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Nitrogen Biogeochemistry in Lakes – Chemical and Biological Drivers and Developing Techniques to Explicitly Measure Concurrent Processes

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Abstract

NITROGEN BIOGEOCHEMISTRY IN LAKES – CHEMICAL AND BIOLOGICAL DRIVERS AND DEVELOPING TECHNIQUES TO EXPLICITLY MEASURE CONCURRENT PROCESSES

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A benthic chamber system was developed to measure all major nitrogen cycling processes in lakes. The system coupled advantages of flow-through systems common in core incubations with those of *in situ* benthic chambers. The system also coupled advantages of N_2:Ar and isotope pairing techniques for explicit measurement of gross and net denitrification, allowing determination of N_2 fixation. Use of ^{15}N tracer ($^{15}N_2$, $^{15}NO_3^-$, or $^{15}NH_4^+$), in these chambers also allowed explicit and simultaneous *in situ* measurements of other major nitrogen cycling processes including ammonium and nitrate uptake, DNRA, and ammonification.

Nutrient loading ratios (Si:N and P:N) and community composition of herbivores (*Daphnia* and mussels) were studied to determine effects on nitrogen cycling using mesocosms simulating thermally stratified, shallow lakes. Increased Si:N enhanced organic matter remineralization in bottom water, enhancing nitrogen loss by denitrification. Herbivore type had no apparent effect on where N was recycled or on N-loss through denitrification.

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1.0 Introduction

Nitrogen (N) is an important nutrient in aquatic systems as it is a primary element in many metabolic processes. The determination of the rates at which these processes occur within the nitrogen cycle is critical in the assessment of lake ecosystem function, i.e. understanding the flow of nutrients/energy through an aquatic ecosystem. The levels at which these processes occur are a representation of the types of activities occurring in a benthic system at a given period of time. This allows researchers the ability to predict the effect that a stressor may have on an ecosystem and allow for potential remediation options in damaged systems.

The nitrogen cycle is a fundamental set of ecological processes that are an area of focus for benthic system because of the ecological impact of excess nitrogen on these systems and the contribution of gases, such as N_2O, to global warming [Robinson, 2001]. Nitrogen is the limiting element for many primary producers, particularly in marine ecosystems [Ryther and Dunston, 1971], with a shift from P to N limitation occurring along the freshwater to marine gradients [Doering et al., 1995]. Similar shifts have been observed within individual freshwater systems along longitudinal gradients (headwaters to lake) [e.g. McCarthy et al., 2007b; Scott et al., 2008], and have also been seen associated with temporally [McCarthy et al., 2007b] or spatially [Elser, 1999; McCarthy et al., 2007a] explicit events within freshwater systems.

Nitrogen loaded to aquatic systems is naturally derived from three major sources: the weathering of soil minerals, deposition from the atmosphere, and biological nitrogen fixation [Dähnke et. al., 2007]. However, recent alterations to the nitrogen cycle have made anthropogenic sources of nitrogen of relatively greater importance for many aquatic ecosystems [Vitousek et. al., 1997]. Changes to these ecosystems have underlying effects on other systems, causing a domino effect when the nitrogen cycle is out of balance, leading to potential loss in

ecological stability and biological diversity [Hall et. al., 2008]. These ecosystem impacts are most dramatic in marine systems as nitrogen can be seen as a major pollutant in these systems. This nitrogen loading contributes to loss in species diversity, eutrophication and hypoxia/anoxia. These conditions are major culprits in fish kills [Anderson et al., 2008] and harmful algal blooms [Anderson et al., 2008, Flynn, 2010]. While nitrogen is generally not a limiting nutrient in freshwater systems, the processing of nitrogen, specifically the removal by denitrification, can be important in moderating the loading of nitrogen to downstream systems such as estuaries and coastal ecosystems [Seitzinger, 1988], reducing the impacts of anthropogenic activities in watersheds on these vulnerable systems. Recent work from Schindler et al. [2008] suggests that eutrophication in freshwaters can be controlled by limiting P loading, without need for limiting N loading. The rationale is that if N is limiting, cyanobacteria will meet their needs through fixation until P availability is exhausted. However, eutrophication in freshwater systems is a re-emerging problem despite tightened P controls, and there is substantial debate regarding the validity of the phosphorus limitation paradigm in freshwater systems (e.g. Elser et al., 1990; Lewis and Wurtsbaugh, 2008; Sterner, 2008; Conley et al., 2009; Scott and McCarthy, 2010). Creation of effective nutrient management strategies may require reliable budgets to be built on the understanding of nitrogen cycling processes, rather than simply ratchetting up regulation of P loading from point and non-point sources. The thesis seeks to provide the tools needed to develop these budgets, enabling explicit measurement of nitrogen biogeochemical processes in lake systems *in situ*. Further, the thesis investigates biological and chemical (nutrient loading ratio) controls on nitrogen cycling and loss from mesocosms designed to simulate shallow lake ecosystems. Both of these objectives are necessary to developing a better understanding of how

N is processed in lake ecosystems so that, ultimately, more effective nutrient management strategies can be developed.

1.1 Nitrogen Cycling Processes

1.1.1 Denitrification

Denitrification ($NO_3^- \rightarrow NO_2 \rightarrow N_2O \rightarrow N_2$) is an important process because it is the major process by which nitrogen, in the form N_2 gas, is permanently removed from aquatic systems. This process is carried out by many species of bacteria, archaea and fungi. Denitrifying bacteria are diverse and(most) are facultative anaerobes that utilize denitrification as an alternative pathway to oxygen respiration [Wallenstein, 2006]. The rates of denitrification may depend on multiple factors, but in general, this process requires anoxic environments, and a source of organic matter and nitrate [Rabalais, 2002].

This process is given by the reaction:

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O$$

However, intermediate steps in this reaction can produce nitrite, nitrous oxide and nitric oxide.

Denitrification is an environmentally important process as it is the major sink for reactive nitrogen. It is estimated that N-removal carried out by the watersheds globally can account for as much as 31Tg N/yr in lakes and 124Tg N/yr in soil [Seitzinger et al., 2006].

1.1.2 Nitrification

The process of nitrification ($NH_4^+ \rightarrow NO_2 \rightarrow NO_3^-$) is important because it is the major process that creates nitrate, NO_3^-. Nitrification can also be an important autotrophic pathway in aquatic systems, resulting in fixed carbon. In the Mid-Atlantic Bight, for example, nitrification accounts for ~10% of total autotrophic carbon fixation [Laursen and Seitzinger, 2002b]. It is likely of lesser importance in freshwater systems that are nutrient rich, however, it may still play

an important role for some lakes. Nitrate is less readily used than ammonium by many primary producers [Glé, et al., 2008]. Nitrate is a poor nitrogen source for cyanobacteria, for example [e.g. Kudela and Dugdale, 2000; Berg et al., 2003], although it is a better N source than ammonium for diatoms [e.g., Kappers, 1980; Blomqvist et al., 1994]. Diatoms may seasonally dominate the autotrophic community of lakes, but these blooms are generally short-lived. Green algae and cyanobacteria more often dominate the planktonic autotrophic community for most of the spring and summer. Consequently, nitrate tends to accumulate as the most abundant mineral form of N in aquatic systems. In some cases, nitrate concentrations accumulate to potentially toxic concentrations [Atlas and Bartha, 1998].

The process of nitrification (in the soil and aquatic environments) is carried out by three groups of microorganisms: ammonia-oxidizing archaea (AOA), nitrite-oxidizing bacteria (NOB) and ammonia-oxidizing bacteria (AOB). The ammonia-oxidizing bacteria and archaea are the species responsible for the conversion of ammonia to nitrite in freshwater and marine environments [Fortunato et al., 2009].This process occurs in the oxic layer of the sediment and is affected by a variety of factors including: concentration of NH_4^+, pH, dissolved oxygen concentration and light intensity [Zhou, 2007].

1.1.3 Nitrogen-Fixation (N-Fixation)

N-fixation ($N_2 + 8H^+ + 8e^- \rightarrow 2NH_4^+$) is the process by which dissolved N_2 gas is converted to a more useful (reactive) form of nitrogen(NH_4^+), which may then be converted to other forms of N such as nitrate, and nitrite. Without this process to recycle gaseous nitrogen from the water column into a mineral form, aquatic systems would become nitrogen limited due to denitrification (which produces N_2 gas). In aquatic ecosystems, some species of cyanobacteria

are able to convert atmospheric nitrogen into NH_3/NH_4^+, which is the major source of reactive nitrogen in some lakes, coastal and open ocean systems [Humpage et al., 1993].

Anthropogenic sources play a major role in the ecological balance of reactive nitrogen species. Sources of industrial N-fixation are found in fertilizer production, the burning of fossil fuels, and agricultural manipulation (rotation of crops), which will all increase the abundance of reactive nitrogen and alter the equilibrium between denitrification and nitrogen fixation [Galloway et al., 1995; Inwood et al., 2007].

1.1.4 Other Nitrogen Cycling Process

1.1.4.1 Anaerobic ammonia oxidation (ANAMMOX)

ANAMMOX ($NH_4^+ + N_2O \rightarrow N_2$) is a bacterial process, which oxidizes ammonium and nitrite to dinitrogen gas under anoxic conditions [Strous et al., 1999]. ANAMMOX rates are sensitive to increases in nitrite concentrations, and will decrease with concentrations of nitrite>0.1g•L^{-1} increase [Strous et al., 1999]. Aerobic conditions can also inhibit the reaction, instead favouring the nitrification process. In some marine environments, ANAMMOX can account for up to 60% of the nitrogen removal through the N_2 production [Thamdrup and Dalsgaard, 2003], although the process is still generally considered to be of negligible importance in freshwater systems.

1.1.4.2 Dissimilatory Nitrate Reduction to Ammonium (DNRA)

Dissimilatory nitrate reduction to ammonium ($NO_3^- + NO_2^- \rightarrow NH_4^+$) is a process carried out by a few species of bacteria in the terrestrial and aquatic systems. It is a form of nitrate respiration, with nitrate acting as a terminal electron acceptor in the oxidation of organic carbon. Within this process nitrate is converted back to ammonium (under anaerobic conditions) [An and Gardner, 2002]. The process is important in some aquatic environments because it will increase reactive or bioavailable pool of ammonium, which will promote primary production. This

process also favours nitrogen retention within a system, unlike denitrification, because it does not produce nitrogen gas as a final product [Scott et al., 2008].

1.2 Ecological Significance of Denitrification

Depending on the loading rate, community composition and lake size (volume), any external loading of nitrogen may accumulate in the lake or be quickly turned over in the system. Nitrogen entering an aquatic system usually follows a three-stage process, in the first stage, nitrogen is incorporated into primary producer biomass, until their metabolic requirements are satisfied. Second, microbial requirements are fulfilled. Third, excess nitrogen must then be removed through denitrification or it accumulates and causes detrimental effects [Bernot and Dodds, 2005].

Denitrification is the major permanent sink for nitrogen species. Dissimilatory nitrate reduction, and incorporation into biomass may also be important sinks for nitrate, although these two represent conversion into other N-form and are not permanent N sinks. Excess N in the aquatic system can lead to development of algae blooms if N is limiting to primary production. This is particularly true of marine systems [Hall et al., 2008], although, as discussed earlier, may also be true in freshwater systems. The removal of nitrate from the water column through denitrification can ameliorate eutrophication, as most algae and cyanobacteria cannot use N_2 as a nitrogen source [An and Joye, 2001].

1.3 Control of Cultural Eutrophication

Currently, one of the leading cause of pollution in natural waterways is nutrient loading, leading to eutrophication. Most eutrophication is due to human activities and ecologists have coined this cultural eutrophication [Sala and Mujeriego, 2001]. Due to the explosion in human population in the last century, an increase in food production became necessary to meet the new

demand. This has caused a substantial increase in the use of mineral fertilizers (enriched with nitrogen and phosphorus) in agriculture to help meet the demand. The extended use of mineral fertilizer has increased the amount of nitrogen and phosphorus circulating in the environment as most of the nutrients are not assimilated by crops, but rather are transformed in soil and/or leached from agricultural soil into ground water and surface water, and will accumulate in the environment [Sala and Mujeriego, 2001]. To combat the increase of nutrient in receiving water from waste management plants around the world, legislation has been created to reduce nitrogen and phosphorus loading concentrations in effluent water [Meinardi et al., 1995; Bykova et al., 2006]. Controls on cultural eutrophication are made more difficult by the uncontrollable diffuse sources of nutrients from agricultural fields and surface runoff caused by rainfalls washing inorganic and organic wastes from watersheds into bodies of nearby water [Meinardi et al., 1995].

Sala and Mujeriego [2001] suggest water reuse as a method to solve the eutrophication problem. If the water from wastewater management facilities were reused for the purpose of irrigation on agricultural fields, nutrient removal will be from crop uptake. This would help reduce the need for mineral fertilizers and thus prevent runoff into waterways and reduce the processing cost from the treatment of wastewater. A problem of this strategy in the North American context is that the largest volumes of water from wastewater management facilities are generated in or near dense population centres, not in agricultural areas.

1.4 Why is measuring N-cycling so challenging?

Nitrogen measurement is hampered by inadequate measurement techniques [Cornwell et al., 1999]. Much of the data for coastal ecosystems (as an example) prior to the past decade are derived from fundamentally flawed methods of analysis (i.e. acetylene reduction method for the

measurement of denitrification) [Seitzinger et al., 1993]. The acetylene reduction method in particular blocks the reduction of N_2O to N_2, and through the measurement of the N_2O build up, the denitrification rates are determined. This method has the benefits of being rapid and easy to perform, but has many issues associated with it. It has been shown to inhibit not only nitrification (and, therefore, coupled nitrification-denitrification), methanogenesis, and other processes, but also incomplete blockage of N_2O reduction by acetylene will underestimate denitrification rates. Even with these drawbacks, acetylene reduction is still one of the most widely used methods for calculating denitrification rates, particularly in freshwater systems (based on a recent literature survey of techniques used in denitrification measurements by A. Laursen, personal communication).

More recent advances in measuring denitrification such as N_2:Ar [Kana et al., 1994, 1998], extension of this technique to *in situ* measurements [Laursen and Seitzinger 2002b], and stable isotope pairing techniques [Rysgaard et al., 1993, Nielsen et al., 1995] and hybrid methods [Eyre et al., 2002] have been developed to fix the issues that were seen with the acetylene reduction method. In N_2:Ar method, the increase over time in N_2 relative to Argon is measured (via Membrane Inlet Mass Spectrometry), assuming Argon is constant [Kana et al., 1994, 1998], or changes in a predictable manner based on measured gas transfer velocities [Laursen and Seitzinger 2002b]. Stable isotope pairing is performed by spiking a system with $^{15}NO_3^-$ and mass-specific N_2 is measured by a mass spectrometer [Rysgaard et al., 1993; Scott et al., 2008]. From the concentration of $^{28}N_2$ from $^{14}NO_3^-$, $^{30}N_2$ from $^{15}NO_3^-$, and $^{29}N_2$ from $^{14}NO_3^-$ and $^{15}NO_3^-$, potential denitrification, N_2-fixation and other nitrogen processes (that result in the production of N_2) can be calculated. N_2:Ar measurements have the advantages (relative to isotope pairing) of

being rapid, and not requiring manipulation of the system (i.e. spiking with a tracer), but the disadvantage of measuring net N_2 change rather than gross denitrification.

Nitrification rates are usually calculated from isotope dilution [Rysgaard et al., 1993] (usually calculated with denitrification rates) or by inhibition of NH_4^+ or NO_2^- (i.e. Nitrapyrin inhibition method) [Bruesewitz et al., 2009]. The isotope dilution is based on measuring the change in $^{15}NO_3^-$:$^{14}NO_3^-$ over time, assuming all new nitrate produced by nitrification will have a ^{14}N signature (reflecting the ammonium pool), thus diluting the ^{15}N signature of the nitrate pool. This method also accounts for the overall change in the nitrate pool size to determine how much ^{14}N nitrate must have been produced to account for the observed dilution of the ^{15}N signature. The inhibition method, either blocks the ammonium from being converted to nitrite (i.e. by acetylene), and nitrification rates are calculated from the accumulation of ammonium or by inhibiting the oxidation of nitrite (to nitrate by chlorate) and monitoring the increase in nitrite [Bruesewitz et al., 2009].

Nitrogen fixation rates are determined by the acetylene reduction method [Marcarelli and Wurtsbaugh, 2009] and stable isotope techniques [Scott et al., 2008]. The acetylene reduction method measures the conversion of acetylene to ethylene, and applies a conversion factor (commonly 3 N_2 reduced to ammonium for each 1 acetylene reduced to ethylene), assuming a constant and greater specificity of nitrogenase for N_2 versus acetylene. Stable isotope techniques involve measuring the loss of $^{15}N_2$, either dissolved or from the headspace of an incubation vessel. Total fixation rates are then calculated on the basis of $^{15}N_2$:$^{14}N_2$, assuming no discrimination between the forms of N_2 in fixation.

While there are now better techniques for measuring most of the key processes in the cycle, measuring them simultaneously, especially *in situ*, will require modification of, and extension of,

current techniques (i.e. ^{15}N tracer technique and N_2:Ar quantification). One gap in the literature is that very few studies show simultaneous measurements of multiple N-processes. This is a gap that the chamber experiment described in this thesis hopes to fill.

Measurement of N-cycling is further complicated by the fact that there are so many transformative processes that occur in nitrogen biogeochemistry, with many of these processes having shared reactants and products, making it very difficult to explicitly measure each of the nitrogen cycling processes [Cornwall et al., 1999].

Adding to the complexity of the problem, many other factors make it challenging to measure denitrification (and the other N-processes) in an accurate manner at ecologically-relevant scales. Most whole-system measurements of denitrification have been based on nitrogen mass-balance. When determining nitrogen loss (and, presumably denitrification) using N-mass balance within a system, the total input (i.e. external and internal loading) and output (i.e. sinks, movement of water) must be accounted for, and for many experiments performed in the field, it may not be possible to know the influence of every N-source independently [Neilsen et al., 1995; David et al., 1997]. More recently, whole-system measurements of denitrification have been made in rivers [Laursen and Seitzinger 2002a, 2004; Smith et al., 2008] and lakes [Ajambo-Doherty, 2007] based on high precision measurement of changes in dissolved N_2 concentration over time. While these approaches are less constrained than mass-balance by the need to know all N-sources, they also only provide net rates of select N-cycling outcomes (i.e. net N_2 production) rather than explicit measurement of multiple simultaneously-occurring N-cycling processes.

1.5 Unanswered Questions about Denitrification

Challenges in determining and predicting denitrification rates accurately can be attributed to an unclear understanding about which environmental factors have the greatest effect on this

process. To that point, the proper method to be used (once these factors are determined) for measuring these rates is also very difficult to determine. Previous studies have identified that biogeochemical factors such as NO_3^- concentration, organic carbon availability and dissolved oxygen concentrations can regulate denitrification [Kemp and Dodds, 2002; Royer et al., 2004]. Additionally, salinity, temperature, pH, and light attenuation may also have a secondary effect on denitrification rates [Tengberg et al., 1995, Inwood et al., 2007]. Although biogeochemical constraints (nitrate concentration, dissolved oxygen, organic C, temperature) may affect denitrification rates [Laursen and Seitzinger, 2002a; Böhlke et al., 2004; Boyer et al., 2006; Pina-Ochoa and Alvarez-Cobelas, 2006; Bohlke et al., 2009], at the ecosystem-level scale hydraulic characteristics may affect N-retention [Howarth et al., 1996; Alexander et al., 2000; Seitzinger et al., 2002; Boyer et al., 2006; Alexander et al. 2008] by denitrification. While these drivers of denitrification and N-retention are becoming clearer, there is still relatively little understanding of the importance of biological drivers such as community composition on denitrification. Further, it is unclear how elemental loading ratios may affect denitrification through the influence of nutrient ratios on phytoplankton community. It might be expected that the biological community of phytoplankton and primary herbivores will affect how organic matter is processed in a lake and where it is processed (i.e. in the water column or in the sediments). If the biological community drives greater deposition of organic matter (including N) to sediments and recycling there, it might increase N loss through sedimentary denitrification.

1.6 Effect of Biotic Structure on Denitrification

Biological interactions are vital in maintaining ecological balance and diversity within the system. Take for example, the invasion of zebra mussels into the Great Lakes, and the effect that has occurred. Due to the high filter capacity, a substantial amount of planktonic and particulate matter is drawn to the sediment surface from the water column [Bruesewitz et al., 2006].

Through the consumption of this organic matter and overall movement of nutrients to the benthic layer, a decrease in primary production has been observed, turbidity in the water is lower, there is a decrease in dissolved oxygen (DO) in the water column and an increase in macrophyte production [Caraco et al., 2000]. In other words, an overall change in the biological, chemical, and physical structure has been seen, most likely causing changes in sediment redox state and biogeochemical cycles for nitrogen and carbon [Bruesewitz et al., 2006]. With the increase in excretion of ammonium from these mussels, a stimulation of ammonia mineralization and enhancement of nitrification is observed. Coupled to this is a reduced oxygen environment, again caused by the deposition of decaying matter deposited by the mussel. This could cause an increased denitrification rate, thus an increase in the removal of N from the system [Bruesewitz et al., 2006]. Bykova et al. [2006] similarly found an increase in nitrate flux into sediments where zebra mussels were present and a decrease in water column N:P ratios. This decrease in N:P due to enhanced nitrate consumption by sediments, perhaps coupled to selective feeding by mussels, was believed to contribute to an observed shift in the phytoplankton communities from green algae to cyanobacteria where zebra mussels were present. These studies suggest that biotic interaction and ecological structure can be important drivers of how N is recycled in an aquatic system, with implications for total N removal by denitrification.

1.7 Objectives

The major research objectives of this thesis research are:

1) Develop an innovative approach to simultaneously measuring all major benthic nitrogen cycling processes *in situ.* This approach builds on elements of previous benthic chamber designs with important modifications, stable isotope approaches in N-biogeochemical studies, and membrane inlet mass spectrometry approaches to measuring gas evolving processes

2) Explore the importance of biological and chemical drivers (i.e. herbivore feeding modes and elemental loading ratios) of denitrification in lake mesocosms

Section 1.8 provides additional background relevant to Objective 1: Development of an approach to simultaneously measure all major nitrogen cycling processes in a lake. This ability would allow for a more accurate measurement of these processes. From these measurements, better budgets and models can be developed to predict the effects of loading on an aquatic system.

Section 1.9 provides additional background relevant to Objective 2: exploration of the importance of chemical and biological drivers in nitrogen retention by lakes. These drivers may be important in predicting how a lake will process exogenous nitrogen loaded to the system. Such knowledge could be important in determining critical loading levels to prevent eutrophication of these aquatic systems.

Collectively, achieving the two objectives is integral to better understanding how lake ecosystems function in nitrogen retention, and this could be important for developing nutrient management strategies to reduce impact on estuarine systems downstream. How much N can a lake assimilate? And what drivers determine this?

1.8 A flow-through benthic chamber system for explicit and simultaneous measurement of major nitrogen cycling processes

The goal of this experiment was to develop an *in situ* chamber system that can be utilised for simultaneous determination of the rates of benthic nitrogen fixation, denitrification and nitrification in lake systems. This system incorporated traditional methods for measuring the above processes such as, benthic chambers and flow-through core incubation, and attempting to improve on these techniques. This chamber system was used in conjunction with ^{15}N isotope

methods to evaluate nitrogen ratios in a water system using a spike of ^{15}N tracer ($^{15}N_2$, $^{15}NO_3^-$, or $^{15}NH_4^+$). These samples were analyzed using Membrane Inlet Mass Spectrometry (MIMS) for N_2 gas ($^{28}N_2$:$^{29}N_2$:$^{30}N_2$), and microdiffusion for $^{15}NO_3^-$:$^{14}NO_3^-$, or $^{15}NH_4^+$:$^{14}NH_4^+$.

This benthic chamber approach provided the means to address a contested question in nitrogen biogeochemistry, that is, will nitrogen fixation and denitrification occur at the same time (and in close proximity)? When there is a readily available source of nitrogen (e.g. nitrate) in the water column, conventional wisdom would suggest there would be no need for an organism to fix nitrogen (an energetically-expensive process). Recently, Fulweiler et al. [2007] measured high rates of N_2-fixation in Naragansett Bay in a location where Seitzinger and Garber [1987] previously found N_2-fixation negligible, suggesting the occurrence of the process could be temporally variable at this site. Halm et al. [2009] demonstrated that denitrification and nitrogen fixation do co-occur in time and space at the chemocline of a meromictic lake. Similarly, An et al. [2001] demonstrated that N_2-fixation and denitrification co-occur in estuarine sediment cores with an overlying cyanobacterial mat. However, Halm et al. [2009] found that N_2- fixation was approximately two orders of magnitude lower than denitrification, while An et al. [2001] found comparable rates of these two processes (within a factor of 3), with net N_2-fixation. Did Fulweiler et al. [2007] capture a snap-shot of an episodic but ecologically meaningful process, or a meaningless happenstance? Are the rates of denitrification and N_2-fixation in aquatic systems comparable in magnitude (as in An et al., 2001), or are they unbalanced (as in Halm et al., 2009), and what determines the relative magnitudes? It is worth noting that two of these studies were in estuarine environments and one in a meromictic lake, not in systems of direct relevance to the current research. However, they represent the only three studies known to the author of this thesis in which simultaneous measurements of denitrification

and nitrogen fixation were attempted. Therefore, questions concerning the relative importance of these two processes and how commonly they co-occur are wide open for dimictic lakes (and remain so for estuarine systems). The only way to address these questions is with more explicit measurement of these processes concurrently. The ability to quantify these processes is crucial in the construction of nitrogen budgets in aquatic systems.

1.8.1 In situ Benthic Chambers and Sediment Cores

Benthic chambers are apparatuses that can be used to measure surface (at water-sediment interface) and hyporheic (the region in which mixing of shallow water and surface water occurs) metabolism simultaneously [Uzarski et al., 2001]. These chambers can be used to measure dissolved gas flux (e.g. N_2, N_2O, O_2) and inorganic nutrients (e.g. NO_3^-) across the sediment-water interface (or benthic layer) [e.g. Devol, 1991; Laursen and Seitzinger, 2002b].

Benthic chambers were introduced by McIntire and Phinney in 1965, for community metabolism measurements (open system) based on changes in dissolved oxygen. Since then, recirculating chambers have become more frequently used in the estimation of lotic (flowing bodies of water) productivity or whole-system metabolism [Dodds and Brock, 1998].

Two main types of *in situ* benthic chambers are used: recirculating and closed [Tengberg et al., 1995, Uzarski et al., 2001]. Each one of these types of benthic chambers has benefits and limitations. Recirculating chambers, for example, have the benefit of maintaining water flow across the sediment surface (through the use of a fan blade or pump) but may increase the temperature of the water within the chamber (due to stirring motor) [Dodds and Brock, 1998]. Closed chambers are simpler in design than recirculating chambers, but contain the major disadvantages of increasing internal pressure (body of enclosed water pushing down onto the top of sediment surface) during chamber installation - disrupting redox gradients for a period of time

after installation. Further, the lack of water flow across the sediment surface can create diffusion barriers that would limit delivery of reactants to sediments, resulting in underestimates of benthic metabolism [Tengberg et al., 1995; Dodds and Brock, 1998].

Benthic chamber techniques are performed *in situ* to prevent modification of ambient conditions during the experimental period. Chambers are a useful tool in monitoring metabolic activities because they allow for the isolation of the sample from the environment, so changes between the benthos and the water column can be isolated [Dodds and Brock, 1998].

Chambers may introduce some errors or uncertainty into the data because of the restricted area in which they are employed (they cannot include all substratum at once), nutrient limitations, alteration of flow, and changes in light field, temperature and internal pressure within the chamber [Dodds and Brock, 1998]. Some of these problems have been addressed by Uehlinger and Brock [1991] who introduced a flow-through system in which nutrients were replenished from the inflow to prevent nutrient depletion. This was done to prevent alteration of metabolism in the sediment or water column. Pennak and Lavelle [1979] mention that chambers need to be pressed gently into the streambed to minimize disruption of substratum layer (in the area of analysis). By far the greatest obstacle encountered by early chamber work was the inability to mimic natural hydrodynamics or water flow across the sample surface. Vogel and LaBarbera [1978] modified their design to include a large diameter return pipe with a propeller to circulate the water and combat this drawback. This large pipe was cumbersome and the increased volume needed to incorporate it made measurements of slow metabolic rates very difficult. Thus smaller diameter recirculating pump systems have been utilized since [Dodds and Brock, 1998]. Other improvements have been made to reduce some of the uncertainty produced by the use of chambers, such as the use of UV transparent plastics/acrylic to prevent

modification of photosynthesis rates and light attenuation, and by making the chambers more portable to allow for sampling in remote areas where providing power to pump is an issue and to reduce the overall weight of the chamber apparatus [Uzarski et al. 2001].

Another drawback of *in situ* chambers is the inability to produce multiple replicates for a particular time frame which creates substantial uncertainty in the results. The relatively small sample area that traditional benthic chambers encompass limits the ability for data to be extrapolated to an entire lake system.

Core incubations have been used for a variety of purposes, most relevant here, in the determination of net denitrification rates [Kana et al., 1994, 1998; Cornwell et al., 1999]. Core incubations have some advantages and some disadvantages relative to benthic chambers, as will be described below. Two major types of core incubators are the static and continuous (or flow-through). The core incubation method consists of a sample 'core' (usually cylindrical segment of sediment from the sediment surface down to a desired depth) with a volume of overlying water contained within an incubator. The core is incubated *ex situ* at ambient conditions (for light, temperature, pH, etc...), in an effort to mimic *in situ* conditions. Surface water is commonly recirculated through the use of a peristaltic pump. Analytes are measured from the inlet and outlet water flows from the core sediment across the sediment-water interface and the flux rates can be calculated based on known mean residence time [Gardner and McCarthy, 2009].

The static technique consists of placing the core sample (with the overlaying water) within an incubator and allowing it to equilibrate, after which samples can be taken at desired intervals. This method is simple, but will alter the natural hydrology of the core environment due to the lack of water current across the core surface and change in chemical and physical characteristics (e.g. nutrient depletion over time). The flow-through method is similar to the static method, but

with the addition of a recirculating pump. The flow of water allows for a current to pass across the core surface, to a degree mimicking a similar process that occurs in natural lake systems. This type of incubation allows the core to equilibrate to a more consistent level of reactants in the surface water, ideally similar to *in situ* conditions, thus providing a more accurate estimate of benthic processes.

The primary advantages of core incubations over benthic chambers are the ability to better control conditions in the laboratory, and the greater ease in operating in a flow-through mode. As well, the number of replicate cores collected is often larger than the number of benthic chambers that can be installed, maintained, and sampled *in situ*. Commonly benthic chamber installation and sampling will require SCUBA and additional boat time relative to the collection of sediment cores. The primary disadvantage to sediment core incubations relative to benthic chambers is cores may be altered structurally and chemically, as the simple act of removing it causes some disruption of redox gradients (of the core) and modification to the biogeochemical rates of the sample (i.e. the metabolic rates of microbial community). To reduce the impact on the sample, minimal time between removing the core and placing it into an incubator must be observed.

1.8.2 Stable Isotopes in Nitrogen Biogeochemical Studies

Stable isotopes are sometimes used as tracers in biogeochemical studies because of the relative ease of use and the ability to automate analysis of the samples. These techniques, more importantly, have the ability to precisely and unambiguously track the flow of tracer material (^{15}N in this case) from one pool to another pool and through microbially-mediated transformations (e.g. nitrate to ammonium). Stable isotope ratios of elements such as carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), sulphur ($^{34}S/^{32}S$), hydrogen ($^{2}H/^{1}H$), and oxygen ($^{18}O/^{16}O$) can be used to extrapolate information about energy flow, trophodynamics, migration patterns, and

metabolic processes [Jardine and Cunjak, 2005]. ^{15}N is a rare, yet stable isotope of nitrogen, and it is now being used more widely in research on nitrogen cycling in organisms and ecosystems [Kana et al., 1994; Robinson, 2001; Eyre et al., 2002]. Isotopic pairing techniques have been used in measurements of denitrification in aquatic sediments in the laboratory [e.g. Nielsen 1992, Nielsen et al., 1995] and more recently *in situ* [Böhlke et al., 2004, Hamilton, et al., 2007]. Isotope dilution techniques have been used to measure nitrification, as new nitrate will dilute an isotopically enriched pool of nitrate independently of the consumption of this nitrate pool [Rysgaard et al., 1993].

1.8.3 N_2:Ar Measurements in Nitrogen Biogeochemical Studies

N_2 production is difficult to quantify due to the normally high background concentrations in the environment [Groffman et al., 2006]. However, accurate measurement of small changes in dissolved N_2 concentrations can be measured with a high level of precision using membrane inlet mass spectrometry (MIMS) [Kana et al 1994]. This technique has become popular in the study of denitrification because of the ability to process samples quickly and minimize pre-processing without compromising precision [Groffman et al., 2006; Kana et al., 1994]. MIMS involves a high precision quadrupole mass spectrometer that is optimized for dissolved gasses and a capillary bore silicone tube (to allow for gas to diffuse through into the mass spectrometer) under vacuum conditions [Kana et al., 1994]. Lloyd et al. [2002] described a similar MIMS system, with one major difference. The system originally described by Lloyd et al. [1985] used a silicone rubber interface at the end of stainless-steel capillary tube as the inlet in a closed ion source pump system [Lloyd et al., 2002]. This setup leads to some problems as the interface creates a sort of void of gasses around the tip as the sample is pulled through the capillary tube and into the mass spectrometer. This is because as the MIMS samples it strips the gases around the tip faster than the gases can diffuse back. Stirring the capillary tube in the sample vessel can reduce

the void problem, but this stirring action may change the concentration of gases that is being sampled at the tip by entraining atmospheric gases. The rapid stripping at the tip of this MIMS system can lead to lower signal strength over time, because as gases are removed less of the analyte is seen by the mass spectrometer. Thus, a downward curve will be seen during analysis and may actually underestimate the level of gas in the sample. The MIMS system described by Kana et al. [1994] on the other hand is much more applicable for the purpose of measuring dissolved N_2:Ar in water samples as the sample is drawn into the capillary bore silicone tube and sample is constantly replenished across the diffusion membrane and will provide a much stronger signal strength over a given period. The Lloyd et al. [2002] design is much better suited for samples over a range or profile (e.g. temperature) where signal strength is less important. Furthermore, the tip configuration Lloyd et al. [2002] described can be attached on the end of their apparatus and be used in different situations (e.g. for soil samples or sediment pore water profiles). MIMS has been used to measure net denitrification based on changes in dissolved N_2:Ar concentrations in sediment core incubations [Kana et al., 1994, 1998; Cornwell et al., 1999] in benthic chamber incubations [Laursen and Seitzinger, 2002b] and in open systems [Laursen and Seitzinger, 2002a, 2004]. MIMS and stable isotope techniques (i.e. isotope pairing) can be combined to permit greater detail in denitrification measurements such as source of nitrate [e.g. Davidson, 1991, Eyre et al., 2002].

The stable nitrogen isotope microdiffusion technique is used to study nitrification and coupled denitrification occurring within the sediment-water layer [Eyre et al., 2002]. The ^{14}N:^{15}N ratios in samples can be determined using this isotope microdiffusion technique. The theory behind the technique is through the addition of magnesium oxide the sample becomes alkaline. Ammonium, NH_4^+ (mineral form) is converted to ammonia, NH_3 gas. This gas is collected and

bound to an acidified GF/F filter wrapped in gas permeable Teflon tape. Ammonia gas can diffuse through the tape onto the GF/F disc and be trapped as ammonium. Once this gas is trapped, an elemental analyzer with mass spectrometer can be used to provide isotope mass ratios of ^{14}N:^{15}N [Eyre et al., 2002]. Nitrate can also be done with this method with the addition of an electron donor compound (Devarda's alloy), which reduces NO_3^- to NH_4^+.

From the analysis, the ratio of the different isotopic nitrogen species aids in the calculation of the rate of nitrification, and the dilution of $^{15}NH_4^+$ allows for the determination of the rate of nitrogen mineralization [Davidson, 1991]. By combining the data from both the MIMS technique and the microdiffusion technique, the total rates of the individual nitrogen cycling processes may be determined simultaneously.

1.8.4 Chamber Construction

The final design of the benthic chamber system was intended to include the best elements of previous systems including the advantage of flow-through systems (e.g. maintaining constant chemical environment, the advantage of recirculating chambers (i.e. creating well mixed water within chamber to prevent diffusional gradients and to create movement of water over the sediment surface), and the advantage of *in situ* static chambers (i.e. maintaining in situ conditions. Water, while mixed, is not recirculated and should not heat over time). Chamber design was compatible with collection of dissolved gas samples and with introduction of a distinct isotopic tracer to each of the three chambers to permit a combination of measurements based on N_2:Ar, isotope pairing, and isotope dilution. Through the assembly of a three chamber pump system to measure the different processes simultaneously and explicitly, combining the advantages of chambers and flow-through core incubations and making use of ^{15}N-tracer techniques, it was expected that an approach with minimal limitations and restrictions could be

developed. This said, it is worth noting that the design has some of the disadvantages associated with other chamber systems, particularly a relatively small surface area enclosed making extrapolation to a whole system a questionable enterprise, and potential for over-pressuring during chamber installation. This is alleviated to a degree by having a two part-chamber where the core can be installed into the sediment and then capped by the stirring unit. Further, open ports allow pressure to equilibrate during chamber installation. The chamber system also encloses sediment and a parcel of water approximately 3L in volume. Measurements do not explicitly distinguish between benthic transformations of N and water column transformations. This could, however, be corrected by including incubations of bottom water in BOD bottles to determine rates of transformations in the water, or by sampling from the inflow bag over time.

1.9 Effects of Elemental Loading Ratios and Feeding Modes on Nitrogen Cycling in Lake Mesocosms

Previous studies have clearly demonstrated that denitrification rates in aquatic systems are increased as a function of total nitrogen loading [e.g. Seitzinger and Nixon 1985; Seitzinger, 1988, Boyer et al., 2006] while nitrogen retention is negatively related to total nitrogen loading [e.g. Böhlke et al., 2009]. These studies demonstrate that biogeochemical (e.g. N load) drivers can be important in determining N loss from a system by denitrification. What is less clear is how different biogeochemical drivers (P and Si) may interact to influence N loss from aquatic systems through their effects on microphyte community structure. Previous work has also demonstrated that filter-feeding bivalves can increase nitrogen removal from aquatic systems [e.g. Bruesewitz et al., 2006; Bykova et al., 2006]. These studies demonstrate that the introduction of an animal with a particular feeding mode (here benthic filter-feeders) can affect N-cycling and N loss through denitrification. However, it is not clear how different feeding modes compare in their effects on nitrogen cycling. Planktonic herbivores, for example, may

increase the rate of N recycling in the water column, reducing the total deposition of particulate matter (including N) to sediments, whereas benthic filter feeders may increase the movement of particulate matter from the water column to the benthos, enhancing anaerobic processing of nitrogen.

In this study, the effects of elemental loading ratios on N processing were tested. It was predicted that under conditions where N-loading was held constant, high silica loading conditions (0.5N:1Si), would increase the relative abundance of diatoms, increasing the transport of organic material from the epilimnion to the hypolimnion as diatoms are relatively dense given their silica frustules. It was also expected that under high phosphorus loading, nitrogen would become the limiting nutrient, promoting the growth of cyanobacteria that can fix nitrogen. As cyanobacteria are generally less dense than green algae and diatoms, this should reduce organic matter deposition, recycling in sediments, and loss of N through anaerobic processing (denitrification).

This study also compared the effects of two different feeding modes on N-processing. It was expected that grazing by *Daphnia* in a thermally stratified system would promote recycling and retention of nutrients in the epilimnion, limiting deposition of organic matter to sediments, anaerobic processing of N in the sediments and N-loss through denitrification. Filter-feeding mussels, in contrast, were expected to increase deposition of organic matter to the sediment surface and increase loss through denitrification.

1.9.1 Primary Producers

One of the major consequences of eutrophication is overstimulation of phytoplankton in the water. At high population densities these organisms can negatively influence water quality in a lake (through the production of offensive odours or toxins and increased turbidity) [Rinehart et

al., 1994; Hamill, 2001; Bruesewitz et al., 2006]. Phytoplankton blooms of algae, cyanobacteria and diatoms are the results of this nutrient overloading. Under limited nutrient conditions algae are able to outcompete other microorganisms (in terms of abundance) through utilizing nutrients more efficiently [Anderson et al., 2008].

Cyanobacteria are photosynthetic prokaryotic organisms which are found naturally in many freshwater and marine ecosystems. These prokaryotes are unicellular, but may group together to form dense mats which are buoyant and appear on the surface water or within the water column. Public health concerns over cyanobacterial blooms are associated with their ability to negatively affect water quality by giving it a bad taste and odour. In addition, some species are capable of producing cyanotoxins, which can be dangerous to human health, as well as aquatic life [Humpage et al., 1993; Hamill, 2001]. A metabolic capability that some species of cyanobacteria possess is N_2-fixation, which allows the organism to convert N_2 gas to a more bioavailable form, ammonium. Lakes that have a low N:P ratio are usually dominated by N-fixing cyanobacteria species [Rydin et al., 2002].

Diatoms are unicellular organisms and are a major group of algae. A major difference of diatom compare to other species of algae is that they are able to utilize silica as the material for their cell walls (frustules) [Smith et al., 1999].

1.9.2 Effect of Nutrient Limitations and Ratios

A nutrient is considered to be limiting when it is in short supply relative to other essential nutrients based on need. This concept of nutrient limitation in aquatic ecosystems is based on the notion that some essential nutrients are relatively less available; the concentration of these nutrients will eventually inhibit the growth and abundance of photosynthetic organisms. By restricting the loading rates of these essential nutrients, the rates of phytoplankton growth in

aquatic systems can be controlled [Smith et al., 1999]. A number of studies have confirmed that the most important nutrients to control the abundance and species composition of phytoplankton in aquatic ecosystems are nitrogen and phosphorus [Vitousek et al., 1997; Smith et al., 1999; Kemp et al., 2002].

The relative proportion of limiting nutrients in a lentic ecosystem can play a major role in controlling community composition and the overall levels of phytoplankton production. The most commonly accepted nutrient ratio for optimal phytoplankton growth conditions is the Redfield ratio. The Redfield ratio is given as carbon: nitrogen: phosphorus which was found to be conserved across marine phytoplankton at a molar ratio of 106:16:1 [Redfield, 1934, 1958]. The ratio, however, may not always be true for phytoplankton production in freshwater lakes [Elser, 1999] which have greater plasticity in biomass ratios of C:N:P.

Current paradigms of nutrient limitation in aquatic systems are that phosphorus is the limiting nutrient in most freshwater systems [e.g. Schindler 2006, Reynolds and Davies, 2001, Liu et al. 2010] and nitrogen is the limiting nutrient in many marine ecosystems [Ryther and Dunstan 1971]. However, temporal or spatial patterns of N limitation in freshwater systems are not uncommon [e.g. Elser, 1999, McCarthy et al., 2007a,b, Scott et al., 2008]. Suggestions of Schindler et al. [2008] notwithstanding, a reduction in both nutrients may be necessary in order to decrease eutrophication rates in freshwater systems. Further, if excess N-inputs are not controlled, they may eventually migrate into the coastal and marine ecosystems, where nitrogen can stimulate an overgrowth of primary producers in these areas. This is particularly evident in coastal water in the United States, where eutrophication of the region is attributed to an increase in river nitrogen exports to coastal systems [Anderson et al., 2008].

Silica is not commonly a limiting nutrient, and is commonly overlooked in primary production, although an increase in silica loading relative to other nutrients can give a competitive advantage to diatoms, increasing their relative abundance in microphyte communities, at least in marine systems [Officer and Ryther, 1980; Conley et al., 1993]. Similarly, increased phosphorus loading relative to nitrogen can give a competitive advantage to cyanobacteria [Bulgakov and Levich, 1999; Conroy et al., 2005], particularly when the N:P ratio is less than the Redfield ratio of 15:1, as many cyanobacteria are capable of overcoming nitrogen limitation by biological fixation of N_2 (again, recognizing there is some plasticity in the N:P ratio of freshwater microphyte biomass and a N:P <15:1 is not an absolute indicator of nitrogen limitation). This indicates that nutrient loading rates and ratios both can be important to ecosystem function. Loading rates (particularly of the limiting nutrient), can regulate biomass production, while loading ratios can affect the community composition concurrently with other environmental factors such as pH, light, temperature, and grazing [e.g. Søndergaard et al., 1990; Muylaert et al., 2005].

Shifts in community composition will impact sediment denitrification through the redistribution of organic carbon in aquatic systems. For example, systems with nutrient loading ratios that favour diatoms (i.e. high silica loading), may enhance primary production on sediments, thus driving the rates of benthic metabolism higher. This could increase anaerobic processing of organic matter in sediments, such as denitrification, and increase in N_2O and N_2 production as a consequence. Furthermore, microphyte groups differ in buoyancy; green algae, as an example, use starch as their major storage product [Wehr and Sheath, 2003], which increases its density and results in sinking. Dense silica frustules are effective sinking structures [Walsby and Reynolds, 1980] for diatoms, allowing them to sink rapidly as a survival strategy as

nutrients become limiting [Conley et al., 1993]. Cyanobacteria frequently contain gas vacuoles and have the ability to regulate their buoyancy [Walsby and Reynolds, 1980], and remain in the water column rather than sinking to the sediments. Similarly, to what was seen in Walsby and Reynolds [1980], nutrient loading ratios that favour cyanobacteria (i.e. low N:P, low Si:N) should favour the recycling of organic matter (associated with cyanobacteria) in the water column. Loading ratios that favour diatoms or green algae should favour sinking of organic matter to sediments, stimulating benthic processes such as denitrification, and enhance nitrous oxide and nitrogen gas production.

1.9.3 Herbivore Feeding Modes

Many studies have shown that phytoplankton populations are limited by grazing rates of zooplankton within the water column. Daphnia, for example, feed on the phytoplankton and can create periods of clear water [Huber et al, 2008]. The feeding of these animals has the greatest effect on the planktonic community during the two periods of stable thermocline (winter and summer), where nutrient limitation of algal growth also becomes a factor [Lampert et al., 1986; Huber et al., 2008], and the photosynthetic species are not able to overcome the feeding stress, thus resulting in a collapse in their populations. These grazing species are important in maintaining ecological stability, and in the prevention of algal blooms, brought on by overstimulation by nutrients. While some nutrients are lost from the photic zone during stratification due to sinking of particulate matter, the action of zooplankton, feeding and excreting nutrients in the epilimnion, helps retain nutrients in this zone.

filter feeders, such as mussels, are biologically significant because they allow for the transport of nutrients from the water column to the benthos enhancing microbial metabolism in deeper water. Effectively, these organisms may contribute to nutrient stress and limitation in the

epilimnion by stripping nutrients from the upper part of the water column. Such activity will enhance deposition of organic matter and release of ammonium (through excretion and remineralization of organic N) enhancing nitrification. This in turns increases denitrification rates and aids in excess N-removal [Bruesewitz et al., 2006] through the loss by N_2 production. This drawing of organic matter and nutrients to the sediment surface could change the benthic structure and the nutrient concentrations in the overlaying water. An increase in organic matter also would result in an increase in decay of organic matter, which in turns reduces dissolved oxygen and creates potential anoxic zones around the filter feeders [Caraco et al., 2000; Bruesewitz et al., 2006] which may permit denitrification to extend into the water column.

The difference between these two feeding modes in how and where organic nitrogen is recycled may have important implications for nitrogen loss from an aquatic system. A lake ecosystem with a large population of benthic filter feeders may have greater N-retention than a lake where herbivory is dominated by zooplankton.

2.0 Methods and Materials

2.1 Benthic chamber system for explicit and simultaneous measurement of major nitrogen cycling processes

2.1.1 Construction of Chamber System

A conceptual design was conceived based on apparatuses described by Vogel and LaBarbera [1978], and Dodds and Brock [1998] (figure 1). A consideration in design enclosing a volume adequate for sampling but small enough that low rates of nitrogen biogeochemical processes could have measureable effect on concentrations. The chambers (three in total), each consisted of two halves. The bottom half was constructed from acrylic plastic with a height of 53cm and an inner diameter of 15.25cm. Final volume in the chamber when inserted into sediment was approximately 2.5 L with a sediment surface area of 0.019 m^2. Acrylic was chosen for the sampling chambers because it allowed light penetration and was fairly inexpensive, chemically inert, and easy to fabricate. These cylinders had fittings for attachment of inlet (from the pump cylinder) and outlet sampling bags. The inlet and outlet ports within the chamber had tubing connections that allowed water to enter the chamber near the sediment surface and leave the chamber near the top, helping to ensure water mixed within the chamber rather than passing through directly from inlet to outlet. Through pressure from the inlet flow, water was displaced through the outlet into a collection bag (figure 2). The top portion of the sampling chamber housed an electric gear motor (20mA) powered by a 12V gel pack battery (7.2Ah) battery that facilitated the stirring of the enclosed volume in the lower half of the sampling chamber. A drive shaft connected to the gear motor passed through a water-tight bearing into the lower portion of

Figure 1. A sampling chamber fully sealed. Top compartment contains 9V battery to power stirrer motor (underneath battery). Bottom compartment is where water samples will be taken.

the chamber driving an aluminum fan blade. This top portion was constructed of a steel cylinder with a flange on top, sealed with a gasket compressed between the flange and a 6.4 mm steel top plate.

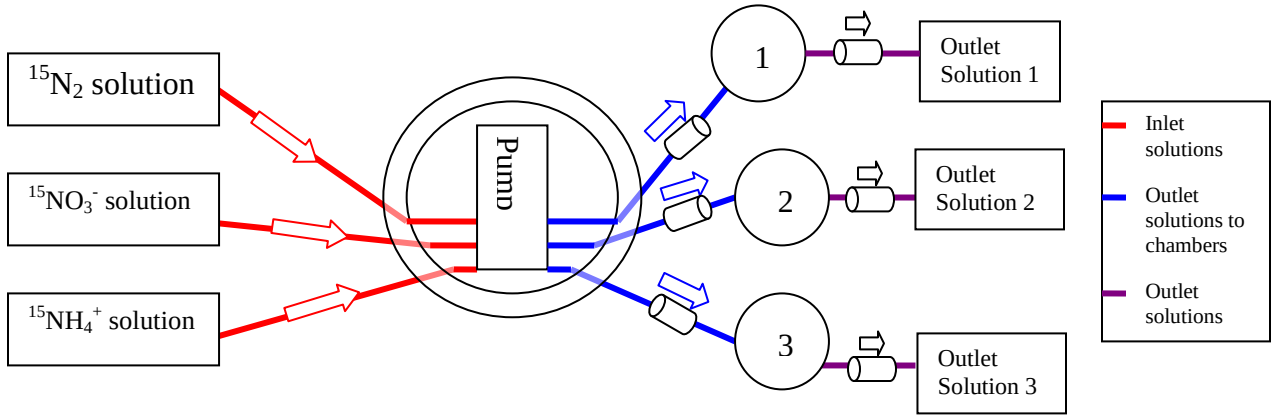


Figure 2. Schematic diagram of the Chamber Experiment Apparatus

Figure 3.Steel cylinder that houses peristaltic pump. 3 Inlet valves (for spike solutions) are on the right side of the cylinder and 3 outlets to the sampling chamber are on the left side.

The housing unit was constructed from a steel cylinder with 6. 4mm wall thickness, 48cm height and internal diameter of 50cm. This cylinder housed a peristaltic pump, which delivered the ^{15}N labelled solutions from inlet bags into the sampling chambers (figure 3).

2.1.2 Artificial Lake and Sediment

An artificial lake was constructed to mimic a shallow lake or pond system, and to provide a location to test the benthic chamber system (figure 4). It was constructed as a 120 x 120 cm frame with a depth of 75 cm in Southern Ontario. A pool liner was stapled to the interior to contain the artificial lake sediment and overlaying water column.

Figure 4. Diagram of artificial lake system

Artificial sediment was made up in accordance with the OECD Guideline [OECD, 1984] with a change in peat moss concentration from 10% to 2% dry weight in order to correspond to the low to moderate values of organic matter found in natural sediments [Suedel and Rodgers, 1993]. Additionally, the cellulose source was changed from Urtica powder to finely ground and dried leaves of sugar maple (*Acer saccharum*). See Table 20 (Appendix B) for detailed composition and mixing procedure. Artificial sediment in this pond experiment was mixed with a slurry of 10L of sediment from a local pond to help inoculate the artificial lake system (approximate a more natural microbial community). Sediment was added to the pond to a depth of 20 cm. Five microphytic organisms, *Oscillatoria*, *Nostoc*, *Synedra*, *Lyngbya sp*. and *Navicula* (Ward's Scientific), as well as *Daphnia magna* were added to further establish a functional community of primary producers and primary grazer. Additional grazers, notably ostracods and various insect larvae were observed during the experiment, likely originating from the pond sediment slurry inoculum or from mature insects laying eggs in the pond. The intention in construction of this pond system was not to replicate any particular lake ecosystem or to establish any specific community composition, but to establish a test location that would have some of the functional ecology of a shallow, temperate, freshwater system.

2.1.3 Seasonal Nutrient Loading

Loading was based on seasonal variation. The assumption made was that N:P tends to vary seasonally with high ratios in Spring, indicative of relative P limitation with N:P decreasing through summer and into Fall, indicative of shifts toward relative N limitation. Absolute loading rates to temperate systems also tend to decrease from Spring through to Autumn. Based on this, loading ratios of 20:1 in Spring decreasing to 10:1 in Summer and Autumn were employed. Total nitrogen and phosphorus load also decreased from Spring to Summer to Autumn, with no loading in Winter during ice cover (Table 1). Again, this was not intended to represent any particular system, but to provide a testing site for the chamber system in a eutrophic pond with seasonally-variable nutrient loading and a functional ecology.

Table 1. Nutrient loading ratio for artificial pond

Season	**Nutrient Ratio (N:P)**	**Nitrogen (mmol N m^{-2} day^{-1})**	**Phosphorus (mmol P m^{-2} day^{-1})**
Fall (11/2010)	5:1	2	0.4
Winter (04/2011)	None[1]	0	0
Spring (05/2011)	20:1	10	0.5
Summer (07/2011)	10:1	2	0.2
Fall (09/2011)	10:1	1	0.1

[1]No loading was performed during winter period because the artificial pond was frozen.

2.1.4 Sampling of Chamber System

Five sampling periods (November 2010, April 2011, May 2011, July 2011, and September 2011) were included for the experiment. There was a sampling for each season starting with Fall 2010 and ending the following Fall season (in 2011). During these sampling points the sampling chambers were lowered into the artificial lake and samples were collected over a 24 hour period.

Due to technical difficulties, there was no $^{15}N_2$ chamber for the Fall 2010 sampling. The other two inlet bags were spiked with 25mL of $^{15}NO_3^-$ (100.1mg/L) or 12mL of $^{15}NH_4^+$ (99.9mg/L). Winter and spring inlet bags were spiked in the same manner, but with different quantities of tracer. Each of the three inlet bags (for both seasons) were spiked with 7mL $^{15}NO_3^-$ (100.1mg/L), 1.4mL $^{15}NH_4^+$ (99.9mg/L), or 100mL $^{15}N_2$ gas (99 atom %). During The summer sampling point the isotope spikes were 2.5mL $^{15}NO_3^-$ (100.1mg/L), 1mL $^{15}NH_4^+$ (99.9mg/L) or 25mL $^{15}N_2$ gas (99% atom%). In each sampling period, the tracer spike was intended to give the ammonium or nitrate pool a ~5 atom% ^{15}N signature based on on-site colorimetric measurements using aquarium test kits. The intention was to label the pools without significantly altering concentrations as this could affect substrate-limited nitrogen cycling processes.

The enriched sample bags were attached (via tygon tubing) to the pumping unit and water was pumped through a stainless steel tube (40 cm x 0.2 cm), into the sampling chamber, out through a second stainless steel tube and into a collection bag. The stainless steel tubes were gas sampling devices and had gas tight valves on each end that can be sealed during sample retrieval. These sampling devices were used for analysis of dissolved N_2 species (m/z 28, 29, and 30) and Argon with the MIMS system. A small volume (50µl) of saturated $HgCl_2$ solution was pipetted into the metal columns to halt metabolic activity and preserve dissolved gas samples.

A mechanical timer was used to control the peristaltic pump (pump rate of 10mL/min) which was activated for 30 minutes of every two hours over the 24 hour run time. The stirring apparatus within each sampling chamber operated continuously for the duration of the sampling period.

2.2 Elemental Loading Ratios and Feeding Modes on Nitrogen Cycling in Lake Mesocosms

2.2.1 Construction of Mesocosms (Columns)

Thirty-five columns of 150x7.75cm (height x diameter) were constructed from acrylic plastic with a rubber cap on one end. Artificial sediment (as above) was added to a depth of 5cm to each of the 35 columns with approximately 6L of dechlorinated tap water overlying the sediment (figure 5).

Figure 5. Acrylic columns containing mesocosms

Different elemental loading ratios were applied to 20 of the 35 columns. In these columns, nitrogen loading was held constant at 3µmol N/m^2/d (equal parts ammonium and nitrate), while silica and phosphorus loading were varied. The other 15 columns were treated with three variations of feeding mode structure (*Daphnia magna*, Mussels – *Ellipitio complanata*, or both), but the sample nutrient loading regime. Five replicates of each treatment were employed (Table 2).

Table 2. Nutrient Ratios for Each Set of Replicates

Treatments	**N:P ratio**	**N:Si ratio**
High N:P, Low N:Si	5:1	2:1
High N:P, High N:Si	5:1	0.5:1
Low N:P, Low N:Si	15:1	2:1
Low N:P, High N:Si	15:1	0.5:1
Daphnia	15:1	2:1
Mussels	15:1	2:1
Both Feeders	15:1	2:1

Nutrient loading began once the columns had been constructed and filled. To inoculate the columns, green algae, diatoms and cyanobacteria were added to each column (as a slurry). The

algae were: *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*, the diatoms were *Navicula pelliculosa*, *Cyclotella meneghiniaua* and *Achnanthes brevipes*, and the cyanobacteria species were *Nostoc sp.*, *Oscillatoria sp.*, *Anabaena sp.*, *Lyngbya sp.* and *Microcystis aeruginosa*. Once the planktonic communities were established, Daphnia and mussels were added to the appropriate columns. The columns were allowed to progress for a week before the first sampling point. It is important to note that the species were chosen to include redundant representation of each of the major taxonomic groups (green algae, cyanobacteria, diatoms). The redundancy was to increase the chances that each group would remain represented in the community should one species collapse. The intention was not to establish a specific type of microphyte community representative of a Southern Ontario lake at any given point in time, but to establish a general community with diversity and representation by each of the three major groups most common in Southern Ontario lakes.

A light bank was setup above the mesocosms with full spectrum fluorescent lighting (T8 VitaLux bulbs, MT-DTC, USA). The light intensity at the water surface of the mesocosms was approximately 18,000lux. The lights were setup on a timer (Intermatic, Mexico), and provided 14 hour light: 10 hour dark cycles.

Three 90L, 30cm tall storage bins (J. Terence Thompson, LLC, US) were set up underneath the columns with cold water circulating among them. The bottom 25 cm of each column was immersed in the cold water bath. Cold water was provided from a freezer with a diaphragm water pump attached to the tubing, pumping water into and from the plastic bins. This created a temperature gradient within the water columns, which was measured daily using an electronic thermometer (HANNA Instruments, Singapore). This allowed for the simulation of the hypolimnion and epilimnion layers of a stratified lake. The lower portion (70cm) of each column

was also covered with black plastic wrapping, in order to create a light gradient separating the photic and aphotic zones. It is recognized that the mesocosms represent a vertically compressed system relative to stratified lakes, with shorter diffusional distances between the epilimnion and the hypolimnion. The mesocosm, then, represents a model for how processes may differ qualitatively in hypolimnia and epilimnia as a function of nutrient loading ratio and feeding mode, but should not be taken as a model for quantitative differences to be expected in stratified lakes.

2.2.2 Sampling

Samples were taken biweekly after day 0 over a 10 week period, starting from December 15, 2010 and ending February 4, 2011. Samples were collected for nutrient concentrations, dissolved organic carbon, and dissolved gases (N_2, O_2, and N_2O).

2.3 Chemical Analysis

Ammonium, nitrate-nitrite, Kjeldahl nitrogen and Membrane Inlet Mass Spectrometry analysis was performed for both experiments. Additionally, microdiffusion was performed for the samples obtained during the chamber experiment, and phosphorus, dissolved organic carbon, pH and nitrous oxide analysis was performed for the mesocosm experiment.

2.3.1 Ammonium Analysis

Ammonium analysis was performed by a modified version of the phenate method [Clesceri et al., 1999; 4500-NH_3 F]. Test tubes were filled with 5ml of each collected sample. To each test tube, 200µl of phenol reagent, 200µl of sodium nitroprusside and 400µl of oxidizing solution were added. Test tube samples were allowed to develop in the dark for 30 minutes. Standards of 0, 10, 25, and 50µM were made from an ammonium chloride (NH_4Cl) standard solution, where the blank (0µM) was made with deionized water. Absorbance of the samples was read on a spectrophotometer (Perkin Elmer UV/Vis Spectrometer, Lambda 20) at 640nm.

2.3.2 Kjeldahl Nitrogen Analysis

A Kjeldahl digestion was carried out in 100ml Kjeldahl flasks, to which 10ml of sample and 5ml of digestion reagent consisting of potassium sulphate, cupric sulphate and sulphuric acid (appendix B) was added. The solution was heated using a semi-micro-Kjeldahl digestion apparatus (with heater element) on a medium-high setting until a white smoke was evolved, at which point the heater was switched to maximum setting. The sample was heated for an additional 30 minutes, then left to cool for 5 minutes, after which 10ml of water was added to dilute the sample residue. The sample was then added to the BUCHI distillation apparatus (BUCHI Labortechnik GmbH, Essen, Germany). Within the distillation apparatus sodium hydroxide (45% (w/w)) was added and the evolved ammonia gas was distilled out and collected into the receiving vessel containing 4% boric acid. The solution was transferred to test tubes, in preparation for ammonium analysis via phenate method.

For the chamber experiment, a second 10ml aliquot of sample was digested (for each sample inlet and outlet) in parallel to the sample from the above digestion to be used for with the microdiffusion technique (determination of isotopic signature in total Kjeldahl N pool). A 50ml volume of water was used for this purpose to dissolve the residue. The resulting solution was distilled similarly to the parallel sample (see above) in the BUSCHI apparatus. The pH of the distillate solutions were adjusted to approximately 9 with 1.8ml of 45% sodium hydroxide (NaOH). Once the NaOH had been added to the sample, regular microdiffusion technique was employed (see below - week #1 of microdiffusion technique).

2.3.3 Nitrate-Nitrite Analysis

Nitrate analysis was by the copperized cadmium reduction method [Clesceri et al., 1999; 4500-NO_3^- E] and consisted of combining 2.5ml of collected sample along with 7.5ml of nitrate buffer. Each sample was fed through a stainless steel column (6.4mm ID) packed with cadmium

shavings coated with copper. Samples were pumped through the column at a rate of 2ml/min for a total of 4 minutes. The first 4ml of sample was discarded (used to flush column of previous sample) and the second aliquot of sample was collected into a test tube. To each test tube 400µl of colour reagent was added (see Appendix B). The test tubes (colour) were allowed to develop for 30 minutes. Absorbance readings of the samples were performed at 543nm on a spectrophotometer (Perkin Elmer UV/Vis Spectrometer, Lambda 20).

2.3.4 Membrane Inlet Mass Spectrometer Analysis (MIMS)

Gas analysis was performed from samples collected in the metal tubes. A membrane inlet mass spectrometer was used for this purpose consisting of a quadrupole mass spectrometer with a Faraday detector. This apparatus was constructed by Dr. Todd Kana of Bay Instruments (Cambridge, Maryland), and based on the design previously described by Kana et al. [1994]. A peristaltic pump pushes a water sample through a stainless steel capillary that passes coaxially through a sealed and evacuated glass tube (the inlet to the mass spectrometer) (figure 6). Within the inlet, the water passes from the stainless steel capillary, into a silicon tube, then back into a second steel capillary and out of the inlet. As the water passes through the silicon tubing, gases are stripped from the water by high vacuum. From this point the gas sample was swept through a glass u-trap submerged in liquid nitrogen (to eliminate water vapour from gas samples), and into the ionizing source of the mass spectrometer. For the chamber samples, a quartz column packed with copper shavings heated to 550°C and a second u-trap (submerged in liquid nitrogen) was placed before the mass spectrometer to eliminate oxygen and any residual moisture/contaminants. Samples were analyzed using the system software designed by Bay Instruments (Cambridge, Maryland). Gas analysis was done on the samples collected in BOD bottles (for mesocosm experiment) and the sample tubes (for chamber experiment).

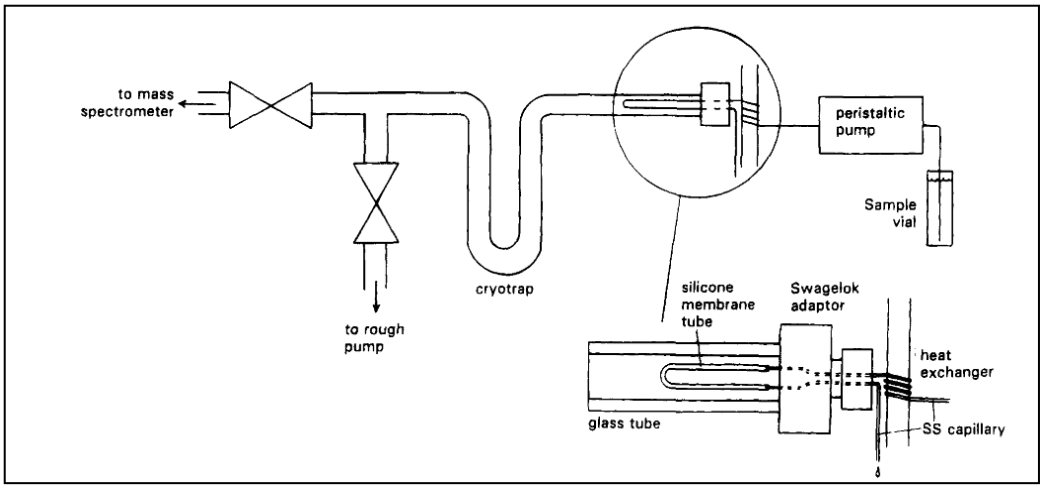


Figure 6. Schematic diagram of MIMS system [Kana et al., 1994]

2.3.5 *Microdiffusion*

The procedure was adapted from Colorado Plateau Stable Isotope (CPSIL) and modified by Monisa Nandi (former Masters student in Ryerson Environmental Applied Science and Management Program), with minor additional revisions to adapt the procedure to current experimental need. Diffusion packets were made by using a 6mm hole punch to create discs from GF/F filters previously ashed at 500°C for 4 hours in a piece of loosely packed aluminum foil. Once GF/F discs were removed from the oven, all surfaces and equipment (tweezers, scissors, glass vial caps, and glass diffusion packet container) was wiped down with ethanol before being used. A GF/F disc was placed in the middle of a 3-4cm piece of Teflon tape. The GF/F discs were acidified by pipetting 10µl of 2M sulphuric acid onto each disc. One end of the tape was folded over the filter disc and the edges were pressed together using the handle of a pair of forceps. Rubbing along the seams sealed the Teflon packet into a water tight pouch enclosing the filter disc.

Each sample for isotopic analysis of nitrate and ammonium was prepared by mixing equal amounts of sample with saturated NaCl solution. Sample volumes differed based on predicted

ammonium concentration. This was to ensure that a minimum requirement (20ng) of analyte for analysis would be present in the sample. The volume required was calculated based on ammonium and nitrate concentration analyses (described above). During the fall sampling period a sample of 25ml was used (performed in 60ml jars). For the winter and spring sampling a 150ml sample volume was utilized in 300ml BOD bottles. A diffusion packet was added to the sample vessel along with 0.9mg of MgO per 150ml (0.15mg for the fall samples) of sample to increase the alkalinity of the solution. The sample containers were incubated at 30°C for 7 days (week #1). After this period, the diffusion packets were removed and placed in a dissector to dry. A second diffusion packet was placed into the sample containers along with 225mg Devarda's alloy (37.5mg for fall samples). The Devarda's alloy reduced nitrate to ammonium which was then captured by microdiffusion. The containers were returned to the incubator for an additional 7 days, after which the packets were removed and placed in a desiccator for a week. Once all packets were dried, the GF/F filters were removed from the Teflon tape and each was placed inside a silver capsule. The silver capsule was compressed into a sphere and placed into a labelled well plate. These samples were shipped to University of California – Davis Stable Isotope Laboratory for analysis with an elemental analyzer coupled to mass spectrometer.

2.3.6 Dissolved Organic Carbon (DOC)

Dissolved organic carbon was determined using a Shimadzu TOC-V Series analyzer. The TOC (total organic carbon) analyzer works by using high temperature combustion (680°C), with a carrier gas being passed at a controlled rate of 150mL/min through an oxidation catalyst-filled TC combustion tube. Samples enter the combustion tube and the total carbon is oxidized to CO_2. The carrier gas transports the combustion products from the combustion tube, where it is cooled and dehumidified before passing to the halogen scrubber. From here, the sample is sent into the sample cell of the non-dispersive infrared detector (NDIR), which is the site of CO_2 detection.

The NDIR signal forms a peak, and the data from the peaks are compared to the calibration curve (which was created using standard solutions of total carbon).

Total carbon is made up of both organic carbon and inorganic carbon (i.e. carbonate and bicarbonate), the inorganic concentrations are determined by adding a small amount of hydrochloric acid to acidify the sample, which was then sparged with compressed air. This converts all inorganic carbon in the sample to carbon dioxide and drives the CO_2 out of the sample solution. By subtracting the concentration of inorganic carbon from the total carbon, the concentration of TOC was estimated.

Each filtered sample was measured twice with one water blank between the samples. The average concentration in the two measurements was recorded as the measured result.

2.3.7 Nitrous Oxide Analysis

A headspace was produced in the glass vial by the insertion of two 0.3mm syringe needles (BD, USA) and purging the liquid out of the bottle using a 10mL syringe (BD, USA) filled with N_2 gas. The gasses within the water column were allowed to equilibrate for 1 hour before carrying out the detection.

The concentrations of N_2O in gas headspaces were measured by gas chromatography coupled with an electron capture detector (GC-ECD) (HP 5890, PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA, USA) with GS-Carbon Plot column (30m, 0.32 mm diameter, 3.00 mm film thickness) (Agilent Technologies, Santa Clara, CA, USA). The ECD uses a beta emitter (^{63}Ni) in order to ionize the gasses and produce a current between a biased pair of electrodes. When molecules of N_2O gas pass by the detector, a reduction in the current between electrodes is measured, thus producing a signal.

The temperature settings were 375°C for the ECD, 35°C for the oven, and 185°C for the injector. Injections were performed using helium gas as a carrier with a split ratio of 0, at a flow rate of 30mL/min. The injection volume was 100μL, with a standard analyzed after every 10 samples.

2.3.8 Phosphorous Analysis

Phosphorus was analyzed using the ascorbic acid method [Clesceri et al., 1999; 4500-P]. Samples were filtered through a membrane filter (0.45μm pore diameter). 5mL of sample was placed into a clean, dry test tube, 1 drop (0.05mL) of phenolphthalein indicator was added. If a red colour developed, just enough drops of 2.5M H_2SO_4 were added to the test tube to remove the colour. To each sample, 0.8mL of combined reagent was added and mixed thoroughly. The samples were allowed to develop for at least 10min (but no more than 30min) before measurements of absorbance were taken of each sample at 880 nm, using a distilled water + reagent blank as the reference solution.

2.3.9 pH Determination

The pH was determined using an electronic pH meter (OAKTON, Singapore). The analysis was carried out by placing the probe in the sample collection tubes and waiting for 20 seconds for the signal to stabilize at which point the measurement was recorded.

2.3.10 Calculations and Statistical Analysis

For the chamber experiment, net denitrification was measured after Laursen and Seitzinger [2002b] based on the change in N_2:Ar, assuming Argon concentrations are constant and at equilibrium with respect to water temperature (based on Weiss [1980] paper). Change in concentration of N_2 was converted to a real rate based on water residence time in the chamber, volume of water in the chamber, and surface area of the sediment. Nitrification was calculated based on the dilution of the $^{15}NO_3^-$ pool after Rysgaard et al. [1993], again converting to a real

rate. Following the logic of the Rysgaard isotope dilution method for nitrification, gross denitrification was measured based on isotopic dilution of the $^{15}N_2$ pool. Nitrogen fixation rates were calculated as the difference between gross and net denitrification. Similarly, ammonification was calculated based on dilution of the $^{15}NH_4^+$ pool. Dissimilatory nitrate reduction to ammonium (DNRA) was calculated by tracing the movement of ^{15}N from the nitrate pool into the ammonium pool. Uptake of nitrate and ammonium was calculated based on movement of the ^{15}N label from the nitrate pool and the ammonium pool into the Kjeldahl nitrogen pool, correcting for the ^{15}N signature of the ammonium pool.

For the mesocosm experiment, statistical analysis was performed on the data collected using SAS 9.2. The data were analyzed using multi-way analysis of variance to determine if there is a significant effect due to treatment, stratum (epilimnion versus hypolimnion) and date. Post-hoc Tukey's tests were performed to compare differences amongst nutrient treatment types and whether the effect of the three different nutrient treatments is significantly different than the reference. A non-parametric (Kruskal-Wallis) approach (using SYSTAT 13) was used to analyse the results for the N_2O (in epilimnion) and N_2 (in hypolimnion) as dissolved gas data failed to conform to the assumptions required for parametric statistical tests (particularly equality of variance among groups). Similar to the statistical analysis used for the nutrient loading data, analysis was performed for the data obtained regarding the primary consumer experiment.

3.0 Results and Discussion

3.1 Benthic chamber system for explicit and simultaneous measurement of major nitrogen cycling processes

3.1.1 Seasonal Variation

The general trend of O_2 consumption rates was decreasing from the Fall, 2010 season to the rest of year and making an increase again the following Fall season (Table 3). The steep drop between the Fall (2010) and the winter was expected as the colder temperature would reduce metabolism. Since the winter and spring sampling periods were very close to each other, it was expected that the levels of primary production was very similar. The increase in O_2 concentrations during the summer period shows an increase in primary production as expected with increased sunlight and water temperature. In the Fall 2011, sampling the O_2 concentration began to decrease again as O_2 consumption was greater than production once again.

Table 3. Process rate measurements in benthic chambers

	Fall, 2010[1]	**Winter, 2011**	**Spring, 2011**	**Summer, 2011**	**Fall, 2011[2]**
Respiration (oxygen consumption)	4640 ± 4504	455 ± 4	376 ± 97	-52 ± 335	187 ± 1430
Gross denitrification	--	159	576	9758	12070 ± 1004
N_2 fixation	--	242	359	9560	11826 ± 420
Net N_2 production	344 ± 270	-83	216	198	248 ± 668
Nitrification	--	9.5	61	4.7	--
NH_4^+ uptake	--	4.1	19.1	nm	--
NO_3^- uptake	--	nm	nm	nm	--
Dissimilatory nitrate reduction to NH_4^+	--	nm	nm	10.0	--
N_2 fixation to NH_4^+ pool	--	0.6	nm	nm	--
N_2 fixation to organic N pool	--	--	2.3	nm	--
%N removal by denitrification	-	--	2.2	9.9	24.8
Mean water temperature (°C)	5.231	4.861	9.107	26.667	--

All rates as μmol (O_2 or N) m^{-2} h^{-1}, unless otherwise indicated.
Note: Values that were not measurable are indicated by "nm", "- -" indicates no result
[1]Due to shortage of $^{15}N_2$ gas, on nitrate and ammonium spiked sampling chambers were used.
[2]Microdiffusion data are still pending from University of California – Davis Laboratory.

Perhaps the most interesting result that was obtained was that the rate of denitrification (gross) was comparable to the rate of nitrogen fixation. This was interesting because it was believed that N-fixation would be non-existent (or very low) in the presence of high nitrogen loading (i.e. under eutrophic conditions). However, from the data, it appears to be occurring at the same magnitude as denitrification, even during the spring and summer with high N loading. This indicates that as denitrification removes N from the system, cyanobacteria are reintroducing it back into the system by fixation. This means that almost no nitrogen is lost from the system, and the system did not retain or remove a large fraction of total loading. Denitrification was the primary fate of nitrate as DNRA was much lower in magnitude (Table 3) and nitrate uptake (incorporation into biomass) was not measurable. Ammonium assimilation was measurable in winter and spring (despite net heterotrophy), although was not quantified in summer due to an unusable sample that prevented this calculation (total N on microdiffusion filter disc too low for analysis). The uptake results are interesting as they suggest that while plenty of N may have been available in the spring-fall, it may not have been in a usable form (nitrate), and the system may have been functionally N-limited, explaining N_2-fixation.

3.1.2 Denitrification

Denitrification rates in aquatic systems are highly variable. More oligotrophic systems (including lakes and coastal marine systems) tend to have rates lower than those measured here. Laursen and Seitzinger [2002b] found denitrification rates (in a continental shelf system) to vary from 78 to > 200 μmol N/m^2/h, while Seitzinger [1988] and van Luijn et al. [1996] reported denitrification rates of 50 to 150 μmol N/m^2/h for oligotrophic lakes, with rates typically lower in fall and winter. The denitrification rates calculated from the experiment were much higher, with Winter and Spring, 2011 rates more consistent with rates reported in mesotrophic to eutrophic lakes [Seitzinger 1988]. The summer and fall (2011) rates were very high, but not

beyond those reported from high N-loaded riverine systems [McCutchan et al., 2003; Laursen and Seitzinger, 2004; Yan et al., 2004]. It should be noted, however, that most of the rates found in literature are net denitrification rates, whereas those calculated in this experiment were gross denitrification rates. That is, the methods previously used to measure denitrification only dealt with net production, and did not account for N_2 consumption. The net N_2 production rates, are consistent with reported rates from other mesotrophic to eutrophic lakes, with rates varying from 216µmol N/m^2/h in the spring, 198µmol N/m^2/h in the summer and ranged between 248µmol N/m^2/h (Fall 2011) to 344µmol N/m^2/h (Fall 2010). Based on the loading into the pond, a net N removal from the system can be calculated. In the spring about 2.2% was removed, in the summer 9.9% was removed and for fall (2011) 24.8% of N was removed by denitrification. This indicates that a significant amount of nitrogen was removed throughout the seasons, especially in the fall.

3.1.3 Nitrification

Nitrification rates were seen to be comparable to those reported in lakes along the oligotrophic to eutrophic spectrum (24 to 70 µmol N/m^2/h) [Rysgaard et al., 1993, van Luijn et al., 1996].

3.1.4 Nitrogen-Fixation

Nitrogen fixation rates were on par to those found with denitrification (as mentioned previously), and as such were very high compared to those previously reported in literature. Presing et al. [2008] found winter and springs rates in eutrophic lakes to be 1.1-1.7µmol N/m^2/h and 4.0-4.7µmol N/m^2/h (respectively). Summer fixation rates were found to be as low as 8.5µmol N/m^2/h [Presing et al., 2008] and high as 14.7µmol N/m^2/h [Rydin et al., 2002] (in eutrophic lake systems). Gettel et al. [2007] found fixation rates to be as low as 1.0µmol N/m^2/h and up to 4.7µmol N/m^2/h in lakes of moderate to high productivity. These studies used

acetylene reduction method to carry out analysis, or net nitrogen-fixation rate (which may underestimate nitrogen fixation if denitrification is co-occurring). The high fixation rates obtained in the experiment may be attributed to the increase in cyanobacterial growth in the pond system fixing N_2 gas coming from the sediment (due to denitrification). From the results, it appears that nitrogen fixation and denitrification are highly co-ordinated, and both rates were prevalent in the system at a very high level. The high levels of both denitrification and nitrogen-fixation show that these processes can co-occur in the same (general area), and that nitrogen fixation does occur at a high rate even in the present of high nitrogen loading.

3.1.5 Importance of Work

This work is important as it developed the capability to explicitly measure each individual nitrogen cycling process simultaneously and *in situ*. This experiment has shown that nitrogen fixation can co-occur in a system with high rates of denitrification, and the rate of fixation is significant portion of the total percentage of nitrogen being transformed.

Furthermore, with the knowledge gained from the experiment (and experimental apparatus), a complete and accurate nitrogen budget can be determined in other systems to allow for better estimates of nitrogen removal. This leads to better modelling of systems, and control over the amount of loading a system can absorb or remove. Being able to create better predictive models, will provide a better understanding of the flow of nutrients from one process (or sink) to another, and allows for the evaluation of the amount of energy in a system and may indeed provide a better picture of micro-nutrient limitations in a system that normally may not be revealed or accounted for by other analyses.

While an effort was made to put the rates measured into context by comparison with literature, it should be restated here that the experimental pond was not intended as a perfect replica of a lake system. The experimental pond suffers the same limitations of ecological

relevance that any mesocosm suffers. However, the experimental pond provided a location for proof-of-concept for this chamber system and allowed an admittedly limited study of how nitrogen cycling rates may vary seasonally with temperature and nutrient loading.

3.2 Elemental Loading Ratios and Feeding Modes on Nitrogen Cycling in Lake Mesocosms

3.2.1 Mesocosm Function

After Day 28, the variation among mesocosms became large, with chemical analytes increasing in some columns and decreasing sharply in others (Appendix A – Tables 4-19). Specifically, sharp decreases in dissolved oxygen concentration and increases in ammonium and phosphate concentrations in some mesocosms suggest senescence of the phytoplankton communities and disruptions of ecosystem function. These deviations were seen by Jeppesen et al. [1990]. The paper described the rapid decline of the algae biomass between day 23 and 25 of the experiment, followed by a rapid recovery 3-4 days later - usually dominated by a different species of algae. Søndergaard et al. [1990] observed a similar pattern during the summer (early to mid-July), where rapid phytoplankton collapse occurred over an 8 day period in mid-July and recovery was seen after 3-5 days (near the end of July). In both studies, multiple algal collapses were observed over the summer months (3-4 per year) in eutrophic (to hypereutrophic) lakes. During these events nutrient concentrations and community composition before and after the event were significantly different indicating that disruption such as this will have substantial effect on the ecosystem.

Mesocosm divergence was indicated by Martinez-Martinez et al. [2006], who found that with the planktonic collapse and recovery occurring independently within the replicates (of mesocosms), within-group variance increased over the period of the experiment, accounting for as much as 91% of the variance in their experiment. In this study, although autotrophic activity

began to recover in some of these mesocosms between Days 42 and 56, the variation among replicates remained large after Day 28, precluding any ability to detect treatment effects beyond Day 28. Further analysis of mesocosms was, therefore, restricted to the first three time-points (Days 0, 14 and 28).

Concentrations of nutrients (DOC, NH_4, and PO_4) were generally higher in the dark, cold hypolimnia than in the warmer epilimnia. Dissolved oxygen was generally above saturation in the epilimnia, and below saturation in the hypolimnia. Further, pH was generally lower in the hypolimnia than in the epilimnia. These results suggest effective separation (i.e. limited mixing) between the two strata, and that organic matter produced by autotrophs in the epilimnia was sinking to the hypolimnia and being remineralized in the deeper layer. In short, the data support these mesocosms operating as expected in representing lake ecosystems, although again it must be acknowledged that any laboratory mesocosm will have limited ecological relevance. In this case, the vertical profile is compressed with little spatial separation between the epilimnion and hypolimnion.

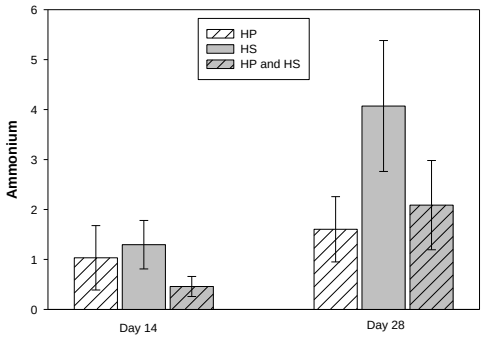
3.2.2 Elemental Loading Ratio

As described in the preceding section, the hypolimnia had greater concentrations of NH_4^+, PO_4^{3-}, and DOC, lower concentrations of dissolved oxygen, and lower pH than the epilimnia ($p = 0.001$, 0.015, 0.048, <0.001 and <0.001, respectively). Accumulation of inorganic nutrients and dissolved organic carbon, and decline of dissolved oxygen are believed to be due to mineralization of organic matter sinking to the sediments from the production zone (epilimnion).

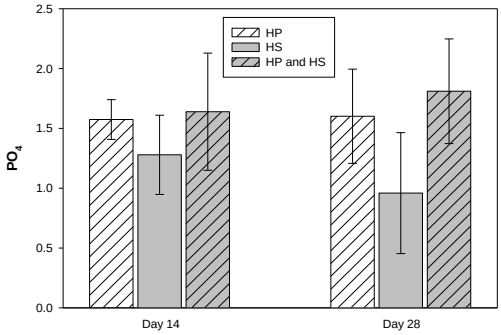
A working hypothesis in this research was that nutrient loading ratios that favoured dense species of phytoplankton (such as diatoms) would increase organic matter export to sediments, remineralization of this organic matter in sediments, and anaerobic processing of the nitrogen

remineralized. Therefore, it would be expected that a high Si:N loading ratio would result in greater export of organic matter (including organic N) from the epilimnion to sediments by sinking of diatoms, and that this should result in greater accumulation of inorganic nutrients (NH_4^+ and PO_4^{3-}) in the hypolimnion over time relative to mesocosms with low Si:N loading. Further, the mesocosms with high Si:N loading would be expected to have higher rates of denitrification fuelled by 1) greater inorganic N liberated by organic matter remineralization and 2) greater availability of organic C as electron donor. Similarly, a high P:N loading ratio was expected to favour cyanobacteria over green algae, resulting in a generally more buoyant microphyte community, less export of organic matter (and with it, organic N and P) to the sediments, lower rates of inorganic nutrient accumulation in hypolimnia, and lower rates of denitrification in sediments.

The expected pattern was observed for high Si:N loading, as increased Si loading did increase NH_4^+ and PO_4^{3-} concentrations in hypolimnia (Figure 7a, b) proportionally to the reference treatment, while decreasing dissolved oxygen concentrations and pH (Figure 7c, d) confirmed the increase in biomass and respiration. Also, as expected, increased Si loading did increase dissolved N_2 concentration by Day 28 ($p = 0.008$) (Figure 7e) and had a marginal effect on N_2O production in hypolimnia by Day 28 ($p = 0.094$). The exception to this trend was the Day 28 data for PO_4^{3-}, where the increased Si did not increase PO_4^{3-} concentration proportionally to reference.



7a)



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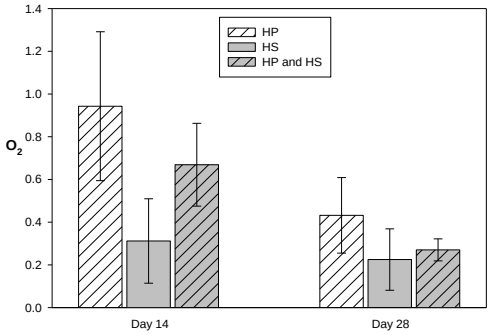

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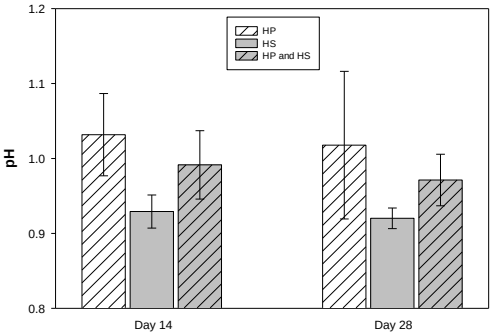
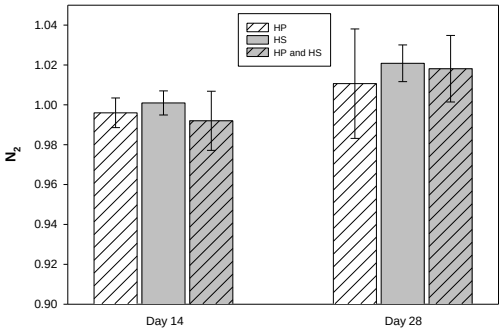


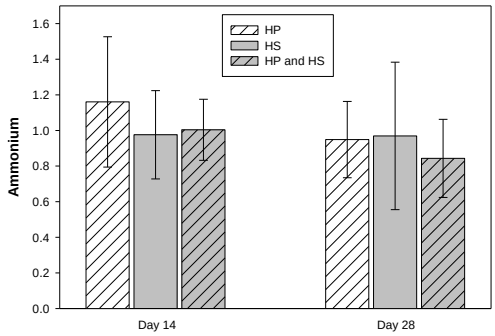
7d)



7e)

Figure 7. Plots of proportional concentration data for a) ammonium, b) phosphorus, c) oxygen, d) pH, and e) dissolved N_2 in hypolimnia for elemental loading ratio experiment.

Under high P:N loading conditions, the proportional data did not support the expected increase in nutrient concentrations (i.e. NH_4^+ and PO_4^{3-}) in the epilimnia (Figure 8), that would be evident as a result of the stimulation of cyanobacterial growth. Instead, high P loading did not show a significant increase in nutrient level relative to other treatments.



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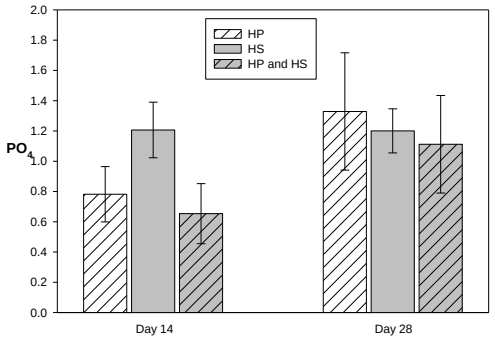

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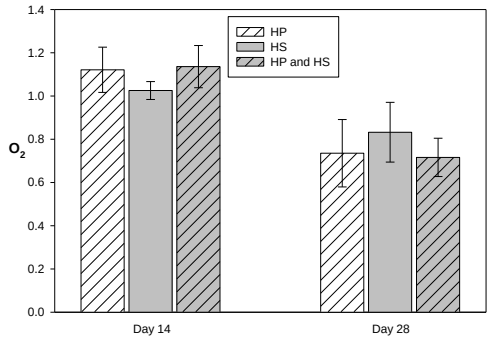

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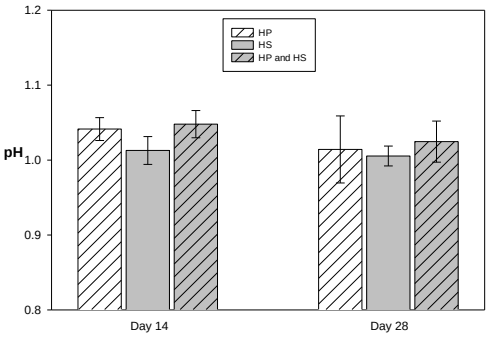

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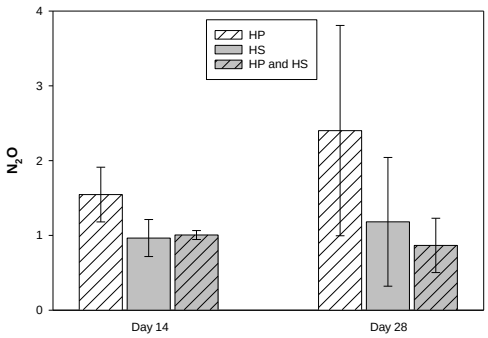


8e)

Figure 8. Plots of proportional concentration data for a) ammonium, b) phosphorus, c) oxygen, d) pH, and e) nitrous oxide in epilimnia for elemental loading ratio experiment.

Overall, the hypothesis of high Si:N loading increasing organic matter production in the hypolimnion was shown to be correct, where as P:N loading (in the epilimnia) did not conform to the expected results. These results may indicate that with the high P:N loading, not only was cyanobacterial growth stimulated, but overall growth of total phytoplankton was observed, fuelling greater delivery of organic matter to the sediments and higher total recycling of organic matter in the hypolimnion. This was partially supported with the results of nutrient analyses in the hypolimnion (Figure 7b-c) where there was a tendency toward increased phosphate, and decreased oxygen in high P:N mesocosms ($p = 0.134$, 0.102, respectively). The effects on total production were not explicitly tested. However, if greater overall production at high P:N is what occurred, then the results of high P:N loading experiments may reflect the effects of greater overall nutrient loading (with N subsidized by fixation) rather than the effects of elemental loading ratios, as intended.

It appears that benthic recycling of organic matter was stimulated by both increased Si:N and P:N, but with Si having relatively greater effect, and with each element stimulating benthic recycling for a different reason (increased Si leading to more dense organic matter, increased P leading to more total organic matter). Interestingly, mesocosms with high Si and P loading had a pattern of response more similar to high P:N than high Si:N, suggesting that the effect of P loading in some way offset the effects of Si loading. This would be consistent with high P loading causing more overall production, but a greater proportion of that production remaining in the water column and being recycled there.

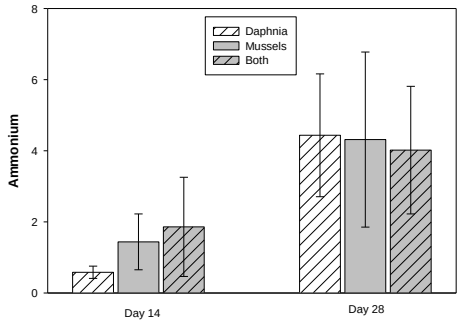
3.2.3 Feeding Modes Experiment

As described previously with the nutrient loading results, a greater concentrations of NH_4^+, PO_4^{3-}, NO_3^- and DOC, lower concentrations of dissolved oxygen, and lower pH than the

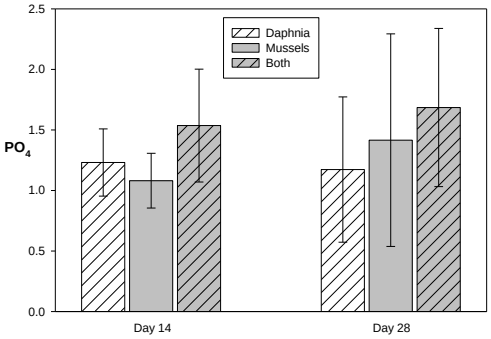
epilimnia ($p < 0.001$ for all measured results) was observed in the hypolimnion. Accumulation of inorganic nutrients and dissolved organic matter, and decline of dissolved oxygen are believed to be due to mineralization of organic matter being drawn to or sinking to the sediment (hypolimnion) from the epilimnion.

The hypothesis for the effect of primary consumers, such as filter feeders (mussels), would draw organic biomass towards their incurrent siphon, thus an increase in organic matter export to sediments, the remineralization of this organic matter in sediments, and anaerobic processing of the nitrogen similarly seen in the nutrient ratio experiment. Therefore, it would be expected that in the presence of mussels, a greater accumulation of inorganic nutrients in the hypolimnion would be observed. This increase would prompt higher rates of denitrification due to the increase in organic (decaying) matter, both as a source of N and as an electron donor. Conversely, primary consumers such as grazers (Daphnia) will tend to recycle nutrients higher in the water column where they feed, allowing for the reuptake by phytoplankton. This would prevent large amounts of nutrients (i.e. organic N and P) from sinking from the epilimnion into the hypolimnion. This would limit denitrification rates, accumulation of inorganic nutrients in the hypolimnion, and stimulate incorporation into planktonic biomass.

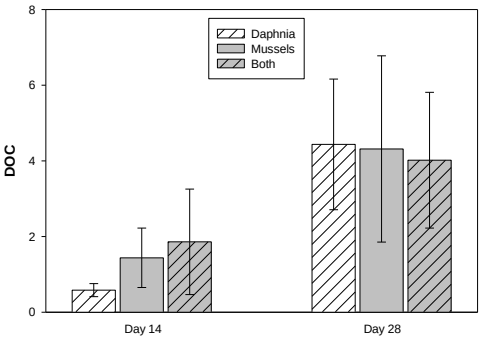
In general, the expected pattern in the experiment was observed for mesocosms containing mussels, as there was an increase in the concentrations of NH_4^+, PO_4^{3-} and DOC (relative to reference mesocosms) in hypolimnia (Figure 9a-c), and a decrease in dissolved oxygen concentrations and pH (Figure 9d,e). However, this apparent increase in benthic (or hypolimnetic) metabolism was seen in all primary consumer treatments. The increase of dissolved N_2 concentration (Figure 9f) in hypolimnia over time in all treatments, further confirms that an increase in denitrification rates occurred regardless of (consumer) treatment.



9a)



9b)



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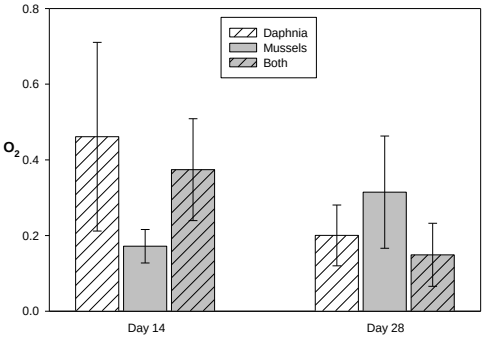
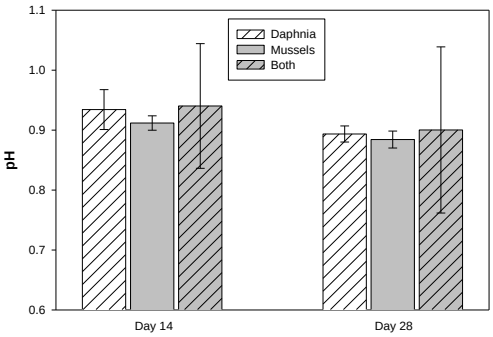


9d)



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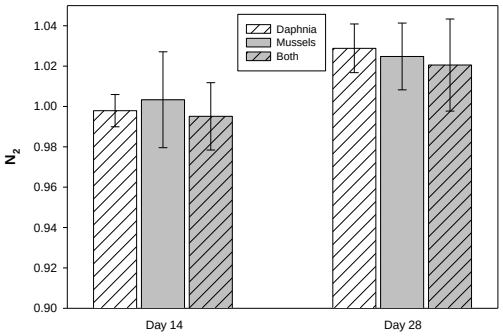
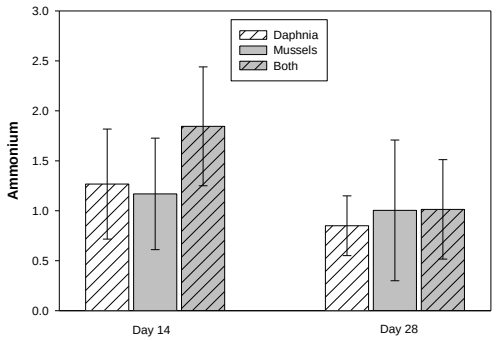


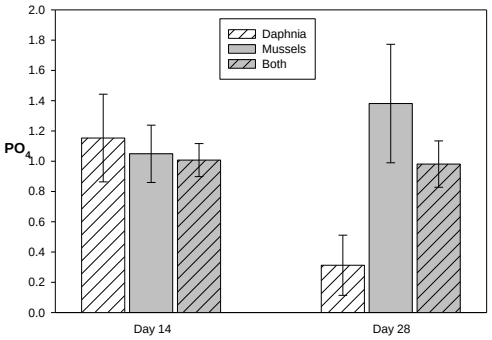
9f)

Figure 9. Plots of proportional concentration data for a) ammonium, b) phosphorus, c) dissolved organic carbon d) oxygen, e) pH, and f) dissolved N in the hypolimnia for feeding modes experiment.

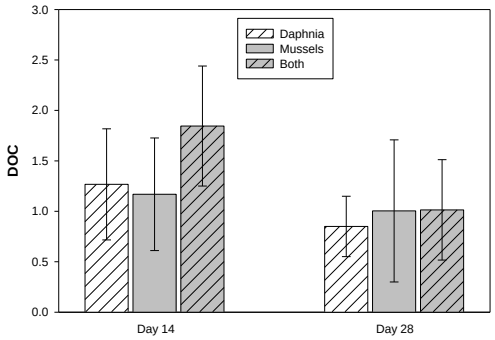
The results from the epilimnion demonstrated a similar pattern, in that all treatments appeared to have the same effect on the mesocosm, where over time a decrease in nutrients (NH_4^+ and DOC) was observed indicating the incorporation of nutrients into phytoplankton biomass (figure 10a and 10c). The decrease in nutrients was unexpected to occur within those mesocosms containing daphnia, where there it was expected nutrient levels would remain constant or slightly higher (if over grazing occurs releasing more nutrients) than reference over time.



10 a)



10b)



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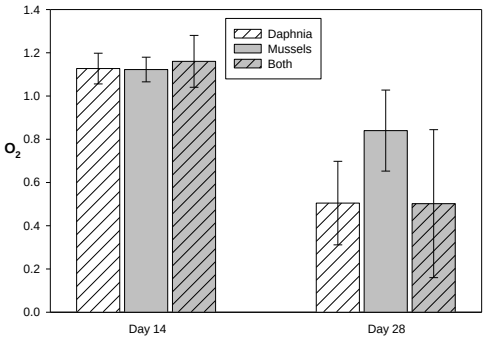
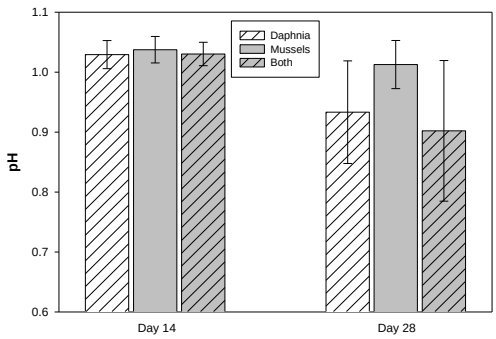
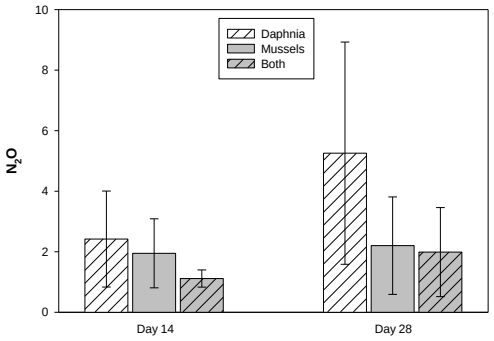


10d)



10e)



10f)

Figure 10. Plots of proportional concentration data for a) ammonium, b) phosphorus, c) dissolved organic carbon d) oxygen, e) pH, and f) nitrous oxide in the epilimnia for feeding modes experiment.

Overall, the results suggest that feeding by either zooplankton or benthic filter feeders would promote the export of carbon to sediments relative to microbial recycling of senescing algae in the water column, in the case of zooplankton. This may occur in production of feces, or with the death and sinking of the zooplankton themselves. The feeding appears more important than the mode of feeding in driving higher rates of benthic metabolism and nutrient recycling.

4.0 Conclusion

One of the major results of the chamber experiment was the fact that despite the availability of inorganic nitrogen, a substantial rate of nitrogen fixation was observed. This disproved the assumption that nitrogen fixation rates are negligible (or much lower) in comparison to denitrification rates when mineral forms of N are available, and shows that net denitrification rates that were previously measured are heavily affected by the nitrogen fixation rates.

The developed chamber system and proof of concept can now be used as a tool in testing hypotheses regarding elemental loading ratio (as an example) effects on nitrogen cycling in real systems. This tool will also allow for better predictive models for evaluating the flow of nutrients through a system and effects of micro-nutrient limitations

Elemental loading ratios (especially Si:N) were shown to be important drivers for nitrogen removal from aquatic systems. This suggests that watershed geology (i.e. abundance of siliceous bedrock and sandy soil) may affect how a lake within the watershed functions for N removal. Furthermore, increased phosphorus loading appears to stimulate overall growth rather than specifically stimulating cyanobacteria as expected. The experiment also suggested that feeding by either zooplankton or benthic filter feeders will stimulate the export of organic matter to the sediment relative to microbial recycling of senescing by algae within the water column. Feeding appears to be more of a driver than the mode of feeding in promoting high rates of benthic metabolism and nutrient recycling. Again, it must be acknowledged that mesocosm studies are limited in representation of real systems, and these hypotheses regarding nutrient loading ratios should be further evaluated in more representative experimental contexts.

Overall, the major research questions were answered and a new tool was generated to be used for future studies in tracing nutrient through a system and allowing for detailed calculation

of the rates of nitrogen biogeochemistry. The apparatus allows for not only net rates of these nitrogen processes, but also allows for each individual process to be measured explicitly.

Appendices

APPENDIX A: Elemental Loading Ratios and Feeding Modes Experiments

Table 4. Ammonium concentrations for elemental loading experiment

Stratum	Treatment	Day 0	Day 14	Day 28	Day 42	Day 56
Top	Reference	1.746 ± 0.723	4.011 ± 3.051	6.123 ± 2.520	8.640 ± 4.190	4.350 ± 2.383
	High P	1.655 ± 0.991	3.224 ± 1.017	4.857 ± 1.096	6.339 ± 2.242	3.969 ± 0.680
	High Si	2.197 ± 0.653	2.712 ± 0.689	4.961 ± 2.119	3.434 ± 1.511	3.435 ± 1.206
	HP +HS	2.001 ± 0.910	2.789 ± 0.477	4.316 ± 1.123	8.401 ± 2.602	6.690 ± 3.250
Bottom	Reference	1.393 ± 0.195	1.171 ± 1.019	9.450 ± 3.348	10.96 ± 3.553	19.76 ± 11.60
	High P	1.881 ± 0.382	1.208 ± 0.755	15.16 ± 6.169	37.36 ± 23.41	22.09 ± 13.34
	High Si	1.718 ± 0.551	3.159 ± 3.706	38.47 ± 12.38	51.36 ± 12.93	40.72 ± 18.24
	HP +HS	1.143 ± 0.624	0.537 ± 0.235	19.73 ± 8.443	80.98 ± 51.52	48.07 ± 16.19

Table 5. Ammonium concentrations for feeding modes experiment

Stratum	Treatment	Day 0	Day 14	Day 28	Day 42	Day 56
Top	Daphnia	2.500 ± 0.769	3.520 ± 1.531	4.350 ± 1.529	5.341 ± 5.779	4.280 ± 2.648
	Mussels	2.102 ± 0.750	3.247 ± 1.551	5.137 ± 3.604	6.243 ± 4.478	7.254 ± 5.409
	Both	2.630 ± 0.715	5.126 ± 1.653	16.61 ± 25.62	15.37 ± 24.87	4.621 ± 2.763
	Reference	1.746 ± 0.723	4.011 ± 3.051	6.123 ± 2.520	8.640 ± 4.190	6.124 ± 4.472
Bottom	Daphnia	1.467 ± 0.439	1.566 ± 1.985	41.902 ± 16.33	55.38 ± 19.04	34.23 ± 20.82
	Mussels	1.534 ± 0.626	1.683 ± 0.920	40.768 ± 23.27	59.66 ± 36.84	12.09 ± 5.603
	Both	1.365 ± 0.892	2.177 ± 1.633	37.958 ± 16.96	232.8 ± 371.1	116.4 ± 167.4
	Reference	1.393 ± 0.195	1.171 ± 1.019	17.414 ± 18.04	18.49 ± 17.12	19.76 ± 11.60

Table 6. Phosphorus concentrations for elemental loading experiment

Stratum	Treatment	Day 0	Day 14	Day 28	Day 42	Day 56
Top	Reference	0.029 ± 0.002	0.054 ± 0.016	0.082 ± 0.034	0.090 ± 0.020	0.072 ± 0.031
	High P	0.024 ± 0.009	0.042 ± 0.010	0.109 ± 0.032	0.099 ± 0.051	0.055 ± 0.050
	High Si	0.090 ± 0.123	0.065 ± 0.010	0.098 ± 0.012	0.084 ± 0.011	0.046 ± 0.030
	HP +HS	0.025 ± 0.006	0.035 ± 0.011	0.091 ± 0.026	0.124 ± 0.102	0.098 ± 0.042
Bottom	Reference	0.028 ± 0.002	0.054 ± 0.016	0.082 ± 0.034	0.085 ± 0.038	0.104 ± 0.028
	High P	0.039 ± 0.012	0.062 ± 0.016	0.026 ± 0.016	0.228 ± 0.100	0.177 ± 0.066
	High Si	0.021 ± 0.003	0.056 ± 0.010	0.113 ± 0.032	0.111 ± 0.045	0.115 ± 0.033
	HP +HS	0.046 ± 0.008	0.054 ± 0.006	0.080 ± 0.013	0.355 ± 0.072	0.281 ± 0.105

Table 7. Phosphorus concentrations for feeding modes experiment

Stratum	Treatment	Day 0	Day 14	Day 28	Day 42	Day 56
Top	Daphnia	0.036 ± 0.010	0.028 ± 0.011	0.059 ± 0.022	0.056 ± 0.049	0.013 ± 0.007

	Mussels	0.045	±	0.016	0.044	±	0.005	0.095	±	0.023	0.086	±	0.065	0.060	±	0.029
	Both	0.041	±	0.017	0.035	±	0.009	0.057	±	0.030	0.140	±	0.198	0.084	±	0.110
	Reference	0.029	±	0.002	0.045	±	0.014	0.108	±	0.026	0.090	±	0.020	0.072	±	0.031
Bottom	Daphnia	0.024	±	0.006	0.028	±	0.011	0.059	±	0.022	0.129	±	0.073	0.167	±	0.138
	Mussels	0.026	±	0.003	0.034	±	0.008	0.070	±	0.036	1.017	±	1.985	3.064	±	3.866
	Both	0.025	±	0.006	0.030	±	0.006	0.084	±	0.052	3.897	±	8.290	4.488	±	6.037
	Reference	0.028	±	0.002	0.043	±	0.013	0.100	±	0.039	0.085	±	0.038	0.104	±	0.028

Table 8. Oxygen concentrations for elemental loading experiment

Stratum	Treatment	Day 0			Day 14			Day 28			Day 42			Day 56		
Top	Reference	-	±	-	677.0	±	51.98	685.2	±	148.7	542.5	±	71.11	630.2	±	189.2
	High P	-	±	-	758.9	±	70.82	503.8	±	106.8	398.1	±	141.3	774.1	±	102.6
	High Si	-	±	-	694.1	±	27.99	570.2	±	94.64	469.7	±	84.84	675.7	±	147.7
	HP +HS	-	±	-	768.9	±	66.04	490.6	±	60.76	355.1	±	83.30	651.2	±	81.06
Bottom	Reference	-	±	-	280.7	±	17.11	272.6	±	85.19	125.8	±	114.9	211.9	±	50.01
	High P	-	±	-	264.7	±	97.83	117.7	±	48.24	75.32	±	53.75	451.3	±	127.3
	High Si	-	±	-	87.58	±	55.48	61.33	±	39.20	50.49	±	11.10	212.7	±	57.76
	HP +HS	-	±	-	187.7	±	54.38	73.64	±	14.05	39.31	±	16.03	216.4	±	24.09

Table 9. Oxygen concentrations for feeding modes experiment

Stratum	Treatment	Day 0			Day 14			Day 28			Day 42			Day 56		
Top	Daphnia	-	±	-	762.8	±	48.13	345.8	±	132.3	425.4	±	133.4	553.2	±	68.40
	Mussels	-	±	-	759.8	±	38.54	575.5	±	128.4	526.6	±	51.40	640.9	±	219.5
	Both	-	±	-	785.5	±	81.26	343.9	±	234.5	394.2	±	129.2	624.2	±	173.9
	Reference	-	±	-	677.0	±	51.98	685.2	±	148.7	542.5	±	71.11	630.2	±	189.1
Bottom	Daphnia	-	±	-	129.5	±	70.03	54.62	±	21.90	57.93	±	45.99	105.7	±	62.04
	Mussels	-	±	-	48.19	±	12.40	85.77	±	40.42	79.45	±	57.15	48.46	±	22.46
	Both	-	±	-	105.0	±	37.81	40.63	±	22.70	89.39	±	152.0	51.70	±	33.97
	Reference	-	±	-	280.7	±	17.11	272.6	±	85.19	125.8	±	114.9	211.9	±	50.01

Table 10. N_2 concentrations for elemental loading experiment

Stratum	Treatment	Day 0			Day 14			Day 28			Day 42			Day 56		
Top	Reference	537.5	±	47.84	-	±	-	552.6	±	28.44	516.6	±	11.44	533.9	±	5.323
	High P	536.9	±	164.9	-	±	-	537.5	±	10.09	526.3	±	31.35	519.9	±	9.830
	High Si	495.2	±	47.08	-	±	-	540.4	±	14.94	525.5	±	41.67	529.1	±	23.62
	HP +HS	530.8	±	25.80	-	±	-	564.1	±	48.65	531.9	±	26.86	521.3	±	10.29
Bottom	Reference	533.6	±	18.50	536.9	±	8.958	535.2	±	12.50	507.7	±	71.17	534.5	±	11.60
	High P	536.0	±	17.71	534.7	±	3.998	540.8	±	14.69	484.8	±	63.67	537.0	±	13.34

	High Si	538.8	±	15.93	537.4	±	3.240	546.3	±	4.932	479.1	±	55.26	535.3	±	18.24
	HP +HS	542.9	±	21.95	532.6	±	7.968	544.8	±	8.940	482.6	±	35.05	538.5	±	16.19

Table 11. N_2 concentrations for feeding modes experiment

Stratum	Treatment	Day 0			Day 14			Day 28			Day 42			Day 56		
Top	Daphnia	498.2	±	28.95	-	±	-	552.6	±	28.44	530.0	±	10.70	528.1	±	5.910
	Mussels	531.2	±	20.43	-	±	-	535.2	±	11.52	530.7	±	24.77	532.2	±	11.42
	Both	551.3	±	20.78	-	±	-	553.1	±	20.71	532.6	±	7.935	511.2	±	26.64
	Reference	537.5	±	47.84	-	±	-	545.1	±	19.77	516.6	±	11.44	533.9	±	5.323
Bottom	Daphnia	544.4	±	24.78	535.8	±	4.285	535.2	±	12.50	508.8	±	85.61	542.3	±	17.75
	Mussels	481.5	±	138.2	538.6	±	12.76	550.6	±	6.459	545.0	±	103.1	539.4	±	29.22
	Both	553.1	±	24.12	534.2	±	8.967	548.4	±	8.854	509.8	±	51.97	553.5	±	31.29
	Reference	533.6	±	18.50	536.9	±	8.958	546.1	±	12.21	507.7	±	71.17	534.5	±	11.78

Table 12. Nitrous oxide concentrations for elemental loading experiment

Stratum	Treatment	Day 0			Day 14			Day 28			Day 42			Day 56		
Top	Reference	90.42	±	22.45	90.20	±	13.48	77.31	±	1.040	185.4	±	36.71	147.4	±	43.76
	High P	129.8	±	21.87	139.4	±	50.97	185.6	±	108.7	168.7	±	28.10	173.0	±	55.24
	High Si	102.0	±	14.43	87.00	±	24.64	91.34	±	66.53	166.0	±	35.83	145.9	±	20.47
	HP +HS	115.0	±	16.31	90.63	±	5.396	66.93	±	28.13	152.2	±	56.86	148.1	±	25.09

Table 13. Nitrous oxide concentrations for feeding modes experiment

Stratum	Treatment	Day 0			Day 14			Day 28			Day 42			Day 56		
Top	Daphnia	110.9	±	19.62	218.1	±	143.0	406.3	±	283.8	201.4	±	21.31	142.3	±	13.74
	Mussels	112.7	±	13.22	175.7	±	102.9	170.1	±	124.5	193.7	±	60.11	143.8	±	32.50
	Both	105.6	±	10.76	100.5	±	25.63	153.7	±	113.7	139.4	±	65.82	170.0	±	81.88
	Reference	90.42	±	22.45	90.20	±	13.48	77.31	±	1.040	185.4	±	36.71	147.4	±	43.76

Table 14. Dissolved carbon concentrations for elemental loading experiment

Stratum	Treatment	Day 0			Day 14			Day 28			Day 42			Day 56		
Top	Reference	1.664	±	0.558	2.090	±	0.524	2.331	±	0.910	1.738	±	1.076	1.341	±	1.061
	High P	1.575	±	0.892	5.877	±	1.448	6.116	±	1.409	3.496	±	0.785	3.483	±	2.088
	High Si	1.577	±	0.385	2.678	±	0.891	2.779	±	0.635	1.950	±	0.990	2.264	±	1.078
	HP +HS	2.157	±	1.216	7.642	±	0.963	8.646	±	1.250	5.741	±	2.710	6.610	±	3.490
Bottom	Reference	13.51	±	3.892	19.63	±	3.293	29.45	±	3.375	26.11	±	13.55	17.07	±	4.835
	High P	15.40	±	2.296	22.65	±	10.66	19.24	±	13.12	39.63	±	12.70	19.83	±	2.847
	High Si	12.90	±	1.480	44.53	±	65.08	24.87	±	7.429	33.70	±	9.016	17.37	±	8.834
	HP +HS	13.08	±	2.594	21.12	±	2.854	25.49	±	1.692	33.14	±	9.761	31.13	±	9.578

Table 15. Dissolved carbon concentrations for feeding modes experiment

Stratum	Treatment	Day 0	Day 14	Day 28	Day 42	Day 56
Top	Daphnia	1.487 ± 0.632	3.009 ± 0.436	2.766 ± 1.754	2.748 ± 2.877	1.674 ± 1.490
	Mussels	1.073 ± 0.740	2.818 ± 0.611	3.262 ± 2.456	2.023 ± 1.278	2.889 ± 1.790
	Both	1.315 ± 0.735	3.062 ± 0.761	5.349 ± 0.932	1.434 ± 1.545	1.515 ± 1.077
	Reference	1.664 ± 0.558	2.090 ± 0.524	2.331 ± 0.910	1.461 ± 1.119	1.146 ± 1.017
Bottom	Daphnia	13.55 ± 1.963	19.82 ± 2.612	23.37 ± 9.116	33.08 ± 10.73	19.97 ± 6.500
	Mussels	15.05 ± 1.945	22.07 ± 2.419	33.39 ± 8.697	43.44 ± 14.53	43.44 ± 14.53
	Both	12.98 ± 2.843	17.84 ± 1.951	46.18 ± 22.83	52.99 ± 19.08	31.35 ± 14.75
	Reference	13.51 ± 3.892	19.63 ± 3.293	29.45 ± 3.375	26.11 ± 13.55	17.07 ± 4.835

Table 16. Nitrate concentrations for elemental loading experiment

Stratum	Treatment	Day 0	Day 14	Day 28	Day 42	Day 56
Top	Reference	3.036 ± 1.534	2.748 ± 0.706	0.738 ± 0.062	2.594 ± 0.630	3.050 ± 0.661
	High P	2.734 ± 0.961	2.922 ± 1.906	0.840 ± 0.116	4.043 ± 3.672	2.559 ± 0.229
	High Si	2.854 ± 1.058	4.143 ± 0.742	1.363 ± 0.584	4.600 ± 1.591	3.822 ± 1.649
	HP +HS	7.561 ± 7.809	3.231 ± 1.779	0.898 ± 0.289	2.866 ± 0.452	2.637 ± 0.113
Bottom	Reference	9.764 ± 2.181	0.780 ± 0.306	1.020 ± 0.335	4.973 ± 2.727	3.290 ± 0.918
	High P	8.690 ± 1.362	0.687 ± 0.044	1.019 ± 0.608	3.161 ± 0.482	3.234 ± 0.896
	High Si	8.074 ± 2.996	0.701 ± 0.153	0.804 ± 0.433	3.770 ± 1.218	3.280 ± 0.940
	HP +HS	11.93 ± 2.435	0.622 ± 0.113	1.577 ± 1.346	3.233 ± 0.831	3.113 ± 1.169

Table 17. Nitrate concentration for feeding modes experiment

Stratum	Treatment	Day 0	Day 14	Day 28	Day 42	Day 56
Top	Daphnia	2.526 ± 1.087	1.351 ± 0.139	0.932 ± 0.464	2.453 ± 0.204	2.641 ± 0.115
	Mussels	4.790 ± 2.595	1.688 ± 0.906	1.070 ± 0.477	2.456 ± 0.168	3.684 ± 2.315
	Both	2.554 ± 0.822	3.820 ± 1.456	0.783 ± 0.061	2.565 ± 0.179	3.317 ± 1.209
	Reference	3.036 ± 1.534	2.748 ± 0.706	0.738 ± 0.062	2.594 ± 0.630	3.050 ± 0.661
Bottom	Daphnia	9.861 ± 1.950	0.753 ± 0.198	0.915 ± 0.388	2.585 ± 0.632	2.873 ± 0.603
	Mussels	6.404 ± 3.320	0.651 ± 0.110	0.729 ± 0.144	3.294 ± 0.420	3.938 ± 2.795
	Both	8.525 ± 3.837	0.742 ± 0.308	1.815 ± 1.102	2.719 ± 0.225	3.447 ± 1.773
	Reference	9.764 ± 2.181	0.780 ± 0.306	1.020 ± 0.335	4.973 ± 2.727	3.290 ± 0.918

Table 18. pH readings for elemental loading experiment

Stratum	Treatment	Day 0	Day 14	Day 28	Day 42	Day 56
Top	Reference	9.640 ± 0.678	9.518 ± 0.285	9.170 ± 0.446	9.426 ± 0.259	9.664 ± 0.405
	High P	10.04 ± 0.115	9.912 ± 0.145	9.300 ± 0.411	9.126 ± 0.652	10.05 ± 0.295

	High Si	9.876	±	0.109	9.64	±	0.177	9.220	±	0.122	9.378	±	0.401	9.886	±	0.313
	HP +HS	9.640	±	0.678	9.974	±	0.173	9.396	±	0.251	9.130	±	0.512	9.964	±	0.284
Bottom	Reference	7.844	±	0.283	7.934	±	0.333	7.743	±	0.428	7.752	±	0.661	8.538	±	0.741
	High P	8.128	±	0.351	8.186	±	0.436	7.880	±	0.763	7.608	±	0.504	8.912	±	0.782
	High Si	8.182	±	0.415	7.372	±	0.175	7.124	±	0.107	7.380	±	0.032	7.912	±	0.414
	HP +HS	7.976	±	0.370	7.866	±	0.363	7.520	±	0.266	7.402	±	0.214	8.080	±	0.704

Table 19. pH readings for feeding modes experiment

Stratum	**Treatment**	**Day 0**			**Day 14**			**Day 28**			**Day 42**			**Day 56**		
Top	Daphnia	9.900	±	0.198	9.796	±	0.223	8.558	±	0.785	9.044	±	0.472	9.646	±	0.189
	Mussels	9.758	±	0.192	9.874	±	0.210	9.286	±	0.368	9.128	±	0.508	9.640	±	0.279
	Both	9.802	±	0.109	9.806	±	0.185	8.272	±	1.076	8.536	±	0.632	9.838	±	0.704
	Reference	9.640	±	0.678	9.518	±	0.285	9.170	±	0.446	9.426	±	0.259	9.664	±	0.405
Bottom	Daphnia	8.008	±	0.382	7.412	±	0.264	6.918	±	0.104	7.380	±	0.180	8.120	±	0.869
	Mussels	8.142	±	0.253	7.234	±	0.096	6.846	±	0.110	7.034	±	0.370	7.584	±	0.918
	Both	7.710	±	0.390	7.460	±	0.825	6.970	±	1.073	7.250	±	1.033	7.434	±	1.281
	Reference	7.844	±	0.283	7.934	±	0.333	7.743	±	0.428	7.752	±	0.661	8.538	±	0.741

APPENDIX B: Reagent List and Experimental Apparatus Setup

Table 20. Percentage dry constituents of artificial sediment [OECD, 1984]

Constituents	Characteristics	% of Sediment
Peat	Sphagnum moss peat (particle size ≤0.5mm)	2±0.5
Quartz Sand	Grain size ≤2mm	76
Kaolinite Clay	Kaolinite content ≥30%	22±1
Dried Maple Leaves	Powered leaves of Maple tree with alpha-cellulose (1:1 ratio)	0.4-0.5
Calcium Carbonate	$CaCO_3$	0.05-1
Deionized Water	Conductivity ≤10µS/cm, in addition to dry sediment	30-50

The peat was air-dried and grounded to a fine powder until no visible plant remains were detected. A suspension of the required amount of peat powder was prepared using deionized water (11.5mL by dry weight of peat). The pH of this suspension was adjusted to 5.5±0.5 with $CaCO_3$. The suspension was conditioned for three days with gentle stirring at room temperature. The pH was measured and adjusted to 6.0±0.5 with additional $CaCO_3$. Next, the suspension was mixed with the other dry constituents and deionized water added to obtain a homogeneous sediment mixture. Again the pH was checked and adjusted to 6.5 with $CaCO_3$ and the quartz sand was mixed in with the sediment. Supplementary to the sediment ingredients, lake sediment from a eutrophic pond was added into the artificial sediments (<0.5% w/w) in order to inoculate artificial sediments and approximate the bacterial community structure of a real lake.

Phosphorus Analysis

2.5M H_2SO_4: 70mL of concentrated $H_2SO_4$was slowly added to approximately 400mL of distilled water in a 500mL volumetric flask. Once the solution cooled, it was diluted to 500mL with deionized water, mixed, and transferred to a plastic bottle for storage.

Ammonium molybdate solution: 20g of $(NH_4)_6Mo_7O_{24}•4H_2O$ was dissolved in 500mL of deionized water and store in a plastic bottle at 4°C.

Potassium antimonyl tartrate solution: Using a 500mL volumetric flask, 1.3715g of $K(SbO)C_4H_4O_6•½H_2O$ was dissolved in approximately 400mL of distilled water. Solution was dilute to mark and transferred to a dark, glass-stoppered bottle for storage.

0.1M Ascorbic acid: Dissolve 1.76g of ascorbic acid in 100mL of distilled water. The solution is stable for about a week if stored in an opaque plastic bottle at 4°C.

Combined reagent: When making the combined reagent, all reagents must be allowed to reach room temperature before they are mixed, and they must be mixed in the following order. To make 100 mL of the combined reagent:

Kjeldahl Nitrogen Analysis

Kjeldahl Digestion Reagent: Consisted of dissolving 134g potassium sulphate and 7.3g of copper sulphate in 800ml of water (may not completely dissolve until addition of acid). Next 134ml of concentrated sulphuric acid was added to the solution. Once the solution had cooled it was

diluted to 1L and mixed well. The digestion reagent was store at room temperature to prevent crystallization.

Ammonium Nitrogen Analysis

Phenol solution: Was prepared weekly by diluting 11.1mg phenol (89%) with 95% (v/v) ethyl alcohol to a final volume of 100mL

Sodium nitroprusside (0.5% (w/v)): 0.5g of sodium nitroprusside was dissolved in 100mL deionized water

Alkaline citrate: The solution was made by dissolving 200g trisodium citrate and 10 g sodium hydroxide in deionized water and diluting it to 1L

Oxidizing solution: 100mL alkaline citrate solution was mixed with 25mL of sodium hypochlorite (commercial bleach ~5%). This was made fresh daily

Stock solution: A stock solution was prepared by dissolving 3.189g anhydrous NH_4Cl (1mg/mL nitrogen) in 1L water

Nitrate-Nitrite Analysis

Preparation of the reduction column: The cadmium column was constructed using a copper cylinder with dimensions length 150mm and a 5mm diameter. Both ends of the column were capped with steel wool and fitted with metal bolts (in order to prevent Cu-Cd granules from leaking out). A plastic aquarium tube adapter was connected to allow the column to be connected to the external setup. Washing of Cu-Cd granules was conducted by rinsing 20g of granules with 6N HCl followed by deionized water. The granules were then rinsed with 50mL 2% $CuSO_4$ (5g $CuSO_4 \bullet 5H_2O$ diluted to 250mL water) for 5 minutes, until blue colour faded. Successive 50mL 2% $CuSO_4$ washings were repeated until a brown colloidal precipitate formed. A water wash was used to remove the precipitated from the washed Cu. These activated granules were then packed into the column using a thin metal rod and a plastic tube filled with the ammonium chloride-EDTA solution. The column was then stored in a plastic case filled with the buffer solution until further use. The washing of the granules was only performed once every 300 samples.

Colour reagent: A colour reagent was made by dissolving 1g of sulphanilamide in 80ml water and 10ml 85% phosphoric acid. Once dissolved, 0.1g N-(1-naphthyl)-ethylenediamine dihydrochloride was added and the whole solution was diluted with water to 100mL. The solution is light sensitive it was therefore stored in a light-proof brown plastic bottle.

Ammonium chloride-EDTA solution: The buffer solution was made by dissolving 13g NH_4Cl and 1.7g disodium ethylenediaminetetraacetate in 900mL water and then adjusting the pH to 8.5 with concentrated NH_4OH and dilute to 1L.

Stock solution: A stock solution was prepared by dissolving 7.22g of KNO_3 (previously dried in an oven at 105ºC for 24 hours) in 1L of water. From this solution, a 100 fold dilution was employed to obtain a 10 $mg \bullet L^{-1}$ working standard solution.

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