Carbon and Nitrogen Mineralization Kinetics in Soil Previously Amended with Sewage Sludge

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ABSTRACT

Microbial mineralization rates of organic carbon (C) and nitrogen (N) were determined on the same sludge-amended and nonamended soil samples. The purpose of this integrated approach was to highlight the long-term dynamics of N release with C stabilization in sludge-affected soil. Three application rates of digested municipal sludge, check, 45 Mg ha^{-1} and 180 Mg ha^{-1}, were incorporated into field plots annually for 8 years, with no addition during the subsequent 3 years. Barley was grown on the site each spring of the 11 years. In an 87-week laboratory incubation experiment conducted on soil samples collected 3 years after the last sludge addition, N and C mineralization rates (k_n, k_c) increased with sludge application rate. Soil nitrogen mineralization potentials (N_o) increased with sludge application, unlike carbon mineralization potentials (C_o) which did not correlate with sludge application. The C/N ratio of the mineralized organic matter decreased with sludge application rate. Three years after field incorporation of sludge, decomposition of the organic fraction can be described as a set of two first-order rate reactions. One fraction is characterized by a large stable element (high N_o, C_o and low k_n, k_c); the second fraction consists of a smaller labile portion which is characterized by low N_o, C_o and high k_n, k_c values. The microbial biomass decreased to less than half of its original amount after 20 weeks of incubation in all soil treatments.

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THE FORMATION of soil organic matter (SOM) is a reversible reaction. Additions of organic waste, such as municipal sludge, has been proposed as one method of maintaining levels of organic matter in agricultural as well as forested and disturbed lands. Data on N mineralization in soils that had recent applications of sludge (Epstein et al., 1978; Lindemann and Cardinas, 1984), as well as studies on land previously amended with sludge (Stark and Clapp, 1980; Griffin and Laine, 1983), have contributed to the understanding of soil N behavior with sludge additions. However there remains a need for models that predict sludge N release especially after the termination of application (Page et al., 1983). Because N turnover in soil is highly dependent on C transformations and microbial biomass (McGill et al., 1981), an integrated approach was taken in this study. A N mineralization indicator (accumulation of NO_3), a C decomposition parameter (CO_2 evolution), and an estimate of microbial bio-

mass ($CHCl_3$ fumigation-incubation) were used to characterize the labile organic fraction while also identifying the interrelationship between N and C mineralization kinetics.

MATERIALS AND METHODS

The 2.4 by 3.0-m sludge-amended field plots are located on the Oxford Tract at the University of California, Berkeley. The Tierra loam soil had an original CEC of 20.1 cmol kg^{-1}, a pH of 5.4, and soil organic C content range of 7 to 10.2 g kg^{-1}. The sludge, from the East Bay Municipal Utility District (Oakland, CA), had been anaerobically digested for 20 d then vacuum-filtered. The final product was a wet-cake slurry that contained 25% solids (Williams et al., 1984). Some chemical properties of the sludge are presented in Table 1. Three application rates of the sludge (check, 45 Mg sludge ha^{-1}, 180 Mg sludge ha^{-1}) were incorporated into triplicate plots annually for 8 consecutive years with no additions for the 3 subsequent years. A barley crop (*Hordeum Vulgare* L.) was grown on the site for each of the 11 years of the study.

Laboratory Incubation Study

Surface soils (0–15 cm) were sampled from two of the triplicated field plots, three years after the last sludge application. Field-moist soils (15 g oven-dried weight) were passed through a 4-mm sieve, then uniformly mixed with 15 g of Ottawa sand (0.59–0.42 mm) and placed in 0.05 L Buckner funnels. To remove any inorganic-N prior to incubation, half of the soil-sand samples were initially leached with 100 mL of 0.1 M $CaCl_2$ and 25 mL of N-free nutrient solution (0.002 M $CaSO_4$, 0.002 M $MgSO_4$, 0.0025 M 0.0025 M K_2SO_4) according to Stanford and Smith (1972); these samples were then leached at week 3, 6, 11, 17, 21, 27, 38, 50, 66, and 87 with $CaCl_2$ and nutrient solution to determine the N mineralization rates. The N mineralization method of Stanford and Smith (1972) was modified by reducing the amount of leaching solution to 1/10 of that recommended by the authors. This reduction in volume was intended to produce a less drastic perturbation, provide a concentrated solution for direct measurement and reduce the amount of organic N leached from the samples (Smith et al., 1980). The other half of the soil-sand samples were not leached but were maintained at constant weight throughout the 87-week incubation period by the addition of water. These nonleached samples were used as controls to determine the effect of N removal on CO_2 evolution.

Into each air-tight Mason jar (0.95 L) which was fitted with a rubber septum, there were placed four 0.05-L Buckner funnels with glass filters and the soil-sand mixtures. Four

Table 1. A chemical analysis of the Oakland sludge (from Williams et al., 1980).

Sludge	Concentration, mg kg^{-1} dry wt
Total N	33 000
Ammonia	2 000
Nitrate-N	500
Total P	16 400
Ca	22 600
Mg	6 200
K	900
NA	1 200
Cd	37
Cr	1 470
Cu	600
Fe	22 100
Hg	14
Mn	300
Ni	180
Pb	1 090
Zn	3 910

jars were filled with 16 funnels from each plot (two plots per sludge application rate), then incubated at 25 °C for 87 weeks.

Leachate Analysis

At each sampling period the funnels were placed under constant vacuum until the soil-sand mixture reached the weight that corresponded to −100 kPa water potential (60% water-holding capacity as determined by a hanging water-column method). Approximately 12.5 mL of the $CaCl_2$ and nutrient solution was passed through the soil-sand mixture, and the leachate was frozen until the following order of analysis was performed: (a) NH_4-N; (b) NO_2-N; and (c) soluble-C. Ammonium, NO_2 plus NO_3, and NO_3 were determined on a flow-through injection system (Am. Public Health Assoc., 1981). Total soluble-C was determined using a Dohrmann DC 80 Carbon Analyzer which used a low temperature, persulfate-UV oxidation procedure.

Soil Analysis

All samples were triplicated and the results expressed on an oven-dried basis (105 °C). Total C determinations were performed on soils using a dry combustion technique (Nelson and Sommers, 1982) and the regular Kjeldahl method was performed for total soil N (Bremner and Mulvaney, 1982).

CO₂ Evolution

Gas samples were obtained from the closed incubation jars by the use of a 1-mL syringe and then measured for CO_2 concentration on a Varian Aerograph Model 920 gas chromatograph (GC). The jars were then opened and allowed to equilibrate with the atmosphere. The frequency of sampling ranged from daily to weekly to insure that the CO_2 concentration did not exceed 0.8 mol m^{-3}.

Data Analysis

A nonlinear least square (NLLS) regression analysis was used to calculate N_o, C_o, and associated rate constants from the 87-week data. The NLLS method was used to reduce the error imposed by the logarithmic transformation of low value mineralization data (Smith et al., 1980).

Microbial Biomass C Determinations

Biomass-C was measured in these soil by the chloroform fumigation incubation method of Jenkinson and Powlson (1976). At week 0, 3, 6, 21, 38, 50, and 87 Buckner funnels containing the soil-sand mixture were removed from the incubation vessels (four funnels per sludge treatment) and transferred to glass beakers for fumigation with distilled $CHCl_3$. The 10-day flush of CO_2 after fumigation was measured on a gas chromatograph. Soil samples were not inoculated after fumigation nor were any controls subtracted from the CO_2 evolution data (Voroney and Paul, 1984). The transfer of the soil-sand samples from the funnels to glass beakers was a precaution to prevent chloroform adsorbed on the plastic walls of the funnel from suppressing microbial activity and the flush of CO_2 after fumigation.

RESULTS AND DISCUSSION

Nitrogen Mineralization

The N mineralization rate, under a particular set of laboratory conditions, is proportional to the quantity of mineralizable substrate in soil (Stanford and Smith, 1972). Nitrogen mineralization potentials (N_o) and N mineralization rate coefficients (k_n) were calculated for each treatment (Table 2). These N_o values determined

Table 2. Soil N and C mineralization potentials (N_o, C_o) and rate constants (k_n, k_c).

	Check	45 Mg ha^{-1}	180 Mg ha^{-1}
N_o (mg kg^{-1})	191	427	579
SE*	2	3	11
k_n (week^{-1})	0.010	0.013	0.020
C_o (mg kg^{-1})	10 010	11 983	11 933
SE	213	91	156
k_c (week^{-1})	0.005	0.007	0.011
N_o/N_{total} (%)	16.6	21.1	17.6
C_o/C_{total} (%)	67.6	55.2	36.2
C_o/N_o	52.4	28.1	20.6
Sludge-soil mineralization data minus control			
N_o (mg kg^{-1})		266	458
SE		2	11
k_n (week^{-1})		0.013	0.020
C_o (mg kg^{-1})		5 573	4 723
SE		321	70
k_c (week^{-1})		0.005	0.019

* SE = standard error of the mean.

Table 3. The effect of multiple leaching on total soil carbon mineralization after the 87-week incubation.

Treatment	Leached	Not leached
	mg CO_2-C kg^{-1} soil	
Check	3615.0	3753.5
SE†	547.9	338.1
45 Mg ha^{-1}	5822.5	5613.5
SE	437.8	328.5
180 Mg ha^{-1}	8014.0	7009.5
SE	506.2	453.0

†SE = Standard error of the mean.

by a NLLS analysis compared well with those reported for soils collected 2 and 4 years after sludge application (Stark and Clapp, 1980). The k_n values increased with sludge application rate but were less than those reported by Stark and Clapp (1980), Lindemann and Cardinas (1984), or Griffin and Laine (1983) because the incubation temperature was kept 10 °C lower.

The increase in k_n values with an increase in sludge application rate was not due to a larger percentage of readily mineralizable N remaining after several years of field decomposition. Although N_o values were higher, the percentage of N_o/N_{total} did not consistently increase with rate of sludge application (Table 2). Stark and Clapp (1980) reported similar N_o/N_{total} values for soils after 2 years of repeated sludge application. In another study by these same authors, soils which were collected 4 years after the last sludge application had a N_o/N_{total} value 12.1% vs. 19.5% for the control. It is assumed that after the termination of the sludge application N_o, k_n, and the percent of labile-N divided by total-N will decrease with increasing age of the site. Results from our experiment indicate that 3 years after the last sludge addition there still remains a substantial labile-N pool in the sludge-treated soils.

Carbon Mineralization

Periodic leaching of available N only slightly increased the rate of organic-C mineralization in the high sludge application rate as measured by CO_2 evolution (Table 3). The results suggest that available-C and not N was probably limiting in the sludge-treated and the nontreated soils.

In order to quantify the C associated with the labile-N fraction (N_o), C mineralization potentials (C_o) were also calculated by the NLLS technique. Soluble-C leached from the soil represented <3% of the C evolved as CO_2 and was not included in computing C_o. The C_o, unlike N_o, did not correlate with sludge additions. In fact, that portion of total soil C that comprised C_o decreased with sludge application rate (Table 2). Although speculative, these results suggest that the total soil C left after 3 years of field decomposition was more recalcitrant than native soil organic-C. The characteristics of this C pool is represented by the sludge application data minus the controls in Table 2.

Stabilization is a term often used to describe soil organic matter in toto, but SOM is composed of many components, each varying in size, rate of decomposition, and proportion to the total organic pool. Although the high sludge application rate doubled the rate constant (k_c), the percentage of mineralizable-C to soil organic-C decreased (C_o/C_{total}). The concept of organic matter stabilization should involve at least three levels of evaluation: the decay rate constant, the absolute size of the mineralizable pool, and the relative size of the "active" fraction.

The Association Between C and N Mineralization

The pool sizes and decay constants in Table 2 suggest that sludge stabilization within soil was more extensive for sludge-C than for sludge-N. These two essential parameters, depicted in Fig. 1 as cumulative mineralized C and N, increased with sludge application rate. The similar shape of the log_e curves indicate that the C/N ratio did not fluctuate over the 87-week incubation. The relationship between the amount of N mineralized per CO_2-evolved was constant for each application rate and increased with sludge application (Fig. 2). Possible factors or combination of factors for the observed decomposition characteristics are:

1. the C/N ratio of microbial byproducts from sludge decomposition was lower than that of the native soil organic fraction;
2. the C stabilized in sludge was primarily derived from anaerobically digested microbial cell walls which may be more recalcitrant than lignin-derived SOM;
3. components within the sludge could be inhibiting mineralization of C more so than N; and/or
4. the labile sludge-N remained in the soil active fraction after the stabilization of sludge-C due to the excess incorporation of N into microbial biomass without a concurrent increase in biomass-C.

Curve Splitting

As seen in Fig. 1, a single first-order function would not accurately describe the N or C mineralization behavior from initiation to completion of the incubation. More than one first-order equation is needed to reflect the heterogeneity of substrate quality. The use of a combination of first-order equations has been employed by Lindemann and Cardinas (1984) to describe sludge decomposition kinetics. To section the mineralization curves into two unique pools with their own rate constants, a curve-splitting technique was used (Paul and Voroney, 1980). The reason for seg-
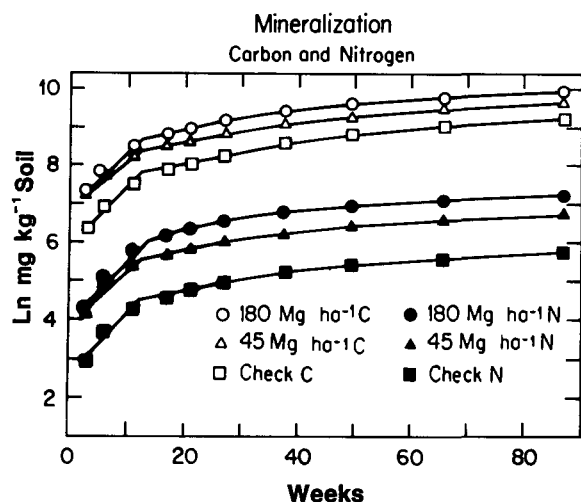
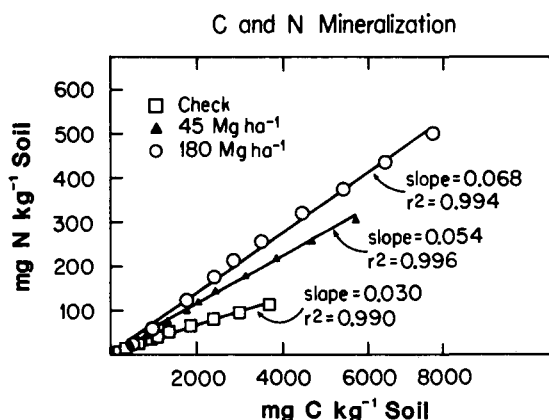Fig. 1. The effect of sludge application rate on soil C and N mineralization.

Fig. 2. The effect of sludge application rate on the amount of soil N mineralized as NO_3 per unit soil C evolved as CO_2.

menting the mineralization curves at 11 weeks can be seen in Fig. 1. The correlation coefficient (r) values from linear regression analysis produces the largest combined r value for all curves when segmented at 11 weeks, except for N with the highest sludge application. The split that gave the highest combined r value for this treatment was at the next sampling date, 17 weeks, suggesting that the high-sludge application prolonged the rapid N mineralization portion of the curve.

To separate out the first 11-week pool sizes of both C and N, the 11-week mineralization data were subtracted out from the original N_o and C_o estimates. Pool sizes were recalculated by NLLS analysis which gave long-term pool sizes for the period between week 11 and week 87 [$N_{o(11-87)}$, $C_{o(11-87)}$]. These long-term pool sizes were then subtracted from the original C_o and N_o estimates to get the short-term pools (first 11 weeks). To determine the short-term rate constants [$k_{n(0-11)}$ or $k_{c(0-11)}$], a linear regression was performed on the $\log_e$ of three values which represent the difference of the mineralized N or C accumulated in the first 11 weeks [$N_{o(0-11)}$, $C_{o(0-11)}$ from the newly calculated $N_{o(0-11)}$ and $C_{o(0-11)}$. The rate constant $k_{n(0-11)}$ for the check soil is represented by the slope of the bottom curve in Fig. 3. In the same graph, the y intercept of the top line represents the $\log_e$ of the long-term pool size [$N_{o(11-87)}$] for the check soil. In Table 4, the original pool
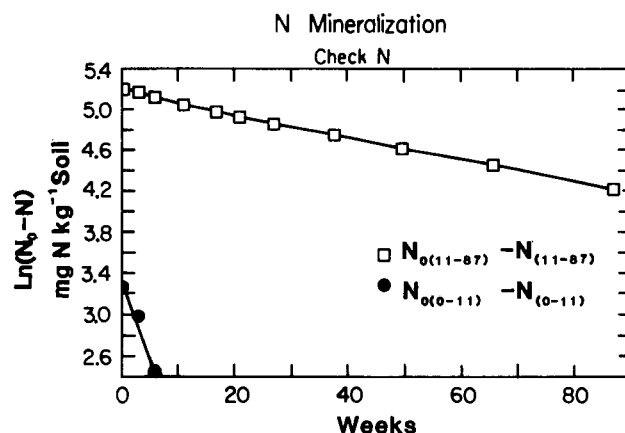

Fig. 3. The use of curve-splitting techniques to segment the check N mineralization data into two distinct rate functions with appropriate pool sizes [$N_{o(0-11)}$, $N_{o(11-87)}$] and rate constants [$k_{n(0-11)}$, $k_{n(11-87)}$].

sizes and rate constants are represented as a two components system for each treatment.

Microbial Biomass

Soil microflora contain substantial quantities of C and N, which upon death are readily mineralized by the surviving microorganisms (Jenkinson and Powlson, 1976; Anderson and Domsch, 1980). In a field study, Lynch and Panting (1980) observed a biomass size fluctuation in an agricultural soil that represented from 1.1 to 2.7% of the total soil C over the course of 1 year. Robertson et al. (1988) attributed 20% of mineralized C to microbial biomass decline in the first 12 weeks of soil incubation. The size of the soil microbial biomass as effected by sludge application over the course of the 87-week incubation is represented in Table 5. The biomass size was initially higher in the sludge-amended soil than the checks but did not consistently increase with application rate. The average decline in the biomass-C decreased to half of its original pool size after 21 weeks.

The decline of all soil treatments can be described by a first-order equation for the first 50 weeks of incubation (each point in Fig. 4 represents an average of 12 measurements). The linear regression of the average $\log_e$ values of biomass-C per time produced a coefficient of determination (r^2) of 0.90 with a slope of -0.06 week^{-1}. Such a decay rate would produce a turnover time, assuming that growth rate was small compared to the death rate, of 16.7 weeks for the first 50 weeks of incubation. The microbial pool of both C and N contributes a small but labile fraction to the mineralizable organic component during this initial period.

The size of the microbial population did not appear to be a limiting factor to decomposition after the initial days of incubation, in light of the observed decrease in microbial biomass time. The biomass size was relatively stable and appeared to achieve steady state after 50 wk.

Although no biomass N measurements were performed during the incubation, the soil microbial biomass C/N ratio were determined initially (Boyle and Paul, 1989). Assuming a biomass C/N ratio of 4.8 for the control soil and 3.9 for the high sludge application

Table 4. Soil C and N mineralization data segmented into two first-order rate equations.

	Check	45 Mg ha^{-1}	180 Mg ha^{-1}
$N_{o(0-11)}$ (mg kg^{-1})	26	83	128
$k_{o(0-11)}$ (week^{-1})	0.073	0.170	0.112
$C_{o(0-11)}$ (mg kg^{-1})	667	1 351	1 821
$k_{c(0-11)}$ (week^{-1})	0.137	0.170	0.137
$N_{o(11-87)}$ (mg kg^{-1})	175	417	613
$k_{n(11-87)}$ (week^{-1})	0.011	0.013	0.017
$C_{o(11-87)}$ (mg kg^{-1})	11 975	11 488	11 368
$k_{c(11-87)}$ (week^{-1})	0.004	0.007	0.011

Table 5. Microbial biomass C content in the sludge-amended and nonamended soils over the length of the 87-week incubation.†

	Weeks						
Treatment	0	3	6	21	38	50	87
				mg C kg^{-1}			
Check	253.0	195.0	283.0	135.0	140.0	116.0	136.0
SE‡	10.6	9.1	4.8	4.5	13.7	9.9	17.6
45 Mg ha^{-1}	419.0	257.0	318.0	157.0	189.0	145.0	154.0
SE	27.3	10.0	16.3	3.6	12.8	13.2	11.8
180 Mg ha^{-1}	409.0	312.0	345.0	225.0	153.0	124.0	147.0
SE	84.4	14.1	6.0	10.9	16.1	13.9	23.6

† Biomass C = (10 day) CO_2-C/0.45.
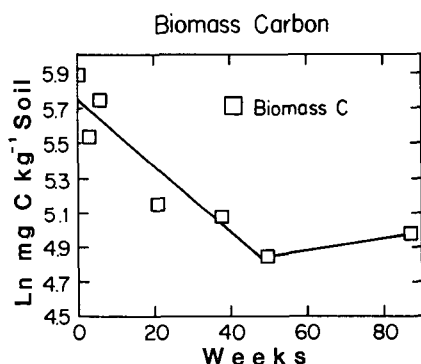‡ SE = standard error of the mean of 4 replicates.

Fig. 4. The effect of incubation time on soil microbial biomass carbon (averaged of the three sludge treatments).

rate, the declining biomass could contribute 47% of the mineralized N for the control soil and 18% for the high sludge amended rate in the first 21 weeks of incubation.

SUMMARY AND CONCLUSIONS

The N mineralization potentials (N_o) were greater in sludge-amended soils 3 years after the last sludge addition than in the control soils. The highest sludge application contained the most labile organic N fraction (largest rate constant). The first-order rate constant (k_n) increased with increased initial substrate concentrations (N_o) and decreased with incubation time, indicating that one first-order equation may not adequately describe long-term organic matter mineralization in these soils.

Carbon mineralization potentials (C_o) did not correspond with sludge application rate. The C_o/C_{total} values decreased with increased sludge application rate, suggesting that the sludge C that remained in soil after deposition had a larger stable pool than C in the control plots.

The C/N ratios of the mineralizable organic fraction decreased with increased sludge application rate, in-

dicating that the soil-sludge C may be more stable than the corresponding N fraction.

With the use of NLLS regression analysis and curve-splitting techniques, the mineralization of both C and N can be described as consisting of a labile short-term pool (first 11 weeks) and a slower decaying organic reservoir (between 11 and 87 weeks).

In all soil treatments, soil microbial biomass decreased to less than half of its original size after 20 weeks of incubation and stabilized after week 50.

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