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Short-Term Denitrification in the Metalimnion of a Eutrophic Reservoir

An Undergraduate Honors Thesis

in the

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by

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I. Summary

Denitrification in metalimnetic water was examined under different conditions to determine how of addition of nitrate (NO$_3^-$) and particulate carbon (PC), aeration, and concentrations of nitrate affect denitrification. In the first experiment, water samples from a metalimnion were treated with different combinations of NO$_3^-$ and PC to find out how addition of NO$_3^-$ and PC changes denitrification occurring in a metalimnion. The treatments included 1) control, 2) samples amended with 1 mg NO$_3^-$ N/L, 3) samples amended with both NO$_3^-$ (1 mg NO$_3^-$ N/L) and PC (5.7 mg C/L), and 4) samples amended with 5.7 mg C/L. Changes in excess N$_2$-N concentration for 10 days were measured using membrane inlet mass spectrometry (MIMS). In the second experiment, the same treatments were used to evaluate the effect of aeration. Besides excess N$_2$-N concentrations, O$_2$ concentrations were measured for 16 days. The third experiment examined how different initial concentrations of NO$_3^-$ influenced denitrification, and changes in the samples with the four different concentrations of 1) control with 0 mg NO$_3^-$ N/L, 2) 1.0 mg NO$_3^-$ N/L, 3) 2.5 mg NO$_3^-$ N/L, and 4) 5.0 mg NO$_3^-$ N/L were measured using MIMS for 42 days. The first experiment indicated that all samples in this experiment had completed denitrification before this experiment, and denitrification occurred only in the samples added with NO$_3^-$. Aeration event in the second experiment resulted in low initial excess N$_2$-N concentrations, and excess N$_2$-N concentrations increased most in the samples containing both PC and NO$_3^-$ and second most in PC amended samples. Excess N$_2$-N concentrations did not increase in other two treatments, indicating the effect of PC on creating anoxic conditions for denitrification. Differences in initial NO$_3^-$ concentrations did not result in differences in denitrification.
II. **Introduction:**

As the human population grows rapidly, the effects resulting from anthropogenic activities have been significantly intensified. Reactive nitrogen is one substance that has been released into the environment with increasing amount in recent years. Since 1970, the creation of reactive nitrogen has increased by 120%, while the human population has grown by 78% (Galloway et al. 2008). Many human activities contribute to the increase of reactive nitrogen at both local and global scales, and the major activities causing this problem are the use of nitrogenous fertilizers, burning of fossil fuels, power generation plants, and industries (Vitousek et al. 1997). Among those activities, the use of nitrogenous fertilizer accounts for the largest amount of newly created anthropogenic nitrogen in the environment (Vitousek et al. 1997). Even though application of nitrogenous fertilizers has significantly increased yields of agricultural crops, at the same time excess nitrogen has caused negative effects in the environment. In addition, reduction in vegetated areas has decreased biological nitrogen fixation by about 10%, contributing to the alteration of nitrogen cycle due to human activities (Seitzinger et al. 2006). The human-induced increase in nitrogen in the environment has brought many negative consequences in the environment.

*Eutrophication and Other Effects Resulting from Nitrogen Enrichment*

There are negative environmental effects resulting from global nitrogen cycle alteration, including the enlarging Gulf of Mexico hypoxia and an increasing concentration of nitrous oxide in the atmosphere (Schlesinger, 2009). Changes in aquatic systems can be more rapid than in other systems and have serious effects on human societies and the whole ecosystem, including animals, fish, and other organisms. Excessive amounts of nitrogen in the environment often lead to eutrophication in aquatic ecosystems, which often is followed by harmful algal blooms,
anoxia/hypoxia, and contamination of drinking water (Galloway et al. 2008). Excess nitrate and resulting eutrophication in the water systems often lead to fish kills, significant loss of sensitive organisms, and loss of biodiversity in aquatic environments and also damage commercial fisheries due to lower oxygen concentrations than the optimum (Rabalais, 2002).

*Denitrification and Nitrogen Elimination in Lakes*

Nitrogen removal from lakes is mostly derived by two processes: sediment burial of particulate nitrogen and atmospheric emission of nitrogen gas via denitrification (Seitzinger, 1988). Denitrification is a process by which facultative anaerobic bacteria reduce nitrate to ultimately nitrogen gas. This process is responsible for about 80% of total nitrogen removal from a lake (Nõges et al. 1998; Galloway et al. 2008). Therefore, denitrification is a potential way to control and mitigate eutrophication in aquatic systems which receive large anthropogenic nitrogen loadings (Seitzinger, 1988). Among aquatic systems, small lakes (<50km²) account for nearly half of the global denitrification amount (Harrison et al. 2009). Additionally, Harrison et al. (2009) also reported that reservoirs remove approximately 33% of total N inputs from lentic systems, while occupying only 6% of the total lentic surface area. Freshwater impoundments have an important role in nitrogen elimination from water bodies, and there are many studies focusing on nitrogen burial and denitrification in the epilimnion and hypolimnion, the upper and lower layers of a lake, respectively. However, comparatively little work has been done to measure the amount of denitrification occurring in a metalimnion, the middle layer of a lake. The objective of this study was to measure the amount of denitrification occurring in the metalimnion of Lake Fayetteville, Arkansas under varying nitrate concentrations.

**III. Research Hypotheses**
I hypothesized that 1) nitrogen gas concentration in the sample water which was closed to atmospheric exchange would increase through time due to denitrification, and 2) nitrogen gas concentration would be higher in the samples with a higher initial nitrate concentration. The first hypothesis was based on the assumption that, without atmospheric exchange, anoxic conditions in the metalimnetic water would be maintained, and therefore microbial denitrification would occur, consuming nitrate and increasing excess N$_2$-N concentrations in the samples through time. The second hypothesis was based on the assumption that denitrification continues as long as the samples contain nitrate in the water. Therefore, samples with a greater initial concentration of nitrate would result in a higher dinitrogen gas concentration in the samples by the end of experiment.

IV. Materials and Methods

Study Site

Lake Fayetteville is a small eutrophic reservoir with an area of 0.604 km$^2$, and is located in northwest Arkansas, USA (36.08 N, 94.08 W). Lake Fayetteville is a flood control impoundment, which is mainly fed by water from urban and agricultural lands. In this study, I focused on denitrification in the metalimnion of Lake Fayetteville. Water samples were obtained from the point which has the deepest depth in the lake, since this point is considered to be most well-stratified and have a thick metalimnion. Also, this point is considered to represent the whole lake because it is close to the outlet of the lake.

To determine the location of the metalimnion, a YSI sonde was used to obtain the vertical temperature and dissolved oxygen profile of Lake Fayetteville. The metalimnion was defined as the layer of water in which a temperature change exceeded 1 °C per m depth (Figure 1). Water was collected from the metalimnion using a Van Dorn sampler and returned to the laboratory for
experimental determinations of denitrification potential. In addition to metalimnion water, a water sample was also collected from the epilimnion to supply the particulate carbon (PC) for Experiment 1. In the three experiments conducted in this study, samples were not replicated except for initial samples, which represented the starting condition of each experiment because of the limited number of BOD bottles for the experiments. Also, this study used membrane inlet mass spectrometry (MIMS) to determine N₂ concentrations in water bodies, according to a study conducted by Deemer et al. (2011).

**Experiment 1: Effects of Nitrate and Particulate Carbon on Denitrification**

Experiment 1 was conducted to evaluate how addition of particulate carbon (PC) and nitrate (NO₃⁻) affects denitrification in the metalimnitic water. BOD bottles were prepared before field sampling to have four groups: 1) bottles with no treatment for control, 2) bottles with NO₃⁻ addition to have 1 mg NO₃⁻ - N/L above the original concentrations of NO₃⁻ and PC in the water (background), 3) bottles with the same concentration of NO₃⁻ above background and PC addition to have 5.7 mg - C/L above background, and 4) bottles with PC of 5.7 mg - C/L above background. Bottles were transported in a cooler with ice. Lake water was collected from the metalimnion by a Van Dorn sampler and directly poured into the bottles. Also, 3 bottles were treated with ZnCl₂ to preserve the condition of the lake water at the time of sampling. All bottles were sealed immediately after the water was poured into the bottles to prevent air exchange, and then stored in an incubator at 15 ºC. The ratios of dissolved nitrogen gas to argon (N₂:Ar), and oxygen gas to argon (O₂:Ar) of one set of bottles were measured by MIMS on days 1, 3, 5, 7, and 10. Detail for the complete MIMS setup was as described in Kana et al. (1994). Since the concentration of Ar only depends on the temperature of the water, the concentrations of N₂ gas in the samples were calculated using the N₂:Ar ratio detected by MIMS and Ar concentration at the
incubated temperature. The N\textsubscript{2} concentrations ([N\textsubscript{2}]\textsubscript{sample}) in the samples were calculated using the equation below according to the study by Grantz et al. (2012),

\[
[N_2]_{sample} = (N_2: Ar_{sample} \times [Ar]_{exp}) \left( \frac{[N_2]: [Ar]_{exp}}{N_2: Ar_{standard}} \right)
\]

where \(N_2: Ar_{sample}\) is the ratio in the sample measured by MIMS, \([Ar]_{exp}\) and \([N_2]: [Ar]_{exp}\) are the theoretical concentration and ratio at the incubated temperature, and \(N_2: Ar_{standard}\) is the ratio of air saturated deionized water. Also, the equation for excess N\textsubscript{2} concentration ([N\textsubscript{2}]\textsubscript{excess}) is as expressed in Grantz et al. (2012),

\[
[N_2]_{excess} = [N_2]_{sample} - [N_2]_{exp} - \text{min}[N_2]_{excess}
\]

where \([N_2]_{sample}\) is the N\textsubscript{2} concentration in the sample which is calculated using the previous equation, \([N_2]_{exp}\) is a theoretical N\textsubscript{2} concentration at the incubated temperature, and \(\text{min}[N_2]_{excess}\) is the lowest N\textsubscript{2} concentration among all the samples in this experiment. Data analysis was done in Statistical Analysis Systems (SAS 9.3) to determine if each treatment had a significantly different result from other treatments.

**Experiment 2: Effects of Nitrate and Particulate Carbon on Denitrification Following an Aeration Event**

Experiment 2 was conducted to observe changes in concentrations after metalimnion water was oxygenated and allowed to go anoxic again. Sample water from the metalimnion was collected in a 20-L container and aerated in the laboratory overnight to oxygenate the water and remove excess N\textsubscript{2} from the sample. Also samples were obtained and preserved using ZnCl\textsubscript{2} to preserve the initial condition. Then, the aerated water was poured into BOD bottles, which consisted of: 1) bottles with no treatment for control, 2) bottles with NO\textsubscript{3}\textsuperscript{−} addition to have 1 mg NO\textsubscript{3}\textsuperscript{−} - N/L higher than the sample water after aeration, 3) bottles with NO\textsubscript{3}\textsuperscript{−} (1 mg NO\textsubscript{3}\textsuperscript{−} - N/L)
and PC addition to have 7.1 mg C/L, and 4) bottles with PC of 7.1 mg C/L. All bottles were stored in the incubator at 15 °C, and the ratios of N2:Ar and O2:Ar of one set of bottles were measured by MIMS at 1, 2, 5, 8, 13, and 16 days after starting the incubation. The concentrations of excess N2-N in the samples were calculated the same way as described for Experiment 1. The O2 concentrations in the samples were also calculated using a similar equation. Data analysis was done in SAS 9.3 to estimate rates of denitrification in different treatments by linear regression analysis on excess N2-N concentrations vs. time.

Experiment 3: Long Term Effect of Nitrate Concentrations on Denitrification

Experiment 3 was conducted to determine how difference NO3− concentration influence denitrification in the water. Sample water from the metalimnion was collected into a 20-L container and aerated for two days. Then, the water was poured into the BOD bottles which consisted of: 1) 0 mg NO3− - N/L, 2) 1 mg NO3− - N/L, 3) 2.5 mg NO3− - N/L, and 4) 5.0 mg NO3− - N/L higher than the NO3− -N concentrations after aeration, respectively. All bottles were amended with PC to have 7.1 mg C/L. Samples for initial conditions were also collected and preserved using ZnCl2. All bottles were stored in a water bath at room temperature. The ratios of N2:Ar and O2:Ar were measured with MIMS at days 1, 5, 10, 15, 25, 33 and 42 after starting incubation. The concentrations of excess N2-N and O2 were calculated as described for Experiments 1 and 2.

V. Results

Experiment 1:

Changes in excess N2-N concentrations in the samples were observed from day 0 to day 10 (Figure 2a) in Experiment 1. The average excess N2-N concentration in all samples was 89.6
µmol/L (± standard deviation 10.0µmol/L) at day 0. Samples with both nitrate (NO₃⁻) and particulate carbon (PC) increased to 142 µmol/L excess N₂-N by the end of the measurement, which was the highest concentration among the four treatments. Samples with only NO₃⁻ had the second most production of N₂ and 121 µmol/L at day 10. The unamended (control) and samples with only particulate carbon resulted in the smallest changes of excess N₂-N concentrations and were 102 µmol/L and 105 µmol/L, respectively.

The mean excess N₂-N concentrations of for the four treatments, F value, and P value were calculated by SAS (Figure 2b). The mean excess N₂-N concentrations of unamended control and PC amended samples were not statistically different from each other, while samples amended with NO₃⁻ and with both PC and NO₃⁻ were significantly different from each other and from unamended control and PC amended samples. The mean excess N₂-N concentrations of the four treatments had F value = 23.2 and p<0.0001.

Experiment 2:

The average initial concentration of excess N₂-N in the samples was 19.6 µmol/L (± 2.4 µmol/L). Samples amended with both NO₃⁻ and PC showed a rapid increase in the concentration of excess N₂-N (Figure 3) with the highest concentration on day 16. Samples with PC resulted in the second highest concentration of excess N₂-N by the end of the experiment. Samples amended with NO₃⁻ and unamended control resulted in 4.1 µmol/L and 0.0 µmol/L, respectively. Dissolved oxygen (O₂) concentrations started to decline immediately after the experiment started (Figure 4). Samples amended with PC and NO₃⁻ and only with PC showed a rapid decline in O₂ concentrations and reached 2.8 µmol/L and 4.0 µmol/L, respectively, which were anoxic conditions. Unamended controls and samples amended with nitrate had slower declines in dissolved O₂ concentrations and did not reach anoxic conditions by day 16. Concentrations of
dissolved O$_2$ in the unamended control and samples amended with NO$_3^-$ were 179 µmol/L and 177 µmol/L. The rate of denitrification was greatest in the samples amended with both PC and NO$_3^-$ and was 3.18 µmol/L/day (t=8.0726, p=0.0013, and r$^2$=0.9422). Samples amended with PC had the rate of denitrification 1.41µmol/L/day (t=3.1290, p=0.0352, and r$^2$=0.7099). The unamended control and sample amended with NO$_3^-$ did not have significant difference due to the treatments (control: t=-2.2538, p=0.0873 and NO$_3^-$: t=-1.1089, p=0.3296).

Experiment 3:

Changes in dissolved O$_2$ concentrations and excess N$_2$-N concentrations in samples with different nitrate concentrations were measured from day 0 to day 42 in Experiment 3. The concentrations of excess N$_2$-N gas rapidly increased in all samples from the initial concentrations (Figure 5) with a simultaneous decline in dissolved O$_2$ concentrations up to day 15 (Figure 6). The excess N$_2$-N gas concentrations reached a peak when the samples became anoxic at day 15 and ranged between 68.7 and 74.1 µmol/L for all treatments. After day 15, the concentrations of excess N$_2$-N gas in all treatments declined rapidly until day 33. Only samples without NO$_3^-$ addition (control) showed a slight increase in excess N$_2$-N concentrations in this period from day 33 to day 42 and resulted in 26.5 µmol/L by day 42. All other treatments continued to show a decline in the excess N$_2$-N concentrations until day 42. The N$_2$ concentrations were 4.2 µmol/L in the samples amended to have a 1 mg/L NO$_3^-$ concentration, 3.7 µmol/L in the 2.5 mg NO$_3^-$/L samples, and 0 µmol/L in the 5 mg NO$_3^-$/L samples. The declines after day 33 were steeper in the samples with additions of higher NO$_3^-$ concentrations. In this experiment, all the samples showed a rapid decline in dissolved O$_2$ concentrations and went anoxic after day 15. After day 15 (Figure 6), dissolved O$_2$ concentrations remained low throughout the experiment. The
changes in dissolved O$_2$ in the four treatments were all similar. Also, the samples of the four treatments showed similar changes of excess N$_2$ production.

VI. Discussion

Factors affecting denitrification

Excess N$_2$-N concentrations increased in the samples which were kept closed to atmospheric exchange and in which dissolved O$_2$ concentration declined to a low enough level for denitrification to occur. In experiment 1, the unamended control and samples amended with PC did not show much increase in excess N$_2$-N concentrations over the 10-day experiment. Since samples were not aerated in this experiment, the water was likely anoxic since the time of sampling and denitrification occurred before this experiment, resulting in high excess N$_2$-N concentrations in all samples throughout the experiment. With low background NO$_3^-$ in the water, only the two treatments with NO$_3^-$ addition resulted in denitrification and increased in excess N$_2$-N concentrations. The slight increase in excess N$_2$-N concentrations from the time 0 which was seen in all samples may have resulted from aeration when the water was transported from the Van Dorn sampler into the bottles.

In Experiment 2, aeration in the beginning of the experiment oxidized dissolved N$_2$ gas into NO$_3^-$ and caused the initial excess N$_2$-N concentrations in the samples to be lower than the samples in Experiment 1 and made nitrate available for denitrification. The unamended control and samples with NO$_3^-$ addition had slower decline in their O$_2$ concentrations and did not become anoxic during the period of this experiment. Since unamended control and NO$_3^-$ amended samples did not develop anoxic conditions required for denitrification, denitrification did not occur in those samples, making their excess N$_2$-N concentrations low. Both treatments amended with PC resulted in faster declines in O$_2$ concentrations than the unamended control.
and samples amended only with NO$_3^-$, which showed that carbon addition resulted in more biological activity, denitrification, in the samples. Concentrations of excess N$_2$-N increased most rapidly in the samples with both NO$_3^-$ and PC addition, in which PC helped the water become anoxic and the higher nitrate concentrations increased the total amount of denitrification.

From this result, it is concluded that the unamended control and samples amended with PC in experiment 1 also did not develop anoxic conditions during the experiment and resulted in low excess N$_2$-N concentrations. In both experiment 1 and 2, samples amended with both NO$_3^-$ and PC resulted in the highest excess N$_2$-N concentrations among the four treatments, indicating the addition of PC and NO$_3^-$ can stimulate denitrification. From Experiment 2, PC not only stimulates denitrification but also was necessary to develop anoxic conditions in water before denitrification started.

**Nitrate concentrations and denitrification**

Initial NO$_3^-$ concentration differences in the samples did not affect excess N$_2$-N concentrations. The changes in excess N$_2$-N concentrations were similar among all treatments, except in the last part of the experimental period in Experiment 3. Since the samples with no nitrate addition (0 mg/L) had almost the same increase in the N$_2$ concentration as other three treatments with addition of NO$_3^-$, it can be concluded that the metalimnetic water either had much more nitrate than the concentrations set in the experiment 3 or had other factors inhibiting consumption of added NO$_3^-$ for denitrification. In Experiment 3, dissolved O$_2$ concentrations declined to anoxic levels in all samples, and concentrations of N$_2$ increased due to denitrification as the dissolved O$_2$ concentrations declined. One possible explanation for the decline in the excess N$_2$-N concentrations after day 15 is that methanogenesis may have occurred in the samples, creating methane gas in the BOD bottles. Since methane is very insoluble in water, it
accumulated in the top of the bottles as bubbles. Excess N₂ gas diffused into the methane bubbles, decreasing excess N₂-N concentrations in the sample water. However, this study did not clear this part and it would have been necessary to measure methane concentrations to confirm if methanogenesis occurred in this condition.

In conclusion, additions of PC and NO₃⁻ can stimulate denitrification and increase excess N₂-N concentration in metalimnetic water because PC enhances biological activity and NO₃⁻ increases the total amount of denitrification. PC was also important in creating anoxic condition in water so that denitrification occurred when water was aerated and initially had oxic condition. To confirm the mechanism of the decline in excess N₂ concentration during the 40-day long experiment, methanogenesis in this condition also needs to be studied in future research. From this study, it is implicated that a metalimnion can be a hotspot of denitrification because a metalimnion can be managed so that metalimnetic water has a repeating cycle between oxic and anoxic conditions by mixing waters of the epilimnion and metalimnion. By repeating the cycle, significant N can be removed in metalimnion through denitrification.
Literature Cited


Fig. 1. A profile of Lake Fayetteville of changes of temperature and dissolved O$_2$ concentration with depth
Fig. 2. a. Excess N$_2$-N concentrations during Experiment 1, b. Mean excess N$_2$-N concentrations during Experiment 1
Fig. 3. Excess N$_2$-N concentrations during Experiment 2
Fig. 4. Excess O₂ concentrations during Experiment 2
Fig. 5. Excess N$_2$-N concentrations during Experiment 3
Fig. 6. Excess $O_2$ concentrations during Experiment 3