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Effects of Cadmium and Simulated Acid Rain on Ammonification and Nitrification in Soil

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Abstract. A perfusion technique was used to study the effects of 1) simulated acid rain on the mineralization and nitrification of glycine (NH_2-N) or ammonium sulfate (NH_4-N) by adjusting the pH of the perfusates to 3.0, 2.5, or 2.0 with H_2SO_4; and 2) 0, 50, 100, 500, and 1,000 μg Cd/g dry weight of soil on the mineralization and nitrification of NH_2-N, both in soil adjusted with H_2SO_4 to pH 3.0 and in soil not exposed to acidity.

Ammonification was relatively insensitive to both Cd and acidity, occurring even in soil exposed to pH 2.0 or 1,000 ppm Cd. Nitrification was more sensitive, being retarded in NH_2-N-supplemented soils exposed to pH 2.5 and inhibited in soil exposed to pH 2.0. In NH_4-N-supplemented soils, nitrification was retarded at pH 3.0 and inhibited at pH 2.5. This retardation and inhibition occurred despite the increase in the pH of the perfusates to a non-inhibitory level. Nitrification was retarded at 500 ppm Cd and to a greater extent at 1,000 ppm Cd, where nitrite accumulated. The combined effect of exposure to Cd and H_2SO_4 at pH 3.0 was additive rather than synergistic, and even with 1,000 ppm Cd at pH 3.0 some nitrification eventually occurred.

Despite wide documentation concerning the importance of acid rain (Hutchinson and Havas 1980) and cadmium (Cd) (Wong *et al.* 1980) on ecosystems, there is little information regarding the effects of these pollutants, either alone or in combination, on the ecology, activity, and population dynamics of microorganisms in soils (Babich and Stotzky 1978a, 1978b, 1978c, 1980; Babich *et al.* 1980). The disruption by these pollutants of biogeochemical cycling, particularly nitrogen transformations, in soils could have serious consequences for ecosystem stability and productivity.

Application of sewage sludge from industrial areas to agricultural land may enrich the soil Cd content (Dudas and Pawluk 1975; Soon *et al.* 1980). Cd is usually found in association with zinc (Zn) (Wong *et al.* 1980). Nitrification was significantly reduced in two out of three soil types, and totally inhibited in all three, by applications of Zn as the sulfate salt, at concentrations of 100 and 1,000 μg Zn g^{-1} soil respectively (Wilson 1977); the pH of these soils ranged from 5.1 to 6.2. However, application of 1,000 ppm Zn to soil as the oxide had no significant effect on nitrogen mineralization or nitrification at pH 6.0, slightly reduced both processes at pH 7.0, and caused a greater reduction at pH 7.7. (Bhuiya and Cornfield 1974). Soil perfusion studies with "garden soil" demonstrated that the nitrification process was reduced by 10 mM $CdSO_4$ and almost completely inhibited by 40 mM $CdSO_4$ (Lees and Quastel 1946). The addition of 500 or 1,000 μg Cd/g soil temporarily depressed ammonification and nitrification rates, with complete suppression of these activities occurring only at 10,000 ppm Cd (Morrissey *et al.* 1974). No investigations have been conducted, however, to determine whether the toxicity of Cd is influenced by the simultaneous presence of acid rain, a situation which may occur frequently under field conditions, as both pollutants are released by various smelting processes (Buchauer 1973; Hutchinson and Havas 1980; Bewley and Campbell 1980).

The heterotrophic process of ammonification appears to be less sensitive to pH than nitrification (Dancer *et al.* 1973; Alexander 1980a). There is controversy as to the pH at which autotrophic ni-

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trification is completely inhibited (Alexander 1980b), although autotrophic bacteria were isolated from acid tea soils ranging in pH from 4.0 to 4.5 (Walker and Wickramasinghe 1979), and in perfusion experiments, active nitrification took place in four soils at pH levels approximating 4.0 (Weber and Gainey 1962). Dry deposition of SO_2 may also reduce ammonification and nitrification (Bozian and Stotzky 1976; Labeda and Alexander 1978) in soil. However, the resultant acidity of soils fumigated with SO_2 may not be the major cause of reduced nitrification (Bozian and Stotzky 1976). Nitrification of added ammonium was reduced following continuous exposure of soil to simulated acid rain of pH 4.1 to 3.2, and at pH 3.2, the process may have been the result of heterotrophic activity (Strayer *et al.* 1981), which may predominate in acidified soils (Ishaque and Cornfield 1972).

In the present investigation, a continuous perfusion technique was employed to study the effects of simulated acid rain and of Cd, alone and in combination, on transformations of glycine-N or ammonium-N in soil. By this method, it was possible to investigate the effect of a high "shock" of acidity, followed by an increase in pH as a result of both the buffering capacity of the soil and the conversion of glycine to ammonium, and of even higher concentrations of H_2SO_4 where subsequent increases in pH were less pronounced.

Materials and Methods

Effects of Simulated Acid Rain on Ammonification and Nitrification

Soil collected at the Kitchawan Research Laboratory of the Brooklyn Botanic Garden at Ossining, NY, was stored at room temperature, sieved, and aggregates larger than 2 mm and smaller than 1 mm were discarded. Physicochemical properties of the soil are given in Table 1. Further details are presented elsewhere (Babich and Stotzky 1977). A modification of the perfusion apparatus described by Audus (1946) was employed (Macura and Stotzky 1980; Kunc and Stotzky 1980) to study the rates of deamination of glycine, the subsequent nitrification of the NH_4-N, and changes in pH. Forty g of air-dried soil was placed in each of 24 perfusion columns (180 × 20 mm, I.D.) and pre-perfused with 200 ml of a glycine (NH_2-N) solution containing 35 μg NH_2-N/ml and a suspension of active "garden soil", in order to enrich for nitrifiers. Continuous perfusion was accomplished by means of three suction pumps ("Neptune" Dynapumps, Model #2). The perfusion rate was maintained equal in all 24 units by the insertion of a matched hypodermic needle (25 gauge) in the suction line of each unit. The perfusion columns were wrapped with aluminum foil to keep out light, and the entire apparatus was maintained at room temperature (25 ± 4°C). After six days, 10 ml of each perfusate was discarded, fresh nitrogen (28 mg, equivalent to 140 μg N/ml) was added as glycine to one-half of the replicates and as $(NH_4)_2SO_4$ to the other half, and the volume of the perfusate was readjusted to 200 ml with water. A few drops of H_2SO_4 (<20% v/v) were added to lower the pH of the perfusates from 6.0 to 3.0, 2.5, or 2.0. Each treatment, plus a control to which no H_2SO_4 was added, was prepared in triplicate for both the NH_2-N and NH_4-N sources. The perfusates were continuously perfused through the soils, and 5 ml samples were removed after 0, 1, 2, 4, 6, 8, 10, 13, 16, 19, 23, 28, 35, and 50 days. After the pH of each sample was measured (Radiometer pH meter), the various forms of nitrogen in the perfusates were determined colorimetrically (Macura and Stotzky 1980; Kunc and Stotzky 1980): one-ml aliquots were analyzed for NH_4-N by nesslerization, NO_2-N by a modification of the Griess-Ilosvay method, and NO_3-N by the 2,4-phenoldisulfonic acid method (Bremner 1965) and, in the case of perfusates supplemented with glycine, NH_2-N by the ninhydrin method (Rosen 1957) (performed after the removal of NH_4-N by heating the sample in an equal volume of 0.1 N HCl to half volume and then repeating the process with 0.1 N NaOH). The total amount of nitrogen (inorganic and NH_2-N, where applicable) per ml of perfusate was calculated by summation, and the relative concentrations of the various forms of nitrogen present were expressed as a percentage of this total. This was a more convenient expression than the actual concentrations, which varied due to loss of perfusates by evaporation and retention in the soil column. At the end of the experimental period, about 20 g soil from each treatment was used for the determination of soil pH on a 1:1 water:soil slurry.

Table 1. Some chemical and physical characteristics of Kitchawan soil

pH	4.8
Organic Matter	5.8%
Cation Exchange Capacity	8.2 meq 100 g^{-1}
Sand	56.8%
Silt	33.8%
Clay	9.4%
N	0.13%
P	27.6 $\mu g\ g^{-1}$
K	61.9 $\mu g\ g^{-1}$
Ca	360.0 $\mu g\ g^{-1}$
Mg	63.1 $\mu g\ g^{-1}$
Na	3.9 $\mu g\ g^{-1}$
Field capacity (⅓ bar water tension)	20.7%

Effects of Cd and Simulated Acid Rain on Ammonification and Nitrification

The experimental design was as described above, with the following modifications: 35 g soil were placed in each perfusion column, and before pre-perfusion of the soils with 35 μg NH_2-N/ml, the perfusates were seeded with a few ml of a suspension derived from the control (non-acidic) soil used in the previous acid rain experiment, in order to enhance the enrichment with ammonifiers and nitrifiers. After pre-perfusion for five days, 28 mg NH_2-N and Cd (as 3 $CdSO_4 \cdot 8H_2O$) was added to the perfusates to yield concentrations of 0, 10, 100, 500, or 1,000 ppm Cd, based on the dry weight of soil. Half of the perfusates were unadjusted with acid (initial pH ranged from 5.4 to 6.0, depending on the Cd concentration), and the other half was adjusted

with a few drops of H_2SO_4 to pH 3.0. Each treatment was prepared in duplicate. Samples were removed on days 0, 1, 2, 4, 10, 16, 24, and 35, and analyses were conducted as described above.

Results and Discussion

Effects of Simulated Acid Rain on Ammonification and Nitrification

The pH of the perfusates in the control soils supplemented with NH_2-N increased from 6.0 to almost 8.0 after the first day of perfusion (Figures 1 and 2). This increase in pH was the result of rapid conversion of NH_2-N to NH_4-N. The subsequent reductions in pH observed in systems supplemented with either NH_2-N or NH_4-N reflected the conversion of NH_4-N to nitrite (NO_2-N) and nitrate (NO_3-N) and the production of associated protons. Most NH_2-N disappeared after the first two days in the perfusates of the control soils, although some NH_2-N was retained in the soil columns and accounted for the fluctuations in levels of NH_2-N in the perfusates at later samplings. NO_2-N was detected in significant amounts only in the perfusates of the NH_2-N supplemented control soils.

The pH of the NH_2-N-supplemented perfusates treated with H_2SO_4 to give an initial pH of 3.0 increased to 7.5 after one day, whereas that of the NH_4-N-supplemented perfusates increased to 5.2. As additional NH_4-N was not produced in the latter system, the increase in pH probably reflected the buffering capacity of the soil. The initial rate of disappearance of NH_2-N was not very much different from that in the control, although NH_2-N was detected longer than in the control. There was less accumulation of NO_2-N compared to the control, and the accumulation of NO_3-N was less, *e.g.*, on day 35, NO_3-N accounted for 48 ± 3.4% of the total-N compared to 60 ± 5.5% in the control, even though the pH of both perfusates was now the same (*i.e.*, 5.2). By day 50, only 59 ± 7.8% of the total-N in the perfusates initially lowered to pH 3.0 was present as NO_3-N as compared to 83 ± 13.8% in the control perfusates. This reduction in nitrification was reflected in the higher levels of NH_4-N that remained in the acidified perfusates (Figure 1).

In the NH_4-N-supplemented treatments, where the "neutralizing effect" of the conversion of NH_2-N to NH_4-N did not occur, the differences between the control and acidified perfusates were more pronounced (Figure 2). There was a longer delay in nitrification, and on day 35, NO_3-N accounted for only 22 ± 5.9% of the total-N in the perfusates originally adjusted to pH 3.0, compared

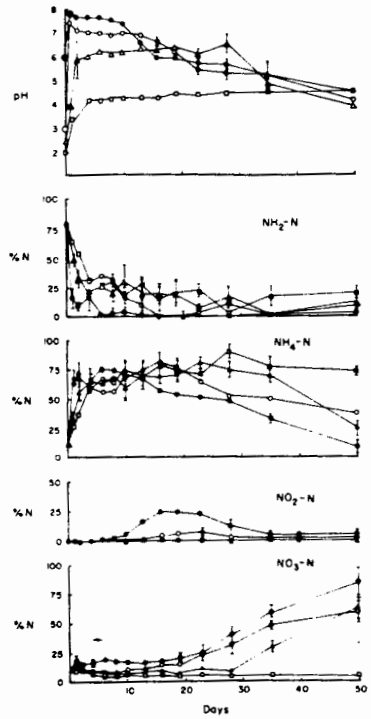

Fig. 1. Changes in pH, and in the relative proportions of NH_2-N, NH_4-N, NO_2-N, and NO_3-N, each expressed as a mean percentage ± SEM of the total-N (inorganic- and NH_2-N), in perfusates supplemented with glycine (NH_2-N) (●) and sufficient H_2SO_4 to lower the pH to 3.0 (○), 2.5 (△), and 2.0 (□)

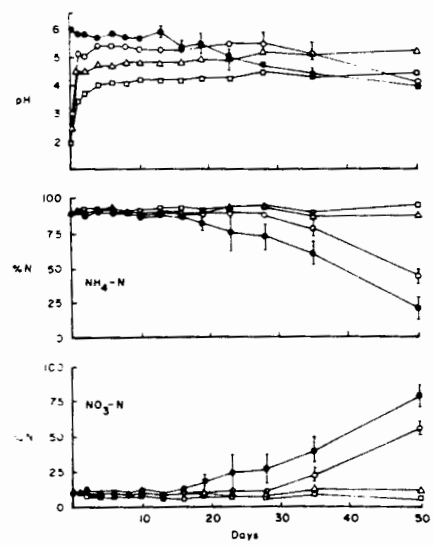

Fig. 2. Changes in pH and in the relative proportions of NH_2-N, NH_4-N, and NO_3-N, each expressed as a mean percentage ± SEM of the total inorganic-N, in perfusates supplemented with $(NH_4)_2SO_4$ (●) and sufficient H_2SO_4 to lower the pH to 3.0 (○), 2.5 (△), and 2.0 (□). NO_2-N accounted for less than 0.25% of the total inorganic-N in all treatments

to 40 ± 8.6% in the control perfusates. On day 50, the respective percentages of the total-N present as NO_3-N were 56 ± 4.5% and 79 ± 8.1%. Similar differences occurred in the levels of NH_4-N that remained in the perfusates.

These results have implications *in situ*, as they suggest that the effects of an acid rain of pH 3.0 on nitrification may be manifest even after subsequent buffering by the recipient environment. Likens and Bormann (1974) have reported frequent individual rainstorms with pH levels between 3 and 4 in the northeastern U.S.A.

When the pH of the perfusates was adjusted initially to pH 2.5, the rate of decomposition of NH_2-N was slower and uneven, and the "neutralizing effect" was reduced as a result of the slower production of NH_4-N. Nitrification, however, was more sensitive than ammonification, as in contrast to both the control and pH 3.0 treatments there was no evidence of its initiation even by day 28 (Figure 1), although the pH of the perfusates was now greater than 6.0, *i.e.*, well above the inhibitory level. Nitrification eventually occurred, however, and although no NO_2-N was detected, the final proportion of NO_3-N in the perfusates on day 50 did not differ significantly from that in the perfusates adjusted initially to pH 3.0, a marked contrast to the perfusates adjusted to pH 2.5 and supplemented with NH_4-N (Figure 2). Despite the increase in the pH of the perfusate to 4.5 after day 1 and to 5.2 by day 50, presumably as a result of the buffering capacity of the soil, there was a total inhibition of nitrification. Although rainstorms of pH 2.5 are probably rare, they have been reported (Likens and Bormann 1974). Consequently, such conditions may have serious consequences for nitrification *in situ*, where the addition of nitrogen in the form of NH_4-N is not uncommon.

Even when the pH of the perfusates was lowered to 2.0, about 80% of the added NH_2-N was eventually converted to NH_4-N, although the process was uneven and slow. As ammonification proceeded, the pH of the perfusates increased to a maximum of 4.5 after 50 days, but nitrification was completely inhibited (Figure 1). The "acid shock" was, therefore, sufficient to eliminate effectively all of the nitrifiers, as they were unable to proliferate even when the low pH conditions were ameliorated. A similar phenomenon occurred in glucose-supplemented soils originally acidified with H_2SO_4 to pH 2.0 or less, in that no CO_2 was evolved, even after the pH of the soils was subsequently raised to non-inhibitory levels of pH 4.1 to 4.3 (Bewley and Stotzky 1983). Inhibition of nitrification also occurred in soils supplemented with NH_4-N when the pH of the perfusates was initially adjusted to 2.0. When the initial pH of the NH_2-N-supplemented perfusates was adjusted to 2.5 or 2.0, there was no accumulation of NO_2-N. Although NO_2-N seldom accumulates in acid soils (Dancer *et al.* 1973), there was no accumulation of NO_2-N in the control perfusates supplemented with NH_4-N. The increase in populations of *Nitrobacter* probably occurred very rapidly in these soils with the initiation of the conversion of NH_4-N to NO_2-N.

The final pH of the soils (the initial pH was 4.8) was the same as that of the perfusates, except for the treatment supplemented with NH_4-N and adjusted to pH 2.5. Following the initial increase in pH, as a probable result of the buffering capacity of the soil, the pH of these perfusates did not subsequently decrease, as nitrification was inhibited, and the final pH of the perfusates was 5.2, whereas that of the soils was 4.6. When the perfusates were initially adjusted to pH 2.0, both the perfusates and soils had a final pH of 4.4 even though no nitrification had occurred, indicating that the buffering capacity of the soil was insufficient to counter this level of acidity.

Effects of Cd on Ammonification and Nitrification; Influence of Simulated Acid Rain

The results indicated that Cd had little inhibitory effect on ammonification, even at 1,000 ppm, and there was no synergistic interaction between H_2SO_4 and Cd.

On day 1, the percentages of total-N present as NH_4-N in perfusates treated with 0, 50, 100, 500, and 1,000 ppm Cd and no H_2SO_4 were 60 ± 1.0%, 62 ± 0.3%, 49 ± 13.1%, 39 ± 2.0%, and 38 ± 3.2%, respectively, and for perfusates whose pH was lowered to pH 3.0 with H_2SO_4, the corresponding values were 50 ± 8.5%, 38 ± 0.2%, 53 ± 6.9%, 60 ± 12.8%, and 63 ± 11.2%. The actual concentrations of NH_4-N present in the perfusates (as μg/ml), however, were 57 ± 3.5, 61 ± 2.2, 61 ± 2.4, 69 ± 0.2, and 69 ± 3.4, respectively, for the soils untreated with H_2SO_4 and 84 ± 3.8, 76 ± 2.4, 86 ± 0.7, 95 ± 0.6, and 103 ± 2.4, respectively, for the soils treated with H_2SO_4. Therefore, although the actual percentage of the total-N present in the perfusate as NH_4-N decreased with increasing Cd concentration (except where the pH was lowered to 3.0), the actual concentrations of NH_4-N increased in the perfusates both treated and untreated with H_2SO_4. The higher concentrations of NH_4-N in perfusates that were treated with the higher concentrations of Cd were probably the result of displacement of NH_4^+ ions from exchange sites in the soils by Cd^{2+}. By day 35 (Figure 3), a significant

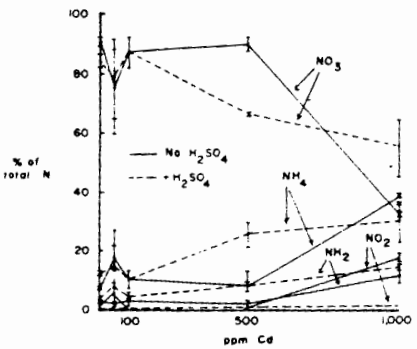

Fig. 3. Effects of Cd on the relative proportions of NH_2-N, NH_4-N, NO_2-N, and NO_3-N, expressed as a mean percentage ± SEM of the total-N (inorganic- and NH_2-N) in the perfusates, 35 days after supplementing the perfusates with glycine (NH_2-N) and with (– – –) or without (——) sufficient H_2SO_4 to lower the pH to 3

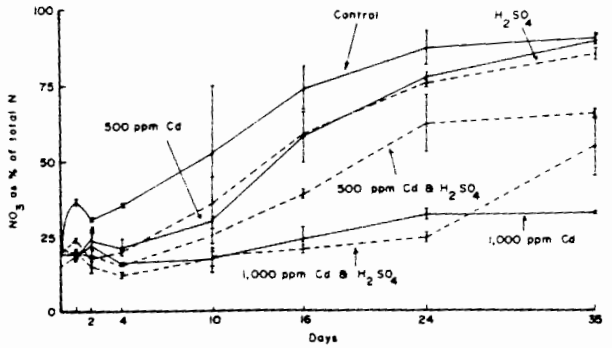

Fig. 4. Effects of Cd on nitrification, expressed as the mean percentage ± SEM of NO_3-N of the total-N (inorganic and NH_2-N) present in perfusates supplemented with glycine (NH_2-N) and with (– – –) or without (——) sufficient H_2SO_4 to lower the pH to 3.0

inhibitory effect on the extent of ammonification was apparent only at a concentration of 1,000 ppm Cd. In perfusates not treated with H_2SO_4, NH_2-N accounted for 3 ± 0.4% of the total-N in the control perfusates and for 12 ± 2.0% in perfusates treated with 1,000 ppm Cd. The corresponding values were 3 ± 0.9% and 15 ± 1.6% in the acidified perfusates.

Nitrification in the control systems commenced earlier than in the previous experiment with only simulated acid rain, probably as the result of the inoculation with active nitrifiers. In the perfusates not treated with H_2SO_4 nor Cd, 35 ± 0.2% of the total-N present in the perfusates on day 4 was in the form of NO_3-N as compared to 27 ± 0.2%, 25 ± 4.1%, 22 ± 2.7%, and 16 ± 0.8% in perfusates treated with 50, 100, 500, and 1,000 ppm Cd, respectively. In the perfusates treated with H_2SO_4, 20 ± 0.2% NO_3-N was present in the perfusates not treated with Cd and 16 ± 2.5%, 19 ± 0.8%, 16 ± 0.3%, and 12 ± 1.1% NO_3-N was present in the perfusates treated with 50, 100, 500, and 1,000 ppm Cd, respectively. These results and those of samplings on subsequent days indicated that although both Cd and reduction in pH to 3.0 with H_2SO_4 retarded the initiation of nitrification, there was no synergistic interaction between the two pollutants.

Concentrations of 50 and 100 ppm Cd had little effect on total nitrification, both in perfusates treated or not treated with H_2SO_4. In perfusates not treated with H_2SO_4, there was a slight accumulation of NO_2-N up to day 10 in most treatments, although mean levels did not exceed 10% of the total-N at any time, except with 1,000 ppm Cd. In one replicate of the perfusates treated only with 50 ppm Cd, there was an accumulation of NO_2-N (maximum of 20% by day 10 and still 12% by day 35), the cause of which is not known. As in the previous experiments, there was little accumulation of NO_2-N in perfusates treated with H_2SO_4, and mean levels did not exceed 3%.

Exposure to both acidity and 500 ppm Cd reduced the extent of nitrification, and the combined effect appeared to be additive rather than synergistic, except on day 35 (Figures 3 and 4) when the percentage of total-N present as NO_3-N was 91 ± 1.2% in the control perfusates, 85 ± 1.7% in the perfusates treated with H_2SO_4, 90 ± 2.1% in the perfusates treated with 500 ppm Cd, but only 66 ± 0.5% in the perfusates treated with both H_2SO_4 and 500 ppm Cd. Why this apparent synergism occurred only at 500 ppm Cd and pH 3.0 is not known, although it is possible that nitrification was autotrophic in systems treated with either 500 ppm Cd or H_2SO_4 but under the simultaneous stress of both pollutants, it was heterotrophic. At 1,000 ppm Cd, however, there was no indication of synergism (Figure 3), and at day 35, a greater proportion of the total-N was present as NO_3-N in systems treated with both Cd and H_2SO_4 (55 ± 9.0%) than in systems treated only with Cd (32 ± 0.4%) (Figures 3 and 4). In the latter but not the former systems, there was a significant accumulation of NO_2-N: 26 ± 0.5% NO_2-N on day 24 (compared to 1% or less in all other treatments, except 50 ppm Cd) and still 18 ± 1.3% on day 35. This suggested that *Nitrobacter* sp., which are mainly responsible for the conversion of NO_2-N to NO_3-N, were more sensitive to the effects of Cd than were *Nitrosomonas* sp., which are mainly responsible for the conversion of NH_4-N to NO_2-N. The effects of acidity on nitrification in the presence of 1,000 ppm Cd, therefore, appeared to be antagonistic, in contrast to 500 ppm Cd. Again, it is possible that the nitrification that did occur in the presence of both 1,000 ppm Cd and H_2SO_4 was heterotrophic, although it is not clear why these or-

ganisms were not able to proliferate when the perfusates were treated only with Cd.

The pH of the perfusates in all treatments reflected the nitrogen transformations which were occurring. After one day, the pH was above 7 in all treatments, and then it subsequently fell as HNO_3 was produced. The mean pH of the perfusates on day 35 ranged from 3.8 to 4.2, and final pH levels of the soils were between 4.0 and 4.1. The one exception was in the system exposed to 1,000 ppm Cd in the absence of H_2SO_4, in which little nitrification had occurred and the final pH of the perfusates was 5.2 and that of the soils was 5.7.

Conclusions

Concentrations of 500 and 1,000 ppm Cd are usually detected only in the immediate vicinity of smelting complexes (Buchauer 1973), although there are reports of much higher levels of contamination in certain areas (Hazen and Kneip 1980). For this reason and as there was no convincing evidence of synergism between Cd and acid rain, it seems that acid rain pollution is of greater concern than Cd pollution to transformations of nitrogen in soil. Heterotrophic ammonification was relatively insensitive to either or both acidity and Cd, confirming other results (Dancer *et al.* 1973; Morrissey *et al.* 1974; Alexander 1980a; Bewley and Stotzky 1983). Conversely, nitrification was considerably more sensitive, in agreement with the results of Bozian and Stotzky (1976) and Strayer *et al.* (1981). Although the pH of the acidified perfusates subsequently increased to non-inhibitory levels, nitrification was nevertheless either retarded or inhibited completely by high concentrations of H_2SO_4, suggesting that exposure to a "shock" of acid rain, as from storms of low pH or melts of acidified snow (Likens and Borman 1974), could have a serious and long-lasting deleterious effect on nitrification in natural environments.

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