

# Differentiation of Soil Nitrogen Fractions Using a Kinetic Approach<sup>1</sup>

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## ABSTRACT

The chemically induced release of  $\text{NH}_4\text{-N}$  from four Delaware soils was monitored over time. The extractants used were  $0.02\text{ M KMnO}_4$ – $0.5\text{ M H}_2\text{SO}_4$  at 297 K and  $0.01\text{ M CaCl}_2$  at 368 K. Data were analyzed using first-order kinetics. The kinetic approach presented does allow direct differentiation of two  $\text{NH}_4^+$  releasing reactions. Two simultaneous first-order reactions were needed to describe three of the soils, while a single equation described the remaining soil. The equations were considered to represent the release of  $\text{NH}_4^+$  from an inorganic and an organic source. Using this approach, estimates of these pools were made and compared with estimates based on traditional batch techniques. Extraction with acid- $\text{KMnO}_4$  yielded kinetic estimates of the inorganic and organic pools, which exceeded the batch estimates of these pools. Kinetic estimates based on extraction with  $\text{CaCl}_2$  were similar to batch estimates of inorganic N, but much less than either acid- $\text{KMnO}_4$  or batch estimates of organic N. Neither extractant gives direct evidence for the existence of a chemically active organic pool; however, there is indirect evidence for such a pool.

*Additional Index Words:* N availability, flow technique,  $\text{N}_2$ , first-order kinetics, soil nitrogen chemistry.

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THE SUBJECT OF SOIL NITROGEN is extremely diverse. It includes the distribution and transformation of organic and inorganic N forms as well as their atmospheric and biospheric interrelations (Stevenson, 1982). Soil N is often broadly classified into four groups: (i) available inorganic N, including exchangeable  $\text{NH}_4^+$ , and soluble  $\text{NO}_2^-$  and  $\text{NO}_3^-$ ; (ii) fixed or nonexchangeable  $\text{NH}_4^+$ ; (iii) easily mineralized N; and (iv) stable organic N associated with the more resistant organic pool. Although useful in understand-

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ing soil N, these have been difficult to quantify in terms of plant availability.

The easily mineralized pool is usually thought to be most important in providing N to a crop (Jenkinson, 1968; Stanford and Smith, 1972); therefore, its quantification has been the focus of much of the N availability research. To this end, numerous chemical extraction techniques have been proposed (Bremner and Mulvaney, 1982; Keeney, 1982; Stanford, 1982). These extractions have been conducted using batch techniques, whereby a known amount of soil is kept in contact with an extractant for a specified period of time. This extraction technique, although simple and rapid, does not provide any information about the reaction itself; it is purely an equilibrium or macroscopic approach.

The research reported in this paper takes a different approach in that we apply a kinetic technique in an attempt to gain information about the chemically induced release of  $\text{NH}_4^+$  from soils. A kinetic approach has been successfully employed to differentiate ionic adsorption-desorption reactions on clay minerals and soils (Jardine and Sparks, 1984). Their work is based on the use of site blocking agents, in conjunction with apparent reaction rate differences, which allow differentiation of reaction sites and mechanisms. The reader is referred to recent reviews for a detailed discussion of kinetics as applied to reactions in soils and clay minerals (Sparks, 1985; Sparks, 1986). Kinetics has also been applied to study the microbial conversion of soil organic N to  $\text{NH}_4^+$ . This application of kinetics, first proposed by Stanford and Smith (1972), provides estimates of potentially available N and apparent rate coefficients for the release of N from soils. Although this approach has been modified and refined in terms of incubation techniques and modeling procedures (Stanford et al., 1973; Stanford and Epstein, 1974; Molina et al., 1980; Smith et al., 1980; Talpaz et al., 1981; Broadbent, 1986; Deans et al., 1986), microbial studies must still be monitored for several months before kinetically based estimates of N availability can be made. Thus, it should be useful to kinetically study

the chemically induced, and therefore more rapid, release of N from soils. The objectives of this research were (i) to develop a methodology that allows a time-monitored, rapid, chemical extraction of soil N, and (ii) to determine if N release reactions can be differentiated through a kinetic analysis of such an extraction. This approach, if successful in differentiating a chemically active organic pool, will not only provide chemical evidence for the existence of an easily mineralized pool, but provide a valuable basis to which rapid chemical indexes of N availability can be compared.

## MATERIALS AND METHODS

Samples from the Ap horizon of several Delaware soils were used in this study. These included: a Chester loam (fine-loamy, mixed, mesic Typic Hapludult), a Leon loamy sand (sandy, siliceous, thermic Aeric Haplaquod), an Elkton loamy sand (clayey, mixed, mesic Typic Ochraqult), and an Evesboro loamy sand (mesic, coated Typic Quartzipsamment). The mineralogy of the <2- $\mu$ m clay fraction of all these soils is dominated by kaolinite, quartz, and chloritized vermiculite. Soils were air-dried and passed through a 2-mm sieve.

### Batch Extractions

Batch extraction techniques were used to characterize the N status of the soils. Exchangeable  $\text{NH}_4^+$  was determined using a 1 h, 2 M KCl extraction (Keeney and Nelson, 1982). The easily mineralized pool was estimated using a 16-h autoclave method (Keeney, 1982) and extraction with 0.02 M  $\text{KMnO}_4$ -0.5 M  $\text{H}_2\text{SO}_4$  (Stanford and Smith, 1978). The  $\text{NH}_4^+$  extracted by these methods was determined using an Orion 95-12  $\text{NH}_3$  electrode and an Orion 901 microprocessor ionalyzer. Baseline recovery problems were minimized by allowing 5-min intervals between samples (Guilbault et al., 1985). Total N was indexed using a standard macro-Kjeldahl procedure, without salicylic acid (Bremner and Mulvaney, 1982). Organic matter was determined using a modified Walkley-Black procedure.

### Kinetic Extractions

Kinetic analyses were conducted with a flow technique utilizing a stirred-flow reaction chamber (Carski and Sparks, 1985); however, a Millipore AP prefilter was used in place of the Nuclepore membrane filter. Operation of this system is simple. A magnetic stir star is placed in the chamber above the plunger, 1 g of air-dry soil is loaded into the chamber, the prefilter and top are attached, and a known volume of starting fluid is added using a hypodermic syringe. The plunger is then used to displace the excess air from within the chamber, thus enabling precise control of the reaction volume. This volume is maintained throughout the extraction. A peristaltic pump is used to maintain a flow rate of 1 mL  $\text{min}^{-1}$  and an LKB Ultrac II 2070 fraction collector is used to monitor the reaction at 2-min intervals. A magnetic stirrer is used to ensure adequate mixing within the chamber.

Separate kinetic extractions using 0.02 M  $\text{KMnO}_4$ -0.5 M  $\text{H}_2\text{SO}_4$  and 0.01 M  $\text{CaCl}_2$  were conducted. The 0.02 M  $\text{KMnO}_4$ -0.5 M  $\text{H}_2\text{SO}_4$  extraction was carried out at 297 K, while the 0.01 M  $\text{CaCl}_2$  extraction was carried out at 368 K. To achieve this elevated temperature, the  $\text{CaCl}_2$  extractant was kept at 372 K, and the chamber was immersed in a 368 K water bath. A heated stir plate was used to stir the bath and the contents of the reaction chamber.

The starting fluid differed for the two extractants. Addition of the acid-permanganate initiated immediate  $\text{CO}_2$  pro-

Table 1. Batch extractions used to characterize the N status of four soils from Delaware.

Soil type	KCl	Auto-clave	Acid-KMnO <sub>4</sub>	Kjeldahl	Organic matter
					g kg <sup>-1</sup>
			mg N kg <sup>-1</sup>		
Chester loam	16.2	32.4	23.7	1811	33.0
Leon loamy sand	17.5	18.7	15.4	851	31.0
Elkton loamy sand	2.7	13.7	12.3	576	14.2
Evesboro loamy sand	1.2	6.8	6.4	233	10.5

duction and thus the reaction volume could not be consistently controlled. This necessitated the use of  $\text{H}_2\text{O}$  as the initial loading solution. Although the acid-permanganate does not reach full strength for approximately 10 min (in the absence of soil), this was not considered to be a problem since each soil was treated precisely the same way. The addition of hot  $\text{CaCl}_2$  did not initiate an evolution of gas, allowing its direct injection into the reaction chamber.

Kinetic extractions were conducted in duplicate and  $\text{NH}_4^+$  was determined using the Orion 95-12  $\text{NH}_3$  electrode. The CV values of the duplicate runs, at each time interval, averaged <5%.

## RESULTS AND DISCUSSION

The batch characterization analyses show that the soils have large differences in total N and organic matter contents, but smaller differences exist for the easily mineralized pools (Table 1). None of the soils have crop histories which include legumes or manure amendments, thus large differences for the easily mineralized pools were not expected.

Typical  $\text{NH}_4^+$  release vs. time curves, presented for acid-permanganate extraction of the Chester and Elkton soils, indicate an initial rapid release followed by a slower sustained release (Fig. 1). Curves for all other extractions, using acid- $\text{KMnO}_4$  and  $\text{CaCl}_2$ , were similar and are not presented.

To determine the conformity of these data to first-order kinetics, an estimate of the total amount of N to be released ( $N_T$ ) must be made. Plotting the inverse of N released vs. the inverse of time (Stanford and Smith, 1972) was not useful since these relationships were not linear. All reactions were monitored for 160 min with little or no additional release of N taking place after 80 min. Thus,  $N_T$  was taken to be the

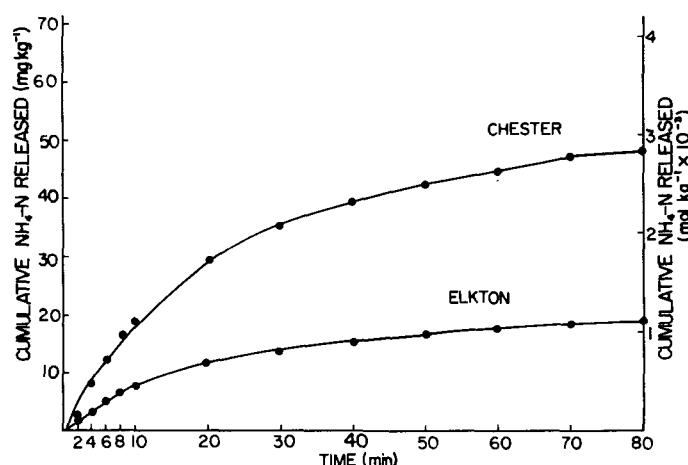
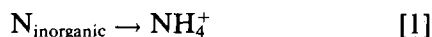


Fig. 1. Cumulative  $\text{NH}_4^+$ -N released on extraction with acid- $\text{KMnO}_4$  as a function of time for Chester and Elkton soils.

amount of N released after 80 min, i.e., well after the initial rapid release phase and after an apparent equilibrium had been reached.

Using these values for  $N_T$ , conformity to first-order kinetics was determined by plotting  $\log(N_T - N_t)$  vs. time, where  $N_t$  is the amount of N released at time  $t$  (Fig. 2). Although not shown, only the Evesboro soil, which had very low values for total N and the easily mineralized pool, displayed conformity to first-order kinetics over the entire reaction period, the other soils showed an initial nonconformity. The data for the latter soils can be further analyzed by assuming that two simultaneous  $\text{NH}_4^+$  releasing reactions are occurring (Moore and Pearson, 1981). Previous  $\text{K}^+$ - $\text{NH}_4^+$  exchange studies conducted in our laboratory indicate that a single first-order equation describes  $\text{NH}_4^+$  exchange on these soils. Thus, the contribution from a nonexchangeable pool is insignificant (unpublished data), and we hypothesize that  $\text{NH}_4^+$  is being released from an inorganic and an organic source as shown below



Given such a situation, the rate equations would be

$$-dN_{\text{inorganic}}/dt = k_{\text{in}} N_{\text{inorganic}} \quad [3]$$

and

$$-dN_{\text{organic}}/dt = k_{\text{or}} N_{\text{organic}} \quad [4]$$

which integrate to

$$N_{\text{inorganic}} = N_{\text{in}} \exp -k_{\text{in}} t \quad [5]$$

and

$$N_{\text{organic}} = N_{\text{or}} \exp -k_{\text{or}} t \quad [6]$$

where  $N_{\text{in}}$  and  $N_{\text{or}}$  represent initial amounts of N in each pool and  $k_{\text{in}}$  and  $k_{\text{or}}$  represent apparent rate coefficients for the release of N from each pool. Since the total amount of N that could be released ( $N_T$ ) equals

$N_{\text{in}}$  plus  $N_{\text{or}}$ , the amount released at any time ( $N_t$ ) equals,

$$N_t = N_T - N_{\text{inorganic}} - N_{\text{organic}} \quad [7]$$

Upon substitution and integration the rate equation becomes

$$\log(N_T - N_t) = \log(N_{\text{in}} \exp -k_{\text{in}} t + N_{\text{or}} \exp -k_{\text{or}} t) \quad [8]$$

Further, since the inorganic pool would likely be depleted first, i.e., simple cation exchange is more rapid than acid-permanganate oxidation or heat-induced hydrolysis, the linear portion of the curve represents conformity to first-order kinetics by the organic source as the rate equation reduces to,

$$\log(N_T - N_t) = \log N_{\text{or}} - k_{\text{or}} t / 2.303 \quad [9]$$

Equation [9] gives an estimate of the initial organic pool and an apparent rate coefficient for the release of  $\text{NH}_4^+$  from the organic source. This equation is also used to find the contribution, towards total N, derived from the organic source at early reaction times. Knowing the total amount and the organic contribution allows calculation of the inorganic contribution. The inorganic values can then be plotted according to a single first-order equation (Fig. 3). Using the inorganic N release equation, an estimate of the initial inorganic N and an apparent rate of release can be obtained.

Employing the above kinetic equations, estimates of the inorganic and organic N pools were calculated for the acid- $\text{KMnO}_4$  extraction (Table 2). The kinetically based values for the inorganic and organic N pools exceed the corresponding batch technique estimates. These findings might be expected for the organic predictions since  $\text{KMnO}_4$  is continually being replenished; however, one would expect the inorganic pools to be similar. Possibly, since the strength of the  $\text{KMnO}_4$  as an oxidant is great and therefore the apparent rate of oxidation high, an easily oxidized or-

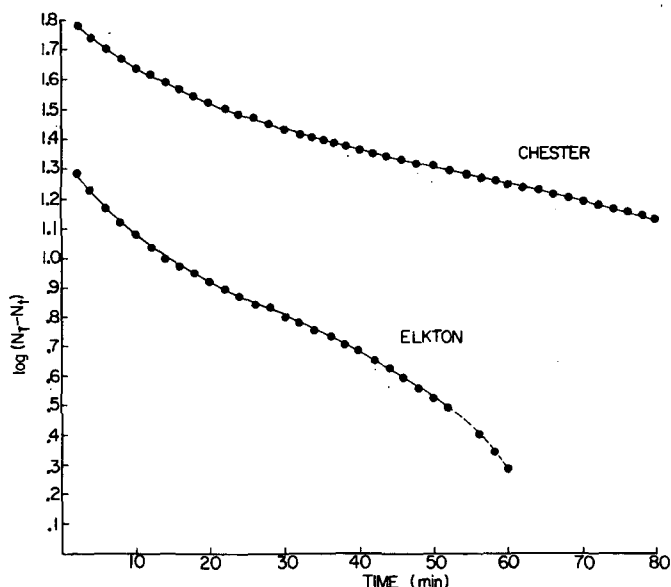


Fig. 2. First-order kinetic plots for  $\text{NH}_4^+$  released from Chester and Elkton soils.

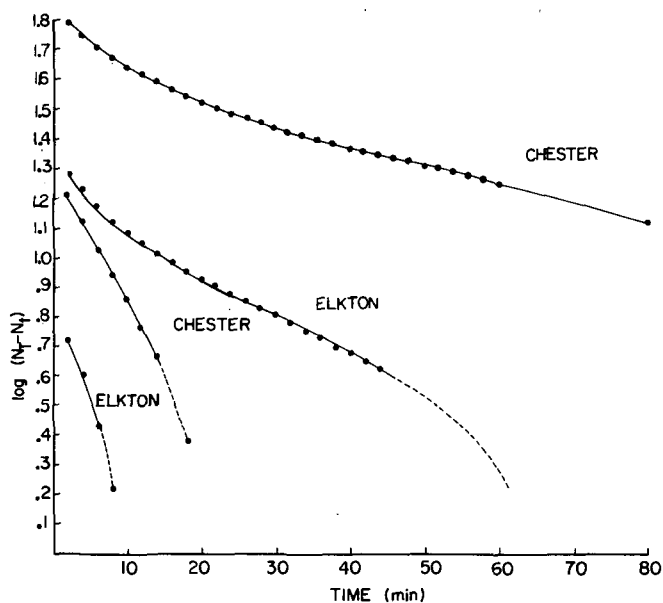


Fig. 3. First-order kinetic plots of  $\text{NH}_4^+$  released from an apparent inorganic source, superimposed on Fig. 2.

**Table 2. Apparent rate coefficients ( $k'$ ) and initial size estimates of the inorganic ( $N_{in}$ ) and organic ( $N_{or}$ ) pools derived from extraction with acid-permanganate.**

Soil	$k'_{in}$	$N_{in}$	$k'_{or}$	$N_{or}$
	$h^{-1}$	$mg\ N\ kg^{-1}$	$h^{-1}$	$mg\ N\ kg^{-1}$
Chester	6.24	24.6	0.84	40.7
Leon	7.32	28.2	2.04	23.4
Elkton	11.08	8.1	1.62	14.4
Evesboro	8.50	9.0		

ganic pool may be included within the inorganic estimate.

The results for the  $CaCl_2$  kinetic extractions are quite different (Table 3). The value for  $N_7$  was again chosen as the amount of  $NH_4^+$  released after 80 min. However, unlike the acid- $KMnO_4$  extraction, the estimates of the initial inorganic pool are quite close to the 2 M KCl batch estimates. The estimates of the initial organic N pool are much lower for the kinetic technique. Interestingly, the  $CaCl_2$  organic N estimates are very similar to the difference between acid- $KMnO_4$  kinetic and KCl batch estimates of the inorganic pool. This implies that the acid- $KMnO_4$  extraction, being more intensive, does not differentiate between the inorganic and an active organic source, thus elevating the acid- $KMnO_4$  inorganic estimate.

Inspection of the apparent rate coefficients for the inorganic reactions supports this conclusion. These coefficients are higher for  $CaCl_2$ , indicating that a second, slower reaction is acting to decrease the observed rate of the inorganic acid- $KMnO_4$  reaction. Additionally, the apparent rates of the  $CaCl_2$  organic reactions are higher than the acid- $KMnO_4$  organic releases, indicating the  $CaCl_2$  differentiated organic fraction is more easily degraded than the acid- $KMnO_4$  differentiated organic fraction.

### CONCLUSIONS

The kinetic approach presented does allow differentiation of two reactions. These reactions appear to be associated with the release of  $NH_4^+$  from an inorganic and an organic source. The acid- $KMnO_4$  extraction differentiates an inorganic plus an active organic fraction from the more resistant organic pool. The  $CaCl_2$  extraction differentiates the inorganic pool from an active organic pool. Thus, although neither extractant gives direct evidence for the existence of a chemically active organic pool, i.e., three pools were not differentiated by a single extraction, indirect evidence does indicate the existence of a chemically active organic pool.

If it is accepted that a chemically active organic pool has been differentiated, several implications concerning N availability can be drawn: (i) the chemically active pool of soil organic N is very small at any given time; (ii) this active pool must be replenished as it is consumed in order to provide N to a crop through a significant portion of the growing season; (iii) traditional, mild extraction techniques that target the active pool extract significantly greater quantities than the actual active pool; and (iv) the ranking of these soils, in terms of N availability, based on traditional techniques is different than the ranking based on the kinetically differentiated active pool. These results may

**Table 3. Apparent rate coefficients ( $k'$ ) and initial size estimates of the inorganic ( $N_{in}$ ) and organic ( $N_{or}$ ) pools derived from extraction with heated  $CaCl_2$ .**

Soil	$k'_{in}$	$N_{in}$	$k'_{or}$	$N_{or}$
	$h^{-1}$	$mg\ N\ kg^{-1}$	$h^{-1}$	$mg\ N\ kg^{-1}$
Chester	11.94	16.3	1.80	6.3
Leon	18.72	15.8	3.45	13.5
Elkton	14.16	6.1	2.28	3.5
Evesboro	12.78	5.2		

help to explain the consistent failure of traditional chemical tests to index the active soil N pool. Further work must be initiated to determine the relationship between the kinetically derived, chemically active fraction and plant growth parameters such as yield and total N uptake.

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