Effects of agricultural components, nitrate and acetate, on nitrification in
Morris Lake, Wisconsin

BIOS 35502: Practicum in Field Biology
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2011

Abstract

Freshwater lakes are vulnerable to nitrogen loading cause by ammonia deposition. Studies of the effects of nitrate (NO_3^-) and acetate (CH_3COO), can affect the nitrification rates within a lake. Water samples of Morris Lake were study in microcosm experiments and nitrapyrin was added to water samples to inhibit nitrifiers for the production of nitrate (NO_3^-), while nitrification was not inhibited in DMSO flasks. Differences between both DMSO and nitrapyrin samples gave us the bacterial nitrification rates. Adding ammonium nitrate ($NH_4^+NO_3^-$) and sodium acetate (CH_3COONa) treatments individually inhibits nitrification rates from 0.55 to 0.58μmol/L/day. We suggested that high differences in C: N ratios could be detrimental to nitrifiers. The combination of acetate and ammonium produced a higher consumption of ammonium and a C: N ratio of 8:1 was proper to allowed nitrification at rates closer to the controls. Adding acetate, a carbon source for bacteria, may inhibit nitrification rates. Decreased nitrification rates are expected to reduced denitrification rates and N_2O production. As less nitrification occurs, less nitrate (NO_3^-) will be produced and potentially less denitrification will occur, which may not inhibit methane production.

Introduction

During the past century, anthropogenic activities have significantly added to the amount of greenhouse gases in the atmosphere through activities such as burning fossils fuels, deforestation, industrial effluents, and agriculture, contributing to an increase in global average temperature and related climate changes. Freshwater lakes are vulnerable to eutrophication due to increased inputs of nutrients, stimulating primary production but also can have a substantial effect in microbial processes (Liikanen and Martikainen, 2003). Approximately 35% to 60% of total nitrogen loading to coastal and fresh waters is estimated to be caused by ammonia (NH_4^+) deposition, which has been a globally major concern (Becker and Graves, 2002).

Eutrophication in water ecosystems has atmospheric importance, because anaerobic conditions and nutrient availability can affect microbial processes that produce greenhouse gases: carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Liikanen and Martikainen, 2003; Stadmark and Leaonardson, 2007).

Nitrogen is found in form of ammonia (NH_4^+) and can easily move in the environment as a gas, dissolved constituent of surface or ground water (Becker and Graves, 2002). Nitrification is the process of ammonium (NH_4^+) interacting with oxygen (O_2) in the epilimnion forming nitrite (NO_2^-) followed by the oxidation of these nitrites into nitrates (NO_3^-) by nitrifying bacteria (Daily et al. 2011). Nitrifiers derive energy from oxidation of ammonia (NH_4) and nitrite (NO_2), and use inorganic compounds for cell synthesis (Carley and Mavinic, 1991). Nitrification is important in the nitrogen cycle in soil and also plays a role with denitrificaton in the removal of nitrogen pollution (Clarens et al. 1998). Denitrification depends on (NO_3^-) availability, which is reduced to nitrous oxide (N_2O) and ultimately dinitrogen (N_2) and low nitrification activity is limiting to denitrification (Liikanen and Martikainen, 2003; Knowles, 1979; Jensen et al., 1993). Annual rates of denitrification have been found to be higher in lakes than rivers, coastal ecosystems and estuaries (EPA, 2010), which makes lakes a significant source of N_2O to our global greenhouse gas budget.

As more nitrification occurs, higher nitrate (NO_3^-) concentrations are produced and potentially more denitrification occur, which may inhibit methane (CH_4) that is produced in anoxic sediments (Liikanen and Martikainen, 2003). Ammonium, which resembles chemical structure of the CH_4 molecule, can interfere with the oxidation of CH_4 because NH_4 competes with CH_4 for the key enzyme in CH_4 oxidation (Bédard and Knowles, 1989). In other hand, acetate ($CH3COO$) that has been observed to contribute in methane production under low-temperature has also been found to inhibit denitrification, but is cleared by methanogens to produce CH_4 (Nozhevnikova et al. 2007).

Our specific study is looking to determine: (1) how ammonium nitrate ($NH_4^+ NO_3^-$) inputs affect nitrification rates, (2) if sodium acetate has a positive effect on NO_3^-

production, (3) and if the combinations of both nutrients have a significant effect on nitrification rates. To do this, water samples from the epilimnion of Morris Lake, in north eastern Wisconsin, were incubated with NH_4^+ NO_3^- and CH_3COONa at 15°C for five days. Nitrapyrin was added to water samples to inhibit nitrifiers for the production of nitrate (NO_3^-), while nitrification was not inhibited in DMSO flasks. The differences between both DMSO and Nitrapyrin samples gave us the bacterial nitrification rates.

Methods

Study lake and water sampling

Morris Lake is an oligotrophic lake located in northeastern Wisconsin. This lake was selected for the low nutrient level and undisturbed status, which would allow for strong contrast between the controls and the amended treatments. On the day of the collection, the lake was profiled by YSI for temp= 5.8° C in the epilimnion water column (1m depth), of 24.1° C going down till 5.8° C in the hypilimniom (6m depth). The DO was 2.02 mg/L in 1m-depth lowering with depth (Figure 2). The pH at the epilimnion was 7.15. Water samples were obtained with a 2-liter Van Dorn bottle from epilimnion water column of Morris Lake from a depth of 1-m.

Nitrification assay

All the water samples were triplicated to each of the treatments: ammonium nitrate effects only; ammonium nitrate and sodium acetate combine effects; sodium acetate only and non-amended controls. Assays were performed to quantify nitrification within water

lake samples, to measure ammonium (NH_4^+) and nitrate (NO_3^-) concentrations within the water column.

Nitrification rates can be measured as the difference in ammonia (NH_4^+) concentrations between incubations in which nitrification is inhibited and those which nitrification is allowed to occur. For the water samples we obtain 24 of 250-mLflasks, which were divided in two groups: microbial activity was inhibited by nytrapyrin (2-chloro-6-[trichloromethyl]-pyridine) in the N flasks and bacterial nitrification was unhibited in the flasks (DMSO). Nutrients were added to each 100ml water sample. For the nitrate treatment we added 357µl of 1g/L NH_4^+ NO_3^-; for the acetate treatment we added 21.8 µl of 100g/L CH_3COONa; we added the same quantities of ammonium nitrate and sodium acetate for the nitrate-acetate treatment. After adding the nutrients we inserted 20 µl of nitrapyrin to all N flasks and 20 µl of DMSO to each D flask. This process was monitored for five days, at the time when the flasks are first set up (day 0) and after the incubation period is complete (day 5).

Ammonium assay

For an accurate measurement of ammonium concentrations a Working Reagent consisting of a preparation of OPA solution (orthophthaldialdehylde) ($40gL^{-1}$), sodium sulphite solution ($10gL^{-1}$) and borate buffer solution. The working reagent was allowed to mature one day before use and stored in the dark at room temperature to keep stable. The standards calculated for the ammonium nitrate measurements were: 15 µl (0.596µl mol/L), 25 µl (0.990 µl mol./L), 50 µl (1.961 µl mol/L), 100 µl (3.846 µl mol/L), 250 µl (9.091µl mol/L). To prepare the standards we add 2.5mL of DI water in each scintillation vials, followed by an appropriated amount of ammonium nitrate ($NH_4NO_3^-$) and 10mL of WR.

For each lake sample we took 2.5mL of water sample and then we added 10mL of WR. We incubated the samples in a dark room temperature for 4 hours until completion of the reaction.

Calculation of nitrification rates

Absorbance readings were taken with a Quantech Fluometer using wavelengths of 360nm excitation and 415nm emission to determine the concentration of NH_4^+ after each incubation day. Given these values we subtracted N treatment (nitrapyrin) from D treatment (DMSO) to quantify how much ammonium (μmol/L/day) was consumed after each day. Rates of nitrification were inferred from these values.

Statistical analyses

One Way ANOVA was performed to test the significance of the ammonium nitrate and sodium acetate addition treatments on nitrification rates. A pos hoc test, Turkey's Honestly Significant Difference Test was conducted to compare mean nitrification rates from the different nutrients microcosm treatments.

Results

Nitrification rates

Controls had the highest nitrification rates during the five day incubation period in comparison with ammonium nitrate and sodium acetate treatments (ANOVA, F=7.089, p= 0.012, df=3,8) (Figure 1). There was a significant difference between $NH_4^+NO_3^-$ and control treatments (p<0.030). There was also a significant difference between CH_3COONa and control treatments (p<0.041). We found a high nitrification rate of 3.02μmol/L/day for

the NA treatment but it was not significant different from the control (p<0.976), which is similar in the consumption rates of ammonium.

Discussion

We observe a significant decrease in nitrification rates (0.556μmol/L/day) in sodium acetate amended treatments and (0.5808 μmol/L/day) in ammonium nitrate treatments when compared to the control (3.336 μmol/L/day) (Figure1). We propose that high differences in C: N ratios could be detrimental to microbial processes. Carley and Mavinic in 1991 tested that adding carbon sources supported a complete denitrification with ratios of 5:9:1 for acetate, 6:2:1 for methanol, but the glucose ratio about 23:1 failed in support the process. We suggest that possibly the amount of ammonium nitrate added change dramatically the ratio of nutrients of the lake water, preventing nitrification.

Acetate, a carbon source for bacteria, may inhibit nitrification rates. Recent studies have revealed that high acetate concentrations can inhibit denitrification and increase methane production (Stadmark and Leonardson, 2007). Carley and Mavinic in 1991 found that nitrification in bioreactors became irregular and decreased up to 40% after adding acetate as a carbon source. This is due because acetate is being consumed by bacteria to produce methane (CH_4). High concentrations of acetate may favor methane production over denitrification and nitrification when NO_3^- and NH_4 are low in concentrations.

Ammonium nitrate ($NH_4NO_3^-$) and sodium acetate (CH_3COONa) combined treatment nitrification rates were 3.0224 μmol/L/day. Acetate is a labile carbon source that can contribute to bacterial growth and abundance (Jones et al. 2009), we suggest that the proper C: N ratio of 8:1 allowed for nitrification to occur at rates closer to the controls.

Lakes with higher carbon sources and ammonium inputs can be subject to increased nitrification rates leading to higher nitrate concentrations available for denitrification. Increased concentrations of acetate in lakes may inhibit nitrification and denitrification. Subsequently high amounts of acetate and low amounts of nitrate are deposited and mixed when turnover occurs. During fall and spring turnover, acetate and nitrate dissolves with the warm surface water and nutrients are mixed evenly throughout the lake. Higher concentrations of acetate versus the lower availability of nitrate in sediments may lead to increased methanogenesis and less denitrification.

Further studies can be about how eutrophication and algal fermentation affect nitrification, denitrification and methanogenesis rates within lakes, including the microbial composition and abundance. Studying microbial processes can improve management of the lakes and help make predictions of whole lake greenhouse gas emissions.

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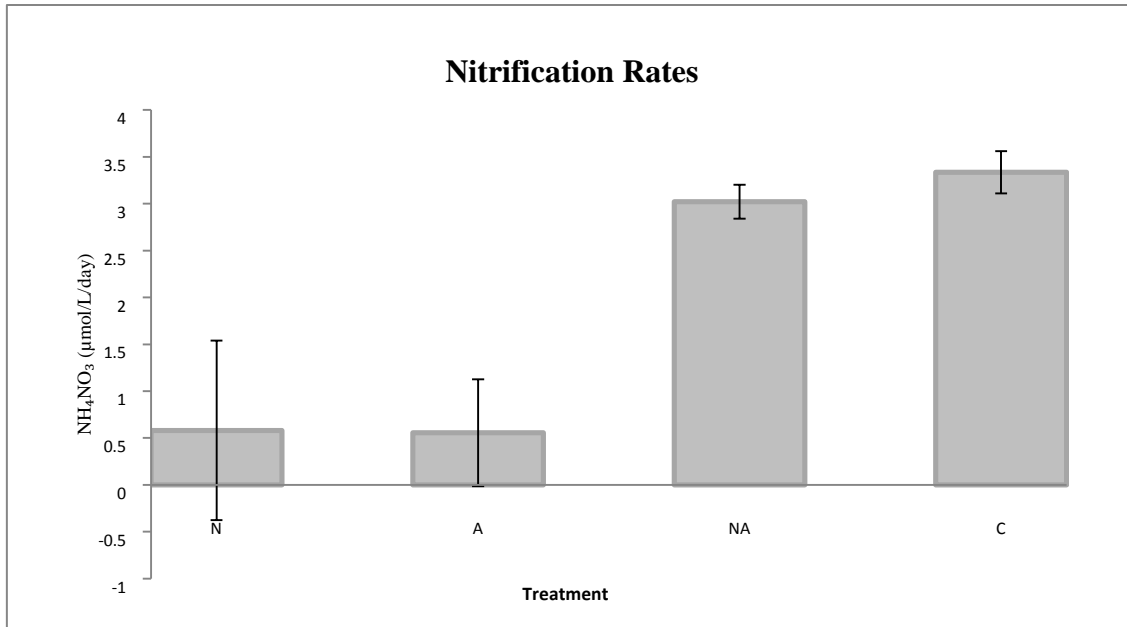
FIGURES

Figure1. Barplot of $NH_4^+NO_3^-$ consumption rates. Nitrification rates are lower in ammonium nitrate (N) and sodium acetate (A) treatments varying from 0.55 to 0.58μmol/L/day in comparison with control (C) that has a higher 3.336μmol/L/day consumption of NH_4NO_3 and nitrate-acetate (NA) treatment with consumption rate of 3.0224μmol/L/day.
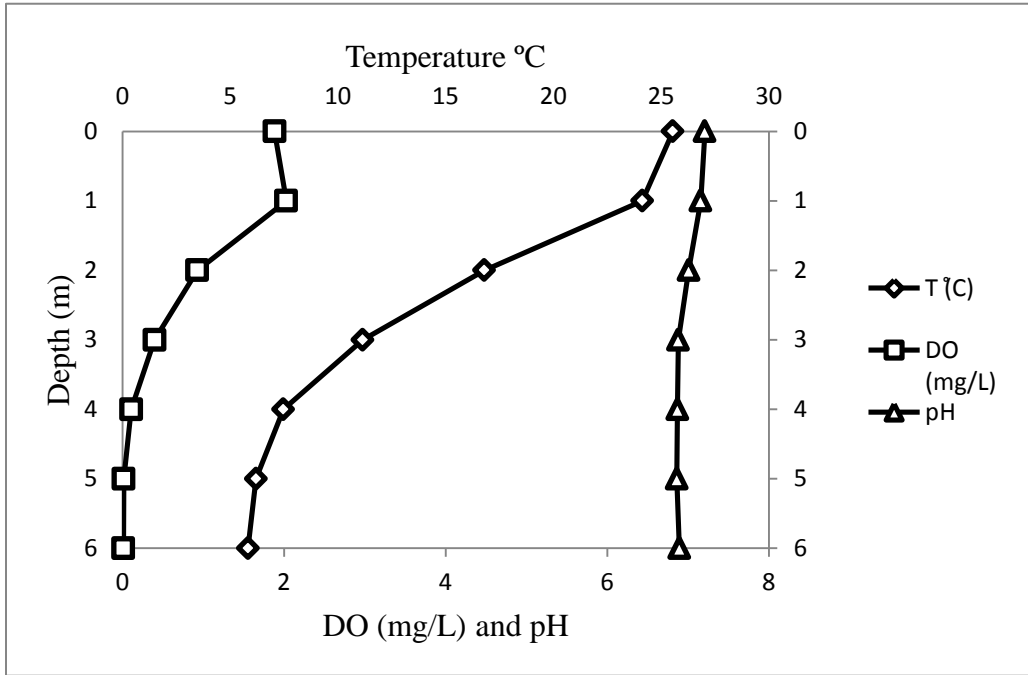
Figure 2. Morris Lake depth profile of T (°C), DO (mg/L), and pH concentrations.