EFFECTS OF ANTHROPOGENIC UREA ON LAKES OF THE NORTH AMERICAN GREAT PLAINS: ECOLOGICAL IMPLICATIONS BASED ON EXPERIMENTAL AND OBSERVATIONAL STUDIES

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SUPERVISORY AND EXAMINING COMMITTEE

Matthew James Bogard, candidate for the degree of Master of Science in Biology**,** has presented a thesis titled, ***Effects of Anthropogenic Urea on Lakes of the North American Great Plains: Ecological Implications Based on Experimental and Observational Studies,*** in an oral examination held on September 16, 2011. The following committee members have found the thesis acceptable in form and content, and that the candidate demonstrated satisfactory knowledge of the subject material.

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ABSTRACT

Urea consumption has increased dramatically since the 1960s and now comprises over 50% of the nitrogen (N)-based fertilizer used globally. Currently, only 30-50% of N-fertilizers applied to cropland are effectively used by crops, while the remainder is lost to the environment. Urea is also a component of livestock and human wastes. In principal, export of urea to lakes as a consequence of human activities may exacerbate ecological problems associated with eutrophication (e.g. increased productivity of aquatic fauna, deep water anoxia, biodiversity loss, fish kills, etc.), especially in phosphorus (P)-rich aquatic ecosystems.

Biweekly measurements of urea content and limnological variables (water chemistry, hydrology, algae, zooplankton) during two summers in a chain of seven productive lakes in central Canada were used to quantify human and environmental influences on temporal and spatial patterns of urea occurrence. Mean ($\pm$ SD) urea concentrations varied between 28.7 $\pm$ 14.0 and 131.7 $\pm$ 64.9 μg N L^{-1}, increased from headwater to downstream sites, and represented 10-50% of bio-available N. Principal components analysis demonstrated that urea concentrations were elevated in agriculturally-impacted lakes with abundant dissolved organic and inorganic nutrients (N, P, C) and low O_2 concentrations, and were inconsistently correlated with plankton abundance and community composition. In contrast, urea concentrations were elevated more than two-fold in lakes receiving N from cities, despite low concentrations of urea in tertiary-treated urban effluent (~50% of lake values). Furthermore, dissolved organic N accounted for ~90% of total dissolved N in a survey of 69 closed basin lakes, suggesting that urea is ubiquitous in regional lakes. These findings suggest a new model for the

regulation of the urea in lakes in which land use practices regulate lotic influx, stimulate regeneration from lake sediments, and influence the balance between planktonic consumption and release of urea.

Differential effects of urea pollution on phytoplankton and heterotrophic bacteria were quantified in three mesocosm experiments conducted in P-rich, hypereutrophic Wascana Lake, Saskatchewan. Urea was added weekly at 0, 1, 3, 8, and 18 mg N L^{-1} to mesocosms (~3000-L) for 21-days each during July, August, and September of 2009. Repeated-measures analysis of variance (RM-ANOVA) revealed all urea concentrations stimulated increases in phytoplankton biomass and productivity to a stable plateau by day 7, afterwards light and P may have limited future autotrophic responses. The magnitude of algal response generally increased with urea loads up to 3 – 5 mg N L^{-1}, but additions beyond that level had little effect on algal abundance, and actually reduced primary production relative to maximum values. In contrast, bacterial abundance and production responded more slowly in a linear fashion to urea amendments, such that bacterial activity was sufficient to deplete oxygen by day 21 in trials with > 8 mg N L^{-1}. These findings suggest that urea pollution at concentrations < 3 mg N L^{-1} may rapidly enhance net autotrophy, while urea additions > 5 mg N L^{-1} decreasingly favor net autotrophy.

Together, these results suggest that the expected 100 million metric ton increase in urea use by ~2050 is likely to alter global N biogeochemistry, ecosystem metabolism, and accelerate water quality degradation in eutrophic aquatic ecosystems across the planet. This thesis concludes with a brief consideration of management strategies which may reduce urea influx to surface waters and favor maintenance of the ecological integrity of aquatic communities.

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DEDICATION

This work is dedicated to my girls. Holly, your love and friendship has carried me through this program and will always be my greatest source of happiness. Kalani, I hope that my scientific efforts here and in the future will contribute to improving the world that you will inherit and at the very least that these efforts inspire you. I love you both.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. GENERAL BACKGROUND

Cultural eutrophication is a global problem that threatens the integrity and use of aquatic ecosystems (Table 1.1). The term eutrophication refers to an increase in the amount, and rate, of biomass production by aquatic organisms beyond normal levels (Wetzel, 2001). Eutrophication occurs under an increased flux of growth-limiting nutrients to receiving waters (Smith, 2003). For aquatic biota, many elements are essential to specific metabolic activities, and if one element is in short supply relative to the others, it typically limits biotic growth and development. Many aquatic ecosystems are limited by either phosphorus (P), nitrogen (N), or a combination of both elements (Wetzel, 2001; Lewis & Wurtsbaugh, 2008), although micronutrients (e.g., Fe, Mo) can sometimes also limit algal productivity (Marino *et al*., 1990; Evans & Prepas, 1997; Wetzel, 2001). Eutrophication due to P and N addition degrades many aquatic systems (Table 1.1) and jeopardizes important ecosystem services (Vitousek *et al*., 1997; Carpenter *et al*., 1998; Schindler, 2006; Smith, 2006).

Human activities (industrial, agricultural, and urban practices) facilitate the transfer of nutrients to aquatic systems and are also closely linked to global population growth (de Jonge *et al*., 2002). Therefore, since the human population has doubled since ~1950 (de Jonge *et al*., 2002), these activities have increased (Tilman *et al*., 2001, 2002) and have resulted in the rapid eutrophication of aquatic ecosystems around the world (Vitousek *et al*., 1997; Carpenter *et al*., 1998; de Jonge *et al*., 2002). For instance, from 1960-1995, the global application rates of N and P fertilizers increased 7, and 3.5-fold respectively (Tilman *et al*., 2002). Yet as fertilization rates increase, the efficiency of

Table 1. Negative effects of eutrophication on freshwater and coastal marine ecosystems. Adapted from Smith (2003).

Eutrophication effects on freshwater and coastal marine systems.
1. Increased productivity and biomass of phytoplankton and suspended algae
2. Shifts in phytoplankton composition to blomm-forming species, many of which may not be consumed effectively by aquatic grazers.
3. Increased productivity, biomass, and species composition of attached microalgae (periphyton) and marine macroalgae.
4. Changes in productivity, biomass, and species composition of aquatic vascular plants.
5. Reduced yields of desirable finfish and shellfish species.
6. Reductions in the health and size of marine coral populations.
7. Threats to endangered aquatic species.
8. Decreases in water column transparency.
9. Taste, odor, and filtration problems in drinking water supplies.
10. Depletion of deep water oxygen.
11. Decreases in the perceived aesthetic value of the water body.
12. Negative economic impacts, including decreased property values and reduced recreational uses.

nutrient uptake by crops rapidly decreases (Ayoub, 1999) to the point where crop use of fertilizer at present accounts for only 30-50 % of N and ~45% of P applications (Tilman *et al*., 2002). Fertilizer that is not taken up by crops is lost to the environment, and largely ends up in aquatic ecosystems (Carpenter *et al*., 1998). Although recent studies have identified different point (localized) and non-point (diffuse) nutrient sources, these sources are very different in nature, and as such there is no unique solution for their regulation. As a result, eutrophication remains a potent cause of water quality degradation despite decades of intensive study (Edmondson, 1970; Schindler, 1974; Vitousek et al. 1997; Carpenter *et al*., 1998; Schindler, 2006).

Eutrophication typically shifts the ratio between N and P available to aquatic biota. As a generality, the ratio of C:N:P in planktonic algae is 40:7:1 by mass (Redfield, 1958). Eutrophication shifts the balance of dissolved aquatic nutrients to lower N:P values because nutrient sources such as sewage, livestock effluent, and chemical fertilizer runoff are typically rich in P relative to N (Downing & McCauley, 1992). Compared to natural terrestrial flora (Elser *et al*., 2000), agricultural crops also have a low N:P ratio, reaching values as low as ~1.5 in some cereal species (Sadras, 2006). Therefore, fertilization with disproportionately large quantities of P are required for crop production, a fact that often increases P influx to lakes (Bennett *et al*., 1999). Furthermore, because crops are readily consumed by livestock and humans (Tilman *et al*., 2001), digestive wastes from both groups often reflect the low N:P ratios of the crops, and deliver comparatively P-rich pollutants to aquatic ecosystems. Once in lakes, P can be retained and recycled for many years (Genkai-Kato & Carpenter, 2005; Jeppesen *et al*., 2005), causing P accumulation and further shifts to lower dissolved N:P ratios.

Aquatic N shortages typically promote the growth of cyanobacterial species capable of fixing atmospheric N_2 and thus compensating for the shortage of dissolved N (Barica *et al*., 1980; Schindler *et al*., 2008). However, the quantity of N fixed by cyanobacteria is variable among lakes (based on light availability, drainage basin characteristics, etc.) and often cannot supply all of the N required by phytoplankton (Ferber *et al*., 2004; Patoine *et al*., 2006; Lewis & Wurtsbaugh, 2008). Under these conditions of high P mass and restricted N fixation, pollution of lakes with N may favor increased algal growth and reduced water quality (Moss *et al*., 2005; Bunting *et al*., 2007). In particular, addition of N to N-limited ecosystems as nitrate (NO_3^-), ammonium (NH_4^+), and dissolved organic N (DON) can stimulate algal biomass production (Lewis & Wurtsbaugh, 2008; Sterner, 2008; Donald *et al*., in press) and shift species composition to favor harmful algal growth (Hall *et al*., 1999; Glibert *et al*., 2004, 2006, 2007; Bronk *et al*., 2007; Finlay *et al*., 2010a).

Although traditionally considered recalcitrant, anthropogenic supply of organic N to aquatic ecosystems is increasingly recognized as an important source of bioavailable N (Seitzinger *et al*., 2002; Berman & Bronk, 2003; Bronk *et al*., 2007). In headwater streams, up to 50% of watershed-derived NO_3^- and NH_4^+ can be taken up by algae, macrophytes, and bacteria, and converted to dinitrogen (N_2) and nitrous oxide (N_2O) gasses through microbial denitrification (Peterson *et al*., 2001; O'Brien *et al*., 2007; O'Brien & Dodds, 2008). The remaining N is exported to lakes and oceans as inorganic (NO_3^-, NH_4^+) and dissolved organic N (DON) (Peierls *et al*., 1991; Bernot & Dodds, 2005; Seitzinger *et al*., 2010), with urea ($[NH_2]CO$), dissolved free amino acids, dissolved combined amino acids such as oligopeptides and proteins, amino sugars,

nucleic acids, and complex humic substances making up a variable portion of the DON pool (Mitamura *et al*., 1994; Stepanauskas *et al*., 2000; Bradley *et al*., 2009; Bronk et al. 2010). Historically, DON species were thought to be minimally available to phytoplankton, only available following bacterial mineralization (Bronk *et al*., 2007); however, recent research suggests that low molecular weight DON (e.g., amino acids) is widely used by phytoplankton and bacteria (Berman & Chava, 1999; Berman & Bronk, 2003; Wiegner *et al*., 2004, 2006; Solomon *et al*., 2010). Further, organic N may selectively promote the growth of toxigenic phytoplankton in both marine and freshwater systems (Glibert *et al*., 2006; Bronk *et al*., 2007; Finlay *et al*., 2010a).

1.1 Thesis rationale

Urea represents a potentially large source of global aquatic N pollution, since it is the most widely used N-fertilizer worldwide (Glibert *et al*., 2006), and is a ubiquitous component of human and livestock wastes (Mitamura *et al*., 1994; Withers, 1998; Petersen *et al*., 2005). To date, increasing societal demands for protein rich crops and meat (Tilman *et al*., 2002) have resulted in a 15-fold increase in N fertilizer use since 1970 (Vitousek *et al*., 1997), and a shift from NH_3 and NO_3^- to more effective urea-based fertilizers. Unlike inorganic fertilizers, urea is less toxic to crop roots, it cannot be used to generate explosives, and is highly water soluble (Glibert *et al*., 2006). As a result, urea fertilizer use has increased from trace levels in the 1960s to ~80 Tg N yr^{-1} at present, and is expected to double again by the year 2050 (Millennium Ecosystem Assessment, 2005; Glibert *et al*., 2006). Further, increased production of human (Mitamura *et al*., 1994;

Bronk *et al*., 2010) and livestock (Petersen *et al*., 2004; Glibert *et al*., 2005) effluents associated with population growth to ~9 billion by 2050 (Millennium Ecosystem Assessment, 2005) is likely to favor elevated urea release to global surface waters.

Although elevated aquatic urea concentrations are linked with urban effluent release (Mitamura *et al*., 1994) livestock production (Glibert *et al*., 2005) and fertilizer applications (Glibert *et al*., 2006), a detailed investigation of the spatial and temporal controls of urea availability in eutrophic lakes has yet to be undertaken. In general, ~5 – 40 % of urea applications can reach aquatic ecosystems intact, depending on a number of factors (Glibert *et al*., 2006). Intact export of urea is enhanced by the use of chemical urease inhibitors, zero-tillage practices, fertilization paired with irrigation or rainfall events, and the fertilization of cold or frozen fields with slow microbial metabolism (Swensen & Singh, 1997; Glibert *et al*., 2006). Given that urea pollution largely occurs in agricultural regions with P-saturated soils (Carpenter, 2005), and P-rich receiving waters (Downing & McCauley, 1992; Donald *et al*., in press), export of terrestrial urea to aquatic ecosystems may be expected to dramatically enhance eutrophication (Finlay *et al*., 2010a). However, biological (Satoh, 1980; Berman *et al*., 1999) and abiotic (Bushaw *et al*., 1996; Bronk *et al*., 2010) production of urea in lakes, combined with rapid planktonic uptake of urea (Mitamura & Saijo, 1986a; Park *et al*., 1997; Solomon *et al*., 2010) have made it difficult to clearly quantify the relative importance of human activity on land and in-situ cycling as controls of ambient urea concentrations in lakes. Consequently, detailed surveys of spatial and temporal urea concentrations in surface waters are needed to elucidate both the internal and external controls on aquatic urea biogeochemistry.

Urea pollution in eutrophic waters may have differential effects on phytoplankton and bacteria, and consequently net ecosystem metabolism, measured as the balance between autotrophic (algal) and heterotrophic (bacterial) community metabolism. In most ecosystems, phytoplankton, rather than bacteria consume the majority of dissolved urea (Glibert *et al*., 2006; Bronk *et al*., 2007) for the synthesis of N-rich biomolecules (Fig. 1 in Finlay *et al*., [2010a]). However, phytoplankton growth may be limited by light availability in eutrophic ecosystems (Park *et al*., 1993; Mallin *et al*., 1999; Roberts & Howarth 2008; Finlay *et al*., 2010a) favoring light-independent bacterial urea uptake (Mitamura *et al*., 1994; Park *et al*., 1997; Finlay *et al*., 2010a). Although the degree of net ecosystem autotrophy generally increases with trophic status (Cottner & Biddanda, 2002; Chróst & Siuda, 2006), excessive pollution with an N-rich, labile organic compound such as urea can potentially enhance bacterial metabolism and net heterotrophy under conditions of heavy organic pollution (e.g., waste waters) (Cloern & Oremland, 1983; Burkholder *et al*., 1997; Prairie *et al*., 2002). Therefore, experiments are needed to identify the conditions under which urea pollution favors phytoplankton and bacterial production, and to identify the implications for net planktonic metabolism.

1.2 Thesis objectives

This study aimed to identify the distribution and effects of anthropogenic urea loading on lakes of the North American Great Plains using a combination of lake surveys and in situ experimentation. Chapter 2 presents results from lake surveys in 2008 and 2009 which quantified the effects of seasonality, land use practices (urban and

agricultural), and environmental conditions (bacterial and algal biomass, nutrient and salt concentrations, temperature, morphometry, etc.) on the spatial and temporal distribution of urea in lakes. Chapter 3 presents the findings from a 2009 mesocosm experiment that builds on previous research (Finlay *et al*., 2010a; Donald *et al*., in press). In particular, the effects of increased urea loading on auto- and heterotrophic communities, and implications for net ecosystem metabolism were quantified. Finally, Chapter 4 combines the findings from previous chapters in a synthesis representing major research findings, discusses implications for the management of aquatic resources, and identifies areas requiring further investigation.

2. DISTRIBUTION AND REGULATION OF UREA CONTENT IN LAKES OF CENTRAL NORTH AMERICA

2.1 Introduction

Urea ($[NH_2]_2CO$) accounts for ~50% of global N fertilizer (Glibert *et al*., 2006) and its use is expected to double by 2050 (Millennium Ecosystem Assessment, 2005); however, little is known of the mechanisms regulating its transfer to, and abundance in, freshwaters (Solomon *et al*., 2010). Together, catchment scale mass-balance studies (Leavitt *et al*., 2006; Bunting *et al*., 2007), whole-ecosystem fertilization experiments (Barcia *et al*. 1980; but see Lathrop 1988), and large-scale mesocosm experiments (Finlay *et al*., 2010a) all demonstrate that pollution of phosphorus (P) -rich lakes (>50 μg dissolved P L^{-1}) with N can promote growth of toxic cyanobacteria (*Microcystis*, *Planktothrix*) at the expense of N_2 fixing taxa (*Anabaena*, *Aphanizomenon*), particularly in regions where soils are saturated with phosphorus (P) and lakes accumulate dissolved P (Bennett *et al*., 2001; Carpenter, 2005). Although these patterns suggest that urea from terrestrial sources may be a potent mechanism regulating water quality, little is known of the controls of spatial and temporal variation of urea concentrations in lakes (Solomon, *et al*., 2010), or whether urea from diffuse or point sources is transmitted to lakes prior to decomposition (Siuda & Chróst, 2006).

Anthropogenically-derived urea may enter surface waters through a number of hydrologic pathways. Under conditions of low lotic discharge, ~5% of urea fertilizer is lost in runoff (Glibert *et al*., 2006), 30-50% is incorporated into crop biomass (Tilman *et*

al., 2002), and the remainder is hydrolyzed to NH_4^+ in soils by the microbial enzyme urease (Glibert*, et al*., 2006) before additional transformation or loss by volatilization, nitrification, or denitrification (Swensen & Singh, 1997; Swensen & Bakken, 1998; Silva *et al*., 2005; Di & Cameron, 2008). In contrast, up to 40% of urea may be exported to groundwater or lotic ecosystems if fertilizer is applied in association with cold temperatures, chemical inhibitors of urease, irrigation, or rainfall (Swensen & Singh, 1997; Glibert *et al*., 2006). In addition, livestock and human wastes may be rich in urea (Mitamura *et al*., 1994; Withers, 1998; Petersen *et al*., 2004), particularly if it is not subject to microbial processing (Mitamura *et al*., 1994; Tilman *et al*., 2001). While urea may also enter lakes via dry or wet deposition, atmospheric sources are thought to be insignificant relative to those controlled by hydrologic runoff (Timperley *et al*., 1985; Cornell *et al*., 1998). However, despite the observation that farming and urbanization are increasing the concentration and bioavailability of dissolved organic N (DON) in rivers (Wiegner *et al*., 2006; Stanley & Maxted, 2008), little is known of whether these patterns record changes in urea content or whether riverine urea accumulates in downstream lakes.

Urea may accumulate in the water column of lakes from internal sources which vary in importance with lake morphometry, food-web structure, and the relative intensity of metabolic and catabolic processes (Solomon *et al*., 2010). Limited prior research suggests that pelagic concentrations of urea are often low (14-56 μg N L^{-1}) and that pelagic regeneration and cellular uptake rates are balanced during summer (range 0.03-4.06 μg N L^{-1} h^{-1}) (Mitamura & Saijo, 1986b; L'Helguen *et al*., 2005). However, eutrophication may both elevate standing stocks to 700 μg N L^{-1} (Siuda & Chróst, 2006)

and selectively stimulate microbial consumption (Park *et al*., 1997; Jorgensen, 2006), including that by cyanobacteria (Berman & Chava, 1999; Finlay *et al*., 2010). In general, phytoplankton consume urea (Mitamura & Saijo, 1986a; Gu *et al*., 1997; Bronk *et al*., 2007), while planktonic bacteria either release urea during decomposition of DON or particulate organic N (PON) (Satoh, 1980; Mitamura & Saijo, 1986a; Berman *et al*., 1999), or acquire urea at <10% the rate of phytoplankton (Solomon *et al*., 2010). In contrast, urea in lake water can be assimilated rapidly by epiphytic algae (Mitamura *et al*., 2010) and benthic biofilms (Thorén, 2007), while zooplankton can account for >50% of pelagic regeneration (Conover & Gustavson, 1999; Lomas *et al*., 2002), particularly when seasonal temperatures are elevated (L'Helguen *et al*., 2005) and invertebrates are N sufficient (Miller & Glibert, 1999). Finally, morphometric and limnological properties can interact to influence urea cycling, because temperature regulates urease activity (Siuda & Chrost, 2006), anoxia reduces both microbial uptake and enzyme-catalyzed decomposition to NH_4^+ (Satoh, 1980; Therkildsen & Lomstein, 1994; Lomas *et al*., 2002), and irradiance may produce urea from DON (Bushaw *et al*., 1996). While several controls of urea biogeochemistry have been studied in isolation, little is known of how these processes might interact to regulate the spatial and temporal variation in water-column concentrations of urea.

In this study I quantified the relationships between urea concentration, limnological conditions, and basin characteristics in seven productive lakes subject to agricultural and urban sources of urea to identify factors regulating spatial and temporal variability of pelagic urea concentrations. My objectives were to: 1) quantify the concentration of urea and its contribution to the dissolved N pool along a landscape

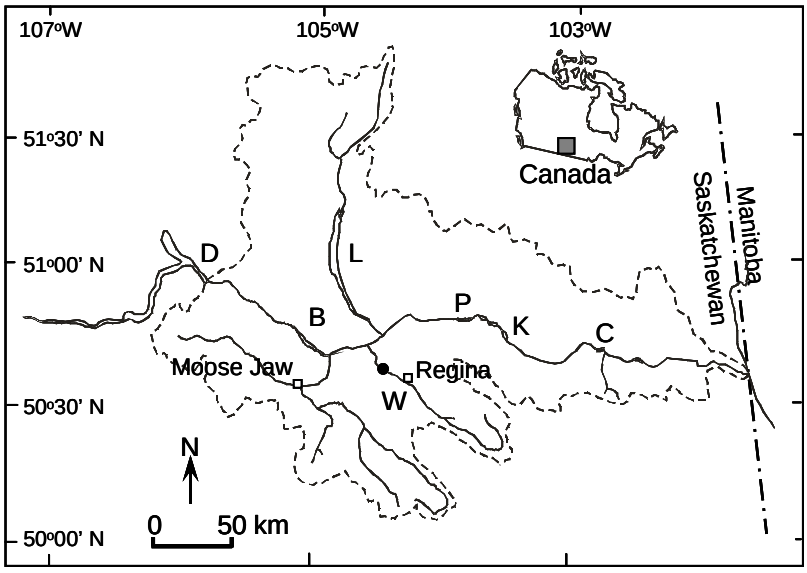
gradient of lake production; 2) identify the mechanisms regulating temporal and spatial variation in urea concentrations, and; 3) evaluate the effect of urban pollution on urea concentrations in lakes. Together these findings allowed development of a new conceptual model for the role of external and in situ processes in regulating urea abundance in lakes. Improved understanding of urea biogeochemistry is essential to mediate potential water quality degradation (Finlay *et al*., 2010a) arising from the two-fold increase in urea applications expected by 2050 (Millennium Ecosystem Assessment, 2005).

2.2 Methods

2.2.1 Study area

Study lakes are located in the Qu'Appelle River catchment, a 52,000 km^2 watershed that spans ~400 km from western headwaters to the confluence of Qu'Appelle and Assiniboine rivers in southern Saskatchewan, Canada (Fig. 2.1). The seven lakes include mesotrophic (Diefenbaker), eutrophic (Buffalo Pound) and hyper-eutrophic reservoirs (Wascana), sub-saline Last Mountain Lake, and a series of highly eutrophic downstream sites (Pasqua, Katepwa, Crooked). Lake Diefenbaker and Wascana Lake reservoirs were formed by impoundment in 1968 and 1883 respectively, while inflow to natural Buffalo Pound Lake has been supplemented with water from the South Saskatchewan River or Lake Diefenbaker since 1958 (Hall *et al*., 1999; McGowan *et al*., 2005). Over 75% of the catchment consists of agricultural land use (crops, forage,

Figure 2.1. Map of the Qu'Appelle River drainage basin, Saskatchewan, Canada. The watershed boundary is delimited by the dashed line, and lakes, from upstream to downstream, include Diefenbaker (D), Buffalo Pound (B), Last Mountain (L), Wascana (W), Pasqua (P), Katepwa (K), and Crooked (C). Open squares represent the cities of Regina and Moose Jaw. Wastewater from Regina is treated at and released from the sewage treatment plant, shown as a black circle downstream from Wascana Lake.



livestock), while the cities of Regina and Moose Jaw are the main sources of urban effluent (Hall *et al*., 1999; Leavitt *et al*., 2006). The regional climate is subhumid continental with long cold winters (mean -16^{o}C in January) and short warm summers (mean 19^{o}C in July) in which mean annual evaporation (~600 mm) exceeds precipitation by two-fold.

Study lakes have been monitored May-Sept since 1994, with the exception of Wascana (1996) and Pasqua lakes (1999), as part of the Qu'Appelle Valley Long Term Ecological Research (LTER) program (Hall *et al*., 1999; Leavitt *et al*., 2006; Finlay *et al*., 2009). Mean limnological conditions during summer vary by two- to 10-fold among sites (Table 2.1), including total dissolved P (TDP) (20 - 240 μg P L^{-1}), total dissolved N (TDN) (325 - 1350 μg N L^{-1}), dissolved organic C (DOC) (5.6 - 16.0 mg C L^{-1}), dissolved inorganic C (DIC) (30 - 60 mg C L^{-1}), and conductivity (420 - 1795 μS cm^{-1}). Lakes have elevated mean summer pH (~8.8) (Finlay *et al*., 2009), but exhibit declining mass ratios of dissolved N : P (from >20:1 to <6:1) and increasing algal abundance (from 5 to 30 μg Chl L^{-1}) along a gradient from headwater to downstream sites. In contrast, mid-reach Pasqua Lake is hypereutrophic due to direct influx of urban effluent from the City of Regina (Hall *et* al., 1999; Leavitt *et al*., 2006). Mean annual water residence time is low in all lakes (0.05-1.3 yr) except Last Mountain Lake (~12 yr), and all sites are polymictic except for occasionally dimictic Katepwa Lake. Despite polymixis, the most productive lakes exhibit seasonal anoxia in deepwaters during summer and under ice, while those in Diefenbaker, Buffalo Pound, and Last Mountain lakes are less commonly anoxic (Patoine *et al*., 2006).

Table 2.1. Morphometric, chemical, and biological characteristics of Qu'Appelle Valley lakes. Data are mean values (± SD, in parentheses) of measurements taken from May – Aug, 1994 – 2007, inclusive, except for Pasqua Lake, where data represent May – Aug, 2004 - 2007. Abbreviations represent total dissolved- and soluble reactive phosphorus (TDP, SRP), total dissolved nitrogen (TDN), dissolved organic and inorganic carbon (DOC, DIC), and chlorophyll *a* (Chl *a*). Lakes are arranged in descending order from headwater to downstream sites, with Last Mountain and Wascana lakes occupying tributary positions upstream of Pasqua Lake (Fig. 2.1).

Lake	Area (km^2)	Volume (m^3 10^{-6})	Water residence (yr)	Mean depth (m)	TDP (μg L^{-1})	SRP (μg L^{-1})	Urea (μg L^{-1})	TDN (μg L^{-1})	DOC (mg L^{-1})	DIC (mg L^{-1})	Conductivity (μS cm^{-1})	Secchi (m)	Chl *a* (μg L^{-1})
Diefenbaker	500.0	9400.0	1.3	33.0	19.9 (40.1)	16.6 (36.0)	28.7 (14.0)	325.2 (135.2)	5.6 (5.0)	33.8 (7.2)	419.9 (341.2)	3.3 (1.3)	4.8 (4.3)
Buffalo Pound	29.1	87.5	0.7	3.0	26.7 (21.7)	50.5 (250.3)	31.8 (15.9)	493.4 (216.5)	6.5 (3.9)	30.9 (9.7)	528.1 (513.7)	1.2 (1.0)	28.3 (39.7)
Last Mountain	226.6	1807.2	12.6	7.9	46.1 (65.3)	30.96 (73.4)	41.3 (24.9)	948.27 (195.6)	12.8 (5.8)	60.4 (11.6)	1794.5 (325.0)	2.2 (0.7)	12.3 (10.2)
Wascana	0.5	0.7	0.05	1.5	242.1 (108.2)	201.4 (168.7)	49.2 (38.5)	1353.8 (766.5)	16.0 (4.3)	40.5 (14.2)	917.7 (414.6)	0.8 (0.5)	35.1 (50.7)
Pasqua	20.2	120.8	0.7	5.8	123.8 (64.7)	105.0 (65.7)	131.7 (64.9)	1129.1 (406.4)	12.27 (1.7)	45.69 (3.1)	1240.68 (316.9)	1.44 (1.1)	27.71 (20.7)
Katepwa	16.2	233.2	1.3	14.3	155.6 (80.4)	112.1 (96.8)	60.5 (63.9)	943.6 (229.2)	11.3 (2.9)	50.6 (10.2)	1096.5 (234.6)	1.8 (0.9)	22.5 (16.2)
Crooked	15.0	120.9	0.5	8.1	128.9 (103.2)	110.6 (143.5)	48.4 (46.5)	874.9 (237.3)	11.0 (3.7)	51.5 (10.0)	1185.7 (279.6)	1.4 (0.7)	27.4 (31.7)

Detailed mass balance budgets constructed for total N (TN) at each site reveal substantial differences in the relative importance of allochthonous and autochthonous N sources for Qu'Appelle Lakes (Leavitt *et al*., 2006; Patoine *et al*., 2006). In general, mean N inputs to lakes receiving only agricultural runoff are regulated by a combination of internal recycling of N (53% of TN influx) and new N influx from rivers (23.5%), while inflow N is retained in sediments (71% of influx) rather than exported in rivers (28.7%). In contrast, N from the City of Regina accounts for 45% of summer N influx to Pasqua Lake, and >70% of total ecosystem N, although sediments still sequester >40% of N influx (Leavitt *et al*., 2006). Given the importance of DON and sediments to N cycling in all lakes (Patoine *et al*., 2006; Leavitt *et al*., 2006), I expected that urea biogeochemistry may also be influenced by similar in-lake processes.

2.2.2 Field methods

Qu'Appelle lakes were sampled biweekly during May - August during both 2008 and 2009 using standard LTER protocols (Patoine *et* al., 2006; Leavitt *et al*., 2006). Water for depth integrated samples was collected using a 6-L Van Dorn bottle deployed at 1-m intervals, pooled, screened through 243-μm mesh netting, and transported to the laboratory within 2 h. Water transparency was estimated using a 20-cm diameter Secchi disk, whereas in situ temperature (^{o}C), dissolved O_2 (mg O_2 L^{-1}), conductivity (μS cm^{-1}) and salinity (g total dissolved solids [TDS] L^{-1}) were measured at 1-m intervals using a Yellow Springs Instruments model YSI 85 meter. Surface pH was measured using a calibrated handheld pH meter (pH Testr 10, Oakton). Zooplankton was collected from

maximum depth to the surface using 20 cm diameter (243-μm mesh) and 50-cm diameter (750-μm mesh) conical nets. All organisms were preserved with a sugar-saturated ethanol solution (Patoine *et al*., 2006).

2.2.3 Laboratory analyses

Water chemistry was quantified using depth integrated samples that were filtered through 0.45-μm nominal pore GF/F filters. Nitrate (NO_3^-) and nitrite (NO_2^-) (together reported as NO_3^- hereafter), ammonia/ammonium (NH_4^+ hereafter), total Kjeldahl nitrogen (TKN = organic N + NH_4^+) (all as μg N L^{-1}), soluble reactive phosphorus (SRP, as orthophosphate) and total dissolved phosphorus (TDP) (both as μg P L^{-1}) were determined following standard methods at the University of Alberta Water Chemistry Laboratory (Stainton *et al*., 1977). A value of 50% of the limit of detection (LOD) was substituted for missing estimates of NH_4^+ (LOD = 1.0 μg N L^{-1}), NO_3^- (0.5 μg N L^{-1}), and SRP (1.0 μg P L^{-1}). Analysis of DIC and DOC concentrations followed standard Environment Canada procedures using a Shimadzu model 5000A total carbon analyzer (Environment Canada, 1979). Dissolved urea concentrations (LOD 0.35 μg N L^{-1}) were measured using a slightly modified version of the diacetyl-monoxime method of Revilla *et al.* (2005).

Algal abundance was estimated by quantifying biomarker pigments using standard trichromatic assays of Chl *a* (Jeffrey & Humphrey, 1975) and high performance liquid chromatographic (HPLC) analysis of carotenoids and Chlorophylls from phototrophs (Leavitt & Hodgson, 2001). Pigments in particulate organic matter (POM)

on filters were extracted using pure acetone for trichromatic assays and a mixture of acetone and methanol (80 : 15, by volume) for HPLC analyses. Pigments were isolated and quantified using a Hewlett Packard model 1100 HPLC system fitted with photo-diode array and fluorescence detectors and calibrated with authentic standards (Leavitt & Hodgson, 2001). Pigments quantified included β-carotene (derived from all algae), fucoxanthin (mainly siliceous algae), diatoxanthin (mainly diatoms), alloxanthin (cryptophytes), Chl *b* (chlorophytes), myxoxanthophyll (colonial cyanobacteria), and aphanizophyll (N_2-fixing cyanobacteria). Trichromatic chlorophyll estimates were expressed as μg Chl *a* L^{-1}, whereas HPLC estimates of other pigments were expressed as nmol pigment L^{-1}, consistent with prior studies (Patoine et al., 2006; Leavitt *et al.*, 2006; Finlay *et al*. 2010a).

2.2.4 Data analysis

Principal components analysis (PCA) was used to summarize the statistical relationship among parameters and identify the best predictors of urea concentration. Environmental variables included major algal groups, zooplankton genera, limnological parameters, lake morphometry (latitude, longitude, depth, area), and hydrology (water residence time, effective drainage area). PCAs were conducted independently using both summer mean values and fully-resolved biweekly data, although there was little difference between analyses. In all analyses, variables were checked for normal distribution and data were normalized using $\log_{10}(x+1)$ transformations where necessary. In addition, a combination of least-squares linear regression and Pearson correlation

analyses were used to quantify the relationship between urea and individual lake parameters, both within years (biweekly data resolution) and at the landscape level (mean of summer), while analysis of variance (ANOVA) was used to compare mean urea concentrations among lakes and years. All statistical analyses were performed using SYSTAT version 10.0.

Nitrogen composition in Qu'Appelle lakes during 2008-2009 was compared to that observed in previously-published surveys of water chemistry in 69 lakes with endorheic (hydrologically-closed) basins (Pham et al. 2009) and decade-long mass-balance analysis of Qu'Appelle lakes (Patoine *et al*., 2006; Leavitt *et al*., 2006) to evaluate the importance of DON, and by inference unmeasured urea, in the N budget of diverse prairie lakes. Lakes within the 100,000 km^2 regional survey were sampled once during a two-week interval in 2004 (centred on day of year, DOY, 190) as described by Pham et al. (2009), whereas N budgets of Qu'Appelle lakes were based on the standard biweekly sampling regime since 1999 (Patoine *et* al., 2006; Leavitt *et al*., 2006). In addition, depth-integrated epilimnetic concentrations of urea were measured once during August 2008 in a subset of 19 closed basin lakes to evaluate inferences based on DON analyses. All chemical determinations in surveys were performed as described above.

2.3 Results

2.3.1 Landscape patterns of water chemistry

Seasonal patterns of water column urea concentrations were relatively invariant in six of seven survey lakes during May-Sept in both 2008 and 2009 (Fig. 2.2). On average, urea concentrations were higher during 2008 than in 2009 on almost every sampling date, a difference which was highly significant ($P = 0.001$) when biweekly time series were analyzed using ANOVA. Furthermore, mean summer concentrations of urea were correlated negatively with longitudinal position in the catchment ($r^2 = 0.66$, $P < 0.05$), and increased from headwater to downstream sites in lakes lacking direct urban pollution. In contrast, Pasqua Lake, the first site to receive urban wastewaters (Fig. 2.1), exhibited a unimodal pattern of urea concentration in both years, with elevated concentrations in during mid-summer (DOY 140-175). In addition, mean summer urea concentrations in Pasqua Lake (131.7 $\pm$ 64.9 μg L^{-1}) were an average of two-fold greater than those of other lakes in both years (Table 2.1).

The soluble N pool of five Qu'Appelle lakes was comprised mainly of non-urea DON, with lesser concentrations of NO_3^-, urea, and NH_4^+ (Fig. 2.3). In Pasqua Lake, and to a lesser extent Lake Diefenbaker, NO_3^- was the most abundant nitrogenous compound, followed by DON, urea, and NH_4^+. In general, there was little temporal variability in concentrations of any dissolved N species, with the exception of NO_3^-, which declined ~50% from spring maxima in Pasqua and Katepwa lakes in both years, and ~75% in Wascana Lake during 2009 (Fig. 2.3).

Urea formed a large proportion of bio-available N, defined as the sum of NO_3^-, NH_4^+ and urea, with mean ($\pm$ SD) values ranging from 10-50% (36.1 $\pm$ 17.4%) and 6-45% (31.8 $\pm$ 19.5%) in 2008 and 2009 respectively (Fig. 2.4). In contrast, urea

Figure 2.2. Biweekly urea concentrations recorded May to August 2008 and 2009 for seven Qu'Appelle lakes. Closed circles represent 2008, while open triangles represent 2009. Error bars represent ± 1 S.D, and $n = 3$. The lakes are arranged by flow pattern (see Fig. 2.1) from headwater (left) to downstream (right) with Wascana (W) and Last Mountain Lake (L) contributing water to mid-reach positions upstream of Pasqua Lake (P). Pasqua Lake is the first lake in the chain to receive the city of Regina's treated sewage effluent. Other lake abbreviations as in figure 2.1.

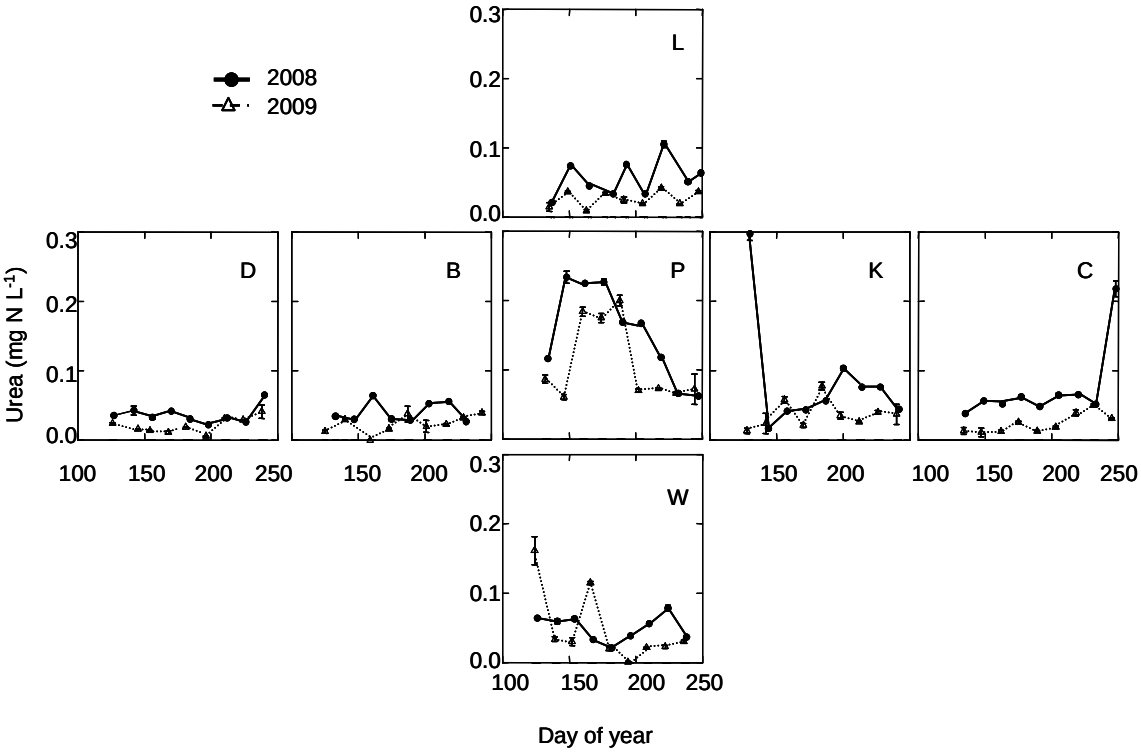
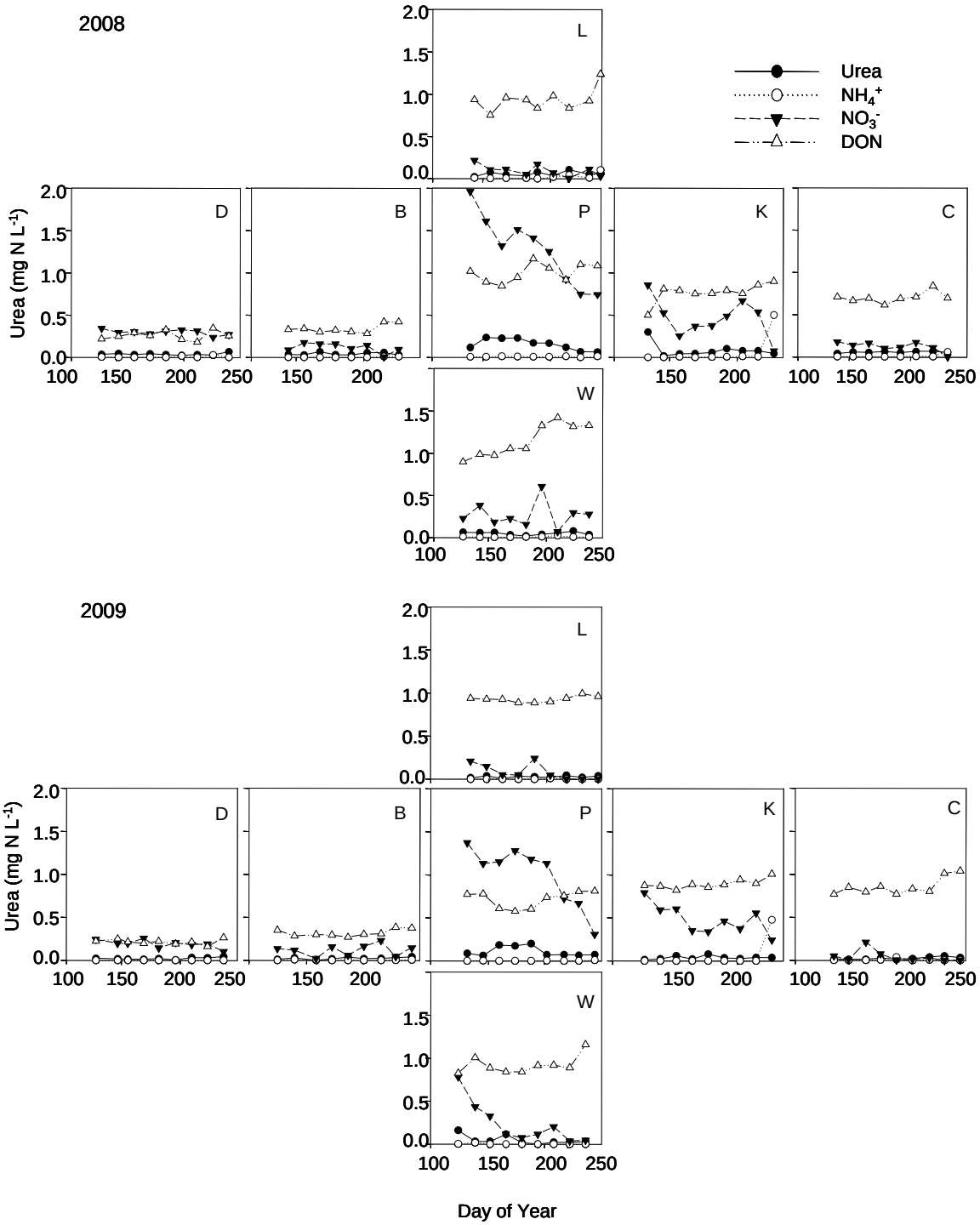


Figure 2.3. Biweekly concentrations of urea (black circles), NH_4^+ (open circles), NO_3^- (black triangles) and DON (open triangles) during May-August 2008 and 2009 in Qu'Appelle lakes. Abbreviations and lake arrangement as in Fig. 2.1.



constituted only 3-8% (6.1 ± 1.6%) and 1-6% (4.0 ± 1.4%) of the TDN pool in 2008 and 2009, respectively, due to elevated concentrations of non-urea DON. No spatial pattern was found for relative (%) contributions of urea to either bio-available or TDN pools, although the relative proportion of dissolved N as urea was consistent among years for each lake.

DON accounted for a high proportion of TDN in both annual mass-balances conducted on Qu'Appelle lakes since 1998 (Fig. 2.5a, b) and a spatial survey of 69 closed basin lakes in 2004 (Fig. 2.5c). Specifically, DON accounted for >75% of TDN in most Qu'Appelle lakes and years, with the exception of both mesotrophic Lake Diefenbaker and urban-impacted Pasqua Lake after ca. 2000. Similarly, DON always accounted for >50% of TDN in closed basin lakes, with particularly elevated mean (89.7 ± 10.0%) and median values (93.0%) observed in study lakes. Assuming that urea accounted for a similar proportion of TDN in the 2004 survey as in Qu'Appelle lakes (see above), urea should also be present at elevated concentrations (176 ± 132 μg N L^{-1}) in closed basin lakes. Consistent with this hypothesis, preliminary estimates of mean urea concentration in a subset of 19 lakes sampled once during 2008 revealed that urea was ubiquitous and abundant (81 ± 48 μg N L^{-1}), albeit at lower concentrations than predicted.

2.3.2 Correlates of urea concentration

Principal components analyses (PCAs) of mean summer limnological characteristics demonstrated that urea concentrations were correlated positively to

Figure 2.4. Water column urea concentrations in Qu'Appelle lakes expressed as relative (%) abundance of total dissolved N (TDN; black bars) and bio-available N (sum of NO_3^-, NH_4^+ and urea; white bars) in 2008 and 2009. Each bar represents the average from May-August, with error bars indicating $\pm$ 1 S.E, and $n = 3$. Lake abbreviations as in Fig. 2.1.

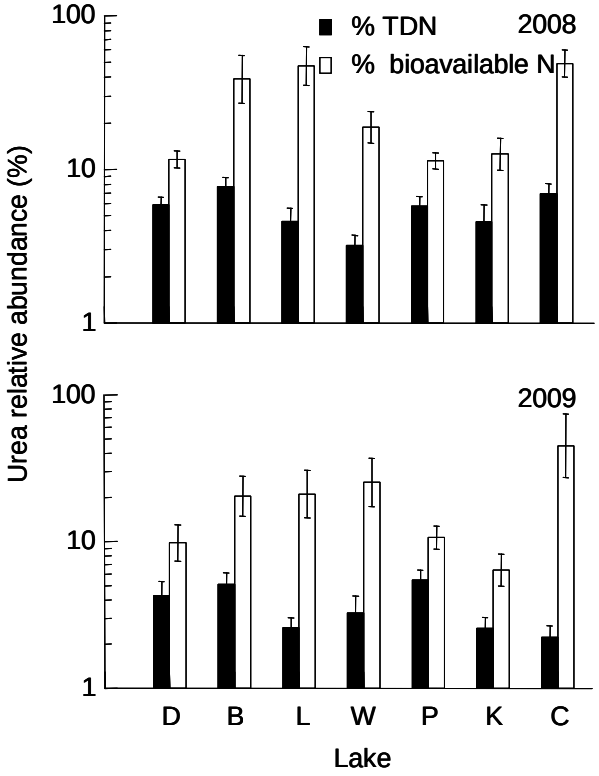
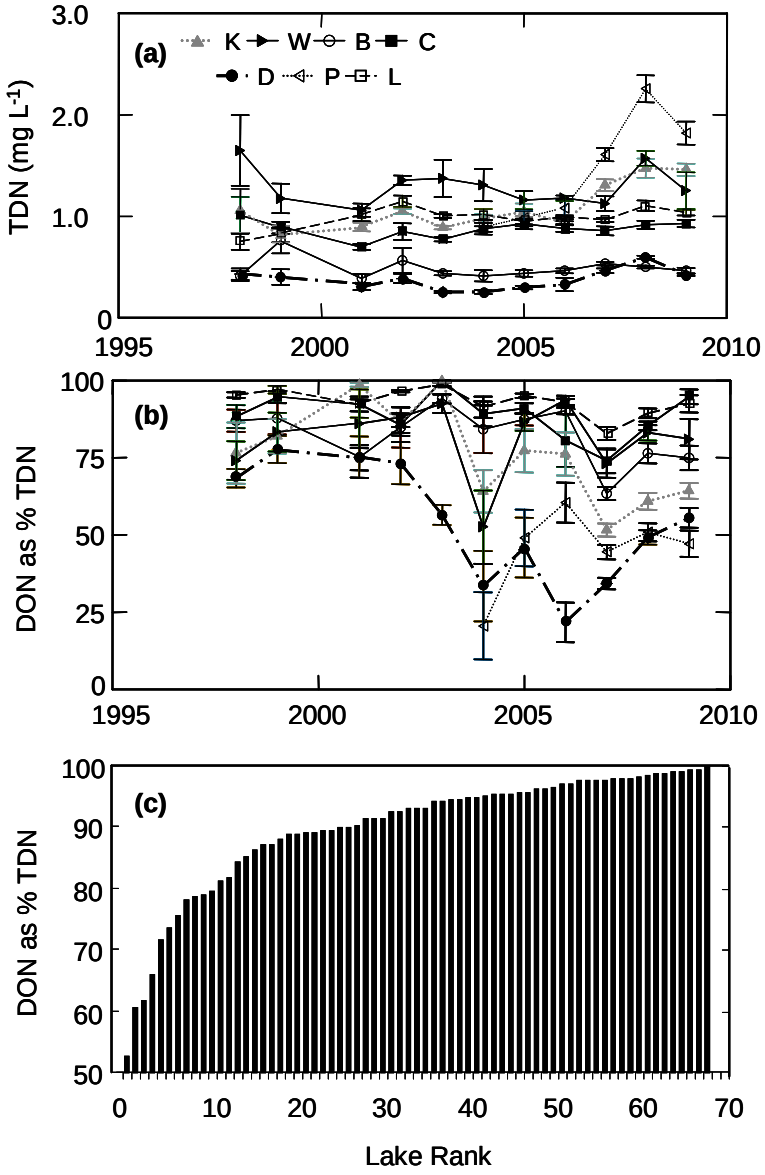


Figure 2.5. Dissolved nitrogen (N) composition of Qu'Appelle and closed basin lakes. (a) Total dissolved N concentration (TDN) as mg N L^{-1}, (b) percent of TDN as dissolved organic N (DON) in Qu'Appelle lakes, and (c) percent of TDN as dissolved organic N (DON) in 69 closed basin lakes in southern Saskatchewan (Fig. 2.1). Data points represent May – August mean values for Qu'Appelle lakes, and a single determination within closed basin lakes (Pham *et al*., 2009). Error bars $\pm$ 1 S.E. Lake abbreviations as in Fig. 2.1.

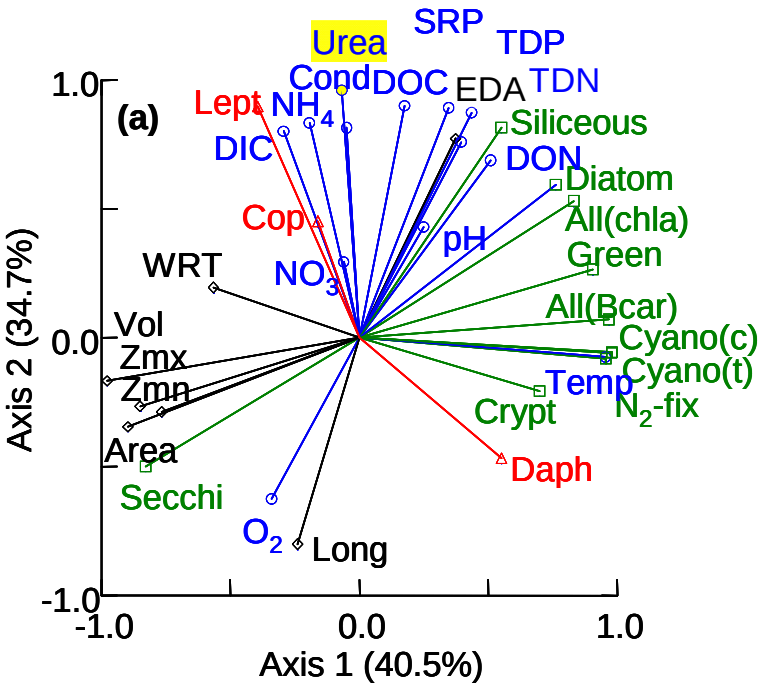


concentrations of many dissolved nutrients (N, P, C) and lake-specific effective drainage area, but were correlated inversely to oxygen content and the longitudinal position of lakes lacking urban inputs in both 2008 and 2009 (Fig. 2.6). In both years, PCA axis 1 and 2 together captured >72.5% of total variation in lake characteristics (Fig. 2.6). Although urea concentrations were correlated positively ($r = 0.62 – 0.99$, $P < 0.05$) with total and group specific algal abundance and negatively correlated with secchi transparency in 2009 (Fig. 2.6b), these relationships were weaker and usually not significant ($r = -0.19 – 0.78$) in 2008 (Fig. 2.5a). In both years, *Leptodora* and siliceous algae were the plankton correlated most strongly with urea concentrations; however, positive relationships were not significant ($P > 0.05$). Results of PCA were not altered significantly by inclusion of urban-polluted Pasqua Lake (data not presented). Furthermore, PCAs of data with biweekly resolution showed similar patterns to those of summer mean values, although total explained variance declined by 20-25% relative to analysis of summer means (data not presented).

2.3.3 Urban sources of urea

Urea was always present in effluent from the City of Regina's sewage treatment plant (STP) (Fig. 2.7), although concentrations in wastewaters were often less than those observed in both upstream Wascana and downstream Pasqua lakes (Fig. 2.2). In general, urea concentrations in the final effluent were greater in winter and spring than during summer. Comparison of urea concentrations at different stage of wastewater processing revealed no consistent difference between raw wastes, secondary-treated water, and

Figure 2.6. Principal components analyses (PCA) of mean lake characteristics from May – August for six agriculturally-impacted lakes (excluding Pasqua) during 2008 (a) and 2009 (b). Water chemistry variables are presented with blue arrows and circles, and include urea (highlighted yellow), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), dissolved oxygen (O_2), dissolved organic nitrogen (DON), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), ammonium (NH_4), nitrate (NO_3), temperature (Temp), conductivity (Cond), and pH. Algal parameters are indicated with green arrows and squares, and include Secchi depth (Secchi) and pigments representing total algae as Chlorophyll *a* (all [Chl]) and β-carotene (all [B-car]), mainly siliceous algae (Siliceous), mainly diatoms (Diatom), chlorophytes (Green), cryptophytes (Crypto), total cyanobacteria (Cyano [t]), colonial cyanobacteria (Cyano [c]), and N_2-fixing cyanobacteria (N_2-fix). Lake parameters are presented with black arrows and diamonds, and include water residence time (WRT), effective drainage area (EDA), mean depth (Zmn), maximum depth (Zmx), lake volume (Vol), lake area (Area), and longitudinal position (Long). Finally, zooplankton were quantified by functional group, presented with red arrows and triangles, and include *Daphnia* spp. (Daph), predaceous *Leptodora* (Lept), and copepods (Cop).


(a)
Urea
SRP
TDP
Cond
DOC
EDA
TDN
Lept
NH4
DIC
Siliceous
DON
Diatom
Cop
All(chla)
pH
Green
WRT
NO3
All(Bcar)
Vol
Cyano(c)
Zmx
Cyano(t)
Zmn
Temp
Crypt
N2-fix
Area
Daph
Secchi
O2
Long
1.0
0.0
-1.0
-1.0
0.0
1.0
Axis 2 (34.7%)
Axis 1 (40.5%)

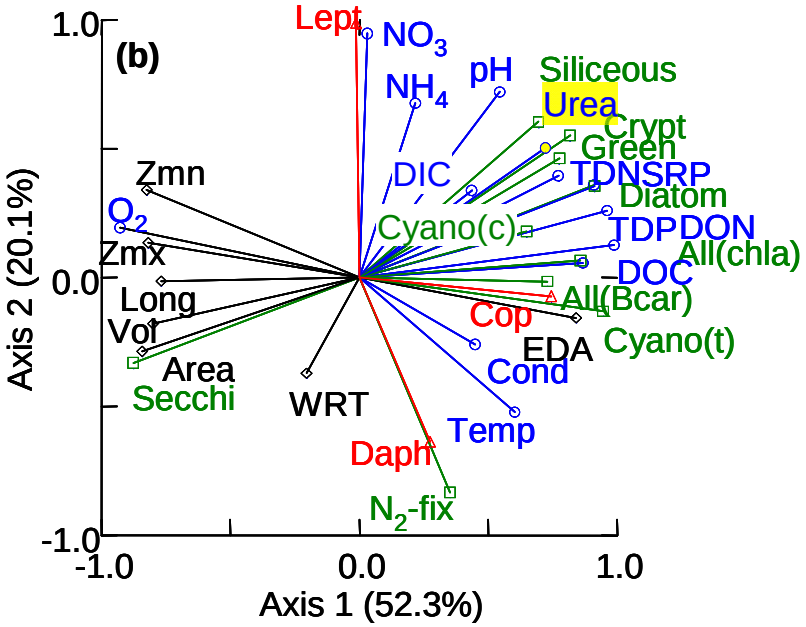
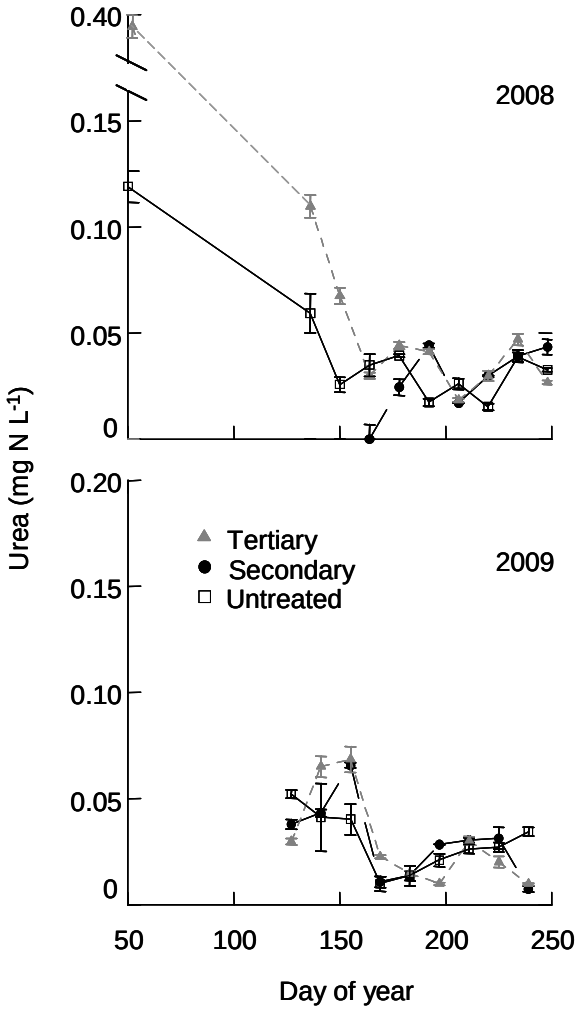

(b)
Lept
NO3
pH
Siliceous
NH4
Urea
Crypt
Green
Zmn
DIC
TDN
SRP
Diatom
O2
Cyano(c)
TDP
DON
Zmx
All(chla)
DOC
Long
All(Bcar)
Cop
Vol
EDA
Cyano(t)
Area
Cond
Secchi
WRT
Temp
Daph
N2-fix
1.0
0.0
-1.0
-1.0
0.0
1.0
Axis 2 (20.1%)
Axis 1 (52.3%)

Figure 2.7. Urea concentrations in effluent subject to processing by the City of Regina wastewater treatment plant. Urea was measured in untreated (open squares), secondary-treated (closed circles) and tertiary-treated (shaded triangles) effluent, from May - August in 2008 and 2009. Winter samples were also taken in 2008, but not 2009. Error bars represent $\pm$ 1 S.D and $n = 3$.



tertiary-treated effluent (Fig. 2.7). Instead, mean water column concentrations of urea were correlated positively ($r^2 = 0.67$, $P = 0.025$) with previous determinations of $\delta^{15}N$ values in surface sediments, a reliable marker of the proportion of N derived from urban waste waters in this catchment (Leavitt *et al*., 2006).

2.4 Discussion

Although urea accounts for ~50% of global N-based fertilizer applications, little is known of its basic biogeochemistry in freshwaters (Glibert *et al*., 2005; Solomon *et al*., 2010). Biweekly analysis of limnological variables in seven polymictic lakes during 2008 and 2009 revealed that urea accounted for ~35% of bio-available dissolved N (Fig. 2.4) and ~5% of TDN, despite 10-fold differences among lakes in algal production, hydrology, and degree of urban pollution (Table 2.1) (Hall *et al*., 1999; Leavitt *et al*., 2006; Patoine *et al*., 2006). Urea concentrations in lakes receiving N from agricultural sources were greatly elevated (Table 2.1) relative to values observed in other lake districts (Glibert *et al*., 2006; Solomon et al., 2010), were correlated with both longitudinal position ($r^2 = 0.66$, $P < 0.05$) and basin-specific catchment area (Fig. 2.6), and varied little through time during summer (Fig. 2.2). PCA also revealed that urea concentrations in agriculturally-influenced lakes were correlated positively with dissolved nutrient content (N, P, C), and inversely with O_2 concentrations, while both algal and invertebrate production and gross community composition were inconsistently correlated with urea abundance (Fig. 2.6). Interestingly, although urea was always present in urban effluent (Fig. 2.7), and water-column levels were correlated strongly (r^2

= 0.67, P <0.05) with stable isotope metrics ($\delta^{15}N$) of urban N pollution (Leavitt *et al*., 2006), concentrations of urea in Qu'Appelle lakes (Fig. 2.2) were usually greater than those observed in urban effluent. Together, these patterns suggest that urea concentrations are regulated by a complex interaction between organic and inorganic N influx in rivers (Stanley & Maxted, 2008), regeneration of dissolved N from sediments (Patoine *et al*. 2006, Leavitt *et al*. 2006), and the balance of urea production and consumption in the water column (Mitamura & Saijo, 1986a,b; L'Helguen *et al*., 2005). Better understanding of the mechanisms regulating urea availability and transformation is essential given its ubiquitous presence in lakes (Figs. 2.2, 2.5c), an anticipated two-fold increase in its use as fertilizer worldwide (Millennium Ecosystem Assessment, 2005), and its potential role in promoting toxic cyanobacteria in P-rich lakes (Finlay *et al*. 2010a).

2.4.1 Importance of urea in productive freshwaters

Urea concentrations in Qu'Appelle lakes (up to 132 $\pm$ 65 μg N L^{-1}) (Table 2.1) were greater than values recorded in unproductive lentic systems (Solomon *et al*., 2010), diverse rivers (2-28 μg N L^{-1}) (Glibert *et al*., 2005; Wiegner *et al*., 2006), and open oceans (4.2–9.8 μg N L^{-1}) (Painter *et al*., 2008), but were consistent with limited prior research on eutrophic lakes (Siuda & Chróst, 2006; Park *et al*., 1997) and anthropogenically-impacted coastal regions (0.1–338 μg N L^{-1}) (Glibert *et al*., 2006). Further, it is inferred here that urea is both ubiquitous and abundant in other lakes of the Northern Great Plains, given that urea accounted for ~5% of DON in Qu'Appelle sites

(Fig. 2.4), DON accounted for >90% of TDN in regional closed basin lakes (Fig. 2.6c), and urea concentrations were elevated in a subset of closed basin lakes (81 $\pm$ 48 μg N L^{-1}). Predominance of dissolved N pools by DON has been reported for diverse ecosystems including temperate rivers (Wiegner *et al.*, 2006; Lutz *et al*., 2011), boreal lakes (Bunting *et al*., 2010), and lotic ecosystems in central North America (Stanley & Maxted, 2008); however, to date few studies have quantified the importance of urea to TDN pools (Solomon *et al*., 2010) and no study has demonstrated that urea is both ubiquitous and abundant in hydrologically diverse lake ecosystems.

Urea accounted for up to ~50% of bio-available dissolved N (as sum of NH_4^+, NO_3^-, and urea) in Qu'Appelle lakes in both years (Fig. 2.4). These values are consistent with the range of proportions (2-42%) observed in nine rivers of the eastern USA (Wiegner *et al*., 2006) and lotic waters downstream of effluent discharge sites (up to 31%) (Mitamura *et al*., 1994). Analysis of rivers in the United States (Stanley & Maxted, 2008; Wiegner *et al*., 2006) suggest that DON composition varies along landscape gradients from forested to human-impacted regions, with recalcitrant wetland-derived DON being augmented or replaced by anthropogenic N which is more labile due to reduced microbial processing during terrestrial export (Seitzinger *et al*., 2002; Wiegner & Seitzinger, 2004) or incomplete N mineralization during wastewater treatment (Glibert *et al*., 2006). In addition, the relative importance of urea to biologically-active N pools may be underestimated in most limnological monitoring studies because urea is converted rapidly (days-weeks) to NO_3^- during storage if aqueous samples are not immediately frozen (Finlay *et al*., 2010a; M. J. Bogard et al. unpublished data). Based on these findings, the ease of urea analysis (Revilla *et al*., 2005), and the stimulatory effect of urea

on the growth and toxicity of planktonic cyanobacteria (Berman & Chava, 1999; Finlay *et al*., 2010a), future limnological studies should include urea determinations.

2.4.2 Effects of land use on urea concentrations

Several lines of evidence suggest that agricultural sources may have contributed significantly to the urea content in lakes of the Northern Great Plains. First, urea use is pervasive on regional lands where >75% of the 52,000 km^2 Qu'Appelle catchment is dedicated to crop and livestock production (Hall *et al*., 1999). Within this region, urea accounts for >50% of total N fertilizer application, a value equivalent to 70% of all urea application in Canada (Glibert *et al*., 2006). Historically, fertilizers are applied in spring, when snowmelt and elevated soil moisture increase hydrologic runoff to annual maxima (Pham *et al*., 2009), and relatively cool temperatures reduce both microbial uptake of urea and extracellular urease activity (Swensen & Singh, 1997; Solomon *et al*., 2010). Elsewhere under similar conditions, up to 40% of applied urea can be exported undegraded from farms to aquatic ecosystems (Thorén *et al*., 2003; Sidua & Chróst, 2006; Glibert *et al*., 2006). Second, mean summer concentrations of urea increased with lake position in the landscape ($r^2 = 0.66$, $P < 0.05$) and with lake-specific estimates of effective drainage area (Fig. 2.6). Solute concentrations commonly increase with distance downstream in diverse lake chains throughout North America (Soranno *et al*.,1999), reflecting increasing ratios of catchment to lake surface area and, consequently, areal nutrient influx. Third, comparison of historical records of climate, resource use, and urbanization with highly resolved paleolimnological time series from the Qu'Appelle

lakes reveal that N from agriculture and livestock sources is correlated with changes in plankton production and community composition since 1900 (Hall *et al*., 1999; Leavitt *et al*., 2006). Livestock wastes, particularly urine, are rich in urea (Withers 1998; Petersen *et a*l., 2004) and can be lost rapidly to aquatic ecosystems during periods of high hydrologic runoff (Tilman *et al*., 2001).

Despite evidence that urea in lakes is derived in part from agricultural sources, temporal patterns of urea abundance during summer (Fig. 2.2) and results of the PCA (Fig. 2.6) were not congruent with the hypothesis the urea content of lakes is regulated mainly by direct hydrologic influx. For example, urea concentrations were not consistently correlated with mean water retention times (Fig. 2.6), an index of river discharge in this region (Vogt *et al*., 2011). Further, because >80% of hydrologic runoff occurs during spring (Pham et al. 2009), it was anticipated that urea abundance would be consistently elevated (e.g., Katepwa Lake 2008) or depressed (Katepwa Lake 2009) during vernal sampling of all lakes, depending on the relative influx of urea and water. Instead, concentrations of urea (Fig. 2.2) and most other N solutes (Fig. 2.3) revealed no common pattern among lakes or years. While it is possible that elevated water discharge perfectly offset increases in urea influx, the findings are more consistent with previous decade-long mass-balance budgets which reveal that river influx accounts for less than 25% of total N inputs to most Qu'Appelle Lakes (Patoine *et al*., 2006; Leavitt *et al*., 2006), and that recycling and transformation of N within lakes may be the paramount controls of dissolved N levels in these ecosystems.

2.4.3 Effects of urban effluent on urea concentrations

Decade-long mass balances demonstrate that nitrogenous wastes from the City of Regina account for 45% of N influx to downstream Pasqua Lake during summer and over 70% of total N in the lake (Leavitt *et al*., 2006), leading to loss of benthic fauna (Quinlan *et al*., 2002) and outbreaks of cyanobacteria (Hall *et al*., 1999; Finlay *et al*., 2009) which increase algal toxins to 10-fold above World Health Organization maxima (Finlay *et al*., 2010a; D.B. Donald *et al*., unpublished data). Further, mean water column concentrations of urea were correlated positively ($r^2 = 0.67$, $P = 0.025$) with the magnitude of N pollution from urban sources, as estimated by stable isotope analysis of surface sediments in Qu'Appelle lakes (Leavitt *et al*., 2006). Despite these patterns, it is inferred that urban effluent is a substantial source of undegraded urea to Pasqua Lake mainly during winter because urea concentrations in final effluent (Fig. 2.7) were 3-to10-fold less than those in Pasqua Lake (Fig. 2.2) during the summer, whereas winter concentrations of urea in effluent exceeded those in Qu'Appelle lakes. Enhanced importance of urea influx during winter is consistent with observations that both microbial uptake and extracellular urease activity are depressed by cold temperatures (Siuda & Chróst, 2006), and that winter flow in Wascana Creek is composed mainly of urban wastewaters (Waiser *et al*., 2011). However, better information is required to test this hypothesis and quantify the proportion of wastewater urea in lakes. Specifically, future studies will require seasonal estimates of the rates of in situ production, degradation, transformation, and of transportation in lotic ecosystems connecting cities and lakes.

Unexpectedly, urea concentrations were not affected by the form of wastewater treatment (untreated, secondary, tertiary) (Fig. 2.7), despite the observation that Regina uses microbial processing in aerated lagoons to remove DOM (City of Regina, http://www.regina.ca/Page438.aspx). In fact, urea concentrations in final effluent during spring were often greater than those observed earlier in the wastewater treatment process (Fig. 2.7), suggesting that despite substantial reductions in total DOM content, internal factors may have regenerated urea later in the effluent processing process. Although speculative, high concentrations of purine-rich DON typical of urban effluent (Berman *et al*., 1999; Bronk *et al*., 2010) may favour production of urea through microbial processes (Solomon *et al*., 2010) and exposure to UV radiation during final sterilization of wastewater (Bushaw *et al*., 1996). Consistent with this view, independent analysis of dissolved N content in Wascana Creek immediately downstream of effluent outfall during 2005-2007 (Waiser *et al*., 2011) reveals greatly elevated concentrations of TDN (~25 mg N L^{-1}) relative to those seen in all Qu'Appelle lakes (<3 mg N L^{-1}) (Fig. 2.3, 2.5a).

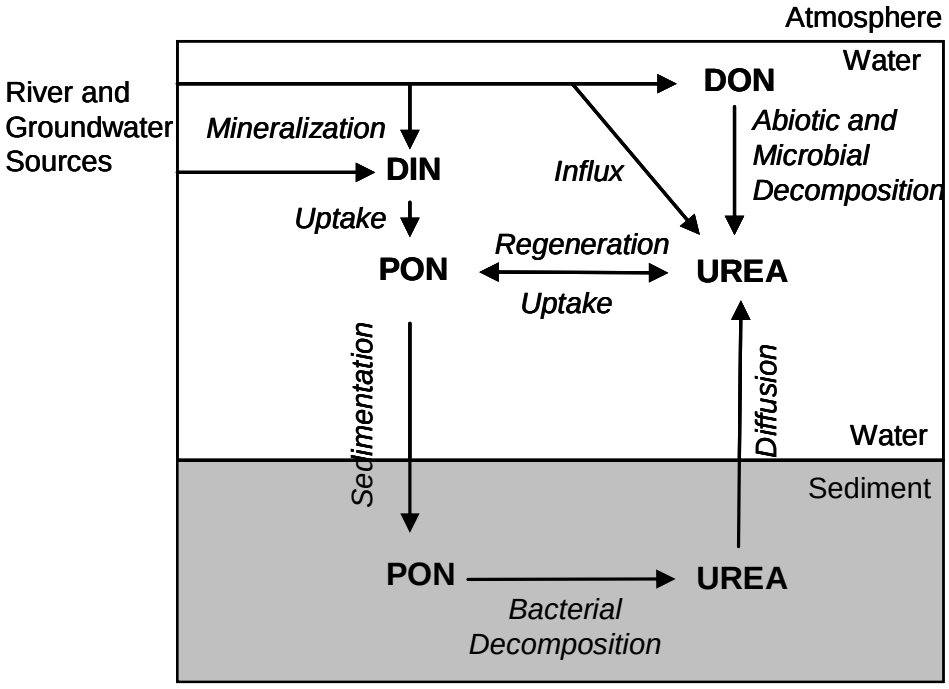
2.4.4 A new model for regulation of urea concentration in lakes

Taken together, limnological monitoring for two years (Figs. 2.2, 2.4, 2.6), decade-long analysis of dissolved N composition (Fig. 2.5b), mass balance budgets (Patoine *et al*., 2006; Leavitt *et al*., 2006), large scale lake survey (Fig. 2.5c), and quantification of urea in urban wastewater (Fig. 2.7) suggest a new model for the regulation of the urea content of lakes (Fig. 2.8). In this model, inputs of allochthonous N to lakes arise mainly from

terrestrial organic and inorganic N in rivers (Patoine *et al*. 2006), as inferred from correlations between lake landscape position and urea content (r^2 = 0.66, P <0.05) (Figs. 2.2, 2.6). In general, allochthonous N influx to agriculturally-impacted lakes is composed mainly of DON (Fig. 2.5b,c) (Patoine *et al*., 2006, Pham et al. 2009), whereas DIN often predominates in lakes influenced by urban wastewaters (Leavitt *et al*., 2006; Waiser *et al*., 2011). In addition, undegraded urea is likely to be present in river inflow (Glibert *et al*., 2005), although it is recognized that further research is required to quantify mechanisms controlling urea production, transformation, and loss in lotic ecosystems (Wiegner *et al*., 2006; Glibert *et al*., 2006; Stanley & Maxted, 2008).

Once in the lake, urea is released into the water column from DOM by microbial action, exposure to irradiance, and other processes (Bushaw *et al*., 1996; Berman *et al*., 1999; Bronk *et al*., 2010), as suggested by positive correlations between concentrations of urea and DON in these lakes (r = 0.51-0.61, P < 0.05) (Fig. 2.6). Although plankton also influence urea concentrations (Satoh, 1980; Lomas *et al*., 2002; Solomon *et al*., 2010), constant levels of urea (Fig. 2.2) despite variable plankton densities (Patoine *et al*., 2006; Vogt *et al*., 2011) and inconsistent relationships between urea and plankton composition (Fig. 2.6) suggest that algae, bacteria, and zooplankton do not represent a net source of urea to these lakes (Mitamura & Saijo, 1986b; L'Helguen *et al*., 2005; Solomon *et al*., 2010). Instead, sediments account for 75% of N sinks and sources in these P-rich, N-limited lakes (Patoine *et al*., 2006; Leavitt *et al*., 2006) suggesting that decomposition of PON in the water or sediments is the main source of urea to lakes (Fig. 2.8). Generally, urea is produced at very high rates in surfacial sediments (Therkildsen & Lomstein, 1994; Therkildsen *et al*., 1996), while urease activity is suppressed by low

Figure 2.8. Conceptual diagram illustrating the main controls of urea concentration in the water column of productive lakes. See Discussion for details.



oxygen availability (Lomas*, et al* 2002; Siuda & Chrost 2006). Although strong inverse correlations between urea concentrations and O_2 levels (Fig. 2.5) are consistent with the importance of deepwater processes, additional experiments should be conducted to evaluate the relative importance of water column and sedimentary mechanisms in controlling urea abundance in freshwaters.

2.4.5 Relevance to global water quality and N biogeochemistry

Pollution of global surface waters with urea has increased logarithmically since 1960 (Glibert *et al*., 2006), reflecting the exponential growth of urban populations, altered social preference for N-rich foodstuffs, and introduction of urea as an agricultural fertilizer (Millennium Ecosystem Assessment, 2005). Furthermore, agricultural use of urea is expected to increase 100% again by 2050, due to its low cost, high solubility in water, benign effects on crops (e.g., avoids root burn), and minimal societal threat (production of explosives) relative to inorganic N compounds. Urea applications will be greatest in regions where natural edaphic or geological conditions have combined with long-term agricultural practices to saturate soils (Bennett *et al*., 1999) and lakes with P (Carpenter, 2005). Under these conditions, subsequent pollution of P-rich waters (>50 μg P L^{-1} as SRP) with urea may suppress growth of diazotrophic cyanobacteria (*Anabaena*, *Aphanizomenon*) while stimulating 400% increases in toxins and biomass of non-N_2-fixing taxa (*Microcystis*, *Planktothrix*) (Finlay et al. 2010a; D.B. Donald *et al*., unpublished data).

Urea pollution can degrade P-rich surface waters through two complimentary pathways. First, analysis of landscape patterns of urea distribution in diverse lake ecosystems (Figs. 2.2, 2.5) and correlates of elevated water column concentrations (Fig. 2.6) suggest that direct runoff from urban and agricultural sources represents a significant source of new undegraded urea to freshwaters. Second, I hypothesize that influx of other dissolved N compounds (DON, DIN) may increase urea availability to cyanobacteria through biotic and abiotic processes in both the water column and sediments (Fig. 2.8). At present, it is uncertain how the relative importance of allochthonous and autochthonous sources varies among lake districts, or whether sedimentary sources are also important in P-limited systems where N sequestration is lower than that observed in Qu'Appelle lakes (Patoine *et al*., 2006; Leavitt *et al*., 2006). Fortunately, development of rapid, reliable analytical methods (Revilla *et al*., 2005) has eliminated a long-standing barrier to urea analysis, and allows a more comprehensive investigation of its potential effects on water quality before agricultural use of urea doubles.

3. DIFFERENTIAL EFFECTS OF UREA FERTILIZATION ON AUTO- AND HETEROTROPHIC COMMUNITIES: IMPLICATIONS FOR PLANKTONIC METABOLISM IN EUTROPHIC WATERS

3.1 Introduction

Application of industrial fertilizers has increased ~500% since 1960 (Vitousek *et al*., 1997) and is expected to nearly double again by 2050 to meet demands of 3 billion more people and a change in societal preference for nitrogen (N)-rich food stuffs (Millennium Ecosystem Assessment, 2005). Rarely used prior to the Green Revolution, urea ($[NH_2]_2CO$) now accounts for ~50% of global N fertilizer use (Glibert *et al*., 2006). Its use is particularly evident in regions where centuries of intensive agriculture have saturated soils with phosphorus (P) (Carpenter, 2005), increased terrestrial P export (Bennett *et al*., 1999), and enriched surface waters to the point that soluble reactive P (SRP) now accumulates to levels >50 μg P L^{-1} and N limits lake production (Leavitt *et al*., 2006; Bunting *et al*., 2007; Finlay *et al*., 2010a). Unfortunately, up to 40% of urea fertilizer can be exported to surface and ground waters, particularly when applied in association with cold temperatures, precipitation, irrigation, or chemical inhibitors of urea decomposition (Swensen & Singh, 1997; Thorén et al., 2003; Glibert *et al*., 2006; Siuda & Chróst, 2006). Further, urea may enter lakes through hydrologic transport of animal and human wastes, especially if no prior microbial decomposition to NH_4^+ has taken place (Mitamura *et al*., 1994; Withers, 1998; Petersen *et al*., 2004; Silva *et al*., 2005). Once in P-rich waters, dissolved N species, including urea, can stimulate total algal

growth and abundance of potentially-toxic cyanobacteria (*Microcystis*, *Planktothrix*), as demonstrated by catchment-scale mass balance studies (Leavitt *et al*., 2006; Bunting *et al*., 2007), whole-ecosystem fertilization experiments (Barcia *et al*., 1980; but see Lathrop, 1988), and large-scale mesocosm experiments (Levine & Schindler, 1999).

Preliminary evidence suggests that pollution of eutrophic lakes with urea may have differential effects on phytoplankton and bacteria. In the absence of fertilization, phytoplankton (Gu *et al*., 1997; Bronk *et al*., 2007) and periphytic algae (Thorén, 2007; Mitamura *et al*., 2010) are the main sinks of urea in surface waters, while pelagic bacteria either release urea from the decomposition of dissolved (DON) or particulate (PON) organic N (Satoh, 1980; Mitamura & Saijo, 1986b; Berman *et al*., 1999), or acquire urea at <10% the rate of phytoplankton (Solomon *et al*., 2010). Moderate eutrophication can elevate urea concentrations in surface waters to >0.7 mg N L^{-1} (Siuda & Chróst, 2006) and selectively stimulate bacterial consumption (Park *et al*., 1997; Jorgensen, 2006), including that by cyanobacteria (Berman & Chava, 1999; Levine & Schindler, 1999). In contrast, the abundance of non-N_2-fixing cyanobacteria (*Planktothrix*, *Microcystis*) and associated toxins (microcystin, MC) can increase two- to four-fold in P-rich lakes fertilized with urea at >5 mg N L^{-1} (Finlay *et al*., 2010a; Donald *et al*., under review), levels which are characteristic in waste-waters of urban and intensive-livestock operations. Finally, extreme nutrient fertilization in aquatic ecosystems can restrict phytoplankton growth due to lack of light or other factors (Mallin *et al*., 1999; Roberts & Howarth, 2006; Yoshiyama & Sharp, 2006), resulting in elevated densities of bacteria and depletion of oxygen (Cloern & Oremland, 1983; Burkholder *et al*., 1997; Paerl *et al*., 1997). Despite these generalities, however, little is known of the precise relationship

between urea pollution and the relative responses of algae and bacteria, including whether thresholds to specific biotic responses exist.

In principle, differential effects of urea fertilization on algal and bacterial communities could influence net ecosystem metabolism and greenhouse gas exchange between aquatic and atmospheric pools. Lakes are important in global carbon (C) cycles because they receive most of their C from terrestrial sources (Cole *et al*., 2007), and permanently bury particulate C (Downing *et al*., 2008). Moreover, lakes exhibit intense microbial processing of dissolved compounds in turn regulating exchanges between organic (OC) and inorganic carbon (IC) pools (Tranvik *et al*., 2009). As a result, ~80% of C influx to boreal lakes is lost atmospherically through CO_2 evasion (Cole *et al*., 2007), a process regulated mainly by autotrophic and heterotrophic metabolic activity in the water column (del Giorgio *et al*., 1997) and sediments (Ask *et al*., 2009). In general, oligotrophic lakes exhibit net heterotrophy (del Giorgio *et al*., 1997; Prairie *et al*., 2002), while eutrophic lakes are often autotrophic due to preferential stimulation of algal growth by nitrogen and phosphorus influx, restriction of bacterial growth by viral infection, grazing, competition for nutrients (with eukaryotes) and OM (with eukaryotic phagotrophs) (Cotner & Biddanda, 2002; Cole *et al*., 2007; Tranvik *et al*., 2009). In contrast, the role of metabolic processes in the C-cycle is less well understood for hard-water lakes (Duarte *et al*., 2008; Tranvik *et al*., 2009), mainly because abiotic variations in pH can regulate CO_2 exchange independent of microbial metabolism (Finlay *et al*., 2009). Better understanding of the role of urea in regulating lake metabolism and gas exchange is urgently needed given the expected 50% in use of urea-based fertilizers by

2050 in agricultural regions with abundant eutrophic and hardwater lakes (Millennium Ecosystem Assessment, 2005; Glibert *et al*., 2006).

In this study, I conducted three 21-day mesocosm experiments to quantify the effects of urea fertilization (1-18 mg N L^{-1}) on phytoplankton, bacteria, and net planktonic metabolism in a hyper-eutrophic lake (>300 μg P L^{-1}). I hypothesized that addition of low concentrations of urea would preferentially favor algal growth and autotrophy for the following reasons: urea uptake in other aquatic ecosystems is conducted primarily by phytoplankton (Mitamura *et al*., 1994; Gu *et al*., 1997; Bronk *et al*., 2007); algal growth in P-rich lakes is often limited by the influx of N (Leavitt *et al*., 2006; Bunting *et al*., 2007); and the predominant phytoplankton in the study lake are N_2-fixing (*Aphanizomenon*, *Anabaena*) and nondiazotrophic cyanobacteria (*Microcystis*, *Planktothrix*) which often prefer urea over other forms of N (Berman & Chava, 1999; Finlay *et al*., 2010a). As well, I expected that greatly elevated urea concentrations (from 1 – 18 mg N L^{-1}) beyond maximal ambient regional levels of ~0.5 mg N L^{-1} (Fig. 2.2) would selectively increase bacterial growth as seen in other experiments (Sanderson *et al*., 2008; Finlay *et al*., 2010a) and eutrophic environments (Park *et al*., 1997; Jorgensen, 2006) receiving anthropogenic dissolved organic matter (DOM) (Cloern & Oremland, 1983; Burkholder *et al*., 1997; Wassenar *et al*., 2010). Improved understanding of the relative effects of urea on autotrophic and heterotrophic communities is essential because global use of urea fertilizer is expected to double in the next 40 years (Millennium Ecosystem Assessment, 2005; Glibert *et al*., 2006), lake eutrophication is caused mainly by agricultural and other diffuse nutrient sources (Carpenter *et al*., 1999; Bunting *et al*.,

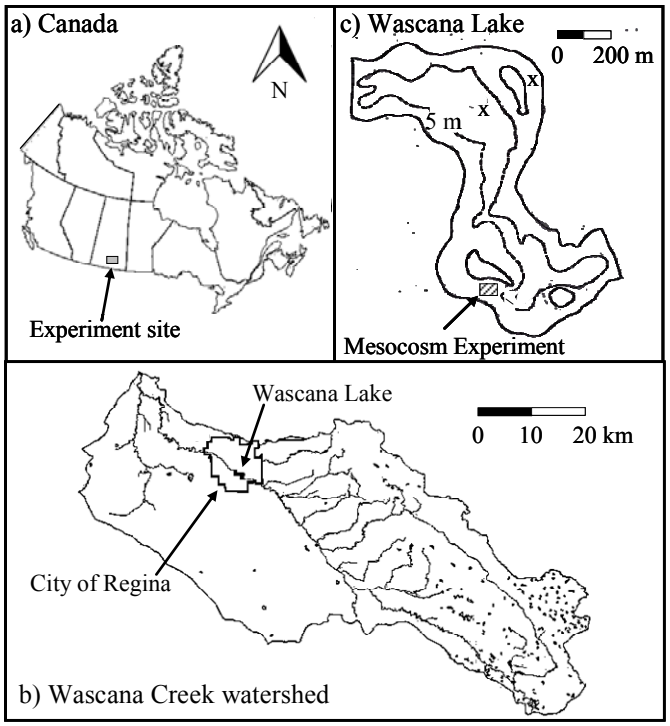
2007), and the metabolic balance of lakes is a critical control on the net flux of CO_2 to the atmosphere in many lake regions (Cole *et al*., 2007; Tranvik *et al*., 2009).

3.2 Methods

3.2.1 Study site

Three 21-day experiments were conducted in Wascana Lake (Fig. 3.1) located in the center of the City of Regina, Saskatchewan, Canada (50°26.17'N, 104°36.91'W). Wascana Lake, created in the 1800s by the impoundment of Wascana Creek (McGowan *et al*., 2005), was deepened to 2 m in the 1930s and 7.5m in 2004 (Hughes, 2004), and is presently a small (0.5 km^2, 7.5 m-deep), hypereutrophic basin (>40 μg Chl *a* L^{-1}) (Finlay *et al*., 2010a). The lake lies within an urban park, receives drainage from a 1400 km^2 agricultural catchment, and exhibits elevated (mean $\pm$ SD; 1998-2007) concentrations of total (TP) (299 $\pm$ 208 μg P L^{-1}) and soluble reactive P (SRP) (200 $\pm$ 169 μg P L^{-1}), with low ratios of total dissolved N (TDN) to SRP (6.7 $\pm$ 6.6, by mass) (McGowan *et al*., 2005; Finlay *et al*., 2010a). Consequently, algal growth is typically limited by the supply of N after mid-July (Finlay *et al*., 2010a) and Wascana Lake experiences regular blooms of N_2-fixing (*Anabaena*, *Aphanizomenon*) and non-N_2-fixing (*Microcystis*, *Planktothrix*) cyanobacteria which elevate concentrations of algal toxins (Patoine *et al*., 2006; Finlay *et al*., 2010a). During summer, zooplankton are composed mainly of small-bodied Cladocera and copepods (Patoine *et al*., 2006), while large- bodied *Daphnia* are common only during the June clear-water phase (Dröscher *et al*., 2009).

Figure 3.1. Map of Wascana Lake showing a) the continental location, b) the gross drainage area (1400 km^2) and lake location, and c) depth contour map with the location of the mesocosm experiment (hatched area) and two long term monitoring sites (x).



3.2.2 Mesocosm experiments

Fifteen mesocosms (2-m diameter, 1-m deep, ~3240 L) were attached to a floating wooden frame and deployed in a sheltered bay of Wascana Lake monthly in July, August, and September 2009 (Fig. 3.1). As detailed in Finlay et al. (2010a), mesocosms were constructed from an opaque white poly-weave plastic held in shape with 3-cm wide black plastic rings at the base and a ring of floatation material at the top of each enclosure. Mesocosms were open to the atmosphere, but closed at the bottom, and did not include lake sediments. Each bag was passively filled by fully submerging to ~1.5 m depth, pulling it to the water surface, affixing floatation material, then filling to capacity by pumping unscreened water from 0.5 m depth. Minnow traps were placed in each enclosure to remove fish, but no attempt was made to modify biotic communities.

To quantify the differential effects of urea supply rate on pelagic communities among seasons, each experiment included triplicate treatments of five urea concentrations (0, 1, 3, 8, and 18 mg N L^{-1}). On days 0, 7, and 14, laboratory grade urea (Fisher Scientific) was dissolved in 0.5 L of lake water in acid-washed polycarbonate bottles and mixed into mesocosms using a paddle. Sampling was conducted immediately before urea addition (10:00-12:00 h), except for day 0 during August and September trials when sampling followed urea addition. On each sampling date, water temperature (^{o}C), conductivity ($\mu S\ cm^{-1}$), and oxygen concentrations (mg $O_2\ L^{-1}$) were measured using either a YSI model 85 (July) or YSI 600XL probe with 650 MDS monitor (August, September). In addition, surface pH was determined using a calibrated handheld Oakton pH meter, while water transparency was estimated using a 20-cm Secchi disk.

Unfortunately, dissolved oxygen (DO) and pH measurements were not available during July for days 7-21 and day 21, respectively, due to equipment failure. Water samples for chemical and biological analyses were collected at 0.5 m with a 6-L Van Dorn water bottle, screened through a 243-μm Nitex mesh to remove large invertebrates, and transported to the lab in 10-L carboys.

3.2.3 Chemical analyses

Water chemistry was determined on samples filtered through 0.45-μm pore cellulose membrane filters. Concentrations of nitrate + nitrite (NO_3^- hereafter), ammonium + ammonia (NH_4^+ hereafter), soluble reactive phosphorus (as orthophosphate), and total dissolved phosphorus (TDP) were determined following standard methods at the University of Alberta Water Chemistry Laboratory (Pfaff, 1993; Stainton *et al*., 1977). Analysis of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) concentrations followed standard Environment Canada (1979) procedures using a Shimadzu model 5000A total carbon analyzer. Dissolved urea concentrations were measured using a slightly modified version of the diacetyl-monoxime method of Revilla et al. (2005). Limits of detection (LOD) were very low (0.35-1.0 μg L^{-1}) relative to observed concentrations.

Particulate organic matter (POM) from each mesocosm was analyzed for stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope content on each sampling date. These analyses were done to investigate whether N and C from urea ($\delta^{15}N_{urea}$ = -1.2 $\pm$ 0.1‰, $\delta^{13}C_{urea}$ = -40.3 $\pm$ 0.9‰) or native sources of dissolved elements (initial $\delta^{15}N_{water}$ = 2-5‰, $\delta^{13}C_{water}$ =

-18‰ to -22‰) were incorporated into biotic communities associated with POM (initial $\delta^{15}N_{POM}$ = 5-7‰, $\delta^{13}C_{POM}$ = -30‰ to -35‰) as described by Finlay et al. (2010a). Briefly, all samples were analyzed using a Thermoquest Delta Plus^{XL} isotope ratio mass spectrometer (IRMS) equipped with a Thermoquest NC2500 Elemental Analyzer. Prior to combustion, POM was concentrated onto glass fiber filters (GFF, 1.2 μm nominal pore size), isolated manually using forceps following 24 h at 40^{o}C (Patoine *et al*., 2006), and ~2 mg dry mass packed into tin capsules. Stable isotope ratios are reported in the conventional δ notation with respect to atmospheric N and organic matter standards.

3.2.4 Estimates of plankton abundance and productivity

Whole water samples for analysis of algal abundance and productivity were collected from 0.5 m depth using a 6-L Van Dorn water bottle. Phytoplankton abundance was estimated by collecting POM onto 1.2 μm GFF filters, extracting pigments using pure acetone, and determining Chl *a* concentrations (μg Chl L^{-1}) using standard spectrophotometric trichromatic equations (Jeffrey & Humphrey, 1975) employed for Wascana Lake since 1996 (McGowan *et al*., 2005). Primary productivity (PP) was measured in situ following slightly modified methods of Waiser & Robarts (2004). Here aliquots of pre-screened water (as above) from each mesocosm were added to 1 light and 1 dark bottle, amended with 400 μL of $NaH^{14}CO_3$ (0.26 MBq), and incubated at 0.5 m for 3 h (10:00 – 13:00 h). Triplicate analyses were conducted for each mesocosm on day 7 of each experiment to verify that PP exhibited only minimal variation within a given mesocosm. Incubations were ended by placing the bottles in a light-proof case until

filtration onto 0.45-μm pore cellulose-nitrate filters (Sartorius), and filters acidified overnight in a fume hood with 500 μL of 1N HCl. Filters were dissolved in 10 ml of Filter Count scintillation fluor and activity determined using a Canberra Packard 1900 CA scintillation counter. Primary productivity (mg C m^{-3} h^{-1}) was determined using methods of Saunders et al. (1962), and converted to daily PP by multiplying hourly rates by 10 (Waiser & Robarts, 2004).

Bacterial abundance in whole water samples was measured using the flow cytometric technique of del Giorgio et al. (1996). Briefly, 0.5 ml of Lugol's preservative was added to 10-ml samples of whole water stored in 20 ml glass vials, samples were stored in the dark at 4^{o}C prior to de-staining with sodium thiosulphate (Waiser & Robarts, 2004) before analysis by flow cytometry. Bacterial productivity (BP) was estimated in triplicate for each mesocosm by adding 15 nM methyl-^{3}H thymidine (TdR) to 10 ml of gently sonicated, screened (as above) lake water in 20-ml glass vials (Waiser & Robarts, 2004). Killed control samples received 500 μL of formaldehyde and 500 μL of 5 N NaOH. Samples were incubated for 30 min in Wascana Lake adjacent to the enclosures and were ended by addition of NaOH and formaldehyde (as above). Samples were transported to the laboratory at 0^{o}C, DNA extracted according to Robarts & Wicks (1989) with subsequent sample counting on a Canberra Packard 1900 CA liquid scintillation spectrometer. Incorporation of TdR into DNA was estimated from sample activities and by converting uptake to cell production assuming 1 mole TdR = 2.0×10^{18} bacterial cells produced and that each cell contained 20 fg C (Lee & Fuhrman, 1987). Finally, BP estimates were adjusted for an assumed 35% growth efficiency typical of eutrophic ecosystems (del Giorgio *et al*., 1997). Rationale for use of conservative

conversion factors in eutrophic prairie ecosystems is provided by Waiser & Robarts (2004). Daily BP was estimated from hourly determinations by multiplying by 24 (Cole *et al*., 1988). Ratios of PP : BP were then used to determine whether mesocosm communities exhibited net autotrophic (PP : BP >1) or heterotrophic (PP : BP <1) metabolism.

3.2.5 Data analysis

Repeated-measures analyses of variance (RM-ANOVA) were used to test the effects of urea fertilization on physical (Secchi depth, temperature), chemical (TDP, SRP, TDN, DOC, DIC, pH, conductivity), and biological responses (Chl *a*, PP, bacterial density, BB, PP : BP, dissolved oxygen). RM-ANOVAs had five treatment levels (urea concentrations) with three replicates per treatment. Day 4 was excluded from RM-ANOVAs so that measurements were all 7 days apart, maintaining equal distribution between the independent variable (time) and meeting the model assumption of homoscedasticity (Field, 2009). Given this design, RM-ANOVAs were conducted using fItime measurements (t = 4), five treatment levels (a = 5), and three replicate enclosures (n = 3). The probability of a significant main effect ($p_{\text{treatment}} < 0.05$) was estimated from the critical F-statistic of $F_{0.05[4,10]} = 3.48$ following Finlay et al. (2010a), by using fI(a - 1) treatment and 10 (a[n – 1]) degrees of freedom. The probability of significant changes in treatment effects through time ($p_{\text{interaction}} < 0.05$) were estimated using a critical F-statistic of $F_{0.05[12,30]} = 2.09$, that had 12 ([a – 1][t – 1]) and 30 (a[n – 1][t – 1]) degrees of freedom (Finlay *et al*., 2010a). Least-squares regression analysis was used to quantify the linear

and non-linear relationships between urea concentration and mean (day 7-21) production parameters, including Chl *a*, PP, bacterial density, BP, PP : BP, and DO. Model fit was evaluated using Akaike's Information Criterion adjusted for small sample sizes (AIC_c) (Johnson & Omland, 2004). RM-ANOVAs were performed using SPSS v. 16, while regression models were generated using Sigma plot v. 11.

Mass balance budgets were calculated for each mesocosm to quantify the relative importance of biological uptake, bacterially-mediated losses or transformations (nitrification, denitrification), and physical processes (NH_3 volatilization) on temporal changes in N concentration during each experiment. Total N lost from the dissolved pool was estimated as the difference between the mass of added N as urea and the sum of observed dissolved N species, corrected for initial N content, on days 7, 14, and 21. On each date, biological assimilation of N was calculated as the sum of particulate N estimated for planktonic bacteria, phytoplankton, and periphyton. Specifically, bacterial N biomass was calculated by multiplying cell abundance with 5.4 fg N $cell^{-1}$ (Lee & Furhman, 1983), although it is recognized that cellular quotas can vary 3-fold (~12 – 35 fg N $cell^{-1}$) according to growth status (Vrede *et al*., 2002). Similarly, N in algae was quantified from water-column estimates of Chl *a*, regression equations relating Chl *a* to phytoplankton biovolume calculated for Wascana Lake (cell volume in mm^3 = 0.578[Chl *a* in μg L^{-1}] + 16.008) (D.B. Donald *et al*., under review), and cellular N quotas of 40 pg N mm^{-3} (Reynolds, 1984). Finally, N in periphyton was estimated as 0.016 × $N_{phytoplankton}$, as determined in previous mesocosm experiments in this lake (Finlay *et al*., 2010a; Donald *et al*. under review). Preliminary analysis suggested that zooplankton

biomass varied little during all experiments and were not included in mass balance calculations.

3.3 Results

3.3.1 Initial conditions

Routine monitoring of Wascana Lake during 2009 revealed little difference in initial chemical conditions among experiments (Fig. 3.2). For example, concentrations of TDP (400-500 μg P L^{-1}), SRP (280-400 μg P L^{-1}), and TDN (0.9-1.4 mg N L^{-1}) were consistently elevated during July-Sept (Fig. 3.2), and were similar to mean ($\pm$ SD) values recorded for Wascana Lake during 1996-2006 (Finlay *et al*., 2010a). TDN was composed mainly (~80%) of non-urea DON, although water column concentrations of urea were lower (~70 μg N L^{-1}) than those measured in past years (Fig. 2.2). Similarly, mean ratios of TDN : SRP were lower (2.7 – 3.5 by mass) than those recorded during the last decade (6.7 $\pm$ 6.6), leading to abundant N_2-fixing cyanobacteria (data not shown) and nutrient enrichment bioassays which demonstrated that N supply limited algal growth (Fig. 3.2c). Although depth-integrated phytoplankton abundance fluctuated 50 - 125 μg Chl *a* L^{-1} throughout the summer (Fig. 3.2b), Secchi disc transparency was low at the onset of each experiment (0.44 - 0.57 m). Overall, DIC content (39 – 45 mg C L^{-1}) and pH (8.9 – 10.8) were consistently elevated, whereas initial DOC concentrations varied from ~10 (July, August) to ~30 mg C L^{-1} (September) among experiments.

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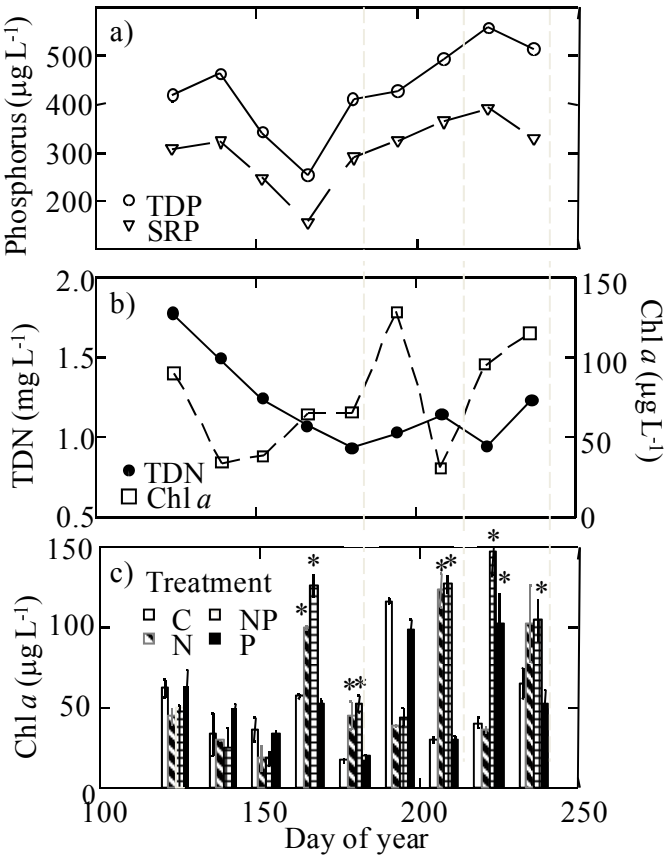
3.3.2 Urea effects on mesocosm environments

Urea amendments resulted in substantial and significant (Table 3.1) accumulation of dissolved N in mesocosms receiving 8 and 18 mg N L^{-1} (Fig. 3.3a) relative to treatments receiving less urea. While urea accounted for an average ($\pm$ SD) 33 $\pm$ 26.8 % of TDN in mesocosms receiving >3 mg N L^{-1}, NO_3^- increased over time to account for 55.1 $\pm$ 27.9% of TDN by the end of each experiment, and ammonia accounted for 10-40% of dissolved N by day 21 in the 18 mg N L^{-1} trials (Fig. 3.4). At the same time, concentrations of TDP (Fig 3.3b) and SRP (Fig 3.3c) declined two- to five-fold in mesocosms receiving >1 mg N L^{-1} (Table 3.1), often to levels below detection (< 0.5 μg SRP L^{-1}) by day 21. Although slightly elevated DOC concentrations occurred in many trials (Fig. 3.3d), differences were not significant or substantial relative to control values, except during the August trial with 18 mg N L^{-1} (Table 3.1). In contrast, amendments with urea at 1-3 mg N L^{-1} resulted in slightly lower DIC concentrations relative to those observed in control and heavily amended mesocosms (Fig. 3.3e), whereas pH increased slightly in trials with low levels of urea enrichment (Fig. 3.3f).

3.3.3 Phytoplankton response to urea

Phytoplankton biomass (as Chl *a*) increased three- to six-fold ($p_{treatment} < 0.001$) above initial concentrations (25-50 μg Chl L^{-1}), with the magnitude of algal response generally increasing as a function of urea concentration (Fig. 3.5a; Table 3.2). Similarly, urea amendments increased PP up to three-fold (Fig. 3.5b), with the greatest response

Figure 3.2. Seasonal limnological trends in Wascana Lake from May – August (inclusive) of 2009, including (a) patterns in total dissolved (TDP) and soluble reactive phosphorus (SRP), (b) total dissolved nitrogen (TDN) and phytoplankton biomass (as Chl *a*), and (c) final concentrations (± 1 S.D. error bars) of Chl *a* after 72-h bottle bioassay incubations of Wascana Lake water receiving no nutrient additions (C; white) or growth-saturating concentrations of NH_4NO_3 (N; hatched), PO_4^{3-} (P; black), or both N and P (NP; checkered). Analysis of variance with Tukey's post hoc tests identified statistically significant (asterisk) phytoplankton biomass response ($p < 0.05$) relative to control bottles. Vertical dashed grey lines show the start dates of the three monthly mesocosm experiments.



usually in mesocosms receiving 3 mg N L^{-1} (Fig. 3.5b, Table 3.2). In most cases, algal abundance and productivity increased within 4-7 days to plateaus of ~80 - 200 μg Chl *a* L^{-1} and ~6 - 8 × 10^3 mg C m^{-3} day^{-1}, respectively (Fig. 3.5a,b). Increased algal biomass resulted in a 0.3 - 0.4 m decline in water transparency in most urea treatments ($p_{treatment} <$ 0.001), while control mesocosms became substantially (~0.5 m) more transparent (Fig. 3.5c). Finally, all amendment levels stimulated photosynthetic activity sufficiently to increase O_2 concentrations from initial values of ~15 mg L^{-1} to supersaturated concentrations of 20-30 mg L^{-1} by day 4 (Fig. 3.5f), although O_2 concentrations declined beyond that time in all experiments. Reductions in O_2 were particularly marked in enclosures receiving 8 or 18 mg N L^{-1}, with O_2 levels being reduced as low as 0.8 mg L^{-1} by day 21.

Mean phytoplankton abundance (Fig. 3.6a) and productivity (Fig. 3.6b) during the last 2 weeks of each experiment increased with the rate of urea addition to a plateau at ~5 mg N L^{-1} $week^{-1}$. Least squares regression models selected using AICc (Table 3.3) suggested that phytoplankton growth was quantified best using two- or three-term exponential models, although declines in PP in mesocosms treated with >3 mg N L^{-1} $week^{-1}$ prevented the fitting of any regression models to PP data during August and September experiments (Fig. 3.6b, Table 3.3). In all other cases, models of non-linear increase to plateau values provided substantially improved fits over linear models relating phytoplankton growth to urea treatment (Table 3.3).

Table 3.1. Results from repeated –measures analysis of variance (RM-ANOVA) of the effects of urea additions (of 0, 1, 3, 8, or 18 mg N L^{-1}) on limnological conditions. Tukey's HSD *post hoc* analyses indicate differences among treatments.

Response Variable	July		August		September	
	F	*Post hoc*	*F*	*Post hoc*	*F*	*Post hoc*
Total dissolved nitrogen						
Treatment	626.25***	18 > 8 > 3,1,0	281.71***	18 > 8 > 3,1,0	523.13***	18 > 8 > 3,1,0
Interaction	419.22***		256.88***		213.66***	
Total dissolved phosphorus						
Treatment	23.93***	0 > 1,3,8,18	164.11***	0 > 1 > 3,8,18	18.55***	0,1 > 1,18 > 18,8 > 8,3
Interaction	37.42***		36.63***		13.11***	
Soluble reactive phosphorus						
Treatment	24.23***	0 > 1,3,8,18	32.57***	0 > 1 > 3,8,18	13.58***	0,1 > 1,18,8 > 18,8,3
Interaction	12.84***		12.93***		6.36**	
Dissolved organic carbon						
Treatment	0.29	----	7.88**	18 > 8,3,1,0	1.00	----
Interaction	0.94		1.35		2.20*	
Dissolved inorganic carbon						
Treatment	9.96**	0,18 > 18,8,1 > 8,1,3	13.42***	0,18,1 > 18,1,8 > 3	28.80***	18 > 0,1,8 > 1,8,3
Interaction	6.58***		7.49***		24.81***	
Temperature						
Treatment	0.88	----	2.24	----	1.67	----
Interaction	0.73		1.17		2.80*	
pH						
Treatment	3.53*	----	26.80***	3 > 8,1 > 18,0	15.67***	3,1,8 > 18,0
Interaction	4.51**		11.76***		13.49***	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 3.3. Limnological conditions through time for July, August and September experiments, including: (a) total dissolved nitrogen (TDN), (b) total dissolved phosphorus (TDP), (c) soluble reactive phosphorus (SRP), (d) dissolved organic carbon (DOC), (e) dissolved inorganic carbon (DIC), and (f) pH. Experimental treatments included weekly amendments of urea to add N at 0 (open circle), 1 (x), 3 (cross), 8 (upward triangle) and 18 mg N L^{-1} (downward triangle). Error bars = $\pm$ 1 S.E, and $n = 3$. Results of statistical analyses presented in Table 1.

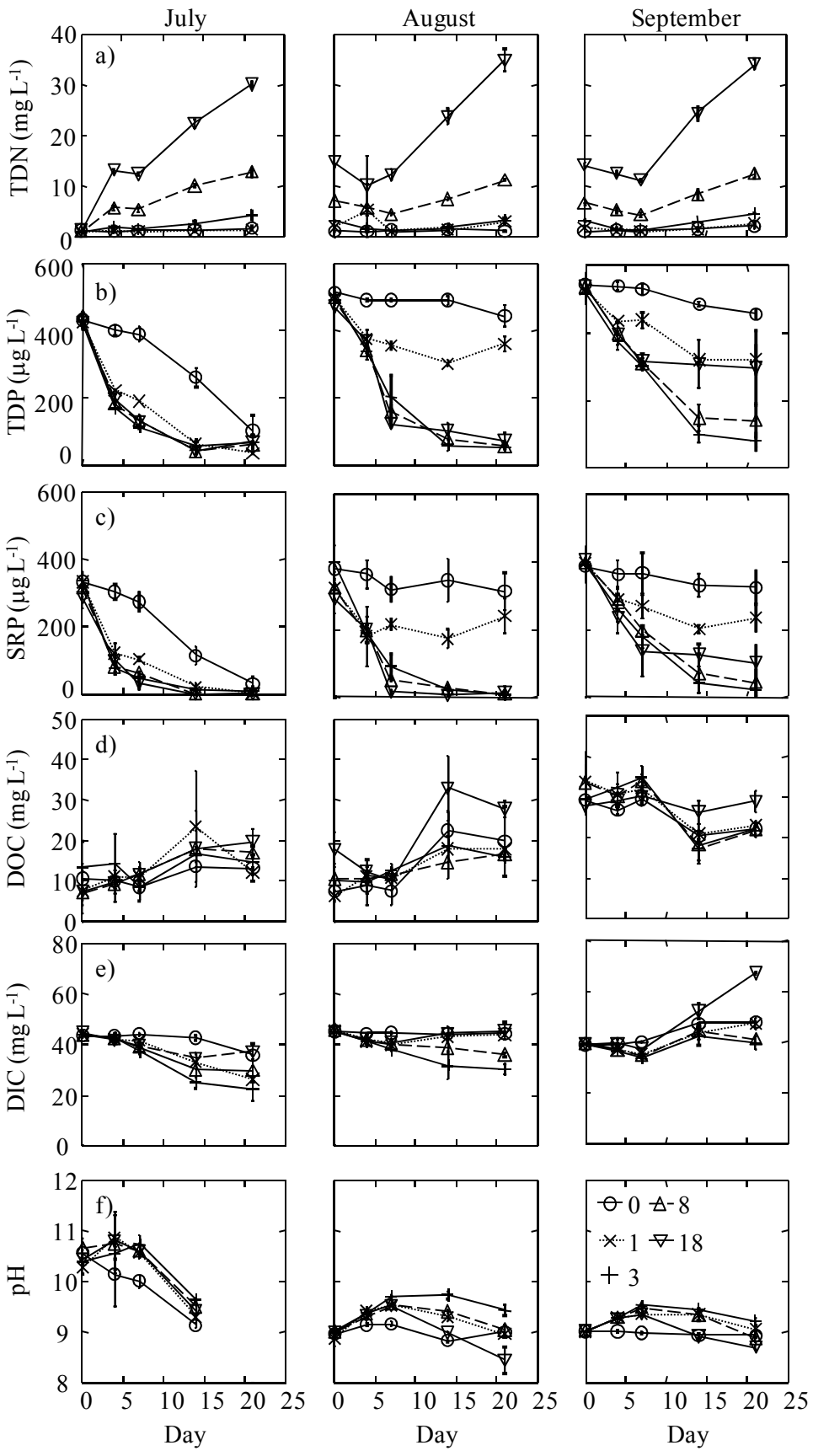

July
August
September
a)
b)
c)
d)
e)
f)
TDN (mg L-1)
TDP (µg L-1)
SRP (µg L-1)
DOC (mg L-1)
DIC (mg L-1)
pH
0 8
1 18
3
Day
Day
Day

Figure 3.4. Temporal patterns in dissolved N species (NO_3^-+ NO_2^-, NH_4^+, urea, and non-urea DON) for July, August and September experiments.

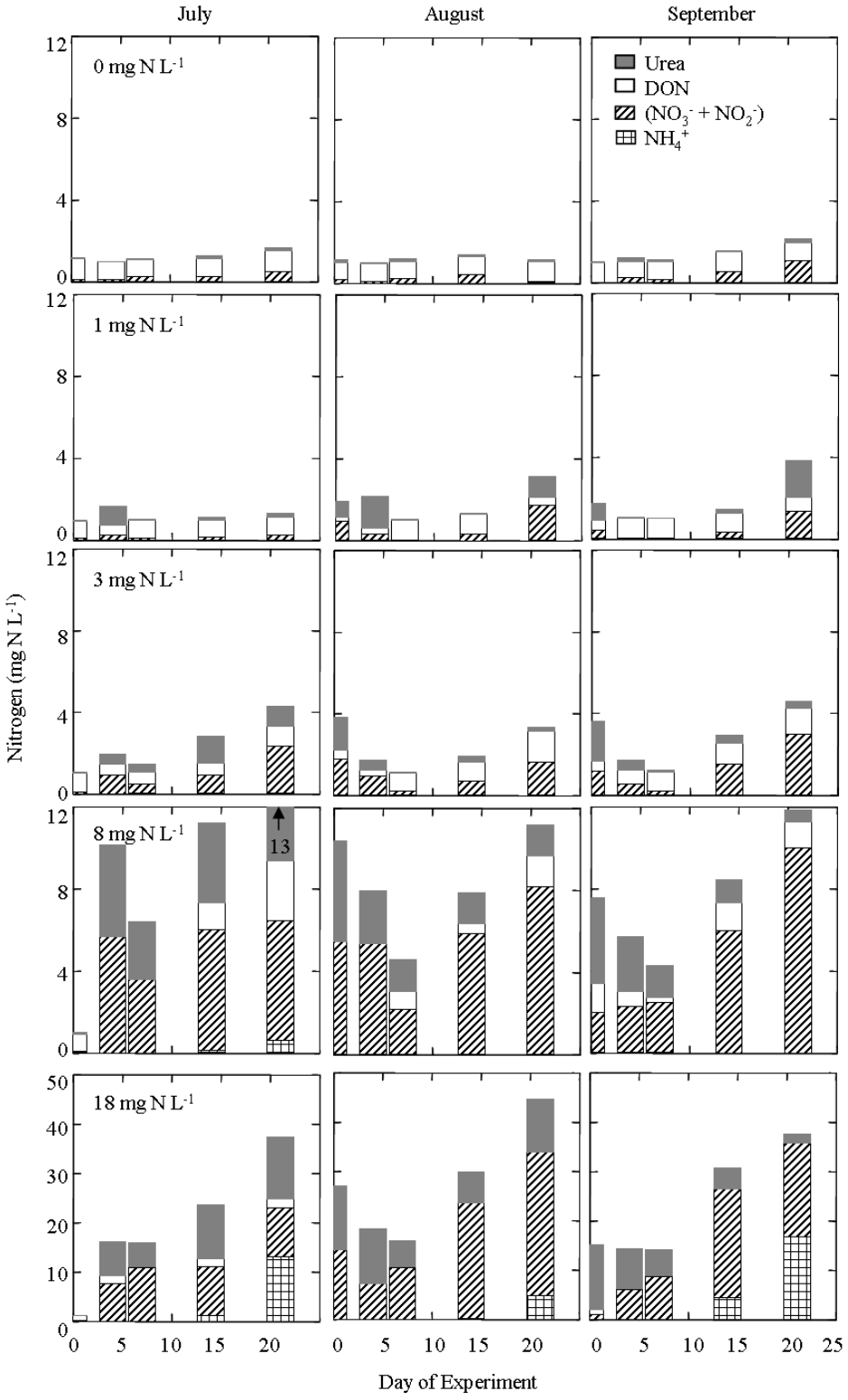


Figure 3.5. Effects of urea on (a) phytoplankton abundance (Chl *a*), (b) primary production (PP), (c) water transparency as Secchi disk depth, (d) bacterial density measured by flow cytometry, (e) bacterial C production (BP), (f) dissolved oxygen (DO), and (g) PP:BP for July, August and September experiments. Symbols used to denote urea treatments as in figure 3.3. Error bars = $\pm$ 1 S.E, and $n = 3$.

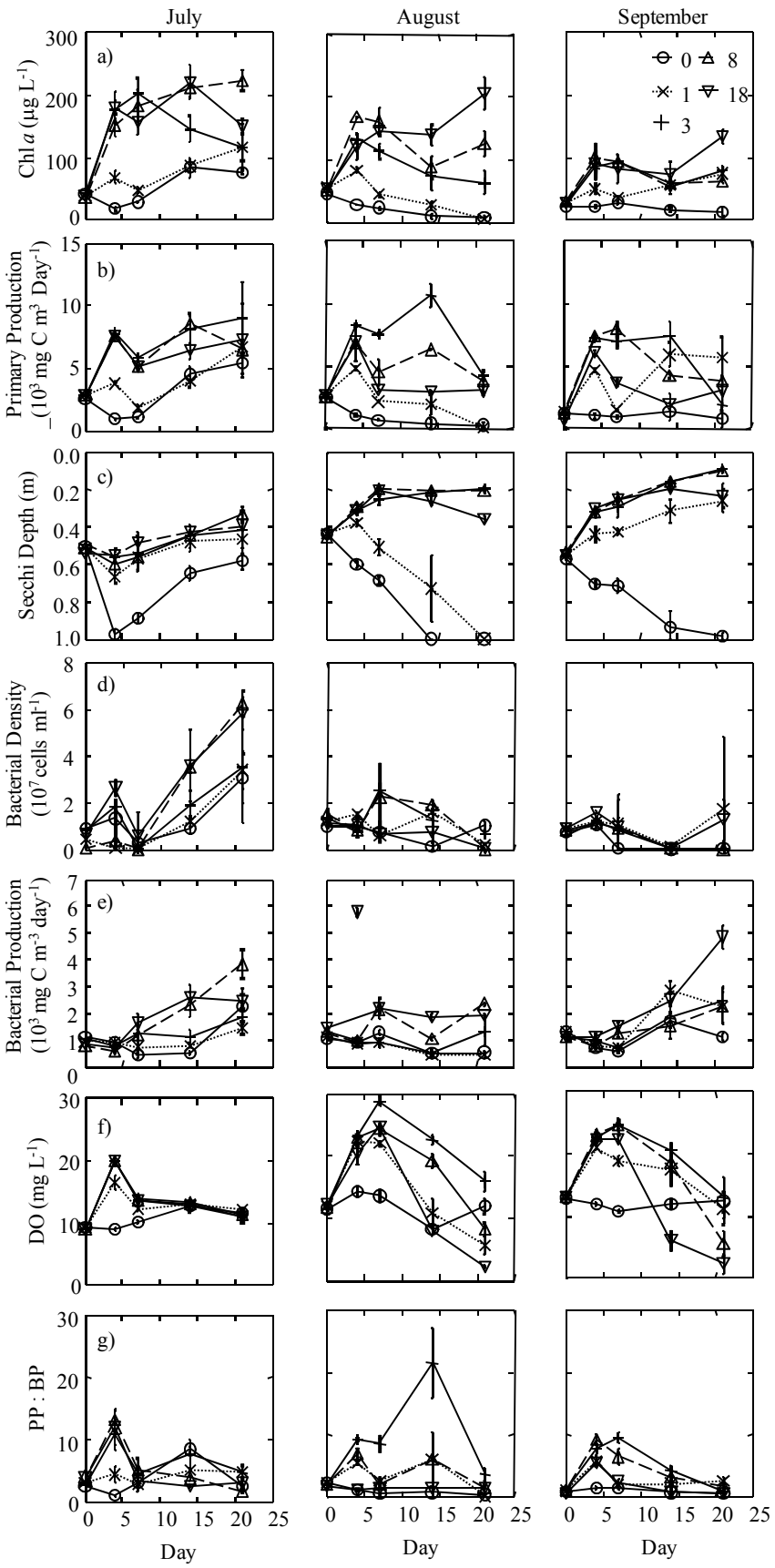

July
August
September
a)
b)
c)
d)
e)
f)
g)
Chl a (µg L-1)
Primary Production (10³ mg C m³ Day-1)
Secchi Depth (m)
Bacterial Density (10⁷ cells ml-1)
Bacterial Production (10³ mg C m-3 day-1)
DO (mg L-1)
PP : BP
0
1
3
8
18
Day

Table 3.2. Repeated –measures analysis of variance (RM-ANOVA) of the effects of urea additions (of 0, 1, 3, 8, or 18 mg N L^{-1}) on biological production and associated limnological variables. Tukey's HSD *post hoc* analyses indicate differences among treatments.

Response Variable	July		August		September	
	F	*Post hoc*	*F*	*Post hoc*	*F*	*Post hoc*
Chlorophyll *a* biomass						
Treatment	16.39***	18,8,3 > 3,1 > 1,0	86.97***	18 > 8 > 3 > 1,0	18.34***	18,3,8 > 3,8,1 > 0
Interaction	3.49**		15.39***		16.57***	
Primary Production (PP)						
Treatment	7.75**	3,8,18 > 8,18,1 > 18,1,0	214.32***	3 > 8 > 18 > 1 > 0	17.33***	3,8,1 > 1,18 > 18,0
Interaction	2.68*		25.02***		19.77***	
Bacterial density						
Treatment	12.01**	18,8,3 > 3,1,0	2.41	n.a.	1.95	n.a.
Interaction	3.13*		1.63		3.57*	
Bacterial Production (BP)						
Treatment	7.31**	8,18,3 > 18,3,0 > 3,0,1	51.74***	18,8 > 3,1,0	13.31**	18 > 1,8,3,0
Interaction	8.26***		8.28***		8.08***	
PP:BP						
Treatment	3.95*	3,8,1,0 > 8,1,0,18	42.75***	3 > 18,8,1,0	23.20***	3,8 > 8,1 > 1,18,0
Interaction	3.71**		7.69**		23.57***	
Dissolved O_2						
Treatment	n.a.		162.60***	3 > 8 > 18,1 > 18,0	17.07***	3,8,1 > 18,0
Interaction	n.a.		29.61***		17.25***	
Secchi depth						
Treatment	18.98***	0 > 1,3,8,18	350.96***	0 > 1 > 3,8,18	179.40***	0 > 1 > 18,3,8
Interaction	2.85*		30.43***		43.04***	
$\delta^{15}N$ (‰)						
Treatment	29.48***	0 > 1,3,8,18	2.27	----	0.69	----
Interaction	3.56*		5.00*		1.41	
$\delta^{13}C$ (‰)						
Treatment	12.80***	3,1,8 > 1,8,18 > 8,18,0	1.36	----	4.02*	3,1,8,18 > 8,18,0
Interaction	4.75**		0.36		5.59	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 3.6. Effects of urea influx rate (mg N L^{-1} wk^{-1}) on mean planktonic parameters during days 7-21. Response variables include (a) phytoplankton (as Chl *a*; open circles) and bacterial (flow cytometry estimate, solid circles) abundance, (b) primary (PP; open circles) and bacterial productivity (BP; solid circles), and (c) water-column metabolism, as PP:BP (solid circles) and dissolved oxygen concentrations (DO; open circles) during July, August, and September experiments. Solid lines indicate fit of statistically significant regression models identified in Table 3, while dashed lines indicate direction of change independent of regression model. Error bars = $\pm$ 1 S.E, and n = 9.

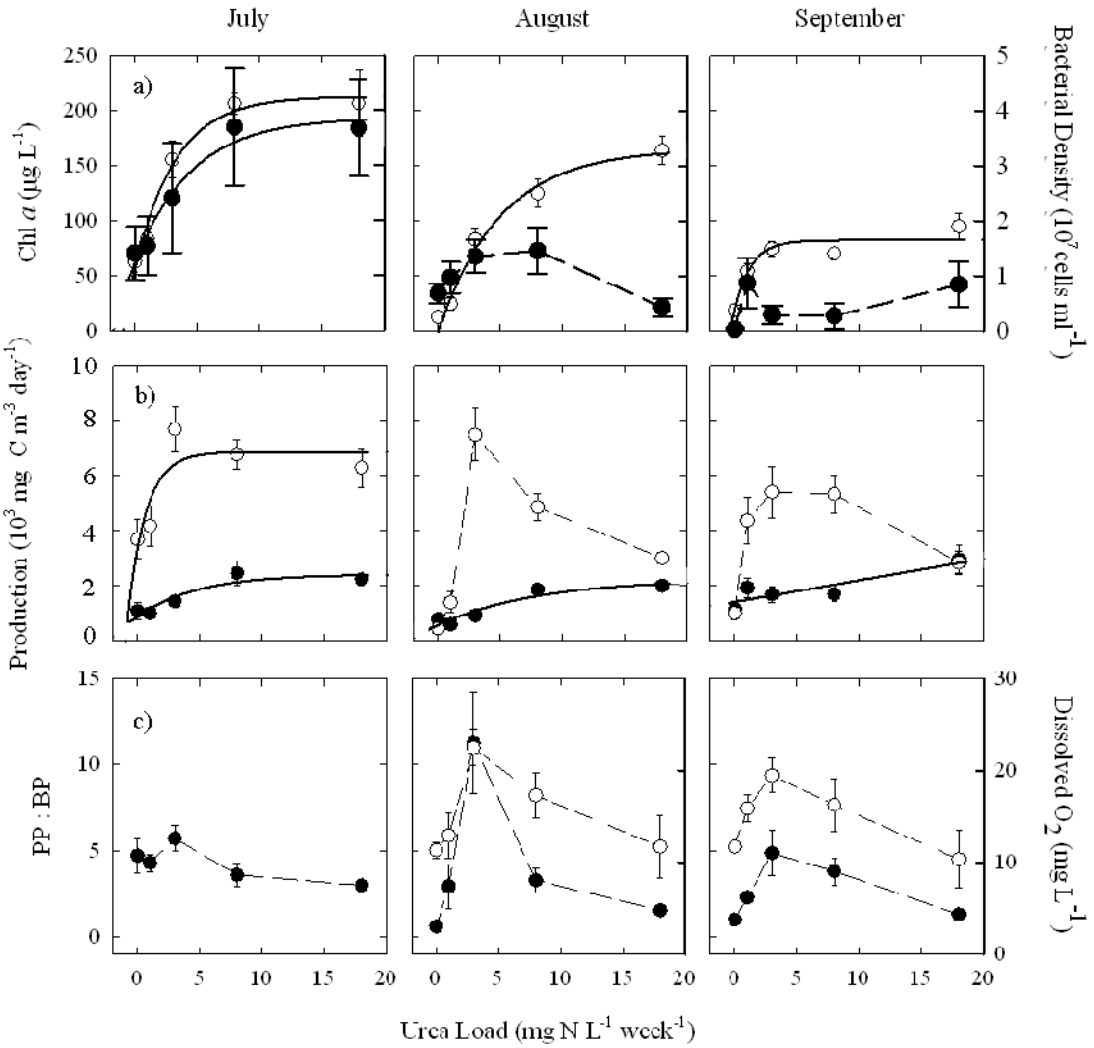


Table 3.3. Least squares regression analysis (n = 15) of phytoplankton and bacterial abundance and production (y) as functions of urea load (x). Models were selected using Akaike information criterion corrected for small sample sizes (AIC_c) (Johnson & Omland 2004), and ranked based on AIC_c score, with best-fitting models in bold. Models with no explanatory power (i.e. $r^2 = 0$) are omitted. See figure 3.5 for graphical representation of best models.

Experiment	Model	RSS	AICc	r^2
	Chlorophyll *a* (mg L^{-1})			
July	**$y = 55.02+157.43(1-1^{(-0.31x)})$**	**338.2**	**61.9**	**0.98**
	$y = 207.46(1-1^{(-0.48x)})$	3941.2	93.4	0.78
	$y = 97.72+7.48x$	5947.3	99.6	0.67
August	**$y = 165.74(1-1^{(-0.20x)})$**	**210.7**	**49.5**	**0.98**
	$y = 8.94+160.64(1-1^{(-0.17x)})$	319.2	61.1	0.99
	$y = 33.40+8.10x$	2313.7	85.4	0.86
September	**$y = 19.17+63.43(1-1^{(-0.76x)})$**	**305.7**	**60.4**	**0.91**
	$y = 81.60(1-1^{(-1.04x)})$	670.4	66.8	0.79
	$y = 44.07+3.09x$	1176.3	75.3	0.64
	Primary Production (mg C m^{-3} day^{-1})			
July	**$y = 3365.66+3485.31(1-1^{(-0.69x)})$**	**2925608**	**197.9**	**0.73**
	$y = 5085.02+105.25x$	9438501.37	210.1	0.203
	Bacterial Abundance (cells ml^{-1})			
July	**$y = 12233695+26453509(1-1^{(-0.22x)})$**	**1.809E+14**	**432.4**	**0.96**
	$y = 17667687+1295526x$	1.293E+14	456.6	0.74
	$y = 37103523(1-1^{(-0.42x)})$	2.110E+14	463.9	0.57
	Bacterial Production (mg C m^{-3} day^{-1})			
July	**$y = 884.69+1524.85(1-1^{(-0.21x)})$**	**225275**	**159.5**	**0.87**
	$y = 1190.77+73.09x$	622761	169.3	0.65
	$y = 2330.29(1-1^{(-0.41x)})$	1287189	180.2	0.28
August	**$y = 572.25+1671.52(1-1^{(-0.12x)})$**	**163337**	**154.6**	**0.9**
	$y = 749.41+79.11x$	316880	159.2	0.81
	$y = 2052.71(1-1^{(-0.24x)})$	658758	170.2	0.61
September	**$y = 1406.17+78.72x$**	**394574**	**162.5**	**0.77**

3.3.4 *Bacterial response to urea*

In contrast to rapid (<7 days) phytoplankton responses, bacterial density increased slowly and irregularly in response to added urea when analyzed by flow cytometry (Fig. 3.5d). Treatment with $\geq$ 8 mg N L^{-1} significantly ($p_{treatment}$ <0.01) increased bacterial densities two-fold during July, but not during August or September experiments (Table 3.2). Similarly, heterotrophic bacterial productivity increased two- to five-fold from initial rates ($p_{treatment} < 0.01$, $p_{interaction} < 0.001$) in all months (Fig. 3.5e), with particularly elevated BP observed in trials with 18 mg N L^{-1} (Table 3.2).

Mean bacterial abundance during days 7-21 increased as a function of the rate of urea addition, although the precise pattern of response varied among months (Fig. 3.6a). During July, bacterial densities followed the pattern observed for Chl *a* (apparent plateau at ~8 mg N L^{-1}). In contrast, bacterial abundance exhibited a unimodal relationship to the mass of urea added during August, and little pattern during the September experiment. In all months, BP exhibited a small increase with urea influx (Fig. 3.6b), with shallow non-linear models providing the best fit to the data during July and August, and a linear model describing change best during September (Table 3.3).

3.3.5 Effects of urea on net planktonic metabolism

The differential responses of phytoplankton and bacterial communities to added urea caused substantial changes in net metabolism of pelagic communities (as PP : BP),

particularly during August and September experiments (Fig. 3.5g, Fig. 3.6c). During these latter two months, mean ratios of PP : BP increased significantly (Table 3.3) by five- to 10-fold, from values observed in unamended mesocosms (PP $\simeq$ BP) to maxima characteristic of highly autotrophic conditions (PP > 5 × BP) in trials receiving 3 mg N L^{-1}, then declined to near-initial ratios in the most heavily amended treatments. Consistent with the increase in net autotrophy, mean O_2 concentrations during day 7-21 increased to maxima of >20 mg O_2 L^{-1} in mesocosms with 3 mg N L^{-1} that were much greater than those seen in both unamended and highly fertilized trials (Fig. 3.6c). Although net autotrophy was also greatest in the 3 mg N L^{-1} trial during July, the magnitude of the PP : BP response to urea addition was much lower (~21%) than that observed during other months (Fig. 3.6c). Oxygen concentrations were not measured at all times during the July experiment due to probe failure.

3.3.6 *N-fluxes*

Comparison of the total mass of added N to the sum of dissolved N pools in each mesocosm revealed that ~40% of added urea was consumed, transformed, and potentially lost to the atmosphere by the end of each experiment, irrespective of the rate of urea amendment (Fig. 3.7a). Interestingly, biotic uptake of urea was sufficient to account for observed declines in TDN concentrations in mesocosms receiving $\leq$ 3 mg N L^{-1}, whereas atmospheric losses (via NH_3 volatilization, denitrification) apparently accounted for > 50% of N export from the dissolved pool in enclosures receiving 18 mg N L^{-1} (Fig. 3.7b). Similarly, biotic uptake could account for all declines in dissolved N content during the

first week of all experiments, but became a progressively less important sink for N later in the experiment (Fig. 3.7c). In all cases, most urea uptake was due to increases in the abundance of phytoplankton (98.42 % $\pm$ 0.01 SD) rather than periphyton (1.57 % $\pm$ 0.001), or suspended bacteria (< 0.01 %).

N isotope ratios of POM declined within 4-7 days from initial values of 5 - 7 ‰ to lower values (0 to -5‰) characteristic of pure urea (-1.2‰) in all experiments (Fig. 3.8a). However, these patterns were statistically significant only during July (Table 3.2) because the $\delta^{15}N$ of POM also declined in control mesocosms during the August experiment, and urea amended treatments returned to baseline values by day 21 in the September experiment. In general, there was little relationship between the magnitude of urea amendment and that of changes in $\delta^{15}N_{POM}$ values, beyond a tendency towards a greater degree of isotopic depletion in mesocosms receiving 18 mg N L^{-1} during July and September. Similarly, while addition of urea to mesocosms generally enriched the $\delta^{13}C$ values of POM relative to both initial values (-30 to -35‰) and reference enclosures (Fig. 3.8b), there was no consistent relationship between the degree of $\delta^{13}C$ enrichment and the mass of added urea (n = 231; r^2 = 0.001; p = 0.46). Interestingly, all mesocosms exhibited increases in $\delta^{13}C$ values through the course of the experiment (Fig. 3.8b), suggesting that atmospheric (ca. -10‰) or respired (ca. -25 to -30‰) sources of CO_2 were more important to algae than was C associated with urea (-40.3‰).

Figure 3.7. Mass balance budget estimates of nitrogen (N) loss from the dissolved pool within mesocosms. (a) Comparison of total dissolved N either measured directly (TDN_{obs}) or predicted (TDN_{pred}) based on total added amounts of urea N for all experiments combined over the full (open circles, main figure) or partial range (solid circles, inset) of TDN concentrations. (b) The percentage of total N lost from the TDN pool (black, solid line) and N losses attributed to biological uptake (grey, dashed line) are presented as a function of urea influx (mg N L^{-1} wk^{-1}), or (c) time (weeks) for each experiment. Symbols are used to denote individual runs in July (circles), August (triangles) and September (squares). Error bars = $\pm$ 1 S.E, and n = 9.

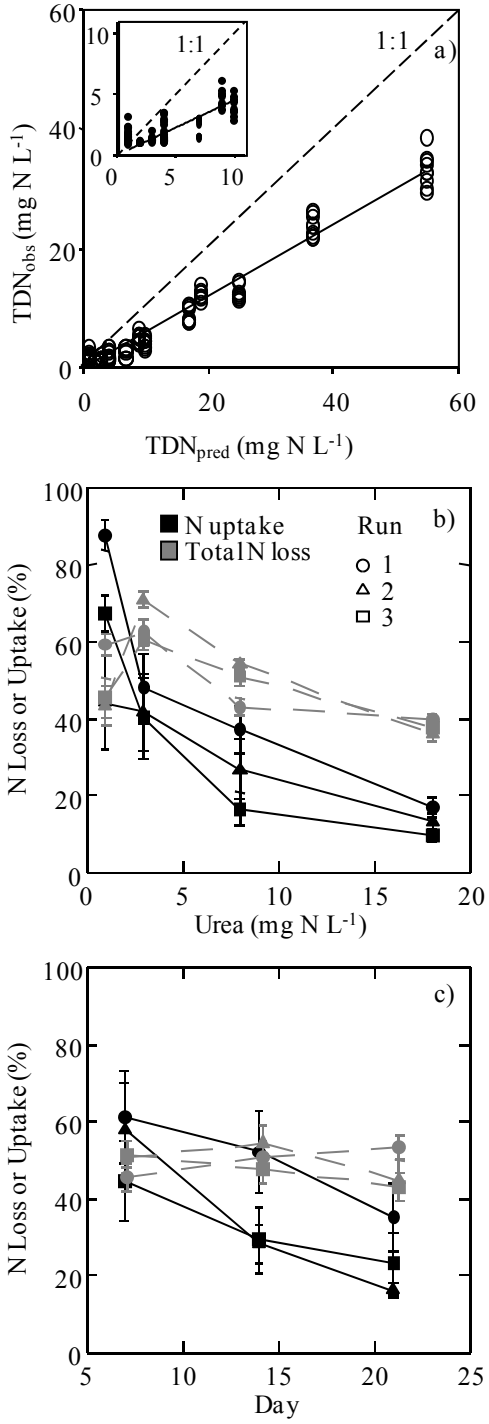
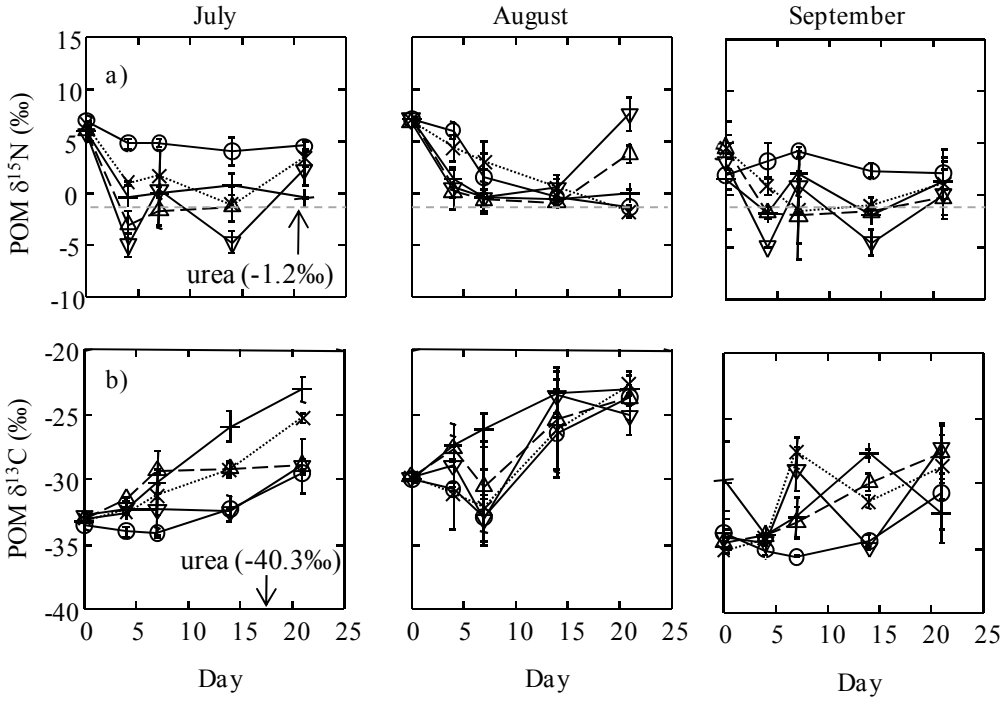

a)
1:1
1:1
TDN$_{obs}$ (mg N L^{-1})
TDN$_{pred}$ (mg N L^{-1})
b)
N uptake
Total N loss
Run
1
2
3
N Loss or Uptake (%)
Urea (mg N L^{-1})
c)
N Loss or Uptake (%)
Day

Figure 3.8. Temporal changes in stable isotope ratios of a) nitrogen (δ^{15}N) and b) carbon (δ^{13}C) observed in particulate organic matter (POM) samples collected during the urea fertilization experiment. Arrows indicate δ^{13}C (-40.3‰) and δ^{15}N (-1.2‰) of added urea. Symbols used to denote urea treatments as in figure 3.4. Error bars = $\pm$ 1 S.E, and n = 3.



3.4 Discussion

Human population growth, increased fertilization for N-rich food stuffs, and disproportionate rates of urban development are expected to double urea influx to the environment within 40 years (Millennium Ecosystem Assessment, 2005; Glibert *et al*., 2006). Here I demonstrate that low levels of urea pollution (1-3 mg N L^{-1}) of P-rich lakes (> 50 μg SRP L^{-1}) can increase phytoplankton biomass and productivity two- to six-fold (Fig. 3.5a, b), including that of toxic cyanobacteria (Berman & Chava, 1999; Finlay *et al*., 2010a), whereas concentrations characteristic of point source pollution (>8 mg N L^{-1}) (Cloern & Omeland, 1983; Burkholder *et al*., 1997; Savage *et al*., 2004) can stimulate growth of heterotrophic bacteria (Fig. 3.5d, e). In general, the magnitude of algal response increased with the mass of urea added to ~5 mg N L^{-1}, beyond which there was little change in production, possibly due to limitation of phytoplankton by light (Fig. 3.5c) (Mitamura *et al*., 1994; Roberts & Howarth, 2006; Yoshiyama & Sharp, 2006), P availability (Fig. 3.3b,c) (Donald *et al*., under review), or other factors. In contrast, heterotrophic bacteria exhibited less sensitivity to the quantity of urea added (Fig. 3.5d,e) and instead increased slowly to biomass and productivity maxima by 3 weeks. Due to the differential response of phytoplankton and heterotrophic bacteria, net planktonic metabolism (as ratios of PP : BP; O_2 concentrations) exhibited a non-linear response to added urea, with up to a 10-fold increase at 3 mg N L^{-1} relative to unamended or heavily fertilized mesocosms (Fig. 3.6c). Analysis of both mass balance budgets (Fig. 3.7) and stable isotope ratios of POM (Fig. 3.8) suggest that biotic uptake of urea was an important control of dissolved N concentration early in the experiments (< 7 days) or at

low levels ($\leq$ 3 mg N L^{-1}) of urea amendment. Together, these data demonstrate that pollution of P-rich surface waters with urea has differential effects on phytoplankton and heterotrophic bacterial assemblages, with preferred growth of potentially-toxic cyanobacteria at <5 mg N L^{-1} (Finlay *et al*., 2010a; Donald *et al*., under review) and increasing heterotrophic metabolism at elevated urea concentrations.

3.4.1 Phytoplankton response to urea

Fertilization of eutrophic surface waters with N resulted in two- to six-fold increases in phytoplankton abundance (Fig. 3.5a) and productivity (Fig. 3.5b) consistent with findings from other laboratory (Turpin *et al*., 1985; Berman & Chava, 1999), mesocosm (Barica *et al*., 1980; Levine & Schindler, 1999; Finlay *et al*., 2010a), whole ecosystem (Barica *et al*., 1980; Lathrop *et al*., 1988), and catchment-scale studies (Leavitt *et al*., 2006; Bunting *et al*., 2007). In general, algal response to added N is greatest when SRP is >50 μg P L^{-1} and mass ratios of TDN : SRP are <20 : 1 (Finlay *et al*., 2010a; Donald *et al*., under review), such as occurred in Wascana Lake during 2009 (Fig. 3.2). Under these N-limited conditions, non-N_2-fixing cyanobacteria (*Microcystis*, *Planktothrix*) may benefit selectively from fertilization with urea (Berman & Chava, 1999; Finlay *et al*., 2010a) because less energy is required for its active uptake, for chemical reduction to NH_4^+, and incorporation into amino acids, relative to NO_3^- or N_2 (Turpin *et al*., 1985; Flores & Herrero, 2005). In contrast, added urea can directly suppress N_2 fixation by diazotrophic cyanobacteria (*Aphanizomenon*, *Anabaena*) because production of heterocysts and nitrogenase enzyme complexes are down-regulated by

uptake of dissolved N (Flores & Herrero, 2005; Finlay *et al*., 2010a). In addition, growth of chlorophyte species is favored by N fertilization, particularly in shallow lakes with substantial phytobenthic communities (Barcia *et al*., 1980; Levine & Schindler, 1999; Finlay *et al*., 2010a). The large increase in algal abundance and production caused by the selective stimulation of urea for chlorophytes and non-diazotrophic cyanobacteria in all experiments supports earlier work demonstrating that added N both increases overall algal abundance and alters phytoplankton community composition (Berman & Chava, 1999; Finlay *et al*., 2010a; Donald *et al*., under review).

Apparent thresholds in the magnitude of phytoplankton response to added N (Fig. 3.6a,b) and the duration of accelerated growth (Fig. 3.5a,b) suggest that phytoplankton assemblages may exhibit a threshold capacity to assimilate urea. As noted in earlier experiments in Wascana Lake (Finlay *et al*., 2010a; Donald *et al*., under review), maximal algal response to urea amendments occurred within ~7 days, after which there was little additional accumulation of biomass either as Chl *a* or cellular biovolume (Fig. 3.5a). This finding is extended by demonstrating for the first time that mean algal abundance and productivity increased to stable plateaus at ~5 mg N L^{-1} (Fig. 3.6a, b). Further, mass balance budgets conducted for each mesocosm confirm that algal uptake was sufficient to account for observed declines in dissolved N during the first week, but that the importance of biological uptake diminished thereafter (Fig. 3.7c). Similarly, δ^{15}N values declined rapidly only during the first week of incubation, particularly in heavily amended treatments (Fig. 3.8a), consistent with rapid assimilation of N from urea followed by a period in which algal growth exhibited steady-state characteristics. Taken

together, these findings demonstrate that pollution of P rich lakes with urea can enhance eutrophication and degrade water quality in less than one week.

Several mechanisms may act in concert to limit algal response to added urea in these experiments. First, rapid decline in SRP concentrations (Fig. 3.3c) due to biological assimilation, combined with elevated N : P ratios following urea amendments (Fig. 3.3a, b), may have reduced the intensity of N limitation of algal growth (Smith, 2006). As noted previously, effects of N pollution on water quality appear to be restricted to environments with elevated P (>50 μg SRP L^{-1} and TDN : SRP < 20 : 1 by mass) (Finlay *et al*., 2010a; Donald *et al*., under review). Second, progressive reduction in water column transparency (Fig. 3.5c) may have induced light limitation of algal production, as seen in other highly eutrophic systems (Mallin *et al*., 1997; Roberts & Howarth, 2006; Yoshiyama & Sharp, 2006), including Wascana Lake (Finlay *et al*., 2010a). Third, rapid increases in algal biomass within mesocosms may have induced micronutrient limitation of growth, including elements which act as cofactors for enzymes involved with active uptake of N (Fe, Mo) (Vitousek & Howarth, 1991; Sterner, 2008) or nickel (Ni^{2+})-dependent urease activity (Solomon *et al*., 2010). In contrast, Iinfer that competition for nutrients with bacteria did not inhibit phytoplankton response to added urea (Stets & Cotner, 2008) because algal production was usually much greater than that of bacteria (Fig. 3.6b) and because heterotrophic microbes accounted for a low proportion of N uptake (< 0.01%). Finally, Iecho the detailed recommendations of Finlay et al. (2010a) and suggest that further experiments be conducted at different experimental scales (larger, longer, open to sediments, whole-lake) to better evaluate the factors regulating effects of urea on planktonic communities and ecosystems.

3.4.2 Bacterial response to urea

Consistent with other nutrient fertilization experiments (Sanderson *et al*., 2008), time series of bacterial response to added urea was muted relative to phytoplankton, with maxima of cell density (Fig. 3.5d) and productivity (Fig. 3.5e) occurring only at the end of experiments. In particular, mean heterotrophic abundance and production increased only two-fold in response to an 18-fold gradient of urea supply (Fig. 3.6a,b). Physiological studies reveal that bacteria assimilate urea via passive diffusion or by energy-dependent membrane transporters during N-replete, and N-deficient conditions, respectively (Siewe *et al*., 1998; Minocha *et al*., 2003; Silberbach *et al*., 2005; Sachs *et al*., 2006). Intracellular urea can then decompose into two NH_4^+ molecules for either amino acid synthesis (Ali *et al*., 1981; Petersen *et al*., 2004; Roslev *et al*., 2004) or for nitrification to NO_2^- and NO_3^- (Burton & Prosser, 2001; Arp *et al*., 2002). Here, direct assimilation of organic N or C likely had little effect on growth of heterotrophic bacteria in Wascana Lake, since DOM levels were elevated significantly (Table 3.1) only in the 18 mg N L^{-1} treatment during July (Fig. 3.3d), DOM concentrations in Wascana Lake already exceed 10 mg C L^{-1} (Fig. 3.3d), and DON composes >80% of TDN throughout the year (Fig. 2.4). Similarly, weak correlations between PP and BP (r^2 = 0.02 – 0.20; p = < 0.0001 – 0.20) suggest that there was little indirect effect of urea additions on bacterial growth through stimulation of autotrophic production and release of labile algal exudates (Baines & Pace, 1991, Chróst & Siuda, 2006). Instead, I hypothesize that the

main effect of urea on heterotrophic bacterial consortia was as an energy substrate for chemolithotrophic growth.

Enhanced bacterial productivity in heavily amended experiments ($\geq$ 8 mg N L^{-1}) (Fig. 3.5e) may have arisen from urea decomposition into NH_3 and selective stimulation of nitrifying bacteria (Fig. 3.4). Consistent with this mechanism, concentrations of NO_3^- increased over time to account for 55.1 $\pm$ 27.9% of TDN, whereas NH_4^+ was >10% of TDN only during the last week of trials with > 8 mg N L^{-1} (Fig. 3.4) despite the fact that urea decomposes only to NH_4^+ (Revilla *et al*., 2005). Similar increases in nitrifying and denitrifying bacteria have been recorded in previous mesocosm (Sanderson *et al*., 2008; Finlay *et al*., 2010a) and whole-lake experiments fertilized with inorganic N species (Barcia *et al*., 1980, Lathrop, 1988). Microbial denitrification was unlikely to be an important control of changes in inorganic N content, as dissolved O_2 content was reduced to near anoxic levels only late in the most heavily amended mesocosms (Fig. 3.5f). Instead, mass balance calculations for 18 mg N L^{-1} treatments show that net oxygen uptake (accounting for oxygen supplied from urea) associated with NO_3 production (35.4 mg O L^{-1}) was sufficient to account for reductions in initial dissolved O_2 content observed by the end of experiments (20.7 mg O L^{-1}). Although further genetic, molecular, or enzymatic analyses are needed to better resolve changes in microbial community structure, these patterns suggest an important role of nitrifying bacteria in N transformation of dissolved N compounds and water-column metabolism (Siuda & Chróst, 2006; Glibert *et al*., 2006; Solomon *et al*., 2010).

3.4.3 Effect of urea on the net metabolism of eutrophic waters

Moderate enrichment of P-rich waters with urea at 1-3 mg N L^{-1} favored net autotrophic conditions in mesocosms, particularly during August and September experiments (Fig. 3.6c). Specifically, ratios of PP : BP increased to nearly 5 : 1 with amendments of 3 mg N L^{-1} (Fig. 3.6c) and resulted in super-saturation of oxygen (~20 mg O_2 L^{-1}) (Fig. 3.6c), 20-40% declines in DIC concentration (Fig. 3.3e), slightly elevated pH (Fig. 3.3f), and up to 5‰ enrichment of POM $\delta^{13}C$ (Fig. 3.8b). Although not all changes were statistically significant (Tables 3.1,3.2), overall patterns are consistent with the effects of elevated algal photosynthesis within alkaline waters, including enhanced HCO_3^- uptake and pH decline (Maberly, 1996) and increased influx of atmospheric CO_2 ($\delta^{13}C_{DIC}$ ~ -10‰) into waters which normally contain depleted C sources due to respiration (-25 to -30‰) or methanogenesis (-40‰) (Finlay *et al*., 2010a; Pennock *et al*., 2010). Further, these findings are congruent with those of whole-lake mass balance studies which demonstrate that P-rich Qu'Appelle lakes are net autotrophic (Finlay *et al*., 2010b), in-gas atmospheric CO_2 during summer (Finlay *et al*., 2009), and are eutrophied substantially by the influx of dissolved N (Leavitt *et al*., 2006) despite abundant N_2-fixing cyanobacteria (Patoine *et al*., 2006). Interestingly, stimulation of algal production by urea occurred at N concentrations (1-3 mg N L^{-1}) (Fig. 3.6b) similar to TDN values recorded in most regional lakes (Pham *et al*., 2008) and urea concentrations seen in other eutrophic ecosystems (~0.5 mg N L^{-1}) (Glibert *et al*., 2006; Solomon *et al*., 2010), suggesting that even modest levels of pollution with urea are capable of increasing the production of P-rich lake ecosystems (Fig. 3.5c) (Leavitt *et al*., 2006; Bunting *et al*., 2007; Finlay *et al*., 2010a).

Urea additions at concentrations ($\geq$ 8 mg N L^{-1}) characteristic of point sources of N pollution (untreated livestock wastes, primary treated urban effluent) (Cloern & Oremland, 1983; Burkholder *et al*., 1997) coincided with declines in PP : BP ratios to baseline values near 1 due to both increased heterotrophic bacterial activity and restrictions on autotrophic production (Fig. 3.5g, 3.6c). Increased heterotrophic metabolism eventually depleted O_2 concentrations and created hypoxic conditions in mesocosms receiving $\geq$8 mg N L^{-1} as urea (data not shown). If confirmed at larger physical scales, this insight can provide important information to urban engineers and resource managers, as it suggests that wastewater treatment processes which fail to reduce urea or NH_4^+ concentrations in effluent may degrade P-rich aquatic ecosystems both by direct stimulation of algal growth (Figs. 3.5a, 3.6a) (Leavitt *et al*., 2006; Bunting *et al*., 2007; Finlay *et al*., 2010a), and by heterotrophic depletion of O_2 due to nitrification (Fig. 3.5g) (Cloern & Oremland, 1983).

3.4.4 Implications for future global change

The human population is expected to reach 9.3 billion within 40 years (United Nations, 2011), necessitating a 30% increase in food production and a doubling of the rate of agricultural fertilizer application (Millennium Ecosystem Assessment, 2005). Unlike the Green Revolution, most N applications in the future will be in the form of urea (Glibert *et al*., 2006). This compound is highly soluble in water, diminishes root damage associated with NH_4^+-based fertilizers, and cannot be used in the production of explosives, thereby reducing societal threat posed by NH_4^+-based fertilizers (Glibert *et*

al., 2006). In general, urea application will be concentrated in areas where long histories of fertilizer application have saturated soils with P (Carpenter, 2005) and have greatly enriched P export from land (Bennett *et al*., 2001), resulting in highly eutrophic surface waters with abundant dissolved P (Foy *et al*., 2002; Waiser & Robarts, 2004; Finlay *et al*., 2010a). Taken in combination with previous N fertilization experiments conducted at mesocosm (Levine & Schindler, 1999; Finlay *et al*., 2010a), whole ecosystem (Barcia *et al*., 1980; Lathrop, 1988) and catchment scales (Leavitt *et al*., 2006; Bunting *et al*., 2007; Savage *et al*., 2004), it is demonstrated here that pollution with urea at near-ambient (i.e. ~ 0.5 mg N L^{-1} [Fig. 2.2]) concentrations is capable of further degrading water quality in P-rich lakes by stimulating algal growth and promoting development of toxic cyanobacteria. Interestingly, these changes may initially enhance autotrophic processes in aquatic ecosystems, leading to uptake and storage of significant quantities of atmospheric CO_2 (Cole *et al*., 2007; Tranvik *et al*., 2009), particularly in hardwater lakes characteristic of lowland agricultural regions (Finlay *et al*. 2009, 2010a). This study also demonstrates that continued pollution with elevated levels of urea associated with influx of urban and intensive livestock operation wastes (Cloern & Oremland, 1983; Burkholder *et al*., 1997), can increase heterotrophic processes and offset the increased C sequestration of enriched aquatic ecosystems (Fig. 3.6c). Further investigation at different physical and temporal scales (discussed in Finlay *et al*., 2010a) will improve our understanding of the mechanisms regulating the production, consumption, transport, and effects of urea (Solomon *et al*., 2010), such that essential food production occurs without obligate environmental degradation.

4. CONCLUSIONS

4.1 Synthesis

Surveys of the temporal and spatial variability of urea in lakes of the northern Great Plains, combined with a series of large-scale mesocosm experiments, revealed that urea pollution from urban and agricultural sources is a potential threat to water quality in many prairie lakes (Fig. 2.2, Table 2.1). Urea concentrations in surface waters are capable of promoting uptake by bloom-forming colonial cyanobacteria and chlorophytes, and increasing net autotrophy of surface waters (Fig. 3.5, 3.6) (Finlay *et al*., 2010a; Donald et al. under review). As summarized in figure 2.8, ambient urea concentrations are influenced by urban effluent (Fig. 2.7) and agricultural land use (*e.g.*, EDA [Fig. 2.6]), as well as by internal regeneration following the breakdown of more complex DON via abiotic and bacterial processes (Fig. 2.6, 2.8). In the next half-century, the expected doubling of urea fertilizer application with the concomitant increase in livestock production (Millennium Ecosystem Assessment, 2005; Glibert *et al*., 2006), and increasing urbanization (Seitzinger *et al*., 2002; Wiegner *et al*., 2006; Stanley & Maxted, 2008) will likely increase the supply of urea to surface waters beyond any ranges experienced in Earth's history. Findings presented herein suggest that such increases in anthropogenic urea loading will favor algal growth and enhance water column net autotrophy in eutrophic systems, although severe pollution with urea ($\geq$ 8 mg N L^{-1}) may also favor elevated bacterial growth and increasing heterotrophy in hypereutrophic systems (Fig. 3.5, 3.6).

Relatively small increases in anthropogenic urea supply to eutrophic lakes may promote rapid increases in net autotrophy and, on a continental scale, may influence freshwater C processing. Although autotrophic conditions typically predominate in eutrophic freshwaters (Cottner & Biddanda, 2001; Waiser & Robarts, 2004; Finlay *et al*., 2010b), observations presented here have shown that phytoplankton rather than bacteria consistently responded to all levels of urea fertilization (Fig. 3.5a,b). Further, added urea usually supported large overall shifts to net autotrophy (Fig. 3.6b,c) and potentially influences the sequestration of large quantities of atmospheric C (Fig. 3.8b), particularly at concentrations of 1 – 3 mg N L^{-1}, which are only moderately greater than levels in regional lakes (Fig. 2.2, Table 2.1). Given that algal communities in eutrophic surface waters of the northern Great Plains are often limited by N or co-limited by N and P (Fig. 3.2) (Hall *et al*., 1999; Pham *et al*., 2008; Salm *et al*., 2009; Donald *et al*., under review), expected increases in urea supply to surface waters with intensified land use (Millennium Ecosystem Assessment, 2005; Glibert *et al*., 2006; Wiegner *et al*., 2006) will likely enhance algal production, net autotrophy, and therefore the storage or downstream export of organic C (Schindler et al. 1972; Cole et al. 2007). Under this scenario, increased algal production would likely increase the export of POC to sediments and coastal ecosystems, ultimately increasing bacterial decomposition of POC (Fig. 2.8) and enhancing the extent, duration, and intensity of benthic hypoxia in terrestrial (*e.g*., Pasqua and Katepwa Lakes [Leavitt et al. 2006]) and coastal waters (Paerl *et al*., 1997; Rabalais *et al*., 2010).

Table 4.1. Urea concentrations, decomposition (sum of uptake and breakdown) and release rates from different freshwater locations. Data are presented as means or range ($\pm$ S.D.). Data are from both tables and graphical interpretation, depending on source. Selection of data was constrained to daytime samples from freshwater sources during spring and/or summer months where available (exceptions are mean groundwater data, which represent an annual range, benthic sediment data, which are marine, and wetland data collected in October). 'Status' refers to the trophic status of ecosystem (O,M,E, H = oligotrophic, mesotrophic, eutrophic, hypereutrophic, respectively). * indicates that values represent uptake only, while ** indicates that sedimentary rates are measured as $\mu g\ N\ m^{-2}\ h^{-1}$.

Location	Urea			Water Body	Status	Sample Period	Source
	Concentration ($\mu g\ N\ L^{-1}$)	Decomposition ($\mu g\ N\ L^{-1}\ h^{-1}$)	Regeneration ($\mu g\ N\ L^{-1}\ h^{-1}$)				
Groundwater	43.1 (± 28.9)			Well & Spring	E-H	Annual	(Washington *et al*., 2006)
	28.8 (± 2.9)			Well	O-M	Jul.	(Washington *et al*., 2006)
All Plankton	52.5	4.2		Lake Nakaumi	O-H	Nov.	(Mitamura *et al*., 2000)
	11.5 - 33.6	1.2		Lake Balaton	M-E	May-Jun.	(Presing *et al*., 2008)
	0.288 - 21.6			17 Lakes	M-H	Jun.	(Siuda & Chróst, 2006)
	173.0	1.1		Pond	H	Jun.	(Park *et al*., 1997)
	0.7 - 3.7		0.03	Lake Biwa	M-H	Jun.	(Mitamura & Saijo, 1986b)
Phytoplankton	39.8	2.7*		Lake Okeechobee	H	Jun.	(Gu *et al*., 1997)
	14.4 - 72.0	49.3*		Smith Lake	H	May-Jun.	(Gu & Alexander, 1993)
Zooplankton	~0.7 - 3.7		0.02	Lake Biwa	M-E	Jun.	(Mitamura & Saijo, 1986b)
Bacteria (pelagic)	0.7 - 3.7		0.01	Lake Biwa	M-E	Jun.	(Mitamura & Saijo, 1986b)
	173.0	0.7		Pond	H	Jun.	(Park *et al*., 1997)
Sediment**	0.006		12734.4	Estuary	H	May-Aug.	(Therkildsen & Lomstein, 1994)
		7207.2 - 13213.4		Wetland		Oct.	(Thorén, 2007)
Macrophyte biofilm	132.5	60.0 - 120.1		Wetland		Oct.	(Thorén, 2007)
Flowing water	36.3 - 256.3	7.8 - 219.4		2 rivers	O	Mar.	(Remsen *et al*., 1972)
	23.2			2 streams	O	May	(Stepanauskas *et al*., 2000)
	9.1	0.1		Han River	M		(Mitamura *et al*., 1994)
	83.4	0.0		Han River	H		(Mitamura *et al*., 1994)

4.2 Implications for watershed management

For nutrient-rich lakes characteristic of the northern Great Plains, future eutrophication management strategies should include the quantification of urea, given its great abundance (mean ~176 $\pm$ 132 μg N L^{-1}) (Fig. 2.2, 2.5), its large contribution to the bioavailable TDN pool (~10-50% [Fig. 2.4]), and its potential to rapidly enhance potentially toxic algal growth and eutrophication (Table 1.1, Fig. 3.5a,b). Historically, urea has been overlooked in many aquatic N budgets since the entire DON pool was assumed to be refractory (Antia *et al*., 1991; Seitzinger *et al*, 2002; Berman & Bronk, 2003). However, as highlighted here (Fig. 2.4, 2.5), the DON pool of lakes of the northern Great Plains is partially made up of extremely bioavailable (Fig. 3.8a) urea-DON, which alone is estimated to contribute ~5% to TDN pools (Fig. 2.4, 2.5). Further, although ambient levels of urea in regional lakes are comparatively high (Table 4.1), mesocosm experiments showed that plankton have the capacity to assimilate much greater quantities of urea (up to ~3 mg N L^{-1} within 4 days [Fig. 3.3a, 3.7b,c]). In particular, urea additions selectively stimulate toxin-producing, non-heterocystous colonial cyanobacteria and chlorophytes (Finlay *et al*., 2010a; Donald *et al*., under review), which result in a up to 8-fold increase in harmful algal abundance and production. As suggested here, the inclusion of urea measurements in programs responsible for water quality monitoring and preservation would provide important insights on phytoplankton N nutrition and anthropogenic influences on aquatic N biogeochemistry.

The management of effects of urea pollution is complicated by its ubiquitous presence (Fig. 2.2), multiple sources (Solomon *et al*., 2010) and its rapid biogeochemical cycling (Table 4.1), yet results from this study suggest a number of potential approaches to minimizing urea influx to surface waters. First, internal urea regeneration (Fig. 2.8) can be slowed by the reduction of total (dissolved + particulate) N loading through diverse methods, (*e.g.*, educated fertilizer applications timed with periods of crop nutrient demand) (Vitousek *et al*., 1997; Carpenter *et al*., 1998). Following the reduction of N loading, rapid internal sedimentary urea recycling (Table 4.1) may slow the permanent burial of PON and cause a lag (annual-decadal) in decline of urea standing stocks, particularly in shallow, well-mixed lakes, as is commonly observed during P reduction efforts (Jepessen *et al*., 2005). However, given that internal N recycling represents a large fraction (up to ~75%) of N loading in regional lakes (Patione *et al*., 2006), the long term benefits to reducing external N supply may be great.

Treatment of urban effluent (http://www.regina.ca/Page438.aspx) with biological (secondary aeration) chemical (tertiary chemical additions) and physical (tertiary UV exposure) methods had little direct effects on effluent urea concentrations (Fig. 2.7) suggesting that permanent diversion of N-rich effluent may be the most effective urea-reduction method. A detailed discussion of urea diversion mechanisms are beyond the scope of this study, yet some examples include effluent reuse (Shon *et al*., 2006), industrial plant-based urea removal (*i.e*., phytoremediation [Converti *et al*., 2006]), biological nutrient removal (Wiessman, 1994; Jokela *et al.,* 2002), and wetland filtration of urea-rich material (Thorén *et al*., 2003). Similarly, increased regulation of agricultural practices involving urea fertilizer application and urea-rich livestock effluent disposal

may provide a third means to reduce urea in terrestrial runoff, as suggested by the positive relationship between urea loads and agricultural intensity surrounding lakes (EDA [Fig. 2.5]) and the fact that 5-40% of fertilizer urea is carried to receiving waters prior to chemical transformation in runoff (Glibert *et al*., 2006). In particular, retention of riparian vegetation (Mayer *et al*., 2006), improved timing of fertilizer applications (Glibert *et al*., 2006), and maintenance of wetlands surrounding lakes may reduce direct urea loading to receiving waters by > 40% (Thorén *et al*., 2003). Finally, although diffuse nutrient pollution has historically been difficult to regulate (Carpenter *et al*., 1998), the methods outlined above can potentially reduce current rates of urea loading, and protect against further degradation of aquatic ecosystems of the northern Great Plains.

4.3 Suggestions for future investigation

Although major pathways regulating aquatic urea biogeochemistry were identified and summarized in figure 2.8, the rates and regulating factors for each urea processing pathway remain unknown. A survey of the literature provides an estimate of the relative importance of different ecosystem processes (Table 4.1), but these estimates come from different systems (*e.g*., wetlands, estuaries, lakes, etc.) and cannot be combined easily to derive a synthetic picture of whole-lake urea dynamics. Further, these processes are typically studied in a piece-meal fashion (but see Thorén, 2007) under different environmental conditions (*e.g*., temperature, light, plankton biomass, etc.) thereby further restricting the potential for cross-study comparison. Although general observations can

be drawn from the survey in Table 4.1 (*e.g.*, role of sediments in urea regeneration) improved insights on urea biogeochemistry will only come from the simultaneous measurement of all major fluxes and transformations, possibly with stable- ($\delta^{13}C$ or ^{15}N) or radioisotope methods (*e.g.*, Mitamura *et al*., 1994; Thorén, 2007). In addition, such data should be placed in the context of external urea loading rates to quantify the relative importance of external versus internal processes, and to estimate total urea loading to lake ecosystems.

This study highlights the need for the characterization and quantification of internally regenerated DON in nutrient budgets for lakes of the northern Great Plains, (Fig. 2.8; Table 4.1). For instance, although it is clear that internal recycling of N can provide up to 75% of a lake's dissolved N (Patoine *et al*., 2006), it remains unresolved as to what proportion of N is released as DON versus DIN, what the composition of DON may be, which plankton species are favored for DON uptake, and how the uptake of DON affects net ecosystem metabolism. Many forms of DON are present in the water column (*e.g*., urea, dissolved free amino acids, oligopeptides, proteins, amino sugars, nucleic acids, humic substances) (Bronk *et al*., 2007), each with different degrees of bioavailability and potential to selectively stimulate unique phytoplankton species (Berman & Chava, 1999). Further, the availability of N-rich organic compounds can stimulate bacterial growth (Therkildsen *et al*., 1996; Berman *et al*., 1999; Prairie *et al*., 2002), and therefore internal cycling of DON has implications for the regulation of overall ecosystem metabolism (Schindler *et al*., 1972; Cole *et al*., 2007). The importance of DON availability in shaping aquatic communities, influencing ecosystem metabolism,

and regulating N biogeochemistry remains poorly understood, and should be considered in the future.

Mesocosm experiments also suggest that further information is needed on how P and N interact to regulate phytoplankton growth and bloom formation in eutrophic lakes. As outlined in chapter 1, many lakes of the northern Great Plains have elevated P concentrations and lower N:P values due to natural soil features (Finlay *et al*., 2009) and anthropogenic influences (Downing & McAuley, 1992; Hall *et al*., 1999), often causing instantaneous limitation of algal growth by either N or N+P (Fig. 3.1) (Salm *et al*., 2009). Not surprisingly, initial urea additions to P rich waters (initial SRP > 300 μg L^{-1} [Fig. 3.2]) caused up to 8-fold increases in algal biomass and production (Fig. 3.5a,b), yet dissolved P supplies were rapidly depleted within ~4 – 7 days of additions, and may constrain long term effects of N on water quality (Fig. 3.3c, 3.5a). In particular, further information is needed on how the mode of nutrient limitation (N vs. P) may shift following snowmelt or rainfall events (Pham *et al*., 2009), as well as how rapid nutrient consumption (*e.g*., algal bloom events) may shift proximate causes of limitation due to differential uptake of N or P. Finally, research is needed on the interaction between planktonic nutrient demand and sedimentary sources of N and P, as declines in water column P concentrations seen in mesocosms (Fig. 3.3b,c) may be offset by continuous release of P from sediments (Scheffer & Jeppesen, 2007). Given the dynamic nature of nutrient limitation in chronically eutrophied lakes (Sterner, 2008), a comprehensive investigation of the factors limiting algal growth through space and time is required to better understand the link between anthropogenic nutrient pollution and water quality loss.

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