Short-Term denitrification in the metalimnion of a eutrophic reservoir

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ABSTRACT

Denitrification in metalimnetic water was examined under different conditions to determine how 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. Changes in excess N_2-N concentration for 10 days were measured using membrane inlet mass spectrometry (MIMS). The second experiment used the same treatments as the first experiment after aeration of water. 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 NO_3^- 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^-. An 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. This study showed denitrification in metalimnion can be enhanced by adding NO_3^- and PC and by repeating oxic and anoxic condition of water, which can be a way to remove nitrogen from aquatic systems.

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MEET THE STUDENT-AUTHOR

I am from Nara, Japan. I have been very interested in global environmental issues since I was in high school, and that was why I decided to study in the United States with a purpose of studying both environmental science and English. I came to Fayetteville four years ago and am finishing my major in Environmental, Soil, and Water Science in May 2013. I struggled with English, but I am glad that I have improved my English through studying in this university. From this fall, I am going to continue my education in the civil engineering program at University of Toronto.

I started this project in the summer of 2009. I learned a lot from this experience and enjoyed doing this research with my mentor, Dr. Thad Scott, and students in his lab. I am very thankful to Dr. Scott for his guidance and support in this research, and Dr. Savin, Dr. Brye, and Dr. Rom for suggestions in revising my thesis.

Aki Kogo

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 the 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 et al., 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 ultimately to 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 (<50 km²) 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.

We 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 water contains nitrate. Therefore, water samples with a greater initial concentration of nitrate would result in a higher dinitrogen gas concentration by the end of experiment.

MATERIALS AND METHODS

Study Site. Lake Fayetteville is a small eutrophic reservoir with an area of 0.604 km², and is located in northwest Arkansas, U.S. (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, we 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 (YSI Incorporated, Yellow Springs, Ohio) 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, which was approximately between 2- and 4-meter depth (Fig. 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 N_2 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 (NO_3^-) affect denitrification in the metalimnetic water. BOD bottles were prepared before field sampling to have four groups: 1) bottles with no treatment for control, 2) bottles with NO_3^- addition to have 1 mg NO_3^--N/L above the original concentrations of NO_3^- and PC in the water (background), 3) bottles with the same concentration of NO_3^- 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 $ZnCl_2$ 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 °C. The ratios of dissolved nitrogen gas to argon (N_2:Ar), and oxygen gas to argon (O_2:Ar) of one set of bottles were measured by MIMS on days 1, 3, 5, 7, and 10. The bottles were kept in water which had the same temperature as incubation to minimize temperature change. 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 N_2 gas in the samples were calculated using the N_2:Ar ratio detected by MIMS and Ar concentration at the incubated temperature. The N_2 concentrations ($[N_2]_{SAMPLE}$) 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_2 concentration ($[N_2]_{excess}$) is as expressed in Grantz et al. (2012),

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

where $[N_2]_{sample}$ is the N_2 concentration in the sample which is calculated using the previous equation, $[N_2]_{exp}$ is a theoretical N_2 concentration at the incubated temperature, and $min[N_2]_{excess}$ is the lowest N_2 concentration among all the samples in this experiment. Data analysis was done in Statistical Analysis Systems (SAS) v. 9.3 (SAS Institute, Inc., Cary, N.C.) to determine if each treatment had a significantly different result from other treatments. In this analysis, samples were considered as being replicated through time, and mean excess N_2-N concentration of each treatment was calculated.

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_2 from the sample. Also samples were obtained and preserved using $ZnCl_2$ 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_3^- addition to have 1 mg NO_3^--N/L higher than the sample water after aeration, 3) bottles with NO_3^- (1 mg NO_3^--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 ºC, and the ratios of N^2:Ar and O_2: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 N_2-N in the samples were calculated the same way as described for Experiment 1. The O_2 concentrations in the samples were also calculated using a similar equation. Data analysis was done in SAS v. 9.3 to estimate rates of denitrification in different treatments by linear regression analysis on excess N_2-N concentrations vs. time.

Experiment 3: Long-Term Effect of Nitrate Concentrations on Denitrification. Experiment 3 was conducted to determine how difference in NO_3^- concentration influences 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 NO_3^--N/L, 2) 1 mg NO_3^--N/L, 3) 2.5 mg NO_3^--N/L, and 4) 5.0 mg NO_3^--N/L higher than the NO_3^--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 $ZnCl_2$. All bottles were stored in a water bath at room temperature. The ratios of N_2:Ar and O_2:Ar were measured with MIMS at days 1, 5, 10, 15, 25, 33 and 42 after starting incubation. The concentrations of excess N_2-N and O_2 were calculated as described for Experiments 1 and 2.

RESULTS AND DISCUSSION

Experiment 1. Changes in excess N_2-N concentrations in the samples were observed from day 0 to day 10 (Fig. 2a) in Experiment 1. The average excess N_2-N concentration in all samples was 89.6 μmol/L (±standard deviation 10.0 μmol/L) at day 0. Samples with both nitrate (NO_3^-) and particulate carbon (PC) increased to 142 μmol/L excess N_2-N by the end of the measurement, which was the highest concentration among the four treatments. Samples with only NO_3^- had the second most production of N_2 of 121 μmol/L at day 10. The unamended (control) and samples with only particulate carbon resulted in the smallest changes of excess N_2-N concentrations and were 102 μmol/L and 105 μmol/L, respectively.

The mean excess N_2-N concentrations of for the four treatments, F value, and P value were calculated by SAS (Fig. 2b). The mean excess N_2-N concentrations of unamended control and PC amended samples were not statistically different from each other, while samples amended with NO_3^- and with both PC and NO_3^- were significantly different from each other and from unamended control and PC amended samples. The mean excess N_2-N concentrations of the four treatments had a F value = 23.2 and $P < 0.0001$.

Experiment 2. The average initial concentration of excess N_2-N in the samples was 19.6 μmol/L with the standard deviation ± 2.4 μmol/L (data not shown). Samples amended with both NO_3^- and PC showed a rapid increase in the concentration of excess N_2-N (Fig. 3) with the highest concentration on day 16. Samples with PC resulted in the second highest concentration of excess N_2-N by the end of the experiment. Samples amended with NO_3^- and the unamended control resulted in 4.1 μmol/L and 0.0 μmol/L, respectively. Dissolved oxygen (O_2) concentrations started to decline immediately after the experiment started (Fig. 4). Samples amended with PC and NO_3^- and only with PC showed a rapid decline in O_2 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 O_2 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,

respectively. 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^-:$t = -0.1089$, $P = 0.3296$).

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 (Fig. 5) with a simultaneous decline in dissolved O_2 concentrations up to day 15 (Fig. 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 N2 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 (Fig. 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.

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, which would allow nitrification to occur in the early incubation, when the water was transported from the Van Dorn sampler into the bottles.

In Experiment 2, initial aeration decreased the dissolved N_2 concentration and increased the dissolved O_2 concentration as dissolved gases in water came into equilibrium with the atmosphere. The increase in dissolved O_2 concentration in the sample allowed nitrification to occur, which converted ammonium (NH_4^+) to NO_3^-. Thus, the samples in Experiment 2 resulted in the lower concentrations in N_2-N than Experiment 1 and had nitrate available for denitrification to occur. 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, keeping 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, consuming the oxygen and promoting the anaerobic process of 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 only amended with PC in experiment 1 also did not have anoxic conditions and nitrate, both of which are required for denitrification, during the experiment. Therefore, those two treatments resulted in no significant increase in 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 stimulated 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 added in 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 N_2 gas could diffuse into the methane bubbles, decreasing excess N_2-N concentrations in the sample water. However, this study did not clarify if this mechanism was responsible for the decrease in excess N_2-N concentrations and it would have been necessary to measure methane concentrations to confirm if methanogenesis occurred in this condition.

In conclusion, additions of PC and NO_3^- can stimulate denitrification and increase excess N_2-N concentration in metalimnetic water because PC enhances biological activity and NO_3^- increases the total amount of denitrification. Particulate carbon was also important in creating anoxic condition in water so that denitrification occurred when water was aerated and initially had an oxic condition. To confirm the mechanism of the decline in excess N_2 concentration during the 40-day long experiment, methanogenesis 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

Deemer, B.R., J.A. Harrison, and E.W. Whitling. 2011. Microbial dinitrogen and nitrous oxide production in a small eutrophic reservoir: An in situ approach to quantifying hypolimnetic process rates. Limnol. Oceanogr. 56: 1189-1199.

Galloway, J.N., A.R. Townsend, J.W. Erisman, M. Bekunda, Z. Cai, J.R. Freney, L.A. Martinelli, S.P. Seizinger, and M.A. Sutton. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320: 889-892.

Grantz, E.M., A. Kogo, and J.T. Scott. 2012. Partitioning whole-lake denitrification using in situ dinitrogen gas accumulation and intact sediment core experiments. Limnol. Oceanogr. 5: 925-915.

Harrison, J.A., R.J. Maranger, R.B. Alexander, A.E. Giblin, P.A. Jacinthe, E. Mayorga, S.P. Seitzinger, D.J. Sobota, and W.M. Wollheim. 2009. The regional and global significance of nitrogen removal in lakes and reservoirs. Biochemistry 93: 143-157.

Kana, T.M., C. Darkangelo, M.D. Hunt, J.B. Oldham, G.E. Bennett, and J.C. Cornwell. 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples. Anal. Chem. 66: 4166-4170.

Nõges, P., A. Järvet, L. Tuvikene, and T. Nõges. 1998. The budgets of nitrogen and phosphorus in shallow eutriphic Lake Võrtsjärv (Estonia). Hydrobiologia 363: 219- 227.

Rabalais, N.N., R.E. Turner, and W.J. Wiseman Jr. 2002. Gulf of Mexico Hypoxia, a. k. a. "the dead zone." Annual Review of Ecology and Systematics 33: 235-263.

Schlesinger, W.H. 2009. On the fate of anthropogenic nitrogen. Proceedings of the National academy of sciences of the United States of America. 106: 203-208.

Seitzinger, S.P. 1988. Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnol. Oceanogr. 33: 702-724.

Seitzinger, S., J.A. Harrison, J.K. Böhlke, A.F. Bouwman, R. Lowrance, B. Peterson, C. Tobias, and G.V. Drecht. 2006. Denitrification across landscaped and waterscapes: a synthesis. Ecological Applications 16: 2064- 2090.

Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and D.G. Tilman. 1997. Human alteration of the global nitrogen cycle: causes and consequences. Ecological Applications 7: 737-750.
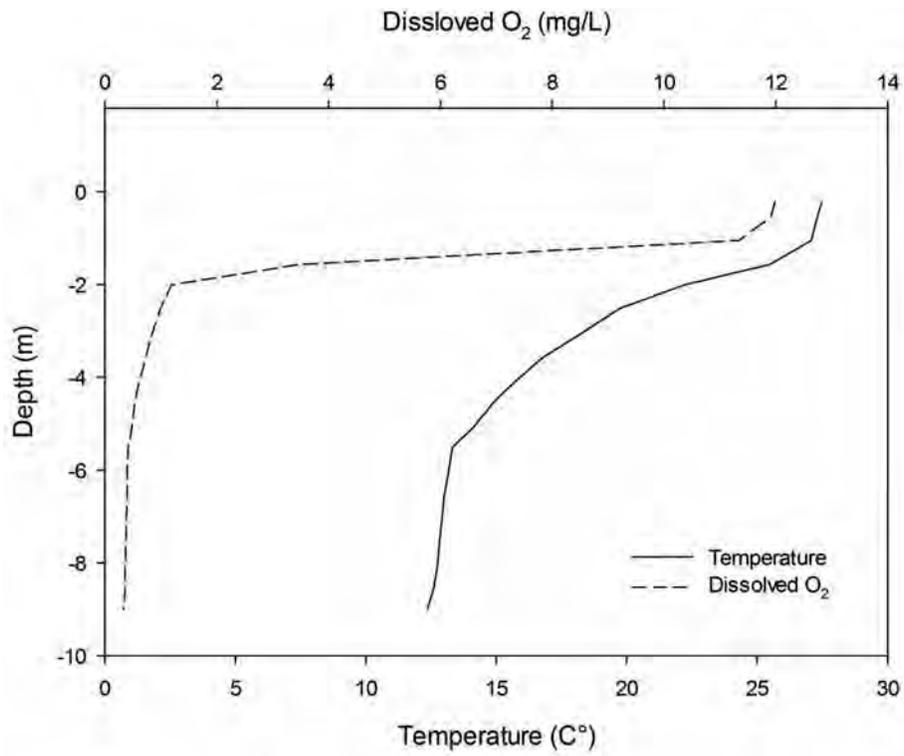
Fig. 1. A profile of Lake Fayetteville of changes of temperature and dissolved O_2 concentration with depth. The metalimnion was located between approximately 2 to 4 meter depth.
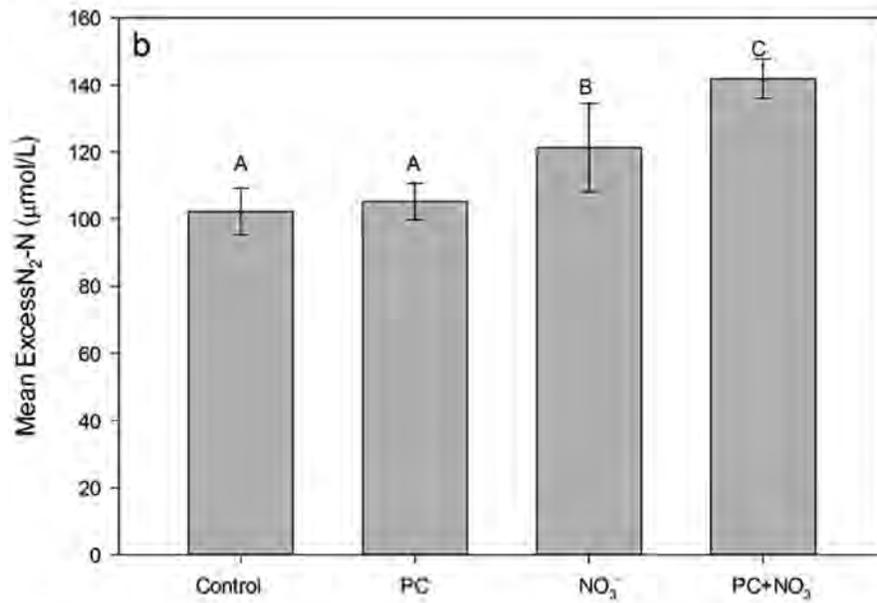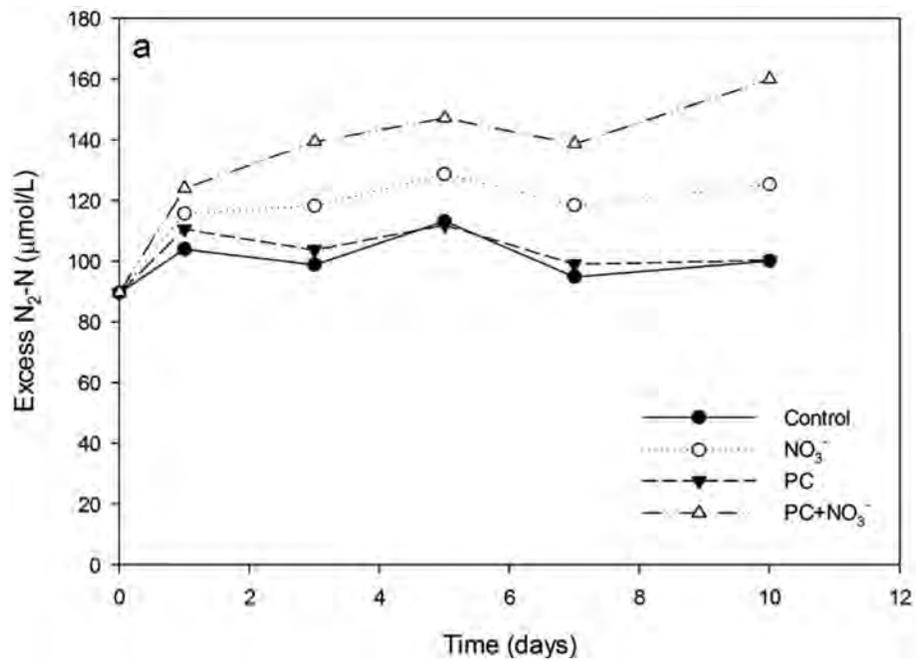
Fig. 2(a) Changes in excess N_2-N concentrations by addition of different combinations of nitrate (NO_3^-) and particulate carbon (PC); **(b)** Mean excess N_2-N concentrations in treatments using different combinations of NO_3^- and PC.
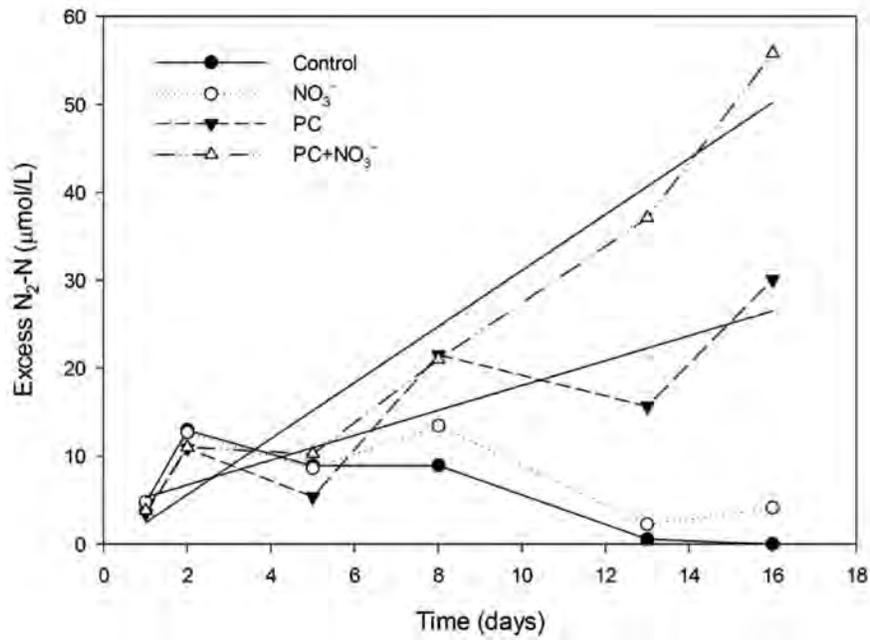
Fig. 3. Changes in excess N_2-N concentrations by different combinations of nitrate (NO_3^-) and particulate carbon (PC) after an aeration event and rates of excess N_2-N concentration increases.
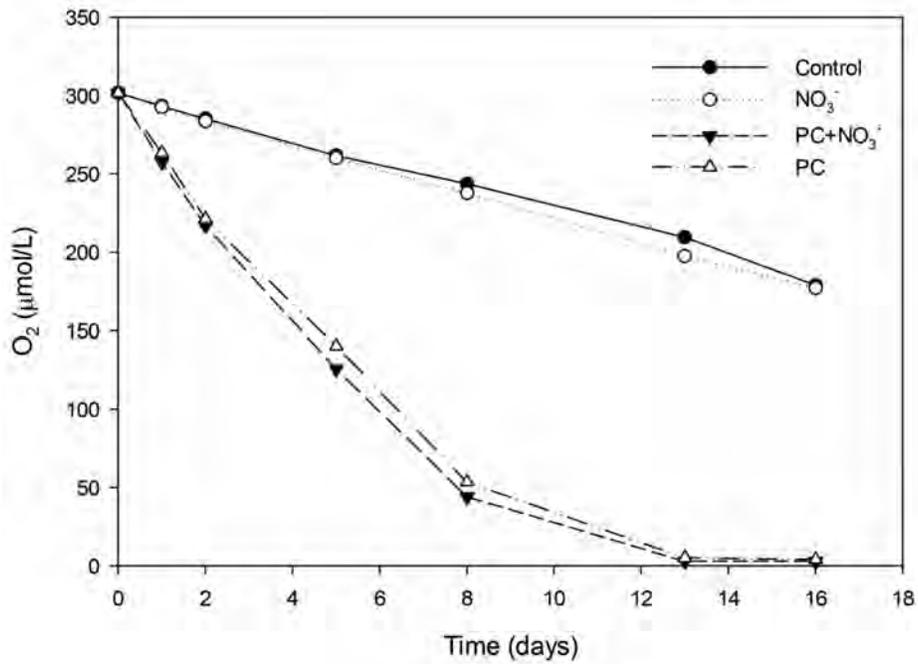
Fig. 4. Changes in excess O_2 concentrations by different combinations of nitrate (NO_3^-) and particulate carbon (PC) after an aeration event.
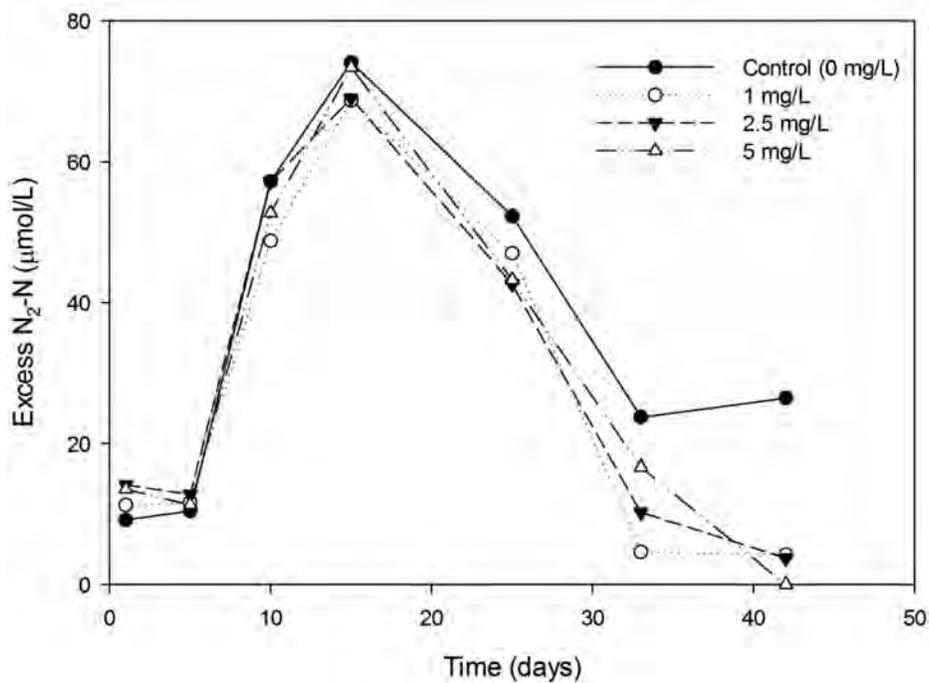
Fig. 5. Changes in excess N_2-N concentrations by addition of particulate carbon and different concentrations of nitrate (NO_3^-) above the background concentration of NO_3^-.

Fig. 6. Changes in excess O_2 concentrations by addition of particulate carbon and different concentrations of nitrate (NO_3^-) above the background concentration of NO_3^-.