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

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Keywords: FACE, grassland, carbon cycle, seasonality, Li-8100, winter climate change, winter dormancy, feedback effect, soil respiration, soil CO_2 efflux

Words: 6707 (main text)

Figures: 6

Tables: 2

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 (corresponds to conditions reached 2035 – 2045) 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 three 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 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 control 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 FACE site under elevated CO_2. This suggests (i) 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 (ii) 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 (*e*CO_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 *e*CO_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 observed 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 & 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 five years) with *e*CO_2, often using open-top chamber 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) 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 *e*CO_2 may be quite different (Luo et al., 2001).

The most suitable approach for conducting ecosystem CO_2 experiments under natural conditions are 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 *e*CO_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 *e*CO_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 durations, 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 *e*CO_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 *e*CO_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 duration 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 five 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 *e*CO_2 on soil respiration. The average increase ranged from 12 % under a sweetgum 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 *e*CO_2 during three consecutive winter seasons (Selsted et al., 2012). Allen et al. (2000) detected a significant effect of *e*CO_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 one hour. 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 ecosystem 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 & Körner (2010) reported that soil respiration from the site was only stimulated when volumetric water content was $\leq$ 40 % at soil temperatures above15 °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 *e*CO_2, and thus the soil respiratory response to *e*CO_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 *e*CO_2, mainly during non-growing season (Lenhart, 2008), we hypothesized that (i) long-term (>10 years) moderate CO_2 enrichment will cause increased soil respiration, (ii) soil respiration will be more enhanced in the growing season than during vegetation dormancy (*winter*) and (iii) soil respiration will still be significantly enhanced in *winter* under *e*CO_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 eight meter 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. Since 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 in 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 µmol 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 beveled edge at one end facilitated the insertion into the soil, which took place on 9th 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 LICOR-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 one month to record the insertion and disturbance effects (Fig. S1). The investigation period spanned over three 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 made weekly in the evening, except from May to July and from October to December 2010, where measurements were carried out every second week.

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 vapor 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 R² 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 5. 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 R² below 0.90 were excluded. This was the case in 0.6 % of all measurements taken in this study throughout the three year investigation period.

Soil moisture was measured in each FACE plot as the volumetric water content (VWC) with time-domain-reflectrometric (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 30-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 observational data of three years for the linear mixed-effect model analysis. 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 analyze 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** = *winter* (November – March); **2** = *start of vegetation period* up to the date of spring fertilizer application (March – middle of April); **3** = *spring* until first biomass harvest (middle of April – end of May); **4** = regrowth and *summer* growing season (end of May – beginning of September); **5** = 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

In order to describe the dependence of soil respiration on temperature, a function was fitted according to Lloyd & 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 *e*CO_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.

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

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 against observed respiration values to test if the linear trend line meets the requested slope of 1 We plotted the temperature relationship of soil respiration of the complete dataset, visualizing the different seasons to show seasonal differences (Fig. 5b) of the relationship. However, we did not include seasonal analyses due to the fact that in some seasons there were not enough data points and statistical power was not sufficient (R^2=0.2) to justify this kind of analysis.

2.5 Gap filling of soil respiration data

To obtain annual sums of soil respiration, a gap filling procedure was applied. Therefore 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 minutes over the three 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. 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 *e*CO_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 mid-summer (Fig. 2c and 2f). 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. 2f).

The relative and absolute change of soil respiration under *e*CO_2 (Fig 2d and 2e) followed a seasonal pattern with greatest increases under *e*CO_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 *e*CO_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 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 *e*CO_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 1.03 (Fig. 4).

Based on the temperature-respiration function by Taylor &Lloyd (1994), soil respiration was significantly correlated to soil temperature under ambient as well as *e*CO_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 *e*CO_2 (Table 1). Soil respiration rates did not differ in their relationship to soil temperature between the treatments (Fig.5a). In Fig. 5b we plotted the temperature relationship of soil respiration, visualizing the different seasons, which indicated that soil CO_2 efflux data from *autumn* imposed a different relationship to soil temperature compared to data from other seasons. During *autumn*, soil temperatures were within the same range as during *spring* and *summer*, but soil respiration was on average lower (Fig. 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, Fig. 6). Mean annual estimates of soil respiration under ambient CO_2 ranged from 1282.48 to 1344.00 g C [CO_2] m^{-2} yr^{-1} and under *e*CO_2 from 1300.15 to 1351.56 g C [CO_2] m^{-2} yr^{-1}.

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, Fig. 6). Mean annual sums of soil respiration were 1316.76 ± 18.10 g C m^{-2} yr^{-1}under ambient CO_2 and 1330.58 ± 15.57 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 been 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 of 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 the 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 & Körner (2010) reported that seven 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 (Fig.6). 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 *e*CO_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 µmol 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 *e*CO_2 plots (Kammann et al., 2005;2008).

In most FACE studies which reported the effect of *e*CO_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 *e*CO_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 treeline 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 *e*CO_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 *e*CO_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 *e*CO_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 three 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 11th – 13th 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 *e*CO_2 (+200 ppm). On average, soil respiration was significantly higher by 23 % under *e*CO_2. Jackson et al. (2009) summarized, after 10 years of CO_2 enrichment, that the greatest stimulation of soil respiration under *e*CO_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 one hour.

After short-term enrichment with *e*CO_2 (550 ppm) on a mixed plantation of *Populus* species (EuroFACE; in the 4th and 5th 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 three 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 & Niklaus (2002) did not observe any wintertime increase in the ecosystem CO_2 efflux from a calcareous grassland in response to three 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 *e*CO_2 being observed in late-summer and autumn (Kammann et al., 2008). These findings support the hypothesis that the driving mechanism of the *e*CO_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 *e*CO_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 *e*CO_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 *e*CO_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 *e*CO_2 in November 2011 (unpublished results). Alternatively, 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 cause of the *autumn* and *winter* soil respiration increase under *e*CO_2 cannot be ruled out.

4.4 Temperature dependence of soil respiration

We observed that the temperature dependence of soil respiration was different in *autumn* compared to other seasons, whereas *e*CO_2 did not change the relationship of soil respiration to temperature. During *autumn*, soil temperatures were at the same range as during *spring* and *summer*, but soil respiration was on average lower (Fig.5a). This pattern could reflect the higher proportion of root respiration (due to active root growth and assimilate allocation to exudates) during *spring* and *summer*, as observed by Lenhart (Lenhart, 2008). Boone et al. (1998) found a greater temperature sensitivity of root respiration than microbial respiration, whereas, Bååth et al. (2003) contradicted this finding in a microcosm experiment where different fractions of soil respiration had the same Q_{10} relationship. They suggested that the intensity of light, and thus the intensity of photosynthetic carbon gain and its availability for root-derived soil-respired C, may co-vary with temperature in field studies, probably explaining different temperature dependencies of soil respiration between seasons. In summary, the lack of a difference between ambient and *e*CO_2 soil respiration temperature functions suggests that there is no need to account for a special "*e*CO_2 temperature sensitivity effect" in larger scale models of temperate-grassland CO_2 exchange under future CO_2-enriched atmospheres.

4.5 N availabilty

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. Moreover, the build-up of stable humus compounds (C:N ratio of 10-11), as a potential negative (dampening) feedback of rising CO_2 atmospheres, requires sufficient quantities of N. Root respiration rates were observed to correlate with tissue nitrogen concentration (Burton et al., 1996, 1998), whereas nitrogen affected microbial respiration in a complex pattern (Magill and Aber, 1998;Saiya-Cork et al., 2002;Ågren et al., 2001). In the Giessen-FACE, *e*CO_2

caused reduced tissue N concentrations and higher C:N-ratios of aboveground plant biomass (Kammann et al., 2008). In line with these findings is the observation of Lenhart (2008), who found a lower fraction of root derived CO_2 on soil respiration with increasing atmospheric CO_2. Furthermore, *e*CO_2 induced a shift of available NO_3^- towards NH_4^+ at the study site (Müller et al., 2009), a typical feature of N-limited ecosystems to retain mineral N (Rütting et al., 2008;Huygens 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 *e*CO_2.

4.6 Microbial community

Multiple observations from the GiFACE indicated that increases in winter soil respiration under *e*CO_2 were largely associated with microbial respiration (including rhizosphere microbiota). Recent studies from other FACE sites detected differences between microbial communities at *e*CO_2 compared to ambient CO_2 (Drigo et al., 2008;Drigo et al., 2009). At the GiFACE, stimulated rhizosphere-C utilization by arbuscular mycorrhizal fungi were found under *e*CO_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 *e*CO_2 plots (K. Brenzinger, personal communication) 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*.

4.7 Soil moisture

Several studies showed that *e*CO_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, based on previous studies, the soil moisture effect is likely not to be large (i.e. soil moisture was not the limiting factor). Therefore, we focused in our study on the soil temperature effect. Moreover, no significant effect of *e*CO_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; but still, enhanced anaerobicity due to enhanced microbial activity, as experimentally produced e.g. by Sehy et al.(2004), cannot completely be ruled out. However any hypothetical aerobicity change, if present at all in the GiFACE, was not large enough to affect the performance and composition of the methanogenic community in the 11th year of CO_2 enrichment (Angel et al., 2012), which is a sensitive indicator for aerobicity changes.

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 *e*CO_2 due to diffusion limitations. Analysis of stable isotopes revealed a distinctive $\delta^{13}CO_2$ gradient in soil during winter with decreasing signatures with depth but a homogenous $\delta^{13}CO_2$ profile during vegetation period at our study site (Lenhart, 2008). The absence of a $\delta^{13}CO_2$ gradient during summer was likely due to improved diffusive mixing of soil air in the profile during aerobic soil conditions. Based on previous studies on this grassland (e.g. Müller et al., (2004) it was shown that during summer, when soil moisture content was relatively low (0.3 cm³ cm^{-3}) in the main rooting zone (top 10 cm) of the GiFACE site, the site of production for gaseous emissions (e.g. N_2O) occurred at deeper soil layers (20-50 cm depth) where the soil moisture content was still high (0.6 cm³ cm^{-3}). 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.. Accordingly, CO_2 diffusion was slowed down at times of high soil moisture, coinciding with limited oxygen supply (Skopp et al., 1990). At these times, soil respiration was likely originating to a major part from the topsoil. However, increased *autumn* soil respiration under *e*CO_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 *e*CO_2 from end-of-season dieback of roots and the root-associated microbiome may explain stimulated soil respiration rates in *autumn*.

4.8 Freeze/thaw cycles

Freeze/thaw cycles are known to mobilize previously inaccessible C- and N-substrates (Goodroad and Keeney, 1984;Kammann et al., 1998;Röver et al., 1998;Müller et al., 2002;Edwards and Cresser, 1992), providing substrates for heterotrophic activity. Frost events occurred during the study at the GiFACE from end of December 2008 to February 2009 (Fig. 2c). The relative change of soil respiration under *e*CO_2 was 17 %,12 % and 5 % from January to March 2009 respectively (Fig. 2d), showing a more pronounced stimulation in these seasons than during the growing season, apart from October 2010 (12 % increase under *e*CO_2).

4.9 Plant community

Another aspect which may have contributed to altered soil respration rates under *e*CO_2 is a shift in the plant community composition. Grüters et al. (2006) observed that summer-greens decreased, whereas evergreens increased under *e*CO_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 *e*CO_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 micro-biota may increase, contributing to the relative increase under *e*CO_2 during *winter*. The higher abundance of evergreens at *e*CO_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 *e*CO_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 demonstrated the importance of winter soil respiration measurements, by showing that soil respiration was increased during *autumn* and *winter* after moderate long-term *e*CO_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 *e*CO_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 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 *e*CO_2 more studies of winter C dynamics under long-term *e*CO_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 *e*CO_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.

Acknowledgements

We are grateful to both, the Hessian Agency for the Environment and Geology (HLUG) for long-term financial support, and to the Hessian Ministry for Science and Arts for financial funding within the LOEWE research project $FACE_2FACE$. The technical assistance of Jochen Senkbeil, Jürgen Franz, Till Strohbusch and Birte Lenz at the Giessen FACE site is gratefully acknowledged, as well as the assistance of Matthias Daum, Christian Eckhard, Christoph von Bredow and Yvette Kühnel. CK and CM gratefully acknowledge the long-term engagement of Prof. H.-J. Jäger († 18.8.2013) who initiated and norished the Giessen FACE study over more than a decade.

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Tables

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

CO_2 treatment	R	Rsqr	Adj Rsqr	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

Table 2

Annual sums of soil respiration under ambient and *e*CO$_2$ from 2008 – 2010. Data are presented as averages (n=3) ± standard error (SE).

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$_2$	4853.93 ± 33.84	1323.80 ± 9.23	1.22	0.17
	Elevated CO$_2$	4913.38 ± 14.20	1340.01 ± 3.87		
2009	Ambient CO$_2$	4928.00 ± 48.34	1344.00 ± 13.18	0.56	0.64
	Elevated CO$_2$	4955.74 ± 39.08	1351.56 ± 10.66		
2010	Ambient CO$_2$	4702.44 ± 36.69	1282.48 ± 10.01	1.38	0.23
	Elevated CO$_2$	4767.22 ± 11.47	1300.15 ± 3.13		

Figure legends

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

Fig. 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 observed and modelled data (d), the absolute mean monthly difference in soil respiration under elevated CO_2 based on observed and modelled data (e) and soil respiration under ambient and elevated CO_2 per measurement from 2008 to 2010 based on observed and modelled data (f). Data are presented as averages (n=3) ± 1 SE.

Fig. 3 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* (for details see methods).

Fig. 4 Observed versus modelled soil respiration rates under ambient and elevated CO_2.

Fig. 5 Relationship between soil respiration rate and soil temperature under ambient and elevated CO_2 **(a)** and temperature dependence of soil respiration under ambient and elevated CO_2 during different seasons **(b)**. Equation of dynamic fit (Lloyd and Taylor, 1994): $f = R10e^{E0\left(\frac{1}{(283.15-T0)}-\frac{1}{(x-T0)}\right)}$

Fig. 6 Annual sums of soil respiration under ambient and elevated CO_2 for 2008 – 2010 based on observed and modelled data. Error bars represent ± 1 SE of the mean.

Figures

Fig. 1

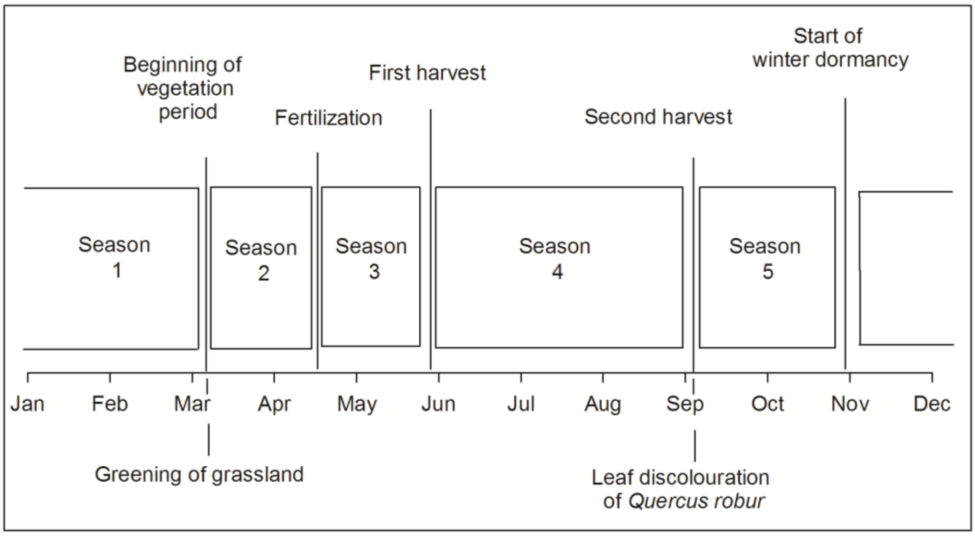
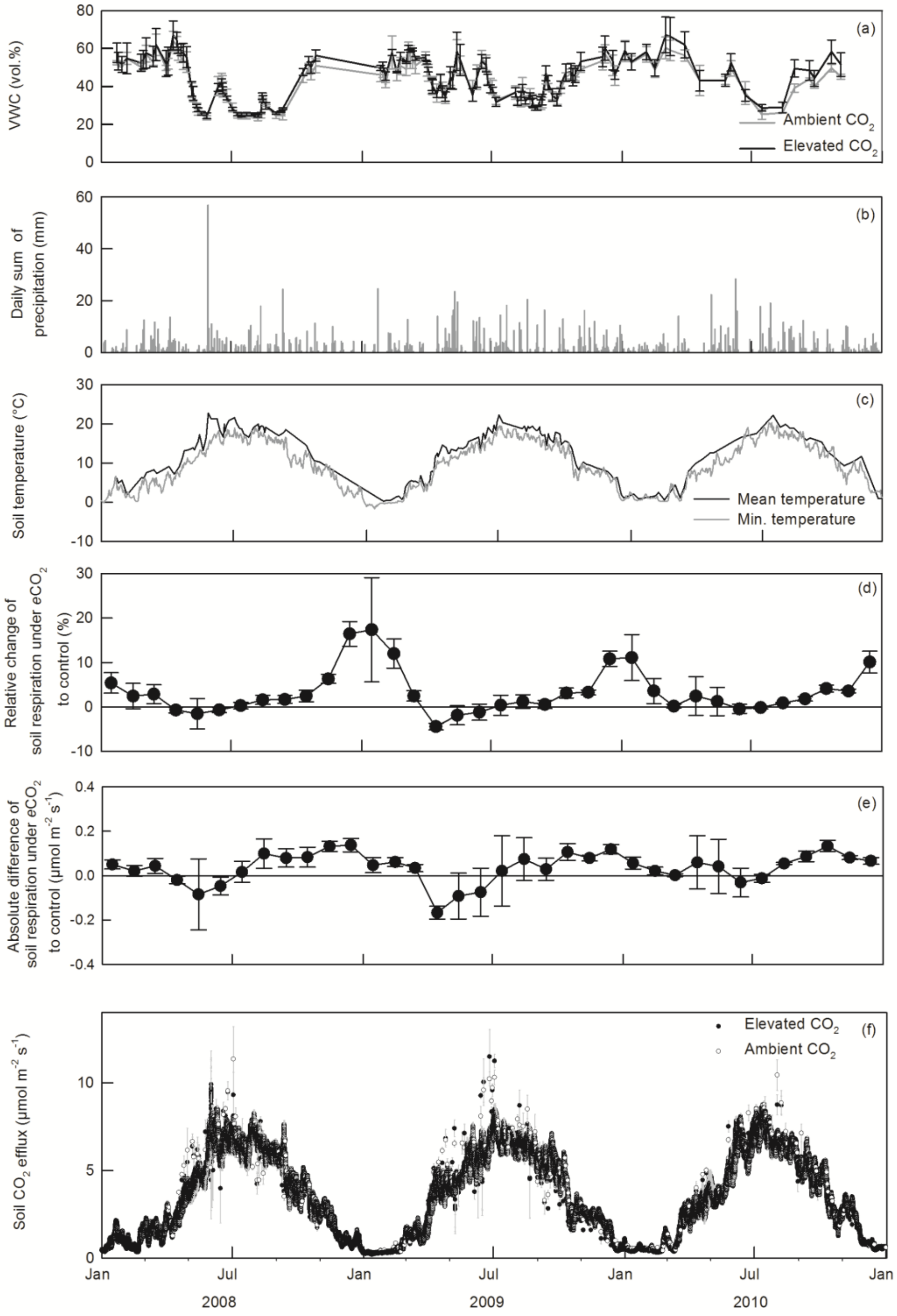
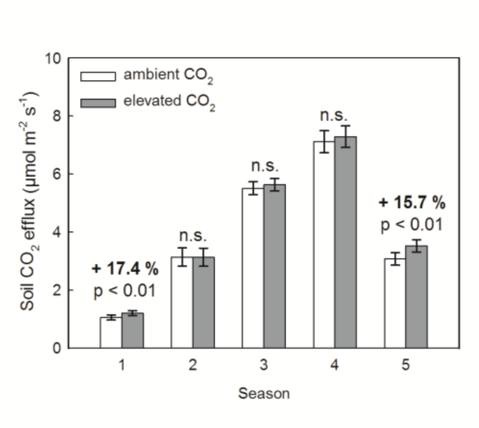


Fig. 2


VWC (vol.%)
80
60
40
20
0
(a)
Ambient CO_2
Elevated CO_2
Daily sum of precipitation (mm)
60
40
20
0
(b)
Soil temperature (°C)
30
20
10
0
-10
(c)
Mean temperature
Min. temperature
Relative change of soil respiration under eCO_2 to control (%)
30
20
10
0
-10
(d)
Absolute difference of soil respiration under eCO_2 to control (µmol m^{-2} s^{-1})
0.4
0.2
0.0
-0.2
-0.4
(e)
Soil CO_2 efflux (µmol m^{-2} s^{-1})
10
5
0
(f)
Elevated CO_2
Ambient CO_2
Jan
Jul
Jan
Jul
Jan
Jul
Jan
2008
2009
2010

1022

Fig. 3



ambient CO_2
elevated CO_2
Soil CO_2 efflux (µmol m^{-2} s^{-1})
Season
0
2
4
6
8
10
1
2
3
4
5
+ 17.4 %
p < 0.01
n.s.
n.s.
n.s.
+ 15.7 %
p < 0.01

Fig. 4

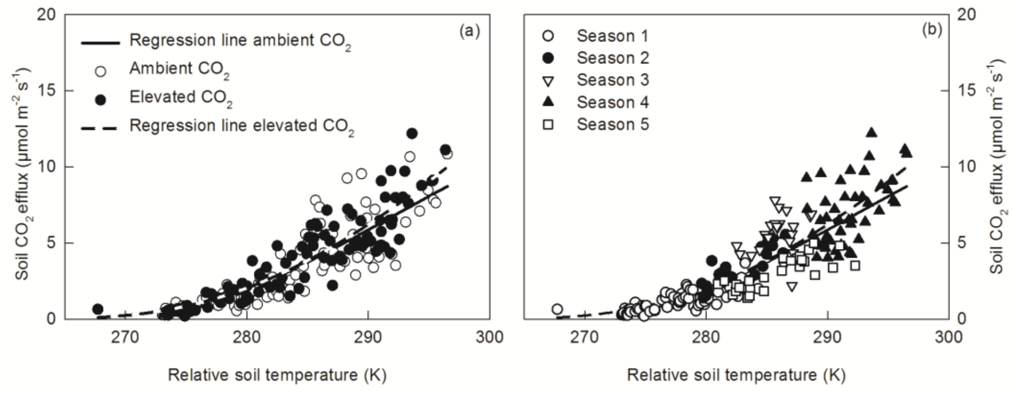

(a)
Regression line ambient CO$_2$
Ambient CO$_2$
Elevated CO$_2$
Regression line elevated CO$_2$
Soil CO$_2$ efflux (µmol m^{-2} s^{-1})
0
5
10
15
20
270
280
290
300
Relative soil temperature (K)
(b)
Season 1
Season 2
Season 3
Season 4
Season 5
Soil CO$_2$ efflux (µmol m^{-2} s^{-1})
Relative soil temperature (K)

Fig. 5

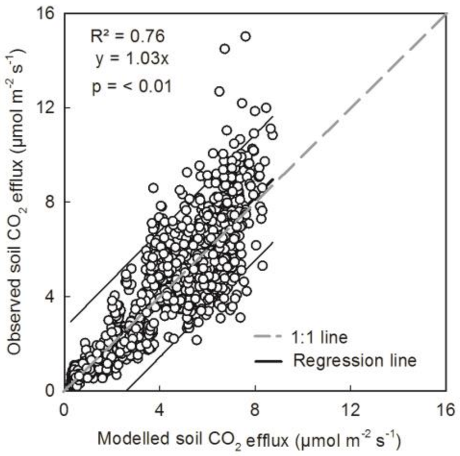
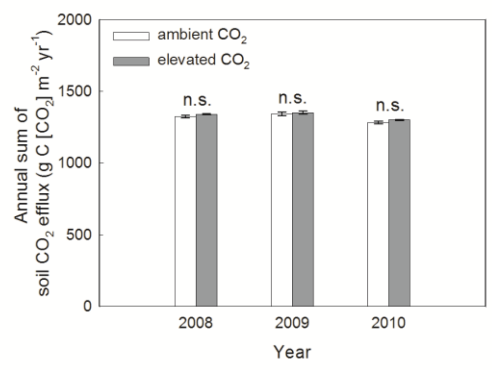

R² = 0.76
y = 1.03x
p = < 0.01
1:1 line
Regression line
Observed soil CO$_2$ efflux (µmol m^{-2} s^{-1})
Modelled soil CO$_2$ efflux (µmol m^{-2} s^{-1})
0
4
8
12
16
0
4
8
12
16

Fig. 6



Kommentar [B1]: Legend was added to Fig. 6

Supporting Information

Fig. S1

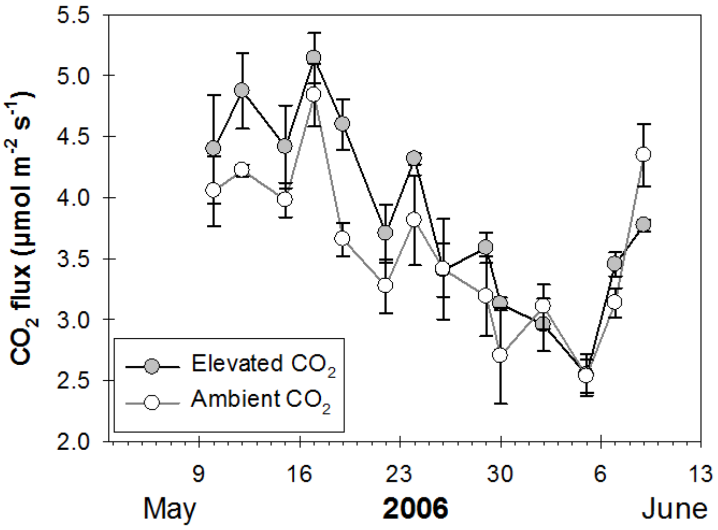


Fig S1: Mean CO$_2$ efflux +/- standard error (n=3) after installation of the frames and removal of the aboveground biomass on 9th May 2006.

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 (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