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Title: Trace gas fluxes of CO_2, CH_4 and N_2O in a permanent grassland soil exposed to elevated CO_2 in the Giessen FACE study

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Short title: Gaseous emissions under elevated CO_2

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Abstract

Long-term field observations showed that N_2O fluxes observed shortly after N application were not significantly affected by elevated CO_2 in the Giessen Free Air Carbon dioxide Enrichment (FACE) study. To further investigate this unexpected result a ^{15}N tracer study was carried out under controlled conditions where in parallel treatments either the NH_4^+ pool ($^{15}NH_4NO_3$) or the NO_3^- pool ($NH_4{}^{15}NO_3$) was enriched with ^{15}N. Fluxes of CO_2, CH_4, and N_2O as well as the ^{15}N enrichment of the N_2O were measured. Denitrifying Enzyme Activity (DEA), total denitrification (N_2+N_2O) and N_2–to–N_2O ratios were quantified in separate experiments. Over the 57 day incubation, N_2O fluxes averaged 0.090 ng N_2O–N g^{-1} h^{-1} under ambient and 0.083 ng N_2O–N g^{-1} h^{-1} under elevated CO_2 (not significantly different). The N_2O production processes were identified by a two-source model. Results showed that N_2O must have also been produced by a third source – possibly related to organic N transformation – which was stimulated by elevated CO_2. Soil CO_2 fluxes were approximately 20% higher under elevated CO_2 than soil from ambient but the differences were not significant. CH_4 oxidation rates were on average −1.75 ng CH_4–C g^{-1} h^{-1} in the elevated and −1.17 ng CH_4–C g^{-1} h^{-1} in the ambient indicating that elevated CO_2 increased the CH_4 oxidation by 49% compared to ambient CO_2 under controlled conditions. N fertilization increased CH_4 oxidation by 3–fold in both CO_2 treatments. CO_2 did not have any significant effect on DEA while total denitrification and N_2-to-N_2O ratios increased by 36 and 33%, respectively. The results indicate that shortly after N application elevated CO_2 must have stimulated both the N_2O production and reduction to N_2 to explain the increased N_2-to-N_2O ratio and at the same time explain the non-responsiveness of the N_2O emissions. Thus, the observed variation of the CO_2 effect on N_2O emissions throughout the year is possibly governed by the dynamics of the N_2O reductase activity.

1 Introduction

The level of earth's atmospheric carbon dioxide (CO_2) concentration has risen from ~280μL L^{-1} at the start of the industrial revolution to greater than 385μL L^{-1} today, and is expected to exceed 700μL L^{-1} by the end of this century (IPCC, 2007). Elevated atmospheric CO_2 increases the plant productivity and aboveground biomass resulting in a substantial allocation of carbon (C) to belowground that may lead to a general increase in C inputs in soil. This additional C is likely to fuel belowground microbial processes and may alter both C and N cycling in soil. Any change in C and N flow and transformation will affect the soil-atmosphere exchange of biogenic trace gases. The accumulation of greenhouse gases (GHG) in the atmosphere does alter the earth's radiative balance and is likely responsible for climate change (Watson et al., 1992; IPCC, 2007; Smith et al., 2010). Although CO_2 is by far the most abundant greenhouse gas, N_2O and CH_4 are important atmospheric trace gases because of their unique radiative properties and their long residence time in the atmosphere resulting in global warming potential of 296 and 21 times that of CO_2, respectively (IPCC, 2007). In addition, N_2O and CH_4 participate in other atmospheric reactions (e.g. stratospheric ozone depletion) of global environmental significance. Their concentration in the atmosphere is continuously rising and since the pre-industrial era it has increased by 15 and 145%, respectively (Watson et al., 1992; Houghton et al., 1996; IPCC, 2007).

Soil plays a major role in the global accounting of C not only due to large amount of C stored in soil, but also since soil contribution to the annual flux of CO_2 to the atmosphere is 10 times that contributed by fossil fuel burning (Post et al., 1990). Respiration fluxes of CO_2 in grassland ecosystems under elevated CO_2 varied from a 10% decline to a 162% increase with a mean response of 51% increase (Zak et al., 2000). Reich et al. (2001) found a 13% greater CO_2 fluxes per unit mass under elevated atmospheric CO_2. Similarly, Smith et al. (2010) reported that soil CO_2 flux in an arable soil was significantly greater under elevated CO_2 being in the range of 15% to 50% compared to ambient CO_2.

In addition to soil CO_2 flux, elevated atmospheric CO_2 can affect other greenhouse and reactive trace gases i.e. CH_4 and N_2O and studies so far provide contradictory results. Ineson et al. (1998) measured fluxes of N_2O, CH_4 and CO_2 from soils under ambient and elevated CO_2 at the Swiss FACE experiment in plots of *Lolium perenne* and reported increased N_2O emissions by 27% under elevated CO_2 while ambient plots oxidized consistently more CH_4 than the elevated plots indicating that elevated CO_2 may result in the inhibition of CH_4 oxidation. Cheng et al. (2006) reported a 58% increase in CH_4 flux from rice paddies under elevated CO_2. This increase was attributed to greater root exudates and numbers of tillers, resulting in more surface area for the release of CH_4 to the atmosphere (Ziska et al., 1998; Inubushi et al., 2003). In another study, Arnon and Bohlen (1998) and Baggs et al. (2003a) reported that both N_2O and CO_2 fluxes under elevated CO_2 were 2–3 times higher than those observed under ambient CO_2. This increase was attributed to increased belowground C allocation in elevated CO_2 providing energy for denitifiers or that there is increased O_2 consumption under elevated CO_2. However, Mosier et al. (2002) conducted an open-top-chamber CO_2 enrichment study in the Colorado shortgrass steppe and reported that even though both C_3 and C_4 plant biomass increased and soil moisture content was typically higher under elevated CO_2, none of the trace gas fluxes were significantly altered by CO_2 enrichment over the 43 months period of observation. Similarly, N_2O fluxes were not affected by elevated CO_2 in a paddy, arable and grassland fields (Cheng et al., 2006; Smith et al., 2010; Dijkstra et al., 2010). However, Kettunen et at. (2006) showed that elevated CO_2 increased both N_2O flux from soil and soil water content.

A significant increase of N_2O emissions under elevated atmospheric CO_2 has been observed in the Giessen FACE study (Kammann et al., 2008). The more than 9-year data set allowed for the first time the investigation of different time periods throughout the year. Unexpectedly, N_2O stimulation by elevated CO_2 in this N limited grassland ecosystem occurred throughout the vegetation period when mineral N supply was limited, while in the period following N application no significant difference in N_2O emissions was detected. Differences in N cycling and/or stimulation of different microbial groups under elevated CO_2 were made responsible for the observed results. A ^{15}N tracing study with soil taken from the Giessen FACE study showed that under elevated CO_2 the turnover of N changed towards a higher N cycling speed (Müller et al., 2009). To explain the N_2O response to CO_2 it is particularly important to study in detail the periods following N fertilizer application because these are times when high N_2O emissions occur and when most of the annual N_2O is produced. Thus the objective of this study was to determine the extent to which elevated CO_2 concentration may change soil-atmosphere exchange of GHG (CO_2, CH_4 and N_2O) from grassland soil that had been under the influence of elevated CO_2 for more than 6 years.

2 Material and Methods

2.1 Site description

The grassland site (Environmental Monitoring Climate Impact Research Station) is located 50°32′N and 8°41.3′E at an elevation of 172 m above sea level near Giessen, Germany. The semi-natural non-grazed grassland has been managed extensively as a meadow for at least 50 years, fertilized with 40 kg N ha^{-1} $annum^{-1}$ as calcium ammonium nitrate and mown twice per year. The annual mean precipitation and temperature (last 35 years) are 644 mm and 9.9°C. The vegetation, an *Arrhenatheretum elatioris* Br.Bl. *Filipendula ulmaria* sub-community, is dominated by 12 grass species, 2 legumes and 15 non-leguminous herbs. The soil is classified as a Fluvic Gleysol and has a sandy clay loam texture over a clay layer, with a mean C and N content of 4.5% and 0.45%, respectively and a pH of 6.2 (Müller et al., 2009, note the organic C content was not significantly different between the two CO_2 treatments, see Lenhart (2008). In May 1998, the long-term Giessen FACE system was established (Jäger et al., 2003).

2.2 Soil sampling and experimental set-up

Soil for the experiments reported here was sampled from the top 12 cm of the old grassland soil. The soil was taken from the ambient and elevated FACE rings where also soil had been sampled for the ^{15}N tracing study described by Müller et al. (2009) (see this publication for more details). Fresh soil was sieved (5 mm) and sub-samples were taken for determining initial gravimetric moisture content at 105°C for 24 h. The soil was stored for a week at 4°C before the start of the incubation experiment. A set of twelve jars (Weck®) was arranged according to the treatments: i) two soils i.e. elevated CO_2 soil and ambient soil; ii) two N sources i.e. $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ (60 atom%) with three repetitions per treatment. Soil portions of 200 g (fresh wt. equivalent) were weighed out and filled into each jar. The soil was adjusted to a water content of 0.40 g H_2O g^{-1} dry soil with distilled water and incubated for a week at 20 °C prior to fertilizer application. Both the soils (either from plots under elevated or ambient CO_2) were labelled with ^{15}N at a rate of 100 µg N g^{-1} fresh soil in 10.5 mL per jar using a seven-needle applicator to assure an even distribution of the applied N in soil. The resulting water content was on average 0.45 g H_2O g^{-1} dry soil. The jars were covered with parafilm that was perforated with a needle to ensure natural gas exchange and incubated at 20 ^{0}C. Samples were weighed at regular intervals during the incubation; water loss under present experimental set-up was almost negligible (~0.2 mL).

2.3 Gas samplings and measurements

In total, 13 gas samplings were carried out at day 0 (shortly after N application) and 1, 2, 4, 9, 14, 18, 24, 29, 35, 39, 48 and 57 days after N application. Four samplings were carried out (3, 4, 5, 7 days) before fertilizer application (control). At each sampling time the jars were closed for 0.5 to 2 hours with a glass lid. Gas samples were taken through a septum in the lid with 60 ml disposable syringes at time zero and at the end of the incubation period. A 12 ml sub sample at the end of the incubation samples were transferred to evacuated exetainer (Labco, England) for ^{15}N analysis. Gas samples were analyzed on a gas chromatograph equipped with ECD (N_2O, CO_2) and FID (O_2, CH_4) detector by standard gas chromatographic method (Mosier and Mack, 1980). The gas chromatograph (Shimadzu 14a) was equipped with a ^{63}Ni-electron capture detector ECD for N_2O and CO_2 (oven, valve and detector temperatures were operated at 65, 100 and 280°C) and flame ionization detector (FID) for O_2 and CH_4 estimation. The ^{15}N excess in N_2O was determined in separate samples by isotope-ratio mass-spectrometry (Stevens et al., 1993). The determination of the relative contribution of denitrification to the overall N_2O flux was calculated based on the Method by Stevens et al (1997). Briefly, the procedure assumes that N_2O is produced either by nitrification (NH_4^+ oxidation) and/or denitrification (NO_3^- reduction) using the following equation: $d = (a_m - a_n)/(a_d - a_n)$ with d, the fraction derived from the denitrification pool; (1-d) = fraction derived from nitrification, a_d, ^{15}N fraction of the NO_3^- pool; a_n, ^{15}N fraction of the NH_4^+ pool, a_m, ^{15}N fraction of the N_2O (mixture).

If the calculation results in a negative value then the ^{15}N abundance of the N_2O must have been lower than the ^{15}N abundance from either the NO_3^- or the NH_4^+ pool. Thus providing an indication that N_2O was produced by a third process that is not associated with the turnover of NH_4^+ and/or NO_3^- (see also, Rütting et al., 2010).

2.4 Denitrification Enzyme activity (DEA)

A set of twelve flasks (Brand) per sampling date (total of 8 sets) was arranged according to the treatments: i) two soils i.e. elevated CO_2 soil and ambient soil; ii) two C_2H_2 levels ($-C_2H_2$; $+C_2H_2$) with three repetitions per treatment. Acetylene was used to inhibit the reduction of N_2O to N_2 during denitrification and thus allowing estimation of total denitrification by measurement of the accumulated N_2O (Abbasi and Adams, 2000a). Prior to DEA analysis, twenty grams of soil at a moisture content of 0.41 g H_2O g^{-1} dry soil was pre-incubated at 20°C for 7 days after adding 100 µg N g^{-1} fresh soil (as NH_4NO_3) following experiment 1. DEA analysis was carried out in 250 mL flasks (Brand) with a septum fitted in the lid for gas sampling, using an anaerobic slurry technique as described by Müller et al. (2002). At the start of the assay 50 mL of a nitrate-glucose solution were applied to each flask resulting in concentrations of 50 µg NO_3^-–N g^{-1} (as KNO_3) and 300 µg C g^{-1} soil (as glucose). The bottles were immediately closed, evacuated and the headspace flushed (to atmosphere pressure) with pure N_2 with a double needle. Each evacuation and/or flushing lasted for 2 min and the internal atmosphere did not contain detectable oxygen, as confirmed by gas chromatography. In C_2H_2 treated flasks, 10% of headspace gas was removed and replace by adding 10 mL of C_2H_2 with a syringe and internal pressure was equilibrated to atmospheric pressure. The samples were placed at 20 °C on a rotary shaker at 120 rmp for a total of 40 min. The headspace atmosphere was removed (first sample) with 60 mL gas-tight syringes at 20 min. The extracted gas after the first sample was replaced by the same amount of N_2. Following continuous shaking, a second sample was taken after 40 min. Gas samples were analyzed for O_2, CH_4, CO_2, and N_2O on a gas chromatograph (GC) equipped with an FID and ECD detector (Mosier and Mack, 1980). DEA was calculated as the difference in N_2O concentration increase during a 20 min incubation (40-20 min), accounting for bottles, soil, media and water volume. The concentrations of the sampling were adjusted for dissolved gas in soil solution using the Bunsen coefficient (Moraghan and Buresh, 1977).

2.5 Statistical analysis

Statistical analysis was carried out with Sigmaplot in combination with Sigmastat (version 3.1, SPSS, Inc.). During the analysis test for normality and equal variance are carried out before running the ANOVA and tests to determine significant differences via the Holm-Sidak test.

3. Results

3.1 Effect of elevated atmospheric CO_2 on CO_2 emissions

Soil carbon dioxide fluxes before N application were 1.00–1.47 µg CO_2–C g^{-1} under ambient and 1.13–1.42 under elevated CO_2 (Fig. 1). During 7 days samplings (average), the fluxes were 1.22 and 1.27 µg CO_2-C g^{-1} in ambient and elevated CO_2 soils, respectively showing a non-significant response of elevated CO_2. Application of N fertilizer did not alter the CO_2 fluxes in both the soils: The maximum fluxes occurred during the first 14 days and thereafter CO_2 fluxes continuously decreased with incubation time. Over 57 days' sampling, CO_2 fluxes were on average 0.77 µg CO_2–C g^{-1} and 0.93 µg CO_2–C g^{-1} in ambient and elevated CO_2 soil, respectively indicating approximately 20% higher soil CO_2 emissions under elevated CO_2 than soil from ambient CO_2 but the differences were not significant ($p>0.05$).

3.2 Effect of elevated atmospheric CO_2 on CH_4 fluxes

Net CH_4 oxidation was observed in all samplings before and after N application (Fig. 1). The CH_4 oxidation rates before N application were −0.29 to −0.34 ng CH_4–C g^{-1} h^{-1} in ambient and -0.46 to –0.76 ng CH_4–C g^{-1} h^{-1} in elevated CO_2 soil indicating about a 22% higher oxidation rate in soil that had been under elevated CO_2. After N application, the rate of CH_4 oxidation increased from−0.21 to −3.1 ng CH_4–C g^{-1} h^{-1} in ambient and -0.45 to –4.26 ng CH_4–C g^{-1} h^{-1} in elevated CO_2. Maximum oxidation rates were observed 1 day after fertilizer application and increased steadily till 18–24 days of incubation. During this period the oxidation rates in the ambient control were –1.19 to –3.07 ng CH_4-C g^{-1} h^{-1} while under elevated CO_2 the rates were –1.79 to –4.18 ng CH_4–C g^{-1} h^{-1}. After day 24, the oxidation potential of soil decreased consistently to background level till the end of the incubation. On average over the incubation time, CH_4 oxidation rates before N application were –0.40 ng CH_4–C g^{-1} h^{-1} and became –1.46 ng CH_4–C g^{-1} h^{-1} after N application indicating a substantial increase in CH_4

oxidation with N fertilization. Average rates over sampling dates revealed that CH_4 oxidation in elevated CO_2 soil was –1.75 ng CH_4–C g^{-1} h^{-1} while the CH_4 oxidation in the ambient soil was –1.17 ng CH_4–C g^{-1} h^{-1} indicating a 49% higher CH_4 oxidation under elevated compared to ambient CO_2.

3.3 Effect of elevated atmospheric CO_2 on N_2O emissions

In the week before fertilizer N application N_2O emissions were 0.019 ng N_2O–N g^{-1} h^{-1} in the ambient and 0.023 ng N_2O–N g^{-1} h^{-1} in the elevated CO_2 soils (Fig. 2). N_2O fluxes did not show any consistent pattern with time. Likewise, N_2O fluxes did not differ between elevated CO_2 and ambient treatments and both showed similar fluxes. After N application the flux rates increased substantially and reached 0.280 and 0.240 ng N_2O–N g^{-1} h^{-1} at day 0. Over the 57 days, N_2O fluxes averaged 0.090 ng N_2O–N g^{-1} h^{-1} in ambient and 0.083 ng N_2O–N g^{-1} h^{-1} in elevated CO_2 (not significantly different) resulted in a 3- to 4-fold increase after N application. The highest fluxes of 0.281 and 0.240 ng N_2O-N g^{-1} h^{-1} were measured from ambient and elevated CO_2 treatments, respectively just after N application (day 0). The increase in emissions was short-lived (3-4 days) with fluxes returning to ‘background’ levels 30 days after N application.

3.4 ^{15}N enrichment of the N_2O

The ^{15}N enrichment of the N_2O in the soil increased one day after N fertilizer application together with the increase in N_2O concentrations (Fig. 3). Ten days after fertilizer N application, the enrichment of the N_2O was close to the enrichment in the applied N, indicating that the observed N_2O originated from the applied fertilizer. Comparing the ^{15}N enrichments in the N_2O from the ambient and elevated CO_2 soils, no significant difference was observed between the two soils labeled either with $NH_4{}^{15}NO_3$ or $^{15}NH_4NO_3$. The ^{15}N enrichment of the N_2O in the treatments where NO_3^- was labelled, were relatively higher than the treatment where NH_4^+ was labeled. The contribution of denitrification for N_2O production estimated by the 2-pool model of Stevens et al. (1997) indicated on day 1 after ^{15}N application a contribution of 16 and 32% under ambient and elevated CO_2 respectively. Negative values after 15 days showed that apart from N_2O contribution related to NH_4^+ and NO_3^- turnover a third process must have been in operation which was responsible for a dilution of the ^{15}N N_2O abundance below the ^{15}N abundance of NH_4^+ and NO_3^-.

3.5 Denitrification enzyme Activity, total denitrification and ratio of N_2–to–N_2O

The measurement of denitrification enzyme activity (DEA) by measuring N_2O emissions during short incubation periods (anaerobic), total denitrification (N_2O+N_2) and N_2/N_2O ratios was carried-out from both CO_2 treatments (Fig. 4). Before N application, one measurement was taken and DEA rates were 0.137 in ambient and 0.172 µg N_2O-N g^{-1} h^{-1} in elevated CO_2 soil while total denitrification (N_2O+N_2) was 0.456 in ambient and 0.514 µg N_2O-N g^{-1} h^{-1} in elevated CO_2 soil. The N_2/N_2O ratios were 3.33 for ambient and 2.99 for elevated CO_2 treatment. After N application, DEA rates (both N_2O and N_2O+N_2) increased in the first two samplings (day 0 and 1) but thereafter the rates continuously declined over time. DEA rates (N_2O fluxes) in the elevated CO_2 treatment were on average (20 days incubation) 16 % higher (0.149 vs 0.128 µg N_2O-N g^{-1} h^{-1}) than N_2O fluxes in the ambient CO_2 treatment. But the values of both treatments across different sampling days were not-significantly different. Total denitrification rates (N_2O+N_2) indicated significantly higher fluxes (36%) in elevated CO_2 treatment than in ambient CO_2 ($P \leq 0.05$). Similarly, the N_2 production was consistently higher under elevated CO_2 treatment and on average 54% higher than the N_2 production in the ambient CO_2 treatment (Fig. 5). The N_2/N_2O ratio was 1.02 in the ambient and 1.36 in the elevated CO_2 treatment showing a 33% higher ratio under elevated CO_2. Contribution of *d* (NO_3^-reduction) to total N_2O production at ambient and elevated CO_2 is shown in Fig. 5. Results indicated that shortly after N application N_2O production and reduction to N_2 substantially increased both in ambient and elevated CO_2 and the emissions decreased sharply with time. Elevated CO_2 stimulated both the N_2O production and reduction to N_2 compared to ambient CO_2.

4 Discussion

4.1. CO_2 production and methane oxidation

Over the 57-day period of observation, CO_2 flux between the ambient and elevated CO_2 treatments was not significant suggesting that CO_2 flux was not affected by elevated atmospheric CO_2. This was unexpected because in the field 25% higher CO_2 fluxes were observed under CO_2 enrichment possibly caused by the enhanced biomass and root biomass production and general higher activity under elevated CO_2 (Kammann et al., 2008). In this laboratory study soil from both ambient and elevated CO_2 treatments was incubated under similar conditions thus, any discrepancy between laboratory and field studies can be associated with plant effects which was also confirmed by ^{13}C studies in the FACE rings (Lenhart, 2008). Higher CO_2 fluxes under elevated CO_2 were also be observed in a number of other studies (Hungate et al. 1997; Arnone and Bohlen, 1998; Ambus and Robertson, 1999; Reich et al., 2001; Smith et al., 2010). However, there are also reports showing that ecosystem respiration (CO_2 flux) was not affected by elevated CO_2 (Ineson et al., 1998; Mosier et al., 2003). Hu et al. (2001) suggested that in the long term, soil microbial decomposition is slowed under elevated CO_2 because of N limitation and CO_2 production is either not affected or limited. In our study it was not the N limiting factor affecting CO_2 production in elevated CO_2 soils but some other unknown control factors which affected soil respiration.

Throughout the course of the experiment, the CH_4 oxidation potential was significantly greater in the elevated CO_2 (49%) than the ambient CO_2. These results were in contrast to the earlier studies where CH_4 consumption i.e. oxidation was lowered by an average of 17μg CH_4–C m^{-2} h^{-1} under elevated CO_2 (Ineson et al., 1998), more oxidation in ambient than elevated CO_2 soil (Mosier et al., 2003), or no effect of elevated CO_2 on CH_4 oxidation was observed (Mosier et al., 2002; Smith et al., 2010). Most of these studies were conducted under field conditions where two possibilities may tend to increase CH_4 production and decrease CH_4 oxidation i) increased soil moisture under elevated CO_2 constrain and slow down the diffusive CH_4 (and O_2) transport from the atmosphere to the water-film covered microbial population and therefore inhibit CH_4 oxidation (Dorr et al., 1993), ii) increased inhibition of CH_4 oxidation under elevated CO_2 or increased CH_4 production due to greater C availability in the soil under elevated CO_2 (van Kessel et al., 2000). In our study similar atmospheric conditions to both ambient and elevated CO_2 soils in the laboratory were maintained so that conditions for gas diffusion in the two soils were similar. Thus, the results from the study showed that the mechanism responsible for inhibiting CH_4 oxidation under elevated CO_2as observed under field conditions, were not operative under laboratory conditions.

Average net CH_4 oxidation rates after N application were 3-fold compared to fluxes before N application. These results are in contrast to earlier findings that the application of NH_4^+ reduced CH_4 oxidation rates almost immediately (forest soils, Steudler et al., 1989; short-grass steppe, Mosier et al. 1991; laboratory incubations, Hütsch, 1998; Tlustos et al., 1998; Ullah et al., 2008). The reduced CH_4 oxidation in the field was attributed to suppression in the population growth and lower abundance of methane oxidizers and to an inhibition of de-novo enzyme synthesis (Kolb et al., 2005). The higher oxidation rates by N addition in the present study are difficult to explain but they show that either the activity of methanotrophic bacteria was enhanced under elevated CO_2 possibly increased methanogenesis under conditions when mineral N was available (Kammann et al., 2001). Therefore, the kinetics of CH_4 oxidation/production is complex and their dependence on soil N status or moisture are crucial for an accurate prediction of net CH_4 oxidation.

4.2 N_2O emissions

The present investigation indicated a substantial increase in N_2O emissions after N application in both CO_2 treatments. Furthermore, results indicated that N_2O emissions in both the treatments (ambient and elevated) appeared to be limited by available N as fluxes in N fertilized soils increased 3-to 4-fold. Application of fertilizer N had a direct effect on N_2O production by supplying N for both nitrification and denitrification (e.g. Mosier, 1994; Clayton et al., 1997; Abbasi and Adams, 2000b) which may occur simultaneously (Abbasi and Adams, 2000a,b). Calculations of the contribution of denitrification on the total N_2O was carried out according to the method of Stevens et al. (1997). Results indicated that in the first 8 days of the experiment (when calculated contributions were still positive) the average contribution of denitrification to N_2O production was 17% higher under elevated compared to ambient CO_2 (i.e. ambient CO_2: 8% and under elevated CO_2: 25%) (Fig. 5). This method is based on the assumption that only nitrification and dentirification contribute to the observed N_2O production. However, as shown by Rütting et al. (2010) via a ^{15}N tracing study in the New Zealand grassland FACE not only nitrification and denitrification but also heterotrophic processes, metabolizing organic N, may contribute to N_2O production. This is also the reason for negative values found in this study from day 15–20 onwards (Fig. 5), which occur in situation when the N_2O ^{15}N abundance is below the ^{15}N abundance of NH_4^+ and NO_3^-, thus indicating that a third source at natural or low abundance contributed to the N_2O emissions. Rütting et al. (2010) showed that denitrification increased from 4.7% to 8% under elevated CO_2, a similar trend was observed in our study.

The N_2O emissions observed before and after N application showed that elevated CO_2 did not show any significant effect on N_2O fluxes, rates of fluxes (average) were almost similar. The pre-existing organic fractions and resulting differences in microbial activity and dynamics under elevated

CO_2 treatment (Kammann et al., 2008) could have had an effect on N_2O production but did not contribute to higher N_2O fluxes under elevated CO_2. This finding is in line with Rütting et al. (2010) who found no statistical evidence that elevated CO_2 stimulated N_2O production in grassland soil exposed to elevated CO_2 for 10 years. Similarly, Barnard et al. (2005) concluded from a review of 20 experiments that field N_2O fluxes were not altered by elevated CO_2. However, in several studies it has been shown that N_2O flux rates were increased by elevated CO_2. Ineson et al. (1998) found 27% higher N_2O emissions in grassland exposed to elevated CO_2. Similarly, in perennial grassland N_2O fluxes under elevated CO_2 were double than those observed under ambient CO_2 (Arnone and Bohlen, 1998). In contrast, there are also reports that elevated CO_2 either did not alter N_2O fluxes, or even reduced N_2O emissions (Hungate et al., 1997; Mosier et al., 2002, 2003; Welzmiller et al., 2008). Baggs and Blum (2004) reported that the response of N_2O emissions to elevated CO_2 in grass swards depend on the rate of N application. The response was non-significant at low rates while N_2O emissions significantly increased under elevated CO_2 when high rates of N fertilizer were applied. Observations in the Giessen FACE study were unexpected because enhanced N_2O emissions in the elevated CO_2 treatment (vs ambient) were only observed during times of low N availability (Kammann et al., 2008). After N fertilizer application N_2O emissions were not different between ambient and elevated CO_2. As highlighted by Kammann et al. (2008) it is important to take the temporal dynamics of N_2O emissions into account, which may identify time periods when N_2O emissions are significantly higher and those which are not. Thus our results from the laboratory study and from other studies are only representative for certain time periods and are not representative of general response patterns.

N_2O production and its concentration in atmosphere are linked to the soil N turnover (mineralization, nitrification, denitrification) (Müller et al., 2009; Rütting et al., 2010). The net and gross nitrification rates decreased while DEA (which is carried out under non-N limiting conditions) did not show any significant increase under elevated CO_2 (Kammann et al., 2008). Thus the potential for net N_2O production is not affected by elevated CO_2. Together with the increase in the contribution of denitrification to the overall N_2O flux this result indicates that the N_2O reductase activity must have been higher under elevated CO_2. Thus to confirm this, the N_2-to-N_2O ratio was determined under optimum conditions for denitrification.

4.3 Total denitrification and N_2–to–N_2O ratio

Stimulation of denitrification and N_2-to-N_2O ratios was also observed from the soil incubation studies. However, apart from CO_2 the magnitude of emissions varies depending on type and timing of inorganic fertilizer application, soil temperature, moisture content, soil type which will vary throughout the year (Baggs et al., 2003a; Kammann et al., 2008).

Denitrification enzyme activity (DEA) was on average 16% higher in the elevated CO_2 than in the ambient treatment but the difference between the two CO_2 treatments was not significantly different, suggesting that elevated CO_2 had only a limited effect on the quantity of active denitrifying enzymes present in the soil. These results were in line with findings of Barnard et al. (2004) who also reported very little response of DEA to CO_2 treatment in German grassland soils. However, the total denitrification (N_2O+N_2) and the ratio of N_2–to–N_2O were significantly higher (36% and 33%) under elevated CO_2 compared to the ambient treatment. In general the ratio under elevated CO_2 (average 1.358) was similar to the ranges reported by Rolston et al. (1976) (0.1–40) but were lower than the ratios (345 and 410) reported by Baggs et al. (2003b).Thus, under elevated CO_2 and under non-N limiting conditions most likely a stimulation of the N_2O reductase occurred while under N limiting conditions the higher N_2O emissions under elevated CO_2 may be related to a higher nirK/nosZ ratio (nitrite/nitrous oxide reductase) as observed by Regan et al. (2011) for this soil. Thus our studies provide indirect evidence that the kinetics of the reductase systems during denitrification in this grassland soils are linked to the enhanced C input in connection to N-oxide availability (Dendooven et al., 1994).

The maximum ratios in both the treatments were found shortly after N application at day 0 in contrast to Baggs et al. (2003b) who found very low N_2–to–N_2O ratios till 8 days after fertilizer application and proposed different times lag for N_2 and N_2O production. Our results are in line with observations by Welzmiller et al. (2008) who also found constantly higher N_2 –to- N_2O ratios under elevated CO_2. The higher N_2–to–N_2O ratios under elevated CO_2 emphasize the need for the consideration of N_2 measurements in future denitrification studies and showed that despite a non significant response to N_2O total denitrification may be altered. This might be due to a shift of the denitrifier community under elevated CO_2 which exhibit different of the different reductase dynamics during denitrification (Regan et al., 2011).

5. Conclusions

Most of the studies conducted so far have suggested higher N_2O emissions under elevated CO_2 while very few reported no response. We observed no statistically significant CO_2 enrichment effect on fluxes of CO_2 and N_2O in the laboratory study which was carried out under non-N limiting conditions. However, the relative rate of N_2O from denitrification and the N_2-to-N_2O ratio changed under elevated CO_2. Thus, elevated CO_2 appears to have an impact on the denitrification kinetics in this grassland soil which was also confirmed by molecular studies of this soil (Regan et al., 2011) The enhanced CH_4 oxidation under elevated CO_2 is surprising and shows that the potential for CH_4 oxidation may increase in this soil. However, this effect was not observed in the field suggesting that the combination of N application in combination with the environmental regulators (e.g. moisture, temperature) which were held constant in the current study have an impact under field conditions.

The understanding of the stimulation of the microbial populations and activity of methanogenics and methanotrophic bacteria in response to changing substrate and abiotic factors are essential to predict the net CH_4 oxidation in terrestrial ecosystem under elevated CO_2. A mechanistic understanding of changes in the N cycle and associated GHG production under elevated atmospheric CO_2 concentrations is essential to predict GHG dynamics under climate change. Therefore, while this study does not directly contribute to a better understanding of atmospheric processes, it still can elucidate some of main drivers governing the exchange of GHGs between soil and the atmosphere which will aid the development of models that are aiming to simulate GHG dynamics.

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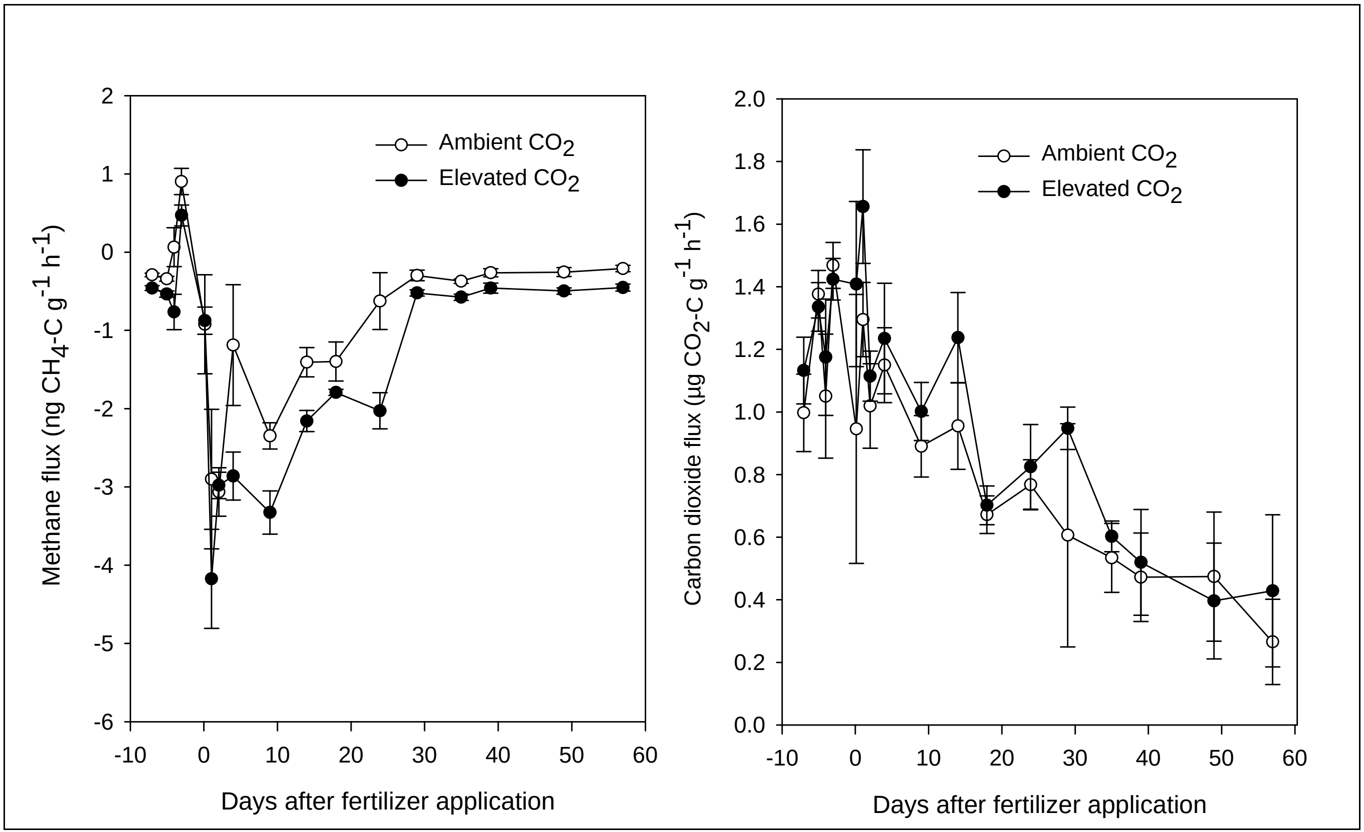

Fig. 1. Daily fluxes of CH_4 (ŋg CH_4–C g^{-1} h^{-1}) and CO_2 (µg CO_2- g^{-1} h^{-1}) (Avg. ±SD) from temperate grassland soil exposed to elevated CO_2 and soil without elevated CO_2 treatment i.e. ambient incubated under controlled laboratory conditions following the application of $NH_4{}^{15}NO_3$ and $^{15}NH_4NO_3$

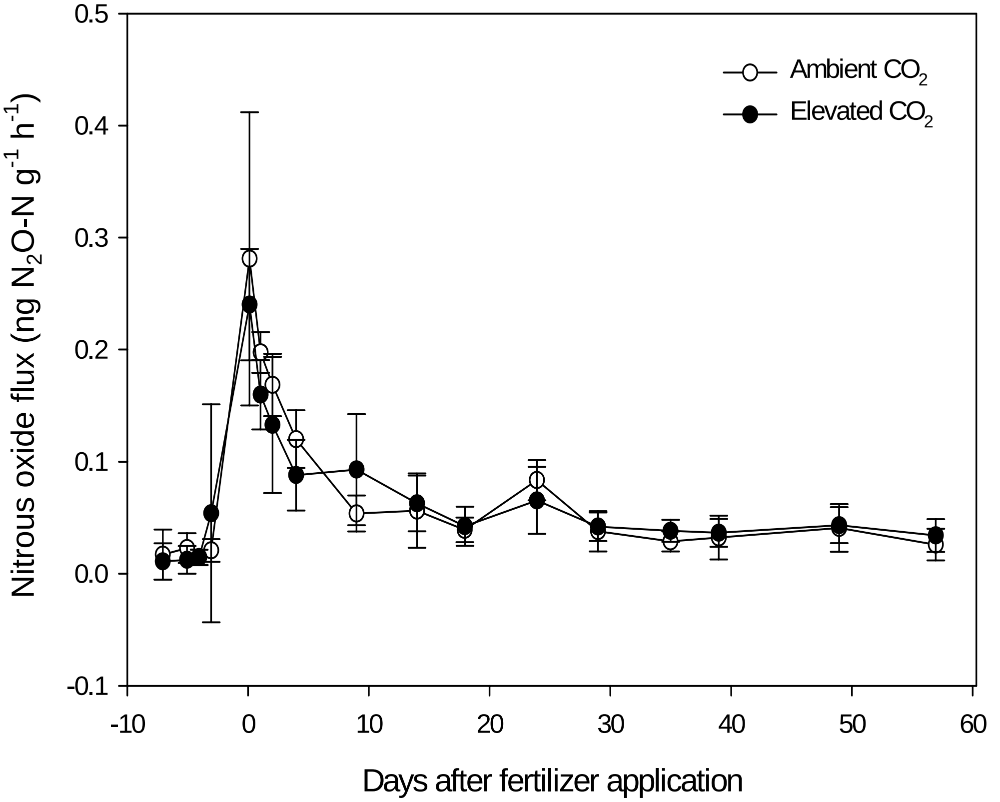

Fig. 2. Daily fluxes of N_2O (ŋg N_2O–N g^{-1} h^{-1}) (Avg. ±SD) from temperate grassland soil exposed to elevated CO_2 and soil without elevated CO_2 treatment i.e. ambient incubated under controlled laboratory conditions following the application of $NH_4{}^{15}NO_3$ and $^{15}NH_4NO_3$

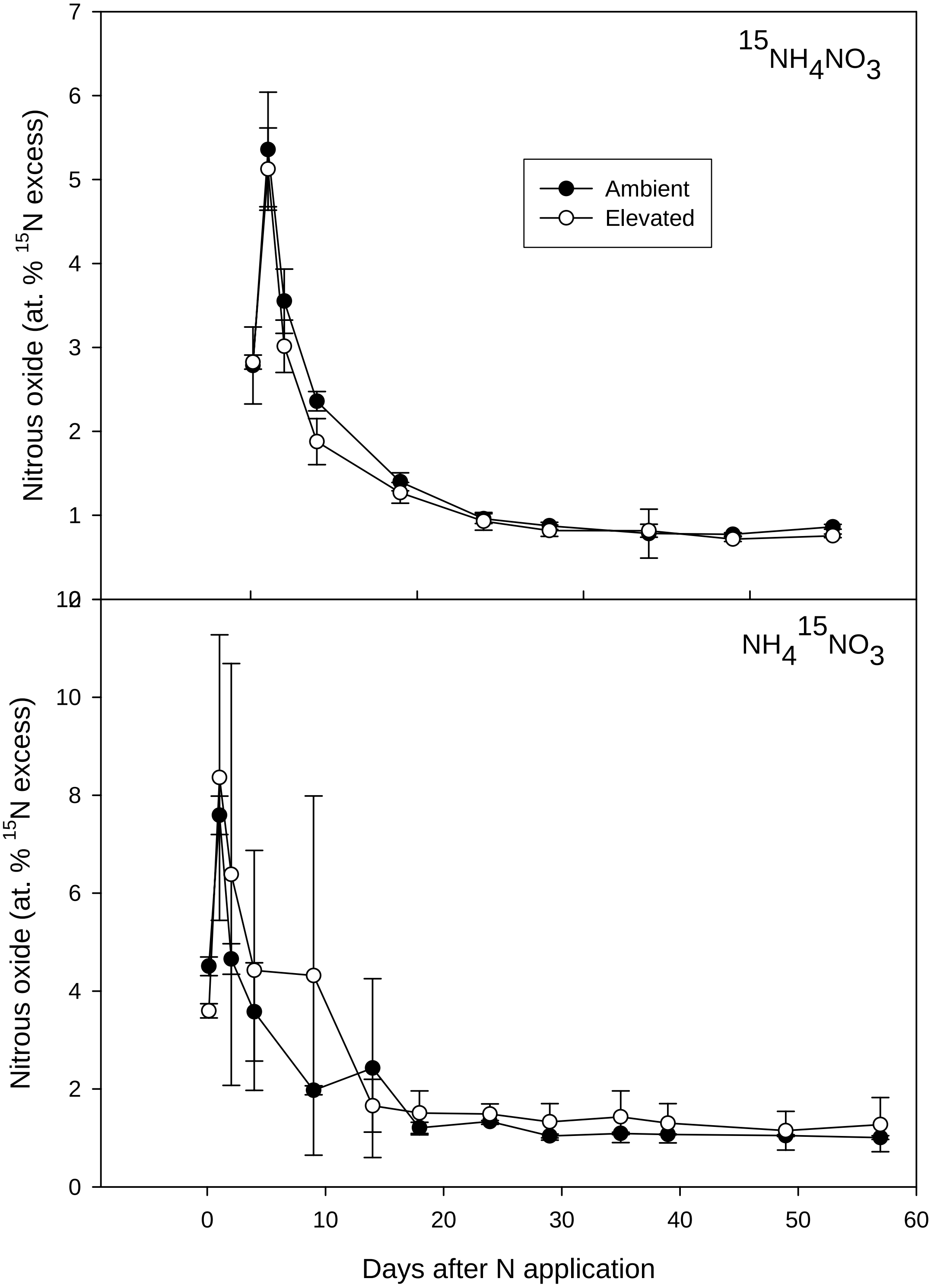

Fig. 3. Nitrous oxide (N_2O) enrichments (Avg. ±SD) in a temperate grassland soil exposed to elevated CO_2 and soil without elevated CO_2 treatment i.e. ambient following N fertilizer application where the nitrate pool ($NH_4{}^{15}NO_3$) and the ammonium pool ($^{15}NH_4NO_3$) were labelled with ^{15}N at 60 atom% excess

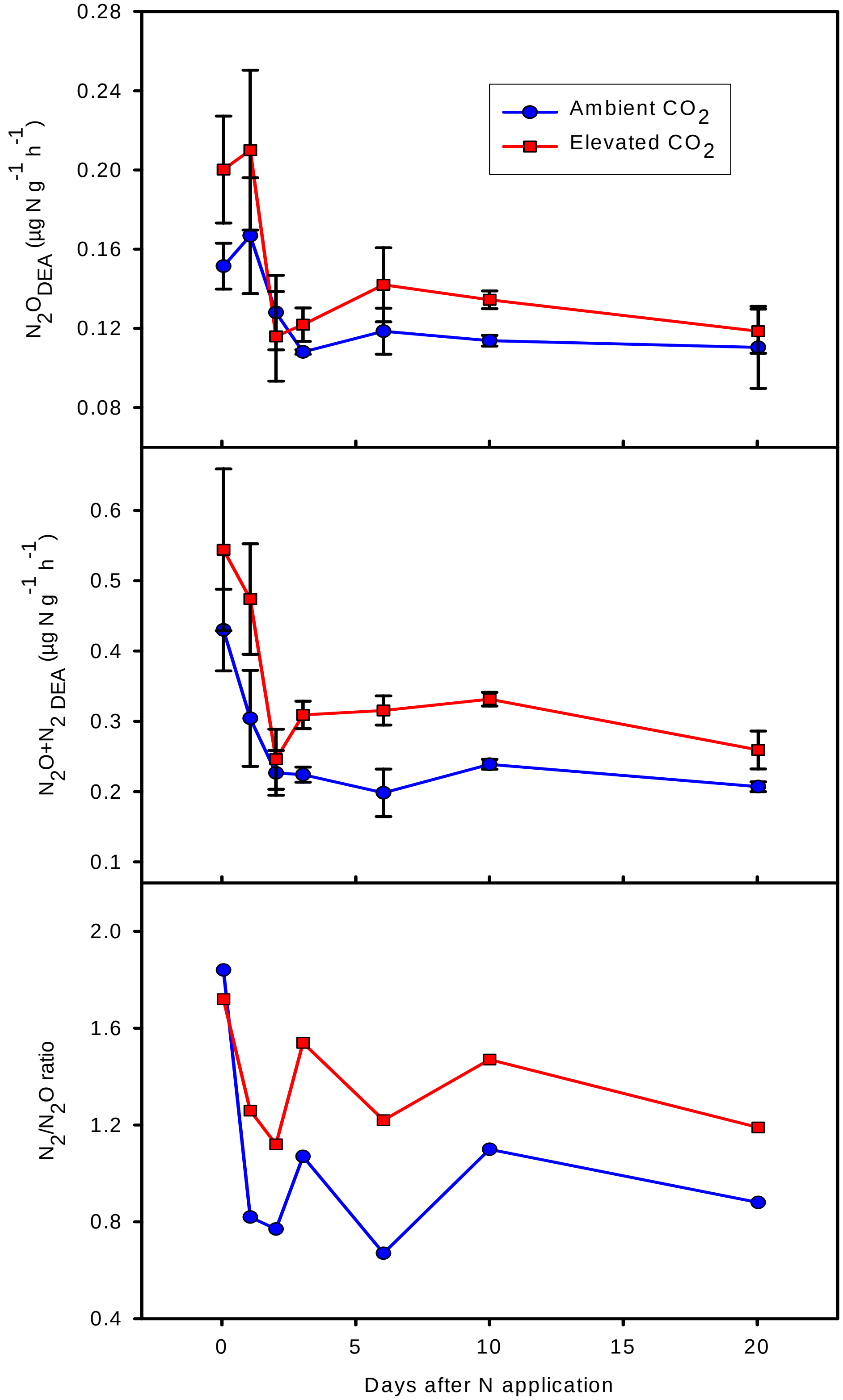

Fig. 4. Emission of N_2O, total denitrification (N_2O+N_2) (µg N g^{-1}h^{-1}) and N_2/N_2O ratio (AVG ±SD) from temperate grassland soil exposed to elevated CO_2 and soil without elevated CO_2 treatment i.e. ambient incubated under controlled laboratory conditions following the application of $NH_4{}^{15}NO_3$ and $^{15}NH_4NO_3$

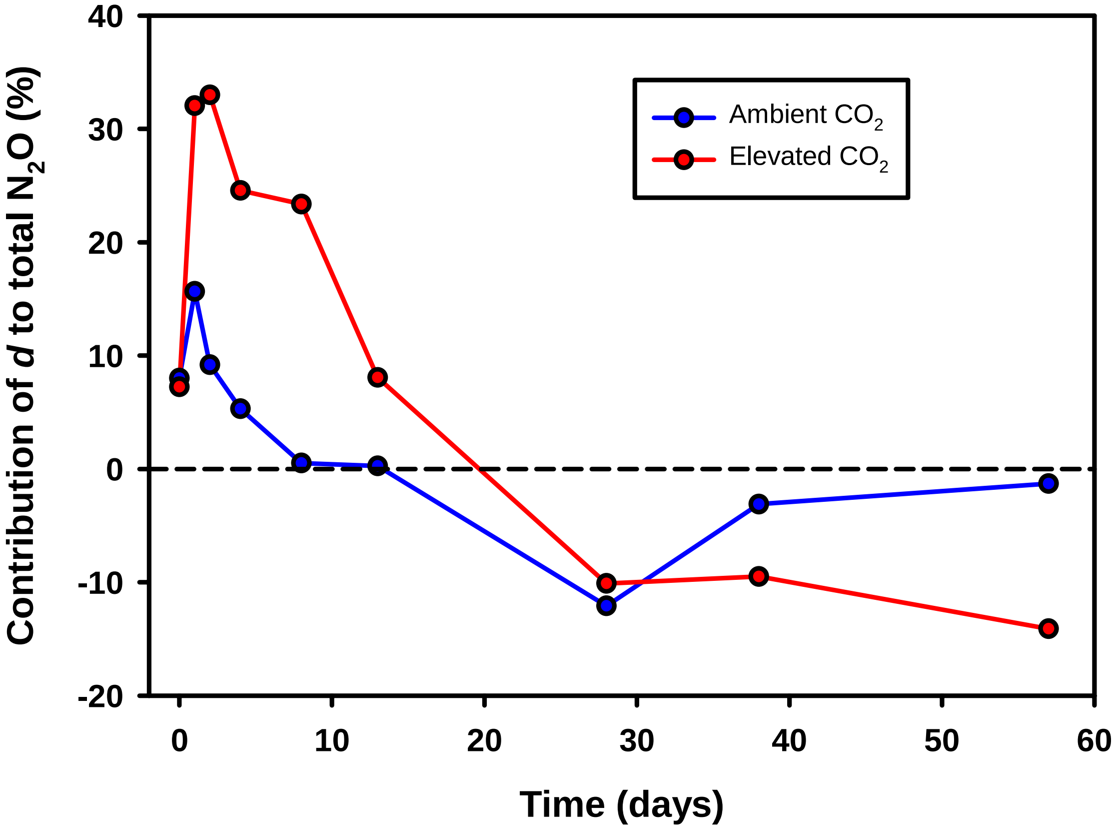

Fig 5. Contribution of *d* (NO_3^-reduction) to total N_2O production in grassland soil at ambient and elevated CO_2.