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**Reservoir mixing effect on dinitrogen gas distribution within the metalimnion
of a man-made lake**

An Undergraduate Honors Thesis

in the

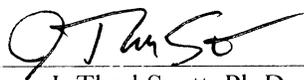
Department of Crop, Soil, and Environmental Science

Submitted in partial fulfillment of the requirements for the
University of Arkansas
Dale Bumpers College of Agricultural, Food and Life Sciences
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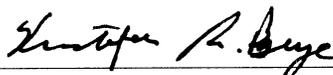
By

Mary Young

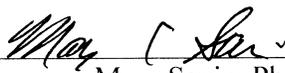
December 2013



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Abstract

The increasing environmental distribution of nitrogen has not only lead to changes in the global nitrogen cycle, but has also lead to many complex environmental consequences. Aquatic systems are particularly sensitive to high nutrient loading, and denitrification occurring in small lakes accounts for half the global release of dinitrogen. The objective of this study was to assess whether mixing events within the metalimnion increase denitrification rates. It was hypothesized that mixing events bring oxygen from the epilimnion and ammonium from the hypolimnion, simulating a coupled nitrification-denitrification process in the metalimnion. The dinitrogen-nitrogen (N_2-N) concentration for three treatments of 0, 50, and 90% hypolimnion water samples represented mixing within the metalimnion and were measured to determine denitrification rates. The nitrate (NO_3^-) concentration was tested in order to determine if the insurgence of oxygen from a mixing event would increase nitrification and lead to a coupled nitrification-denitrification cycle. The results showed that denitrification occurred within the metalimnion; however, the 0% and 50% hypolimnion treatments had similar N_2-N concentrations. The 90% hypolimnion treatment declined in N_2-N concentration. Lack of increases in NO_3^- concentrations indicates that any products of nitrification proceeded directly to denitrification or microbial assimilation. The results show that the metalimnion of a reservoir can be a hotspot for denitrification, and provides opportunities for future studies to investigate the potential of metalimnetic denitrification. Metalimnetic water management can be a possible resolution for nitrogen removal from aquatic systems.

Introduction

Humans have dramatically altered global biogeochemical cycles, and this change is manifested at local and regional scales. Nitrogen (N) concentration in the terrestrial landscape has considerably increased resulting from human activities in agricultural, industrial, and urban settings. For example, the increasing demand for agricultural production has intensified N fertilizer application to meet global market demands (Galloway et al., 2008). Compared to natural N distribution, areas that produce agricultural goods have high N input, and after harvest and exportation, have high N loss. These activities have greatly redistributed nutrients and skewed the N cycle (Vitousek et al., 1997; Galloway et al., 2008).

The amount of N in the terrestrial N cycle has doubled due to human activities causing a rise in greenhouse gases, such as nitrous oxide (N₂O), and smog. Nitrogen is essential to ecosystem functioning, but the expansion of the N pool has led to a decline in N efficient plants and therefore a loss in the species that require those plants (Vitousek et al., 1997). The overabundance of N transferred throughout systems has led to acidification in terrestrial and aquatic systems (Vitousek et al., 1997). High amounts of N have led to eutrophication, algal blooms, hypoxia, fish kills, and an overall loss in biodiversity (Vitousek et al., 1997). Aquatic systems are able to retain up to half the watershed N input, transferring excessive amounts of nutrients to downstream waterways that are highly receptive (Wollheim et al., 2008).

Lakes and reservoirs have greater N retention than streams due to longer water residence time (Saunders and Kalff, 2001; Kozelnik et al., 2007). Longer residence time helps enable the process of denitrification, and the releasing of dinitrogen back into the atmosphere (Grantz et al.,

2012). Identifying N cycling rates and N sources can help to find solutions that will minimize N inputs without production loss, and help reduce N loading in aquatic ecosystems.

Denitrification

Denitrification occurs when inorganic N is transformed into N₂ gas, releasing N from aquatic ecosystems (Seitzinger, 1988). Dinitrogen gas can be released through anaerobic ammonium oxidation (anammox) or denitrification. Denitrification in lakes is estimated to release 80% of total N, and in small lakes close to 50% of global denitrification (Galloway et al., 2008; Harrison et al., 2009). This would make denitrification a conceivable option for restoring and preventing eutrophication in waterways (Seitzinger, 1988). Reservoirs, which are man-made lakes, remove 33% of total N input within lake systems (Harrison et al., 2009). Due to functionality, reservoirs have greater nutrient loading and increased carbon burial which creates hot spots for denitrification in sediments (Downing et al., 2008; Kozelnik et al., 2007).

Denitrification rates are increased in reservoirs with seasonal thermal stratification because there is more microbial activity occurring during times of seasonally greater temperatures and greater NO₃⁻-N concentrations (David et al., 2006; Groffman et al., 2009; Nowicki et al., 1997). Seasonal temperature fluctuations are positively related to N availability and are a determining factor for NO₃⁻ concentration. Seasonal thermal stratification, therefore, influences the rate of denitrification occurring in aquatic ecosystems.

During the summer stratification, the lake is split into three distinct sections, each characterized by different temperatures, oxygen concentrations, and oxidation-reduction potentials. The epilimnion is a well-mixed upper zone of the lake that has the greatest amount of atmospheric oxygen exchange, therefore the greatest concentration of O₂ gas. The metalimnion is

the middle part of the lake that separates the epilimnion from the lower part of the lake, the hypolimnion. The hypolimnion, because of the thermocline, remains relatively undisturbed. This causes the hypolimnion to be restricted from atmospheric oxygen, and therefore has negligible O_2 gas available. The epilimnion contains negligible amounts of inorganic N because of nutrient uptake by phytoplankton. In the hypolimnion, there are greater concentrations of inorganic N available, in part, because of mineralization. During summer stratification mixing events such as wind or rain may cause an expansion of the metalimnion where epilimnetic waters are driven downward into the more reduced hypolimnetic waters. This phenomenon could stimulate coupled nitrification-denitrification.

Coupled Nitrification-Denitrification

Nitrification is the process that occurs when inorganic ammonium (NH_4^+) is oxidized and creates nitrate (NO_3^-). Ammonium is made available through mineralization, where extracellular enzymes release monomers through depolymerization. The enzymes that nitrify ammonium require O_2 which is why nitrification is generally restricted to the epilimnion. However, some studies have shown that *Nitrosomonas* sp., the organism that achieves ammonium oxidation, can oxidize ammonia to nitrite and nitrous oxide at extremely low oxygen concentrations (Downes, 1988). The insurgence of oxidized water in sediment samples enables a coupled process of nitrification-denitrification to occur during mixing events when oxygen is introduced to anaerobic conditions making NO_3^- available for denitrification (Risgaard-Peterson, 1994). The idea of mixing events occurring in the metalimnion, where the coupled nitrification-denitrification process can occur, has not been well tested. The metalimnion acts as an epilimnion and hypolimnion interface. Because the epilimnion is O_2 -rich and the hypolimnion is

NH₄-rich (Grantz et al., 2012), disturbance to the metalimnion may bring these resources together to stimulate coupled nitrification-denitrification event.

The overall objective of this study was to determine if mixing events provide conditions that enhance denitrification in the metalimnion of a reservoir. Simulated summer metalimnion mixing was achieved combining different volumes of epilimnetic and hypolimnetic water and then isolating this water from the atmosphere. The experiment was designed to test the following hypothesis:

Hypothesis: Mixing events which bring together oxygen from the epilimnion and ammonium from the hypolimnion will enhance coupled nitrification-denitrification in the metalimnion.

Materials and Methods

Study Sites

The study was conducted at Lake Fayetteville (36°08'11.5"N, 94°07'46.7"W) in Fayetteville, Arkansas (Scott and Grantz, 2013). Lake Fayetteville is a small (surface area < 1 km²), eutrophic, man-made lake within the Lake Fayetteville Watershed which is approximately 30-40x greater in surface area than the lake (Scott and Grantz, 2013). Lake Fayetteville has a water residence time between 0.8 and 1.2 years. Lake Fayetteville watershed is a sub-watershed of the Illinois River watershed. Lake Fayetteville's drainage basin is characterized by 41% urban and 37% agricultural land use (Scott and Grantz, 2013). Seasonal thermal stratification occurs within this reservoir over summer months and anoxia occurs within the hypolimnion and metalimnion.

Measuring N₂ Gas

On August 16, 2013 at Lake Fayetteville, dissolved oxygen (DO), temperature, and oxidation-reduction potential (ORP; Ag/AgCl reference electrode) were measured at 0.5 to 1 m intervals using a Yellow Spring, Inc. 600XLM multi-parameter datasonde. Samples were collected using a Van Dorn horizontal sampler which was moved back and forth to ensure only water from the desired depth (Gardner et al., 2006). Sample depths were determined from the YSI sonde to define the depth of the thermocline. Epilimnion water samples were collected at ~2 m and hypolimnion samples were collected at ~7 m. Six initial water samples from the epilimnion and hypolimnion were transferred into 300-mL Wheaton biological oxygen demand (BOD) bottles, making sure to slowly fill from the bottom up to prevent additional atmospheric exchange. Samples were immediately preserved with 4.0-mL of 50% (w:v) zinc chloride (ZnCl₂) solution and sealed with a ground-glass stopper wrapped with parafilm to avoid atmospheric gas exchange. Samples were returned to the laboratory and refrigerated at 4°C prior to gas analysis.

To test the hypothesis about the effect of mixing on metalimnetic denitrification, three combinations of epilimnetic and hypolimnetic water (0% hypolimnion water, 50% hypolimnion water, and 90% hypolimnion water) were mixed into 300-mL Wheaton BOD bottles and incubated in the laboratory for 10 days under a simulated metalimnetic temperature. A total of 72 samples, 12 epilimnion and hypolimnion initials and 20 for each treatment, were collected at Lake Fayetteville on August 16, 2013 and prepared for each treatment combination. Four bottle replicates from each treatment were harvested on days 1, 3, 5, 7, and 10 after the start of experimental conditions. All samples were preserved and stored as described previously and gas analysis was performed on all samples.

Dissolved oxygen gas-to-argon ratio (O₂: Ar) and N₂: Ar was measured using a membrane inlet mass spectrometer (MIMS) with a Pfeiffer Prisma mass spectrometer and a Bay Instruments Dissolved Gas Analyzer membrane inlet S-25-75 similar to that described by Deemer et al. (2011), Grantz (2011), and Grantz et al. (2012). The description of the MIMS arrangement is detailed in Kana et al. (1994). Briefly, samples were returned to in-situ sample temperature (19 °C) before MIMS testing. The MIMS standard solution was changed to match the experimental sample temperature. Since argon is a conservative element, the MIMS methods assumes that any changes in temperature or salinity will cause Ar saturation to vary, enabling gas concentrations to be measured

In order to calculate the N₂ concentration of the samples, the following equation was used:

$$[N_2]_{sample} = (N_2:Ar_{sample} \times [Ar]_{sample}) \left(\frac{[N_2]:[Ar]_{exp}}{N_2:Ar_{standard}} \right) \quad (1)$$

Where N₂: Ar_{sample} represented the ratio of the measured sample. Ar_{sample} and $N_2:Ar_{exp}$ are the saturated ratio adjusted for in-situ temperature (Grantz et al., 2012). $N_2:Ar_{standard}$ is the ratio of deionized water open to the atmosphere at in-situ temperature. The O₂ concentrations were also calculated using this equation.

In order to calculate the N₂ concentration that was over 100% saturation, we used the following equation:

$$[N_2]_{excess} = [N_2]_{sample} - [N_2]_{exp} - \min[N_2]_{excess} \quad (2)$$

Where N₂_{exp} represented N₂ saturation at the in-situ temperature (Grantz et al., 2012). The min N₂_{excess} was the lowest net N₂ sample measured. Some N₂ samples were below the N₂_{exp},

therefore creating N₂ excess that was negative. To get equilibrium between the field sample and the standard, the minimum N₂ excess was included.

Dinitrogen excess was adjusted to quantify the amount of N₂ excess that exceeded the epilimnion initial (EI) and hypolimnion initial (HI) samples' N₂ excess. Corrected values for 0% hypolimnion samples were calculated using the following equation:

$$\text{corrected } [N_2]_{\text{excess}} = [N_2]_{\text{excess}} - EI [N_2]_{\text{excess}} \quad (3)$$

Corrected values for 50% hypolimnion samples were calculated using the following equation:

$$\text{corrected } [N_2]_{\text{excess}} = [N_2]_{\text{excess}} - (HI [N_2]_{\text{excess}} * 0.5) + (EI [N_2]_{\text{excess}} * 0.5) \quad (4)$$

Corrected values for 90% hypolimnion samples were calculated using the following equation:

$$\text{corrected } [N_2]_{\text{excess}} = [N_2]_{\text{excess}} - (HI [N_2]_{\text{excess}} * 0.9) + (EI [N_2]_{\text{excess}} * 0.1) \quad (5)$$

Water samples were immediately filtered after MIMS testing with an acid-washed Whatman GFF filter and frozen for later nitrate+nitrite-N (NO₃⁻-N) analysis. Epilimnion and hypolimnion initial samples, and 50% hypolimnion samples were tested for NO₃⁻-N. Nitrate-N was measured colorimetrically using the cadmium reduction method (APHA, 2005). Turner Designs Trilogy Lab Fluorometer with a spectrophotometer adaptor containing a 600-nm filter cell was used for the NO₃⁻-N analysis.

Statistics

Differences between O₂ and N₂ concentrations in the initial samples and day 1 samples were compared with a one-way analysis of variance (ANOVA). The corrected excess N₂ (μM) was investigated using a two-way ANOVA. The independent factors used in the analysis were

time, treatment, and the interaction. Corrected excess N_2 was the dependent factor. Two-way ANOVA using PROC GLM in SAS 9.3 (SAS Institute, Cary, North Carolina) and REGWQ multiple comparison procedure was used to detect differences among specific treatments. Statistical differences in the omnibus F-test for an independent factor (time or treatment) were distinguished by having a p-value less than 0.05.

Results

Preliminary Conditions

The average in-lake temperature of the epilimnion was approximately 24°C, with the thermocline estimated between 2.5 and 6.5 m depth (Fig. 1). The hypolimnion average temperature was approximately 15°C (Fig. 1). The measured DO concentration within the epilimnion was 270 to 305 μM , but decreased to 30 μM in the upper metalimnion and 10 μM in the lower metalimnion and hypolimnion (Fig. 1). Oxidation-reduction potential had metalimnetic maxima, but rapidly decreased with decreasing oxygen concentrations in the lower metalimnion and hypolimnion (Fig. 1).

Oxygen gas concentration was significantly greater in the epilimnion than in the hypolimnion ($p < 0.001$; Fig. 2). The average O_2 in the epilimnion was 352 (± 13.49) μM and 6 (± 3.24) μM in the hypolimnion (Fig. 2). The N_2 gas concentration in the epilimnion was significantly lower in concentration than the N_2 gas in the hypolimnion ($p < 0.001$; Fig. 3). The epilimnion initial samples averaged 485 (± 2.81) μM of N_2 gas and the hypolimnion initial samples averaged 630 (± 10.09) μM of N_2 gas (Fig. 3). Nitrate concentrations averaged 4.29 μM in the epilimnion and 2.75 μM in the hypolimnion. Ammonium nutrient testing was attempted, however the use of $ZnCl_2$ as a preservative interfered with the salicylate method.

Effect of Mixing on Denitrification in Metalimnion

The results of the mixing experiment from day 0 to day 10 are presented in Figure 5 and Table 1. Excess N_2 increased over the 10-day experiment for 0% and 50% hypolimnion treatments, but decreased in the 90% hypolimnion treatment (Fig. 5). The 0% hypolimnion treatment showed N_2 excess by day 1 indicating immediate denitrification in epilimnetic water. The 0% hypolimnetic samples showed that denitrification was not inhibited by the presence of oxygen, however N_2 production increased as samples became hypoxic. The average corrected excess of N_2 (μM) for the 0% hypolimnion treatment on day 1 increased from ~ 0 to ~ 20 μM and was ~ 30 μM by day 10 (Fig. 5). The 50% hypolimnion treatment stayed at ~ 0 μM until day 3 when it averaged ~ 20 μM , which was the largest excess concentration for days 3 to 7 among the three treatments and was ~ 28 μM by day 10 (Fig. 5). The 90% hypolimnion treatment started at ~ 0 μM at day 0 and immediately went to ~ -10 μM by day 1. It continued to decrease and was ~ -25 μM by day 10 (Fig. 5).

Average O_2 decreased over the 10-day experiment for all three treatments. On day 1 all three treatments were significantly different from each other ($p < 0.001$; Fig. 2). The average O_2 for the 0% hypolimnion treatment had a concentration of ~ 335 μM on day 1 and decreased to ~ 93 μM by day 10 (Fig. 2 & 4). The 50% hypolimnion treatment started at ~ 90 μM O_2 at day 1 then decreased to ~ 2 μM on day 3 and was ~ 0.59 μM on day 10 (Fig. 4). The 90% treatment started at ~ 5 μM on day 1 and increased to a maxima of ~ 8 μM on day 3, then decreased to ~ 1 μM by day 10 (Fig. 4).

Excess N_2 concentration was significantly affected by mixing treatment over time ($p < 0.001$; Table 1). Day alone was not a significant source of differentiation in data result (Table

1). The differences in N_2 due to treatment were primarily driven by the difference between 0% and 50% hypolimnion treatments versus the 90% hypolimnion treatment (Table 1) because of the negative excess N_2 observed in the 90% hypolimnion treatment. The 50% hypolimnion samples showed that $NO_3^- -N$ was low in both the epilimnion and hypolimnion and did not increase during the duration of the experiment.

Discussion

Expected denitrification rates measured by N_2 concentration occurred in the 50% hypolimnetic water samples and represented an ideal mixing event in the metalimnion. However unexpected results occurred in the 0 and 90% hypolimnetic samples. The 50% hypolimnion water, showed the expected lag where N_2 excess did not occur until day 3 after the onset of anoxia. This is similar to the results of Kogo et al. (2013), who reported that aeration could cause low initial N_2 excess; however, only after the addition of NO_3^- and the development of the necessary anoxic conditions could aeration have enabled denitrification to occur. The 50% treatment appropriately represents the metalimnion, which can be a hotspot for denitrification to occur because of the mixing of hypolimnetic and epilimnetic water (Kogo et al., 2013). The repeated mixing of oxidized and reduced water enables the coupled nitrification-denitrification effect to occur. The interaction of inorganic N and O_2 in this study uniquely shows how important the metalimnion is for releasing significant amounts of N from lentic systems.

Given measured O_2 patterns, it was expected that 0% hypolimnion samples would show a longer lag with N_2 concentration increasing after conditions became anoxic. However, the 0% hypolimnetic samples that were comprised of 100% epilimnetic water, showed positive excess of N_2 gas in the presence of O_2 . Oxygen concentrations in the 0% treatment did not go anoxic but

went hypoxic at day 10 allowing N₂ gas concentrations to increase (Fig. 4 & 5). After the initial increase on day 1, the corrected N₂ excess for the 0% hypolimnion samples stayed the same throughout the time trial until day 10 when it increased (Fig. 5). It is uncertain if N₂ excess would have continued to increase after day 10. The availability of organic carbon may have limited O₂ consumption and therefore, denitrification in the 0% hypolimnetic samples. What remains unclear is why N₂ gas concentrations increased by day 1 in the 0% hypolimnion samples. These samples were oxic, which should have precluded denitrification. Further investigation is needed to explain these results.

It was expected that the 90% hypolimnetic samples would show the greatest amount of N₂ concentration because denitrification would be able to occur in the anaerobic conditions. However, the 90% hypolimnetic treatment showed a negative excess of N₂ gas with low concentration of O₂. This could be due to methanogenesis, which can only occur in extremely reduced conditions. The presence of insoluble methane bubbles formed in BOD bottles after collection can trap N₂ gas (Kogo et al., 2013). This mechanism would explain the substantial decrease, and perceived consumption of N₂, within the 90% treatment samples throughout the 10 days of the study. Methane concentration was not measured in this study, which would be needed to know if methanogenesis occurred. Since this study only measured O₂ and N₂ gas concentrations, it is unclear if the denitrification process was able to completely convert NO₃⁻ to N₂ gas in the 90% treatment samples. It is possible that instead of N₂, intermediate products such as N₂O or NO_x were released.

This study, and previous studies showed how man-made lakes can significantly contribute to denitrification and help lower excessive N loading into streams when water leaves lentic systems. Since a majority of total N removal from lakes is due to denitrification, the

process is extremely important in controlling the many environmental consequences that are accompanied with high N sequestering. Past studies have shown that reservoirs, specifically, create hot spots for denitrification, but the metalimnetic potential for releasing N_2 back into the atmosphere is underrepresented. This study showed that metalimnetic mixing and repeated epilimnetic water movement into the metalimnion could enable denitrification to occur. The coupled nitrification-denitrification process in the metalimnion can contribute to N_2 removal. Mixing events within the metalimnion are important because during summer stratification, biological processes, such as denitrification, are able to occur that otherwise would not because of the zonation caused by thermal stratification. Therefore, the metalimnion of reservoirs has the potential to significantly contribute to N removal from man-made lakes on local and global scales.

While this study demonstrated how the metalimnion is a zone for denitrification, the mixing effect that occurred in this study was not well defined. This was due to the 0 and 50% hypolimnion treatments being similar in N_2 concentrations. The 90% hypolimnion treatment samples showed similar negative N_2 concentrations observed previously by Kogo et al. (2013). The amount of N denitrified in the 90% hypolimnion samples was not certain possibly because conditions became too anaerobic for denitrification to occur or intermediate products were released. More testing should be done in future studies to identify the possible mechanism that is consuming N_2 gas in 90% hypolimnion water samples, and the mechanism allowing denitrification to occur in 100% epilimnetic water samples. Future studies should be conducted in order to reveal the effect of mixing events within the metalimnion of reservoirs. Testing how different lake sizes with different inputs, loading capacities and retention rates could greatly impact local and regional communities.

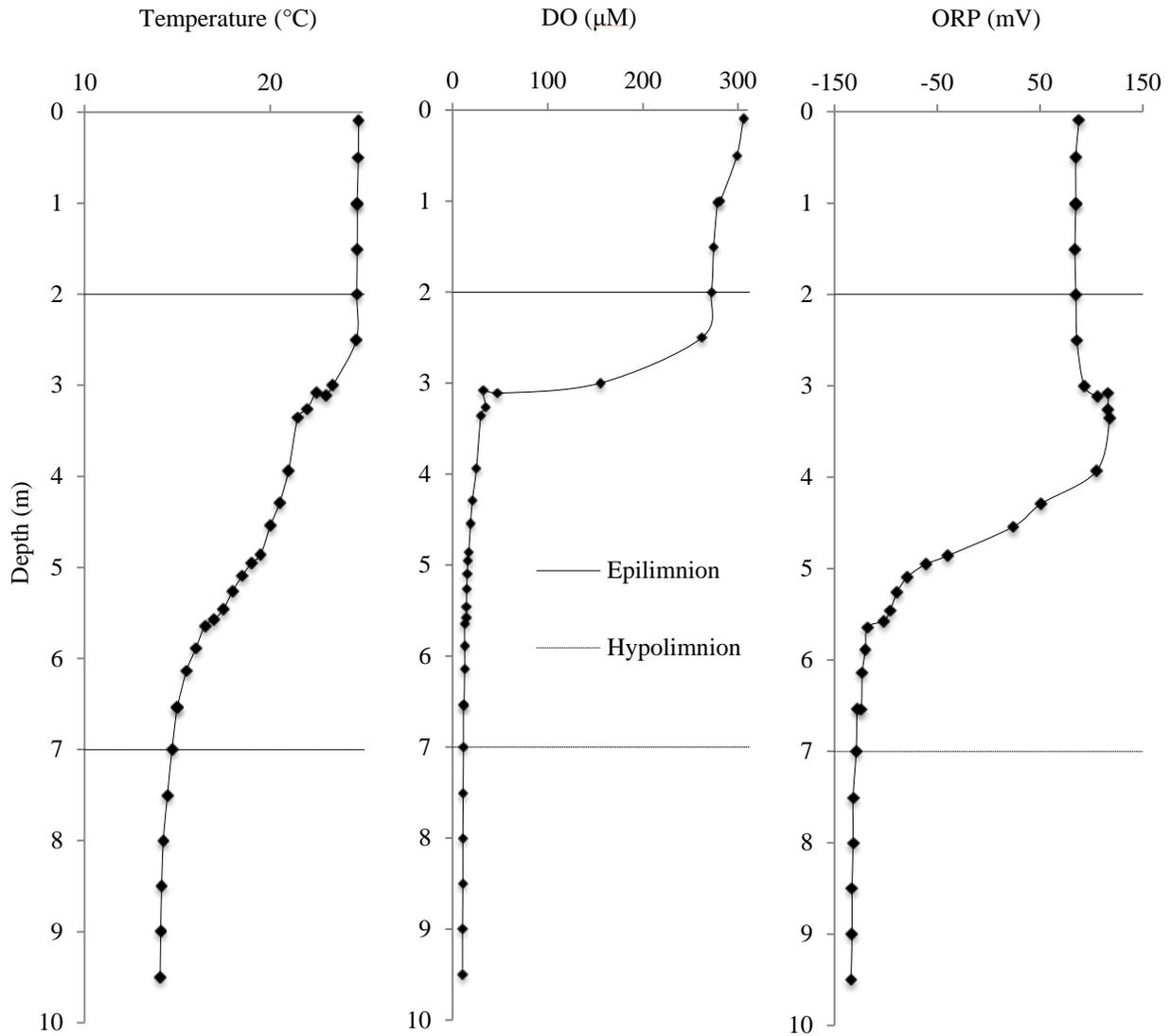


Fig. 1. Temperature in °C, dissolved oxygen concentration in µM, and oxidation-reduction potential in mV of Lake Fayetteville depicting where the epilimnion and hypolimnion samples were taken with depth in meters.

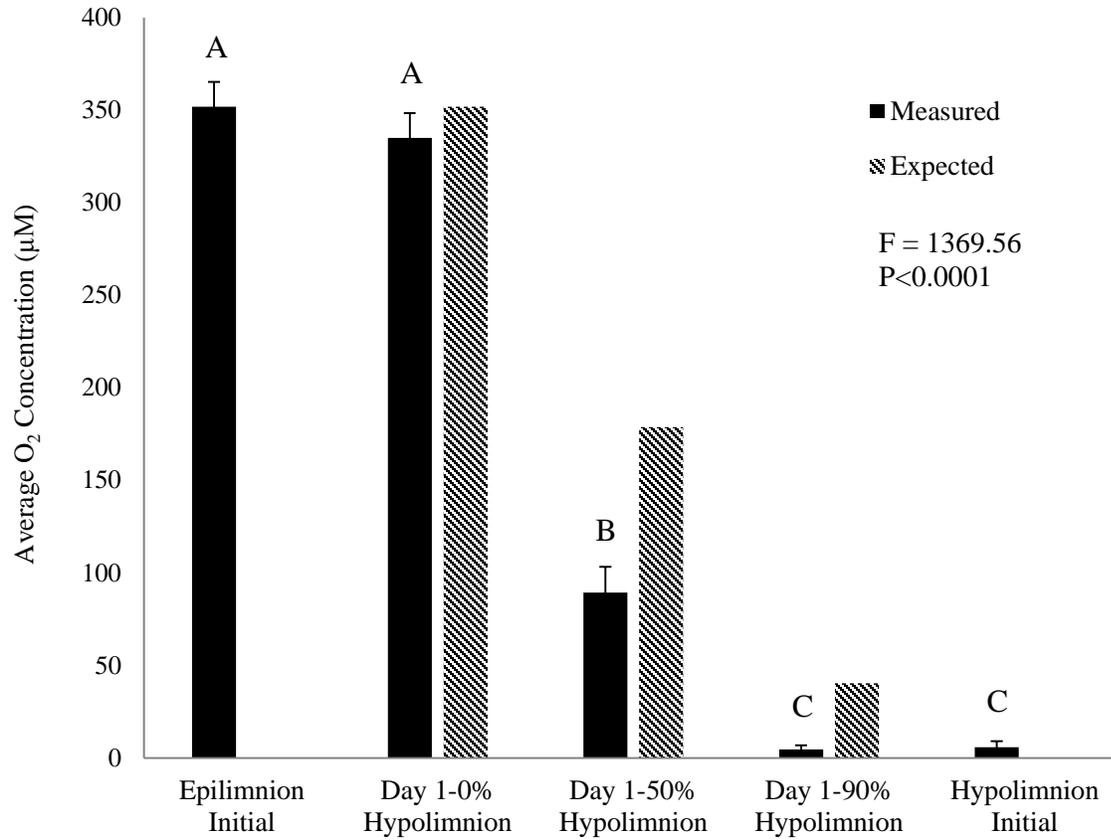


Fig. 2. Measured and expected average O₂ concentrations for epilimnion & hypolimnion initials and 0, 50, & 90% hypolimnion samples from day 1 with standard deviation bars. Expected average values calculated with equations 3, 4, & 5 using the epilimnion and hypolimnion initial averages without considering excess. REGWQ grouping indicates significant differences.

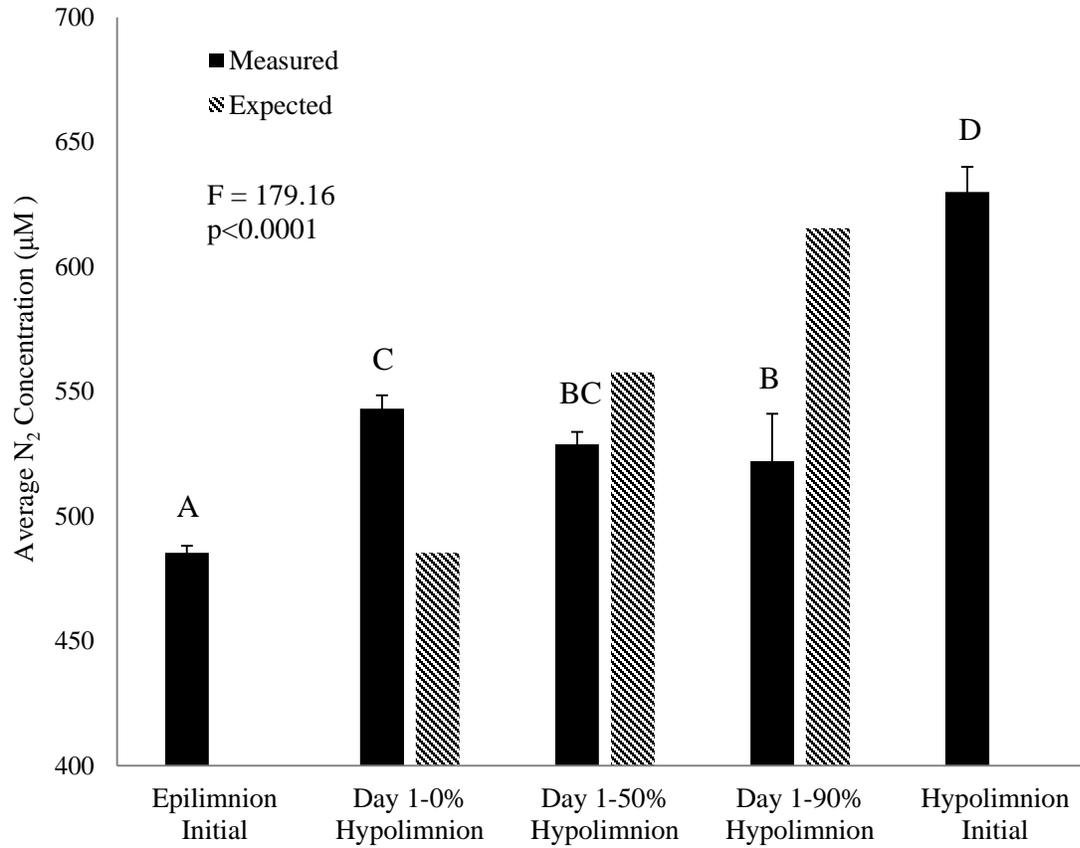


Fig. 3. Measured and expected average N_2 concentrations for epilimnion & hypolimnion initials and 0, 50, & 90% hypolimnion samples from day 1 with standard deviation bars. Expected average values calculated with equations 3, 4, & 5 using the epilimnion and hypolimnion initial averages without considering excess. REGWQ grouping indicates significant differences.

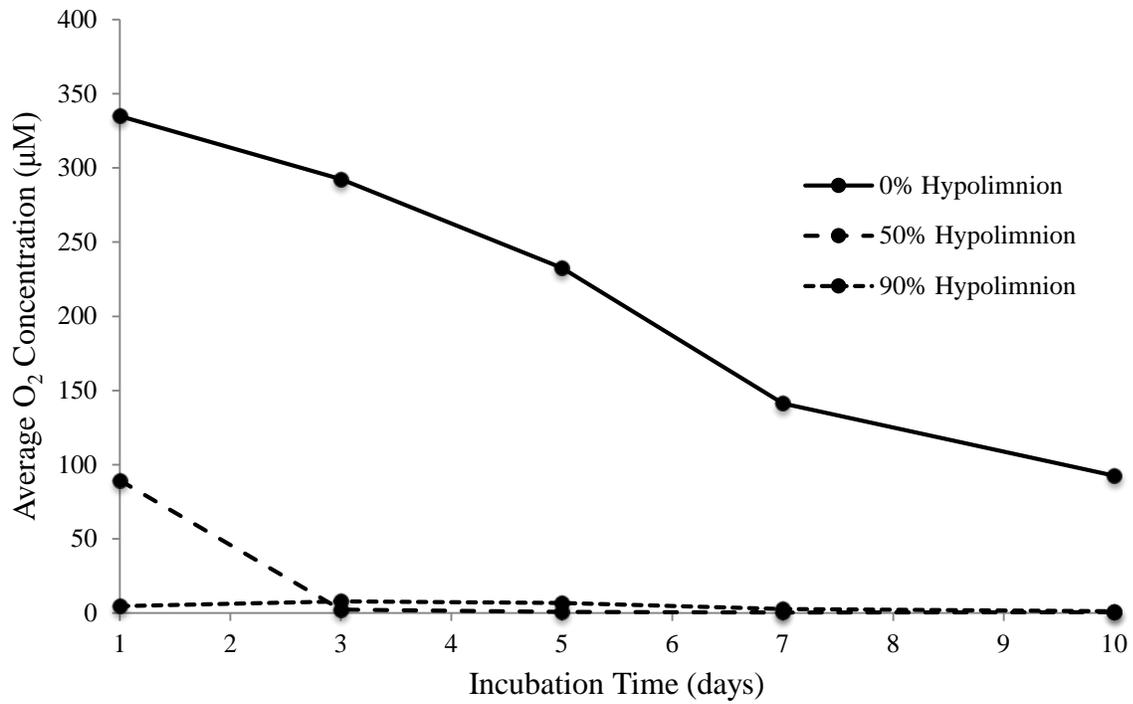


Fig. 4. The average O₂ concentration for 0, 50, and 90% hypolimnion treatments samples which represented metalimnetic mixing through time.

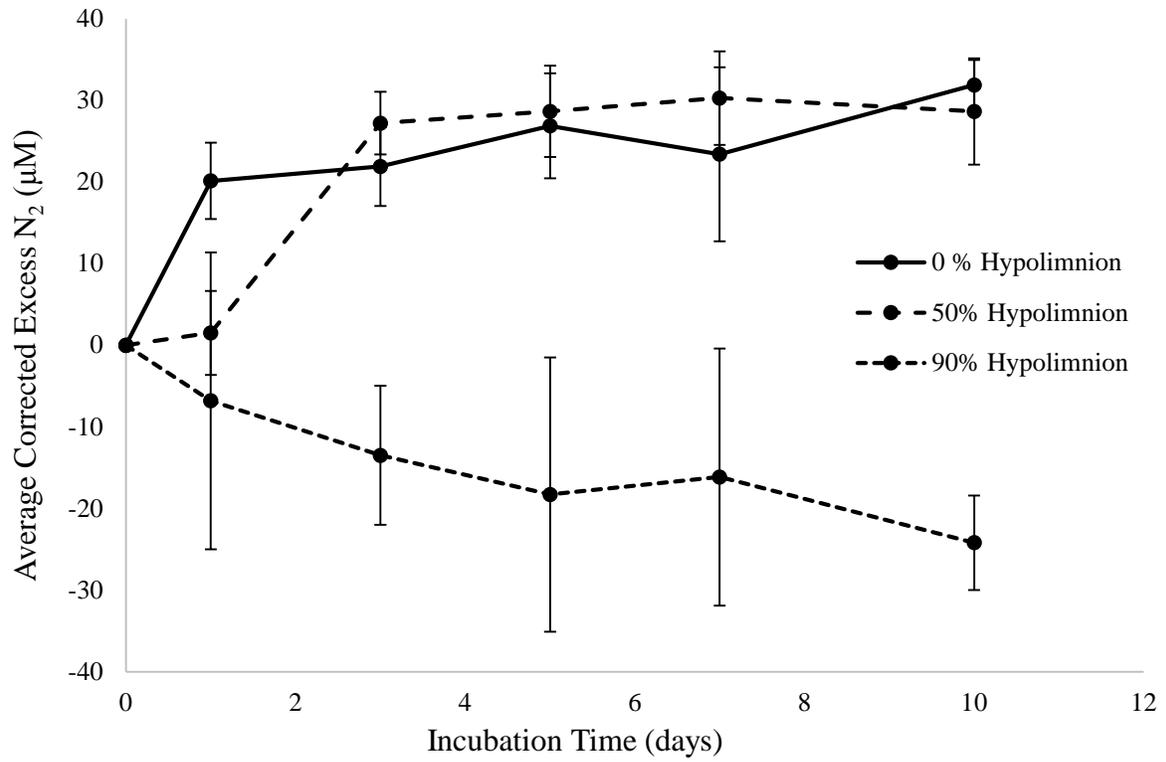


Fig. 5. The average corrected excess of N₂ through time for 0, 50, and 90% hypolimnion treatments samples that represented metalimnetic mixing, with standard deviation bars.

Table 1. The statistical analysis showing the differences in measured N₂ concentrations among different sources.

Source	F Value	P Value
Day	1.5	0.2333
TRT	119.9	< 0.0001
Day*TRT	4.1	0.0011

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