Nitrate transformations and loss in an agricultural stream

by

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GENERAL INTRODUCTION

The complex nitrogen cycle in streams affects and is affected by many different processes including aerobic and anaerobic respiration, carbon and nitrogen loads and primary production. The processes important to nitrogen transformations in streams include are illustrated in Figure 1. Nitrogen fixation may occur, although this process is inhibited by the presence of inorganic nitrogen. Inorganic nitrogen may be assimilated by both autotrophic and heterotrophic organisms and immobilized to organic nitrogen. Organic nitrogen may be mineralized to NH_4^+ which, under aerobic conditions, may be converted to NO_2^- or NO_3^- by nitrification. Reduction of nitrate may be assimilatory, in which the nitrogen is incorporated into cell biomass, or dissimilatory, in which the functional role is to act as a terminal electron accepter to support an energy yielding reaction. Respiring organisms use various electron accepters to oxidize organic compounds. The highest energy yield is obtained when O_2 is the oxidizing agent (National Research Council 1979). When oxygen is exhausted, alternate electron accepters, such as nitrate, must be utilized, with lower energy yield. Dissimilatory nitrate reduction can be of two types, denitrification, which produces N_2 and N_2O, and dissimilatory nitrate reduction to ammonium, where NH_4^+ is the product. The relative importance of denitrification and

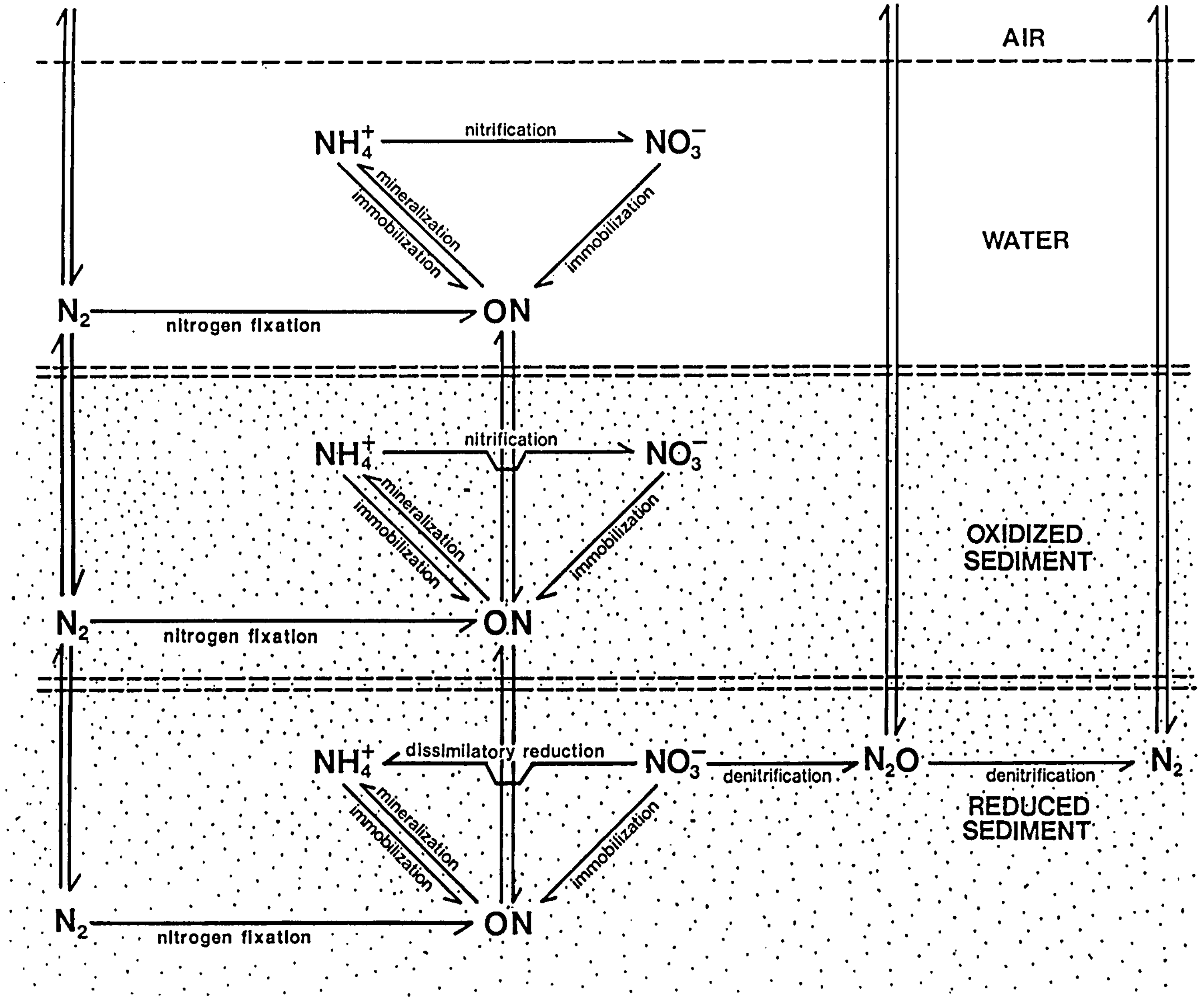

Figure 1. Nitrogen transformations in streams

dissimilatory nitrate reduction to ammonium is of particular interest. These processes both occur under similar conditions but one process results in the loss of combined nitrogen (denitrification), whereas the other conserves nitrogen (dissimilatory reduction).

There is continual input, conversion and loss of nitrogen in stream systems. Several studies of nitrogen mass balances have shown considerable losses of nitrate during transport in well-oxygenated streams. Most of these studies have identified microbial denitrification in anaerobic stream sediments as the major mechanism of nitrate depletion (Hill 1981, 1983, 1988; Kaushik et al. 1975; Robinson et al. 1979; van Kessel 1977a). Nitrate losses in some New Zealand streams were attributed to macrophyte uptake (Cooper and Cooke 1984). Others studies (Crumpton and Isenhart 1987, Crumpton and Hersh 1987) concluded that algal assimilation of inorganic nitrogen may be an important loss process in streams receiving high nitrogen loads.

Laboratory investigations, using tracer or acetylene inhibition techniques, have generally confirmed that microbial denitrification is a major nitrate loss process in stream sediments (Chatarpaul and Robinson 1979, Hill 1983, Hill and Sanmugadas 1985, van Kessel 1977b). Although dissimilatory nitrate reduction to ammonium may be an important process in some marine systems (Koike and Hattori 1978, Sorensen 1978, Cole and Brown 1980), there is little evidence for this process in freshwater systems. Chatarpaul and Robinson (1979) and van Kessel (1977b) found ^{15}N labelled ammonium after incubation of lake sediments amended with ^{15}N labelled nitrate but they attributed this finding to the mineralization of previously immobilized nitrogen. Hill and Sanmugadas (1985), however, trying to account for lost nitrate that did not show up as N_2O using acetylene inhibition, concluded that nitrate reduction to ammonium may be important in some stream sediments.

Temperature, oxygen, nitrate concentration of the overlying water, and the thickness of the sediment layer may all affect nitrate loss rates in streams (Sain et al. 1977, van Kessel 1977a). Loss rates were found to be positively correlated with temperature and nitrate concentration of the overlying water and slightly reduced by increasing the dissolved oxygen concentration in the overlying water from 0 to approximately 2 mg L^{-1}. A further increase of the dissolved oxygen concentration had no further inhibitory effect on the denitrification rate. Depth of the sediment column was a factor in disappearance only at lower temperatures with sediment depths less than 5 cm, under which conditions the disappearance was retarded (van Kessel 1977b).

Chemical and physical properties of stream sediments also have significant effect on nitrate loss rate (Wyer and Hill

1984, Hill and Sanmugadas 1985, Hill 1988). Positive correlations have been found between nitrate loss rate and sediment organic matter, total nitrogen, organic carbon and water soluble organic carbon. The presence of tubificid worms in stream sediments has been shown to enhance nitrate loss rate, presumably due to mixing of sediment and increased exchange rate (Chatarpaul et al. 1979, 1980).

Increased inputs of ammonium and nitrate could effect the balance of these nitrogen transformation processes in streams. Therefore, non-point source loading of inorganic nitrogen to surface and groundwaters and subsequent effects on water quality is of great concern in many heavily agricultural regions. In Iowa, approximately 60 percent of the total land area is in row crop cultivation which receives agricultural chemical applications (Hallberg 1987). Across the Corn Belt area of the U.S., annual nitrogen application rates have increased from about 50 kg N ha^{-1} (45 lbs N ac^{-1}) in 1965 to 153 kg N ha^{-1} (135 lbs N ac^{-1}) in 1982 (Hargett and Berry 1983). In Iowa, the state average fertilizer-N rate on corn increased from 50 kg N ha^{-1} (45 lbs N ac^{-1}) in 1964 to 160 kg N ha^{-1} (143 lbs N ac^{-1}) in 1984.

Agricultural practices at the land surface affect the quality of both groundwater and surface waters. In Iowa, groundwater discharge into master streams provides perennial flow. This is why streams continue to flow, even after many weeks with no precipitation. The high nitrate concentrations that appear in Iowa streams are related to periods of high recharge from shallow groundwater. The nitrate is mobilized by water infiltrating through soil, recharging shallow groundwater which then discharges into surface waters. This interconnection is best exemplified by data on nitrate nitrogen losses in tile effluent in relation to fertilizer nitrogen (Baker and Johnson 1981, Kanwar et al. 1983, Hallberg 1987). Kanwar et al. (1983) concluded that nearly half of the fertilizer nitrogen applied is discharged with tile drainage water. As a result of these agricultural practices, non-point nitrogen loads in surface waters in Iowa are among the highest in the country (Omernick 1977) and nitrate-nitrogen concentrations in surface waters frequently reach 10 mg L^{-1} (Bushong 1985, Crumpton and Hersh 1987).

The studies reported here are part of ongoing research into the transformation, fate and transport of nitrogen in agricultural streams. The specific objectives of the two studies were:

1. To determine in-stream losses of nitrate nitrogen and to estimate the importance of the various nitrate loss mechanisms in a stream receiving non-point agricultural inputs of inorganic nitrogen.

2. To estimate the influence of temperature, aeration and sediment characteristics on nitrate transformations in sediments of agricultural streams.

SECTION I. IN-STREAM LOSS OF NITRATE IN AN AGRICULTURAL STREAM

INTRODUCTION

The complex nitrogen cycle in streams affects and is affected by many different processes including aerobic and anaerobic respiration, carbon and nitrogen loads and primary production. Nitrogen fixation may occur in streams, however, this process is inhibited by the presence of organic or inorganic nitrogen. Inorganic nitrogen may be assimilated by both autotrophic and heterotrophic organisms and immobilized to organic nitrogen. Organic nitrogen may be mineralized to NH_4^+ which, under aerobic conditions, may be converted to NO_2^- or NO_3^- by nitrification. Reduction of nitrate may be assimilatory, in which the nitrogen is incorporated into cell biomass, or dissimilatory, in which the functional role is to act as a terminal electron accepter to support an energy yielding reaction. Respiring organisms use various electron accepters to oxidize organic compounds. The highest energy yield is obtained when O_2 is the oxidizing agent (National Research Council 1979). When oxygen is exhausted, alternate electron accepters, such as nitrate, must be utilized, with lower energy yield. Dissimilatory nitrate reduction can be of two types, denitrification, which produces N_2 and N_2O, and dissimilatory nitrate reduction to ammonium, where NH_4^+ is the product. The relative importance of denitrification and dissimilatory nitrate reduction to ammonium is of particular interest. These processes both occur under similar conditions but one process results in the loss of combined nitrogen (denitrification), whereas the other conserves nitrogen (dissimilatory reduction).

There is continual input, conversion and loss of nitrogen in stream systems. Several studies of nitrogen mass balances have shown considerable losses of nitrate during transport in well oxygenated streams. Most of these studies have identified bacterial denitrification in anaerobic stream sediments as the major mechanism of nitrate depletion (Hill 1981, 1983, 1988; Hill and Sanmugadas 1985; Kaushik et al. 1975; Robinson et al. 1979; van Kessel 1977a). Nitrate losses in some New Zealand streams were attributed to macrophyte uptake (Cooper and Cooke 1984). Others have demonstrated that algal assimilation of nitrogen is an important loss process in a stream receiving secondary treatment effluent (Crumpton and Isenhart 1987). Laboratory investigations have generally indicated that microbial denitrification is a major nitrate loss process in stream sediments (Chatarpaul and Robinson 1979, Hill 1983, Hill and Sanmugadas 1985, van Kessel 1977b). Some studies have demonstrated that microbial dissimilatory nitrate reduction to ammonium may be an important process in marine systems (Koike and Hattori 1978, Sorenson 1978, Samuelsson and Ronner 1982).

Increased inputs of ammonium and nitrate could effect the balance of these nitrogen transformation processes in streams.

Therefore, non-point source loading of inorganic nitrogen to surface and groundwaters is of great concern in many heavily agricultural regions. The role of agriculture and fertilizer nitrogen has been demonstrated as many studies show a direct relationship between nitrate leaching to groundwater and nitrogen fertilization rates and/or fertilization history (Hallberg 1987, Klepper 1978). In Iowa, groundwater discharges into master streams provide perennial flows, providing the interconnection affecting stream water quality (Hallberg 1987). As a result, non-point nitrogen loads in the region are among the highest in the country (Omernick, 1977) and nitrate-nitrogen concentrations in surface waters frequently reach 10 mg L^{-1} (Bushong 1985, Crumpton and Hersh 1987).

This paper reports the results of a study of nitrate nitrogen mass balance in a small agricultural stream in central Iowa. Additionally, the results of a laboratory investigation measuring nitrate nitrogen loss rates from water overlying intact sediment cores are reported. The primary objectives were to determine in-stream losses of nitrate nitrogen and to estimate the importance of the various nitrate loss mechanisms in a stream receiving non-point agricultural inputs.

MATERIALS AND METHODS

Bear Creek is a shallow, productive stream which enters the South Skunk River north of Ames, IA (Figure 1). Bear Creek does not receive secondary wastewater treatment effluent but does receive high nitrate loads due to intensive row crop agriculture in its watershed.

Nitrate loads entering and leaving a stream reach were used to calculate nitrogen mass balance budgets for Bear Creek during several low flow periods in 1986. Two diel studies were conducted during periods of base flow when discharge was less than 0.3 m^3s^{-1}. Two sites were established, approximately 1600 meters apart. During each study, triplicate samples were collected from each site approximately every two hours and preserved for analyses of ammonium and of nitrate plus nitrite nitrogen combined, hereafter referred to as nitrate nitrogen (Crumpton et al. 1987). Stream temperature ($^{+}0.1\ ^{o}C$) was measured with a thermistor and pH ($^{+}0.01$ pH) with a Markson pH probe in the field using portable meters. Dissolved oxygen ($^{+}0.05$ mg O_2 L^{-1}) was measured in the field using a Yellow Springs Instruments Co., model 5700 polarographic dissolved oxygen probe and model 57A meter.

During the diel studies, stream velocity and discharge were determined at three sites within the stream reach. At

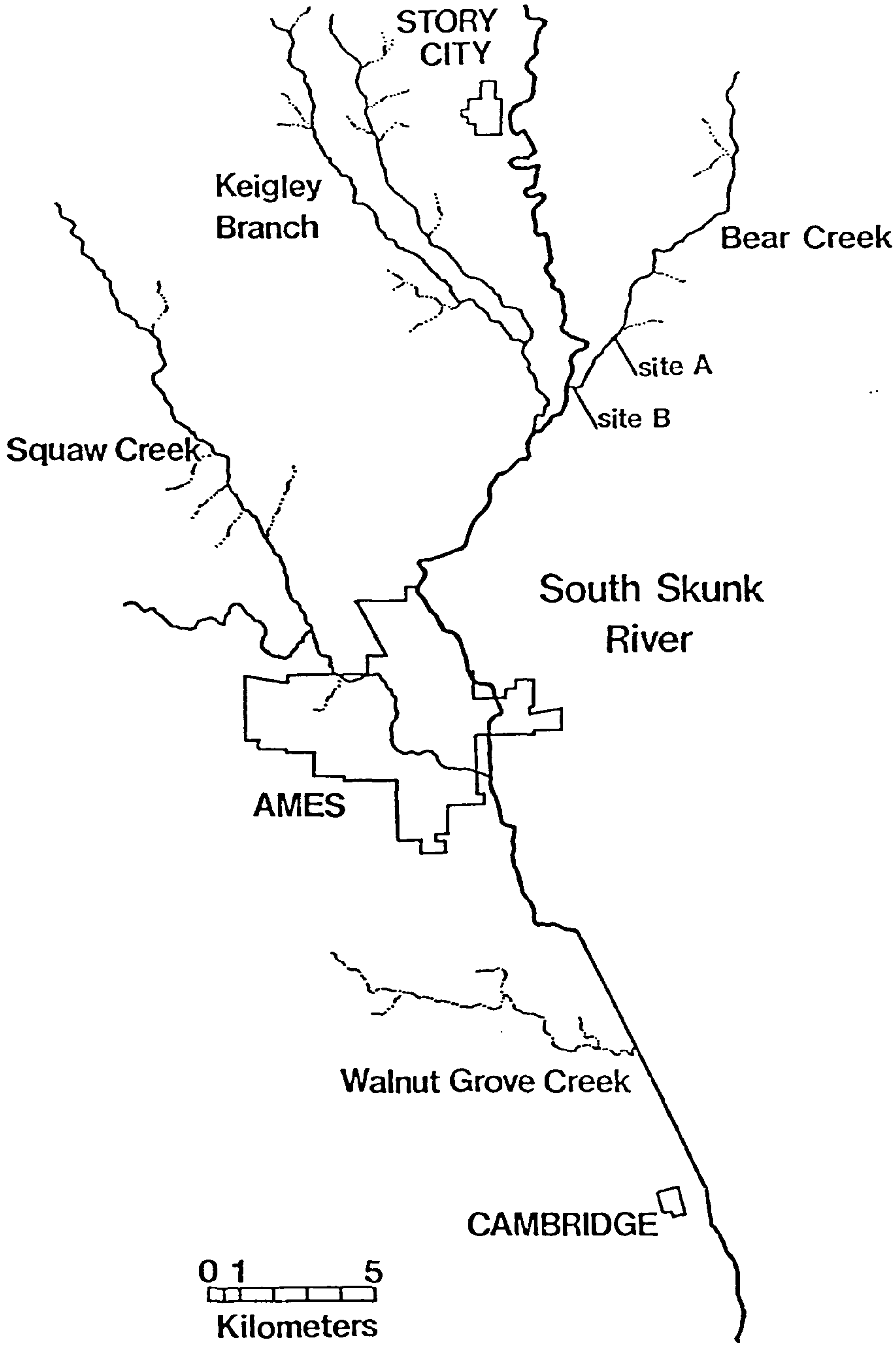

Figure 1. Upper South Skunk River Basin including Bear Creek, with sampling sites

each site, average stream velocity was measured at 0.5 meter intervals across the stream using a Pygmy Gurley meter. Discharge was calculated by incorporating stream cross section estimated by measuring depth at each point velocities were taken, multiplied by stream width. Discharge estimates used in nitrate flux calculations were the average of the three calculated on the day of interest.

Detailed stream surveys were conducted on Bear Creek in order to estimate stream channel morphometry during the study periods. At 100 m intervals along the study reach, a surveyors level was used to calculate stream cross section between the bank tops, stream stage and average depth at that site. These measurements were referenced to a benchmark established on a bridge abutment. It was assumed that an increase in stage would be uniform along the stream reach, which is typical for these low gradient streams. During the study periods, stage was measured relative to the benchmark, and using the data from the stream surveys, an estimate was made of cross section and average depth at each point surveyed. Averaging these cross sections and depths within the stream reach studied gives a much better estimate of these parameters than relying only on the estimates from discharge calculations. Volume was calculated by multiplying average cross section by reach length. Residence time was determined as reach volume/discharge.

Orion model 95-12 Gas-sensing ammonia probes were used to measure total ammonia nitrogen in basified samples and to measure nitrate nitrogen after alkaline reduction with $TiCl_3$ as described by Crumpton et al. (1987).

Daily nitrate losses were determined based on the mass balance of nutrient loads entering and leaving the stream reach between the two sampling sites. Daily nitrate flux rates at the upstream and downstream stations were calculated as the integral of the individual flux rates for each two hour sampling interval during a 24-hour period. For the downstream station the period integrated was displaced to account for travel time between stations. Incorporating discharge gives nitrate nitrogen flux rates at each station in kilograms nitrogen per day.

Gross primary production and community respiration were estimated from analyses of diurnal changes in dissolved oxygen and temperature (Owens 1974, Hall and Moll 1975). Oxygen production and respiration were calculated and converted to carbon equivalents assuming an $O_2:CO_2$ photosynthetic quotient of 1.2, an $O_2:CO_2$ respiratory quotient of 1.0 (Wetzel and Likens 1979) and a temperature coefficient for respiration of 2.0.

Based on the results of the mass balance studies, nitrate transformations and loss in water overlying stream sediment were investigated using experimental enclosures in the laboratory. Intact sediment cores were collected in March, 1987 from a depositional area in Bear Creek using polycarbonate cylinders (5.1 cm ID, 30.5 cm long). The cylinders were pushed into the sediment to a 14 cm depth, the top closed with a rubber stopper, the columns pulled out with the intact core of sediment, and the lower end stoppered. The cores, with approximately 250 ml of overlying stream water, were incubated in environmental chambers immediately upon return to the laboratory (Figure 2). Cores were bubbled with air via a cannula inserted through a serum stopper in an airtight plexiglass plunger stoppering the cylinder. The air bubbles were introduced close to the sediment-water interface in order to create some turbulence, in an effort to simulate stream conditions. Initial concentrations of nitrate nitrogen and ammonium nitrogen in the overlying stream water were 11.95 and 0.02 mg L^{-1} respectively. Treatments were initiated after all cores were acclimated for 30 hrs at $20^{o}C$ on a 12:12 hour light-dark cycle while being aerated.

To estimate the influence of photoautotrophic nitrogen demand on nitrate loss, some cores were incubated on a 12:12 hour light-dark cycle under aeration while other cores were incubated in the dark under aeration. Additional cores were also amended with 0.5 mg L^{-1} ammonium nitrogen and treated as above. Ammonium concentration increased during incubation

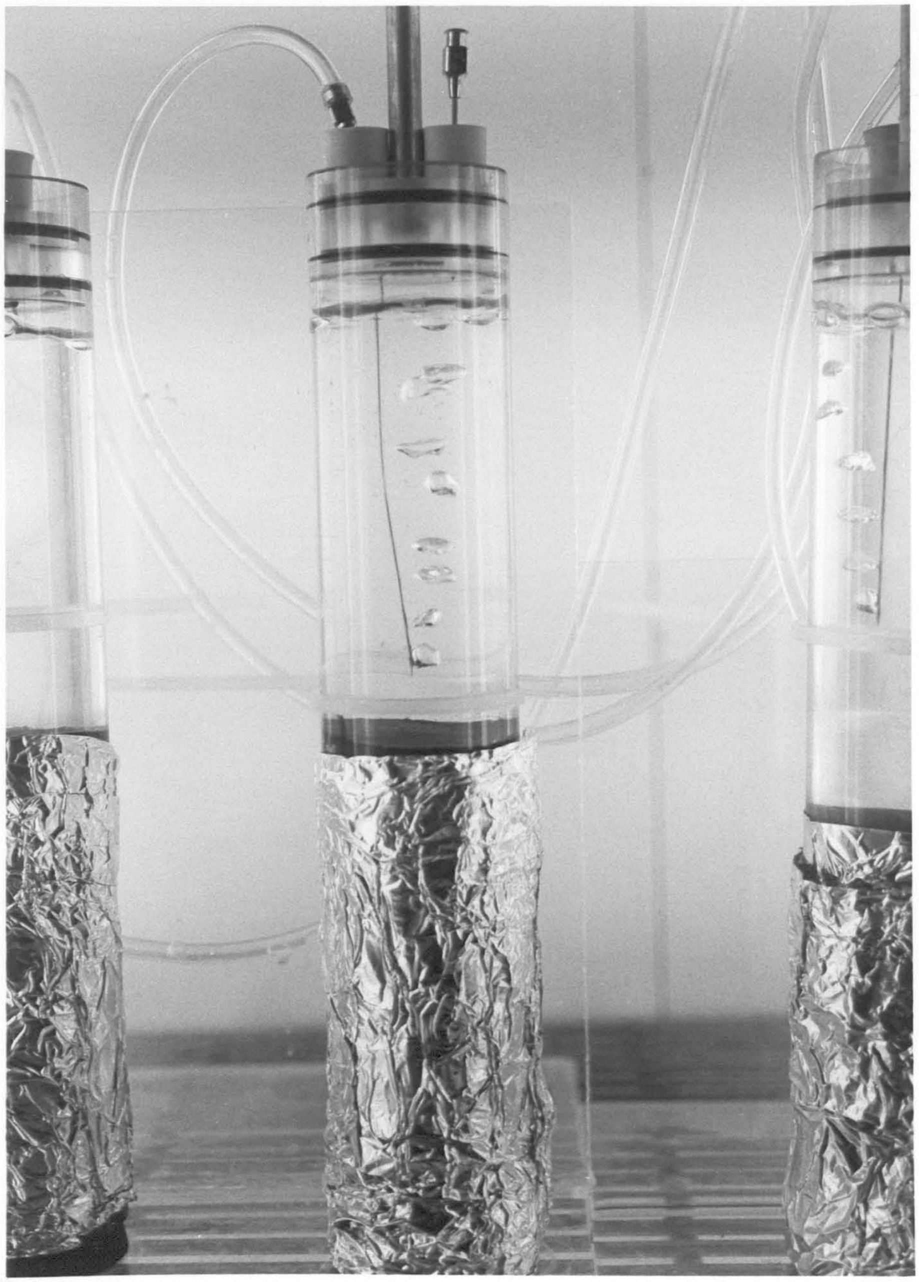

Figure 2. Experimental enclosure used to estimate nitrogen transformations in stream sediment-water systems

however, overwhelming the ammonium treatment addition. Therefore the results from this treatment were pooled with those with no addition. To determine the effect of aeration on nitrate loss, some cores were incubated in the dark and anaerobically by introducing nitrogen instead of air, through the cannula. Duplicate 5 ml samples were collected with a pipette from mid-depth in the overlying water of each core at 24 hour intervals and preserved for analyses of ammonium and nitrate as described by Crumpton et al. (1987).

RESULTS AND DISCUSSION

On the dates samples were taken, stream discharge varied less than 10% between study sites. There were no substantial inputs from tributaries or from surface runoff, and dilution from groundwater was considered insignificant based on discharge variance between study sites. Average stream velocity ranged between 0.16 and 0.22 m s^{-1} and discharge ranged between 0.23 and 0.25 m^3 s^{-1}. Data for the reach studied on Bear Creek on the two sample dates is shown in table 1.

Ammonium nitrogen concentrations in Bear Creek seldom reached 0.1 mg L^{-1}, while nitrate nitrogen concentrations were several orders of magnitude higher, ranging between 8 and 14 mg L^{-1}. Figures 3 and 4 illustrate concentrations of ammonium and nitrate nitrogen over time at a single sampling site for each of the two study dates. There were significant losses of nitrate nitrogen in Bear Creek during the low flow periods studied (Table 2). Average nitrate nitrogen loss between stations was 7.4 Kg day^{-1} during the August 11-12 sampling period and 5.3 Kg day^{-1} during the August 29-30 sampling period. Loss rates per unit area of stream bottom averaged 0.66 g N m^{-2} day^{-1} for Bear Creek. Stream bottom area was calculated based on the length of the reach and the average stream width estimated using stream survey data The loss

Table 1. Data for study reach on Bear Creek for study dates in 1986

	Aug 11-12	Aug 28-29
Discharge (m^3 s^{-1})	0.25	0.23
Average depth (cm)	23.8	19.5
Avg. cross section (m^2)	1.53	1.16
Velocity (cm s^{-1})	16.9	20.7
Length (m)	1601	1601
Volume (m^3)	2406	1829
Travel time (hrs)	2.60	2.13

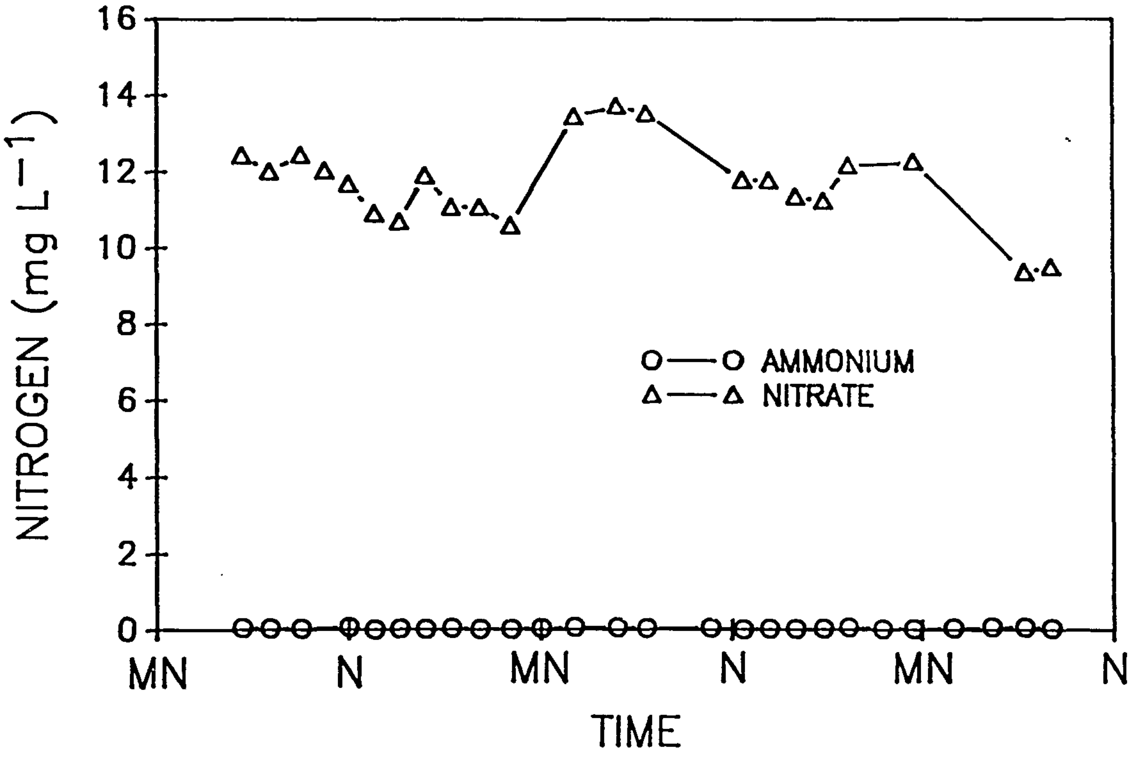

Figure 3. Nitrate nitrogen and ammonium nitrogen concentrations (mg L^{-1}) at one sampling site on Bear Creek on Aug 11-12, 1986 (N = noon, MN = midnight)

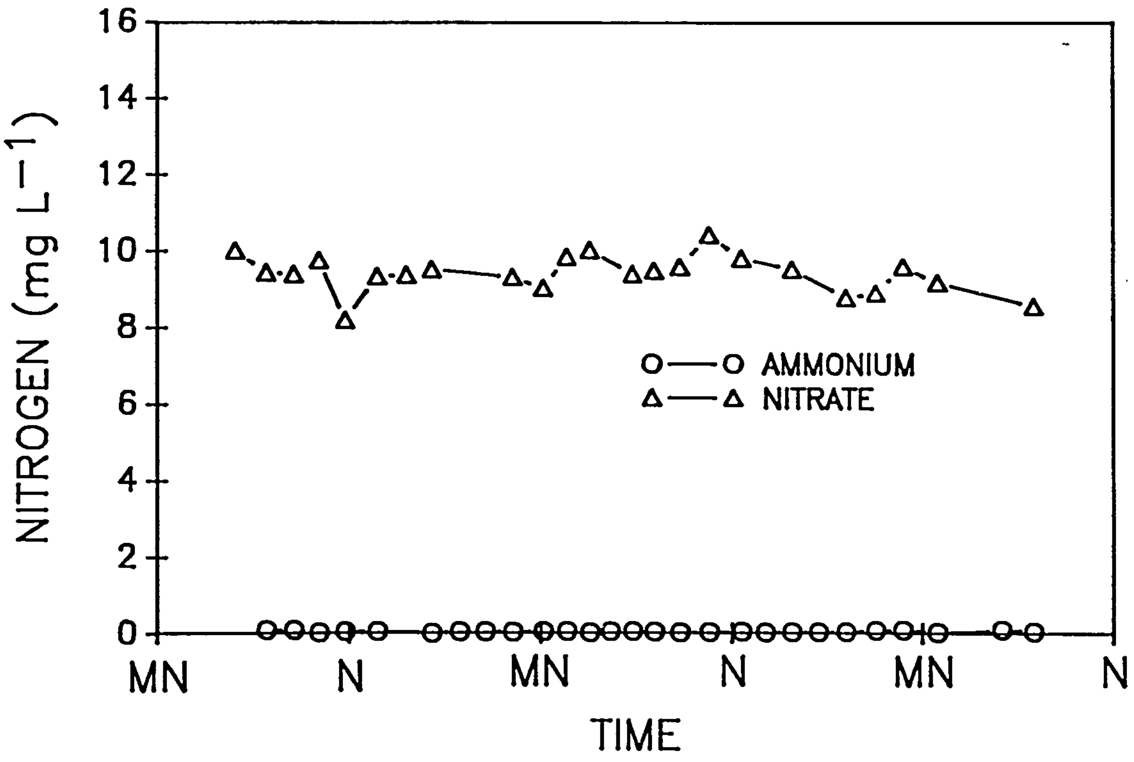

Figure 4. Nitrate nitrogen and ammonium nitrogen concentrations (mg L^{-1}) at one sampling site on Bear Creek on Aug 29-30, 1986 (N = noon, MN = midnight)

Table 2. Nitrate nitrogen mass balance in Bear Creek for several dates in 1986

Date	kg NO_3-N day^{-1}		Difference
	upstream	downstream	
Aug 11	280.9	276.8	- 4.1
Aug 12	251.9	241.2	-10.7
Aug 28	199.1	195.2	- 3.9
Aug 29	186.4	179.7	- 6.7

rates per area of stream bottom are in the low range of those found during previous studies on the South Skunk River below the Ames Water Pollution Control Plant outfall (Crumpton and Isenhart 1987), but in the high range of those measured in streams not receiving point source loads (Table 3). The nitrate losses can be taken as minimal estimates of assimilatory and dissimilatory nitrate reduction but do not include in-stream production of nitrate due to nitrification.

Algal assimilation of inorganic nitrogen has been shown to be important in the nitrogen balance of Ames area streams receiving secondary wastewater treatment effluent, in some cases accounting for all observed ammonium losses and for up to 85% of the nitrate loss in stream reaches (Crumpton and Isenhart 1987). One objective of this study was to contrast these findings and estimate the importance of algal assimilation in the nitrogen balance of stream reaches in the absence of point source loads. It was expected that algal assimilation might also be important in the nitrogen balance of Bear Creek. This stream is very productive, as are many of the streams in this heavily agricultural region. Under summertime base flow conditions, these low gradient streams are broad and shallow with high nutrient loads, low turbidity, and high light exposure. Although primary production of suspended algae is low, benthic algal production can be

Table 3. Nitrate nitrogen loss rates (g m^{-2} day^{-1}) of replicate cores, treatment means and standard error of the mean

Treatment	NO_3-N Removal g N m^{-2} day^{-1}	Mean	SEM
Light, aerobic	0.214	0.164	0.016
	0.142		
	0.106		
	0.180		
	0.194		
	0.148		
Dark, aerobic	0.063	0.065	0.006
	0.036		
	0.054		
	0.057		
	0.081		
	0.081		
	0.080		
Dark, anaerobic	0.281	0.197	0.029
	0.149		
	0.191		
	0.170		

extremely high (Kortge 1984, Robertson 1986).

During this study, rates of gross primary productivity in Bear Creek ranged between 1.5 and 3.5 g C m^{-2} day^{-1}. Rates of community respiration ranged between 0.9 and 2.2 g C m^{-2} day^{-1}. Production:respiration ratios ranged between 0.8 and 2.2 (Table 4). These rates of production are much lower than those found in previous studies on the South Skunk River below the Ames Water Pollution Control Plant outfall (Crumpton and Isenhart 1987), but are comparable to rates reported for small, agricultural streams in the absence of point source loads (Kortge 1984). Algal demand of inorganic nitrogen was estimated based on the nitrogen required to support observed rates of algal production, an estimate which is independent of the nitrogen mass balance calculations. Net primary production was estimated from gross primary production, assuming a ratio of net-to-gross primary production of 60%. This ratio is lower than might be expected for such high rates of photosynthesis (Peterson 1980), and is thus a conservative estimate of net primary production. The nitrogen required to support net primary production was calculated assuming a molar ratio for algal carbon-to-nitrogen uptake of 8.0. This is near the middle of the range considered by Warwick and McDonnell (1985) in developing a model for simultaneous in-stream nitrogen and dissolved oxygen balancing. However,

Table 4. Gross primary production, community respiration and production/respiration ratio in Bear Creek for several dates in 1986

Date	GPP g C/m^2/day	Respiration g C/m^2/day	P/R
Aug 11	1.82	2.24	0.81
Aug 12	1.50	0.93	1.61
Aug 28	2.80	2.04	1.36
Aug 29	3.53	1.72	2.20

lower ratios are often reported for well nourished algal cultures (McCarthy 1980), so a ratio of 8.0 probably yields a conservative estimate of nitrogen uptake in highly productive, nutrient rich streams.

Calculated rates of algal nitrogen demand ranged between 1.5 and 2.6 g N m^{-2} day^{-1}. Ammonium concentrations in Bear Creek were low, and loss rates were insignificant in comparison to either algal demand or nitrate loss rates. Without some regeneration of ammonium, it is probable that algae in Bear Creek are instead utilizing other sources of inorganic nitrogen. Direct assimilation of nitrate, however, would probably be repressed by the concentrations of ammonium found in Bear Creek (Syrett 1981). It seems more likely that algae are acting as a sink for ammonium produced within stream sediments. The calculated rates of algal nitrogen demand are sufficient to account for between 20% and 49% of the nitrate loss observed in Bear Creek on the dates studied.

As was observed in Bear Creek, there were significant losses of nitrate from water overlying sediment cores in the laboratory. Nitrate nitrogen levels in experimental enclosures showed progressive declines over the incubation period for all treatments (Figure 5). Decreases in concentration were most rapid in those cores incubated on the light-dark cycle and in those incubated anaerobically. Cores

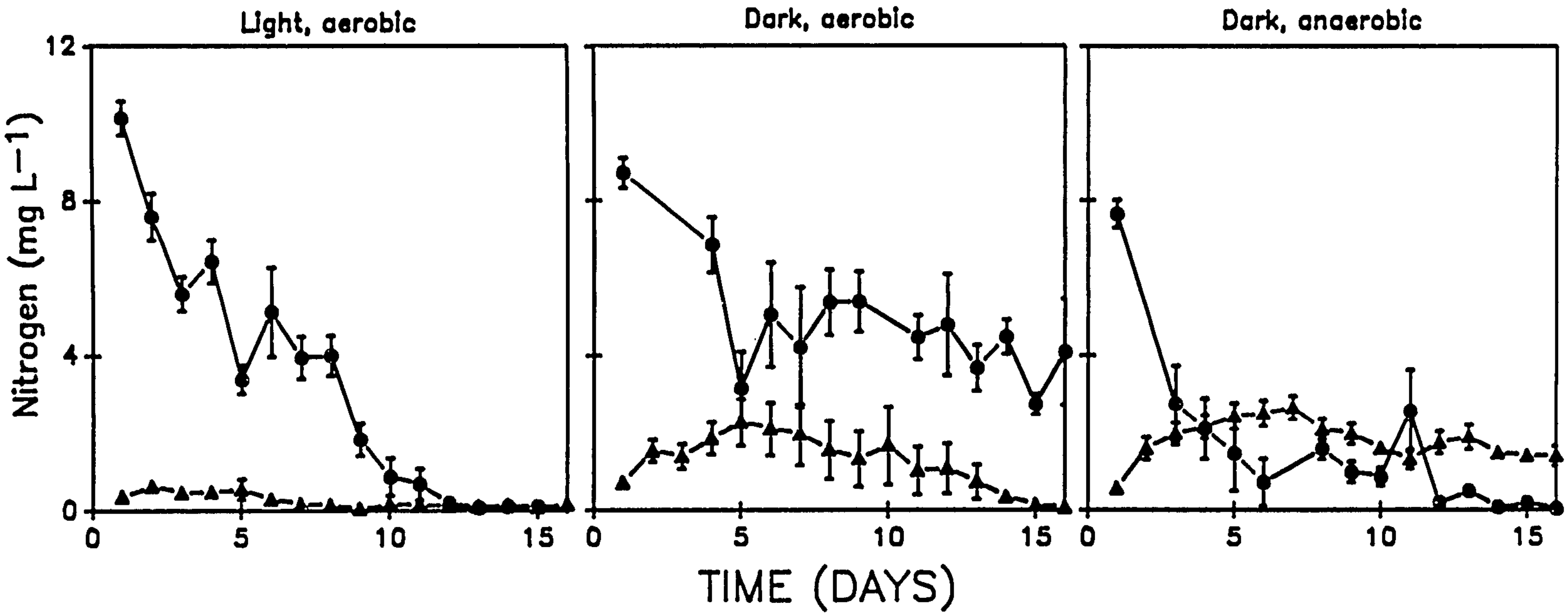

Figure 5. Nitrate nitrogen (circles) and ammonium nitrogen (triangles) concentrations (mg L^{-1}) in stream water overlying sediment cores with standard error of mean

incubated aerobically in the dark and aerobically showed the slowest decline in nitrate levels.

The rate of nitrate disappearance on a sediment area basis was calculated using regression analysis to determine the linear equation best describing the data. As the estimate of interest was the loss rate most closely approximating in situ rates, only the first six days were included in the regression analysis for those cores on the light-dark cycle and those cores treated anaerobically. In the case of the cores under the dark, aerobic treatment, nitrate concentrations declined much more slowly, so the regression analysis included all days of the study.

The average rate of nitrate disappearance observed for all treatments in the laboratory incubation studies was 0.065-0.197 g m^{-2} day^{-1} (Table 5). Analysis of variance suggests that the null hypothesis that there was no difference between treatments may be rejected ($F = 20$, $p < 0.01$). Additionally, a Student-Newman-Keuls multiple range test indicated a significant difference ($p < 0.01$) between the dark, aerobic treatment mean and either the dark, anaerobic mean or the light-dark aerobic mean. Nitrate loss rates observed in those cores incubated aerobically on the light-dark cycle could be considered most comparable to field observations. In those cores the mean loss rate observed (0.164 g m^{-2} day^{-1}) was of

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	0.142		
	0.106		
	0.180		
	0.194		
	0.148		
Dark, aerobic	0.063	0.065	0.006
	0.036		
	0.054		
	0.057		
	0.081		
	0.081		
	0.080		
Dark, anaerobic	0.281	0.197	0.029
	0.149		
	0.191		
	0.170		

the same order of magnitude as that observed in Bear Creek in August of 1986 (0.66 g m^{-2} day^{-1}). However, these laboratory experiments were not intended to mimic in situ conditions, but rather to determine whether photoautotrophic nitrogen demand and anaerobic conditions have any effect on nitrate loss in a sediment water system.

Previous laboratory incubation studies have also demonstrated rapid nitrate loss from water overlying stream sediments (Table 6). These studies have generally identified bacterial denitrification as the mechanism for the nitrate loss observed although some research has indicated that dissimilatory nitrate reduction to ammonium by bacteria can also be important in anaerobic sediments or within the water column (Hill and Sanmugadas 1985, Koike and Hattori 1978, Samuelsson and Ronner 1982, Sorensen 1978). The relative importance of denitrification and of dissimilatory nitrate reduction to ammonium are of particular interest. These processes occur under similar conditions but one process results in the loss of combined nitrogen (denitrification), whereas the other conserves nitrogen (dissimilatory reduction). If dissimilatory nitrate reduction to ammonium is favored, this may be an important pathway in the recycling of inorganic nutrients to support autotrophic production in these systems.

Table 6. Nitrate loss rates (g m^{-2} day^{-1}) from stream water overlying sediment cores

Location	NO_3-N Removal g N m^{-2} day^{-1}	Reference
Duffin Creek (Ontario)	0.02-0.25	Hill 1981
Swifts Brook (Ontario)	0.06-0.17	Sain et al. 1977
Canal (Holland)	0.10-0.16	van Kessel 1977b
Several Streams (Ontario)	0.04-0.13	Wyer and Hill 1984
Canagagigue Cr. (Ontario)	0.14	Robinson et al. 1978
Purukohukohu Basin (New Zealand)	0.07-0.49	Cooper and Cooke 1984
Nottawasaga R. (Ontario)	0.01-0.19	Hill 1983
Several Streams (Ontario)	0.03-0.22	Hill and Sanmugadas 1985
Canagagigue Cr. (Ontario)	0.05-0.09	Chatarpaul and Robinson 1979
Bear Creek (Iowa)	0.07-0.20	This study

The concentration of ammonium increased initially in all cores with the exception of those incubated aerobically on a light-dark cycle. In cores incubated aerobically in the dark, ammonium nitrogen concentrations peaked at 1-2.5 mg L^{-1} between days 4 and 8 and declined to near zero by the end of the experiment. In those cores incubated anaerobically, ammonium nitrogen increased rapidly to 2-3.5 mg L^{-1} and fluctuated in this range for the duration of the experiment.

In this study, the greater nitrate loss rate in cores treated aerobically on the light-dark cycle than those treated aerobically in the dark indicates that photoautotrophic demand of inorganic nitrogen is important and contributes to the overall nitrate decline in these systems. It is unclear whether algae are acting as a sink for ammonium produced by mineralization or dissimilatory reduction of nitrate or are assimilating nitrate directly. Ammonium concentrations in these cores on the light-dark cycle were depressed throughout the incubation, probably due to assimilation and immobilization. Depletion of ammonium produced initially in the dark, aerobic cores was probably due to a combination of immobilization, nitrification, and/or decline of ammonium production. The higher nitrate loss rate in cores under the dark, anaerobic treatment than those under the dark, aerobic treatment indicates that nitrate loss processes in the dark, denitrification or dissimilatory nitrate reduction, are promoted by lowered oxygen tension in the overlying water.

The analysis of inorganic nitrogen in stream water overlying sediments cannot completely delineate nitrogen transformations. The data do not provide estimates of internal recycling such as mineralization, nitrification, and dissimilatory nitrate reduction. However, these studies, combined with field mass balance studies, provide critical information regarding the dynamics and potential importance of nitrate loss processes in these agricultural streams. Future research should address the possible role of algae as sink for ammonium produced via mineralization and dissimilatory reduction of nitrate and as mediators of oxygen tensions in sediment systems.

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SECTION II. FACTORS INFLUENCING NITRATE TRANSFORMATIONS AND LOSS IN AN AGRICULTURAL STREAM

INTRODUCTION

The complex nitrogen cycle in streams affects and is affected by many different processes including aerobic and anaerobic respiration, carbon and nitrogen loads and primary production. Nitrogen fixation may occur in streams, however, this process is inhibited by the presence of organic or inorganic nitrogen. Inorganic nitrogen may be assimilated by both autotrophic and heterotrophic organisms and immobilized to organic nitrogen. Organic nitrogen may be mineralized to NH_4^+ which, under aerobic conditions, may be converted to NO_2^- or NO_3^- by nitrification. Reduction of nitrate may be assimilatory, in which the nitrogen is incorporated into cell biomass, or dissimilatory, in which the functional role is to act as a terminal electron accepter to support an energy yielding reaction. Respiring organisms use various electron accepters to oxidize organic compounds. The highest energy yield is obtained when O_2 is the oxidizing agent (National Research Council 1979). When oxygen is exhausted, alternate electron accepters, such as nitrate, must be utilized, with lower energy yield. Dissimilatory nitrate reduction can be of two types, denitrification, which produces N_2 and N_2O, and dissimilatory nitrate reduction to ammonium, where NH_4^+ is the product. The relative importance of denitrification and dissimilatory nitrate reduction to ammonium is of particular interest. These processes both occur under similar conditions but one process results in the loss of combined nitrogen (denitrification), whereas the other conserves nitrogen (dissimilatory reduction).

There is continual conversion and loss of nitrogen in stream systems. Several studies of nitrogen mass balances have shown considerable losses of nitrate during transport in streams. Most of these studies have identified bacterial denitrification in anaerobic stream sediments as the major mechanism of nitrate depletion (Hill 1981, 1983, 1988; Hill and Sanmugadas 1985; Kaushik et al. 1975; Robinson et al. 1979; van Kessel 1977a). Laboratory investigations have generally confirmed that microbial denitrification in stream sediments is a major nitrate loss process (Chatarpaul and Robinson 1979, Hill 1983, Hill and Sanmugadas 1985, van Kessel 1977b). Others have demonstrated that microbial dissimilatory nitrate reduction to ammonium may be an important or even the dominant nitrate loss process in some marine systems (Koike and Hattori 1978, Sorenson 1978, Cole and Brown 1980). Hill and Sanmugadas (1985) conclude that dissimilatory reduction may be important in stream sediments.

Temperature, oxygen and nitrate concentration of the overlying water may all affect nitrate loss rates in streams (Sain 1977 et al., van Kessel 1977b). Nitrate loss rates are also related to the chemical and physical properties of stream sediments (Wyer and Hill 1984, Hill and Sanmugadas 1985, Hill

1988). Hill and Sanmugadas (1985) conclude that stream sediment properties may influence the relative amounts of nitrate which are denitrified or are removed by other nitrate reduction processes.

Increased inputs of ammonium and nitrate could effect the balance of these nitrogen transformation processes in streams. Therefore, non-point source loading of inorganic nitrogen to surface and groundwaters is of great concern in many heavily agricultural regions. The role of agriculture and fertilizer nitrogen has been demonstrated as many studies show a direct relationship between nitrate leaching to groundwater and nitrogen fertilization history (Hallberg 1987, Klepper 1978). In Iowa, groundwater discharges into master streams provide perennial flows, providing the interconnection affecting stream water quality (Hallberg 1987). As a result, non-point nitrogen loads in the region are among the highest in the country (Omernick 1977) and nitrate-nitrogen concentrations in surface waters frequently reach 10 mg L^{-1} (Bushong 1985, Crumpton and Hersh 1987).

This paper presents the results of a study designed to estimate the influence of temperature, aeration and sediment characteristics on nitrate transformations in agricultural stream sediments. An additional objective of this study was to examine ammonium production under aerobic and anaerobic conditions in an effort to elucidate the role of this process in nitrogen transformations in these systems.

MATERIALS AND METHODS

The effects of temperature, aeration, and sediment characteristics on nitrate transformations in sediment water systems from an agricultural stream were investigated using experimental enclosures in the laboratory. Intact sediment cores were collected in December, 1987 from Bear Creek, a shallow, productive stream in central Iowa. Polycarbonate cylinders (5.1 cm ID, 30.5 cm long) were pushed into the sediment to a depth of approximately 14 cm, the top closed with a rubber stopper, the columns pulled out with the intact core of sediment, and the lower end stoppered. Twenty cores high in organic matter were collected from a single depositional area of the stream and twenty cores with coarse textured sediment low in organics were collected from a sandy section. These cores were evenly divided into treatments of aerobic versus anaerobic conditions and incubation at 15°C and 25°C. The cores, with approximately 250 ml of overlying stream water, were incubated in environmental chambers immediately upon return to the laboratory. Air or nitrogen, providing aerobic or anaerobic conditions, was introduced via a cannula inserted through a serum stopper in an air tight plexiglass plunger which replaced the top rubber stopper. The gas bubbles were introduced close to the sediment-water interface in order to create some turbulence in an effort to simulate stream conditions. All cores were incubated in the dark.

Every 24 hr. samples were collected from the overlying water of each core. Duplicate two ml samples were filtered and immediately analyzed for ammonium nitrogen using a phenol-hypochlorite method (Scheiner 1976). Two additional one ml samples were immediately analyzed for nitrate nitrogen using second derivative, ultraviolet spectroscopy (Simal et al. 1985, Crumpton and Isenhart Ca 1988). Concentration values were corrected for evaporation losses during the incubation period.

To determine organic carbon and nitrogen content of stream sediments, five replicate cores of each sediment type were collected from the same areas as above using a polycarbonate cylinder (1.6 cm ID) in a manner similar to above. A threaded plunger inserted from the bottom was used to extrude the core and section the sediment by depth. Cores were sectioned by 0.5 cm for the first two cm, and by 1.0 cm from two to ten cm. Sections were frozen, freeze dried and inorganic carbon removed by acidification as described by Hedges and Stern (1984). Organic carbon and nitrogen were determined using a Carlo Erba NA 1500 carbon-nitrogen-sulfur analyzer.

RESULTS AND DISCUSSION

There were significant differences in organic carbon and nitrogen content both between sediment types and by depth within each sediment type (Table 1). Organic carbon and nitrogen content decreased with depth in both sediment types. Organic carbon content at 10 cm in the silt cores was only 14% and organic nitrogen was only 9% of that in the first 0.5 cm. In the sand cores, organic carbon and nitrogen at 10 cm was 39% and 41% respectively of that in the first 0.5 cm. The average organic carbon content within the first two centimeters of sediment was over six times greater in silt cores compared to sand cores while the organic nitrogen content was ten times greater (Table 2). The organic content from the top two centimeters was pooled for comparison as it is thought that the majority of biological activity in these systems occurs above this depth. Organic carbon and nitrogen also declined rapidly between depths of two and three centimeters.

Analysis of variance of the means of organic carbon and nitrogen content within the first two centimeters of each sediment type suggests that the null hypothesis that there is no difference between the sand and silt sediment types may be rejected ($F=11$, $p < 0.05$ for carbon; $F=12$, $p < 0.01$ for nitrogen). This difference in organic carbon and nitrogen

Table 1. Mean organic carbon and nitrogen values (mg ml^{-1}) by depth for silt and sand cores with standard error of mean (n=5)

Depth cm	Carbon mg ml^{-1}	SEM	Nitrogen mg ml^{-1}	SEM
<u>Silt sediments</u>				
0.5	0.128	0.0282	0.0133	0.0033
1.0	0.191	0.0460	0.0207	0.0054
1.5	0.127	0.0344	0.0132	0.0039
2.0	0.087	0.0342	0.0087	0.0038
3.0	0.022	0.0089	0.0018	0.0008
4.0	0.022	0.0060	0.0017	0.0007
5.0	0.029	0.0074	0.0023	0.0007
6.0	0.026	0.0058	0.0020	0.0006
7.0	0.026	0.0067	0.0022	0.0007
8.0	0.022	0.0064	0.0017	0.0006
9.0	0.021	0.0064	0.0016	0.0006
10.0	0.018	0.0035	0.0012	0.0003
<u>Sand sediments</u>				
0.5	0.030	0.0040	0.00127	0.00034
1.0	0.028	0.0044	0.00126	0.00026
1.5	0.029	0.0041	0.00134	0.00029
2.0	0.023	0.0041	0.00108	0.00053
3.0	0.016	0.0013	0.00058	0.00018
4.0	0.010	0.0005	0.00043	0.00009
5.0	0.010	0.0009	0.00030	0.00012
6.0	0.011	0.0021	0.00039	0.00008
7.0	0.009	0.0005	0.00020	0.00006
8.0	0.009	0.0005	0.00034	0.00008
9.0	0.010	0.0014	0.00043	0.00018
10.0	0.012	0.0010	0.00054	0.00011

Table 2. Organic carbon and nitrogen content (mg ml^{-1}) in the first two cm for replicate silt and sand cores, means and standard error of mean (n=5)

Carbon mg ml^{-1}	Mean	SEM	Nitrogen mg ml^{-1}	Mean	SEM
<u>Silt sediments</u>					
0.1644	0.1331	0.0323	0.0170	0.0140	0.0035
0.1369			0.0134		
0.0126			0.0007		
0.1468			0.0159		
0.2046			0.0230		
<u>Sand sediments</u>					
0.0336	0.0271	0.0035	0.0023	0.0012	0.0003
0.0301			0.0013		
0.0344			0.0015		
0.0192			0.0006		
0.0182			0.0005		

content is striking in that the two sediment types were collected within several meters of each other and is indicative of the heterogeneity of substrates within these systems.

The mean carbon/nitrogen ratio for the pooled top two centimeters of sediment was 10 for the silt cores and 26 for the sand cores (Table 2). This ratio generally increased with depth. In silt sediments the C/N ratio was 10 in the first 0.5 cm, increasing to a maximum of 15 at 10 cm (Table 3). Rosenfield (1981) observed a similar increase of the C/N ratio of the organic matter of up to 15-17 as a function of depth within the sedimentary column in a marine system. Carbon/nitrogen ratios for the sand sediments were much more variable, due mostly to the organic nitrogen content being low and variable in the replicate cores.

Initial concentrations of nitrate nitrogen and ammonium nitrogen in the overlying stream water were 9.6 and 0.2 mg L^{-1} respectively. Nitrate concentrations decreased rapidly in all treatments of silt sediment cores, which were high in organic sediment (Figure 1). Both anaerobic conditions and higher temperature increased the rate of nitrate loss in silt sediment cores. In contrast, nitrate concentrations declined much more slowly in sand cores and treatment effects were less obvious (Figure 2).

Table 3. Mean organic carbon/organic nitrogen ratios by depth for silt and sand sediment cores with standard error of mean (n=5)

Depth cm	C/N	C/N
	Silt sediments	Sand sediments
0.5	10	24
1.0	9	22
1.5	10	22
2.0	10	21
3.0	12	28
4.0	13	23
5.0	13	33
6.0	13	28
7.0	12	45
8.0	13	27
9.0	15	23
10.0	15	22

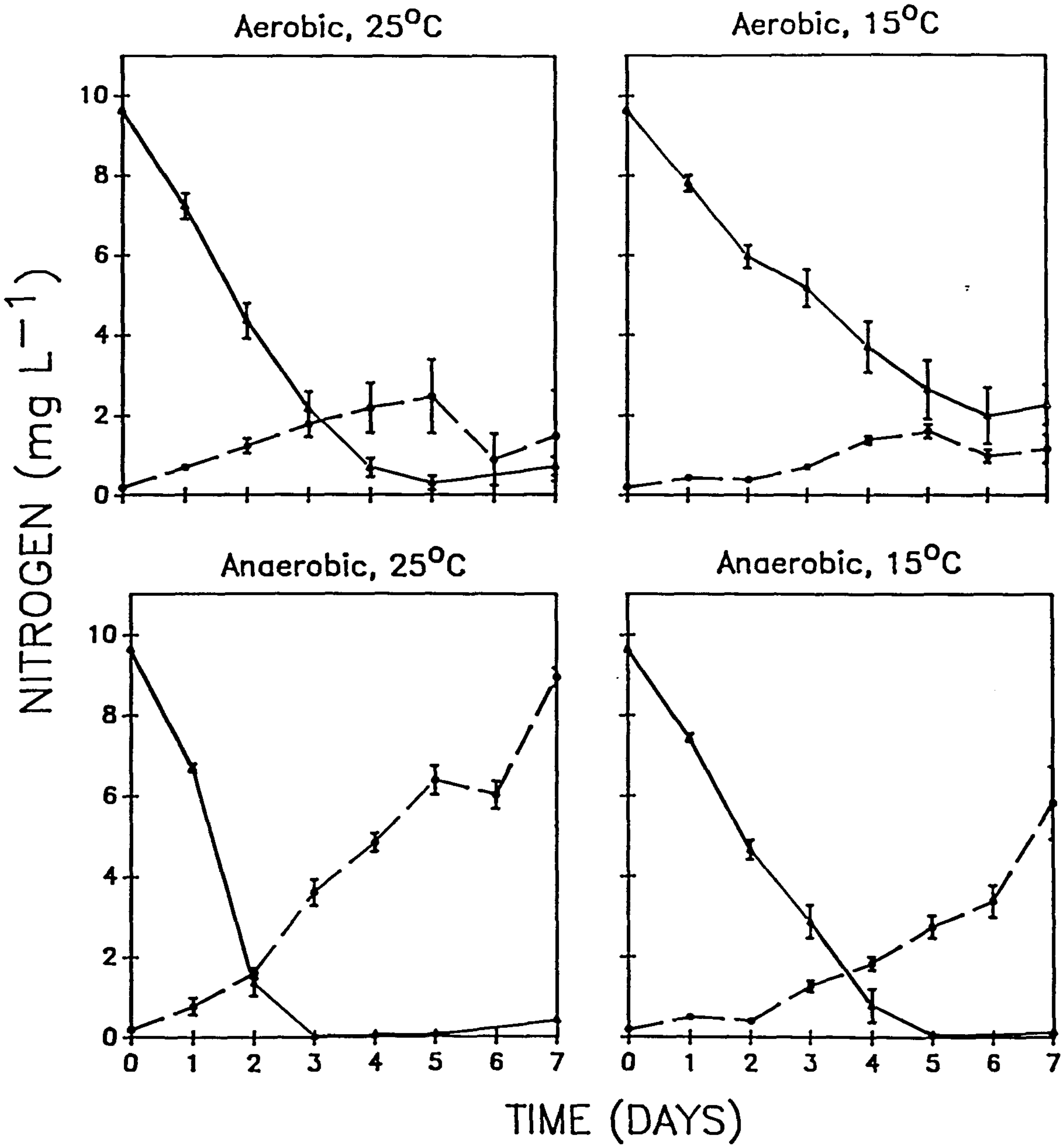

Figure 1. Nitrate nitrogen (solid line) and ammonium nitrogen (dashed line) concentrations (mg L^{-1}) in water overlying silt sediments with standard error of mean (n=5)

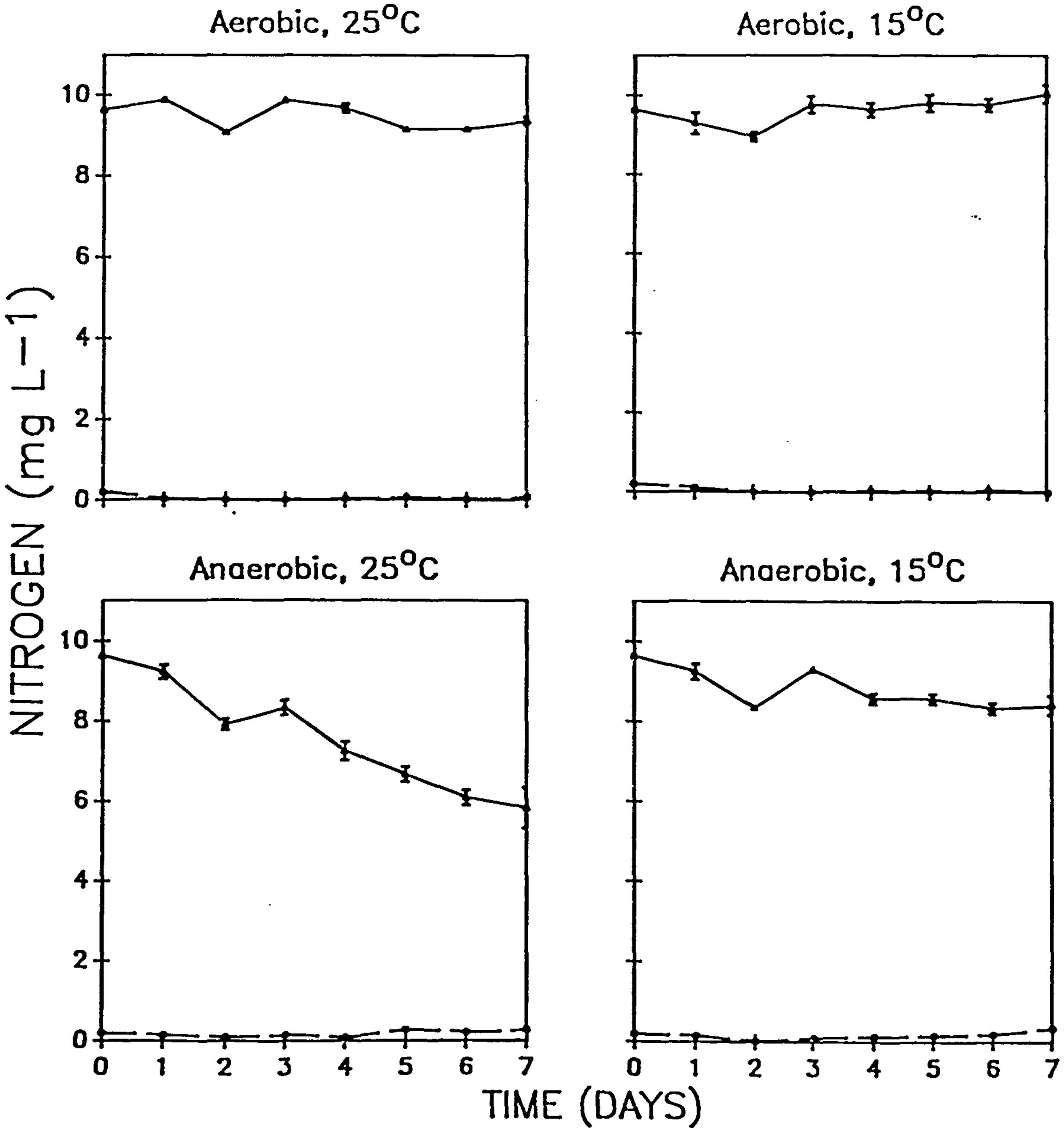

Figure 2. Nitrate nitrogen (solid line) and ammonium nitrogen (dashed line) concentrations (mg L^{-1}) in water overlying sand sediments with standard error of mean (n=5)

The rate of nitrate disappearance on a sediment area basis was calculated using regression analysis to determine the linear equation best describing the data. Only the first three days were included in the regression analysis, since nitrate concentrations had approached zero by this time in silt cores in the 25°C, anaerobic treatment. The average rate of nitrate disappearance ranged between 0.032-0.407 g N m^{-2} day^{-1} (Tables 4 and 5, Figure 3). Analysis of variance of treatment means suggests that the null hypothesis that there was no difference between treatments may be rejected ($F=177$, $p <0.001$). A Student-Newman-Keuls multiple range test indicated significant differences ($p <0.01$) between average loss rates in silt sediments at each temperature, and aerobic vs anaerobic treatments. Within sand cores, the only significant difference was between average loss rate in those cores treated anaerobically at 25°C and sand cores treated otherwise.

Stream sediment quality greatly affected the nitrate loss rate. In every case with temperature and aeration the same, silt cores had significantly higher nitrate loss rates than did sand cores. It is presumed that this is because the large amount of carbon available in silt sediments provides for a large microbial population. This agrees with results presented by Hill and Sanmugadas (1985), who found a

Table 4. Nitrate nitrogen loss (g m^{-2} day^{-1}) in replicate silt cores, treatment means and standard error of mean

Treatment	NO_3-N Removal g N m^{-2} day^{-1}	Mean	SEM
<u>Silt Sediments</u>			
25°C, anaerobic	0.380	0.407	0.010
	0.425		
	0.415		
	0.430		
	0.385		
25°C, aerobic	0.356	0.327	0.009
	0.326		
	0.306		
	0.336		
	0.311		
15°C, anaerobic	0.346	0.293	0.018
	0.316		
	0.252		
	0.296		
	0.257		
15°C, aerobic	0.227	0.197	0.016
	0.148		
	0.173		
	0.203		
	0.232		

Table 5. Nitrate nitrogen loss rates (g m^{-2} day^{-1}) for replicate sand cores, treatment means and standard error of mean

Treatment	NO_3-N Removal g N m^{-2} day^{-1}	Mean	SEM
<u>Sand Sediments</u>			
25°C, anaerobic	0.099	0.106	0.008
	0.084		
	0.109		
	0.133		
	0.104		
25°C, aerobic	0.030	0.039	0.005
	0.054		
	0.030		
	0.044		
	0.035		
15°C, anaerobic	0.054	0.062	0.007
	0.049		
	0.089		
	0.054		
	0.064		
15°C, aerobic	0.049	0.032	0.007
	0.020		
	0.025		
	0.015		
	0.049		

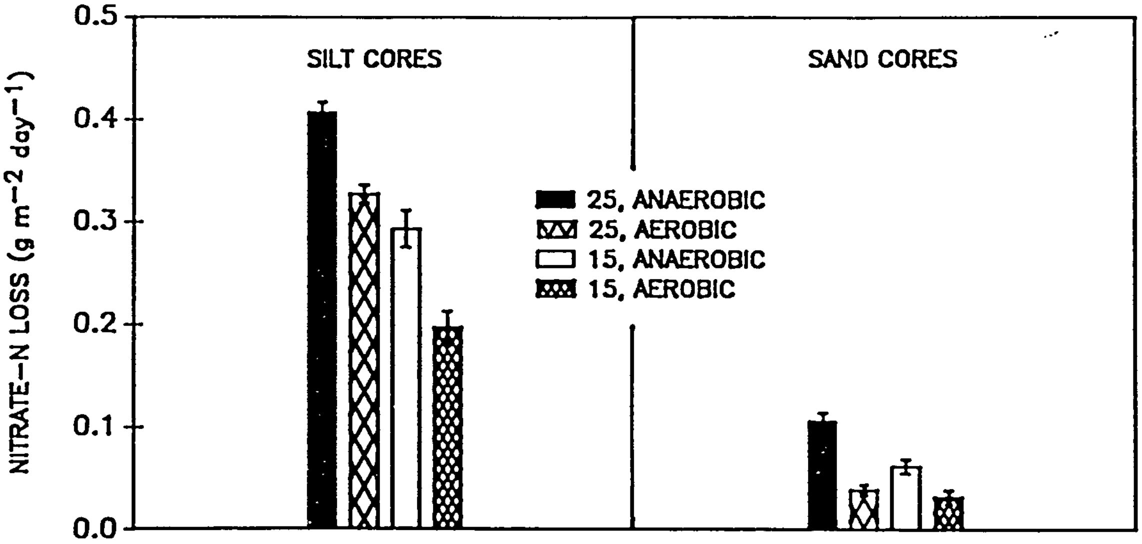

Figure 3. Nitrate nitrogen loss rates (g m^{-2} day^{-1}) in water overlying stream sediments with standard error of mean (n=5)

significant correlation between sediment organic carbon and nitrate loss rate from cores overlying stream sediment.

Incubation at higher temperature significantly increased the rate of nitrate loss in silt cores and in sand cores incubated anaerobically (Tables 4 and 5). This is consistent with the results of Sain et al. (1977) and van Kessel (1977b). Q_{10} values calculated for nitrate loss ranged from 1.4 in silt cores incubated anaerobically to 1.7 in sand cores incubated anaerobically and silt cores incubated aerobically (Table 6). Additionally, higher temperature seems to have increased the rate of ammonium production in both aerobic and anaerobic treatments (Figures 1 and 2).

The effect of aeration on nitrate loss processes was also apparent. In sand cores incubated aerobically at either temperature, nitrate concentrations varied little throughout the experiment. Sand cores incubated anaerobically at 15°C also showed no significant increase in loss rate compared with similar cores incubated aerobically. However, anaerobic incubation significantly increased the rate of nitrate loss in silt cores at both temperatures and in sand cores at 25°C. van Kessel (1977b) concluded that the thickness of the sediment layer into which oxygen penetrates increases with increase in dissolved oxygen concentration in the overlying water, thus increasing the length of the supply route for

Table 6. Q_{10} values for nitrate nitrogen loss rates in sediment cores incubated at 25°C and 15°C

Treatment	25°C Loss Rate $g\ m^{-2}\ day^{-1}$	15°C Loss Rate $g\ m^{-2}\ day^{-1}$	Q_{10}
Silt, anaerobic	407	293	1.4
Silt, aerobic	327	197	1.7
Sand, anaerobic	106	62	1.7

nitrate before reducing conditions are encountered. Anaerobic incubation also seemed to increase the rate at which ammonium concentrations increased in silt cores (Figure 1).

These results confirm those of previous laboratory studies which have demonstrated rapid nitrate loss from water overlying stream sediments and the effects of sediment organic content, temperature, and O_2 availability (Hill 1981, Sain et al. 1977, van Kessel 1977b, Wyer and Hill 1984, Chatarpaul and Robinson 1979). An additional objective of this study was to examine ammonium production under aerobic and anaerobic conditions in an effort to elucidate the role of this process in nitrogen transformations in these systems. Previous studies have generally identified microbial denitrification as the mechanism for the nitrate loss with dinitrogen gas or nitrous oxide as endproducts. Hill and Sanmugadas (1985), however, suggested that nitrate reduction to ammonium may be important in some stream sediments. Dissimilatory reduction of nitrate to ammonium has been demonstrated in some marine systems (Koike and Hattori 1978, Samuelsson and Ronner 1982, Sorensen 1978). To date, however, there is little evidence that dissimilatory nitrate reduction to ammonium is an important process in freshwater systems. Chen et al. (1972), in a study using freshwater lake sediments, reported as much as 68% of labeled nitrate present as ammonium after anaerobic incubation. They concluded, however, that this result indicates rapid mineralization of labelled nitrogen incorporated into organic-N. Chatarpaul and Robinson (1979) and van Kessel (1977b) found ^{15}N labelled ammonium after incubation with the addition of ^{15}N labelled nitrate but they also attributed this finding to the mineralization of previously immobilized nitrogen.

In this study, the concentration of ammonium in sand cores was low throughout the experiment. However, ammonium concentrations increased over time in all cores high in organics (Figure 1). The increase in concentration of ammonium in silt cores incubated anaerobically at $25^{o}C$ was nearly stoichiometric with the nitrate loss observed, reaching over 8 mg L^{-1} by day 7 (Figure 1). In silt cores incubated aerobically, ammonium concentrations leveled off at about 2 mg L^{-1} and declined slightly by the end of the experiment.

Mineralization of organic nitrogen and dissimilatory reduction of nitrate to ammonium are possible sources of this increase in ammonium in the overlying stream water. However, mineralization of organic nitrogen would not be expected at rates sufficient to account for the increases in ammonium observed in the silt cores under anaerobic conditions. Under anaerobic conditions, with NO_3-N as the electron accepter, mineralization of organic nitrogen would not be expected to produce NH_4-N at near this ratio. Furthermore, as the average C/N ratio in the cores ranged between 9 and 15, immobilization rather than mineralization of organic nitrogen would be expected (Lancelot and Billen 1985).

Tiedje *et* al. (1982) examined the outcome of competition for available nitrate between denitrification and dissimilatory reduction in terms of energetics and kinetics. They stated that if evaluated on energy yield per electron donor (H_2), then denitrification provides more potential energy. However, if evaluated on energy available per nitrate consumed, then the dissimilatory reduction is slightly more favorable. More important, though, could be the capacity of the dissimilatory reduction for accepting eight electrons versus only five for denitrification. In strongly anaerobic habitats, the lack of electron accepters is probably the most limiting factor for growth. Thus dissimilatory reduction could be more favorable simply because of its capacity for accepting an additional three electrons. Tiedje et al. (1982) postulated that the partitioning observed is a function of the ratio of available carbon to electron accepters. The maximum energy-yielding steps are favored when carbon is limiting, but the eight electron reduction is favored when electron accepters are limiting. This prediction is consistent with results from this study. The treatments under which a large increase in ammonium concentration was observed were cores containing a high amount of organic matter (silt cores) incubated anaerobically. It is quite conceivable, therefore, that much of this observed increase in ammonium is the result of microbial dissimilatory nitrate reduction to ammonium. If so, this may be an important pathway in the recycling of inorganic nutrients to support autotrophic production in these systems.

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SUMMARY DISCUSSION

Non-point source loading of inorganic nitrogen to surface and groundwaters in Iowa are among the highest in the nation. Consequently, concentrations of nitrate-nitrogen in Iowa surface waters are also among the highest found, consistently reaching 10 mg L^{-1}. Streams do not act as simply as conduits for this material. There is continual conversion and loss of nitrogen in stream systems.

Mass balance studies on Bear Creek in central Iowa during low flow periods of 1986 demonstrated significant losses of nitrate-nitrogen, ranging from 5.3 to 7.4 kg day^{-1} over the reach studied. Autotrophic nitrogen demand, based on observed rates of primary production, was estimated to be 20 and 49 percent, respectively, of these losses. It is unclear whether this demand is manifested through assimilation and immobilization of nitrate directly or of ammonium produced through dissimilatory nitrate reduction. The remainder of the nitrate losses can be taken as minimal estimates of denitrification and/or dissimilatory nitrate reduction to ammonium.

Experimental enclosures containing intact sediment cores were used to investigate various factors affecting nitrogen transformations and loss in agricultural streams. Nitrate nitrogen levels in water overlying stream sediments showed progressive decline over the incubation period for all treatments with silt sediments but only under anaerobic conditions at higher temperature with sand sediments. An increase in nitrate loss rate in cores on a light-dark cycle over those in the dark gives further evidence that photoautotrophic nitrogen demand contributes to the overall nitrate decline in these systems. Temperature, aeration and sediment characteristics were all found to affect significantly the rate of nitrate decline in experimental enclosures.

A nearly stoichiometric increase in the concentration of ammonium was observed in enclosures with silt cores incubated anaerobically. Mineralization of organic nitrogen and dissimilatory reduction of nitrate to ammonium are possible sources of this increase. It is unlikely, however, that mineralization could account for the ratio of ammonium production to nitrate decline with nitrate as the electron accepter. Additionally, the carbon/nitrogen ratios in the upper two centimeters of sediment were high, and it is likely that immobilization of mineral nitrogen should precede mineralization. With strongly anaerobic conditions and high amounts of available carbon, it is conceivable, therefore, that much of the observed ammonium increase is the result of microbial dissimilatory nitrate reduction to ammonium. If so, this may be an important pathway in the recycling of inorganic nutrients to support autotrophic production in these systems.

Suitable conditions for nitrate transformation and removal during transport exist in agricultural streams. However, more detailed investigations are needed to estimate the relative importance of the various nitrate transformation and loss processes, particularly with respect to the support of autotrophic production.

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