

**Soil-atmosphere exchange of CH₄, CO₂, NO_x, and N₂O in the Colorado Shortgrass
Steppe following Five Years of Elevated CO₂ and N Fertilization**

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Soil-atmosphere exchange of CH₄, CO₂, NO_x, and N₂O in the Colorado Shortgrass Steppe following Five Years of Elevated CO₂ and N Fertilization

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Abstract. An open-top-chamber (OTC) CO₂ enrichment study was conducted in the Colorado shortgrass steppe to determine the effect of elevated CO₂ (~720 μmol mol⁻¹) on plant production, photosynthesis, and water use of this mixed C₃/C₄ plant community, soil nitrogen (N) and carbon (C) cycling and the impact of changes induced by CO₂ on trace gas exchange. Weekly measurements of CO₂, CH₄, NO_x and N₂O fluxes within control (unchambered), ambient CO₂ and elevated CO₂ OTCs and soil water and temperature were measured at each flux measurement time from early April 1997, year round, through October 2001. Even though both aboveground plant biomass increased under elevated CO₂ and soil moisture content was typically higher than under ambient CO₂ conditions, none of the trace gas fluxes were significantly altered by CO₂ enrichment over the 55 month period of observation. During early summer of 2002, following the removal of the open-top-chambers from the CO₂ 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 CO₂ 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 g m⁻² of ammonium nitrate-N was applied to the soil surface. Fluxes of CO₂, CH₄, NO_x and N₂O fluxes within control (unchambered), ambient CO₂ and elevated CO₂ OTCs soils 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 N₂O fluxes lower in elevated CO₂ soils. Adding water and mineral N resulted in increased CO₂ emissions, increased CH₄ uptake and decreased NO emissions in elevated CO₂ 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.

1 Introduction

During the past few decades the atmospheric concentration of CO₂ has increased at historically unprecedented rates, as have N₂O and CH₄ concentrations (IPCC, 2001). Increasing CO₂ 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 CO₂ 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 CH₄, and producers of N₂O (Mosier et al. 1991; 1996; 1997). The impact of elevated CO₂ on the production and consumption of other trace gases (NO_x, CO₂ and CH₄) is not well understood and had not been assessed in semiarid grasslands. The few measurements of NO_x, N₂O, CH₄ and CO₂ fluxes in CO₂ enrichment studies give contradictory results, and long term measurements had not been made within any ecosystem before the shortgrass steppe studies reported in Mosier et al. (2002a).

Growth chamber studies suggest that plant C/N ratios, nitrogen use efficiency and water use efficiency all increase under elevated CO₂ (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 CO₂ would induce increased soil moisture and increased N mineralization rates. As a result, CO₂, NO and N₂O 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, the soil-atmosphere exchange of CO₂, NO_x, N₂O and CH₄ were monitored weekly, year-round, April 1997 to November 2001 on unchambered control, ambient CO₂ and ~720 μmol mol⁻¹ CO₂ experimental plots in the Colorado shortgrass steppe. We observed no statistically significant CO₂ enrichment effect on ecosystem respiration, oxidation of atmospheric CH₄, or emissions of NO_x or N₂O using our closed chamber technique (Mosier et al. 2002a). Methane oxidation tended to be higher under elevated CO₂ while NO_x and N₂O tended to be lower, but not significantly in either case. Above ground biomass production was higher under elevated CO₂ (Morgan et al. 2001), which utilized more soil N (King et al. 2003). However, soil N mineralization was probably somewhat enhanced under elevated CO₂ because of moister soils (Hungate et al. 1997a,b,c). The two opposing processes apparently offset each other because NO_x and N₂O 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 CO₂.

By analyzing the concentration of soil CO₂ at different depths in the OTCs and

calculating soil respiration, Pendall et al. (2003) and found that elevated CO₂ 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. $\delta^{13}\text{C}$ analyses of soil CO₂ revealed that soil organic matter decomposition rates were more than doubled under elevated CO₂ 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 CO₂ during dry years, but may be in wet years (Pendall et al., in review).

During the 5 years of the study trace gas exchange measurements suggested that soil microbial processes were not greatly altered under double CO₂ concentrations, at least in the short term. We were, however, interested to determine if residual effects on microbial processes persisted following CO₂ enrichment. During early summer of the year following the removal of the open-top chambers from the CO₂ 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 CO₂ concentrations during the growing season for the past five years. The response of emissions of CO₂, NO_x and N₂O and the uptake of atmospheric CH₄ to water addition and water and mineral nitrogen fertilization to soils that had or had not been exposed to elevated CO₂ are reported in this paper.

2 Materials and Methods

The CO₂ 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' north, 104 42' west). The semiarid grassland site is at 1650 m elevation and has a long term average annual precipitation of ~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⁻² 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* (C₄, accounts for approximately 42% of total aboveground biomass), *Pascopyrum smithii* (C₃, 21% of total aboveground biomass) and *Stipa comata* (C₃, 26% of total aboveground biomass). 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 ~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 CO₂ source for elevating CO₂ 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 CO₂ 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 June 11, 2002, water (1-cm) or water plus N-fertilizer (1-cm of water containing the equivalent of 10 g N m⁻² of ammonium nitrate) was added to each microplot. We measured fluxes of NO_x, N₂O, CH₄ and CO₂, two hours after water addition and then at one to three day intervals until July 12, 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 N₂O, CO₂ and CH₄ 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-NO₂-NO_x analyzer that is housed in the instrument trailer (Mosier et al. 1998). NO_x 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 CO₂ 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, weekly measurements of CO₂, CH₄, NO_x and N₂O fluxes within control (unchambered), ambient CO₂ and elevated CO₂ OTCs and soil water and temperature were measured at each flux measurement time from early April 1997, year round, through October 2001 (Mosier et al. 2002 a, b). Even though both aboveground plant biomass increased under elevated CO₂ (Morgan et al. 2001) and soil moisture content was typically higher than under ambient CO₂ conditions (Ferretti et al., 2003), none of the trace gas fluxes were significantly altered by CO₂ enrichment. Over the 55 month period of observation NO_x flux averaged 4.3 in ambient and 4.1 μg 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 μg N m⁻² hr⁻¹, CH₄ flux rates averaged -31 and -34 μg C m⁻²hr⁻¹ and ecosystem respiration averaged 43 and 44 mg C m⁻²hr⁻¹ under ambient and elevated CO₂, 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 CO₂ remained during the growing season following the five years of CO₂ fumigation and found that some important changes in

soil microbial responses resulting from CO₂ enrichment.

NO and N₂O Emissions. As in the long term study, NO emissions from the control soils were higher when the soils were irrigated, and in this study N₂O emissions were significantly lower ($P < 0.05$) as well (Table 2). This reflects the N depleted state of the soils under elevated CO₂ due to enhanced plant production (King et al. 2003). Both N₂O and to a lesser extent NO emissions increased following water application (Fig. 1 a & b). Over the measurement period NO/N₂O ratios ranged between 2 and 5 with the highest in the elevated CO₂ soils. When NH₄NO₃ was added N₂O flux tripled in elevated CO₂ soils ($P < 0.05$), and increased slightly in control and ambient chamber soils ($P > 0.05$). NO emissions and N₂O emissions in N-fertilized soils increased markedly following each irrigation and precipitation event (Figs. 1 a, b, & e). Nitric oxide fluxes increased almost 10 fold with N addition in control and ambient CO₂ soils but only about 5-fold in elevated CO₂ soils. NO emissions were significantly lower from elevated CO₂ soils than from control or ambient CO₂ soils, again indicating the N depleted state of the elevated CO₂ soils. Hungate et al. (1997 b,c) found that, during wet up, NO emissions were depressed by 55% in high nutrient conditions under elevated CO₂ (ambient + 360 $\mu\text{mol mol}^{-1}$) while there was no difference among treatments in N₂O emissions. They attributed the decreased NO emissions under elevated CO₂ 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 nonexistent because of the very low amount of precipitation that had fallen in the preceding year. Ecosystem CO₂ 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 CO₂ (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.

The rate of uptake of atmospheric CH₄ was significantly greater ($P < 0.05$) in elevated CO₂ soils than either control or ambient CO₂ soils (Fig. 1c; Table 2). CH₄ uptake rates were not measurably enhanced with N addition in control or ambient CO₂ soils but tended to be greater in elevated CO₂ soils ($P > 0.05$). During the 5-years of CO₂ enrichment CH₄ uptake rates tended to be higher under elevated CO₂. This short term study suggests that a microbial population developed under elevated CO₂ which tended to increase utilization of atmospheric CH₄. Ineson et al. (1998) observed lower CH₄ uptake rates under elevated CO₂ within a free-atmosphere CO₂-enrichment (FACE) study in Switzerland. They also observed lower CO₂ respiration rates and increased N₂O emissions under elevated CO₂. McLain et al. (2002) also observed lower CH₄ consumption rates under elevated CO₂ in a pine plantation. The decrease in CH₄ consumption was attributed, in part to wetter soils under elevated CO₂. 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 CO₂ in the semiarid grassland likely produced more favorable conditions for methanotrophic activity, rather than limiting CH₄ diffusion into the soil in the Swiss grassland (Ineson et al. 1998) and the pine forest (McLain et al. 2002). Hu et al. (2001) suggest that over

the long term, soil microbial decomposition is slowed under elevated CO₂ because of N limitation. Conversely, Hungate et al. (1997c) found that higher soil water contents under elevated CO₂ in an annual grassland stimulated soil N mineralization and resulted in greater plant N uptake. The N-addition study confirms Pendall et al. (2003) observations that soil respiration is enhanced under elevated CO₂ and N immobilization is increased, thereby decreasing NO emissions, despite the fact that we observed no general CO₂-induced effect on NO_x and N₂O flux during the 5-year observation period (Mosier et al. 2002 a, b).

Summary. In a semi-arid shortgrass steppe, addition of water and ammonium nitrate to soils that had been exposed to double-ambient CO₂ concentrations during the growing season of the previous five years increased ecosystem respiration and atmospheric CH₄ oxidation and decreased NO emissions. These observations suggest that methanotroph populations were enhanced under elevated CO₂ while soil N supply was depleted by increased plant growth (King et al. 2003; Morgan et al. 2001). Soil respiration was higher in elevated CO₂ soils following irrigation and N addition, suggesting that microbes were becoming N limited. Although decomposition rates were twice as high under elevated as ambient CO₂ 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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Table 1. Selected soil properties* of the soil at the Open-Top-Chamber study site.

Soil Depth (cm)	Sand -----%-----	Silt	Clay	pH	Total N -----%-----	Total C	Bulk Density g cm ⁻³
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

*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).

Table 2. Mean mean trace gas flux rates within the shortgrass steppe open-top-chamber CO₂ enrichment study area the summer following 5-years of CO₂ enrichment, June 11-July 12 2002.

CO ₂ Treatment	CO ₂	CH ₄	NO _x	N ₂ O
	mg C m ⁻² hr ⁻¹	µg C m ⁻² hr ⁻¹	----- µg N m ⁻² hr ⁻¹	
<u>Water Only</u>				
Control	52a	-19b	25a	6.5a
Ambient	53a	-22b	13b	6.6a
High	49a	-28a	13b	2.6b
<u>Water + N</u>				
Control	50b	-22b	160a	9.1a
Ambient	57b	-24ab	121a	7.4a
High	64a	-34a	63b	8.6a

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

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Figure 1. Trace gas flux, air temperature and soil water content in ambient and elevated CO₂ chambers: a) NO_x flux; b) N₂O flux; c) uptake of atmospheric CH₄ by soil micro-organisms; d) dark chamber CO₂ 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.

