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# Effect of water addition and nitrogen fertilization on the fluxes of $CH_4$ , $CO_2$ , $NO_x$ , and $N_2O$ following five years of elevated $CO_2$ in the Colorado Shortgrass Steppe

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Abstract. An open-top-chamber (OTC) CO<sub>2</sub> enrichment  $(\sim 720 \,\mu \text{mol mol}^{-1})$  study was conducted in the Colorado shortgrass steppe from April 1997 through October 2001. Aboveground plant biomass increased under elevated CO<sub>2</sub> and soil moisture content was typically higher than under ambient CO<sub>2</sub> conditions. Fluxes of CH<sub>4</sub>, CO<sub>2</sub>, NO<sub>x</sub> and N<sub>2</sub>O, measured weekly year round were not significantly altered by CO<sub>2</sub> enrichment over the 55 month period of observation. During early summer of 2002, following the removal of the open-top-chambers from the CO<sub>2</sub> enrichment sites in October 2001, we conducted a short term study to determine if soil microbial processes were altered in soils that had been exposed to double ambient CO<sub>2</sub> concentrations during the growing season for the past five years. Microplots were established within each experimental site and 10 mm of water or 10 mm of water containing the equivalent of  $10 \,\mathrm{g}\,\mathrm{m}^{-2}$  of ammonium nitrate-N was applied to the soil surface. Fluxes of CO<sub>2</sub>, CH<sub>4</sub>, NO<sub>x</sub> and N<sub>2</sub>O fluxes within control (unchambered), ambient CO2 and elevated CO2 OTC soils were measured at one to three day intervals for the next month. With water addition alone, CO2 and NO emission did not differ between ambient and elevated CO2 soils, while CH4 uptake rates were higher and N<sub>2</sub>O fluxes lower in elevated CO<sub>2</sub> soils. Adding water and mineral N resulted in increased CO<sub>2</sub> emissions, increased CH<sub>4</sub> uptake and decreased NO emissions in elevated CO<sub>2</sub> soils. The N addition study confirmed previous observations that soil respiration is enhanced under elevated CO2 and N immobilization is increased, thereby decreasing NO emission.

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#### 1 Introduction

During the past few decades the atmospheric concentration of CO<sub>2</sub> has increased at historically unprecedented rates, as have N<sub>2</sub>O and CH<sub>4</sub> concentrations (IPCC, 2001). Increasing CO<sub>2</sub> concentrations will have a direct effect on plant production and plant communities and indirectly feed back into a number of soil biotic systems that influence long term ecosystem viability (Hungate et al., 1997a, b, c; Owensby et al., 1993a). The impact of elevated CO<sub>2</sub> on the shortgrass steppe, which is used extensively for grazing and is similar to regions which occupy about 8% of the U.S. and about 11% of global land area (Bailey, 1979) has not been previously addressed. These interactive feedbacks on the soil C and N cycles and their influence on trace gas fluxes have potentially important impacts on the global atmospheric budgets of the gases and the long term sustainability of the grassland. Earlier studies within the shortgrass steppe have demonstrated that such grasslands play an important role as consumers of atmospheric CH<sub>4</sub>, and producers of N<sub>2</sub>O (Mosier et al., 1991; 1996; 1997). Doubling CO<sub>2</sub> had little impact on trace gas fluxes in the shortgrass steppe (Morgan et al., 2001; Mosier et al., 2002a). The few measurements of NO<sub>x</sub>, N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> fluxes in CO<sub>2</sub> enrichment studies in other ecosystems have given contradictory results.

Growth chamber studies suggest that plant C/N ratios, nitrogen use efficiency and water use efficiency all increase under elevated CO<sub>2</sub> (Drake et al., 1996; Morgan et al., 1994; Rogers et al., 1994). In the short term, increases in soil moisture content resulting from higher water use efficiency (Hungate et al., 1997a, b, c) may accelerate rates of C and N mineralization, increasing N availability for plant uptake. Over the long term, however, decreased litter quality is expected to increase N immobilization rates and reduce N availability for plant uptake. From these observations we hypothesized that initially elevated CO<sub>2</sub> would induce increased soil

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Table 1. Selected soil properties\* of the soil at the Open-Top-Chamber study site

Soil Depth (cm)	Sand	Silt %	Clay	pН		Total C	Bulk Density g cm <sup>-3</sup>
0–10	76	14	10	7.3	0.101	0.891	1.28
10-20	74	15	11	8.1	0.077	0.606	1.42
20-30	74	13	13	8.0	0.076	0.584	1.48

<sup>\*</sup>Gravimetric soil water content for the 0-15 cm depth at water filled pore space of 0.2, 0.4 and 0.6 is 7.2, 14.4 and 21.6 kg kg $^{-1}$ , respectively (Mosier et al., 1998).

moisture and increased N mineralization rates (Hunt et al., 1988). As a result, CO<sub>2</sub>, NO and N<sub>2</sub>O emissions should increase on the short term under elevated CO2, while CH4 uptake should decrease. Over the longer term, however, increased C/N ratios in plant litter and roots would result in longer term decreases in N mineralization rates, and decreased NO and N2O fluxes would be observed. To test these responses, Mosier et al., (2002a) monitored the soilatmosphere exchange of CO<sub>2</sub>, NO<sub>x</sub>, N<sub>2</sub>O and CH<sub>4</sub> weekly, year-round, April 1997 to November 2001 on unchambered control, ambient CO<sub>2</sub> and  $\sim$ 720  $\mu$ mol mol<sup>-1</sup> CO<sub>2</sub> experimental plots in the Colorado shortgrass steppe. Mosier et al., (2002a, b) observed no statistically significant CO<sub>2</sub> enrichment effect on ecosystem respiration, oxidation of atmospheric CH<sub>4</sub>, or emissions of NO<sub>x</sub> or N<sub>2</sub>O. Methane oxidation tended to be higher under elevated CO<sub>2</sub> while NO<sub>x</sub> and N<sub>2</sub>O tended to be lower, but not significantly in either case. Above ground biomass production was higher under elevated CO<sub>2</sub> (Morgan et al., 2001), which utilized more soil N (King et al., 2003). However, soil N mineralization was probably enhanced under elevated CO2 because of more moist soils (Hungate et al., 1997a, b, c). The two opposing processes apparently offset each other because NO<sub>x</sub> and N<sub>2</sub>O emissions, which reflect system N mineralization and nitrification, did not differ. Ecosystem respiration, which included soil and aboveground plant respiration, was not generally higher under elevated CO<sub>2</sub> (Mosier et al., 2002a). During the 5 years of the study trace gas exchange measurements suggested that soil microbial processes were not greatly altered under double CO<sub>2</sub> concentrations, at least in the short term. Plant production during a year of above average rainfall did, however, appear to be limited by N availability (King et al., 2003). By analyzing the concentration of soil CO2 at different depths in the OTCs and calculating soil respiration, Pendall et al., (2003) found that elevated CO<sub>2</sub> increased soil respiration by about 25% in a moist growing season and by about 85% in a dry season. Significant increases in soil respiration rates occurred only during dry periods.  $\delta^{13}$ C analyses of soil CO<sub>2</sub> revealed that soil organic matter decomposition rates were more than doubled under elevated CO<sub>2</sub> whereas rhizosphere respiration rates were not changed. Estimates of net ecosystem production, which account for both inputs and losses of

carbon, suggest that soil carbon sequestration is not increased under elevated CO<sub>2</sub> during dry years, but may be in wet years (Pendall et al., accepted).

We were interested to determine if residual effects on microbial processes persisted following CO<sub>2</sub> enrichment. During early summer of the year following the removal of the open- top chambers from the CO<sub>2</sub> enrichment sites we conducted a short term study to determine if soil microbial processes were altered in soils that had been exposed to double ambient CO<sub>2</sub> concentrations during the growing season for the past five years. The response of emissions of CO<sub>2</sub>, NO<sub>x</sub> and N<sub>2</sub>O and the uptake of atmospheric CH<sub>4</sub> to water addition and water and mineral nitrogen fertilization to soils that had or had not been exposed to elevated CO<sub>2</sub> are reported in this paper.

#### 2 Materials and Methods

The CO<sub>2</sub> enrichment studies were conducted at the USDA/ARS Central Plains Experimental Range (CPER) on which is located the Shortgrass Steppe long-term-ecological-research (LTER) project, about 60 km NE of Fort Collins, CO, USA (40°50' N, 104°42' W). The semiarid grassland site is at 1650 m elevation and has a long term average annual precipitation of  $\sim$ 320 mm with mean summer air temperatures of 15.6°C and 0.6°C mean winter air temperatures (Morgan et al., 2001). Annual mineral nitrogen input is an estimated 0.5 g N m<sup>-2</sup> mainly through wet and dry atmospheric deposition (Mosier et al., 1996).

### **Experimental Site**

In the fall of 1995, vegetation and soil surveys were conducted in native shortgrass steppe at the experimental site. The survey results enabled a selection of relatively similar experimental plots on the basis of soil and plant community information and documented plot differences before the treatments were implemented. The grassland community is comprised of over 25 species of forbs and grasses, but dominated by three grass species, Bouteloua gracilis (C<sub>4</sub>, accounts for approximately 42% of total

aboveground biomass), Pascopyrum smithii ( $C_3$ , 21% of total aboveground biomass) and Stipa comata ( $C_3$ , 26% of total aboveground biomass). After 5-years of exposure to double ambient  $CO_2$  concentrations the plant species composition had changed to approximately 50% Stipa comata (Morgan et al., 2003). Nine experimental plots of similar plant species composition were selected on the basis of this initial survey. The soil within the study site is a Remmit fine sandy loam (Ustollic camborthids) which holds 18% (gravimetric) water at field capacity, and 4% at the permanent wilting point (Table 1).

On six of the nine plots, open-top chambers (4.5 m diameter by 3 m height) of similar design to Owensby et al., (1993a, b) were installed in March 1997 (Morgan et al., 2001a). All chambers were equipped with blowers to exchange  $\sim$ 1.5 air volumes per minute. Three of the chambers had precision valve outlets located in-line between the blowers and the chambers and attached to a compressed  $\rm CO_2$  source for elevating  $\rm CO_2$  to approximately 720 mol mol  $^{-1}$ . Carbon dioxide in the chambers was controlled only during the growing season, from early April to late October. Chambers were removed in the winter. Daily precipitation was measured at a meteorological station located about 50 m from the OTCs. The  $\rm CO_2$  enrichment was terminated in late October of 2001.

## Establishment of Microplots and Trace Gas Flux Measurements

In late April, 2002 two microplots and gas flux sampling locations were established by driving flux chamber anchors (20 cm diameter, 10 cm high PVC cylinder) 8 cm into the soil within each OTC and control (unchambered) plot. In the morning of 11 June 2002, water (1-cm) or water plus N-fertilizer (1-cm of water containing the equivalent of  $10\,\mathrm{g}\,\mathrm{N}\,\mathrm{m}^{-2}$  of ammonium nitrate) was added to each microplot. Soil moisture was the same in all treatments at the beginning of this study and emissions of all trace gases were small at this time. We measured fluxes of NO<sub>x</sub>, N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub>, two hours after water addition and then at one to three day intervals until 12 July 2002. Measurements were typically made midmorning of each sampling day using vented closed chambers (Hutchinson and Mosier, 1981; Mosier et al., 1991) where the changes in concentration of N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub> within the chamber were measured by withdrawing samples from the chamber by syringe at three time periods and analyzing the gas concentrations by gas chromatography (Mosier et al., 1996; 1997; 1998). Nitric oxide flux was monitored from the same chamber anchors on the same day using a flow-through chamber system (Martin et al., 1998) and a Thermo Environmental Instruments model 42C chemiluminescence NO-NO<sub>2</sub>-NO<sub>x</sub> analyzer that is housed in the instrument trailer (Mosier et al., 1998). NO<sub>x</sub> emissions from the soil are typically >90% NO, so fluxes will generally be discussed in terms of NO only (Martin et al., 1998; Mosier et al., 1998). Air and soil temperatures were monitored at each flux measurement using a hand held digital thermometer.

#### **Statistical Analyses**

Gas flux measurements, soil moisture and soil temperatures within each treatment replicate (OTC or unchambered location) were averaged (n=3) for each observation time. Over the month long analysis period the eleven flux measurements were averaged and single factor analysis of variance was performed (Microsoft Excel). Individual CO<sub>2</sub> treatments were compared for a designated time interval using a paired t-test. Significance levels of 0.05 were used unless specifically noted.

#### 3 Results and Discussion

#### **Trace Gas Fluxes**

During the five year study prior to the N-addition experiment  $NO_x$  flux averaged 4.3 in ambient and 4.1  $\mu g\,N\,m^{-2}\,hr^{-1}$  in elevated chambers .  $NO_x$  flux was negatively correlated to plant biomass production. Nitrous oxide emission rates averaged 1.8 and 1.7  $\mu g\,N\,m^{-2}\,hr^{-1}$ , CH<sub>4</sub> flux rates averaged -31 and  $-34\,\mu g\,C\,m^{-2}\,hr^{-1}$  and ecosystem respiration averaged 43 and 44 mg C m $^{-2}\,hr^{-1}$  under ambient and elevated CO<sub>2</sub>, respectively, over the same time period (Mosier et al., 2002a, b). We conducted the short-term N-addition to see if any residual effects of elevated CO<sub>2</sub> remained during the growing season following the five years of CO<sub>2</sub> fumigation and found that some important changes in soil microbial responses resulting from CO<sub>2</sub> enrichment.

#### NO and N<sub>2</sub>O Emissions

Under the hot and very dry conditions at the study site when this study was initiated NO and N2O emissions are typically very low (Mosier et al., 1998). NO emissions from the control soils were higher than from soils that had been under ambient or CO<sub>2</sub> enriched chambers when the soils were irrigated (Table 2). This response was similar to the long term NO fluxes observed where control soil NO emissions averaged  $11 \mu g m^{-2} hr^{-1}$  compared to 4.3 and 4.1 in ambient and CO2 enriched chambers (Mosier et al., 2002a). Plant production and uptake of nitrogen was opposite this trend with plant biomass being greater under elevated CO<sub>2</sub>>ambient CO<sub>2</sub>>unchambered control (Morgan et al., 2001). This suggests that N availability for NO production by nitrifiers is partly regulated by plant uptake (Mosier et al., 2002a). Nitrous oxide emissions were significantly lower (P<0.05) from soils that had been exposed to elevated CO<sub>2</sub> (Table 2). This may also reflect the lower N availability to soil microbes under elevated CO2 due

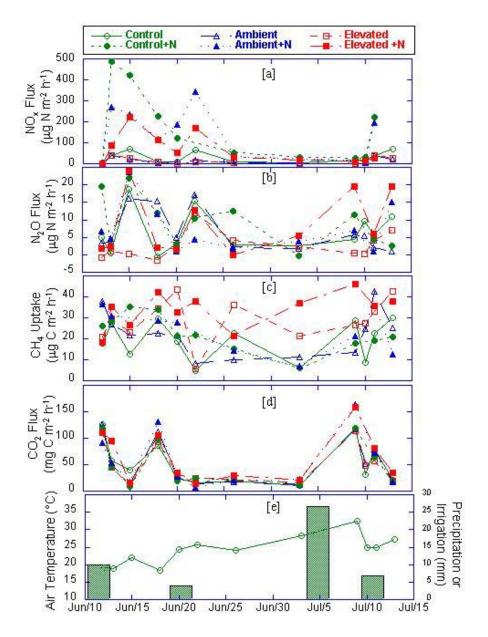


Fig. 1. Trace gas flux, air temperature and soil water content in ambient and elevated  $CO_2$  chambers: (a)  $NO_x$  flux; (b)  $N_2O$  flux; (c) uptake of atmospheric  $CH_4$  by soil micro-organisms; (d) dark chamber  $CO_2$  flux which includes above ground plant respiration, plant root respiration and soil microbial respiration; (e) air temperature at time of flux measurements and water addition and precipitation.

to enhanced plant production (King et al., 2003). Soil mineral N concentrations were typically very low ( $<1\,\text{mg/kg}$ ) in all treatments (Mosier, unpublished data). Both N<sub>2</sub>O and to a lesser extent NO emissions increased following water application (Figs. 1a and b). Over the measurement period when water alone was added, NO/N<sub>2</sub>O ratios ranged between 2 and 5 with the highest in the elevated CO<sub>2</sub> soils.

When  $NH_4NO_3$  was added  $N_2O$  flux tripled in elevated  $CO_2$  soils (P<0.05), and increased slightly in control and ambient chamber soils (P>0.05). NO emissions and  $N_2O$  emissions in N-fertilized soils increased markedly following

each irrigation and precipitation event (Figs. 1a, b, and e). Nitric oxide fluxes increased almost 10 fold with N addition in control and ambient  $CO_2$  soils but only about 5-fold in elevated  $CO_2$  soils. NO emissions were significantly lower from elevated  $CO_2$  soils than from control or ambient  $CO_2$  soils, again indicating the lower availability of N in the elevated  $CO_2$  soils. Hungate et al., (1997b, c) found that, during wet up, NO emissions were depressed by 55% in high nutrient conditions under elevated  $CO_2$  (ambient+360  $\mu$ mol mol<sup>-1</sup>) while there was no difference among treatments in N<sub>2</sub>O emissions. They attributed the

Table 2. Mean mean trace gas flux rates within the shortgrass steppe open-top-chamber  $CO_2$  enrichment study area the summer following 5-years of  $CO_2$  enrichment, 11 June–12 July 2002

CO <sub>2</sub> Treatment	$CO_2 \ mg C m^{-2} hr^{-1}$	$CH_4$ $\mu$ g C m <sup>-2</sup> hr <sup>-1</sup>	NO <sub>x</sub> μg N m	$N_2O$ $n^{-2}hr^{-1}$
Water Only				
Control	52a	-19b	25a	6.5a
Ambient	53a	-22b	13b	6.6a
High	49a	-28a	13b	2.6b
Water + N				
Control	50b	-22b	160a	9.1a
Ambient	57b	-24ab	121a	7.4a
High	64a	-34a	63b	8.6a

<sup>\*</sup>Numbers in each column followed by the same letter are not significantly different (P>0.05).

decreased NO emissions under elevated CO<sub>2</sub> to increased N immobilization. Increased utilization of added N by soil microbes, thus a decrease in NO emissions, appears to be the case in this study as well (Table 2).

#### CO2 and CH4 Fluxes

Plant growth during the time of the study was virtually nonexistant because of the very low amount of precipitation that had fallen in the preceding year. Ecosystem CO<sub>2</sub> flux (dark chamber respiration which includes plant, root and soil microbial respiration) increases following water addition were similar in all soils (Fig. 1d; Table 2). Only with water+N addition did CO2 fluxes from elevated CO2 soils exceed those from control and ambient soils (P < 0.05). Microbial respiration appears to be enhanced under elevated CO<sub>2</sub> (Pendall et al., 2003), especially when microbes are not limited by water or N availability. N addition appeared to stimulate soil microbial respiration while decreasing NO emissions because of increased microbial immobilization of added N. Hu et al., (2001) suggest that over the long term, soil microbial decomposition is slowed under elevated CO<sub>2</sub> because of N limitation. The rate of uptake of atmospheric CH<sub>4</sub> was significantly greater (P<0.05) in elevated CO<sub>2</sub> soils than either control or ambient CO2 soils (Fig. 1c; Table 2). CH<sub>4</sub> uptake rates were not measurably enhanced with N addition in control or ambient CO<sub>2</sub> soils but tended to be greater in elevated CO<sub>2</sub> soils (P>0.05). During the 5-years of CO<sub>2</sub> enrichment CH<sub>4</sub> uptake rates tended to be higher under elevated CO<sub>2</sub>. This short term study suggests that a microbial population developed under elevated CO<sub>2</sub> which tended to increase utilization of atmospheric CH<sub>4</sub>. Ineson et al., (1998) observed lower CH<sub>4</sub> uptake rates under elevated CO<sub>2</sub> within a free-atmosphere CO<sub>2</sub>-enrichment (FACE) study in Switzerland. They also observed lower CO<sub>2</sub> respiration rates and increased N<sub>2</sub>O emissions under elevated CO<sub>2</sub>. McLain et al., (2002) also observed lower  $CH_4$  consumption rates under elevated  $CO_2$  in a pine plantation. The decrease in  $CH_4$  consumption was attributed, in part to wetter soils under elevated  $CO_2$ . Soil conditions in the pine forest were likely much more comparable to the grassland soils in Switzerland (Ineson et al., 1998) than to the much drier conditions in the Colorado shortgrass steppe. The wetter soil conditions under elevated  $CO_2$  in the Colorado grassland (Ferretti et al., 2003) likely produced more favorable conditions for methanotrophic activity, rather than limiting  $CH_4$  diffusion into the soil in the Swiss grassland (Ineson et al., 1998) and the pine forest (McLain et al., 2002).

#### **Summary**

In a semi-arid shortgrass steppe, addition of water and ammonium nitrate to soils that had been exposed to doubleambient CO<sub>2</sub> concentrations during the growing season of the previous five years increased ecosystem respiration and atmospheric CH<sub>4</sub> oxidation and decreased NO emissions. These observations suggest that methanotroph populations were enhanced under elevated CO<sub>2</sub> while soil N supply was depleted by increased plant growth (King et al., 2003; Morgan et al., 2001). Soil respiration was higher in elevated CO<sub>2</sub> soils following irrigation and N addition, suggesting that microbes were becoming N limited. Although decomposition rates were twice as high under elevated as ambient CO<sub>2</sub> mid-way through the 5-year experiment (Pendall et al., 2003), the N fertilization response observed here suggests that eventually microbial decomposition rates will slow, as predicted by Hu et al., (2001), leading to increased C sequestration potential.

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