CARBON DIOXIDE CONCENTRATION IN SOIL EFFECTS ON NITRIFICATION, DENITRIFICATION AND ASSOCIATED NITROUS OXIDE PRODUCTION

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Summary—Experiments were conducted to study the effects of a range of CO_2 concentrations (ambient to 100%) on nitrification, denitrification and associated nitrous oxide (N_2O) production in a silt loam soil It was found that increase in CO_2 concentration from 0 3 to 100% CO_2 increasingly retarded the rate of nitrification No nitrification occurred at 100% CO_2 Nitrous oxide production associated with nitrification increased as CO_2 increased from 0 3 to 2 6% and tended to be greater as CO_2 concentration increased to 73% At 100% CO_2, no N_2O was produced during 7 days at 25°C

Carbon dioxide did not affect N_2O production or reduction in a saturated NO_3^--amended soil or the rate of N_2O reduction in anaerobic environments

INTRODUCTION

Carbon dioxide, a product of both aerobic and anaerobic metabolism, can have a direct influence on microbial growth It is usually the sole C source for chemoautotrophs and photoautotrophs and apparently is also required, in addition to organic C, for most heterotrophs (Alexander, 1977) While CO_2 is rarely limiting in soil due to its continual evolution from plant roots and during organic matter decomposition, excess CO_2 may be toxic to some soil fungi (Alexander, 1977) High CO_2 in the soil atmosphere will also affect solution pH, nutrient availability, aeration status (Bohn et al, 1979) and root growth (Buyanovsky and Wagner, 1983)

However, little attention has been paid to possible effects of CO_2 on soil microbial activities Clark (1968) found more rapid nitrification in the range 0 6–2 9% CO_2 than at higher and lower CO_2 concentrations (see also Singh and Kanehiro, 1972) Yeomans and Beauchamp (1978) noted that the rate of reduction of N_2O in the absence or presence of C_2H_2 was delayed when the CO_2 produced during incubation was removed by sorption in an alkali trap

Measurements of CO_2 in atmospheres of field soils and animal feedlots have given values ranging from ambient (0 3% CO_2) to as high as 100% (Enoch and Dasberg, 1971, Wood and Greenwood, 1971, Stevens and Cornforth, 1974, Focht et al, 1979, Buyanovsky and Wagner, 1983, Norstadt and Porter, 1984), although values below 20% CO_2 are more commonly observed However, these are bulk soil atmospheres and microsite CO_2 concentrations probably will be higher than those in large voids

Our objective was to evaluate the effects of different CO_2 concentrations on nitrification, denitrification and N_2O production and reduction associated with a silt loam soil This study was prompted by the previously cited findings of Yeomans and Beauchamp

(1978) A further effect of CO_2 was suggested by Sherlock and Goh (1983), who stated that CO_2 could be involved in creation of anaerobic microsites, leading to increased N_2O evolution from soils

MATERIALS AND METHODS

Soil

A bulk sample of the Ap (0–20 cm) horizon of a Plano silt loam (Typic Argiudoll) was obtained from a site previously cropped to maize (*Zea mays* L) and had a pH (soil water ratio of 1 2) of 7 0 and 14 mg organic C g^{-1} (determined as described in Harris and Adams, 1979) Ammonium-N and NO_3^--N (determined by extraction with 2 M KCl and steam distillation, Keeney and Nelson, 1982) were 7 and 10 mg kg^{-1}, respectively Before use in the incubations, the sample was air-dried and crushed (< 2 mm)

Gas analyses

Flask atmospheres were analyzed for N_2O with a Perkin-Elmer Sigma 3 GC equipped with a high temperature electron capture detector (Goodroad and Keeney, 1984) CO_2 in the soil atmosphere in the flasks was analyzed with a Tracor 150G gas chromatograph fitted with dual ultrasonic detectors (Blackmer and Bremner, 1977)

Incubation procedures

In all experiments, 10 g dry wt of soil were added to a 125 ml Erlenmeyer flask fitted with a rubber septum and the flasks were incubated at 25°C Water content was adjusted to either 2 5 ml 10 g^{-1} (-33 kPa water tension) or 10 ml 10 g^{-1} In experiments involving varying concentrations, the appropriate volume of flask air was removed by syringe and replaced with high purity CO_2 or N_2O Experiments involving the addition of inorganic N used $(NH_4)_2SO_4$ or KNO_3 as amendments All experiments were made in triplicate

Unless otherwise stated, flask atmospheres were sampled using a syringe and analyzed after 4 and 7

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Table 1. Nitrogen transformations in an aerobic soil under varying concentrations

CO$_2$ (%)	NH$_4^+$	NO$_3^-$	CO$_2$ 7 days (%)	4 days	7 days	NH$_4^+$	NO$_3^-$	Sum
	Treatment (μg N g^{-1})			N$_2$O (ng N g^{-1})		Inorganic N (μg N g^{-1})		
Air	0	0	3.3	96 (1)	117 (2)	2 (0)	44 (2)	46 (1)
22	0	0	25	65 (12)	83 (14)	16 (0)	29 (1)	45 (1)
50	0	0	49	132 (5)	154 (11)	26 (1)	17 (7)	43 (1)
73	0	0	70	329 (38)	544 (55)	30 (1)	13 (0)	43 (0)
100	0	0	100	0	0	22 (6)	0	22 (6)
Air	0	95	3.1	94 (8)	123 (10)	0	140 (2)	140 (1)
23	0	95	25	112 (15)	168 (23)	14 (1)	127 (2)	141 (1)
50	0	95	47	133 (6)	250 (28)	24 (0)	115 (0)	139 (0)
73	0	95	71	516 (102)	716 (303)	28 (1)	113 (0)	141 (0)
100	0	95	100	62,000 (2740)	46,400 (1350)	20 (0)	26 (1)	46 (1)
Air	90	0	3.6	205 (21)	396 (126)	48 (1)	78 (2)	126 (1)
2.6	90	0	6.2	474 (141)	1120 (208)	65 (2)	69 (3)	134 (2)
5.2	90	0	8.6	389 (90)	972 (88)	66 (1)	70 (2)	136 (1)
11	90	0	14	538 (202)	1616 (637)	67 (2)	61 (0)	128 (1)
23	90	0	26	399 (16)	1111 (104)	80 (4)	50 (1)	130 (1)
50	90	0	49	298 (45)	1034 (152)	104 (3)	24 (1)	128 (2)
73	90	0	71	609 (260)	1270 (562)	121 (4)	13 (5)	134 (4)
100	90	0	100	0	0	113 (1)	0	113 (1)

[a]Plano silt loam (10 g) was incubated at 25°C with 2.5 ml of water or with 2.5 ml of a solution containing either 900 μg NO$_3^-$-N (as KNO$_3$) or 950 μg NH$_4^+$-N [as (NH$_4$)$_2$SO$_4$]. The soil initially contained 7 and 11 μg NH$_4^+$-N and NO$_3^-$-N g^{-1} soil, respectively. Figures in parentheses are standard deviations.

days of incubation, and the flasks were not flushed between measurements. Soil was analyzed for NH$_4^+$-N, and (NO$_2^-$ + NO$_3^-$)-N at 7 days. Nitrite-N was never > 0.5 mg kg^{-1} and usually was below detection limits.

RESULTS

The CO$_2$ levels in the flasks did not increase markedly during incubation (Table 1). With the exception of the 100% CO$_2$ treatment, O$_2$ was still present in all flasks at the end of 7 days. The presence of CO$_2$ affected both net N$_2$O and NO$_3^-$ production. In the nonamended system, 22% CO$_2$ lowered the amount of NO$_3^-$ produced by *ca.* 45%. This effect continued so that by 73% CO$_2$ only 2 μg NO$_3^-$-N g^{-1} soil was formed (control, 33 μg NO$_3^-$-N g^{-1}; 22%, 18 μg). However, with the exception of the 100% CO$_2$ treatment, net N mineralization was not greatly affected as evidence by the nearly constant value for

Table 2. Effect of CO$_2$ on the ratio of N$_2$O produced to NO$_3^-$ produced

CO$_2$ (%)	NH$_4^+$	NO$_3^-$	N$_2$O-N to NO$_3^-$-N
	Treatment (μg g^{-1})		($\times 10^3$)
Air	0	0	3.4
23	0	0	4.4
50	0	0	22
73	0	0	181
Air	0	95	3.6
23	0	95	7
50	0	95	25
73	0	95	90
Air	90	0	6
2.6	90	0	19
5.2	90	0	16
11	90	0	32
23	90	0	28
50	90	0	74
73	90	0	423

(NH$_4^+$ + NO$_3^-$-N. This was true for the control and for the N-amended soils where NH$_4^+$-N produced increased and NO$_3^-$-N produced decreased as CO$_2$ concentrations increased.

Nitrous oxide production tended to increase with CO$_2$ concentration in the unamended and NO$_3^-$-amended soils to 50% CO$_2$, increased dramatically at 73% CO$_2$ and declined to nil at 100% CO$_2$ with the nonamended soil. It would appear that at least with the air and 22% CO$_2$ treatments, the source of N$_2$O was largely from nitrification (Bremner and Blackmer, 1978) since the ratio of N$_2$O-N produced to NO$_3^-$-N produced (Table 2) was in the range of 3×10^{-3} to 7×10^{-3}. Goodroad and Keeney (1984) found with the Plano at 0.2 m^3 H$_2$O m^{-3} (the same water ratio as used here) the N$_2$O-N to NO$_3^-$-N ratio was 1×10^{-3} at 20° to 30°C. The ratio increased at higher water contents and at lower soil pH's.

The N$_2$O-N to NO$_3^-$-N ratio increased at 50 and 73% CO$_2$, indicating either (a) that more of the N$_2$O was being released during nitrification; (b) that some denitrification was occurring at microsites due to lower pO$_2$ in the high pCO$_2$ flasks, or (c) chemical reactions with NO$_2^-$ (Nelson, 1982) due to the low pH in the CO$_2$-saturated systems. It is not possible with the data presented here to ascertain which of these mechanisms may be dominating. Soil pH could not be measured in the closed systems, but was generally about 6.4 after incubation. The pH of a pure water–H$_2$CO$_3$ system would be 4.3 at 20% and 3.4 at 100% CO$_2$ (Stumm and Morgan, 1970).

The pattern of N$_2$O evolution from the NH$_4^+$-amended soils was more erratic than it was in the control and NO$_3^-$-amended treatments. However, there is little doubt that CO$_2$, even at concentrations commonly occurring in soil profiles, yet sufficiently low that soil pH or aeration would be little affected (up to 11% CO$_2$), markedly increases N$_2$O production during nitrification.

At 100% CO$_2$, lack of O$_2$ would prevent nitrification and denitrification would be rapid. N$_2$O

Table 3. Effect of CO_2 on reduction of N_2O[a]

Treatment	Initial	4 days	7 days
		($\mu g\, N_2O$-$N\, g^{-1}$ soil)	
Control	0	0.1	0.2
CO_2	0	0	0
N_2O-air	189 (9)	149 (6)	137 (5)
N_2O-air-C_2H_2	190 (3)	190 (3)	156 (15)
N_2O-He	190 (2)	0	0
N_2O-CO_2	191 (2)	18 (16)	0
N_2O-CO_2-C_2H_2	190 (5)	144 (2)	85 (49)
N_2O-He-C_2H_2	191 (4)	152 (2)	132 (0)

[a]Plano silt loam (10 g) was treated with 10 ml H_2O and with CO_2 (100%) or He (100%) with or without N_2O (ca 1%). Acetone-free C_2H_2 was added at 0.1%. Figures in parentheses are standard deviations.

formed with the nonamended and NH_4^+-amended treatments was rapidly reduced to N_2. The NO_3^- treatment at 100% CO_2 resulted in a large evolution of N_2O with subsequent reduction of a portion of this to N_2.

Under aerobic conditions, N_2O disappeared from the aerobic system although C_2H_2 inhibited this loss for at least 4 days (Table 3). Reduction of N_2O was rapid under CO_2 or He. In the presence of C_2H_2, N_2O reduction was slower but still significant. Loss of effectiveness of C_2H_2 in blocking N_2O reduction has been reported by others (Yeomans and Beauchamp, 1978). There is some indication that high CO_2 may accelerate the loss of N_2O in the presence of C_2H_2, but the effect was not significant. CO_2 also had no effect on N_2O production from a saturated soil (data not reported). An additional experiment (data not reported) to evaluate the effect of prior treatment of soil with 100% CO_2 for 4 days showed that there was no significant effect of this treatment on nitrification, denitrification, or N_2O production rates.

DISCUSSION

These results indicate that CO_2 increase in soils, particularly arable soils containing high amounts of nitrifiable N, could result in increased N_2O production during nitrification. The effect is not large, however, until CO_2 comprises >50% of the soil atmosphere (Tables 1 and 2), a situation not normally encountered in the field. There appears to be little effect of CO_2 concentrations on N_2O reduction or denitrification (Table 3).

REFERENCES

Alexander M. (1977) *Introduction to Soil Microbiology*, 2nd edn. Wiley, New York.
Blackmer A. M. and Bremner J. M. (1977) Gas chromatographic analysis of soil atmospheres. *Soil Science Society America Journal* **41**, 908–912.
Bremner J. M. and Blackmer A. M. (1978) Nitrous oxide: emission from soils during nitrification of fertilizer nitrogen. *Science* **199**, 295–296.
Bohn H., McNeal B. and O'Conner G. (1979) *Soil Chemistry*. Wiley, New York.
Buyanovsky G. A. and Wagner G. H. (1983) Annual cycles of carbon dioxide level in soil air. *Soil Science Society America Journal* **47**, 1139–1145.
Clark F. E. (1968) The growth of bacteria in soil. In *The Ecology of Soil Bacteria* (T. R. G. Gray and D. Parkinson, Eds), pp. 441–457. University of Toronto Press.
Enoch H. and Dasberg S. C. (1971) The occurrence of high CO_2 concentrations in soil air. *Geoderma* **6**, 17–21.
Focht D. D., Stolzy L. H. and Meek B. D. (1979) Sequential reduction of nitrate and nitrous oxide under field conditions as brought about by organic amendments and irrigation management. *Soil Biology & Biochemistry* **11**, 37–46.
Goodroad L. L. and Keeney D. R. (1984) Nitrous oxide production in aerobic soils under varying pH, temperature and water content. *Soil Biology & Biochemistry* **16**, 39–43.
Harris R. F. and Adams S. S. (1979) Determination of the carbon-bound electron composition of microbial cells and metabolites by dichromate oxidation. *Applied and Environmental Microbiology* **37**, 237–243.
Keeney D. R. and Nelson D. W. (1982) Inorganic forms of nitrogen. In *Methods of Soil Analysis*, Part 2 (2nd edn). Agronomy **10**, 643–698.
Nelson D. W. (1982) Gaseous losses of nitrogen other than through denitrification. In *Nitrogen in Agricultural Soils*. Agronomy **22**, 327–363.
Norstadt F. A. and Porter L. K. (1984) Soil gases and temperature: a beef cattle feedlot compared to alfalfa. *Soil Science Society of America Journal* **48**, 783–789.
Sherlock R. R. and Goh K. M. (1983) Initial emission of nitrous oxide from sheep urine applied to pasture soil. *Soil Biology & Biochemistry* **15**, 615–617.
Singh B. R. and Kanehiro Y. (1972) Interaction of CO_2 tension and salt concentration on mineral nitrogen release in an allophanic soil. *Soil Science* **114**, 401–403.
Stevens R. J. and Cornforth I. S. (1974) The effect of pig slurry applied to a soil surface on the composition of the soil atmosphere. *Journal of Science of Food & Agriculture* **25**, 1263–1272.
Stumm W. and Morgan J. J. (1970) *Aquatic Chemistry. An Introduction Emphasizing Chemical Equilibria in Natural Waters*. Wiley, New York.
Wood J. T. and Greenwood D. J. (1971) Distribution of carbon dioxide and oxygen in the gas phase of aerobic soils. *Journal of Soil Science* **22**, 281–288.
Yeomans J. C. and Beauchamp E. G. (1978) Limited inhibition of nitrous oxide reduction in soil in the presence of acetylene. *Soil Biology & Biochemistry* **10**, 517–519.