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The effect of sodium chlorate and nitrapyrin on the nitrification mediated nitrosation process in soils

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Summary

The influence of total nitrification to nitrate or partial nitrification to nitrite on the soil organic nitrogen status was examined. NH_4^+-^{15}N was added to the soil in the absence and the presence of $NaClO_3$, respectively nitrapyrin. The first chemical inhibits only nitrate formation, the second inhibits total nitrification. The accumulation of nitrite nitrogen in the soil at levels up to 5 mg kg^{-1} increased the loss of nitrogen. Yet, it did not increase the binding of mineral nitrogen into soil organic matter, relative to the control soil. The data suggest that the biochemistry of the nitrite formation process, rather than the levels of nitrite ions formed, are of primary importance in the role of nitrification mediated nitrosation of soil organic matter.

Introduction

Recently a link has been proposed between the nitrification process at one hand and the soil organic nitrogen status on the other hand. It has been shown that the nitrification process mediated the reaction of its intermediate nitrite with phenolic compounds present in the soil humic matter, to form organic nitrogen (Boudot and Chone, 1985; Azhar *et al.*, 1986abc).

By using NH_4^+ – ^{15}N in the presence and absence of the nitrification inhibitor nitrapyrin, Azhar *et al.* (1986c) showed that up to 55 percent of the nitrogen added as labelled fertilizer could be immobilized, whereas in the nitrapyrin treatment a net mineralization process occurred.

Specific inhibitors of nitrification have been useful and are of considerable potential for the experimental study of the nitrification process and the ecology and biochemistry of the nitrifying bacteria. Of the many inhibitors that specifically affect the NH_4^+-oxidizers, nitrapyrin has been used most frequently in soil related research (Bremner *et al.*, 1978; Hooper and Terry, 1973).

The Na^+ or K^+ salt of chlorate was shown by Lees and Simpson (1957) to be a sensitive and specific inhibitor of Nitrobacter. This inhibitor is used in determinations of nitrification activity and permits monitoring the accumulation of NO_2^- rather than the accumulation of NO_3^- as a convenient measure of the nitrification rate (Belser and Mays, 1980).

In this study, the effect of inhibiting the total nitrification process, or only the nitrate formation, on the mineralization—immobilization interactions was investigated. The increase of the nitrite nitrogen levels in the soil, by blocking the second step of the nitrification process, was studied in relation to the binding of nitrite nitrogen in soil organic matter and the loss of NO_2^--N via volatilization mechanisms.

Materials and methods

Soil incubation

The soil used in this study is a grassland soil (location Gistel, Belgium). The soil has the following characteristics : pH_{H_2O} 6.5, sandy clay, CEC

14,4 meq/100 g and 4% C. One kg aliquots of soil were put in plastic containers. The moisture content was adjusted to 3/4 field capacity. A 100 mL beaker, half filled with water, was put in each container to maintain a constant soil moisture level. The containers were covered with plastic foil to reduce moisture evaporation and were incubated at 25°C. The treatments were (A) 200 mg $(NH_4)_2SO_4$ – ^{15}N kg^{-1} soil, (B) 200 mg $(NH_4)_2SO_4$ – ^{15}N kg^{-1} soil together with 2.13 g $NaClO_3$ kg^{-1} soil and (C) 200 mg $(NH_4)_2SO_4$ – ^{15}N kg^{-1} soil together with 80 mg nitrapyrin kg^{-1} soil.

Measurements

The inorganic nitrogen was determined by the distillation method of Bremner and Keeney (1965). Twenty grammes of soil were taken from each of the treatments and suspended in to 40 mL KCl 1*N*. The mixture was shaken for 2 hours and centrifuged. Twenty-five mL of the supernatant was taken for NH_4^+-N and (NO_2^- + NO_3^-)-N determination. NO_2^--N was measured by the colorimetric method of Montgomery and Dymock (1961). The total organic nitrogen was determined by the Kjeldahl method.

Determination of ^{15}N

The ^{15}N content was measured by emission spectrometry as described by Fiedler and Proksch (1975). The modified Dumas method was used to convert the NH_4^+-N into N_2 gas, which was then measured in quartz discharge tubes. The unknown samples were compared with standard samples of known enrichment measured under the same conditions.

Results

The influence of nitrification on the soil organic nitrogen status was examined by applying ammonium nitrogen to the soil in the absence and the presence of either a selective inhibitor of total nitrification such as nitrapyrin or a selective inhibitor of nitrite oxidation such as $NaClO_3$.

Table 1. Effect of different $NaClO_3$ concentrations on NO_2^--N accumulation in the soil after 1 day incubation

Amount of 1 *M* $NaClO_3$ (mL)	NO_2^--N mg kg^{-1} soil	
	Soil as such	Soil + 200 mg NH_4^+-N kg^{-1} soil
0	0.18	0.49
10	0.79	0.76
15	0.78	1.62
20	0.76	4.59
30	0.79	4.33

For the soil used, different $NaClO_3$ concentrations were tested in order to verify which concentrations were appropriate. Ten mL of $NaClO_3$ 1 *M* per kg soil were not effective in blocking nitrite oxidation in the presence of 200 mg NH_4^+-N kg^{-1} soil. Such concentration was effective for the soil, without NH_4^+ addition (Table 1). Twenty and 30 mL $NaClO_3$ 1 *M* yielded a maximal accumulation of nitrite. Accordingly, a concentration of 20 mL $NaClO_3$ 1 *M*, equivalent to 2.13 g $NaClO_3$ kg^{-1} soil, was used.

Fig. 1a presents the NH_4^+-N levels in the three treatments. After 10, 20 and 30 days of incubation the levels of NH_4^+-N had already decreased strongly in the no-nitrapyrin treatments (A) and (B). In the presence of the nitrification inhibitor nitrapyrin (treatment (C)) an increase in NH_4^+-N as a function of time was observed. In treatment (A) the NO_3^--N level increased strongly due to the ongoing nitrification process (Fig. 1b). For treatments (B) and (C) the NO_3^--N levels showed some increase during the first 10 days only. Fig. 1c presents the NO_2^--N levels. Treatment (A) showed a constant low level of nitrite nitrogen. Treatment (B) showed accumulation of NO_2^--N as a result of the selective inhibition of *Nitrobacter* sp. In treatment (C) nitrite was minimal at all times.

Fig. 2a presents the total mineral nitrogen in the three treatments during the incubation time. Total mineral nitrogen decreased in both treatments (A) and (B) as a function of time. On the other hand, it increased in treatment (C). However, the organic nitrogen level (Fig. 2b) showed an increase as a function of time for treatments (A) and (B) while it decreased for treatment (C) as a result of the ongoing mineralization process.

The pH values of the three treatments are presented in Fig. 2c. Treatment (A) showed a significant decrease as a result of the nitrate formation. Yet, in none of the soils the pH dropped to acidic values.

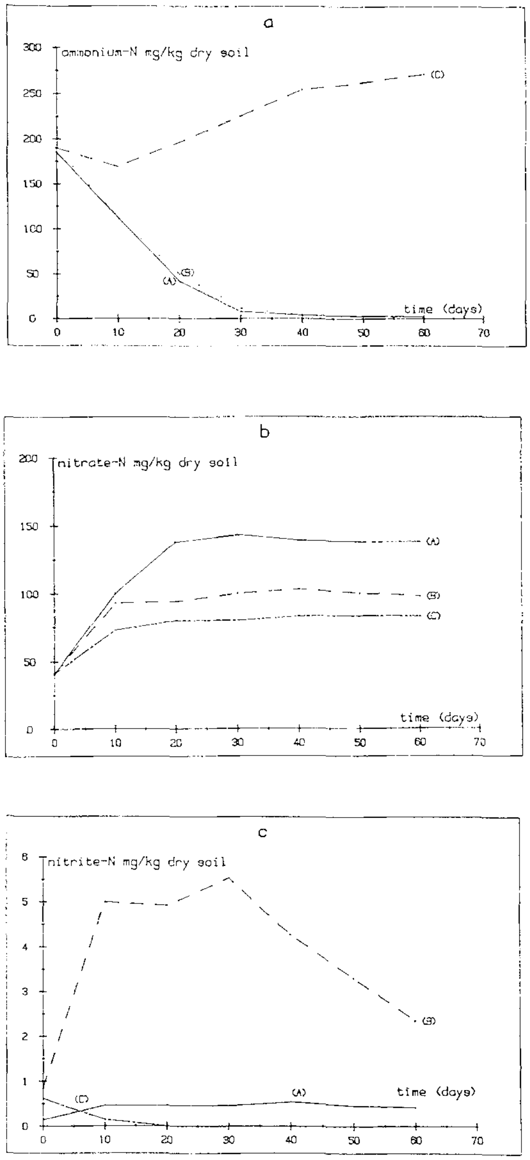

Fig. 1. **a** Nitrification patterns. Evolution of the NH_4^+-N levels of soil treated with $NaClO_3$ or nitrapyrin compared to untreated soil. A. soil + 200 ppm NH_4^+-N kg^{-1} soil. B. soil + 200 ppm NH_4^+-N kg^{-1} soil + 2,13 g $NaClO_3$ kg^{-1} soil. C. soil + 200 ppm NH_4^+-N kg^{-1} soil + 80 mg nitrapyrin kg^{-1} soil.

b. Nitrification patterns. Evolution of the NO_3^--N levels of soil treated with $NaClO_3$ or nitrapyrin compared to untreated soil. A. soil + 200 ppm NH_4^+-N kg^{-1} soil. B. soil + 200 ppm NH_4^+-N kg^{-1} soil + 2,13 g $NaClO_3$ kg^{-1} soil. C. soil + 200 ppm NH_4^+-N kg^{-1} soil + 80 mg nitrapyrin kg^{-1} soil.

c. Nitrification patterns. Evolution of the NO_2^--N levels of soil treated with $NaClO_3$ or nitrapyrin compared to untreated soil. A. soil + 200 ppm NH_4^+-N kg^{-1} soil. B. soil + 200 ppm NH_4^+-N kg^{-1} soil + 2,13 g $NaClO_3$, kg^{-1} soil. C. soil + 200 ppm NH_4^+-N kg^{-1} soil + 80 mg nitrapyrin kg^{-1} soil.

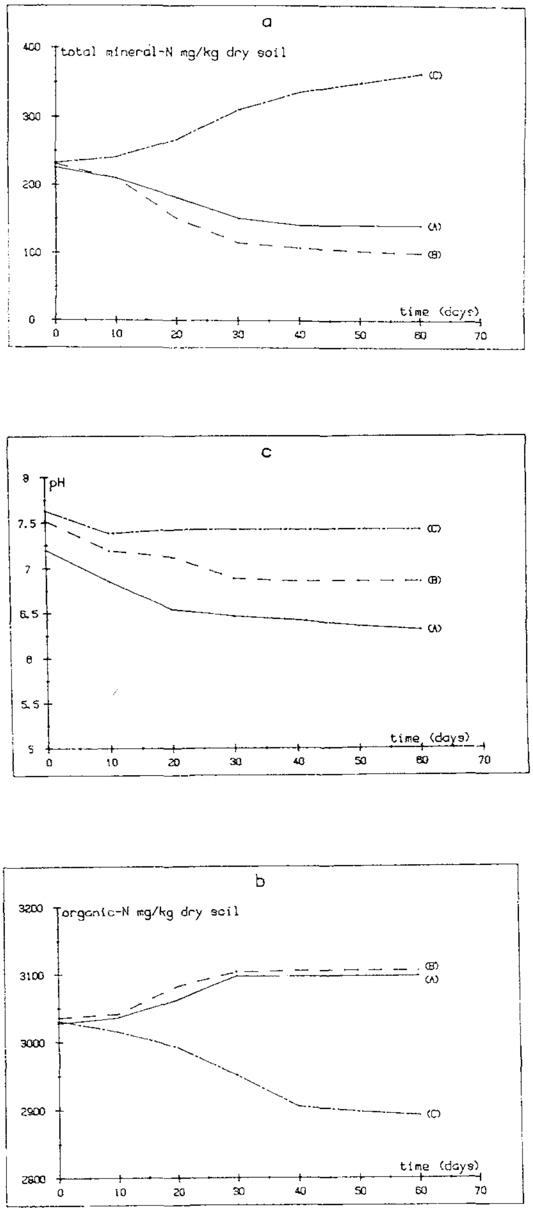

Fig. 2. **a** Total mineral nitrogen evolution of soil treated with $NaClO_3$ or nitrapyrin compared to untreated soil. A. soil + 200 ppm NH_4^+-N kg^{-1} soil. B. soil + 200 ppm NH_4^+-N kg^{-1} soil + 2,13 g $NaClO_3$ kg^{-1} soil, C. soil + 200 ppm NH_4^+-N kg^{-1} soil + 80 mg nitrapyrin kg^{-1} soil.

b. Organic nitrogen evolution of soil treated with $NaClO_3$ or nitrapyrin compared to untreated soil. A. soil + 200 ppm NH_4^+-N kg^{-1} soil. B. soil + 200 ppm NH_4^+-N kg^{-1} soil + 2,13 g $NaClO_3$ kg^{-1} soil. C. soil + 200 ppm NH_4^+-N kg^{-1} soil + 80 mg nitrapyrin kg^{-1} soil.

c. pH values during the incubation period. A. soil + 200 ppm NH_4^+-N kg^{-1} soil. B. soil + 200 ppm NH_4^+-N kg^{-1} soil + 2,13 g $NaClO_3$ kg^{-1} soil. C. soil + 200 ppm NH_4^+-N kg^{-1} soil + 80 mg nitrapyrin kg^{-1} soil.

^{15}N data

The results of ^{15}N analyses are given in Table 2. The % ^{15}N atom excess showed an increase as a function of time for both treatments (A) and (B) as compared to treatment (C) where nitrification was totally inhibited and where the % ^{15}N atom excess did not show any increase in the organic nitrogen during the incubation period (data not shown). At the end of the incubation, up to 4.7% of the organic nitrogen in the soil was derived from the added nitrogen fertilizer in both treatments (A) and (B) as a result of the fact that biochemical oxidation of ammonium was allowed to take place.

The specific changes in the soil organic nitrogen status are given in Table 3. The total amount of organic nitrogen which had been formed in treatments (A) and (B) or mineralized in treatment (C) were calculated in relation to zero time data. The values of the differences, given in Table 3, column 3, reflect the total amount of organic nitrogen which had been immobilized in treatments (A) and (B). These values are higher than the values obtained from the ^{15}N analyses. Probably recycling of mineralized soil nitrogen via the nitrification—nitrosation route, was responsible for this.

The amount of nitrogen lost from the system during the incubation time of 60 days amounted to 16.24 mg N kg^{-1} soil in treatment (A). This corresponds to 0.30% of the NH_4^+-N nitrified per day within the first 30 days. For treatment (B) the loss was about 42.53 mg N kg^{-1} soil which corresponds to 0.76% of the NH_4^+-N nitrified per day within the first 30 days. Treatment (C) showed also a loss of 13.08 mg N kg^{-1} soil which is about 0.07% of the NH_4^+-N formed per day.

Table 3. Changes in the organic nitrogen status (mg N kg^{-1} soil) of the soil as a consequence of the nitrification-nitrosation phenomena

Time (days)	Parameter 1[a]	2	3	4	5
Treatment A					
10	9.28	− 13.34	22.62	0	22.62
20	35.94	− 41.35	77.29	45.98	31.31
30	69.31	− 81.45	150.76	129.22	21.54
40	70.41	− 123.55	193.96	140.74	53.22
60	68.80	− 141.41	210.21	145.90	64.31
Treatment B					
10	10.25	− 13.34	23.59	24.36	0
20	47.38	− 41.35	88.73	57.94	30.79
30	72.16	− 81.45	153.61	136.08	17.53
40	80.96	− 123.55	204.51	144.58	59.93
60	76.00	− 141.41	217.41	146.64	70.77

[a] 1. Amount of organic nitrogen in the treatment at the given time of incubation minus the amount of organic nitrogen at zero time.

2. Amount of organic nitrogen in treatment C at the given time of incubation minus the amount of organic nitrogen at zero time.

3. The difference between 1 and 2; this corresponds to the total amount of nitrogen immobilized in the respective treatment.

4. Organic nitrogen derived from added labelled fertilizer (see Table 2).

5. Organic nitrogen derived from recycled soil nitrogen *i.e.* 3–4.

Discussion

Nitrification has been shown to bring about the

Table 2. The influence of total *versus* partial nitrification on the incorporation of fertilizer nitrogen into soil organic nitrogen

Time (days)	% ^{15}N atom excess		Org. N derived from labelled fertilizer					
			mg kg^{-1} soil		% of fertilizer N added		% of total soil N	
	(A–C)	(B–C)	(A)	(B)	(A)	(B)	(A)	(B)
0	0	0.18	0	23.06	0	11.53	0	0.76
10	0	0.19	0	24.36	0	12.18	0	0.80
20	0.36	0.45	45.98	57.94	22.99	28.97	1.50	1.88
30	1.00	1.05	129.22	136.08	64.61	68.04	4.17	4.38
40	1.09	1.11	140.74	144.58	70.37	72.29	4.54	4.64
60	1.13	1.13	145.90	146.64	72.92	73.92	4.71	4.70

A: soil + 200 ppm NH_4^+ − ^{15}N kg^{-1} dry soil,
B: A + $NaClO_3$.
C: A + nitrapyrin.

binding of mineral nitrogen into an organic form (Azhar *et al.*, 1986abc; Boudot and Chone, 1985).

Nitrite-N is considered to be the key intermediate in the reaction between the mineral nitrogen and the phenolic compounds in the soil to form new forms of organic nitrogen (Azhar *et al.*, 1986c). In the latter studies, no accumulation of nitrite nitrogen was observed during the nitrification process. In the present study, nitrite nitrogen accumulated up to levels of 5 mg kg^{-1} in treatment (B) as a result of blocking the second step of nitrification. Yet, the accumulation of nitrite nitrogen did not bring about a higher amount of ^{15}N fixed in the organic form, than in case of total nitrification. Hence these data suggest that, somehow the biochemistry of the nitrite-forming microorganisms is of predominant importance in the nitrification mediated nitrosation process.

The total mineral nitrogen levels decreased strongly in both treatments (A) and (B) in which the nitrification and the nitrosation process took place (Fig. 2a). This is postulated to be due to immobilization reactions. In contrast herewith, treatment (C) showed a net increase in the total mineral nitrogen as a result of the mineralization. Immobilization of reduced mineral nitrogen in soils has been reported before, particularly in organic soils (Cochran *et al.*, 1980; Power and Legg, 1984; Rice and Smith, 1985; Smith and Patrick, 1983). Boudot and Chone (1985) were the first to suggest that such immobilization was linked to the occurrence of the nitrification process.

The organic nitrogen levels are presented in Fig. 2b. Treatments (A) and (B) must be considered to have an equilibrium between mineralization and nitrification mediated immobilization. In treatment (C) where nitrification was inhibited, the mineralization process resulted in a net decrease of the organic nitrogen level. Accordingly it appears that the nitrification through the nitrosation gives rise to a net increase of the organic nitrogen in the soil (Azhar *et al.*, 1986abc).

It must be noted that the enhanced rates of immobilization in the treatments where nitrifiers are not inhibited (Fig. 2b) could be due to nitrapyrin's inhibition of immobilization of NH_4^+ by heterotrophs in addition to its blocking of autotrophic nitrification. Yet, in the previous work (Azhar *et al.*, 1986c), it was clearly shown by means of infrared and mass spectrometry that indeed nitrosated compounds were formed and thus most probably at the basis of the organic nitrogen increase.

The amount of fixed nitrogen can be calculated from the amount of ^{15}N present in the organic nitrogen fraction. The % ^{15}N atom excess in treatments (A), (B) and (C) increased to different extents. If it is assumed that some immobilization or chemical fixation into clay minerals of the added ^{15}N into the soil microbial biomass has taken place, then it must be about the same for the three treatments (A), (B) and (C). Yet only treatments (A) and (B) showed an increase in the % ^{15}N atom excess as a function of time. By substracting the % ^{15}N atom excess which has been observed in treatment (C), from that of treatments (A) and (B), one obtains the net % ^{15}N atom excess, which can be considered to be due to the presence of the complete nitrification process in case of treatment (A) and of the partial nitrification process in case of treatment (B).

The deficit of mineral nitrogen in treatments (A) and (B) is quite acceptable (Clarke *et al.*, 1960; Clark, 1962). The deficit of mineral nitrogen in the case of treatment (B) *i.e.* 0.76% per mg NH_4^+-N nitrified per day, is considered to be due to the accumulation of nitrite nitrogen in the soil. The accumulation of nitrite nitrogen provides a mechanism for gaseous nitrogen loss because of the tendency of NO_2^- to react with soil components to form nitrogenous gases *e.g.* N_2, N_2O, NO, and NO_2 (Bremner and Nelson, 1968; Gerretsen and De Hoop, 1957; Nelson and Bremner, 1970; Porter, 1969; Smith and Chalk, 1980; Soulides and Clark, 1958; Van Cleemput, 1984; Van Cleemput and Baert, 1984). Some authors have proposed that part of the deficit may be due to the evolution of methyl nitrite from the soil. This gas can be evolved during reaction of NO_2^- with methoxyl groups in lignin preparations under acid conditions (Bremner, 1957; Bremner and Fuhr, 1966; Edwards and Bremner 1966; Stevenson and Kirkman, 1964; Stevenson and Swaby, 1964). It is evident that further investigation of the loss mechanisms is required. Identification, measurement and analysis of the isotope ratio of all gaseous-N products is necessary.

The changes in the soil organic nitrogen status in the three treatments (A), (B) and (C) are given in Table 3. In treatment (C) a decrease due to the mineralization process has occurred. This decrease is quite acceptable when compared to the previous study by Azhar *et al.* (1986c). The mineralization process can be expected to proceed with a similar rate for all treatments. Accordingly the (A–C) and (B–C) values are reflecting the total amount of organic nitrogen formed in case of treatments (A) and (B). These values are higher than the amounts of immobilized nitrogen derived from the added mineral labelled fertilizer. This difference could be due to recycling of some mineralized soil nitrogen. That is, soil nitrogen set free as ammonium and subsequently nitrified and re-immobilized as a consequence of the nitrification process (Azhar *et al.*, 1986c) Hence the values for immobilized nitrogen as given in Table 3 can be divided into two sources. First the fraction derived from the added mineral ^{15}N (145.90 and 146.64 mg N kg^{-1} soil for treatments (A) and (B) respectively) and second the fraction derived from mineralized soil nitrogen (64.30 and 70.77 mg N kg^{-1} soil for treatments (A) and (B) respectively).

The data suggest that the biochemistry of the nitrite formation process rather than the levels of nitrite ions formed, are of primary importance in the role of nitrification mediated nitrosation of soil organic matter.

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