sodium selenite when both sources were fed at equivalent selenium levels. Selenomethionine, presumed to be the predominant form of selenium in plant materials, has been shown by using a similar bioassay to be less biologically available than sodium selenite (Cantor et al., 1975b). The results of Cantor et al. (1975b) indicate that selenomethionine or a form of selenium with similar biological availability fed at high levels as in this study would effectively protect against selenium deficiency.

Although several studies have shown that selenium in fly ash or in plants grown on fly ash is deposited in the tissues of animals consuming such materials, the present study constitutes the first demonstration of the nutritional value of that selenium. Tissue selenium levels do not correlate with the nutritional value of selenium in fly ash. Therefore, tissue residue analyses may not be a valid indicator of the biologic availability of selenium for animals fed fly ash. Nevertheless, it can be inferred from these results that fly ash selenium can be of only limited nutritional significance for animals. Plants grown on fly ash appear to be able to convert its poorly available form(s) of selenium to a form(s) with superior biological availability, or they may be able to concentrate low levels of soluble selenium present in fly ash.

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Fate of Amide Nitrogen Added to Soils

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The fate of nitrogen in amides (formamide, acetamide, and propionamide) was studied in five soils. The amounts of inorganic N ion species and NH_3 produced from each compound were compared with those produced from soils treated with $(NH_4)_2SO_4$ or urea and incubated under aerobic conditions at 30 °C for 14 days. Recovery of the inorganic N ion species and NH_3 produced was affected by the compound and soil used. More than 25% of the N added as formamide was evolved as NH_3 from one sandy soil. The nitrification rate of the amide N was the greatest with formamide, and those of acetamide and propionamide were similar to those of urea and $(NH_4)_2SO_4$. The total recovery of inorganic ion species of N and NH_3 produced in the five compounds and five soils studied ranged from 62 to 103%. The amounts of NH_3 voltatilized from the amide- and urea-treated soils were significantly but negatively correlated with cation-exchange capacity, organic C, and clay contents of the soils examined.

Numerous studies have been conducted on the manipulation of biochemical processes in soils for increasing the efficiency of N fertilizers, but there seems to be little progress in achieving that goal. A number of problems are encountered when N fertilizers are employed. Upon application to soils, these fertilizers may be subjected to (1) leaching and runoff losses, (2) denitrification losses through biological and chemical mechanisms, (3) dissolution rates too slow to keep pace with daily and normal crop requirements, and (4) NH_3 volatilization losses during or shortly after application.

One of the more popular solid N fertilizers available today is urea, an organic compound containing 46% N. The advantages of urea are manifold and include (1) a high analysis, (2) safety in handling, (3) application as either a solid or solution, and (4) relatively low cost (Gasser,

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Table I. Properties of Surface Field-Moist Soils Used

soil	pH	organic C, %	total N, %	CEC[a]	clay, %	sand, %	urease[b] act.	amidase act.[b] form-amide	amidase act.[b] acet-amide	amidase act.[b] propion-amide
Clarion	5.9	1.50	0.159	14.6	19.0	53.4	41	143	15	29
Chelsea	7.2	0.64	0.057	5.6	3.6	92.8	33	105	10	25
Downs	7.5	3.08	0.289	24.2	25.9	3.3	165	374	42	91
Canisteo	7.8	4.66	0.464	30.1	31.5	23.1	220	449	51	129
Harps	7.9	3.73	0.367	27.4	28.0	30.4	139	229	36	81

[a] Cation-exchange capacity (mequiv/100 g of soil). [b] Activity is expressed in microgram of NH_4 N released per gram of soil per 2 or 24 h when urea and formamide or acetamide and propionamide, respectively, were used as substrates.

1964). Several problems, however, result from the rapid hydrolysis of urea by soil urease. These include gaseous loss of urea N as NH_3 (Chin and Kroontje, 1963), NO_2^- toxicity (Chapman and Liebig, 1952), and free NH_3 damage to seedlings and young plants (Court et al., 1964). Consequently, many approaches have been used to control and retard urea hydrolysis in soils. These inlcude coating the urea granules with elemental S, enzyme inhibitors, and use of urea derivatives (Beaton et al., 1967, Parr, 1967; Gasser and Penny, 1967; Pugh and Waid, 1969; Bremner and Douglas, 1971; Gould et al., 1978). The problems often encountered with these approaches include uneven coatings of the urea granules and nonspecificity of the enzyme inhibitors. The need for a slow-releasing N source that is economical and has a relatively high N content should be of interest for future research on N nutrition of plants.

Recently, we detected amidase activity in soils (acylamide amidohydrolase, EC 3.5.1.4), an enzyme that catalyzes the hydrolysis of a family of amides producing NH_3 and their corresponding carboxylic acids ($R{\cdot}CONH_2 + H_2O = NH_3 + R{\cdot}COOH$) (Frankenberger and Tabatabai, 1980). Amidase acts on C–N bonds other than peptide bonds in linear amides. Amides, particularly formamide, seem to have potentials as N fertilizers because of their high water solubility, favorable crop yields in greenhouse tests and field trials when compared with urea (Brown and Reid, 1937; Rehling and Taylor, 1937; Terman et al., 1968; Hunter, 1974), and the possibility for economical large-scale production through new methods of synthesis (Jones et al., 1966). One such amide (oxamide) already has been evaluated as a slow-release N fertilizer (DeMent et al., 1961).

Also, it seems feasible to synthesize organic N fertilizers in which both nonspecific and specific metabolic inhibitor groups are a part of the whole molecular configuration. Such functional groups would provide the molecule with resistance to degradation by enzyme attack through properties such as isomerism, chain length, asymmetry, steric hindrance, and resonance. Through selective inhibition, a controlled release of N would be possible. Synthesis of such compounds with amides seems possible because several amides and their derivatives already are available. But before recommendations for application to soils, the fates of N in amides should be elucidated. Therefore, this study was carried out to determine the amounts of NH_4 N, NO_2 N, NO_3 N, and NH_3 N produced from formamide, acetamide, and propionamide in comparison with those produced from $(NH_4)_2SO_4$ and urea added to soils, to assess the effect of hydrolysis of these amides on soil pH, and to examine the relationship between NH_3 volatilization from amide-treated soils and soil properties.

MATERIALS AND METHODS

Materials. The soils used (Table I) were field-moist surface samples (0–15 cm) selected to obtain a range in pH (5.9–7.9), organic C (0.64–4.66%), and texture (4–32% clay and 3–93% sand). Before use, each soil was sieved and passed through a 2-mm screen. In the analyses reported in Table I, pH, organic C, total N, cation-exchange capacity (CEC), percentage clay, and percentage sand were performed as described by Neptune et al. (1975). Amidase and urease activities (Table II) were assayed by the methods of Frankenberger and Tabatabai (1980) and Tabatabai and Bremner (1972), respectively. The Downs soil was under the influence of forest vegetation, and the other four soil samples used were obtained from fields under mixed grasses.

Table II. Amides and Other Nitrogen Compounds Studied

compd	formula	formula wt	N, %
ammonium sulfate	$(NH_4)_2SO_4$	132.1	21
urea	NH_2CONH_2	60.1	46
formamide	$HCONH_2$	45.0	31
acetamide	CH_3CONH_2	59.1	24
propionamide	$C_2H_5CONH_2$	73.1	19

All the reagents used (Table II) were reagent-grade chemicals. Urea and $(NH_4)_2SO_4$ were obtained from Fisher Scientific Co., formamide and propionamide from Aldrich, and acetamide from Sigma Chemical Co.

Experimental Methods. Field-moist soil samples (10 g on an oven-dry basis) were placed in 8-oz (~250-mL) French square bottles and treated with 2 mL of a solution containing 2 mg of N as $(NH_4)_2SO_4$, urea, formamide, acetamide, or propionamide (the moisture contents of the incubated soils ranged from 40 to 60% of their water-holding capacities). The bottles were then fitted with an aeration device having an acid trap for absorption of NH_3 evolved on incubation of the soil samples. This device consisted of a rubber stopper having a central hole fitted with a glass tube (length, 130 mm; diameter, 5 mm) that had a glass vial (10-mL beaker) containing 5 mL of 0.5 N H_2SO_4 attached to its lower end, the tube being sealed to the inside wall of the beaker. The design of this stopper–tube–vial assembly was such that the bottom of the vial was ~1.5 cm above the surface of the soil sample in the bottle, the lower end of the glass tube was ~5 above the surface of the acid in the vial, and the upper end of the glass tube was ~2 cm above the top of the stopper. The end of the tube above the stopper was sealed with a rubber septum. The rubber septum was removed every 3 days for 20 min for aeration. The stoppered bottles were incubated at 30 °C, and after 14 days, the contents of their acid beakers were analyzed for NH_4^+ by steam distillation after treatment with 5 mL of 1 M NaOH (Bremner and Edwards, 1965). The incubated soil samples were extracted with 100 mL of 2 M KCl, and the extracts thus obtained were analyzed for NH_4 N and NO_3 N (Bremner and Keeney, 1966) and for NO_2 N (Barnes and Folkard, 1951). Controls were performed on all the soil samples to allow for NH_3 N, NH_4 N, NO_3 N, and NO_2 N not derived from the N sources added. The procedure described for

Table III. Inorganic N Recovered from $(NH_4)_2SO_4$, Urea, and Amides Added to Soils[a]

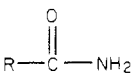
soil	treatment[b]	pH[c]	NH_3 N		NH_4 N		NO_2 N		NO_3 N		total, %
			μg/g of soil	%	μg/g of soil	%	μg/g of soil	%	μg/g of soil	%	
Clarion	AS	5.2	0.5	0.3	122.3	61.2	0	0	52.6	26.3	87.8
	U	5.5	0.5	0.3	25.0	12.5	0	0	155.2	77.6	90.4
	FA	6.0	2.5	1.3	26.1	13.1	0	0	172.6	86.3	100.7
	AA	5.6	1.3	0.7	24.4	12.2	0	0	128.9	64.5	77.4
	PA	6.0	0.8	0.4	18.7	9.4	0	0	141.6	70.8	80.6
Chelsea	AS	6.1	4.6	2.3	1.9	1.0	0	0	182.3	91.2	94.5
	U	5.6	33.7	16.9	3.0	1.5	11.5	5.8	107.8	53.9	78.1
	FA	6.0	56.6	28.3	18.0	9.0	42.3	21.2	75.9	38.0	96.5
	AA	5.3	27.0	13.5	1.7	0.9	0.7	0.4	114.9	57.5	72.3
	PA	5.6	26.3	13.2	1.5	0.8	0.4	0.2	129.4	64.7	78.9
Downs	AS	6.6	3.7	1.9	0.7	0.4	0	0	156.5	78.3	80.6
	U	6.3	8.9	4.5	0.2	0.1	0	0	160.0	80.0	84.6
	FA	7.4	14.9	7.5	0.7	0.4	0	0	183.8	91.9	99.8
	AA	5.9	5.3	2.7	1.2	0.6	0	0	150.6	75.3	78.6
	PA	6.4	5.6	2.8	7.0	3.5	0.3	0.2	130.4	65.2	71.7
Canisteo	AS	6.0	3.3	1.7	0.5	0.3	0	0	171.3	85.7	87.7
	U	6.2	3.7	1.9	1.2	0.6	0	0	170.8	85.4	87.9
	FA	6.1	7.8	3.9	1.2	0.6	0	0	196.4	98.2	102.7
	AA	6.2	2.8	1.4	1.4	0.7	0	0	157.5	78.8	80.9
	PA	6.1	2.3	1.2	0.0	0.0	0.3	0.2	153.0	76.5	77.9
Harps	AS	6.9	4.0	2.0	1.0	0.5	0	0	159.9	80.0	82.5
	U	6.3	5.8	2.9	1.0	0.5	0	0	161.8	80.9	84.3
	FA	6.3	7.5	3.8	0.7	0.4	0	0	194.6	97.3	101.5
	AA	6.3	4.4	2.2	2.1	1.0	0	0	137.7	68.9	72.1
	PA	6.5	4.1	2.1	14.3	7.2	2.2	1.1	103.5	51.8	62.2

[a] 2 mg of N was added to a 10-g soil sample and incubated at 30 °C for 14 days. [b] AS = ammonium sulfate; U = urea; FA = formamide; AA = acetamide; PA = propionamide. [c] pH of soil (soil/water ratio, 1:2.5) after incubation.

incubation of N-treated soil samples was followed to perform controls, but 2 mL of deionized water was added instead of the solution containing the N sources. All values reported are averages of duplicate determinations expressed on a moisture-free basis, moisture being determined from loss in weight after drying at 105 °C for 24 h.

RESULTS AND DISCUSSION

The structural formula, molecular weight, and percentage of N for each of the N compounds used in this study are shown in Table II. The percentage of N content of the compounds used ranged from 19% in propionamide to 46% in urea. Both urea and the amides are similar in their formula and chemical composition. These substrates contain the same basic unit, a carbonyl and an amine group. The common structural formula between these substrates is



where R may represent H as in formamide, CH_3 as in acetamide, C_2H_5 as in propionamide, and NH_2 as in urea. Because the structural formulas of these substrates are similar and amidase has a relative specificity for its substrates, one must ask if the hydrolysis of urea could be catalyzed by soil amidase. Tests showed, however, that pure crystalline urease (B-grade jack bean meal, Calbiochem, San Diego, CA) will not catalyze the hydrolysis of aliphatic amides. Because of lack of availability of purified amidase, the effect of urea on the catalytic action of this enzyme could not be studied. The information available indicates, however, that urea is a noncompetitive inhibitor of a partially purified amidase when using propionamide as a substrate (Clarke, 1970). Other studies have shown that amidase is protected from inhibition by urea in the presence of hydroxylamine. It has been suggested that the inhibition by urea is due to its known effect on the hydrogen bonding of proteins, and it is possible that, when hydroxylamine is present and bound to the amidase, the change in conformation of the enzyme protein may make it less vulnerable to attack by urea (Clarke, 1970). It is difficult to study these reactions in a system, such as soils, containing both urease and amidase activities.

All the soils studied exhibited both urease and amidase activities (Table I). The rates of hydrolysis of urea and the amides followed the same order in all soils. Both urease and amidase activities were greatest in the Canisteo soil and least in the Chelsea soil. Amides hydrolyzed by soil amidase showed the following order of activities: formamide > propionamide > acetamide. Urease activity in these soils is comparable to the activity resulting from the hydrolysis of propionamide catalyzed by soil amidase.

Table III shows the inorganic N recovery of the compounds added to soils after incubation at 30 °C for 14 days undere aerobic conditions. Total recovery of inorganic N derived from the added treatments ranged from 62 to 103%. All soils showed the greatest recovery of inorganic N derived from the formamide treatment.

Small amounts (0.5–2.5 μg of N/g of soil) of NH_3 were evolved from the acid soil (Clarion) used, but no NO_2^- was formed in this soil when treated with the N compounds studied (Table III). Most of the N recovered from $(NH_4)_2SO_4$ was exchangeable NH_4^+ (122 μg/g; 61% of the total recovery). Recovery of exchangeable NH_4^+ was greater in this soil than in any other soils when all treatments were considered. A large percentage of NH_4 N released from the hydrolysis of urea and the amides was nitrified to NO_3^-. Nitrate recovery was greatest with the formamide treatment.

The Chelsea soil was chosen for this investigation because of its high sand content (93%) and alkaline pH. Of all the soils, Chelsea showed the greatest amount of NH_3 N volatilization (Table III). The amount of N loss through volatilization ranged from 5 μg/g of soil (2% of the total recovery) with $(NH_4)_2SO_4$ to 57 μg/g of soil (28% of the

Table IV. Correlation Coefficients (r) between NH_3 N Volatilized and Soil Properties

substrate	r			
	organic C	CEC	clay	sand
urea	−0.97[a]	−0.99[b]	−0.99[b]	0.91
formamide	−0.96[a]	−0.99[b]	−0.99[b]	0.90
acetamide	−0.96[a]	−0.99[b]	−0.99[b]	0.93
propionamide	−0.96[a]	−0.99[b]	−0.99[b]	0.92

[a] Significant at 5% level. [b] Significant at 1% level.

total recovery) with the formamide application. A considerable amount of NO_2 N was released from urea (12 μg/g of soil; 6% of the total recovery) and formamide (42 μg/g of soil; 21% of the total recovery) in this soil. These findings suggest that the classifical *Nitrobacter* reaction (oxidation of nitrite) was inhibited. Chapman and Liebig (1952) also have reported that nitrite frequently will accumulate in alkaline soils when fertilized with NH_3 or urea. Exchangeable NH_4 N did not accumulate in any appreciable amounts except with the formamide treatment (18 μg/g of soil; 9% of the total recovery). Most of the added N was recovered as NO_3 N, particularly when $(NH_4)_2SO_4$ was added.

Recovery of N volatilized from the Downs soil was considerably greater when derived from formamide than from any other treatment applied (Table III). Very little exchangeable NH_4^+ and no NO_2^- (except for trace amounts with the propionamide treatment) was recovered in this forest soil. Most of the NH_4 N produced was nitrified to NO_3^-.

The amounts of NH_3N volatilized from the two calcareous soils (Canisteo and Harps) ranged from 2 μg/g of soil (1% of the total recovery) to 8 μg/g of soil (4% of the total recovery) under aerobic conditions (Table III). Very little N was recovered in the exchangeable NH_4^+ form. Most of the NH_4 N was nitrified to NO_3^-. Nitrification was enhanced in these soils because the nitrifiers have an optimum pH in the vincinity of 7.5–8.0 (Alexander, 1965), the same as the soils' initial pH.

The pH of soils treated with $(NH_4)_2SO_4$, urea, formamide, acetamide, and propionamide is shown in Table III. Almost all treatments produced a decrease in soil pH after incubation. Nitrification of NH_4N resulted in a release of H^+ ions, creating an acidifying effect on the soil reaction:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$$

Both carboxylic acids and NH_3 are products of amide hydrolysis, and the former also may have some influence on the final soil pH.

A high percentage of NH_3 N derived from urea and the amides was volatilized when the initial soil pH exceeded 7.0. The acid soil (Clarion) showed very little NH_3 N volatilized, whereas the other four soils had substantial losses, the greatest loss being in the Chelsea sandy soil. Similar findings have been reported by Ernst and Massey (1960).

Statistical analysis indicated that the amounts of NH_3 N volatilized from urea and the amides in all soils except the acidic soil (Clarion) correlate signficantly but negatively with organic C, cation-exchange capacity (CEC), and clay content (Table IV). The amount of NH_3 volatilized was correlated with organic C at the <5% probability level and with the cation-exchange capacity and clay content at the <1% probability level. Although the NH_3 volatilized is highly correlated with these soil properties, there is an intercorrelation within the properties themselves. The CEC of a soil is, however, dependent upon the clay content and organic matter; therefore, a strong relationship between CEC and NH_3 volatilization should exist. The capacity of soils to retain NH_4^+ in exchange sites and pH will influence the rate at which gaseous NH_3 is volatilized. The soils with lower cation-exchange capacities showed the greatest amounts of NH_3 N volatilization. The amount of NH_3 loss and its relationship to the soils CEC also is supported by the findings of Loftis and Scarsbrook (1969).

Hydrolysis and nitrification in the soils of the amides reported coupled with the informtion available on uptake by plants of the mineral N produced indicate that amides should have potentials as fertilaizers. The possible synthesis of other amides with a relatively high N content and containing P and (or) S deserves investigation.

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