Reprinted from the *Soil Science Society of America Journal*
Volume 56, no. 5, September–October 1992
677 South Segoe Rd., Madison, WI 53711 USA

Sewage Sludge Proteins as Labile Carbon and Nitrogen Sources

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ABSTRACT

The study of specific, organic sewage sludge constituents is necessary to augment our knowledge of C and N mineralization in sludge-amended soils. A laboratory incubation study of seven sewage sludges was initiated to test the hypothesis that sewage sludge proteins are labile C and N sources. Sewage sludge proteins were extracted with H_2O, 10% (v/v) Triton X-100, and 1.0 *M* NaOH and determined by the Lowry assay. Sewage sludges were mixed with Bresser sandy loam soil (fine-loamy, mixed, mesic Aridic Argiustoll) at a rate of 10 g dry sludge kg^{-1} dry soil and incubated at 25 °C and 0.111 kg kg^{-1} soil water content for 12 wk to determine sludge C and N mineralization. Extractable sludge proteins were highly correlated to C mineralization (r^2 = 0.94–0.96), but they were poorly correlated to N mineralization (r^2 = 0.40–0.41). This supported the hypothesis that sludge proteins were a labile C source but not a labile N source. However, low molecular weight primary amines (assumed to be predominately protein degradation products) combined with the sludge C/N ratios were highly correlated to sludge N mineralization rates (r^2 = 0.91). Nitrogen mineralization of sludge-amended soil followed either zero- or first-order kinetics. Kinetic models of the first-order systems showed that N mineralization was best described as the decomposition of two distinct organic-N pools. Sewage sludge proteins appear to be significant sources of labile C, and their degradation products apparently are critical N sources.

To improve our knowledge of C and N mineralization in sludge-amended soil, it is necessary to relate the quantities of specific, labile organic sludge constituents to these microbial processes. Proteins represent potentially labile C and N sources because of their abundance in sludges (Hattingh et al., 1967; Hattori and Mukai, 1986) and low C/N ratio. Furthermore, proteolytic microorganisms are ubiquitous in soils and sludges (Hobson et al., 1974; Hankin and Hill, 1978; Loll and Bollag, 1983) indicating the importance of proteins as substrate for microbial growth.

Carbon and N mineralization of sludge-amended soils has been studied extensively (Miller, 1974; Barbarika et al., 1985; Hattori and Mukai, 1986; Hattori, 1988; Wiseman and Zibilske, 1988). The effect, however, of specific sludge organic-C constituents on C and N mineralization in sludge-amended soil has received little attention. Hattori and Mukai (1986) showed that the total organic C, organic N, and crude protein (measured as organic N times 6.25 from sequential extractions) concentrations of six sludges positively correlated to the C and N mineralization rate in amended soil. Hemicellulose and lignin concentrations of the sludges were reported to be negatively correlated to C and N mineralization rate (Hattori and Mukai, 1986). Soil microbial activity and growth measured in sludge-amended soil showed that changes in protease activity and bacterial populations were significantly correlated to C and N mineralization in 12-wk incubations (Hattori, 1988). Hattori (1988) concluded that proteolytic bacteria significantly contributed to the rapid decomposition immediately following sludge application, while fungi and actinomycetes were important to the gradual decomposition that followed.

Descriptions of N mineralization kinetics of soils and sludge-amended soils have mainly focused on the use of first-order models (Stanford and Smith, 1972; Lindemann and Cardenas, 1984; Garau et al., 1986; Lindemann et al., 1988). These models were based on the assumption that the rate of N mineralization is proportional to the quantity of substrate N present (Stevenson, 1965),

$$dN/dt = -kN \quad [1]$$

where N = substrate N, k = rate constant, and t = time. From this assumption, two forms of the first-order model have been developed. The first model developed was derived from the solution to Eq. [1] for the disappearance of substrate N,

$$N_m = N_o(1 - e^{-kt}) \quad [2]$$

where N_m = the net N mineralized at any time t, and N_o = the potentially mineralizable N in the system. This model has been referred to in the literature as the single exponential model. Two major assumptions are associated with this model: (i) the decomposition rate constant (k) of all N_o is the same; and (ii) given an infinite amount of time, the net mineralized N would equal the potentially mineralizable N (i.e., $N_m = N_o$). This last assumption, which implies that all replicates of an experiment would converge to the same value, has been considered to be unacceptable by some researchers (Talpaz et al., 1981). In an effort to address the assumption that only one form of potentially mineralizable N exists, Molina et al. (1980) proposed the use of a double exponential model,

$$N_m = N_oS(1 - e^{-ht}) + N_o(1 - S)(1 - e^{-kt}) \quad [3]$$

where S = the rapidly decomposable N fraction, $1 - S$ = the slowly decomposable N fraction, and h and k are the rate constants for the rapidly and slowly decomposable fractions, respectively. A noted weakness of this model is the time dependence of the parameter estimates (Cabrera and Kissel, 1988a).

By contrast, some researchers have reported zero-order N mineralization kinetics (Tabatabai and Al-Khafaji, 1980; Addiscott, 1983; Wiseman and Zibilske, 1988). This observation has generally been associated with the use of field-moist soil samples. This suggests that the observation of first-order kinetics may

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Published in Soil Sci. Soc. Am. J. 56:1470–1476 (1992).

Abbreviations: ASP, Aspen, CO; CHY, Cheyenne, WY; CHI, Chicago, IL; DEN, Denver, CO; FTC, Ft. Collins, CO; L/E, Littleton/Englewood, CO; RSP, Rock Springs, WY; DTPA, diethylenetriamine pentaacetic acid; R–NH_2, primary amines; TNBS, trinitrobenzenesulfonic acid; MW, molecular weight; DNA, deoxyribonucleic acid.

be an artifact of the perturbation resulting from soil prepared by air drying. However, studies comparing various soil preparation techniques, including field moist soil, have reported first-order N mineralization kinetics (Beauchamp et al., 1986; Cabrera and Kissel, 1988b).

While a great deal of research has been conducted on the mineralization of C and N in sludge-amended soil, there are no reported data correlating sludge protein concentrations (as measured by protein-specific assays) to these processes. Furthermore, N mineralization kinetics have often been assumed to be first-order for sludge-amended soils, particularly freshly amended soils. Therefore, our objectives were two-fold: (i) to test the hypothesis that sewage sludge proteins are a labile C and N source by correlating extractable sludge proteins to C and N mineralization of sludge-amended soils; and (ii) to evaluate kinetic models that describe net N mineralized in sludge-amended soil systems.

MATERIALS AND METHODS

Soils and Sludges

Bresser sandy loam soil was sampled on 3 Apr. 1990 from the Ap horizon (top 10–15 cm). The field had been in a dryland winter wheat (*Triticum aestivum* L.)–fallow cropping system for ≈10 yr. The Bresser sandy loam soil was composed of 733 g kg^{-1} sand, 158 g kg^{-1} silt, and 108 g kg^{-1} clay with a pH of 5.4 and electrical conductivity of 0.49 dS m^{-1} (elemental composition listed in Table 1). Soil texture was measured by the hydrometer method. Soil pH was measured on saturated pastes after equilibration for 24 h, and electrical conductivity was measured from the extracts of the saturated pastes. The soil was sieved to 4 mm and large pieces of wheat residue were removed. The soil was maintained at field-moist conditions (0.111 kg kg^{-1} soil water content) by storage in covered, plastic containers at ≈2 to 4 °C for 15 d prior to initiation of the incubation study. The field-moist condition of the soil was approximately the same as field capacity (0.108 kg kg^{-1} soil water content); therefore, at the initiation of the incubation experiment, no soil moisture adjustments were made.

Seven sludges were studied: Aspen, CO (ASP); Cheyenne, WY (CHY); Chicago, IL (CHI); Denver, CO (DEN); Ft. Collins, CO (FTC); Littleton/Englewood, CO (L/E); and Rock Springs, WY (RSP). Sludges were prepared by oven drying at 55 °C followed by extensive grinding and mixing to produce homogeneous samples. The sludges were stored at −20 °C prior to incubation, and triplicate subsamples were used for chemical analyses (Table 1). The sludge preparation and chemical characteristics of the sludges were described in detail by Lerch (1991).

Incubation Design

Sewage sludges were throroughly mixed with Bresser sandy loam soil at a rate of 0.5 g of dry sludge to 50 g of dry soil and placed in sealed 0.5-L canning jars. The treatments (each of the seven sludges and a control of soil only) were arranged in the incubator using a completely randomized design. Environmental conditions were 25 ± 1 °C, 0.111 kg kg^{-1} soil water content (monitored gravimetrically), and no light. Separate experimental units were established for measurement of C and N mineralization.

For C mineralization determination, triplicate samples of each treatment were established plus two blanks (i.e., empty jars with alkali traps only). In order to trap the evolved CO_2, NaOH (see below for details) was added to glass vials placed at an angle to the soil surface, and they were replaced and analyzed at 1, 2, 4, 8, and 12 wk. At each sampling date, the jars were left open for approximately 1 h, and the soil moisture content checked to restore to field capacity.

For determination of N mineralization, triplicate samples of each treatment were also established, and a nonleached, destructive sampling procedure was used. At each sampling date (1, 2, 4, 8, and 12 wk), 24 jars (three per treatment) were randomly selected for inorganic-N analysis. Because a nonleached procedure was used, the quantity of inorganic N existing at time zero was subtracted from the quantity of inorganic N extracted at each sampling date to determine net N mineralized. Sample jars were opened to allow for aeration at 12, 28, and 56 d for ≈1 h. Soil moisture was monitored about every 10 d.

Analytical Methods

Sludges and Soil

Ammonium was extracted with 2 *M* KCl followed by NH_4^+ analysis with a Technicon Autoanalyzer II (Technicon, Tarrytown, NY). Nitrate was extracted with H_2O (containing 0.01 *M* $CuSO_4 \cdot 5H_2O$ and 0.001 *M* $AgSO_4$ to inhibit microbial growth) followed by colorimetric analysis using the NAS Szechrome azo dye (Polysciences, Warrington, PA). Organic N was calculated as the difference between total N, determined by Kjeldahl digestion (Bremner and Mulvaney, 1982), and NH_4–N concentrations. Organic C was determined by the modified Mebius procedure (Nelson and Sommers, 1982) with the samples heated using aluminum digestion blocks. Elemental analyses were conducted by soil extraction with 1 *M* NH_4HCO_3–0.005 *M* DTPA(Soltanpour et al., 1982) and by digestion of sludge in concentrated HNO_3 (Havlin and Soltanpour, 1980) followed by analysis with the inductively coupled plasma–optical emission spectrophotometer. Carbon mineralization

Table 1. Composition of sewage sludges and Bresser sandy loam (BSL).

Sludge /soil†	Organic C	Organic N	C/N	NH_4–N	NO_3–N	P	Cd	Cu	Ni	Pb	Zn
	g kg^{-1}			mg kg^{-1}							
ASP	424	73.4	5.8	3230	<1.00	1570	4.40	1010	15.5	59.5	868
CHY	416	31.6	13.2	3670	<1.00	1870	12.6	1110	33.8	255	1520
CHI	431	16.0	26.9	27.8	1020	1440	252	1650	673	1170	4940
DEN	383	59.6	6.4	3710	<1.00	1550	17.7	609	69.9	200	1350
FTC	432	67.2	6.4	5520	<1.00	1990	7.44	936	31.8	205	865
L/E	221	53.8	4.1	3400	<1.00	7210	10.1	644	97.2	298	1010
RSP	405	25.5	15.9	3550	237	1600	8.75	566	47.1	223	921
BSL	10.5	0.625	16.8	2.50	2.89	4.60	0.11	1.90	1.12	1.22	3.00

† ASP = Aspen, CO; CHY = Cheyenne, WY; CHI = Chicago, IL; DEN = Denver, CO; FTC = Fort Collins, CO; L/E = Littleton/Englewood, CO; RSP = Rock Springs, WY.

was measured by CO_2 evolution from the sludge-amended soil treatments (Anderson, 1982). The CO_2 was trapped by 5 mL of standardized 3.58 *M* NaOH. To the NaOH traps, 10 mL of 0.5 *M* $BaCl_2$ was added to precipitate CO_3. The alkaline solution was then titrated with standardized 1.33 *M* HCl using phenolphthalein as an indicator. All analyses were performed in triplicate.

Sludge Protein and Primary Amine Extractions

All extractions were performed in capped 50-mL polypropylene centrifuge tubes using a horizontal shaker with a stroke length of 4 cm and shaking speed of 180 oscillations min^{-1}. Sludge proteins were extracted by deionized-distilled H_2O for 16 h, 10% (v/v) Triton X-100 (polyoxyethylene [9.5] p-t-octylphenol) (Sigma Chemical Co., St. Louis, MO) for 6 h, and 1 *M* NaOH for 12 h. Protein was measured by the Lowry assay (Lowry et al., 1951) using bovine serum albumin standards. Sodium dodecyl sulfate (10% w/v) was added to the Triton X-100 extracts to prevent interferences (Ji, 1973). Specific details of the protein extraction procedure were detailed by Lerch (1991).

Primary amine analysis of the sludges was conducted using TNBS (Habeeb, 1966) as described by Lerch (1991) Briefly, the TNBS procedure was used to determine the R–NH_2 content of water extracts (as described above) before and after dialysis (membrane exclusion limit of 14 000 MW), yielding the total and high MW fractions, respectively. The low MW fraction was then determined by the difference between the total R–NH_2 and high MW R–NH_2 fraction. All analyses for R–NH_2 were performed in duplicate.

Nitrogen Mineralization Kinetic Models

Parameter estimations for both exponential models (Eq. [2] and [3]) were performed by a nonlinear least squares algorithm. For each of the first-order models, initial parameter estimates were based on reported values from the literature, and they were modified as needed to facilitate convergence. A zero-order kinetic model for the appearance of mineral N in a system was also considered:

$$N_m = kt \qquad [4]$$

where N_m = net N mineralized (mg N kg^{-1}), t = time (wk), and k = the zero-order rate constant (mg N kg^{-1} wk^{-1}).

RESULTS AND DISCUSSION

Carbon Mineralization

The cumulative CO_2–C mineralized showed first-order kinetics for all treatments (Fig. 1), but the curvilinear nature of mineralized C with time was less marked for the control and CHI treatments. All treatments had the highest rate of C mineralization during the first week of incubation with decreasing mineralization rates thereafter. The observed cumulative C mineralization data are typical of sludge-amended soils (Miller, 1974; Hattori, 1988) because of stimulation of microbial activity from the added labile sludge C. The curvilinear nature of the control however, suggests that some redistribution of labile C may have resulted from the soil sampling and preparation, which contributed to the flush of mineralized C in the first week of incubation. Carbon mineralization rates of the sludge treatments ranged from 2 to 34% of the added sludge C. The increased C mineralization of the sludge

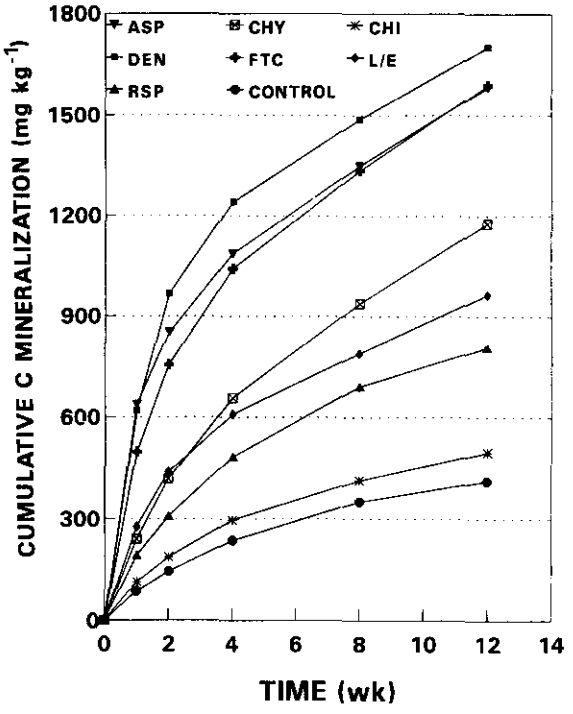

Fig. 1. Cumulative C mineralization as a function of time for all sewage sludge treatments (incubation conditions: sludge application rate of 10 g dry sludge kg^{-1} dry soil, 25 °C, 0.111 kg kg^{-1} soil water content).

treatments compared with the control indicated a significant enhancement of microbial activity or growth as a result of sludge addition to the soil, and this has been reported by Hattori (1988).

Nitrogen Mineralization

The net N mineralized at all sampling dates was greatest for the ASP, DEN, FTC, and L/E treatments, and all four treatments had very high rates of N mineralized during the first week of incubation (Fig. 2). The flush of N mineralized was rapid and extensive for these treatments, with N mineralization rates for the first week ranging from 16 to 33% of the added sludge organic N. Thereafter, N mineralization was generally slower and steadier, suggesting the presence of two distinct pools of mineralizable N for these four sludges. The CHY, CHI, RSP, and control treatments showed low total net N mineralization and little or no flush of N mineralized in the early stages of incubation. These treatments showed a linear accumulation of mineral N with time — implying the presence of a single pool of mineralizable N. Nitrogen mineralization rates for all sludge treatments ranged from −2.4 to 55.4% of added sludge organic N (Table 2) and, with the exception of the CHI sludge, were within previously reported ranges for aerobic or anaerobic sludges (Lindemann and Cardenas, 1984; Barbarika et al., 1985; Garau et al., 1986; Hattori and Mukai, 1986).

Correlation of Extractable Protein to Carbon Mineralization

Linear regressions of extractable protein vs. cumulative sludge CO_2–C mineralized (at 12 wk) showed

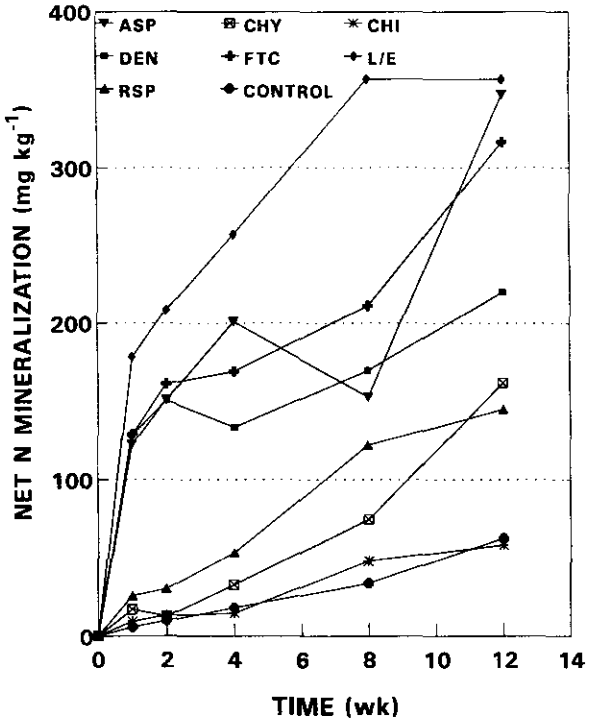

Fig. 2. Net N mineralization as a function of time for all sewage sludge treatments (incubation conditions: sludge application rate of 10 g dry sludge kg^{-1} dry soil, 25 °C, and 0.111 kg kg^{-1} soil-water content).

high correlation for all extractable protein fractions (Fig. 3a–3c). The extraction procedures provided rapid methods (compared with lengthy incubations) for the prediction of cumulative C mineralized in sludge-amended soil. The high correlations provided support for the hypothesis that proteins were a labile C component in the sludges studied. The potential for extensive C mineralization of bacterial protein C, which presumably comprises a significant proportion of sludge proteins, has been reported. Verma et al. (1975) showed that 82% of (noncomplexed) bacterial protein C added to soil was evolved as CO_2 in 12 wks of incubation. In addition, protease activity of sludge-amended soil has also been shown to be significantly correlated to C mineralization (Hattori, 1988). The high correlations, however, may have resulted, in part, from the mineralization of nonproteinaceous C compounds because of stimulation of microbial activity by the proteins. Throughout the incubations, the coefficients of simple determination (r^2) increased with time to their maximum values at 12 wk. This suggests that during the early weeks of incubation more labile C sources, such as glucose or free amino acids (Verma et al., 1975), were being mineralized in addition to proteins. As the incubation proceeded, proteins apparently became the predominant C substrate as the most labile components were depleted.

Correlation of Extractable Protein to Nitrogen Mineralization

Linear regressions of extractable protein vs. net sludge N mineralized indicated low correlation for all

Table 2. Prediction of sludge N mineralization rates from Eq. [5].

Sludge†	$LMWRNH_2$‡	Sludge C/N	Actual rate	Predicted rate	Residuals
	mg kg^{-1}		%		
ASP	43.2	5.8	39.2	40.7	−1.5
CHY	43.0	13.2	31.9	34.3	−2.4
CHI	1.6	26.9	−2.4	−5.0	2.6
DEN	32.4	6.4	26.8	33.0	−6.2
FTC	42.8	6.4	38.3	39.9	−1.6
L/E	47.3	4.1	55.4	44.9	10.5
RSP	46.4	15.9	33.0	34.3	−1.3

† ASP = Aspen, CO; CHY = Cheyenne, WY; CHI = Chicago, IL; DEN = Denver, CO; FTC = Fort Collins, CO.; L/E = Littleton/Englewood, CO; RSP = Rock Springs, WY.
‡ $LMWRNH_2$ = low molecular weight primary amines.

extracts (Fig. 4a–4c). The correlation of extractable protein with sludge N mineralization rate gave even lower coefficients of determination. There was no statistical support, therefore, for the hypothesis that sewage sludge proteins were also labile N components. In addition, correlation of N mineralization (either net sludge N mineralized at 12 wk or N mineralization rate) with sludge organic N contents was not significant. The immobilization of N or the presence of another labile sludge N pool could have accounted for the low correlations. On the basis or reported activity and abundance of proteolytic microorganisms in sewage sludges and sludge-amended soils (Hobson et al., 1974; Loll and Bollag, 1983; Hattori, 1988), protein degradation products (e.g., oligopeptides, short-chain peptides, and free amino acids) were considered as a possible labile N pool. Other nonproteinaceous sources such as hexosamine-N or DNA were improbable choices, given their low levels in either sludges or sludge extracts (Brown and Lester, 1980; Hattori and Mukai, 1986).

The low MW $R–NH_2$ content of the water extracts was measured because it was assumed that these compounds would be water soluble. From these results, multiple linear regression analysis showed that the low MW $R–NH_2$ fraction and the sludge C/N were significantly correlated to the N mineralization rate of the sludges:

$$N_R = 16.6 + 0.670(LMWRNH_2) - 0.843(C/N) \quad [5]$$

$$R^2 = 0.91 (P = 0.009)$$

where N_R = N mineralization rate (% of sludge organic N mineralized), $LMWRNH_2$ = low MW primary amines, and C/N = sludge C/N ratio. The predicted N mineralization rates generally had residuals <3%, except for the DEN and L/E sludges (Table 2). For the DEN sludge, the low C/N ratio caused an overprediction of the actual mineralization rate, but the L/E sludge N mineralization rate was considerably underestimated despite having the highest $R–NH_2$ content and lowest C/N ratio. The model, however, resulted in generally accurate prediction of a broad range of N mineralization rates. The significant correlation of the low MW $R–NH_2$ fraction to sludge N mineralization rates indicates the importance of this organic-N form as a labile N source in sewage sludges.

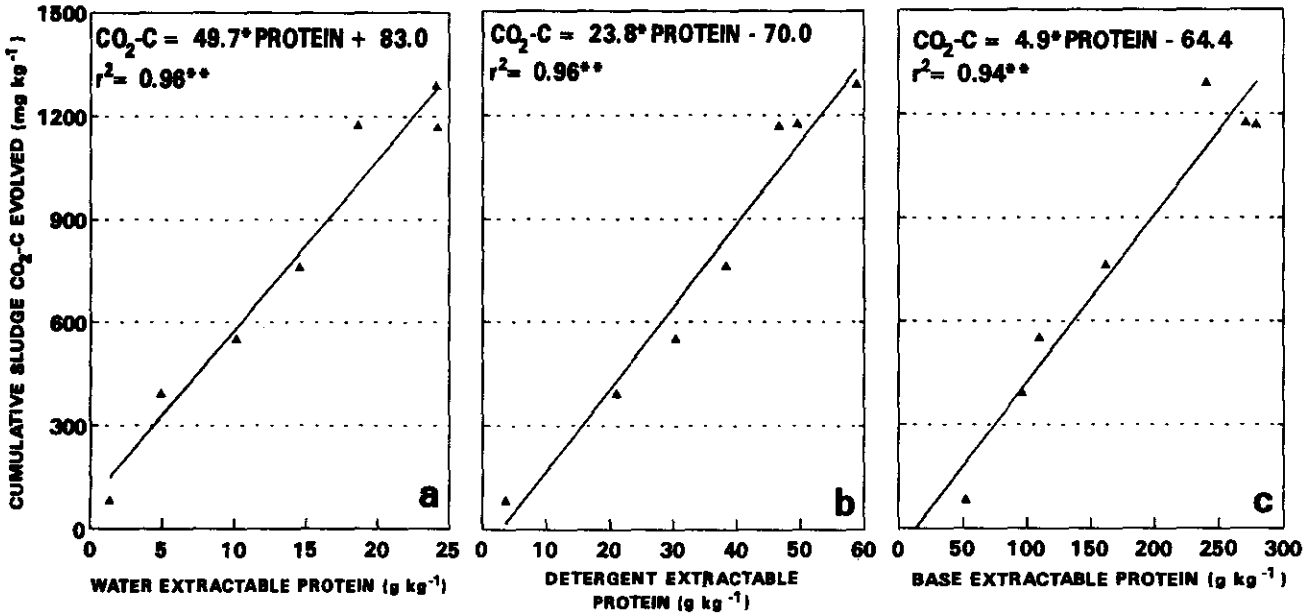

Fig. 3. Correlation of extractable sewage sludge proteins to total cumulative sludge CO_2–C evolution from 12-wk incubations: (a) water; (b) detergent; (c) base (symbols denote mean extractable protein levels for each of the sludges).

Kinetics of Net Nitrogen Mineralization

The sewage sludge treatments fell into two categories with respect to the kinetics of net N mineralized (Fig. 2). The first category was zero-order kinetic systems exhibited by the CHI, CHY, and RSP sludges and the control. These sludges had been stored from 0.5 to 12 yr prior to sampling, and this, apparently, resulted in organic-C stabilization. As discussed above, these treatments had no marked flush of mineralized N in the first week of incubation, and the accumulation of mineralized N was linear with time. The slopes of the lines represent the rate constants, k, for each treatment, and the rates ranged from 4.9 to 13.2 mg N mineralized kg^{-1} wk^{-1} (Fig. 5). Other researchers have reported zero-order kinetics for net N mineralized (Tabatabai and Al-Khafaji, 1980; Addiscott, 1983; Wiseman and Zibilske, 1988). By estimation from the graphs of Wiseman and Zibilske (1988), rates of N mineralization of either municipal or domestic sludges appeared to be ≈20 mg N kg^{-1} wk^{-1} for most treatments. Sludge treatments displaying zero-order kinetics were characterized by high C/N ratios (>13:1),

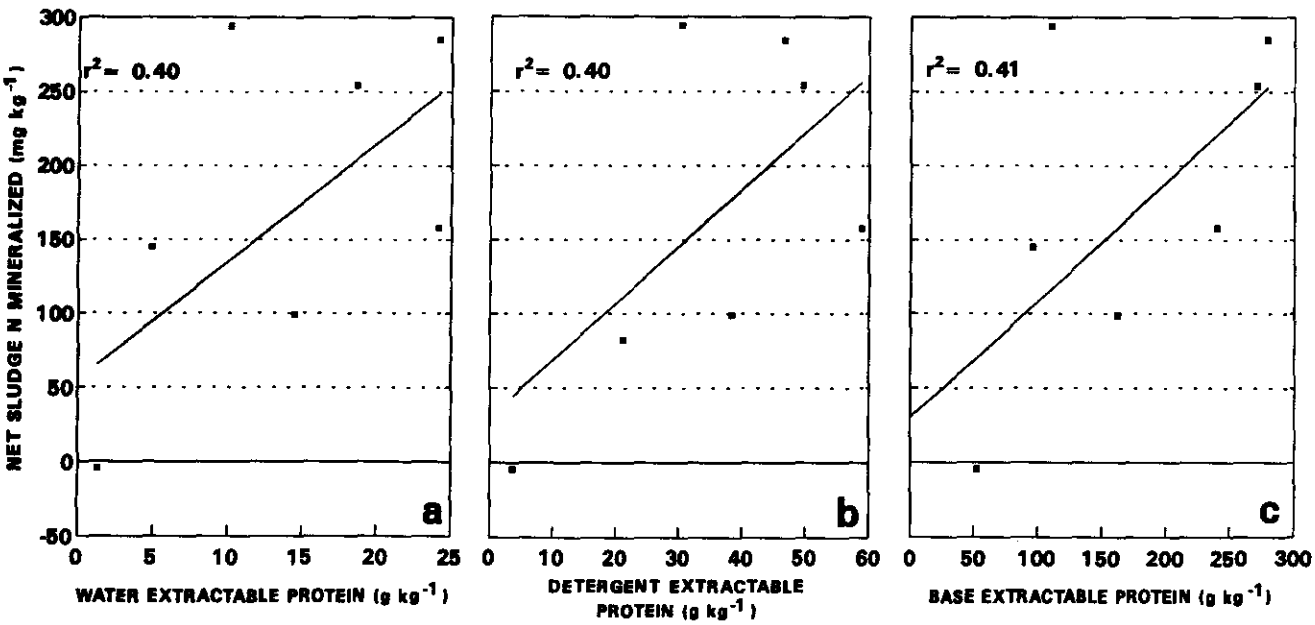

Fig. 4. Correlation of extractable sewage sludge proteins to net sludge N mineralization at Week 12 of the incubations: (a) water; (b) detergent; (c) base (symbols denote mean extractable protein levels for each of the sludges).

Table 3. Parameter estimates of first-order models.

	Single exponential†			Double exponential‡				
Sludge§	N_o	k	Pseudo R-square¶	N_o	S	h	k	Pseudo R-square
	mg kg^{-1}	wk^{-1}		mg kg^{-1}		wk^{-1}		
ASP	353	0.27	0.82	564	0.17	4.34	0.06	0.90
DEN	179	0.91	0.86	821	0.16	1.07	0.01	0.93
FTC	264	0.39	0.56	866	0.17	1.10	0.02	0.81
L/E	349	0.49	0.79	394	0.28	14.3	0.20	0.89

† $N_m = N_o(1 - e^{-kt})$ where N_m = net mineralized at time, t; N_o = potentially mineralizable N; k = first-order rate constant.
‡ $N_m = N_oS(1 - e^{-ht}) + N_o(1 - S)(1 - e^{-kt})$ where S = rapidly decomposable organic-N fraction; $1 - S$ = slowly decomposable organic-N fraction; h = rate constant for the rapidly decomposable fraction; k = rate constant for the slowly decomposable fraction.
§ ASP = Aspen, CO; DEN = Denver, CO; FTC = Fort Collins, CO. L/E = Littleton/Englewood, CO;
¶ Pseudo R-Square = $1 - (RSS/(n - 1)s_N)$ where RSS = residual sum of squares; n = number of observations; s_N = variance of the mean net N mineralized for each sludge.

low organic N and extractable protein, and storage for at least 0.5 yr. The organic C of these sludges was apparently stabilized during storage and, therefore, it was more resistant to microbial degradation.

The other four sludges (ASP, DEN, FTC, and L/E) all displayed first-order kinetics, and their graphs of net N mineralized vs. time were curvilinear (Fig. 2). These sludges all had a flush of mineralized N in the first week, indicating the presence of a rapidly decomposable N pool. These four sludges were characterized by low C/N, high organic-N levels, high amounts of extractable protein (except L/E), and no storage prior to acquisition. The measure of goodness of fit of the first-order models was referred to as the pseudo R-square (Table 3). The interpretation of the pseudo R-square is analogous to linear or multiple linear regression coefficients of determination (i.e., the percentage of the variability in the Y data explained by the X data).

The single exponential model (Eq. [2]) resulted in curves that predict rapid N mineralization through the first 2 to 4 wk but, thereafter, N mineralization rates were predicted to be very low. This resulted in low estimates of N_o and high estimates of the rate constants, k (Table 3). This was most notable for the DEN sludge, for which the fitted curve was almost flat after 4 wk. Actual net N mineralization data for all four sludges showed that, by 12 wk, the estimated N_o had been reached or exceeded — indicating underestimation of N_o values by this model. The range of N_o and k estimates were within previously reported values for sludge-amended soil incubations in which the single exponential model was used (Lindemann and Cardenas, 1984; Garau et al., 1986).

The double exponential model (Eq. [3]) predicted much greater N mineralization from 4 to 12 wk than the single exponential model. The parameter estimates from the double exponential model also reflect this difference with higher N_o and lower k estimates in all cases (Table 3). Similar differences between parameter estimates of these models were reported by Deans et al. (1986). The greatest differences in N_o estimates between models were observed for the DEN and FTC sludges. Partitioning of N_o into rapidly and slowly decomposable fractions showed that the L/E sludge had the largest rapidly decomposable pool, and its decomposition rate was notable higher than the other sludges as well (Table 3). Rate-constant estimates,

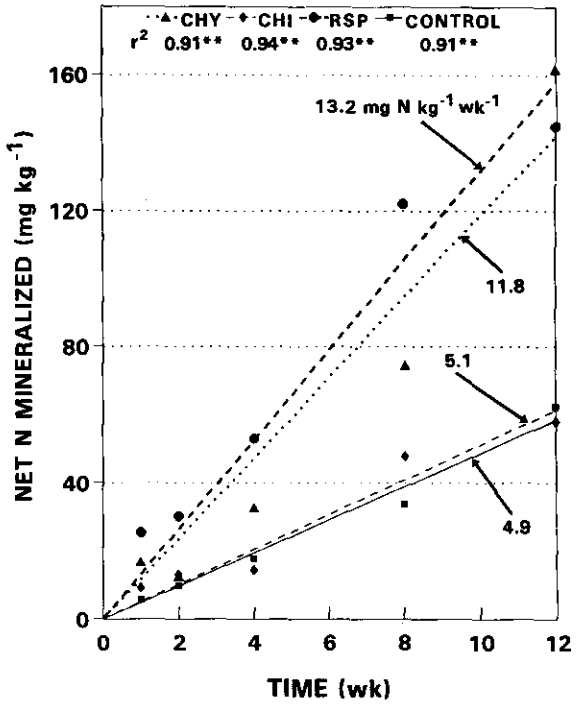

Fig. 5. Sewage sludge treatments exhibiting zero-order net N mineralization kinetics during 12-wk incubations (arrows denote zero-order rate constants in milligrams of N per kilogram per week).

except for the L/E sludge, were within ranges previously reported (Lindemann and Cardenas, 1984; Deans et al., 1986).

Overall, the double exponential model appeared to provide N_o estimates that were more accurate than the single exponential model. The single exponential model was unrealistic in its prediction that 12 wk of incubation was sufficient to reach complete degradation of all potentially mineralizable N. Furthermore, the double exponential model was the best-fit model on the basis of lower residual sum of squares and higher pseudo R-square values (Table 3). Deans et al. (1986) also reported better fits to the data for the double compared with the single exponential model. The underlying theory of the double exponential model also provided a more realistic description of N mineralization based on the observed net N mineralization data. Therefore,

the better fit to the data, for sludges demonstrating first-order kinetics, appears to arise from a more mechanistic, rather than solely empirical, relationship.

CONCLUSIONS

The sludges studied covered a broad range of C and N mineralized in 12 wk of incubation. Four of the sludges studied had both rapidly and slowly decomposable labile N pools while three of the sludges had only slowly decomposable labile N pools. Extractable sludge proteins were highly correlated, for all extractants used, to sludge C mineralization. This was considered support of the hypothesis that proteins are a labile C source in sludges. The protein extraction methods employed offer rapid alternatives to incubations for assessing C mineralization in sludge-amended soil. Extractable sludge proteins showed low correlations to sludge N mineralization. Water-extractable primary amines (presumably protein degradation products), however, were shown to be potentially significant labile N sources in sewage sludges.

Net N mineralization of the sludges showed either zero- or first-order kinetics. The distinction between the kinetics was delineated by sludge C/N ratio and the quantity of organic N and extractable protein of the sludges. In general, sludges with high C/N and low organic-N and extractable-protein levels exhibited zero-order kinetics, whereas sludges with low C/N and high organic-N and extractable-protein levels exhibited first-order kinetics. First-order systems were best described by the double exponential model due to the distinction of two decomposable organic-N fractions.

ACKNOWLEDGMENTS

We thank the cities of Littleton and Englewood, CO, and the Colorado Agricultural Experiment Station (Project no. 15–2924) for the financial support of this project.

REFERENCES

Addiscott, T.M. 1983. Kinetics and temperature relationships of mineralization and nitrification in Rothamsted soils with differing histories. J. Soil Sci. 34:343–353.

Anderson, J.P.E. 1982. Soil Respiration. p. 841–845. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. ASA and SSSA, Madison, WI.

Barbarika, A., L.J. Sikora, and D. Colacicco. 1985. Factors affecting the mineralization of nitrogen in sewage sludge applied to soils. Soil Sci. Soc. Am. J. 49:1403–1406.

Beauchamp, E.G., W.D. Reynolds, D. Brasche-Villeneuve, and K. Kirby. 1986. Nitrogen mineralization kinetics with different soil pretreatments and cropping histories. Soil Sci. Soc. Am. J. 50:1478–1483.

Bremner, J.M., and C.S. Mulvaney. 1982. Nitrogen — total. p. 610–616. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. ASA and SSSA, Madison, WI.

Brown, M.J., and J.N. Lester. 1980. Comparison of bacterial extracellular polymer extraction methods. Appl. Environ. Microbiol. 40:179–185.

Cabrera, M.L., and D.E. Kissel. 1988a. Length of incubation time affects the parameter values of the double exponential model of nitrogen mineralization. Soil Sci. Soc. Am. J. 52:1186–1187.

Cabrera, M.L., and D.E. Kissel. 1988b. Potentially mineralizable nitrogen in disturbed and undisturbed soil samples. Soil Sci. Soc. Am. J. 52:1010–1015.

Deans, J.R., J.A.E. Molina, and C.E. Clapp. 1986. Models for predicting potentially mineralizable nitrogen and decomposition rate constant. Soil Sci. Soc. Am. J. 50:323–326.

Garau, M.A., M.T. Felipo, and M.C. Ruiz de Villa. 1986. Nitrogen mineralization of sewage sludges in soils. J. Environ. Qual. 15:225–229.

Habeeb, A.F.S.A. 1966. Determination of free amino groups in proteins by trinitrobenzenesulfonic acid. Anal. Biochem. 14:328–336.

Hankin, L., and D.E. Hill. 1978. Proportion of bacteria in agricultural soils able to produce degradative enzymes. Soil Sci. 126:40–43.

Hattingh, W.H.J., P.G. Thiel, and M.L. Siebert. 1967. Determination of protein content of anaerobic digesting sludge. Water Res. 1:185–189.

Hattori, H. 1988. Microbial activities in soil amended with sewage sludges. Soil Sci. Plant Nutr. 34:221–232.

Hattori, H., and S. Mukai. 1986. Decomposition of sewage sludges in soil as affected by their organic matter composition. Soil Sci Plant Nutr. 32:421–432.

Havlin, J.L., and P.N. Soltanpour. 1980. A nitric acid plant tissue digest method for use with inductively coupled plasma spectroscopy. Commun. Soil Sci. Plant Anal. 11:969–980.

Hobson, P.N., S. Bousfield, and R. Summers. 1974. Anaerobic digestion of organic matter. CRC Crit. Rev. Environ. Control. 4:131–191.

Ji, T.H. 1973. Interference by detergents, chelating agents, and buffers with the Lowry protein determination. Anal. Biochem. 52:517–521.

Lerch, R.N. 1991. Characterization of sewage sludge proteins. Ph.D. diss. Colorado State Univ., Fort Collins (Diss. Abstr. 92–07333).

Lindemann, W.C., and M. Cardenas. 1984. Nitrogen mineralization potential and nitrogen transformations of sludge-amended soil. Soil Sci. Soc. Am. J. 48:1072–1077.

Lindemann, W.C., G. Connell, and N.S. Urquhart. 1988. Previous sludge addition effects on nitrogen mineralization in freshly amended soil. Soil Sci. Soc. Am. J. 52:109–112.

Loll, M.J., and J. Bollag. 1983. Protein transformations in soil. Adv. Agron. 36:351–382.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.

Miller, R.H. 1974. Factors affecting the decomposition of an anaerobically digested sewage sludge in soil. J. Environ. Qual. 3:376–380.

Molina, J.A.E., C.E. Clapp, and W.E. Larson. 1980. Potentially mineralizable nitrogen in soil: the simple exponential model does not apply for the first 12 weeks of incubation. Soil Sci. Soc. Am. J. 44:442–443.

Nelson, D.W., and L.E. Sommers. 1982. Total carbon, organic carbon, and organic matter. p. 571–572. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. ASA and SSSA, Madison, WI.

Soltanpour, P.N., J.B. Jones, and S.M. Workman. 1982. Optical emission spectrometry. p. 54–55. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. ASA and SSSA, Madison, WI.

Stanford, G., and S.J. Smith. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. Proc. 36:465–472.

Stevenson, F.J. 1965. Origin and distribution of nitrogen in soil. p. 1–42. *In* W.V. Bartholomew and F.E. Clark (ed.) Soil nitrogen. Agron. Monogr. 10. ASA, CSSA, and SSSA, Madison, WI.

Tabatabai, M.A., and A.A. Al-Khafaji. 1980. Comparison of nitrogen and sulfur mineralization in soils. Soil Sci. Soc. Am. J. 44:1000–1006.

Talpaz, H., P. Fine, and B. Bar-Yosef. 1981. On the esimation of N-mineralization parameters from incubation experiments. Soil Sci. Soc. Am. J. 45:993–996.

Verma, L., J.P. Martin, and K. Haider. 1975. Decomposition of carbon-14-labeled proteins, peptides, and amino acids; free and complexed with humic polymers. Soil Sci. Soc. Am. Proc. 39:279–284.

Wiseman, J.T., and L.M. Zibilske. 1988. Effects of sludge application sequence on carbon and nitrogen mineralization in soil. J. Environ. Qual. 17:334–339.