PRODUCTION OF DINITROGEN AND NITROUS OXIDE IN SOIL SUSPENSIONS AS AFFECTED BY REDOX POTENTIAL

MARIE KRALOVA, P. H. MASSCHELEYN*, C. W. LINDAU, and W. H. PATRICK, JR.

Laboratory for Wetland Soils and Sediments, Center for Wetland Resources, Lousiana State University, Baton Rouge, LA 70803-7511, U.S.A.

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Abstract. The effect of soil redox potential on N_2O and N_2 emission from soil suspensions was studied under laboratory conditions. Crowley silt loam soil suspensions were equilibrated under controlled (-200, -100, 0, +100, +200, +300, and +400 mV) redox levels, and the amounts of N_2 and N_2O evolved quantified. At higher redox levels (+300, and +400 mV) nitrification was the dominant soil biological process controlling N chemistry. A small amount of N_2O evolved during nitrification. A redox value between +300 and +200 mV was found critical for denitrification to occur. Both N_2 and N_2O were produced during denitrification. The maximum amount of N_2O evolved at a redox value of 0 mV. Dinitrogen emission increased at lower redox levels. The highest N_2/N_2O evolution ratio was observed at -200 mV and the ratio decreased with increasing redox. A lack of N-balance during denitrification at redox levels of +100, and +200 mV is also reported.

1. Introduction

Concern over destruction of the stratospheric O_3 layer by N_2O has led to a great interest in the sources of N_2O in the atmosphere. Nitrous oxide is also reported to be the most important N-containing gas with respect to the climate warming trend. The atmospheric lifetime of N_2O is estimated to be greater than 100 yr and the only known removal process is stratospheric photolysis. The 1980 atmospheric N_2O concentration was 303 ppbv and is increasing at a rate of 0.2 to 0.3% yr^{-1} (Weiss, 1981; NASA, 1988). Ramananthan *et al.* (1985) estimates that by the year 2030 N_2O concentrations will be in the range of 350 to 450 ppbv.

Present-day global N_2O fluxes appear to be dominated by industrial (McElroy *et al.*, 1977; Liu *et al.*, 1977) and soil-biological (Blackmer and Bremner, 1978, 1979; Firestone and Tiedje, 1979; Firestone *et al.*, 1980; Letey *et al.*, 1980a; Smith *et al.*, 1983; Nommik *et al.*, 1984) processes. Two distinct soil-biological processes are reported to contribute to N_2O emissions: denitrification and nitrification. Denitrification of native soil N and applied fertilizer N are the major sources of N_2O from soil-biological processes (Letey *et al.*, 1980a, b, c; Smith *et al.*, 1983). Although emissions due to nitrification are reported (Bremner and Blackmer 1978, 1979; Blackmer *et al.*, 1980), they appear to be small in comparison to denitrification reactions. Both processes could be important in regulating global atmospheric N_2O concentrations.

The intensity of soil reduction, as measured by redox potential (Eh) has been

* Author for all correspondence.

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shown to be an important factor affecting both denitrification and nitrification rates and, therefore, could be important in assessing the potential hazard for O_3 layer destruction. Soils can experience redox conditions ranging from -200 to +600 mV. In oxidized (aerobic) soils, redox potential is reported to range from about +400 to +600 mV. When soils are inundated with water, continued O_2 demand of micro-organisms and plant roots rapidily deplete the O_2 content and reduced conditions usually result. Upon flooding, various chemical and biological transformations take place resulting in a decrease in Eh. In most reduced (anaerobic) soils the Eh ranges from around -300 to +100 mV. Moderately reduced soils are characterized by an Eh between +100 and +400 mV. (Gambrell and Patrick, 1978). Under anaerobic conditions, denitrification is the dominant biological process producing gaseous N products. Nitrous oxide is an obligatory intermediate in the dissimilatory reduction of NO_3^- to N_2 (Nommik *et al.*, 1984). The importance of soil redox potential for N_2O production during denitrification has been reported by Letey *et al.* (1980b) and by Smith *et al.* (1983). In both studies it was observed that the N_2O production rate decreased as the redox potential increased. Firestone and Tiedje (1979) found temporal changes in N_2 and N_2O evolution during denitrification. The staggered synthesis of enzymes in response to anoxia led to an initial production of N_2O. After a certain period of anaerobiosis, the produced N_2O was reduced to N_2. Similar results were obtained in studies by Letey *et al.* (1980a, b). Initial high N_2O evolution rates decreased with time of incubation and after a short time approached zero.

Previous studies have shown a relationship between redox potential and N_2O production (Letey *et al.*, 1980b; Pedrazzini and Moore, 1983), but no control of the redox potential was maintained in these experiments. The objective of the laboratory experiments reported here was to determine the relative amounts of N_2 and N_2O evolved from soil suspensions equilibrated under precisely controlled redox potentials.

2. Materials and Methods

A Crowley silt loam soil (Typic Albaqualf), collected from the Rice Experiment Station, Crowley, LA (U.S.A.) was used in the present study. Upon arrival in the laboratory the soil was air dried, and grounded to pass a 1 mm sieve. After thorough mixing the soil was stored in 4-L polyethylene containers until use. The air-dried soil had a pH of 5.3, and a total C and N content of 0.7 and 0.08 %, respectively.

Soil suspensions were equilibrated under controlled redox conditions, using the redox control system developed by Patrick *et al.* (1973). Suspensions were prepared by mixing 375 g of air dry soil with distilled water so that the final soil to water ratio was 1 to 4. Organic matter (ground dried soybean straw) was added 0.3% w/w on a dry soil basis as an energy source to promote microbial activity. Suspensions were equilibrated during a 3-week period at the desired redox potentials. Equilibrations were performed at redox levels of -200, -100, 0, +100, +200, +300, and

+400 mV. In the system the redox potential is maintained at a preselected level automatically. Platinum electrodes in the suspensions were connected to a millivolt meter to give continuous measurements of the redox potential of the soil suspension. The recorder output of the millivolt meter was connected in turn to a meter relay that activated an air pump. Whenever the redox potential dropped below the desired potential, a small amount of air was pumped into the system to maintain the desired level. During the equilibrations the flasks were continuously purged with O_2-free Ar gas. Argon gas was effective in purging excess air at the end of the aeration cycle and in preventing a buildup of gaseous decomposition products such as CO_2 and H_2S. Using this system we could maintain the desired Eh within ± 20 mV. After the pre-equilibration period, the purge gas flow was stopped and a KNO_3 solution containing 60 atom % ^{15}N excess was added to the suspensions. The total amount of KNO_3 added increased the NO_3 content of the soil suspension with 50 ppm. Soil suspensions were withdrawn by means of a plastic syringe and needle. After centrifugation and filtration, under an inert argon atmosphere for reduced treatments (Patrick and Henderson, 1981), the amount of water soluble NO_3^-, NO_2^-, and NH_4^+ were determined. Immediately following the initial sampling, the original stoppers of the flasks were replaced by rubber stoppers equipped with a septum and thermometer. The system was continuously stirred during both the preincubation and equilibration period.

The emission of N_2O and N_2 from the soil suspensions was determined in gas samples withdrawn from the headspace of the flasks. Gas samples were taken 1, 2, 3, and 4 days after $^{15}NO_3^-$ application. Samples were withdrawn with a 15 mL gas-tight syringe and injected into evacuated gas sampling vials. Knowing the initial headspace volume and the total amount of gas withdrawn allowed for the calculation of the volume of the headspace gases before each sampling. The temperature from both the headspace and the surrounding atmosphere was also recorded. The temperature stayed relatively constant over the period of the experiment (30±2 °C). After the final gas sampling, Pt electrodes and a reference electrode were placed into the suspensions, and the Eh of the suspensions before and after the experiment were compared. During the experiment the redox levels, obtained during the pre-equilibration period, remained constant within ± 50 mV. Final suspension aliquots were withdrawn and analyzed for water soluble N compounds.

The total amount of N_2 evolved from the soil suspensions was determined according to Mulvaney and Boast (1986). In our experiments the N_2 gas samples were purified through a liquid N_2 trap. The purified gas samples were analyzed on a Finnigan MAT Delta E mass spectrometer equipped with a dual intlet and triple collector. Based on the N isotopic abundances (28, 29 and 30 peaks) the total amount of N_2 produced was calculated. Total N_2O evolved was determined with a Perkin Elmer gas chromatograph (GS 8410) equipped with an electron capture detector (Lindau *et al.*, 1988). A standard curve was constructed (0, 1, 5.3, 10.5, and 24.7 ppm N_2O) and the amount of N_2O-N in the sample gases calculated. Water-soluble N species were determined by steam distillation techniques (Bremner, 1965). Magnesium

oxide was added to the NH_4^+ fraction to raise the solution pH and Devarda's alloy reduced NO_3^- to NH_3. The NH_4^+ from each fraction was collected in a boric acid solution and titrated to determine the amount of N present. Nitrite-N was analyzed separately by colorimetry according to Kralova *et al.* (1978).

3. Results and Discussion

The concentration changes of water soluble N-species during the 4-day equilibration period (after NO_3^- application) under different redox conditions are presented in Table I. The amount of N_2 and N_2O evolved from the soil suspensions as affected by redox potential are shown in Table II.

The redox effects were linked to the types of microbial metabolism they permit. Biological processes performed by microorganisms influenced N-transformations. The significant decrease in NO_3^- concentration observed during the time period of the experiment (Table I) clearly illustrates that denitrification was the dominant biological process controlling N chemistry under moderately and strongly reducing conditions (–200, –100, 0, +100, and +200 mV). Denitrification also led to a slight increase in NO_2^- concentrations. At the lowest redox potential (–200 mV), 54.5 μg NO_3^--N g^{-1} dry soil was denitrified while at a redox potential of +200 mV only 22.7 μg NO_3^--N g^{-1} dry soil was reduced.

Under oxidized conditions (+300, and +400 mV) increased NO_2^- concentrations, due to nitrification, were observed. At an Eh of +300 mV, a substantial amount of NO_3^- was produced. This was even more evident at an Eh value of +400 mV where the greatest increase of NO_3^- (126 μg N g^{-1} dry soil) occurred.

TABLE I

Concentration changes of watersoluble N-species in soil suspensions during a 4-day equilibration period under different redox potentials

Eh (mV)	pH	Time (days)	NO_3^-	NO_2^-	NH_4^+
			Watersoluble	(μg N g^{-1} dry soil)	
−200	7.5	0	61.4	0.12	59.7
		4	6.88	0.23	56.9
−100	7.3	0	51.1	< 0.05	14.2
		4	2.00	< 0.05	20.6
0	7.1	0	54.9	0.19	63.4
		4	9.63	8.32	57.8
+100	6.7	0	54.0	< 0.05	14.8
		4	25.8	7.08	22.3
+200	6.5	0	52.2	2.88	8.00
		4	29.5	3.60	2.80
+300	6.0	0	92.0	19.4	5.60
		4	90.7	< 0.05	3.40
+400	5.2	0	60.1	0.37	33.5
		4	186	3.48	1.23

TABLE II

Dinitrogen and N_2O production in soil suspension as affected by redox potential

Eh (mV)	pH		Days after KNO_3 application 1	2	3	4
			gaseous N (μg N g^{-1} soil)			
−200	7.5	N_2O	N.D.	N.D.	N.D.	N.D.
		N_2	25.9	32.4	38.7	52.7
−100	7.3	N_2O	14.1	3.40	3.91	4.17
		N_2	5.44	15.8	28.0	45.6
0	7.1	N_2O	15.6	2.89	6.11	8.24
		N_2	4.93	9.01	14.9	24.5
+100	6.7	N_2O	N.D.	0.30	0.99	8.98
		N_2	N.D.	0.30	0.96	1.01
+200	6.5	N_2O	4.39	6.58	8.29	7.54
		N_2	N.D.	N.D.	1.92	1.14
+300	6.0	N_2O	N.D.	N.D.	N.D.	N.D.
		N_2	1.09	1.12	1.12	1.09
+400	5.2	N_2O	1.18	2.83	4.61	6.04
		N_2	N.D.	N.D.	N.D.	N.D.

N.D.: Not detected above background levels

The data in Table II illustrate the time dependency of both N_2 and N_2O evolution as affected by soil redox potential. At −200 mV, no N_2O was detected and the initial high N_2 production rate decreased over time. At −100 mV we found N_2O to be the dominant early product of denitrification (14.1 μg N g^{-1} dry soil d^{-1}). After the first day of high N_2O evolution, however, a substantial decrease in N_2O with a steady increase in N_2 (>10 μg N g^{-1} dry soil d^{-1}) was observed. At 0 mV, the high amount of N_2O evolved (15.6 μg N g^{-1} dry soil d^{-1}) during the first day decreased significantly during the following two days, and was accompanied by an increase in N_2. At +100 mV, both the N_2O and N_2 production increased gradually to reach a maximum of 8.98 and 1.01 μg N g^{-1} dry soil respectively, at the fourth day after KNO_3 application. A redox potential of approximately +200 mV appeared to be critical in order for denitrification to occur in the Crowley soil suspensions. This value is in good agreement with the observation made by Patrick (1960) that NO_3^- in Crowley soil suspensions becomes unstable between a redox potential of +200 and +300 mV. Our results demonstrate that N_2O is the initial and dominant gaseous N-species produced at the +200 mV redox level. Three days after KNO_3 application, a small amount of N_2 was produced (1.92 μg N g^{-1} dry soil). Firestone and Tiedje (1979) and Letey *et al.* (1980b) also reported N_2O production during denitrification to be time dependent. They found that, while the dissimilatory nitrate reductase develops rapidly the dissimilatory N_2O reductase only develops after a certain period of anaerobic conditions.

At +300 mV, the N_2 production rates were small and remained nearly constant

over the time of the experiment. No N_2O could be detected at +300 mV. This suggests that a redox potential of +300 mV was critical for N_2O production and reduction in the Crowley soil suspensions. Using a Hanford sandy soil, Letey *et al.* (1980b) found a redox potential in the range of +200 to +300 mV to be critical for N_2O production and reduction. A redox potential of +250 mV was found to be the critical value for N_2O production in a Mhoon silt loam soil by Smith *et al.* (1983).

At +400 mV, N_2O production rates were low but increased slightly over time. The detectable amounts of N_2O under these oxidized (+400 mV) conditions are somewhat surprising and probably result from N_2O emission during nitrification. Bremner and Blackmer (1978) showed that the oxidation of NH_4^+ during nitrification can lead to a release of N_2O. They found that oxidation of NH_4^+ to N_2O by intact cells or cell-free extracts of *Nitrosomonas europaea* led to the production of N_2O. The observed high amounts of nitrates (186 $\mu g\ N\ g^{-1}$ dry soil) and low concentrations of water-soluble NH_4^+ (1.23 $\mu g\ N\ g^{-1}$ dry soil) at the end of the 4-day equilibration period (Table I) indicate the importance of nitrification at the high Eh values. The high NO_3^- concentration in solution under the oxidized conditions could have been another important factor affecting N_2O production since the presence of high NO_3^- concentrations has been reported to retard the reduction of N_2O to N_2 (Nommik *et al.*, 1984). On the other hand, Blackmer and Bremner (1978, 1979) found that nitrates could inhibit as well as stimulate N_2O reduction. In experiments conducted by Firestone *et al.* (1980), an increase in soluble (NO_3^- + NO_2^-) – N led to an increased N_2O production. Also, even though the suspension was stirred and frequently received additions of O_2 to maintain the redox potential, there may have been anaerobic microzones or short time periods of anaerobiosis. This would stimulate denitrifiers and, under the oxidized conditions, N_2O would have been the dominant gaseous end product.

Figure 1 shows the average amounts of N_2O and N_2 evolved from the soil suspensions during denitrification at the different Eh levels. The ratio $N_2/$ (N_2 + N_2O) evolved from soil suspensions as affected by redox potential is also included. Dinitrogen was the major evolved N species under strongly reduced conditions (–200, –100, and 0 mV). Under more oxidized conditions (+100 and +200 mV), N_2O became the dominant N species produced during denitrification. This led to high $N_2/$ (N_2+ N_2O) evolution ratios under low redox values (–200, –100, and 0 mV). The $N_2/$ (N_2+N_2O) ratio decreased with increasing redox potential. It is interesting to note that, in the range of –200 to +100 mV, a linear relationship exists between the amount of N_2O or N_2 produced and the redox level. Correlation coefficients are 0.98 and 0.97 for N_2 and N_2O, respectively. The slopes of the lines represent the change in the amount of N_2 or N_2O produced per mV change in redox. For every mV increase in redox potential, the daily amount of N_2O evolved increased with 7.7 ng $N\ g^{-1}$ dry soil. Dinitrogen production decreased at a rate of 44.0 ng $N\ g^{-1}$ dry soil d^{-1} for every mV increase in redox.

Calculated denitrification rates are shown in Figure 2. The denitrification rate

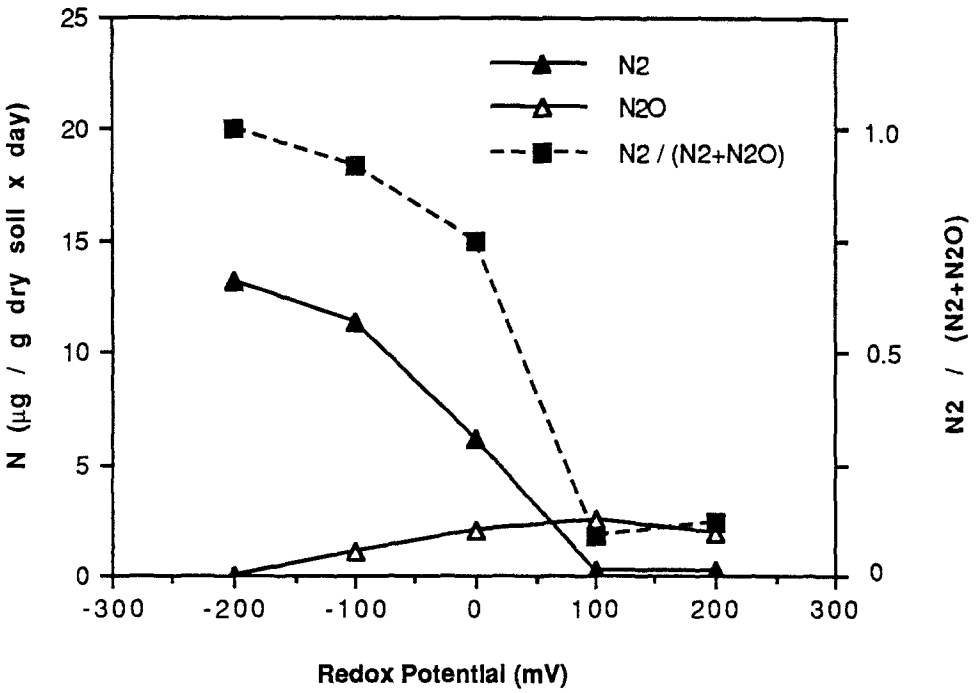

Fig. 1. Average daily amounts of N_2O and N_2 evolved from soil suspensions, and the N_2 / (N_2+N_2O) ratio as affected by redox potential.

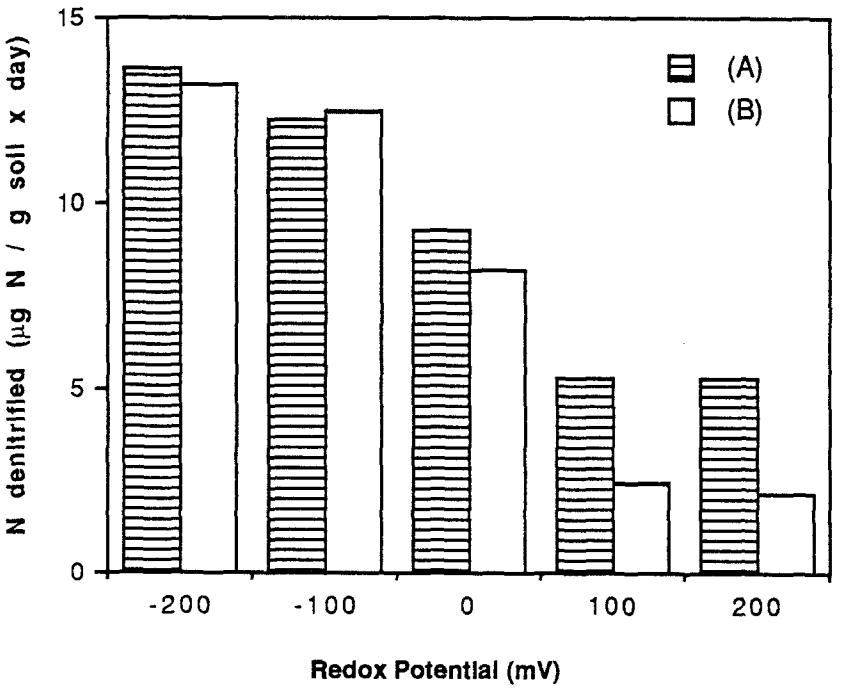

Fig. 2. Effect of soil redox potential on denitrification rates. Denitrification rates were calculated based on (A) differences in NO_3^- and NO_2^- concentrations, and (B) amount of N-gases evolved.

was calculated from A) differences in NO_3^--N and NO_2^--N concentrations before and after gas samplings as well as from B) the amount of gases evolved. It is evident that the reduction rate of nitrates increased with a decrease of soil redox potential. Under strongly reduced conditions (-200, and -100 mV), results of both calculations are very similar. At redox levels of +100 and +200 mV, however, there was a discrepancy between the calculated denitrification rates. Considerable more NO_3^--N was lost from the soil suspensions than could be accounted for by gaseous losses. The amounts of N_2O and N_2 evolved account for less than 50% of the observed decrease in NO_3^--N. Several mechanisms may be responsible for the observed discrepancy.

It seems likely that the NO_3^--N not accounted for by (N_2O + N_2)-N was used by heterotrophic microorganisms as a N source. The NO_3^--N assimilated by microorganisms would have been metabolized into nitrogenous constituents of their cells.

The following schematic pathway for denitrification has been generally accepted (Focht and Verstraete, 1977; Payne, 1973): $NO_3^- -> NO_2^- -> NO -> N_2O -> N_2$. The importance of NO as an intermediate in the sequence reduction is still unclear (Nommik *et al.*, 1984). It is unlikely that all of the NO_3^- unaccounted for would be present as NO. Nevertheless, this mechanism could be important. As demonstrated in in-vitro studies by Payne (1973), the presence of NO can inhibit N_2O reduction and could be responsible for the higher N_2O concentrations observed in our experiments conducted at a redox potential of +100, and + 200 mV. It is also possible that the N_2O measurements in the laboratory microcosms did not provide a quantitative estimation of the amount of N_2O produced. Letey *et al.* (1980a) showed that N_2O retention increases with increasing soil water content. The difference in solubility or diffusion rate between N_2 and N_2O may have affected the relative amounts of N_2O and N_2 that escaped into the headspace from the soil suspensions. Nitrous oxide is up to 25 times more soluble in water than N_2 (The Merck Index, 1960). This indicates that N_2O will be retained to a greater extent in the soil solution. Apparently N_2 was not retained since results of calculations of denitrification rates based on differences in NO_3^- and NO_2^- concentrations and on the amount of N-gases evolved were similar under reduced conditions (Figure 2). Clearly, further studies are needed to better understand the mechanisms behind the observed effects.

In summary, our experiments illustrate that redox potential is an important factor determining the composition of gaseous N-products evolved from soils. A redox level of +200 mV was found to be critical for denitrification to occur. During denitrification both N_2 and N_2O were produced. The redox potential of the soil-water system determined the relative amounts of N_2O and N_2 released. Under strongly reduced conditions (-200 mV) the produced N_2O appears to be immediately reduced to N_2 since no N_2O could be detected. At higher redox values both N_2O and N_2 were produced during denitrification. Our results also indicated a lack of N-balance during denitrification at redox levels of +100 and +200 mV. A small amount of N_2O is produced during nitrification.

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