Microb Ecol (1995) 30:269–284

MICROBIAL ECOLOGY

Estimation of Nitrification Rates in Flooded Soils

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Received: 13 July 1994; Revised: 19 January 1995

Abstract. Three techniques for estimating nitrification rates in flooded soils were evaluated in short-term incubation experiments using three soils. The techniques were based on inhibition of either ammonium or nitrite oxidation and ^{15}N isotope dilution. Of four inhibitors of ammonium oxidation evaluated, one (allylthiourea) was ineffective and two (2-ethynylpyridine or phenyl acetylene dissolved in ethanol) promoted immobilization of ammonium. Emulsified 2-ethynylpyridine and acetylene were equally effective inhibitors of ammonium oxidation and had little or no effect on gross rates of N mineralization and immobilization. Four inhibitors of nitrite oxidation were evaluated, but this approach was compromised by the nonspecificity of three of the compounds—potassium cyanide, 2-ethylamino-4-isopropylamino-6-methylthio-s-triazine (ametryne) and 3-(3,4-dichlorophenyl)-1-methylurea (DMU)—and by the partial effectiveness of another (potassium chlorate). Two methods based on isotope dilution gave similar estimates of nitrification rates. These rates were similar to those estimated by inhibition of ammonium oxidation in one soil but were lower in the other two soils. In the latter two soils, nitrification of labeled ammonium derived from dissimilatory nitrate reduction resulted in underestimation of nitrification rates by isotope dilution.

Introduction

Nitrification occurs in flooded soils in a thin oxidized layer at the soil–water interface [30]. Nitrate produced in this aerobic layer may diffuse to the soil below, where anaerobic conditions are conducive to biological denitrification. The potential for loss of N via this pathway is greatly enhanced by addition of fertilizer N to the floodwater. The recovery of fertilizer N by the rice crop is often low [13], with

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indirect estimates of losses that are due to denitrification ranging from 0 to 34% of the applied N [15]. Information on nitrification rates in flooded soils would assist in the development of management strategies to increase the agronomic efficiency of N fertilizers.

Estimation of the nitrification rate by measuring the rate of production of nitrate is precluded in flooded soil by the simultaneous occurrence of nitrification and nitrate reduction in close proximity. Several techniques have been developed for estimating nitrification rates in freshwater and marine sediments, but few applications in flooded soils have been reported. Nitrification rates in sediments have been estimated by (a) measuring the difference in ammonium concentration in the presence and absence of inhibitors of ammonium oxidation (e.g., nitrapyrin, allylthiourea) [17, 19]; (b) measuring the difference in nitrite concentration in buffered slurries incubated for 24 h in the presence and absence of an inhibitor of nitrite oxidation (e.g., chlorate) [4]; (c) measuring short-term (2–5 h) incorporation of ^{14}C-bicarbonate in the dark, in the presence and absence of nitrapyrin [5]; and (d) measuring the change in the size and isotopic composition of the nitrate pool labeled with ^{15}N [24].

The objective of the present investigation was to evaluate methods with potential for estimating in situ rates of nitrification in flooded soils. The ^{14}C-bicarbonate method was not chosen, because of perceived difficulties associated with both calibration (i.e., variable ratios of C fixed to NH_4^+-N oxidized) [21] and in situ application. On the other hand, the efficacy of techniques that employ chemical inhibition of nitrification is entirely dependent on inhibitor effectiveness and specificity. We report the results of a comparison of ^{15}N isotope dilution and inhibition techniques for assessing short-term nitrification rates in three flooded soils. Several inhibitors of either ammonium or nitrite oxidation were evaluated. A comparison of gross rates of N mineralization and immobilization in control and inhibitor-treated soils was used to assess the effect of inhibitors of ammonium oxidation on heterotrophic activity.

Materials and Methods

Soils

Two alkaline soils (Narrabri and Griffith, New South Wales, Australia) and an acid soil (Tatura, Victoria, Australia), were selected to provide a range in nitrification rates. Composite samples taken to a depth of 0.15 m were air dried, ground to pass through a 2-mm sieve, and well mixed before use. Some properties of the soils are given in Table 1.

Incubation Studies

Preliminary experiments were conducted using methods based on inhibition of both ammonium and nitrite oxidation using a single soil (Narrabri) incubated in a glasshouse under a variable day/night temperature regimen of 25/12°C for 10 days. A greater degree of control was exercised in subsequent experiments that were of shorter duration (up to 54 h) at a constant temperature of 30°C. These laboratory-based studies were designed to provide wider applicability of results through the use of a wider range of soils (Table 1) and methods, with a narrowed focus on the use of methods based on selective inhibition of nitrification.

Table 1. Properties of the soils

Soil			CEC[d]	Nitrogen (g kg^{-1})			Clay
Location[a]	Taxonomy[b]	pH[c]	(cmol kg^{-1})	Total	NH_4^+	NO_3^-	(g kg^{-1})
Griffith	Typic Pelloxerert	8.4	36	1.1	0.0043	0.0082	500
Narrabri	Typic Pellustert	8.2	35	1.0	0.0026	0.0088	360
Tatura	Typic Haplustalf	6.8	13	0.8	0.0019	0.1320	300

[a]Griffith (34°21′S;146°02′E); Narrabri (30°20′S;149°49′E); and Tatura (36°24′S;145°14′E).
[b]SSS [34].
[c]1:5, Soil/water.
[d]Cation exchange capacity.

Air-dried soil (20 g) was placed in 120-ml bottles (25-mm radius and 80-mm high), and 40-ml water was added to give a floodwater depth of ~40 mm. Bottles were left open to the atmosphere. Water lost by evaporation was replenished every day. Samples were preincubated for 7 days to establish stratification in O_2 status within the soil before treatments were applied. The alkaline Narrabri and Griffith soils were adjusted to pH 6.5–7.0 at time zero (and every 2 days during incubation in the glasshouse) to minimize error resulting from NH_3 volatilization when estimating nitrification rates by inhibiting the oxidation of ammonium.

Each treatment was replicated three times, and sufficient bottles were prepared to allow for periodic sampling during incubation for determination of inorganic N and also for analysis of organic N when labeled N was added.

Inhibition of Ammonium Oxidation Four inhibitor treatments—phenylacetylene (PA), 2-ethynylpyridine (2EP), allylthiourea (ATU), acetylene (C_2H_2), and a control (distilled water)—were included in the glasshouse experiment. Inhibitor solutions (0.02 M) were prepared using ethanol (95%) as the solvent for PA and 2EP and distilled water for ATU. The required concentration of inhibitor (0.2 mmol kg^{-1} soil) was provided by adding 0.2 ml solution per bottle. The inhibitor concentration (approximately 20 mg kg^{-1} soil) was within the range of 10–50 mg kg^{-1} soil used by McCarty and Bremner [27]. The soils treated with PA and 2EP contained 10 μl ethanol g^{-1} (4.7 mg C g^{-1} soil). C_2H_2 was bubbled into the flooded soil for 5 min on one occasion only at the beginning of the incubation. C_2H_2 was passed through traps of concentrated H_2SO_4 and water to remove residual acetone [35]. Labeled ammonium sulfate (20.118 atom % ^{15}N) was added at 150 μg N g^{-1} soil.

Two inhibitor treatments (C_2H_2 and emulsified 2EP) and a distilled water control were included in the laboratory experiment. The inhibitor concentrations were the same as in the glasshouse experiment. The effect of concentration of added ammonium (25, 50, 100, and 150 μg N g^{-1} soil) on production of nitrate in the Narrabri soil was determined in the control treatment. Based on the results of this experiment, labeled ammonium sulfate (20.118 atom % ^{15}N) was added at 25 μg N g^{-1} soil in the inhibitor experiment.

Inhibition of Nitrite Oxidation Four compounds reported to inhibit nitrite oxidation were used: potassium chlorate [25], potassium cyanide [32], 2-ethylamino-4-isopropylamino-6-methylthio-s-triazine (ametryne, AMT) [14], and 3-(3,4 dichlorophenyl)-1-methylurea (DMU) [9]. The inhibitors were dissolved in distilled water, and $KClO_3$, KCN, AMT, and DMU were applied at 20, 2, 1, and 1 mmol kg^{-1} soil, respectively. A distilled water control was included in the experiment, which was conducted only in the glasshouse.

Isotope Dilution Unlabeled ammonium sulfate and labeled potassium nitrate (27.990 atom % ^{15}N) were added in solution at 25 and 2.5 μg N g^{-1} soil, respectively. The experiment was conducted only in the laboratory. The measured changes in the size and ^{15}N enrichment of the nitrate pool were used to calculate rates of nitrate production (nitrification) and nitrate reduction by the model of Koike and Hattori [24]. The model proposed by Barraclough et al. [3] was also used to estimate nitrification rates.

Chemical Analysis

In the inhibition of ammonium oxidation experiments, exchangeable $NH_4^+ + NO_2^- + NO_3^-$ was extracted by shaking the sample for 1 h with 40-ml 4 M KCl (analytical grade). In the inhibition of nitrite oxidation and isotope dilution experiments, $NO_2^- + NO_3^-$ was extracted with 40 ml 0.4 M KCl. The KCl solutions contained 10 $\mu g\ ml^{-1}$ phenylmercuric acetate to inhibit microbial activity. Suspensions were centrifuged at 3000 rpm for 5 min, the supernatant was decanted, and the soil extracted with 4 M KCl was washed three times with 50 ml 0.4 M KCl. The soil was dried at 60°C and then ground to <0.15 mm.

Ammonium in an aliquot of the extract was determined by steam distillation [22]. Nitrate in extracts was reduced to nitrite on a copperized cadmium column, using procedures described for extracts containing labeled [8] and unlabeled [22] nitrate. Nitrite was determined colorimetrically by the Griess-Ilosvay method.

Total soil N was determined by semimicro Kjeldahl digestion and steam distillation [6]. Labeled distillates and eluents were dried, and isotope ratios were determined on N_2 generated by hypobromite oxidation (distillates) or reaction with sulphamic acid (eluents) [8]. Isotope ratios were measured on a VG Isogas (Sira 10) mass spectrometer equipped with dual inlets and triple collectors.

Nomenclature and Calculations

Nitrogen pools and N transformation rates were identified by the nomenclature used by Smith et al. [33]: *AL*, labeled exchangeable ammonium-N; *AT*, total (i.e., labeled + unlabeled) exchangeable ammonium-N; *NL*, labeled nitrate-N; *NT*, total (i.e., labeled + unlabeled) nitrate-N; *OL*, labeled organic-N; *m*, gross N mineralization rate; *i*, gross N immobilization rate; *n*, nitrification rate; *r*, nitrate reduction rate. The subscripts $_{1,2}$ and $_a$ denote the initial, final, and arithmetic mean of a pool at two consecutive sampling times, respectively. Δt denotes an interval of time.

Gross N Mineralization. A zero-order model was used to estimate gross rates of N mineralization in samples labeled with $^{15}NH_4^+$. The model, developed by Kirkham and Bartholomew [23], was expressed by Smith et al. [33] as

$$m = [(AT_1 - AT_2)/\Delta t] \log (AL_1AT_2/AL_2AT_1)/\log (AT_1/AT_2) \quad (1)$$

Gross N Immobilization. Gross rates of N immobilization were estimated by a zero-order model in samples labeled with $^{15}NH_4^+$. The model was based on changes in the *AL*, *AT*, and *OL* pools [31] and was expressed by Smith et al. [33] as

$$i = [(OL_2 - OL_1)/\Delta t]\ (AT_a/AL_a) \quad (2)$$

Nitrification and Nitrate Reduction. The nitrification rate in the method based on inhibition of ammonium oxidation was calculated as

$$n = [(AT_2 - AT_1)_{+\text{inhibitor}} - (AT_2 - AT_1)_{-\text{inhibitor}}]/\Delta t$$

The isotope-dilution model of Koike and Hattori [24] was expressed as

$$N_2 - N_1 = Z - Y \quad (3)$$

and

$$N_2X_2 - N_1X_1 = Z\overline{X}_a - Y\overline{X} \quad (4)$$

where Y is the amount of nitrate reduced during the period $t_2 - t_1$; Z is the amount of nitrate produced by nitrification during the same period; N_1 and N_2 are the amounts of nitrate-*N* present at t_1 and t_2, respectively; X_1 and X_2 are the ^{15}N abundances (atom %) of the nitrate pool at times t_1 and t_2, respectively; $\overline{X}_a$ is the ^{15}N abundance of the ammonium pool (assumed to be 0.366 atom %); $\overline{X}$ is the

arithmetic mean ^{15}N abundance (atom %) of the nitrate pool during the period $t_2 - t_1$ [i.e., $\overline{X} = (X_1 + X_2)/2$].

The solution of these simultaneous equations for rates of nitrification and nitrate reduction, expressed in the standard nomenclature, follows:

If E_f = the ^{15}N enrichment of the labeled nitrate source (atom % excess),
E'' = the ^{15}N enrichment of the sample nitrate (atom % excess), and
V = the proportion of nitrate-N derived from the labeled source,
then $V = NL/NT = E''/E_f$

Expressing Eq. 4 in terms of ^{15}N enrichment (atom % excess),

$$N_2E_2'' - N_1E_1'' = -Y(E_2'' + E_1'')/2$$

Dividing E_1'' and E_2'' by E_f,

$$N_2V_2 - N_1V_1 = -Y(V_2 + V_1)/2$$

Transforming to the standard nomenclature and rearranging,

$$r = [(NL_1 - NL_2)/\Delta t]/(NL/NT)_a \tag{5}$$

Transforming Eq. 3 to the standard nomenclature,

$$NT_2 - NT_1 = (n - r)\,\Delta t \tag{6}$$

Substituting Eq. 5 into Eq. 6 and rearranging,

$$n = \{(NT_2 - NT_1) - [(NL_2 - NL_1)/(NL/NT)_a]\}/\Delta t \tag{7}$$

The model proposed by Barraclough et al. [3] to estimate nitrification rates was expressed by Barraclough [2] as

$$N_t^* = N_0^*/(1 + \phi\, t/N_0)^{n/\phi} \tag{8}$$

where $\phi = (N_t - N_0)/t$; N_0 and N_0^* are the size and ^{15}N enrichment (atom % excess) of the nitrate pool at time zero, respectively; N_t and N_t^* are the size and ^{15}N enrichment of the nitrate pool at time t, respectively; t is elapsed time, and n is the nitrification rate. The expression of Eq. 8 in standard nomenclature follows. Rearranging Eq. 8,

$$n = [(N_t - N_0)/t]\log(N_0^*/N_t^*)/\log(N_t/N_0)$$

Dividing N_0^* and N_t^* by E_f,

$$n = [(N_t - N_0)/t]\log(V_0/V_t)/\log(N_t/N_0)$$

Transforming to the standard nomenclature and rearranging,

$$n = [(NT_1 - NT_2)/\Delta t]\log(NL_1NT_2/NL_2NT_1)/\log(NT_1/NT_2) \tag{9}$$

Analysis of Data. Data were analyzed statistically using the analysis of variance procedure of Minitab, where time was included as an independent variable.

Results and Discussion

Nitrification

Inhibition of Ammonium Oxidation. Nitrate concentrations were significantly lower in the Narrabri soil treated with C_2H_2, PA, and 2EP compared to the control after

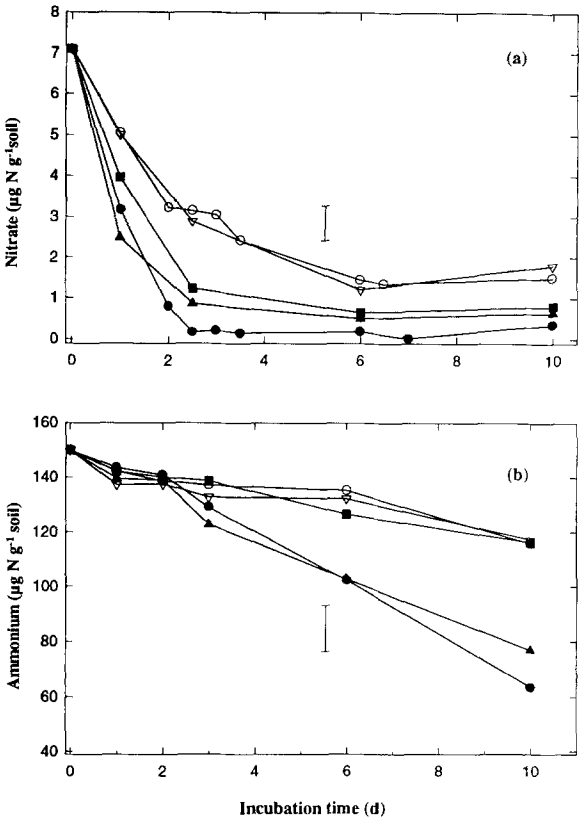

Fig. 1. Concentrations of **(a)** nitrate and **(b)** exchangeable ammonium in flooded Narrabri soil in the control (○), 2EP (●), PA (▲), ATU (▽), and C_2H_2 (■) treatments. Bars represent least significant differences ($P < 0.05$).

incubation for 1 day in the glasshouse (Fig. 1a), indicating that nitrification was inhibited by these compounds. Little or no difference was observed between 2EP, C_2H_2, and PA in their ability to inhibit ammonium oxidation. Nitrate concentrations in the control and ATU treatments were similar (Fig. 1a), indicating that ATU was ineffective in inhibiting nitrification. The rapid decline in nitrate concentration in the first few days was followed by little change, indicating equilibrium between production and reduction of nitrate (Fig. 1a).

Concentrations of ammonium declined in all treatments during incubation of the Narrabri soil in the glasshouse (Fig. 1b). Although 2EP and PA inhibited nitrification, less ammonium was present in these treatments than in the control, ATU, and C_2H_2 treatments after incubation for 2 days (Fig. 1b). Concentrations of ammonium in the PA and 2EP treatments were only about one half of those in the other treatments at day 10 (Fig. 1b). Enhanced immobilization of the added ammonium by microorganisms capable of using the ethanol solvent as a carbon source could explain this result. This was confirmed by the recoveries of added $^{15}NH_4^+$ in the organic N pool. More than 60% of added ammonium was immobilized in the presence of 2EP and PA after incubation for 10 days, whereas less than 30% was immobilized in the control treatment (Table 2). The enhanced immobilization of ammonium in 2EP and PA treatments precluded estimation of the nitrification rate.

Table 2. Recovery (%)[a] of applied $^{15}NH_4^+$ as exchangeable ammonium and organic N (data in parentheses) in flooded Narrabri soil with and without addition of nitrification inhibitors

Time (days)	Control	2EP	PA	ATU	C_2H_2	LSD[b]
0	95.8 (1.7)	95.8 (1.7)	95.8 (1.7)	95.8 (1.7)	95.8 (1.7)	0.0 (0.0)
1	87.8 (4.3)	85.7 (12.4)	81.7 (8.2)	82.5 (4.2)	85.2 (4.6)	11.7 (2.1)
2	80.9 (10.7)	78.5 (18.4)	75.5 (13.9)	ND[c]	79.3 (10.1)	5.4 (6.8)
3	76.3 (12.0)	71.5 (27.1)	69.5 (24.0)	75.7 (ND)	76.2 (11.8)	7.5 (7.1)
6	72.0 (17.2)	55.9 (41.1)	55.7 (38.5)	71.4 (ND)	70.6 (16.2)	9.7 (5.9)
10	59.0 (28.1)	33.2 (60.6)	38.2 (60.6)	59.8 (23.4)	60.6 (25.1)	9.8 (13.3)

[a]Data are means of three replicates.
[b]LSD, least significant difference ($P < 0.05$).
[c]ND, not determined, because of sample loss.

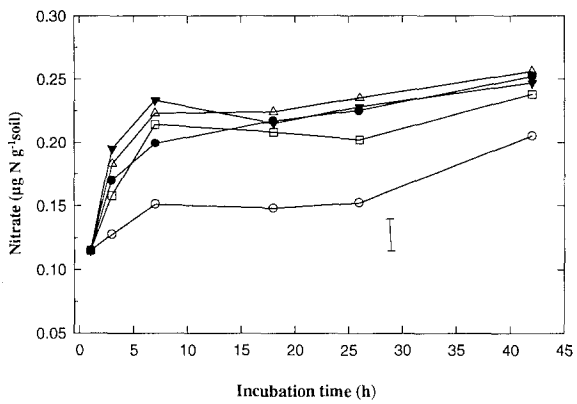

Fig. 2. Concentrations of nitrate in flooded Narrabri soil following addition of nil (○), 25 (●), 50 (□), 100 (△), and 150 (▼) μg NH_4^+-N g^{-1} soil. Vertical bar represents the least significant difference ($P < 0.05$).

Acetone has been employed as a solvent for inhibitors that were used to estimate nitrification rates in sediments by blockage of ammonium oxidation [17, 19]. The present study in flooded soil and previous work in well-aerated soils [10] have shown that neither ethanol nor acetone should be used to dissolve nitrification inhibitors when they are used to estimate nitrification rates, because the organic solvents promote immobilization of ammonium.

C_2H_2 inhibited nitrate production (Fig. 1a) but did not significantly affect the ammonium concentration relative to the control (Fig. 1b). The results therefore suggest that the nitrification rate was low, being masked by the high background concentration of substrate ammonium. C_2H_2 did not promote immobilization of labeled ammonium (Table 2). Previous incubation studies with acetone-free $^{14}C_2H_2$ under anaerobic conditions showed that very little C_2H_2 was oxidized to CO_2 within 3 days [16] or 7 days [35]. Therefore, C_2H_2, unlike ethanol, does not provide an immediate source of carbon for the heterotrophs. It is essential, however, that residual acetone be removed from the C_2H_2 before use, because the contaminant can promote immobilization of ammonium [10].

A decrease in the concentration of added ammonium from 150 to 25 μg N g^{-1} soil did not affect nitrate production in the Narrabri soil (Fig. 2). Hence, 25 μg NH_4^+-N g^{-1} soil was chosen for the laboratory incubation study to achieve greater

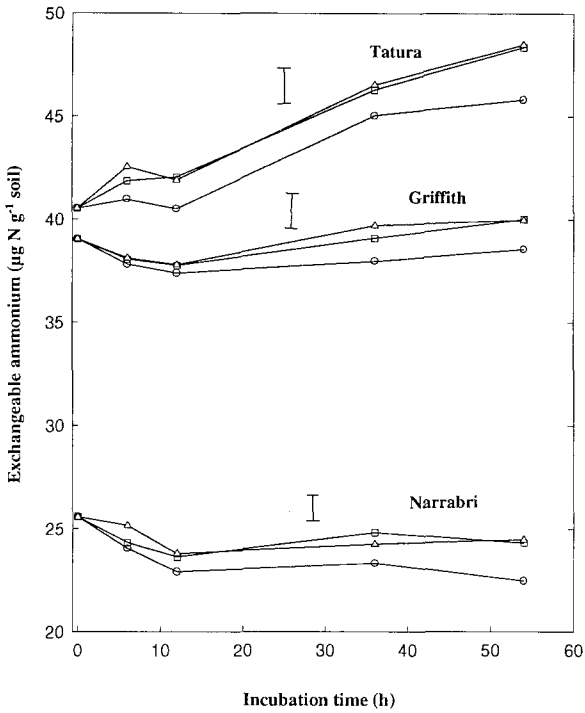

Fig. 3. Concentrations of exchangeable ammonium in three flooded soils in the control (○), emulsified 2EP (△), and C_2H_2 (□) treatments. Vertical bars represent least significant differences ($P < 0.05$).

sensitivity. Additional modifications included the discarding of organic solvents, the use of a wider range of soils, and the use of fewer inhibitors.

A significant increase in the ammonium concentration was observed in the Tatura soil after 30 h of incubation in all treatments (Fig. 3). In contrast, little or no change in the ammonium concentration was observed in the Griffith soil, and a significant decrease was observed in the Narrabri soil. Nevertheless, higher concentrations of ammonium were present in all soils at the end of incubation in emulsified 2EP and C_2H_2 treatments compared to the control, with no difference between inhibitor treatments.

Estimated nitrification rates ranged from 19 to 96 ng N g^{-1} h^{-1} (Table 3), with similar estimates for the 2EP and C_2H_2 treatments. Nitrification rates declined throughout incubation in the Griffith and Narrabri soils and during 0–36 h in the Tatura soil (Table 3). The trend may reflect the microbial depletion of oxygen that was dissolved when the system was set up. Nitrification rates were undoubtedly constrained by the slow diffusion of O_2 in the flooded system.

Inhibition of Nitrite Oxidation. Nitrite accumulated after 1 day of incubation in all treatments, including the control, but thereafter the concentration fell (Fig. 4a). Nitrite concentrations were generally very low, however (<2.5 μg N g^{-1} soil), even when inhibitors were added (Fig. 4a).

Concentrations of both nitrite (Fig. 4a) and nitrate (Fig. 4b) in the DMU treatment were consistently lower than in the control, suggesting that this compound inhibited the oxidation of ammonium to nitrite. While Corke and Thompson [9] reported

Table 3. Nitrification rates (n) (ng N g^{-1} soil h^{-1})[a] estimated by inhibition of ammonium oxidation and ^{15}N dilution techniques in three flooded soils

		Nitrification inhibitors[b]			^{15}N dilution	
Soil	Time (h)	C_2H_2	Emulsified 2EP	Time (h)	Barraclough et al. [3][c]	Koike and Hattori [24][d]
Griffith	0–6	72 ± 16	77 ± 10	0–6	34 ± 6	37 ± 6
	6–12	47 ± 18	44 ± 14	6–12	18 ± 2	20 ± 4
	12–24	ND[e]	ND	12–24	18 ± 2	19 ± 1
	12–36	39 ± 16	39 ± 9	24–36	ND	ND
	36–54	27 ± 10	26 ± 8	36–54	ND	ND
Narrabri	0–6	91 ± 18	80 ± 22	0–6	33 ± 9	35 ± 10
	6–12	67 ± 17	72 ± 13	6–12	12 ± 4	12 ± 3
	12–24	ND	ND	12–24	12 ± 3	13 ± 3
	12–36	46 ± 13	26 ± 11	24–36	12 ± 1	13 ± 1
	36–54	21 ± 12	19 ± 6	36–54	11 ± 2	12 ± 2
Tatura	0–6	84 ± 14	96 ± 16	0–6	93 ± 13	96 ± 14
	6–12	64 ± 17	63 ± 14	6–12	77 ± 10	79 ± 11
	12–24	ND	ND	12–24	70 ± 9	72 ± 11
	12–36	28 ± 8	39 ± 13	24–36	68 ± 14	69 ± 10
	36–54	29 ± 9	34 ± 11	36–54	37 ± 8	38 ± 13

[a] Data are means of three replicates ± standard deviation.
[b] $[(AT_2 - AT_1)_{+\text{inhibitor}} - (AT_2 - AT_1)_{-\text{inhibitor}}]/\Delta t$.
[c] $[(NT_1 - NT_2)/\Delta t]$ log $(NL_1\ NT_2/NL_2\ NT_1)$/log (NT_1/NT_2).
[d] $\{(NT_2 - NT_1) - [NL_2 - NL_1)/(NL/NT)_a]\}/\Delta t$.
[e] ND, not determined, because of absence of 24-h sampling (inhibitor treatments) or negligible nitrate concentrations (^{15}N dilution).

marked inhibition of *Nitrobacter* by DMU (as seen by nitrate accumulation), evidence indicated that *Nitrosomonas* was also inhibited as a result of a lag in nitrite formation. The extent to which DMU inhibited nitrite oxidation in the present study cannot be ascertained.

Concentrations of nitrite in the KCN and AMT treatments initially fall below, but then increased above, the control (Fig. 4a). Concentrations of nitrate in the KCN and AMT treatments were consistently lower than the control (Fig. 4b). Taken together, these two sets of data suggest that both compounds inhibited both *Nitrosomonas* and *Nitrobacter*. Previous work demonstrated that *Nitrobacter* grown in culture medium was inhibited by KCN [32] and that oxidation of nitrite in soils was inhibited by AMT [14]. The present study confirms these results but also shows that AMT is not a specific inhibitor of *Nitrobacter* as previously claimed [14].

Higher concentrations of nitrite (Fig. 4a) and lower concentrations of nitrate (Fig. 4b) were measured in the $KClO_3$ treatment compared to the control. $KClO_3$ was not completely effective in blocking *Nitrobacter*, as the concentration of nitrite fell between day 1 and day 2, and only increased beyond day 6. The extent to which $KClO_3$ may have inhibited ammonium oxidation cannot be gauged from the data, although it was claimed that *Nitrosomonas* would be inhibited by chlorite (ClO_2^-), formed from the reduction of ClO_3^- in flooded systems [20].

The $KClO_3$-induced nitrite accumulation at day 10 was modest (1.8 μg N g^{-1} soil, or an average of 7.5 ng N g^{-1} soil h^{-1}). By comparison, the average 2EP-induced ammonium accumulation at 54 h was 39 ng N g^{-1} soil h^{-1} (Table 3).

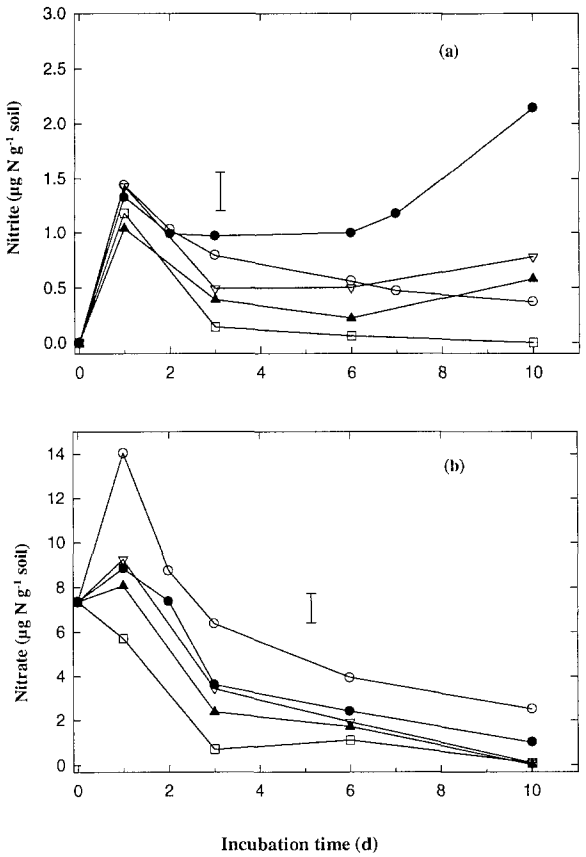

Fig. 4. Concentration of **(a)** nitrite and **(b)** nitrate in flooded Narrabri soil in the control (○), DMU (□), AMT (▽), KCN (▲), and $KClO_3$ (●) treatments. Vertical bars represent least significant differences ($P < 0.05$).

Thus, none of the inhibitors of nitrite oxidation that were tested were satisfactory. The nonspecificity of DMU, KCN, and AMT preclude their use in this technique. The failure of $KClO_3$ to completely inhibit *Nitrobacter*, and concerns about its specificity, also cast doubt on the efficacy of this inhibitor in flooded systems.

Isotope Dilution. Nitrification rates calculated by the models of Barraclough et al. [3] and Koike and Hattori [24] were not significantly different ($P < 0.05$) (Table 3). Nitrification rates were faster at the beginning of incubation than toward the end and ranged from 96 to <11 ng N g^{-1} soil h^{-1} (Table 3). Previous isotope dilution estimates of nitrification rates in unfertilized paddy soils ranged from between 4 and 10 ng N g^{-1} soil h^{-1} [18, 26] to between 10 and 30 ng N g^{-1} soil h^{-1} [37]. Much faster rates have been measured in N-fertilized soil. For example, a rate of 218 ng N g^{-1} soil h^{-1} (assuming a bulk density of 1 g cm^{-3}) was measured during 4–19 days after urea addition [26].

Nitrification rates estimated by isotope dilution were lower than estimates based on inhibition of ammonium oxidation in the Griffith and Narrabri soils, but similar estimates were obtained by the two methods during three of the four measurement intervals in the Tatura soil (Table 3). The reason for the inconsistency between

Table 4. Gross N mineralization ($m\ \Delta t$), immobilization of labeled ammonium ($\Delta\ OL$) and gross immoblization ($i\ \Delta t$) in three flooded soils incubated for 54 h in the presence and absence of two nitrification inhibitors

Soil	Treatment	N transformation (μg N g^{-1} soil)[a]		
		($m\ \Delta t$)[b]	($\Delta\ OL$)[c]	($i\ \Delta t$)[d]
Griffith	Control	8.7 ± 1.1	3.9 ± 0.2	6.8 ± 0.3
	C_2H_2	8.7 ± 0.7	3.4 ± 0.3	5.8 ± 0.6
	2EP	7.2 ± 1.1	3.7 ± 0.2	6.1 ± 0.3
Narrabri	Control	2.2 ± 0.4	4.0 ± 0.1	4.1 ± 0.1
	C_2H_2	1.8 ± 0.3	4.1 ± 0.2	4.2 ± 0.2
	2EP	1.5 ± 0.4	4.0 ± 0.4	4.0 ± 0.4
Tatura	Control	12.1 ± 1.1	2.0 ± 0.1	3.8 ± 0.2
	C_2H_2	10.7 ± 0.1	1.7 ± 0.3	3.5 ± 0.6
	2EP	11.6 ± 0.2	2.1 ± 0.1	4.1 ± 0.2

[a] Data are means of three replicates ± standard deviation.
[b] $(AT_1 - AT_2)\ \log\ (AL_1AT_2/AL_2AT_1)/\log\ (AT_1/AT_2)$.
[c] $OL_2 - OL_1$.
[d] $(OL_2 - OL_1)\ (AT_a/AL_a)$.

methods is not immediately apparent. If the inhibitor was ineffective, or if export of ammoniacal N was enhanced in inhibitor-treated samples through processes such as immobilization, volatilization, and fixation by clay and organic matter, the inhibitor method would underestimate nitrification. On the other hand, the isotope dilution method would underestimate nitrification if either organic N or ammonium derived from reduction of $^{15}NO_3^-$ were biologically oxidized to nitrate during incubation.

Mineralization and Immobilization

Marked differences were observed between soils in the amounts of gross N mineralization during incubation in the laboratory for 54 h (Table 4). The inhibitors had minimal effects on gross N mineralization in the three soils, confirming results obtained for emulsified 2EP [10] and for C_2H_2 [29] in soils incubated aerobically.

Substantial immobilization of labeled ammonium was measured in all three control soils after 54 h of incubation (16, 16, and 8% of applied ammonium in the Griffith, Narrabri, and Tatura soils, respectively) (Table 4). The nitrification inhibitors had little or no effect on immobilization of labeled ammonium, which was consistent with results obtained for nitrapyrin, emulsified 2EP, and 4-amino-1,2,4-triazole in soils incubated aerobically [7, 10].

Gross N immobilization was similar in the Narrabri and Tatura soils and was less than that in the Griffith soil (Table 4). Gross N immobilization exceeded gross N mineralization in the Narrabri soil, but not in the Griffith and Tatura soils (Table 4). Thus, the decrease in ammonium observed in the control treatment of the Narrabri soil during incubation (Figs. 1b, 3) was due to net N immobilization. Net N immobilization was also reported in the same soil during aerobic incubation [10]. Emulsified 2EP and C_2H_2 had little or no effect on gross N immobilization in all soils (Table 4), which is also consistent with previous results obtained for

2EP [10] and C_2H_2 [1, 29]. The results therefore confirm previous observations that nitrification inhibitors have insignificant effects on immobilization of soil and fertilizer N when the supply of available carbon is limiting heterotrophic activity.

Methods based on inhibition of ammonium oxidation assume that nitrification is an autotrophic process and that inhibition of autotrophic nitrification is specific and completely effective. It is assumed that the inhibitor does not affect other processes (e.g., mineralization, immobilization, ammonia volatilization) that interact with the ammonium pool. Thus, the lack of a significant effect of emulsified 2EP and acetone-free C_2H_2 on heterotrophic activity in incubated samples supports the use of these inhibitors of ammonium oxidation to estimate short-term nitrification rates, provided heterotrophic nitrification is not a significant process in flooded soils. Some evidence, however, indicates that in situ application of the method may be compromised by the presence of plant roots. For example, immobilization of ammonium was enhanced when ammonium oxidation was inhibited in soil planted with wheat [11], which was possibly due to an abundant supply of carbon in planted soil. The effects of heterotrophic nitrification and plant roots on the efficacy of the method based on inhibition of ammonium oxidation remain to be determined. The isotope dilution approach, on the other hand, can accommodate both autotrophic and heterotrophic nitrification, and it is independent of processes that remove N from the nitrate pool.

Nitrate Reduction

The concentrations of nitrate in the Griffith and Narrabri soils at the beginning of the isotope dilution experiment were low (<3.5 μg N g^{-1}), but approximately 70 of the initial 132 μg N g^{-1} soil (Table 1) remained in the Tatura soil following preincubation (Fig. 5a). Concentrations of nitrate were negligible (<0.5 μg N g^{-1}) after 20 h or 35 h in the Griffith and Narrabri soils, respectively, whereas a considerable amount remained in the Tatura soil at 54 h (Fig. 5a).

Rates of nitrate reduction varied from 200 (Narrabri soil) to >800 ng N g^{-1} soil h^{-1} (Tatura soil) during the first 6 h of incubation (Fig. 5b). The rates declined in all soils after 6 h. The nitrate reduction rate in the Tatura soil was maintained at >300 ng N g^{-1} soil h^{-1} during the 54 h of incubation. However, rates were <100 ng N g^{-1} soil h^{-1} in the Narrabri and Griffith soils after 6 h (Fig. 5b). Low substrate concentrations may have been a significant factor limiting nitrate reduction rates in these soils. Rates of nitrate reduction were between six and eight times faster than nitrification rates (Table 3) during incubation. Similarly, Lindau et al. [26] found that the rate of nitrate reduction (14 ng N g^{-1} soil h^{-1}) was between three and four times faster than the nitrification rate in unfertilized soil. Although the rate of nitrate reduction increased in urea-fertilized soil (25 ng N g^{-1} soil h^{-1}), it was only 11% of the nitrification rate measured in this treatment [26].

A fast rate of nitrate reduction may indirectly affect the accuracy of an isotope dilution estimate of the nitrification rate, because it has the potential to quickly reduce the size of the nitrate pool. Although a large change in the size of the labeled pool during the measurement interval is desired [12], large errors in estimating small pool sizes can have a profound effect on the accuracy of estimates of the N transformation rate, particularly when the rate is low [12]. Thus, accurate estimation

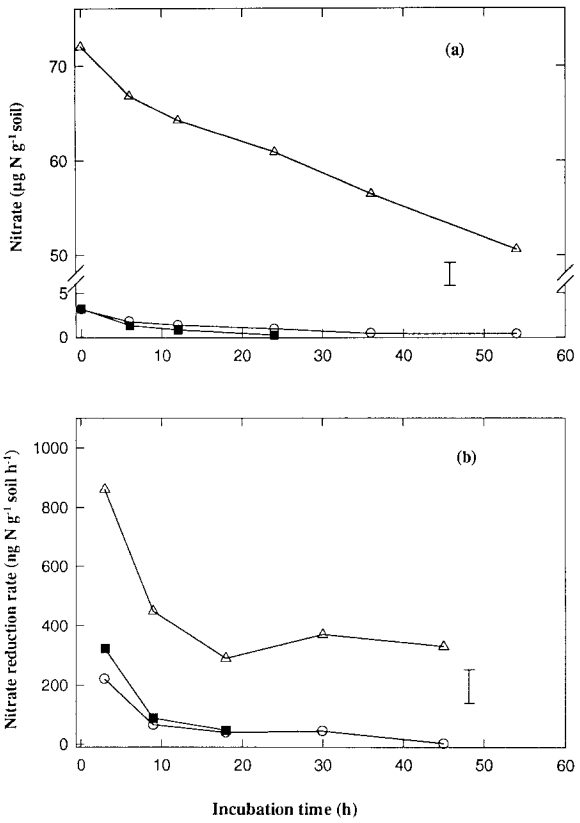

Fig. 5. Concentrations of **(a)** nitrate and **(b)** rates of nitrate reduction in flooded Griffith (■), Narrabri (○), and Tatura (△) soils. Vertical bars represent least significant differences ($P < 0.05$).

of n in flooded soils by isotope dilution may be of necessity limited to short periods of measurement, depending on the initial size of the nitrate pool and the respective rates of nitrate reduction and nitrification (Table 3, Fig. 5a).

Labeled N was measured in the ammonium and organic N pools in all soils after 6 h of incubation (Table 5). The percentages of labeled nitrate reduced to ammonium and organic N at 54 h varied between 0.6 and 14.5% and between 2.2 and 6.6%, respectively (Table 5). The amount of labeled nitrate reduced to ammonium was either greater (Griffith and Narrabri soils) or less (Tatura soil) than the amount reduced to organic N. The amounts of labeled nitrate reduced to ammonium at 54 h were between 16 (Narrabri soil) and 24 (Griffith soil) times greater than that in the Tatura soil.

The rapid and relatively greater amounts of labeled nitrate reduced to ammonium in the Griffith and Narrabri soils are important in respect of the estimation of nitrification by isotope dilution. Rates of nitrification in these soils estimated by isotope dilution were lower than rates estimated by inhibition of ammonium oxidation (Table 3). Labeled nitrate reduced to ammonium would be nitrified, resulting in the underestimation of n by the isotope dilution technique. Although rates of nitrate reduction were faster in the Tatura soil (Fig. 5b), less labeled nitrate was reduced to ammonium and organic N compared to that in the Griffith and Narrabri

Table 5. ^{15}N-labeled nitrate recovered in ammonium and organic N pools in three flooded soils

Soil	Time (h)	Ammonium N[a] ($\mu g\ g^{-1}$ soil)	Ammonium N[a] (%)	Organic N[a] ($\mu g\ g^{-1}$ soil)	Organic N[a] (%)
Griffith	6	0.14 ± 0.02	5.5	0.20 ± 0.04	7.8
	12	0.26 ± 0.02	10.3	0.13 ± 0.02	5.0
	24	0.34 ± 0.03	13.6	0.13 ± 0.01	5.3
	36	0.38 ± 0.06	15.2	0.15 ± 0.06	6.1
	54	0.37 ± 0.01	14.5	0.17 ± 0.01	6.6
Narrabri	6	0.14 ± 0.02	5.5	0.15 ± 0.01	6.0
	12	0.14 ± 0.01	5.7	0.16 ± 0.04	6.3
	24	0.21 ± 0.03	8.1	0.08 ± 0.02	3.2
	36	0.22 ± 0.01	8.8	0.12 ± 0.03	4.8
	54	0.24 ± 0.01	9.6	0.10 ± 0.02	4.1
Tatura	6	0.01 ± 0.00	0.3	0.05 ± 0.02	2.1
	12	0.01 ± 0.00	0.4	0.06 ± 0.02	2.4
	24	0.02 ± 0.01	0.6	0.02 ± 0.01	0.9
	36	0.03 ± 0.01	1.0	0.05 ± 0.01	2.1
	54	0.02 ± 0.00	0.6	0.06 ± 0.02	2.2

[a]Data are means of three replicates ± standard deviation.

soils, because of the large amount of indigenous unlabeled nitrate in the Tatura soil available for reduction. Consequently, errors in isotope dilution estimates of n induced by reduction of labeled nitrate to ammonium were much lower in the Tatura soil, and agreement between isotope dilution and inhibition of ammonium oxidation techniques was close.

Reduction of labeled nitrate to organic N may have proceeded directly via assimilatory nitrate reduction or indirectly by assimilation of labeled ammonium derived from dissimilatory nitrate reduction. However, the latter pathway is indicated, because assimilation of nitrate would have been inhibited by the relatively high concentrations of ammonium present (25–40 $\mu g\ N\ g^{-1}$ soil) during incubation [36]. Labeled organic N decreased after 6 h in the Griffith soil or 12 h in the Narrabri soil (Table 5). Labeled N assimilated by microorganisms can be remineralized or excreted as ammonium after short periods (1 day) of anaerobic incubation [16, 36]. Recycling of labeled ammonium assimilated by microorganisms would also contribute to errors in isotope dilution estimates of nitrification rates.

An alternative approach can be used to overcome the problem of nitrification of labeled ammonium derived from the various pathways of nitrate reduction, but frequent sampling is required. It involves numerical modeling and nonlinear parameter estimation to estimate the rates of several N-cycle processes occurring simultaneously [28, 33].

Acknowledgments. We thank Mr. R. Teo and Mr. R. Agnew for technical assistance; Ciba-Geigy Australia Ltd. and Dupont Australia Ltd. for gifts of ametryne and DMU, respectively; and the Australian Centre for International Agricultural Research for financial support.

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