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NITROGEN AVAILABILITY AND TRANSFORMATIONS IN MISSISQUOI BAY, LAKE CHAMPLAIN: EFFECTS ON PHYTOPLANKTON COMMUNITY STRUCTURE AND CYANOBACTERIAL BLOOMS

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DISPONIBILITÉ ET TRANSFORMATIONS DE L'AZOTE DANS LA BAIE MISSISQUOI DU LAC CHAMPLAIN: EFFETS SUR LA STRUCTURE DE LA COMMUNAUTÉ DE PHYTOPLANCTON ET SUR LES FLEURS D'EAU DE CYANOBACTÉRIES

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RESUMÉ

L'objectif de l'étude était de déterminer la disponibilité de l'azote (N) et les transformations dans la colonne d'eau (WC) et l'interface eau-sédiments (SWI), et d'établir des liens avec les éclosions de cyanobactéries dans la baie Missisquoi du lac Champlain. Ces paramètres ont été évalués à l'embouchure de la rivière aux Brochets et dans le bassin central, afin d'obtenir un gradient environnemental pour étudier l'effet des décharges de nutriments sur le cycle de l'azote et la structure des communautés de phytoplancton (PCS). Les résultats portent à croire qu'en général, les nutriments ne limitent pas la productivité primaire, mais que l'azote peut la limiter à petite échelle. Contrairement à nos hypothèses, le phytoplancton semblait gérer les concentrations en nutriments, et non l'inverse. La PCS n'affectait pas la consommation en NH_4^+ dans la WC. De même, le taux de régénération du NH_4^+ dans la WC n'était pas relié à la PCS, ce qui réfute l'hypothèse que les cyanobactéries inhibent la régénération. Les sédiments agissaient comme un puits de NO_3^-, tel que prédit, mais étaient aussi une source de NH_4^+ vers la WC, contrairement aux hypothèses. Une biomasse élevée de cyanobactéries était associée avec une relâche subséquente de NO_3^- des sédiments, ce qui pourrait impliquer une stimulation de la nitrification. L'anammox explique de 6 à 10% de la production totale de diazote, mais la dénitrification en était le principal vecteur. Les taux de dénitrification étaient reliés à la concentration instantanée en azote de la WC, mais pas à ses fluctuations. On observe des relations similaires entre les flux de NO_3^- dans les sédiments et la dénitrification avec la biomasse cyanobactérienne antérieure. Cela suggère que des concentrations inférieures en azote dans la WC, à la suite de floraisons de cyanobactéries, ont rendu possibles des taux de dénitrifications inférieurs. Les données ne corroborent pas l'hypothèse selon laquelle la dénitrification mènerait à des conditions propices pour les cyanobactéries fixatrices d'azote. Au contraire, les résultats suggèrent que les conditions propices aux cyanobactéries fixatrices d'azote causent une réduction des taux de dénitrification. Le lac était un puits net d'azote dans un budget préliminaire calculé en utilisant le cycle du NH_4^+ dans la WC et les flux d'azote à l'SWI, comparés aux apports en azote estimés des affluents. Ces calculs révèlent une source d'azote « manquante », qui pourrait être la fixation d'azote dans la WC selon les résultats. Les taux observés de fixation d'azote dans la WC n'étaient pas différents de ceux des contrôles expérimentaux, mais même ces taux négligeables sont d'un ordre de grandeur plus élevé que ceux nécessaires pour combler l'azote « manquant ».

Mots-vedettes : cycle de l'azote, cyanobactéries, dénitrification, eutrophisation, budget d'azote

SUMMARY

The study objective was to determine nitrogen (N) availability and transformations in the water column (WC) and at the sediment-water interface (SWI) and examine linkages to cyanobacterial blooms in Missisquoi Bay, Lake Champlain. These parameters were evaluated at the Pike River discharge into Missisquoi Bay and in the central basin to provide environmental gradients to study the effects of nutrient discharges on N cycling and phytoplankton community structure (PCS). Results suggested that nutrients generally did not limit primary productivity, but there was evidence that N limited productivity on fine scales. Contrary to hypotheses, phytoplankton appeared to control nutrients from the top down. PCS did not affect WC NH_4^+ uptake rates, but the proportion of cyanobacteria to total phytoplankton biomass affected the balance between autotrophic and heterotrophic ammonium (NH_4^+) uptake. WC NH_4^+ regeneration rates were not related to PCS, refuting the hypothesis that cyanobacteria would inhibit regeneration. Sediments were a NO_3^- sink, as predicted, but also were an NH_4^+ source to the WC, contrary to the hypothesis. High cyanobacteria biomass was related to future increases in sediment NO_3^- release, which may reflect a stimulation of nitrification. Anammox may account for 6 – 10% of total N_2 production, but denitrification was the dominant N_2 pathway. Denitrification rates were related to WC N concentrations but did not affect future WC N concentrations. Similar relationships between sediment NO_3^- flux and denitrification with previous cyanobacteria biomass suggest that lower WC N concentrations resulting from cyanobacteria blooms led to reduced denitrification. The data do not support the hypothesis that denitrification would lead to conditions suitable for N fixing cyanobacteria. In contrast, the results indicate that conditions suitable for N fixing cyanobacteria caused lower denitrification. The lake was a net N sink in a preliminary N budget prepared using WC NH_4^+ cycling and SWI N fluxes compared to estimated N load from tributaries. These calculations indicate a 'missing' N source, which results suggest may be WC N fixation. WC N fixation rates were not different from experimental controls, although the insignificant rates were an order of magnitude higher than those required to account for the missing N source.

Key Words: nitrogen cycling, cyanobacteria, denitrification, eutrophication, nitrogen budget

CHAPTER I: GENERAL INTRODUCTION

1.1. Background

Algal blooms are affecting lakes throughout the world (e.g., Bormans et al. 2004, Paerl & Fulton 2006), and these blooms have increased public and government attention to these and other eutrophication-related issues. Some algal blooms produce toxins, which threaten public health and recreational usage of affected waters. Like other lakes in southern Québec, Missisquoi Bay, Lake Champlain, has exhibited annual summer algal blooms in recent years, and some blooms have been toxic, resulting in public health notices being issued (Giani et al. 2005, Rolland et al. 2005). Harmful algal blooms (HABs), and particularly those caused by cyanobacteria (cyanoHABs), generally are associated with anthropogenic eutrophication (Paerl & Fulton 2006). Cyanobacteria are the oldest known oxygenic phototrophs (> 2.5 billion years), and blooms are a well-established indicator of eutrophication in aquatic systems. However, not all eutrophication is anthropogenic in origin (e.g., James et al. 2003). HABs can be a natural phenomenon as well, and historical records suggest that HABs have been described for centuries (Anderson et al. 2002). Harmful effects of eutrophication in aquatic systems include HABs (Heisler et al. 2008), oxygen depletion (e.g., Boesch et al. 2009), and altered food webs leading to decreases in exploitable resources (Vitousek et al. 1997). Global climate change is expected to exacerbate cyanoHABs world-wide (e.g., Jeppesen et al. 2005, Paerl & Huisman 2008, 2009).

Toxins associated with HABs have been linked to wildlife death, seafood poisonings, and, in some rare cases, human death (Anderson et al. 2002). Toxic cyanoHABs also have inhibited water usage for human consumption and recreation (Paerl & Fulton 2006, Wang & Shi 2008). In humans, cyanotoxins have been associated with primary liver tumors (Shen et al. 2003), and microcystins are the most common of these hepatotoxins in aquatic systems (Rolland et al. 2005). Microcystins also have been found in health-food industry dietary supplements formulated using cyanobacteria, prompting advisories to be issued by local health departments (Gilroy et al. 2000). These supplements recently were implicated in the

death from liver failure of a regular consumer (Dietrich et al. 2007). Microcystins also contaminated the water supply of a hemodialysis center in Brasil, and 50 patients receiving treatment at the center subsequently died of liver failure (Jochimson et al. 1998).

Definitions of eutrophication vary, but a common concept involves the increased productivity of a system due to increased nutrient inputs. Eutrophication has been described as "one of the foremost problems in protecting freshwater and coastal marine systems" (Schindler 2006). Sources of excess nutrients to watersheds and aquatic systems include treated and untreated sewage, agricultural runoff containing fertilizers, urban stormwater runoff, atmospheric deposition (e.g., Hicks 2007, Duce et al. 2008, Galloway et al. 2008, Elser et al. 2009), and groundwater inflow (Anderson et al. 2002, Smolders et al. 2010).

Phosphorus (P) and nitrogen (N) are the nutrients associated with eutrophication in aquatic systems, and these nutrients are the targets of watershed management strategies to remediate affected systems. There has been intense debate in the recent literature about the necessity to manage N inputs in addition to the well-established strategy of managing P inputs (Schindler 1974, 1977). While some authors have supported the P-only nutrient management approach (Schindler et al. 2008, Bryhn & Hakanson 2009, Schelske 2009, Smith & Schindler 2009, Wang & Wang 2009, Welch 2009), many others have challenged the wisdom of failing to consider N in nutrient management strategies aimed at alleviating the harmful effects of eutrophication (Paerl et al. 2004, Jeppesen et al. 2005, Howarth & Paerl 2008, Lewis & Wurtsbaugh 2008, Sterner 2008, Conley et al. 2009, McCarthy et al. 2009b, Paerl 2009, Scott & McCarthy 2010). The P-only nutrient management approach is based on the assumption that atmospheric N can be fixed to alleviate any N limitation of primary production (e.g., Schindler & Hecky 2009). The P-only approach fails to account for the fact that N fixation is energetically unfavorable compared to all other N assimilation strategies and, thus, is unlikely to reverse N limitation (e.g., Paerl 1990). This assumption also has been challenged in the very study that led to the theory (Scott & McCarthy 2010). In this challenge, the authors contend that N fixation in the study lake has failed to reverse N limitation. Instead, N limitation has become more severe, phytoplankton biomass has decreased, and total N concentrations have decreased since N fertilization was stopped.

These nutrient limitation paradigms are based on the Redfield ratio, which posits that the nutrient requirements of organisms in nature mirror the ratios of these elements within their biomass and surrounding environment (Redfield 1958). The molar C:N:P ratio in organisms and deep sea waters observed by Redfield was 106:16:1. Carbon control of eutrophication was dismissed due to air/water exchange of carbon dioxide preventing long-term carbon deficiency in natural systems (Schindler 1977). In terms of N and P, a molar ratio above 16 reflects excess N compared to P, and, thus, P would limit the productivity of the system. Conversely, a ratio below 16 reflects excess P versus N and, thus, N limitation of primary production. The P-only nutrient management approach argues that controlling N inputs can lead to N fixing cyanobacteria blooms (e.g., Flett et al. 1980). While this is an intuitive conclusion, it fails to consider that many cyanoHABs are caused by non-N-fixing cyanobacteria genera, such as *Microcystis*, which is often toxic. These genera also are notable for their preference and high competitive abilities for reduced N compounds (Kappers 1980, Blomqvist et al. 1994, Hyenstrand et al. 1998a, 1998b), which are common in anthropogenic runoff and also are produced by organic matter and oxidized N recycling processes.

The Redfield ratio-based nutrient limitation paradigm is not universal (e.g., Elser et al. 1990; Downing & McCauley 1992; Guildford & Hecky 2000; Sagrario et al. 2005; Jeppesen et al. 2007), and recent work stresses evaluation of systems as individual entities rather than potentially representative systems (Moss et al. 1997; Hameed et al. 1999; Kilinc & Moss 2002). N limitation in freshwater systems seems to occur more often in eutrophic systems with high P loading (Downing & McCauley 1992). However, P loading reductions alone do not always lead to improved water quality and mitigation of eutrophication (Sagrario et al. 2005). A review of nutrient limitation studies in North American lakes concluded that the roles of N and P in limiting phytoplankton growth have not been constrained sufficiently and suggested that N has a more important role as a limiting nutrient than previously recognized (Elser et al. 1990). This review also concluded that, "Little support can be found in these results for the conventional wisdom that P is the predominant primary limiting nutrient in freshwater, with N functioning largely as a secondary limiting factor in special situations." Other studies have shown that N has a greater potential than CO_2

and Si to affect phytoplankton community structure (Huszar & Caraco 1998). A recent review of the P limitation paradigm (Sterner 2008) concluded that:

"*...at time and space scales relevant to population growth, multi-resource control is the rule, not the exception and this view should at least be part of the paradigm of nutrient limitation of lakes. Primary producers in lakes are not regulated on day-to-day time scales by solely or even mainly by P. I further believe that though there is logical reasoning behind it, the paradigm for phosphorus limitation of whole lakes at multi-annual scales needs further examination, particularly in reference to eutrophic and other habitats. Many years after the carbon vs. phosphorus controversy was successfully resolved there still are relevant and interesting unanswered questions about limiting factors at different scales in freshwaters.*"

Nutrient recycling and subsequent internal loading from sediments can exacerbate the ecosystem effects of eutrophication (Bailey & Hamilton 1997, Hansen et al. 1997, Berelson et al. 1998), even after external loading reductions have been implemented (Burger et al. 2007, Jeppesen et al. 2007). Non-N-fixing cyanobacteria are capable of storing excess P during sedimentary phases (Pettersson et al. 1993), and this excess P is used during pelagic growth periods, which results in unbalanced and higher N versus P uptake (e.g., Ahn et al. 2002). Other major environmental issues, such as climate change, habitat fragmentation, and biotic exploitation have led to loss of resilience of lakes to increased nutrient inputs and expedited eutrophication (Gulati & van Donk 2002). The efficient recycling of nutrients, complicating factors leading to loss of resiliency, relative rarity of N fixation in nature, and ability of cyanobacteria to out-compete other genera for reduced N argue for consideration of comprehensive nutrient management plans, which include P input reductions and, at a minimum, an evaluation of the feasibility and potential effects of a combined N and P management approach.

The relationships between anthropogenic eutrophication and cyanoHABs have led to extensive research to further our understanding of the consequences of unmitigated nutrient inputs. In marine systems, these studies often focus on N, because it is generally assumed to be the primary limiting nutrient in these systems (e.g., Howarth & Marino 2006, Howarth

2008). However, eutrophication studies in freshwater systems have focused on P, which has created a dichotomy in research along the freshwater to marine continuum (Elser et al. 1990, Gu et al. 1997). This dichotomy has resulted in significant information gaps with respect to P cycling in marine systems and N cycling in freshwater systems. These information gaps invoke numerous questions regarding the importance of N in the eutrophication of freshwater systems, and some of these questions are the focus of this thesis and are addressed in the following chapters:

Chapter II	What are the seasonal dynamics of ambient N forms and phytoplankton community structure in Missisquoi Bay?
Chapter III	What are the seasonal rates of water column N transformations in Missisquoi Bay?
Chapter IV	What are the seasonal rates of sediment-water interface N transformations in Missisquoi Bay?
Chapter V	How do relationships between these various processes interact to determine phytoplankton community structure?

Chapter II involves describing basic characteristics related to the ecology of Missisquoi Bay, such as identifying the dominant phytoplankton groups within the growing season, the seasonal dynamics of in-lake N availability, and the most conducive N form for growth of the dominant phytoplankton group or groups (e.g., von Rückert & Giani 2004, McCarthy et al. 2009b). These basic ecological characteristics form the basis of the questions and hypotheses addressed in subsequent chapters and use data obtained from biweekly sampling of water quality and biological parameters conducted independently of the sampling and experiments described in this thesis.

In Chapter III, water column N transformations are evaluated, primarily with respect to the uptake and regeneration of ammonium (NH_4^+; e.g., McCarthy et al. 2009a), which represents the most bioavailable N form for primary producers (e.g., McCarthy et al. 1977). These transformations were evaluated using isotope dilution techniques and high performance liquid chromatography (HPLC; Gardner et al. 1995a). The potential for N_2 fixation by specialized organisms, particularly during times when N may be limiting system productivity, also was evaluated using a novel combination of water column incubations and dissolved gas analyses via membrane inlet mass spectrometry (MIMS; Kana et al. 1994, 1998, An et al. 2001).

Chapter IV focuses on sediment-water interface (SWI) N transformations mediated by microbes and affecting N forms available to primary producers. These transformations include dissimilatory nitrate (NO_3^-) reduction to NH_4^+ (DNRA; e.g., An & Gardner 2002, Gardner et al. 2006, Burgin & Hamilton 2007), denitrification (e.g., Seitzinger 1988), anaerobic NH_4^+ oxidation (anammox; e.g., Mulder et al. 1995), and heterotrophic N_2 fixation (e.g., Fulweiler et al. 2007). In addition, sediment oxygen (O_2) demand (SOD) and net nutrient fluxes were evaluated. Nutrient fluxes included soluble reactive P (SRP), NO_3^-, nitrite (NO_2^-), and NH_4^+. Nitrification was not measured directly but could be inferred in relation to its coupling to denitrification and from SWI nutrient fluxes. Microbial N transformations, SOD, and nutrient fluxes at the SWI were evaluated using continuous-flow incubations of intact sediment cores (e.g., Lavrentyev et al. 2000) combined with colorimetric nutrient analyses, HPLC, and MIMS.

Chapter V synthesizes the limnological data in Chapter II and the rate measurements in Chapters III and IV to: (1) infer the nutrient limitation status of primary producers; (2) determine relationships between ambient nutrients and phytoplankton community structure; (3) determine relationships between ambient nutrients, phytoplankton community structure, and water column N transformation rates; and (4) determine relationships between ambient nutrients, phytoplankton community structure, and SWI N transformation rates.

The following sections review the state of knowledge associated with each of these chapters and provide a theoretical basis for the hypotheses, methods, and interpretations. The approaches used to examine the questions and hypotheses will be summarized, and a description of the study site and rationale for sampling site selection will be presented.

1.1.1. Seasonal phytoplankton dynamics in lakes --- light, temperature, and N forms

Phytoplankton communities are affected by numerous physical and geochemical properties in aquatic systems. Although phytoplankton often are grouped into taxonomic units based on morphology and functional characteristics (e.g., Reynolds 1997) based on light and nutrient acquisition strategies, others have suggested that cell size and shape may be better predictors of phytoplankton response to physico-chemical conditions (Huszar & Caraco 1998). In the case of diatoms and cyanobacteria, which are often dominant in temperate lakes at different times, cell size and shape and light and nutrient acquisition strategies tend to differ significantly. For example, size-fractionated communities often represent diatoms in larger fractions (> 2 μm) and picocyanobacteria in the small fractions ($<$ 2 μm), particularly in marine systems (e.g., Hutchins et al. 2003, L'Helguen et al. 2008).

Responses of these broad phytoplankton groups also differ with respect to light fluctuations and intensity. For example, freshwater diatoms dominated the phytoplankton community and grew fastest in culture when light intensities fluctuated, particularly at low average light intensities (Litchman 1998). However, cyanobacteria dominated and grew fastest in culture at constant and high light intensities. Similar results have been reported in natural systems (Zhang & Prepas 1996). Diatoms also may assimilate inorganic N in the dark by using excess carbon fixed during light-replete conditions, when NO_3^- assimilation cannot keep up with carbon fixation (Clark et al. 2002). These morphological characteristics provide diatoms with an advantage during the more physically dynamic conditions found in lakes in spring. These same physical conditions in spring (i.e., turbulent water columns) can neutralize the ability of some cyanobacteria to control buoyancy to maximize light acquisition near the water surface (Hunter et al. 2008). Several studies also suggest that light availability and competition plays a role in community succession within the cyanobacteria.

For example, when *Microcystis* is dominant in summer, toxic strains can be out-competed by non-toxic strains for light (Kardinaal et al. 2007), which leads to a succession from toxic to non-toxic strains due to self-shading by the bloom. Also, N-fixing cyanobacteria were more numerous than non-N-fixing cyanobacteria during low light conditions and vice versa in a Brasilian reservoir (Soares et al. 2009), but this finding is opposite most of the literature regarding light and cyanobacteria community structure (e.g., Havens et al. 2003).

Temperature also affects phytoplankton community dynamics, with diatoms better able to initiate or continue growth at temperatures below 15 °C (Zhang & Prepas 1996, Butterwick et al. 2005) and cyanobacteria dominating at temperatures > 21 °C (McQueen & Lean 1987). Diatoms are known to take up excess NO_3^- and grow faster than other groups at lower temperatures (Lomas & Glibert 1999b), and it was hypothesized that the non-nutritional NO_3^- uptake could be reduced intracellularly to NO_2^- or NH_4^+ to modulate photosynthetic electron flow during turbulent conditions (Lomas & Glibert 1999a). In contrast, low temperatures inhibit cyanobacteria growth by inactivation of transporters required for oxidized N to traverse the cell membrane prior to assimilation (Sakamoto & Bryant 1999). At high temperatures, toxic *Microcystis aeruginosa* strains grew faster than non-toxic strains (Davis et al. 2009), which may give these toxic strains an advantage by reducing grazing pressure (Gobler et al. 2007) and maximizing growth rates in a warming climate (Paerl & Huisman 2008, 2009, Wagner & Adrian 2009).

The N form available to phytoplankton communities plays a major role in determining phytoplankton community structure (Gligora et al. 2007, McCarthy et al. 2009b). In the case of diatoms and cyanobacteria, there is extensive evidence in the literature suggesting that diatoms are more competitive for oxidized N forms (i.e., NO_3^- and NO_2^-) than cyanobacteria (e.g., Presing et al. 1999, Kudela & Dugdale 2000, Hutchins et al. 2003, Ornolfsdottir et al. 2004, van der Grinten et al. 2004, Horgan 2005). Conversely, cyanobacteria are more competitive for reduced N forms (i.e., NH_4^+ and urea) than diatoms (e.g., Kappers 1980, Blomqvist et al. 1994, Hyenstrand et al. 1998a, Hyenstrand et al. 1998b, Horgan 2005). Analysis of a long-term dataset from Lake Okeechobee (Florida), a very large, shallow, eutrophic lake, revealed that the proportion of cyanobacteria to total phytoplankton

was related positively to the ratio of NH_4^+ to NO_x (McCarthy et al. 2009b). At the same time, diatom proportion was negatively related to NH_4^+:NO_3^-.

Despite the vast literature on these differences in N assimilation capacity, there have been very few studies conducted where cyanobacteria and diatoms were in direct competition for nutrient resources. These few studies support the circumstantial observations described above. For example, diatoms out-competed cyanobacteria in N-limited biofilms (i.e., lowest NO_3^- treatment; van der Grinten et al. 2005). In Lake Erken (Sweden), cyanobacteria were unable to compete with eukaryotic algae (i.e., diatoms) for NO_3^-, while NH_4^+ availability promoted cyanobacteria dominance over other phytoplankton groups (Blomqvist et al. 1994). Another compelling example of this phenomenon was reported for Acton Lake (Ohio), where diatom-dominated phytoplankton communities used more NO_3^- relative to NH_4^+, and cyanobacteria-dominated communities used more NH_4^+ relative to NO_3^- (Horgan 2005). This pattern also holds for marine phytoplankton. Continuous inputs of NO_3^- caused a phytoplankton community shift from cyanobacteria to diatoms, and urea availability resulted in an increase in picocyanobacteria proportions (Hutchins et al. 2003). In the Baltic Sea, the disappearance of diatoms coincided with undetectable NO_3^- concentrations and lowest NO_3^- uptake rates, while cyanobacteria were associated with highest NH_4^+ and urea uptake rates (Berg et al. 2003). Other than diatoms, none of the other phytoplankton groups evaluated (dinoflagellates, cyanobacteria, cryptophytes) appeared to be using NO_3^- at all. Most recently, a mesocosm study found that reduced N additions stimulated non-N-fixing cyanobacteria and cyanotoxin production (Donald et al. 2011).

With very few exceptions, bioassays conducted to evaluate nutrient limitation in aquatic systems have used NO_3^- as the N source (e.g., Aldridge et al. 1995, Phlips et al. 1997, Piehler et al. 2009). This trend is presumably based on the frequent observation that oxidized N concentrations exceed reduced N concentrations and the subsequent misinterpretation that relative abundance equates to importance in the system. In fact, the opposite is probably true, whereby those substrates present in lowest quantities are the most important (e.g., Glibert et al. 1982). Therefore, the focus of research efforts should be on substrates present in lower concentration, because these substrates are assimilated and recycled the fastest. The result of

this misapplication of von Liebig's Law of the Minimum (c.f., de Baar 1994) has likely been a gross under-estimate of potential N limitation in aquatic systems, particularly freshwater systems, where cyanobacteria represent dominant or significant proportions of total phytoplankton. However, using only NH_4^+ as the N source in nutrient limitation bioassays may select against diatoms, so a mixed NO_3^- and NH_4^+ addition (and perhaps organic N forms, such as urea) would be ideal for evaluating nutrient limitation.

These differences in competitive outcomes have a physiological basis and result from a genetic cascade that must be initiated and completed before cyanobacteria can assimilate oxidized N forms. First, iron is a vital component of NO_3^- and NO_2^- reductases in phytoplankton (North et al. 2007), and these enzymes are required to reduce oxidized N to NH_4^+, which can then be assimilated into biomass (Syrett 1981). Thus, iron deficiency can inhibit NO_3^- and NO_2^- assimilation in phytoplankton, and approximately five times as much energy is required for oxidized N assimilation versus NH_4^+ (Vallino et al. 1996). This higher energy requirement is primarily due to the need for active transport of oxidized N forms across the cell membrane and intracellular reduction to NH_4^+ (Lindell & Post 2001). In fact, some cyanobacteria lack (e.g., Garcia-Fernandez et al. 2004) or have lost (e.g., Miller & Castenholz 2001) the ability to assimilate oxidized N altogether.

Nitrogen control in cyanobacteria in regulated by NtcA, a 222-amino acid protein homologous to *E. coli* CAP (catabolite activator protein) and a transcriptional regulator of genes involved in N metabolism and cell differentiation (Herrero et al. 2001). NtcA is encoded by *ntcA*, a positive-acting element required for expression of genes regulated by N. Nitrogen status in cyanobacteria is sensed by the cells as changes in the 2-oxoglutarate pool, which reflects the cellular carbon-nitrogen balance, rather than the presence of NH_4^+ (Herrero et al. 2001). 2-oxoglutarate is involved in cellular N incorporation within the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway responsible for incorporating NH_4^+ into carbon skeletons (Flores & Herrero 2005). Thus, positive control of *ntcA* on other genes first requires cellular recognition of insufficient NH_4^+ assimilation into cell tissue and subsequent increases in *ntcA* gene transcription above basal levels (Lindell & Post 2001). NtcA has been found in all cyanobacteria genera evaluated to date, and quantification of *ntcA*

gene expression has been used to evaluate the N status of wild cyanobacteria populations (Lindell & Post 2001, Lindell et al. 2005, Penno et al. 2006, Junier et al. 2007).

Once the cell senses insufficient NH_4^+ assimilation, a N deficiency response is initiated, which starts with transcription of *ntcA* above basal levels (Lindell & Post 2001). The next step is *ntcA*-promoted induction of genes encoding high affinity transport mechanisms involving NH_4^+ and methyl-NH_4^+ permeases, which allow passive diffusion across the cell membrane (Muro-Pastor & Florencio 2003). These permeases allow cellular recapture of NH_4^+ released or leaked into the surrounding water. Activation of genes encoding proteins required for assimilation of NO_2^-, NO_3^-, urea, and amino acids follows at higher transcription levels of *ntcA* (Herrero et al. 2001). In response to the higher energy requirements for assimilation of alternate N sources, cell growth decreases in favor of synthesis of the proteins required for continued assimilation of these non-NH_4^+ substrates. All of these cellular responses to N deficiency are ceased if NH_4^+ becomes available (Lindell & Post 2001). Thus, it could be argued that cyanobacteria assimilating any N form other than NH_4^+ are actually experiencing N limited growth.

The final response to N deprivation depends on the ability of the cyanobacteria to fix atmospheric N_2 or not. Once all external, readily available N sources are exhausted, N-fixing genera undergo cellular differentiation to produce heterocytes, which are specialized cells that provide the anoxic environment required for nitrogenase-mediated N fixation (Zhang et al. 2006). Heterocyte differentiation is controlled by *ntcA* in cyanobacteria, and resumption of assimilation of other N sources, especially NH_4^+, will halt formation and activity of heterocytes (Valladares et al. 2008). For non-N-fixing cyanobacteria genera, the cell begins to degrade phycobiliproteins, resulting in chlorosis and loss of chlorophyll (Wyman et al. 1985). Chlorosis can sustain minimal cell growth for a short period of time, but cell growth ceases once all internal and external N sources are exhausted.

It is clear from the literature that the N form available to phytoplankton plays a critical role in determining the competitive outcomes and phytoplankton community structure observed in aquatic systems. The purpose of Chapter II is to evaluate ambient N availability

and phytoplankton community structure relative to literature-predicted outcomes (i.e., what is the preferred N form for the phytoplankton community present at temporally and spatially explicit sampling events?). However, the effects of other factors, such as light, temperature, and micronutrients, also must be considered but are mostly beyond the scope of this project.

1.1.2. Importance of water column NH_4^+ uptake and regeneration processes

Primary producers convert atmospheric or dissolved carbon dioxide to organic matter. Phytoplankton and bacteria assimilate nutrients as part of this process, and measurements of nutrient uptake are key components of primary productivity models and estimates. The concept of nutrient limitation began with von Liebig's Law of the Minimum (reviewed by de Baar 1994):

"When a given piece of land contains a certain amount of all the mineral constituents in equal quantity and in an available form, it becomes barren for any one kind of plant when, by a series of crops, only one of these constituents – as for example soluble silica – has been so far removed, that the remaining quantity is no longer sufficient for a crop."

This concept was extended by Brandt (1899; cited in de Baar 1994), who chose N as the common limiting nutrient for marine algae. This single-limiting nutrient hypothesis was quickly challenged by Nathansohn (1908; cited in de Baar 1994), who suggested that there is a difference between "maximal rate of production" (i.e., production is controlled by a single chemical factor) and "the rate at which production actually proceeds", which could be controlled by "all possible components". The subsequent interpretations of this debate reflected that phytoplankton production is controlled by several nutrients and maintained by the equilibrium between growth and loss terms (e.g., grazing; de Baar 1994). It could be argued that the P limitation paradigm for freshwater lakes (Schindler 1977) follows a Brandtonian view, while the Nathansohnian view would challenge the assumptions made to formulate the paradigm (Lewis & Wurtsbaugh 2008, Scott & McCarthy 2010).

Primary production in aquatic systems controlled by nutrient availability is referred to as "bottom-up" control, while grazing on primary producers by secondary consumers is called "top-down" control (e.g., Glibert 1998). As might be argued by Nathansohn, the "rate at which production actually proceeds" depends on the relative balance between these growth and loss terms. However, these controls are intricately linked, and changes in one factor will cause changes in the other (Glibert 1993).

Some studies have documented a gradual shift from P to N limitation along freshwater to estuarine to marine gradients (Doering et al. 1995). While coastal marine systems are usually considered N sinks (via denitrification), recent studies suggest that sediment N fixation also may be an important nutrient source for phytoplankton in these systems (Gardner et al. 2006; Fulweiler et al. 2007). These shifts in nutrient limitation status are not unique to the freshwater to marine gradient, however. Similar shifts are observed within the boundaries of individual freshwater systems as well. In Taihu Lake (China), a very large (~2400 km^2), shallow (mean depth = 1.9 m), and highly eutrophic system, TN:TP ratios > 16 (Redfield ratio) have suggested that the lake is P limited (Vant et al. 1998; Dokulil et al. 2000). However, evaluation of N transformations and microbial food web community structure along a gradient from river discharge to the main lake (a distance of ~20 km) during a summer cyanobacteria bloom revealed numerous characteristics consistent with N limitation in the main lake. These characteristics included low dissolved inorganic N (DIN) concentrations (< 0.5 μM), low molar DIN:PO_4^{3-} (12), low molar TN:TP (8 – 12), measurable SWI N fixation, no significant SWI N_2 efflux (i.e., lack of denitrification), net PO_4^{3-} flux into sediments, and presence of known N fixers in the water column (McCarthy et al. 2007c). Low NH_4^+:NO_x (0.2) also was observed in the main lake during this sampling event. These observations all suggested that the main lake became N limited (or at least co-limited) late in the growing season, leading to an observed shift from non-N-fixing cyanobacteria (e.g., *Microcystis*) to N fixing genera (e.g., *Anabaena* and *Aphanizomenon*). Low TN:TP ratios in the central basin have been observed in previous studies as well (Dokulil et al. 2000), and bioassays have confirmed seasonal N limitation (Xu et al. 2010).

Nutrient limitation status in freshwater systems may be related to distance from nutrient inflow sources (McCarthy et al. 2007c; Scott et al. 2008), temporally and spatially explicit events (McCarthy et al. 2007c), and hydrodynamic flow regimes (Elser 1999; McCarthy et al. 2007b). In the Taihu Lake example, distance from the nutrient source was likely an issue in the shift from apparent P limitation to N limitation. The lake area between the river inflow and the main lake is commonly impacted by severe cyanobacteria blooms, and the area close to the river inflow has very high N removal rates, both from water column phytoplankton uptake and denitrification in the sediments (McCarthy et al. 2007c). These factors, in addition to simple dilution over the ~20 km distance from the river inflow to the main lake, likely led to the observed N deficiency in the central basin of the lake. Distances from nutrient inputs to apparently N limited areas in the same system are not always long, however. In a small, constructed wetland in central Texas (USA), nutrient inputs from the source river were depleted rapidly along the flow path, with the system exhibiting evidence of severe N limitation within only a few hundred meters (Scott et al. 2008). As in Taihu Lake, the Texas wetland adjusted for N deficiency by development of N fixing communities, mostly as benthic cyanobacterial mats, and other N recycling mechanisms.

In small systems, such as the central Texas constructed wetland, where distances from the nutrient source are not great, system hydrology may play an important role in nutrient dynamics and limitation patterns (e.g., Clausen & Johnson 1990). This point is illustrated in a small, Great Lakes coastal wetland (Old Woman Creek, Lake Erie; OWC). Wave action and along-shore currents in Lake Erie lead to development of a sand barrier, which shuts down water exchange between OWC and Lake Erie, particularly in summer (Tomaszek et al. 1997). This barrier can be breached by storm events leading to high runoff into the wetland and, subsequently, Lake Erie (Klarer & Millie 1992). Residence time in the wetland is short (i.e., a few hours) when runoff events occur, which provides a nearly direct conduit for nutrient exchange between the mostly agricultural OWC watershed and Lake Erie. However, when conditions stabilize, and the sand barrier is in place, denitrification and nutrient uptake by primary producers leads to severe N depletion in the stagnant water column (McCarthy et al. 2007b). The system responded to this depletion in the same way as

was observed in the Texas wetland – by development of N fixing communities and increased importance of water column and sediment nutrient recycling mechanisms.

These examples illustrate why aquatic systems, regardless of salinity, must be evaluated individually to determine whether or not established nutrient limitation paradigms apply (Conley et al. 2009). As shown in these examples, it is premature to assume that P limits primary production because the systems are not saline. Another issue worthy of consideration in these cases is background nutrient concentrations and the possibility that nutrients are replete and do not limit primary production, regardless of availability ratios (e.g., Heath 1992). In all of the examples described above, areas of the system closest to the nutrient source are nutrient replete, especially during runoff events. While nutrient availability ratios may suggest P or N limitation, the capacity of the primary producers using these nutrients may be exceeded, thus leading to potential limitation by other factors, such as light (e.g., Aldridge et al. 1995; Phlips et al. 1997) or micronutrients (North et al. 2007).

As described above, NH_4^+ is the most efficient N source for photoautotrophs. This is also the case for bacterial primary producers, which are much more efficient at assimilating NH_4^+ over other N sources (Vallino et al. 1996, Vrede et al. 1998). Bacterial NH_4^+ uptake can account for up to 60% of total NH_4^+ uptake in aquatic systems (Glibert 1993). Phytoplankton photosynthesis is generally associated with light energy, but nutrient uptake by phytoplankton in the absence of light can occur in nutrient limited phytoplankton (Cochlan et al. 1991). While this phenomenon complicates differentiation of autotrophic (light-dependent) and heterotrophic (light-independent) nutrient uptake, it is still reasonable in most cases to use light/dark incubations as a proxy for distinguishing phytoplankton versus bacterial uptake. This is particularly the case when longer incubations are used (e.g., 24 hours), since phytoplankton incubated in the dark for more than a few hours would not be able to sustain dark uptake, whereas bacterial NH_4^+ uptake would not be light-dependent (Glibert 1988). It should be noted that dissolved organic N (DON) often is a significant N source for primary producers (e.g., Glibert 1993), particularly as urea and amino acids. The role of DON in phytoplankton and bacterial nutrition and community structure is beyond the intended scope of this project, but its potential importance as a N source is acknowledged.

Nutrient limited primary production often is sustained by nutrient regeneration processes occurring in the water column, such as bacterial remineralization and protist excretion (e.g., Dugdale & Goering 1967; Eppley & Peterson 1979; Glibert et al. 1982; Flint 1984; Fisher et al. 1988; Glibert 1988; Kirchman 2000; Gardner et al. 2004; Raimbault & Garcia 2008). Regeneration processes become more important in systems where inputs of new nutrients are low or episodic and dependent on terrestrial runoff, in which case regeneration can sustain production between episodic discharges (Glibert 1988; Dham et al. 2002). In the Mississippi River plume, high NO_3^- discharges in spring lead to a diatom bloom associated with high NO_3^- uptake rates and low importance of NH_4^+ regeneration (Bode & Dortch 1996). However, in summer, low river inputs of NO_3^- result in low primary productivity fueled primarily by NH_4^+ regeneration. A similar trend was observed in Chesapeake Bay, where N demand in spring was higher than regeneration, but N regeneration exceeded N demand by three times in fall (Bronk et al. 1998). These results suggest that autotrophic production dominates when nutrient inputs are high in spring, but heterotrophic regeneration sustains system productivity throughout the summer dry season.

The purpose of Chapter III is to investigate water column NH_4^+ regeneration and uptake and N fixation rates in Missisquoi Bay within the growing season. These rates may be dependent on and play a role in determining phytoplankton community structure, particularly when cyanobacteria comprise a large proportion of the total phytoplankton.

1.1.3. Sediment-water interface nutrient fluxes and N transformations

Nutrient regeneration in sediments underlying the water column also can be an important nutrient source for primary producers (Flint & Kamykowski 1984; Flint 1985, Tobias et al. 2003). In some stratified systems, sedimentation of diatoms may decrease nutrient remineralization rates in the water column (Poister & Armstrong 2004), which may lead to an increasing role for sediment recycling in these cases. Sediments are the primary location for organic matter degradation and remineralization in shallow aquatic systems (Tobias et al. 2003). Sustained external nutrient loading into a system results in high water column productivity and, ultimately, high organic matter deposition and degradation rates in

sediments (Beman et al. 2005). Remineralized nutrients in the sediments can enter the water column as a result of physical forcing (e.g., sediment resuspension; Fan et al. 2001), chemical remobilization (e.g., anoxia), or diffusion. In some cases, internal nutrient regeneration and release from sediments can exceed external nutrient inputs and delay system recovery from nutrient input reductions (e.g., Burger et al. 2007). In systems where diatoms dominate, sediments are expected to play a more important role in system nutrient recycling (Poister & Armstrong 2004), while water column recycling may be more important when cyanobacteria are dominant (e.g., McCarthy et al. 2007c; McCarthy et al. 2009a).

In eutrophic aquatic systems, sediment nutrient regeneration is dominated by organic matter remineralization to NH_4^+, or ammonification (Tobias et al. 2003). The fate of this regenerated NH_4^+ plays a critical role in system nutrient status, phytoplankton and bacterial productivity, phytoplankton community structure, and organic matter processing. Potential fates for regenerated NH_4^+ are summarized in Figure 1.1 (taken from Brandes et al. 2007), although it should be pointed out that release or diffusion into the overlying water column is a potential fate with important consequences for system productivity. The most direct fate involves re-assimilation into biomass. The classical paradigm proceeds to nitrification, which involves NH_4^+ oxidation to NO_2^- (via N_2O), then NO_3^-. The two steps of nitrification are carried out by different bacteria, since no known organism can perform both reactions (Ward et al. 2007). Nitrate from nitrification then can be denitrified, which is the heterotrophic reduction of NO_3^- to N_2 (via NO_2^-, NO, and N_2O; e.g, Seitzinger 1988, Zumft 1997). Denitrification is a poorly constrained (Galloway et al. 2004) N sink in the global N budget. It may drive systems toward N limitation and also may be a natural eutrophication defense (Seitzinger 1988; Bartkow & Udy 2004). The importance of this coupling increases when microphytobenthic communities are photosynthesizing at the sediment-water interface (e.g., Dong et al. 2000; An & Joye 2001). DIN sources for denitrification in sediments include remineralization of organic matter and diffusion from the water column (Cornwell et al. 1999). Sediments accumulating easily degraded organic matter tend to have higher denitrification rates, particularly from water column NO_3^- diffusion (Dong et al. 2000; Dahllöf & Karle 2005). Both nitrification and denitrification often proceed at rates far below their potential in freshwater wetlands (Bowden 1987).

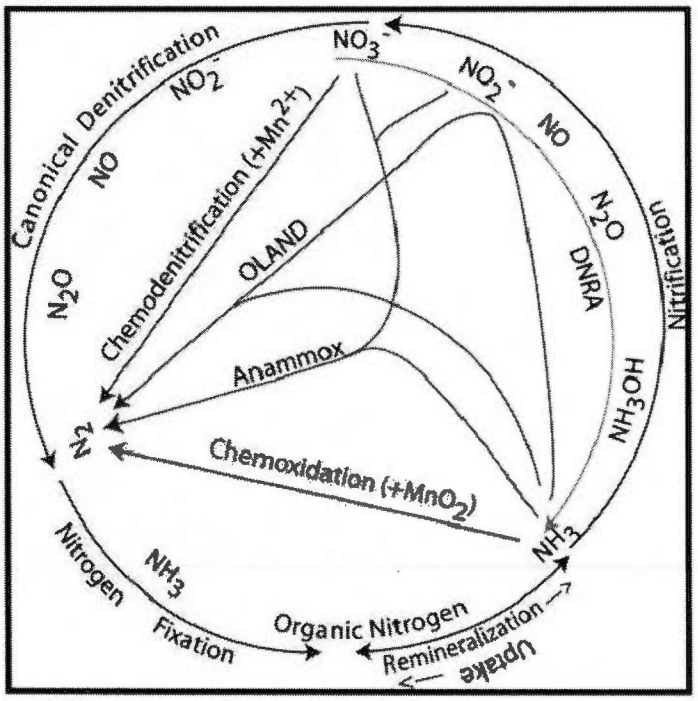

Figure 1.1. Conceptual diagram of the nitrogen cycle (from Brandes et al. 2007).

This simplistic view of the nitrogen cycle dominated in the literature until the 1990's. Recent discoveries have added to our understanding of the N cycle and have characterized additional pathways beyond the nitrification/denitrification regime. The DNRA pathway has been recognized for some time, but difficulties in measuring the process have inhibited our understanding of its importance and distribution in aquatic systems. DNRA usually is associated with sulfidic estuarine or marine sediments but also can account for 30% of NO_3^- reduction in lake sediments (Tiedje 1988; Brunet & Garcia-Gil 1996). DNRA, previously called reammonification (Bowden 1987), has been evaluated in only a few freshwater systems (Burgin & Hamilton 2007) and accounted for varying degrees of internal N recycling. In two lakes on the North Island of New Zealand, DNRA was not observed in the shallower lake (Lake Rotorua) but accounted for 7 – 12 % of total sediment NH_4^+ flux in the deeper lake (Lake Rotoiti; McCarthy et al. 2007a). Previous investigators working in Lake Rotoiti observed NH_4^+ accumulation in the hypoxic hypolimnion during stratification periods

(Priscu et al. 1986), and DNRA may have accounted for some of this NH_4^+ accumulation. DNRA accounted for a similar fraction of total sediment NH_4^+ flux (~10 %) in a Great Lakes coastal wetland (McCarthy et al. 2007b). DNRA also was observed, at varying degrees of importance, in a constructed wetland in central Texas (Scott et al. 2008).

The relative partitioning between DNRA and denitrification is an important factor for aquatic systems. The processes are not mutually exclusive and often occur simultaneously (e.g., Bonin et al. 1998; Kelly-Gerreyn et al. 2001; An & Gardner 2002; Gardner et al. 2006; Gardner & McCarthy 2009). However, some systems exhibit only one process, such as a Danish estuary, which only reduced NO_3^- to N_2 (Binnerup et al. 1992). The presence or absence of free sulfide (H_2S) or the occurrence of sulfate reduction in sediments has been shown to favor DNRA or denitrification, respectively (Brunet & Garcia-Gil 1996). Temperature also is an important factor determining the fate of reduced NO_3^-, with extreme temperatures (i.e., < 14 and > 17 °C) favoring DNRA (Kelly-Gerreyn et al. 2001). The different end-products of each process, NH_4^+ and N_2, respectively, have different and significant ramifications for the system N budget (Bowden 1987; Kemp et al. 1990; Bonin et al. 1998). Denitrification results in N loss from the system, and this N is not available to most organisms until it is fixed back into organic matter, which is an energetically unfavorable process (Capone 2000). However, DNRA returns reduced N to the system in a bioavailable form (NH_4^+) favored by most primary producers (Bowden 1987; Bonin et al. 1998).

Denitrification usually is considered the only biological process capable of removing fixed N from aquatic systems and returning it to the atmosphere (Seitzinger 1988; Blackburn & Blackburn 1992). Recent discoveries have shown that this paradigm is not accurate (Hulth et al. 2005; Burgin & Hamilton 2007). Anammox is an alternate pathway to N_2 (Rysgaard et al. 2004), but its significance in freshwater systems is unknown (Burgin & Hamilton 2007). Potential anammox was evaluated recently in Lake Tanganyika, where it may have accounted for 7 – 13% of total N_2 production (Schubert et al. 2006). Anammox in marine systems is better understood, and evidence suggests that water depth and temperature are factors determining anammox activity in marine systems (Dalsgaard et al. 2005). The relationship to

water depth may be due to the correlation with water temperature, since anammox bacteria have a lower optimum temperature (12 °C) than denitrifiers (24 °C; Jetten 2001).

The anammox mechanism was proposed in 1941 (Hamm & Thompson 1941) and again in the mid-1970's (Barnes et al. 1975) but not confirmed until later in a denitrifying fluidized bed reactor treating effluent from a methanogenic reactor (Mulder et al. 1995). Anammox is inhibited by simple organic compounds (e.g., pyruvate, ethanol, and glucose; Jetten et al. 1999). Therefore, it has been hypothesized that anammox may be most important in low labile carbon sediments (Burgin & Hamilton 2007). Potential anammox can be evaluated by adding $^{15}NH_4^+$ in the presence of a sufficient $^{14}NO_x$ pool (e.g., Rysgaard et al. 2004) and measuring $^{29}N_2$ production using mass spectrometry. However, there are alternative explanations for $^{29}N_2$ production in this case, and molecular techniques may be required to confirm anammox. While the mechanism of N_2 formation and effects on carbon cycling are interesting, the end product of both processes is the same; i.e., N is removed from the system and must be fixed to become available to non-diazotrophic primary producers.

Sediment-water interface nutrient fluxes and N transformations are expected to play a significant role in mediating phytoplankton in Missisquoi Bay, Lake Champlain, and these transformations are investigated in Chapter IV. A thorough characterization of N fluxes and transformations at the sediment-water interface, including potential N sources (N fixation), sinks (denitrification/anammox), and links (recycling, including DNRA; Gardner et al. 2006), is needed to determine if these processes contribute to conditions favorable for cyanobacteria bloom formation, maintenance, species succession, and senescence (e.g., Elser 1999).

1.2. Study site description

Lake Champlain is located along the New York/Vermont border in the northeastern United States and also reaches into Québec, Canada (Figure 1.2). The lake is 170 km long, has a mean depth of 22.8 m (maximum depth ~ 122 m), and a surface area of 1,130 km^2 (Smeltzer & Quinn 1996). The watershed covers about 20,000 km^2, and outflows from the

lake discharge to the St. Lawrence River. Most (56%) of the watershed is in Vermont, with smaller proportions located in New York (37%) and Québec (7%; Stickney et al. 2001). Average annual precipitation is about 1 m (Weller et al. 1996). The main axis of the lake is meso- to oligotrophic, but shallow bays in the northeastern and southern sections are eutrophic (Smeltzer & Quinn 1996, Mihuc et al. 2005).

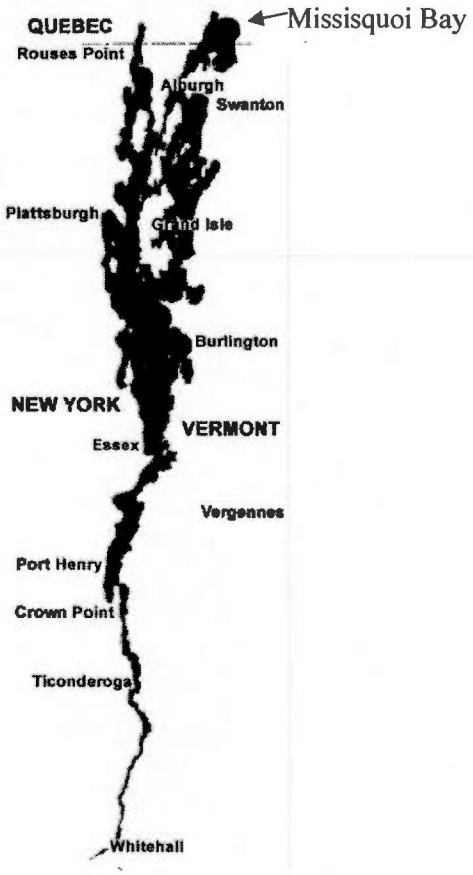

Figure 1.2. Map of Lake Champlain showing the location of Missisquoi Bay.

Like most temperate lakes, nutrient research in Lake Champlain has focused on P (e.g., Smeltzer & Quinn 1996, Weller et al. 1996), and very little is known about N cycling. A survey of limnological conditions in the lake (not including Missisquoi Bay) in 1970 reported NO_3^- concentrations ranging from 0.7 – 106 µM (Gruendling & Malanchuk 1974).

Lake Champlain phytoplankton were determined not to be limited by nutrients in spring but were limited by P in summer (Levine et al. 1997). However, these authors noted that N exerts an influence worthy of additional investigation. It also should be noted that this study was conducted in areas south of Missisquoi Bay. Another interesting finding of the Levine et al. study was the apparent absence of NH_4^+ (< 1.4 μM, the analytical detection limit), with DIN comprised entirely of $NO_3^- + NO_2^-$. Another study estimated wet and dry N deposition from the atmosphere to Lake Champlain and concluded that wet deposition of NO_3^- and NH_4^+ is a significant N source to Lake Champlain, particularly to the watershed (Hicks 2007). A lake-wide survey of phytoplankton community structure in 2002 revealed that, in contrast to previous surveys, cyanobacteria communities were dominated by non-N-fixing genera, such as *Microcystis* (Mihuc et al. 2005). This was particularly the case in northern sections of the lake, including Missisquoi Bay. The authors suggested that this structural shift may be the result of several factors, including changes in nutrient regimes, invasion by zebra mussels, and climate warming. However, it should be noted that zebra mussels have not invaded Missisquoi Bay in large numbers to date (Lake Champlain Basin Program; http://www.lcbp.org/nuissum.htm#zebramus).

Missisquoi Bay is a small (~77.5 km^2), shallow bay in northeast Lake Champlain (Mendelsohn et al. 1997; Figure 0.2). Maximum depth is approximately 4 m, mean depth is 2.8 m, and major tributaries into the bay include the Missisquoi, Rock, and Pike Rivers. The Missisquoi and Rock Rivers drain watersheds located mostly in the United States, while the Pike River drains an agricultural watershed located mostly in Québec. Approximately 40% of the watershed for Missisquoi Bay is located in Québec (Smeltzer & Simoneau 2008). Despite a cross-border agreement to reduce P loading, recent evaluations suggest that total P loads have increased by 13% over 1991 loads (Smeltzer & Simoneau 2008). Hydrodynamic models showed that a bridge separating the bay from Lake Champlain does not significantly inhibit water exchange or flushing times (Mendelsohn et al. 1997). Missisquoi Bay has had annual, late-summer, toxic cyanobacteria blooms consisting primarily of *Microcystis* in recent years (Mihuc et al. 2005), although this was not the case in 2006, when a late summer bloom was dominated by *Anabaena* (Davis et al. 2009). In 2006, elevated nutrient levels (as DIN and dissolved inorganic P) in Missisquoi Bay preceded peak bloom densities, which then

coincided with lower nutrient levels, likely due to phytoplankton uptake (Davis et al. 2009). Maximum bloom densities also coincided with maximum water temperatures, and N additions significantly increased the growth rates of toxic *Microcystis* strains.

Water column and sediment-water interface N cycling rates were measured at two sampling sites in Missisquoi Bay (Figure 1.3). These two sites were chosen to represent spatial differences between major inflows (PRM; Pike River mouth) and open water conditions (MB; middle bay). PRM was expected to have higher nutrient concentrations and coarser sediments (Mendelsohn et al. 1997) due to its proximity to riverine discharges, while MB was expected to have lower nutrient concentrations and fine, muddy sediments. The distance between these sampling sites was approximately 3 km. Water depths at these sites vary depending on lake levels but were expected to be about 1 m at PRM and about 4 m at MB. Water clarity was expected to be greater at MB than PRM due to suspended solids discharged by the river.

Sampling events included the growing seasons (approximately May to October) in 2006, 2007, 2008, and 2009. In 2007, water column and sediment core samples were collected on June 12 (PRM only), June 25, and August 27. The sampling regime was expanded in 2008 and included field collections on May 12, June 2, June 25, July 2, August 4, August 11, and October 7. The 2009 program focused on attempting water column N fixation measurements and included water collections from both sites on May 26, June 17, July 3, September 9, and September 23. In addition, water column NH_4^+ cycling and sediment core incubations were conducted on July 9 and September 23, 2009.

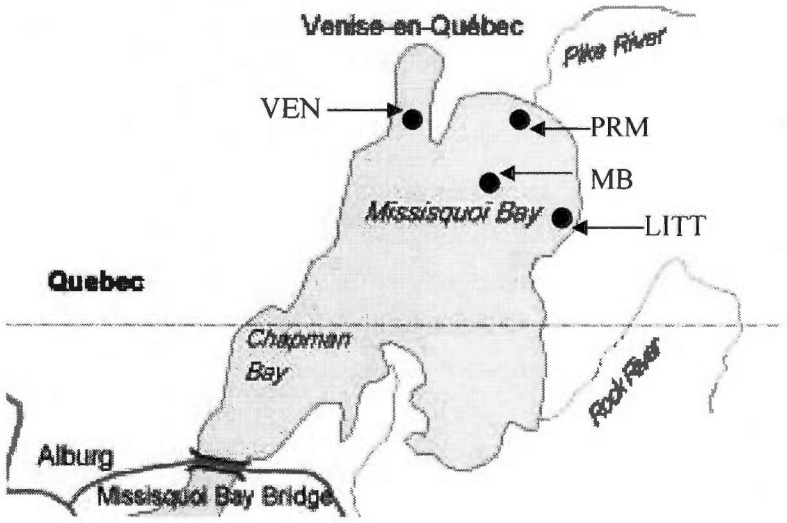

Figure 1.3. Map of Missisquoi Bay showing location of sampling sites PRM (Pike River mouth) and MB (middle bay). LITT and VEN are the locations of additional sampling sites from the Missisquoi biweekly monitoring program. Dashed line approximates the USA/Canada border.

1.3. Approaches used

1.3.1. Ambient nutrients and phytoplankton community structure

In Chapter II, ambient nutrient concentrations measured during the project at the two focus sites were evaluated for seasonal and spatial trends, particularly relative to the concentrations of various N forms available for assimilation by phytoplankton. Individual nutrients evaluated included dissolved P (as soluble reactive P; SRP) and dissolved N forms (NH_4^+, NO_2^-, and NO_3^-). Missisquoi Bay data from an ongoing sampling program in southern Québec lakes also were used (D.F. Bird, unpublished data) in combination with measurements associated with this project. Similarly, seasonal trends in phytoplankton community structure within the growing season from samples collected during this project and the southern Québec lakes sampling program were analyzed by grouping individual

species by their N acquisition and competitive strategies. Phytoplankton groups included diatoms, non-N-fixing cyanobacteria, and N-fixing cyanobacteria, although other groups (e.g., cryptophytes, chlorophytes, and dinoflagellates) were present.

1.3.2. Water column NH_4^+ regeneration, potential NH_4^+ uptake, and N fixation

In Chapter III, water column NH_4^+ regeneration and potential uptake rates measured in Missisquoi Bay were evaluated for seasonal trends relative to ambient N concentrations and forms. Light and dark incubations provided information on the importance of autotrophic and heterotrophic organisms, respectively. Theoretically, autotrophic production by phytoplankton is light dependent, while heterotrophic production by bacteria or mixotrophs should remain active in the dark. Measurements of these processes were achieved using the isotope dilution technique (Blackburn 1979, Caperon et al. 1979, McCarthy et al. 2009a). This technique involved the addition of excess substrate as $^{15}NH_4^+$, so uptake rates are qualified as "potential", although the isotope additions, in some cases, were significantly lower than ambient NH_4^+ concentrations. Regeneration rates for NH_4^+ are not "potential" because the added isotope represents the end-product of regeneration, rather than the substrate. However, this approach required the assumption that turnover times for added $^{15}NH_4^+$ are longer than the incubation time (~ 24 hours). Water column regeneration rates were depth-averaged (e.g., McCarthy et al. 2007a, 2007b) to allow comparison with areal sediment-water interface regeneration rates described in Chapter III.

A novel idea for measuring water column N fixation using MIMS was developed and tested. This technique involved incubating site water in gas-tight vessels (i.e., ground-glass stoppered tall test tubes used for other dissolved gas measurements) with an intact phytoplankton assemblage and monitoring N_2:Ar over time. Since gas solubility in water is temperature dependant, and N fixation often requires light energy, these physical parameters were controlled throughout the incubation. Filtered site water incubated in an identical manner was evaluated as a control. If N fixation occurred in the water column, a decrease in N_2:Ar was expected, and a rate was determined given sample volume and incubation time.

1.3.3. Sediment-water interface nutrient fluxes, DNRA, O_2 demand, and N transformations

Sediment nutrient fluxes, DNRA, SOD, and N transformations at the SWI were evaluated using continuous-flow incubations of intact sediment cores collected from the two sampling sites in Missisquoi Bay. This technique is based on the system described by Lavrentyev et al. (2000) and has been used in numerous other shallow aquatic systems (e.g., An et al. 2001; An & Gardner 2002; Gardner et al. 2006; McCarthy et al. 2007a, b, and c; Scott et al. 2008; Gardner et al. 2009; Gardner & McCarthy 2009).

SWI nutrient fluxes were determined as the concentration difference between inflowing and outflowing water samples normalized for system flow rate and sediment surface area in the cores. These results determined whether the sediments acted as a source or sink for the various bioavailable nutrients. These fluxes may be important determinants of the nutrient pool available to primary producers in the water column. In contrast, these fluxes may be affected by changes in water column nutrient concentrations from uptake and regeneration processes and external inputs.

Potential DNRA was determined as $^{15}NH_4^+$ production from $^{15}NO_3^-$ added to inflow water. $^{15}NH_4^+$ production rates were determined using HPLC (An & Gardner 2002; Gardner et al. 2006) on samples also used to measure total NH_4^+ concentrations. Inflow and outflow samples collected from the core incubations were analyzed for total NH_4^+ concentration and isotope ratio (Gardner et al. 1995a), and DNRA rates were calculated as $^{15}NH_4^+$ concentration differences in inflow and outflow samples given flow rate and sediment surface area. Potential DNRA rates in $^{15}NO_3^-$ additions were compared to control cores, and DNRA was considered significant only if the $^{15}NH_4^+$ production was significantly higher in the $^{15}NO_3^-$ addition treatment than the control cores. DNRA rates were qualified as "potential" because substrate ($^{15}NO_3^-$) was added in excess of ambient concentrations. However, these rates actually may be conservative because they did not include DNRA occurring with NO_3^- formed during sediment nitrification. In addition, cation exchange processes in the sediments may result in preferential adsorption of $^{15}NH_4^+$ onto sediment particles, resulting in increased total NH_4^+ efflux from sediments (Gardner et al. 2006). This "nitrate-induced ammonium

flux" (NIAF) was compared to measured $^{15}NH_4^+$ fluxes, and discrepancies were attributed to cation exchange anomalies.

Net O_2 flux into sediments reflects the O_2 consumed during organic matter degradation. Sediment cores were incubated in the dark (foil wrapped), so benthic photosynthesis producing O_2 was assumed to be insignificant. Net O_2 flux in intact sediment core incubations was measured using MIMS in dissolved gas samples collected from inflow and outflow water. Results from unamended cores were used as estimates of in situ SOD. In spring and early summer, sediment organic matter concentrations generally are high due to spring runoff, sinking diatomaceous material, and accumulation of waste excretions from grazers feeding on spring phytoplankton. Warming temperatures accelerate decomposition and SOD. Organic matter decomposition also results in a complex network of sharp redox gradients within the sediments, including anoxic microsites (e.g., Rao et al. 2008). These gradients are important for N transformations occurring at or just below the SWI, such as nitrification (aerobic) and denitrification (anaerobic), which, in turn, are important factors determining nutrients available for primary producers in the water column. DNRA and anammox, anaerobic processes, also are expected to depend on these sharp redox gradients. If the water column becomes stagnant and/or stratifies, SOD also may result in bottom-water hypoxia, but this situation is not common in Missisquoi Bay (Mendelsohn et al. 1997). However, in the absence of large external inputs of fresh organic material during summer in Missisquoi Bay, sediment organic matter available for decomposition may become limiting, and SOD may decrease as summer progresses. Organic matter limitation also would affect N transformations, such as nitrification and denitrification. Therefore, denitrification rates and SOD were expected to be positively related, and the importance of denitrification coupled to nitrification was expected to decrease as summer progresses.

The core incubation technique presented two possible avenues for identifying the importance of N fixation occurring at the SWI. First, net N_2 flux measured in control cores (i.e., no isotope additions) represents the balance between denitrification (N_2 efflux) and N fixation (N_2 influx). The direction of sediment N_2 flux identifies which of these processes is more important during the incubation, and a negative net N_2 flux would suggest that N

fixation rates exceed denitrification. Second, isotope pairing techniques allow estimation of sediment N fixation occurring simultaneously with denitrification (An et al. 2001). Sediment N fixation, presumably carried out by bacteria without requirements for light energy (e.g., Fulweiler et al. 2007), has been observed in several aquatic systems where N deficiency persists or develops seasonally. Fixation of atmospheric N_2 represents a new source of organic material for microbial decomposition, and this new material can affect ambient nutrient concentrations and forms available for primary producers. N fixation can help alleviate N deficiency caused by denitrification and high N uptake rates. In Missisquoi Bay, expected late summer N deficiency may be partially mitigated by N fixation occurring in the water column by phototrophic cyanobacteria and at the SWI by bacteria.

Similar to sediment N fixation, the methods used offer a few avenues for evaluating the importance of denitrification, and isotope pairing of N_2 formed via denitrification of added isotopes ($^{15}NH_4^+$ or $^{15}NO_3^-$) quantified denitrification potential and identified the substrate for denitrification (i.e., NO_3^- produced via nitrification versus denitrification of water column NO_3^-). First, net N_2 flux provided a direct measurement of denitrification in the absence of N fixation. If isotope pairing calculations in the $^{15}NO_3^-$ addition treatment failed to quantify any sediment N fixation occurring simultaneously with denitrification, then net N_2 flux in control cores was assumed to be the best estimate of ambient denitrification occurring in Missisquoi Bay sediments. If isotope pairing calculations showed a significant sediment N fixation rate, then that rate was added to the net N_2 flux in control cores to determine the best estimate of sediment denitrification rate.

Second, denitrification potential, as the sum of $^{28,\ 29,\ \text{and}\ 30}N_2$ production in cores enriched with $^{15}NO_3^-$, quantifies maximum denitrification rates if substrate (i.e., NO_3^-) is not limiting. If net N_2 flux in control cores is not significantly different from potential denitrification, then it can be concluded that some other factor (i.e., organic matter, temperature, or competition for NO_3^- with DNRA) is limiting N removal from the system. Potential anammox was evaluated by measuring $^{29}N_2$ production from $^{15}NH_4^+$ added to inflowing water. This technique does not conclusively identify anammox as a N sink, because other combinations of N transformations could explain the measured $^{29}N_2$. The absence of

$^{29}N_2$ production in the presence of added $^{15}NH_4^+$ and a sufficient $^{14}NO_x$ pool would, however, suggest that anammox is not occurring. Molecular techniques likely would be required to confirm anammox, but these methods are beyond the scope of this study.

The ion source within the quadrupole mass spectrometer used in MIMS ionizes gases prior to detection and produces O^+ ions, which can react with N_2, forming NO (mass 30; Jensen et al. 1996). This scavenging of N_2 is more significant at low O_2 concentrations, such as those found in sediments, and may result in over-estimation of denitrification rates via interference with ratio measurements (Eyre et al. 2002; Kana and Weiss 2004). This error appears to be machine-specific and has not been observed on the MIMS at Université du Québec à Montréal (UQÀM; McCarthy and Bird, unpublished data). This effect can be monitored in control cores (i.e., no isotope addition) by measuring mass 30 production, and subsequent denitrification estimates can be corrected by subtracting the control mass 30 production from these denitrification estimates. However, this procedure was not necessary in any case experienced with the MIMS instrument at UQÀM.

1.3.4. Relationships between N transformations, ambient nutrients, and phytoplankton community structure

In Chapter V, ambient N concentrations were compared to phytoplankton community structure for the time period of study. Specifically, the hypothesis that NH_4^+ availability relative to oxidized N (as NH4:NOx) would drive the relative proportions of cyanobacteria versus diatoms to total phytoplankton was tested. When NH_4^+ is high relative to NOx (NH4:NOx > 1), it was anticipated that cyanobacteria would dominate the phytoplankton community (as biomass), while diatoms would dominate at low NH4:NOx (c.f., McCarthy et al. 2009b). When both of these inorganic N forms are at low concentrations, cyanobacteria species capable of N fixation should be at a competitive advantage over other phytoplankton groups. It was further hypothesized that water column and SWI N transformations affect observed NH4:NOx and, subsequently, phytoplankton community structure.

If Missisquoi Bay follows a standard phytoplankton progression from spring diatoms to summer cyanobacteria, as observed in 2002 (Mihuc et al. 2005), then NO_x may be depleted relative to NH_4^+ (i.e., NH4:NOx > 1) by NO_3^-competitive diatoms (and denitrification of water column NO_3^-) by mid to late summer, when cyanobacteria are expected to become more competitive for scarce, reduced N forms. As described previously, NH_4^+ is assimilated preferentially over other DIN forms by phytoplankton when it is available. Therefore, NH_4^+ uptake over the course of the growing season in Missisquoi Bay would lead to a lower NH4:NOx in the absence of substantial external inputs or regeneration. As the cyanobacteria community depletes NH_4^+ (i.e., NH4:NOx < 1), those species capable of fixing atmospheric N_2 (e.g., *Anabaena*), while remaining competitive for N made available by regeneration or external sources, would be expected to have a competitive advantage over those that cannot fix N (e.g., *Microcystis*). Similarly, excess NH_4^+ relative to NO_x was expected to result in higher NH_4^+ uptake rates.

As aquatic systems become N deficient, some phytoplankton (Jochem 1999), and especially cyanobacteria (Shi et al. 2007), are capable of dark nutrient uptake (Cochlan et al. 1991; Flynn et al. 2002). In fact, *Microcystis* showed increased metabolic activity, no cell death, and no degradation of *chlorophyll-a* fluorescence, whereas a green algae was unable to survive, under dark, anaerobic conditions (Shi et al. 2007). Therefore, it was expected that light versus dark NH_4^+ uptake differences would be smaller when cyanobacteria dominate the phytoplankton community in Missisquoi Bay.

Cyanobacteria blooms can depend on and affect water column nutrient regeneration processes (Sörensson and Sahlsten 1987). The relative unpalatability of cyanobacteria, including *Microcystis* (e.g., Sellner et al. 1993; Vanderploeg et al. 2001), may decrease water column NH_4^+ regeneration rates and NH4:NOx. However, the importance of nutrients regenerated in the water column, relative to sediments, increases during cyanobacteria blooms (Presing et al. 2001). Therefore, conditions where nutrients are made available to phytoplankton primarily via regeneration processes in the water column were expected to be more suitable for cyanobacteria. In contrast, low NH4:NOx favoring diatoms also favors grazers, since diatoms generally are more palatable than cyanobacteria. Grazing activity

results in higher regeneration. Thus, low NH4: NOx was expected to result in higher regeneration and lower uptake rates.

Various data groupings also were evaluated. For example, data collected over the course of the project in multiple years were pooled by month, which proved valuable in evaluations of datasets from Lakes Okeechobee and Taihu (McCarthy et al. 2009b). Another technique that proved valuable in these evaluations was comparing phytoplankton community structure at given times with ambient nutrients in preceding or subsequent months. This exercise may identify cause/effect relationships between phytoplankton community structure and nutrients.

If sediment organic matter concentrations are high and the material is sufficiently labile, degradation and ammonification (resulting in NH_4^+ efflux from sediments, including any DNRA) combined with denitrification of water column NO_3^- (observed as NO_3^- influx into sediments and verified by direct measurements of N_2 production; see below) would lead to higher water column NH_4^+ concentrations and conditions more suitable for non-N-fixing cyanobacteria. However, as water column NO_3^- concentrations decrease, denitrification may continue via coupled nitrification-denitrification as long as there is still sufficient organic matter availability. This situation may result in a reversal of sediment NH_4^+ flux (efflux to influx, to fuel nitrification) and subsequent water column NH_4^+ depletion, which would favor a shift from non-N-fixers to N-fixing cyanobacteria in Missisquoi Bay.

Denitrification removes bioavailable N and returns it to the atmosphere in a form available only to diazotrophs. This N sink can drive systems to N limitation if external inputs (or N fixation) do not replace the bioavailable N. Denitrification can be driven by NO_3^- produced via NH_4^+ oxidation (coupled nitrification-denitrification) or by NO_3^- diffusing into sediments from the overlying water column. The source of the substrate for denitrification can affect ambient nutrient concentrations and forms. Coupled nitrification-denitrification results in a net loss of two NH_4^+ molecules ($NH_4^+ \rightarrow NO_3^-$ via nitrification followed by $NO_3^- \rightarrow N_2$) and lower NH_4^+ concentrations relative to NO_3^-, which should allow diatoms to be more competitive for available NOx. However, if nitrification is dependent on NH_4^+ from

ammonification of organic matter, then there would be no net effect on NH4:NOx, except that nitrified NH_4^+ is not available to diffuse into the water column.

Missisquoi Bay often exhibits very high water column NO_x concentrations (D.F. Bird, UQÀM, unpublished data), which can decline to near or below detection limits in summer. Given this observation, combined with consistent water column NH_4^+ concentrations until later summer, it was hypothesized that denitrification in Missisquoi Bay sediments is fueled primarily by diffusion of water column NO_3^-. In this case, denitrification would lead to higher NH_4^+ concentrations and conditions suitable for development of non-N-fixing cyanobacteria blooms. In turn, subsequent NH_4^+ depletion by these cyanobacteria would lead to system N deficiency and a community shift to N-fixing cyanobacteria.

Anammox would not affect ambient N ratios, in terms of NH_4^+ and NO_x, unless the required NO_2^- substrate originates from the first step of nitrification ($NH_4^+ \rightarrow NO_2^-$), in which case the result would be the same as that from coupled nitrification-denitrification. Very little is known about the distribution of anammox in freshwater systems (Burgin & Hamilton 2007). However, anammox generally is associated with cold, deep-water marine sediments and open ocean O_2-minimum zones (e.g., Dalsgaard et al. 2005), and its importance as a N removal pathway has been minimal in most shallow systems where it has been evaluated. Therefore, it was hypothesized that anammox, like coupled nitrification-denitrification, would not be an important process in Missisquoi Bay. If anammox were found to be a potentially important process, the isotopic techniques used here could not distinguish between anammox fueled by NO_2^- from partial nitrification, incomplete denitrification (NO_2^- is an intermediate between NO_3^- reduction and NO formation), or ambient NO_2^-.

1.4. Summary: objectives and hypotheses

This study aimed to describe the N dynamics in Missisquoi Bay relative to ambient nutrients and phytoplankton community structure. Little is known about N cycling in Missisquoi Bay and most other freshwater systems, while P dynamics have been studied

extensively. Measurements of ambient nutrients, phytoplankton community structure, and N transformation rates in the water column and at the SWI were used to answer questions and address hypotheses formulated based on the theory that reduced N favors the development and maintenance of cyanobacteria blooms.

In Chapter II, questions to be answered were: 1. What are the seasonal dynamics of ambient nutrient concentrations?; 2. What are the dominant phytoplankton genera in each season?; and 3. Based on what is known about these genera, which N form is more conducive to support their dominance?

In Chapter III, questions to be answered were: 1. What are water column NH_4^+ regeneration and potential uptake rates in the light and dark?; 2. What are water column N fixation rates?; and 3. What are depth-averaged water column NH_4^+ regeneration rates?

In Chapter IV, questions to be answered were: 1. What are sediment O_2 consumption rates?; 2. What are SWI nutrient fluxes?; 3. Is DNRA an NH_4^+ source to the water column, and, if so, what are the rates?; 4. What are sediment denitrification and N fixation rates?; 5. Do in situ denitrification estimates differ from denitrification potential rates?; and 6. Is anammox a possible pathway for N loss?

In Chapter V, questions and hypotheses synthesizing the results from the previous chapters were addressed. These include:

A. Are phytoplankton community structure and ambient N concentrations related?

Hypothesis: Higher NH4:NOx in summer will coincide with or precede higher cyanobacteria proportion of total phytoplankton biomass, and lower NH4:NOx in spring will coincide with higher proportions of diatoms. If true, is the N availability ratio (i.e., NH4:NOx) a cause or effect of phytoplankton community succession? In other words, is phytoplankton community structure driven by the N availability ratio, or is the N availability

ratio driven by the phytoplankton community structure? Cause/effect relationships can be evaluated by comparing ambient nutrient concentration and ratio data to phytoplankton community structure in previous or following sampling events (McCarthy et al. 2009b).

B. **Are water column NH_4^+ regeneration/uptake and/or N fixation rates related to phytoplankton community structure and/or ambient N concentrations?**

Hypotheses: 1. Water column NH_4^+ regeneration will be higher if more palatable phytoplankton (i.e., not cyanobacteria) are dominant, and potential NH_4^+ uptake will be higher when diatoms are not dominant (diatoms are more competitive for NO_3^-). 2. Water column N fixation rates will coincide with presence of known N fixers (e.g., *Anabaena*), low NH4:NOx, and low NH_4^+ concentration. These conditions are expected to occur in Missisquoi Bay in late summer, when NH_4^+ concentrations are depleted.

C. **Are sediment-water interface nutrient fluxes related to phytoplankton community structure and/or ambient N concentrations?**

Hypothesis: Sediments will be a net sink for water column NO_3^- (via denitrification), especially during summer. NH_4^+ flux (and DNRA) will be insignificant, leading to higher water column NH4:NOx from NO_3^- removal via denitrification and maintenance of ambient NH_4^+ concentrations via water column regeneration processes. Sediment regeneration, as nutrient fluxes from sediments, will decrease in importance subsequent to cyanobacteria bloom events, while water column regeneration processes will increase in importance for driving primary production.

D. **Does denitrification contribute to seasonal N limitation and increased proportion of N fixing cyanobacteria?**

Hypothesis: Higher denitrification rates will drive late-summer N limitation and lead to a cyanobacteria community shift to N-fixing species. Denitrification leads to removal of N

available for primary producers. As available N decreases, primary producers become N limited, and phytoplankton capable of gaseous N fixation become more competitive, especially if they are also effective competitors for scarce, reduced N forms (i.e., cyanobacteria).

E. **Is denitrification (and anammox) related to N species concentrations and ratios?**

Hypotheses: 1. Water column NO_3^- will drive sediment denitrification and lead to higher NH4:NOx. If denitrification is driven by and coupled to nitrification (oxidation of NH_4^+ to NO_3^-), then sediment NH_4^+ flux would decrease and lead to lower NH4:NOx. Isotope pairing of added $^{15}NO_3^-$ will provide information on the substrate for denitrification. 2. Anammox will not be a significant N removal mechanism in Missisquoi Bay. Anammox involves the oxidation of NH_4^+ with NO_2^- to form N_2. If anammox is important, the resulting effect on NH4:NOx will depend on the source of the NO_2^-. If the NO_2^- originates from the ambient NOx pool, then there would be no net effect on NH4:NOx, since one NH_4^+ and one NO_2^- will cancel in terms of the ratio. If the NO_2^- originates from the first step of nitrification (NH_4^+ oxidized to NO_2^-), then the net effect on the ratio would be the same as that from coupled nitrification-denitrification; i.e., two NH_4^+'s would be lost as N_2, resulting in a lower NH4:NOx. The NO_2^- source for anammox cannot be determined using isotopic tracer techniques without supporting, quantitative molecular techniques, which are beyond the scope of this project. However, anammox generally is not important in shallow systems (Hulth et al. 2005) and is not expected to be important in Missisquoi Bay.

CHAPTER II: AMBIENT NUTRIENTS AND PHYTOPLANKTON COMMUNITY STRUCTURE IN MISSISQUOI BAY, LAKE CHAMPLAIN, 2006-2009

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2.1. Summary

Nutrients and phytoplankton in Missisquoi Bay, Lake Champlain, were measured as part of two separate sampling efforts. First, a sampling program monitored limnological data in the lake approximately biweekly from 2006 – 2009. These results were combined with data obtained during sediment core and water column sampling for incubations to determine nitrogen (N) transformation rates during 2007-2009. The combined database was evaluated to identify spatial and seasonal trends and establish a limnological basis for interpretation of N transformation rates in later chapters. With a few exceptions in late summer, nutrients were replete in Missisquoi Bay and likely did not limit primary production. Colonial cyanobacteria (individual cell size < 2 μm) dominated phytoplankton cell counts but did not contribute significantly to total phytoplankton biomass. Cryptophytes dominated most sampling events in May, June, and October, while diatoms dominated in July and September. Mixed cyanobacteria assemblages dominated in August and were comprised mostly of potentially N-fixing genera with only a few heterocytes per 100 cells. Replete bioavailable N and phosphorus concomitant with low heterocyte abundances suggested that fine-scale N scarcity must occur to induce dominance of N-fixers and heterocyte differentiation in August. However, the sampling regime used in this study may not have had sufficient resolution to capture these spatially and/or temporally explicit events. The data suggest that other factors, such as light and temperature, were more important than nutrients in controlling primary production and phytoplankton community structure.

2.2. Introduction

Aquatic system productivity often is controlled from the "bottom-up". That is, nutrient availability controls primary productivity, which is then transferred to higher trophic levels. "Top-down" control refers to grazing from higher trophic levels controlling populations of primary producers and, thus, system productivity (Glibert 1998). Regardless of the ultimate controlling factors, system productivity reflects the balance between bottom-up (i.e., growth) and top-down (i.e., loss) controls. The main nutrients governing bottom-up effects on system productivity are phosphorus (P) and nitrogen (N). In marine systems, N is thought to usually limit primary production (e.g., Ryther & Dunston 1971), while P has historically been regarded as the more limiting nutrient in freshwater systems (e.g., Schindler 1974). Unlike P, N occurs in several bioavailable forms, which can be assimilated into biomass, regenerated and returned to the system, or lost as a result of various physical and microbial processes (e.g., denitrification; Seitzinger 1988). Bioavailable N forms include organic (e.g., urea) and inorganic N, including nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+). Of these inorganic forms, NH_4^+ is the most favorable for bacterial (Vallino et al. 1996) and phytoplankton assimilation (e.g., Syrett 1981), and NO_3^- is the least energetically favorable. In the case of the ubiquitous picocyanobacteria (e.g., *Synechococcus*; Lindell & Post 2001) and most other primary producers, oxidized N forms must be actively transported across cell membranes, then converted intracellularly to NH_4^+ before the N can be used for protein synthesis and growth (Syrett 1981).

Physiological differences in assimilative capacities for inorganic N forms lead to competitive differences between phytoplankton groups for available N forms. For example, diatoms have evolved to take advantage of the lower temperatures and higher oxidized N concentrations (e.g., Lomas & Glibert 1999a & 1999b) common during spring in lakes. Conversely, cyanobacteria have evolved to maximize their growth in the warm, relatively quiescent summer months (e.g., Paerl & Huisman 2008 & 2009), which often are characterized by low nutrient inputs and high microbial remineralization of organic matter generated during the spring bloom. By summer, algal uptake by diatoms and N sinks, such as denitrification, often have exhausted much of the N available for new production, and system

productivity becomes dependent on nutrients regenerated in sediments and the water column. Cyanobacteria have adapted to these conditions and are superior competitors for reduced N compounds (i.e., NH_4^+ and urea) produced by remineralization of degraded organic matter (Kappers 1980, Blomqvist et al. 1994, Hyenstrand et al. 1998a & 1998b). As cyanobacteria and microbial N sinks deplete bioavailable N forms, cyanobacteria capable of fixing atmospheric N_2 often increase (e.g., McCarthy et al. 2007c).

The N form available to primary producers thus directly affects the phytoplankton community assimilating the available resource. For example, in Lake Okeechobee (Florida, USA), which is a very large, shallow, subtropical lake influenced by annual cyanobacteria blooms, the ratio of NH_4^+ to oxidized N (NH4:NOx) was significantly correlated to the relative abundances of cyanobacteria (positive slope) and diatoms (negative slope; Figure 2.1; McCarthy et al. 2009b). In fact, these relationships improved significantly when phytoplankton community structure from one month was compared to NH4:NOx in the previous month, suggesting a diagnostic capacity for this relationship.

While P has been studied extensively, little is known about N availability and its effects on phytoplankton community structure in freshwater systems. Nutrient patterns in lakes often are characterized by total N (TN) and total P (TP) concentrations (e.g., Downing et al. 2001) and ratios (TN:TP; Smith 1983), leaving information gaps relative to seasonal and spatial trends in bioavailable, dissolved inorganic nutrients. The relationship between P and chlorophyll in lakes has been studied for decades, and a plot of TP concentration versus chlorophyll (Dillon & Rigler 1974) has been used to predict summer chlorophyll from a single TP measurement in spring. However, total nutrient concentrations (TN and TP) are autocorrelated with simultaneous measurements of phytoplankton biomass, including chlorophyll (Lewis & Wurtsbaugh 2008), because analyses for total nutrients consume phytoplankton biomass during digestion steps needed to quantify total nutrient concentrations. Therefore, the variables are not independent and must be correlated unless P is present in amounts far exceeding biological requirements. This issue is controversial and the subject of continued debate, but strong cases for the tautologous relationships between total nutrients and phytoplankton biomass have been made (e.g., Lewis & Wurtsbaugh 2008).

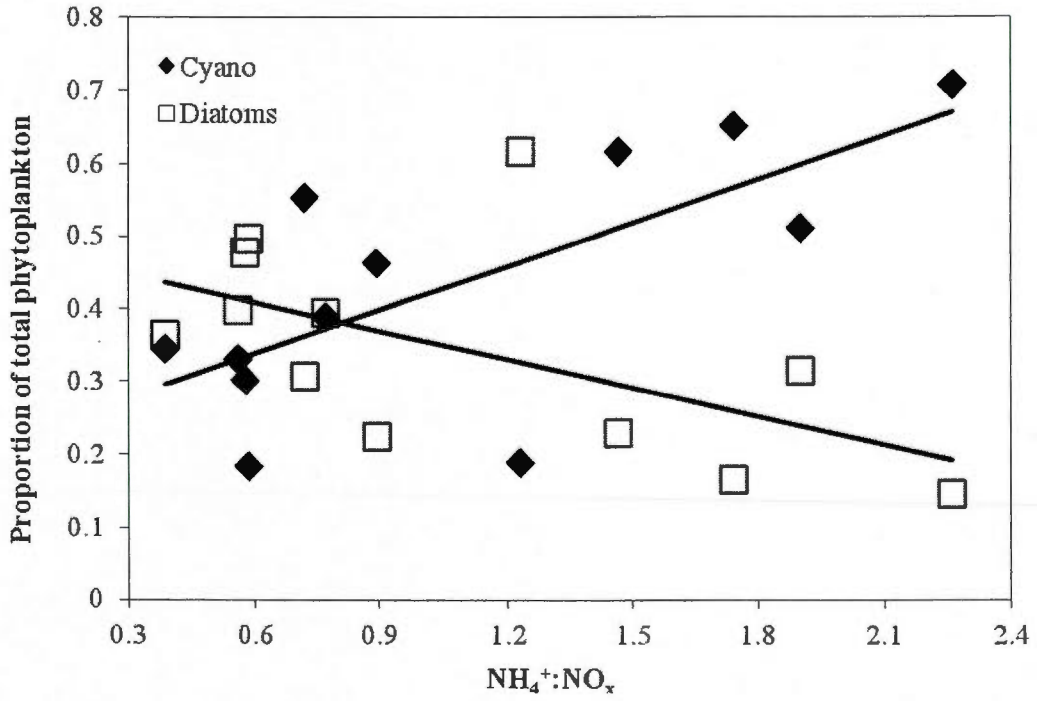

Figure 2.1. Relationships between monthly mean proportions of cyanobacteria (cyano; filled diamonds) and diatoms (open squares) to total phytoplankton biomass versus the ratio of ammonium (NH_4^+) to oxidized N (NO_x) in Lake Okeechobee, Florida, USA (taken from McCarthy et al. 2009b).

In this chapter, phytoplankton and nutrient monitoring data collected from Missisquoi Bay, Lake Champlain, from 2006 – 2009 were combined with data collected during sediment core and water collection events from 2007 – 2009 and evaluated to answer the following questions: (1) what are the trends of ambient nutrient concentrations during the growing season (May through October)?; (2) what are the dominant phytoplankton groups during the growing season?; (3) based on available literature, which N form present in Missisquoi Bay is most conducive to growth of the observed dominant phytoplankton group? Phytoplankton community structure was described in terms of broad functional groups and focused on cyanobacteria and diatoms. Where appropriate, cyanobacteria were considered

relative to their ability to fix atmospheric N. The primary objective of this effort was to form the limnological basis for hypotheses addressed in subsequent chapters by characterizing seasonal and spatial patterns of nutrients and phytoplankton in Missisquoi Bay.

2.3. Materials & Methods

Nutrient and phytoplankton data from Missisquoi Bay were collected as part of two separate sampling efforts. First, a monitoring program collected data from two locations in the lake, a pelagic (MB) and littoral (LITT; Figure 1.3) site, approximately biweekly during the growing seasons of 2006 – 2008 and weekly in 2009. In 2009, a third site was added near Venice-en-Québec (VEN; Figure 1.3). Depth-integrated water samples were collected using a pump and preserved for phytoplankton enumeration with Lugol's iodine. Phytoplankton counts were conducted using an inverted microscope and converted to biomass (Menden-Deuer & Lessard 2000). Nutrient samples were filtered on site using 0.45 µm syringe filters in 2009 and analyzed using standard colorimetric and autoanalyzer techniques. Unfortunately, nutrient sampling protocols varied each year, with some nutrient samples not filtered until return to the laboratory. Effects of this methodological change on results are not known. SRP and NO_2^- were not measured as part of the regular monitoring program.

The second sampling effort was a sediment coring and water column incubation program aiming to measure N transformation rates, which are described in subsequent chapters. These efforts were conducted in 2007 (3), 2008 (7), and 2009 (6) at two sites in Missisquoi Bay: one at the pelagic site described above for the monitoring program (MB) and the other at the mouth of the Pike River (PRM; Figure 1.3). Water samples for phytoplankton and nutrient analyses were collected from surface waters (~0.5 m depth) using a site-water rinsed 60 mL syringe. Phytoplankton samples were preserved with Lugol's iodine for enumeration as described above. Nutrient samples were filtered immediately on site using 0.2 µm syringe filters and frozen until analysis using standard colorimetric and autoanalyzer

methods, with the exception of NH_4^+, which was analyzed using high performance liquid chromatography (HPLC; Gardner et al. 1995a) at the University of Texas Marine Science Institute (UTMSI). SRP and NO_2^- were measured as part of this sampling effort.

Phytoplankton data were grouped into the following categories: chlorophytes, cryptophytes, chrysophytes, cyanobacteria, diatoms, dinoflagellates, and euglenophytes. The biomasses of organisms in each group were summed to determine proportions to total phytoplankton biomass for each sampling event and station. Proportions of diatoms and cyanobacteria to total phytoplankton biomass and actual biomasses were the focus of this study. Where appropriate, one-way analysis of variance (ANOVA) and linear regression were used to determine significant differences and trends, respectively, and a p-value < 0.05 was deemed significant. Means are presented plus/minus one standard error (SE). Two sampling events in early November 2006 were pooled with October data.

2.4. Results

2.4.1. Phosphorus in Missisquoi Bay

Monthly mean TP concentrations in Missisquoi Bay from 2006 – 2009 ranged from 1.46 ± 0.09 µM (n = 24) in June to 3.13 ± 0.46 µM (n = 30) in August (Figure 2.2). The overall mean TP concentration during the course of the study was 2.09 ± 0.11 µM (n = 166; median = 1.80 µM), and individual measurements ranged from 0.75 µM on 16 May 2006 at LITT to 14.8 µM on 11 August 2008, also at LITT. TP concentration was highest in Missisquoi Bay in 2006 and 2008 and lowest in 2009 (Table 2.1). LITT had the highest mean concentration for all years followed by MB and VEN. TP was not analyzed at PRM.

Monthly mean total dissolved P (TDP) concentrations ranged from 0.66 ± 0.05 µM in June (n = 22) to 1.02 ± 0.06 µM in August (n = 31; Figure 2.2). Individual measurements

ranged from 0.31 µM on 17 June 2009 at the pelagic station (MB) to 2.67 µM on 23 May 2007, also at MB. Median TDP for all observations was 0.82 µM (n = 162), which was similar to the overall mean of 0.88 ± 0.03 µM. On average, TDP accounted for 45% of TP. TDP concentration was highest in 2008 and lowest in 2009 (Table 2.1). TDP exhibited the same spatial pattern as TP, with highest TDP at LITT, followed by MB and VEN, but these differences were not significant. TDP was not measured at PRM.

Table 2.1. Summary of phosphorus (P) measurements in Missisquoi Bay in 2006 – 2009 by year and sampling site. All concentrations are in µmol P L^{-1}. SE = standard error. n = number of measurements. TP = total P. TDP = total dissolved P. SRP = soluble reactive P. N/A = not measured. LITT = littoral site. MB = pelagic site. VEN = Venice-en-Québec site. PRM = Pike River mouth site.

Year/Site	n	TP	SE	n	TDP	SE	n	SRP	SE
2006	42	2.43	0.18	42	0.95	0.07	N/A		
2007	26	2.03	0.18	26	0.83	0.08	4	1.18	0.39
2008	27	2.55	0.50	22	1.26	0.10	14	0.81	0.24
2009	71	1.74	0.08	72	0.76	0.03	16	0.37	0.07
LITT	81	2.48	0.19	79	0.93	0.05	2	0.17	0.01
MB	63	1.81	0.08	60	0.86	0.06	16	0.40	0.07
VEN	22	1.47	0.08	23	0.78	0.05	N/A		
PRM	N/A			N/A			15	1.01	0.23

SRP was only measured in sampling for sediment core and water column incubations. Thus, the total number of measurements is small compared to TP and TDP. Overall mean SRP was 1.21 ± 0.58 µM (n = 35), but the median concentration (0.40 µM) was lower. Individual measurements exhibited a wide range from 0.11 µM on 17 June 2009 at MB to 20.5 µM on 25 June 2007 at PRM. The next highest SRP concentration (3.39 µM) was

measured on 4 August 2008, also at PRM. Monthly mean SRP concentrations ranged from 0.26 µM in May (± 0.08; n = 8) and September (± 0.07; n = 4) to 3.05 ± 2.19 µM in June (n = 8; Figure 2.2). Excluding the highest measurement from 25 June 2009 resulted in an overall mean SRP of 0.65 ± 0.12 µM (n = 34) and a median concentration of 0.38. Mean SRP concentration for June becomes 0.87 ± 0.27 µM (n = 7) when the outlier is excluded. All analyses involving SRP from here forward also will exclude the outlier. Annual SRP concentrations were highest in 2007 and lowest in 2009 (Table 2.1). Only the 2009 value was significantly different from the others. SRP was highest at PRM and lowest at LITT. No SRP measurements were made in 2006 or at VEN.

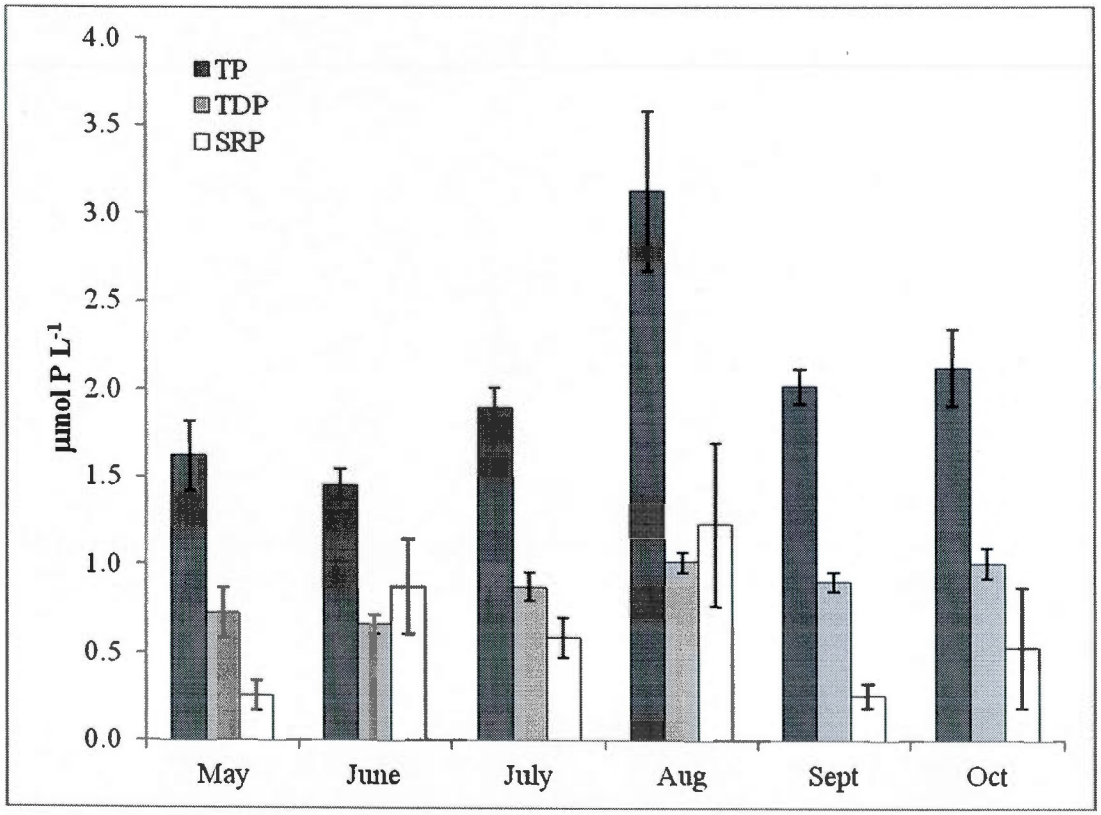

Figure 2.2. Monthly mean phosphorus concentrations in Missisquoi Bay from 2006 - 2009. Error bars are standard error. Note that the number of observations for soluble reactive phosphorus (SRP) is much fewer than for total P (TP) and total dissolved P (TDP). Graph does not include the SRP outlier discussed in the text.

2.4.2. Nitrogen in Missisquoi Bay

Overall mean TN concentration in Missisquoi Bay was 56.0 ± 4.46 μM (n = 164). The highest individual measurement was 733 μM at LITT on 11 August 2008, and the next highest measurement was 136 μM on 31 October 2006, also at LITT. The lowest individual TN measurement was 11.6 μM at MB on 30 May 2006. Excluding the high outlier from 11 August 2008, overall mean TN concentration was 51.9 ± 1.70 μM (n = 163; median = 44.4 μM). Monthly mean TN concentrations (excluding the outlier) ranged from 40.3 ± 1.95 μM (n = 35) in September to 59.7 ± 4.97 μM (n = 24) in June (Figure 2.3). Including the outlier gives a mean TN concentration for August of 79.8 ± 22.3 μM (n = 30). Annual mean TN concentrations were lowest in 2009 and highest in 2006 (Table 2.2). LITT had the highest TN concentrations followed by MB and VEN. TN was not measured at PRM.

Monthly mean TDN concentration ranged from 46.3 ± 4.89 μM (n = 22) in June to 27.4 ± 1.22 μM (n = 35) in September (Figure 2.3). TDN accounted for 73.7% of TN for the entire dataset. Overall mean TDN concentration was 37.7 ± 1.48 μM (n = 159; median = 31.4 μM). The highest and lowest individual TDN measurements were 129 μM on 31 October 2006 and 7.98 μM on 30 May 2006, respectively, both at LITT. There was no significant difference in TDN concentrations at LITT and MB, but TDN was lower at VEN (Table 2.2). The annual TDN pattern was similar to that of TN, with highest concentrations in 2006 and 2008 and lowest concentrations in 2007 and 2009. TDN was not measured at PRM.

Dissolved inorganic oxidized N ($NO_x = NO_3^- + NO_2^-$) exhibited high variability, with values ranging from undetectable on 27 August 2007 at MB to 337 μM on 2 June 2008 at PRM. The highest measurement not from PRM was 119 μM on 31 October 2006 at LITT. Monthly mean NO_x concentrations ranged from 1.86 ± 0.42 μM in September (n = 29) to 48.7 ± 14.7 μM in June (n = 29; Figure 2.3). Overall mean NO_x concentration was 23.6 ± 3.39 μM (n = 166; median = 8.42 μM). For the entire dataset, NO_x accounted for 22.9% of TN when NO_x data from PRM are excluded (no TN measurements were made at PRM). Annual mean NO_x concentrations were highest in 2006 and 2008 and lowest in 2007 and 2009. Mean NO_x concentration was highest at PRM and lowest at VEN.

Table 2.2. Summary of nitrogen (N) measurements in Missisquoi Bay in 2006 – 2009 by year and sampling site. All concentrations are in μmol N L^{-1}. n = number of measurements. SE = standard error. TN = total N. TDN = total dissolved N. NOx = dissolved inorganic oxidized N (NO_3^- + NO_2^-). NH4 = ammonium (NH_4^+). N/A = not measured. LITT = littoral site. MB = pelagic site. VEN = Venice-en-Québec site. PRM = Pike River mouth site.

Year/Site	n	TN	SE	n	TDN	SE	n	NOx	SE	n	NH4	SE
2006	42	67.6	3.87	41	50.6	3.80	42	27.3	4.03	42	5.58	0.54
2007	26	46.7	2.92	25	32.4	2.39	29	16.4	3.96	30	3.41	0.37
2008	24	59.2	4.44	22	43.8	3.02	24	47.2	16.5	26	4.16	0.71
2009	72	42.1	1.77	71	30.2	1.53	71	16.3	4.58	11	5.58	1.54
LITT	78	59.1	2.76	76	41.0	2.40	64	19.1	2.90	43	4.80	0.40
MB	63	49.6	2.19	60	38.2	2.22	66	13.1	1.86	50	4.07	0.57
VEN	23	33.3	1.26	23	25.2	1.00	20	4.27	1.03	N/A		
PRM	N/A			N/A			16	109	23.5	16	6.02	0.88

In sediment core and water column N cycling sampling events, NO_3^- and NO_2^- were distinguished. Nitrite concentrations ranged from undetectable on 27 August 2007 at MB and 23 September 2009 at PRM to 4.76 μM on 2 July 2008 at PRM. Excluding one sampling event in October 2009 at MB, where NO_2^- comprised 70.5% of DIN (NO_2^- = 0.43 μM; DIN = 0.61 μM), NO_2^- accounted for only 3.55% of DIN (TN was not measured at PRM). Mean NO_2^- concentration was 1.38 ± 0.22 μM (n = 30; median = 1.04 μM).

Overall mean NH_4^+ concentration was 4.64 ± 0.33 μM (n = 109; median = 4.09 μM). The lowest and highest individual NH_4^+ measurements were 0.15 μM on 3 September 2008 and 21.6 μM on 22 August 2006, respectively, both at MB. Monthly mean NH_4^+ concentrations ranged from 2.42 ± 0.39 μM in September (n = 22) to 6.40 ± 1.26 μM in May (n = 13; Figure 2.3). When both NH_4^+ and TN were measured (n = 85), NH_4^+ accounted for 8.3% of TN. PRM had the highest mean NH_4^+ concentration and MB and LITT had similar

values (Table 2.2). High NH_4^+ concentrations were measured in 2006 and 2009, while lower concentrations were measured in 2007 and 2008. Ammonium measurements from the monitoring program in 2009 were not used because the sample analyses were unreliable and inaccurate. All NH_4^+ data from 2009 resulted from sediment core and water column sampling for N cycling incubations, with subsequent analyses via HPLC at UTMSI. Ammonium was not measured at VEN.

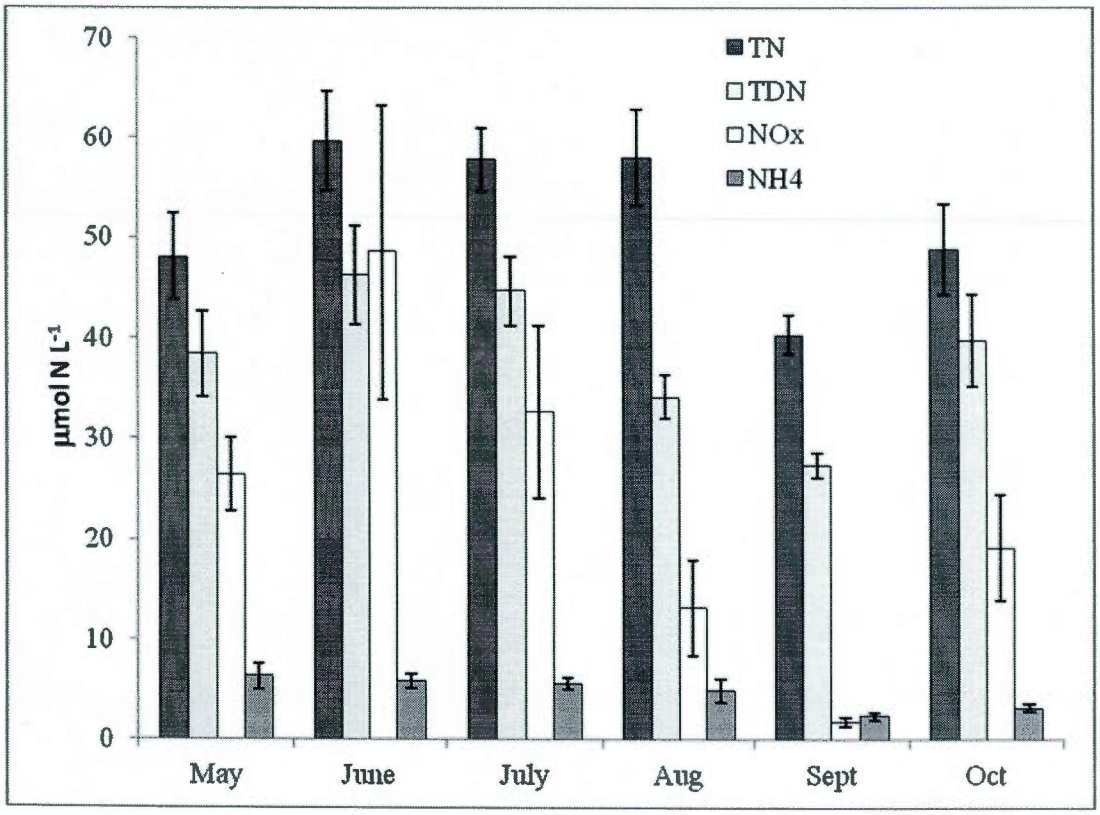

Figure 2.3. Monthly mean nitrogen concentrations in Missisquoi Bay from 2006 - 2009. Error bars are standard error. TN = total nitrogen. TDN = Total dissolved N, NOx = inorganic oxidized N (NO_3^- + NO_2^-). NH4 = ammonium. Graph does not include the TN outlier discussed in the text.

Using the monthly mean TN and TP concentrations, molar TN:TP ranged from 18.6 in August to 41.0 in June and averaged 27.1 ± 3.42 (n = 6). This value was not significantly different from the mean TN:TP calculated monthly from individual measurements (29.1 ± 3.10; n = 6). TN:TP ratios in 2006 (32.3 ± 2.42; n = 42) and 2008 (35.5 ± 3.67; n = 21) were significantly higher than ratios in 2007 (25.1 ± 1.97; n = 26) and 2009 (26.0 ± 1.01; n = 71). TN:TP at VEN (23.8 ± 1.28; n = 22) was significantly lower than the ratios at MB (31.0 ± 1.92; n = 61) and LITT (28.4 ± 1.35; n = 77). In the dissolved fractions, molar TDN:TDP from monthly means ranged from 30.5 in September to 70.0 in June and averaged 46.3 ± 6.01 (n = 6). This mean value did not differ significantly from the mean of individual measurements (50.5 ± 7.57; n = 6) within each month. TDN:TDP was significantly higher than TN:TP at all sites and in all years except 2008 (41.3 ± 4.43; n = 17). DIN was dominated by NO_x (i.e., NH4:NOx < 1) in all months except September. All other monthly mean NH4:NOx ratios were < 0.38, and all annual and site mean NH4:NOx ratios were < 0.35.

2.4.3. Phytoplankton in Missisquoi Bay

Monthly mean chlorophyll *a* concentrations (*chl*) were lowest in October (7.64 ± 0.92 μg L^{-1}; n = 33) and highest in August (31.5 ± 9.81 μg L^{-1}; n = 28; Figure 2.4). Overall mean *chl* was 15.5 ± 1.93 μg L^{-1} (n = 166; median = 8.60 μg L^{-1}). The highest individual *chl* (260 μg L^{-1}) was on 11 August 2008 at LITT during a cyanobacteria bloom consisting of a mixed assemblage dominated by *Microcystis flos-aquae* and *Anabaena flos-aquae*. The next highest observation (103 μg L^{-1}) was one week later at LITT but dominated by *Anabaena* spp., with decreased *Microcystis* biomass. Annual average *chl* concentrations were highly variable, with the lowest value in 2007 (3.73 ± 0.15 μg L^{-1}; n = 26), intermediate values in 2006 (11.0 ± 1.61 μg L^{-1}; n = 42) and 2009 (14.4 ± 1.91 μg L^{-1}; n = 63), and the highest mean in 2008 (31.0 ± 7.51 μg L^{-1}; n = 36). VEN had the lowest mean *chl* (8.52 ± 1.14 μg L^{-1}; n = 21), LITT had the highest (19.2 ± 3.77 μg L^{-1}; n = 80), and MB (13.0 ± 1.61 μg L^{-1}; n = 63) and PRM (15.3 ± 6.57 μg L^{-1}; n = 3) had intermediate values.

Colonial cyanobacteria (individual cells < 2 μm) dominated the cell counts in nearly all cases, and the most abundant species was *Aphanothece clathrata brevis*. However, these

cells rarely contributed significantly to total phytoplankton biomass. This pattern was observed at all sites and in all years and months, with only a very few exceptions, particularly during cyanobacteria blooms. As a group, cyanobacteria accounted for 88.5% of all cells counted in 2007 – 2009 (cell counts were not available for 2006).

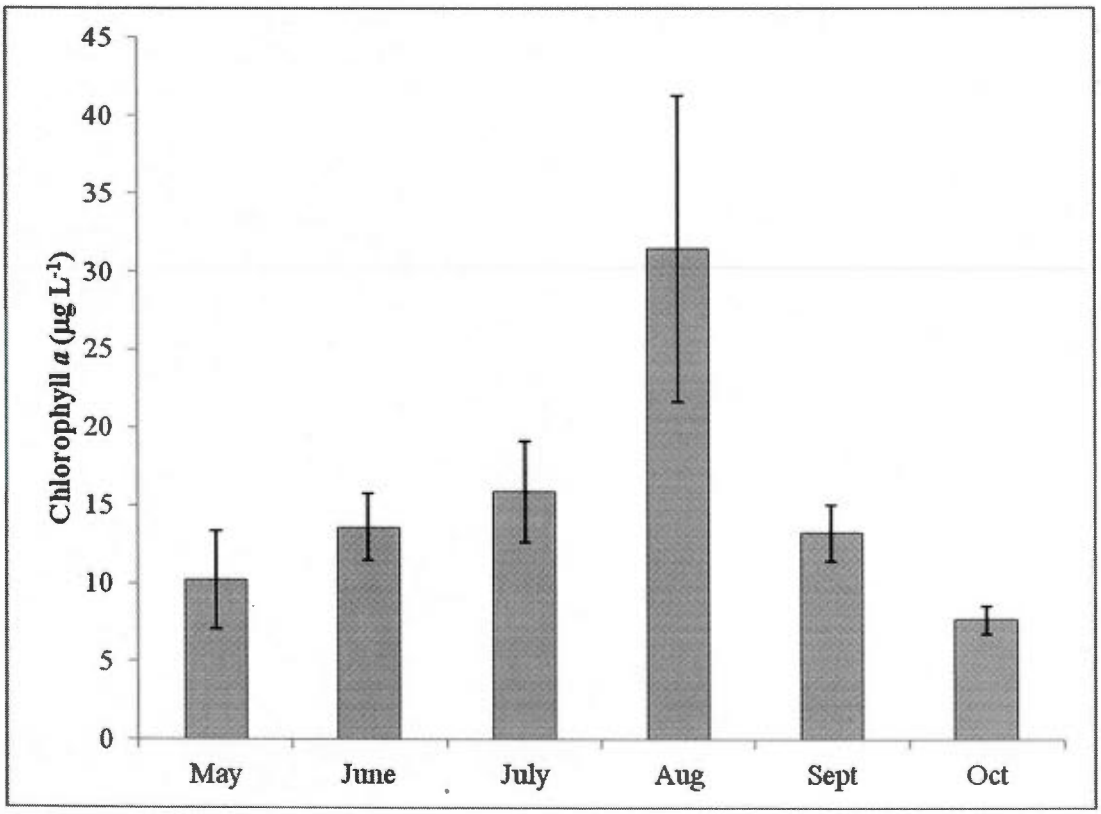

Figure 2.4. Monthly mean chlorophyll a concentrations in Missisquoi Bay from 2006 – 2009. Error bars reflect one standard error.

Lakewide, Missisquoi Bay phytoplankton biomass was dominated most often by diatoms (32.6%) followed by cryptophytes (31.4%), cyanobacteria (23.3%), and dinoflagellates (9.3%). LITT was dominated most often by cryptophytes (32.9%) followed by diatoms and cyanobacteria (25.3% each) and dinoflagellates (12.7%). MB was dominated

most often by diatoms (40.3%) followed by cyanobacteria (25.8%), cryptophytes (24.2%), and dinoflagellates (8.1%). Diatoms also dominated at VEN (47.8%), followed by cryptophytes (26.1%), and cyanobacteria (17.4%). Cyanobacteria never dominated phytoplankton biomass at PRM, but cryptophytes dominated seven of 10 sampling events, followed by diatoms twice and chlorophytes once.

Annually, cyanobacteria dominated phytoplankton biomass most often only in 2006 (16 times), followed closely by cryptophytes (15). Cyanobacteria dominated biomass only once (5 September at MB) in 2007, which was dominated by cryptophytes (11) and dinoflagellates (10). Diatoms most commonly dominated biomass in 2008 (17 times), followed by cyanobacteria (8) and cryptophytes (7). Diatoms also dominated most sampling events in 2009 (30 times), followed by cryptophytes (21) and cyanobacteria (15). Monthly mean biomasses for cyanobacteria (bC) and diatoms (bD) are presented in Figure 2.5, and monthly mean proportions of cyanobacteria (pC) and diatom (pD) biomass to total phytoplankton biomass are presented in Figure 2.6. Monthly mean bD and bC at each sampling site are shown in Figures 2.7 and 2.8, respectively, and corresponding proportions of total phytoplankton biomass are shown in Figures 2.9 and 2.10, respectively.

Phytoplankton biomass in May was dominated by cryptophytes on 12 of 15 sampling events, with diatoms dominating on the other 3 occasions (all in 2009). Mean bD in May was lowest at LITT and highest at VEN (n = 1). Diatoms accounted for 49.4% of total biomass at VEN on the only sampling occasion in May, ~25% at PRM and MB, and only 12.8% at LITT. Cyanobacteria comprised < 1% of total phytoplankton biomass at all sites in May except LITT. Cryptophytes also dominated phytoplankton biomass in June (20 of 26 sampling events). Diatoms dominated on five sampling events. Diatom biomass in June was lowest at PRM and highest at VEN, but these values were not different from intermediate values at LITT or MB. Diatom proportion of total phytoplankton biomass was higher in June versus May for LITT and MB but lower for PRM and VEN. Cyanobacteria did not account for more than 2.5% of total phytoplankton biomass at any site in June. Diatoms dominated phytoplankton biomass in July on 20 of 35 occasions, and mean bD ranged from only 182 μg C L^{-1} on the only sampling event in July at PRM to > 4000 μg C L^{-1} at all other sites. Except

for PRM, pD was highest in July at all sites. Again with the exception of PRM, bC and pC increased at all sites in July. Mean bC's were highly variable at all sites in July, and pC's ranged from 16.5 ± 6.1% at MB to 26.5 ± 7.4% at LITT.

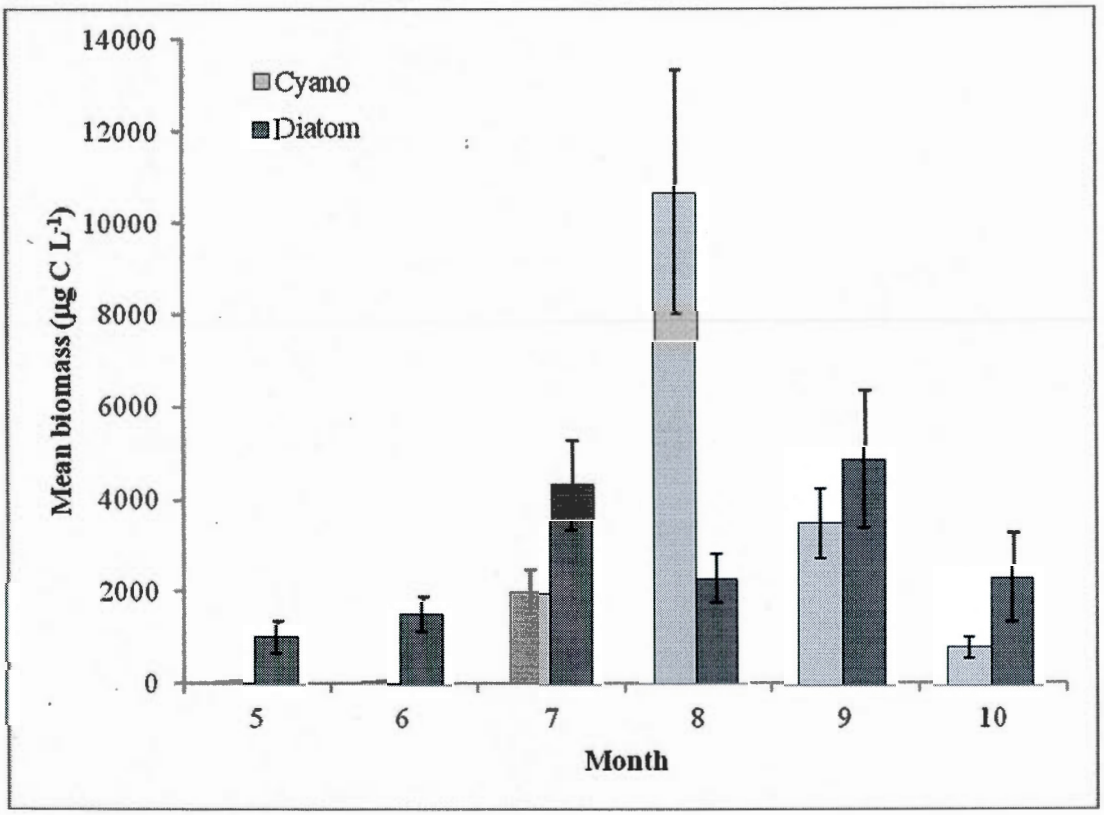

Figure 2.5. Monthly mean biomass (µg C L^{-1}) for cyanobacteria (Cyano) and diatoms in Missisquoi Bay from 2006 – 2009. Error bars reflect one standard error.

Monthly mean bC was highest in August at all sites except PRM, while bD decreased at all sites except PRM. Cyanobacteria (63.3%) most often dominated the phytoplankton community followed by diatoms (16.7%) and dinoflagellates (13.3%). At LITT, mean bC was 16600 ± 5320 µg C L^{-1} (n = 14), accounting for 63.4 ± 9.5% of total phytoplankton biomass. Cyanobacteria also dominated at MB in August with mean biomass of 6490 ± 1770

μg C L^{-1} (n = 12) and accounting for 46.5 ± 8.6% of total biomass. On the one sampling event in August where phytoplankton were counted at PRM, bC was 669 μg C L^{-1} and accounted for 23.1% of total phytoplankton, and pD was 73.8%. Diatoms and cyanobacteria were co-dominant at VEN in August, with each accounting for > 40% of total phytoplankton.

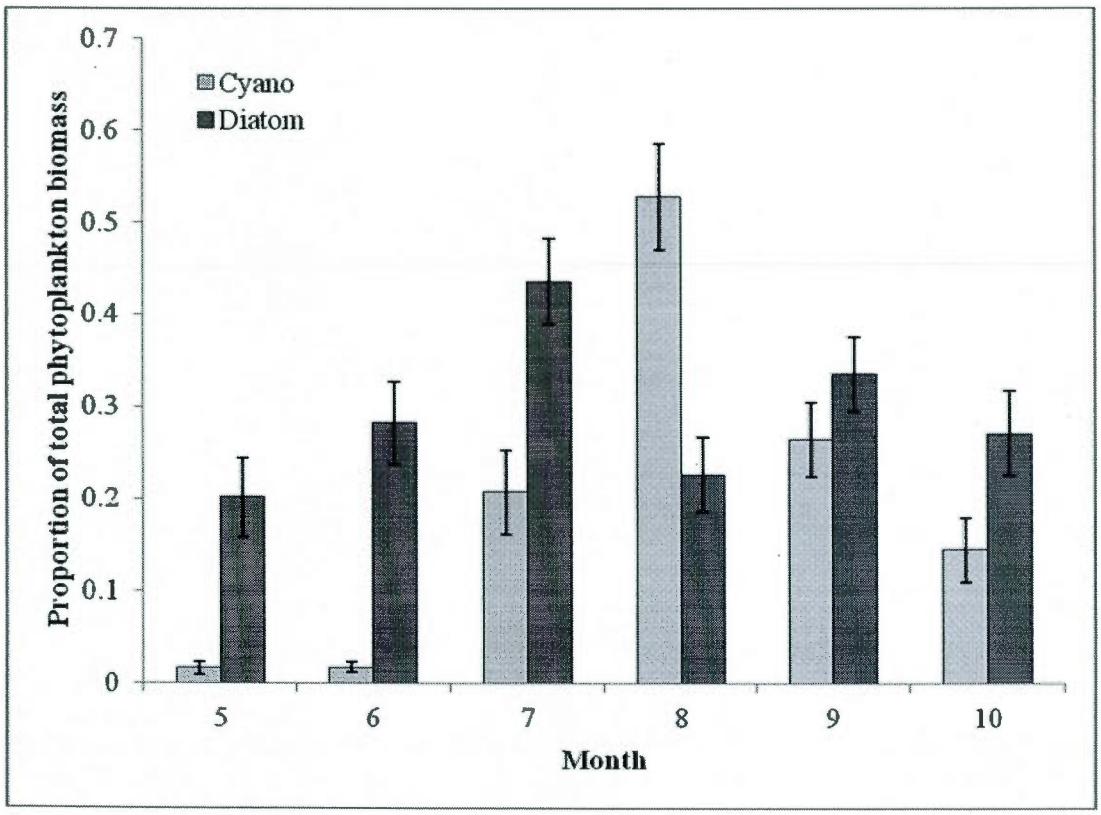

Figure 2.6. Monthly mean proportions of total phytoplankton biomass for cyanobacteria (Cyano) and diatoms in Missisquoi Bay from 2006 – 2009. Error bars reflect one standard error.

Cyanobacteria biomass decreased in September, mostly in favor of diatoms, which most often dominated the phytoplankton community (44.4%), followed by cyanobacteria (25%) and dinoflagellates (22%). At LITT, mean bC dropped to 3770 ± 1270 μg C L^{-1} (n =

15) and accounted for 26.7 ± 6.3% of total phytoplankton, while bD increased to 6600 ± 3160 µg C L^{-1} (32.3 ± 7.0%). A similar pattern was observed at MB, where diatoms accounted for 36.8 ± 5.2% of total phytoplankton and bD increased. At PRM, bC and bD did not change significantly from August. However, the cyanobacteria population at VEN collapsed in September, with biomass dropping to only 91.1 ± 51.1 µg C L^{-1} (n = 5). Diatom biomass also decreased, but not significantly.

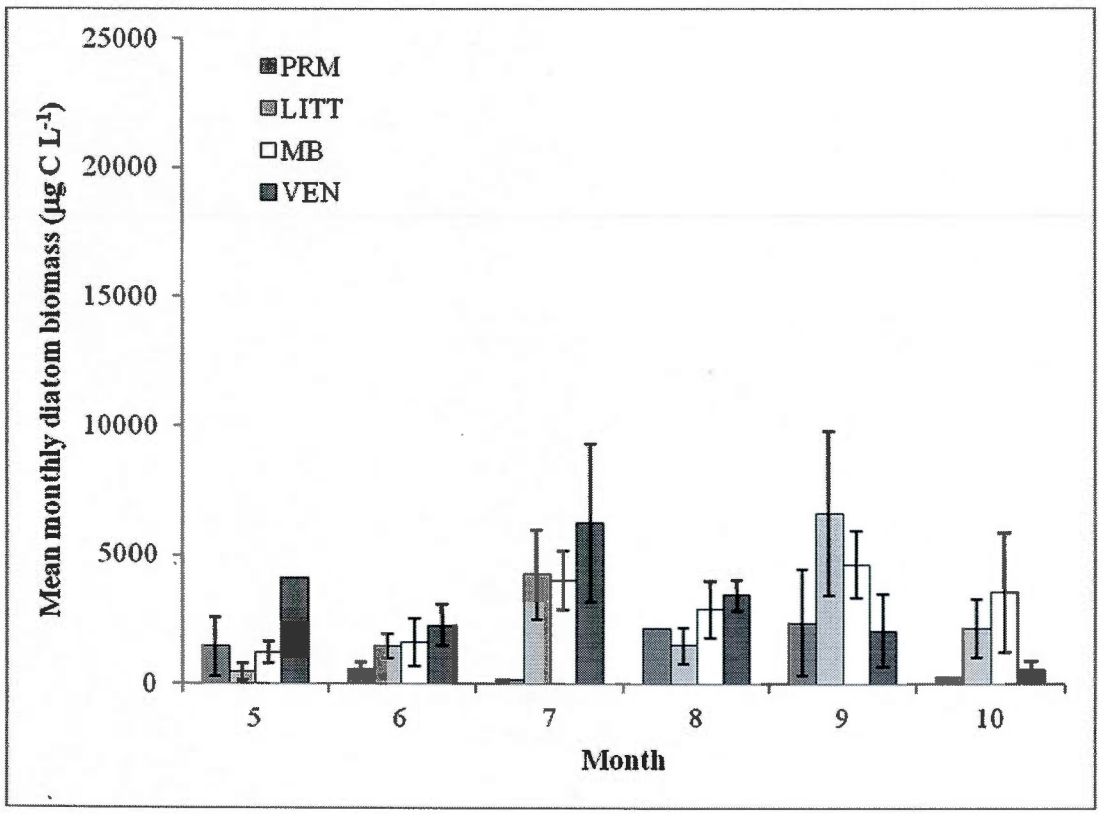

Figure 2.7. Mean monthly diatom biomass (µg C L^{-1}) at sampling sites in Missisquoi Bay from 2006 – 2009. PRM = Pike River mouth. LITT = littoral site near Philipsburg. MB = pelagic site offshore from Philipsburg. VEN = pelagic site offshore from Venice-en-Québec. Error bars reflect one standard error. Note that the y-axis scale is the same as Fig 2.8.

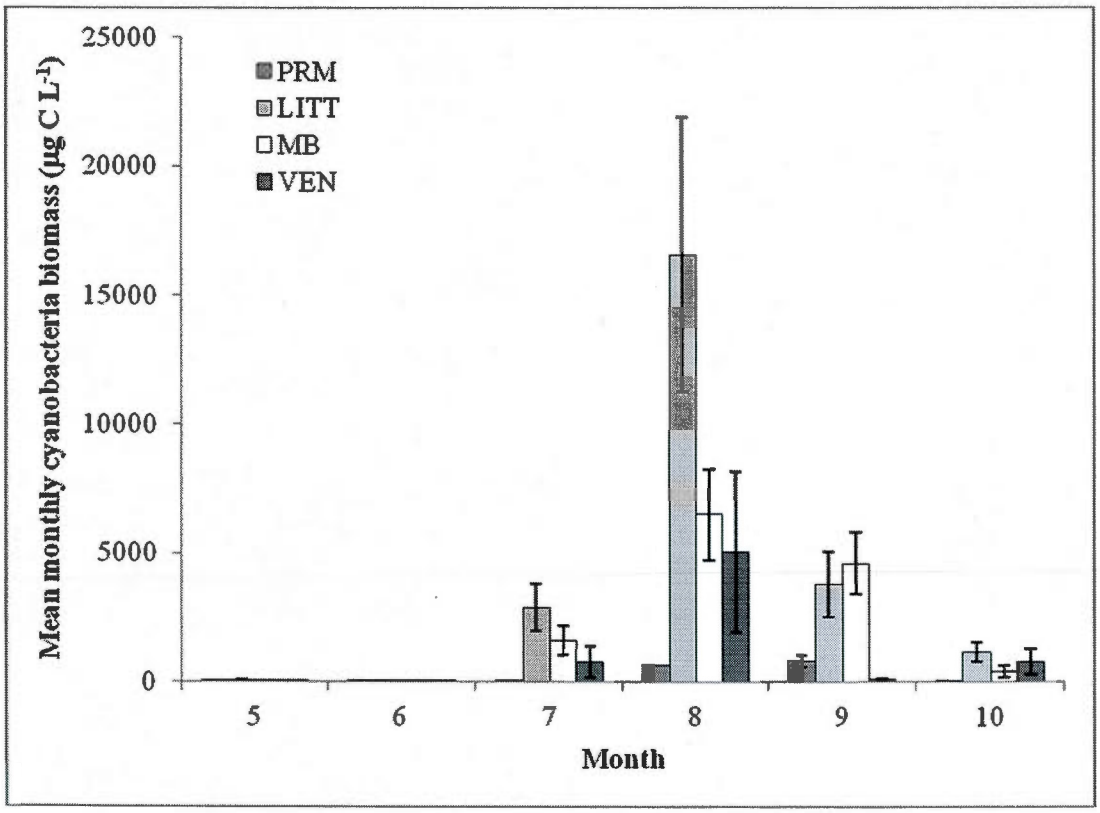

Figure 2.8. Mean monthly cyanobacteria biomass (µg C L^{-1}) at sampling sites in Missisquoi Bay from 2006 – 2009. See Figure 2.7 legend for site names. Error bars reflect one standard error. Note that the y-axis scale is the same as Figure 2.7.

Remnant cyanobacteria populations remained at all sites except PRM in October and dominated the phytoplankton biomass 16.1% of sampling events. Cryptophytes most often dominated in October (35.5%) followed by diatoms (25.8%) and dinoflagellates (12.9%). Mean bC ranged from only 13.4 µg C L^{-1} (n = 1) at PRM to 1160 ± 390 µg C L^{-1} (n = 16) at LITT. MB had a large decline in bC (429 ± 231 µg C L^{-1}; n = 10) and pC (8.8 ± 5.7%) from September to October, while bD (3530 ± 2310 µg C L^{-1}) and pD (34.7 ± 10.5%) remained unchanged. At VEN, bD (568 ± 303 µg C L^{-1}; n = 4) and pD (18.3 ± 9.3%) decreased, but variability was high. A different phytoplankton group dominated biomass at each of the four sampling events at VEN in October (all in 2009).

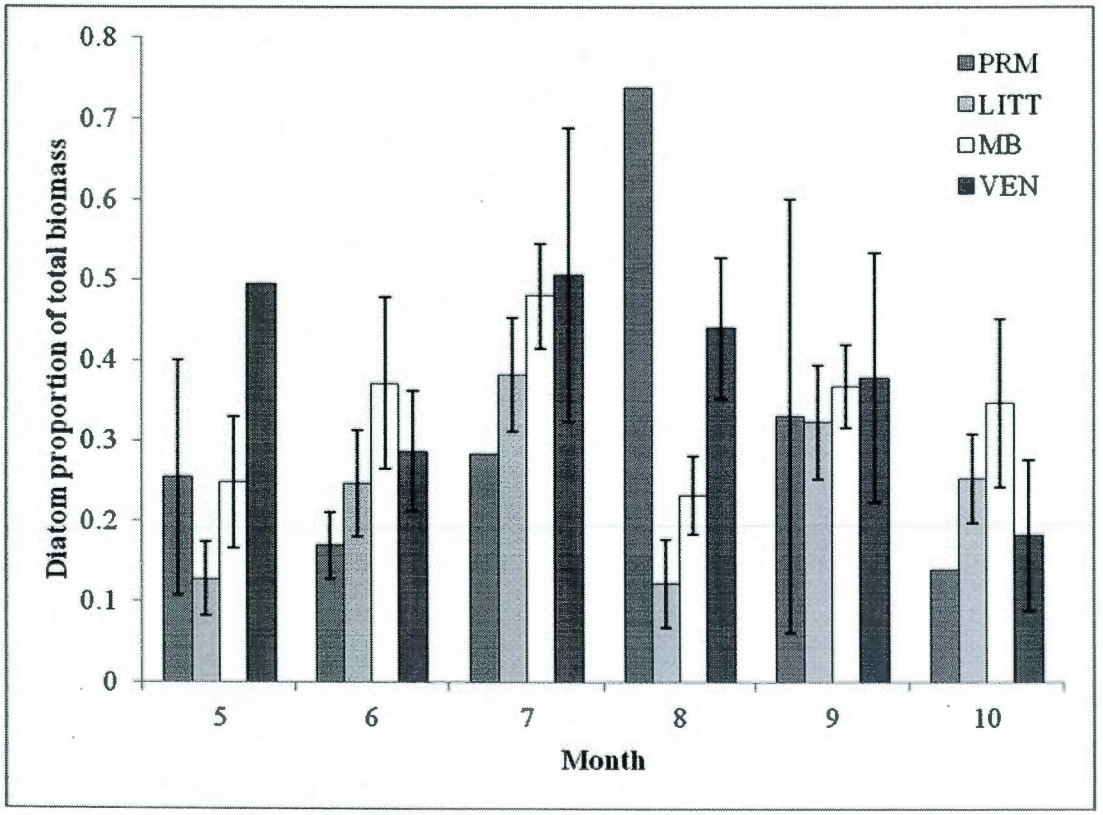

Figure 2.9. Mean monthly proportions of diatoms to total phytoplankton biomass at sampling sites in Missisquoi Bay from 2006 – 2009. See Figure 2.7 legend for site names. Error bars reflect one standard error.

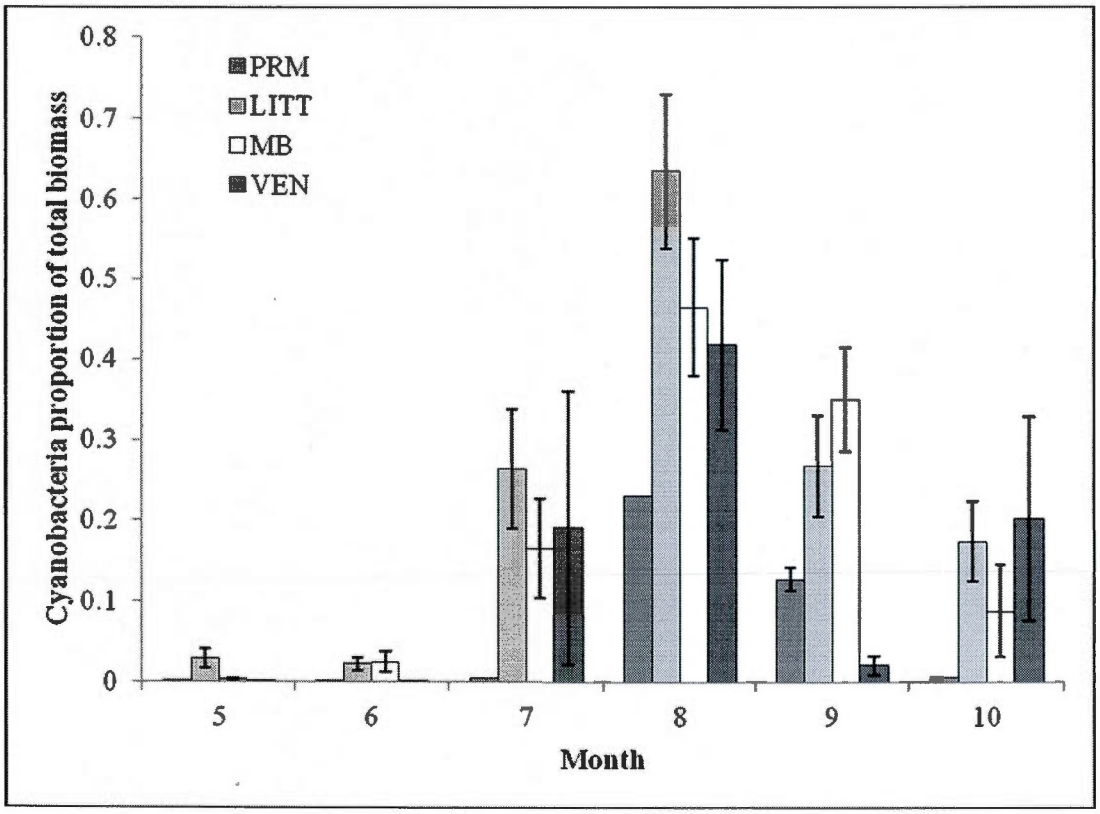

Figure 2.10. Mean monthly proportions of cyanobacteria to total phytoplankton biomass at sampling sites in Missisquoi Bay from 2006 – 2009. See Figure 2.7 legend for site names. Error bars reflect one standard error. Note that the y-axis scale is the same as for Figure 2.9.

2.5. Discussion

This study characterized spatial and temporal nutrient and phytoplankton dynamics in Missisquoi Bay, Lake Champlain, to form the limnological basis for extensive N cycling studies occurring in 2007 – 2009 and described elsewhere. Cyanobacteria blooms have been reported in the few studies that mention Missisquoi Bay (Mihuc et al. 2005; Davis et al.

2009), and occasional blooms were observed in the present study as well. Data collected from various sites on the Canadian side of Missisquoi Bay during the growing seasons (May to October) of 2006 – 2009 revealed that nutrient and phytoplankton dynamics exhibit considerable spatial and temporal variation. Some of the patterns observed are predictable based on well-known paradigms (e.g., temperature and cyanobacteria biomass). However, exceptions to many common assumptions regarding nutrient and phytoplankton dynamics in lakes were often observed. Results from the present study are discussed below relative to the basic limnological questions identified above.

In general terms, the results of this study suggest an alternating cycle with respect to nutrients. Higher nutrient concentrations were observed in 2006 and 2008 than 2007 and 2009. Other related parameters did not always follow expected paradigms based on annual nutrient patterns. For example, while 2008 had the highest annual mean *chl* concentration, the other high nutrient year (2006) had the second lowest *chl*. The highest *chl* year (2008), also one of the two high nutrient years, was the lowest annual precipitation year. The highest precipitation and temperature year (2007) also was one of the low nutrient years and was by far the lowest *chl* year. The only parameter exhibiting the same trend as nutrients was annual mean air temperature obtained from the Environment Canada online database for Philipsburg, QC (Environment Canada; 8.69 and 7.67 °C for 2006 and 2008, respectively, versus 7.02 and 7.12°C for 2007 and 2009, respectively).

These results illustrate the value of long term monitoring programs, but only if useful parameters are monitored at regular intervals with sufficient temporal resolution. An example is the Lake Champlain Long-Term Water Quality and Biological Monitoring Program (http://www.vtwaterquality.org/lakes/docs/lcmonitoring/lp_lclongtermprogdesc.pdf), which no longer monitors any dissolved inorganic N or soluble reactive P concentrations in the sampling program. The omission of these environmental parameters from the monitoring program complicates relating nutrients to the various biological parameters, which are still part of the monitoring program.

2.5.1. What are the trends of ambient nutrient concentrations during the growing season (May through October)?

2.5.1.1. Phosphorus

Total P concentrations followed a predictable spatial pattern in Missisquoi Bay. LITT had a significantly higher mean TP concentration than the two pelagic sites (MB and VEN; Table 2.1). LITT also had a significantly higher mean TDP concentration than VEN, but a higher mean TDP concentration at LITT versus MB was not significant. LITT is located adjacent to the town of Philipsburg and may be subject to nutrient runoff from residential fertilizer, septic systems, and agriculture. This area of the lake is located several kilometers from the nearest tributary input (Pike River), but prevailing surface and bottom water currents tend to converge in this area (Mendelsohn et al. 1997). In contrast to TP and TDP, LITT had the lowest mean SRP concentration of the applicable sites (SRP was never measured at VEN), but this observation is based on only two SRP observations at LITT (in May 2009). If this trend were to hold throughout the growing season, a likely explanation would be the higher mean *chl* observed at this site.

There are inherent problems with SRP measurements (e.g., Dodds 2003), but SRP represents, in theory, the most bioavailable P forms for phytoplankton assimilation. Thus, it might be expected that high phytoplankton biomass would deplete SRP, while the digestion techniques used to measure TP would also digest the phytoplankton biomass and result in high TP concentrations (Lewis & Wurtsbaugh 2008). Indeed, monthly mean TP was correlated to *chl* ($r^2 = 0.66$, $p = 0.0497$, positive slope), even in the small dataset used here. However, this relationship falls apart ($r^2 = 0.03$, $p = 0.79$) when monthly mean TP is regressed with *chl* in the following month, which suggests that TP is tautologous with *chl* and cannot be used to predict future *chl* concentrations in this lake.

Phosphorus concentrations did not differ dramatically from year-to-year. There was no relationship between either annual or monthly mean TP, TDP, or SRP with precipitation

data obtained from Environment Canada (not shown). This finding suggests that loss terms for P, such as sediment burial or washout, are similar in magnitude to inputs, including internal P inputs from sediments (Smith 2009), throughout the growing season. Annual mean P concentrations also did not correlate to total precipitation in the preceding winter and spring (November through May) in any form (TP, TDP, or SRP), which suggests that the P status of the lake is statistically independent of spring runoff from snowmelt.

Phosphorus was measurable in all forms at all times and sampling sites where analyses were conducted (Figure 2.2), and no discernible monthly trends were apparent. Phosphorus loads to Missisquoi Bay have increased since the early 1990's, despite a cross-border agreement in 2002 to substantially reduce P loads to the bay (Smeltzer & Simoneau 2008). The Pike River is the largest tributary discharging into Missisquoi Bay from Québec and represents a significant P load to the lake (Adhikari et al. 2010). Accordingly, PRM exhibited the highest SRP concentrations during this study. PRM did not have the highest *chl* levels, however. Phytoplankton growth in eutrophic systems often is limited by factors other than nutrients, such as light or temperature (Heath 1992). This may be the case for P in Missisquoi Bay; i.e., TDP and SRP concentrations, which should not be tautologous with *chl*, would be expected to be lowest at times when *chl*, and thus bioavailable P uptake, is highest. However, the lack of any relationship between TDP or SRP and *chl*, combined with the ubiquitous presence of bioavailable substrate, suggested that some factor, or factors, other than P availability limited phytoplankton growth at the times and locations sampled.

This interpretation contrasts with results from bioassays conducted in 2007 in Missisquoi Bay, which found increased *chl* in bottle incubations with N and P additions (Gonzalez-Rueda 2008). It should be noted, however, that 2007 was an exceptional year in Missisquoi Bay. Mean *chl* in 2007 was by far the lowest of any year (3.73 ± 0.15 $\mu g\ L^{-1}$), and the zooplankton population also crashed, resulting in high water clarity with no major cyanobacteria blooms (Dunlap et al. 2008). Ambient nutrient concentrations were not anomalous in 2007. Mean water temperature in 2007 was the highest during the study period, but it was not significantly different from mean temperatures in any other year. Total precipitation also was highest in 2007, but this value (1154 mm) was only slightly higher

than other years (range 993 to 1132 mm). Thus, there is no obvious explanation for the unusual observations in the bay in 2007, including the stimulation of phytoplankton growth by nutrient additions to bottles despite nutrient replete conditions and low biomass in situ.

2.5.1.2. Nitrogen

Like TP, TN concentration exhibited a predictable pattern in Missisquoi Bay. Significantly higher annual mean TN concentrations were measured proximal to the input source (LITT > MB > VEN; TN was not measured at PRM; Table 2.2). Differences in TDN at LITT and MB were not significant, but both of those sites had higher TDN than VEN (TDN was not measured at PRM). Dissolved inorganic N species followed the same pattern, with NO_x concentrations decreasing with distance from the river input (PRM >> LITT > MB > VEN). Ammonium concentration at PRM was significantly higher than MB, but the high variability of the relatively few NH_4^+ measurements at PRM prevented the difference between PRM and LITT from being significant. Higher mean NH_4^+ at LITT versus MB was not significant, but the spatial trend in the lake follows the same general trend as the other N analyses, with higher concentrations nearest the tributary input and decreasing with distance (no NH_4^+ measurements were made at VEN).

None of the N components (TN, TDN, NO_x, or NH_4^+) measured in this study correlated with *chl* on any timescale evaluated in this study, even considering time lags of one month in the regressions. No annual or monthly nutrient ratios correlated with *chl* either. All of the p values in the regressions improved with the shifting procedure, but not enough to reach significance. The dataset used here may not be large and complete enough to allow relationships to emerge from the data, but the improved relationships after shifting do suggest a potential for predictive capabilities in a larger dataset. This situation also was observed in an evaluation of long-term datasets from Taihu Lake (China) and Lake Okeechobee (Florida, USA), where better relationships, both "straight" and shifted, were observed in the more extensive Okeechobee data (10 years) than the smaller Taihu dataset (four years; McCarthy et al. 2009b). The Taihu and Okeechobee datasets also included data from all 12 months, whereas Missisquoi data was only collected from May to October due to winter ice cover.

These results, like those from the P analyses, suggest that other factors besides nutrients controlled phytoplankton growth in Missisquoi Bay during this study. Also like P, none of the monthly or annual means of N forms measured correlated to precipitation or temperature, which indicates that N concentrations in the lake are driven by internal processes, at least during the growing season. However, unlike the P results, both NO_x and NH_4^+ decreased as the growing season progressed (Figure 2.3). All monthly mean NO_x concentrations from May to July were > 25 μM, while all NO_x concentrations from August to October were < 20 μM. Primary producer uptake and/or permanent NO_x removal processes, such as denitrification, therefore must be progressing more rapidly than external inputs and internal transformations producing NO_x, such as nitrification. Monthly mean NH_4^+ decreased in each month from May to September, then increased slightly in October. Like with NO_x, this finding suggests that primary producer NH_4^+ uptake and/or transformation processes converting NH_4^+ to oxidized N forms are occurring at higher rates than internal production (e.g., regeneration and organic matter remineralization) and external inputs. The general ubiquity of measurable levels of DIN (and P) on monthly and annual timescales does not preclude the possibility that nutrients could have limited phytoplankton growth on a more temporally explicit scale. For example, NO_x was undetectable, and NH_4^+ concentration was 0.23 μM at MB on 27 August 2007. SRP concentration was still high (0.74 μM), and the cyanobacteria community was dominated by a N fixing genera (*Anabaena*). These observations suggest that N may have been limiting at this site on that date.

Ammonium samples within the monitoring program from 2006 – 2008 were collected and handled in varying ways, none of which represent the ideal technique for NH_4^+ measurements. Further, NH_4^+ analyses of 2009 monitoring samples were not successful and lacked reproducibility. Thus, they were not used in the present study. Early NH_4^+ sampling techniques within the monitoring program did not involve sample filtering in the field, which subjects the final measured concentration to uptake and regeneration processes in the sample bottle from collection until analysis. Later techniques implemented field filtration, but these filters were 0.45 μm pore-size, which allows some picoplankton, including small bacteria and cyanobacteria, to pass into the sample bottle. As shown above, picocyanobacteria were the most numerous phytoplankton cells in almost all samples. These cells, as well as presumably

numerous bacterial cells, are capable of significant NH_4^+ uptake, even in the dark (Cochlan et al. 1991), and NH_4^+ regeneration processes (e.g., grazing and cellular exudation) are not light dependent. Therefore, storing unfiltered or 0.45 µm filtered samples in the dark prior to analysis does not prevent biologically-driven changes in NH_4^+ concentration in sample bottles. Field-collected samples collected as part of the sediment core and water column incubation sampling events were filtered immediately after collection in the field using thoroughly rinsed 0.2 µm Nylon syringe filters. These filters are the best available way to prevent biological activity from changing the concentration of rapidly cycled analytes (including SRP) between collection and analysis.

The TN outlier result from 11 August 2008 at LITT was confirmed in discrete samples collected using both horizontal (surface water only) and vertically integrated techniques and in duplicate measurements of each sample. The possibility that the analysis of these samples was flawed cannot be excluded, but other measurements from 4 and 18 August 2008 were not outliers. Separate samples collected on 11 August 2008 from PRM and MB did not show any DIN components with unusually high concentrations. However, this TN outlier coincided with the highest *chl* and TP measurements, which also were outliers. Thus, digestion of phytoplankton cells provides the most reasonable explanation for the very high TN and TP measurements on this occasion and illustrates why total nutrient concentrations should be analyzed with caution for nutrient studies in aquatic systems.

2.5.2. What are the dominant phytoplankton groups during the growing season?

Colonial cyanobacteria (individual cell size < 2 µm) dominated most phytoplankton cell counts in this study, regardless of the sampling site or time, but rarely accounted for a meaningful proportion of total phytoplankton biomass. The most common organism from this group was *Aphanothece clathrata brevis*. Despite their relatively small biomass, these cells do contribute to significant nutrient uptake (e.g., Hutchins et al. 2003) and are readily grazed by microzooplankton (e.g., Christaki et al. 1999). Therefore, the contributions of these organisms to N uptake and regeneration processes cannot be ignored. On the few occasions where these cells did not dominate cell counts, other unicellular or colonial cyanobacteria

(e.g., *Anabaena* and *Microcystis*) were most numerous. In these cases, these cyanobacteria also comprised a large proportion of total phytoplankton biomass (i.e., during blooms).

Phytoplankton biomass in May, June, and October generally was dominated by cryptophytes at all sites. The most common genus was *Cryptomonas*. These flagellates are rarely found at temperatures > 20°C and are common in higher latitude and temperate lakes and marine systems (Alexander et al. 1980; Klaveness 1988). Monthly mean water temperatures in May (mostly late in May) and October were < 20°C, but mean water temperature in June was > 20°C. Cryptophytes also dominated phytoplankton biomass on a few occasions in July and August and were present in all samples, even when water temperatures exceeded 20°C. Cryptophytes are well-adapted to low light conditions since they use red or blue phycobiliproteins (Gervais 1997), and they also are readily grazed by microzooplankton (Tirok & Gaedke 2007). Cryptophytes generally have a low assimilation capacity for NO_3^- and a high affinity for NH_4^+ (Klaveness 1988; Semeneh et al. 1998).

These transitional months also exhibited large diatom populations, although they did not usually dominate phytoplankton biomass in these months. Common genera were *Aulacoseira*, *Cyclotella*, *Fragilaria*, and *Stephanodiscus*. As mentioned previously, diatoms are well adapted to lower water temperatures and light conditions (Lomas & Glibert 1999a, 1999b), but they were usually out-competed by cryptophytes in these conditions. Diatoms were present in all samples (Figures 2.7 and 2.9), but lowest biomasses were observed in May and June, followed by August and October. The seasonal pattern of diatoms observed in this study (Figures 2.5 and 2.9) was opposite that reported by other investigators for Missisquoi Bay, who observed higher diatom densities (cells per liter; biomass was not reported) in May than July or September (Mihuc et al. 2005). In the present study, mean bD was highest in July and September and lowest in May (Figure 2.5). Other shallow, eutrophic areas of Lake Champlain had low diatom abundances in July (Levine et al. 1997), unlike the results from Missisquoi Bay. Maximum pD also was observed in July, but pD in September was not significantly higher than June or October (Figure 2.6). No significant differences in bD or pD between sampling sites were observed either (i.e., LITT versus MB; Figures 2.7 and 2.9, respectively). These results suggest that typical phytoplankton succession patterns

observed in many temperate lakes, including other areas of Lake Champlain, did not hold for Missisquoi Bay during the 2007 – 2009 growing seasons.

Cyanobacteria in Missisquoi Bay exhibited a very clear maximum in August, both in terms of biomass (Figures 2.5 and 2.8) and proportion of total phytoplankton biomass (Figures 2.6 and 2.10). High cyanobacteria coincided with high water temperature and low precipitation, environmental conditions known to favor cyanobacteria (e.g., Paerl & Fulton 2006), although these relationships were not statistically significant over the entire dataset (precipitation relationships were nearly significant; $p = 0.054$ for monthly mean precipitation and cyanobacteria biomass; $p = 0.076$ for monthly mean precipitation and cyanobacteria proportion). Cyanobacteria did not comprise a large proportion of total phytoplankton biomass until July (Figures 2.6 and 2.10), and both bC (Figures 2.5 and 2.8) and pC decreased to near-July levels in September. Cyanobacteria decreases in September occurred in favor of diatoms, especially at LITT, where mean bD tripled from August to September (Figure 2.7). This late season diatom maximum coincided with lowest NO_x concentrations in September (Figure 2.3), probably due in part to increased uptake from diatoms.

Common cyanobacteria genera were *Anabaena*, *Aphanizomenon*, *Aphanocapsa*, *Aphanothece*, *Chroococcus*, *Coelosphaerium*, *Merismopedia*, *Microcystis*, and *Pseudanabaena*. These genera are mostly colonial with individual cell sizes < 2 μm, but bC and pC maxima observed in August were clearly driven by large *Microcystis* and *Anabaena* blooms (and *Aphanizomenon* on one occasion). Of the ten highest bC measurements for individual species, all occurred in August, eight occurred in 2008 (the other two in 2009), eight were observed at LITT (two at MB), eight involved N-fixing genera (seven *Anabaena*, one *Aphanizomenon*), and two involved *Microcystis*. The relative lack of non-N-fixing genera (i.e., *Microcystis*) was surprising given the high DIN and P concentrations observed in Missisquoi Bay. The lack of non-N-fixers also is different from previous observations, which suggested *Microcystis* dominance of phytoplankton and cyanobacteria populations in Missisquoi Bay (Mihuc et al. 2005). Later studies, however, reported a similar dominance of N-fixing genera (*Anabaena*) over *Microcystis* in Missisquoi Bay (Davis et al. 2009).

In cases where highest bC's were observed, the total cyanobacteria community was represented by large populations of both N-fixers and non-N-fixers. For example, at the littoral site on 11 August 2008, *Microcystis*, *Anabaena*, and *Aphanizomenon* (the latter two are potential N-fixers) represented nearly equal proportions of the phytoplankton community and are all included in the ten highest individual species biomass observations mentioned above. All of the various N and P forms were present in relatively high concentrations during August (Figures 2.2 and 2.3), so nutrient limitation or competitive abilities for a more available nutrient form do not appear to be factors in the dominance of any particular group, at least on a monthly basis (see below). High phytoplankton, and especially cyanobacteria, biomass in August preceded lowest NO_3^- and NH_4^+ concentrations, which were measured in September (Figure 2.3). This observation suggests that the phytoplankton may have driven the observed nutrient concentrations, rather than the observed nutrients explaining the phytoplankton community dynamics.

The presence of N-fixing genera and heterocytes during these August blooms (and others) suggest that N was limiting at more temporally or spatially explicit scales than captured in the sampling regime used here. Heterocyte differentiation in N-fixing cyanobacteria is the result of a cascading genetic response to N deficiency (Muro-Pastor et al. 1999). This genetic cascade is controlled by cellular recognition of insufficient NH_4^+ assimilation into the cell (e.g., Lindell & Post 2001), and assimilation of any N form other than N_2 gas will inhibit heterocyte differentiation in cyanobacteria (Muro-Pastor et al. 1999). The presence of heterocytes indicates that these cells were severely N deficient at some point, but some form of DIN was observed in all discrete samples collected during this study. Heterocytes numbered fewer than two cells per 100 N-fixer cells (in *Anabaena* and *Aphanizomenon*) in 2008, which suggests that, as a community, these N-fixers were not relying heavily on N fixation for growth. This situation is similar to Shelburne Pond in Vermont, where potential N fixers dominated the phytoplankton community but acquired only 2 – 9 % of their N demand via N fixation (Ferber et al. 2004). Less than three heterocytes per 100 N-fixers were observed in Shelburne Pond, which also suggested a small reliance on N fixation. Instead, the heterocyteous cyanobacteria-dominated phytoplankton community satisfied their N demand with NH_4^+ (82 – 98%) and NO_3^- (< 5% in summer, 15 –

18% in spring and fall; Ferber et al. 2004). In Missisquoi Bay, it is apparent that N was deficient on temporally and spatially explicit scales and allowed those genera capable of N fixation to out-compete the non-N-fixing cyanobacteria on a system-wide basis.

2.5.3. Which N form in Missisquoi Bay is most conducive to growth of the dominant phytoplankton group?

High nutrient concentrations in most samples from Missisquoi Bay suggested that nutrients were replete throughout most of the growing season. In these cases, it is likely that other environmental factors, such as light and temperature, limited primary productivity and controlled phytoplankton community structure (e.g., Heath 1992). However, higher populations of N-fixing versus non-N-fixing cyanobacteria genera in July through September, and the presence of some heterocytes in these cells, suggested that N may be scarce on smaller scales during mid-summer than captured by the sampling regime used here. P was never depleted, and the only evidence of possible N scarcity was observed in September, when DIN concentrations were lowest. Cryptophytes dominated in May, June, and October, and available literature suggests that this phytoplankton group does not compete well for oxidized N (e.g., Klaveness 1988). Ammonium and NO_x were replete in these months and suggested that cryptophytes were better able to compete for available light at ambient temperatures and replete nutrients. Diatom dominance in July, again with replete nutrients, including diatom-conducive NO_x, also suggested a physical rather than chemical explanation for phytoplankton community dynamics. Diatom dominance in September appeared to contribute to NO_x depletion, but factors other than nutrients appear to be more important for constraining primary production and phytoplankton community structure in Missisquoi Bay.

CHAPTER III: WATER COLUMN NITROGEN CYCLING IN MISSISQUOI BAY, LAKE CHAMPLAIN

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3.1. Summary

Water column ammonium (NH_4^+) regeneration and potential uptake rates were measured in Missisquoi Bay, Lake Champlain, during the growing seasons of 2007 – 2009 using isotope dilution techniques. In addition, water column nitrogen (N) fixation rates were assessed using membrane inlet mass spectrometry on six occasions in 2009, and these incubations also provided O_2 respiration data. These N transformation rates were evaluated at a site near the Pike River discharge into Missisquoi Bay (PRM) and in the central basin (MB). Process rate measurements were integrated with phytoplankton community structure and nutrient data. The most bioavailable nutrients (NH_4^+ and soluble reactive phosphorus) were present at measurable levels at both sites at all times, suggesting that nutrients do not limit primary production. Nutrient concentrations were generally higher at PRM relative to MB, as expected. Colonial cyanobacteria (individual cell size < 2 μm), particularly *Aphanothece clathrata brevis*, were the most abundant phytoplankton at both sites but never accounted for more than 5% of phytoplankton biomass. Cyanobacteria never dominated at PRM, where cryptophytes were the dominant phytoplankton group (as biomass). The phytoplankton community at MB was more complex, and cyanobacteria only dominated during infrequent summer blooms comprised of potential N fixing (*Anabaena* and *Aphanizomenon*) and non-N-fixing genera (*Microcystis*). During non-bloom conditions, cyanobacteria accounted for < 6% of the phytoplankton biomass at MB. Water column potential NH_4^+ uptake rates averaged 0.205 ± 0.022 μmol N L^{-1} h^{-1} lake-wide, and autotrophic (light minus dark) and heterotrophic (dark) uptake rates generally were balanced in the lake.

The mean NH_4^+ regeneration rate was 0.085 ± 0.009 μmol N L^{-1} h^{-1}, and no light/dark incubation differences were observed. Generally higher NH_4^+ cycling rates early in the growing season at PRM and in mid-summer at MB suggest allochthonous nutrient and organic matter sources at PRM versus autochthonous sources at MB. Water column N fixation was not detected at either site, and low heterocyte abundances suggested that N-fixing cyanobacteria did not rely heavily on atmospheric N_2 to satisfy N requirements. Basin-scale NH_4^+ regeneration rates were about 700,000 mol N d^{-1} in Missisquoi Bay, which is almost twice the estimated N load from tributaries. Comparisons of regeneration and uptake rates in the lake suggest that primary production cannot be sustained by water column regeneration alone and that external inputs and benthic regeneration must be important.

3.2. Introduction

Nitrogen (N) is an important nutrient regulating productivity in aquatic systems. Over-enrichment with N, alone or in combination with P, can lead to eutrophication and algal blooms, but most research and management efforts are focused on phosphorus (P) in lakes. Unlike P, N occurs in several bioavailable forms, and ammonium (NH_4^+) requires the least energy of these forms for assimilation into biomass (e.g., Syrett 1981). Primary producer preferences for NH_4^+ are well-established, and NH_4^+ also is known to inhibit assimilation of other N forms in some cases (e.g., McCarthy et al. 1977). Accurate determination of NH_4^+ availability to primary producers requires ambient concentration and turnover rate data. These turnover rates may determine phytoplankton growth rates more so than external inputs (e.g., Glibert 1998). The nutrient status of aquatic ecosystems often is evaluated based on analysis of discrete water samples, which represent "snapshots" of nutrient concentrations in space and time. The inadequacy of snapshot nutrient data is compounded by insufficient sample collection and handling protocols, which may not prevent substantial changes in nutrient concentrations between sample collection and analysis. Biological activity occurring in water samples after collection and before analysis can affect measured nutrient concentrations in short periods of time, particularly in eutrophic systems. These changes are especially important in the case of NH_4^+, which is the most bioavailable of inorganic N forms

(e.g., Syrett 1981) and also controls all N-related biochemical functions in cyanobacteria (e.g., Flores & Herrero 2005).

In the Mississippi River plume, high nitrate (NO_3^-) discharges in spring fueled a diatom bloom associated with high NO_3^- uptake rates, and NH_4^+ regeneration was less important (Bode & Dortch 1996). By summer, diatoms had depleted available NO_3^- and river inputs decrease, which led to lower primary productivity and increased importance of regenerated N. In Chesapeake Bay, spring N demand exceeded NH_4^+ regeneration, but regeneration exceeded N demand by a factor of three in fall (Bronk et al. 1998). These results suggested that autotrophic production dominated heterotrophic metabolism in spring, when nutrient inputs were high, and heterotrophic regeneration sustained productivity later in the year. There are few reports on the importance of water column regeneration in freshwater systems, but NH_4^+ regeneration rates were related at times to hydrology and microbial food web structure in a small Great Lakes wetland (McCarthy et al. 2007b), Lake Michigan (Gardner et al. 2004), and a hypereutrophic lake (Taihu Lake, China; McCarthy et al. 2007c).

Grazing on phytoplankton and subsequent excretion of wastes by zooplankton is one NH_4^+ regeneration pathway. Other pathways include cellular exudation from phytoplankton, bacterial remineralization of organic material, photo-degradation of organic matter (e.g., Moran & Zepp 1997, sloppy feeding by grazers (e.g., Kirchman 2000), and viral lysis (Wilhelm & Suttle 1999). In cases of severe N depletion, water column N fixation by cyanobacteria may be an important N source (e.g., Paerl 1990), and resulting organic matter can be remineralized to NH_4^+ to fuel additional primary production. However, N fixation is an energy intensive process and likely represents a last resort for phytoplankton capable of converting atmospheric N to biomass, which provides a niche for these algae to thrive during N deficiency. The importance of these pathways for ecosystem productivity can be magnified in environments with episodic external inputs (e.g., McCarthy et al. 2007b) and may be related to cyanobacteria blooms (McCarthy et al. 2007c, 2009a). Cyanobacteria are highly competitive for reduced, inorganic N forms resulting from regeneration processes (Blomqvist et al. 1994, Hyenstrand et al. 1998a & b), and the presence of NH_4^+ relative to oxidized N forms may contribute to phytoplankton community structure (McCarthy et al. 2009b). On the

other hand, phytoplankton community structure may regulate rates and pathways of NH_4^+ regeneration. Cyanobacteria, particularly those forming large colonies and producing toxins, may not be suitable forage for grazers (e.g., Blomqvist et al. 1994). Thus, if cyanobacteria are abundant relative to other phytoplankton groups, then lower regeneration rates may be expected. In contrast, diatoms are readily grazed by zooplankton and may be associated with higher regeneration rates.

Light and dark incubations provide information on the importance of autotrophic versus heterotrophic organisms with respect to NH_4^+ uptake dynamics. Autotrophic uptake generally is light dependent, while heterotrophic uptake by bacteria or mixotrophs should remain active in the dark. However, phytoplankton nutrient uptake and storage in the dark can occur when nutrients are scarce (Cochlan et al. 1991). Conditions necessary for dark uptake by phytoplankton revolve around low N-to-carbon ratios due to N stress (Clark et al. 2002; Flynn et al. 2002). This phenomenon complicates the differentiation between autotrophic and heterotrophic uptake, but this problem can be addressed to some degree with longer incubation times. Phytoplankton incubated in the dark should not be able to sustain high uptake rates for long time periods, while heterotrophic uptake should proceed normally. Longer incubations (e.g., 24 hours) also prevent biasing results toward specific diurnal trends in uptake rates (Gardner et al. 2004).

As with phytoplankton, bacteria assimilate NH_4^+ more efficiently than other N forms (Vallino et al. 1996, Vrede et al. 1998). Bacterial NH_4^+ uptake can dominate total NH_4^+ uptake in some systems, including those representing "links" to other N forms. For example, nitrification is a two-step, bacterial (and archaeal) process that converts NH_4^+ to nitrite (NO_2), and then to NO_3^- (Ward et al. 2007). The end-products of both nitrification steps can be reassimilated, converted to biomass, and remineralized to NH_4^+. Dissolved organic N (DON) also may represent an important N source for primary producers (e.g., Glibert 1993), but its potential importance in Missisquoi Bay is beyond the scope of this project.

Instantaneous "snapshots" of ambient nutrient concentrations, without knowledge of turnover rates, are not accurate reflections of ambient conditions or useful for generating

predictive relationships about phytoplankton and nutrients. The objective of this project was to quantify NH_4^+ regeneration and uptake rates in Missisquoi Bay, Lake Champlain, which has experienced nearly annual cyanobacteria blooms (Mihuc et al. 2005; Davis et al. 2009). It was hypothesized that NH_4^+ uptake and regeneration rates would be highest near a river outflow and lowest in the central basin due to differences in ambient nutrient concentrations. NH_4^+ uptake and regeneration rates and ratios were expected to be related to phytoplankton community structure, with higher rates and ratios coinciding with higher proportions of cyanobacteria. Additional water column incubations were conducted to quantify N fixation rates, which were expected to be low in this eutrophic lake. Quantifying these N cycling rates is needed to constrain factors affecting phytoplankton community structure and cyanobacteria bloom dynamics in this shallow, temperate lake. In addition, descriptions of the fate of N in the water column will benefit management efforts seeking to reduce nutrient inputs.

3.3. Materials & Methods

Water column N cycling rates were determined at two sites in Missisquoi Bay, Lake Champlain. These sites were located at the Pike River discharge into the lake (PRM) and in the central basin (MB; Figure 1.3). PRM is shallow (~1 m), turbid from the river discharge, and expected to have higher nutrient concentrations. MB is deeper (~4 m), less turbid, and expected to have lower nutrient concentrations due to distance from external nutrient sources. Temperature and dissolved oxygen (DO) concentration depth profiles were determined using a YSI sonde. Phytoplankton samples collected from depth-integrated water were preserved in Lugol's iodine, and algal cells were counted using an inverted microscope. Heterocytes in cyanobacteria colonies also were counted. Cell counts and size measurements were converted to biomass using standard equations (Menden-Deuer & Lessard 2000). Ambient nutrient samples were collected in site-water rinsed, 60-mL syringes and filtered immediately in the field with 0.2 µm Nylon syringe filters. Nutrients were analyzed using standard autoanalyzer techniques (NO_3^- + NO_2^-, NO_2^-, and soluble reactive phosphorus (SRP)) and, for NH_4^+, high performance liquid chromatography (HPLC; Gardner et al. 1995a).

Ammonium regeneration and potential uptake rates were determined during the growing season on three dates in 2007 (12 June, 25 June, and 27 August), seven dates in 2008 (12 May, 2 June, 25 June, 2 July, 4 August, 11 August, and 7 October), and two dates in 2009 (8 July and 23 September). Regeneration and uptake incubations were conducted in triplicate light and dark (foil wrapped), clear Polystyrene culture bottles (70 ml; Corning). Water from each site was amended with 8 µM (final concentration) $^{15}NH_4Cl$ (Sigma), distributed into culture bottles, and initial samples were filtered into 8 ml Wheaton vials and frozen. Culture bottles were incubated in a laboratory incubator set at near-ambient temperature and photoperiod. Final samples were collected after ~24 hours, filtered, and frozen until analysis. Total NH_4^+ concentration and NH_4^+ isotope ratios were determined using HPLC (Gardner et al. 1995a) at the University of Texas Marine Science Institute. Regeneration and uptake rates were calculated using a modified isotope dilution model (Blackburn 1979, Caperon et al. 1979). Amendment concentration was selected to saturate the NH_4^+ pool with $^{15}NH_4^+$. Isotope dilution incubations often are conducted with tracer level amendments (i.e., < 10% of ambient NH_4^+; Glibert et al. 1982). Potential problems with tracer level additions are magnified in eutrophic systems, where the entire NH_4^+ pool can be depleted before final sampling (Gardner et al. 2004). In Missisquoi Bay, previous NH_4^+ measurements ranged from two to 13 µM (unpublished data). Therefore, a saturating level amendment (8 µM) was chosen for these incubations.

Water column N fixation (and O_2 respiration) was determined only in 2009 on six dates (26 May, 17 June, 3 July, 8 July, 9 September, and 23 September). Nitrogen fixation was evaluated by measuring changes in N_2:Ar over time using membrane inlet mass spectrometry (MIMS; Kana et al. 1994). Water samples were incubated in 15 ml ground-glass stoppered test tubes (Chemglass) custom-made for dissolved gas analyses. These tubes have a very low surface area-to-volume ratio and minimize atmospheric contamination during sample analysis. Unfiltered water from both sites was placed in 24 light and dark tubes in the field. Three tubes from each treatment were killed with 200 µl of 50% $ZnCl_2$ solution immediately in the field to stop biological activity and characterize initial N_2:Ar. The rest of the tubes were capped in the field but not killed. This procedure was repeated with water filtered in the field (control) using 0.2 µm Nylon syringe filters for evaluation of

incubation and preservation effects. All filled tubes from each site were submerged in either a foil-wrapped (dark) or unwrapped (light) acrylic tube filled with lake water to maintain ambient temperature. Upon return to the laboratory, the acrylic tubes were placed in an incubator set at near-ambient temperature and photoperiod. The exact light level experienced by the natural phytoplankton community in the incubation tubes is unknown, but the Chemglass and acrylic tubes used in the incubations were clear, and this incubator also was used to culture cyanobacteria. Therefore, it was assumed that N fixation was not limited by light in these incubations. Triplicate light and dark sample tubes were uncapped, preserved with $ZnCl_2$, and recapped at various time-points ranging from five to 53 hours after initial capping. During sample analysis, water was pumped directly from the bottom of the tubes into the MIMS to avoid potentially atmosphere-contaminated water at the top of the tube.

MIMS allows calculation of N_2 concentrations assuming that Ar is inert and at equilibrium saturation given constant water temperature (Kana et al. 1994). Changes in N_2 during the incubation determined net N_2 fluxes, which represent the balance between gas uptake and production. Since the well-mixed Missisquoi Bay water column is oxic, water column denitrification was assumed to be near-zero. Thus, decreases in N_2:Ar during the incubation were assumed to be from N fixation. N_2 changes in dark controls, which also limit O_2 production by phytoplankton, were used to establish detection limits for the rate measurements. Some tubes developed bubbles during the incubation, and these results were discarded.

Where appropriate, differences between rates and sampling sites were evaluated using a one-way analysis of variance (ANOVA). Significant relationships between variables were evaluated using linear regressions. Differences and relationships were deemed significant at $p < 0.05$.

3.4. Results

3.4.1. Water column characteristics and hydrochemistry

Mean water depths were 1.3 m at PRM and 4.8 m at MB, and fluctuations were more severe at PRM than MB (Table 3.1), presumably due to changes in river flow volume and bottom morphology. Water column stratification was not observed at either site except on two occasions at MB (17 June and 3 July 2009), when bottom water DO was < 1.0 mg L^{-1} (Table 3.1). Highest water temperatures were measured in August, and lowest water temperatures were measured in October. Ammonium and SRP were present in all samples, even when NO_x was not detected (Table 3.2). All nutrients were generally higher in early to mid-summer (June – July) than late in the season. As expected, PRM had higher nutrient concentrations than MB. Mean NO_x and NH_4^+ concentrations (May-July) at PRM were lower in 2007 (51.3 and 4.78 µM, respectively) than the same period for both 2008 (229 and 10.1 µM, respectively) and 2009 (152 and 8.29 µM, respectively). In contrast, SRP was higher at PRM for this period in 2007 (11.3 µM) than either 2008 (1.15 µM) or 2009 (0.72 µM). No similar trends were apparent for MB.

3.4.2. Phytoplankton

Phytoplankton counts and biomass data were not available for all sampling events corresponding to water column N cycling incubations. No phytoplankton data were available at PRM in 2007, and, in some cases, phytoplankton counts from within a few days before or after the N cycling incubations are reported for MB (Table 3.3). Colonial cyanobacteria (individual cell sizes < 2 µm), particularly *Aphanothece clathrata brevis*, were the most abundant phytoplankton in cell counts. However, these cyanobacteria never comprised > 5% of total phytoplankton biomass.

Table 3.1. Station depth, temperature, and dissolved oxygen (DO) concentrations at the Pike River mouth (PRM) and central basin (MB) sites in Missisquoi Bay, Lake Champlain. Temperature and DO are given for the water surface (s) and near-bottom (b). Low water depth (#) and DO (*) are noted. ND = no data.

Date	Station	Depth m	Temp(s) °C	Temp(b) °C	DO(s) mg/l	DO(b) mg/l
5/12/08	PRM	2.0	16.0	13.5	8.0	6.7
	MB	5.5	14.5	13.4	3.8	2.6
5/26/09	PRM	2.2	18.0	16.1	8.8	9.7
	MB	4.5	16.4	15.5	10.5	10.1
6/2/08	PRM	2.5	14.6	14.2	9.0	8.3
	MB	4.9	16.0	15.8	9.3	8.4
6/12/07	PRM	ND	ND	ND	ND	ND
6/17/09	PRM	1.0	21.3	20.6	7.7	7.0
	MB	5.0	19.9	19.6	8.6	0.97*
6/25/07	PRM	1.0	21.2	20.6	6.6	5.6
	MB	4.5	20.7	20.2	8.8	7.7
6/25/08	PRM	0.9	20.4	20.0	7.5	6.9
	MB	4.5	21.5	20.6	12.7	8.0
7/2/08	PRM	0.6	23.2	23.2	8.4	7.8
	MB	4.5	23.1	23.0	9.6	9.7
7/3/09	PRM	2.1	21.7	20.8	8.3	5.9
	MB	5.0	22.1	21.9	7.8	0.56*
7/8/09	PRM	1.5	19.8	19.3	8.6	7.3
	MB	4.3	20.9	20.5	8.7	4.9
8/4/08	PRM	0.08#	23.9	23.9	9.4	9.2
	MB	4.8	23.7	23.6	9.6	9.0
8/11/08	PRM	1.5	ND	ND	ND	ND
	MB	4.7	22.7	22.0	11.2	9.4
8/27/07	PRM	ND	ND	ND	ND	ND
	MB	ND	ND	ND	ND	ND
9/9/09	PRM	0.6	20.9	20.7	8.2	8.4
	MB	ND	20.7	ND	ND	ND
9/23/09	PRM	0.5	ND	ND	ND	ND
	MB	4.8	ND	ND	ND	ND
10/7/08	PRM	0.5	ND	ND	ND	ND
	MB	4.7	11.4	12.8	9.6	10.7

Table 3.2. Ambient nutrient concentrations (in μmol L^{-1}) at the Pike River mouth (PRM) and central basin (MB) sites in Missisquoi Bay, Lake Champlain. SRP = soluble reactive phosphorus. NO3 = nitrate. NO2 = nitrite. NH4 = ammonium. ND = no data.

Date	Station	SRP	NO3	NO2	NH4
5/12/08	PRM	0.16	18.8	0.49	2.36
	MB	0.15	17.8	0.47	1.00
5/26/09	PRM	0.85	57.5	1.05	11.3
	MB	0.21	18.8	0.39	15.9
6/2/08	PRM	0.93	334	3.07	9.26
	MB	0.36	6.26	1.11	5.07
6/12/07	PRM	2.15	60.5	2.03	3.64
6/17/09	PRM	0.34	217	3.23	10.2
	MB	0.11	0.70	0.43	1.60
6/25/07	PRM	20.5	42.2	1.76	5.91
	MB	0.41	9.85	0.65	3.60
6/25/08	PRM	1.91	195	3.69	10.9
	MB	0.77	27.1	1.38	0.28
7/2/08	PRM	0.60	156	4.76	10.3
	MB	0.20	22.1	1.06	10.6
7/3/09	PRM	0.73	191	2.86	6.66
	MB	0.32	10.2	0.65	ND
7/8/09	PRM	0.98	144	1.81	5.03
	MB	0.70	37.0	0.89	6.39
8/4/08	PRM	3.39	122	2.70	4.97
	MB	1.06	14.8	1.62	4.09
8/11/08	PRM	0.35	38.5	1.02	1.18
	MB	0.40	10.1	1.00	0.78
8/27/07	PRM	1.43	77.5	1.45	5.46
	MB	0.74	< 0.05	< 0.05	0.23
9/9/09	PRM	0.19	1.01	0.13	2.56
	MB	0.14	1.43	ND	0.55
9/23/09	PRM	0.25	1.14	< 0.05	0.43
	MB	0.45	1.73	0.18	0.78
10/7/08	PRM	0.87	58.5	1.04	6.26
	MB	0.19	< 0.05	0.43	0.18

Cryptophytes dominated the phytoplankton community at PRM on seven of 10 occasions, and cyanobacteria never dominated (Table 3.3). Cyanobacteria biomass (bC) was < 35 μg C L^{-1} on all but three occasions, which had bC of 600 – 1,050 μg C L^{-1}. Cyanobacteria proportion of total phytoplankton biomass (pC) never exceeded 0.23 on these three occasions and did not exceed 0.01 on any other occasion. The heterocyteous genus *Anabaena* and the non-heterocyteous genus *Microcystis* comprised the majority of cyanobacteria on the three high biomass dates (not shown). No heterocytes were observed in any of the cyanobacteria filaments at PRM. Highest diatom biomass (bD) and proportion of diatoms to total phytoplankton biomass (pD) at PRM were observed in May and August 2008 and late September 2009 (Table 3.3). Diatoms dominated the total phytoplankton biomass at PRM on 11 Aug 2008 (pD = 0.74) and 23 Sept 2009 (pD = 0.60).

The phytoplankton community at MB was more complex, with each of diatoms (5x), cryptophytes (4x), cyanobacteria (3x), and dinoflagellates (2x) dominating the community at least twice (Table 3.3). All three instances when cyanobacteria dominated at MB occurred in August 2008 or early September 2009. These cyanobacteria blooms were dominated by heterocyteous genera (*Anabaena* and *Aphanizomenon*), but non-heterocyteous *Microcystis* also accounted for a substantial proportion of phytoplankton biomass (not shown). Heterocytes were observed in some cyanobacteria filaments but at low abundance (4.4 heterocytes per 100 cells). Dinoflagellates only dominated at MB in late August 2007, but biomasses were low (< 250 μg C L^{-1}). Cryptophytes never dominated at MB after July 1 in any year. Diatoms never dominated when their biomass was < 1,000 μg C L^{-1}. On 7 Oct 2008, a large diatom bloom occurred (bD = 24,000 μg C L^{-1}) and comprised 92% of the phytoplankton biomass. This bloom was a near monoculture of *Aulacoseira ambigua*, which comprised 85% of the total phytoplankton community by itself.

Table 3.3. Biomass (b; in μg C L^{-1}) and proportions (p) of total phytoplankton biomass for cyanobacteria (C) and diatoms (D) in Missisquoi Bay, Lake Champlain. The dominant phytoplankton group (Dom) is included. c = cryptophytes. p = dinoflagellates. d = diatoms. b = cyanobacteria. v = chlorophytes. * denotes data obtained within a few days before and/or after N cycling incubations.

Date	Site	bC	pC	bD	pD	Dom
5/23/07*	MB	3.04	0.006	43.8	0.08	c
7/1/07*	MB	13.4	0.023	89.2	0.16	c
8/22/07*	MB	78.0	0.122	55.2	0.09	p
8/30/07*	MB	75.9	0.141	117	0.22	p
5/12/08	PRM	12.4	0.002	2590	0.40	c
6/2/08	PRM	4.43	0.003	407	0.24	c
6/25/08	MB	35.3	0.004	133	0.01	c
6/25/08	PRM	2.49	0.002	226	0.17	c
7/2/08	MB	103	0.018	3760	0.65	d
8/7/08*	MB	7320	0.780	1610	0.17	b
8/11/08	MB	18400	0.603	9630	0.31	b
8/11/08	PRM	669	0.231	2140	0.74	d
10/7/08	MB	941	0.036	24000	0.92	d
10/7/08	PRM	13.4	0.007	260	0.14	c
5/26/09	MB	17.8	0.004	2100	0.47	d
5/26/09	PRM	4.62	0.002	284	0.11	c
6/17/09	MB	10.8	0.002	1420	0.28	c
6/17/09	PRM	33.0	0.003	1090	0.10	v
7/8/09	MB	2600	0.257	5940	0.59	d
7/8/09	PRM	3.77	0.006	182	0.28	c
9/9/09	MB	8040	0.672	2660	0.22	b
9/9/09	PRM	602	0.114	324	0.06	c
9/23/09	MB	141	0.060	1040	0.44	d
9/23/09	PRM	1050	0.142	4420	0.60	d

3.4.3. Water column potential NH_4^+ uptake

Since NH_4^+ was added in excess of tracer levels, NH_4^+ uptake rates in this study are considered 'potential' rates. For the entire dataset, light NH_4^+ uptake rates (0.205 ± 0.022 μmol N L^{-1} h^{-1}) were significantly higher than dark rates (0.104 ± 0.015 μmol N L^{-1} h^{-1}; $p < 0.001$). There was no significant difference between light and dark uptake rates on two occasions; once at PRM on 8 July 2009, and once at MB on 4 Aug 2008. The ratio of light to dark uptake at PRM (2.26 ± 0.33; Fig. 3.1) was not significantly different from that at MB (3.00 ± 0.82; Fig. 3.2). Note that this ratio was negative for PRM on 4 Aug 2008 due to a negative dark uptake value (Fig. 3.1), and this ratio was excluded from all data analyses. The mean light NH_4^+ uptake rate at PRM (0.224 ± 0.036 μmol N L^{-1} h^{-1}) was not significantly different from that at MB (0.185 ± 0.024 μmol N L^{-1} h^{-1}). Mean dark NH_4^+ uptake also was not significantly different between the two sites (0.105 ± 0.020 and 0.103 ± 0.022 μmol N L^{-1} h^{-1} at PRM and MB, respectively). Both the highest and lowest light NH_4^+ uptake rates were measured at PRM in early July 2008 and 2009, respectively (Fig. 3.1). At PRM, light NH_4^+ uptake rates were generally higher early in the season (i.e., before July 3; 0.276 ± 0.050 μmol N L^{-1} h^{-1}) than later (after July 3; 0.171 ± 0.047 μmol N L^{-1} h^{-1}), but this trend was not statistically significant. The opposite trend was apparent at MB for dark NH_4^+ uptake, with higher rates after July 3 (0.128 ± 0.037 μmol N L^{-1} h^{-1}) versus early in the growing season (0.074 ± 0.012 μmol N L^{-1} h^{-1}). Again, this trend was not statistically significant.

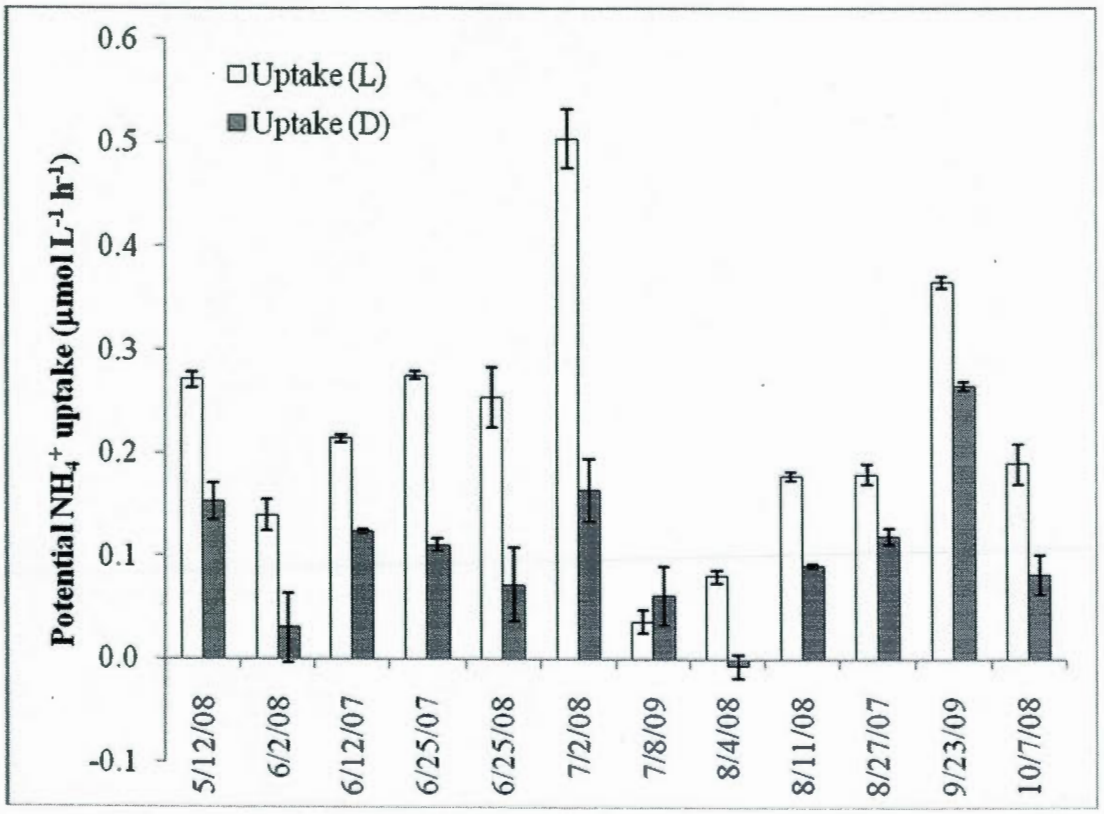

Fig. 3.1. Potential NH_4^+ uptake rates in light (L) and dark (D) water column incubations at the Pike River mouth (PRM) in Missisquoi Bay, Lake Champlain. Error bars are one standard error.

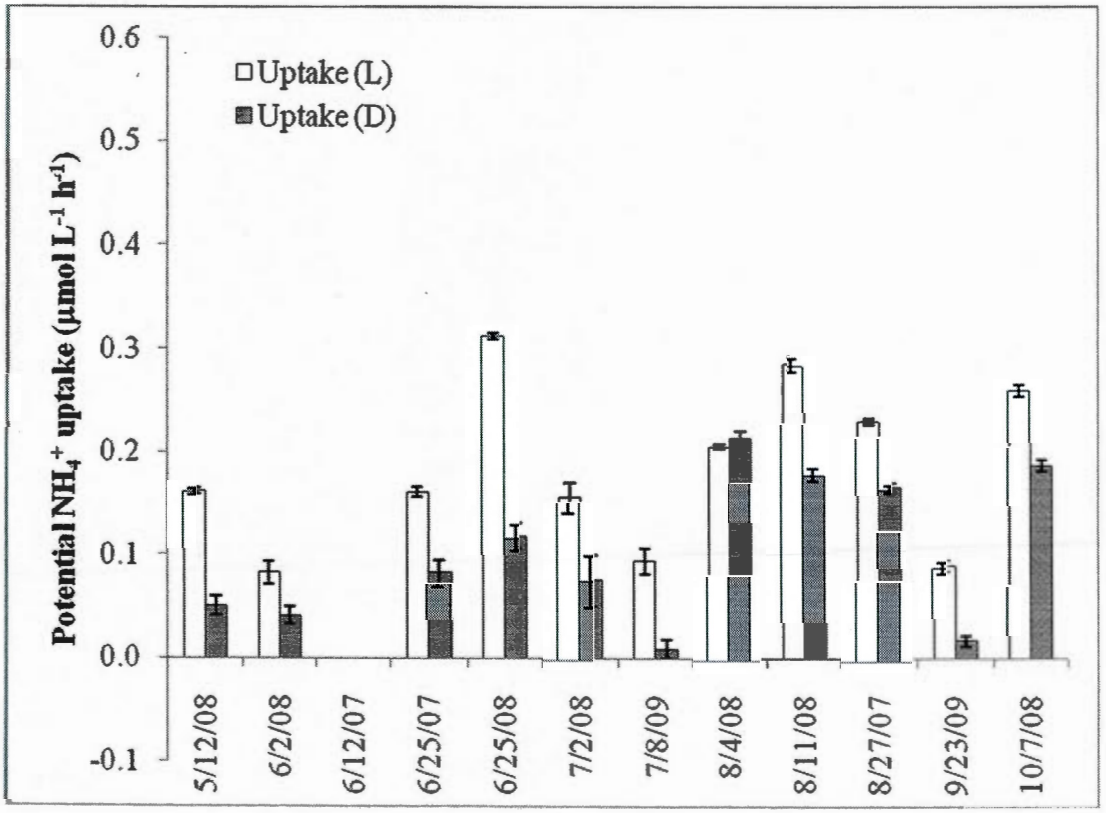

Fig. 3.2. Potential NH_4^+ uptake rates in light (L) and dark (D) water column incubations in the central basin (MB) of Missisquoi Bay, Lake Champlain. Error bars are one standard error. Note that the y-axis scale is the same as in Fig. 3.1.

3.4.4. Water column NH_4^+ regeneration

Mean light NH_4^+ regeneration rates were not significantly different between PRM (0.076 ± 0.014 µmol N L^{-1} h^{-1}; Fig. 3.3) and MB (0.070 ± 0.019 µmol N L^{-1} h^{-1}; Fig. 3.4). These rates were significantly lower ($p < 0.005$) than light NH_4^+ uptake rates at both sites. There also was no difference between dark NH_4^+ regeneration rates at PRM (0.097 ± 0.016 µmol N L^{-1} h^{-1}) and MB (0.094 ± 0.025 µmol N L^{-1} h^{-1}). Unlike with light rates, there was no difference between dark NH_4^+ regeneration and uptake rates at either site. As expected, there was no difference between light and dark NH_4^+ regeneration rates at either site, and only the

mean of light and dark NH_4^+ regeneration rates (PRM = 0.087 ± 0.010 and MB = 0.082 ± 0.015 μmol N L^{-1} h^{-1}) at each site will be discussed from here forward.

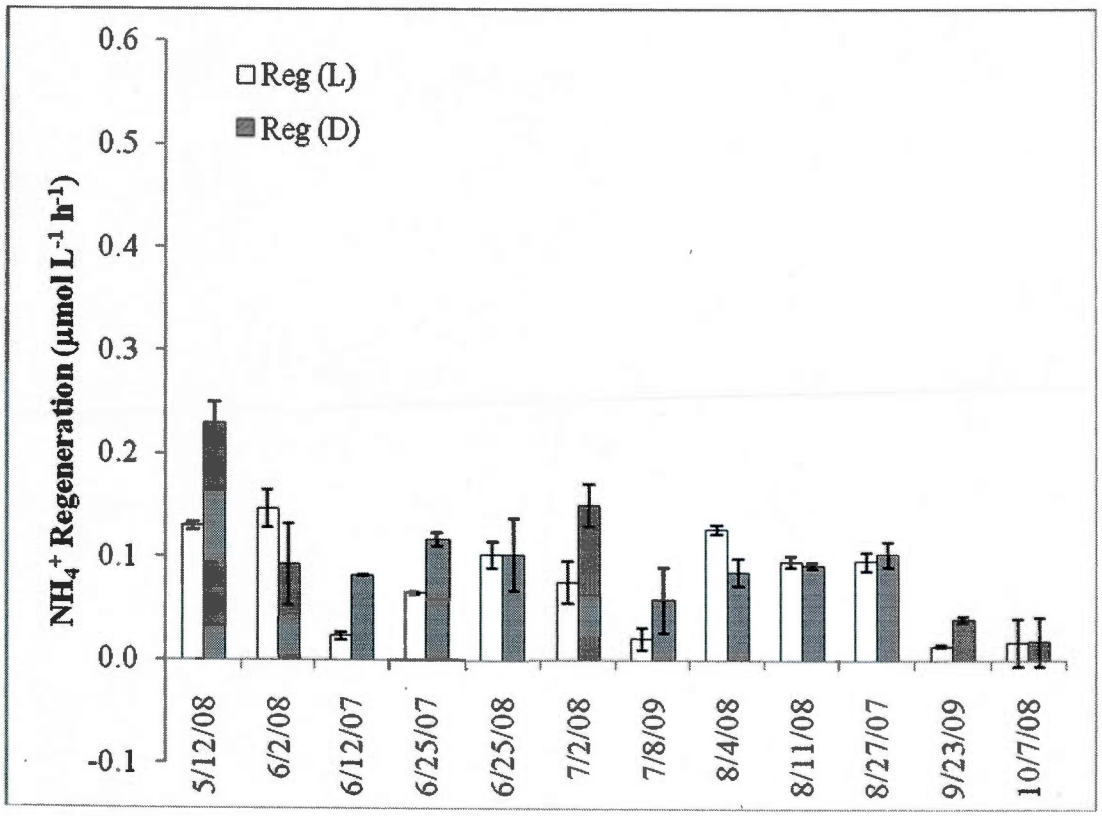

Fig. 3.3. NH_4^+ regeneration rates in light (L) and dark (D) water column incubations at the Pike River mouth (PRM) in Missisquoi Bay, Lake Champlain. Error bars are one standard error. Note that the y-axis scale is the same as in Fig. 3.1.

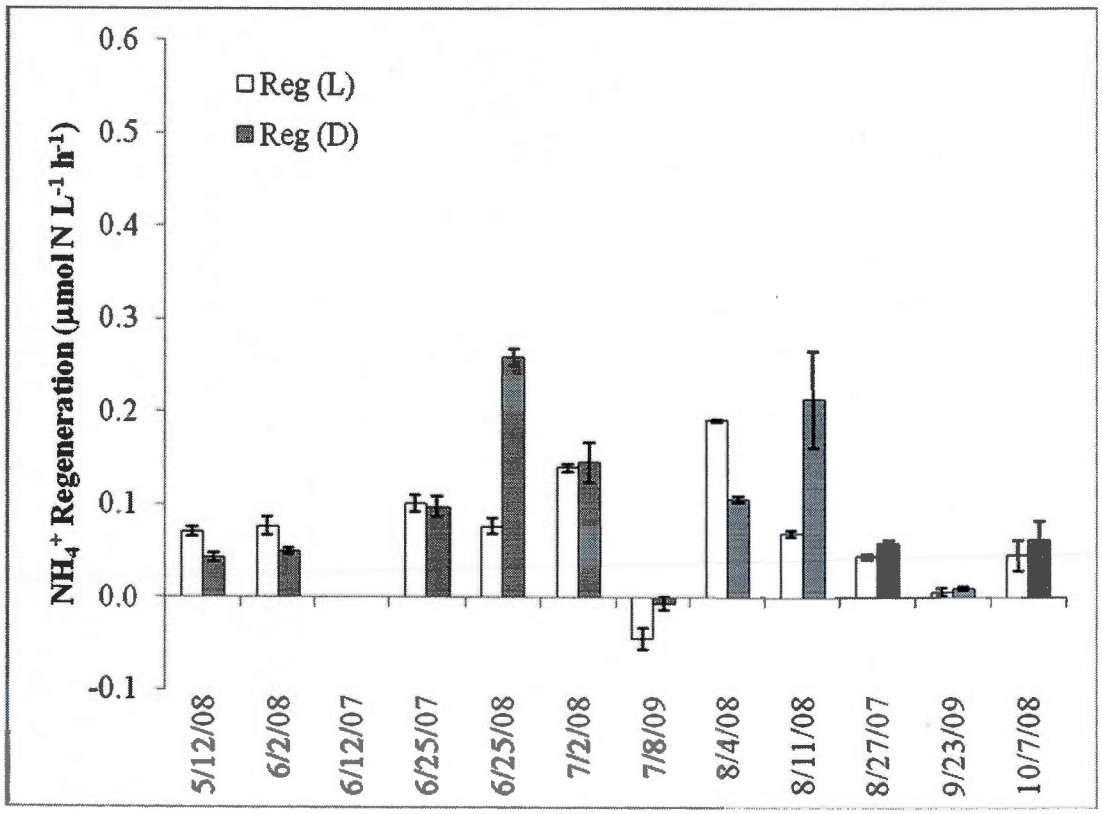

Fig. 3.4. NH_4^+ regeneration rates in light (L) and dark (D) water column incubations in the central basin (MB) in Missisquoi Bay, Lake Champlain. Error bars are one standard error. Note that the y-axis scale is the same as in Fig. 3.1.

Ammonium regeneration rates at PRM generally decreased as the growing season progressed (Fig. 3.3), with highest measurements occurring in May (> 0.2 µmol N L^{-1} h^{-1}) and the lowest rates (< 0.04 µmol N L^{-1} h^{-1}) in late September and early October. Regeneration rates at PRM clustered around 0.1 µmol N L^{-1} h^{-1} for most of the growing season, with a notable exception in July 2009, when regeneration was low (< 0.06 µmol N L^{-1} h^{-1}). At MB, highest regeneration rates were measured in mid-summer (Fig. 3.4), with July 2009 again being a notable exception. Regeneration was barely detectable in late September 2009 at MB. The highest individual NH_4^+ regeneration rate (0.259 µmol N L^{-1} h^{-1}) in this study was observed at MB on 25 June 2008. The ratio of light NH_4^+ uptake to the mean of light and dark NH_4^+ regeneration (UL:Reg) at PRM exhibited a large maximum late in the

growing season (UL:Reg > 10) and a secondary maximum in mid to late June (UL:Reg > 2; Fig. 3.5). This ratio was between one and two prior to mid-June and in mid to late August and below one in July and early August. MB also had a late season UL:Reg maximum (> 4), and all other UL:Reg were between one and three. Both light and dark NH_4^+ regeneration rates were calculated as negative values at MB on 8 July 2009, so no ratio was considered for this sampling event. There was no significant difference in UL:Reg between PRM and MB.

3.4.5. Water column N fixation

Water column N fixation rates (in light incubations only) at PRM in 2009 ranged from -0.758 on 3 July to 0.958 μmol N L^{-1} h^{-1} on 23 September (mean = 0.100 ± 0.272 μmol N L^{-1} h^{-1}; Fig. 3.6). This mean rate was not significantly different from that at MB (0.253 ± 0.145 μmol N L^{-1} h^{-1}), which ranged from 0.023 on 9 September to 0.810 μmol N L^{-1} h^{-1} on 3 July (Fig. 3.7). Rates at both sites were not significantly different from those in the filtered (0.2 μm) water incubations (control), and the rate at PRM was not significantly different from zero. High apparent N fixation rates in the control incubation on 23 Sept 2009 are nonsensical, but the measured concentrations in this incubation were repeated in replicate samples (note the error bars in Figs. 3.6 and 3.7). Therefore, there is no obvious explanation for these results. However, the apparent N fixation rate at MB was not significantly different from zero anyway, and N fixation at the nutrient replete PRM site would not be expected.

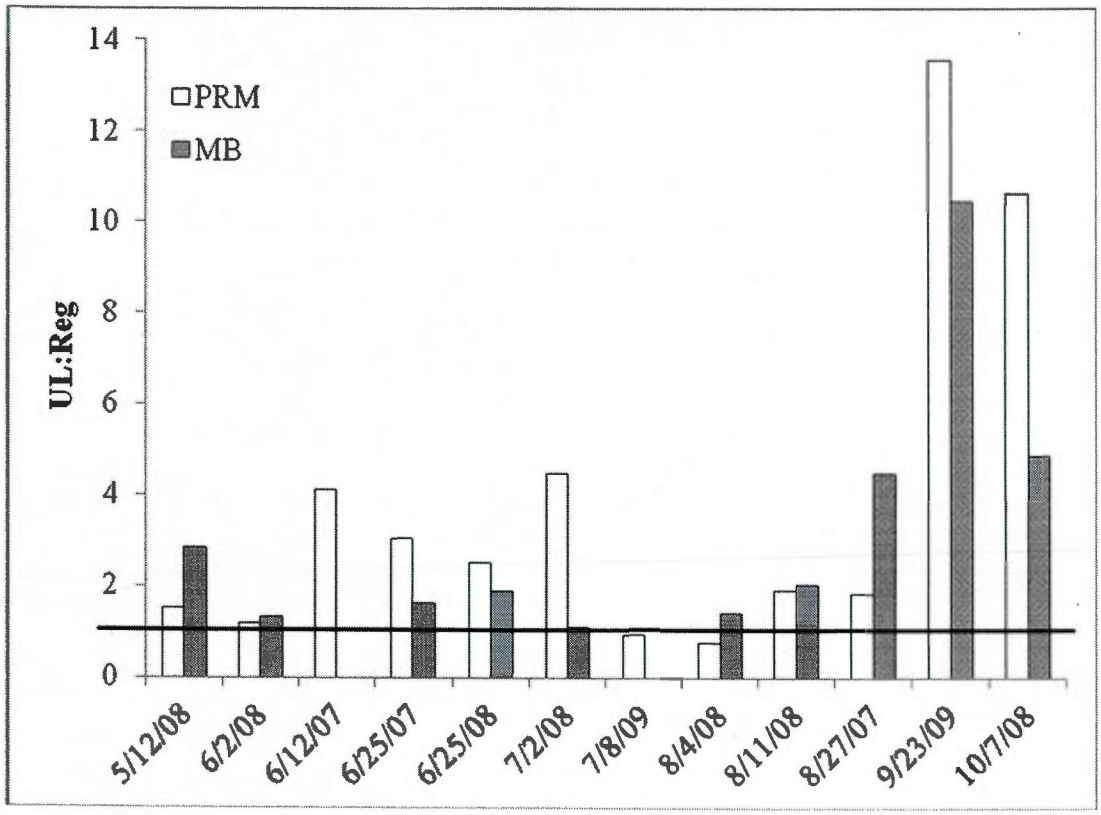

Figure 3.5. The ratio of light NH_4^+ uptake (UL) to the mean of light and dark NH_4^+ regeneration (Reg), which were not significantly different from each other, at the Pike River mouth (PRM) and central basin (MB) of Missisquoi Bay, Lake Champlain. Line is drawn at one, which reflects balanced uptake and regeneration. No incubation was conducted at MB on 12 June 2007, and regeneration was negative at MB on 8 July 2009.

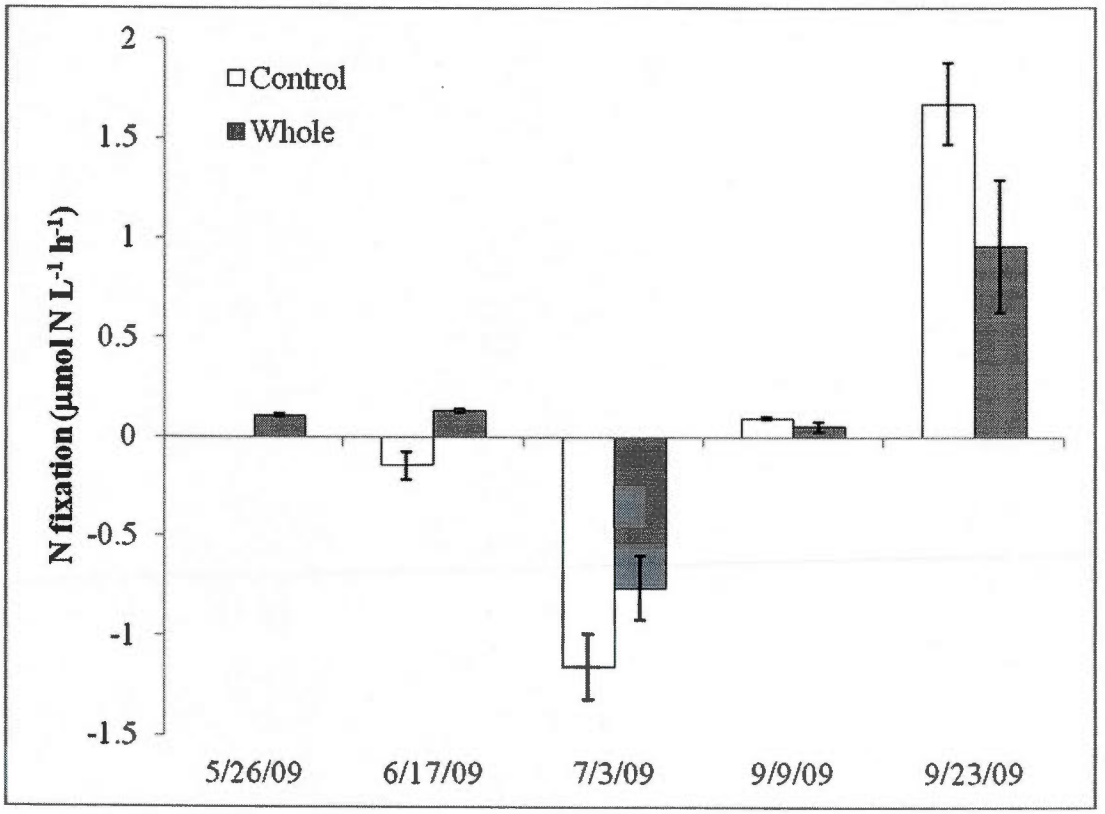

Figure 3.6. Water column N fixation rates in filtered (0.2 µm syringe filter; Control) and whole water samples from the Pike River mouth (PRM) in Missisquoi Bay, Lake Champlain. The rates in whole water incubations are not significantly different from those in the filtered controls, and the mean rate (0.10 ± 0.27 µmol N L^{-1} h^{-1}) is not significantly different from zero.

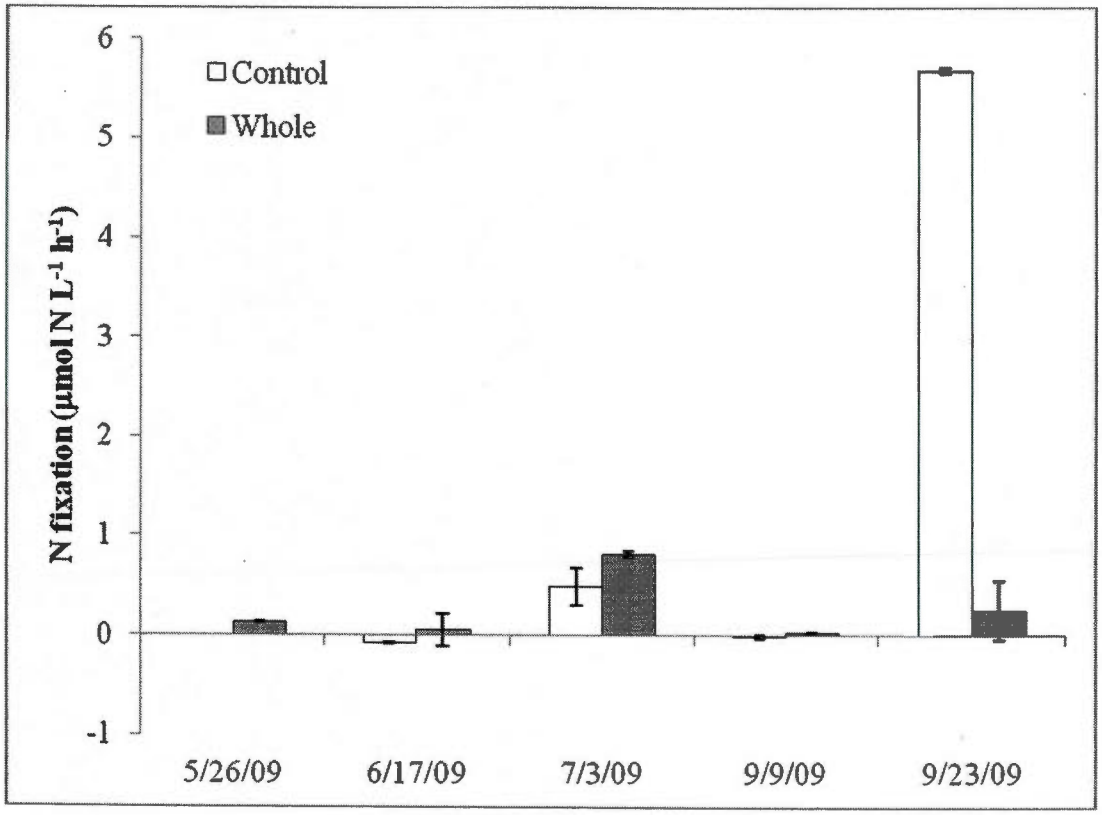

Figure 3.7. Water column N fixation rates in filtered (0.2 µm syringe filter; Control) and whole water samples from the central basin (MB) in Missisquoi Bay, Lake Champlain. The rates in whole water incubations are not significantly different from those in the filtered controls.

3.5. Discussion

3.5.1. Relationships between ambient conditions and phytoplankton

The cyanobacteria proportion of total phytoplankton biomass (pC) was weakly related to surface water temperature (r^2 = 0.225; p = 0.063; positive slope), but several missing datapoints for temperature, particularly late in the growing season (Table 3.1), were

likely detrimental to the significance of the relationship. The relationship between temperature and cyanobacteria blooms is well documented (e.g., McQueen & Lean 1987; Paerl & Huisman 2008, 2009), and the near-significant relationship between these parameters in Missisquoi Bay is consistent with the literature. Likewise, the diatom proportion of total phytoplankton biomass (pD) also was weakly related to water temperature ($r^2 = 0.217$; $p = 0.069$), but with a negative slope. This result also was predicted from the literature, which suggests that diatoms are better adapted to cooler water temperatures than some other phytoplankton groups (e.g., Lomas & Glibert 1999a, 1999b).

The presence of SRP and NH_4^+, which are the most bioavailable of the inorganic nutrient forms, in all samples suggested that nutrients may not limit primary production in Missisquoi Bay. Nutrient maxima generally occurred in early to mid-summer (June – July; Table 3.2), which coincided with the agricultural planting and fertilizing season in the watershed. With the exception of 2007, NO_x and NH_4^+ concentrations at PRM decreased from very high levels across the growing season, and NO_x was sometimes undetectable (e.g., Oct 2008). This trend is consistent with primary producer uptake within the lake and N sinks, such as denitrification. However, SRP remained high throughout most of the sampling events with no discernible trend, suggesting that internal and external sources were balanced with primary producer requirements.

Further evidence for lake productivity not being nutrient limited was the lack of lake-wide relationships between phytoplankton (as biomass or proportions of total biomass) and nutrients (as concentrations or ratios) in Missisquoi Bay. The only exception to this finding was a weak, negative relationship ($r^2 = 0.19$, $p = 0.04$) between pD and SRP concentration. The next closest relationship was between pC and NH_4^+ concentration ($r^2 = 0.11$, $p = 0.12$), which is intuitive given the expected cyanobacteria competitiveness for NH_4^+. No nutrients or ratios were related to any phytoplankton parameters at MB. However, NH_4^+ concentration was related negatively to all phytoplankton parameters at PRM, with stronger relationships for cyanobacteria than diatoms (Table 3.4). This pattern supports previous work describing phytoplankton preference for NH_4^+ over other inorganic N forms (e.g., McCarthy et al. 1977). The negative slope of these relationships also suggests that higher phytoplankton biomass

results in lower NH_4^+ concentrations (i.e., top down control). PRM is shallow but very turbid from suspended solids in the river discharge, and light is a probable factor limiting phytoplankton growth. No light measurements are available from PRM, but Secchi depths were < 0.5 m on most occasions.

Table 3.4. Summary of linear regression statistics for NH_4^+ concentration with phytoplankton parameters at the Pike River mouth (PRM) in Missisquoi Bay, Lake Champlain. pC = proportion of cyanobacteria biomass to total phytoplankton biomass. bC = cyanobacteria biomass. pD = proportion of diatom biomass to total phytoplankton biomass. bD = diatom biomass. All relationships have negative slopes.

Variable	r^2	p
pC	0.44	0.025
bC	0.47	0.020
pD	0.37	0.046
bD	0.36	0.051

3.5.2. Water column potential NH_4^+ uptake and autotrophy versus heterotrophy

Water column NH_4^+ uptake rates in Missisquoi Bay were within the range of rates observed in other freshwater systems. Missisquoi Bay rates were higher than rates in oligo- and mesotrophic Lakes Lugano (Switzerland; M.F. Lehmann, unpublished data) and Michigan (Gardner et al. 2004) but lower than in more eutrophic lakes Okeechobee (James et al., 2011), Taihu (Paerl et al., 2011), and Old Woman Creek (Ohio; McCarthy et al. 2007b). Missisquoi Bay uptake rates were similar to those from Saginaw Bay (Lake Huron; Gardner et al. 1995b) and coastal Lake Erie (McCarthy et al. 2007b). These results suggest that trophic status (as *chl*) could be a reasonable predictor of uptake rates, as expected. Uptake rates in Missisquoi Bay generally increased with available *chl* data ($p = 0.10$), but a more complete *chl* dataset may be required to constrain this possible relationship.

No spatial or temporal trends were observed in the NH_4^+ uptake data in Missisquoi Bay. Light NH_4^+ uptake includes heterotrophic uptake, which remains active in dark incubations, so balanced autotrophic and heterotrophic uptake would have a light:dark ratio of two. The mean ratio of light to dark NH_4^+ uptake (UL:UD = 2.63 ± 0.44) suggests that autotrophic, light-dependent uptake plays a slightly larger role than heterotrophic, light-independent (i.e., bacterial) uptake. However, this mean ratio is biased by a few events where light uptake was much higher than dark uptake. When sampling events were considered individually, dark uptake accounted for 51.6 and 55.8% of total NH_4^+ uptake at PRM and MB, respectively. These values suggest balanced autotrophic and heterotrophic uptake. Bacterial uptake can account for up to 60% of total uptake in some systems (Glibert 1993), and the values in Missisquoi Bay are consistent with this report. Some phytoplankton exhibit opportunistic dark uptake when nutrients are limiting (e.g., Cochlan et al. 1991). However, low N-to-carbon ratios resulting from N stress are required for phytoplankton to process DIN in darkness (Clark et al. 2002; Flynn et al. 2002). Replete nutrients in Missisquoi Bay suggest that dark uptake likely is performed by heterotrophic bacteria and/or mixotrophs, rather than luxury uptake by phytoplankton.

Uptake rates in Missisquoi Bay were not related to temperature or ambient NH_4^+ (or any other inorganic, dissolved nutrient) concentrations at either site or lake-wide. This result is not surprising since NH_4^+ (and other nutrients) was generally replete throughout the study period and is consistent with the idea that some factor other than nutrients generally were limiting primary productivity. Uptake rates for NH_4^+ were not related to the proportion of DIN present as NH_4^+ (NH4:NOx). Uptake rates also were not related to proportions or biomasses of diatoms or cyanobacteria. However, pC ($r^2 = 0.24$, $p = 0.045$) was related to the ratio of light to dark NH_4^+ uptake with a positive slope. As discussed previously, cyanobacteria are superior competitors for NH_4^+ (e.g., Blomqvist et al. 1994, Hyenstrand et al. 1998a, 1998b), and high cyanobacteria proportion would be expected to account for a large proportion of measured light NH_4^+ uptake and affect light versus dark rates. These results suggest that high cyanobacteria populations in Missisquoi Bay may affect the balance between autotrophic and heterotrophic NH_4^+ uptake during blooms, presumably by out-competing heterotrophic bacteria for NH_4^+.

3.5.3. Water column NH_4^+ regeneration

Regeneration of NH_4^+ via zooplankton grazing and excretion and organic matter remineralization should not be light-dependent, and the data from this study support this notion. There was no significant difference between light and dark regeneration rates at either site, and these rates were then averaged for each sampling event at each site. Highest regeneration rates at PRM (Fig. 3.3) generally were measured early in the season (i.e., 2 July and before; $p = 0.08$). In contrast, highest regeneration rates at MB were measured from late June to mid-August (not significant). A notable exception to the latter trend was the 8 July 2009 sampling event at MB, where pC and bC were high (Table 3.3). All water column NH_4^+ cycling rates tended to be higher at PRM early versus late in the season, and rates were higher in mid-summer than early and late in the season at MB. The apparent lag between highest rates at the two sites may reflect the longer distance from the nutrient source for MB, despite a lack of relationships with ambient nutrient concentrations. Instead, allochthonous organic matter being discharged by the river, particularly early in the season, may stimulate regeneration processes earlier at PRM than MB, which may be more dependent on autochthonous organic matter.

Regeneration rates were not related to any nutrient parameters or temperature. Regeneration rates also were not significantly related to proportions or biomasses of cyanobacteria or diatoms at either site. Cyanobacteria generally are not favorable forage for grazers due to toxin production, mucilaginous sheaths, colony formation, and other factors (e.g., Sellner et al. 1993; Vanderploeg et al. 2001). This theory suggests that cyanobacteria may exclude competitors directly by out-competing them for NH_4^+ and indirectly by focusing grazing pressure on those competitors (e.g., Blomqvist et al. 1994). Therefore, it is surprising that low regeneration rates were not associated with high cyanobacteria, but the small dataset gathered for this study may not have been sufficient to allow relationships to emerge.

Depth-averaged, areal water column NH_4^+ regeneration rates were calculated from volumetric rates to allow comparison with sediment-water interface rates reported in subsequent chapters of this thesis. This calculation assumes that measured regeneration rates

are not dependent on depth. While the water column in Missisquoi Bay is well-mixed, the photic zone likely does not extend to the sediment surface in deeper areas of the lake, and forage for grazers are likely more concentrated in the photic zone. Secchi depths in pelagic areas of the lake range from 1.5 to 3 m (unpublished data), and mean depth is about 2.8 m (Mendelsohn et al. 1997). For this exercise, it was assumed that the entire lake volume was euphotic to the mean depth. In those cases where water depth measurements were not available, the calculation was performed using the mean depth for that site.

Depth-averaged NH_4^+ regeneration rates were higher ($p = 0.004$) at MB (398 ± 79.8 μmol N m^{-2} h^{-1}) than PRM (111 ± 32.0 μmol N m^{-2} h^{-1}), which was expected given the large difference in water depth and lack of difference in volumetric rates between the sites. If the estimated surface area surrounding PRM is 1.5 km^2, and there are two other areas near rivers entering Missisquoi Bay, then the water column regeneration rate for river discharge areas can be scaled-up to 12,000 mol N d^{-1}. Missisquoi Bay surface area is 77.5 km^2 (Mendelsohn et al. 1997) less 4.5 km^2 for river discharge areas gives a pelagic surface area of 73 km^2. Spatially extrapolating the regeneration rates from MB yields a daily regeneration rate of 696,000 mol N d^{-1}. Combined, these lake areas contribute an estimated 708,000 mol N d^{-1} via regeneration in the water column. No estimates of N loading into Missisquoi Bay could be found in the scientific or gray literature, but total N load was estimated from tributary monitoring (http://www.vtwaterquality.org/cfm/champlain/lp_longterm-lakes.cfm) and flow data (Smeltzer & Simoneau 2008). This estimate, including the Pike, Rock, and Missisquoi Rivers, is ~372,000 mol N d^{-1}, which is less than half the estimated water column NH_4^+ regeneration rate. This comparison suggests that regeneration pathways play an important role in supporting primary productivity in the lake. Further, the total biomass turnover time in Missisquoi Bay (range 3.6 to 45.8 days; mean = 15.3 ± 4.24 days; not shown) supports other results suggesting some level of resource limitation and top-down control of productivity (e.g., Dufour & Torreton 1996).

The light NH_4^+ uptake to regeneration ratio (UL:R) at PRM (mean = 3.86 ± 1.17) was not significantly different from MB (mean = 3.19 ± 0.91), and these values suggest that water column regeneration cannot fully support NH_4^+ uptake. At PRM, there were two occasions (8

July 2009 and 4 Aug 2008) in mid-summer when regeneration exceeded uptake and could supply sufficient NH_4^+. Neither cyanobacteria nor diatoms dominated the phytoplankton community on 8 July 2009 at PRM (cryptophytes dominated), and no phytoplankton data are available for 4 Aug 2008 at PRM. Thus, it cannot be speculated that cyanobacteria or diatoms benefitted from regeneration exceeding uptake in these cases. Regeneration never exceeded uptake at MB, suggesting that primary producers must supplement their nutrient requirements with N released from sediments (see Chapter III) or advected from other parts of the lake.

3.5.4. Water column N fixation

N fixation rates were not detected in the water column in Missisquoi Bay using the water column incubation technique. This finding was supported qualitatively by independent measurements of NO_3^- isotopic ratios, which yielded $\delta^{15}N\text{-}NO_3^-$ values > 5 ‰ (mean = 9.61 ± 1.27 ‰; M.F. Lehmann, unpublished data). These values are higher than would be expected if N fixation represented a significant input of new N to the lake. N fixation generates fixed N with a $\delta^{15}N\text{-}NO_3^-$ near 0 ‰ (e.g., Sigman et al. 2005). Since DIN was measurable at both sites at all times, it is not surprising that heterocyteous cyanobacteria were not relying heavily on N fixation, which is an energy intensive process (e.g., Paerl 1990). No heterocytes were observed in cyanobacteria at PRM, and less than five heterocytes per 100 cells were observed at MB. These results support the lack of measured N fixation, but the presence of heterocytes suggests that extreme N limitation occurred on fine spatial or temporal scales. Heterocyte differentiation in cyanobacteria occurs at the expense of cell growth and reproduction (Attridge & Rowell 1997) and only when the cell senses severe N deficiency (Muro-Pastor et al. 1999). Therefore, the presence of heterocytes in cyanobacteria cells at MB, despite the presence of DIN in all samples collected, suggests that extreme N limitation occurred on scales not captured during the sampling. The ability to fix atmospheric N during these episodes of N scarcity, while also being capable of efficient DIN assimilation the rest of the time, likely provides these genera a competitive advantage over non-N-fixing cyanobacteria, other phytoplankton, and obligate N fixers.

3.5.5. Conclusion

Water column NH_4^+ uptake and regeneration rates in Missisquoi Bay were within ranges of rates measured in other eutrophic freshwater systems. The environmental conditions in Missisquoi Bay were not such that extreme rates would be expected. No significant spatial or temporal trends were apparent in either uptake or regeneration, and NH_4^+ cycling rates were not related to nutrients or temperature. Results suggest that autotrophic and heterotrophic NH_4^+ uptake were balanced, except during cyanobacteria blooms, when most NH_4^+ uptake was autotrophic. Generally higher NH_4^+ cycling rates early in the growing season at PRM and in mid-summer at MB suggest allochthonous nutrient and organic matter sources at PRM versus autochthonous sources at MB. More data are needed to confirm or refute this pattern. Basin-scale extrapolation of regeneration rates revealed that ~708,000 mol N d^{-1} was regenerated in the Missisquoi Bay water column. Estimated N loading to Missisquoi Bay is ~372,000 mol N d^{-1} based on tributary mean annual TN concentrations and flow rates, and this comparison suggests a primary role for regeneration mechanisms in fueling primary productivity. Light uptake to regeneration ratios implied that NH_4^+ regeneration in the water column alone cannot sustain measured NH_4^+ uptake rates. This result suggests that primary producers in Missisquoi Bay must supplement their nutrient requirements with N released from sediments (see Chapter IV) or advected from other parts of the lake. No significant N fixation was measured in the lake, which was not a surprising result due to replete nutrients measured at both sites at all times. However, the presence of heterocytes, albeit at low abundances, suggested that extreme N limitation occurred at temporally and spatially explicit scales not captured in the sampling regime from this study. This conclusion is supported by numerous studies showing that cyanobacteria do not differentiate heterocytes unless subjected to extreme N limitation (e.g., Muro-Pastor et al. 1999). The ability to fix atmospheric N during these events and return to competing for N sources as they become available provides N fixing cyanobacteria with a competitive advantage over non-N-fixing cyanobacteria, other phytoplankton, and obligate N fixers.

CHAPTER IV: SEDIMENT-WATER INTERFACE NITROGEN TRANSFORMATIONS IN MISSISQUOI BAY, LAKE CHAMPLAIN

MARK J. McCARTHY, WAYNE S. GARDNER, MORITZ F. LEHMANN & DAVID F. BIRD

4.1. Summary

Nitrogen (N) transformations and fluxes at the sediment-water interface (SWI) were measured using continuous-flow, intact sediment core incubations in Missisquoi Bay, Lake Champlain, during the 2007 – 2009 growing seasons. These incubations were combined with stable isotope techniques to evaluate N sources (e.g., N fixation), sinks (e.g., denitrification and anammox), and "links" (e.g., dissimilatory NO_3^- reduction to NH_4^+; DNRA) at two sampling sites: one near the Pike River discharge into Missisquoi Bay (PRM), and one in the central basin of the lake (MB). Results from this study suggested that organic matter (OM) limitation at PRM affected SWI nutrient fluxes and N transformation rates and pathways. MB sediments were less dynamic and did not exhibit evidence of OM limitation. Sediments were a more effective NO_3^- sink at PRM (-416 ± 144 μmol N m^{-2} h^{-1}) than at MB (-24.4 ± 20.3 μmol N m^{-2} h^{-1}), and both sites switched from being a net NO_3^- sink early in the season to a NO_3^- source late in the season. The opposite trend was measured for NH_4^+, for which sediments at both sites were a net source (PRM = 210 ± 61.9 μmol N m^{-2} h^{-1}; MB = 79.9 ± 19.2 μmol N m^{-2} h^{-1}). However, PRM sediments switched to being a net NH_4^+ sink late in the season. DNRA was not a consistent NH_4^+ regeneration pathway in sediments at either site, but cation exchange in sediments may have complicated DNRA estimates. Sediment O_2 demand (SOD) measurements supported the idea that OM limitation developed in PRM sediments by decreasing from > 2,000 in July to < 1,000 μmol O_2 m^{-2} h^{-1} in fall. In contrast, no temporal trend in SOD was apparent at MB. Further evidence of OM limitation at PRM

was the reversal of net N_2 production occurring through late August to net N_2 fixation in fall. MB sediments also produced N_2 for most of the season, but rates were lower than at PRM.

Higher potential denitrification versus net N_2 fluxes suggested that denitrification was limited by NO_3^-, especially at MB, where ambient NO_3^- concentrations were lower. Anammox may have contributed 6 – 10% of total N_2 production in lake sediments, on average, but molecular techniques are needed to confirm whether anammox is occurring. N fixation occurred simultaneously with denitrification in most cases, but these rates could offset only 25 – 30% of total N losses from microbial N_2 production. Actual denitrification was balanced by dissolved inorganic N (DIN) fluxes at PRM but exceeded water column NH_4^+ regeneration, confirming PRM as a net N sink. Sediment N removal at MB was balanced by DIN influxes but was 7-fold lower than water column NH_4^+ regeneration, which could support only ~80% of measured potential NH_4^+ uptake. These results support the hypothesis that pelagic areas of the lake may be N limited some parts of the year.

A bottom-water hypoxia event at MB in early July 2009 led to an altered N cycle, with lower actual denitrification rates and higher NH_4^+ effluxes, while SRP flux was unaffected. The earliest observed cyanobacteria bloom in this study occurred less than one week after this hypoxia event, and this finding contradicts a parallel study (Smith 2009), which concluded that sediment redox dynamics were controlled by cyanobacterial blooms. However, the present study showed that there were few cyanobacteria in the water column before the hypoxia event, which suggested that the altered N transformation pathways may have contributed to stimulation of the bloom observed five days later.

4.2. Introduction

Eutrophication involves increased biological productivity in an aquatic system due to increased nutrient inputs. Eutrophication has been described as "one of the foremost problems in protecting freshwater and coastal marine systems" (Schindler 2006). Sources of excess nutrients to watersheds and aquatic systems include treated and untreated sewage,

agricultural runoff containing fertilizers, urban stormwater runoff, atmospheric deposition (e.g., Hicks 2007, Duce et al. 2008, Galloway et al. 2008, Elser et al. 2009), and groundwater inflow (Anderson et al. 2002, Smolders et al. 2010).

Nutrient recycling and subsequent internal loading from sediments can exacerbate the ecosystem effects of eutrophication (Bailey & Hamilton 1997, Hansen et al. 1997, Berelson et al. 1998), even after external loading reductions have been implemented (Burger et al. 2007, Jeppesen et al. 2007). Nutrient regeneration in sediments underlying the water column can supply nutrients to primary producers (Flint & Kamykowski 1984; Tobias et al. 2003). Sediments are the primary location for organic matter degradation and remineralization in shallow aquatic systems (Tobias et al. 2003). Sustained external nutrient loading into a system results in high water column productivity and, ultimately, high organic matter deposition and degradation rates in sediments (Beman et al. 2005). Nutrients remineralized in sediments can enter the water column from physical forcing (e.g., sediment resuspension; Fan et al. 2001), chemical remobilization (e.g., anoxia), or diffusion. In some cases, internal nutrient regeneration and release from sediments can exceed external nutrient inputs and delay system recovery from nutrient input reductions (e.g., Burger et al. 2007). In systems where diatoms dominate, sediments may play a more important role in system nutrient recycling (Poister & Armstrong 2004), while water column recycling may be more important when cyanobacteria are dominant (e.g., McCarthy et al. 2007c; McCarthy et al. 2009a).

In eutrophic systems, sediment nutrient regeneration is dominated by organic matter remineralization to NH_4^+, or ammonification (Tobias et al. 2003). The fate of regenerated NH_4^+ is critical to system nutrient status, primary productivity, phytoplankton community structure, and organic matter processing. The most direct fate involves re-assimilation into biomass, but the classical paradigm proceeds from NH_4^+ to nitrification, which is the step-wise oxidation of NH_4^+ to NO_2^- (via N_2O), then NO_3^-. Nitrate from nitrification then can be denitrified, which is the step-wise heterotrophic reduction of NO_3^- to N_2 (via NO_2^-, NO, and N_2O; e.g, Seitzinger 1988, Zumft 1997). Denitrification is a poorly constrained (Galloway et al. 2004) N sink in the global N budget. It may drive systems toward N limitation and be a natural eutrophication defense (Seitzinger 1988; Bartkow and Udy 2004). DIN sources for

denitrification in sediments include remineralization of organic matter and diffusion from the water column (Cornwell et al. 1999). Sediments accumulating easily degraded organic matter tend to have higher denitrification rates, especially driven by water column NO_3^- diffusion (Dong et al. 2000; Dahllöf and Karle 2005). Both nitrification and denitrification often proceed at rates below their potential in freshwater systems (Bowden 1987).

Recent discoveries have added to our understanding of the N cycle and characterized additional pathways beyond the nitrification/denitrification regime. These pathways include dissimilatory NO_3^- reduction to NH_4^+ (DNRA) and anaerobic NH_4^+ oxidation (anammox). The DNRA pathway has been recognized for some time, but difficulties in measuring the process have impeded our understanding of its importance and distribution in aquatic systems. DNRA usually is associated with sulfidic estuarine or marine sediments but can account for up to 30% of total NO_3^- reduction in lake sediments (Tiedje 1988; Brunet and Garcia-Gil 1996). DNRA, previously called reammonification (Bowden 1987), has been evaluated in only a few freshwater systems (Burgin & Hamilton 2007), where it accounted for varying degrees of internal N recycling. In two lakes on the North Island of New Zealand, DNRA was not observed in the shallower lake (Lake Rotorua) but accounted for 7 – 12 % of total sediment NH_4^+ flux in the deeper lake (Lake Rotoiti; McCarthy et al. 2007a). Previous studies in Lake Rotoiti noted NH_4^+ accumulation in the hypoxic hypolimnion during stratified periods (Priscu et al. 1986), and DNRA may explain some of this NH_4^+ accumulation. DNRA accounted for a similar fraction of total sediment NH_4^+ flux (~10 %) in a Great Lakes wetland (McCarthy et al. 2007b). DNRA also was observed, at varying degrees of importance, in a constructed wetland in central Texas (Scott et al. 2008). The relative partitioning between DNRA and denitrification is an important constraint in the N budget of aquatic systems. The different end-products of each process, NH_4^+ and N_2, respectively, have different and significant ramifications for the system N budget (Bowden 1987; Kemp et al. 1990; Bonin et al. 1998). Denitrification results in N loss from the system, and this N is not available to most organisms until it is fixed back into organic matter, which is an energetically unfavorable process (Capone 2000). However, DNRA returns reduced N to the system in a bioavailable form (NH_4^+) favored by most primary producers (Bowden 1987; Bonin et al. 1998).

Historically, denitrification was considered the only biological process removing fixed N from aquatic systems and returning it to the atmosphere (Seitzinger 1988; Blackburn and Blackburn 1992). Anammox is an alternate pathway to N_2 and oxidizes NH_4^+ with NO_2^- to form N_2 (Rysgaard et al. 2004), but its significance in most freshwater systems is unknown (Burgin & Hamilton 2007). Potential anammox was evaluated recently in Lake Tanganyika, where it may have accounted for 7 – 13% of total N_2 production in the suboxic water column (Schubert et al. 2006). Anammox in marine systems is better understood, and evidence suggests that water depth and temperature are important factors determining anammox activity (Dalsgaard et al. 2005). The relationship to water depth may be due to the correlation with water temperature, since anammox bacteria have a lower optimum temperature (12 °C) than denitrifiers (24 °C; Jetten 2001). This characteristic suggests that there may be a seasonal pattern to the importance of anammox relative to denitrification in systems where temperature fluctuates seasonally. Anammox is inhibited by some simple organic compounds (e.g., pyruvate, ethanol, and glucose; Jetten et al. 1999). Therefore, anammox may be most important in low labile carbon sediments (Burgin & Hamilton 2007). Anammox can be assessed by adding $^{15}NH_4^+$ in the presence of replete $^{14}NO_x$ (e.g., Rysgaard et al. 2004) and measuring $^{29}N_2$ production using mass spectrometry. However, there are alternative explanations for $^{29}N_2$ production in this case, and molecular techniques may be required to confirm anammox. While the mechanism of N_2 formation and effects on carbon cycling are interesting, the end result of anammox and denitrification is the same; i.e., N is removed from the system and must be fixed to become available to non-diazotrophic primary producers.

The primary objective of this chapter was to quantify sediment-water interface (SWI) O_2 demand, nutrient fluxes (including DNRA), and microbial N sources (heterotrophic N fixation) and sinks (denitrification and anammox). Another objective was to determine whether denitrification in Missisquoi Bay was supported more by NO_3^- from nitrification or water column diffusion. Constraining these rates is valuable for understanding controls on phytoplankton dynamics and constructing nutrient budgets, which are imperative for successful eutrophication management efforts. It was hypothesized that denitrification rates would be positively related to sediment O_2 demand (SOD) and decrease over the growing season, ultimately reversing to net N fixation late in the season. DNRA and anammox were

hypothesized to be minor in Missisquoi Bay. Sediments were hypothesized to be a net nutrient source to the water column via organic matter remineralization. With the exception of inconsistent monitoring of total and dissolved N concentrations, little or no information exists on N cycling in Missisquoi Bay.

4.3. Materials & Methods

SOD and N cycling rates were determined at two sites in Missisquoi Bay, Lake Champlain. These sites were located at the Pike River discharge into the lake (PRM) and the central basin (MB; Figure 1.3). PRM is shallow (~1 m), turbid from the river discharge, and expected to have higher ambient nutrient concentrations. MB is deeper (~4 m), less turbid, and expected to have lower ambient nutrient concentrations. Depth profiles of water temperature and dissolved oxygen (DO) concentration were determined using a YSI sonde. Ambient nutrient samples were filtered immediately after collection in the field with 0.2 μm Nylon syringe filters and analyzed using standard autoanalyzer colorimetric techniques (NO_3^- + NO_2^-, NO_2^-, and soluble reactive phosphorus (SRP)) and, for NH_4^+, high performance liquid chromatography (HPLC; Gardner et al. 1995a).

Intact sediment core incubations were conducted on cores collected on three dates in 2007 (12 June, 25 June, and 27August), seven dates in 2008 (12 May, 2 June, 25 June, 2 July, 4 August, 11 August, and 7 October), and two dates in 2009 (8 July and 23 September). Only cores from PRM were incubated on 12 June 2007 due to the inability to collect cores from depth at MB. Adjustments were made to the coring apparatus after this date to allow core collections from greater depths. Sediment nutrient fluxes, DNRA, SOD, and N transformations at the SWI were evaluated using continuous-flow incubations of intact sediment cores collected from the two sampling sites in Missisquoi Bay. This technique is based on the system described by Lavrentyev et al. (2000) and has been used in numerous other shallow aquatic systems (e.g., An et al. 2001; An & Gardner 2002; Gardner et al. 2006; McCarthy et al. 2007a, b, and c; Scott et al. 2008; Gardner et al. 2009; Gardner & McCarthy 2009). The incubation system (Fig. 4.0) consisted of 20 L carboys with bottom water

collected from each site, a Rainin peristaltic pump, acrylic core tubes (7.6 cm ID), a custom plunger assembly with Teflon inflow and outflow tubing, and sample collection vessels.

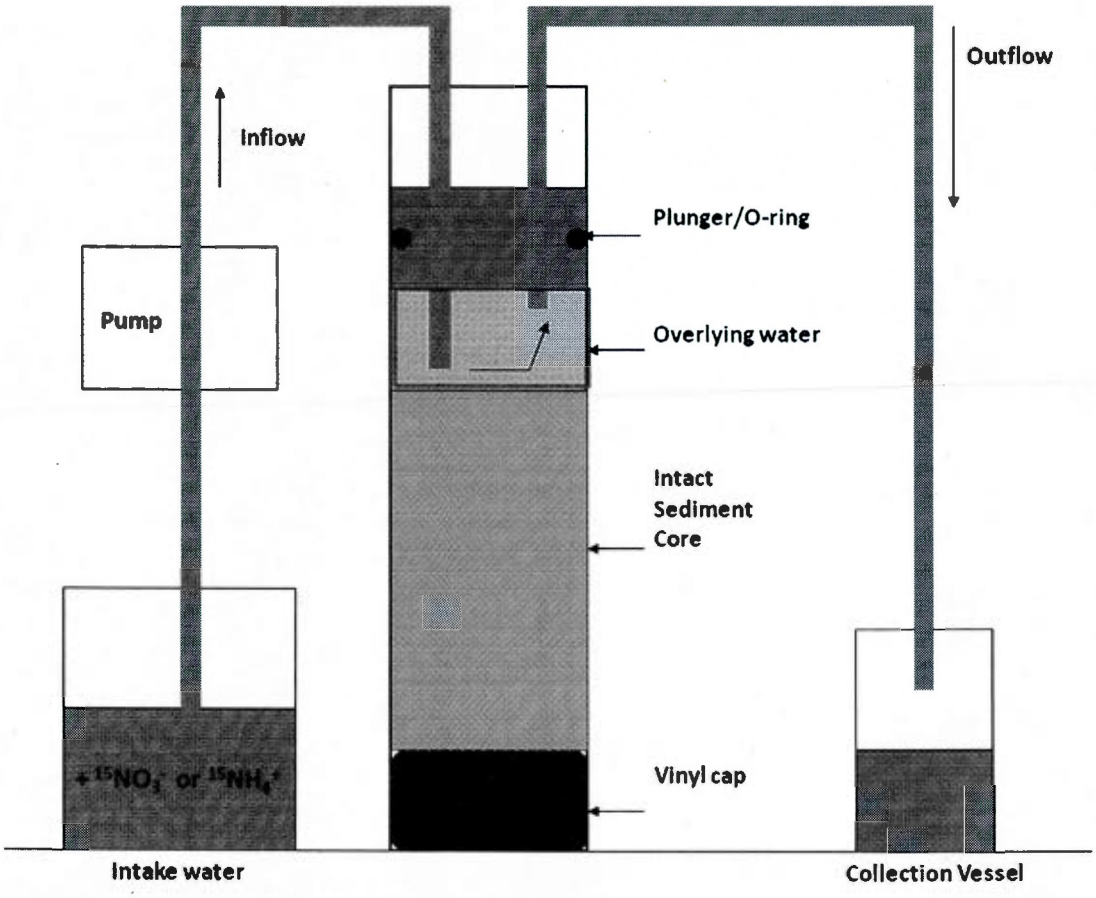

Figure 4.0. Schematic diagram of the continuous-flow, intact sediment core incubation system (based on Lavrentyev et al. 2000).

Intact cores (10 – 15 cm depth) were collected from a boat using a custom one-way valve fitted onto the core tube and attached to a 6 m long aluminum pole. Cores with overlying water were capped immediately using vinyl caps sealed with electrical tape and stored in a cooler for transport to the lab. Bottom water was collected using a submersible pump. Cores were wrapped with aluminum foil and installed into the continuous-flow incubation system immediately upon return to the lab and allowed to flow (~1.2 ml min^{-1}) for

at least 18 hours before sampling to allow re-establishment of steady-state conditions. Plungers were positioned about 5 cm above the SWI, leaving an overlying water volume of approximately 230 ml (Gardner & McCarthy 2009). Three treatments from each site were conducted on duplicate cores, for a total of six cores per site. Control cores were unamended, and $^{15}NH_4^+$ (~5 μM final concentration in inflow water) and $^{15}NO_3^-$ (~50 μM final concentration) were spiked into the inflow water of respective duplicate cores. Cores were incubated for four days total at room temperature and sampled on days two, three, and four.

SWI nutrient fluxes, SOD, and N transformations were assessed as concentration differences between inflow and outflow water samples normalized for system flow rate and sediment surface area in the cores (Lavrentyev et al. 2000). All nutrient samples were filtered and analyzed as described above for ambient nutrients. Dissolved gas samples were collected in 15 mL tall, ground-glass stoppered test tubes (Chemglass) and analyzed immediately using MIMS for O_2:Ar, $^{28}N_2$:Ar, $^{29}N_2$, and $^{30}N_2$ (Kana et al. 1994; An et al. 2001). The test tubes were allowed to overflow for several tube volumes to prevent atmospheric contamination.

Potential DNRA was determined as $^{15}NH_4^+$ production from $^{15}NO_3^-$ added to inflow water. $^{15}NH_4^+$ production rates were determined using HPLC (An & Gardner 2002; Gardner et al. 2006) on samples also used to measure total NH_4^+ concentrations. Inflow and outflow samples collected from the core incubations were analyzed for total NH_4^+ concentration and isotope ratio (Gardner et al. 1995), and DNRA rates were calculated as $^{15}NH_4^+$ concentration differences in inflow and outflow samples given flow rate and sediment surface area. Potential DNRA rates in $^{15}NO_3^-$ additions were compared to control cores, and DNRA was considered significant only if the $^{15}NH_4^+$ production was significantly higher in the $^{15}NO_3^-$ addition treatment than in the control cores. DNRA rates were qualified as "potential" because substrate ($^{15}NO_3^-$) was added in excess of ambient concentrations. However, these rates actually may be conservative estimates because they did not include DNRA fueled by NO_3^- from sediment nitrification. In addition, cation exchange processes in the sediments may result in preferential adsorption of $^{15}NH_4^+$ onto sediment particles, resulting in masking of DNRA measured by $^{15}NH_4^+$ production from added $^{15}NO_3^-$ (Gardner et al. 2006). If $^{15}NO_3^-$ addition stimulated DNRA and leads to increased total NH_4^+ efflux, then this "nitrate-induced

ammonium flux" (NIAF) was compared to measured $^{15}NH_4^+$ fluxes to evaluate discrepancies, which were attributed to cation exchange anomalies.

Sediment cores were incubated in the dark (foil wrapped), so benthic photosynthesis producing O_2 was assumed to be insignificant. Results from unamended control cores were used for in situ SOD estimates. The core incubation technique offered two possible avenues for evaluating the importance of benthic N fixation. First, net N_2 flux measured in control cores represents the balance between denitrification (N_2 efflux) and N fixation (N_2 influx). The direction of sediment N_2 flux identifies which of these processes is more important during the incubation, and a negative net N_2 flux would suggest that N fixation rates exceed denitrification. Second, isotope pairing techniques allow estimation of gross sediment N fixation occurring simultaneously with denitrification (An et al. 2001).

Similar to sediment N fixation, the methods used offer multiple avenues to evaluate the importance of denitrification. Isotope pairing of N_2 formed via denitrification (Nielsen 1992) of added isotopes ($^{15}NH_4^+$ or $^{15}NO_3^-$) allows quantification of potential denitrification (DNF) and identifies the substrate for denitrification (i.e., NO_3^- produced via nitrification versus denitrification of water column NO_3^-). Net N_2 flux in control cores provides a direct measurement of denitrification in the absence of N fixation. If isotope pairing calculations in the $^{15}NO_3^-$ addition treatment failed to quantify any sediment N fixation occurring simultaneously with denitrification (An et al. 2001), then net N_2 flux in control cores is the best estimate of ambient denitrification occurring in Missisquoi Bay sediments. If isotope pairing calculations showed a significant sediment N fixation rate, then that rate was added to the net N_2 flux in control cores to determine the best estimate of sediment denitrification rate.

Possible anammox was evaluated by measuring $^{29}N_2$ production from $^{15}NH_4^+$ added to inflowing water. This technique does not conclusively identify anammox as a N sink, because other combinations of N transformations could explain the measured $^{29}N_2$. For example, $^{15}NH_4^+$ oxidation to $^{15}NO_3^-$ via nitrification combined with an ambient $^{14}NO_3^-$ via denitrification to form $^{29}N_2$ also is a possible explanation. The absence of $^{29}N_2$ production in

the presence of added $^{15}NH_4^+$ and a sufficient $^{14}NO_x$ pool would, however, suggest that anammox is not occurring. Molecular techniques are required to confirm anammox.

The ion source within the quadrupole mass spectrometer used in MIMS ionizes gases prior to mass separation and detection and produces O^+ ions, which can react with N_2, forming NO (mass 30; Jensen et al. 1996). This scavenging of N_2 is more significant at low O_2 concentrations, such as those found in sediments, and may result in over-estimation of denitrification rates via interference with ratio measurements (Eyre et al. 2002; Kana and Weiss 2004). This error appears to be machine-specific and was not observed on the MIMS at Université du Québec à Montréal (UQÀM; McCarthy and Bird, unpublished data). This effect can be monitored in control cores (i.e., no isotope addition) by measuring mass 30 production, and subsequent denitrification estimates can be corrected by subtracting the control mass 30 production from these denitrification estimates. However, this procedure was not necessary in any case with the MIMS instrument at UQÀM or results from this study. Differences between rates and sampling sites were evaluated using one-way analysis of variance (ANOVA). Significant relationships between variables were evaluated using linear regressions. Differences and relationships were deemed significant at $p \leq 0.05$.

4.4. Results

4.4.1. Ambient conditions

Water depths, surface and bottom temperatures, and surface and bottom dissolved O_2 concentrations from the sampling events at PRM and MB in 2007 – 2009 were presented previously in Table 3.1. Relative to the SWI, noteworthy observations include very low water depth (0.08 m) at PRM on 4 Aug 2008 and bottom-water hypoxia at MB on 3 July 2009 (also observed on 17 June 2009). Sediment core sampling occurred five days after this hypoxia event on 8 July 2009. Ambient nutrient concentrations from these sampling events were presented previously in Table 3.2. As expected, water column nutrient concentrations were higher at PRM than MB. No temporal patterns were observed for SRP in the water column,

but both NO_3^- and NH_4^+ exhibited a decreasing trend over the course of the growing season. SRP and NH_4^+ were measurable at all sites at all times during this project, but NO_x was undetectable at MB on 27 Aug 2007 and < 0.5 µM on 7 Oct 2008 (Table 3.2).

Sediment porosity and composition were not characterized in detail for this project, but PRM sediments were tightly packed, large grain sands with intact plant material (twigs and leaves) often embedded or resting on the sediment surface. The sand layer was usually about 5 cm thick and underlain by a clay/sand/gravel conglomerate. Sediments at MB were loose muds with a "fluff" layer about 2 - 4 cm thick and no visible plant material. Occasional large bivalves (not zebra mussels) and small, benthic crustaceans were encountered. These organisms were removed from cores prior to incubations. No sulfur or methane was detected by smell during core collection or after incubations were complete at either site.

4.4.2. SWI nutrient fluxes

Missisquoi Bay sediments were a SRP source in all cases at MB and about half the time at PRM (Fig. 4.1), where sediments were a SRP source early in the season and usually a sink late in the season. For the entire dataset, SRP flux at PRM was not significantly different from zero (mean = -0.15 ± 1.72 µmol P m^{-2} h^{-1}), but MB sediments were a significant SRP source (mean = 7.63 ± 1.33 µmol P m^{-2} h^{-1}). These means were significantly different from each other ($p = 0.002$). SRP flux was not related to water column SRP concentration at either site, suggesting that their dynamics may be occurring on different timescales. SRP flux was not related to SOD (or bottom water DO) at either site or to water temperature at PRM. SRP flux was related to water temperature at MB, however ($r^2 = 0.43$; $p = 0.03$; slope = 0.71). Nitrogen isotope additions had no effect on SRP fluxes at either site.

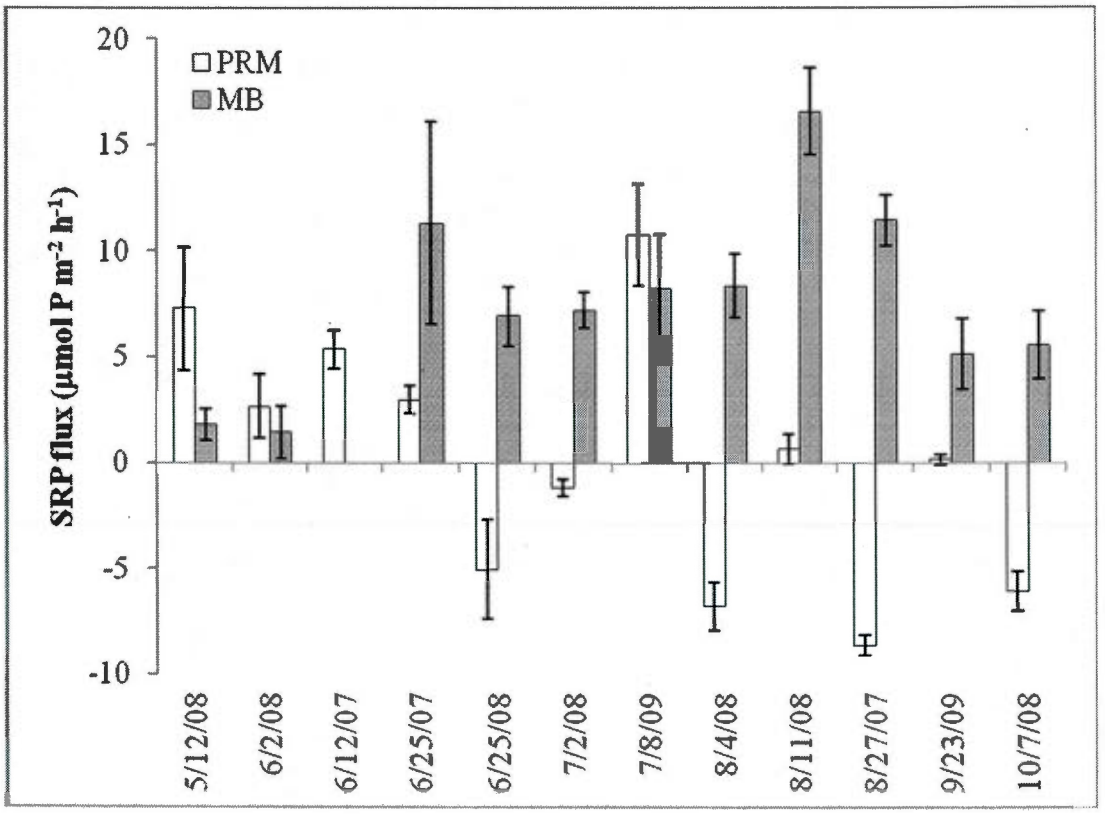

Figure 4.1. Soluble reactive phosphorus (SRP) flux from sediments in Missisquoi Bay, Lake Champlain, at the Pike River mouth (PRM) and central basin (MB) in 2007 – 2009. Positive bars represent flux out of sediments, and negative bars represent flux from the water column into sediments. Error bars represent one standard error.

Missisquoi Bay sediments also were a net source of NO_2^- at both sites, which implies incomplete nitrification and/or denitrification. Mean NO_2^- flux at PRM (20.7 ± 8.25 µmol N m^{-2} h^{-1}) was almost significantly higher ($p = 0.06$) than at MB (3.89 ± 2.16 µmol N m^{-2} h^{-1}). A July-August maximum was observed at PRM (Fig. 4.2) with rates > 30 µmol N m^{-2} h^{-1}, and a smaller August maximum was observed at MB with rates > 10 µmol N m^{-2} h^{-1}. This trend at PRM was nearly related to water temperature ($r^2 = 0.289$; $p = 0.071$) and SOD ($r^2 = 0.285$; $p = 0.074$), but NO_2^- flux was not related to net $^{28}N_2$ flux, NH_4^+ flux, or water column NO_x

concentration at either site. Nitrite flux at MB was related to NO_3^- flux ($r^2 = 0.36$; $p = 0.05$; positive slope). Nitrogen isotope additions had no effect on NO_2^- fluxes at either site.

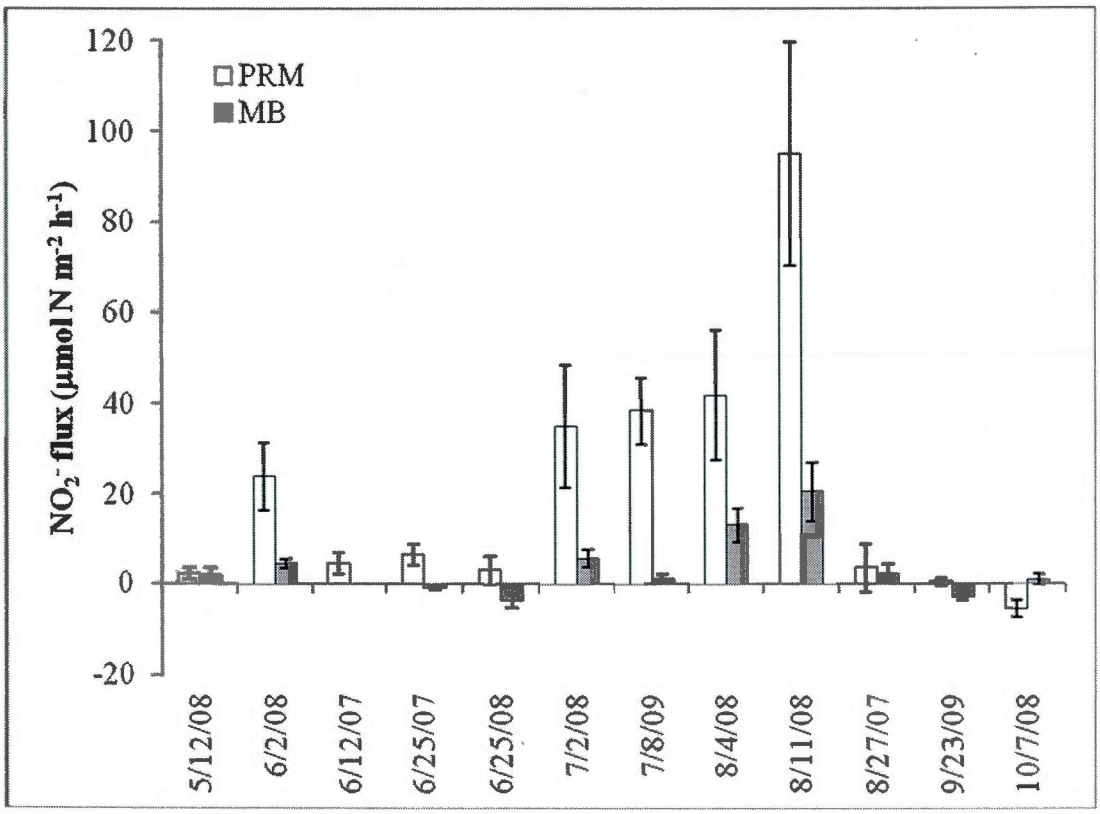

Figure 4.2. Nitrite (NO_2^-) fluxes from sediments in Missisquoi Bay, Lake Champlain, at the Pike River mouth (PRM) and central basin (MB) in 2007 – 2009. Positive bars represent flux out of sediments, and negative bars represent flux from the water column into sediments. Error bars represent one standard error.

PRM sediments were a significantly ($p = 0.02$) more effective NO_3^- sink (-416 ± 144 µmol N m^{-2} h^{-1}) than MB sediments for the entire dataset (-24.4 ± 20.3 µmol N m^{-2} h^{-1}). However, sediments at both sites switched from being a net NO_3^- sink early in the season to being a small NO_3^- source later (Fig. 4.3). This switch occurred in August at MB but not until September at PRM. Nitrate flux at PRM was negatively correlated with NO_3^- concentration

($r^2 = 0.84$; $p << 0.001$), $^{28}N_2$ flux ($r^2 = 0.78$; $p < 0.001$), NH_4^+ flux ($r^2 = 0.70$; $p < 0.001$), and SOD ($r^2 = 0.65$; $p = 0.002$) and weakly correlated with DNF ($r^2 = 0.28$; $p = 0.076$). Nitrate flux at MB was negatively correlated to NO_3^- concentration ($r^2 = 0.60$; $p = 0.005$) and positively correlated to NO_2^- flux ($r^2 = 0.36$; $p = 0.05$). To clarify the former relationship, high water column NO_3^- concentrations were associated with high NO_3^- uptake by sediments, which caused the negative slope. Excluding the 25 June 2007 data (the only time when NH_4^+ flux was into the sediments at MB) produces a negative relationship between NO_3^- and NH_4^+ fluxes at MB ($r^2 = 0.54$; $p = 0.015$). Isotopic N additions did not stimulate or inhibit NO_3^- flux at PRM, and $^{15}NH_4^+$ addition had no effect on NO_3^- flux at MB. However, $^{15}NO_3^-$ addition stimulated NO_3^- uptake by sediments at MB (mean NO_3^- flux in control cores = -24.4 ± 20.3 versus -228 ± 31.9 μmol N m^{-2} h^{-1} in $^{15}NO_3^-$ enriched cores; $p << 0.001$). This pattern suggests that denitrifiers were limited by NO_3^-, which will be discussed further below.

Missisquoi Bay sediments were generally an NH_4^+ source at both sites, with higher mean efflux from PRM (210 ± 61.9 μmol N m^{-2} h^{-1}) than MB (79.9 ± 19.2 μmol N m^{-2} h^{-1}). This difference was not statistically significant ($p = 0.066$), but this is likely due to the small number of observations. With the exception of 25 June 2007, MB sediments were an NH_4^+ source with rates ranging from 24 to 205 μmol N m^{-2} h^{-1} (Fig. 4.4). Ammonium efflux from PRM sediments was high (120 to 670 μmol N m^{-2} h^{-1}) until early August, then decreased, even becoming an NH_4^+ sink on 27 Aug 2007 and 7 Oct 2009 (Fig 4.4). Ammonium flux at PRM was positively correlated to $^{28}N_2$ flux ($r^2 = 0.75$; $p < 0.001$), DNF ($r^2 = 0.68$; $p = 0.001$), and SOD ($r^2 = 0.57$; $p = 0.004$). However, NH_4^+ flux at PRM was not related to water column NH_4^+ concentration or water temperature. At MB, NH_4^+ flux also was not related to water temperature or water column NH_4^+ concentration but was related to SOD ($r^2 = 0.47$; $p = 0.02$; positive slope). In contrast to PRM, NH_4^+ flux at MB was not related to $^{28}N_2$ flux or DNF. On average, isotope additions had no significant effect on SWI NH_4^+ fluxes at either site.

4.4.3. DNRA and NIAF

DNRA rates in $^{15}NO_3^-$ enriched cores were low in Missisquoi Bay (not shown; range = 0 to 29.4 μmol N m^{-2} h^{-1}) and were only significantly different from those in control cores

on four occasions at PRM (all before July) and two occasions at MB (both after July). Mean DNRA at PRM and MB were 20.3 ± 5.21 (n = 4) and 10.6 ± 4.88 μmol N m^{-2} h^{-1} (n = 2), respectively. NIAF was observed on seven occasions at PRM and averaged 105 ± 26.7 μmol N m^{-2} h^{-1}. At MB, NIAF was observed three times and averaged 164 ± 50.7 μmol N m^{-2} h^{-1}. DNRA and NIAF were not observed on enough occasions to attempt to determine relationships with other parameters or site differences.

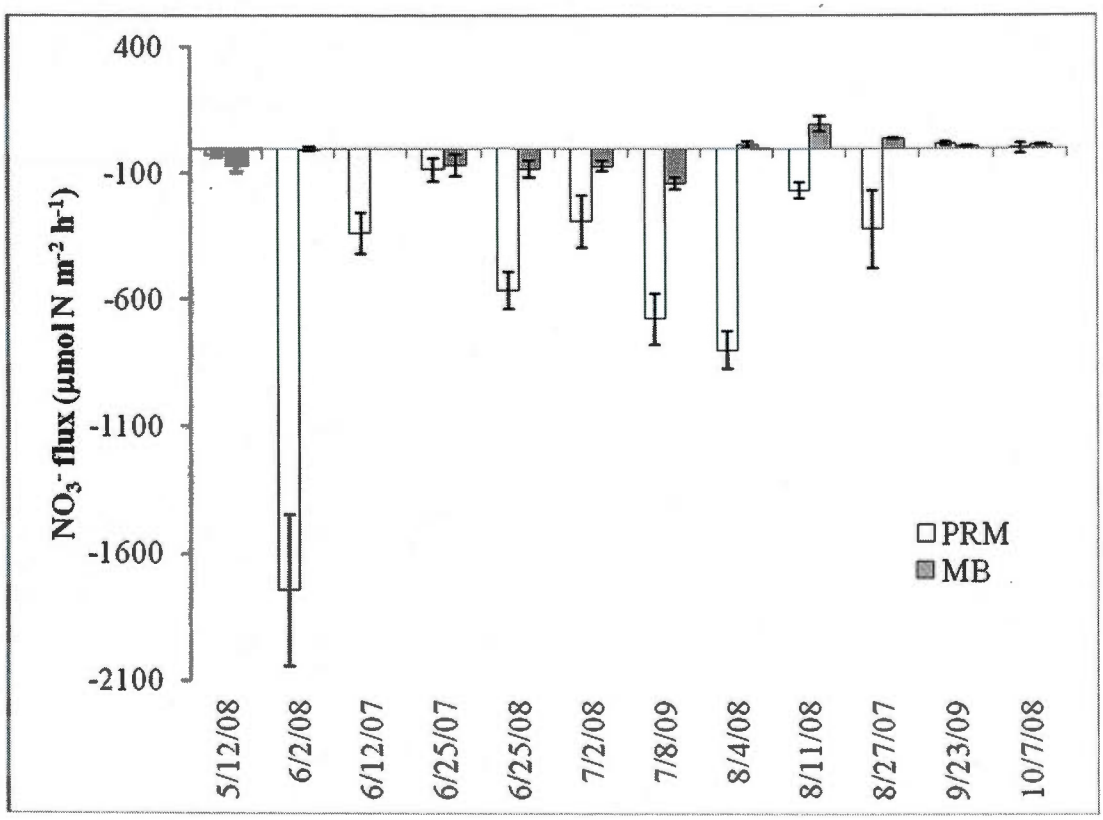

Figure 4.3. Nitrate (NO_3^-) fluxes from sediments in Missisquoi Bay, Lake Champlain, at the Pike River mouth (PRM) and central basin (MB) in 2007 – 2009. Positive bars represent flux out of sediments, and negative bars represent flux from the water column into sediments. Error bars represent one standard error.

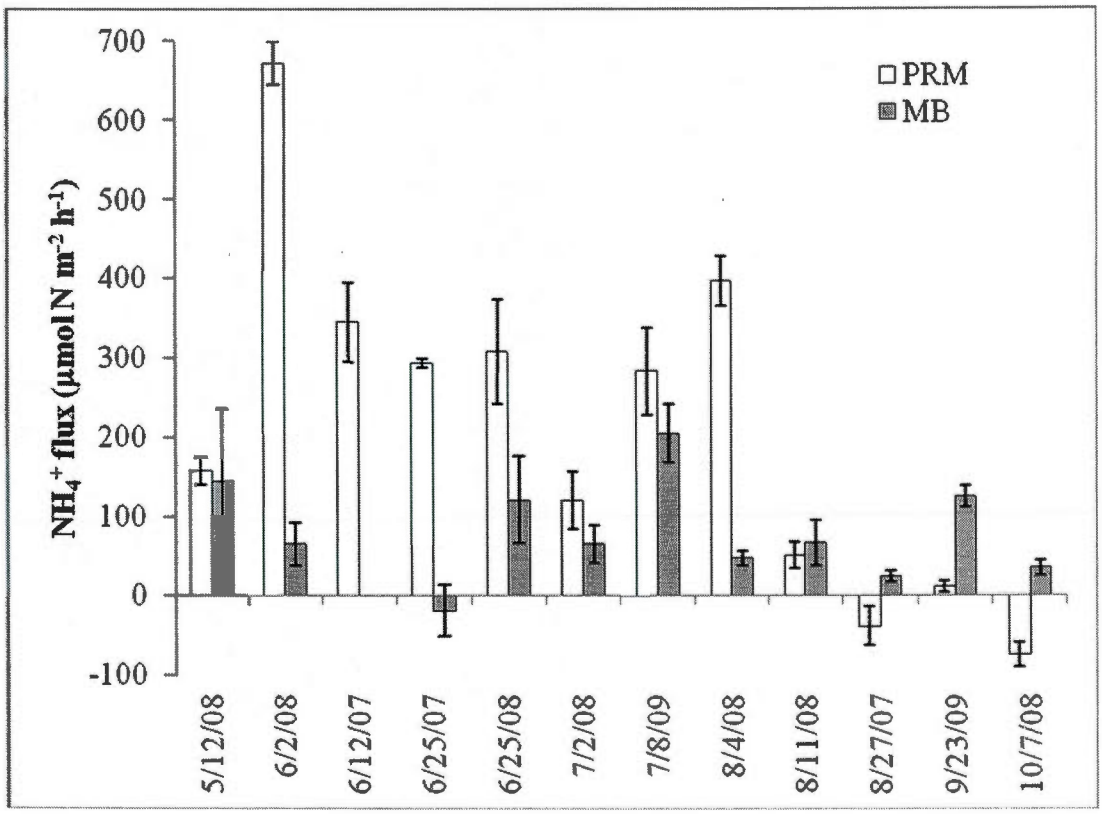

Figure 4.4. Ammonium (NH_4^+) fluxes from sediments in Missisquoi Bay, Lake Champlain, at the Pike River mouth (PRM) and central basin (MB) in 2007 – 2009. Positive bars represent flux out of sediments, and negative bars represent flux from the water column into sediments. Error bars represent one standard error.

4.4.4. SOD

Mean SOD at PRM (1,700 ±186 µmol O_2 m^{-2} h^{-1}) was not significantly higher than mean SOD at MB (1,500 ± 142 µmol O_2 m^{-2} h^{-1}) for the entire dataset. SOD was not related to water temperature or water column nutrient concentrations at MB. SOD was related to water column NO_3^- concentration at PRM ($r^2 = 0.50$; $p = 0.01$; positive slope) but not to water temperature or other nutrients. As mentioned previously, SOD was negatively related to NO_3^- at PRM and positively to NH_4^+ fluxes at PRM and MB. Highest SOD at both sites occurred

early in the season. At PRM, SOD declined from the July maximum (>2,000 μmol O_2 m^{-2} h^{-1}) to <1,000 μmol O_2 m^{-2} h^{-1} in fall (Fig. 4.5). While the lowest SOD at MB also occurred in fall, a temporal trend was not apparent between the early summer maximum and fall minimum. Isotope enrichments had no significant effect on SOD at either site.

4.4.5. Net $^{28}N_2$ flux

Note that all N_2 results have been converted to single N units to allow comparison with nutrient fluxes and DNRA. For the entire dataset, mean net $^{28}N_2$ flux in control cores at PRM (159 ± 52.9 μmol N m^{-2} h^{-1}) was significantly higher ($p = 0.02$) than at MB (1.38 ± 28.2 μmol N m^{-2} h^{-1}). These rates suggest that PRM sediments were a net N sink, and the maximum rate was ~600 μmol N m^{-2} h^{-1} in early June (Fig. 4.6). From this maximum, net $^{28}N_2$ flux at PRM decreased through the growing season and was either negative or not different from zero from late August onward. Negative net N_2 flux indicates that N fixation exceeded denitrification at that time. At MB, sediments were generally a net N sink (Fig. 4.7), but the $^{28}N_2$ measurement from 12 May 2008 (-260 ± 24.2 μmol N m^{-2} h^{-1}) skewed the mean. Excluding this measurement, net $^{28}N_2$ flux at MB was 27.5 ± 11.7 μmol N m^{-2} h^{-1}, and the significant difference between the two sites was upheld ($p = 0.04$).

As mentioned previously, net $^{28}N_2$ flux in control cores was related positively to NH_4^+ flux and negatively to NO_3^- flux at PRM. Net $^{28}N_2$ flux at PRM also was related positively to water column NO_3^- concentration ($r^2 = 0.82$; $p < 0.001$), SOD ($r^2 = 0.50$; $p = 0.01$), DNF ($r^2 = 0.40$; $p = 0.03$), water column NH_4^+ concentration ($r^2 = 0.37$; $p = 0.035$), and SWI N fixation ($r^2 = 0.355$; $p = 0.04$). At MB, net $^{28}N_2$ flux was only related to the ratio of SWI N fixation to potential denitrification (NF:DNF; $r^2 = 0.71$; $p = 0.001$) and SWI N fixation ($r^2 = 0.53$; $p = 0.01$), both with negative slopes. However, excluding the very high negative $^{28}N_2$ flux measured on 12 May 2008 at MB results in a breakdown of these two relationships. Isotope additions had no significant effect on net $^{28}N_2$ flux at either site, despite the mean flux at PRM being about half in $^{15}NO_3^-$ enriched cores versus control cores.

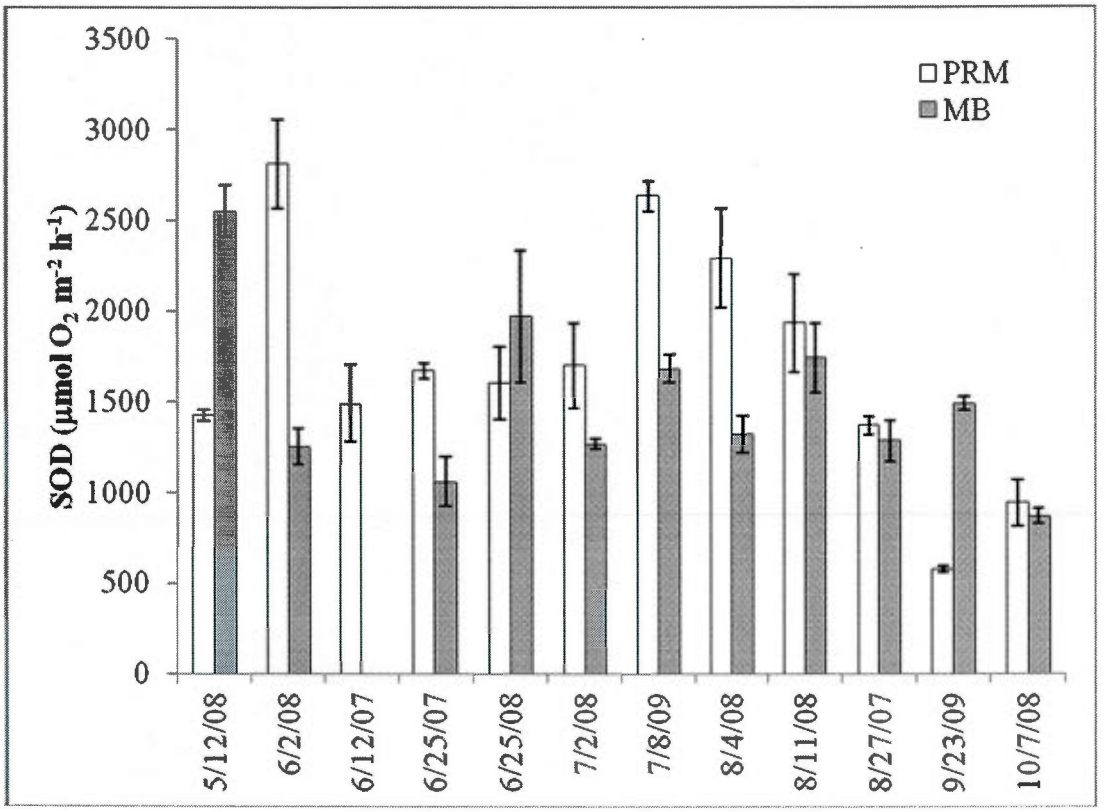

Figure 4.5. Sediment O_2 demand (SOD) in Missisquoi Bay, Lake Champlain, at the Pike River mouth (PRM) and central basin (MB) in 2007 – 2009. Error bars represent one standard error.

4.4.6. Potential denitrification (DNF)

DNF was calculated as the sum of all isotopic N_2 fluxes ($^{28+29+30}N_2$) in $^{15}NO_3^-$ enriched cores and includes any N_2 produced by anammox. Mean DNF at PRM (360 ± 58.2 µmol N m^{-2} h^{-1}) was significantly higher ($p = 0.03$) than at MB (214 ± 16.3 µmol N m^{-2} h^{-1}). DNF was > 200 µmol N m^{-2} h^{-1} at PRM through mid-August and < 200 µmol N m^{-2} h^{-1} from late August (Fig. 4.6). DNF at MB, however, was highest in mid-summer but remained within a smaller range (149 – 296 µmol N m^{-2} h^{-1}) than at PRM (49 – 631 µmol N m^{-2} h^{-1}). Mean DNF was significantly higher ($p < 0.02$) than mean net N_2 flux in control cores at both

sites. DNF also was significantly higher than net N_2 flux in control cores for all sampling events at MB and all but three sampling events (all occurring before August) at PRM.

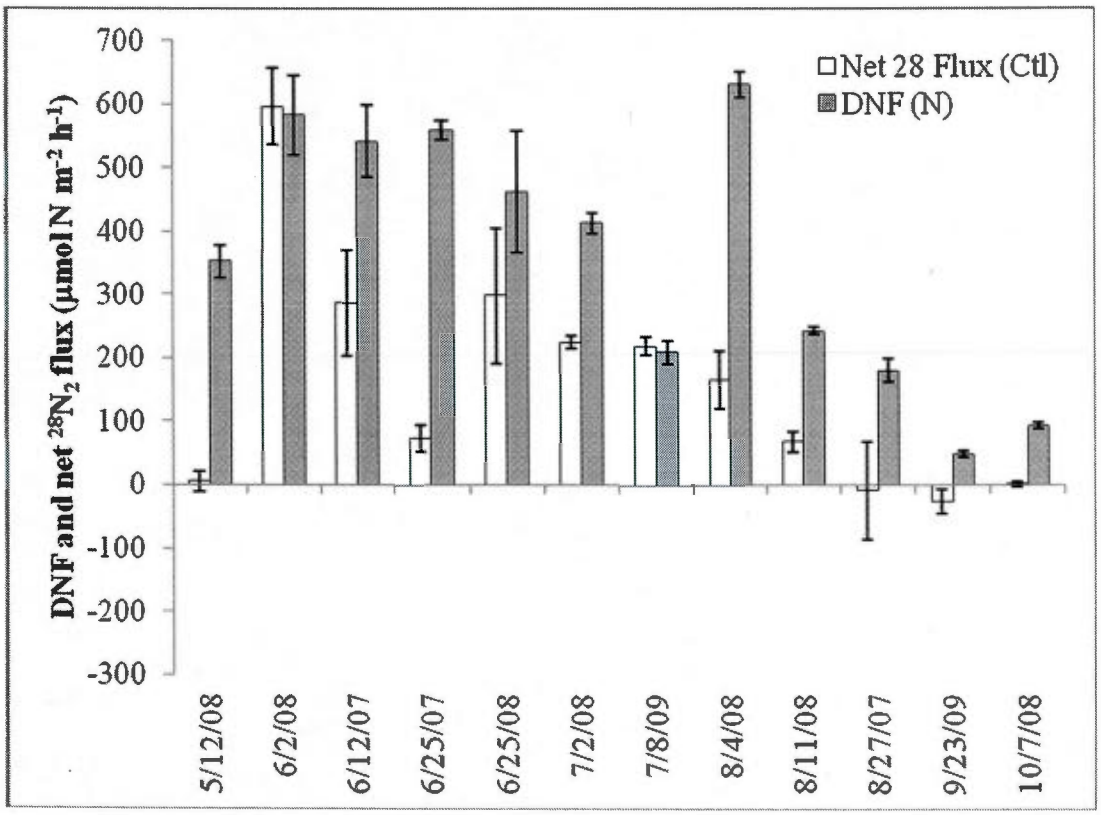

Figure 4.6. Net $^{28}N_2$ flux in control (Ctl) and potential denitrification (DNF) in $^{15}NO_3^-$ enriched (N) cores from the Pike River mouth (PRM) in Missisquoi Bay, Lake Champlain. Positive net $^{28}N_2$ flux suggests that denitrification exceeds nitrogen fixation, while negative flux suggests the opposite. Error bars represent one standard error. Note that the y-axis scale is the same as for Fig. 4.7.

As mentioned previously, DNF at PRM was related to NH_4^+ and net N_2 fluxes and nearly related to NO_3^- flux in control cores. DNF at PRM was nearly related to SOD in control cores ($r^2 = 0.31$; $p = 0.058$) and was related strongly ($r^2 = 0.63$; $p = 0.002$) to SOD in $^{15}NO_3^-$ enriched cores, which were the same cores used for DNF measurements. The same

was true with total N_2 flux in $^{15}NH_4^+$ enriched cores, with very similar statistics. DNF at PRM was not related to any other water column or sediment parameters. At MB, DNF was related positively to water temperature ($r^2 = 0.44$; $p = 0.026$) and water column NO_3^- concentration ($r^2 = 0.37$; $p = 0.047$) but not to any other parameters in control or $^{15}NO_3^-$ enriched cores. Addition of $^{15}NH_4^+$ resulted in significantly lower total N_2 flux than DNF in $^{15}NO_3^-$ enriched cores at both sites ($p < 0.02$).

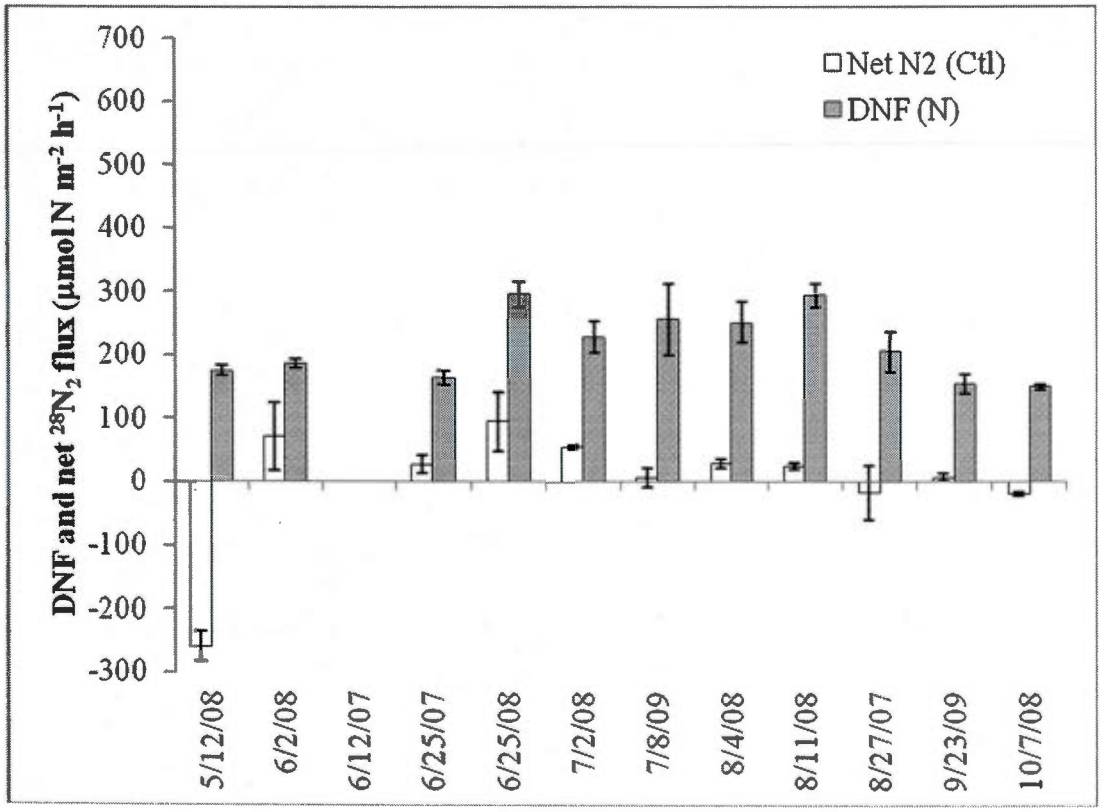

Figure 4.7. Net $^{28}N_2$ flux in control (Ctl) and potential denitrification (DNF) in $^{15}NO_3^-$ enriched (N) cores from the central basin (MB) in Missisquoi Bay, Lake Champlain. Positive net $^{28}N_2$ flux suggests that denitrification exceeds nitrogen fixation, while negative flux suggests the opposite. Error bars represent one standard error. Note that the y-axis scale is the same as for Fig. 4.6.

Table 4.1. Ratio of N_2 production from $^{15}NO_3^-$ versus $^{14}NO_3^-$ (15:14) in $^{15}NO_3^-$ enriched cores from the Pike River mouth (PRM) and central basin (MB) of Missisquoi Bay, Lake Champlain. Values represent the mean of three timepoints, and SE is the standard error. ND = no data.

Date	PRM	SE	MB	SE
5/12/2008	13.3	1.33	5.37	0.35
6/2/2008	0.46	0.03	6.15	0.10
6/12/2007	1.52	0.07	ND	ND
6/25/2007	4.37	0.28	3.41	0.98
6/25/2008	0.66	0.14	2.77	0.04
7/2/2008	1.05	0.01	4.36	0.23
7/8/2009	0.89	0.03	3.00	0.12
8/4/2008	1.54	0.02	9.84	0.24
8/11/2008	2.07	0.01	6.97	0.19
8/27/207	1.95	0.38	4.36	1.67
9/23/2009	13.3	1.67	16.6	0.91
10/7/2008	1.22	0.05	6.74	0.28

4.4.7. NO_3^- source for denitrification

Isotope pairing calculations can identify the NO_3^- source used for denitrification in $^{15}NO_3^-$ enriched cores. The ratio of N_2 production from $^{15}NO_3^-$ versus $^{14}NO_3^-$ (15:14) averaged 3.53 ± 1.35 at PRM and 6.32 ± 1.20 at MB, but this difference was not significant ($p = 0.14$). The ratio was < 1 on three occasions at PRM, all in June and July, but always > 2.75 at MB (Table 4.1). A ratio < 1 suggests that $^{14}NO_3^-$ was the main source for denitrification, while a ratio > 1 means that $^{15}NO_3^-$ was most often used to produce N_2. There was no temporal trend in this ratio at PRM, with high and low values each occurring throughout the growing season. However, all values < 1 occurred in either June or July. At MB, the lowest values generally

occurred in late June and July, and highest values occurred early and late in the season. The 15:14 ratio was not related to ambient NO_3^- or NH_4^+ concentrations at either site.

4.4.8. Anammox

Possible anammox was estimated by $^{29}N_2$ production in $^{15}NH_4^+$ enriched cores and ranged from 1.98 – 29.2 μmol N m^{-2} h^{-1} at PRM. Anammox rates at MB were less variable and ranged from 9.17 – 37.9 μmol N m^{-2} h^{-1}. Mean possible anammox at MB (20.6 ± 2.42 μmol N m^{-2} h^{-1}) was significantly higher ($p = 0.02$) than at PRM (12.6 ± 2.18 μmol N m^{-2} h^{-1}). Possible anammox at PRM was not related to any water column or sediment parameters, but water column NO_3^- concentration ($r^2 = 0.59$; $p = 0.006$) and sediment NH_4^+ flux were related positively to $^{29}N_2$ production in $^{15}NH_4^+$ enriched cores at MB. As a proportion of total N_2 production (29:DNF), anammox may have accounted for 5.9 ± 2.0% and 9.8 ± 1.0% of DNF at PRM and MB, respectively (Fig. 4.8). This ratio was always higher than 5% at MB but never exceeded 16%. At PRM, the ratio was 8% or less on all occasions except 23 Sept 2009, when DNF was low, but $^{29}N_2$ production was near average.

4.4.9. N fixation

Isotope pairing calculations resolved N fixation occurring simultaneously with denitrification (see An et al. 2001 for calculation details) in the sediments on all but one occasion at each site (12 June 2007 at PRM, and 27 Aug 2007 at MB). These rates were variable at both sites and not significantly different from each other (mean = 66.6 ± 14.4 and 59.1 ± 15.4 μmol N m^{-2} h^{-1} at PRM and MB, respectively). No clear temporal trends were apparent (Fig. 4.9), and rates were not related to water temperature at either site. Sediment N fixation rates at PRM were related positively to NO_3^- flux ($r^2 = 0.64$; $p = 0.002$), SOD ($r^2 = 0.54$; $p = 0.006$), NH_4^+ flux ($r^2 = 0.38$; $p = 0.03$), and $^{28}N_2$ flux ($r^2 = 0.36$; $p = 0.04$) in control cores. These rates at PRM also were related negatively to water column NO_3^- concentration ($r^2 = 0.56$; $p = 0.005$). At MB, N fixation rates were related positively to SOD ($r^2 = 0.59$; $p = 0.006$) and NH_4^+ flux ($r^2 = 0.46$; $p = 0.02$) and negatively to $^{28}N_2$ flux ($r^2 = 0.54$; $p = 0.01$).

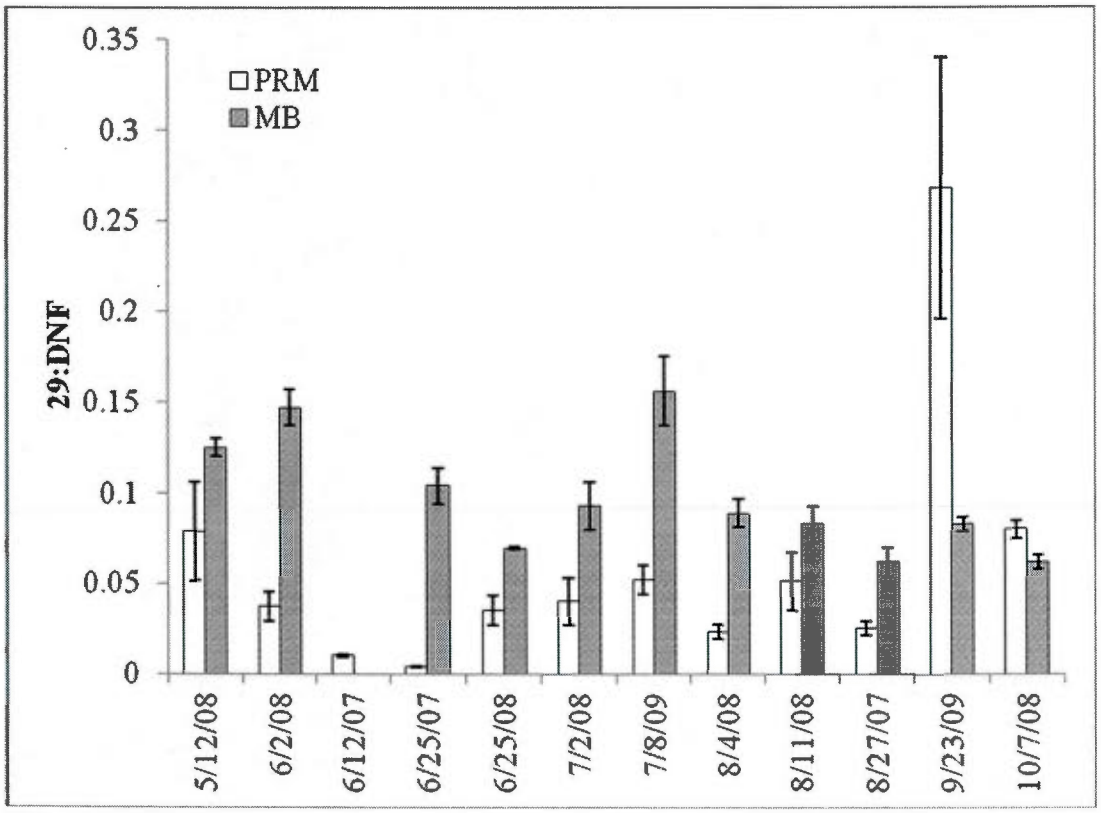

Figure 4.8. The ratio of $^{29}N_2$ production (29) in $^{15}NH_4^+$ enriched cores (possible anammox) to potential denitrification (DNF) in $^{15}NO_3^-$ enriched cores from the Pike River mouth (PRM) and central basin (MB) of Missisquoi Bay, Lake Champlain. This ratio estimates the proportion of total N_2 production that may be from anammox. Error bars represent one standard error of three timepoints and duplicate cores (n = 6).

The ratio of N fixation to DNF (NF:DNF) for simultaneous measurements in $^{15}NO_3^-$ enriched cores allows speculation on whether sediment N fixation can offset losses from microbial N sinks (denitrification and anammox). In Missisquoi Bay, this was the case only once (12 May 2008 at MB), although NF:DNF was 0.81 on 23 Sept 2009 at PRM (Fig. 4.10). With the exception of the two events mentioned, all other NF:DNF values were < 0.3 at MB and < 0.52 at PRM. The mean ratio was very similar at both sites (0.27 ± 0.07 at PRM and

0.29 ± 0.09 at MB), suggesting that sediment N fixation offsets about 25 – 30% of N losses from microbial N sinks, on average, over the course of the growing season.

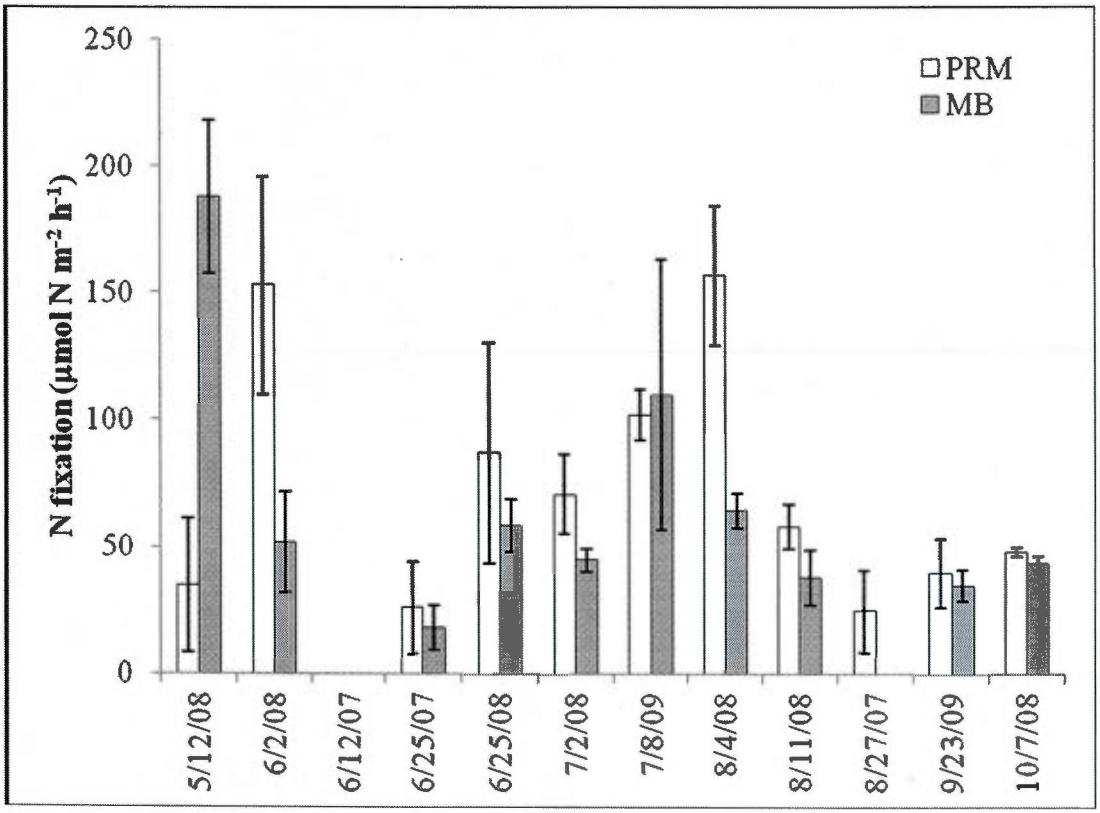

Figure 4.9. Nitrogen fixation rates calculated from isotope pairing in $^{15}NO_3^-$ enriched cores from the Pike River mouth (PRM) and central basin (MB) of Missisquoi Bay, Lake Champlain. Error bars represent one standard error. Calculations did not result in positive values on 12 June 2007 at PRM and 27 Aug 2007 at MB.

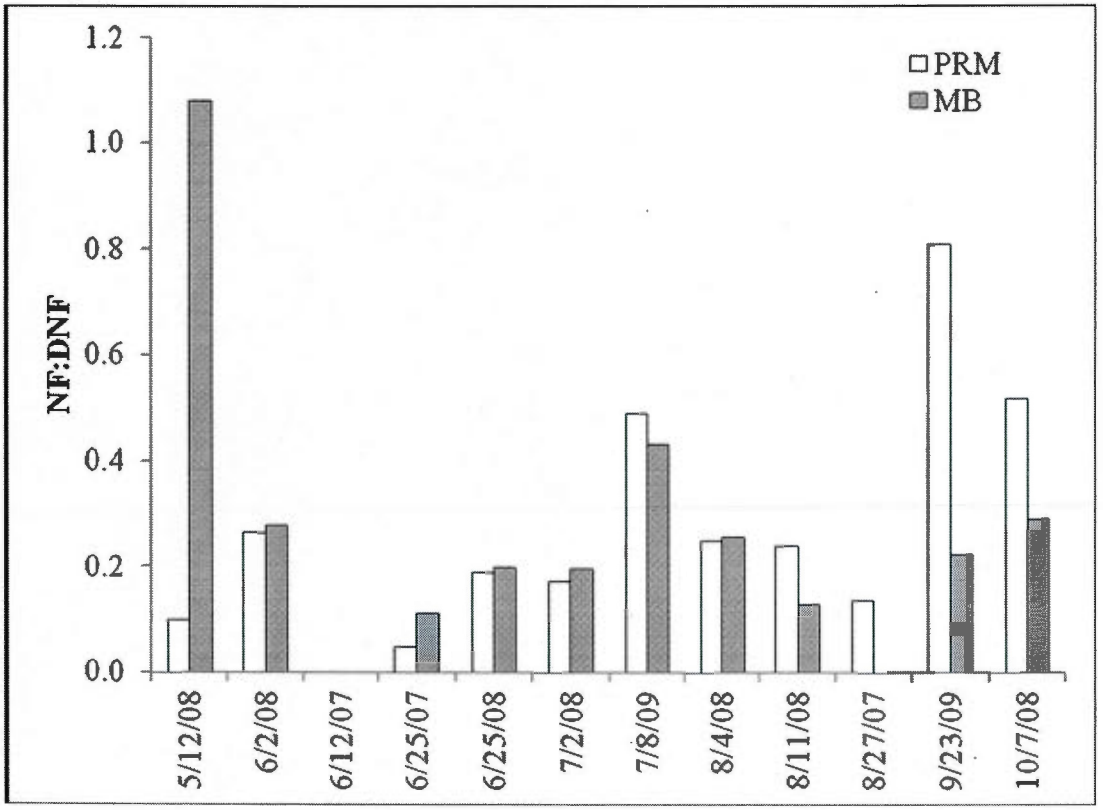

Figure 4.10. Ratio of N fixation (NF) to potential denitrification (DNF) in $^{15}NO_3^-$ enriched cores from the Pike River mouth (PRM) and central basin (MB) in Missisquoi Bay, Lake Champlain. A ratio > 1 implies that sediment NF can offset losses from microbial N sinks (denitrification and anammox). Isotope pairing calculations did not return positive values for NF on 12 June 2007 at PRM or 27 Aug 2007 at MB.

4.5. Discussion

In lakes with episodic inputs from tributaries, sediments can drive primary productivity in the water column between discharge events. Sediments generally are the most important site for organic matter remineralization and nutrient recycling in shallow aquatic systems (Tobias et al. 2003). Results from the present study will help clarify the role of sediments in processing N and help identify important mechanisms in Missisquoi Bay. These

results confirm that sediments play an important role in determining how nutrients are processed and cycled in lakes, but results relative to previously stated hypotheses were mixed. The occurrence of bottom water hypoxia in the central basin on one sampling date provided an interesting opportunity to observe the effects of low overlying water oxygen concentrations on SWI N transformation rates and pathways in this lake, which normally does not experience hypoxia. The discussion of results will begin with this event and continue with individual nutrient fluxes followed by N transformations. All results are discussed relative to implications for the nutrient status of the lake, particularly in the case of the various N transformation pathways and relative to cyanobacteria, when possible.

4.5.1. Effects of bottom water hypoxia on SWI processes in July 2009

Bottom water hypoxia was observed at MB in mid-summer 2009, and implications of this event on the nutrient status of the lake may enhance our understanding of the conditions stimulating cyanobacteria blooms in lakes. No evidence of widespread hypoxia or anoxia in the bottom water of Missisquoi Bay has been noted in the literature, and results from this study suggest that the lake generally remains well-mixed and oxygenated to the sediment surface. The lake is shallow and experiences high winds (Mendelsohn et al. 1997), so chronic hypoxia is not expected. Other investigators have noted anoxic conditions in water immediately overlying (5 mm above the SWI) sediments in Missisquoi Bay (Smith 2009), and two sampling events at MB (17 June and 3 July 2009) during the present study exhibited bottom water hypoxia (dissolved $O_2 < 1$ mg L^{-1}). Unfortunately, sediment core incubations did not coincide with either of these hypoxia events, although core incubations were conducted within a week of the 3 July event. This lag in sampling was fortuitous, because it provided an interesting opportunity to observe the subsequent effects of this event on the SWI processes already being quantified in this study.

Net $^{28}N_2$ flux was not different from zero during the incubation starting five days after the hypoxia observation on 3 July 2009 (Fig. 4.7), which was the only time between mid-May and late August that there was not a positive net $^{28}N_2$ flux at MB. This finding suggests that nitrification may have been inhibited by the low bottom water O_2 concentration,

thus limiting coupled nitrification-denitrification. Potential DNF was not affected, and this result is logical because NO_3^- reduction via denitrification is an anaerobic process. If denitrifiers rely on nitrification for substrate, then low bottom water O_2 concentration and anoxic sediments would shut off this NO_3^- source and force denitrifiers to rely more heavily on NO_3^- diffusion from overlying water. In this case, where the contribution from nitrification was expected to be less than the proportion of denitrification fueled by NO_3^- diffusion, then the 15:14 denitrification ratio (15:14) was expected to be highest. However, the 15:14 ratio was 3.00 during this hypoxia event (Table 4.1), which was one of the lowest values measured at MB. Despite the zero net $^{28}N_2$ flux, calculated N fixation (Fig 3.9) and NF: DNF (Fig. 4.10) were maximal on this date at MB. N fixation is assumed to mask denitrification of ambient NO_3^-, whether it originates from nitrification or overlying water diffusion (An et al. 2001). Therefore, the estimate for the "14" portion of the ratio was higher than at other times, leading to a lower 15:14. Highest negative NO_3^- flux measured at MB in control cores (Fig. 4.3) also was observed at this time, and this result, combined with the lack of response from DNF and high N fixation, explains the surprisingly low 15:14 observation.

This event also corresponded to the highest positive NH_4^+ flux (Fig. 4.4) and proportion of possible anammox (29:DNF; Fig. 4.8) measured at MB. DNRA apparently was not contributing to this NH_4^+ flux. The actual $^{29}N_2$ production rate in $^{15}NH_4^+$ enriched cores (37.9 ± 4.26 μmol N m^{-2} h^{-1}), which would represent the actual anammox rate if this pathway could be confirmed, also was highest at this time. Anammox is inhibited by the presence of O_2 (Mulder et al. 1995), so, as with potential denitrification, this is an intuitive observation. Interestingly, SRP flux was not affected by the bottom water hypoxia event (Fig. 4.1). Taken together, these results suggest that chronic bottom water hypoxia, if it were to develop in Missisquoi Bay, could lead to changes in the N transformations in the sediments, higher reduced N concentrations in the water column, and possible stimulation of non-N-fixing cyanobacteria blooms. Indeed, the 8 July 2009 sampling event did exhibit high water column NH_4^+ concentration (Table 3.2) and high cyanobacteria biomass (Table 3.3), particularly *Microcystis*. In this study, the appearance of any cyanobacteria bloom prior to August only occurred subsequent to this bottom water hypoxia event. The physical characteristics of Missisquoi Bay make chronic hypoxia unlikely, but the occasional occurrence of bottom

water hypoxia in this and other lakes may promote cyanobacteria migration into the reduced-N-rich water column. The conditions favoring development of hypoxia in this and other shallow, well-mixed lakes (e.g., low wind speeds, increasing temperatures, high SOD rates) also favor cyanobacteria blooms, especially buoyant, non-N-fixing genera (e.g., Paerl 1990).

4.5.2. Sediment SRP flux, bottom-water hypoxia, cyanobacteria, and nutrient limitation

Sediments at MB were a continuous P source to the water column throughout the growing season (Fig. 4.1), consistent with results from other work in Missisquoi Bay (Smith 2009). However, these investigators concluded that cyanobacteria blooms in 2008 contributed to bottom water and sediment redox dynamics, and P mobility from sediments. In contrast, results from the present study suggest that the altered redox dynamics occurred first and may have stimulated the bloom independent of P mobility, which was not significantly different during hypoxia. For example, *Microcystis* was not observed in samples collected on 3 July 2009 at MB or LITT (see Chapter II). By 8 July, *Microcystis* biomass had reached bloom levels at both sites (bC > 4,000 μg C L^{-1} at LITT; bC ≈ 2,000 μg C L^{-1} at MB). Doubling times as fast as 1.4 days have been reported for *Microcystis* in natural systems where N is not limiting (e.g., Moisander et al. 2009), such as Missisquoi Bay. Thus, five days is enough time for a bloom this size to appear at MB if an initial inoculum of about 500 μg C L^{-1} were provided from sediment migration or advected from other parts of the lake.

Bottom water hypoxia was observed on 3 July, before the appearance of *Microcystis*, and altered N transformation rates and fluxes were measured in cores collected less than a week later. As discussed above, these altered N transformations conformed to expected responses to bottom water hypoxia. In contrast, there was no evidence of enhanced SRP release from sediments as a result of bottom water hypoxia (Fig. 4.1), SRP flux was not related to SOD or water column SRP concentrations, and water column SRP concentrations remained low (< 0.7 μM; Table 3.2) on both 3 July and 8 July 2009. These results suggest that the bloom may have been initiated by the consequences of sediment redox dynamics, especially enhanced NH_4^+ release from sediments, rather than sediment redox dynamics acting to proliferate and sustain the bloom, as concluded by Smith (2009).

The pattern of SRP fluxes observed at PRM (Fig. 4.1) is consistent with the hypothesis that Missisquoi Bay becomes more N limited as the growing season progresses. Early in the season (May and June), PRM sediments were a source of SRP to the water column, but this reversed in most cases from July to October, with PRM sediments becoming a P sink. This result suggests that P was present in excess of the capabilities of primary producers in the water column to assimilate it, thus allowing influx to sediments via microbial processes or burial. The ubiquitous presence of measurable SRP in the water column, combined with decreasing DIN concentrations with time, also supports this conclusion. Sediment SRP release at MB throughout the season, independent of all measured limnological variables other than water temperature, suggest that internal P loading likely will delay system recovery from P loading reductions (e.g., Burger et al. 2007), particularly in a warming climate.

4.5.3. Sediment NO_x fluxes relative to N transformation pathways

At MB, NO_2^- and NO_3^- fluxes in control cores were related with a positive slope and intercept ($y = 0.06x + 5.44$; NO_2^- flux was the dependent variable). This relationship suggests that high NO_3^- flux is associated with higher NO_2^- fluxes. Nitrite is an intermediate of both nitrification and denitrification, but NO_3^- is the product of nitrification and the substrate for denitrification. Thus, this relationship has different interpretations depending on whether NO_3^- flux is positive or negative. If NO_3^- flux is positive, then this suggests that nitrification is producing more NO_3^- than denitrification is reducing. Corresponding positive NO_2^- flux, then, would imply that NO_2^- is being released as an intermediate of incomplete nitrification. Nitrification is an aerobic process, and NO_2^- oxidizing bacteria are more sensitive to low O_2 concentrations than NH_4^+ oxidizing bacteria (Bernet et al. 2001). Therefore, NO_2^- efflux (Fig. 4.2) and accumulation in overlying water (Table 3.2), observed in mid-summer at both sites, may be explained by inhibition of NO_2^- oxidizing bacteria by low O_2 concentrations near the SWI, which has been observed in Missisquoi Bay in summer (Smith 2009).

If NO_3^- flux is negative (i.e., into sediments), then a positive NO_2^- flux would suggest that NO_2^- is being released as an intermediate of incomplete denitrification. Sulfide inhibits

both nitrification (Joye & Hollibaugh 1995) and denitrification (Sørensen et al. 1980) and can cause NO_2^- release and accumulation. Anoxic freshwater sediments may be sulfidic (Brunet & Garcia-Gil 1996) and cause limitation of NO_2^- reduction to N_2. However, there is no mention of sulfide being present in Missisquoi Bay sediments in a recent study on sediment redox dynamics (Smith 2009), and no hydrogen sulfide odor was detected during core sampling or after incubations in the present study. Since sulfide was not quantified in the present study, this mechanism cannot be ruled out in Missisquoi Bay as an explanation for high NO_2^- fluxes in mid-summer (Fig. 4.2), but other possible explanations, such as organic matter limitation and enzyme activity (e.g., Tiedje 1988), may be more plausible.

Negative relationships between NO_3^- flux and water column NO_3^- concentration, net $^{28}N_2$ flux, NH_4^+ flux, and SOD at PRM are consistent with our understanding of sediment N cycling. High NO_3^- flux into sediments is a strong indication of denitrification, which depends on anaerobic conditions and organic matter availability (Seitzinger 1988). Denitrifiers remineralize organic matter and produce NH_4^+ and N_2 gas (Tiedje 1988), so high NO_3^- flux into sediments (a negative value) associated with high NH_4^+ and N_2 fluxes from sediments (positive values) is expected. High SOD due to organic matter decomposition leads to redox conditions favorable for denitrification, so the negative relationship between NO_3^- concentration and SOD also is expected (note that SOD is a positive value when O_2 is consumed by sediments). At MB, NO_3^- flux was only related to water column NO_3^- concentration and, after excluding the 25 June 2007 datapoint, NH_4^+ flux. Addition of $^{15}NO_3^-$ to overlying water stimulated total NO_3^- flux at MB, but not at PRM. These results suggest that denitrification at MB was NO_3^- limited and an effective sink for water column NO_3^-. When water column NO_3^- concentrations were low late in the season, sediment NO_3^- flux reversed at both sites, and nitrification exceeding denitrification is the likely explanation. However, NO_3^- effluxes in late summer were small relative to the influxes earlier in the season (Fig. 4.3), which suggests that nitrification also was limited indirectly by organic matter (via remineralization to NH_4^+) late in the season.

4.5.4. Importance of sediment NH_4^+ flux to the N budget in Missisquoi Bay

Late season organic matter limitation of microbial processes in Missisquoi Bay also is suggested by NH_4^+ fluxes, which decrease dramatically at PRM, even reversing to become an NH_4^+ sink (Fig. 4.4). The seasonal decrease is less obvious at MB, and there was no reversal of flux direction. These patterns may reflect the proximity to and variability in allochthonous organic matter discharges from the Pike River, which are highest in spring due to a combination of snowmelt, high precipitation rates, and the start of the agricultural growing season (Adhikari et al. 2010). Positive relationships between NH_4^+ flux and SOD at both sites, and net $^{28}N_2$ flux and DNF at PRM, are expected for the same reasons described above for NO_3^- flux. The lack of a relationship between sediment NH_4^+ flux and water column NH_4^+ concentration at either site suggests that microbial processes drive NH_4^+ release to overlying water independently of water column NH_4^+ concentration.

Sediment NH_4^+ fluxes in Missisquoi Bay were similar to those measured in a Great Lakes coastal wetland (McCarthy et al. 2007b) and Saginaw Bay (Lake Huron; Gardner et al. 2001) but only about half of rates measured along a river-to-pelagic gradient in hypereutrophic Taihu Lake (China; McCarthy et al. 2007c). Average NH_4^+ release from sediments was 210 ± 61.9 μmol N m^{-2} h^{-1} at PRM and 79.9 ± 19.2 μmol N m^{-2} h^{-1} at MB. At PRM, this value is about twice the areal NH_4^+ regeneration rate (111 ± 32.0 μmol N m^{-2} h^{-1}) estimated from water column incubations (see Chapter III for details of this extrapolation). The combined sediment and water column areal NH_4^+ regeneration estimates easily account for the depth-averaged, areal NH_4^+ uptake estimate (234 ± 41.1 μmol N m^{-2} h^{-1}). The remaining ~100 μmol N m^{-2} h^{-1}, in addition to any NH_4^+ from external sources, would then be surplus available for transport to pelagic areas of the lake. At MB, the mean NH_4^+ release rate from sediments is only ~20% of estimated areal NH_4^+ regeneration rate (398 ± 79.8 μmol N m^{-2} h^{-1}) in the water column.

The depth-averaged, areal NH_4^+ uptake estimate for MB is 680 ± 102 μmol N m^{-2} h^{-1}. Thus, the combined sediment and water column areal NH_4^+ regeneration rate is only ~70% of the depth-averaged NH_4^+ uptake rate. This comparison suggests that total water column NH_4^+

uptake in pelagic areas of the lake cannot be supported entirely by system regeneration processes. Transport of excess NH_4^+ regenerated from riverine areas (e.g., PRM), combined with tributary inputs, must support the remaining 30% of estimated total NH_4^+ uptake. Sediment NH_4^+ regeneration also was insufficient to support pelagic NH_4^+ demand in Lake Balaton, a large, shallow lake in Hungary (Presing et al. 2008). No other comparisons of direct sediment NH_4^+ flux measurements and water column NH_4^+ uptake were found for lakes. In the present study, this comparison is complicated by the fact that NH_4^+ uptake rates are 'potential' rates because of high isotope spikes used in the isotope dilution incubations (see Chapter III for water column incubation details). However, actual and potential uptake rates tend to converge in eutrophic systems with high ambient NH_4^+ concentrations (Glibert et al. 1982), and Missisquoi Bay exhibited replete NH_4^+ in most cases in this study. Therefore, 'potential' and 'actual' NH_4^+ uptake rates probably are similar in this lake and suggest that these comparisons have some validity.

As noted in Chapter III, the depth-averaged NH_4^+ uptake estimate for pelagic areas assumed that the entire water column was euphotic, and that NH_4^+ uptake rates did not change with depth. Without rates at discrete depths, these assumptions likely cause an over-estimate of total pelagic NH_4^+ uptake, so the spread between total NH_4^+ uptake and regeneration may be closer than estimated here. However, cation exchange mechanisms in sediments may counteract or minimize the error described above. Cation exchange involves NH_4^+ adsorption to sediment particles and, therefore, retention of NH_4^+ in freshwater sediments rather than release to overlying water (Gardner et al. 1991; Seitzinger et al. 1991; Zimmerman & Benner 1994; Gardner et al. 2006). The implications of cation exchanges, if present, are that measured NH_4^+ release rates from sediments may be under-estimated. The cation exchange effect may be more important when porewater NH_4^+ is removed via coupled nitrification/denitrification. Otherwise, NH_4^+ likely would saturate cation exchange sites and be released in the isotopic proportion in which they are produced (W.S. Gardner, pers. comm.). The magnitudes of these potential errors (possibly over-estimated depth-averaged NH_4^+ uptake and sediment cation exchange) are unknown, but they would counteract each other if both errors were present.

4.5.5. Does DNRA contribute to NH_4^+ regeneration in Missisquoi Bay?

In this study, measurable $^{15}NH_4^+$ production in cores (i.e., significantly different from $^{15}NH_4^+$ production in control cores) was rare, occurring only four times at PRM and twice at MB. In these cases, DNRA represented ~7% and 25% of total NH_4^+ flux at PRM and MB, respectively. Mean DNRA rates were higher at PRM, when measured, but accounted for a smaller proportion of total NH_4^+ flux. Cation exchange mechanisms in freshwater sediments also may affect the ability to measure DNRA using $^{15}NH_4^+$ production, which may replace $^{14}NH_4^+$ already sorbed to sediment particles, particularly in sediments with an oxic surface layer (Gardner & McCarthy 2007). If NH_4^+ concentrations in sediments are higher than in overlying water, as is usually the case in aquatic sediments, then $^{15}NH_4^+$ produced via DNRA from $^{15}NO_3^-$ enrichments should exchange readily with $^{14}NH_4^+$ sorbed to sediment particles. This new equilibrium may prevent measurement of $^{15}NH_4^+$ in overlying water and effectively mask DNRA. However, $^{14}NH_4^+$ replaced by $^{15}NH_4^+$ in the sediments should diffuse to the overlying water, creating a NO_3^- induced NH_4^+ flux (NIAF), defined as the difference between total NH_4^+ flux in control versus $^{15}NO_3^-$ enriched cores. Therefore, NIAF includes any DNRA that may be occurring and is being masked by cation exchanges.

Comparison of DNRA estimates with NIAF suggested that $^{15}NH_4^+$ production from $^{15}NO_3^-$ addition under-estimated DNRA on seven of 12 occasions at PRM and three of 11 occasions at MB, including those where no $^{15}NH_4^+$ production was measured. In those cases where both DNRA and NIAF were observed, NIAF was 12.0 ± 3.9 (n = 6) times higher than DNRA, suggesting that DNRA generally was under-estimated by an order of magnitude using the $^{15}NH_4^+$ production approach. The relative partitioning between DNRA and denitrification has important consequences for aquatic systems, since DNRA returns bioavailable N to the system, while denitrification removes N from the system (Tiedje 1988). In Missisquoi Bay, the NIAF:DNF ratio was 0.35 ± 0.09 (n = 7) at PRM and 0.85 ± 0.15 (n = 3) at MB. These values suggest that, when DNRA occurs (as estimated by NIAF), DNRA and DNF are nearly balanced at MB, but DNRA accounts for only about one third of total NO_3^- reduction at PRM. A review of DNRA and denitrification stated that DNRA could account for 10 – 30% of NO_3^- reduction in lake sediments (Tiedje 1988), and the results at

PRM are similar to this estimate, although DNRA was only detected (as either $^{15}NH_4^+$ production or NIAF) in ~ 60% of core incubations. Apparently balanced DNRA and denitrification at MB is not consistent with the review cited above, but DNRA was only detected (as NIAF) in < 30% of core incubations in Missisquoi Bay. It has been hypothesized that DNRA is more likely to be important in high carbon, low NO_3^- systems (Tiedje 1988; Burgin & Hamilton 2007). However, NIAF was not related to NO_3^- concentration or SOD at either site. Together, these results suggest that DNRA occurred on some occasions but likely is not a significant NH_4^+ regeneration pathway on a lake-wide or seasonal scale.

4.5.6. SOD patterns relative to N transformations

SOD was not related to water temperature at either site, and this was a surprising result, since temperature is one of the most important abiotic factors affecting biological activity. Exclusion of the low temperature, high SOD data points on 2 June 2008 at PRM and 12 May 2008 at MB also failed to produce significant relationships. Positive relationships between SOD and NH_4^+ flux at both sites (Fig. 4.11) are consistent with O_2 consumption during organic matter decomposition and remineralization, which produces NH_4^+. The large decline in SOD at PRM from > 2,000 μmol O_2 m^{-2} h^{-1} in July to < 1,000 μmol O_2 m^{-2} h^{-1} in September and October (Fig. 4.5) supports the DIN flux interpretations above suggesting that organic matter became increasingly limiting late in the season. The physically and biogeochemically dynamic nature of the river discharge lends itself to rapid decomposition of fresh, allochthonous organic matter deposited at the SWI. Consistent delivery of allochthonous organic matter from the river, combined with the frequent mixing of surface sediments and rapid re-establishment of redox gradients, allows the bacterial community a consistent supply of labile organic material. Combined with lower river flows during the summer (Adhikari et al. 2010), these characteristics also support the organic matter limitation hypothesis for PRM. The lack of a temporal trend in SOD at MB suggests that mid-lake sediments are less dynamic and not limited by organic matter.

Mean SOD rates in Missisquoi Bay (40.8 ± 4.46 and 36.0 ± 3.40 mmol O_2 m^{-2} d^{-1} at PRM and MB, respectively) exceeded the range of rates (1.6 – 33 mmol O_2 m^{-2} d^{-1}) compiled

by Pace & Prairie (2005). It is unlikely that this reflects any more than a difference in measurement techniques. Most SOD measurements have been conducted using benthic chamber and static sediment core incubations. These techniques fail to account for the effect of decreasing O_2 concentrations with time on measured SOD rates. That is, as O_2 is consumed in a static incubation, SOD rates will decrease due to O_2 limitation. In the present study, consumed O_2 was continuously replaced throughout the incubation via the continuous-flow incubation technique. It is also notable that most static incubations are short-term, lasting a few hours, while the continuous-flow technique used here was continued for four days. Therefore, SOD rates reflect the equilibrium achieved within the continuous-flow incubation, rather than a continuous decrease in ambient O_2 concentration with time in short-term, static incubations.

The positive relationship between SOD and net $^{28}N_2$ flux and potential DNF (Fig. 4.12) suggests a coupling between organic matter decomposition in sediments and denitrification. Nitrification consumes O_2 during NH_4^+ oxidation to NO_2^- and NO_3^- (Ward 1986) and may contribute to development of hypoxia in aquatic systems (Dagg et al. 2008). Nitrification in oxic surface sediments was expected to stimulate SOD in $^{15}NH_4^+$ enriched cores. However, no enhancement of SOD was observed with isotope additions at either site, suggesting that nitrifiers were NH_4^+ replete and limited by other factors. Consistent sediment NH_4^+ efflux and the presence of NH_4^+ in all water column samples support this interpretation.

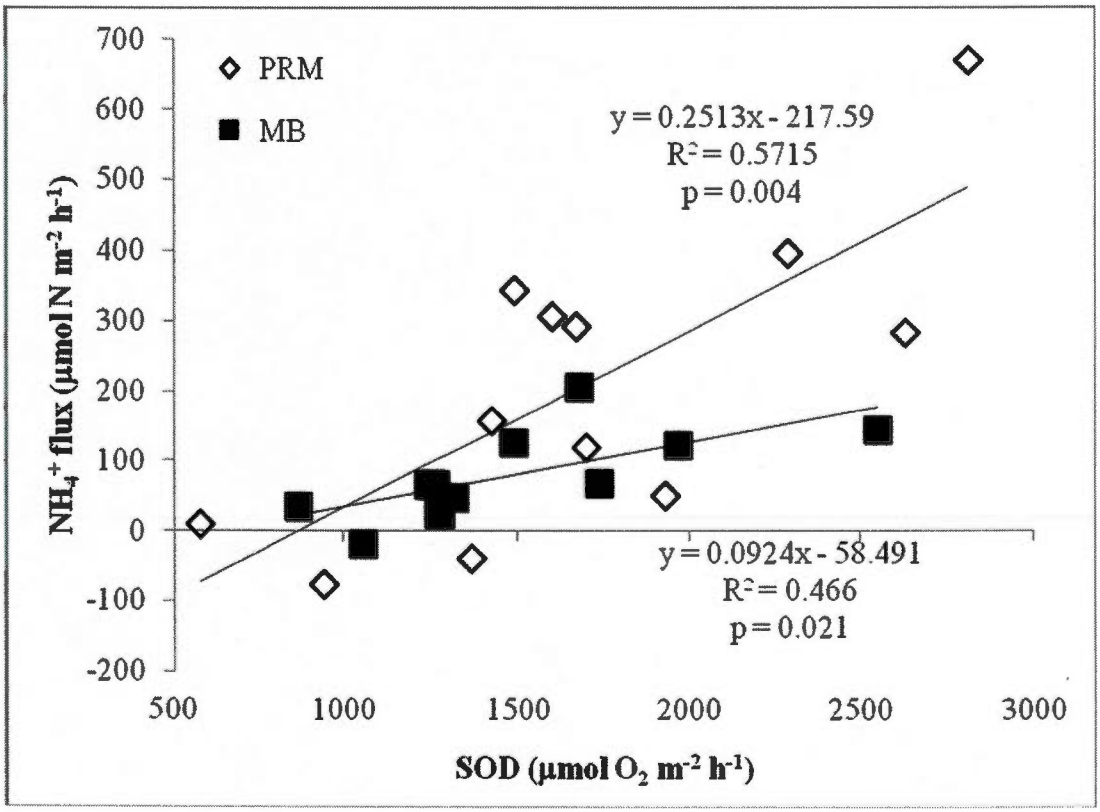

Fig. 4.11. Relationships between sediment oxygen demand (SOD) and net NH_4^+ flux at the Pike River mouth (PRM) and central basin (MB) of Missisquoi Bay, Lake Champlain.

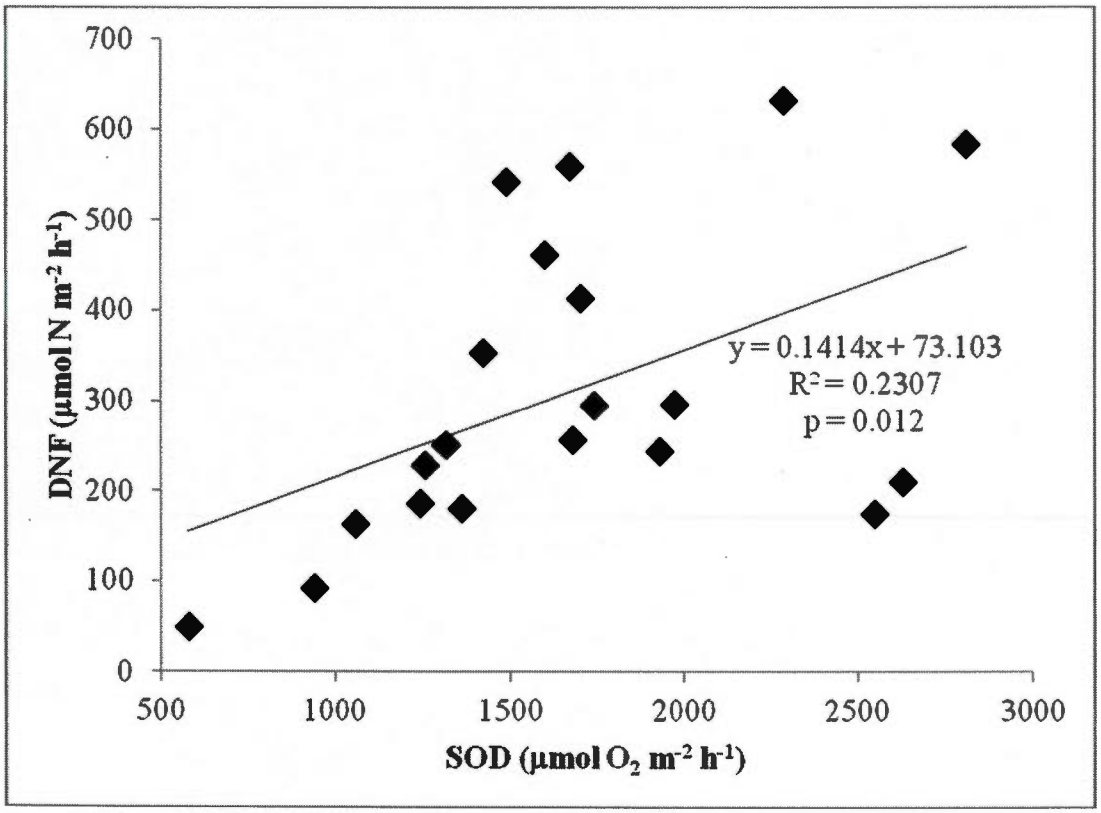

Fig. 4.12. Relationship between sediment oxygen demand (SOD) and potential denitrification (DNF) in Missisquoi Bay, Lake Champlain (both sites are included). Note that SOD also was related to net $^{28}N_2$ flux (not shown).

4.5.7. N_2 dynamics – denitrification, anammox, and N_2 fixation

Denitrification is a microbial N sink in aquatic systems, can lead to N limitation of primary production, and can act as a natural defense against eutrophication (Seitzinger 1988). Anammox is an alternate pathway to N_2 production, but these microbes are slow-growing (Mulder et al. 1995) and have the same net effect on ecosystem N budgets as denitrification; i.e., conversion of bioavailable N to atmospheric N_2 gas, which is bioavailable only to diazotrophs (Howarth et al. 1988a). The general importance of anammox in freshwater systems is unknown (Burgin & Hamilton 2007), but evidence for anammox has been

observed in the anoxic water columns of some lakes (e.g., Schubert et al. 2006; Hamersley et al. 2009). Nitrogen fixation in lake sediments tends to be minor compared to total N inputs from other sources (Howarth et al. 1988b). Results from Missisquoi Bay sediment core incubations will be interpreted below based on these basic tenets of N_2 dynamics in lakes.

Sediments at PRM were a significant net N sink until mid-August, when the effects of organic matter limitation led to either no significant N_2 flux or a reversal to net N_2 fixation (Fig. 4.6). Sediments at MB also were a net N sink at most times, with notable exceptions in May 2008 and late in the season (Fig. 4.7). Isotope pairing calculations produced positive N fixation rates in all cases at both sites except two (Fig. 4.9). These rates ranged from about 24 – 157 μmol N m^{-2} h^{-1} at PRM and 18 – 188 μmol N m^{-2} h^{-1} at MB. Net N_2 fluxes in control cores represent the balance between N_2 production via denitrification and anammox and N_2 uptake via N fixation. Therefore, N fixation rates must be added to net N_2 fluxes to determine the best estimate for denitrification rates, which are presented in Table 4.2. Combining net N_2 flux and N fixation results in positive values in all cases at PRM ranging from 13.8 – 749 μmol N m^{-2} h^{-1} (mean = 225 ± 62.0; Table 4.2). Scaling this rate up to include the other two river mouth areas in Missisquoi Bay (see Chapter III) produces a daily estimate of 24,300 mol N d^{-1} removed via denitrification (and any anammox). Sediment NH_4^+ and NO_2^- effluxes are exceeded by NO_3^- influxes at PRM, leaving PRM sediments as a net DIN sink (mean DIN flux = -185 ± 96.8 μmol N m^{-2} h^{-1}). Scaling this DIN influx up to include the other two river mouth areas gives a total DIN sink value of 20,000 mol N d^{-1}, which is similar to the N removal estimate by N_2 production. The N removal estimate is about twice the daily estimated water column NH_4^+ regeneration rate (12,000 mol N d^{-1}), suggesting that river mouth areas in the lake are significant N sinks over the growing season and contribute to water column N limitation of primary production, especially in late summer and fall.

At MB, calculated N fixation rates were not sufficient to account for negative net N_2 flux on 12 May 2008 and 27 Aug 2007 (Table 4.2). However, calculated N fixation and net N_2 fluxes on these dates were not significantly different. These results suggest that there was little or no N_2 production at MB on these dates. Both $^{15}NO_3^-$ and $^{15}NH_4^+$ additions to overlying water stimulated N_2 production on these dates, which suggests that denitrifiers

were limited by substrate. In August 2007, NO_x was not detected in the water column (Table 3.2), which supports the substrate limitation explanation on this date. NO_x was present in the water column in May 2008 at a level above mean NO_x concentration at MB, so there is no obvious explanation for the lack of N_2 production in this case.

Table 4.2. Estimated N removal rates (μmol N m^{-2} h^{-1}) via denitrification and anammox at the Pike River mouth (PRM) and central basin (MB) in Missisquoi Bay, Lake Champlain, determined by the sum of net N_2 flux in control cores (28) and isotope pairing calculations of N_2 fixation in $^{15}NO_3^-$ enriched cores (NF). Isotope pairing calculations did not return a positive value for NF on 12 June 2007 at PRM and 27 Aug 2007 at MB. ND = no data.

Date	PRM	MB
5/12/08	40.3	-72.0
6/2/08	749	122
6/12/07	287	ND
6/25/07	98.5	44.8
6/25/08	385	153
7/2/08	296	99.4
7/8/09	321	116
8/4/08	322	93.3
8/11/08	126	61.9
8/27/07	15.6	-17.6
9/23/09	13.8	41.2
10/7/08	48.9	23.3

Net N_2 flux plus N fixation rates results in a mean N removal rate of 60.5 ± 19.9 μmol N m^{-2} h^{-1} at MB (range -72.0 – 153 μmol N m^{-2} h^{-1}; Table 4.2). Scaling up this rate to encompass the entire pelagic area of the lake (see Chapter III) results in an estimated N removal rate of 106,000 mol N d^{-1}. This estimate is about 7-fold lower than the daily estimated water column NH_4^+ regeneration rate in pelagic areas (696,000 mol N d^{-1}). Also considering the mean sediment DIN flux at MB (59.4 ± 20.7 μmol N m^{-2} h^{-1}) and scaling it up

adds another 104,000 mol N d^{-1} as an internal N source. The DIN efflux estimate, which includes NO_3^- flux into sediments and NH_4^+ efflux, is nearly identical to the N removal estimate at MB, which suggests that DIN released to the overlying water is balanced by N removal by denitrification (and any anammox). The depth-averaged NH_4^+ uptake at MB was 1,190,000 mol N d^{-1}, which is almost twice the depth-averaged NH_4^+ regeneration rate. Therefore, DIN fueling primary production in the lake relies on external inputs and is supplemented by water column regeneration.

Water column N fixation was not measureable at MB (see Chapter III), so external inputs must account for the balance. As mentioned previously, there are no estimates of N loads into Missisquoi Bay in the scientific or gray literature, but N load was estimated from tributary flows (Smeltzer & Simoneau 2008) and TN concentrations (http://www.vtwaterquality.org/cfm/champlain/lp_longterm-lakes.cfm) to be ~372,000 mol N d^{-1}. Ammonium regeneration and external N loading estimates still fail to account for all (~90%) of the estimated NH_4^+ uptake at MB, and these results support the hypothesis that N limits phytoplankton production in pelagic areas of the lake, at least on some scale. The presence of heterocyteous cyanobacteria also supports this hypothesis. It is likely that N starvation occurs on temporally and spatially explicit scales not captured by the sampling regime in this study. The ability to differentiate heterocytes and fix atmospheric N_2, despite the high energetic costs and consequences for growth (e.g., Attridge & Rowell 1997), provides a competitive advantage for these cyanobacteria over other phytoplankton that cannot supplement their N demand via N fixation during episodes of extreme N deficiency.

A central assumption of the P limitation paradigm is that N deficiency can be offset by N fixation in lakes (e.g., Schindler et al. 2008). For this assumption to be valid, water column and sediment N fixation rates must balance microbial N sinks, burial, and downstream export (Scott & McCarthy 2010). This study did not address sediment N burial or export, but the ratio of sediment N fixation to potential denitrification (NF:DNF), in the absence of water column N fixation (see Chapter III), can provide evidence for whether N loss terms are balanced with N sources. In Missisquoi Bay, NF:DNF suggests that sediment N fixation only offsets about 25 – 30% of N losses via microbial N sinks. Therefore, N

fixation in Missisquoi Bay did not balance microbial N removal during this study. This result is consistent with results from other lakes compiled by Seitzinger (1988), where only the artificially eutrophied Lake 227 in the Experimental Lakes Area exhibited higher N fixation versus N removal. The results from Lake 227 also have been questioned recently, particularly after cessation of N fertilization in 1990 (Scott & McCarthy 2010).

Significantly higher potential denitrification rates (DNF) than net N_2 fluxes during almost all sampling events at both sites suggests that denitrifiers could remove more NO_3^- if it were available. Higher DNF in $^{15}NO_3^-$ enriched versus $^{15}NH_4^+$ enriched cores suggests that nitrifiers in sediments do not supply most of the NO_3^- reduced to N_2 via denitrification in Missisquoi Bay. The mean ratios of N_2 production from $^{15}NO_3^-$ versus $^{14}NO_3^-$ (15:14) were much higher than one at both sites (Table 4.1), and this result supports the hypothesis that nitrification is not the primary NO_3^- source for denitrification. This ratio was expected to be dependent on ambient NO_3^- concentrations, since the final substrate ratio for NO_3^- should affect the proportions of heavy and light N_2 production. However, 15:14 was not related to ambient NO_3^- concentration at either site. Nitrification in Missisquoi Bay sediments may be limited by low O_2 concentrations near the SWI (e.g., Smith 2009), especially during episodic bottom-water hypoxia events. The lack of significant differences in NO_3^- fluxes between $^{15}NH_4^+$ enriched and control cores supports this interpretation. Nitrate flux should be higher in $^{15}NH_4^+$ enriched cores than control cores if nitrification were limited by substrate (i.e., NH_4^+). Nitrification generally is considered the primary NO_3^- source for denitrification in aquatic systems (Seitzinger 1988), but Missisquoi Bay does not appear to follow this pattern.

Studies quantifying anammox in freshwater systems are rare. A literature search resulted in only two published studies regarding anammox in lakes, and both of those were focused on suboxic layers in the water column (Schubert et al. 2006; Hamersley et al. 2009). The results presented here, therefore, represent the first known quantification of possible anammox in freshwater sediments. Anammox was estimated as $^{29}N_2$ production in $^{15}NH_4^+$ enriched cores, but there are other possible explanations for $^{29}N_2$ production in this case. For example, $^{15}NH_4^+$ oxidation to $^{15}NO_3^-$ via nitrification combined with an ambient $^{14}NO_3^-$ via denitrification to form $^{29}N_2$ also is a plausible explanation. Therefore, the isotopic techniques

used here cannot conclusively distinguish denitrification from anammox, and anammox rates presented here are thus qualified as "possible anammox". Molecular techniques quantifying abundance and expression of anammox-specific genes, such as hydrazine oxidoreductase (*hzo*; Schmid et al. 2008), are needed to confirm anammox in this case.

In Missisquoi Bay sediments, possible anammox may have accounted for up to 27% (at PRM on 23 Sept 2009) of total N_2 production. On average, however, anammox may have accounted for ~6 and 10% of total N_2 production at PRM and MB, respectively. These relative proportions are on the low end of anammox proportions determined in marine sediments (Dalsgaard et al. 2005). However, many of the anammox measurements in sediments presented by Dalsgaard et al. (2005) were conducted in artificially anoxic, homogenized sediment incubations. These experimental conditions are not representative of natural sediments, and the results should be interpreted cautiously. In suboxic water column layers in Lake Tanganyika, anammox was estimated to account for 7 – 13% of total N_2 production, which is similar to the estimates from Missisquoi Bay sediments. Anammox is inhibited by simple organic compounds, such as ethanol, glucose, and pyruvate (Jetten et al. 1999), and it has been hypothesized that anammox may be more important in sediments with low labile organic matter. The proportional and absolute anammox results from Missisquoi Bay support this hypothesis if it is assumed that PRM sediments have higher labile proportion and total organic matter levels than MB due to proximity to the river discharge.

4.5.8. Conclusions

Nitrogen transformations in Missisquoi Bay sediments were dynamic, and the importance of the various pathways exhibited spatial and temporal variability. A bottom-water hypoxia event in the central basin in early July 2009 resulted in an altered N status in sediments and overlying water and was followed within one week by the earliest cyanobacteria bloom observed during this study. These observations contrast with a parallel study (Smith 2009), which concluded that cyanobacterial bloom dynamics controlled sediment redox conditions. Sediment P flux was not related to SOD, and there was no enhancement of SRP flux during the hypoxia event. Sediment SRP flux patterns were

consistent with the hypothesis that Missisquoi Bay becomes more N limited as the growing season progresses. Total NH_4^+ regeneration could account for water column NH_4^+ demand at PRM but not at MB, and DNRA in sediments did not appear to be a significant mechanism of NH_4^+ regeneration in most cases. Cation exchange mechanisms in the sediments may have caused an underestimation of sediment NH_4^+ release and DNRA. Decreasing NH_4^+, SOD, and N_2 fluxes at PRM suggested that organic matter became limiting for N transformations in sediments, but this was generally not the case at MB. Positive relationships between SOD and sediment NH_4^+ flux and SOD and potential denitrification confirm a coupling of remineralization and N removal processes. Isotope pairing calculations yielded positive benthic N fixation values in all but two cases during the study, but these rates did not offset N losses by N_2 production pathways. Actual N_2 production rates, considering N fixation effects on net N_2 flux, were balanced by net DIN influx to sediments at PRM but exceeded water column NH_4^+ regeneration rates. Thus, PRM sediments were a net N sink and may contribute to late season N deficiency in the lake. Sediment DIN efflux was balanced by N_2 removal at MB, leaving water column regeneration and external inputs to support primary production. However, water column regeneration and external input estimates could not account for estimated NH_4^+ uptake at MB. This result at MB supports the hypothesis that N limits phytoplankton production in pelagic areas of the lake. Nitrification did not appear to be the primary NO_3^- source for denitrification, contrary to most aquatic systems. Anammox may have accounted for 6 – 10% of total N_2 production, consistent with relative anammox proportions in marine sediments and suboxic waters in Lake Tanganyika.

CHAPTER V: SYSTEM NITROGEN TRANSFORMATIONS AND THEIR EFFECTS ON NUTRIENTS AND PHYTOPLANKTON COMMUNITY STRUCTURE IN MISSISQUOI BAY, LAKE CHAMPLAIN: SYNTHESIS OF RESULTS

MARK J. McCARTHY

5.1. Review of significant findings

In preceding chapters, basic limnological characteristics (Chapter II), water column nitrogen (N) cycling rates (Chapter III), and sediment-water interface (SWI) nutrient fluxes and N transformation rates (Chapter IV) were described for Missisquoi Bay. Sampling and experimental incubations occurred over three growing seasons (2007 – 2009) at two contrasting sites; one at the mouth of a major tributary (Pike River mouth; PRM), and the other in the center of the main lake basin (MB). These sites were selected to provide gradients in distance from external nutrient inputs, sediment type, and water depth. Station selection, sampling, and methodological details are introduced in the General Introduction (Chapter I) and preceding chapters. Water quality and phytoplankton data from a parallel monitoring program also were used in Chapter II.

In Chapter II, questions asked were: (1) what are the seasonal dynamics of ambient nutrient concentrations?, (2) what are the dominant phytoplankton genera in each season?, and (3) based on relevant literature about these genera, which N form is more conducive to support their dominance? The results presented in Chapter II show that Missisquoi Bay was generally nutrient replete, particularly with respect to phosphorus (P) but also for N in most cases. The phytoplankton community was dynamic, with temperature-tolerant and reduced-N-competitive cryptophytes dominating early and late in the season (May, June, and October). Oxidized-N-competitive diatoms dominated in July and September, and reduced-

N-competitive cyanophytes dominated in August. Despite high bioavailable nutrients in the lake, the mid-summer cyanobacteria community was dominated by genera capable of N fixation (e.g., *Anabaena* and *Aphanizomenon*), although heterocyte counts were low. However, the presence of heterocytes suggests that extreme N deficiency occurred on a spatial and/or temporal level not captured by the sampling regime employed here. The ability to shift to N fixation during these episodes of N deficiency would provide a competitive advantage for these genera over non-N fixing cyanobacteria, like *Microcystis*. In general, though, the results from Chapter II suggest that factors other than nutrients, such as light and/or temperature, are most important in determining phytoplankton community structure.

In Chapter III, the objectives were to: (1) assess water column NH_4^+ regeneration and potential uptake rates in the light and dark, (2) measure water column N fixation rates, and (3) determine depth-averaged water column NH_4^+ uptake and regeneration rates. Water column NH_4^+ cycling rates measured using isotope dilution were similar to those measured in other freshwater systems, and no significant spatial or temporal trends were observed. Phytoplankton dominance by cyanobacteria was associated with a higher proportion of autotrophic NH_4^+ uptake, but autotrophic and heterotrophic NH_4^+ uptake otherwise were balanced. Higher NH_4^+ cycling rates early in the season at PRM suggest a reliance on allochthonous nutrient and organic matter sources, while mid-summer rate maxima at MB suggest the importance of autochthonous sources in the central basin. Water column N fixation was not detected in the lake, despite the presence of some heterocytes in cyanobacteria filaments at MB. This finding supports the conclusion from Chapter II that extreme N deficiency occurred at fine spatial and/or temporal scales not captured in the sampling regime implemented here. Depth-averaged and extrapolated NH_4^+ regeneration rates were used to estimate a lake-wide, daily regeneration rate of 708,000 mol N d^{-1} for the Missisquoi Bay water column, which was nearly twice the estimated N load from tributaries (372,000 mol N d^{-1}). This comparison suggests a primary role for internal recycling mechanisms in fueling primary production in the lake. Biomass turnover times were slower (~19 days) than observed in eutrophic areas of the tropical, northeastern Atlantic Ocean (Dufour & Torreton 1996) and support the other conclusions that production in Missisquoi Bay is controlled from the top down.

Chapter IV focused on answering the following questions related to SWI N transformations: (1) What is the sediment O_2 demand? (2) What are SWI nutrient fluxes, and which pathways are important for N transformations? (3) Is DNRA an NH_4^+ source to the water column, and, if so, what are the rates? (4) What are sediment denitrification and N fixation rates? (5) Do in situ denitrification estimates differ from denitrification potential rates (i.e., is denitrification substrate limited)? (6) Is anammox a possible pathway for N loss?

A bottom-water hypoxia event at MB in July 2009 resulted in an altered N status in sediments and overlying water and was followed closely by the earliest cyanobacteria bloom observed during this study. Contrary to a parallel study on sediment redox characteristics in the lake, the present study showed that the cyanobacteria bloom came after the change in sediment redox status. However, doubling times for *Microcystis* generally are not sufficient to explain the absence of any cells on 3 July 2009 to the bloom conditions observed 5 days later, unless there was an inoculum from either vertical migration from sediments or horizontal advection from other areas of the lake. Sediments were a consistent soluble reactive P (SRP) source to the water column in the central basin, which suggests that internal sediment loads may delay recovery from P loading reductions. SRP and NH_4^+ fluxes supported the hypothesis that the lake became increasingly N limited as the growing season progressed. Sediment NH_4^+ regeneration rates could support water column NH_4^+ uptake rates at PRM but not at MB. DNRA estimates suggest that this pathway is an inconsistent but potentially important mechanism for sediment N recycling. When it occurred, DNRA accounted for about 35 and 85% of total NO_3^- reduction at PRM and MB, respectively. However, DNRA occurred in only about half of the incubations at PRM and less than a third of the incubations at MB. Decreasing SOD, NH_4^+ fluxes, and N_2 fluxes at PRM indicated organic matter limitation at PRM. Calculated sediment N fixation rates were not sufficient to offset N losses via denitrification/anammox. Actual N losses were balanced by dissolved inorganic N (DIN) effluxes from sediments at MB, leaving water column regeneration and external inputs to support pelagic primary production. However, these N sources, as estimated in this study, could not account for water column NH_4^+ uptake rates. At PRM, actual N_2 production was balanced by DIN flux into sediments but exceeded water column regeneration, suggesting that PRM sediments were a net N sink and may contribute to late

season N deficiency. Water column NO_3^-, rather than benthic nitrification, was the primary NO_3^- source for denitrification, contrary to most aquatic systems. Anammox may have accounted for 6 – 10% of total N_2 production, but molecular studies quantifying anammox gene expression are needed to confirm that anammox occurred in these sediments.

The objective of this chapter is to synthesize the background conditions reported in Chapter II and the rate measurements in Chapters III and IV to: (1) make inferences about the nutrient limitation status of primary producers, (2) determine any relationships between ambient nutrients and phytoplankton community structure, (3) determine any relationships between ambient nutrients, phytoplankton community structure, and water column N transformation rates, and (4) determine any relationships between ambient nutrients, phytoplankton community structure, and SWI N transformation rates.

5.2. Evidence for N limitation in Missisquoi Bay

With respect to objective (1), generally replete nutrients in the lake suggested that primary producers were limited by other factors, such as light, temperature, or top-down effects (e.g., Heath 1992). However, as mentioned previously, the presence of heterocytes in some cyanobacteria filaments at MB argue for spatially and temporally explicit episodes of extreme N deficiency. The ability to respond to these episodes by fixing N until a DIN source materializes may be the most important factor determining cyanobacteria community structure when the physicochemical conditions favor this group over other phytoplankton groups. Heterocyte differentiation and N fixation come at the expense of growth and biomass accumulation (e.g., Rhee & Lederman 1983, Attridge & Rowell 1997, De Nobel et al. 1997), and N fixation is limited by numerous environmental factors (Paerl 1990). Further, heterocyte differentiation in cyanobacteria occurs only at the end of a genetic cascade stimulated by exhaustion of all other energetically more favorable N sources (e.g., Valladares et al. 2008). Thus, the energetic costs of N fixation are such that the presence of heterocytes in cyanobacteria filaments can be used as a proxy for N limitation in aquatic systems. Sampling events occurring on broad time frames and incorporating large water volumes are unlikely to resolve fine scale episodes of N deficiency, and this premise is supported by the results

presented in Chapter II, which failed to identify any episode where DIN, especially NH_4^+, was not present (Table 3.2). The methods used to measure water column N fixation also were unable to resolve any evidence of active N fixation, which further supports the occurrence of fine scale, rather than widespread, N deficiency in the lake.

Other results from this study support the hypothesis that N limited productivity on some scale in Missisquoi Bay. Sediment SRP fluxes reversed from positive (efflux) to negative (influx) at PRM late in the season (Fig. 4.1), suggesting that water column P demand was insufficient to prevent sediment P uptake by microbes. If other nutrients (i.e., N supply) were sufficient, then P would be depleted in the water column due to primary producer uptake. The resulting hypothetical SRP concentration gradient between sediment porewaters and overlying water would lead to SRP efflux from sediments, as was observed early in the season at PRM, when there was no evidence of N limitation. In addition, water column NH_4^+ regeneration combined with external N loading was insufficient to support water column NH_4^+ uptake in the pelagic zone, and DIN release from sediments at MB was balanced by N_2 production and subsequent loss to the atmosphere. These results also suggest some level of N limitation in the central basin of the lake. Other studies have observed a significant response from experimental nutrient enrichments in Missisquoi Bay (Gonzalez-Rueda 2009), but these experiments were not designed to identify the nutrient limiting primary production.

5.3. Do nutrient dynamics control phytoplankton community structure?

The lowest observed monthly mean DIN concentrations in September (Figure 2.3) were preceded by the highest phytoplankton biomass in August, which was dominated by cyanobacteria (Figure 2.5). NH_4^+ concentration at PRM also was related negatively to all phytoplankton parameters (Table 3.5), especially for cyanobacteria. These results imply that phytoplankton drove nutrients, rather than nutrients driving phytoplankton from the bottom-up. It was hypothesized that the ambient NH_4^+ to NO_x ratio (NH4:NOx) would be useful to predict phytoplankton community structure. The rationale for this hypothesis was that cyanobacteria are more competitive for reduced N (i.e., NH_4^+) and would be favored at high

NH4:NOx. In contrast, oxidized-N-competitive diatoms were hypothesized to be favored at low NH4:NOx. However, neither diatom biomass (bD) nor diatom proportion of total phytoplankton biomass (pD) were related to NH4:NOx, even when bD and pD were compared to NH4:NOx from the preceding or subsequent month. The hypothesis also was rejected for cyanobacteria, which were not related to NH4:NOx as either biomass (bC) or proportion of total phytoplankton biomass (pC). Comparing bC and pC to NH4:NOx in the previous month also resulted in no significant relationships, suggesting that NH4:NOx could not predict cyanobacteria dominance. However, bC was related ($r^2 = 0.89$, $p = 0.015$; Fig. 5.1) and pC was nearly related ($r^2 = 0.75$, $p = 0.056$) to NH4:NOx in the following month, and these relationships were driven by cyanobacteria dominance in August and high NH4:NOx in September. Mean NH_4^+ concentration in September was < 50% of the concentration in August, in accordance with high cyanobacteria NH_4^+ uptake in August. As such, monthly mean NH_4^+ concentrations were related to bC ($r^2 = 0.83$, $p = 0.031$; Fig. 5.2) and pC ($r^2 = 0.90$, $p = 0.014$) from the previous month.

Given the competitive abilities of cyanobacteria for NH_4^+, the apparent top-down control of nutrients by phytoplankton is counter-intuitive unless an alternate explanation for very low NO_x concentrations (and thus high NH4:NOx) in September can be proposed. Denitrification rates (Figures 4.6 & 4.7) and diatom biomass (Figure 2.7) were not high in August and do not appear to be suitable explanations. One possible explanation for low NO_x concentration, and thus high NH4:NOx, in September is related to the rationale for fine-scale N deficiency discussed above. While NH_4^+ is consistently regenerated in the water column and sediments, NO_x would only be "regenerated" via nitrification, which would come at the expense of NH_4^+. If nitrification were converting large amounts of NH_4^+ to NO_x, we would expect lower NH4:NOx, so it is unlikely that any microbial processes were replacing a significant amount of the NO_x consumed by sediment denitrification and phytoplankton uptake. If fine-scale N deficiency occurred and led to heterocyte differentiation in some filaments, despite the presence of NH_4^+ in the discrete samples collected at an insufficient resolution, then NO_x would be depleted by those cyanobacteria before differentiating heterocytes. Without a replacement mechanism for assimilated or converted NO_x, it is plausible that the cyanobacteria-dominated phytoplankton community depleted all DIN forms

in August, but regeneration processes were able to replace some of the NH_4^+ when biomasses decreased into September. Another consideration is that diatom biomass more than doubled from August to September. Low NO_x concentration in September (and thus high NH4:NOx), combined with the high cyanobacteria biomass in August and the relationships of bC and pC with NH4:NOx in the following month, may have driven the observed relationship. If this is the case, then there would be no obvious limnological value to this relationship. Continuation of the monitoring program and accumulation of long-term data at higher spatial and temporal resolution may allow this relationship to be dismissed or understood more completely, whatever the case may be.

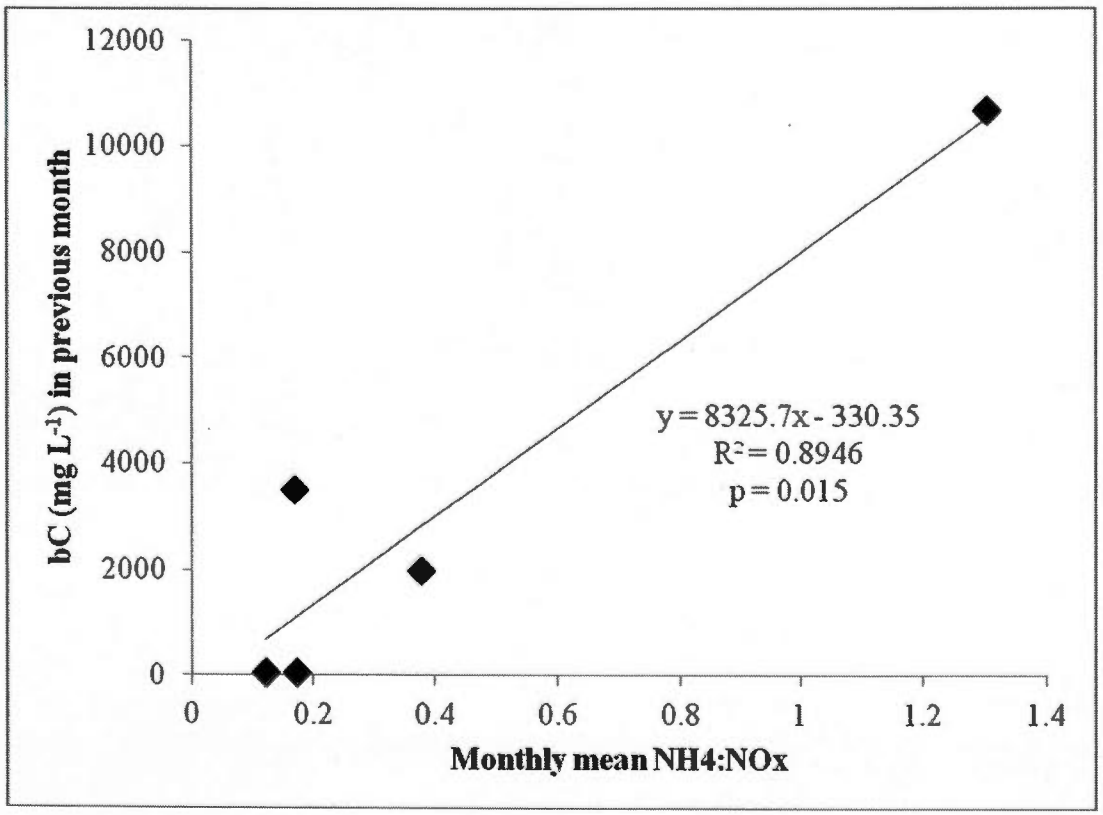

Fig. 5.1. Relationship between monthly mean NH4:NOx and cyanobacteria biomass (bC) in the previous month in Missisquoi Bay, Lake Champlain.

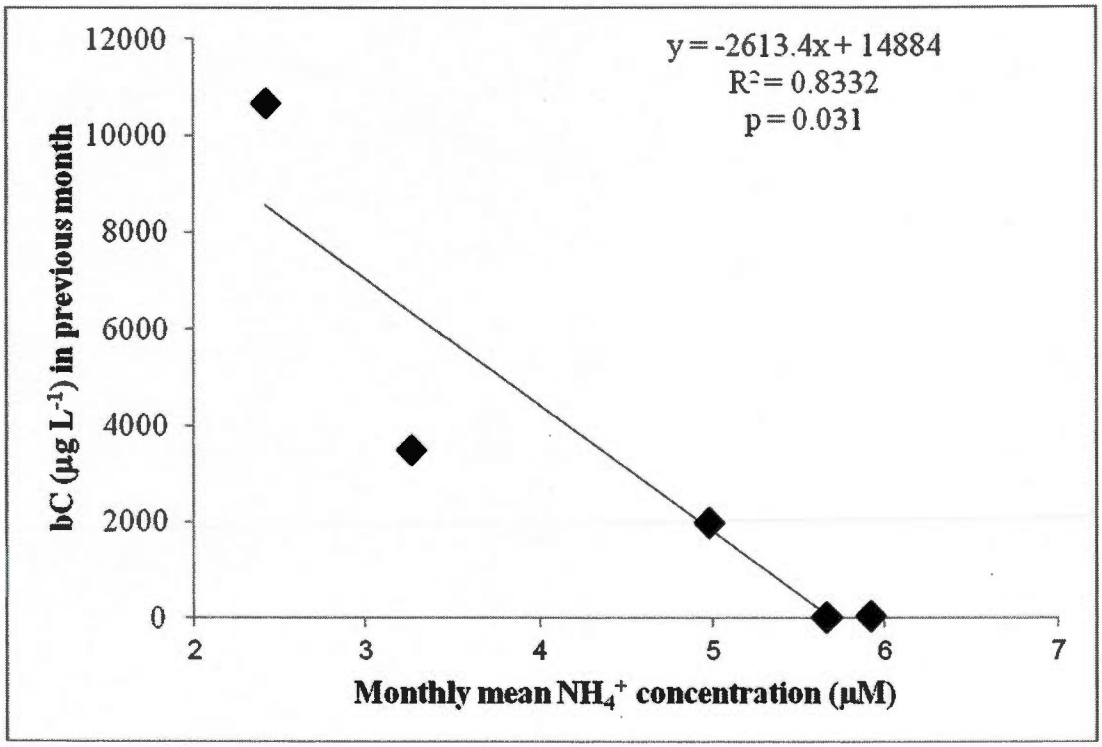

Fig. 5.2. Relationship between monthly mean NH_4^+ concentration (µM) and cyanobacteria biomass (bC) in the previous month in Missisquoi Bay, Lake Champlain.

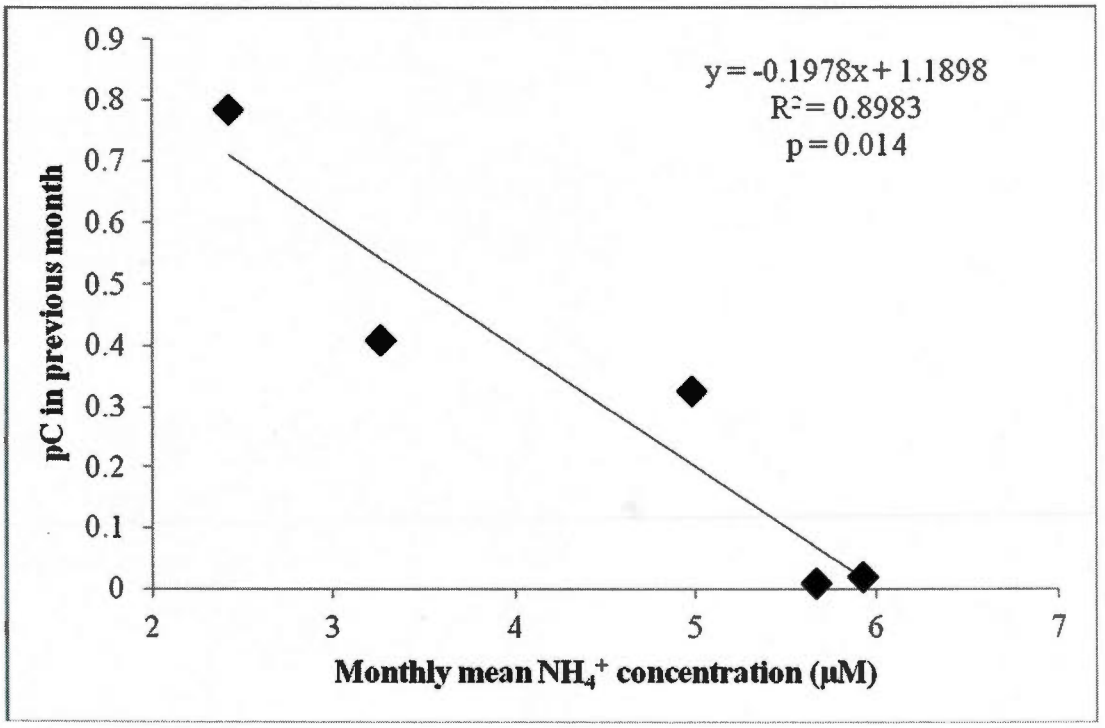

Fig. 5.3. Relationship between monthly mean NH_4^+ concentration (µM) and proportion of cyanobacteria biomass to total phytoplankton biomass (pC) in the previous month in Missisquoi Bay, Lake Champlain.

5.4. Water column N cycling relationships with phytoplankton and ambient nutrients

It was hypothesized that water column N fixation rates would be related to low DIN concentrations and the presence of known N fixers. However, incubations failed to measure significant N fixation at any time in Missisquoi Bay. Further, low DIN concentrations in discrete samples appeared to be related to preceding phytoplankton biomass, rather than driving the phytoplankton community composition. As discussed above, these patterns may not hold at fine spatial and temporal scales, and studies designed to capture these patterns are needed to confirm that conditions suitable for heterocyte differentiation and N fixation occur at scales not measurable using conventional techniques. Molecular characterization of gene expression in cells presents an intriguing possibility to accomplish this task. A study in the

Red Sea used this approach to determine that picocyanobacteria were assimilating regenerated NH_4^+ by quantifying *ntcA* gene expression in wild cells (Lindell & Post 2001). A similar design may be feasible for combining *ntcA* quantification with *nifH*, which controls N fixation, and *hetC*, which controls heterocyte differentiation (e.g., Muro-Pastor et al. 1999). Fine-scale N deficiency at the cellular level can be evaluated with *ntcA* transcription levels (Lindell & Post 2001), and resulting heterocyte development and N fixation could be followed with time to constrain the pathways to N fixation in wild cyanobacteria cells.

Cyanobacteria are more competitive than other phytoplankton groups for reduced N forms, such as NH_4^+ (e.g., Blomqvist et al. 1994), while diatoms are more competitive for oxidized N forms, such as NO_3^- (e.g., Hutchins et al. 2003, Horgan 2005). Therefore, it was hypothesized that highest NH_4^+ uptake rates would coincide with high cyanobacteria biomass and proportion of total phytoplankton biomass. This hypothesis was not confirmed in Missisquoi Bay, since no significant relationships occurred between phytoplankton community structure and NH_4^+ uptake rates at either site. Dark NH_4^+ uptake at PRM was related to NH4:NOx with a positive slope (Fig. 5.4), however, and this result supports the idea that heterotrophic primary producers remained active in the dark when NH_4^+ concentrations were high. The possibility that dark uptake included phytoplankton uptake cannot be excluded, especially in cases where cyanobacteria accounted for a large proportion of the phytoplankton community (Shi et al. 2007) or during N deficiency (Cochlan et al. 1991, Jochem 1999, Flynn et al. 2002). However, PRM did not exhibit signs of N deficiency, and cyanobacteria never dominated at PRM. Therefore, the idea that heterotrophic (i.e., bacteria) NH_4^+ uptake explains the relationship between dark uptake and NH4:NOx seems to be the most plausible in this case. This idea also is supported by the positive relationship between pC and the ratio of light-to-dark NH_4^+ uptake, which suggests that high cyanobacteria proportion of total phytoplankton biomass affects the balance between heterotrophic and autotrophic uptake in the lake.

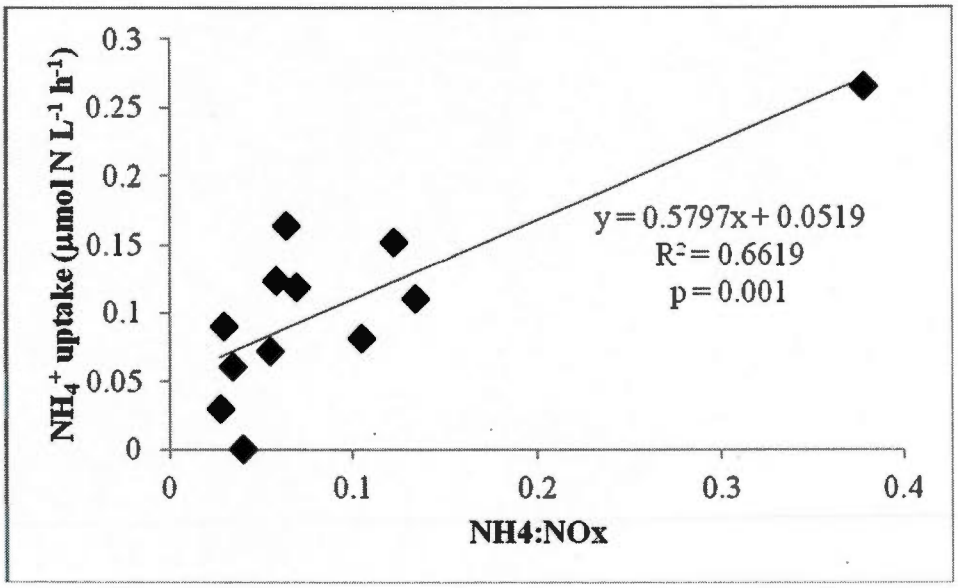

Fig. 5.4. Relationship between NH4:NOx and dark NH_4^+ uptake (µmol N L^{-1} h^{-1}) at the Pike River mouth (PRM) in Missisquoi Bay, Lake Champlain.

Cyanobacteria accumulated into colonies generally are not preferred forage for grazers (e.g., Sellner et al. 1993, Vanderploeg et al. 2001). Thus, it was hypothesized that water column NH_4^+ regeneration rates would be lowest when cyanobacteria represent a large proportion of the phytoplankton community biomass. However, no significant relationship was observed between regeneration rates and either pC or bC at either site. Like other potential relationships involving phytoplankton community structure, this hypothesis likely suffered from a lack of data, particularly for phytoplankton community structure. Only eight data points for PRM and nine for MB were available where both water column NH_4^+ cycling and phytoplankton community structure were available for comparison. A larger dataset may be needed to adequately test these hypotheses.

5.5. Links between SWI nutrient fluxes and phytoplankton community structure

It was hypothesized that Missisquoi Bay sediments would be a sink for oxidized N and not be a significant NH_4^+ source. Sediments were indeed a sink for NO_3^- at both sites

(Figure 4.3), and these rates were not offset by low NO_2^- effluxes (Figure 4.2). Lake-wide SWI NO_3^- fluxes were related strongly ($r^2 = 0.87$, $p << 0.001$; Fig. 5.5) to water column NO_3^- concentrations and also related to NH4:NOx ($r^2 = 0.18$, $p = 0.048$), suggesting that water column DIN concentrations were driving SWI fluxes. Contrary to the hypothesis, sediments at both sites were a significant NH_4^+ source (Figure 4.4). In fact, NH_4^+ efflux exceeded NO_3^- influx at MB, where net DIN flux was from the sediments to the water column. SWI NH_4^+ fluxes were not related to NH4:NOx at either site but was nearly related on a lake-wide basis ($r^2 = 0.17$, $p = 0.058$). The negative slope of this near-significant relationship implies that water column NH_4^+ concentration may affect SWI fluxes based on concentration gradients between the water column and sediment porewater. These concentration gradients likely are generated by differences in the magnitudes of uptake and regeneration mechanisms. This interpretation is supported by another near-significant, lake-wide relationship between water column NH_4^+ concentration and SWI NH_4^+ flux ($r^2 = 0.15$, $p = 0.066$, positive slope). These results suggest that sediment processes indeed play an important role in determining DIN available to primary producers in the water column.

Results presented in previous chapters suggested that both phytoplankton and sediment N fluxes influenced ambient N concentrations. Do these sediment DIN fluxes also relate to phytoplankton community structure? It was hypothesized that sediment DIN fluxes would decrease after cyanobacteria bloom events based on results from other aquatic systems (McCarthy et al. 2007c, 2009a). At MB, SWI DIN flux was related significantly to bC ($r^2 = 0.40$, $p = 0.05$) with a positive slope, but no other relationships between SWI N fluxes and phytoplankton were found at either site or lake-wide. There also were no relationships between SWI N fluxes and phytoplankton from the next sampling event, suggesting that there was no lag effect on phytoplankton. However, SWI NO_3^- flux at MB was related to pC from the previous sampling event ($r^2 = 0.66$, $p = 0.007$; Fig. 5.6), in contrast to the hypothesis. This result is consistent with a stimulation of NO_3^- production via nitrification and a decrease in NO_3^- reduction via denitrification, possibly resulting from the low water column DIN concentrations after cyanobacteria blooms. Together, these results imply a more indirect effect for SWI nutrient fluxes on phytoplankton community structure, but more data is needed to clarify the role of sediment nutrient release in Missisquoi Bay.

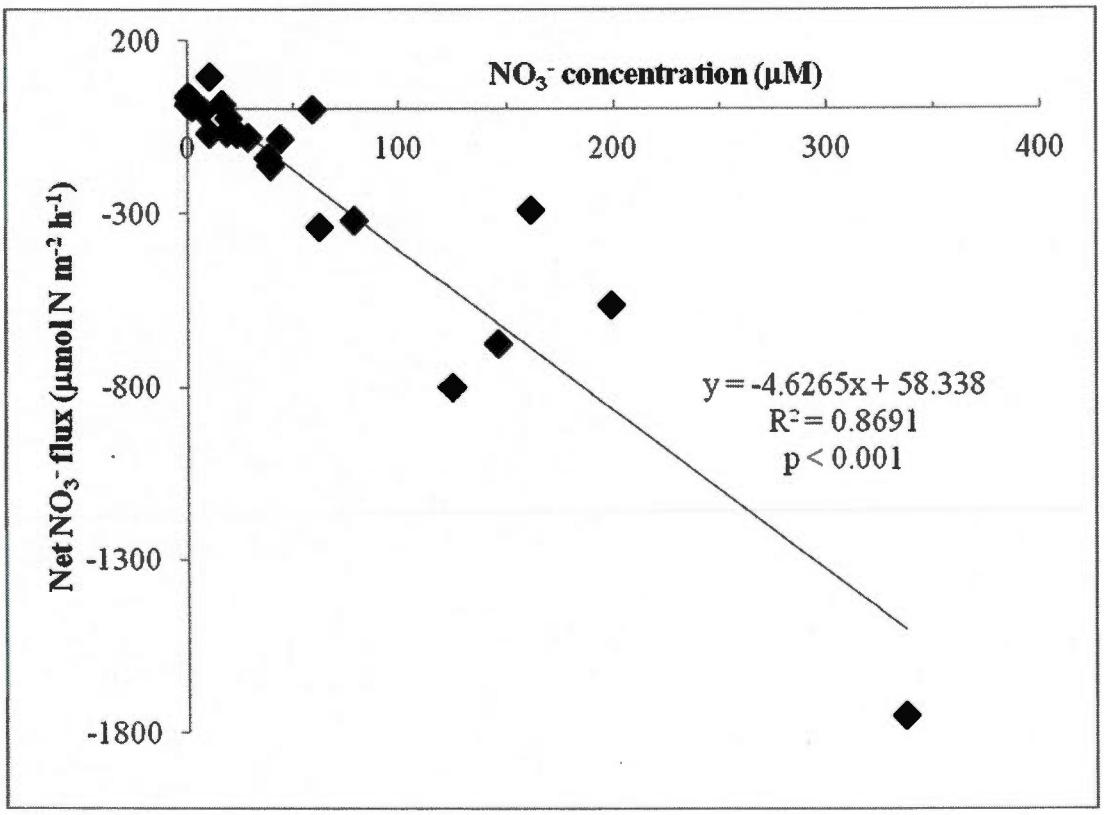

Fig. 5.5. Relationship between water column NO_3^- concentration (µM) and net NO_3^- flux across the sediment-water interface in Missisquoi Bay, Lake Champlain (both sites are included). Note that negative rates indicate flux from the water column into sediments.

5.6. Does denitrification affect N concentrations and ratios and contribute to N fixing cyanobacteria blooms?

It was hypothesized that high denitrification rates (including any anammox) in summer would lead to reduced DIN concentrations in the water column and subsequent development of N fixing cyanobacteria. It was also hypothesized that denitrification of water column NO_3^- would lead to lower NH4:NOx. Anammox may have accounted for about 6 – 10% of total N_2 production, but it will not be addressed separately in this section. Estimated

denitrification (sum of net $^{28}N_2$ flux and sediment N fixation; Table 4.2) was nearly related to NH4:NOx at PRM ($r^2 = 0.26$, $p = 0.088$) and lake-wide ($r^2 = 0.14$, $p = 0.084$) with negative slopes, but not to NH4:NOx measured on the previous or subsequent sampling events. Estimated denitrification was related to DIN concentration lake-wide ($r^2 = 0.86$, $p << 0.001$; Fig. 5.7) and at PRM ($r^2 = 0.87$, $p << 0.001$) with positive slopes. Therefore, it can be concluded that denitrification was related to ambient nutrients, and the positive slopes for the latter relationships and negative slopes for the former relationships can be interpreted as more significant relative to NO_3^- than NH_4^+. However, the results indicate that denitrification is driven by ambient nutrients, and the related processes driving them (i.e., organic matter decomposition), rather than denitrification affecting observed nutrient concentrations.

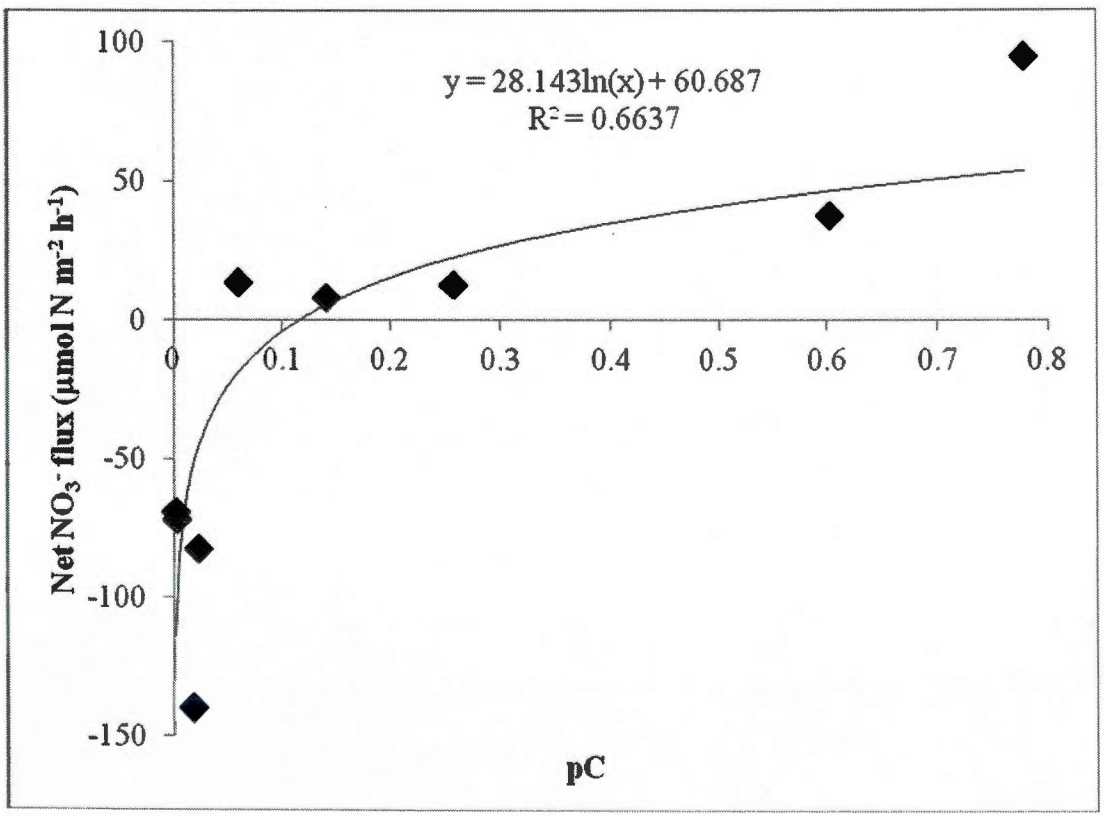

Fig. 5.6. Log relationship between sediment-water interface NO_3^- flux and the proportion of cyanobacteria to total phytoplankton biomass (pC) from the previous sampling event in the central basin (MB) of Missisquoi Bay, Lake Champlain.

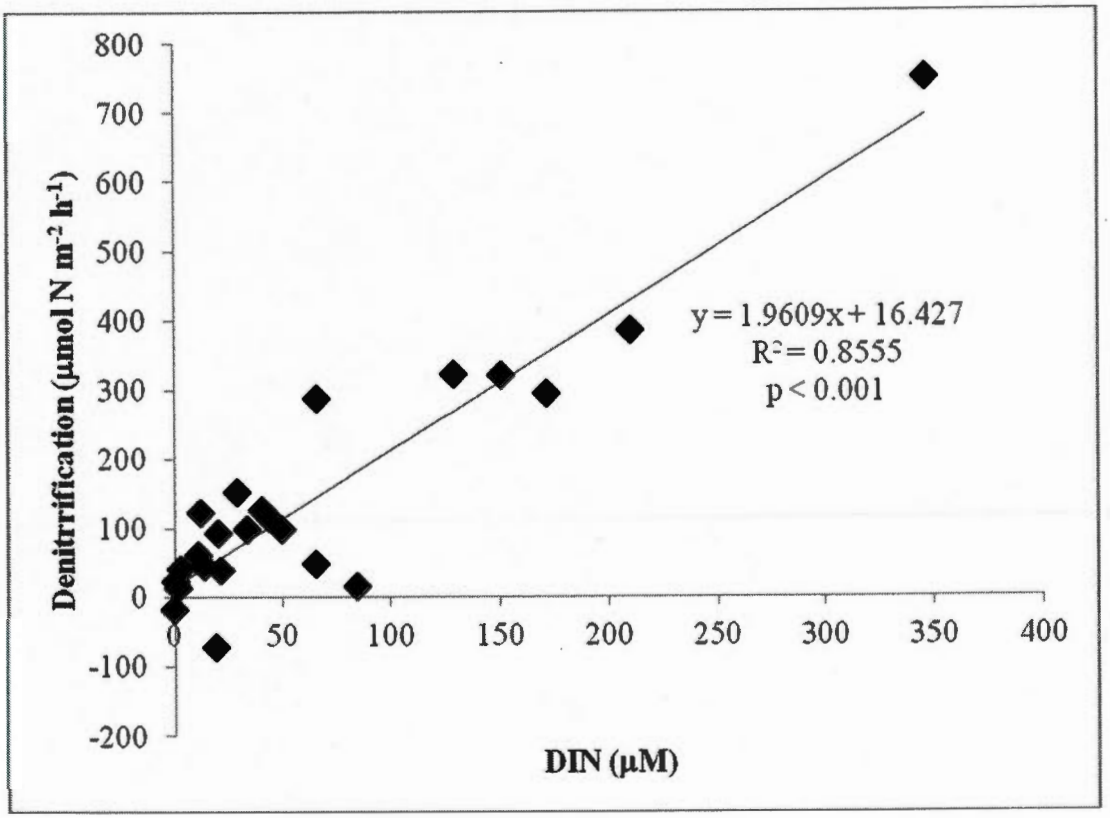

Fig. 5.7. Relationship between dissolved inorganic N (DIN) concentration in the water column and estimated denitrification (sum of net N_2 flux and N_2 fixation) in sediments in Missisquoi Bay, Lake Champlain (both sites are included).

As mentioned in Chapter II, cyanobacteria accounted for large proportions of phytoplankton biomass only during blooms, and these blooms were dominated by potential N fixers. Thus, cyanobacteria biomass (bC) was used as a proxy for N fixing cyanobacteria. No significant relationships were discovered between denitrification and bC at either site or lake-wide. However, comparing estimated denitrification to bC measured on the previous sampling event resulted in improved, but still insignificant, relationships at both PRM (r^2 = 0.39, p = 0.133) and MB (r^2 = 0.37, p = 0.084) with negative slopes. Therefore, high cyanobacteria biomass on one sampling event may be associated with lower denitrification rates on the following sampling event. This result is similar to the previously described relationship between pC and NO_3^- flux at MB, where high pC was followed by increased

NO_3^- efflux. Despite not being statistically significant due to the small dataset, this observation is intuitive and fits with conclusions from previous chapters. If N fixing cyanobacteria dominate because of N deficiency beyond sampling resolution, and denitrification is driven by water column DIN, then it would follow that conditions suitable for N fixing cyanobacteria also would lead to lower denitrification rates. Therefore, the data do not support the hypothesis that denitrification leads to N limitation and a subsequent phytoplankton community shift to potential N fixers. Rather, the data suggest that denitrification and phytoplankton community structure are instead controlled by ambient nutrient concentrations.

5.7. Preliminary N budget for Missisquoi Bay

Total N load to Missisquoi Bay from tributaries was estimated to be 372,000 mol N d^{-1}. Internal sources and microbial sinks at each site were measured and extrapolated for each site (see Chapter III for extrapolation details) and the whole lake for comparison with total estimated N load from tributaries (Table 5.1). At PRM, water column NH_4^+ uptake (18,830 mol N d^{-1}), sediment DIN removal (20,000 mol N d^{-1}), and N_2 production (24,300 mol N d^{-1}) are loss terms. However, if it is assumed that all sediment DIN influx is denitrified, then the net N loss at PRM is 4,300 mol N d^{-1}. It also could be argued that NH_4^+ uptake is not a loss term because of regeneration processes. However, planktonic primary producers may not remain in the river discharge area long enough to be regenerated. It is more likely that these primary producers are advected to pelagic areas of the lake. Indeed, these ideas are supported by the low water column NH_4^+ regeneration estimate (i.e., source term; 12,000 mol N d^{-1}) relative to uptake. Considering these sources and loss terms, PRM is a net N sink of 11,130 mol N d^{-1}. This value suggests that river discharge areas in the lake remove about 3% of total N loading from tributaries. Possible loss terms not quantified include sediment N burial, ammonia volatilization, incorporation into live biomass, and physical advection to other areas of the lake. Source terms not quantified include atmospheric deposition and nutrient advection from other lake areas, and water column N fixation rates were not detectable. Any sediment N fixation is incorporated into the net N_2 loss term.

Table 5.1. Preliminary N budget for the Pike River mouth (PRM) and central basin (MB) in Missisquoi Bay, Lake Champlain. Loss terms are water column (WC) NH_4^+ uptake and estimated denitrification (DNF = net N_2 flux + N_2 fixation). At PRM, DNF was adjusted on the assumption that all of the net DIN flux into the sediments was due to denitrification. This adjustment was not needed at MB because net DIN flux was out of the sediments. Source terms were WC NH_4^+ regeneration for each site and estimated tributary load for the whole lake. Net DIN flux was a source term at MB. Rates are expressed in mol N d^{-1}.

Term	PRM	MB
WC Uptake	-18830	-1190000
WC Regeneration	12000	696000
DNF	-24300	-106000
SWI DIN flux	(-20000)	104000
Adjusted DNF	-4300	N/A
Total	-11130	-496000
Lake-wide		-507130
Tributary N load		372000
N deficit		-135130

At MB, water column NH_4^+ uptake (1,190,000 mol N d^{-1}) and N_2 production (106,000 mol N d^{-1}) are loss terms. Sediments were a net DIN source (104,000 mol N d^{-1}), and water column NH_4^+ regeneration added 696,000 mol N d^{-1}. These results suggest that pelagic lake areas are a net N sink of 496,000 mol N d^{-1}. Possible loss terms not quantified include those mentioned for river discharge areas, in addition to discharge into the main body of Lake Champlain. If it is assumed that river discharge areas of the lake remove 3% of total N loading from tributaries, and the remainder (360,870 mol N d^{-1}) is advected to pelagic areas, then a 'missing' N source of ~135,000 mol N d^{-1} is needed to balance this preliminary N budget for Missisquoi Bay. Water column N fixation rates would need to be only 0.026 µmol N L^{-1} h^{-1} in the lake, on average, to be this 'missing' N source. As mentioned in Chapter

III, measured water column N fixation rates were not significantly different from controls and, at PRM, not significantly different from zero. The insignificant rate at MB, however, was 0.25 ± 0.15 μmol N L^{-1} h^{-1} and is an order of magnitude higher than the estimated N fixation rate needed to balance this preliminary budget. Thus, N fixation may be more important than the measured results in this study suggest, and this possibility is supported by other measurements and observations presented in this study. This preliminary N budget for Missisquoi Bay has many uncertainties and should be interpreted cautiously. Future work could refine the incubation techniques used for the water column N fixation measurements. In particular, the technique may benefit from incubation vessels with a larger volume to reduce the high variability in rates.

5.8. Conclusion

Synthesis of results from this study generally suggested that factors other than nutrients limit primary productivity in Missisquoi Bay, but there also were multiple lines of evidence supporting speculation that N limits productivity on fine spatial and temporal scales not captured in the sampling regime. Contrary to the hypotheses tested, phytoplankton in the lake may control nutrient concentrations from the top down. Phytoplankton community structure did not affect water column NH_4^+ uptake rates, but the proportion of cyanobacteria to total phytoplankton biomass affected the balance between presumed autotrophic and heterotrophic NH_4^+ uptake by causing an increase in the ratio of light to dark NH_4^+ uptake. Water column NH_4^+ regeneration rates were not related to phytoplankton community structure, refuting the hypothesis that cyanobacteria would inhibit N regeneration. As predicted, sediments were a significant NO_3^- sink in the lake, but the sediments also were a significant NH_4^+ source to the water column, contrary to the hypothesis. However, this study confirmed that sediments are important in determining water column nutrient concentrations. High cyanobacteria biomass was related to future increases in NO_3^- release from sediments, which may reflect a stimulation of nitrification in the sediments. Anammox may be present in the lake and account for 6 – 10% of total N_2 production, but denitrification appears to be the dominant N_2 pathway. Molecular gene expression characterizations are needed to confirm and quantify anammox presence and activity. Denitrification rates were related positively to

water column DIN concentration, but denitrification did not affect future water column DIN concentrations. Similar relationships between sediment NO_3^- flux and denitrification with cyanobacteria biomass on the previous sampling event suggest that lower water column DIN concentrations resulting from cyanobacteria blooms led to reduced denitrification rates. The data do not support the hypothesis that denitrification would lead to conditions suitable for N fixing cyanobacteria. In contrast, the results indicate that the conditions suitable for N fixing cyanobacteria cause lower denitrification rates. The lake was a net N sink according to a preliminary N budget prepared using the water column NH_4^+ cycling and SWI N fluxes compared to estimated N load from tributaries. These preliminary calculations indicate that there is a 'missing' N source, which results suggest may be water column N fixation. Water column N fixation rates measured in this study were not significantly different from experimental controls, although the insignificant rates were an order of magnitude higher than those required to account for the missing N source. Improved incubation techniques for water column N fixation measurements are needed reduce the variability in results from the method used here. More thorough quantification of N sources (e.g., water column N fixation) and loss terms (e.g., sediment N burial and washout) are needed to build on the results presented here and further constrain the N budget in the lake.

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