Published May, 1989

Nitrogen Transformations in Soils Previously Amended with Sewage Sludge

Michael Boyle* and E. A. Paul

ABSTRACT

This short-term (10-d) incubation experiment established the rates of nitrogen (N) transformations occurring in sludge-amended and nonamended soil. Utilizing a nitrification block (C_2H_2) with $(^{15}NH_4)_2SO_4$, first-order rate constants were calculated for N immobilization, ammonification, nitrification, and denitrification. These rate constants were compared to values obtained after a long-term (87-wk) incubation performed on soils sampled from the same field plots. The short-term rates of ammonification were still higher than the controls 4 yr after the last sludge addition. Sludge applications over an 8-yr period (180 Mg ha^{-1} yr^{-1}) reduced soil nitrification potential compared to the controls when spiked with ^{15}N. Denitrification did not cause a significant loss of N during either a short- or long-term incubation period. The microbial biomass in the sludge-amended soil contained more N, which resulted in a microbial C/N ratio of approximately 4:1 vs. 5:1 for the controls. Initial (short-term) N immobilization rate constants were 0.43 for the sludge-amended and 0.35 for the nonamended soil.

SOIL MICROORGANISMS function as organic "microprocessors" of the terrestrial ecosystem by facilitating the nutrient flow and decomposition of organic residues. Currently, there is a need to improve the integration of data concerning these microbes and the soil N processes they control.

The hub of N transformation in soil is the NH_4^+ pool, for it lies at the crossroads of three major N processes: nitrification, ammonification and immobilization. Soil N mineralization rate, a measurement of gross ammonification minus immobilization, has traditionally been determined by the accumulation of inorganic N, mainly NO_3^-. However, acetylene (C_2H_2), which inhibits nitrifying bacteria such as *Nitrosomonas spp.*, can be employed to determine the ammonification rate by measuring the accumulation of ammonium. Acetylene also allows for the determination of N uptake by the microbial biomass in the absence of the competing nitrification reaction. With the use of ^{15}N in conjunction with the chloroform fumigation incubation method (CFIM), the flux of N into soil microbial biomass can be measured (Jenkinson and Powlson, 1976; Voroney and Paul, 1984). An estimate of the denitrification rate can also be obtained by measuring the increase in N_2O over a short-time period when C_2H_2 is used to inhibit nitrous oxide reductase (Smith et al., 1978).

The purpose of this work was to evaluate the effects of sludge on long-term N processes utilizing a short-term N transformation study. The objectives were to: (i) measure microbial-N and determine the short-term flux of N into the soil microbial biomass; (ii) estimate soil ammonification and nitrification rates; (iii) determine if N mineralization (net ammonification) constant (k) is indeed constant; and (iv) determine the significance of denitrification during aerobic incubations.

M. Boyle, Lab. of Microbial Ecology, Div. of Applied Sciences, Harvard Univ., Cambridge, MA 02138; and E.A. Paul, Dep. of Crop and Soil Sciences, Michigan State Univ., East Lansing, MI 48824. Contribution from the Dep. of Plant and Soil Biology, Univ. of California, Berkeley. Received 20 June 1988. *Corresponding author.

Published in Soil Sci. Soc. Am. J. 53:740–744 (1989).

MATERIALS AND METHODS

Field Study

The 2.4- by 3.0-m plots are located on the Oxford Tract at the Univ. of California, Berkeley. The Tierra loam soil had an original CEC of 20.1 cmol kg^{-1} and a pH of 5.4. A municipal sludge (Oakland) was incorporated into triplicate plots annually (180 Mg ha^{-1} yr^{-1}) for 8 yr with no addition in the subsequent 4 yr. A crop of barley was grown on the site each of the 12 yr of the study. The sludge was anaerobically digested for 20 d then vacuum-filtered. When applied to the field, the sludge was a wetcake slurry that contained 25% solids (Williams et al., 1984).

Short-Term Laboratory Incubation Study

Surface soils (0–15 cm) from two control (check) and two sludge-amended plots were collected 4.5 yr after the last sludge application. The field moist soils were sieved (<4 mm) and bulked into either check or sludge-treated samples. These soil samples (20-g oven-dry weight) were mixed with 20 g of Ottawa sand (0.59–0.42 mm) to aid filtration. Labeled $(^{15}NH_4)_2SO_4$ (70.5 atom % ^{15}N excess) solution was then applied with a syringe at a rate of 20.8 mg (^{14}N + ^{15}N)–N kg^{-1} soil to each soil/sand sample. Water was added to bring the soil-sand mixture up to −100 kPa water potential (60% water-holding capacity). The soil-sand mixtures were placed into 236-mL Mason jars fitted with a gas sampling septum.

Half of the jars were injected with C_2H_2 (30 cm^3) to inhibit both nitrification and nitrous oxide reductase. Measurements of N_2O were taken 1, 3, 5, and 24 h after injection of C_2H_2 (5 mol m^{-3}) on a Varian Model 3700 gas chromatograph (Varian, Palo Alto, CA) equipped with a ^{63}Ni electron capture detector (ECD) (Smith et al., 1978; Strauss, 1983).

Triplicated soil samples, with and without the nitrification block (15 cm^3 C_2H_2), were then incubated for an additional 2 or 9 d at 25 °C. The N pool sizes were measured at 0, 3 and 10 d in an attempt to frame the most important changes in N uptake and nitrification. At the end of the incubation, the soils were shaken for 30 min with 75 mL of 0.5 *M* KCl and extracted to determine inorganic-N content. The soils were rinsed with 25 mL of deionized water, in an attempt to remove excess salt, and were then vacuum-filtered back to −100 kPa.

Half of the samples were fumigated with $CHCl_3$ for 24 h and allowed to incubate at 25 °C for an additional 10 d to determine microbial biomass C from the evolved CO_2 according to Jenkinson and Powlson (1976). The CO_2 evolved from the nonfumigated soils was not subtracted from the fumigated samples because an appropriate control for biomass C has yet to be determined (Voroney and Paul, 1984). To determine biomass N, the soils were extracted 10 d after fumigation with 75 mL of 2.0 *M* KCl and measured for inorganic N (Voroney and Paul, 1984). Also at this time the microbial N extract was measured for ^{15}N content to determine the N immobilization rate.

Soil extracts were also performed on soils immediately after the addition of $(^{15}NH_4)_2SO_4$ to determine the recovery efficiency of the labeled N and the standing pool sizes of inorganic N. The efficiency of recovery of the added $^{15}NH_4^+$ was found to be 91.3% in the sludge-amended soil.

Total NH_4^+-N and NO_3^--N in the KCl extracts were determined by steam distillation followed by autotitration on a Fisher Model 381 (Fisher Scientific, Pittsburg, PA) using 0.0128 *M* H_2SO_4. The following sequence was followed to avoid cross contamination of the isotope ratio measurements during steam distillation:

1. 25 mL of 5% acetic acid was distilled then discarded;
2. 25 mL of 90% ethyl alcohol was distilled then discarded;
3. 80 mL of 0.5 *M* KCl extract was distilled with MgO;
4. 40 mL of distillate was collected in a glass beaker with 5 mL of 2% H_3BO_3;
5. step 1 and 2 were repeated;
6. Devarda's alloy was added to the 0.5 *M* KCl extract in step 3 distilled again and the 40 mL of distillate was collected in 5 mL of 2% H_3BO_3;
7. the 2.0 *M* KCl extracts were analyzed similarly, except that MgO and Devarda's alloy were added together and distilled only once to determine combined NH_4^+-N and NO_3^--N content;
8. the samples were acidified with 2 mL of 0.04 *M* H_2SO_4 and dried in a 60 °C oven that was flushed with acid-scrubbed air; and
9. the samples were transferred to test tubes with successive washings of methanol and water. The samples were again dried then sent to Los Alamos for ^{15}N mass spectrometric analysis (Hauck, 1982).

RESULTS AND DISCUSSION

Biomass Nitrogen

More N was found in the sludge-amended biomass than in the check biomass, reflecting the higher availability of inorganic N in the sludge-amended soil (Fig. 1). The C/N ratio of the microbial biomass was lower in the sludge-amended soils, averaging 3.9 vs. 4.8 for the checks. These biomass C/N ratios are lower than those reported by Anderson and Domsch (1980) but are consistent with the ratios of Voroney and Paul (1984). The low microbial C/N ratios suggest that the immobilized N conserved in the microbial biomass could allow for inorganic N to be released slowly over time as the microbial C/N ratio increases and the general microbial biomass decreases.

After a long-term incubation (initiated 87 wk earlier), the C/N ratio of the microbial biomass of the check soil rose to 6.2 and in sludge-amended soil to 5.7 (Boyle, 1986). A decline in sludge microbial biomass C from 409 to 260 mg kg^{-1} was also observed in the field during this time (Boyle and Paul, 1989).

The short-term immobilization of $^{15}NH_4^+$ and $^{14}NH_4^+$ into the microbial biomass of both soils is depicted in Fig. 2. The immobilization of N was greater in the check soil in the presence of C_2H_2 than when nitrification was allowed to proceed, which is consistent with results of Nishio et al. (1985). Nitrification seemed to be competing with N immobilization in the check soil, and in the sludge-amended soil after 3 d.

Inorganic Nitrogen

The distribution of soil N among the biomass and inorganic pools is depicted in Fig. 3 and 4. In both soil treatments, the size of the NH_4^+ pool increased from day 3 to day 10 with the nitrification block. The increase can be attributed to the mineralization of or-

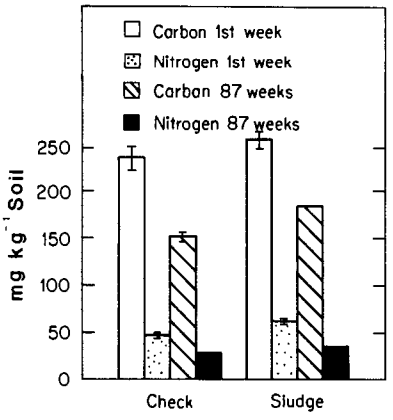

Fig. 1. The microbial biomass C and N after the short-term incubation as compared to a long-term incubation (Boyle and Paul, 1989). (Error bars represent the standard error of the mean).

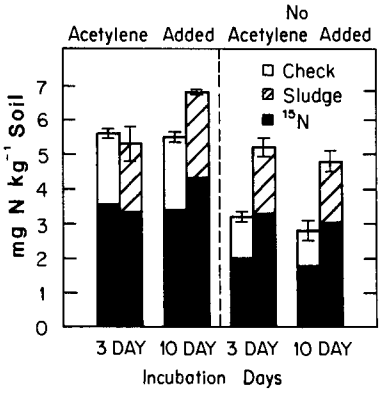

Fig. 2. The short-term uptake of N ($^{14}NH_4^+$ + $^{15}NH_4^+$) into the soil microbial biomass.

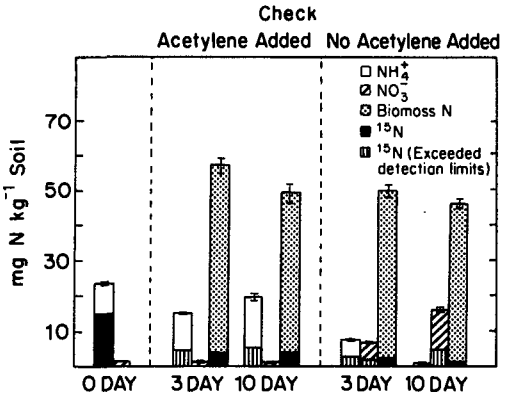

Fig. 3. The N pool sizes of the check soil with and without acetylene at 3 and 10 d.

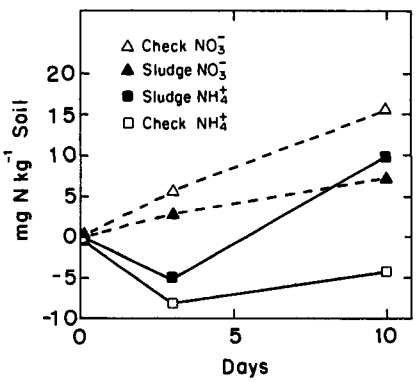

Fig. 5. The short-term production of NH_4^+ (with C_2H_2) and NO_3^- (without C_2H_2).

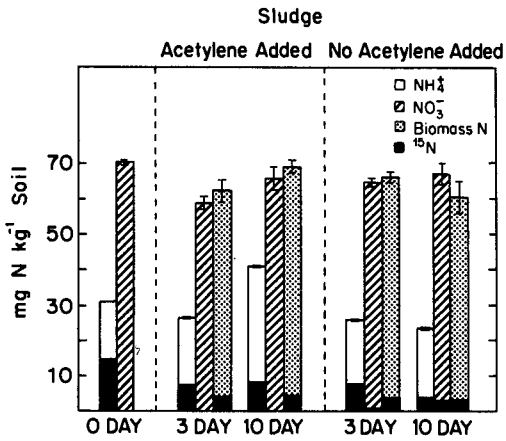

Fig. 4. The N pool sizes of the sludge-amended soil with and without acetylene at 3 and 10 d.

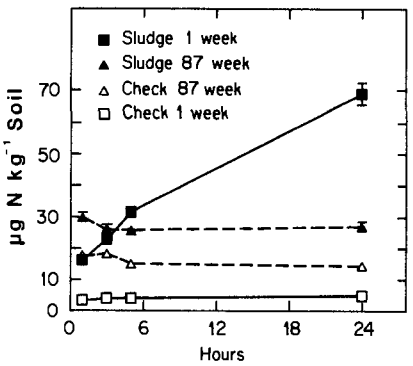

Fig. 6. The production of N_2O–N (with C_2H_2) at day 1 and after 87 wk of incubation.

ganic N to NH_4^+. Without the C_2H_2, the NH_4^+ pool size decreased significantly in both soils between 0 and 10 d.

The NO_3^- analysis of the 0.5 *M* KCl solution indicates that the C_2H_2 block was more effective for the check soil, in which there was little change in NO_3^- present after 10 d, than for the sludge-treated soil in which the NO_3^- concentration was as high with the C_2H_2 block as without the block (Fig. 3 and 4). The sludge-treated soil had initially (day 0) higher concentration of NO_3^- than the check soil which may have obscured the response of the block. However, the effectiveness of the nitrification block was confirmed by the paucity of $^{15}NO_3^-$ found in the presence of C_2H_2. Reliable $^{15}NO_3^-$ results could not be obtained from the check soil, which contained a small unlabeled NO_3^- pool, because the high ^{15}N enrichment of this pool approached or exceeded the detection limit of the mass spectrometer (approx. 30%).

The term "mineralization" includes both ammonification and nitrification processes, however in most soils the limiting step is the conversion of organic N to NH_4^+. The 10-d increase in net production by both these processes is depicted in Fig. 5 (accumulation − initial concentration). The NH_4^+ production in soils treated with C_2H_2 is represented by the solid lines. Both soils displayed a net negative production of NH_4^+ at day 3 which demonstrated the initial dominant effect of immobilization.

The dashed lines in Fig. 5 represent "potential" nitrification because both soils were spiked with 20.8 mg NH_4^+–N kg^{-1} soil. The reduction in nitrification potential in the sludge-amended soil could be due to the lower pH (pH 4.9 vs. 5.6 for the check soil), greater metal content (422 mg Zn kg^{-1} soil vs. 114 mg kg^{-1} for the check) or a combination of the two.

It has been suggested that denitrification is a major cause for the loss of N in some aerobic incubations (Ryan et al., 1973). Lindemann and Cardenas (1984) reported up to 65% of mineralized-N lost in sludge-treated soils through denitrification. These authors attributed the nonlinear increase of NO_3^- production with increased sludge additions to be due in part to denitrification. The data presented here (Fig. 6) does not support this route as a significant loss of N during aerobic incubation in these soils. Denitrification as determined by N_2O accumulation with C_2H_2 block was essentially zero for the check soil, while the sludge-amended soil produced 68 μg N kg^{-1} in the first 24 h of the short-term incubation (Fig. 6). Because denitrification was measured by the amount of N_2O produced from the NO_3^- soil pool (each point represents an average of eight measurements), the low initial NO_3^- pool size of the short-term check soil (0.5 mg N kg^{-1}) could have caused the low rates. However, for the long-term 87-wk incubation, both the check and sludge-soil were naturally enriched with higher levels of NO_3^- (approximately 30 and 70 mg N kg^{-1}, respectively), but neither soil produced an increase in N_2O levels in the 24-h period. These results suggest that the low production of N_2O was due to factors other

Table 1. The comparison of first-order rate constants for four soil N transformations.

	Short-term (10 d)		Long-term (87 wk)	
	Check	Sludge	Check	Sludge
	(week^{-1})			
Immobilization	0.64	0.42	0.26	0.24
without C_2H_2	0.35	0.43	ND‡	ND
Ammonification				
net	†	0.06	0.01	0.02
gross	0.03	0.10	ND	ND
Nitrification				
potential	0.69	0.18	ND	ND
Denitrification	0.00	0.04	0.00	0.00

† = between 0 and 10 d the net ammonification rate remain less than zero, but between 3 and 10 d the rate was 0.19 (0.12 for the sludge soil).
‡ ND = not determined.

than low NO_3^- content, possibly the scarcity of anaerobic sites or available C.

Nitrogen Transformation Rates

Because first-order kinetics have been successfully used to characterize soil N processes (Stanford and Smith, 1972; Myrold and Tiedje, 1986), first-order rate constants (k) for N transformations during the short incubation period (10-d) and the constants obtained from the long-term (87 wk) incubation sampled a year and a half earlier from the same field plots (Boyle and Paul, 1989) were calculated and are presented in Table 1.

Nitrogen immobilization rates were calculated from 0- to 3-d incorporation of $^{15}NH_4^+$ into the microbial biomass (Voroney and Paul, 1984). The k value was found to be slightly greater for the check soil than the sludge-soil if nitrification was blocked by C_2H_2 (Table 1). The lower k value for the check soil in the absence of C_2H_2 corresponds to the lower N content in the microbial biomass (Fig. 1). After 87 wk, the value of k decreased to about 0.25 wk^{-1} for both soils (Table 1).

The check soil exhibited a lower NH_4^+ production than the sludge soil (Fig. 5), in fact after 10 d this soil did not attain its initial (0-d) NH_4^+ pool size. For both check and sludge-amended soil, the net and gross ammonification rate constants were determined between day 0 and day 10. The gross ammonification rate was determined by adding the immobilization uptake of N to the net production of NH_4^+. To determine short-term ammonification rate, the initial pool size has to be indirectly measured because it represents the portion of the organic N that is potentially mineralizable. An estimate of this short-term potentially mineralizable N pool size was calculated by fractionating the long-term (87-wk) incubation data into two pools (Boyle and Paul, 1989). The short-term (10-d) net ammonification rate constants were similar to the k values obtained from curve splitting the long-term data into an 11-wk pool (Boyle and Paul, 1989). These short-term ammonification k values are also similar to the mineralization values reported by Myrold and Tiedje (1986). After 87-wk, the ammonification constants were significantly lower (Table 1) indicating that organic N mineralization should be characterized as having more than one first-order rate constant.

The nitrification potential rate constant was greater for the check soil than for the sludge-amended soil (Table 1). The check rate of nitrification was determined by 3-d (^{14}N + ^{15}N) NO_3^- accumulation while the sludge nitrification was calculated by the less variable $^{15}NO_3^-$ data.

The term "production rate" represents the initial pool size minus the change in pool size over the 3-d incubation due to a particular N transformation [production rate = $N_{(initial)}$ $(1 - e^{-kt})$]. For N immobilization, the production rate indicates the amount of decay of the NH_4^+ reservoir due to microbial accumulation. Microbial immobilization of NH_4^+ was higher in the check soil than the sludge-amended soil only if nitrification was blocked. The production rate of NH_4^+ from organic N (with C_2H_2) was greater in the sludge-amended than the check soil which did not display a positive rate between 0 and 10 d. The potential for the production of NO_3^- from the spiked NH_4^+ pool was lower in the sludge-treated soil than the check. Although it was noted that overall production of NO_3^- is greater in these sludge-amended soils than the checks (Boyle and Paul, 1989), this is assumed to be due to the greater production of NH_4^+ from a larger organic N fraction, and not due to a greater rate in the nonlimiting nitrification step. The rate of denitrification and the production of N_2O production was initially greater in the sludge-amended soil, but this would represent less than 0.5 mg N kg^{-1} lost from the soil per week, which is minimal compared to NO_3^- production.

SUMMARY

Previous applications of sludge (4 yr earlier) increased the short-term (10-d) soil ammonification rate.

The rate constant for ammonification (N mineralization) decreased between 10 d and 87 wk of incubation, indicating a need for more than one mineralization constant.

Eight years of sludge application (a total of 1440 Mg sludge ha^{-1}) decreased soil nitrification potential.

Denitrification was not found to be a significant loss of inorganic N from these soils during either a short or long-term incubation.

The initial short-term N immobilization rate constant was slightly higher (without C_2H_2) in the sludge-amended than the check soil.

The sludge-amended soil had a lower biomass C/N ratio than the check soil. Excess storage of N in microbial cells in the sludge-amended soil could provide a means of retaining N in the elastic labile fraction over an extended period of time (at least 4 yr).

ACKNOWLEDGMENTS

Facilities and funds were provided by the Western Regional Project W-170 grant and the Dep. of Plant and Soil Biology, Univ. of California, Berkeley. The authors also thank Dr. M.K. Firestone for her comments.

REFERENCES

Anderson, J.P.E., and K.H. Domsch. 1980. Quantities of plant nutrients in the microbial biomass of selected soils. Soil Sci. 130:21–216.

Boyle, M. 1986. Carbon and nitrogen transformations in soil previously amended with sewage sludge. Ph.D. diss. Univ. of California, Berkeley (Diss. Abstr. 87-17090).

Boyle, M., and E.A. Paul. 1989. Carbon and nitrogen mineralization kinetics in soil previously amended with sewage sludge. Soil Sci. Soc. Am. J. 53:99–103.

Hauck, R.D. 1982. Nitrogen—isotope-ratio analysis. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agronomy 9:735–776.

Jenkinson, D.S., and D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8:209–213.

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.

Myrold, D.D., and J.M. Tiedje. 1986. Simultaneous estimation of several nitrogen cycle rates using ^{15}N: Theory and application. Soil Biol. Biochem. 18:559–568.

Nishio, T., T. Kanamori, and T. Fujimoto. 1985. Nitrogen transformations in an aerobic soil as determined by a $^{15}NH_4^+$ dilution technique. Soil Biol. Biochem. 17:149–154.

Ryan, J.A., D.R. Keeney, and L.M. Walsh. 1973. Nitrogen transformations and availability of an anaerobically digested sewage sludge in soil. J. Environ. Qual. 4:489–492.

Smith, M.S., M.K. Firestone, and J.M. Tiedje. 1978. The acetylene inhibition method for short-term measurement of soil denitrification and its evaluation using ^{13}N. Soil Sci. Soc. Am. J. 42:611–615.

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

Strauss, R.B. 1983. Denitrification in coniferous forest soil. M.S. thesis, Univ. of California, Berkeley.

Voroney, P. and E.A. Paul. 1984. Determination of k_c and k_n *in situ* for calibration of chloroform fumigation-incubation method. Soil Biol. Biochem. 16:9–14.

Williams, D.E., J. Vlamis, A.H. Pukite, and J.E. Corey. 1984. Metal movement in sludge-treated soils after six years of sludge additions: I. Cadmium, copper, lead and zinc. Soil Sci. 137:351–359.