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Measurement of Nitrification Rates in Lake Sediments: Comparison of the Nitrification Inhibitors Nitrapyrin and Allylthiourea

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Abstract. A method for measuring rates of nitrification in intact marine sediment cores has been modified and adapted for use in freshwater sediments. The technique involves subsampling a sediment core into mini-cores. Half of these cores are treated with an inhibitor of chemolithotrophic nitrification and, after incubation, differences in ammonia and nitrate concentration between inhibited and uninhibited systems are calculated. The within-treatment variability of ammonia and nitrate concentrations could be reduced by storing the cores overnight prior to subsampling. Estimates of the nitrification rate using the difference in ammonia concentrations between the inhibited and uninhibited mini-cores were always greater than the rate estimate using the difference in nitrate concentrations. Comparison between the results using the nitrification inhibitors allylthiourea (ATU) and nitrapyrin (N-Serve) indicated that the former appeared to give larger values for the nitrification rate than did the latter. Differences in the efficiency of these inhibitors in the control of nitrification under the conditions used partly explain these results. Data are also presented on the effect of N-Serve and ATU on some other nitrogen transformations affecting ammonia and nitrate concentrations.

Introduction

The inorganic nitrogen species nitrate and ammonia have a central role in the nitrogen cycle of all ecosystems. Attempts, therefore, to obtain a direct measure of the activity of a particular microbial transformation of either of these nutrients is problematical. In the case of nitrification, this difficulty has been partly overcome by the use of nitrification inhibitors. These allow differences in activity between inhibited and uninhibited subsamples to give an estimate of nitrifying activity in the sample. Activities which have been measured are dark O_2 [11, J. Cirello, Ph.D. thesis, Rutgers University, New Brunswick, N.J., 1975] and CO_2 [3, 8, 15, 33, 35] uptake rates and changes in both ammonia and nitrate concentrations [7, 14, 18].

Nitrification, which occurs in the hypolimnetic waters of many lakes may be important both as an oxygen sink and in providing a substrate for subsequent

nitrogen removal from the system [8, 15, 34]. However, many authors consider the sediment water interface to be the primary site of nitrifying activity [4, 13, 14, 35] although there are no measurements of *in situ* nitrification rates in freshwater sediments. Deficiencies in the existing methodology used to estimate nitrification rates in sediments include disruption of concentration gradients, particularly ammonia and oxygen, within intact sediments by the use of slurry techniques [Hall, Ph.D. thesis, Brunel University, Uxbridge, Middlesex, England, 1981] and the ability of lithotrophic nitrifying organisms to survive periods of anaerobiosis [14, 19]. These "inactive" organisms become active on exposure to oxygen during slurry preparation and therefore contribute to the measured activity. Using redox potential measurements attempts have been made to restrict the use of slurry techniques to oxidized sediments [Hall, Ph.D. thesis, 1981]. However, Eh may not be directly related to oxygen concentrations [31] and interpretation of some results was hindered by variability of the data [Hall, Ph.D. thesis, 1981]. Estimation of the difference in ammonia and/or nitrate concentrations between inhibited and uninhibited subsamples has obvious advantages over indirect estimates of nitrification rates using dark O_2 or CO_2 uptake rates. The latter require conversion to values of nitrogen oxidation.

Recently, a method has been described to measure nitrification rates directly in undisturbed marine sediment cores [18]. This paper describes a modification of this technique for freshwater sediments. The nitrification inhibitor N-Serve has been used in most research investigations [3, 7, 8, 15, 19, 33, 35]; ATU has been used less frequently [11, 25, Cirello, Ph.D. thesis, 1975]. The performance of the 2 inhibitors is compared here.

Materials and Methods

The study site was Grasmere, a lake whose basic limnological characteristics and sampling sites have been described [16, 23]. Sediment samples were collected from either the 20 m or 5 m sites (A & D respectively) using a Jenkin corer. The samples were returned to the laboratory and stored overnight a 4°C.

Nitrification Rate Measurement

(a) Each sediment core sample was subsampled into 6 mini-cores using the extrusion apparatus which has been previously described [30]. The mini-cores were 30 ml, central luer, polypropylene syringes (2.1 cm id) with the flanges removed and the cut edge beveled to approximately 60°. The overlying water volume of each mini-core was adjusted to 15 ml by inserting the syringe piston. Four cores were subsampled on each sampling date. (b) The nitrification inhibitor, either N-Serve (Dow Chemical Co.) or ATU (BDH Ltd), was injected into the surface sediment (1.0 cm) of 3 of the mini-cores to obtain a final inhibitor concentration of 10 mg/liter. The N-Serve was dissolved in acetone and ATU in double distilled water to stock solution concentrations of 10 g/liter. The total volume of inhibitor solution added was 3.5 μl in seven 0.5 μl aliquots. The additions were made 0.5 cm into the sediment at regular spacings to minimize diffusion distances. The inhibitors were also added to the overlying water of these mini-cores to final inhibitor concentrations of 10 mg/liter. The remaining 3 mini-cores were used as controls and were treated with equivalent volumes of acetone (N-Serve series) or double distilled water (ATU series).

Each set of 6 mini-cores was permanently labeled to ensure that the inhibitors were always added to the same mini-core tubes. This prevented any sorbed N-Serve being released into uninhibited samples [5]; it is not known if ATU has similar properties. The mini-cores were incubated in a water bath at *in situ* temperatures(6–7°C) for 1.5–2.5 days with constant aeration of the overlying water. Two cores were subsampled for each inhibitor on each sampling date. (c) After incubation, the overlying water of each mini-core was removed, filtered through previously ignited and washed Whatman GF/F filters, and stored frozen (−10°C) for analysis. The surface 1.0 cm of each mini-core was similarly removed into a preweighed vial. After removal of an aliquot for dry weight analysis (70°C for 48 hours), the vial was reweighed. Ammonia was extracted from the sediment by agitation (10 min) in the presence of a known volume of 1% (w/v) KCl solution; all nitrate was assumed to be soluble and removed in the extract. Increasing the KCl concentration, or agitation time, did not increase the recovery of ammonia. The extract was filtered through Whatman GF/F filters and stored frozen for analysis. Such prepared samples could be stored for at least 5 days without change in ammonia and nitrate concentrations. (d) As the mechanisms underlying the exchange of inorganic nitrogen species between the sediments and overlying water are not fully understood, it was assumed that the ammonia and nitrate fractions in these 2 phases could not be treated separately. Therefore, for statistical analysis of differences in concentrations between treatments, the individual replicates for each analysis (see below) of sediment extract and overlying water were summed. This resulted in 3 replicate values of ammonia or nitrate concentration in each mini-core. Differences were tested using a 2-level nested analysis of variance. Errors may occur in this analysis as it was not determined if the assumptions required for the use of parametric statistical tests were valid for these data. (e) When significant differences were detected between inhibited and uninhibited cores, the actual differences were calculated and reported as dNH_3 or dNO_3 values. These represent 2 independent estimates of the same nitrification rate. (f) Periodically, overlying water from a Jenkin core sample was enriched with ammonia and incubated on an orbital incubator at 20°C. Samples were removed for nitrate analysis every 24 hours for a maximum of 4 days. This covered the maximum incubation time of the sediment mini-cores. On no occasion was nitrite or nitrate produced during this period.

Chemical Analysis

All chemical analyses were performed on a Pye-Unicam ACI chemistry unit coupled to a Beckman 6-550 spectrophotometer. Printout was obtained via an interface with a Hewlett-Packard 9815A calculator.

Ammonia was determined by the cyanurate-salicylate method [9] with the final concentration of reagents in the reaction mixture modified as follows: sodium salicylate 10.5 g/liter, sodium nitroprusside 0.285 g/liter, sodium cyanurate 0.39 g/liter, and sodium hydroxide 0.05 M. Colour was developed for 8 min at 33°C and absorbance read at 660 nm. Throughout this paper, ammonia in aqueous solution will be referred to as ammonia, although at environmental pH (6.0–7.6), very little would be present in the undissociated form.

Nitrate was determined by reduction to nitrite by hydrazine in the presence of Zn^{2+} and Cu^{2+} ions [10]. Final reagent concentrations in the reduction mixture were as follows: sodium hydroxide 0.09 M, hydrazine 0.059 g/liter, Zn^{2+} 0.016 g/liter, Cu^{2+} 0.078 g/liter. A reduction time of 7 min at 33°C was allowed. The nitrite formed was subsequently determined by diozatization [2] with the following reagent: sulphanilamide 5 g/liter, H_3PO_4 (conc.) 50 ml/liter, *N-1*-napthylethylenediamine dihydrochloride 0.25 g/liter. Colour development was allowed for 3 min at 33°C and absorbance read at 540 nm. Comparison of nitrate and nitrite standard solutions indicated a stoichiometric reduction under these conditions.

Three replicate determinations were made per sample and 2 more contained internal standard ammonia or nitrate solutions to estimate the recovery efficiency of the determinations. The mean percentage recoveries of both ammonia and nitrate standard solutions, in the presence of KCl, the nitrification inhibitors and acetone, throughout the study period are shown in Table 1. The absorbance of the sample blank for the determination of ammonia in the presence of KCl was found

Table 1. The effect of extractant, solvents, and inhibitors on the recovery of ammonia and nitrate from standard solutions

Standard solution made up with	% recovery of standard solutions	
	Ammonia	Nitrate
1% KCl	100	64
10 mg/liter ATU	120	71
10 mg/liter N-Serve	79	9
Acetone control	77	8

to increase in relation to that obtained in water. However, blank corrected values for standard ammonia concentrations were not significantly different. The increased absorbance for the ammonia analysis in the presence of ATU necessitated a correction for all analyses in which ATU was present. All reported results are corrected in this way. The low recovery of nitrate, particularly in the presence of acetone, indicates the importance of internal standard addition to estimate the recovery efficiency of chemical determinations. Failure to include such controls could lead to considerable errors in the data. Nitrite was not determined on any samples.

Inhibitor Studies

Throughout this report, volumes of acetone equal to that used in the addition of N-Serve were used as a control. It was assumed that double distilled water, used as a solvent for ATU, would have no effect on any of the nitrogen transformations studied. In each of the following experiments, the treatments refer to the following additions, each in triplicate: (a) 10 mg/liter N-Serve, (b) the acetone control, and (c) 10 mg/liter ATU. Control flasks receiving no additions were also included.

Nitrification. Enrichment cultures of chemolithotrophic nitrifying bacteria were prepared by diluting Grasmere surface sediments 1:10 with overlying water and dispensing 200 ml aliquots into 250 ml Erhlenmeyer flasks. After addition of 1 mg NH_3-N, incubation was continued at room temperature (~20°C) with or without aeration. Samples for nitrate and nitrite analysis were taken every 24 hours. When nitrate was being produced and nitrite was undetectable, the inhibitors and acetone were added. Incubation was continued for 96 hours with daily analysis of nitrate concentrations.

Nitrogen Mineralization (Ammonification). The overlying water from a Jenkin core was used to prepare an 0.025% (w/v) solution of gluten (BDH Ltd) which was incubated at room temperature. High concentrations of ammonia were detected in this culture after 48 hours. The enrichment was subcultured into fresh sterile medium and incubation continued with daily analysis of ammonia concentrations. When ammonia was being produced, the culture was thoroughly mixed and 100 ml aliquots dispensed into 150 ml Erhlenmeyer flasks. The treatments were applied and incubation continued for 72 hours with daily analysis of ammonia concentrations.

Nitrate Removal. Nitrate removal could be either assimilatory or dissimilatory as metabolic end products were not determined. Grasmere sediment was diluted 1:10 with overlying water and 7 ml volumes dispensed into 10 ml Hypo vials (Pierce Chemical Co.). After addition of 1 mg NO_3-N, the vials were degassed by bubbling with oxygen-free helium for 15 min. The vials were sealed with neoprene septa and the treatments applied. Incubation was continued for 2–3 days at 10°C. Aliquots were removed from each vial, filtered through Whatman GF/F filter paper, and analysed for the nitrate remaining.

Table 2. Comparison of nitrification rate estimates using the inhibitors N-Serve and ATU on 3 separate occasions

Inhibitor used	Core	3rd February '82		25th February '82		11th March '82	
		dNH_3[a]	dNO_3[a]	dNH_3	dNO_3	dNH_3	dNO_3
N-Serve	1	4.896	0.777	4.039	NS	3.851	1.070
	2	4.122	NS	4.446	NS	3.755	0.876
ATU	1	6.676	1.728	6.380	2.267	5.417	3.492
	2	8.280	1.793	9.289	4.600	4.677	3.032

NS = not significant
[a] rates in mgN m^{-2} day^{-1}

Results

Nitrification Rate Estimates

Before reporting any data on rates of nitrification, some general observations must be made on the effect of nitrification inhibition on ammonia and nitrate concentrations in the mini-core systems. Nitrate concentration in the sediment extracts were often below the sensitivity of the analytical technique (the interstitial waters were diluted approximately 1:7). This is in keeping with other observations on Grasmere sediments which indicated that nitrate reductase activity is the main controlling factor of nitrate concentrations in the interstitial water [23]. Therefore, dNO_3 values for the nitrification rate esimates are almost totally composed of changes in the nitrate concentrations in the overlying water. Samples with the inhibitor added showed almost a complete loss of nitrate from the overlying water. Assuming nitrate reduction does not occur to any extent in the aerobic water column, the nitrate must diffuse into the sediments. Ammonia concentrations in the overlying water of inhibited cores increased with time. Although organisms capable of producing ammonia from gluten could be enriched from the overlying water, there are no available data on *in situ* activity. As the overlying water showed no potential for nitrification and, moreover, ammonia concentrations in the overlying water of uninhibited cores showed little change from time zero values, the sediments are the most likely source of this ammonia. The increase in overlying water ammonia concentrations was concommitant with an increase in exchangeable ammonia concentrations in the sediments. The failure to enrich nitrifying organisms from the overlying water of the sediment cores over the time course of the rate measurement (1.5–2.5 days) also indicates that any change in ammonia and nitrate concentrations between inhibited and uninhibited mini-cores must be due to sediment nitrifying activity.

Storing the sediment samples overnight had a considerable effect on the within treatment variability of both ammonia and nitrate concentrations. Analysis of data from cores which had been subsampled immediately indicated that, on average, the treatments accounted for 47% (range 2%–92%) of the variability of the data. After overnight storage, this figure increased to a mean value of 79% (range 51%–99%).

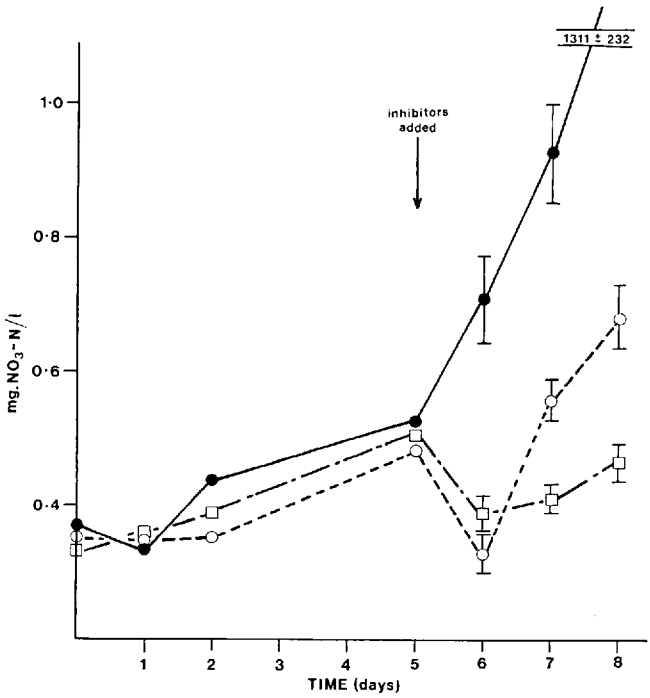

Fig. 1. Effect of 10 mg/liter N-Serve (○- - -○) and ATU (□- - -□) on nitrate production by enrichment cultures of chemolithotrophic nitrifying organisms under aerated conditions. Vertical bars indicate 95% confidence limits. Control ●——●.

Results from nitrification rate estimates, using both inhibitors, on 3 sampling dates are shown in Table 2. Duplicate cores were used for each inhibitor on each occasion. Although the data are limited, it is possible to make some general observations. On all occasions, with either inhibitor, the nitrification rate estimate based on dNO_3 values is always lower than the estimate based on dNH_3. Secondly the estimate based on either dNH_3 or dNO_3 appears to be higher with ATU than with N-Serve as the inhibitor.

Inhibitor Studies

The relative ability of both N-Serve and ATU, at concentrations of 10 mg/liter, to inhibit chemolithotrophic nitrifiers was tested on enrichment cultures of these organisms. The production of nitrate in cultures with active aeration is shown in Fig. 1. These results are presented as mean nitrate concentration in the 3 replicate flasks for each treatment. All cultures showed similar rates of nitrate production up to day 5 of incubation (confidence limits have been omitted to preserve clarity). The rate of nitrification in control cultures between days 5 and 8 of incubation was 0.26 mg N/liter/day. Both N-Serve and ATU inhibited nitrate production for 24 hours after addition. The decrease in nitrate concentrations during this time could be due to rapid assimilation, or dissimilation in anaerobic microsites. After this initial inhibition phase, however,

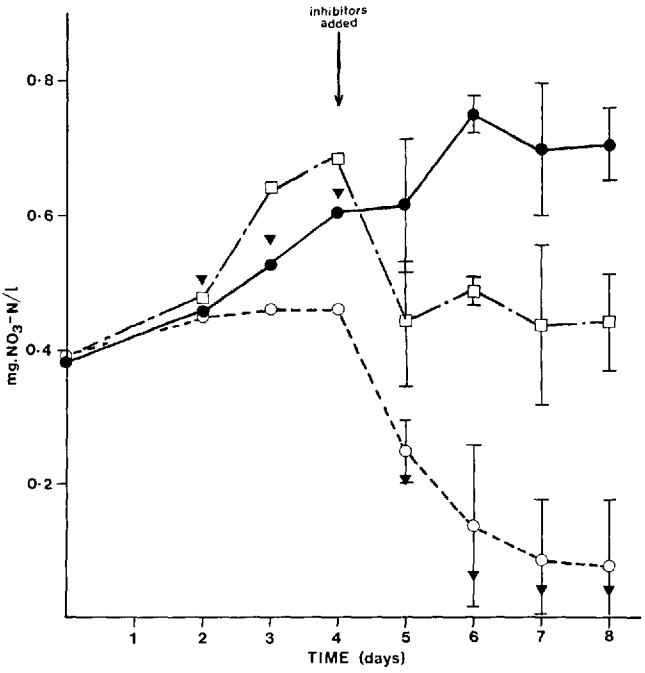

Fig. 2. The effect of 10 mg/liter N-Serve (○---○) and ATU (□---□) and the acetone control (▼) on nitrate production by enrichment cultures of chemolithotrophic nitrifying organisms under static conditions. Vertical bars indicate 95% confidence limits. Control ●——●.

nitrate production resumed in both inhibitor treatments. The rate in the N-Serve treatments recovered to 0.185 mg N/liter/day whereas that in the ATU treatment was only 0.035 mg N/liter/day. Although nitrification resumed 24 hours after the addition of both inhibitors, the reduced rate of nitrate production observed with the ATU treatment indicates that this was the most persistant of the 2 inhibitors over the maximum incubation time of 2.5 days. To preserve clarity, the results of the acetone control treatment have not been included although inhibitory effects were noted, there being no significant difference ($P = 0.05$) between acetone and N-Serve treatments. N-Serve is known to be volatile and this is one of the factors affecting its use as an inhibitor of nitrification [12, 24]. The recovery of nitrification noted in Fig. 1 could be due to the rapid loss of N-Serve, or the carrier solvent acetone, during aeration. The results of a similar experiment, but without aeration, are shown in Fig. 2. Both inhibitors and the acetone control inhibited nitrification; moreover, the inhibitory effect was prolonged over the full incubation period. Again the decrease in nitrate concentrations for 24 hours after the addition of the inhibitor was observed. This decrease was greater than the initial fall noted under the aerated conditions. After this phase, nitrate concentrations remained stable in the ATU treatment but continued to decrease in both the N-Serve and acetone treatments. The control cultures probably become oxygen or ammonia limited after 6 days incubation.

Table 3. Production of ammonia by gluten enrichment cultures (incubated at room temperature for 72 hours) after treatment with (a) 10 mg/liter N-Serve, (b) the acetone control, and (c) 10 mg/liter ATU (values are expressed as percentage production of control)

	N-Serve	Acetone control	ATU
1	83	86	98
2	90	89	100

Table 4. Removal of nitrate from anaerobic sediment slurries (incubated at 10°C for 48 hours) in the presence of (a) 10 mg/liter N-Serve, (b) the acetone control, and (c) 10 mg/liter ATU (values are expressed as a percentage of removal in control)

	N-Serve	Acetone control	ATU
1	198	318	145
2	198	158	152

It is apparent that, at the concentration added (0.79 g/liter), acetone inhibits nitrification and that the persistence of both N-Serve and acetone is increased under static conditions. Further evidence for the loss of N-Serve and acetone from the aerated cultures came from the recovery efficiencies of the nitrate analysis of the cultures. In the aerated cultures, the recovery of nitrate 24 hours after the addition of N-Serve and acetone was 30%; this increased to between 75 and 80% after 48 hours. In the unaerated cultures, the recovery in the presence of N-Serve or acetone never exceeded 20% over the incubation period. Under the conditions of the nitrification rate measurement, ATU appears to be the more efficient inhibitor of nitrification.

The results of 2 experiments showing the effect of 10 mg/liter N-Serve, the acetone control, and 10 mg/liter ATU on ammonification of gluten in an enrichment culture are shown in Table 3. The production of ammonia is expressed as a percentage of the production in control cultures. These percentages were only calculated if the ammonia concentrations between treatments and controls were significantly different ($P = 0.05$). In the first experiment, all treatments showed a significant reduction in ammonia production over the control cultures. The N-Serve and acetone control treatments were not significantly different but both were significantly lower ($P > 0.05$) than the ATU treatment. Similar results were obtained in the second experiment except that ATU had no effect on ammonia production. From these results it appears that acetone has an inhibitory effect on nitrogen mineralization from gluten by the organisms present in this enrichment culture. Allylthiourea may have a slight inhibitory action but this appears to be variable.

The effect of N-Serve, the acetone control, and ATU on nitrate removal

from anaerobic sediment is shown in Table 4. The results are expressed as a percentage of the removal in control vials. All treatments showed a stimulation of nitrate metabolism but the magnitude of this stimulation did not show any consistent trends.

Discussion

The observation that ammonia accumulated in aerobic water overlying sediments in which nitrification was inhibited was interesting. In his classic paper, Mortimer surmised that the bulk of ammonia released from the sediments was associated with the reduction of ferric to ferrous iron and the subsequent increase in solubility of base complexes. Therefore, an oxidized surface mud presented a barrier to ammonia release due mainly to its cation binding potential and also, to a smaller extent, the occurrence of nitrification [27]. In sediment cores which maintained an aerobic sediment water interface but received a nitrification inhibitor, ammonia was released into the overlying water. It would be interesting to compare ammonia release due to chemical inhibition of nitrification and that due to the onset of reducing conditions. The latter would cause chemical changes in the sediment as well as inhibiting nitrification. The lower estimate of the nitrification rate using dNO_3 as opposed to dNH_3 was not unexpected as nitrate could diffuse deeper into the sediments where it might be dissimilated. The maintenance of high oxygen concentrations in the overlying water would cause the redox discontinuity layer, a region of maximal nitrate reductase activity [27], to be structured at some depth into the sediments. The present studies on the effects of the inhibitors on anaerobic nitrate metabolism indicate that this activity will be stimulated.

There have been a number of studies on the effect of N-Serve on denitrification [17, 28, 29, W. H. Cribbs, Ph.D. thesis, University of Georgia, Athens, U.S.A., 1978]. N-Serve concentrations of up to 10 ppm were found to have no effect on denitrification by a precultured *Pseudomonas* spp. whereas up to 50 ppm had no effect on soil denitrification. The carrier solvent (ethanol, final concentration 1%) had been removed from the soil before the test began [17]. High concentrations of N-Serve (50 ppm) initially stimulated N_2O evolution from soil [Cribbs, Ph.D. thesis, 1978] whereas field level application (0.2–20 ppm) was inhibitory to N_2O and N_2 evolution [28]. An accelerated loss of nitrate has been observed with N-Serve in sand culture experiments. Similar results were obtained if the carrier solvent, acetone, was added alone [29].

It is difficult to compare published results on the bio-activity of N-Serve as many factors are involved. The extent to which individual factors are manifest also depends on the chemical composition of the environment. These have been discussed in more detail elsewhere [24]. Also various carrier solvents have been used and the final concentrations of these in the samples could also affect microbial activity in the sample [5]. The author is unaware of any studies on the bioactivity of ATU except towards the nitrifying bacteria and apparently related organisms [6, 20]. However, the effect of thiourea on anaerobic nitrate removal has been shown to be variable [21].

The apparent inhibition of nitrogen mineralization from gluten, particularly

by acetone, is difficult to interpret in terms of its effect on the nitrification-rate estimate. As acetone is added to both the inhibited and uninhibited mini-core series, the inhibition would be the same in all cores. Therefore, any effect on the nitrification rate would only occur if the rate was dependent upon the ammonia concentration. Different genera of ammonia oxidizing organisms appear to have different kinetics [1] but little is known on the kinetics of ammonia oxidase. As the nitrification rate estimated never accounted for more than 40% of the total extractable ammonia pool, the effect of the solvent on nitrogen mineralization might be negligible. In addition, it is not known whether the total KCl extractable ammonia pool is available to the nitrifying organisms and whether, during the incubation times used in these experiments, only the interstitial water ammonia fraction would be involved.

Using an isotope dilution technique 20 mg/liter N-Serve, and the acetone equivalent, was found to have no effect on ammonia production in aerobic marine sediment slurries [18]. The present results indicate that 10 mg/liter N-Serve, and the acetone equivalent inhibited this activity in freshwater enrichment cultures. The N-Serve stock solution concentrations were the same in both investigations. This could indicate that different species of ammonia-producing microorganisms exhibit different sensitivity towards N-Serve and acetone.

There is little doubt that both N-Serve and ATU, at concentrations of 10 mg/liter, are inhibitory towards the initial oxidation of ammonia to nitrite [6, 15, 21, 26, 33, 36]. However, many factors are known to affect the degree of inhibition affected by N-Serve and these have been reviewed [24]. There have been few studies on the factors that affect the control of nitrification by ATU [6, 26, 36] and, as yet, little is known, for example, on the effect of pH, temperature, or species specificity. In the present work, it is apparent that the volatility of N-Serve is a major factor affecting its activity. In addition, the carrier solvent, acetone, at the concentration applied, also inhibits nitrification.

These effects partly explain the higher estimates of nitrification rates obtained with ATU. However, as diffusion is relied upon to disperse the inhibitor through the sediment, it would be expected that a water soluble compound would be more effective than N-Serve which requires an organic carrier solvent. N-Serve is now available in a water soluble form [32] and comparisons between this formulation and ATU would clearly be useful. Alternatively, regular additions of N-Serve could be made to maintain inhibitory concentrations [18]. However, the present results on the effects of acetone on nitrogen transformations in Grasmere sediments indicate that this would not be satisfactory. The effects of ATU on the general activity of the heterotrophic microflora is, however, less well documented than those of N-Serve and more research in this area would be desirable.

The mini-core method used to estimate nitrification rates in the present work is time consuming. Also, aeration of the overlying water, which is necessary to maintain oxidizing conditions, may increase the oxygen concentration and therefore, due to increasing the concentration gradient, cause the redox potential discontinuity layer to be structured deeper into the sediments. This could result in overestimation of the actual nitrification rate [18]. However, the ease of manipulation of the subsamples does allow great flexibility. The ability to

control the *in situ* environment for nitrification provides a useful research tool for investigating not only the *in situ* rates of activity but also the factors that affect the ecology of the chemolithotrophic nitrifiers in sediments.

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