1 Comments from the Editor

Dear Dr. Subke,

Thank you for your comments on our revisions. Below you find our answers point-by point and revisions under each of your comments.

Best regards,

Lisa Keidel

Dear Dr. Keidel,

I think you have managed to address most of my concerns, and think that the manuscript is now more concise than previously. I have two comments that I would like you to address before final acceptance:

1.1 Table 3: I assume that you have added this following my request to clarify what is modelled and what is measured. What I had meant was that it is clad in the data, where you have filled gaps with the model, and where you used the measured data. It seems to me that you now present data as measured results independently form the modelled data. That is ok, and should illustrate to the reader what you did. It is not strictly gap-filling, which spies that you use measured data whenever available and modelled data for data gaps. This should be adjusted in the text. Table 3 is not in fact needed.

Response: Thank you for clarifying your previous request. We omitted Table 3 and changed the heading of 2.5 from "Gap filling of soil respiration data" to "Annual estimates of soil respiration". We adjusted the text according your suggestion:" To obtain annual sums of soil respiration, measured data were used whenever available, and modelled data for data gaps." We also added to 2.3: "We used all measured data of three years for the linear mixed-effect model analysis to obtain seasonal estimates of soil respiration."

2.1 For the figure showing modelled fluxes, I am surprised that you have apparent outliers. Temperature is your only driver, and the presented results show no outliers, so why are calculated fluxes different? Again, this should be clarified before publication.

Best regards,
Jens-Arne Subke

Response: Thank you for pointing out that figure 2f is showing outliers that are not explained by the model. We checked all modelled data and found that the outliers were due to a measuring error of one soil temperature sensor at the dates where the outliers were shown in the figure. We removed the invalid data.

Title: **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 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 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 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 (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 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 eCO_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 eCO_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 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 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

158 FACE study but not during the previous growing season beneath the loblolly pine forest. Andrews
159 and Schlesinger (2001) reported from the same site greater increases of soil respiration during
160 fumigation periods (26-59 %) than during non-fumigated periods (8-15 %). Fumigation was
161 stopped when ambient air temperature dropped below 5 °C for more than one hour. In line with
162 these results, much larger percentage enhancements of the soil CO_2 efflux were observed during
163 the growing season (up to 111 %) than during dormant season (40 %) from a mixed plantation of
164 *Populus* species exposed to *e*CO_2 (EuroFACE) (Lagomarsino et al., 2013). CO_2 enrichment was
165 provided from bud burst to leaf fall at this site.
166 Out of six long-term studies on soil respiration (Carol Adair et al., 2011;Pregitzer et al.,
167 2008;Jackson et al., 2009;Pendall et al., 2001;Bader and Körner, 2010;Dawes et al., 2013), only
168 one study reported measurements throughout the dormant season, showing that after 10 years of
169 *e*CO_2 during the growing season at a loblolly pine forest (Duke FACE) soil respiration was
170 consistently higher in midsummer to early fall and diminished or disappeared in winter (Jackson
171 et al., 2009). This was explained by a reduction in assimilation and hence available root exudate
172 during dormancy. If the fumigation may continue during the dormant season in an ecosystem with
173 a green canopy e.g. in a permanent grassland, the stimulation may theoretically continue on a
174 higher level.
175 Reports from other long-term FACE studies in temperate ecosystems (disregarding the dormant
176 season) were consistent by reporting an increase in soil respiration under *e*CO_2, with the exception
177 of the Swiss Canopy Crane experiment in an old-growth, mixed deciduous forest. Bader & Körner
178 (2010) reported that soil respiration from the site was only stimulated when volumetric water
179 content was $\leq$ 40 % at soil temperatures above15 °C.
180 In summary, only fragmented information is available on how soil respiration responds to *e*CO_2
181 during vegetation as well as dormant periods after long-term *e*CO_2. To our knowledge, no long-
182 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 (i) long-term (>10 years) moderate CO$_2$ enrichment causes increased soil respiration, (ii) soil respiration is more enhanced in the growing season than during vegetation dormancy (*winter*) and (iii) 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.

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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).

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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

228 calcium-ammonium-nitrate fertilizer at the rate of 40 kg N ha^{-1} yr^{-1}. Before 1996, fertilizer
229 was applied at a rate of 50–100 kg N ha^{-1} yr^{-1} (Kammann et al., 2008).
230 The soil of the study site is classified as a Fluvic Gleysol (FAO classification) with a texture
231 of sandy clay loam over a clay layer (Jäger et al., 2003).
232 Observations in this study were carried out from January 2008 - December 2010 (i.e. more
233 than 9 years after the onset of CO_2 enrichment). During the observation period the mean
234 annual temperature was 9.2 °C and mean annual precipitation was 562 mm which was
235 identical to the average rainfall since the beginning of recording in 1995. Rainfall was
236 recorded at the site in 30-min intervals with 20 randomly distributed "Hellmann" samplers.
237 Air temperature was recorded continuously at two locations at the site in 2 m height and
238 averaged 9.5 °C since 1995.

239 2.2 Measurement of soil CO_2 fluxes at the field site

240 In each of the six FACE plots, soil respiration rates were measured using an automated closed
241 dynamic chamber system with an infrared gas analyzer (LI-COR 8100, LI-COR, Inc.,
242 Lincoln, Nebraska, USA) with a patented vent for pressure equilibration between the closed
243 chamber and the atmosphere (McDermitt et al., 2005). Carbon dioxide fluxes were reported in
244 μmol CO_2 m^{-2} s^{-1}. The measurements were performed at four permanently installed PVC soil
245 collars per FACE ring, to cover the spatial heterogeneity within each ring. The soil collars had
246 a diameter of 20.3 cm (8 inch) and were about 11 cm high. A beveled edge at one end
247 facilitated the insertion into the soil, which took place on 9th May 2006 and the vegetation
248 cover, including surficial rhizomes, was removed manually. Subsequently, the surface was
249 held vegetation-free by removing germinated seedlings weekly. Due to uneven soil
250 conditions, soil collars varied +/- 1 cm in their insertion depth. Generally, the insertion was
251 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 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 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 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 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 R^2 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 15~~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~~ measured data of three 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 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

We applied a temperature response model to fill gaps in the measured data set. Therefore 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 eCO_2) by using the dynamic fit function in the SigmaPlot 11.0

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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 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 ~~Gap filling of soil respiration data~~Annual estimates of soil respiration

To obtain annual sums of soil respiration measured data was used whenever available and modelled data for data gaps.~~, a gap filling procedure was applied. Therefore m~~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 ~~(Table 3)~~ 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 *e*CO_2 were calculated in comparison to soil respiration under ambient CO_2, based on observational and modelled data ~~(Table 3)~~. For calculating the relative change ambient soil respiration was set to 0 %.

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349 **3 Results**

350 3.1 Annual variability of soil respiration

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

357 The relative and absolute change of soil respiration under *e*CO_2 (Fig 2d and 2e) followed a
358 seasonal pattern with greatest increases under *e*CO_2 during *autumn* and *winter*. During
359 midsummer, when the largest absolute soil respiration rates occurred, the relative increase due
360 to the CO_2 enrichment was lowest or non-existent. A linear mixed effect model analysis
361 confirmed that soil respiration rates under *e*CO_2 were significantly higher compared to rates
362 under ambient CO_2 during *autumn* (15.7 %) and *winter* (17.4 %) (Fig. 3). During all other
363 seasons (*beginning of vegetation period* (season 2), *spring* (season 3) *and summer* (season 4)),
364 covering most of the vegetation period, a trend towards higher soil respiration, but no
365 significant CO_2 effect was observed with *e*CO_2 (Fig. 3).

366 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 &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).

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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 g C [CO_2] m^{-2} yr^{-1} 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 1331 ± 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 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

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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.

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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.

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The majority of long-term FACE studies reported significantly increased soil respiration under *e*CO_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 *e*CO_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.

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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 *e*CO_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 *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.,

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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 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 11^{th} – 13^{th} 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

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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.

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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.

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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).

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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). 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 eCO_2 cannot be ruled out.

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. Root respiration rates were observed to correlate with tissue nitrogen concentration (Burton et al., 1996, 1998).In the Giessen-FACE, eCO_2 caused reduced tissue N concentrations and higher C:N-ratios of aboveground plant biomass (Kammann et al.,

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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, the soil moisture effect is likely not

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548 to be large . Moreover, no significant effect of *e*CO_2 on the soil water content was observed
549 either during the first 5 years of enrichment (Kammann et al., 2005) or after 13 years of
550 enrichment (Meine, 2013). Consequently, a CO_2-induced soil moisture effect is unlikely
551 governing increased soil respiration rates.

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

568 4.9 Plant community

569 Another aspect which may have contributed to altered soil respration rates under *e*CO_2 is a
570 shift in the plant community composition. Grüters et al. (2006) observed that summer-greens

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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 micro-biota 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.

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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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882 **Tables**

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

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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

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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). 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		

Table 3

Results of the study based on observed and/or modelled data

Results	**Observed soil respiration**	**Modelled soil respiration**
Relative mean monthly change of soil respiration under eCO_2	X	X
Absolute mean monthly difference in soil respiration under eCO_2	X	X
Mean soil respiration rates during the five defined seasons under ambient and elevated CO_2 averaged over three years from 2008 – 2010	X	-
Annual sums of soil respiration under ambient and eCO_2 from 2008 – 2010.	X	X

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 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~~easured~~ soil respiration under ambient and elevated CO_2 from 2008 to 2010 (f) and measured~~odelled~~ soil respiration under ambient and elevated CO_2 from 2008 to 2010 (g). 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. Error bars show ± 1 SE associated by averaging across the three replicates per treatment (n=3) (1) = *winter dormancy*; (2) = *start of vegetation period*; (3) = *spring*; (4) = *summer*; (5) = *autumn* (for details see methods). P-values indicate the difference between treatments obtained by a linear mixed-effect model analysis.

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

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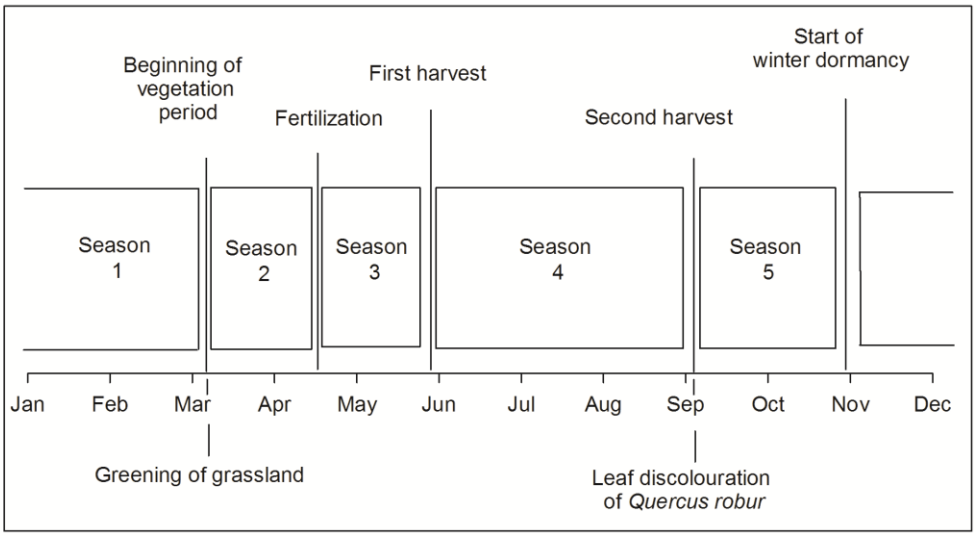
Fig. 5 Observed versus modelled soil respiration rates under ambient and elevated CO_2.

965 **Figures**

966 Fig. 1



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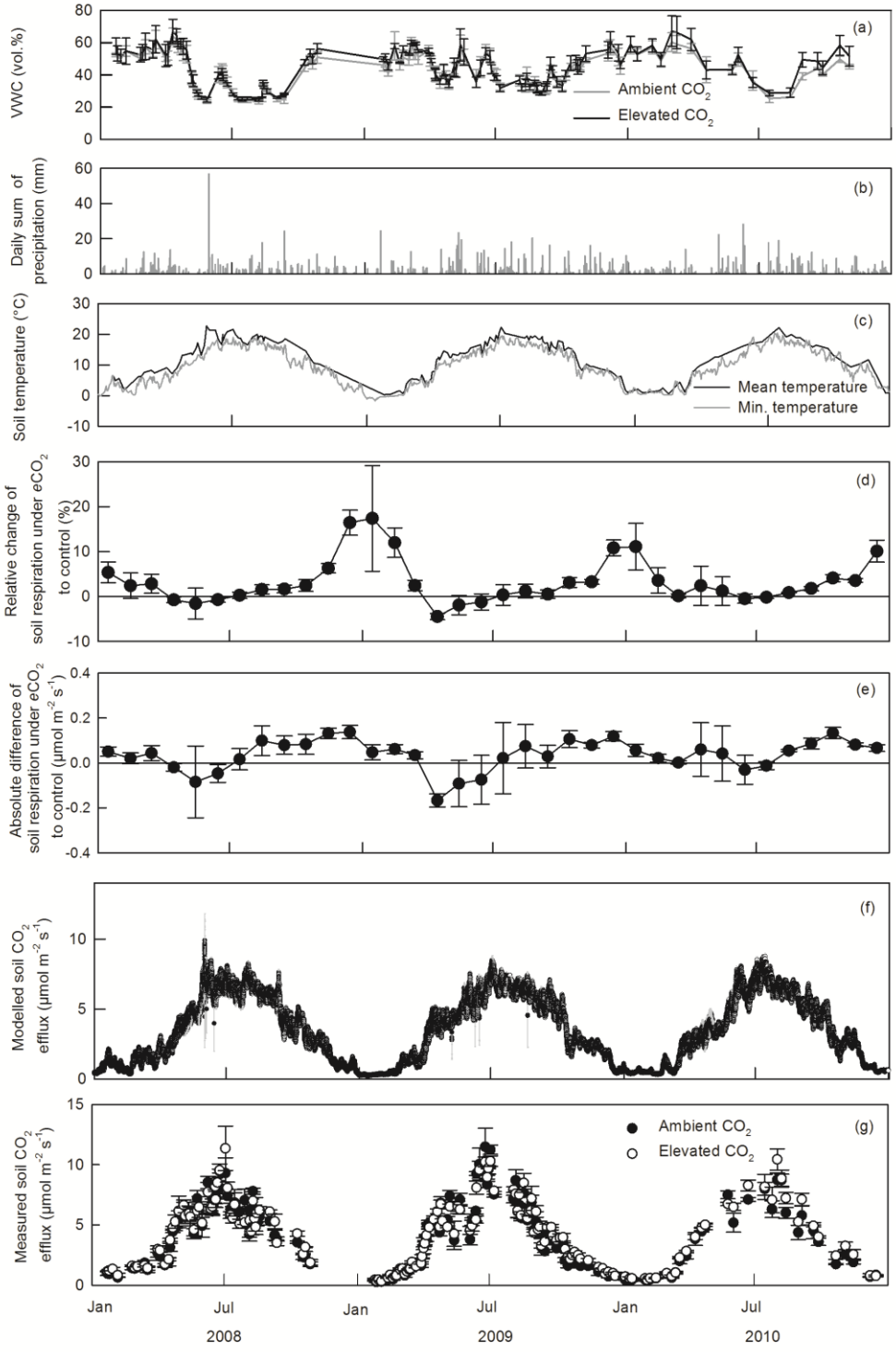
979 Fig. 2


VWC (vol.%)
(a)
Ambient CO2
Elevated CO2
Daily sum of precipitation (mm)
(b)
Soil temperature (°C)
(c)
Mean temperature
Min. temperature
Relative change of soil respiration under eCO2 to control (%)
(d)
Absolute difference of soil respiration under eCO2 to control (µmol m-2 s-1)
(e)
Modelled soil CO2 efflux (µmol m-2 s-1)
(f)
Measured soil CO2 efflux (µmol m-2 s-1)
(g)
Ambient CO2
Elevated CO2
Jan
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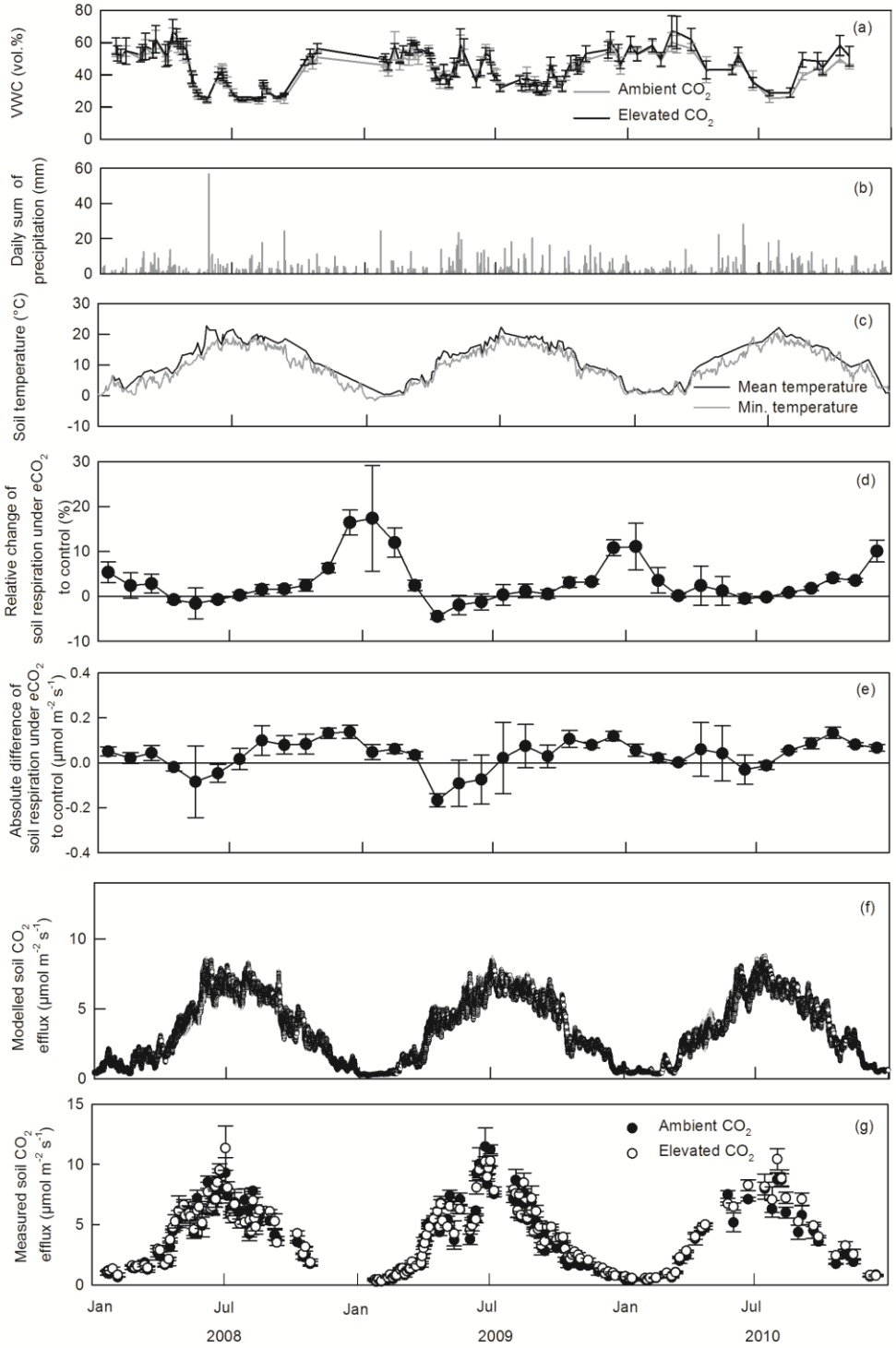
VWC (vol.%)
(a)
Ambient CO2
Elevated CO2
Daily sum of precipitation (mm)
(b)
Soil temperature (°C)
(c)
Mean temperature
Min. temperature
Relative change of soil respiration under eCO2 to control (%)
(d)
Absolute difference of soil respiration under eCO2 to control (µmol m-2 s-1)
(e)
Modelled soil CO2 efflux (µmol m-2 s-1)
(f)
Measured soil CO2 efflux (µmol m-2 s-1)
Ambient CO2
Elevated CO2
(g)
Jan
Jul
Jan
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2008
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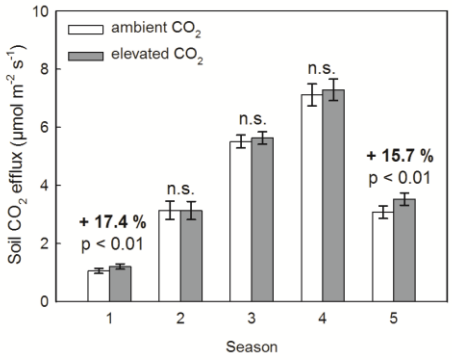
982 Fig. 3



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1000 Fig. 4

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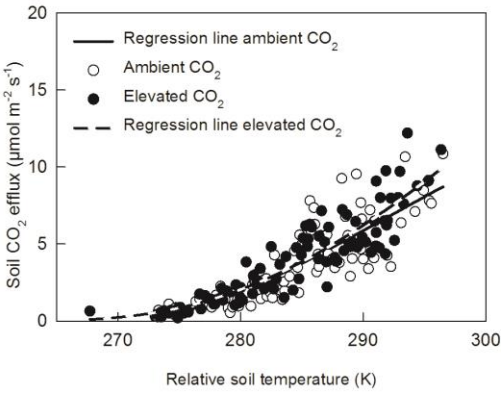

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1017 Fig. 5

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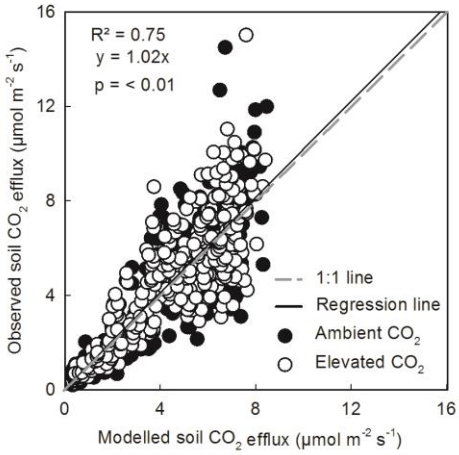

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Supporting Information

Fig. S1

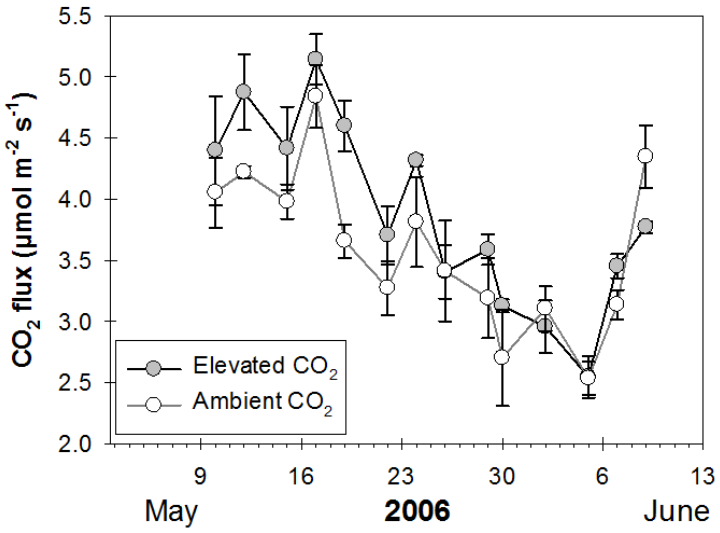

Fig S1: Mean CO_2 efflux +/- standard error (n=3) after installation of the frames and removal of the aboveground biomass on 9th May 2006.
On 11 out of 14 measurement occasions all three E-plot fluxes where higher than those of their corresponding A-plot partner. A mixed Model analysis (SPSS version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

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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