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# Nitrous Oxide Emission Associated with Autotrophic Ammonium Oxidation in Acid Coniferous Forest Soil

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**Aerobic $N_2O$ production was studied in nitrifying humus from urea-fertilized pine forest soil. Acetylene and nitrapyrin inhibited both $NH_4^+$ oxidation and $N_2O$ production, indicating that $N_2O$ production was closely associated with autotrophic $NH_4^+$ oxidation. $N_2O$ production was enhanced by low soil pH; it was negligible above pH 4.7. When soil pH decreased from 4.7 to 4.1, the relative amount of $N_2O$-N produced from $NH_4^+$-N oxidized increased exponentially to 20%. There was also some evidence that $N_2O$ formation was stimulated by salts (potassium sulfate and sodium phosphates). The maximum rate of $N_2O$-N production was 0.17 μg of $N_2O$-N per g of soil per h. When humus was treated with $NO_2^-$, $N_2O$ evolved immediately, indicating chemical formation, but no $N_2O$ was formed on the addition of $NO_3^-$. The amount of $N_2O$-N evolved was 0.6 to 4.6% of $NO_2^-$-N added. A high concentration of $NO_2^-$ and low soil pH enhanced chemical production of $N_2O$. There was no accumulation of $NO_2^-$ during nitrification. The calculations indicated that chemical formation of $N_2O$ was not the main source of $N_2O$ during $NH_4^+$ oxidation. After the addition of inhibitors of $NH_4^+$ oxidation the soils contained $NO_3^-$, but no $N_2O$ was produced. The results suggest that enhanced autotrophic $NH_4^+$ oxidation is a potential source of $N_2O$ in fertilized acid forest soil.**

Besides being produced during biological denitrification $N_2O$ is also formed during $NH_4^+$ oxidation. Results of studies with pure cultures of $NH_4^+$ oxidizers have revealed that 0.05 to 25% of the nitrogen from $NO_2^-$ ($NO_2^-$-N) produced evolved as $N_2O$-N when $NH_4^+$-N is used as substrate (13, 16, 17, 41). $N_2O$ production from $NH_2OH$, which is an intermediate in $NH_4^+$-N oxidation (1), can be much higher. *Nitrosomonas europaea* can produce over 90% of $N_2O$-N from $NH_2OH$-N that is oxidized (41). In well-aerated soils $N_2O$-N production ranges from 0.01 to 2% of $NH_4^+$-N added or $NO_3^-$-N produced (2, 3, 5, 8, 12). When $NO_2^-$-N accumulates in soil during $NH_4^+$-N oxidation, even higher amounts of $N_2O$-N may be formed (33) via chemodenitrification (9), in addition to direct $N_2O$ emission from cells of $NH_4^+$-oxidizing bacteria. Although $N_2O$ emission during nitrification may not be of general agronomic importance, it nevertheless is a source of $N_2O$ postulated to be linked to the catalytic destruction of stratospheric ozone (10, 40).

Most studies of $N_2O$ emission from soil, using aerobic atmosphere, have been made with agricultural soils, and only a few results are available from acid forest soils. Goodroad and Keeney (12) have measured $N_2O$ fluxes in deciduous and coniferous forest sites and have found that significantly more $N_2O$ is emitted from coniferous than from deciduous forest floors. They concluded that the lower pH of the coniferous forest soil might cause the difference. Robertson and Tiedje (30) have studied $N_2O$ production in forest soils using intact soil cores in a recirculating aerobic atmosphere. Their results suggest that in some acid forest soils mechanisms other than denitrification might be important in $N_2O$ production. This report presents results from laboratory experiments in which $N_2O$ production was studied in an aerobic atmosphere of fertilized acid coniferous forest humus.

## MATERIALS AND METHODS

Soil samples were collected in 1983 from an experimental Myrtillus pine forest site located at Tammela in southern Finland. The soil and the fertilization treatments have been described previously (18). The urea fertilization applied in autumn 1978 was 200 kg of N/ha. Some soil characteristics are given in Table 1. The samples were taken from the organic horizon of both unfertilized and urea-fertilized soils. The sampling techniques and the soil homogenization method have been described previously (18). Storage and laboratory manipulations are described below.

**Analytical methods.** Soil pH was measured in soil-water suspensions (1/2 [vol/vol]). When exchangeable ammonium, nitrate, and nitrite were determined after the pH was measured, 4 M KCl was added to the suspensions to obtain 2 M KCl for extraction. Ammonium and combined nitrate plus nitrite were determined by steam distillation (6). Nitrite was measured by the method of Montgomery and Dymock (21). Total N was determined by the Kjehldahl method (7), and total C was determined with a Hewlett-Packard 185 C-H-N analyzer. $N_2O$ and $O_2$ were determined with a Carlo Erba 4200 gas chromatograph equipped with an electron capture detector and a Porapak Q 80/100-mesh column (length, 2 m). Detector, injector, and oven temperatures were 200 to 300, 130, and 50°C, respectively. The flow of carrier gas (either $N_2$ or 90% argon–10% methane) was 30 ml/min. Analyses are expressed on an oven dry weight basis. The results were treated by the analysis of variance or correlation. The differences between means of various treatments were tested, subsequent to analysis of variance, by the Tukey honestly significant difference method (34).

**Soil incubations for measuring nitrification and $N_2O$ production.** Incubations were performed at 14°C in 100-ml bottles, stopped with rubber septa, containing 1.5 to 2 g of soil (oven dry weight). Both fresh and air-dried soils were used. In all experiments soil moisture content during incubation was 60% of the water-holding capacity (WHC). Periodically, the bottles were aerated after determination of the amount of $N_2O$ produced. At intervals three replicate bottles for every treatment were analyzed for nitrate and nitrite (after measurement of soil pH; see above). The partial pressure of $O_2$ was always > 0.18. Autotrophic ammonium oxidation was repressed by adding nitrapyrin (31) in water

TABLE 1. Description of soils

| Previous fertilizer treatment[a] | Soil parameter | | | |
|---|---|---|---|---|
| | pH (in $H_2O$) | Total C (%) | Total N (%) | C/N ratio |
| None | 4.0–4.2 | 31.9 ± 6.6[b] | 0.83 ± 0.13[b] | 38.3 ± 4.7[b] |
| Urea | 4.4–4.7 | 35.0 ± 5.1[c] | 1.00 ± 0.12[c] | 35.1 ± 1.0[c] |
| UABM | 4.6–4.7 | 27.7 ± 5.8[c] | 0.80 ± 0.11[c] | 34.2 ± 4.6[c] |

[a] For the amounts, see Martikainen (18).
[b] Values are the mean ± standard deviation for nine determinations.
[c] Values are the mean ± standard deviation for three determinations.

solution (50 μg/g of soil; Dow N-serve [18]) or $C_2H_2$ (10% [vol/vol]) (15, 16). All nutrients were added in solution. Soil pH was adjusted by adding $CaCO_3$.

In the first experiment samples were taken from the plot fertilized with urea, apatite, biotite, and micronutrients (UABM). It has already been shown that, on fertilization, nitrification activity is highest in the plot fertilized with UABM (18). Soil samples for this experiment were taken in mid-January 1983 (soil temperature at a depth of 5 cm was 0 to 0.5°C). After homogenization soil was amended with urea (200 μg of N per g of soil) and stored in polyethylene bags for 3 weeks at 4°C to activate nitrification. Fresh soil (2 g oven dry weight) was placed in bottles, some of which were supplemented with $CaCO_3$ (18 mg/g of soil) to obtain a higher pH.

In the second experiment the same UABM soil as described above was used, but 40 μg of urea-N was added prior to a 3-week storage at 4°C. The effects of the addition of $K_2SO_4$ on nitrification and $N_2O$ production were tested in this experiment. It previously has been found that in UABM-fertilized soil nitrification is highly inhibited on the addition of 53.5 μmol of $K_2SO_4$ per g of soil (19). Here, $K_2SO_4$ was added at 5.35 μmol per g of soil.

The experiments described above were done with UABM soil. $N_2O$ production during nitrification was also measured in urea-fertilized soil not treated with apatite, biotite, and micronutrients. Samples for this experiment were taken in May 1983. Lime, ammonium, phosphate, and micronutrients were tested by the treatments shown in Table 2. Because nutrients were added in water solutions, soil was air dried (after homogenization with a Moulinex homogenizer), so that all solutions could be added to obtain a final soil moisture content of 60% of the WHC. Soil pH was adjusted by adding $CaCO_3$, after which the soil was added to 100-ml incubation flasks, and the nutrient solutions were then added.

**$N_2O$ production from added nitrite.** Chemical production of $N_2O$ from $NO_2^-$ was tested by adding $NO_2^-$ to fresh humus samples. Aerobic soil incubations were performed as described above, except that each bottle contained 3 g of soil (oven dry weight). Soils were not sterilized before the addition of $NO_2^-$. The possibility that evolution of $N_2O$ was due to biological denitrification was tested by determining the amount of $N_2O$ produced from added $NO_3^-$. $NO_2^-$ additions were 0, 50, and 200 μg of N per g of soil. Chemical $N_2O$ evolution at low soil pH was studied by supplementing samples with $H_2SO_4$. Additions of $H^+$ were 0, 57, and 210 μmol of $H^+$ per g of soil. The highest $H^+$ level was equivalent to an oxidation by autotrophic ammonium oxidizers of 1,500 μg of $NH_4^+$-N to $NO_2^-$-N (2 mol of $H^+$ is produced for every 1 mol of $NH_4^+$ oxidized). An amount of 1,500 μg of N is equivalent to an $NH_4^+$-N application of 200

TABLE 2. Effects of calcium carbonate, ammonium sulfate, sodium phosphate, and micronutrients on nitrification and nitrous oxide formation[a]

| Treatment | Effects of the additions after the following times (days)[b]: | | | | | | | | | | | | μg of $N_2O$-N/g of soil during days 65-74[d] | (100 × $N_2O$-N)/($NO_3^-$ + $N_2O$)-N (65-74 days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 | | | 39 | | | 65 | | | 74 | | | | |
| | pH | $NH_4^+$-N | $NO_3^-$-N[c] | pH | $NH_4^+$-N | $NO_3^-$-N | pH | $NH_4^+$-N | $NO_3^-$-N | pH | $NH_4^+$-N | $NO_3^-$-N | | |
| None | 4.74 ± 0.02 | 191 ± 11 | <1 | 4.71 ± 0.02 | 259 ± 9 | <1 | 4.79 ± 0.02 | 319 ± 39 | <1 | 4.69 ± 0.02 | 616 ± 19 | 13 ± 12 | 0 | 0 |
| Ca[e] | 6.79 ± 0.25 | 345 ± 36 | <1 | 6.26 ± 0.44 | 323 ± 17 | 126 ± 6 | 4.94 ± 0.07 | 24 ± 4 | 354 ± 22 | 4.34 ± 0.09 | 154 ± 32 | 697 ± 54 | 0.98 ± 0.58 | 0.29 |
| Ca + P[f] | 6.12 ± 0.12 | 387 ± 14 | <1 | 5.78 ± 0.08 | 416 ± 41 | 61 ± 12 | 4.87 ± 0.15 | 37 ± 26 | 327 ± 24 | 4.34 ± 0.04 | 190 ± 36 | 474 ± 91 | 1.83 ± 0.54 | 1.23 |
| Ca + N[g] | 6.32 ± 0.06 | 576 ± 33 | <1 | 5.78 ± 0.19 | 562 ± 24 | 68 ± 12 | 4.65 ± 0.02 | 264 ± 52 | 312 ± 24 | 4.24 ± 0.01 | 262 ± 83[h] | 565 ± 174 | 9.58 ± 2.09 | 3.65 |
| Ca + N + P | 6.05 ± 0.08 | 615 ± 25 | <1 | 5.72 ± 0.05 | 606 ± 14 | 55 ± 7 | 4.58 ± 0.08 | 198 ± 73 | 375 ± 15 | 4.23 ± 0.06 | 268 ± 36[h] | 698 ± 43 | 13.31 ± 1.11 | 3.96 |
| Ca + N + P + M[i] | 6.33 ± 0.23 | 573 ± 10 | <1 | 5.81 ± 0.09 | 649 ± 100 | 52 ± 5 | 4.59 ± 0.02 | 151 ± 47 | 366 ± 113 | 4.23 ± 0.01 | 261 ± 26[h] | 602 ± 43[j] | 9.58 ± 0.01 | 3.90 |

[a] Formation in samples of the organic horizon of soil fertilized 5 years previously with urea. Each value is the mean ± standard deviation for three replicate incubations at 14°C (60% WHC). Average standard deviation 2% (pH), 24% ($N_2O$-N), 13% ($NO_3^-$-N) and 15% ($NH_4^+$-N). N fractions are expressed per gram of oven dry soil.
[b] Levels of $NH_4^+$-N are in micrograms of nitrogen per gram of soil.
[c] Including $NO_2^-$-N.
[d] $N_2O$ formation began after 60 days of incubation (Fig. 3).
[e] 0.0123 g of $CaCO_3$ per g of soil.
[f] 100 μg of P per g of soil ($Na_2HPO_4 \cdot 2H_2O$ + $NaH_2PO_4 \cdot H_2O$: pH of solution, 6.4).
[g] 250 μg of $(NH_4)_2SO_4$-N per g of soil.
[h] 250 μg of $(NH_4)_2SO_4$-N per g of soil was added after 67 days of incubation.
[i] 50 μg of micronutrient mixture per g of soil (see Martikainen [18] for composition).
[j] $C_2H_2$ was added after 72 days of incubation (Fig. 3).

kg/ha to the O horizon (depth, 5 cm; bulk density, 0.26 g/cm$^3$).

## RESULTS

**Nitrification and $N_2O$ production.** In the first experiment the lag period before nitrification was shorter in soils treated with $CaCO_3$ than in nontreated soils (Fig. 1). Also the maximum rate of nitrification and the amount of ($NO_3^-$ + $NO_2^-$)-N accumulated was higher in $CaCO_3$-amended soils ($P < 0.001$; Fig. 1). $NO_2^-$-N did not accumulate during incubation (its concentration remained at <1 μg/g of soil). After a 2-week incubation, when nitrification activity was still low (Fig. 1), soil pH was 5.2 and 6.8 without and with $CaCO_3$, respectively. During nitrification soil pH decreased but was always higher in soils treated with $CaCO_3$ than in nontreated soils ($P < 0.001$; Fig. 1). Although accumulation of ($NO_3^-$ + $NO_2^-$)-N was higher in soils with lime, production of $N_2O$ was lower in these soils than in soils without lime ($P < 0.001$; Fig. 1). The addition of $C_2H_2$ inhibited nitrification and $N_2O$ production (Fig. 1). The maximum rate of $N_2O$ production in soils without lime was 0.064 μg of $N_2O$-N per g of soil per h.

In the second experiment ammonification increased soil pH (Fig. 2) during the lag phase in nitrification. When nitrification began, soil pH decreased slightly less in soils receiving $K_2SO_4$ ($P < 0.01$), probably because of the lower nitrification activity of these soils ($P < 0.05$; Fig. 2). In contrast, the addition of $K_2SO_4$ increased $N_2O$ production ($P < 0.01$; Fig. 2). Nitrapyrin inhibited both nitrification and $N_2O$ formation in $K_2SO_4$-treated soils (Fig. 2). Inhibition was not tested in soils receiving no $K_2SO_4$. In nontreated and $K_2SO_4$-treated soils the maximum rate of $N_2O$ production

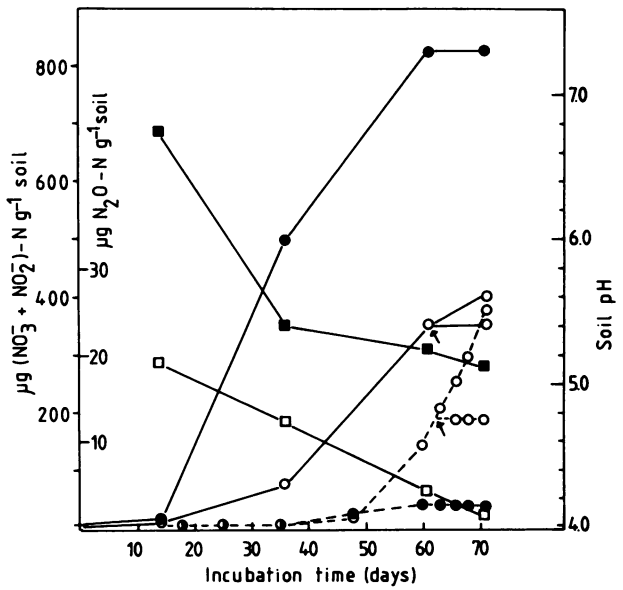


FIG. 1. ($NO_3^-$ + $NO_2^-$)-N accumulation, $N_2O$ production and soil pH in UABM soil without lime (−Ca) and with lime (+Ca). Each value is the mean for three replicate incubations at 14°C (60% of the WHC). The average standard deviations are 4%, 2% ($N_2O$-N), and 1% (pH). Symbols: □, pH (−Ca); ■, pH (+Ca); ○—○, ($NO_3^-$ + $NO_2^-$)-N (−Ca); ●—●, ($NO_3^-$ + $NO_2^-$)-N (+Ca), ○- - -○, $N_2O$-N (−Ca); ●- - -●, $N_2O$-N (+Ca). The addition of $C_2H_2$ is shown by arrows.

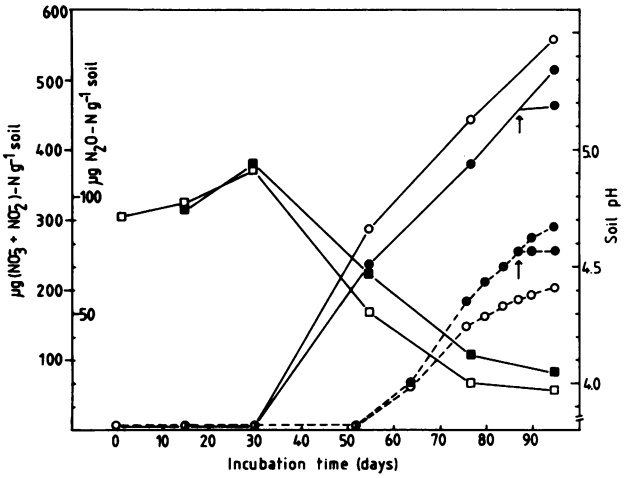


FIG. 2. ($NO_3^-$ + $NO_2^-$)-N accumulation, $N_2O$ production, and soil pH in UABM soils treated or untreated with $K_2SO_4$ (5.35 μmol/g of soil). Each value is the mean for three replicate incubations at 14°C (60% of the WHC). The average standard deviations are 9%, 8% ($N_2O$-N), and 1% (pH). Symbols: □, pH (−$K_2SO_4$); ■, pH (+$K_2SO_4$), ○—○, ($NO_3^-$ + $NO_2^-$)-N (−$K_2SO_4$); ●—●, ($NO_3^-$ + $NO_2^-$)-N (+$K_2SO_4$); ○- - -○, $N_2O$-N (−$K_2SO_4$); ●- - -●, $N_2O$-N (+$K_2SO_4$). The addition of nitrapyrin is shown by arrows.

was 0.091 and 0.121 μg of $N_2O$-N per g of soil per h, respectively.

In the third experiment nitrification activity in soils without lime was low, and no $N_2O$ production was found (Table 2 and Fig. 3). In soils with lime nitrification activity began after a 15-day incubation (Table 2). After incubation for 39 days the soils treated only with lime had the highest ($NO_3^-$ + $NO_2^-$)-N content ($P < 0.01$) among the soils with lime, but after further incubation no significant differences in nitrate accumulation were detected in these soils. There were differences ($P < 0.01$) in $N_2O$ production in soils with lime (Fig. 3). When $N_2O$ production began after 60 days of incubation (Fig. 3), soil pH was different ($P < 0.01$) in these soils (Table 2).

In soils with lime the addition of ammonium sulfate increased the amount of $N_2O$ production ($P < 0.01$). The addition of phosphates increased $N_2O$ formation in soils treated with ammonium sulfate and lime ($P < 0.05$) and in those receiving only lime (not significant; Fig. 3). Micronutrients did not have a statistically significant effect on $N_2O$ production. The rate of $N_2O$ production was highest (0.165 μg of $N_2O$-N per g of soil per h) in soils treated with lime, ammonium sulfate and phosphates (Fig. 3). Inhibition of both $N_2O$ production and nitrification by $C_2H_2$ was also demonstrated in this experiment (Fig. 3 and Table 2).

When $N_2O$ production began (after 65 days of incubation; Fig. 3) the pH was lower in soils treated with ammonium than in soils receiving only lime ($P < 0.05$; Table 2). At the end of the experiment (after 74 days of incubation; Table 2) no statistically significant differences in pH were apparent in soils with lime. Micronutrients did not have statistically significant effects on soil pH, nor did phosphates after 15 days of incubation.

Since no nitrate or nitrite was added before soil incubation, $N_2O$-N was also derived from oxidized ammonium. This is equal to ($NO_3^-$ + $NO_2^-$ + $N_2O$)-N, provided that no other nitrogenous gas is produced besides $N_2O$. The gas chromatographic method used did not detect other N oxides.

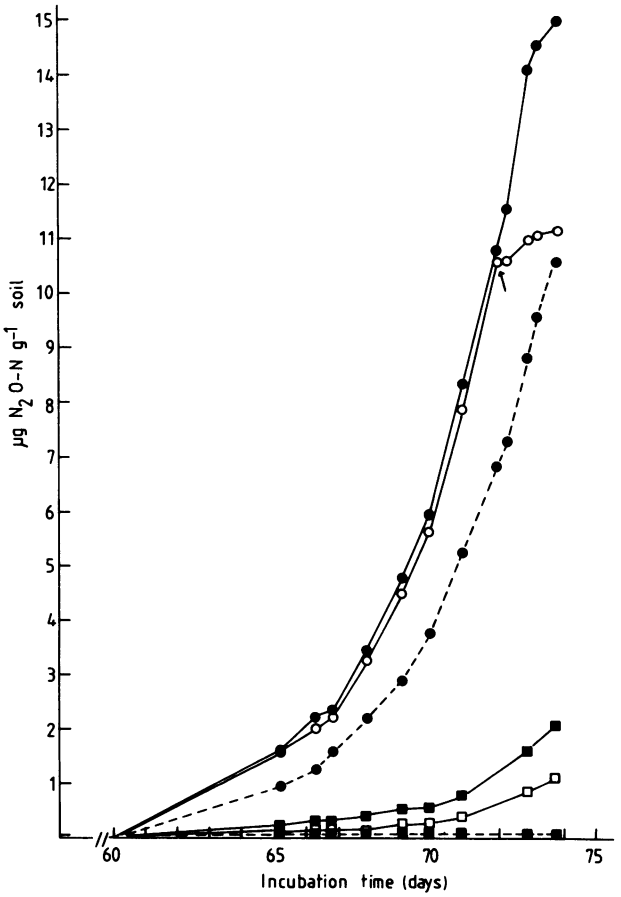


FIG. 3. Effects of calcium carbonate (Ca), ammonium sulfate (N), sodium phosphates (P), and micronutrients (M) on $N_2O$ production in urea-fertilized soils. For application rates, see Table 2. Each value is the mean for three replicate incubations at 14°C (60% of the WHC). The average standard deviation is 22%. Symbols: ■- -■, no additions; □—□, Ca; ■—■, Ca + P; ●- - -●, Ca + N, ●—●, Ca + N + P; ○—○, Ca + N + P + M. The addition of $C_2H_2$ is shown by an arrow.

Also $NO_2^-$ was not found. The amount of $N_2O$-N produced was 0.29 to 3.96% of the $NH_4^+$-N oxidized (Table 2).

**$N_2O$ production and soil pH.** The results of the three experiments described above suggest that $N_2O$ production is enhanced by low soil pH. The ratio of $N_2O$-N evolved to $NH_4^+$-N oxidized (see above) was plotted against soil pH for different incubation periods of the three experiments. The ratio of $N_2O$-N evolved to $NH_4^+$-N oxidized was determined because of variations in the nitrification rates. Average soil pHs for the measurement period were used. $N_2O$ production was negligible above soil pH 4.7 (Fig. 4). The ratio of $N_2O$-N to $NH_4^+$-N oxidized increased exponentially with a decrease in the soil pH from 4.7 to 4.1 (Fig. 4). Soils receiving $K_2SO_4$ were excluded from this analysis. The addition of $K_2SO_4$ enhanced $N_2O$ production, even though $K_2SO_4$-treated soils had a higher pH (Fig. 2).

**Chemical production of $N_2O$ from added $NO_2^-$.** On the addition of $NO_2^-$, $N_2O$ production was manifest in both unfertilized and UABM-fertilized soils (Fig. 5). Soil pH below 4.7 had negligible effects on $N_2O$-N production at a $NO_2^-$–N concentration of 50 μg/g of soil but enhanced it at a concentration of 200 μg of $NO_2^-$-N per g of soil (Fig. 5 and 6). No $N_2O$ was produced from added $NO_3^-$. The amount of $N_2O$-N produced from added $NO_2^-$-N during the experiment (Fig. 5) ranged from 0.63 to 2.05% and 2.55 to 4.58% when 50 and 200 μg of $NO_2^-$–N per g of soil, respectively, were added. Maximum rates of $N_2O$-N production occurred immediately after the addition of $NO_2^-$ (Fig. 5), and these were much higher than the maximum rates in experiments in which $N_2O$ was produced during nitrification (see above). The rates of chemical $N_2O$ production decreased over the experimental period (Fig. 5). After incubation the amount of $NO_2^-$-N remained below 0.05 μg/g of soil.

## DISCUSSION

The results of this study show that $N_2O$ may form in nitrifying forest humus under well-aerated conditions. $N_2O$ production was closely associated with autotrophic $NH_4^+$ oxidation and was enhanced by low soil pH. These results support the observations of Robertson and Tiedje (30), who found that in some forest soils $N_2O$ might not be produced by denitrifiers. They have found that in some intact forest soil cores $N_2O$ is produced only in the absence of $C_2H_2$. It is interesting that these soils had lower pHs than those that produced $N_2O$ only in the presence of $C_2H_2$ (4.6 versus 5.2). Goodroad and Keeney (12) have found that a coniferous forest site produces more $N_2O$ in situ than does a deciduous site. Soil pH was lower in the coniferous site (4.5 versus 5.0).

The effects of nutrients on $N_2O$ production must be considered in terms of nutrient effects on nitrification and soil pH. A low soil pH favored $N_2O$ production (Fig. 4). In $K_2SO_4$-treated soils $N_2O$ production and soil pH were higher, but ($NO_3^-$ + $NO_2^-$)-N accumulation was lower than that in nontreated soils (Fig. 2). These results indicate that $K_2SO_4$ has a stimulative salt effect on $N_2O$ production. $(NH_4)_2SO_4$ also increased $N_2O$-N production (Fig. 3 and Table 2), possibly by decreasing soil pH and not by stimulating nitrification (Table 2; see above). It has already been found that the addition of $K_2SO_4$ and $(NH_4)_2SO_4$ to acid soils inhibits ($NO_3^-$ + $NO_2^-$)-N accumulation (19). In this study, sodium phosphates had no statistically significant effect on soil pH and nitrification during $N_2O$ production (see above). The slight increase in $N_2O$ production because of phosphates might thus be a salt or nutrient effect.

$N_2O$ production by $NH_4^+$ oxidizers may vary with cultural

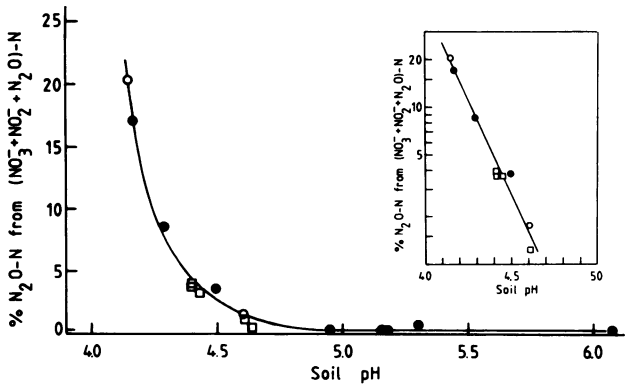


FIG. 4. Percentage production of $N_2O$-N from $NH_4^+$-N oxidized at pH 6.1 to 4.1. The values were calculated by the results shown in Fig. 1 (●), Fig. 2 (○), Fig. 3 (□), and Table 2 (□). The insert shows the percentage of production of $N_2O$-N from $NH_4^+$-N oxidized at pH 4.6 to 4.1. In $y = -2.387\ x + 11.184$, $y$ = log% $N_2O$-N from $NH_4^+$-N oxidized, $x$ = soil pH, $r = -0.985$ ($P < 0.001$, $df = 7$).

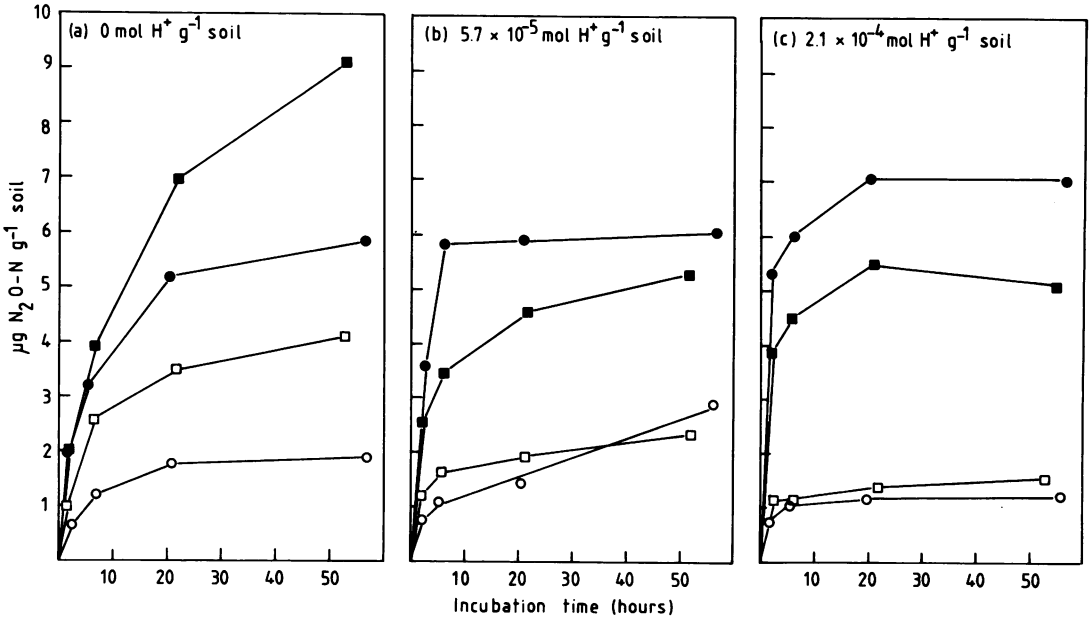


FIG. 5. Cumulative production of $N_2O$-N from $NO_2^-$-N in unfertilized (○, ●) and UABM-fertilized (□, ■) soils after different additions of $H^+$ as $H_2SO_4$. Open symbols, 50 μg of $NO_2^-$-N per g of soil; closed symbols, 200 μg of $NO_2^-$-N per g of soil. Added $NO_3^-$-N (50 μg of N per g of soil) produced no $N_2O$. Each treatment had two replicates (means are shown). Incubations were at 14°C (60% of the WHC).

conditions, because it is dependent on the buffer systems used (16, 41) and on cell age. Old cells produce more $N_2O$ than young ones (41). This observation may be of some importance in this study, in which $N_2O$ production occurred at the end of the experiments when most cells were aged. However, at or above soil pH 4.7, $N_2O$ production was negligible even after a long incubation. Thus, low soil pH is essential to $N_2O$ production.

Autotrophic $NH_4^+$ oxidizers have been reported to produce $N_2O$ in both oxidative and reductive processes (16, 29). It has been suggested that $N_2O$ evolves from a labile intermediate between $NH_2OH$ and $NO_2^-$ (possibly $N_2O_2H_2$) during $NH_4^+$ oxidation (1, 29). A low oxygen level supports

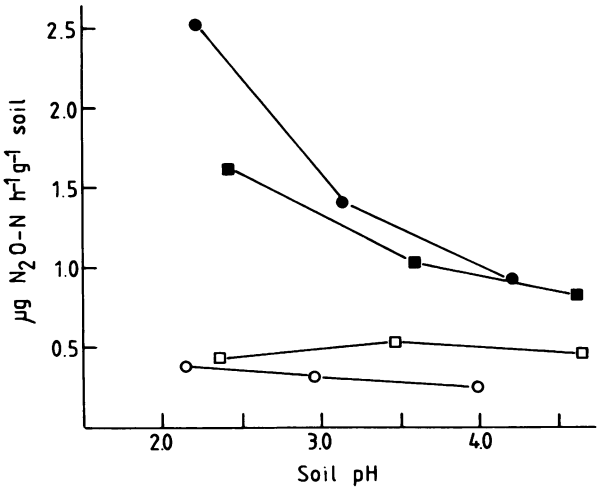


FIG. 6. Maximum rates of chemical $N_2O$-N formation from added $NO_2^-$-N at different soil pH calculated by the results shown in Fig. 5. Symbols as described in the legend to Fig. 5.

$N_2O$ formation (13, 16, 17), indicating participation of a reductive enzyme system. Recently, Poth and Focht (27) have shown that *Nitrosomonas europaea* produces $N_2O$ mainly by $NO_2^-$ reduction (nitrifier denitrification) and have pointed out that simultaneous $NH_4^+$ oxidation is needed to transfer electrons from $NH_4^+$ to $NO_2^-$ ($NO_3^-$ was not reduced). This coupling of $NO_2^-$ reduction to oxidative processes can explain why $C_2H_2$, which inhibits the first step in $NH_4^+$ oxidation, has been reported to inhibit $N_2O$ production from $NH_4^+$ in the presence of $NO_2^-$ (16). $C_2H_2$ does not inhibit $NH_2OH$ oxidation (15, 16), which explains why it does not inhibit $N_2O$ production from $NH_2OH$ (16).

The observations from this study that no $N_2O$ is produced on the addition of inhibitors, even when the soil contains $NO_3^-$ for possible denitrification by heterotrophic bacteria, support nitrifier denitrification. However, the favorable effects of low soil pH and salts cannot be unambiguously explained by these results. Enhanced $N_2O$ production at a low pH was not detected in studies with pure cultures of *Nitrosomonas europaea*, but sterile soil was found to greatly stimulate this process (16). It is not known what effect low pH or salts have on $N_2O$ production by *Nitrosospira* spp., which probably is responsible for $NH_4^+$ oxidation in fertilized forest soil (20). It is possible that *Nitrosospira* enzymes that catalyze the oxidation of the intermediate between $NH_2OH$ and $NO_2^-$ are sensitive to low pH and some salts at high concentrations. If the intermediate ($N_2O_2H_2$) accumulates in cells, $N_2O$ may be formed during hydrolysis of $N_2O_2H_2$ (29). The labile intermediate theory is not supported by the results of Poth and Focht (27). They have pointed out that the use of $NO_2^-$ as a terminal electron acceptor conserves $O_2$ for the first step of $NH_4^+$ oxidation. This oxidation needs molecular oxygen. Nitrite, which is a toxic end product, can also be removed by its reduction to $N_2O$. In this study the oxygen hypothesis did not fit well into the observed $N_2O$ production pattern. For example, in the first experiment (Fig. 1), oxygen depletion probably was greater in soils with lime because of their higher level of nitrification

(and respiration of heterotrophic microbes?), and $N_2O$ formation thus must be higher in soils with lime than in soils without lime. It is more likely that under the extreme conditions caused by low soil pH (or salts) *Nitrosospira* spp. are sensitive to $NO_2^-$ and will eliminate $NO_2^-$ by reducing it to $N_2O$.

Besides direct evolution from cells of $NH_4^+$ oxidizers, other mechanisms also exist for the production of $N_2O$ in aerated soils. Biological denitrification in anaerobic microsites, even during incubation of nonwaterlogged soil in an aerobic atmosphere, may occur. This possibility could not be totally excluded in this study because nonsterile soil was used. However, when nitrification was inhibited by nitrapyrin or $C_2H_2$, $N_2O$ production ceased, although the soil still contained $NO_3^-$. It was to be expected that $NO_3^-$ would diffuse from soil solution into anaerobic microsites. The possibility that both $C_2H_2$ and nitrapyrin inhibit denitrification is unlikely. It has been shown that the addition of 50 μg of nitrapyrin per g of soil does not inhibit denitrification (14). One possible explanation is that denitrifiers in the microsites use $NO_2^-$ but not $NO_3^-$ as electron acceptor (37, 39). A $K_m$ value as low as 0.20 mg of $O_2$ per liter at 27°C has been found for *Nitrosospira* sp. in forest soil (20). It is possible that $NO_2^-$ is produced by $NH_4^+$ oxidizers in a microenvironment and that part of it is reduced to $N_2O$ by denitrifiers in the same niche. Denitrification is known to occur at $O_2$ concentrations of 0.1 to 0.2 mg/liter in sewage, ocean, and pure cultures (11). Some denitrifiers can use both $O_2$ and $NO_2^-$ as electron acceptors simultaneously (39). Pang and Cho (25) recently have reported that biological denitrification of added $NO_2^-$ can occur in the L-H layer of coniferous forest soil, even under aerobic conditions.

In soil, $N_2O$ is also produced from $NO_2^-$ by chemical denitrification (9). High organic matter content and low soil pH favor this process (22, 23); forest soil may thus be a good environment for chemical denitrification. In this study the amount of $N_2O$-N was 0.6 to 4.6% of the amount of $NO_2^-$-N added. This agrees with observations made with other types of soil (4, 23, 28, 32). In this study a high concentration of $NO_2^-$ at low soil pH enhanced chemical $N_2O$ formation. The rates of chemical $N_2O$ formation immediately after the addition of $NO_2^-$ were much higher than those of $N_2O$ formation during nitrification, but when $NO_2^-$ concentrations had decreased (final concentrations, <0.05 μg of N per g of soil) the rates were of the same magnitude.

In the second experiment (Fig. 2), chemical $N_2O$ formation could not explain the rate of $N_2O$ production. In soils that received $K_2SO_4$, $N_2O$-N and $NO_3^-$-N productions averaged 2.2 and 7.0 μg of N per g of soil per day, respectively. The amount of $N_2O$-N produced from $NO_2^-$-N did not exceed 5% (see above). As estimated by the amounts of $N_2O$ and $NO_3^-$ formed, the average rate of $NO_2^-$-N production should be at least $(100/5 \times 2.2) + 7.0 = 51$ μg of N per g of soil per day, to provide $NO_2^-$ for chemical denitrification and nitrite oxidation during the whole 40-day period. Over 2,000 μg of $NH_4^+$-N thus must be oxidized during this period. In some $K_2SO_4$-treated soils ammonium oxidation and $N_2O$ production were inhibited by nitrapyrin during the 95 days of incubation (data not shown in Fig. 2). The ammonium accumulation rates determined for these soils showed that the highest possible amount of $NH_4^+$-N oxidation was about 600 μg of $NH_4^+$-N per g of soil. Furthermore, after 95 days of incubation the cumulative amount of ($NH_4^+ + NO_3^- + NO_2^- + N_2O$)-N was higher in soils without nitrapyrin than in soils with nitrapyrin (673 and 609 μg of N per g of soil, respectively). With a high chemical $N_2O$ production, the amount of N should be lower in the former than in the latter soils because $N_2O$ is a minor component of the nitrogenous gases that evolve during chemical denitrification (4, 23, 28, 35). It must also be mentioned that the reduction of $NO_3^-$ to $NO_2^-$ could not provide enough $NO_2^-$ to make the observed $N_2O$ formation a chemical process. So, in these soils, the chemical $N_2O$ formation seems to be of relatively limited importance in the total $N_2O$ flux.

The long-term laboratory incubations in this study indicate a potential for aerobic $N_2O$ production in nitrifying acid soil. A comparison of this potential with the denitrification potential in the UABM soil with anaerobic incubations and an optimum $NO_3^-$ level (38) showed that the maximum aerobic $N_2O$-N production (0.17 μg/g of soil per h) was about 50% of the denitrification potential. The production of $N_2O$ by forest soil *Nitrosospira* spp. must be examined at various conditions for better understanding of the mechanisms of $N_2O$ production. Additional work must also be done to evaluate increases in in situ evolution of $N_2O$ in terms of fertilization, which stimulates nitrification in acid forest soil. Evidence from other types of soil with higher pHs indicated that ammonium or urea fertilization increases $N_2O$ emission in situ and that this $N_2O$ is produced by ammonium oxidizers (5, 9). Urea fertilization is likely to increase $N_2O$ evolution from coniferous forest soil because urea can stimulate nitrification for many years (18). It recently has been discussed how lime applied to forest soil to neutralize acid deposition affects tree growth (26) and soil microbes (24, 36). It is known that the addition of lime can stimulate nitrification in acid forest soil (24). The results of this study indicate that the addition of lime may be environmentally harmful because of an increase in $N_2O$ evolution, especially when nitrification is stimulated by lime but the soil pH remains below 5.

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