Soil Biol. Biochem. Vol. 16, No. 4, pp. 351–356, 1984
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0038-0717/84 $3.00 + 0.00

ACETYLENE AND N-SERVE EFFECTS UPON N_2O EMISSIONS FROM NH_4^+ AND NO_3^- TREATED SOILS UNDER AEROBIC AND ANAEROBIC CONDITIONS

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(*Accepted* 25 *October* 1983)

Summary—N_2O emissions from soils treated with NH_4^+-N under aerobic conditions in the laboratory were 3- to 4-fold higher than those from controls (no extra N added) or when NO_3^--N was added. Although the emission of N_2O-N in these field and laboratory experiments represented only 0.1–0.8% of the applied fertilizer NH_4^+-N and are therefore not significant from an agronomic standpoint, these studies have conclusively demonstrated that the oxidation of applied ammoniacal fertilizers (nitrification) could contribute significantly to the stratospheric N_2O pool.

Like N-serve, acetylene was shown to be a potent inhibitor of nitrification as it stopped the oxidation of NH_4^+-N to (NO_3^+-N + NO_2^-)-N and hence reduced the evolution of N_2O from nitrification within 60 min after its addition.

Although high amounts of NO_3^--N were present, the rate of denitrification was very low from soils with moisture up to 60% saturation. The further increase in the degree of saturation resulted in several-fold increase of denitrification which eventually became the predominant mechanism of gaseous N losses under anaerobic conditions.

INTRODUCTION

The major source of gaseous N losses ($N_2O + N_2$) from soils, is denitrification of nitrate under anaerobic soil conditions (reaction 1, given below). However, the evolution of N_2O also occurs during the oxidation of ammonium (nitrification, reaction 2) under aerobic conditions (Bremner and Blackmer, 1978; Freney *et al.*, 1979).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O\uparrow \rightarrow N_2\uparrow \qquad (1)$$

$$\begin{array}{l} NH_4^+ \rightarrow NH_2OH \rightarrow (\text{e.g. } H_2N_2O_2) \rightarrow NO_2^- \rightarrow NO_3^- \\ \qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\quad N_2O\uparrow \end{array} \qquad (2)$$

The acetylene inhibition technique which has provided a relatively simple method to assess gaseous N losses is based on the blocking of the reduction of N_2O to N_2 by C_2H_2 in reaction (1). However, C_2H_2 inhibits ammonium oxidase, a most sensitive enzyme in nitrification (Suzuki, 1978) and therefore stops nitrification (Hynes and Knowles, 1978; Walter *et al.*, 1979; Berg *et al.*, 1982). Thus under aerobic conditions, N_2O evolution resulting from reaction (2) was reduced in a C_2H_2 environment (Bremner and Blackmer, 1979; Mosier, 1980). Again like that of Mosier (1980) our earlier study (Aulakh *et al.*, 1982), also showed 1 day after the addition of C_2H_2 that N_2O emissions in an aerobic environment were stopped.

We have conducted laboratory and field experiments to (a) study how quickly C_2H_2 inhibits nitrification and hence reduces the emission of N_2O due to reaction (2) during the first 4 h after addition of C_2H_2, and (b) elucidate relative contribution of nitrification and denitrification to the emissions of N_2O under various degrees of moisture saturation.

MATERIALS AND METHODS

In the first laboratory experiment and in the field study, the soil used was Hamlin clay loam (Orthic Black Chernozem) the plow layer (0–15 cm) of which had a pH of 7.3, a total N content of 0.38% and an organic C content of 4.31%.

In a second laboratory experiment, the soil used was an Elstow clay loam (Orthic Dark Brown Chernozem) with a pH of 7.1, a total N content of 0.25% and an organic C content of 3.01% (Aulakh *et al.*, 1984b).

For both laboratory experiments, the bulk soil samples (0–15 cm) were collected and sieved (< 2 mm) in the field-moist condition.

The first laboratory experiment was designed to assess the effect of C_2H_2 on nitrification. The treatments included ambient air (which contains about 21%O_2), N-serve[2-chloro-6-(trichloromethyl)-pyridine] and C_2H_2. Field-moist soil samples (each 100 g on oven-dry basis) were added to six 600 ml conical flasks and 40 μg NH_4^+-N g^{-1} soil as $(NH)_2SO_4$ was added in solution. Two flasks received an application of 10 μg N-serve g^{-1} soil in solution form. Deionized water was added to bring the soil moisture content to 22%, w/w (equivalent to 40% of the amount required to saturate the soil) and the soil was mixed. A glass vial containing 5 ml of 2 N NaOH was placed in each flask (to absorb CO_2) and the flasks were stoppered with rubber corks with serum caps in

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the center. In another set of two flasks, 5% (v/v) of C_2H_2 was injected after removing equal amount of air from each flask. The flasks were sealed and placed in an incubator at 26 ± 1°C. One cm^3 air sample was withdrawn (after thoroughly mixing the air in the flasks) and periodically the concentration of N_2O was analyzed using a 5710-A H.P. gas chromatograph, as described earlier (Aulakh *et al.*, 1982). The data were corrected for the solubility of N_2O in soil water (Moraghan and Buresh, 1977).

In a second laboratory experiment the treatments included control (no extra N) and 50 μg N g^{-1} soil in the form of $(NH_4)_2SO_4$ or KNO_3. Three levels of moisture saturation (60, 90 and 120% of the amount required to saturate the soil) were maintained. Using duplicate treatments and the same procedure as described in the first experiment, the samples were incubated for 96 h with and without C_2H_2 (5% v/v). Periodically gas samples were taken and N_2O was measured. At the end of the experiment, the samples were immediately extracted with 2 M KCl and were analyzed for NH_4^+-N and (NO_3^- + NO_2^-)-N using an Autoanalyzer (Technicon Autoanalyzer Systems, 1973, 1975).

A field experiment was conducted to study the effect of nitrate and ammoniacal fertilizers on emission of N_2O from soil. The experiment was established on a wheat stubble field. The 100 m^2 experimental site was cultivated twice in late September 1980. All loose wheat trash was removed by raking. The treatments included a control and 50 kg N ha^{-1} applied as KNO_3, urea or urea + N-serve and were replicated twice in 2 × 4 m plots. The fertilizers were broadcast and raked into the soil. The urea + N-serve was prepared by spraying the N-serve (1% of active ingredient per weight of fertilizer N) with chromatograph spray gun onto the urea in an enclosed container, the urea was then shaken 1 h and sealed until applied (Guthrie and Bomke, 1980).

Using the "acetylene inhibition-soil core technique", the emissions of N_2O were measured during October and November 1980 (till the soil was frozen) by removing four pairs of undisturbed soil cores from each treatment (Aulakh *et al.*, 1984a). Soil moisture and temperature were also measured. The residual effect of fertilizer N on N_2O emissions from the four treatments was measured during the following spring (April and May 1981). At each sampling, soil samples were analyzed for NH_4^+-N and (NO_3^- + NO_2^-)-N.

RESULTS

Laboratory studies

Where favourable conditions were created for reaction (2) (i.e. ammonium-treated soil was incubated under aerobic conditions), N_2O emission continued to accumulate only under an ambient air environment (Fig. 1). In contrast, where N-serve or C_2H_2 was added, the production of N_2O was stopped within 60 min of their addition.

In the second laboratory experiment where the soil was incubated without added N (control) and with 50 μg N g^{-1} soil added in NO_3^--N and NH_4^+-N forms at 60, 90 and 120% saturation, the emission of N_2O was maximum from the NH_4^+-N treated soil when incubated at 60% saturation without C_2H_2 (Fig. 2). The addition of C_2H_2 reduced the emission of N_2O to the level of the control and the NO_3^--N treatments. This can be attributed to inhibition of reaction (2); this is confirmed from the data in Table 1.

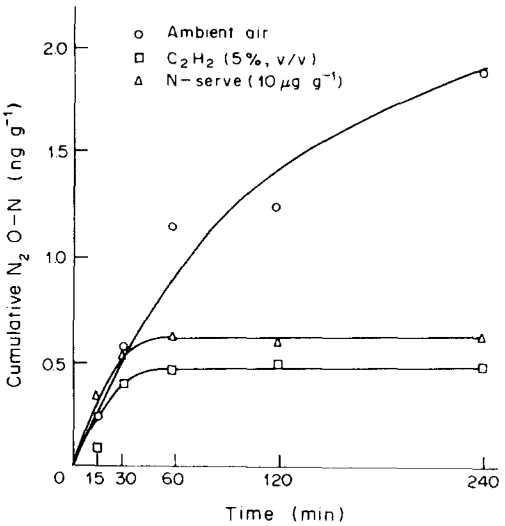

Fig. 1. Nitrous oxide production from the ammonium-treated Hamlin cl soil incubated in the presence of ambient air, N-serve and C_2H_2 at 22% moisture (40% saturation).

On the other hand the rate of denitrification (reaction 1) which was negligible at 60% saturation increased several-fold at 90% saturation, and the presence of C_2H_2 blocked the further reduction of N_2O to N_2 and therefore increased the accumulation of N_2O. Further increase in the degree of saturation to 120% did not increase the rate of denitrification over 90% saturation but that for the control was sharply increased.

It is interesting to note that the increased concentration of NO_3^--N from applied KNO_3 or that accumulated through the nitrification of added NH_4^+-N did not appreciably increase the rate of denitrification over and above the control in spite of the favourable anaerobic soil conditions at 120% saturation. This is presumably because of the very large amount of NO_3^--N present in the soil (Table 1) in comparison to the quantities of NO_2^--N evolved in this short-term incubation.

Field study

An experiment was set out in October 1980 to study the effect of different forms of fertilizer N on the patterns of N_2O emission with and without C_2H_2 under field conditions. the "without C_2H_2" incubation of undisturbed soil cores taken at different dates showed that the application of urea lead to marked increases in the emissions of N_2O over and above the unfertilized (control) treatment, while the addition of equal amounts of N as KNO_3 or "urea + N-serve" did not (Fig. 3). The data in Table 2, however, confirm that the N-serve virtually blocked the oxidation of NH_4^+-N to (NO_3^- + NO_2^-)-N, while in the "urea only" treatment, the oxidation of NH_4^+-N continued over time, and this resulted in N_2O emissions arising primarily from the nitrification reaction.

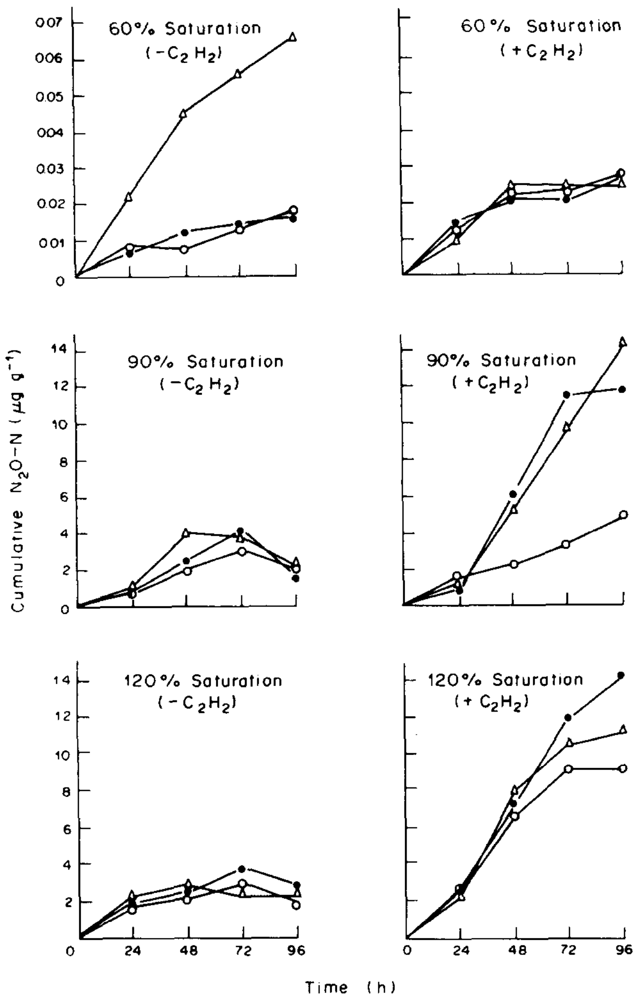

Fig. 2. Nitrous oxide production from Elstow cl soil incubated without added N(○), with additional 50 μg NO_3^--N g $soil^{-1}$(●), and 50 μg NH_4^+-N g $soil^{-1}$ (△) at 60, 90 and 120% saturation in the absence or presence of 5% C_2H_2 (v/v). Note differences in the scale on y-axis.

Table 1. Effect of C_2H_2 on the nitrification of added NH_4^+-N in Elstow cl soil incubated for 96 h at various degrees of moisture saturation

N source	C_2H_2	Degree of moisture saturation (%) 60	 90	 120
	NH_4^+-N, μg g^{-1}			
Control	−	2.5a[1]	4.7a	4.0a
	+	4.7a	5.5a	10.3a
KNO_3	−	2.2a	3.9a	3.8a
	+	2.2a	4.1a	7.3a
$(NH_4)_2SO_4$	−	21.6a	24.3a	28.9a
	+	43.0b	46.7b	49.0b
	$(NO_3^- + NO_2^-)$-N, μg g^{-1}			
Control	−	44.6a	25.3a	13.6a
	+	46.7a	24.0a	7.2a
KNO_3	−	98.2a	74.7a	56.8a
	+	91.1a	68.7a	51.7a
$(NH_4)_2SO_4$	−	61.2b	36.0b	26.6b
	+	45.9a	15.9a	10.0a

[1] In the same column within each N source separately for NH_4^+-N and $(NO_3^- + NO_2^-)$-N, the values in the absence and presence of C_2H_2 are significantly different at $P \leqslant 0.05$ when not followed by the same letter.

The residual effect of the different treatments on N_2O emissions were measured during the following spring (30 April and 6 May, 1981); The data show that the N_2O emissions from the different treatments were not substantially different than the emissions from the corresponding unfertilized plots (Fig. 3). Although high levels of $(NO_3^- + NO_2^-)$-N were present (Table 2), there was negligible denitrification, presumably due to very low soil moisture content, i.e. only 26–37% of saturation (Fig. 3). This field study has again confirmed that the presence of C_2H_2 in the incubation of soil cores inhibited the reaction (2) and reduced the N_2O emissions from the oxidation of applied NH_4^+-N. Similarly N-serve reduced the conversion of NH_4^+-N to $(NO_3^- + NO_2^-)$-N (Table 2) and thus very low N_2O fluxes were noted in its presence (Fig. 3).

DISCUSSION

These results obtained under laboratory and field conditions show that the addition of an NH_4^+ form

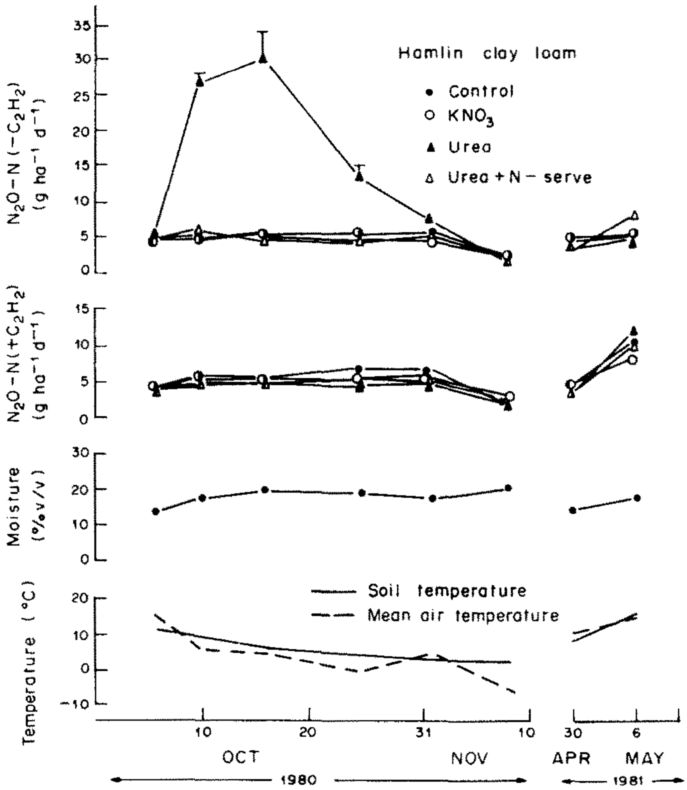

Fig. 3. Emission of N$_2$O-N measured and with C$_2$H$_2$, soil moisture, and air and soil temperature of Hamlin cl soil for the period 6 October–10 November, 1980, and on 30 April and 6 May, 1981. Bars indicates standard deviation.

of N under aerobic conditions ($\leqslant 60\%$ saturation) resulted in a 3–4-fold increase in N$_2$O evolution (due to reaction 2) in comparison to a control or where an equivalent amount of NO$_3^-$-N was added (Table 3). However, the losses represented only $\leqslant 0.1\%$ in the laboratory studies (4 days) to 0.8% in the field study (19 days) of the applied NH$_4^+$-N. These results are consistent with other laboratory and field studies (Bremner and Blackmer, 1978; Breitenbeck *et al.*, 1980) where it was found that the loss of fertilizer N as N$_2$O gas represents only 0.1–0.5% of the added NH$_4^+$-N. Accordingly it can be concluded that reaction (2) (nitrification) does not lead to appreciable gaseous losses of N but it could make a substantial contribution to the stratospheric N$_2$O pool where it may destroy the ozone catalytically and increase indirectly the health hazards (CAST, 1976; Crutzen and Ehhalt, 1977).

Bremner and Blackmer (1979), Mosier (1980), and Aulakh *et al.* (1982) have shown that C$_2$H$_2$ inhibits nitrification and stops the evolution of N$_2$O through reaction (2). However, the first measurement of N$_2$O$_2$ after the addition of C$_2$H$_2$ in their studies was taken after 24–48 h. Our experiment illustrated very clearly that C$_2$H$_2$ is a potent inhibitor of N$_2$O evolution either equal to or greater than N-serve, a well-known nitrification inhibitor, and stops the evolution of N$_2$O in less than 1 h.

The rate of denitrification (reaction 1) was very low from soils with moisture up to 60% saturation but

Table 2. Amounts of NH$_4^+$-N and (NO$_3^-$ + NO$_2^-$)-N in plots of Hamlin cl soil (0–15 cm). Treated with KNO$_3$, urea and urea + N-serve on 6 October, 1980[1]

Treatment	10 Oct. 1980	16 Oct. 1980	25 Oct. 1980	1 Nov. 1980	8 Nov. 1980	30 Apr. 1981	6 May 1981
			NH$_4^+$-N, μg g^{-1}				
Control	2.5 ± 0	2.1 ± 0.3	3.9 ± 0.5	1.9 ± 0.5	2.6 ± 0.8	4.1 ± 0.3	4.1 ± 1.0
KNO$_3$	2.8 ± 0.3	2.3 ± 0.3	3.8 ± 0.3	2.1 ± 0.9	2.1 ± 0.3	3.8 ± 1.0	3.1 ± 0.6
Urea	26.1 ± 2.8	19.1 ± 3.9	16.6 ± 3.4	13.6 ± 2.3	13.6 ± 2.4	2.3 ± 0.5	3.3 ± 1.5
Urea + N-serve	30.9 ± 4.3	27.6 ± 3.4	26.0 ± 6.7	23.9 ± 1.4	22.0 ± 3.2	8.8 ± 1.1	5.0 ± 0.8
			(NO$_3^-$ + NO$_2^-$)-N, μg g^{-1}				
Control	4.2 ± 1.6	5.9 ± 1.8	7.1 ± 1.3	7.3 ± 1.5	7.2 ± 1.1	15.6 ± 1.0	14.3 ± 2.0
KNO$_3$	29.2 ± 3.5	32.3 ± 2.2	32.9 ± 4.0	31.5 ± 3.0	33.1 ± 2.6	38.1 ± 2.0	39.3 ± 2.8
Urea	12.4 ± 2.3	19.3 ± 3.6	20.7 ± 3.0	23.4 ± 3.1	23.7 ± 1.8	44.5 ± 1.8	45.3 ± 2.2
Urea + N-serve	5.2 ± 1.1	9.6 ± 2.0	10.1 ± 1.3	11.6 ± 1.8	12.2 ± 2.3	49.3 ± 2.5	54.2 ± 1.6

[1] NH$_4^+$-N and (NO$_3^-$ + NO$_2^-$)-N contents of soil on 6 October, 1980 were 3.0 and 1.1 μg g^{-1}, respectively.

Analysis of variance: Treatments are significant for both NH$_4^+$-N and (NO$_3^-$ + NO$_2^-$)-N at $P \leqslant 0.01$.

Table 3. Amounts of N_2O evolved from the soils treated with different forms of fertilizer N

Form of N added	C_2H_2	Laboratory experiments at degree of saturation[1] 40%[2]	60%[3]	90%[3]	120%[3]	Field experiment[1] at 26–37% saturation
None	–	ND[4]	18	198	192	63
	+	ND	27	493	924	71
NO_3^--N	–	ND	17 (0)	179 (0)	287 (0.19)	64 (0)
	+	ND	27 (0)	1182 (1.38)	1434 (1.02)	70 (0)
NH_4^+-N[5]	–	28 (0.07)	67 (0.10)	219 (0.04)	229 (0.07)	269 (0.82)
	+	9 (0.02)	25 (0)	1436 (1.89)	1124 (0.40)	60 (0)
NH_4^+-N[5] + N-serve	–	10 (0.03)	ND	ND	ND	62 (0)
	+	ND	ND	ND	ND	62 (0)

[1] Amount of N_2O-N evolved (ng N_2O-N g soil^{-1}) during initial 4 days in laboratory experiments and 19 days in field experiments. The figures in parentheses indicate fertilizer-induced emission of N_2O calculated as % of fertilizer N added, i.e. = (N_2O-N evolved from fertilized soil – N_2O-N evolved from control soil)/amount of N added × 100.

[2] Calculated from the data reported previously (Aulakh *et al.*, 1982).

[3] Calculated from the data of second laboratory experiment reported in the present study.

[4] ND = No data, as the particular treatment was not included.

[5] Either ammonium sulfate or urea was used as a source of NH_4^+-N.

increased very rapidly beyond this moisture content. Bremner and Shaw (1958) and Burford and Stefanson (1973) have demonstrated that denitrification is usually negligible at moisture contents below 60% of the maximum water holding capacity of soils regardless of the C supply and NO_3^--N concentration. Craswell and Martin (1974) have further shown that even in soils containing a relatively high moisture content (90% w/w), denitrification can be relatively low due to the high rates of O_2 diffusion rate in well structured soils.

Bremner and Blackmer (1978) on the basis of laboratory experiments, and McKenney *et al.* (1978) and Breitenbeck *et al.* (1980) from field experiments have shown that increased amounts of N_2O are evolved from soils fertilized with urea or ammonium sulphate for periods of about 2–3 weeks after application. Results of our field study show that the gaseous losses of N, through oxidative reaction, continued for approximately 4 weeks.

The data of these experiments show that N_2O fluxes measured in the presence of C_2H_2 can be equal to, higher or lower than those measured in its absence depending upon the soil conditions (favourable for reaction 1 or 2). While it is not possible to differentiate between the N_2O produced through reactions (1) and (2), respectively, in the light of results discussed above it is reasonable to assume that when soil moisture is high (anaerobic conditions), the N_2O was primarily produced as a result of reaction (1). The data in Fig. 2 support this in that the N_2O from the C_2H_2 system was greater than the N_2O without C_2H_2. In general, when soil moisture was average or low ($\leq 60\%$ saturation), a substantial amount of N_2O emission was probably a result of reaction (2); the N_2O flux in the presence of C_2H_2 was either equal to or less than the flux in its absence. Thus, these findings further the support the assumption made by us (Aulakh *et al.*, 1982) that the contribution of reaction (2) (nitrification) be taken into account by estimating the total gaseous N losses from the higher values of N_2O evolved in the presence or absence of C_2H_2 when the "acetylene inhibition technique" is used to assess gaseous N losses.

Acknowledgements—We gratefully acknowledge the financial assistance received from the Natural Sciences and Engineering Research Council of Canada.

Saskatchewan Institute of Pedology Publication R348.

REFERENCES

Aulakh M. S., Rennie D. A. and Paul E. A. (1982) Gaseous nitrogen losses from cropped and summerfallowed soils. *Canadian Journal of Soil Science* **62,** 187–195.

Aulakh M. S., Rennie D. A. and Paul E. A. (1984a) Field studies on gaseous nitrogen losses from soils under continuous wheat versus a wheat-fallow rotation. *Plant & Soil* **75,** In press.

Aulakh M. S., Rennie D. A. and Paul E. A. (1984b) Gaseous N losses from soils under zero-till as compared to conventional-till management systems. *Journal of Environmental Quality* **12,** In press.

Berg. P., Klemedtsson L. and Rosswall T. (1982) Inhibitory effect of low partial pressures of acetylene on nitrification. *Soil Biology & Biochemistry* **14,** 301–303.

Breitenbeck G. A., Blackmer A. M. and Bremner A. M. (1980) Effects of different nitrogen fertilizers on emission of nitrous oxide from soil. *Geophysical Research Letters* **7,** 85–88.

Bremner J. M. and Blacker A. M. (1978) Nitrous oxide: emission from soils during nitrification of fertilizer nitrogen. *Science* **199,** 295–296.

Bremner J. M. and Blackmer A. M. (1979) Effects of acetylene and soil water content on emission of nitrous oxide from soils. *Nature* **280,** 380–381.

Bremner J. M. and Shaw K. (1958) Denitrification in soil. II. Factors affecting denitrification. *Journal of Agricultural Science* **51,** 40–52.

Burford J. R. and Stefanson R. C. (1973) Measurement of gaseous losses of nitrogen from soils. *Soil Biology & Biochemistry* **5,** 133–141.

CAST (Council for Agricultural Science and Technology) (1976) Effect of increased nitrogen fixation on stratospheric ozone. Report No. 53, Iowa State University, Ames, Iowa.

Craswell E. T. and Martin A. E. (1974) Effect of moisture content on denitrification in a clay soil. *Soil Biology & Biochemistry* **6,** 127–129.

Crutzen R. J. and Ehhalt D. H. (1977) Effects of nitrogen fertilizers and combustion on the stratospheric ozone layer. *Ambio* **6,** 112–117.

Freney J. R., Denmead O. T. and Simpson J. R. (1979) Nitrous oxide emission from soils at low moisture contents. *Soil Biology & Biochemistry* **11,** 167–173.

Guthrie T. F. and Bomke A. A. (1980) Nitrification inhibition by N-serve and ATC in soils of varying texture. *Soil Science Society of America Journal* **44,** 314–320.

Hynes R. K. and Knowles R. (1978) Inhibition by acetylene of ammonia oxidation in *Nitrosomonas europaea*. *FEMS Microbiology Letter* **4**, 319–321.

McKenney D. J., Wade D. L. and Findlay W. I. (1978) Rates of N_2O evolution from N-fertilized soil. *Geophysical Research Letters* **5**, 777–780.

Morahan J. T. and Buresh R. (1977) Correction for dissolved nitrous oxide in nitrogen studies. *Soil Science Society of America Journal* **41**, 1201–1202.

Mosier A. R. (1980) Acetylene inhibtion of ammonium oxidation in soil. *Soil Biology & Biochemistry* **12**, 443–444.

Suzuki I. (1978) Microbial inorganic oxidations with special reference to NH_4. Paper presented at the *12th International Congress on Microbiology*, Munich, FRG, September, 1978.

Technicon AutoAnalyzer Systems (1973) *Nitrate and Nitrite in Water and Waste Water*. Technicon Industrial Systems, New York.

Technicon Autoanalyzer Systems (1975) *Ammonia by Acid Digests*. Technicon Industrial Systems, New York.

Walter H. M., Keeney D. R. and Fillery I. R. (1979) Inhibition of nitrification by acetylene. *Soil Science Society of America Journal* **43**, 195–196.