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Ammonium oxidizer numbers, potential and actual oxidation rates in two swedish arable soils

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Summary. The number of ammonium-oxidizing bacteria was measured with the most probable number (MPN) method while potential ammonium oxidation rates were determined with a chlorate inhibition technique in two arable soils. A new method for measuring actual in situ ammonium oxidation in soil cores is presented.

One soil was cropped for 4 years with one of four crop-fertilizer combinations: Unfertilized lucerne ley, unfertilized barley, nitrate-fertilized grass ley, or nitrate-fertilized barley. The highest ammonium oxidizer numbers and potential rates were found in the grass ley. The unfertilized barley had one-third the number and activity of the grass ley. Actual rates were in general 5–25 times lower than potential rates.

The other soil was that undergoing a 27-year-old field trial with a fallow and four different cropping treatments: No addition, nitrate, nitrate + straw, or manure. Ammonium oxidizer numbers were highest in the manure and straw treatments. MPN numbers and potential rates were lowest in the fallow treatment. Typical specific potential rates were 30 ng N oxidized $cell^{-1}$ h^{-1}. Actual rates were in general 40 times lower than potential rates.

Actual ammonium oxidation measurements seem to correspond to actual in situ activity at the moment of sampling, whereas the MPN technique and the potential measurements reflect events that occurred weeks to months before the sampling.

Key words: Nitrification – MPN of ammonium oxidizers – Chlorate inhibition – Arable soil

Nitrification rates are important to nitrogen cycling in arable soil because it regulates nitrate formation. Once formed nitrate may be easily lost through leaching or denitrification, especially in arable ecosystems. Methods are thus needed for measuring the actual in situ nitrification rate. This has been a difficult task, however, and methods employed so far have mainly concentrated on counting ammonium oxidizers and on measuring their potential activity in laboratory experiments.

Specific strains of nitrifiers may be counted by the fluorescent antibody technique (Schmidt 1973). The isolation work is time consuming, however, and the fluorescent antibody stain is specific with respect to species and even strains (Belser and Schmidt 1978b). Isolation of all occurring strains thus has to precede the actual population studies if all nitrifying cells are to be counted. Provided fluorescent antibodies can be produced for all the major species of nitrifiers, this method can be used for population-dynamic studies in field experiments that are carried out for several years on the same site of well-characterized soil.

Total numbers of autotrophic ammonium oxidizers are generally counted with the most probable

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number (MPN) technique by diluting extracts in selective media. This has been done both in test tubes (Alexander and Clark 1965; Belser and Schmidt 1978a) and in microtiter plate wells (Rowe et al. 1977). These methods are time consuming (5–8 weeks incubation), give large standard errors and may underestimate real numbers (Belser and Mays 1982). MPN counts will only detect strains of nitrifiers that are readily enriched in the liquid medium used.

Theoretically it is possible to estimate the numbers of nitrifiers indirectly by measuring potential nitrification rates, provided the specific potential oxidation rate per cell is known (Belser and Mays 1982).The most common way of measuring nitrification activity is to incubate soil samples for several weeks in the laboratory (at standard conditions) or in the field by measuring the accumulated nitrate. Robertson (1982) reviewed incubation measurements of nitrification for a large number of forest soils. Since nitrogen mineralization is generally the rate-limiting step rather than nitrification, such incubations give more information about mineralization rates than about the conditions that limit and regulate nitrification. If potential nitrification is measured in short-term experiments, the process is influenced very little by mineralization. Potential nitrification can be measured with the chlorate inhibition technique (Belser and Mays 1980) in short-term (10-h) experiments under optimal conditions with respect to oxygen partial pressure, substrate concentration, pH and temperature. The accumulation of nitrite in a soil slurry is determined after the addition of sodium chlorate (10 m*M*), which inhibits nitrite oxidation, whereas ammonium oxidation is not affected according to Belser and Mays (1980). They observed complete inhibition of nitrite oxidation in pure cultures, sediments and certain soils, although other soils only showed partial inhibition.

When soil samples are collected this disturbs the microenvironment of the soil organisms. Nitrifiers may be affected by the sampling. Most measurements of nitrifying activity involve mixing of the soil to obtain a representative subsample, which will increase mineralization rates and thus nitrifier substrate concentration. If a more realistic measurement of nitrification activity is needed, soil should be left intact as far as possible with the object of conserving in situ soil conditions. If chlorate solution is added to intact soil cores, disturbance of the soil is minimized and the nitrifiers obtain access to an amount of ammonium that is actually produced in the soil. In a substrate-unamended soil core, the accumulation of nitrite after chlorate addition could thus be a measure of the actual ammonium oxidation rate at the moment of sampling.

In the present paper three methods for measuring ammonium oxidizer numbers and oxidation rates were used to investigate a total of nine different soil treatments in two soils – one with high and one with low clay content. In the first soil ammonium oxidation was studied in a 3-year-old field experiment with four different treatments within the project: "Ecology of arable land – the role of organisms in nitrogen cycling" (Persson and Rosswall 1983; Rosswall and Paustian 1984; Steen et al. 1984). In the second soil the effect of organic manure, crop residues and nitrogen fertilizer on ammonium oxidation was studied in a field experiment established in 1956. Data from this long-term field experiment were also used by Parton et al. (1983) to evaluate a simulation model for nitrogen mineralization and organic matter changes and by Schnürer et al. (1985) for a study of microbial biomass and activity.

Materials and methods

Sites and soils

Two soils from Uppsland, central Sweden, were used. One soil was taken from Kjettslinge (40 km north of Uppsala) at the experimental site of the "Ecology of arable land" project (Steen et al. 1984). The soil is a loam with a clay content of 19%, pH of 6.3 and organic carbon content of 2.2%. Four cropping systems were investigated:

1. Barley (*Hordeum distichum*) without fertilizer addition (BO)
2. Barley receiving 120 kg N ha^{-1} $year^{-1}$ as calcium nitrate (B120)
3. Grass ley (meadow fescue, *Festuca pratensis*) receiving 120 + 80 kg N ha^{-1} $year^{-1}$ (GL)
4. Lucerne ley (*Medicago sativa*) without fertilizer addition (LL)

The other soil (at Ultuna near Uppsala) belongs to an old field experiment established in 1956 by the Swedish University of Agricultural Sciences. The soil is a sandy clay loam with a clay content of 35%. At the start of the experiment, the soil organic carbon content was 1.50% and the pH was 6.5 (Nilsson 1980). The crops have been annuals, mostly cereals. All above-ground residues have been removed after harvest. The properties of this soil have been determined previously (Nilsson 1980; Schnürer et al. 1985). Five of the original 15 treatments (Nilsson 1980) were included in this study:

1. Bare fallow; 1.13% C, pH 6.6
2. Cropped; no additions; 1.27% C, pH 6.7
3. Cropped; 80 kg N ha^{-1} $year^{-1}$ as $Ca(NO_3)_2$, 1.41% C, pH 6.8
4. Cropped; 80 kg N ha^{-1} $year^{-1}$ as $Ca(NO_3)_2$ and 1800 kg ha^{-1} $year^{-1}$ C as straw; 1.79% C, pH 6.9
5. Cropped; 80 kg N and 1800 kg C ha^{-1} $year^{-1}$ as farmyard manure; 1.86% C, pH 6.8

Straw and manure were added every 2 year at twice the rates given above. Additions were made in the beginning of October of 1981 and 1983.

MPN counts of ammonium oxidizers

Preparations and dilutions of samples for MPN counts were performed in the same way for both soils. The soil extraction procedure was modified from Clarholm and Rosswall (1980). Ten grams of top soil (0–10 cm) were mixed with 250 ml 2% Calgon (sodium hexametaphosphate) solution and mixed for 2 min in a Braun multimixer MX 32 (Braun, Frankfurt, FRG) at maximum speed, allowed to rest for 5 min, followed by 1 min of high-speed mixing. The slurry was transferred to a 250-ml measuring cylinder and soil particles were allowed to settle for 1 min, after which 20 ml was extracted from the 90-ml mark of the cylinder and transferred to a Petri dish. The microtiter plate technique was a modification of that used by Rowe et al. (1977) and the ammonium oxidizer medium was taken from Sarathchandra (1979). Microtiter plates with 96 wells (12 × 8 rows) were filled with 200 μl ammonium oxidizer medium in each well. Of the soil extract in the Petri dish, 50 μl was added to the first row of 12 wells in a number of microtiter plates with a multi channel pipette, and the extract was then diluted in fivefold steps with a Flow Multidiluter (Flow, Irvine, UK). The plates were stored in double plastic bags and incubated in the dark for 8 weeks at 24°C.

Calculation of MPN statistical tables (12 parallels, fivefold dilutions) was based on Parnow (1972).

Potential ammonium oxidation rates

Potential nitrification was measured according to Belser and Mays (1980) with minor modifications. The first two experiments were designed to find out at what concentrations of sodium chlorate nitrite oxidation was inhibited in soil slurries. Topsoil (0–10 cm) was taken from the B120 plot in Kjettslinge. Six replicate samples of 25 g soil were mixed with 100 ml 4 m*M* (di-)ammonium sulphate in 300-ml capped bottles, which were incubated on a shake table (200 rpm) in a 24°C thermostat. In the first experiment chlorate was added to the medium to give concentrations of 0, 5, 10, 15 or 25 m*M*. Samples of 1 ml were taken every other hour from 0 to 22 h after chlorate addition and put into test tubes with 1 ml 4 *M* potassium chloride to stop the ammonium oxidizer activity. Samples were centrifuged in a Wifug table centrifuge at 3000 g for 2 min, filtered through filter paper and analysed for accumulated nitrite. The second experiment was carried out as above but chlorate was added at lower concentrations: 0, 0.025, 0.050, 0.25, 0.50, 1.0, 5.0 and 15 m*M*. Three replicate samples were taken at 0, 3, 5 and 7 h after chlorate addition and treated as above. In all further potential ammonium oxidation measurements 15 m*M* sodium chlorate and 4 m*M* (di-)ammonium sulphate were used and samples were taken at 0, 3 and 6 h after chlorate addition. Procedures were otherwise as described above.

Actual ammonium oxidation rates

In both Kjettslinge and Ultuna soils, soil cores were taken between plant rows with a 50-cm-long steel soil corer with an outer diameter of 3.5 cm. The corer contained plastic cylinders (8 cm long and 3 cm in diameter), which after sampling were put into Plexiglass cylinders with tight bottoms (10 cm high and 3.3 cm in diameter). A chlorate solution (20 ml) was added to give a final concentration of 15 m*M*. In order to find out if nitrite accumulated linearly, soil cores were taken in the B120 plot at Kjettslinge. Soil from each of 10 replicate soil cores were incubated for 1, 4, 6, 8, 10 and 11 h after addition of chlorate. After incubation the soil was mixed in 300-ml plastic bottles with 100 ml 2 *M* potassium chloride. The bottles were shaken vigorously for 1 min to extract the nitrite formed. The slurries were allowed to sediment for 1 h and at least 20 ml of the supernatant was removed, centrifuged at 3000 g for 2 min in a Wifug table centrifuge, filtered through filter paper and analysed for accumulated nitrite.

A similar evaluation experiment was carried out in the Ultuna soil, where 35 soil cores were taken and 5 replicate cores were incubated for 0, 2, 4, 6, 8, 10 and 12 h after the addition of chlorate.

In all further experiments it was assumed that nitrite was produced linearly over time. Nitrite concentrations were determined at 0 and 6 h after chlorate addition.

Inorganic nitrogen analysis

In situ ammonium, nitrite and nitrate concentrations at sampling occasions were determined as follows: Soil (10 g) was mixed in 300-ml bottles with 50 ml 2 *M* potassium chloride and shaken on a shake table at 200 rpm for 1 h. Of the extract, 20 ml was centrifuged in a Wifug table centrifuge (3000 g for 2 min) and filtered through a filter paper. Nitrite and nitrate were analysed by flow injection analysis (FIA, Tecator, Höganäs, Sweden). Flow injection principles were discussed in Ružička and Hansen (1975). Detection limit for nitrite-N was 2 ppb. Ammonium was determined by the indophenol blue method (Runge 1971).

Sampling occasions

Potential and actual ammonium oxidation rates as well as the in situ nitrite, nitrate and ammonium concentrations were determined in the four cropping systems at Kjettslinge in July and September 1983. MPN counts of ammonium oxidizers were determined in September 1983.

Numbers of ammonium oxidizers, potential and actual ammonium oxidation rates and in situ nitrite, nitrate and ammonium concentrations were measured in the five soil treatments at Ultuna in November 1982. The same parameters were determined in September and November 1983, with the exception of actual ammonium oxidation rates and ammonium concentrations.

Results

Evaluation of chlorate inhibition in soil slurries

Nitrite oxidation was completely inhibited, and ammonium oxidation was unaffected in soil slurries by chlorate concentrations ranging from 5 to 25 mM $NaClO_3$ (Fig. 1). The control gave no accumulation of nitrite. Production of nitrite in chlorate-inhibited samples was linear over time for all concentrations of chlorate.

In the second experiment (Table 1), 1, 5 and 15 m*M* $NaClO_3$ resulted in total inhibition in soil slurries. Concentrations between 0.025 and 0.5 m*M* resulted in partial inhibition of nitrite oxidation in soil slurries during 7 h of incubation. The production of nitrite was linear over time in 5- and 15-m*M* samples. For further investigations, 15 m*M* $NaClO_3$ was used to ensure a linear nitrite production over an incubation time of 6 h.

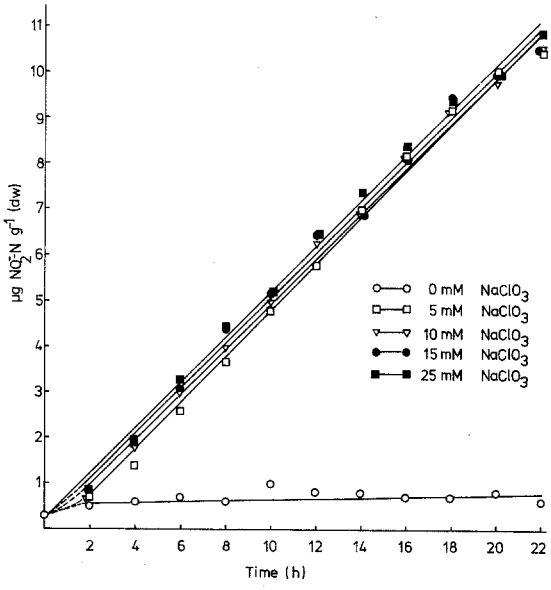

Fig. 1. Nitrite accumulation in Kjettslinge soil incubated in ammonium sulphate solution at different concentrations of sodium chlorate. $N = 6$ at each point and standard error $\leq 2\%$ of the mean. The average nitrite accumulation rate was 502 ± 6 ng N g^{-1} dry soil h^{-1} ($r = 0.996$, $P < 0.001$) for the four chlorate concentrations investigated

Table 1. Nitrite accumulation rates (*V*) in ng N g^{-1} dry soil h^{-1} in the Kjettslinge soil (slurries) incubated in ammonium sulphate solution at different concentrations of sodium chlorate. Values are means of three replicates

	Concentrations of $NaClO_3$ (m*M*)							
	0	0.025	0.050	0.25	0.50	1	5	15
V	12	48	66	83	119	207	209	189
SE	12	0	20	10	9	12	21	12

SE, standard error

Evaluation of chlorate inhibition in soil cores without ammonium added

Nitrite accumulated linearly over time (Fig. 2) in soil cores from Kjettslinge to which 15 m*M* $NaClO_3$ had been added. The ammonium oxidation rate was 1.8 ng N g^{-1} dry soil h^{-1}. Nitrite also accumulated linearly in Ultuna soil cores (Fig. 3), although the variation was greater. The mean ammonium oxidation rate was 1.3 ng N g^{-1} dry soil h^{-1}. One of the 12-h samples was disregarded as it showed a remarkably high nitrite concentration (100 ng NO_2^- N dry soil), which could have been caused by the core being sampled very close to a wooden frame that bound the plot area resulting in a waterlogged soil. This condi-

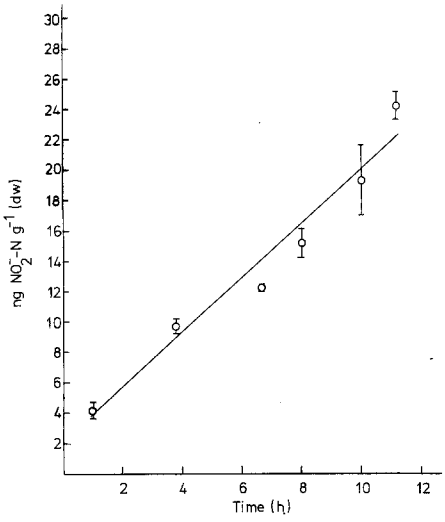

Fig. 2. Nitrite accumulation in Kjettslinge soil (cores) with $NaClO_3$ added to give a final concentration of 15 m*M*. Bars indicate standard error of 10 replicate samples (except at 1 and 8 h where $N = 5$); $r = 0.868$ ($N = 50$); $P < 0.001$

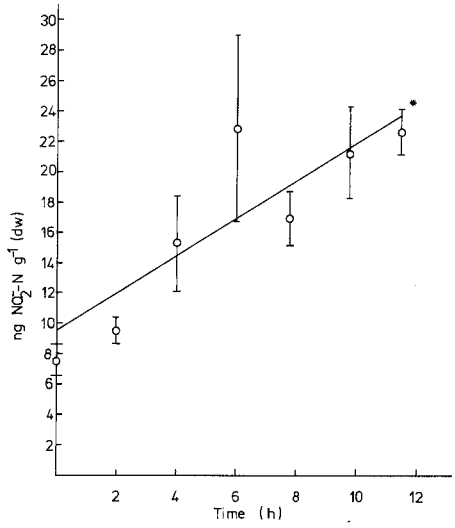

Fig. 3. Nitrite accumulation in Ultuna soil (cores) with $NaClO_3$ added to give a final concentration of 15 m*M*. Bars indicate standard error of five replicate samples (except at 0 and 12 h where $N = 3$); $r = 0.502$ ($N = 31$), $P < 0.01$, for the regression line. *) Note that one extreme value was neglected (100 ng NO_2^- N g^{-1} dry wt. at the 12 h sampling)

Table 2. MPN counts, potential and actual activities of ammonium oxidizers and inorganic ions in the Kjettslinge top-soil (0–10 cm). Values are means of four replicates ± standard errors (SE). All samples were taken in 1983

Treatment		MPN counts (10^3 g^{-1} dry wt.)	Potential activity (ng N g^{-1} dry wt. h^{-1})		Actual activity (ng N g^{-1} dry wt. h^{-1})		Nitrate (μg N g^{-1} dry wt.)		Nitrite (μg N g^{-1} dry wt.)		Ammonium[a] (μg N g^{-1} dry wt.)	
		Sept	July	Sept	July	Sept	July	Sept	July	Sept	July	Sept
B0	Mean	8.2	150	206	34	0	0.55	2.70	0.14	0.20	1.73	3.30
	SE	1.7	4	6	5	0	0.03	0.05	0.01	0.003	0.28	1.36
B120	Mean	6.2	200	501	49	21	28.70	2.60	0.12	0.23	3.00	2.40
	SE	0.8	5	10	4	3	2.60	0.44	0.01	0.01	0.32	0.68
LL	Mean	10.7	131	261	42	11	1.00	2.60	0.13	0.19	2.98	4.80
	SE	2.1	13	24	3	2	0.17	0.65	0.004	0.01	0.64	1.03
GL	Mean	20.2	374	483	283	7.3	67.90	0.75	0.14	0.28	3.90	3.50
	SE	2.6	19	10	62	0.5	0.75	0.08	0.01	0.01	0.44	0.90

B0, unfertilized barley; B120, barley fertilized with 120 kg NO_3^- N ha^{-1} $year^{-1}$; LL, lucerne ley; GL, grass ley receiving 120 + 80 kg NO_3^- N ha^{-1} $year^{-1}$

[a] Data obtained from Bergström (personal communication)

tion could have inhibited the nitrite oxidizers (but not the ammonium oxidizers), as they are more sensitive to oxygen depletion. Nitrite may thus have accumulated prior to sampling.

Ammonium oxidation in the Kjettslinge soil

MPN counts were highest in the grass ley and lowest in fertilized and unfertilized barley (Table 2).

Potential ammonium oxidation rates were highest in September in the grass ley and the fertilized barley plots and lowest in the lucerne ley and unfertilized barley (Table 2). The same pattern was seen in the July samples. Actual ammonium oxidation rates were highest in the grass ley in the July samples. Actual activities were lowest in unfertilized barley in July and September samples (Table 2). Actual rates were close to the potential ammonium oxidation rates in the July samples in the grass ley. Soil nitrite

Table 3. MPN counts, potential and actual activities of ammonium oxidizers and inorganic ions in the Ultuna topsoil (0–10 cm). Values are means of four replicates ± standard errors (SE)

Treatments	MPN counts (10^3 g^{-1} dry wt.)			Potential activity (ng N g^{-1} dry wt. h^{-1})			Actual activity (ng N g^{-1} dry wt. h^{-1})	Nitrate (μg N g^{-1} dry wt.)			Nitrite (μg N g^{-1} dry wt.)			Ammonium (μg N g^{-1} dry wt.)	C (%)[a]
	Nov 82	Sep 83	Nov 83	Nov 82	Sep 83	Nov 83	Nov 82	Nov 82	Sep 83	Nov 83	Nov 82	Sep 83	Nov 83	Nov 82	
Fallow															
Mean	1.4	5.4	6.1	216	188	162	4.5	3.19	2.79	1.61	<0.01	0.29	0.09	0.83	1.13
SE	0.2	1.0	1.2	24	5	5	1.2	0.80	0.29	0.22	–	0.01	0.01	0.12	–
0															
Mean	4.1	6.4	7.1	266	302	344	4.9	2.75	0.95	2.29	<0.01	0.32	0.08	0.85	1.27
SE	1.0	1.2	1.4	20	3	24	0.8	0.13	0.07	0.22	–	0.05	0.02	0.07	–
Nitrogen															
Mean	2.1	26.3	16.4	470	483	508	16.8	3.88	1.06	1.70	<0.01	0.33	0.08	0.63	1.41
SE	0.4	8.4	7.1	16	21	11	2.9	0.49	0.15	0.22	–	0.01	0.004	0.00	–
Straw															
Mean	5.8	21.3	22.9	628	597	688	18.0	3.56	1.23	1.06	<0.01	0.37	0.14	0.54	1.79
SE	2.0	7.0	5.3	28	8	10	4.0	0.43	0.13	0.24	–	0.04	0.01	0.08	–
Manure															
Mean	30.0	56.2	27.1	648	558	652	13.5	6.25	1.07	4.22	<0.01	0.34	0.11	0.73	1.86
SE	2.2	11.0	4.1	24	19	4	0.8	1.25	0.16	0.72	–	0.01	0.02	0.06	–

Fallow, bare fallow, no additions; O, cropped, no additions; Nitrogen, cropped, fertilized with 80 kg NO_3^- N ha^{-1} $year^{-1}$; Straw, cropped, fertilized with 80 kg NO_3^- N and 1800 kg straw C ha^{-1} $year^{-1}$; Manure, cropped, fertilized with 80 kg N and 1800 kg C as farmyard manure; C, organic carbon content of the soil

[a]Persson 1983 (personal communication

concentrations were high at both sampling occasions for all treatments (Table 2). Ammonium concentrations were generally highest in the grass ley and lowest in the unfertilized barley (Table 2).

Ammonium oxidation in the Ultuna soil

The ammonium oxidizer numbers and potential activities were generally highest in the manure and straw treatments, intermediate in the nitrate treatment and lowest in the fallow treatment (Table 3).

Actual ammonium oxidation rates were highest in the straw treatment and lowest in the fallow plot. Due to high nitrite background concentrations in the September and November samples of 1983 (see below), no actual ammonium oxidation activity could be detected. Nitrite concentrations were 10 ng N g^{-1} dry soil or less for the 1982 November samples, whereas the 1983 samples had nitrite concentrations between 100 and 400 ng N g^{-1} dry soil. Ammonium concentrations were highest in the fallow and no addition treatments.

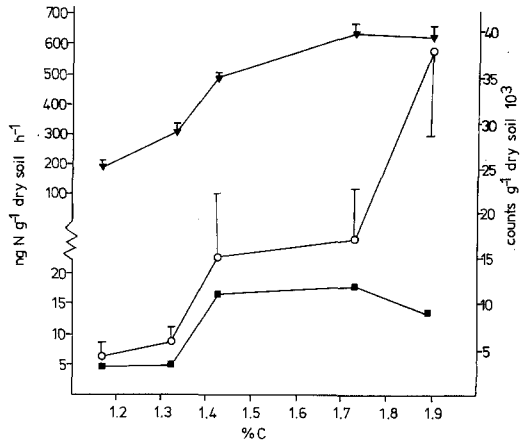

Fig. 4. Actual (■–■) and potential (▼–▼) ammonium oxidation rates and MPN counts (o–o) in Ultuna soil at different organic carbon contents. Bars indicate standard error of three replicate sampling occasions (Nov 1982, Sept and Nov 1983). Actual rates were from Nov 1982

Discussion

MPN counts of ammonium oxidizers

Ammonium oxidizer numbers varied over a greater range between different treatments in the Ultuna soil (1.4–56 × 10^3 cells g^{-1} dry soil) as compared with the Kjettslinge soil (6–20 × 10^3 cells g^{-1} dry soil). This is probably a result of the Ultuna plots having received their respective treatment, ranging from fallow to manure addition, for a longer time (27 versus 4 years), which caused the considerable differences in organic carbon contents (Table 3). The high MPN counts found in the manure and straw treatments in the Ultuna soil were probably due to the higher organic matter content in those treatments. This resulted in higher mineralization rates (Schnürer et al. 1985), which would supply more substrate to the nitrifiers. MPN counts were higher in the manure treatment than in the straw treatment, probably because of the higher C/N ratio in the straw, resulting in immobilization of nitrogen. MPN counts increased with increasing carbon content (Fig. 4) but showed no significant linear correlation ($r = 0.77$, $N = 5$). In Kjettslinge ammonium concentrations were consistently highest in the grass ley during 1983 and 1984 (L. Bergström, personal communication). This was probably due to a higher mineralization rate than in other treatments. The grass ley contained a larger root biomass, at least compared with the barley treatments (L. Hanson, personal communication), which would support a greater microbial turnover.

MPN counts were in accordance with previous reports for soils, although a few investigations have shown higher values, especially for organic soils and soil under permanent pasture and grazing. In other investigations MPN counts varied between 3 and 19 × 10^3 cells g^{-1} dry soil (Molina and Rovira 1963; Rowe et al. 1977; Belser and Mays 1982; Stout et al. 1984). Sarathchandra (1979) found 74 × 10^3 cells g^{-1} dry soil in a New Zealand sandy loam and 58 × 10^3 cells g^{-1} dry soil in a clay loam. Both soils were under permanent pasture. The importance of organic matter content was seen in the same investigation, in which ammonium oxidizers in a peaty loam were about 40 times more numerous (2.7 × 10^6 cells g^{-1} dry soil) than in the other two soils.

The specific activity of ammonium oxidizers calculated from MPN data and potential ammonium oxidation rates are shown in Table 4. The high frequency of specific potential activities around 30 pg N $cell^{-1}h^{-1}$ is worth noting. Since the specific activities in the present investigation were about 100 times higher than the pure culture activities of Belser and Mays (1982), and potential activities were comparable to their data (see below), this would imply that our MPN counts underestimated real numbers of ammonium oxidizers in the Kjettslinge and Ultuna soils.

When the MPN technique is used, one encounters difficulties such as large standard errors, possible problems with dispersion of ammonium oxidizer cells during preparation (Molina et al. 1979), as well as possible selectivity of the ammonium oxidizer medium for certain ammonium-oxidizing species and

Table 4. Potential ammonium oxidation activity in pg NH_4 N h^{-1} $cell^{-1}$ in Kjettslinge and Ultuna soils

		Soil treatments			
Kjettslinge		B0	B120	LL	GL
September 1983		25	81	24	24
Ultuna	Fallow	0	Nitrogen	Straw	Manure
November 1982	154	65	229	108	22
September 1983	35	47	18	28	10
November 1983	27	49	31	30	24

B0, unfertilized barley; B120, barley, fertilized with 120 kg NO_3 N ha^{-1} $year^{-1}$; LL, lucerne ley; GL, grass ley, fertilized with 120 + 80 kg NO_3 N ha^{-1} $year^{-1}$; Fallow, bare fallow, no additions; 0, cropped, no additions; Nitrogen, cropped, fertilized with 80 kg NO_3 N ha^{-1} $year^{-1}$; Straw, cropped, fertilized with 80 kg NO_3 N ha^{-1} $year^{-1}$ and 1800 kg straw C ha^{-1} $year^{-1}$; Manure, cropped, 80 kg N and 1800 kg C ha^{-1} $year^{-1}$ as farmyard manure

thus underestimation of counts (Belser and Mays 1982). In spite of these problems the MPN technique gives acceptable data on the effect of different crops and different soil treatments on numbers of ammonium oxidizers, as was shown for the investigated soils. Belser and Mays (1982) stated that potential nitrification rates were more reliable in estimating numbers of nitrifiers than the MPN technique. They used specific growth rates of pure cultures to determine the numbers corresponding to a specific activity. Even if the potential rate measurements do reflect the activity of all ammonium-oxidizing bacteria, some problems remain. First, the soil slurry is remote from the field situation, since substrate and oxygen are non-limiting. The aqueous slurry medium allows free exchange of nutrients and dilute metabolic products, which may affect growth. It thus resembles a batch culture, with the possible exception that a batch culture contains individual cells. A soil slurry probably contains nitrifiers distributed as microcolonies on soil particles (Molina et al. 1979), since stirring is so mild and nitrifiers can adhere strongly to particle surfaces (Bazin et al. 1982). A second problem could be that the specific activities of the individual cells of a pure culture and the cells in microcolonies of a soil slurry may not be the same, although Belser and Mays (1982) took it for granted.

Potential rates of ammonium oxidation

In general, potential ammonium-oxidation rates varied according to the same pattern as MPN counts, although the range of variations was smaller. The stimulation of heterotrophic microorganisms in fertilized treatments through stimulation and growth of plant roots was believed to cause the higher activity observed in fertilized barley plots when compared with the unfertilized. However, MPN counts were low in the fertilized barley plots, implying that conditions were not favourable enough for multiplication of ammonium oxidizers. This could be a result of a stronger competition by the plant root for available ammonium nitrogen. Compared with plant roots nitrifiers seem to have a fairly high K_m value for ammonium uptake (Rosswall 1982). In Kjettslinge, the grass ley showed the highest activities. In the Ultuna soil manure and straw treatments had the highest activities because of the higher amounts of organic matter in these cropping systems.

Potential ammonium oxidation rates were in accordance with earlier investigations by Belser and Mays (1982). They measured the potential ammonium oxidation rate in two grazed Minnesota permanent clover-grass pastures and found rates between 0.3 and 1.4 μg N g^{-1} dry soil h^{-1}.

A striking feature about our potential ammonium oxidation measurements is the similarity of potential rates between different treatments, between different sampling occasions and even between different soils (Tables 1, 2). The results showed that potential ammonium-oxidation rates varied over a small range in both soils: about four times between lowest and highest values (150–690 ng N g^{-1} dry soil h^{-1}), whereas MPN counts varied 40 times between lowest and highest values. The correlation between potential rates and MPN counts was nevertheless good (r = 0.59, $P < 0.01$, N = 19), in contrast to Belser and Mays (1982).

The evenness of ammonium oxidizer potential in the two soils could have at least three explanations. Firstly, nitrifiers due to their autotrophy can grow in microsites where easily degradable carbon is absent and might therefore be evenly distributed throughout all parts of the topsoil. Secondly, nitrifiers like many other soil bacteria have different means of resisting adverse conditions, which will prevent drastic reductions of the nitrifying population and consequently of the potential activity. They can survive drought periods, adverse temperatures and substrate shortages by forming zoogloea (Hofman and Lees 1953), by adhering to soil particle surfaces and producing slime (Bazin et al. 1982) and by growing in microcolonies (Molina et al. 1979). Thirdly, the population of ammonium oxidizer colonies at a given moment seems to exhibit their activity asynchronously. Molina et al. (1979) concluded that the activity of ammonium oxidation in the soil represents the resultant average

of pulses of activity from small isolated and asynchronously active ammonium oxidizer clusters. This would even out variations in the potential oxidation rates between different sampling occasions.

But why do MPN counts vary more than potential rates? One reason may be that the MPN enrichment method selects some strains of ammonium oxidizers, whose numbers vary greatly in the soil under normal conditions. Another reason could be that while the potential activity method measures the activity of microcolonies, the MPN method gives the numbers of the individual cells. It is probable that the specific activity of a cell in a microcolony would be different from the activity of a free individual cell.

Actual ammonium oxidation rates

The present paper describes the first attempt to measure actual in situ nitrification in intact soil cores. The method is a development of the potential ammonium oxidizer rate method, but with unperturbed soil and without substrate addition. The only addition was sodium chlorate (15 m*M* final concentration), which was added to inhibit nitrite oxidation. As nitrite accumulated linearly in both Kjettslinge and Ultuna soils, the oxidation should not be limited by chlorate or oxygen diffusion rates.

A problem with the method is in the apparent negative correlation between nitrite concentrations and the rate of nitrite accumulation, at least in the Ultuna soil. It is possible that nitrite can inhibit ammonium oxidation at substantially lower concentrations (e.g., 200 ng $NO_2^-N\ g^{-1}$ dry soil) than what can be expected from measuring potential ammonium oxidation in soil slurries. This could be a result of high local nitrite concentrations in the microcolony environment. In an intact microsite without the diluting action of a slurry system, nitrite would locally reach concentrations that might inhibit ammonium oxidation.

Comparisons of methods

It is obvious that the methods used measure different aspects of ammonium oxidation. With increasing organic carbon content in the soil, and thus increasing mineralization activity, numbers of ammonium oxidizers increase exponentially, whereas the actual and potential activities increase somewhat less (Fig. 4).

The stable, asynchronously working population of ammonium oxidizers is thought to be the same in potential (slurry) and actual (cores) rate measurements. The difference is that the core method can probably detect short-term variations in substrate concentrations (hours–days). Accordingly, the actual activity could vary considerably between sampling occasions. The slurry method and the MPN technique, on the other hand, probably reflect long-term changes in temperature, moisture and substrate availability of the soil. The reason for actual rates being higher in the Kjettslinge than in the Ultuna soil was probably the higher ammonium concentration in the former. In both Kjettslinge and Ultuna, potential and actual ammonium oxidation rates showed a similar pattern between the different treatments, although actual rates were one or two orders of magnitude lower. This lower activity was considered to reflect the actual in situ available substrate for the ammonium oxidizer population.

Several methods can be used to follow nitrification in soils. Both the MPN method and the potential nitrification slurry technique suffer from the drawback of perturbing the soil. The advantage of short-term incubations used in potential and actual ammonium oxidation measurements is that no growth of ammonium oxidizers will occur during the time of the experiment. Although many uncertainties remain, the actual measurement method is an attempt to bring an undisturbed soil into the laboratory for short-term experiments to determine the actual rate at the particular sampling occasion. After further development this method will become valuable in assessing the role of nitrification in the biogeochemical cycling of nitrogen.

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