

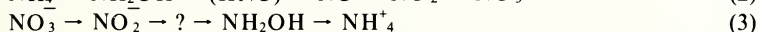
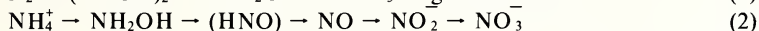
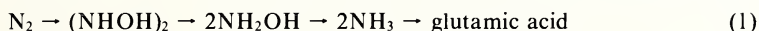
Transformations of Hydroxylamine in Soils

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Introduction

Although hydroxylamine (NH_2OH) is considered to be an intermediate in biological nitrogen fixation (Equation 1), nitrification (Equation 2), and nitrate reduction (Equation 3) in various ecological systems (Alexander, 1961), few investigations have been concerned with NH_2OH transformations in soils.



Duisberg and Buehrer (1954) found that NH_2OH was not converted to nitrite and nitrate when added to soils. Bremner and Shaw (1958) could not detect the presence of NH_2OH during studies of denitrification in soils; furthermore, they reported that NH_2OH could not be recovered soon after addition to soils. They speculated that higher oxides of manganese and iron reacted with NH_2OH to produce gaseous N compounds. Relatedly, Arnold (1954) observed that N_2O was produced when NH_2OH was added to wet soil which he attributed to reactions with nitrite. Nommik (1956) reported that N_2 and N_2O were evolved when NH_2OH was added to soil maintained in an argon atmosphere.

The lack of quantitative information on NH_2OH transformations in soils coupled with the potential importance of these transformations in the loss of inorganic N from soils has prompted the work reported here. The objective of the work was to determine the fate of NH_2OH in soils and to characterize the products of NH_2OH reactions in representative soils.

Materials and Methods

The soils used (Table 1) were surface (0-15 cm) samples representing a wide range in physical and chemical properties. Samples were air-dried (20 to 22° C for 48 hours) and ground to pass an 80-mesh sieve. Organic C was determined by the method of Mebius (1960), total N by a semimicro Kjeldahl procedure (Bremner, 1965a), pH by glass electrode (soil:water ratio 1:2.5), clay by pipette analysis (Kilmer and Alexander, 1949) after dispersion by Na-saturated Amberlite IRC-50 resin (Edwards and Bremner, 1965) and cation exchange capacity by the procedure of Edwards (1967).

In Experiment 1, 3 g samples of steam-sterilized soil were treated with 600 μg of $\text{N}^{15}\text{H}_2\text{OH-N}$ in 1 ml of solution. Treated samples were immediately extracted with 2 M KCl or incubated for 4 days (25° C, 100% relative humidity) before extraction. The amount of $\text{NH}_2\text{OH-N}$ fixed and amounts converted to NH_4^+ , NO_2 , and NO_3 were determined. In Experiment 2, 10 ml of pH 5 acetate buffer (2 M containing 10 g of soil, 0.5 g MnO_2 , 0.5 M FeCl_3 , or 0.057 M NaNO_2) were treated with 6 ml of a $\text{N}^{15}\text{H}_2\text{OH} \cdot \text{HCl}$ solution containing 8 mg of $\text{NH}_2\text{OH-N}$.

TABLE 1. *Characteristics of soils used in the investigation.*

Soil		pH	Organic	Total	Clay	Cation-exchange capacity
No.	Type*		C	N		
			%	%	%	me/100 g
1	Pershing sil	5.1	1.79	0.164	19	7.0
2	Clyde sil	5.5	4.30	0.402	25	29.9
3	Sac sicl	6.8	2.48	0.237	34	28.4
4	Thurman sa	6.8	0.64	0.056	1	4.4
5	Glencoe sic	6.8	8.92	0.860	41	48.8

*sil, silt loam; silty clay loam; sa, sand; silty clay.

N. Treatments were performed in a sealed gas analysis unit (helium-oxygen atmosphere) described by Nelson and Bremner, 1970) containing KMnO_4 solution in the center well. After 48 hours of incubation at 25°C , the amount of added $\text{NH}_2\text{OH-N}$ fixed and the amounts converted to NH_4^+ , NO_2^- , NO_3^- , $\text{NO} + \text{NO}_2$, N_2 , and N_2O were determined.

The amounts of NH_4^+ , NO_2^- , and NO_3^- in 2 M KCl extracts of soils or in acetate buffer were estimated by the extraction-distillation procedure of Bremner and Keeney (1966). Hydroxylamine was estimated by a steam distillation procedure which involved recovery of inorganic N in soil extracts or acetate buffer before and after treatment with FeCl_3 solution. High FeCl_3 concentrations oxidized NH_2OH to gaseous forms of N. Details of the method will be published elsewhere. The amount of N fixed (i.e. rendered nonextractable) on addition of N^{15} enriched NH_2OH to soil was determined by total N analysis and isotope-ratio analysis of the total N digest after removal of inorganic forms of N by extraction with 2 M KCl. Nitrogen isotope-ratio analyses were performed as described by Bremner (1965b) using a Consolidated Electrodynamics Corporation Model 21-620 mass spectrometer fitted with an isotope-ratio accessory. Amounts of NH_2OH converted to N_2 and N_2O were estimated by gas chromatographic analysis of a 1 ml sample of the atmosphere within gas analysis units as described by Nelson and Bremner (1970). Amounts of NO plus NO_2 formed during NH_2OH reactions were determined by absorbing these gases in a 0.1 M K MnO_4 : 1 M K OH solution and subsequent analysis of this solution for inorganic N as described by Nelson and Bremner (1970).

Results and Discussion

Data in Table 2 establish that added NH_2OH rapidly reacts with soil constituents. The total recovery of added $\text{NH}_2\text{OH-N}$ immediately after addition averaged 55% in the five soils investigated. Only small amounts of added $\text{NH}_2\text{OH-N}$ were converted to NH_4^+ , NO_2^- , or NO_3^- , whereas an average of 25% was "fixed" by soil constituents in a form which could not be extracted by KCl solutions. The fixation process was very rapid and the amount of $\text{NH}_2\text{OH-N}$ fixed was directly related to the organic C content of the soil. This suggests that the site of fixation of NH_2OH in soils is organic matter. Recovery data suggests

TABLE 2. *Recovery of hydroxylamine N after treatment of soils with hydroxylamine solution for 0 and 4 days.*

Soil no.	Treatment time (days)	Recovery of $\text{NH}_2\text{OH-N}$ (%)				Total
		As NH_2OH	As NH_4^+	As $\text{NO}_2^- + \text{NO}_3^-$	As fixed N	
1	0	4	0	0	9	13
	4	1	0	0	10	11
2	0	43	1	5	30	79
	4	5	2	8	34	49
3	0	19	0	0	19	38
	4	4	0	4	21	29
4	0	67	0	3	10	80
	4	2	3	21	22	48
5	0	5	0	0	55	60
	4	2	0	0	56	58
Ave.	0	28	0	2	25	55
	4	3	1	7	29	40

that an average of 45% of the added N^{15} could not be accounted for and was presumably lost from the system in gaseous form.

Recovery of $\text{NH}_2\text{OH-N}$ four days after addition to soils showed that only small amounts of added N were present as NH_2OH (Table 2). Limited amounts of added $\text{NH}_2\text{OH-N}$ were converted to NH_4^+ , NO_2^- , or NO_3^- (significant amounts of NO_3^- were formed in two soils). The amounts of added $\text{NH}_2\text{OH-N}$ fixed increased slightly after four days of incubation as compared to values for immediate fixation. Only an average of 40% of added N was recovered in soils following four days of incubation, suggesting that 60% was lost from soil in gaseous form.

In an attempt to determine which components of soil were responsible for gaseous loss of added $\text{NH}_2\text{OH-N}$ and what types of gaseous N compounds are released upon addition of NH_2OH to soils, a model system was used which allowed measurement of all NH_2OH reaction products. Addition of NH_2OH to acetate buffer containing two soils demonstrated that a portion of the added N is fixed, and substantial amounts of N are evolved as N_2 and N_2O (Table 3). The proportion of added N evolved from soils as N_2 is about equal to that evolved as N_2O . This finding is somewhat surprising because most investigators have believed that N_2O is the major gaseous product of NH_2OH reactions in soils. In an attempt to determine which soil constituents may be responsible for conversion of NH_2OH to N_2 and N_2O , inorganic substances present in soils were reacted with NH_2OH under controlled conditions. Nitrite and MnO_2 oxidized NH_2OH to N_2O with little formation of N_2 (Table 3). Reaction of FeCl_3 with NH_2OH resulted in significant production of N_2 along with large amounts of N_2O . These findings suggest that inorganic materials may be responsible for decomposition of NH_2OH in soils with subsequent release of gaseous forms of N.

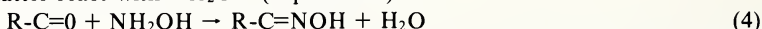
TABLE 3. *Recovery of hydroxylamine N added to pH 5 buffer containing various materials (25° C)*.*

Material in buffer	Recovery of added NH ₂ OH-N (%)						
	As NH ₂ OH	As NH ₄ ⁺	As NO ₂ ⁻ + NO ₃ ⁻	As NO + NO	As N ₂	As N ₂ O	As fixed N
Soil no. 1 (10g)	2	0	0	1	41	46	10
Soil no. 2 (10g)	6	2	10	0	21	23	32
MnO ₂ (0.5g)	0	1	0	0	2	96	0
0.5 M FeCl ₃	0	0	0	<1	29	71	0
0.057 M NaNO ₂	0	0	5	0	5	90	0

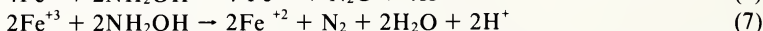
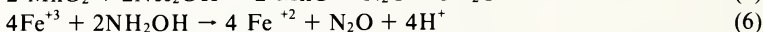
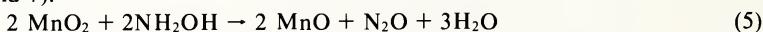
*Ten ml. of pH 5 acetate buffer (2M) containing the material specified was treated with 6 ml of hydroxylamine hydrochloride solution containing 8 mg of hydroxylamine N. Treatments were performed in sealed gas analysis units (helium-oxygen atmosphere) with KMnO₄ solution in center chamber.

Conclusions

Hydroxylamine reacted rapidly with soil constituents after addition to soils. A small portion of added NH₂OH was converted to other inorganic forms of N in soils (NH₄⁺, NO₂⁻, NO₃⁻) whereas substantial amounts were fixed by soil organic matter and evolved as gaseous forms of N. Fixation of NH₂OH likely occurs through the formation of oximes when carbonyl groups in soil organic matter react with NH₂OH (Equation 4):



Schnitzer and Skinner (1965) and Porter (1969) have observed oxime formation upon treating humic acid materials with NH₂OH. Gaseous N compounds are likely formed through the reaction of NH₂OH with common inorganic constituents in soils such as ferric iron and manganese dioxide (Equations 5, 6 and 7):



Mann and Quastel (1946) observed that NH₂OH reacted rapidly with MnO₂ in soils. Porter (1969) reported that NH₂OH reacts with NO₂ to liberate large amounts of N₂O, however, NO₂ is seldom detected in soils.

The finding that NH₂OH is rapidly decomposed and fixed when added to soils explains why this compound has never been detected in soil systems. Furthermore, it seems likely that NH₂OH is not released into the soil environment during N transformation carried out by microorganisms because if this were the case large unexplained losses of N would occur. A more likely situation is that NH₂OH is an intermediate in the transformations of inorganic N in cells of soil organisms, however, NH₂OH is probably bound to the enzymes involved in oxidation-reduction of N compounds. Little NH₂OH is released from the cell before more stable N compounds are formed enzymatically. The more stable N forms such as NH₄⁺ or NO₃⁻ are then released from the cells and are available for use by plants or microorganisms.

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