DENITRIFICATION IN FOUR STREAMS IN BALTIMORE, MARYLAND, USA: A STUDY OF HYPORHEIC ZONES

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Abstract . This study examined hyporheic zone denitrification in four streams in Baltimore, Maryland. Hyporheic zone denitrification is ecologically important because it decreases nitrate (NO_3^-) levels in streams. High NO_3^- levels can cause eutrophication in coastal (salt) waters, which kills fish and other aquatic life forms. The objectives of the study were 1) to determine if some stream physical features are more conducive to denitrification than others and 2) if potential denitrification rates differ between pristine, semi-pristine, semi-degraded, and highly degraded streams that have different organic matter content and NO_3^- levels. The Baltimore Ecosystem Study has been recording NO_3^- levels in the streams studied. These streams show evidence of hyporheic zone denitrification, because NO_3^- levels upstream are higher than those downstream. Sediments were taken from five different stream physical features: organic debris dams, vegetated gravel bars, gravel bars, pools, and riffles. Both sediment % organic matter content and stream NO_3^- levels controlled denitrification rates. Organic debris dams, which had the highest % organic matter content (average 19%), also had the highest potential denitrification rates (average 2,495 ng N/g soil/ hour). Organic matter content and potential denitrification rates in the rest of the stream physical features were less than 5% and under 250 ng N/g soil/ hour respectively. Hyporheic zone denitrification was higher in streams with high NO_3^- levels. The highly degraded stream had lower than expected hyporheic zone denitrification rates because its stream physical features have been altered, which has affected its stream chemical processes.

INTRODUCTION

Excess levels of nitrogen, especially in the form of nitrate (NO_3^-), in water can cause eutrophication (Edwards 1998), especially in coastal (salt) waters, which can kill fish and other forms of aquatic life. Some bacteria found in stream sediments oxidize organic carbon using NO_3-N as an energy source. The product of this denitrification process reaction is often nitrous oxide (N_2O) and dinitrogen gas (N_2) (Martin et al. 2001), thus nitrogen is removed from the stream (Triska et al. 1993). Denitrification in hyporheic zones has been found to lower NO_3^- levels in streams and rivers, thus preventing eutrophication (Martin et al. 2001; Clement et al. 2002).

As part of a long-term study, the Baltimore Ecosystem Study (BES), NO_3^- levels in various streams in Baltimore have been recorded. It has been observed that in some of the streams NO_3^- levels are higher upstream than they are downstream. It is believed that this decrease in NO_3^- levels is caused by hyporheic zone denitrification. These streams are of particular interest because they are a part of watersheds that feed into the Chesapeake Bay, which is highly susceptible to NO_3^--induced eutrophication. In order for denitrification to occur various conditions must be met. Three of those conditions are that there be a carbon source (such as leaf litter), that there be a NO_3^- source, and that there are anaerobic conditions (Thompson et al. 2000; Clement et al. 2002). Based on these conditions, it is hypothesized that physical features in streams with higher % organic matter content will have higher potential denitrification rates. Also, streams with higher levels of NO_3^- should have higher potential denitrification rates. The purpose of this study was to determine if some physical features are more conducive to denitrification than others, and if potential denitrification rates differ in pristine, semi pristine, semi-degraded, and highly degraded streams due to differences in organic matter content and NO_3^- levels. There is great interest in, and concern about, the ability of urban streams to consume NO_3^-. Changes in hydrology associated with urbanization have been shown to degrade stream and riparian ecosystems. High storm water flows remove stream features with high organic matter content and cause streams to be incised and isolated from their riparian zones (Groffman et al. 2002).

SAMPLING AND METHODS

Site Description

All sites were located in Baltimore County, Maryland, USA. Four streams were included in the study. Pond Branch (39º28’49.1”, 76°41’15.0”) is a pristine stream draining a 0.38 km^2 watershed, with a broad riparian zone, located in a forested park. Baisman’s Run (39º28’46.1”, 76º40’40.9”) is a semi-pristine stream, draining a 3.82 km^2 watershed, with a forested riparian zone (drier than Pond Branch), and with evidence of incision (0.5 - 1 m). The land use in the area surrounding the stream is 35% urban-residential, 1% urban-other, and 62% forested. Glyndon (39º28’18.1”, 76º49’00.8”) is a semi-degraded stream, draining a 0.81 km^2 watershed, with a forested riparian zone and evidence (smell) of sewage contamination. The land use of the area surrounding this stream is 43% urban-residential, 46% urban-other, 9% forested, and 2% agricultural. Mine Bank Run (39º 25’00.0”, 76º 32’46.7”) is a partially restored highly degraded stream, draining a 7.8 km^2 watershed, with a grassed riparian zone, deeply incised stream banks (3 m), and evidence of sewage contamination.

Sections of each stream near existing BES sampling sites were examined for the presence of gravel bars, vegetated gravel bars, organic debris dams, pools, and riffles. Sediment samples for each feature were taken at two different sites along each stream. The physical characteristics of each stream influenced the type and number of features sampled (Table 1). When necessary, sediment was dug to a level deep enough so that the sample taken was covered with water (to ensure that we were sampling features that were in contact with stream water).

All samples were collected July 1st, 2002. Samples were collected in zip-lock bags and kept at ambient temperature on site. Samples were kept on ice when transported, and were refrigerated at the lab until analyzed (within 1 month). All samples were sieved through 6mm mesh in order to remove large objects (rocks, sticks, etc.), to homogenize the samples, and to break up saturated, decomposing leaves.

Laboratory Analysis

Soil moisture content was measured gravimetrically. Duplicate samples of 10g were heated at 105ºC for 48 hours until weight was constant (Parry et al. 1999).

Percentage organic matter content was measured using the loss on ignition method (Felmer et al. 1998). Dried samples were heated at 550ºC for one hour and allowed to come to room temperature in the oven. Cooled samples were placed in a dessicator and weighed.
Sediment NO_3 - N and NH_4-N were determined by KCL extraction as described in (Lou et al. 2000). Duplicate 5g samples were amended with 10 ml of 2M KCL, placed on a rotary shaker for an hour, and filtered using Whatman #42 filters. Extracts were analyzed colormetrically (Perstorp Analytical).

Potential denitrification was assessed by a Denitrification Enzyme Assay (DEA) method (Tiedje et al. 1989; Groffman et al. 1999). Duplicate samples of 5g of wet sediment were placed in 125ml Erlenmeyer flasks with 10 ml of DEA solution containing KNO_3, glucose, and chloramphenicol. The chloramphenicol prevents the growth of bacteria during the incubation period (Gilbert et al. 1998) so the denitrification rate reflects that of the sample at the time it was taken (Groffman et al. 1999). Flasks were stoppered and alternately evacuated and flushed with N_2 gas 3 times for a total of 12 minutes to make samples anaerobic, thus promoting denitrification. Samples were brought to atmospheric pressure by puncturing the stopper with a hypodermic needle. 10 ml of acetylene (C_2H_2) was added to each sample. The final concentration of C_2H_2 was 10% (10kPa) in the gas phase. The syringe was pumped to ensure the C_2H_2 was evenly distributed in the flask. Flasks were placed on a rotary shaker for 24 hours. The gas inside the flasks was mixed before the samples were taken. A 9 ml sample of the headspace gas was taken from each sample an injected into a pre-evacuated 9 ml vial. Samples were analyzed for N_2O gas using an electron capture gas chromatograph (Tracor 540).

Conversion of N_2O to N_2 in soils, when incubated in the presence of C_2H_2, is inhibited. In atmospheres of 0.1-10% v/v C_2H_2, N_2O is the sole gaseous product of denitrification. In this case moles of N_2O produced with C_2H_2 equals moles of $N_2O + N_2$ produced without C_2H_2 (Yoshinari et al. 1977). This means that denitrification can be estimated by a single measurement of N_2O using a gas chromatograph (Lou et al. 2000).

Acetylene inhibition methods have problems that must be considered in any study. If there is an insufficient amount of C_2H_2 present, inhibition can be ineffective (especially when organic matter is high and/or nitrate concentrations are low) (Tiedje et al. 1989). However, complete inhibition has been shown with as little as 0.01 ATM C_2H_2 (Yoshinari and Knowles 1976). The % organic matter content in the samples did not exceed 30% and nitrate levels in all of the streams, except Pond Branch were high. C_2H_2 can be biodegraded (but only after exposure for ~ one week) (Tiedje et al. 1989). The samples were incubated for a 24-hour period only. If the C_2H_2 is contaminated it can affect denitrification (Tiedje et al. 1989). The acetylene that was used was purified. Dispersal of the C_2H_2, recovery of N_2O, and significant water solubility of N_2O can all affect the results (Tiedje et al. 1989). An incubation period of 24 hours allows for diffusion of the C_2H_2 throughout the sample (Ineson et al. 1991). N_2O levels in solution were corrected for by the Bunson absorption coefficient (Mahmood et al. 1999). The incubation period used can also affect results by the development of organisms that can reduce N_2O in the presence of C_2H_2 (Rolston 1986). This is unlikely to happen in a 24-hour incubation period. The NO_3^- pool in the sample may become depleted because concurrent nitrification is blocked by C_2H_2 (Ryden 1982). In our DEA method, sufficient NO_3^- was added to prevent this problem. In our incubation method (described below), nitrification was allowed to proceed for 10 days before treatment with C_2H_2 for one day, ensuring a sufficient NO_3^- pool.

Denitrification, respiration, potential net mineralization, and potential net nitrification rates were measured in incubations of unamended soil in the laboratory. Duplicate 20g samples of sediment were placed in mason jars that were sealed with screw top lids with septa to allow for gas sampling. Jars were allowed to incubate in the dark at room temperature (~ 20ºC) for 10 days. The air in the jars was mixed by pumping a 10ml syringe 4 times before extracting 9ml of gas from the headspace and injecting it into evacuated 9 ml vials. Jars were then opened to return the air inside the jar to that of the atmosphere. 50ml of C_2H_2 was then added to each jar. The gas inside the jar was mixed by pumping a 60ml syringe twice. After a 24-hour incubation period the gas inside the jar was pumped 4 times with a 10ml syringe and 9ml gas samples from the headspace of the jar were taken and injected into 9 ml evacuated vials. Samples were tested for N_2O and CO_2 (without C_2H_2) and N_2O (with C_2H_2) by using an electron capture gas chromatograph (Tracor 540). Sediment NO_3-N and NH_4-N were extracted with KCL and analyzed colorometrically as described above. Mineralization was calculated as the accumulation of total inorganic N (NO_3-N plus NH_4-N), nitrification was calculated as the accumulation of NO_3^- and respiration was calculated as the accumulation of CO_2 over the course of the 10-day incubation. Denitrification was calculated as the accumulation of N_2O over the course of the 24-hour incubation with C_2H_2.

Statistical analysis was done using Pearson correlation and the SAS program.

RESULTS

Percentage organic matter content (Figure 2) was greatest in organic debris dams (average 19, range 14.2 – 32.5), followed by vegetated gravel bars (average 3, range 0.9 – 8.8), pools (average 1.5, range 0.6 – 3.2), riffles (average 1.5, range 0.6 – 2.8), and gravel bars (average 1.3, range 0.7 – 2.3). There was variability in organic matter content between sites at the same stream, especially when % organic matter content was high. Organic debris dams at Glyndon had 23% and 13% organic matter content, Baisman's Run had 20% and 32%, and Pond Branch had 11% and 18%.

Potential denitrification rates (Figure 3) were greatest in organic debris dams (average 2495 ng N/g soil/hour, range 153-7414), followed by pools (average 67, range 3.6 – 435), vegetated gravel bars (average 39, range 1.4 – 125), riffles (average 28, range 1.1 – 141.5), and gravel bars (average 12, range 0.2 – 36.8). Potential denitrification rates were extremely variable between sites, in different stream physical features, and in different streams. (Figures 4 and 5)

Initial nitrate values were consistently low at all sites. Values ranged from 0.2 to 1.9 ug N/g dry soil. Initial ammonium values were variable between sites, physical features, and streams. This variability was greatest at the sites where % organic matter content variability was greatest (in organic debris dams). Values at Glyndon were 63 and 31 ug N/g dry soil, 19 and 45 at Baisman's Run, and 19 and 58 at Pond Branch.

Potential net nitrification rates were low (0.0005 to 2 ug N/g dry soil/ day), and showed some variability. Potential net mineralization rates were low (-5 to 1.6 ug N/g dry soil /day), with the most variability in values in organic debris dams. At Glyndon the rates were -5 and -0.8 ug N/g dry soil/day, -1 and -4 at Baisman's Run, and -0.9 and -3.7 at Pond Branch.

Respiration rates were variable between sites, in different physical features, and across streams. High and low values were 69 and 2 ug C/g soil/day respectively. N_2O production was also variable between sites, in different physical features, and across streams. High and low values were 33 and 1.4 ng N/g soil day respectively. Denitrification rates varied somewhat by site, with all values being low (0.7 to 18 ng N/g/hr). Note that there was only one site with a rate of 18 ng N/g/hr, with the next highest rate being 8 ng N/g/hr.

A positive correlation was found between % organic matter content, potential denitrification rates, initial ammonium levels, and respiration rates. A positive correlation was also found between denitrification rates, initial ammonium levels, and N_2O production. Potential net mineralization rates were negatively correlated with all of the above factors. No correlation was found between initial nitrate levels or potential net nitrification rates and any other factor measured.

DISCUSSION

Because organic matter is required for denitrification to take place, features with higher % organic matter content were expected to have higher denitrification rates. The positive correlation between organic matter content and potential denitrification rates explains the variability in the potential denitrification rates between sites, in different physical features, and in different streams. Organic debris dams, which had particularly high organic matter content likely function as “hot spots” of denitrification in these streams, as has been observed in other studies (Hill et al. 2000; Martin et al. 2001).

Although denitrification in stream features is preferred, because it consumes NO_3^-, nitrification, the production of NO_3^-, is another reaction that may occur in hyporheic zones (Burns 1998; Duff and Triska 2000). Nitrification is a two-step process mediated by autotrophic bacteria that convert ammonium (NH_4^+) to nitrite (NO_2^-) and nitrite (NO_2^-) to nitrate (NO_3^-). For this reaction to take place ammonium, oxygen, and a high carbon/nitrogen ratio are needed (Brady and Weil 1999).

Nitrification rates are of interest because in some hyporheic zones denitrification and nitrification reactions are coupled (Holmes et al. 1996). This is especially important where NO_3^- levels in the stream are low (such as in pristine streams), because nitrification can then provide a source of NO_3^-, thus raising NO_3^- concentrations in the stream water (Holmes et al. 1996; Kemp et al. 1990). The nitrification produced NO_3^- is then consumed in the subsequent denitrification reaction. The nitrification rates in all streams and features were low, thus, in these streams, nitrification does not provide a source of NO_3^-.

Those streams with higher nitrate levels (Table 2) also had higher potential denitrification rates, except for Mine Bank Run (Figure 5). Mine Bank Run is a highly incised stream (up to 10 ft. in some places). As the stream has cut into the streambed its geomorphology has changed. There were two major effects from this change. The first was the smaller accumulation of organic matter. The reason for this is two-fold: there was less organic matter to be accumulated and stream physical features capable of collecting organic matter were eliminated. The distance between the streamside vegetation and the stream increased, making it less likely that branches and other organic matter would fall into the stream. Also, the flow of water after precipitation would be greater, washing away any organic matter that may have collected. The second major effect would be the physical change in relationship between ground and surface water (Edwards 1998; Wondzell and Swanson 1999). The stream water would now flow at a different level, thereby changing its location in relation to the ground water. Hyporheic zone denitrification depends on the exchange of surface and ground water (Dahm et al. 1998; Duff and Triska 2000). If the nitrate found in the surface water does not get mixed with the ground water it will not be transferred to the hyporheic zone where it can be denitrified. Also, the geomorphology and physical features in a stream affect the depth and lateral extent of hyporheic zones (Triska et al. 1993; Grimaldi and Chaplot 2000). We expect that hyporheic denitrification will not be an important process in Mine Bank Run.

Ultimately, the ability of stream features to remove nitrate by denitrification depends on the amount and rate of water flowing through these stream features. Most likely organic debris dams and riffles will have the highest degree of water flowing through them, because they are generally found in the middle of or across the stream, where water flow would be greatest. Gravel bars and vegetated gravel bars probably have less water flowing through them than organic debris dams or riffles, because they tend to cover a larger area and are denser. Pools probably have the least water flowing through them, because they are where the water in the stream collects and is still. Quantifying flow rates through different features was beyond the scope of this study.

Potential denitrification rates are not true measures of the denitrification actually occurring in the stream. Rather, it is a measure of how much denitrification might happen under ideal conditions. We also measured denitrification under conditions without the addition of NO_3^- or carbon. Although these unamended rates were much lower than the potential rates, they showed the same patterns as the potential rates, suggesting that denitrification is consuming NO_3^- in sediments in these streams.

CONCLUSION

This study has confirmed that stream features with higher levels of organic matter content have higher potential denitrification rates, and that high nitrate levels in stream waters can stimulate hyporheic zone denitrification. The study has also shown that urbanization can change a stream’s physical features and chemical processes causing its hyporheic zones to function less efficiently than those found in less disturbed streams.

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APPENDIX

TABLE 1. Physical features where sediments were collected at each stream

Stream	Pond Branch	Baisman's Run	Glyndon	Mine Bank Run
Physical Feature				
organic debris dams	yes	yes	yes	no
riffles	yes	yes	yes	yes
vegetated gravel bars	no	yes	no	yes
gravel bars	yes	yes	no	yes
pools	yes	yes	yes	yes

TABLE 2. Stream nitrate levels

Stream	Nitrate Levels mg N/L
Pond Branch	0.01
Baisman's Run	1.5
Glyndon	1.9
Mine Bank Run	1.1

TABLE 3. Mean and Standard Error of Measured Variables by Site

Stream	Baisman's Run		Glyndon		Mine Bank Run		Pond Branch	
Feature Variable	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
Gravel Bar								
feature	4.00	0.00			4.00	0.00	4.00	0.00
replicant	1.50	0.50			1.50	0.50	1.50	0.50
moisture content	0.17	0.00			0.20	0.00	0.25	0.00
% organic matter content	0.80	0.15			1.36	0.13	1.02	0.12
potential denitrification	21.35	15.47			15.70	15.49	8.07	5.51
initial NO_3^-	0.65	0.10			1.15	0.74	0.50	0.08
initial NH_4	0.48	0.01			0.66	0.20	1.54	0.56
potential net mineralization	0.24	0.06			0.19	0.03	-0.03	0.03
potential net nitrification	0.16	0.04			0.20	0.04	0.07	0.03
respiration	2.66	0.94			3.84	2.41	3.08	0.56
N_2O production	2.07	0.58			2.94	1.37	2.19	0.51
denitrification	0.78	0.08			1.18	0.37	1.01	0.10
Organic Debris Dam								
feature	1.00	0.00	1.00	0.00			1.00	0.00
replicant	1.50	0.50	1.50	0.50			1.50	0.50
moisture content	0.68	0.06	0.67	0.05			0.64	0.04
% organic matter content	26.40	6.06	16.91	3.87			14.22	3.62
potential denitrification	1604.49	75.84	4954.87	2459.58			184.61	31.39
initial NO_3^-	1.34	0.60	1.31	0.12			0.69	0.14
initial NH_4	31.99	12.82	46.83	16.04			38.64	19.47
potential net mineralization	-2.73	1.54	-3.03	2.20			-2.33	1.40
potential net nitrification	0.05	0.05	1.10	0.86			0.05	0.04
respiration	68.48	0.49	45.86	8.24			55.02	5.46
N_2O production	3.51	0.06	9.38	3.54			19.57	13.45
denitrification	1.35	0.29	5.00	2.97			10.37	7.85
Pool								
feature	5.00	0.00	5.00	0.00	5.00	0.00	5.00	0.00
replicant	1.50	0.50	1.50	0.50	1.50	0.50	1.50	0.50
moisture content	0.23	0.03	0.27	0.03	0.25	0.01	0.26	0.01
% organic matter content	0.92	0.31	2.73	0.47	1.05	0.12	1.50	0.42
potential denitrification	36.41	29.67	219.28	215.72	21.26	15.39	48.15	33.35

initial NO_3^-	0.55	0.21	0.43	0.06	0.43	0.02	0.53	0.14	
initial NH_4	0.67	0.09	1.42	0.23	4.88	1.11	1.22	0.32	
potential net mineralization	0.19	0.08	0.04	0.04	1.11	0.54	-0.01	0.04	
potential net nitrification	0.08	0.04	0.11	0.06	1.44	0.61	0.03	0.01	
respiration	4.10	0.73	8.87	4.51	8.81	3.40	5.75	0.33	N_2O
production	2.03	0.47	3.42	0.32	4.07	1.69	2.45	0.50	
denitrification	0.86	0.09	1.70	0.01	2.45	0.13	1.77	1.00	
Riffle									
feature	2.00	0.00	2.00	0.00	2.00	0.00	2.00	0.00	
replicant	1.50	0.50	1.50	0.50	1.50	0.50	1.50	0.50	
moisture content	0.20	0.01	0.23	0.01	0.20	0.01	0.20	0.01	
% organic matter content	0.67	0.10	2.21	0.61	2.10	0.14	0.86	0.00	
potential denitrification	17.59	7.47	72.57	68.96	7.62	6.51	15.13	8.85	
initial NO_3^-	0.64	0.10	0.53	0.29	0.61	0.05	0.60	0.03	
initial NH_4	0.61	0.15	4.32	0.14	3.22	1.43	1.48	0.97	
potential net mineralization	0.25	0.18	-0.23	0.02	0.48	0.07	0.03	0.18	
potential net nitrification	0.16	0.05	-0.01	0.03	0.69	0.07	0.13	0.08	
respiration	3.50	0.15	9.40	0.72	6.81	0.61	4.72	0.67	
N_2O production	2.92	0.08	3.69	0.56	2.83	0.50	2.46	0.70	
denitrification	1.81	0.64	1.80	0.51	3.75	0.37	1.24	0.19	
Vegetated Gravel Bar									
feature	6.00	0.00			6.00	0.00			
replicant	1.50	0.50			1.50	0.50			
moisture content	0.32	0.14			0.21	0.01			
% organic matter content	4.86	3.91			1.28	0.12			
potential denitrification	64.91	60.14			45.36	22.35			
initial NO_3^-	0.33	0.14			0.26	0.05			
initial NH_4	7.65	6.13			2.73	0.48			
potential net mineralization	0.07	0.31			-0.03	0.04			
potential net nitrification	0.25	0.23			0.17	0.10			
respiration	6.87	4.29			10.64	0.94			
N_2O production	5.44	3.66			5.98	3.76			
denitrification	2.59	1.87			3.85	0.60			

FIGURE 1 Stream Nitrate levels

FIGURE 2 Average % Organic Matter Content by Stream Feature

FIGURE 3 Average Potential Denitrification Rates by Stream Feature

FIGURE 4 Average Potential Denitrification Rates of Organic Debris Dams by Stream

FIGURE 5 Average Potential Denitrification Rates of Riffles by Stream