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Denitrification measurements in aquatic sediments: A comparison of three methods

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Abstract. Measurements of denitrification using the acetylene inhibition, ^{15}N isotope tracer, and N_2 flux methods were carried out concurrently using sediment cores from Vilhelmsborg sø, Denmark, in an attempt to clarify some of the limitations of each technique. Three experimental treatments of overlying water were used: control, nitrate enriched, and ammonia enriched water. The N_2 flux and ^{15}N tracer experiments showed high rates of coupled nitrification/denitrification in the sediments. The acetylene inhibition method did not capture any coupled nitrification/denitrification. This could be explained by acetylene inhibition of nitrification. A combined ^{15}N tracer/acetylene inhibition experiment demonstrated that acetylene inhibition of N_2O reduction was incomplete and the method, therefore, only measured approximately 50% of the denitrification due to nitrate from the overlying water. Similar rates of denitrification due to nitrate in the overlying water were measured by the N_2 flux method and the acetylene inhibition method, after correcting for the 50% efficiency of acetylene inhibition. Rates of denitrification due to nitrate from the overlying water measured by the ^{15}N tracer method, however, were only approximately 35% or less of those measured by the acetylene inhibition or N_2 flux methods.

Introduction

The importance of denitrification in the N cycle in marine and freshwater ecosystems has been the focus of an increasing number of studies during the past 15 years. Denitrification occurs in sediments in lakes (e.g. Andersen 1977; Keeney et al. 1971; Messer & Brezonik 1983; Gardner et al. 1987), rivers and streams (e.g. Chatarpaul et al. 1980; Hill 1981; Billen et al. 1985; Christensen & Sørensen 1988), estuaries and coastal bays (e.g. Sørensen 1978a; Nishio et al. 1983; Kaspar et al. 1985; Smith et al. 1985), and continental and deep sea sediments (e.g. Wilson 1978; Koike & Hattori 1979; Christensen et al. 1987; Goloway & Bender 1982). In addition, denitrification occurs in anoxic waters (e.g. Keeney et al. 1971; Billen et al. 1985; Codispoti & Packard 1980). Denitrification removes, as gaseous N, a substantial amount of N cycled through sediments in many coastal marine and freshwater environments (e.g. Kaspar et al. 1985; Seitzinger 1988; Kemp et al. 1990) and is a major sink in the overall N budget of many aquatic ecosystems (e.g. Smith et al. 1985; Billen et al. 1985; Christensen et al. 1987; Seitzinger 1988; Degobbis & Gilmartin 1990).

The major end product of denitrification in aquatic sediments is N_2. However, due to the high background concentrations of N_2 in both the atmosphere and in water, simple measurements of N_2 production in sediments are difficult to obtain at *in situ* conditions. During the years, a number of methods have been developed to quantify denitrification rates in sediments, including the acetylene inhibition technique (Balderston et al. 1976; Sørensen 1978a), ^{15}N tracer techniques (Nishio et al. 1982, 1983; Jenkins & Kemp 1984), N_2 flux methods (Seitzinger et al. 1980; Devol 1991), N mass balance approach (Ahlgren 1967; Hill 1983; Billen et al. 1985) and measurements of nitrate disappearance (Andersen 1977; Robinson et al. 1979), among others.

There has been considerable discussion regarding the efficacy of the various techniques for measuring denitrification (see reviews by Knowles 1982 and 1990; Taylor 1983; Koike & Sørensen 1988); all have potential problems and limitations. A few studies have concurrently used two or more techniques in an aquatic sediment and compared the resultant denitrification rates (Messer & Brezonik 1983; Smith & DeLaune 1983; Kemp et al. 1990), but these studies have not generally been designed to specifically identify or clarify problems or limitations. Some studies in homogenized agricultural soil samples have compared denitrification methods (e.g. Smith et al. 1978; Parkin et al. 1985). The current study was undertaken specifically to examine some of the potential problems and limitations of three of the most commonly used techniques for measuring denitrification in aquatic sediments: the acetylene inhibition, ^{15}N tracer, and N_2 flux method. These three techniques use different approaches and are based on different underlying assumptions.

Assumptions of the applied techniques

The acetylene inhibition technique is a relatively easy and rapid technique and has been one of the most frequently used methods for measuring denitrification in aquatic sediments (e.g. Sørensen 1978a; Sørensen et al. 1979; Kaspar et al. 1985; Christensen & Sørensen 1986, 1988; and others). Acetylene blocks the reduction of N_2O to N_2 during denitrification (Balderston et al. 1976; Yoshinari & Knowles 1976) and accumulations of N_2O in samples exposed to acetylene are equated to denitrification rates. However, a number of complicating interactions of acetylene have been demonstrated. For example, acetylene inhibits nitrification (Hynes & Knowles 1978), methanogenesis (Oremland & Taylor 1975; Knowles 1979) and the growth of sulfate-respiring bacteria (Payne & Grant 1982), among other effects (see review by Knowles 1990). In addition, acetylene is not always an effective block of N_2O reduction to N_2 (Tam & Knowles 1979; Van Raalte & Patriquin 1979; Kaspar 1982; Oremland et al. 1984; Christensen et al. 1989; Slater & Capone 1989). The significance of these effects for the measurement of denitrification in aquatic sediments has not been fully evaluated, although the method continues to be used extensively in aquatic sediments.

The ^{15}N tracer technique was developed to measure both denitrification of nitrate diffusing into the sediments from the overlying water and denitrification coupled to nitrification within the sediments (Nishio et al. 1983; Jenkins & Kemp 1984). Two overlying water treatments are used. One set of cores is incubated with $^{15}N\text{-}NO_3^-$ to measure denitrification of nitrate from the overlying water. If NO_3^- concentrations higher than field concentrations are used, a linear correction is made to estimate denitrification rates at ambient field NO_3^- concentrations (Nishio et al. 1983). Coupled nitrification/denitrification is measured in cores incubated with high concentrations of $^{15}N\text{-}NH_4$ in the overlying water. It is assumed that the increased ammonia concentrations in the overlying water do not alter the rates of coupled nitrification/denitrification. It is also assumed that the measured ^{15}N enrichment of NH_4^+ in the pore waters of the upper cm is the same as the ^{15}N enrichment of the NO_3^- produced by nitrification in the sediments and subsequently denitrified. The total ambient denitrification in the sediments is calculated as the sum of the N_2 production due to nitrate from the overlying water (NO_3^- treatment corrected for ambient field NO_3^- concentrations) plus the coupled nitrification/denitrification measured in the NH_4^+ enriched cores.

Using the N_2 flux technique, the background N_2 concentration is decreased from 79% N_2 to approximately 1% N_2 by incubating the cores with an overlying water and gas which is flushed with 79% He and 21% O_2 (Seitzinger et al. 1980). In the applied set up, a 10-day preincubation is necessary to decrease the background N_2 in the pore water. The low background N_2 concentration then makes it possible to measure increases in N_2 concentration in the overlying water and gas phase due to denitrification over approximately 24-h time intervals. This technique requires incubation chambers and gas sampling techniques which are free from room air contamination. A major assumption is that the 10-day preincubation of the sediments does not appreciably change the denitrification rates from ambient field rates. A recent modification has been made to the N_2 flux method which reduces the pre-incubation time to 2—3 days (Nowicki 1993). This modification has been used at a number of locations; denitrification rates measured during days 3—5 are not different than rates measured during days 7—11 (Nowicki 1993).

Our study focussed on the ability of the three techniques to measure denitrification of the two major sources of nitrate for denitrification, nitrate from the overlying water and nitrate produced from nitrification in the sediments. The experiments were not designed to test the ability of the techniques to measure ambient field rates at the study site, although the results provide considerable insight into reasons why each technique is or is not accurately measuring denitrification.

Methods

Site selection and sediment collection

To test the ability of the techniques to measure denitrification of the two major sources of nitrate, we chose sediments with high nitrification activity and then experimentally manipulated the overlying water nitrate concentrations. Sediment cores from the lake Vilhelmsborg sø, Denmark, were collected in September 1990 using hand held plexiglass tubes. The sediments were silt-clay with some sand and had 6% C, a C/N ratio of 20, and a porosity of 0.86. The cores were maintained in the laboratory at approximately 23 °C, in the dark, and with overlying water that was continuously mixed and kept aerobic. The details of the three denitrification techniques as applied are described below.

Treatments

Three different overlying water treatments were used: control cores with low concentrations ($\leqslant 1$ μM) of both NO_3^- and NH_4^+, nitrate enriched cores with approximately 100 μM NO_3^-, and ammonia enriched cores with approximately 100 μM NH_4^+ (Fig. 1). The control and nitrate treatments were chosen to examine the rates of denitrification obtained from each technique when nitrification in the sediments is the major source of nitrate for denitrification, and when there is an additional source of nitrate in the overlying water, respectively. The ammonia enriched treatment was chosen to test the effect of high ammonia concentrations on coupled nitrification/denitrification.

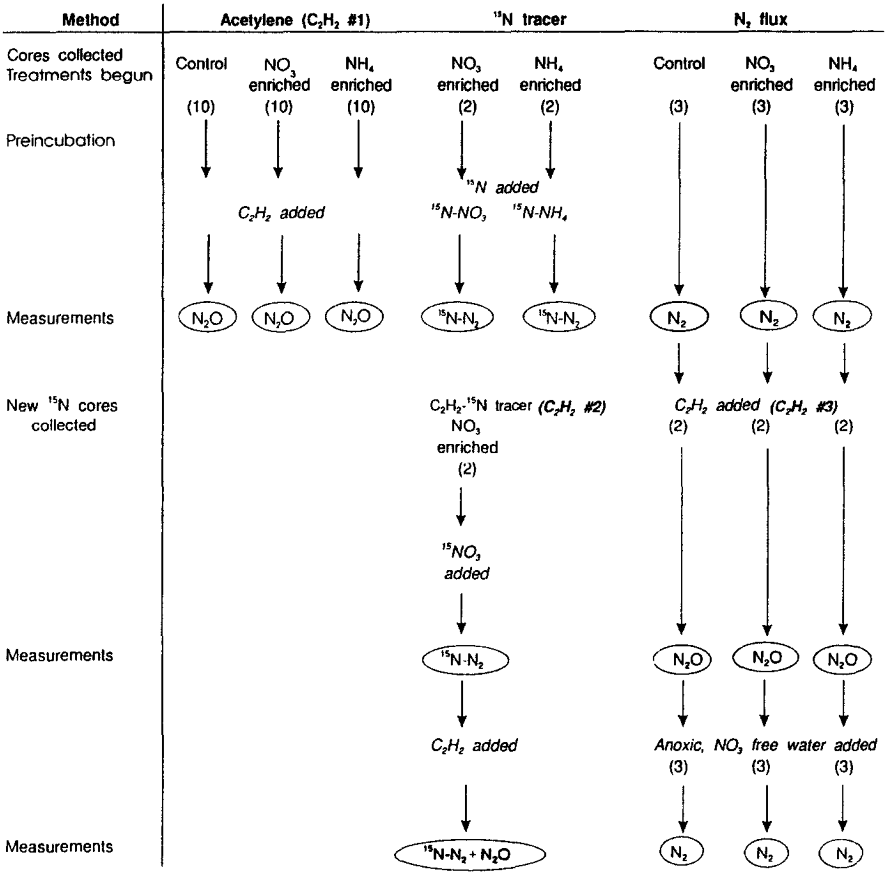

Fig. 1. Flow chart of experimental set-up. Values in parentheses indicate number of cores.

Artificial freshwater (modified from Lehman 1980) was used over all cores to obtain the necessary nitrate and ammonia concentrations. All cores were preincubated for 10 days to 2 weeks with one of the three treatment waters before denitrification measurements were made (Fig. 1); the water was changed daily over the cores. During the preincubation period as well as during the denitrification measurements, the oxygen concentration in the water overlying all cores was above approximately 50% saturation.

Denitrification measurements

Detailed descriptions of the three methods have been published previously (Christensen et al. 1990; Nishio et al. 1982, 1983; Seitzinger 1987 and Seitzinger et al. 1980). Therefore, only the general outline and modifications to those methods are presented below.

Acetylene inhibition technique

Ten sediment cores (3.6 cm diameter, 5 cm deep, approx. 50 ml overlying water) in plexiglass tubes were used for each of the three overlying water treatments. Ten percent of the water was replaced with C_2H_2 saturated treatment water to give a final concentration of 10 kPa acetylene. The first core was sampled immediately (2 minutes) after C_2H_2 additions, and individual cores were subsequently sampled at 15 minute intervals. Samples of the gas phase were taken for N_2O analysis after vigorously shaking the cores to equilibrate the N_2O in the sediments, water and gas phases (Christensen et al. 1990). Samples were analyzed for N_2O by ECD gas chromatography, and the total N_2O in each core was calculated (Christensen et al. 1990). The denitrification rate for each treatment was calculated from linear regression analysis of total N_2O per core versus incubation time. Water samples were collected from each core for NO_3^- and NH_4^+ analysis (Armstrong et al. 1967; Solorzano 1969) prior to shaking. (This acetylene inhibition experiment is referred to as C_2H_2# 1 below, to avoid confusion with other secondary experiments with acetylene.)

^{15}N tracer method

Four cores (7 cm diameter, 6 cm of sediment, and 10 cm of overlying water) were incubated in glass chambers with the overlying water continuously exchanged with a turnover time of approximately 1.3 hours (5 ml/min flow rate); there was no gas phase in these chambers. Two cores received nitrate enriched water (cores 1 and 2) and two received ammonia enriched water (cores 3 and 4). No control treatment cores were run because the ^{15}N tracer method requires relatively high concentrations of ^{15}N NO_3^- or NH_4^+ in the overlying water. During a preincubation period of 17 days, NO_3^- (100 μM) and NH_4^+ (100 μM) in the influent water were unlabeled. On day 18, the influent water was changed to 50 atom % ^{15}N NO_3^- or 100 atom % ^{15}N NH_4^+, respectively (concentration 100 μM N). Approximately 12 hours later, time series samples of the effluent water were taken and analyzed for ^{15}N enrichment of the N_2, NH_4^+, and NO_3^- (Bremner 1965; Blackburn 1979; Binnerup et al. 1992). At the end of the experiment, the cores were sectioned vertically (0—0.5, 0.5—1, 1—2, 2—4, 4—6 cm) and analyzed for ^{15}N enrichment of the total NH_4^+ (extractable plus pore water), pore water NH_4^+, and total N (Blackburn 1979). Denitrification rates in the nitrate and ammonia enriched cores were calculated according to Nishio et al. (1982; 1983). In the nitrate enriched cores, denitrification of the added NO_3^- was estimated as:

$$D = [(^{15}N\text{-}N_2\%) \times (N_2\text{conc}) \times F]/[A \times (^{15}N\text{-}NO_3^-)] \quad (1)$$

where D is denitrification, ^{15}N-N_2% is ^{15}N-N_2 excess in the effluent water (atom %), N_2conc is the N_2 concentration in the overlying water, F is the flow rate, A is the sediment surface area and ^{15}N-NO_3^- is the ^{15}N-NO_3^- atom % excess in the overlying water.

In the ammonia enriched cores, denitrification coupled to nitrification in the sediments was estimated as:

$$D = [(^{15}\text{N-N}_2\%) \times (\text{N}_2\text{conc}) \times F]/[A \times (^{15}\text{N-NH}_4^+)] \qquad (2)$$

where ^{15}N-NH_4^+ is the atom % excess in the porewater from 0—1 cm.

^{15}N tracer/acetylene inhibition

Two additional cores were collected (3 weeks after the cores for the above experiment were collected) to examine the effect of acetylene on N_2O and $^{15}N_2$ production in cores incubated with 100 μM NO_3^- (50 atom % ^{15}N). The cores (designated cores A and B) were sampled and treated as in the above experiment except that 18 hours after additions of ^{15}N-NO_3^-, acetylene was also added to a final concentration of 10 kPa in the influent water, and effluent water samples were analyzed for N_2O by ECD gas chromatography (Christensen et al. 1990).

N_2 flux method

Triplicate sediment cores (6.7 cm diameter, 6 cm deep) for each of the three treatments were incubated in gas tight glass incubation chambers (Seitzinger 1987). The water (~ 750 ml) was changed daily with treatment water flushed with a gas mixture of 79% He and 21% oxygen to lower the background N_2 concentration, beginning the day after cores were collected from the field. The overlying gas phase (approx. 70 ml) was also flushed with the He/O_2 gas mixture. N_2 flux measurements began on the same day that the C_2H_2 # 1 experiment began. Duplicate samples (75 μl) of the gas phase were taken for N_2 and O_2 analysis from each chamber approximately 6 and 24 h after the water was changed and analyzed immediately by TCD gas chromatography (Seitzinger et al. 1980; Seitzinger 1987). Net N_2 and O_2 fluxes across the sediment-water interface were calculated based on the measured changes in concentration between sequential samples during the repeated 24 h incubations, the volume of the gas phase in each chamber, and the surface area of the sediment. Two to four separate denitrification rate (N_2 flux) measurements were made on each core. Initial and final water samples were collected during selected water changes for nitrate and ammonia analysis (Armstrong et al. 1967; Solorzano 1969).

After N_2 flux measurements were completed, we added acetylene (final concentration 10 kPa) to the N_2 flux chambers (2 out of 3 chambers in each treatment) and compared the rates of N_2O production in the presence of acetylene in the N_2 flux chambers with those in the acetylene inhibition experiment (C_2H_2 # 1) described above. Time series samples of the gas phase were taken 6, 24, and 46 h after acetylene addition for N_2O analysis by ECD gas chromatography (Christensen et al. 1990). Rates of N_2O production were calculated based on the rate of change of N_2O concentration in the gas phase, volume of the gas phase, volume of water and solubility of N_2O in water (Weiss & Price 1980), and the surface area of sediment.

After denitrification (N_2 flux) measurements and acetylene inhibition experiments were completed as described above, we quantified atmospheric contamination that was introduced to the N_2 flux chambers due to incubation and sampling procedures. To do this, we removed all sources of nitrate for denitrification by replacing the water in all nine chambers (sediment cores still in chambers) with anoxic NO_3^--free control water (obtained by flushing with only He). With no oxygen for nitrification and no nitrate in the overlying water, N_2 increases would be due to contamination. N_2 fluxes were measured in the anoxic chambers using the procedures described above.

Results

Acetylene inhibition technique

The nitrous oxide content of cores from the nitrate enriched treatment increased linearly with time (Fig. 2); calculated denitrification rates from these data are 99 ± 5 μmol N m^{-2} h^{-1} (mean ± S.E.) (Table 1). There was no significant increase (α = 0.02) in nitrous oxide content in cores from either the control or high ammonia treatments (Fig. 2; Table 1).

^{15}N tracer method

Time series samples of the effluent water from the ^{15}N-NH_4^+ treatment cores (cores 1 and 2) were analyzed for ^{15}N-N_2 beginning approximately 12 hours after $^{15}NH_4^+$ was added. There was no significant change in ^{15}N-N_2 excess between 12 and 60 hrs (α = 0.05), indicating steady state conditions (Fig. 3a). The mean (± S.D.) ^{15}N-N_2 excess in the two replicate cores was 0.0433 (± 0.0088) and 0.0138 (± 0.0084) atom %, respectively. Variability in the ^{15}N-N_2 excess numbers between replicate cores and among time series samples within a core was high compared to

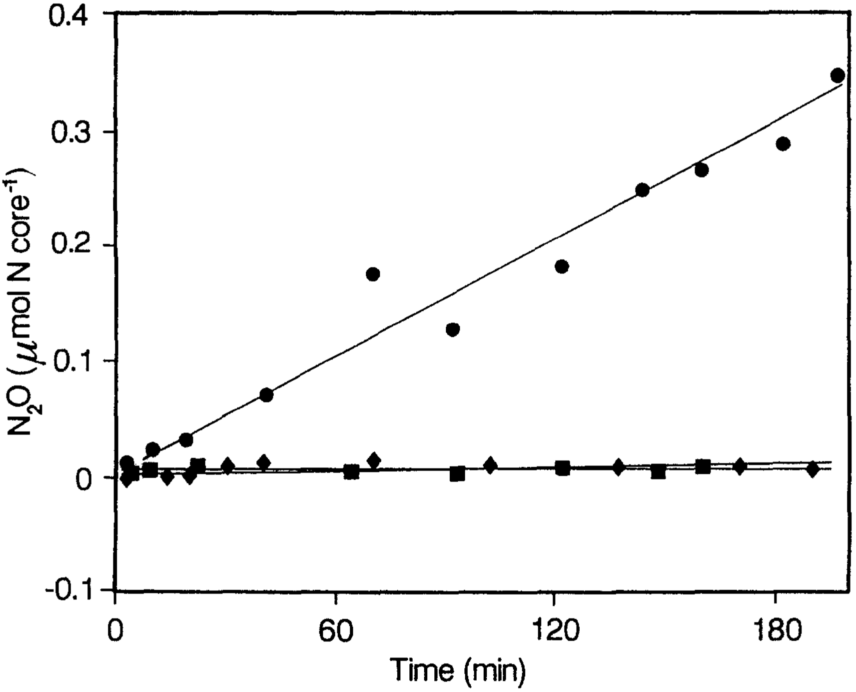

Fig. 2. N_2O accumulation in control (■), nitrate enriched (●) and ammonia enriched (◆) cores incubated with 10 pKa acetylene in overlying water (C_2H_2 # 1 experiment). X-axis is minutes after acetylene was added.

Table 1. Calculated rates of denitrification in Vilhelmsborg sø sediments based on results of acetylene inhibition, ^{15}N tracer, and N_2 flux methods. Replicate cores were incubated with different overlying water treatments: ammonia enriched and nitrate enriched cores had approximately 100 μM NH_4^+ or NO_3^-, respectively, in the overlying water; control cores had low NH_4^+ and NO_3^- concentrations in the overlying water (see text). units: μmol N m^{-2} h^{-1}; *n* = number of cores per treatment.

Overlying water treatment	Acetylene inhibition (x ± S.E.) (*n* = 10)	^{15}N Tracer (range) (*n* = 2)	N_2 Flux (x ± S.D.) (*n* = 3)
Control	2 (± 1)		260 (±65)
NH_4^+ Enriched	0 (±1)	77—220	490 (±120)
NO_3^- Enriched	99 (±5)	121—290[1]	395 (±45)

[1] Sum of denitrification of NO_3^- from overlying water measured in ^{15}N-NO_3^- enriched cores (44 and 70 μmol N m^{-2} h^{-1}) and coupled nitrification/denitrification rates obtained from ^{15}N-NH_4^+ enriched cores (77 and 220 μmol N m^{-2} h^{-1}).

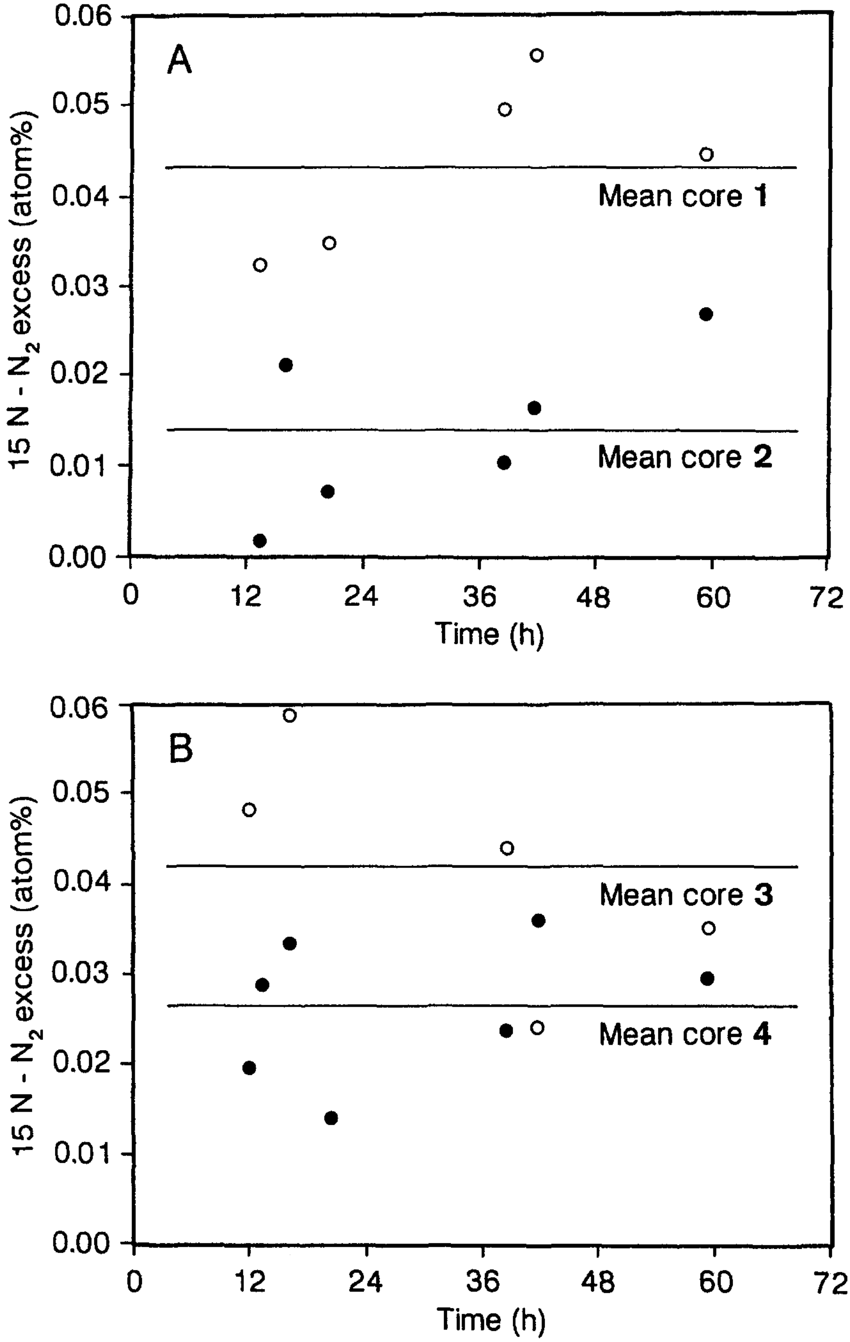

Fig. 3. $^{15}N\text{-}N_2$ enrichment in effluent water beginning 12 h after (a) $^{15}N\text{-}NH_4^+$ or (b) $^{15}N\text{-}NO_3^-$ additions to influent water.

similar studies (Binnerup et al. 1992). $^{15}N\text{-}NH_4^+$ excess in the porewater from 0—1 cm was 14.1 and 14.9 atom % in the two cores. The denitrification rate due to coupled nitrification/denitrification in the sediments was calculated from equation 2 as 220 and 77 μmol N m^{-2} h^{-1} for the two cores, respectively (Table 1).

Time series samples of the effluent water from the nitrate enriched cores were analyzed for $^{15}N\text{-}N_2$ and $^{15}N\text{-}NO_3^-$ beginning 12 hours after $^{15}NO_3^-$ addition. No significant change ($\alpha = 0.05$) in $^{15}N\text{-}N_2$ with time was observed, indicating steady state conditions (Fig. 3b). For the two replicate cores, the mean (± S.D.) $^{15}N\text{-}N_2$ excess was 0.0420 (± 0.0127) atom % and 0.0264 (± 0.0071) atom % ^{15}N, respectively; the mean $^{15}NO_3^-$ excess was 48.9 (± 8.8) and 46.0 (± 7.9) atom %, respectively. The denitrification rate due to NO_3^- diffusion from the overlying water was calculated from equation 1 as 70 and 44 μmol N m^{-2} h^{-1} for the two cores. Adding this to the range of coupled nitrification/denitrification rates from the $^{15}NH_4^+$ treatment (77 and 220 μmol N m^{-2} h^{-1}) yielded a total denitrification rate for the NO_3^- enriched cores of between 121 and 290 μmol N m^{-2} h^{-1} (Table 1).

There was enrichment of $^{15}N\text{-}NH_4^+$ in the total NH_4^+ (extractable plus porewater) in the sediments of the nitrate enriched cores (data not shown), indicating dissimilatory reduction of NO_3^- to NH_4^+ (Sørensen 1978b; Koike & Hattori 1978). However, the calculated rates of NO_3^- reduction to NH_4^+ from the $^{15}N\text{-}NH_4^+$ data were low (aver. 5 μmol N m^{-2} h^{-1}) compared to NO_3^- reduction to N_2 (44—70 μmol N m^{-2} h^{-1}).

^{15}N tracer/acetylene inhibition

In the second ^{15}N tracer experiment, the effect of C_2H_2 on $^{15}N\text{-}N_2$ and N_2O production was measured using two cores incubated with 100 μM $^{15}N\text{-}NO_3^-$. The average $^{15}N\text{-}N_2$ excess in the effluent water before acetylene addition was 0.0573 (± 0.0217) and 0.1189 (± 0.0083) atom % excess in cores A and B, respectively (Fig. 4a). After acetylene was added, the $^{15}N\text{-}N_2$ enrichment decreased and then remained relatively constant at 0.0296 (± 0.0065) and 0.0409 (± 0.0039) atom % excess for cores A and B, respectively (Fig. 4a), demonstrating that some $^{15}N\text{-}N_2$ was still being produced by denitrification in the presence of acetylene. N_2 production rates supported by NO_3^- diffusion from the overlying water decreased from 93 to 182 μmol N m^{-2} h^{-1} in cores A and B before acetylene addition to 43 and 64 μmol N m^{-2} h^{-1} after acetylene addition. Rates of N_2O production increased from zero to 35 and 74 μmol N m^{-2} h^{-1} after acetylene addition (Fig. 4b). The calculated rates of denitrification in this

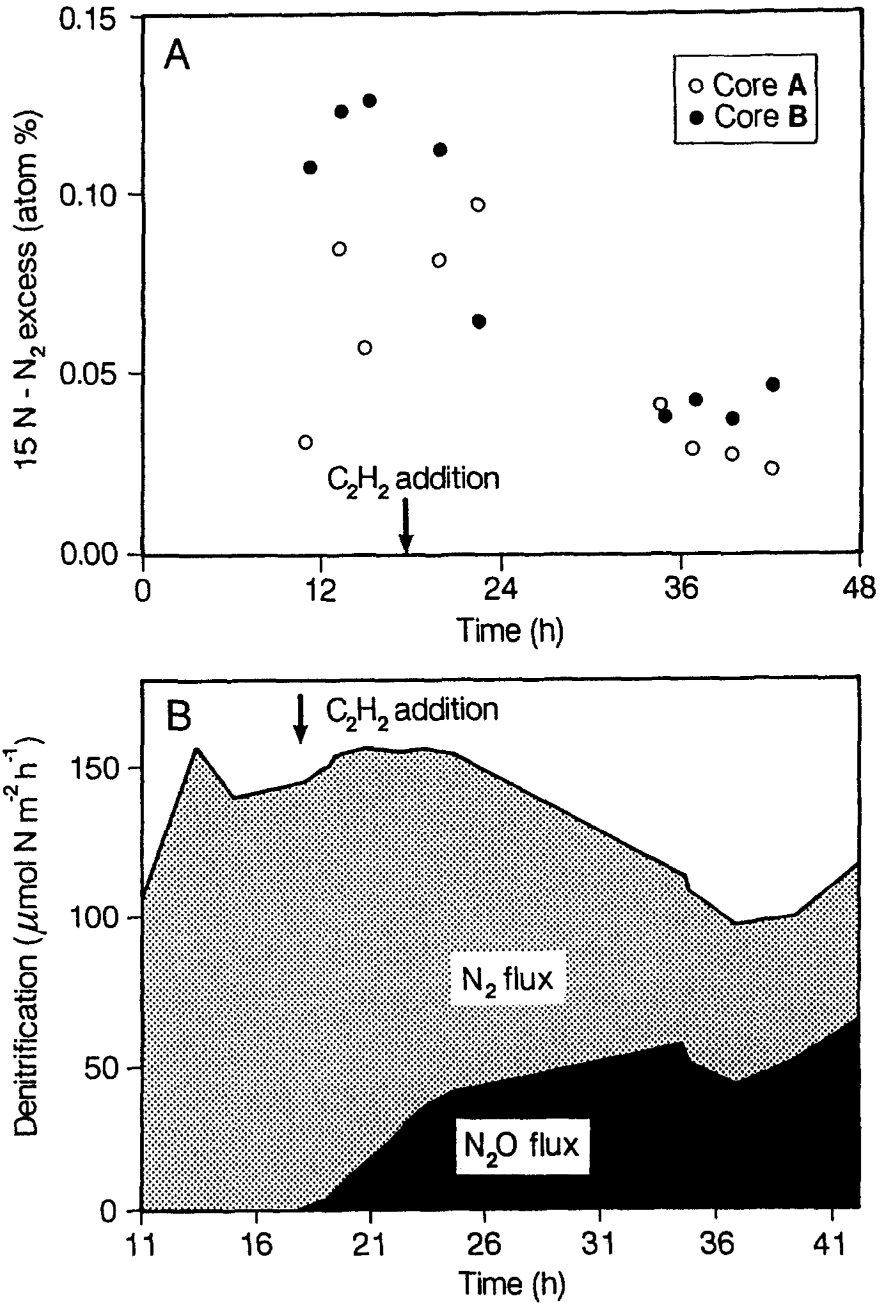

Fig. 4. Combined ^{15}N tracer/acetylene inhibition experiment: (a) ^{15}N-N_2 enrichment in effluent water beginning 12 h after ^{15}N-NO_3^- was added to the influent water. Acetylene was added to chambers at time indicated by the arrow. (b) Average rate of N_2 and N_2O flux from sediments of nitrate enriched cores before and after incubation with acetylene.

experiment are not directly compared to rates obtained during C_2H_2 # 1 as the cores were collected 3 weeks after those for C_2H_2 # 1.

N_2 flux method

Cores from all three treatments showed an increase in N_2 concentration over the repeated 18-h incubations (Fig. 5). Denitrification rates in the nitrate enriched cores were significantly (α = 0.05) greater (395 ± 45 μmol N m^{-2} h^{-1}) (mean ± S.D.) than in the control cores (260 ± 65 μmol N m^{-2} h^{-1}) (Table 1). Denitrification rates in the ammonia enriched cores (490 ± 120 μmol N m^{-2} h^{-1}) were also significantly (α = 0.05) greater than in the control cores (Table 1), but not significantly different than rates in the nitrate enriched cores.

When acetylene was added to the N_2 flux chambers (2 out of 3 per treatment) at the end of the N_2 production measurements, there was a linear increase in N_2O concentration in the nitrate enriched treatment (data not shown). Rates of N_2O production in the nitrate enriched cores

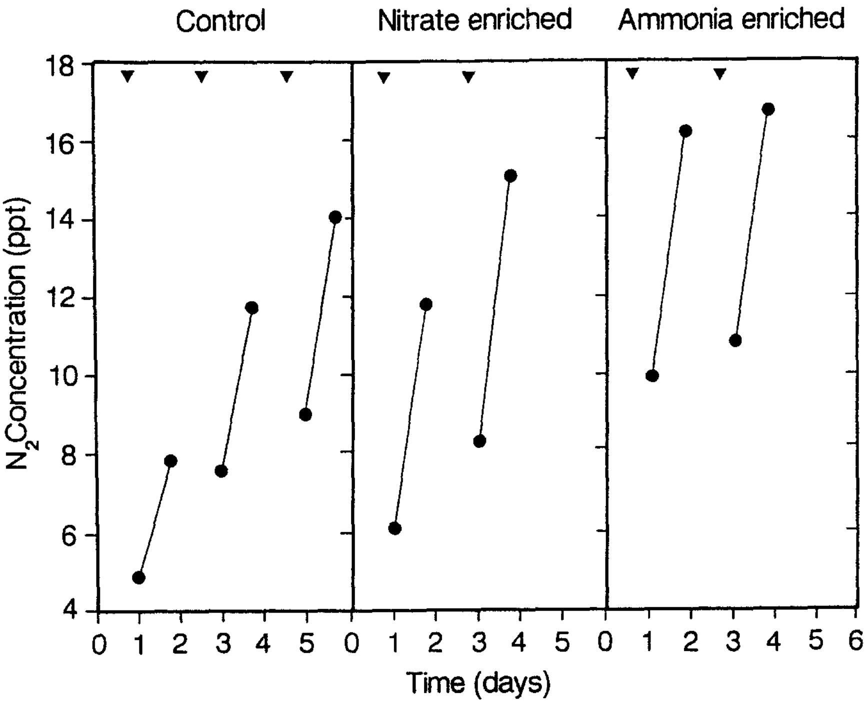

Fig. 5. N_2 concentration versus time in gas phase of N_2 flux chambers over repeated 18-h incubation intervals. Data from one out of 3 cores from control, nitrate enriched and ammonia enriched cores. Arrows indicate water changes.

averaged 130 μmol N m^{-2} h^{-1} which was similar to the rate in the acetylene inhibition experiment (C_2H_2 # 1) (99 μmol N m^{-2} h^{-1}). As was found in the acetylene inhibition experiment (C_2H_2 # 1), there was no N_2O production in the control or ammonia enriched cores after addition of C_2H_2 to the N_2 flux chambers.

When the water in the N_2 flux chambers was replaced with anoxic, NO_3^--free water, N_2 production decreased to 10% or less than the rates measured during the denitrification experiment. Rates with anoxic, NO_3^--free water ranged between 6 and 37 μmol N m^{-2} h^{-1} (average ± S.D. = 19 ± 10 μmol N m^{-2} h^{-1}) (Table 2). This demonstrates that the N_2 increases measured under the experimental conditions were due primarily to denitrification and not to air contamination during incubation or sampling procedures.

Discussion

The current study was undertaken specifically to examine some of the potential problems and limitations of three of the most commonly used

Table 2. Rate of N_2 increase in N_2 flux chambers with sediments during denitrification experiment with aerobic overlying treatment water and at end of experiment with anoxic NO_3^--free water to check gas tightness of chambers and ability to sample chambers without contamination. Units: μmol N m^{-2} h^{-1}.

Treatment/chamber #	Aerobic treatment water (x ± S.D.)	Anoxic NO_3^--free water
Control treatment		
#1	234 ± 49	12*
#2	343 ± 77	6*
#3	191 ± 52	19
NO_3^- Enriched		
#1	332	26*
#2	437 ± 75	23*
#3	416 ± 78	37
NH_4^+ Enriched		
#1	640 ± 35	7*
#2	352 ± 34	17*
#3	476 ± 48	23
	Average ± S.D.	19 ± 10

* Prior treatment with acetylene.

techniques for measuring denitrification in aquatic sediments. The results clearly demonstrated that the acetylene inhibition technique failed to measure denitrification coupled to nitrification in Vilhemsborg sø sediments. Substantial rates of coupled nitrification/denitrification were indeed occurring in the sediments as demonstrated both by the ^{15}N tracer experiment where $^{15}N_2$ was produced in ammonia enriched cores (Fig. 3a and Table 1) and by the N_2 flux experiment where high rates of N_2 production were found both in the control and ammonium enriched cores (Table 1). In contrast, denitrification was not detected by the acetylene inhibition technique in either the control or ammonium enriched cores where nitrification was the only source of nitrate; denitrification was only detected in nitrate enriched cores by this technique (Table 1).

Numerous studies have shown that acetylene blocks nitrification (e.g. Hynes & Knowles 1978, 1984; Hyman & Wood 1985; Bremner & Blackmer 1979). The degree to which the acetylene technique will underestimate coupled nitrification/denitrification depends on the ratio of the turnover time of the nitrate pool from nitrification in the sediments relative to the incubation time. As demonstrated in the present study, the turnover time of the nitrate pool from nitrification in the sediments may be very short (less than 15 minutes) and thus almost impossible to determine with the acetylene technique alone, even when there are high rates of nitrification. Investigators have often assumed that if the rate of N_2O production is constant over the time course of an experiment then the nitrate pool in the sediments, due both to nitrate from the overlying water and from sediment nitrification, is not depleted. However, a constant N_2O production does not necessarily indicate that the nitrate pool from sediment nitrification is not depleted since the production can also be due to denitrification of NO_3^- from the overlying water.

In addition to failing to measure any coupled nitrification/denitrification, the acetylene inhibition method detected only about half of the denitrification due to nitrate diffusion from the overlying water. In the nitrate enriched cores, acetylene (10 kPa) was only about 50% effective at blocking N_2O reduction to N_2 as demonstrated by the combined ^{15}N tracer/acetylene inhibition experiment (C_2H_2 # 2) (Fig. 4). Previous studies have shown acetylene to be 60% to 100% effective at blocking N_2O reduction to N_2 in an estuarine sediment (Binnerup et al. 1992) and 70% effective in a stream sediment (Nielsen et al. 1990). Concentrations of acetylene between 1 and 5 kPa or higher are required to completely block nitrous oxide reductase especially at low nitrate concentrations (< 10 μM) (e.g. Kaspar 1982; Oremland et al. 1984; Slater & Capone 1989; Nielsen et al. 1990). The acetylene concentration in our experiments was 10 kPa and the nitrate concentration in the overlying water of the ^{15}N/acetylene experiment was 100 μM. The average nitrate concentration in the porewater would have been considerably lower. In stream sediments, Christensen et al. (1989) showed that even at high nitrate concentrations N_2O can be consumed when it diffuses into the deeper layers of sediment where NO_3^- is absent. Taking into account the ~ 50% efficiency of acetylene inhibition and using the N_2O production rate in the C_2H_2 # 1 experiment from the nitrate enriched cores (99 μmol N m^{-2} h^{-1}; Table 1), we estimated that the total rate of denitrification due to 100 μM NO_3^- in the overlying water was 200 μmol N m^{-2} h^{-1}.

The N_2 flux data also can be used to separate denitrification activity due to water column nitrate and coupled nitrification/denitrification. The concentration of nitrate in the overlying water of the N_2 flux control treatment cores was approximately 25 μM due to a net flux of NO_3^- out of the sediments from sediment nitrification (Nielsen et al. in prep.). In the nitrate enriched cores, the NO_3^- concentration averaged 85 μM due to a net uptake of NO_3^- by the sediments (Fig. 6). When denitrification rates are plotted as a function of NO_3^- concentrations (Fig. 6), the rate of

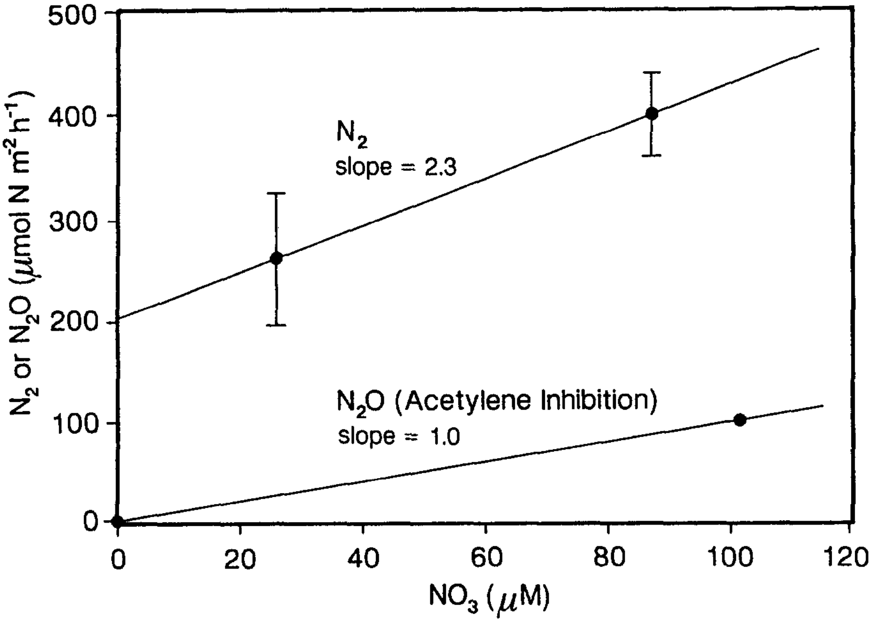

Fig. 6. N_2 production from N_2 flux method and N_2O production from acetylene method (C_2H_2 # 1), versus overlying water NO_3^- concentration. The y-intercept of the N_2 flux data (0 μM NO_3^-) represents coupled nitrification/denitrification. The y-intercept of the acetylene data is 0 due to acetylene inhibition of nitrification. The lower slope of N_2O production versus NO_3^- concentration relative to that of N_2 production is due to the inefficiency of acetylene inhibition of N_2O reduction.

coupled nitrification-denitrification is indicated as the N_2 production at 0 μM NO_3^- (y-intercept = 205 μmol N m^{-2} h^{-1}), while the slope indicates denitrification due to water column nitrate. The slope of the regression line (2.3 ± 0.92; slope ± S.E.) was significantly different from 1 and not significantly different from 2 (α = 0.1). The slope of the line of N_2 production vs NO_3^- concentration for the N_2 flux method is approximately twice that of the N_2O production vs NO_3^- concentration for the acetylene method (Fig. 6), supporting the ~ 50% efficiency of acetylene inhibition of N_2O reduction demonstrated in the ^{15}N tracer/C_2H_2 # 2 experiment. It follows that the denitrification rate due just to 100 μM NO_3^- in the overlying water obtained from the C_2H_2 # 1 experiment (200 μmol N m^{-2} h^{-1} after correcting for the efficiency of the block) and from the N_2 flux method (230 μmol N m^{-2} h^{-1}) were similar.

Based on the ^{15}N tracer experiment, the denitrification rate due to 100 μM NO_3^- in the overlying water ranged from 44 to 70 μmol N m^{-2} h^{-1} (see Results). This is less than half the rates calculated from either the C_2H_2 # 1 data (corrected rate 200 μmol N m^{-2} h^{-1}) or the N_2 flux data (230 μmol N m^{-2} h^{-1}). These ^{15}N data were also more variable than found in other studies using the same mass spectrometer and incubation chambers (Binnerup et al. 1992). At this time we have no explanation for the variability or lower rates obtained with the ^{15}N tracer method.

Estimates of coupled nitrification/denitrification using the ^{15}N tracer data depends on the assumption that the high ammonia concentrations do not alter the rates of coupled nitrification/denitrification. However, the rate of denitrification in the ammonia enriched N_2 flux cores was considerably greater than in the control N_2 flux cores indicating that the high ammonia concentrations enhanced nitrification. Another possible explanation for the discrepancy between the N_2 flux and ^{15}N tracer rates in the ammonia enriched treatment is that there may have been growth of nitrifiers on the walls of the N_2 flux chambers which would provide an additional source of NO_3^-; the walls of the ^{15}N chambers were cleaned prior to the ^{15}N additions which would have eliminated that potential source. Additional experiments beyond the scope of the present study are required to clarify the validity of the assumptions of the ^{15}N tracer technique. Recent modifications to the ^{15}N tracer technique (Nielsen 1991) eliminate some assumptions of the technique used here.

Room air contamination of the N_2 flux chambers was demonstrated to be insignificant relative to measured rates of N_2 production due to denitrification. Laboratory measured denitrification rates obtained from the N_2 flux and ^{15}N tracer methods still need to be verified with ambient field N_2 fluxes.

The results of the present experiment demonstrated that the acetylene inhibition method dramatically underestimated denitrification rates in an aquatic sediment. The acetylene inhibition method did not capture any coupled nitrification/denitrification in Vilhelmsborg sø sediments, although high rates of nitrification and coupled nitrification/denitrification were occurring as demonstrated by the ^{15}N tracer and N_2 flux methods. In addition, the acetylene technique only measured approximately 50% of the denitrification from water column nitrate due to incomplete inhibition of N_2O reduction. Previously reported denitrification rates obtained by this technique, particularly in nitrate poor systems, therefore, may have severely underestimated the denitrification activities and we advise that the acetylene inhibition technique not be used in such systems when the goal is to obtain realistic *in situ* denitrification rates.

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