increase, but that expected combined effects were poor predictors of observed combined effects. In their study, there were no significant interactions on N_2O emissions if the combined effects of CO_2, heating, precipitation change and N addition were tested, while significant interactions were found for precipitation change plus N addition, and for the combination of heating, precipitation change and N addition. These authors found that denitrification was the dominant microbial source of N_2O, and responded to increased soil water content and higher labile C availability. But, the findings suggest, that N_2O emissions are unlikely to be a simple function of effects observed in single-factor experiments. Cantarel et al. (2012) reported from an upland grassland that not only warming alone, but also the simultaneous application of warming, summer drought and elevated CO_2 had a positive effect on N_2O fluxes, nitrification and N_2O release by denitrification, which was explained by shifts in the microbial community and population size. This is in line with results from our grassland site where warming stimulated N_2O production pathways related to the turnover of organic N (Jansen-Willems et al., 2016). Therefore, pathways for N_2O production that are not normally considered (such as heterotrophic nitrification coupled to nitrite reduction) will most likely play an important role under climate change and determine whether N_2O emissions will increase under climate change. That this pathway is important, is understandable because both eCO_2 and warming, will accelerate C transformations and stimulate the processes of the mineralization-immobilization turnover in soils.

Ecosystem responses to changes in several abiotic parameters is not necessarily the sum of the ecosystem response to a single parameter changing (Larsen et al., 2011). Thus, it remains a great challenge to

design, instal and run long-term multifactor global change experiments that allow realistic simulation of the changed biotic and abiotic parameters to provoke realistic ecosystem responses to multifactorial global change (Templer & Reinmann, 2011).

In summary, this field ^{15}N tracing study confirms that elevated CO_2 causes a more than twofold increase in N_2O emissions from the GiFACE grassland. We showed that field studies of intact ecosystems are essential to evaluate the effect of climate change on N_2O emissions, because we found that intact atmosphere-plant-soil interactions under field conditions revealed different results than pure soil incubations studies in the laboratory. Although, the total gross NH_4^+ production and consumption rates decreased, their ratio stayed the same under eCO_2 and had only minor effect on N_2O emissions. Higher NO_3^- production and less NO_3^- consumption under eCO_2 had also only small effects on increased N_2O emissions. We found that the source of most of the additional N_2O emissions under eCO_2 was the oxidation of organic N and incomplete reduction in NO_2^-, emitting N_2O instead of N_2. We presume that increased root exudation under eCO_2 provided an additional source of bioavailable supply of energy that triggered the stimulation of microbial SOM mineralization and an increased activity of bacterial nitrite reductase, which caused the shift in N_2O:N_2 ratio via incomplete denitrification. If this positive feedback reaction, via a doubling of N_2O emissions from grassland ecosystems, takes place during future climate change, we will face a significantly faster temperature rise than predicted by current climate projections within this century.

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REFERENCES

Ainsworth, E. A., & Long, S. P. (2005). What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist*, *165*, 351–371.

Andresen, L. C., Yuan, N., Seibert, R., Moser, G., Kammann, C. I., Luterbacher, J., … Müller, C. (2017). Biomass responses in a temperate European grassland through 17 years of elevated CO_2. *Global Change Biology*, 1–11, in press. https://doi.org/10.1111/gcb.13705

Asensio, J. S. R., Rachmilevitch, S., & Bloom, A. J. (2015). Responses of *Arabidopsis* and wheat to rising CO_2 depend on nitrogen source and nighttime CO_2 levels. *Plant Physiology*, *168*, 156–163. https://doi.org/10.1104/pp.15.00110

Baggs, E. M., Richter, M., Hartwig, U. A., & Cadisch, G. (2003). Nitrous oxide emissions from grass swards during the eighth year of elevated atmospheric pCO_2 (Swiss FACE). *Global Change Biology*, *9*, 1214–1222. https://doi.org/10.1046/j.1365-2486.2003.00654.x

Bloom, A. J., Asensio, J. S. R., Randall, L., Rachmilevitch, S., Cousins, A. B., & Carlisle, E. A. (2012). CO_2 enrichment inhibits shoot nitrate assimilation in C3 but not C4 plants and slows growth under nitrate in C3 plants. *Ecology*, *93*, 355–367. https://doi.org/10.1890/11-0485.1

Bloom, A. J., Burger, M., Kimball, B. A., & Pinter, J. P. Jr (2014). Nitrate assimilation is inhibited by elevated CO_2 in field-grown wheat. *Nature Climate Change*, *4*, 477–480. https://doi.org/10.1038/nclimate2183

Bloom, A. J., Burger, M., Rubio-Asensio, J. S., & Cousins, A. B. (2010). Carbon dioxide enrichment inhibits nitrate assimilation in wheat and *Arabidopsis*. *Science*, *328*, 899–903. https://doi.org/10.1126/science.1186440

Bloom, A. J., Smart, D. R., Nguyen, D. T., & Searles, P. S. (2002). Nitrogen assimilation and growth of wheat under elevated carbon dioxide. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 1730–1735. https://doi.org/10.1073/pnas.022627299

Brenzinger, K., Kujala, K., Horn, M. A., Moser, G., Guillet, C., Kammann, C., … Braker, G. (2017). Soil conditions rather than long-term exposure to elevated CO_2 affect soil microbial communities associated with N-cycling. *Frontiers in Microbiology*, *8*, 1976. https://doi.org/10.3389/fmicb.2017.01976

Brown, J. R., Blankinship, J. C., Niboyet, A., van Groenigen, K. J., Dijkstra, P., Le Roux, X., … Hungate, B. A. (2012). Effects of multiple global change treatments on soil N_2O fluxes. *Biogeochemistry*, *109*, 85–100. https://doi.org/10.1007/s10533-011-9655-2

Cantarel, A. A. M., Bloor, J. M. G., Pommier, T., Guillaumaud, N., Moirot, C., Soussana, J.-F., & Poly, F. (2012). Four years of experimental climate change modifies the microbial drivers of N_2O fluxes in an upland grassland ecosystem. *Global Change Biology*, *18*, 2520–2531. https://doi.org/10.1111/j.1365-2486.2012.02692.x

Chen, Z., Zhang, J., Xiong, Z., Pan, G., & Müller, C. (2016). Enhanced gross nitrogen transformation rates and nitrogen supply in paddy field under elevated atmospheric carbon dioxide and temperature. *Soil Biology and Biochemistry*, *94*, 80–87. https://doi.org/10.1016/j.soilbio.2015.11.025

Cheng, L., Booker, F. L., Tu, C., Burkey, K. O., Zhou, L., Shew, H. D., … Hu, S. (2012). Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. *Science*, *337*, 1084–1087. https://doi.org/10.1126/science.1224304

Denef, K., Bubenheim, H., Lenhart, K., Vermeulen, J., Van Cleemput, O., Boeckx, P., & Müller, C. (2007). Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO_2. *Biogeosciences*, *4*, 769–779. https://doi.org/10.5194/bg-4-769-2007

Dlugokencky, E., & Tans, P. (2017). NOAA/ESRL. Retrieved from www.esrl.noaa.gov/gmd/ccgg/trends/

Feng, Z., Rütting, T., Pleijel, H., Wallin, G., Reich, P. B., Kammann, C. I., … Uddling, J. (2015). Constraints to nitrogen acquisition of terrestrial plants under elevated CO_2. *Global Change Biology*, *21*, 3152–3168. https://doi.org/10.1111/gcb.12938

Gelman, A., Carlin, J. B., Stern, H. S., & Rubin, D. B. (2003). *Bayesian data analysis* (2nd ed., p. 668). Boca Raton, MA: Chapman & Hall/CRC.

Gerstner, J. (2014). Influence of abiotic factors like carbon dioxide, soil water and nitrogen content on the abundance of arbuscular mycorrhiza fungi (AMF) in the GiFACE study in Leihgestern. Unpublished Master Thesis, Justus-Liebig-University Giessen, Giessen, 56 pp.

Griffis, T. J., Chena, Z., Bakera, J. M., Wooda, J. D., Milleta, D. B., Leed, X., … Turnera, P. A. (2017). Nitrous oxide emissions are enhanced in a warmer and wetter world. *Proceedings of the National Academy of Science of the United States of America*, *114*, 12081–12085. https://doi.org/10.1073/pnas.1704552114

Guenet, B., Lenhart, K., Leloup, J., Giusti-Miller, S., Pouteau, V., Mora, P., … Abbadie, L. (2012). The impact of long-term CO_2 enrichment and moisture levels on soil microbial community structure and enzyme activities. *Geoderma*, *170*, 331–336. https://doi.org/10.1016/j.geoderma.2011.12.002

Hungate, B. A., Chapin, F. S. III, Zhong, H., Holland, E. A., & Field, C. B. (1997). Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. *Oecologia*, *109*, 149–153. https://doi.org/10.1007/s004420050069

Hungate, B. A., Dijkstra, P., Johnson, D. W., Hinkle, C. R., & Drake, B. G. (1999). Elevated CO_2 increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. *Global Change Biology*, *5*, 781–789. https://doi.org/10.1046/j.1365-2486.1999.00275.x

Hungate, B. A., Lund, C. P., Pearson, H. L., & Chapin, F. S. III (1997). Elevated CO_2 and nutrient addition alter soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland. *Biogeochemistry*, *37*, 89–109. https://doi.org/10.1023/A:1005747123463

Inselsbacher, E., Wanek, W., Strauss, J., Zechmeister-Boltenstern, S., & Müller, C. (2013). A novel ^{15}N tracer model reveals: Plant nitrate uptake governs nitrogen transformation rates in agricultural soils. *Soil Biology & Biochemistry*, *57*, 301–310. https://doi.org/10.1016/j.soilbio.2012.10.010

IPCC (2007) Climate Change - The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC, Cambridge, UK: Cambridge University Press.

IPCC (2013) Climate Change 2013: The Physical Science Basis. Working Group I contribution to the IPCC fifth assessment report (AR5), Cambridge, UK: Cambridge University Press.

Jäger, H. J., Schmidt, S. W., Kammann, C., Grünhage, L., Müller, C., & Hanewald, K. (2003). The University of Giessen Free-Air Carbon Dioxide Enrichment study: Description of the experimental site and of a new enrichment system. *Journal of Applied Botany*, *77*, 117–127.

Jansen-Willems, A. B., Lanigan, G. J., Clough, T. J., Andresen, L. C., & Müller, C. (2016). Long-term elevation of temperature affects organic N turnover and associated N_2O emissions in a permanent grassland soil. *Soil*, *2*, 601–614. https://doi.org/10.5194/soil-2-601-2016

Kammann, C., Müller, C., Grünhage, L., & Jäger, H.-J. (2008). Elevated CO_2 stimulates N_2O emissions in permanent grassland. *Soil Biology & Biochemistry*, *40*, 2194–2205. https://doi.org/10.1016/j.soilbio.2008.04.012

Keidel, L., Kammann, C., Grünhage, L., Moser, G., & Müller, C. (2015). Long term CO_2 enrichment in a temperate grassland increases soil respiration during late autumn and winter. *Biogeosciences*, *12*, 1257–1269. https://doi.org/10.5194/bg-12-1257-2015

Keiluweit, M., Bougoure, J. J., Nico, P. S., Pett-Ridge, J., Weber, P. K., & Kleber, M. (2015). Mineral protection of soil carbon counteracted by root exudates. *Nature Climate Change*, *5*, 588–595. https://doi.org/10.1038/nclimate2580

Kettunen, R., Saarnio, S., Martikainen, P. J., & Silvola, J. (2006). Increase of N_2O fluxes in agricultural peat and sandy soil under elevated CO_2 concentration: Concomitant changes in soil moisture, groundwater table and biomass production of *Phleum pratense*. *Nutrient Cycling in Agroecosystems*, *74*, 175–189. https://doi.org/10.1007/s10705-005-6239-3

Kettunen, R., Saarnio, S., Martikainen, P. J., & Silvola, J. (2007). Can a mixed stand of N_2-fixing and non-fixing plant restrict N_2O emissions with increasing CO_2 concentrations? *Soil Biology & Biochemistry*, *39*, 2538–2546. https://doi.org/10.1016/j.soilbio.2007.04.023

Knohl, A., & Veldkamp, E. (2011). Global change: Indirect feedbacks to rising CO_2. *Nature*, *475*, 177–178. https://doi.org/10.1038/475177a

Larsen, K. S., Andresen, L. C., Beier, C., Jonasson, S., Albert, K. R., Ambus, P. E. R., … Ibrom, A. (2011). Reduced N cycling in response to elevated CO_2, warming, and drought in a Danish heathland: Synthesizing results of the CLIMAITE project after two years of treatments. *Global Change Biology*, *17*, 1884–1899. https://doi.org/10.1111/j.1365-2486.2010.02351.x

Laughlin, R. J., Stevens, R. J., & Zhuo, S. (1997). Determining nitrogen-15 in ammonium by producing nitrous oxide. *Soil Science Society of America Journal*, *61*, 462–465. https://doi.org/10.2136/sssaj1997.03615995006100020013x

Leakey, A. D. B., Ainsworth, E. A., Bernacchi, C. J., Rogers, A., Long, S. P., & Ort, D. R. (2009). Elevated CO_2 effects on plant carbon, nitrogen, and water relations: Six important lessons from FACE. *Journal of Experimental Botany*, *60*, 2859–2876. https://doi.org/10.1093/jxb/erp096

Loftfield, N., Flessa, H., Augustin, J., & Beese, F. (1997). Automated gas chromatographic system for rapid analysis of the atmospheric trace gases methane, carbon dioxide, and nitrous oxide. *Journal of Environmental Quality*, *26*, 560–564. https://doi.org/10.2134/jeq1997.00472425002600020030x

Luo, Y., Hui, D., & Zhang, D. (2006). Elevated CO_2 stimulates net accumulation of carbon and nitrogen in land ecosystems: A meta-analysis. *Ecology*, *87*, 53–63. https://doi.org/10.1890/04-1724

Luo, Y., Su, B. O., Currie, W. S., Dukes, J. S., Finzi, A., Hartwig, U., … Pataki, D. E. (2004). Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *BioScience*, *54*, 731–739. https://doi.org/10.1641/0006-3568(2004)054[0731:PNLOER]2.0.CO;2

Morgan, J. A., Pataki, D. E., Körner, C. H., Clark, H., Grosso, S. J., & Grünzweig, J. M. (2004). Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO_2. *Oecologia*, *140*, 11–25. https://doi.org/10.1007/s00442-004-1550-2

Mosier, A. R., Delgado, J. A., & Keller, M. (1998). Methane and nitrous oxide fluxes in an acid oxisol in western Puerto Rico: Effects of tillage, liming and fertilization. *Soil Biology & Biochemistry*, *30*, 2087–2098. https://doi.org/10.1016/S0038-0717(98)00085-6

Müller, C., Laughlin, R. J., Spott, O., & Rütting, T. (2014). Quantification of N_2O emission pathways via a ^{15}N tracing model. *Soil Biology & Biochemistry*, *72*, 44–54. https://doi.org/10.1016/j.soilbio.2014.01.013

Müller, C., Martin, M., Stevens, R. J., Laughlin, R. J., Kammann, C., Ottow, J. C. G., & Jäger, H.-J. (2002). Processes leading to N_2O emissions in grassland soil during freezing and thawing. *Soil Biology & Biochemistry*, *34*, 1325–1331. https://doi.org/10.1016/S0038-0717(02)00076-7

Müller, C., Rütting, T., Abbasi, M. K., Laughlin, R. J., Kammann, C., Clough, T. J., … Stevens, R. J. (2009). Effect of elevated CO_2 on soil N dynamics in a temperate grassland soil. *Soil Biology & Biochemistry*, *41*, 1996–2001. https://doi.org/10.1016/j.soilbio.2009.07.003

Müller, C., Stevens, R. J., & Laughlin, R. J. (2006). Sources of nitrite in a permanent grassland soil. *European Journal of Soil Science*, *57*, 337–343. https://doi.org/10.1111/j.1365-2389.2005.00742.x

Müller, C., Stevens, R. J., Laughlin, R. J., & Jäger, H.-J. (2004). Microbial processes and the site of N_2O production in a temperate grassland soil. *Soil Biology & Biochemistry*, *36*, 453–461. https://doi.org/10.1016/j.soilbio.2003.08.027

Myhre, G., Shindell, D., Bréon, F. M., Collins, W., Fuglestvedt, J., Huang, J., … Nakajima, T. (2013) Anthropogenic and natural radiative forcing. In Q. D. Stocker, G.-K. Plattner, M. Tignor, S. K. Allen, J.

Boschung, A. Nauels, Y. Xia, V. Bex & P. M. Midgley (Eds.), *The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 659–740). Cambridge, UK: Cambridge University Press.

Niklaus, P. A., Alphei, J., Ebersberger, D., Kampichler, C., Kandeler, E., & Tscherko, D. (2003). Six years of in situ CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland. *Global Change Biology*, *9*, 585–600. https://doi.org/10.1046/j.1365-2486.2003.00614.x

Obermeier, W. A., Lehnert, L. W., Kammann, C. I., Müller, C., Grünhage, L., Luterbacher, J., … Bendix, J. (2017). Reduced CO_2 fertilization effect in temperate C3 grasslands under more extreme weather conditions. *Nature Climate Change*, *7*, 137–141. https://doi.org/10.1038/nclimate3191

Pepper, D. A., Del Grosso, S. J., McMurtrie, R. E., & Parton, W. J. (2005). Simulated carbon sink response of shortgrass steppe, tallgrass prairie and forest ecosystems to rising [CO_2], temperature and nitrogen input. *Global Biogeochemical Cycles*, *19*, GB1004.

Phillips, R. P., Bernhardt, E. S., & Schlesinger, W. H. (2009). Elevated CO_2 increases root exudation from loblolly pine (Pinus taeda) seedlings as an N-mediated response. *Tree Physiology*, *29*, 1513–1523. https://doi.org/10.1093/treephys/tpp083

Phillips, R. P., Finzi, A. C., & Bernhardt, E. S. (2011). Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecology Letters*, *14*, 187–194. https://doi.org/10.1111/j.1461-0248.2010.01570.x

Rachmilevitch, S., Cousins, A. B., & Bloom, A. J. (2004). Nitrate assimilation in plant shoots depends on photorespiration. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 11506–11510. https://doi.org/10.1073/pnas.0404388101

Regan, K., Kammann, C., Hartung, K., Lenhart, K., Müller, C., Philippot, L., … Marhan, S. (2011). Can differences in microbial abundances help explain enhanced N_2O emissions in a permanent grassland under elevated atmospheric CO_2? *Global Change Biology*, *17*, 3176–3186. https://doi.org/10.1111/j.1365-2486.2011.02470.x

Richter, M., Hartwig, U. A., Frossard, E., Nösberger, J., & Cadisch, G. (2003). Gross fluxes of nitrogen in grassland exposed to elevated atmospheric pCO_2 for seven years. *Soil Biology & Biochemistry*, *35*, 1325–1335. https://doi.org/10.1016/S0038-0717(03)00212-8

Rütting, T., Clough, T. J., Müller, C., Lieffering, M., & Newton, P. C. D. (2010). Ten years of elevated atmospheric carbon dioxide alters soil nitrogen transformations in a sheep-grazed pasture. *Global Change Biology*, *16*, 2530–2542. https://doi.org/10.1111/j.1365-2486.2009.02089.x

Rütting, T., & Müller, C. (2007). ^{15}N tracing models with a Monte Carlo optimization procedure provide new insights on gross N transformations in soils. *Soil Biology & Biochemistry*, *39*, 2351–2361. https://doi.org/10.1016/j.soilbio.2007.04.006

Singh, B. K., Bardgett, R. D., Smith, P., & Reay, D. S. (2010). Microorganisms and climate change: Terrestrial feedbacks and mitigation options. *Nature Reviews Microbiology*, *8*, 779–790. https://doi.org/10.1038/nrmicro2439

Stevens, R. J., & Laughlin, R. J. (1994). Determining nitrogen-15 nitrite or nitrate by producing nitrous oxide. *Soil Science Society of America Journal*, *58*, 1108–1116. https://doi.org/10.2136/sssaj1994.03615995005800040015x

Stevens, R. J., Laughlin, R. J., Atkins, G. J., & Prosser, S. J. (1993). Automated determination of nitrogen-15-labelled dinitrogen and nitrous oxide by mass spectrometry. *Soil Science Society of America Journal*, *57*, 981–988. https://doi.org/10.2136/sssaj1993.03615995005700040017x

Templer, P. H., & Reinmann, A. B. (2011). Multi-factor global change experiments: What have we learned about terrestrial carbon storage and exchange? *New Phytologist*, *192*, 797–800. https://doi.org/10.1111/j.1469-8137.2011.03959.x

Tricker, P. J., Pecchiari, M., Bunn, S. M., Vaccari, F. P., Peressotti, A., Miglietta, F., & Taylor, G. (2009). Water use of a bioenergy plantation increases in a future high CO_2 world. *Biomass and Bioenergy*, *33*, 200–208. https://doi.org/10.1016/j.biombioe.2008.05.009

van Groenigen, K.-J., de Graaff, M.-A., Six, J., Harris, D., Kuikman, P., & vanKessel, C. (2006). The impact of elevated atmospheric [CO_2] on soil C and N dyanamics: A meta-analysis. In J. Nösberger, S. P. Long, R. J. Norby, M. Stitt, G. R. Hendrey & H. Blum (Eds.), *Managed ecosystems and CO_2 case studies, processes, and perspectives* (pp. 373–391). Berlin-Heidelberg: Springer.

van Groenigen, K. J., Osenberg, C. W., & Hungate, B. A. (2011). Increased soil emissions of potent greenhouse gases under increased atmospheric CO_2. *Nature*, *475*, 214–216. https://doi.org/10.1038/nature10176

Wrage, N., Velthof, G. L., Van Beusichem, M. L., & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology & Biochemistry*, *33*, 1723–1732. https://doi.org/10.1016/S0038-0717(01)00096-7

Wu, K., Chen, D., Tu, C., Qiu, Y., Burkey, K. O., Reberg-Horton, S. C., … Hu, S. (2017). CO_2-induced alterations in plant nitrate utilization and root exudation stimulate N_2O emissions. *Soil Biology and Biochemistry*, *106*, 9–17. https://doi.org/10.1016/j.soilbio.2016.11.018

Yoccoz, N. G. (1991). Use, overuse, and misuse of significance tests in evolutionary biology and ecology. *Bulletin of the Ecological Society of America*, *72*, 106–111.

Zak, D. R., Pregitzer, K. S., King, J. S., & Holmes, W. E. (2000). Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: A review and hypothesis. *New Phytologist*, *147*, 201–222. https://doi.org/10.1046/j.1469-8137.2000.00687.x

Zhang, J., Müller, C., & Cai, Z. (2015). Heterotrophic nitrification of organic N and its contribution to nitrous oxide emissions in soils. *Soil Biology & Biochemistry*, *84*, 199–209. https://doi.org/10.1016/j.soilbio.2015.02.028

Zhong, L., Bowatte, S., Newton, P. C. D., Hoogendoorn, C. J., & Luo, D. (2018). An increased ratio of fungi to bacteria indicates greater potential for N_2O production in a grazed grassland exposed to elevated CO_2. *Agriculture, Ecosystems and Environment*, *254*, 111–116. https://doi.org/10.1016/j.agee.2017.11.027

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References

Accoe, F., Boeckx, P., van Cleemput, O., Hofman, G., Hui, X., Bin, H., Chen, G., 2002. Characterization of soil organic matter fractions from grassland and cultivated soils via C content and $\delta^{13}C$ singature. Rapid Communications in Mass Spectrometry 16, 2157-2164.

Adair, E.C., Reich, P.B., Trost, J.J., Hobbie, S.E., 2011. Elevated CO_2 stimulates grassland soil respiration by increasing carbon inputs rather than by enhancing soil moisture. Global Change Biology 17, 3546-3563.

Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytologist 165, 351-372.

Allard, V., Newton, P.C.D., Lieffering, M., Soussana, J.-F., Carran, R.A., Matthew, C., 2005. Increased Quantity and Quality of Coarse Soil Organic Matter Fraction at Elevated CO_2 in a Grazed Grassland are a Consequence of Enhanced Root Growth Rate and Turnover. Plant and Soil 276, 49-60.

Allard, V., Robin, C., Newton, P.C.D., Lieffering, M., Soussana, J.-F., 2006. Short and long-term effects of elevated CO_2 on Lolium perenne rhizodeposition and its consequences on soil organic matter turnover and plant N yield. Soil Biology & Biochemistry 38, 1178-1187.

Amundson, R., 2001. The carbon budget in soils. Annual Review of Earth and Planetary Sciences 29, 535-562.

Andresen, L.C., Yuan, N., Seibert, R., Moser, G., Kammann, C.I., Luterbacher, J., Erbs, M., Müller, C., 2018. Biomass responses in a temperate European grassland through 17 years of elevated CO2. Global Change Biology 24, 3875-3885.

Angers, D.A., Arrouays, D., Saby, N.P.A., Walter, C., 2011. Estimating and mapping the carbon saturation deficit of French agricultural topsoils. Soil Use and Management 27, 448-452.

Arndal, M.F., Tolver, A., Larsen, K.S., Beier, C., Schmidt, I.K., 2018. Fine Root Growth and Vertical Distribution in Response to Elevated CO2, Warming and Drought in a Mixed Heathland–Grassland. Ecosystems 21, 15-30.

Arnone, J.A., III, Zaller, J.G., Spehn, E.M., Niklaus, P.A., Wells, C.E., Körner, C., 2000. Dynamics of root systems in native grasslands: effect of elevated atmospheric CO_2. New Phytologist 147, 73-85.

Baggs, E.M., Richter, M., Cadisch, G., Hartwig, U.A., 2003. Denitrification in grass swards is increased under elevated atmospheric CO_2. Soil Biology & Biochemistry 35, 729-732.

Barnard, R., Leadley, P.W., Hungate, B.A., 2005. Global change, nitrification, and denitrification: a review. Global Biogeochemical Cycles 19, GB1007, doi:1010.1029/2004GB002282.

Bernhardt, E.S., Barber, J.J., Pippen, J.S., Taneva, L., Andrews, J.A., Schlesinger, W.H., 2006. Long-term Effects of Free Air CO2 Enrichment (FACE) on Soil Respiration. Biogeochemistry 77, 91-116.

Bitzer, J., Schröder, P.J.H., Zhou, X., Yiqi, L., 2010. Soil Respiration and the Environment. Elsevier Science & Technology, San Diego, United States.

Bloom, A.J., Burger, M., A. Kimball, B., J. Pinter, J.P., 2014. Nitrate assimilation is inhibited by elevated CO_2 in field-grown wheat. Nature Climate Change 4, 477.

Bollmann, A., Conrad, R., 1998. Influence of O_2 availability on NO and N_2O release by nitrification and denitrification in soils. Global Change Biology 4, 387-396.

Bouwman, A.F., Boumans, L.J.M., Batjes, N.H., 2002a. Emissions of N_2O and NO from fertilized fields: summary of available measurement data. Global Biogeochemical Cycles 16, 2001GB001811.

Bouwman, A.F., Boumans, L.J.M., Batjes, N.H., 2002b. Modeling global annual N_2O and NO emissions from fertilized fields. Global Biogeochemical Cycles 16, 2001GB001812.

Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., Zechmeister-Boltenstern, S., 2013. Nitrous oxide emissions from soils, how well do we understand the processes and their controls. Philosophical Transactions of the Royal Society London B368, 16-21.

Cambardella, C.A., Elliott, E.T., 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal 56, 777–783.

Cantarel, A.A.M., Bloor, J.M.G., Pommier, T., Guillaumaud, N., Moirot, C., Soussana, J.-F., Poly, F., 2012. Four years of experimental climate change modifies the microbial drivers of N2O fluxes in an upland grassland ecosystem. Global Change Biology 18, 2520-2531.

Cardon, Z.G., 1996. Influence of rhizodeposition under elevated CO_2 on plant nutrition and soil organic matter. Plant and Soil 187, 277-288.

Cardon, Z.G., Hungate, B.A., Cambardella, C.A., Chapin, F.S., III, Field, C.B., Holland, E.A., Mooney, H.A., 2001. Contrasting effects of elevated CO_2 on old and new soil carbon pools. Soil Biology & Biochemistry 33, 365-373.

Casella, E., Soussana, J.F., 1997. Long-term effects of CO2 enrichment and temperature increase on the carbon balance of a temperate grass sward. Journal of Experimental Botany 48, 1309-1321.

Castellano,M., Poffenbarger, H.,Cambardella,C., Liebman,M.,Mallarino,A., Olk, D.,.Six, J., 2017. Evaluation of carbon saturation across gradients of cropping systems diversity and soil depth. EGU General Assembly Conference Abstracts.19, p. 10357.

Chapin, F.S., III, Matson, P.A., Mooney, H.A., 2002. Principles of terrestrial ecosystem ecology. Springer, New York.

Chen, H., Mothapo, N.V., Shi, W., 2014. The significant contribution of fungi to soil N_2O production across diverse ecosystems. Applied Soil Ecology 73, 70-77.

Chen, S., Martin, M.P., Saby, N.P.A., Walter, C., Angers, D.A., Arrouays, D., 2018. Fine resolution map of top- and subsoil carbon sequestration potential in France. Science of the Total Environment 630, 389-400.

Cheng, L., Booker, F.L., Tu, C., Burkey, K.O., Zhou, L., Shew, H.D., Rufty, T.W., Jr, Hu, S., 2012. Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. Science 337, 1084-1087.

Christensen, B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. European Journal of Soil Science 52, 345-353.

Coskun, D., Britto, D.T., Kronzucker, H.J., 2016. Nutrient constraints on terrestrial carbon fixation: The role of nitrogen. Journal of Plant Physiology 203, 95-109.

Dawes, M.A., Hagedorn, F., Handa, I.T., Streit, K., Ekblad, A., Rixen, C., Körner, C., Hättenschwiler, S., 2013. An alpine treeline in a carbon dioxide-rich world: synthesis of a nine-year free-air carbon dioxide enrichment study. Oecologia 171, 623-637.

De Graaff, M.-A., Six, J., Van Kessel, C., 2007. Elevated CO_2 increases nitrogen rhizodeposition and microbial immobilization of root-derived nitrogen. New Phytologist 173, 778-786.

De Graaff, M.-A., Van Groenigen, K.-J., Six, J., Hungate, B., Van Kessel, C., 2006. Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. Global Change Biology 12, 2077-2091.

Díaz, S., 1995. Effects of elevated [CO2] at the community level mediated by root symbionts. Plant and Soil 187, 309-320.

Dijkstra, F.A., Pendall, E., Mosier, A.R., King, J.Y., Milchunas, D.G., Morgan, J.A., 2008. Long-term enhancement of N availability and plant growth under elevated CO_2 in a semi-arid grassland. Functional Ecology 22, 975-982.

Drake, B.G., Gonzàles-Meler, M.A., Long, S.P., 1997. More efficient plants: a consequence of rising atmospheric CO_2? Annual Reviews of Plant Physiology and Plant Molecular Biology 48, 609-639.

Dukes, J.S., Chiariello, N.R., Cleland, E.E., Moore, L.A., Shaw, M.R., Thayer, S.S., Tobeck, T., Mooney, H.A., Field, C.B., 2005. Response of grassland produciton to single and multiple global environmental changes. PLOS Biology 3, 1829-1837.

Edwards, G.R., Clark, C., Newton, P.C.D., 2001. The effects of elevated CO_2 on seed production and seedling recruitment in a sheep-grazed pasture. Oecologia 127, 383-394.

Ellsworth, D.S., Reich, P.B., Naumburg, E.S., Koch, G.W., Kubiske, M.E., Smith, S.D., 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated CO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. Global Change Biology 10, 2121-2138.

Eviner, V.T., Chapin, F.S., III, 2002. The influence of plant species, fertilization and elevated CO_2 on soil aggregate stability. Plant and Soil 246, 211-219.

Feng, Z., Rütting, T., Pleijel, H., Wallin, G., Reich, P.B., Kammann, C.I., Newton, P.C.D., Kobayashi, K., Luo, Y., Uddling, J., 2015. Constraints to nitrogen acquisition of terrestrial plants under elevated CO_2. Global Change Biology 21, 3152-3168.

Finzi, A.C., Schlesinger, W.H., 2003. Soil–Nitrogen Cycling in a Pine Forest Exposed to 5 Years of Elevated Carbon Dioxide. Ecosystems 6, 444-456.

Fitter, A.H., Graves, J.D., Wolfenden, J., Self, G.K., Brown, T.K., Bogie, D., Mansfield, T.A., 1997. Root production and turnover and carbon budgets of two contrasting grasslands under ambient and elevated atmospheric carbon dioxide concentrations. New Phytologist 137, 247-255.

Friedlingstein, P., Fung, I., Holland, E., John, J., Brasseur, G., Erickson, D., Schimel, D., 1995. On the contribution of CO_2 fertilization to the missing biospheric sink. Global Biogeochemical Cycles 9, 541-556.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vörösmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153-226.

Galloway, J.N., Townsend, A.R., erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. Science 320, 889-893.

Gifford, R.M., Barrett, D.J., Lutze, J.L., 2000. The effects of elevated CO_2 on the C:N and C:P mass ratios of plant tissues. Plant and Soil 224, 1-14.

Gill, R.A., Anderson, L.J., Polley, H.W., Johnson, H.B., Jackson, R.B., 2006. Potential nitrogen constraints on soil carbon sequestration under low and elevated atmospheric CO_2. Ecology 87, 41-52.

Gill, R.A., Polley, H.W., Johnson, H.B., Anderson, L.J., Maherall, H., Jackson, R.B., 2002. Nonlinear grassland response to past and future atmospheric CO_2. Nature 417, 279-282.

Grace, P.R., Ladd, J.N., Robertson, G.P., Gage, S.H., 2006. SOCRATES—A simple model for predicting long-term changes in soil organic carbon in terrestrial ecosystems. Soil Biology and Biochemistry 38, 1172-1176.

Groenigen, K.J., Osenberg, C.W., Terrer, C., Carrillo, Y., Dijkstra, F.A., Heath, J., Nie, M., Pendall, E., Phillips, R.P., Hungate, B.A., 2017. Faster turnover of new soil carbon inputs under increased atmospheric CO2. Global Change Biology 23, 4420-4429.

Hassink, J., 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant and Soil 191, 77-87.

Hendrey, G.R., Ellsworth, D.S., Lewin, K.F., Nagy, J., 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. Global Change Biology 5, 293-309.

Higgins, P.A.T., Jackson, R.B., Des Rosiers, J.M., Field, C.B., 2002. Root production and demography in a california annual grassland under elevated atmospheric carbon dioxide. Global Change Biology 8, 841-850.

Hofmockel, K.S., Gallet-Budynek, A., McCarthy, H.R., Currie, W.S., Jackson, R.B., Finzi, A., 2011. Sources of increased N uptake in forest trees growing under elevated CO_2: results of a large-scale ^{15}N study. Global Change Biology 17, 3338-3350.

Hovenden, M.J., Newton, P.C.D., Wills, K.E., 2014. Seasonal not annual rainfall determines grassland biomass response to carbon dioxide. Nature 511, 583.

Hungate, B.A., Dukes, J.S., Shaw, M.R., Luo, Y., Field, C.B., 2003. Nitrogen and climate change. Science 302, 1512-1513.

Hungate, B.A., Groenigen, J.W., Six, J., Jastrows, J.D., luo, Y., Graaff, A.M., Kessel, C., Osenbergq, 2009. Assessing the effect of elevated carbon dioxide on soil carbon: a comparison of four meta-anaylses. Global Change Biology 15, 2020-2034.

Hungate, B.A., Holland, E.A., Jackson, R.B., Stuart Chapin, F., III, Mooney, H.A., Field, C.B., 1997. The fate of carbon in grasslands under carbon dioxide enrichment. Nature 388, 576-579.

Hungate, B.A., Jackson, R.B., Field, C.B., Chapin, F.S., 1996. Detecting changes in soil carbon in CO_2 enrichment experiments. Plant and Soil 187, 135-145.

Hungate, B.A., Stiling, P.D., Dijkstra, P., Johnson, D.W., Ketterer, M.E., Hymus, G.J., Hinkle, C.R., Drake, B.G., 2004. CO_2 elicits long-term decline in nitrogen fixation. Science 304, 1291.

IPCC, 2007. Climate change 2007: The physical science basis - Summary for policymakers. IPCC Secretariat, Geneva, Switzerland, p. 21.

IPCC, 2013. Working Group I Contribution to the IPCC Fifth Assessment Report Climate Change 2013: The Physical Science Basis Summary for Policymakers. Intergovernmental Panel for Climate Change, p. 36.

Iversen, C.M., Keller, J.K., Garten, C.T., Norby, R.J., 2012. Soil carbon and nitrogen cycling and storage throughout the soil profile in a sweetgum plantation after 11 years of CO2-enrichment. Global Change Biology 18, 1684-1697.

Jackson, R.B., Cook, W.C., Pippen, J., Palmer, S.M., 2009. Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-termperate forest. Ecology 90, 3352-3366.

Jäger, H.-J., Schmidt, S.W., Kammann, C., Grünhage, L., Müller, C., Hanewald, K., 2003. The University of Giessen Free-Air Carbon Dioxide Enrichment Study: Description of the experimental site and of a new enrichment system. Journal of Applied Botany 77, 117-127.

Janssens, I.A., Ceulemans, R., 2000. The response of soil CO_2 efflux under trees grown in elevated atmospheric CO_2: A literature review. Phyton-Annales Rei Botanicae 40, 97-101.

Jastrow, J.D., Boutton, T.W., Miller, R.M., 1996. Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. Soil Science Society of America Journal 60, 801-807.

Jastrow, J.D., Miller, R.M., Owensby, C.E., 2000. Long-term effects of elevated atmospheric CO_2 on below-ground biomass and transformation to soil organic matter in grassland. Plant and Soil 224, 85-97.

Jenkinson, D.S., Rayner, J.H., 1977. The turnover of soil organic matter in some of the Rothamsted classical experiments. Soil Science 123, 298-305.

Jobbagy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications 10, 423-436.

Jones, D.L., Nguyen, C., Finlay, R.D., 2009. Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant and Soil 321, 5-33.

Jones, M.B., Donnelly, A., 2004. Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO_2. New Phytologist 164, 423-439.

Kalbitz, K., Solinger, S., Park, J.-H., Michalzik, B., Matzner, E., 2000. Controls of the dynamics of dissolved organic mater in soils: a review. Soil Science 165, 277-304.

Kammann, C., Grünhage, L., Grüters, U., Janze, S., Jäger, H.-J., 2005. Response of aboveground grassland biomass and soil moisture to moderate long-term CO_2 enrichment. Basic and Applied Ecology 6, 351-365.

Kammann, C., Müller, C., Grünhage, L., Jäger, H.-J., 2008. Elevated CO_2 stimulates N_2O emissions in permanent grassland. Soil Biology & Biochemistry 40, 2194-2205.

Kandeler, E., Tscherko, D., Bardgett, R.D., Hobbs, P.J., Kampichler, C., Jones, T.H., 1998. The response of soil microorganisms and roots to elevated CO_2 and temperature in a terrestrial model ecosystem. Plant and Soil 202, 251-262.

Keeling, C.D., Chin, J.F.S., Whorf, T.P., 1996. Increased activity of northern vegetation inferred from atmospheric CO_2 measurements. Nature 382, 146.

Kleber, M., Nico, P.S., Plante, A., Filley, T., Kramer, M., Swanston, C., Sollins, P., 2011. Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. Global Change Biology 17, 1097-1107.

Klironomos, J.N., Allen, M.F., Rillig, M.C., Piotrowski, J., Makvandi-Nejad, S., Wolfe, B.E., Powell, J.R., 2005. Abrupt rise in atmospheric CO_2 overestimates communitiy response in a model-plant soil system. Nature 433, 621-624.

Kool, D.M., Chung, H., Tate, K.R., Ross, D.J., Newton, P.C.D., Six, J., 2007. Hierarchical saturation of soil carbon pools near a natural CO_2 spring. Global Change Biology 13, 1282-1293.

Körner, C., 2000. Biosphere responses to CO_2 enrichment. Ecological Applications 10, 1590-1619.

Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept & review. Soil Biology and Biochemistry 83, 184-199.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal of Plant Nutrition and Soil Science 163, 421-431.

Lal, R., Negassa, W., Lorenz, K., 2015. Carbon sequestration in soil. Current Opinion in Environmental Sustainability 15, 79-86.

Langley, J.A., McKinley, D.C., Wolf, A.A., Hungate, B.A., Drake, B.G., Megonigal, J.P., 2009. Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO_2. Soil Biology & Biochemistry 41, 54-60.

Laughlin, R.J., Stevens, R.J., 2002. Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. Soil Science Society of America Journal 66, 1540-1548.

Leadley, P.W., Drake, B.G., 1993. Open top chambers for exposing plant canopies to elevated CO_2 concentration and for measuring net gas exchange. Vegetatio 104, 3-15.

Leakey, A.D.B., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P., Ort, D.R., 2009. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany 60, 2859-2876.

Lee, T.D., Barrott, S.H., Reich, P.B., 2011. Photosynthetic responses of 13 grassland species across 11 years of free-air CO_2 enrichment is modest, consistent and independent of N supply. Global Change Biology 17, 2893-2904.

Ley, M., Lehmann, M.F., Niklaus, P.A., Luster, J., 2018. Alteration of nitrous oxide emissions from floodplain soils by aggregate size, litter accumulation and plant–soil interactions. Biogeosciences 15, 7043-7057.

Liao, J.D., Boutton, T.W., Jastrow, J.D., 2006. Organic matter turnover in soil physical fractions following woody plant invasion of grassland: evidence from natural ^{13}C and ^{15}N. Soil Biology & Biochemistry 38, 3197-3210.

Liu, S., Ji, C., Wang, C., Chen, J., Jin, Y., Zou, Z., Li, S., Niu, S., Zou, J., 2018. Climatic role of terrestrial ecosystem under elevated CO2: a bottom-up greenhouse gases budget. Ecology Letters 21, 1108-1118.

Lorenz, K., Lal, R., 2018. Carbon Sequestration in Grassland Soils, Carbon Sequestration in Agricultural Ecosystems. Springer International Publishing, Cham, pp. 175-209.

Luo, Y., 2001. Transient ecosystem responses to free-air CO_2 enrichment (FACE): experimental evidence and methods of analysis. New Phytologist 152, 3-8.

Luo, Y., Currie, W.S., Dukes, J.S., Finzi, A.C., Hartwig, U., Hungate, B.A., McMurtrie, R.E., Oren, R., Parton, W.J., Pataki, D.E., Shaw, M.R., Zak, D.R., Field, C.B., 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. BioScience 54, 731-739.

Luo, Y., Field, C.B., Mooney, H.A., 1994. Predicting responses of photosynthesis and root fraction to elevated [CO_2]a: interactions among carbon, nitrogen, and growth*. Plant, Cell & Environment 17, 1195-1204.

Luo, Y., Hui, D., Zhang, D., 2006. Elevated CO_2 stimulates net accumulation of carbon and nitrogen in land ecosystems: a meta-analysis. Ecology 87, 53-63.

Luo, Y., Wu, L., Andrews, J.A., White, L., Matamala, R., Schäfer, K.V.R., Schlesinger, W.H., 2001. Elevated CO_2 differentiates ecosystem carbon processes: deconvolution analysis of Duke forest data. Ecological Monographs 71, 357-376.

Lützow, M.v., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. European Journal of Soil Science 57, 426-445.

Miglietta, F., Peressotti, A., Vaccari, F.P., Zaldei, A., de Angelis, P., Scarascia-Mugnozza, G., 2001. Free-air CO_2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. New Phytologist 150, 465-476.

Milchunas, D.G., Lauenroth, W.K., 2001. Belowground primary production by carbon isotope decay and longterm root biomass dynamics. Ecosystems 4, 139-150.

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.-C., Vågen, T.-G., van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. Geoderma 292, 59-86.

Monastersky, R., 2013. Global carbon dioxide levels near worrisome milestone. Nature 497, 13-14.

Moss, R.H., Edmonds, J.A., Hibbard, K.A., Manning, M.R., Rose, S.K., Van Vuuren, D.P., Carter, T.R., Emori, S., Kainuma, M., Kram, T., Meehl, G.A., Mitchell, J.F.B., Nakicenovic, N., Riahi, K., Smith, S.J., Stouffer, R.J., Thomson, A.M., Weyant, J.P., Wilbanks, T.J., 2010. The next generation of scenarios for climate change research and assessment. Nature 463, 747-756.

Müller, C., Laughlin, R.J., Spott, O., Rütting, T., 2014. Quantification of N_2O emission pathways via a ^{15}N tracing model. Soil Biology & Biochemistry 72, 44-54.

Myhre, G., D. Shindell, F.-M. Bréon, W. Collins, J. Fuglestvedt, J. Huang, D. Koch, J.-F. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura and H. Zhang, 2013: Anthropogenic and Natural Radiative Forcing. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Mystakidis, S., Davin, E.L., Gruber, N., Seneviratne, S.I., 2016. Constraining future terrestrial carbon cycle projections using observation-based water and carbon flux estimates. Global Change Biology 22, 2198-2215.

Newton, P.C.D., Clark, H., Edwards, G.R., Ross, D.J., 2001. Experimental confirmation of ecosystem model predictions comparing transient and equilibrium plant responses to elevated atmospheric CO_2. Ecology Letters 4, 344-347.

Nguyen, C., 2009. Rhizodeposition of Organic C by Plant: Mechanisms and Controls, In: Lichtfouse, E., Navarrete, M., Debaeke, P., Véronique, S., Alberola, C. (Eds.), Sustainable Agriculture. Springer Netherlands, Dordrecht, pp. 97-123.

Niklaus, P.A., Spinnler, D., Körner, C., 1998. Soil moisture dynamics of calcareous grassland under elevated CO_2. Oecologia 117, 201-208.

Nitschelm, J., Lüscher, A., Hartwig, U., Van Kessel, C., 1997. Using stable isotopes to determine soil carbon input differences under ambient and elevated atmospheric CO_2 conditions. Global Change Biology 3, 411-416.

Norby, R.J., O'Neill, E.G., Hood, W.G., Luxmoore, R.J., 1987. Carbon allocation, root exudation and mycorrhizal colonization of Pinus echinata seedlings grown under CO(2) enrichment. Tree Physiology 3, 203-210.

Norby, R.J., Zak, D.R., 2011. Ecological lessons from Free-Air CO2 Enrichment (FACE) experiments. Annual Review of Ecology, Evolution, and Systematics 42, 181-203.

O'Mara, F.P., 2012. The role of grasslands in food security and climate change. Annals of Botany 110, 1263-1270.

Okada, M., Lieffering, M., Nakamura, H., Yoshimoto, M., Kim, H.Y., Kobayashi, K., 2001. Free-air CO_2 enrichment (FACE) using pure CO_2 injection: system description. New Phytologist 150, 251-260.

Paustian, K., Andrén, O., Janzen, H.H., Lal, R., Smith, P., Tian, G., Tiessen, H., van Noordwijk, M., Woomer, P.L., 1997. Agricultural soils as a sink to mitigate CO_2 emissions. Soil Use and Management 13, 230-244.

Pendall, E., Leavitt, S.W., Brookes, T., Kimball, B.A., Pinter, P.J., Jr, Wall, G.W., LaMorte, R.L., Wechsung, G., Wechsung, F., Adamsen, F., Matthias, A.D., Thompson, T.L., 2001. Elevated CO_2 stimulates soil respiration in a FACE wheat field. Basic and Applied Ecology 2, 193-201.

Pendall, E., King, J.Y., 2007. Soil organic matter dynamics in grassland soils under elevated CO_2: insights from long-term incubations and stable isotopes. Soil Biology & Biochemistry 39, 2628-2639.

Pendall, E., Mosier, A.R., Morgan, J.A., 2004. Rhizodeposition stimulated by elevated CO_2 in a semiarid grassland. New Phytologist 162, 447-458.

Phillips, R.P., Meier, I.C., Bernhardt, E.S., Grandy, A.S., Wickings, K., Finzi, A.C., 2012. Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. Ecology Letters 15, 1042-1049.

Poirier, V., Angers, D.A., Whalen, J.K., 2014. Formation of millimetric-scale aggregates and associated retention of 13C–15N-labelled residues are greater in subsoil than topsoil. Soil Biology and Biochemistry 75, 45-53.

Pregitzer, K.S., Burton, A.J., King, J.S., Zak, D.R., 2008. Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3. New Phytologist 180, 153-161.

Pregitzer, K.S., Zak, D.R., Curtis, P.S., Kubiske, M.E., Teeri, J.A., Vogel, C.S., 1995. Atmospheric CO_2, soil nitrogen and turnover of fine roots. New Phytologist 129, 579-585.

Pritchard, S.G., Strand, A.E., McCormack, M.L., Davis, M.A., Oren, R., 2008. Mycorrhizal and rhizomorph dynamics in a loblolly pine forest during 5 years of free-air-CO_2-enrichment. Global Change Biology 14, 1252-1264.

Raich, J.W., Potter, C.S., 1995. Global patterns of carbon dioxide emissions from soils. Global Biogeochemical Cycles 9, 23-36.

Raich, J.W., Schlesinger, W.H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus 44B, 81-99.

Rastetter, E.B., Ågren, G.I., Shaver, G.R., 1997. Responses of N-limited ecosystems to increased CO_2:a balanced-nutrition, coupled-element-cycles model. Ecological Applications 7, 444-460.

Rastetter, E.B., Ryan, M.G., Shaver, G.R., Melillo, J.M., Nadelhoffer, K.J., Hobbie, J.E., Aber, J.D., 1991. A general biogeochemical model describing the response of the C and N cycles in terrestrial ecosystems to changes in CO_2, climate, and N deposition. Tree Physiology 9, 101-126.

Raynaud, D., Barnola, J.M., 1985. An Antarctic ice core reveals atmospheric CO2 variations over the past few centuries. Nature 315, 309.

Regan, K., Kammann, C., Hartung, K., Lenhart, K., Müller, C., Philippot, L., Kandeler, E., Marhan, S., 2011. Can differences in microbial abundances help explain enhanced N_2O emissions in a permanent grassland under elevated atmospheric CO_2? Global Change Biology 17, 3176-3186.

Reich, P.B., Hobbie, S.E., Lee, T., Ellsworth, D.S., West, J.B., Tilman, D., Knops, J.M.H., Naeem, S., Trost, J., 2006a. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. Nature 440, 922-925.

Reich, P.B., Hungate, B.A., Luo, Y., 2006b. Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. Annual Review of Ecology, Evolution, and Systematics 37, 611-636.

Reich, P.B., Tilman, D., Craine, J., Ellsworth, D., Tjoelker, M.G., Knops, J., Wedin, D., Naeem, S., Bahauddin, D., Goth, J., Bengtson, W., Lee, T.D., 2001. Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO_2 and N availability regimes? A field test with 16 grassland species. New Phytologist 150, 435-448.

Reis, C.E.S.D., Dick, D.P., Caldas, J.D.S., Bayer, C., 2014. Carbon sequestration in clay and silt fractions of Brazilian soils under conventional and no-tillage systems. Sci. Agric. 71, 292–301.

Rice, C.W., Garcia, F.O., Hampton, C.O., Owensby, C.E., 1994. Soil microbial response in tallgrass prairie to elevated CO_2. Plant and Soil 165, 67-74.

Rillig, M.C., Wright, S.F., Allen, M.F., Field, C.B., 1999. Rise in carbon dioxide changes soil structure. Nature 400, 628.

Rogers, H.H., Runion, G.B., Krupa, S.V., 1994. Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. Environmental Pollution 83, 155-189.

Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. Plant and Soil 338, 143-158.

Rütting, T., Andresen, L.C., 2015. Nitrogen cycle responses to elevated CO_2 depend on ecosystem nutrient status. Nutrient Cycling in Agroecosystems 101, 285-294.

Rütting, T., Clough, T.J., Müller, C., Lieffering, M., Newton, P.C.D., 2010. Ten years of elevated atmospheric carbon dioxide alters soil nitrogen transformations in a sheep-grazed pasture. Global Change Biology 16, 2530-2542.

Schlesinger, W.H., 1997. Biogeochemistry : an analysis of global change. Second edition. San Diego, Calif. : Academic Press, [1997] ©1997.

Schortemeyer, M., Dijkstra, P., Johnson, D.W., Drake, B.G., 2000. Effects of elevated atmospheric CO_2 concentration on C and N pools and rhizosphere processes in a Florida scrub oak community. Global Change Biology 6, 383-391.

Schrumpf, M., Kaiser, K., Guggenberger, G., Persson, T., Kögel-Knabner, I., Schulze, E.D., 2013. Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. Biogeosciences 10, 1675-1691.

Selsted, M.B., van der Linden, L., Ibrom, A., Michelsen, A., Larsen, K.S., Pedersen, J.K., Mikkelsen, T.N., Pilegaard, K., Beier, C., Ambus, P., 2012. Soil respiration is stimulated by elevated CO_2 and reduced by summer drought: three years of measurements in a multifactor ecosystem manipulation experiment in a temperate heathland (CLIMAITE). Global Change Biology 18, 1216-1230.

Shahzad, T., Chenu, C., Genet, P., Barot, S., Perveen, N., Mougin, C., Fontaine, S., 2015. Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. Soil Biology and Biochemistry 80, 146-155.

Sillen, W.M.A., Dieleman, W.I.J., 2012. Effects of elevated CO2 and N fertilization on plant and soil carbon pools of managed grasslands: a meta-analysis. Biogeosciences 9, 2247-2258.

Six, J., Carpentier, A., van Kessel, C., Merckx, R., Harris, D., Horwath, W.R., Lüscher, A., 2001. Impact on elevated CO_2 on soil organic matter dynamics as related to changes in aggregate turnover and residue quality. Plant and Soil 234, 27-36.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil 241, 155-176.

Six, J., Jastrow, J.D., 2002. Organic matter turnover, Encyclopedia of Soil Science. Marcel Dekker, New York, pp. 936-942.

Smith, M.S., 1982. Dissimilatory Reduction of NO_2^- to NH_4^+ and N_2O by a soil Citrobacter sp. Applied and Environmental Microbiology 43, 854-860.

Soussana, J.-F., Allard, V., Pilegaard, K., Ambus, P., Amman, C., Campbell, C., Ceschia, E., Clifton-Brown, J., Czobel, S., Domingues, R., Flechard, C., Fuhrer, J., Hensen, A., Horvath, L., Jones, M., Kasper, G., Martin, C., Nagy, Z., Neftel, A., Raschi, A., Baronti, S., Rees, R.M., Skiba, U., Stefani, P., Manca, G., Sutton, M., Tuba, Z., Valentini, R., 2007. Full accounting of the greenhouse gas (CO_2, N_2O, CH_4) budget of nine European grassland sites. Agriculture, Ecosystems & Environment 121, 121-134.

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2007. Soil carbon saturation: concept, evidence and evaluation. Biogeochemistry 86, 19-31.

Spott, O., Russow, R., Stange, C.F., 2011. Formation of hybrid N2O and hybrid N2 due to codenitrification: First review of a barely considered process of microbially mediated N-nitrosation. Soil Biology & Biochemistry 43, 1995-2011.

Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchia, N., Jenkins, M., Minasny, B., McBratney, A.B., de Remy de Courcelles, V., Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, B.C., Chenug, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell, A.G., Parton, W.J., Whitehead, D., Zimmermann, M., 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agriculture, Ecosystems & Environment 164, 80-99.

Syakila, A., Kroeze, C., 2011. The global nitrous oxide budget revisited. Greenhouse Gas Measurement and Management 1, 17-26.

Taub, D.R., Wang, X., 2008. Why are Nitrogen Concentrations in Plant Tissues Lower under Elevated CO_2? A Critical Examination of the Hypotheses. Journal of Integrative Plant Biology 50, 1365-1374.

Thaysen, E.M., Reinsch, S., Larsen, K.S., Ambus, P., 2017. Decrease in heathland soil labile organic carbon under future atmospheric and climatic conditions. Biogeochemistry 133, 17-36.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science 33.

Treseder, K.K., 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytologist 164, 347-355.

Treseder, K.K., Allen, M.F., 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition. New Phytologist 147, 189-200.

UNFCCC, 2015. Paris Agreement. United Nations Framework Convention on Climate Change.

van Groenigen, K.-J., Harris, D., Horwath, W.R., Hartwig, U.A., van Kessel, C., 2002. Linking sequestration of ^{13}C and ^{15}N in aggregates in a pasture soil following 8 years of elevated atmospheric CO_2. Global Change Biology 8, 1094-1108.

van Groenigen, K.-J., Six, J., Hungate, B.A., De Graaff, M.-A., Van Breemen, N., Van Kessel, C., 2006. Element interactions limit soil carbon storage. Proceedings of the National Academy of Sciences of the United States of America 103, 6571-6574.

van Groenigen, K.J., Osenberg, C.W., Hungate, B.A., 2011. Increased soil emissions of potent greenhouse gases under increased atmospheric CO_2. Nature 475, 214.

Van Kessel, C., Boots, B., De Graaff, M.-A., Harris, D., Blum, H., Six, J., 2006. Total soil C and N sequestration in a grassland following 10 years of free air CO_2 enrichment. Global Change Biology 12, 2187-2199.

van Kessel, C., Nitschelm, J., Horwath, W.R., Harris, D., Walley, F., Lüscher, A., Hartwig, U., 2000. Carbon-13 input and turn-over in a pasture soil exposed to long-term elevated atmospheric CO_2. Global Change Biology 6, 123-135.

Whitehead, D., 2000. Nutrient elements in soil, Nutrient elements in grassland: soil–plant–animal Relationships. CAB International, Wallingford, pp. 15-40.

Wieder, W.R., Cleveland, C.C., Smith, W.K., Todd-Brown, K., 2015. Future productivity and carbon storage limited by terrestrial nutrient availability. Nature Geoscience 8, 441.

Wrage, N., Velthof, G.L., van Beusichem, M.L., Oenema, O., 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology & Biochemistry 33, 1723-1732.

Wu, K., Chen, D., Tu, C., Qiu, Y., Burkey, K.O., Reberg-Horton, S.C., Peng, S., Hu, S., 2017. CO_2-induced alterations in plant nitrate utilization and root exudation stimulate N_2O emissions. Soil Biology and Biochemistry 106, 9-17.

Zak, D.R., Holmes, W.E., Finzi, A.C., Norby, R.J., Schlesinger, M.E., 2003. Soil nitrogen cycling under elevated CO_2: a synthesis of forest FACE experiments. Ecological Applications 13, 1508-1514.

Zak, D.R., Pregitzer, K.S., Curtis, P.S., Teeri, J.A., Fogel, R., Randlett, D.L., 1993. Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant and Soil 151, 105-117.

Zak, D.R., Pregitzer, K.S., King, J.S., Holmes, W.E., 2000. Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytologist 147, 201-222.

Zhang, J., Müller, C., Cai, Z., 2015. Heterotrophic nitrification of organic N and its contribution to nitrous oxide emissions in soils. Soil Biology & Biochemistry 84, 199-209.

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Erklärung

Ich erkläre hiermit, dass ich die vorgelegte Dissertation selbstständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt habe, die ich in der Dissertation angegeben habe.

Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Lisa Keidel

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