

**Gaseous emissions
under elevated CO₂**

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Trace gas fluxes of CO₂, CH₄ and N₂O in a permanent grassland soil exposed to elevated CO₂ in the Giessen FACE study

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Abstract

Long-term field observations showed that N₂O fluxes were not significantly affected by elevated CO₂ shortly after N application in the Giessen Free Air Carbon dioxide Enrichment (FACE) study. To further investigate this unexpected result a ¹⁵N tracer study was carried out under controlled conditions where in parallel treatments either the NH₄⁺ pool (¹⁵NH₄NO₃) or the NO₃⁻ pool (NH₄¹⁵NO₃) was enriched with ¹⁵N. Fluxes of CO₂, CH₄, and N₂O as well as the ¹⁵N enrichment of the N₂O were measured. Denitrifying Enzyme Activity (DEA), total denitrification (N₂ + N₂O) and N₂-to-N₂O ratios were quantified in separate experiments. Over the 57 day incubation, N₂O fluxes averaged 0.090 ng N₂O-N g⁻¹ h⁻¹ under ambient and 0.083 ng N₂O-N g⁻¹ h⁻¹ under elevated CO₂ (not significantly different). Based on the ¹⁵N enrichments of the N₂O the N₂O production processes were identified by a two-source model. Results showed that N₂O must have also been produced by a third source – possibly related to organic N transformation – which was stimulated by elevated CO₂. Soil CO₂ fluxes were approximately 20% higher under elevated CO₂ than soil from ambient but the differences were not significant. CH₄ oxidation rates were on average -1.75 ng CH₄-C g⁻¹ h⁻¹ in the elevated and -1.17 ng CH₄-C g⁻¹ h⁻¹ in the ambient indicating that elevated CO₂ increased the CH₄ oxidation by 49% compared to ambient CO₂. N fertilization increased CH₄ oxidation by 3-fold in both CO₂ treatments CO₂ did not have any significant effect on DEA while total denitrification and N₂-to-N₂O ratios increased by 36 and 33%, respectively. The results indicate that shortly after N application elevated CO₂ must have stimulated both the N₂O production and reduction to N₂ to explain the increased N₂-to-N₂O ratio and at the same time explain the non-responsiveness of the N₂O emissions. Thus, the observed variation of the CO₂ effect on N₂O emissions throughout the year is possibly related to the dynamics of the N₂O reductase activity.

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1 Introduction

The level of earth's atmospheric carbon dioxide (CO_2) concentration has risen from $\sim 280 \mu\text{L L}^{-1}$ at the start of the industrial revolution to greater than $385 \mu\text{L L}^{-1}$ today, and is expected to exceed $700 \mu\text{L L}^{-1}$ by the end of this century (Intergovernmental Panel on Climate Change, 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. Accumulation of radiatively active gases in the atmosphere could alter the earth's atmosphere energy balance, and has been linked to recent warming trends in global climate (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 stratospheric ozone depletion and 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). 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 seasonal soil CO_2 flux in an arable soil was significantly greater under elevated CO_2 being in the range of 15% to 50% compare to ambient CO_2 .

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In addition to soil CO₂ flux, elevated atmospheric CO₂ can affect other greenhouse and reactive trace gases i.e. CH₄ and N₂O and studies so far provide contradictory results. Ineson et al. (1998) measured fluxes of N₂O, CH₄ and CO₂ from soils under ambient and elevated CO₂ at the Swiss FACE experiment in plots of *Lolium perenne* and reported increased N₂O emissions by 27% under elevated CO₂ while ambient plots oxidized consistently more CH₄ than the elevated plots indicating that elevated CO₂ may result in the inhibition of CH₄ oxidation. Cheng et al. (2006) reported a 58% increase in CH₄ flux from rice paddies under elevated CO₂. This increase was attributed to greater root exudates and numbers of tillers, resulting in more surface area for the release of CH₄ 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 N₂O and CO₂ fluxes under elevated CO₂ were 2–3 times higher than those observed in ambient CO₂. This increase was attributed to increased belowground C allocation in elevated CO₂ providing energy for denitrifiers or that there is increased O₂ consumption under elevated CO₂. However, Mosier et al. (2002) conducted an open-top-chamber CO₂ enrichment study in the Colorado shortgrass steppe and reported that even though both C₃ and C₄ plant biomass increased and soil moisture content was typically higher under elevated CO₂, none of the trace gas fluxes were significantly altered by CO₂ enrichment over the 43 months period of observation. Similarly, N₂O fluxes was not affected by elevated CO₂ in a paddy, arable and grassland fields (Cheng et al., 2006; Smith et al., 2010; Dijkstra et al., 2010). However, Kettunen et al. (2006) showed that elevated CO₂ increased both N₂O flux from soil and soil water content.

A significant increase of N₂O emissions under elevated atmospheric CO₂ 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, the N₂O stimulation in this N limited grassland ecosystems occurred throughout the vegetation period when mineral N supply was limited, while in the period following N application no significant difference in N₂O emissions was detected. Differences in N cycling and/or stimulation of different microbial groups under

elevated CO₂ were made responsible for the observed results. A ¹⁵N tracing study with soil taken from the Giessen FACE study showed that under elevated CO₂ the turnover of N changed towards a higher N cycling speed (Müller et al., 2009). To explain the CO₂ response on N₂O it is particularly important to study in detail the periods following N fertilizer application because these are times when high N₂O emissions occur. Thus the objective of this study was to identify the effect of CO₂ on N₂O emissions and identify the processes of N₂O production as well as the effect on the N₂-to-N₂O ratios.

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 a.s.l. near Giessen, Germany. The semi-natural non-grazed grassland has been managed extensively as a meadow for at least 50 years, fertilized with 50–80 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 Stagnofluvic Gleysol on loamy-sandy sediments over clay (Kammann et al., 1998). 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 (organic C 6.6; pH 6.2). The soil was taken from the ambient and elevated FACE rings where also soil had been sampled for the ¹⁵N tracing study described by Müller et al. (2009) (see this publication for more details). Fresh soil was sieved

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(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 CO₂ soil and ambient soil; (ii) two N sources i.e. ¹⁵NH₄NO₃ or NH₄¹⁵NO₃ (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 H₂O 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 CO₂) were labelled with ¹⁵N 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 H₂O g⁻¹ dry soil. The jars were covered with parafilm that was perforated with a needle to facilitate gas exchange and incubated at 20 °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). Gas samples were analyzed on a gas chromatograph equipped with ECD (N₂O, CO₂) and FID (O₂, CH₄) detector by standard gas chromatographic method (Mosier and Mack, 1980). The gas chromatograph (Shimadzu 14a) was equipped with a ⁶³Ni-electron capture detector ECD for N₂O and CO₂ (oven, valve and detector temperatures were operated at 65, 100 and 280 °C) and flame ionization detector (FID) for O₂ and CH₄ estimation. The ¹⁵N excess in N₂O was determined in separate samples by isotope-ratio mass spectrometry (Stevens et al., 1993). The procedure assumes that N₂O is produced either via nitrification (NH₄⁺ oxidation) and/or denitrification (NO₃⁻ reduction). A negative

value would indicate that the enrichment of the N_2O is lower than the enrichment of the NH_4^+ and the NO_3^- 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^- .

2.4 Denitrification Enzyme Activity (DEA)

5 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 ($-\text{C}_2\text{H}_2$; $+\text{C}_2\text{H}_2$) with three repetitions per treatment. Prior to DEA analysis, twenty grams of soil at a moisture content of 41% (vol/vol) was pre-incubated at 20°C for 7 days after adding $100\ \mu\text{g}\ \text{N}\ \text{g}^{-1}$ fresh soil (as NH_4NO_3) following experiment

10 1. DEA 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 $50\ \mu\text{g}\ \text{NO}_3^- \text{N}\ \text{g}^{-1}$ (as KNO_3) and $300\ \mu\text{g}\ \text{C}\ \text{g}^{-1}$ soil (as glucose). The bottles were immediately closed, evacuated and the headspace flushed

15 (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 occasionally confirmed by gas chromatography. In C_2H_2 treated flasks, 10% of headspace gas was removed and replaced by adding 10 mL of C_2H_2 with a syringe and internal pressure was equilibrated to atmospheric pressure. The samples were placed

20 at 20°C on a rotary shaker at 120 rpm 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 analysed 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).

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2.5 Statistical analysis

Statistical analysis was carried out with Sigmaplot in combination with Sigmastat (version 3.1, SPSS, Inc.).

3 Results

3.1 Effect of elevated atmospheric CO₂ on CO₂ emissions

Soil carbon dioxide fluxes before N application were 1.00–1.47 $\mu\text{g CO}_2\text{-C g}^{-1}$ under ambient and 1.13–1.42 under elevated CO₂ (Fig. 1). During 7 days samplings (average), the fluxes were 1.22 and 1.27 $\mu\text{g CO}_2\text{-C g}^{-1}$ in ambient and elevated CO₂ soils, respectively showing a non-significant response (3.6%) of elevated CO₂. Application of N fertilizer did not alter the CO₂ fluxes in both the soils: The maximum fluxes occurred during the first 14 days and thereafter CO₂ fluxes continuously decreased with incubation time. Over 57 days' sampling, CO₂ fluxes were on average 0.77 $\mu\text{g CO}_2\text{-C g}^{-1}$ and 0.93 $\mu\text{g CO}_2\text{-C g}^{-1}$ in ambient and elevated CO₂ soil, respectively indicating approximately 20% higher soil CO₂ emissions under elevated CO₂ than soil from ambient CO₂ but the differences were not significantly different ($p > 0.05$).

3.2 Effect of elevated atmospheric CO₂ on CH₄ fluxes

Net CH₄ oxidation was observed in all samplings before and after N application (Fig. 1). The CH₄ oxidation rates before N application were -0.29 to -0.34 $\eta\text{g CH}_4\text{-C g}^{-1} \text{h}^{-1}$ in ambient and -0.46 to -0.76 $\eta\text{g CH}_4\text{-C g}^{-1} \text{h}^{-1}$ in elevated CO₂ soil indicating about a 22% higher oxidation rate in soil that had been under elevated CO₂. After N application, the rate of CH₄ oxidation increased from -0.21 to -3.1 $\eta\text{g CH}_4\text{-C g}^{-1} \text{h}^{-1}$ in ambient and -0.45 to -4.26 $\eta\text{g CH}_4\text{-C g}^{-1} \text{h}^{-1}$ in elevated CO₂. Maximum oxidation rates were observed 1 day after fertilizer application and occurred at constant rates till

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18–24 days of incubation. During this period the oxidation rates in the ambient control were -1.19 to $-3.07 \text{ ng CH}_4\text{-C g}^{-1} \text{ h}^{-1}$ while in elevated CO_2 the rates were -1.79 to $-4.18 \text{ ng CH}_4\text{-C g}^{-1} \text{ h}^{-1}$. 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 $-0.40 \text{ ng CH}_4\text{-C g}^{-1} \text{ h}^{-1}$ and became $-1.46 \text{ ng CH}_4\text{-C g}^{-1} \text{ h}^{-1}$ 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 $-1.75 \text{ ng CH}_4\text{-C g}^{-1} \text{ h}^{-1}$ while the CH_4 oxidation in the ambient soil was $-1.17 \text{ ng CH}_4\text{-C g}^{-1} \text{ h}^{-1}$ 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 $0.019 \text{ ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ in the ambient and $0.023 \text{ ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ 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 i.e. 0.019 and $0.023 \text{ ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ (average). After N application the fluxes rates increased substantially and reached 0.280 and $0.240 \text{ ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ at day 0. Over the 57 days, N_2O fluxes averaged $\text{ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ in ambient and $0.083 \text{ ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ 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 $0.240 \text{ ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ 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.

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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 $\text{NH}_4^{15}\text{NO}_3$ or $^{15}\text{NH}_4\text{NO}_3$. 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 ($\text{N}_2\text{O} + \text{N}_2$) and $\text{N}_2/\text{N}_2\text{O}$ 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 $\mu\text{g N}_2\text{O-N g}^{-1} \text{h}^{-1}$ in elevated CO_2 soil while total denitrification ($\text{N}_2\text{O} + \text{N}_2$) was 0.456 in ambient and 0.514 $\mu\text{g N}_2\text{O-N g}^{-1} \text{h}^{-1}$ in elevated CO_2 soil. The $\text{N}_2/\text{N}_2\text{O}$ ratios were 3.33 for ambient and 2.99 for elevated CO_2 treatment. After N application, DEA rates (both N_2O and $\text{N}_2\text{O} + \text{N}_2$) 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

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were on average (20 days incubation) 16% higher (0.149 vs. $0.128 \mu\text{g N}_2\text{O-N g}^{-1} \text{h}^{-1}$) 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 ($\text{N}_2\text{O} + \text{N}_2$) 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 $\text{N}_2/\text{N}_2\text{O}$ 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 averaged 0.77 in ambient and $0.93 \mu\text{g N}_2\text{O-N g}^{-1} \text{h}^{-1}$ in elevated CO_2 treatment showing a 20% increase in CO_2 fluxes under elevated atmospheric CO_2 . But the differences between the two soils were non-significant suggesting that CO_2 flux was not affected by elevated atmospheric CO_2 . The observed effect from the soil having 25% high CO_2 concentration and under CO_2 enrichment for the last 6 years was unexpected since the amount of C entering the soil is generally considered to be higher because of higher rhizodeposition and microbial activity. Although soil from both ambient and elevated CO_2 treatments was incubated under similar conditions, yet the pre-existing organic fractions and microbial differences may have had a substantial effect on CO_2 emissions which was not the case in the present study. This is not in agreement with the previous observations

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where CO₂ fluxes under elevated CO₂ were significantly higher compared to ambient CO₂ (Hungate et al., 1997; Arnone and Bohlen, 1998; Ambus and Robertson, 1999; Reich et al., 2001; Smith et al., 2010). However, there are reports showing that ecosystem respiration (CO₂ flux) was not affected by elevated CO₂ (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 CO₂ because of N limitation and CO₂ production is either not affected or limited. In our study there was no difference in N supply because equal amount of N fertilizer was applied in both the soils. Both the soils had shown similar CO₂ production potential indicating that it is not the N limiting factor affecting the CO₂ production in elevated CO₂ soils but some other unknown control factors.

Throughout the course of experiment, net CH₄ oxidation was observed in all samplings before and after N application. In both the cases, CH₄ oxidation potential was significantly greater in the elevated CO₂ (49%) than the ambient CO₂. These results were in contrast to studies where either reduced CH₄ emissions (Ineson et al., 1998; Cheng et al., 2006) more oxidation in ambient than elevated CO₂ soil (Mosier et al., 2003), or no effect of elevated CO₂ on CH₄ oxidation was observed (Mosier et al., 2002; Smith et al., 2011). Most of these studies were conducted under field conditions where two possibilities may tend to increase CH₄ production and decrease CH₄ oxidation (i) increased soil moisture under elevated CO₂ constrain and slow down the diffusive CH₄ (and O₂) transport from the atmosphere to the water- film covered microbial population and therefore inhibit CH₄ oxidation (Dorr et al., 1993), (ii) inorganic N pools tended to be higher in the elevated CO₂ soil than in the ambient CO₂ soil. The higher concentration of either NH₄⁺ or NO₃⁻ may inhibit CH₄ oxidation (Stendler et al., 1989; Reay and Nedwell, 2004). We provided similar atmospheric conditions to both ambient and elevated CO₂ soils in the laboratory and oxidation of CH₄ throughout the experiment supported the idea that evidently the mechanism responsible for inhibiting CH₄ oxidation in response to elevated CO₂ in the field was not operative under laboratory conditions. Reduced CH₄ oxidation in response to elevated CO₂ soil generally linked to lower diffusion rates. However, under field conditions in the same study site,

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Kammann et al. (2001) explained that moisture level does not play major role on CH₄ oxidation/production but the depth of the CH₄ producing horizon in combination with the duration of sub-surface CH₄ production contribute to the overall CH₄ oxidation rate at the soil surface, conditions which are not present under laboratory conditions.

5 Average of both -N and +N soils over time indicated oxidation rates of -0.4 $\eta\text{g CH}_4\text{-C g}^{-1}\text{ h}^{-1}$ in control (-N) and -1.46 $\eta\text{g CH}_4\text{-C g}^{-1}\text{ h}^{-1}$ in N added (+N) soil indicating a 3-fold increase in CH₄ oxidation following N application. These results are in contrast to earlier findings that the application of NH₄⁺ reduced CH₄ oxidation rates almost immediately (forest soils, Steudler et al., 1989; short-grass steppe, Mosier et al., 1991; 10 Laboratory incubations, Hütsch, 1998; Tlustos et al., 1998; Ullah et al., 2008). They attribute this delay to suppression in the population growth of methane oxidizers and to an inhibition of de-novo enzyme synthesis. Kammann et al. (2001) found no relationship between the N fertilizer and CH₄ oxidation rates during field study of the same site. The high oxidation rates by N addition in the present study might be that 15 after 6–7 years of FACE establishment, it is unlikely to have any inhibitory effect by elevated CO₂ (Kammann et al., 2001). Most of the researchers reported inhibition of CH₄ oxidation by NH₄⁺ not by NO₃⁻ and accumulation of NO₃⁻ after nitrification might slow down the inhibitory effect of N fertilizer. Steudler et al. (1989) reported that nitrification is responsible for CH₄ oxidation and nitrifying bacteria including the dominant 20 soil ammonium-oxidizing bacterium *Nitrosomonas europaea*, have the ability to oxidize CH₄. The hypothesis of higher CH₄ oxidation by nitrification could not be justified in our study. We in our mineral N study found significantly more accumulation of NO₃⁻ in ambient than elevated CO₂ while CH₄ oxidation were 49% higher under elevated CO₂. Reay and Nadwell (2004) found a differential reduction in CH₄ oxidation by NO₃⁻, 25 rather than NH₄⁺. Therefore, the kinetics of CH₄ oxidation/production is complex and their dependence on soil N status or moisture remains an area of some controversy.

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4.2 N₂O emission

Results of the previous study on the Giessen FACE site indicated significant increase in N₂O emissions throughout the vegetation period when mineral N supply was limited, while in the period following N application no significant difference in N₂O emissions was detected (Kammann et al., 2008). However, estimation of N₂O emission was only one time period in the Kammann et al. (2008) study. Moreover, ¹⁵N tracing study showed that the N turnover changed towards a higher N cycling speed under elevated CO₂ (Müller et al., 2009). To explain the CO₂ response of N₂O it is particularly important to study in detail the periods following N fertilizer application because these are times when high N₂O emissions occur. The experimental conditions resemble the period after N fertilizer application. Kammann et al. (2008) showed that N₂O emissions from field observations of the Giessen FACE study were not significantly different during the period following N application which is in line with the results from this laboratory incubation.

Long-term incubation studies in the present investigation indicated a substantial increase in N₂O emissions after fertilizer N application. Results indicated that N₂O 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 would have had a direct influence on N₂O production by provision of N for both nitrification and denitrification. The two processes can occur simultaneously and produce N₂O in ecosystem (Abbasi and Adams, 2000a, b). Emissions of N₂O have previously been shown to increase after application of inorganic fertilizer (e.g. Mosier, 1994; Clayton et al., 1997; Abbasi and Adams, 2000b). However, the magnitude of emissions varies depending on type, method and timing of inorganic fertilizer application, soil temperature, moisture content, soil type (Baggs et al., 2003a; Khalil et al., 2009; Sistani et al., 2010). Calculations of the contribution that denitrification had on the total N₂O gas carried out according to Stevens et al. (1997). Results showed that the contribution of denitrification was higher under elevated CO₂. Furthermore results showed that

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apart from nitrification and denitrification a third source for N₂O, which was not related to NH₄⁺ and/or NO₃⁻ turnover, must have been active. A recent study on the effect of CO₂ on N cycling showed that oxidation of organic N may be an important process for N₂O production in permanent grassland soils (Rütting et al., 2010). They showed that denitrification increased from 4.7 to 8%. A similar trend was observed in our study. Stimulation of denitrification and N₂-to-N₂O ratios was also observed from the soil incubation studies. However, apart from CO₂ 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).

The N₂O emissions observed before and after N application showed that elevated CO₂ did not show any significant effect on N₂O fluxes and rates of fluxes (average) were almost similar. Both (elevated and ambient) soils were incubated under similar moisture and temperature condition, so that pre-existing organic fractions and resulting differences in microbial activity and dynamics could have had an effect on N₂O production. But this was not the case and elevated CO₂ showed no evidence for any significant altered fluxes of N₂O. In many other investigations, elevated CO₂ increased N₂O flux rates. Ineson et al. (1998) reported 27% higher N₂O emissions in grassland exposed to elevated CO₂. Similarly, in perennial grassland N₂O fluxes under elevated CO₂ were found double than those observed under ambient CO₂ (Arnone and Bohlen, 1998). Baggs and Blum (2004) reported that response of elevated CO₂ to N₂O emissions from grass swards depend on the rate of N application. Elevated CO₂ had no significant effect on emissions following low N application rates while N₂O emissions significantly increased under elevated CO₂ when high rates of N fertilizer were applied to the same grass swards. Observations in the Giessen FACE study are contrary because increasing N₂O emissions were only observed during times of low N availability. After N fertilizer application N₂O emissions were not different between ambient and elevated CO₂. There are reports that elevated CO₂ either did not alter N₂O fluxes or even lower down (decreased) N₂O emissions (Hungate et al., 1997; Mosier et al.,

2002, 2003; Welzmilller et al., 2008). The arguments in favor of increased N₂O emissions under elevated CO₂ are (i) improved soil moisture (ii) increase in C supplies in soil which affect N transformation processes (Ineson et al., 1998; Arnone and Bohlen, 1998; Baggs and Blum, 2004). In contrast, the lower N availability of N in the mineral N pool for soil microbes because of enhance plant uptake and altered N dynamics under elevated CO₂ are responsible for lower N₂O emissions (Mosier at al., 2003).

Increasing concentration of atmospheric CO₂ is likely to affect the production of N₂O. Greater above ground biomass production under elevated CO₂ may lead to increase N uptake, reducing the potential for production of N₂O during nitrification and or denitrification. Alternately, the increased belowground C allocation under elevated CO₂ may increase the potential for denitrification by providing energy for this process. However, the effects of elevated CO₂ on N availability are uncertain, with suggestion of increased availability (Zak et al., 1993), reduced availability of N due to high rate of microbial immobilization of soil N (Diaz et al., 1993; Hartwig et al., 1996) or no significant effect (Gloser et al., 2000). The N₂O production and its concentration in atmosphere depend more on soil N turnover (mineralization, nitrification, denitrification) (Müller et al., 2009; Rütting et al., 2010). The net and gross nitrification rates even decreased while DEA did not show any significant increase under elevated CO₂. Therefore, higher fluxes of N₂O under elevated CO₂ conditions in our experiment were unlikely and the similarity of the N₂O fluxes from the two soils could be expected. The soil collected from a grassland field exposed to FACE since 1998 and the tendency of soil to respond to elevated CO₂ over 7–8 years of exposure in this FACE experiment may be different to the soil exposed for shorter period of time. In the first 3 years of the FACE study higher N₂O emissions were observed from the same field exposed to elevated CO₂ (Kammann et al., 2008). Mosier et al. (2002) explained that soil moisture and C, N turnover increased substantially in the earlier stages of CO₂ establishment. But over the long-term, N transformation processes remain unchanged and response to elevated CO₂ becomes limited. A similar, conclusion was also reported by Baggs and Blum (2004). Zak et al. (2003) reported that increased in substrate quantity by elevated CO₂ did

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not alter microbial processes of C and N in soil (e.g. supply and demand of N). Our gaseous measurements suggested that at least trace gases fluxes were not significantly changed by elevated atmospheric CO₂. Rather than changing N₂O emissions an effect on the N₂-to-N₂O ratio could lead to the same result.

5 4.3 Total denitrification and N₂-to-N₂O ratio

Denitrification enzyme activity (DEA), total denitrification (N₂ + N₂O) and the ratio of N₂-to-N₂O were determined over a 20'day incubation using an anaerobic slurry technique as described by Müller et al. (2002). DEA was on average 16% higher in the elevated CO₂ than in the ambient treatment but the difference between the two CO₂ treatments was not significant suggesting that elevated CO₂ had only a limited effect on the quantity of active denitrifying enzymes present in the soil. Despite the large potential effects of elevated CO₂ on DEA, our study showed a small response. This small response is attributed to the absence of CO₂ effects on DEA drivers e.g. soil water content and level of CO₂ production. In the same soil under field condition, Kammann et al. (2008) found no change in moisture content. These results were in line with findings of Barnard et al. (2004) who reported very little response of DEA to CO₂ treatment in German grassland soils. However, total denitrification (N₂O + N₂) and the ratio of N₂-to-N₂O were significantly higher under elevated CO₂. Total denitrification was 36% higher under elevated CO₂ than the ambient treatment while elevated CO₂ had shown a 33% higher N₂-to-N₂O ratio. The ratio under elevated CO₂ (average 1.358) were similar to the ranges typically reported (0.1–40) (Rolston et al., 1976) but lower than the ratios of 345 and 410 measured by Baggs et al. (2003b). Baggs et al. (2003b) found very low N₂-to-N₂O ratios till 8 days after fertilizer application and proposed a different lag phases for N₂ and N₂O production. The ratios in their study increased substantially only in the elevated CO₂ during 8-10 days period and decreased to as low as 0 at day 15 while ambient showed no effect with time. We found different pattern of changes. Both ambient and elevated CO₂ treatments exhibited similar trend and the ratios ranged from 0.77–1.84 in ambient and 1.12–1.72 in the elevated CO₂. The

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maximum ratios in both the treatments were found shortly after N application at day 0. We also considered the long-term measurements proposed by Baggs et al. (2003b) but found no consistent pattern of changes in ratios with time. The constantly higher concentration of N_2 relative to N_2O throughout the measurements was observed similar to those reported by Welzmler et al. (2008). The higher N_2 -to- N_2O ratios under elevated CO_2 emphasized the need for the consideration of N_2 measurements in the denitrification studies in the future and shows that despite a non significant response to N_2O total denitrification may be altered. This is supported by a recent microbial study in the Giessen FACE study (Kandeler et al, unpublished results) which strongly indicated a shift towards denitrifier community patterns that could explain an increased N_2 -to- N_2O ration. The results indicate that shortly after N application elevated CO_2 stimulated both the N_2O production and reduction to N_2 to explain the increased N_2 -to- N_2O ratio. Thus, the observed variation of the CO_2 effect on N_2O emissions throughout the year is possibly related to the dynamics of the N_2O reductase activity.

5 Conclusions

Most of the studies conducted so far suggesting higher N_2O emissions under elevated CO_2 while very few reported no response. Over 57 days measurement of trace gas exchange in ambient and elevated CO_2 , we observe no statistically significant CO_2 enrichment effect on fluxes of CO_2 and N_2O . It is possible that exposure of soil to FACE over long period diminished its enhancing effects on microbial processes including nitrification and denitrification thereby did not show any significant effect on trace gas fluxes. The larger CH_4 oxidation under elevated CO_2 is surprising and shows that potential for CH_4 oxidation may increase under field where reduced net CH_4 uptake was observed under elevated CO_2 previously. Despite several previous studies suggesting N fertilizer to be a key determinant of CH_4 oxidation capacity in soil, our findings suggest that N fertilization do not have any inhibitory effect on CH_4 oxidation rather it increases CH_4 oxidation potential of soil. The understanding of the stimulation of

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population and activity of methanogenics and methanotrophic bacteria is essential to predict the net CH₄ oxidation in terrestrial ecosystem. The higher N₂-to N₂O ratios found in our study under elevated CO₂ demonstrate that enzyme dynamics that govern the production and consumption of N₂O are most likely affected by long-term elevated CO₂ enrichment. Changes in the N cycle and GHG production due to increasing atmospheric CO₂ concentrations are also important to consider in process-based models that try to simulate atmospheric GHG dynamics under climate change. Therefore, while this study does not directly contribute to a better understanding of atmospheric processes, it can elucidate indirectly some of the main drivers of changing GHGs and therefore can contribute to the development of models that are aiming to simulate GHG dynamics in the atmosphere.

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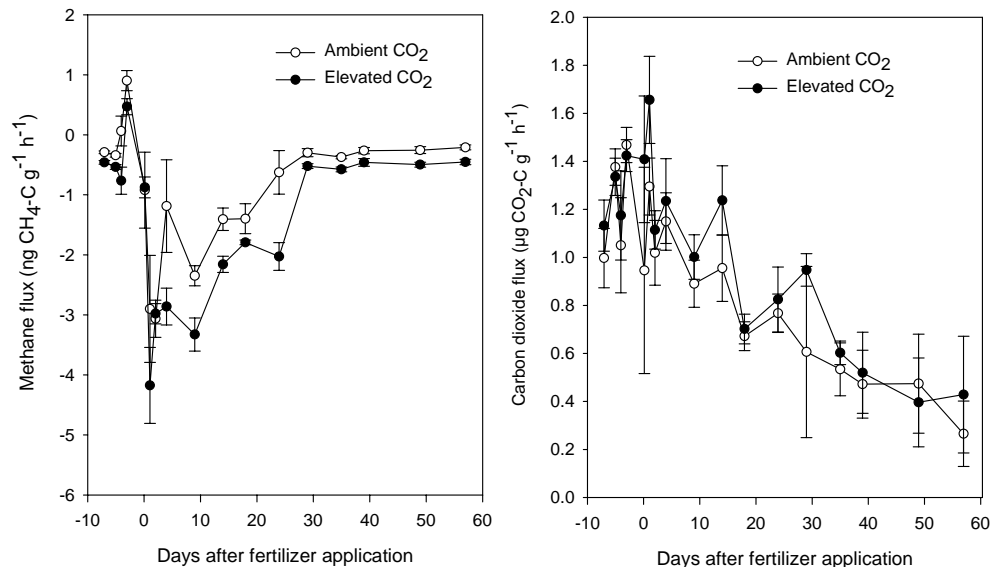
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Fig. 1. Daily fluxes of CH₄ ($\text{ng CH}_4\text{-C g}^{-1} \text{h}^{-1}$) and CO₂ ($\mu\text{g CO}_2\text{-g}^{-1} \text{h}^{-1}$) (Avg. \pm SD) from temperate grassland soil expose to elevated CO₂ and soil without elevated CO₂ treatment i.e. ambient incubated under controlled laboratory conditions following the application of NH₄¹⁵NO₃ and ¹⁵NH₄NO₃.

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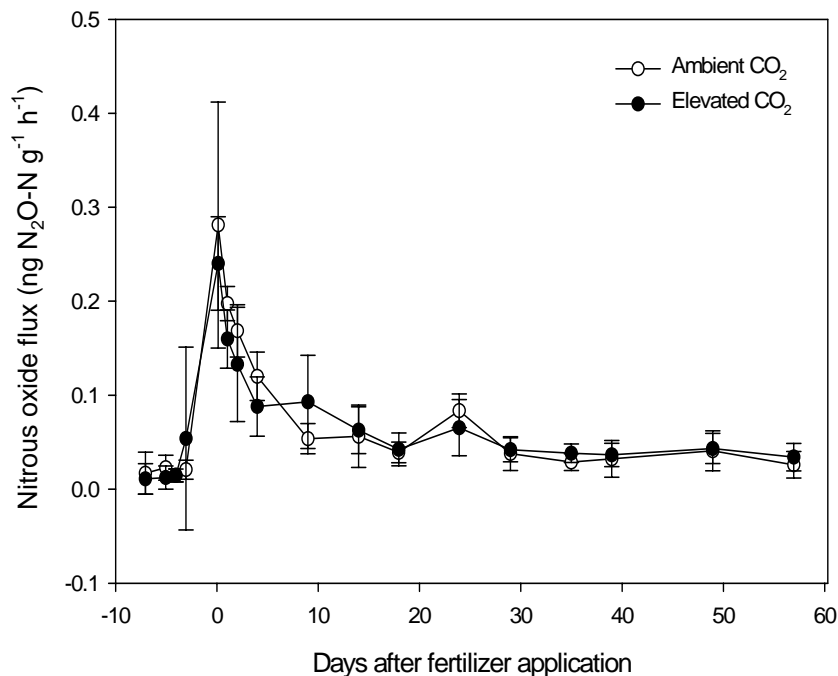


Fig. 2. Daily fluxes of N₂O (ng N₂O-N g⁻¹ h⁻¹) (Avg. ± SD) from temperate grassland soil exposed to elevated CO₂ and soil without elevated CO₂ treatment i.e. ambient incubated under controlled laboratory conditions following the application of NH₄¹⁵NO₃ and ¹⁵NH₄NO₃.

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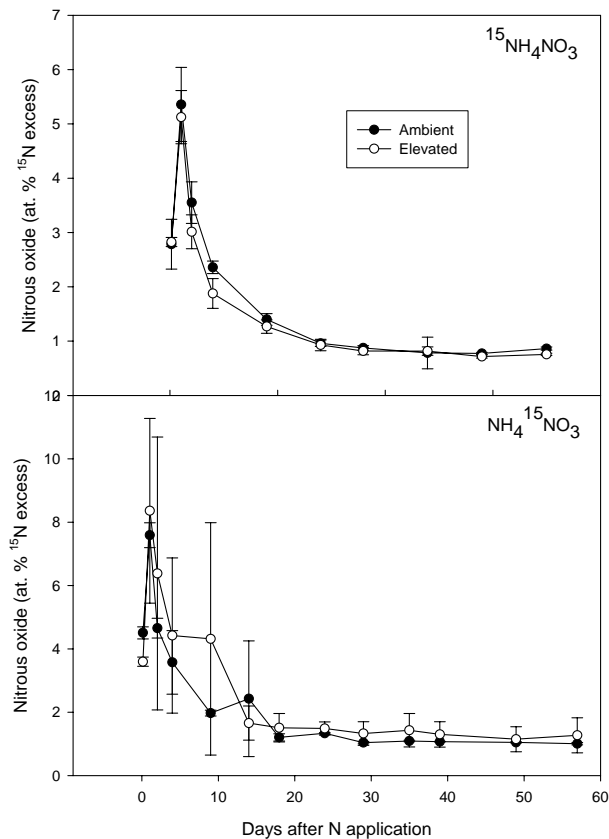

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Fig. 3. Nitrous oxide (N_2O) enrichments (Avg \pm SD) in a temperate grassland soil expose to elevated CO_2 and soil without elevated CO_2 treatment i.e. ambient following N fertilizer application where the nitrate pool ($\text{NH}_4^{15}\text{NO}_3$) and the ammonium pool ($^{15}\text{NH}_4\text{NO}_3$) were labelled with ^{15}N at 60 atom% excess.

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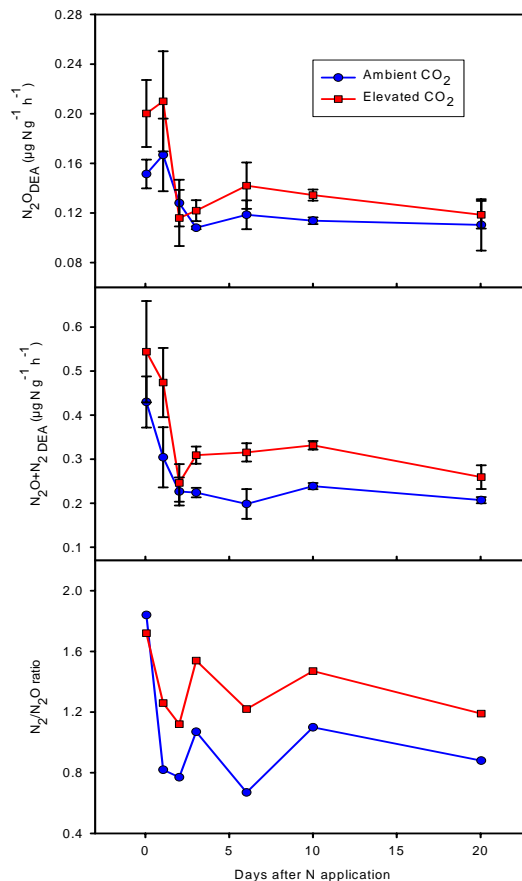
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Fig. 4. Emission of N₂O, total denitrification (N₂O + N₂) (µg N g⁻¹ h⁻¹) and N₂/N₂O ratio (AVG ±SD) from temperate grassland soil expose to elevated CO₂ and soil without elevated CO₂ treatment i.e. ambient incubated under controlled laboratory conditions following the application of NH₄¹⁵NO₃ and ¹⁵NH₄NO₃.

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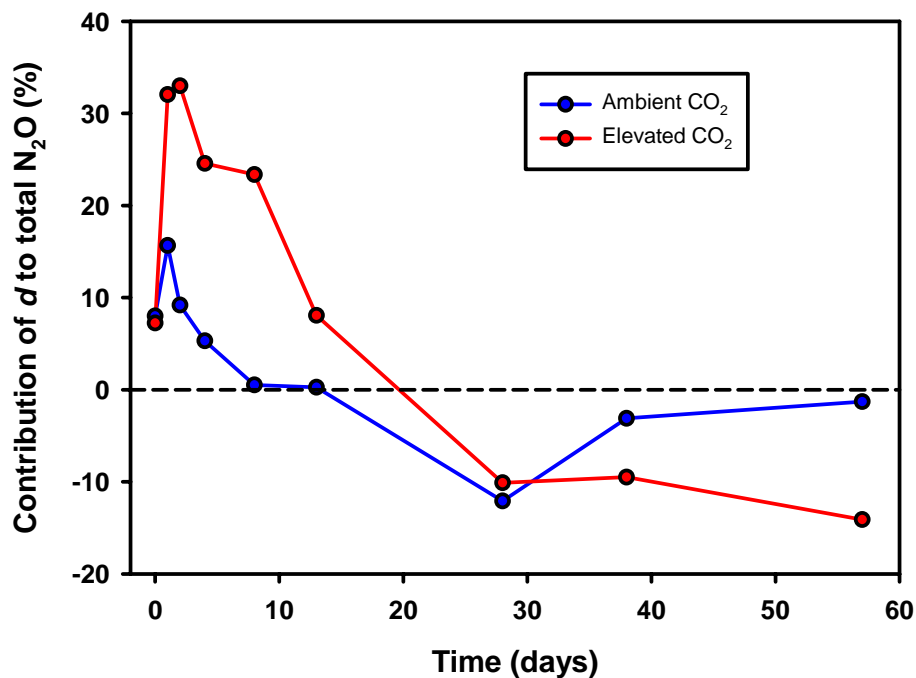
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Fig. 5. Contribution of d (NO₃⁻ reduction) to total N₂O production in grassland soil at ambient and elevated CO₂.

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