

**Soil-atmosphere exchange of CH<sub>4</sub>, CO<sub>2</sub>, NO<sub>x</sub>, and N<sub>2</sub>O in the Colorado Shortgrass  
Steppe following Five Years of Elevated CO<sub>2</sub> and N Fertilization**

**A.R. Mosier<sup>1</sup>, E. Pendall<sup>2</sup>, J.A. Morgan<sup>1</sup>**

**<sup>1</sup>USDA/ARS, Fort Collins, CO; <sup>2</sup>Department of Botany, University of Wyoming,  
Laramie WY**

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Corresponding author: Arvin R. Mosier  
USDA/ARS  
301 S. Howes, Fed Bldg. Rm. 420  
Fort Collins, CO 80521  
USA

Phone: 1-970-490-8250

Fax: 1.970-490-8213

Email: [amosier@lamar.colostate.edu](mailto:amosier@lamar.colostate.edu)

## Soil-atmosphere exchange of CH<sub>4</sub>, CO<sub>2</sub>, NO<sub>x</sub>, and N<sub>2</sub>O in the Colorado Shortgrass Steppe following Five Years of Elevated CO<sub>2</sub> and N Fertilization

A.R. Mosier<sup>1</sup>, E. Pendall<sup>2</sup>, J.A. Morgan<sup>1</sup>

<sup>1</sup>USDA/ARS, Fort Collins, CO; <sup>2</sup>Department of Botany, University of Wyoming, Laramie WY

**Key Words:** global change, greenhouse gases, grasslands

**Abstract.** An open-top-chamber (OTC) CO<sub>2</sub> enrichment study was conducted in the Colorado shortgrass steppe to determine the effect of elevated CO<sub>2</sub> (~720 μmol mol<sup>-1</sup>) on plant production, photosynthesis, and water use of this mixed C<sub>3</sub>/C<sub>4</sub> plant community, soil nitrogen (N) and carbon (C) cycling and the impact of changes induced by CO<sub>2</sub> on trace gas exchange. Weekly measurements of CO<sub>2</sub>, CH<sub>4</sub>, NO<sub>x</sub> and N<sub>2</sub>O fluxes within control (unchambered), ambient CO<sub>2</sub> and elevated CO<sub>2</sub> 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 CO<sub>2</sub> and soil moisture content was typically higher than under ambient CO<sub>2</sub> conditions, none of the trace gas fluxes were 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, 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 g m<sup>-2</sup> 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 CO<sub>2</sub> and elevated CO<sub>2</sub> OTCs soils at one to three day intervals for the next month. With water addition alone, CO<sub>2</sub> and NO emission did not differ between ambient and elevated CO<sub>2</sub> soils, while CH<sub>4</sub> 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 CO<sub>2</sub> and N immobilization is increased, thereby decreasing NO emission.

## 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). The impact of elevated CO<sub>2</sub> on the production and consumption of other trace gases (NO<sub>x</sub>, CO<sub>2</sub> and CH<sub>4</sub>) is not well understood and had not been assessed in semiarid grasslands. 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 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 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 moisture and increased N mineralization rates. As a result, CO<sub>2</sub>, NO and N<sub>2</sub>O emissions should increase on the short term under elevated CO<sub>2</sub>, while CH<sub>4</sub> 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 N<sub>2</sub>O fluxes would be observed. To test these responses, the soil-atmosphere exchange of CO<sub>2</sub>, NO<sub>x</sub>, N<sub>2</sub>O and CH<sub>4</sub> were monitored weekly, year-round, April 1997 to November 2001 on unchambered control, ambient CO<sub>2</sub> and ~720 μmol mol<sup>-1</sup> CO<sub>2</sub> experimental plots in the Colorado shortgrass steppe. We 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 using our closed chamber technique (Mosier et al. 2002a). 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 somewhat enhanced under elevated CO<sub>2</sub> because of moister 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>.

By analyzing the concentration of soil CO<sub>2</sub> at different depths in the OTCs and

calculating soil respiration, Pendall et al. (2003) and 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.  $\delta^{13}\text{C}$  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., in review).

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. We were, however, 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' 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<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<sub>3</sub>, 21% of total aboveground biomass) and *Stipa comata* (C<sub>3</sub>, 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 CO<sub>2</sub> source for elevating CO<sub>2</sub> to approximately 720 mol mol<sup>-1</sup>. 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 CO<sub>2</sub> 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<sup>-2</sup> of ammonium nitrate) was added to each microplot. 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 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 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, weekly measurements of CO<sub>2</sub>, CH<sub>4</sub>, NO<sub>x</sub> and N<sub>2</sub>O fluxes within control (unchambered), ambient CO<sub>2</sub> and elevated CO<sub>2</sub> 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 CO<sub>2</sub> (Morgan et al. 2001) and soil moisture content was typically higher than under ambient CO<sub>2</sub> conditions (Ferretti et al., 2003), none of the trace gas fluxes were significantly altered by CO<sub>2</sub> enrichment. Over the 55 month period of observation NO<sub>x</sub> flux averaged 4.3 in ambient and 4.1 μg N m<sup>-2</sup> hr<sup>-1</sup> in elevated chambers. NO<sub>x</sub> flux was negatively correlated to plant biomass production. Nitrous oxide emission rates averaged 1.8 and 1.7 μg N m<sup>-2</sup> hr<sup>-1</sup>, CH<sub>4</sub> flux rates averaged -31 and -34 μg C m<sup>-2</sup>hr<sup>-1</sup> and ecosystem respiration averaged 43 and 44 mg C m<sup>-2</sup>hr<sup>-1</sup> 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.* As in the long term study, NO emissions from the control soils were higher when the soils were irrigated, and in this study N<sub>2</sub>O emissions were significantly lower ( $P < 0.05$ ) as well (Table 2). This reflects the N depleted state of the soils under elevated CO<sub>2</sub> due to enhanced plant production (King et al. 2003). Both N<sub>2</sub>O and to a lesser extent NO emissions increased following water application (Fig. 1 a & b). Over the measurement period 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<sub>4</sub>NO<sub>3</sub> was added N<sub>2</sub>O flux tripled in elevated CO<sub>2</sub> soils ( $P < 0.05$ ), and increased slightly in control and ambient chamber soils ( $P > 0.05$ ). NO emissions and N<sub>2</sub>O 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 CO<sub>2</sub> soils but only about 5-fold in elevated CO<sub>2</sub> soils. NO emissions were significantly lower from elevated CO<sub>2</sub> soils than from control or ambient CO<sub>2</sub> soils, again indicating the N depleted state of the elevated CO<sub>2</sub> soils. Hungate et al. (1997 b,c) found that, during wet up, NO emissions were depressed by 55% in high nutrient conditions under elevated CO<sub>2</sub> (ambient + 360  $\mu\text{mol mol}^{-1}$ ) while there was no difference among treatments in N<sub>2</sub>O emissions. They attributed the 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).

*CO<sub>2</sub> and CH<sub>4</sub> 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 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 CO<sub>2</sub> fluxes from elevated CO<sub>2</sub> 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.

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 CO<sub>2</sub> 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<sub>4</sub> consumption rates under elevated CO<sub>2</sub> in a pine plantation. The decrease in CH<sub>4</sub> consumption was attributed, in part to wetter soils under elevated CO<sub>2</sub>. 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<sub>2</sub> in the semiarid grassland likely produced more favorable conditions for methanotrophic activity, rather than limiting CH<sub>4</sub> 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 CO<sub>2</sub> because of N limitation. Conversely, Hungate et al. (1997c) found that higher soil water contents under elevated CO<sub>2</sub> 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 CO<sub>2</sub> and N immobilization is increased, thereby decreasing NO emissions, despite the fact that we observed no general CO<sub>2</sub>-induced effect on NO<sub>x</sub> and N<sub>2</sub>O 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 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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**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 <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

\*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<sup>-1</sup>, respectively (Mosier et al. 1998).

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

CO <sub>2</sub> Treatment	CO <sub>2</sub>	CH <sub>4</sub>	NO <sub>x</sub>	N <sub>2</sub> O
	mg C m <sup>-2</sup> hr <sup>-1</sup>	µg C m <sup>-2</sup> hr <sup>-1</sup>	----- µg N m <sup>-2</sup> hr <sup>-1</sup>	
<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).

## List of Figures

**Figure 1.** Trace gas flux, air temperature and soil water content in ambient and elevated CO<sub>2</sub> chambers: a) NO<sub>x</sub> flux; b) N<sub>2</sub>O flux; c) uptake of atmospheric CH<sub>4</sub> by soil micro-organisms; d) dark chamber CO<sub>2</sub> 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.

