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Author(s): D. LASKOWSKI and J. T. MORAGHAN

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THE EFFECT OF NITRATE AND NITROUS OXIDE ON HYDROGEN AND METHANE ACCUMULATION IN ANAEROBICALLY-INCUBATED SOILS

by D. LASKOWSKI and J. T. MORAGHAN

Department of Soils, North Dakota State University, Fargo, North Dakota

INTRODUCTION

The gases methane (CH_4) and hydrogen (H_2) are common gaseous products resulting from anaerobic decomposition of organic matter. In exhaustive investigations, Harrison and Aiyer [10] demonstrated that large amounts of CH_4 and smaller amounts of H_2 are common products of waterlogged soils. Alexander [2], Aomine [3] and Koyama [11] have recently discussed the production of these gases in soil. An interest in CH_4 and H_2 production in soil has arisen in connection with the use of the manometric technique to study denitrification in soils [7 9 12 13 14 25 26]. In this technique, the evolved carbon dioxide (CO_2) and other acidic gases are absorbed in alkali, and the increase in pressure in the system is assumed to result from the accumulation of nitrogen (N_2) and nitrous oxide (N_2O). However, if gases other than these should accumulate during the denitrification studies the results would be invalidated.

It is well known that nitrate (NO_3^-) can control the production of nuisance odors and the reduction of iron, manganese and sulfate in soil. The literature concerning these effects as well as the effect of NO_3^- in stabilizing soil redox potentials at high values has been reviewed by Ponnamperuma [19 20]. The growth of *Methanobacterium omelianskii* has been reported to be almost completely prevented at a NO_3^--N content of between 0.0111 to 0.0139% [4]. However, the effect of NO_3^- on the production of CH_4 and H_2 in soil does not appear to have been extensively investigated. The possibility that NO_3^- might suppress the production of CH_4 and H_2 in soil is sug-

gested by the work of Takai *et al.*[22] and Yamane [27]; these workers demonstrated that small amounts of native NO_3^- disappeared from certain incubated waterlogged soils before the production of H_2 and CH_4 was observed. Their results also suggest that the redox potential of a soil must be greatly reduced before CH_4 and H_2 production will occur.

The objective of the work presented in this paper was to determine the effect of added NO_3^- and its reduction product N_2O on the production of CH_4 and H_2 in soil during incubation under anaerobic conditions. The research has relevance in connection with the use of the manometric technique to study denitrification.

MATERIALS AND METHODS

Soils and incubation experiments

Some characteristics of the soils used in this study are given in Table 1. The Tetonka and Cavour loams which were obtained from eastern North Dakota are examples of a Planosol and a Solodized Solonetz, respectively. The soils were taken from the surface 0 to 6 inches and were air dried, ground sufficiently fine to pass a 60-mesh sieve and then stored in an air-dried condition until required.

TABLE 1

Some chemical characteristics of the soils used to investigate the effect of NO^-_3 and N_2O on H_2 and CH_4 accumulation

Soil	pH*	C %	N %	NO_3^-–N ppm	SO_4^{2-}–S ppm	Specific conductivity mhos/cm
Tetonka	5.8	4.8	0.460	3.1	21.1	0.22
Cavour	7.5	2.6	0.215	4.4	5.6	0.24

* Soil/water = 1/2.

The effect of KNO_3- and N_2O-N (0 and 500 ppm) on the accumulation of H_2, N_2, and CH_4 during anaerobic incubation of the Tetonka soil was first examined. In this investigation, the effect of NO_3^- on the accumulation of NO_2^- and N_2O was also determined. The effect of KNO_3–N (0 and 500 ppm) on the soil pH during anaerobic incubation was studied in a separate indentical experiment. The NO_3^- and N_2O treatments were added to both the air-dried soil and soil actively producing CH_4 as a consequence of an anaerobic pre-incubation (69 and 72 hours for the N_2O and NO_3^- treatments respectively) prior to the addition of the N–treatments. The moisture added to 5 g soil for the pre-incubation was 6 ml. At the end of the pre-incubation all flasks were opened and treated identically apart from the addition of the N-treatments.

The gases formed during the pre-incubation were removed during the re-establishment of the anaerobic atmosphere.

The effect of KNO_3–N (0 and 250 ppm) on the accumulation of CH_4, H_2, NO_2^- and N_2 during anaerobic incubation of the air-dried Cavour soil was next studied. A lower amount of NO_3^- was applied to this soil because a preliminary experiment had indicated that its readily oxidizable carbon content was markedly lower than of the Tetonka soil.

The effects of KNO_3 and KCl at equivalent K^+ rates (0.179 me K^+-Tetonka, 0.0893 me K^+–Cavour) on CH_4 production were investigated in the final experiment. The amounts of K^+ were equivalent to those applied to the appropriate soils in the earlier experiments.

The weight of soil used in all experiments was 5 g and the total volume of water added was 11 ml. The soils were incubated in 150 ml Kjeldahl digestion flasks provided with ground glass joints. The contents of each flask were sealed from the atmosphere by inserting in the flask neck an inner ground glass joint with an attached stopcock. An anaerobic atmosphere was obtained by placing the incubation flask on a manifold and removing the original atmosphere by a series of seven evacuations and flushings with He. The NO_3^- was added with the water, while the N_2O was added immediately at the end of the seventh evacuation. The He was U.H.P. grade obtained from the Matheson Co., Joliet, Illinois. The O_2 content was 0.1 ppm (v/v). The N_2O was also obtained from Matheson and was of 98% minimum purity. To remove traces of O_2 the N_2O from the cylinder was stored for several days in a 2-liter all-glass flask which contained 100 ml alkaline pyrogallol. For the addition of N_2O, the glass reservoir was attached to a manifold outlet on which the incubation flasks were evacuated. All treatments were replicated twice and the incubations were carried out in a water-bath at 30°C.

Analytical methods

The methods used were as follows:

Inorganic-N – The NH_4^+–, NO_2^-–, and NO_3^-–N were extracted from the incubated soil by shaking for 60 minutes with 50 ml of 2*N* KCl. NO_2^-–N was estimated in an aliquot of the 2*N* KCl extract by means of the modified diazotization technique described by Barnes and Folkard [5]. NH_4^+–N was released from another aliquot of the extract by alkaline steam distillation using MgO as a base. The distillation proceeded for 4 minutes at a rate of 7 ml per minute. To the contents of the distillation flask left after the alkaline steam distillation with MgO, 1 g of finely divided Devarda's alloy was added, and the NH_4^+–N produced from an additional 4-minute steam distillation was determined by either titration with standard acid or nesslerization. This NH_4^+–N was assumed to result from the NO_2^-– and NO_3^-–N of the aliquot. NO_3^-–N was determined by difference.

Soil pH – The pH of the soil suspension (soil/water = 1/2.2) was read immediately after the sample was taken out of the water bath. Sufficient solid KCl was then added to the flask to make the soil solution 1*N* with respect to this reagent. The flask and its contents were shaken for 15 minutes before suspension pH readings were taken again.

Gas analysis – Gas mixtures containing H_2, N_2, N_2O and CH_4 were analyzed. These gas samples were analyzed on a Model 29 Fisher-Hamilton Gas Partitioner using He as a carrier gas at a pressure of 21 pounds per square inch and a flow rate of 40 ml per minute. The gas chromatograph had two Al columns in series with hot wire filament detectors at the exhaust end of each column. Signals from the two detectors were recorded with a Sargent SR recorder equipped with a Disc intergator. The first column consisted of 18 inches ascarite and 2 feet of silica gel, while the second column consisted of 6.5 feet of Molecular Sieve 5A. The diameter of the first column was 1/4 inch while that of the second was 3/16 inch. The order of elution of the gases as indicated on the recorder chart was similar to that described by Otterstein[17] for the above columns with the exception that the peaks resulting from the passage of H_2 past the second detector and CH_4 past the first detector were confounded. N_2 and CH_4 were estimated from the responses produced by the passage of the gases past the second detector, while N_2O and H_2 were estimated from the responses resulting from their passage past the first detector. Peak areas recorded by the integrator unit were used in determining the quantities of the gases N_2, N_2O and CH_4. Linear peak height was used for H_2 determinations. For calibration purposes standard gas mixtures obtained from the Matheson Co. were passed through the chromatograph at regular intervals.

Before analysis of its gas mixture, a flask was gently shaken and then it was attached to one of the sample loop outlets on the instrument; the other sample loop outlet was attached to a vacuum line containing a manometer. The 5-ml sample loop of the gas chromatograph and its accessory tubing leading to the vacuum line and the flask were evacuated for at least 6 minutes prior to opening the flask stopcock and allowing the gas mixture to expand into the evacuated sample loop. The He carrier gas was then diverted through the instrument's sample loop. From a knowledge of the volumes of the flask and its contents and of the sample loop and its accessory tubing the fraction of the flask atmosphere sampled was determined. The N_2O value was corrected for dissolved gas at 30°C by assuming that the Henry's Law constant for N_2O dissolved in soil water was identical with that in distilled water.

RESULTS

The addition of NO_3^- directly to the air-dried Tetonka soil (Fig. 1) as well as to the pre-incubated Tetonka soil that was actively producing CH_4 at the time of treatment application (Fig. 2) resulted in a decrease in the rate of accumulation of CH_4. As expected, the check (0 ppm NO_3^--N) pre-incubated soil produced CH_4 at a faster rate than the check remoistened air-dried soil. However, in the presence of NO_3^-, the accumulation of CH_4 was slower in the pre-incubated soil. A small amount of CH_4 (0.5 ppm) was found in the NO_3^- treated pre-incubated soil at 12 hours. Whether this was the

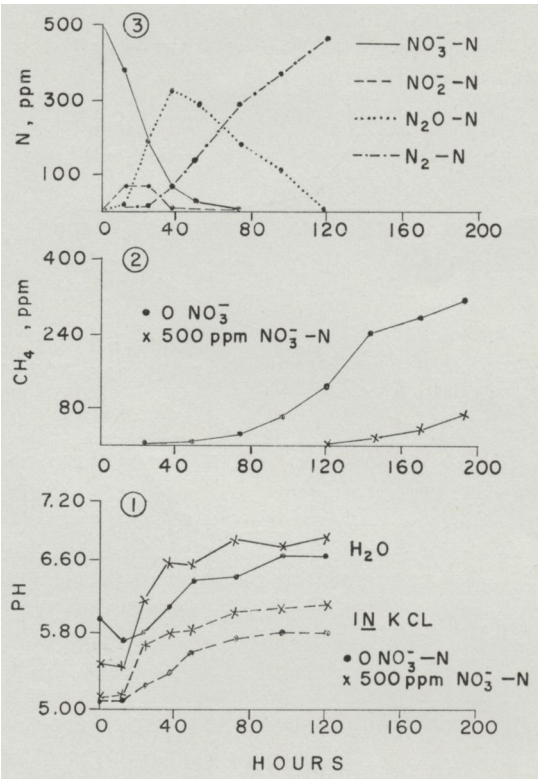

Fig. 1. Effect of 0 and 500 ppm added NO_3^-–N on soil pH (1) and the accumulation of CH_4 (2) during anaerobic incubation of a remoistened air-dried Tetonka loam soil together with the denitrification pattern of the soil treated with 500 ppm NO_3^-–N (3).

result of an incomplete removal of CH_4 from the soil matrix during the evacuation process or the result of production subsequent to NO_3^- addition was not determined.

In both the pre-incubated and remoistened air-dried Tetonka soils the added NO_3^- rapidly disappeared and after a transient accumulation of NO_2^- and N_2O large quantities of N_2 were produced. The denitrifiction pattern of the pre-incubated soil different from that of the remoistened air-dried soil in that the disappearance of NO_3^- and N_2O was more rapid; in addition, NO_2^- accumulated to a larger extent in the pre-incubated soil.

Both in the presence and absence of NO_3^- the pH of the remoistened air-dried and pre-incubated soils increased during incu-

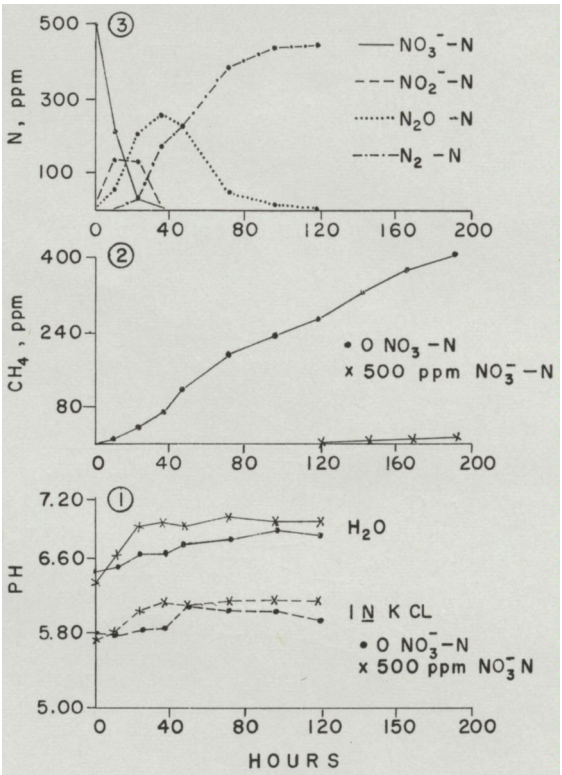

Fig. 2. Effect of 0 and 500 ppm added NO_3^-–N on soil pH (1) and the accumulation of CH_4 (2) during anaerobic incubation of a Tetonka loam soil together with the denitrification pattern of the soil treated with 500 ppm NO_3^-–N (3). The soil was anaerobically pre-incubated for 72 hours prior to NO_3^- addition.

bation. The pH values of the soil suspensions in which the soil solution was 1N with respect to KCl were markedly lower than the values found in the corresponding water suspensions. The pH values of the NO_3^--treated soils at a given time period were generaly higher than those of the soil to which no NO_3^- was added.

Table 2 shows the effect of NO_3^- on H_2 production in the remoistened air-dried Tetonka soil. NO_3^- suppressed H_2 accumulation completely, and after an initial build-up in the check series, the H_2 content gradually decreased. No H_2 was found to accumulate in the pre-incubated Tetonka soil that was actively producing CH_4 at the time of treatment application.

TABLE 2

Effect of 500 ppm NO_3^-–N on H_2 accumulation (ppm) by a remoistened air-dried Tetonka loam during anaerobic incubation

Treatment	Incubation period, hours							
	0	12	24	37	48	72	96	120
Check	0	8.6	6.0	8.7	5.3	3.3	3.1	1.8
500 ppm NO_3–N	0	0	0	0	0	0	0	0

The data in Tables 3 and 4 show that 500 ppm N_2O-N reduced CH_4 production in both the remoistened air-dried and pre-incubated Tetonka soils respectively. As in the case of the NO_3^- experiment, CH_4 production proceeded at a faster rate in the check pre-incubated soil. However, the CH_4 production pattern in the presence of N_2O was rather similar in both incubation systems. N_2O prevented H_2

TABLE 3

Effect of 0 and 500 ppm N_2O–N on CH_4 and H_2 production by a remoistened, air-dried Tetonka loam during anaerobic incubation

Treatment	Incubation, hours	% N_2O remaining	CH_4, ppm	H_2, ppm
Check	29		1.0	10.0
	47		4.5	8.9
	71		19.2	7.3
	96		59.1	6.9
500 ppm N_2O–N	29	88.0	0.5	0
	47	49.9	3.4	0
	71	0	14.2	0
	96	0	31.5	0

TABEL 4

Effect of 0 and 500 ppm N_2O–N on CH_4 and H_2 production by an anaerobically-incubated Tetonka loam pre-incubated anaerobically for 69 hours prior to treatment application

Treatment	Incubation, hours	% N_2O remaining	CH_4, ppm	H_2, ppm
0 ppm N_2O–N	22		25.8	0
	47		92.5	0
	71		198	0
	96		263	0
500 ppm N_2O–N	22	96.0	4.5	0
	47	62.3	7.4	0
	71	0	12.1	0
	96	0	29.0	0

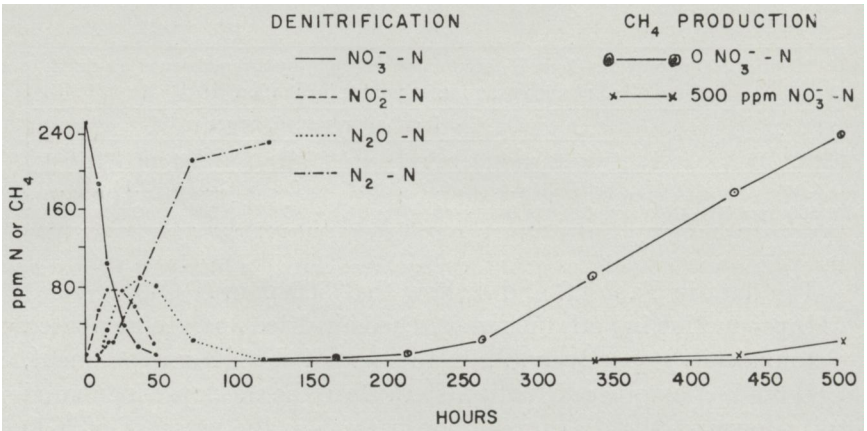

Fig. 3. Effect of 0 and 250 ppm NO_3^-–N on CH_4 accumulation by a Cavour loam together with the denitrification pattern of the soil treated with 250 ppm NO_3^-–N.

accumulation in the remoistened air-dried soil; no H_2 accumulated in the pre-incubated soil. Although not exhaustively investigated, it appeared that N_2O reduction was slower in the pre-incubated soil.

As illustrated by the data given in Figure 3, the addition of NO_3^- to the Cavour soil also resulted in an appreciable reduction in CH_4 accumulation during anaerobic incubation. In comparison to the results obtained with the Tetonka soil, CH_4 was produced in the check Cavour soil only after an appreciable lag phase. In this soil, N_2 was the ultimate denitrification product and no H_2 accumulated at any incubation period, even in the absence of added NO_3^-.

TABEL 5

Effect of different K-salts (0.179 meq K^+ Tetonka loam, 0.0893 meq K^+ Cavour loam) on CH_4 production during anaerobic incubation of Tetonka and Cavour loams

Soil	Incubation, days	CH_4, ppm		
		Check	KCl	KNO_3
Tetonka	6	213	208	17
Cavour	14	198	166	9

As indicated in Table 5, KNO_3 was much more repressive to CH_4 production during anaerobic incubation of the Tetonka and Cavour soils than was KCl. KCl had a slight depressing effect on CH_4 production by the Cavour soil. However, this effect was very minor as compared to the effect of KNO_3.

DISCUSSION

KNO_3 repressed CH_4 production in the Tetonka and Cavour soils much more than did KCl. Alexander [1], in discussing the sparing effect of NO_3^- on Fe^{2+} formation from Fe^{3+} and S^{2-} formation from SO_4^{2-}, stressed the difficulty of interpreting such effects. The same comment applies to the effect of NO_3^- in suppressing soil CH_4 production by the Tetonka and Cavour soils. The suppression could be the result of either direct or indirect effects of NO_3^- on the CH_4-producing bacteria. Barker [4] has observed that the growth of a CH_4-producing species in pure culture was inhibited by NO_3^-, and this direct effect could be operative in soils also. In most soils, NO_3^- is not likely to suppress permanently CH_4 formation since the concentration decreases during anaerobic incubation as a result of denitrification. An indirect effect of NO_3^- could result from NO_3^- affecting the supply of substrate to the CH_4-formers or by NO_3^- creating an unfavorable environment for the growth of these organisms. Studies with pure cultures of denitrifying bacteria have revealed that a large proportion of the H donor is converted into CO_2 [21 23 24 25]. Broadbent [6] observed that in soil under anaerobic conditions a larger amount of CO_2 was produced in the presence than in the absence of NO_3^-. Greenwood and Lees [8] found that the application of NO_3^- to anaerobic soils reduced the accumulation of volatile fatty acids. Because certain species of CH_4-producing bacteria require volatile fatty acids as substrates [2], a reduction in the volatile fatty acid content could lower the CH_4-producing potential of a soil. CH_4-producing bacteria require a low redox potential for their growth [15 22]; hence the ability of NO_3^- to poise the redox potential of an anaerobic system at a high potential is another way in which NO_3^- could suppress CH_4 production.

Both direct and indirect effects resulting from the reduction of N_2O in soil could suppress CH_4 production. CO_2 production would be favored by N_2O reduction[23], and the redox potential of the soil could be poised at a level sufficiently high to suppress the CH_4-producing bacteria. A study to determine whether the growth of CH_4-producing bacteria in pure culture is suppressed by N_2O would be of interest. Because of its lower oxidation state, 500 ppm N_2O-N would have less of an effect on available substrate for the CH_4-producing bacteria than would 500 ppm NO_3^--N.

The nature of the denitrification products in both the Tetonka and Cavour soils was similar to those reported elsewhere [16]. The more rapid rate of NO_3^- disappearance and the smaller accumulation of N_2O observed in the pre-incubation Tetonka soil as compared to that in the remoistened air-dried soil probably resulted from the higher pH of the soil at the time of NO_3^- application. As discussed by Piper [18], the pH of suspensions 1N with respect to KCl were lower than comparable water suspensions. The NO_3^- treated soil tended to have a higher pH than the corresponding check soil, but in both cases the pH values increased during incubation. Ponnamperuma [19 20] has reviewed the roles of NH_3 formation and Fe and Mn reduction as possible causes contributing to an increase in pH of submerged soils. The production of CH_4 was probably an additional factor which favored an increase in pH.

H_2 was produced in measurable amounts only by the remoistened airdried Tetonka soil and both N_2O and NO_3^- completely suppressed its accumlation. Whether the addition of these N treatments resulted in a more rapid consumption or the non-production of H_2 is uncertain.

In the manometric technique for studying denitrification in soil it is assumed that NH_3, CH_4 and H_2 do not accumulate in appreciable amounts as gases in the flask atmosphere. In closed flasks it is unlikely that NH_3 would be present in more than trace amounts in the flask atmospheres when the pH of the incubated soil is less than 8. Large amounts of CH_4 and sometimes H_2 can result from the anaerobic decomposition of soil, but the evidence shown suggests that NO_3^- and N_2O retard their production. The manometric technique would therefore appear to be a suitable method for studying soil denitrification provided any such work is terminated soon after a stationary phase is reached in gas production.

SUMMARY

The effect of KNO_3 and N_2O on the accumulation of CH_4, H_2 and denitrification products in two North Dakota soils during anaerobic incubation at 30°C was studied by means of gas chromatography. KNO_3 and N_2O (500 ppm N) reduced the rate of accumulation of CH_4 by a Tetonka soil regardless of whether the soil was in an air-dried condition or had been pre-incubated and actively producing CH_4 prior to the treatment application. Both KNO_3 and N_2O completely suppressed H_2 accumulation by the remoistened air-

dried soil; no H_2 either in the presence or absence of added KNO_3 or N_2O was accumulated by the pre-incubated Tetonka soil subsequent to the treatment application. KNO_3 (250 ppm N) reduced the rate of accumulation of CH_4 by a Cavour loam during anaerobic incubation. No H_2 was accumulated by this soil during anaerobic incubation. At equivalent K^+ concentrations, KNO_3 suppressed CH_4 accumulation by the Tetonka and Cavour soils to a greater extent than did KCl.

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