Prairie potholes as transformers on the landscape: Exploring the rates of planktonic nitrogen uptake, DNRA, and denitrification

A Thesis Submitted to

the College of Graduate and Postdoctoral Studies

In Partial Fulfillment of the Requirements

For the Degree of Master of Environment and Sustainability

In the School of Environment and Sustainability

University of Saskatchewan

Saskatoon

By

Amy E. Hergott

PERMISSION TO USE STATEMENT

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

DISCLAIMER

Reference in this thesis to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes.

Requests for permission to copy or to make other uses of materials in this thesis in whole or part should be addressed to:

Director of School of Environment and Sustainability
University of Saskatchewan
117 Science Place, Kirk Hall
Saskatoon, S7N 5C8, Canada

OR

Dean of College of Graduate and Postdoctoral Studies
University of Saskatchewan,
107 Administration Place,
Saskatoon, S7N 5A2, Canada

ABSTRACT

The Prairie Pothole Region of Canada is under substantial anthropogenic stress associated with climate change, land-use modification, and pollutant release. As food production demands increase in concert with the global population, the need for agricultural fertilizers to supplement plant growth has also increased. This, combined with other anthropogenic activity, has caused an accumulation of nitrogen (N) compounds in managed and natural aquatic ecosystems which pose a significant threat to biodiversity, water quality, and human health. Prairie pothole wetlands have high nutrient holding and transformation capacity and may help to offset the risk of downstream nutrient export and pollution through many transformative biogeochemical processes, thereby retaining N on the local landscape. The goal of this research was to quantify the rates of planktonic uptake, denitrification, and dissimilatory nitrate reduction to ammonium (DNRA) across a gradient of parameters observed in pothole wetlands. Planktonic uptake was rapid, reaching a maximum rate of 16,100 $\mu g\ N\ L^{-1}\ hr^{-1}$ at ambient concentrations, and displayed a preference for NH_4^+. Additionally, uptake was prevalent across light and dark conditions, suggesting bacteria may play a larger role in pelagic N cycling than previously thought. Benthic NO_3^- reduction was dominated by DNRA, reaching a maximum rate of 0.756 $\mu g\ N\ g^{-1}\ hr^{-1}$, while comparatively low rates of denitrification were occurring, reaching a maximum of 0.014 $\mu g\ N\ g^{-1}\ hr^{-1}$. This research is one of the first to quantify pelagic and benthic N cycling in prairie potholes. The rapidity at which N cycling via uptake and DNRA occurs highlights the importance of potholes as transformers on the landscape, capable of harnessing and recycling N within the system. However, anthropogenic inputs and modifications can alter the rates of transformations and retention capacity of potholes. With the looming threat of climate change that may bring more extreme weather events, continued nutrient inputs, and widespread wetland drainage that increases the frequency of hydrologic connectivity necessary for nutrient export, it is imperative that we recognize the role of pothole wetlands for healthy water resources, including their capacity to transform N on the landscape.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Ar   Argon

C   Carbon

Chl. *a*   Chlorophyll a

DNRA   Dissimilatory nitrate reduction to ammonium

DO   Dissolved oxygen

H_2S Sulfide

LOI   Loss on ignition

N   Nitrogen

N_2 Dinitrogen

NH_3 Ammonia

NH_4^+ Ammonium

NO_2 Nitrite

NO_3^- Nitrate

OM   Organic matter

P   Phosphorus

PPR   Prairie Pothole Region

SO_4^{2-} Sulfate

TDP   Total dissolved phosphorus

TN   Total nitrogen

TP   Total phosphorus

1.0 Introduction

1.1 Overview

Freshwater wetlands trap major ions, nutrients, and pollutants (Craft and Casey 2000), which is beneficial for downstream water quality. However, increased human activity has begun to overwhelm the functioning of these ecosystems. Today, more nitrogen (N) is converted from atmospheric to reactive forms by anthropogenic activity than by all natural processes in terrestrial ecosystems (Galloway and Cowling 2002). The excessive production of reactive N has led to an accumulation of N compounds in the natural environment. Anthropogenically altered N compounds are either released into the atmosphere through industrial emissions, discharged in wastewater, or applied directly to the landscape as agricultural fertilizer. As a result, anthropogenic N is transported in runoff, and can degrade water quality, promote eutrophication of surface water, introduce health hazards for humans and animals, and reduce biodiversity in aquatic ecosystems (Saunders and Kalff 2001; Galloway et al. 2003). Increased nutrient loading is among the most significant stressors to aquatic biodiversity and ecosystem health (Glibert et al. 2016). Nitrogen that would ordinarily pollute surface water bodies and groundwater, however, can be trapped, transformed, and stored in depressional wetlands. The Prairie Pothole Region (PPR) covers 780,000 km^2 throughout the Canadian prairies and the upper-midwest United States and contains 5–60 wetlands per km^2 (National Wetlands Working Group 1988), making up one of the most extensive wetland landscapes in North America (Biswas et al. 2012). These pothole wetlands can reduce catchment-scale N export through the many transformative biogeochemical processes. Although scientists are aware potholes could be significant in transforming N, little work has explored the role of specific biogeochemical processes within these ecosystems. This research will focus on three N cycling processes: planktonic uptake, dissimilatory nitrate reduction to ammonium, and denitrification. Understanding the rates and controls on N cycling will contribute to further understanding of aquatic biogeochemistry of prairie pothole wetlands and support an improved understanding of controls on pothole nutrient concentration and downstream transport.

The expanse of nitrogen cycling throughout the Prairie Pothole Region is much too large to cover here. Rather, this introduction will give a brief synthesis of key information, aspects,

and influences regarding N cycling. To begin, the dynamics of freshwater wetlands will be discussed, followed by an introduction into prairie pothole hydrology, chemistry, and anthropogenic impacts. Next, the N compounds of focus will be introduced, and the pathways of interest identified. Lastly, the research rationale and gaps, significance, and objectives will be laid out.

1.2 Literature review

1.2.1 Freshwater wetlands

Freshwater is crucial to all forms of life, provides habitat, and supports the production of energy, materials, and food. As human demand for food, goods, and services increases, more pollutants are released, threatening freshwater systems. Lakes, rivers, and other freshwaters are becoming increasingly degraded and more sensitive to environmental and anthropogenic stressors. One freshwater system that is particularly vulnerable to environmental stress is wetlands (Marton et al. 2015). The Canadian Wetland Classification System by the National Wetlands Working Group (1997) defines a wetland as "land that is saturated with water long enough to promote wetland or aquatic processes as indicated by poorly drained soils, hydrophytic vegetation, and various kinds of biological activity which are adapted to a wet environment".

Wetlands are among the most productive ecosystems on Earth (Whittaker and Likens 1973) and provide invaluable services such as habitat for wildlife, aesthetically pleasing views, and have cultural or religious significance (Ramsar Convention 2007; Ghermandi et al. 2010). Wetlands also provide considerable benefits through water storage, maintenance of water tables, nutrient cycling, pollutant retention, timber production, energy sources (peat, plant matter), agriculture, fishing and hunting, wildlife resources, biodiversity, and recreation and tourism opportunities (Ramsar Convention 2007; Ghermandi et al. 2010). They are important biogeochemical reactors distributed across watersheds (Evenson et al. 2018). Wetlands have an important role as a sink or transformer for many chemical compounds (Johnston 1991; Crumpton and Goldsborough 1998) and remarkably, wetlands may remove over 90% of incoming nutrients (Deghi and Ewel 1984; Dierberg and Brezonikt 2017). One estimate suggests global ecosystem services provided by wetlands amount to an average of USD $14,785 \text{ ha}^{-1} \text{ yr}^{-1}$ (Costanza et al. 1997).

Despite these many services, wetlands have been lost at alarming rates. Wetlands are lost to urbanization, land-use changes, infrastructure development, climate change, and agricultural practices (Arya and Syriac 2018). It is estimated that the world has already lost 56–65% of its wetlands due to intensive agriculture in Europe and North America (Galbraith et al. 2005). Land modification in conjunction with high agrochemical applications are catalysts for polluted runoff, which threatens surrounding aquatic and terrestrial ecosystems.

1.2.2 Prairie Pothole Region

The Prairie Pothole Region (PPR) of North America is a region where wetland loss has been considerable, and those that remain are under stress. Since European settlement, the PPR has already lost over 71% of wetlands (Rubec 1994; National Wetlands Working Group 1997), and this number is projected to increase (Doherty et al. 2013). Additionally, the use of fertilizers has also increased, and these chemicals can easily be transported into nearby wetlands. However, there are few reliable measurements of the effects of agrochemical loading on the metabolism of wetlands receiving non-point source agricultural loads (Phipps and Crumpton 1994), which raises concern over potential degradation of wetlands and adjacent water bodies in the PPR. Exploring how prairie wetlands contribute to N cycling is critical to further understanding N biogeochemistry, wetland function, controls on catchment N export, and their role in the global N cycle.

The unique characteristics of the PPR contribute to it being one of the richest, most diverse wetland-grassland ecosystems in the world (Baldassarre and Bolden 2006; Doherty et al. 2013). The PPR contains an average of 20 wetlands per km^2 (Huel 2000) with some areas having triple that number (National Wetlands Working Group 1988), comprising the largest wetland landscape in North America (Biswas et al. 2012). This region spans 780,000 km^2 of central North America, covering three Canadian provinces and the north-central United States (Minke et al. 2010; Biswas et al. 2012). Formed by the Laurentide ice sheet retreating during the Wisconsin age glacial period ~12,000 years ago (Dyke 2004; Skopec and Evelsizer 2017), the movement, temporary halts, and disintegration of glaciers formed hummocky moraines with knob and kettle topography (Kiel et al. 1972). During slow glacial retreat, glacio-lacustrine, fluvial, and morainal materials rich in minerals and nutrients were deposited (Coupland 1983). Left behind are 'potholes' composed of dark brown and black clay-loam soils of the humic gleysol and gleyed

chernozem suborders, and areas of calcareous salinity (Henderson 2013). Glacial deposits have also made this region of the prairie rich in sulfate (SO_4^{2-}), calcium, and magnesium (Van Stempvoort et al. 1994), with pyrite in the underlying glacial till linked to SO_4^{2-} accumulation in stagnant, isolated ponds of the PPR (Rosenberry and Winter 1997; Goldhaber et al. 2014). The mineral-rich deposits provide exceptional conditions for the production of small grains, legumes, and livestock (Rashford et al. 2011) and has resulted in one of the most biologically diverse and productive ecosystems on Earth (Reddy and Delaune 2008; van der Kamp and Hayashi 2009; Rover and Mushet 2015).

Features of prairie wetlands include natural ponds with temporary or permanently anaerobic conditions, hosting hydrophytic vegetation (Reddy and Delaune 2008). However, the transient water regime of the PPR creates ambiguity in the features and terminology of prairie pothole wetlands (van der Kamp et al. 2016). A prairie pothole was defined by Eisenlohr et al. (1972) as "a depression in the prairie capable of storing water, that is the result of glacial processes". More recently, prairie pothole wetlands are described as "persistent features on the landscape regardless of whether or not there is ponded surface water" (van der Kamp et al. 2016). Prairie pothole wetlands are classified by wetland vegetation (Stewart and Kantrud 1971; Millar 1976) and the extent of hydric soils which define their boundaries (Richardson et al. 1994; Euliss et al. 2002). This definition of prairie pothole wetlands, "potholes" herein, will be used in this study. The modifier "prairie" should be assumed throughout.

The unique conditions of potholes support aquatic organisms and influence the surrounding plant and animal communities. Many species take advantage of the wet conditions, such as hydrophytic vegetation that has adapted to anaerobic conditions (Richardson et al. 1994; Reddy and Delaune 2008; Arya and Syriac 2018), or animals such as waterfowl, semiaquatic rodents, and mammals that benefit from the pothole for food, water, and habitat (Naugle et al. 2001; Higgins et al. 2002). Remarkably, these potholes are vital habitat for 50–75% of North America's waterfowl, provide critical resting and feeding zones during migrations (Batt et al. 1989) and provide habitat for resident birds and mammals (Rashford et al. 2011). The uniqueness of this region is attributed to the extent of highly productive potholes associated with the silty and clayey glacial till deposits and the lack of hydrological connectivity that influences their shapes, sizes, and functions (Kantrud et al. 1989; Leibowitz and Nadeau 2003).

1.2.2.1 Wetland classifications

Pothole wetlands are part of a complex mosaic of closed system catchments across a landscape, where each pothole is unique in permanence, size, and hydrological connectivity. Stewart and Kantrud (1971) developed a wetland classification for the PPR. Their system uses the presence and distributional pattern of vegetational zones, saturation phases, and plant species composition to characterize seven major classes of wetlands (Stewart and Kantrud 1971). Stewart and Kantrud (1971) also integrated into their wetland classification more precise descriptions such as subclass, determined by salinity (fresh to subsaline), and cover type, referring to the spatial relation of water or exposed soil to emergent vegetation. The scope of this research focuses on classes I–V to capture a range of wetland size and permanence from small ephemeral snowmelt ponds to permanently flooded systems, the latter of which are thought to be under less pressure from agricultural stressors such as drainage, tillage, sedimentation, and pesticide and agrochemical overspray (Detenbeck 2002).

1.2.2.2 Pothole hydrology

The climate of the PPR is typically semi-arid, whereby evapotranspiration exceeds precipitation, and thus, limits the development of surface flow through distinguished and integrated inlet or outlet drainage systems (Richardson et al. 1994; Biswas et al. 2012). The resultant hydroperiod of potholes ranges from temporarily saturated ponds, which hold water for only a few days, to permanently flooded ponds (Stewart and Kantrud 1971). These stagnant ponds can be supplemented by groundwater movement, which drives movement of water horizontally (Shaw et al. 2012; Mekonnen et al. 2014) and results in subsurface and groundwater connectivity (Evenson et al. 2018), and some may also function as sites of groundwater recharge (van der Kamp and Hayashi 2009).

In winter, potholes collect blowing snow as it becomes trapped in the surrounding vegetation (Mekonnen et al. 2014). This snow makes up 20% of the water balance and will largely control the water balance of the pothole (van der Kamp and Hayashi 2009). As the temperatures warm and snow melts, water collects in the pothole depression until the soil begins to thaw (van der Kamp and Hayashi 1998; Mekonnen et al. 2014). After the soil thaws, the surrounding soil acts as a sponge absorbing water in the pore space and making up 75–80% of summer water loss from the pothole (van der Kamp and Hayashi 2009). But, before the soil

5

thaws, 80% of the annual runoff can be produced within ~2 weeks of the snowmelt season (Gray and Landine 1988; Coles and McDonnell 2018). The frozen ground beneath has minimal topographic slopes and deep, permeable soils, which contributes to the difficulty in modeling the melt runoff in the PPR (Coles and McDonnell 2018). With this lack of predictability and the compromised infiltration capacity due to frost, coupled with the high volume of runoff during the snowmelt period, it's especially important to consider the constituents carried and their fate once pooled within these potholes.

During very wet conditions, such as snowmelt (Shook et al. 2013), when the ground is frozen, or all soil storage pores are full, and water depths increase, potholes may become temporarily connected to other potholes, open waters, or streams (Vanderhoof et al. 2017) through fill-and-spill events (Shaw et al. 2012; van der Kamp et al. 2016). During "fill" events, potholes are hydrologically disconnected, but fed by rain, and snowmelt. This transient state creates an opportunity for hydrological, chemical, and biological transformations (Evenson et al. 2018; Lane et al. 2018). Spill events occur once the water level exceeds the outlet or bank heights, thereby generating surface flow and temporarily connecting adjacent ponds (Shaw et al. 2012; Evenson et al. 2018), until water levels recede back to the spill point. Spill events are the primary mechanism of connectivity between depressional potholes (Evenson et al. 2018). These events, supplemented with groundwater connections, are crucial for flood management as water is pooled rather than occupying and dispersing over valuable land. Water is also lost from the potholes through water evaporation, transpiration, and anthropogenic drainage events (Shook et al. 2013), and as these water levels fluctuate, so can pothole chemistry.

1.2.2.3 Pothole chemistry and nutrient transport

Prairie potholes are subject to ever-changing landscapes, hydrological dynamics, seasonal fluctuations, and external nutrient import from both natural and synthetic sources. These conditions are rarely duplicated across the landscape and consequently, create completely unique ecosystems. Geological materials beneath the pothole contribute significantly to pond composition. These factors, as well as seasonal and inter-annual variability, influence which vertebrates, invertebrates, and faunal species find the habitat suitable (Stewart and Kantrud 1971; Labaugh and Swanson 1992; Euliss and Mushet 1999), and can further influence pond chemistry (Heagle et al. 2013).

The range in physical and chemical characteristics across the watershed/landscape can be dramatic. Within short distances (<200 m), pond chemistry can vary dramatically between potholes (Goldhaber et al. 2016). Pothole ponds range from fresh to saline, largely due to parent material and recharge vs. discharge ponds (Seelig and DeKeyser 2006). The build-up of salts can also drive a fluctuation in pH, influencing solubility reactions and cation exchange capacities (Seelig and DeKeyser 2006). Concurrent with pH levels, the nutrient pool is also susceptible to changes in the predominant forms available. Forms and concentration of N, phosphorus (P), and carbon (C) can change depending on the pond acidity, salt dynamics (Heagle et al. 2007; Nachshon et al. 2014), geology (Winter 2003; Goldhaber et al. 2014), and macro and microfauna, and can range from undetectable to exceeding health, recreation, and drinking water guidelines,

Moreover, hydrological processes are a major factor in pothole pond chemistry due to the movement of constituents to and from potholes (Labaugh et al. 1987). The primary mode of transport between potholes is through episodic fill-and-spill, as well as groundwater movement (van der Kamp and Hayashi 2009; Heagle et al. 2013). Nutrients that reside in the pond may become increasingly concentrated due to filtration, transpiration, and evapotranspiration (Parsons et al. 2004; Kalkhoff et al. 2016). When the precipitation is lower than evapotranspiration, solutes become concentrated as water levels decrease (Jones and Deocampo 2003). Additionally, as the temperatures drop, ice formation excludes solutes and can concentrate chemicals in the remaining water, or pore water if freezing continues into the sediments (Ficken 1967; Heagle et al. 2013).

1.2.2.4 Anthropogenic impacts

The composition of the soils within the PPR has led to the development of a strongly agriculture-influenced economy (Crumpton and Goldsborough 1998). Many potholes are lost to drainage in efforts to expand agriculturally productive areas and relieve economic pressures (Dahl 2011). Additional stressors impacting potholes include land modification, land use changes, and high agrochemical applications including pesticides and nutrients which hold the potential to degrade the landscape and surrounding ecosystems. Intense land use practices in the region, mainly as row-crop agriculture (Skopec and Evelsizer 2017), have resulted in the conversion of over 95% of native grasslands to crops and pasture (Murkin 1998) and a loss of

7

over 70% of potholes (Thompson and Young 2013). The high loss is attributed to the imposed burden of potholes for production practices. They are commonly seen as obstacles to maneuver large machinery around, delay seeding dates, pose unfavorable conditions for plant growth, harbor disease, and reduce available land for cropland development, along with the associated economic reward (Scarth 1998; Zedler and Kercher 2005; Skopec and Evelsizer 2017). Blackwell et al. (2009) found mechanical modification (e.g., compaction) altered soil structure and porosity, ultimately changing hydrologic regimes and reducing water storage capacity. This increases the severity and frequency of flooding and promotes the runoff and export of agrochemicals and industrial waste responsible for pollution and degradation of aquatic ecosystems. The transport of excess P and N is one of the most common and concerning issues in aquatic pollution (Cole et al. 2014) and fertilizer use in the Prairies has increased by about 20% (as a proportion of cropland fertilized) since the 1980s (Malaj et al. 2020a).

1.2.3 Nitrogen compounds in the environment

1.2.3.1 Synthetic nitrogen

Nitrogen is abundant and required by all living organisms (Bunn 2012); however, less than 1% of this N is bioavailable, and must first be converted into reactive N (Galloway et al. 2003), from the dinitrogen (N_2) that makes up 78% of the earth's atmosphere (Bunn 2012). Dinitrogen is "fixed" through natural phenomena such as lightning, volcanic eruptions, and microbes (Galloway et al. 2003). Once fixed, various organisms are able to use different N compounds for nutrition, reproduction, energy, and growth that form parts of the N cycle.

Since the mid-1900s humans have been altering the amount of N in the environment through exploiting the Haber-Bosch process (Bernhard 2010). The Haber-Bosch process transforms N_2 into bioavailable N (as ammonia (NH_3)) in the presence of iron, high heat, and high pressure (Erisman et al. 2008). The produced NH_3 is then transformed into ammonium (NH_4^+), ammonium nitrate, or nitric acid for use in agricultural fertilizers. This process has been the root of increased global food production, vital to increasing the number of humans supported per hectare from 1.9 in 1908 to 4.3 in 2008 (Erisman et al. 2008; Cassidy et al. 2013). Similarily, nitrate (NO_3^-) can be derived from fossil fuel combustion and other anthropogenic sources which enter the atmosphere and subsequently into our water. Nitrate can be formed in water bodies

through the oxidation of other forms of N including nitrite (NO_2), NH_3, and organic N compounds.

The production of synthetic urea has also proved beneficial for many industries and applications, including livestock production, aquaculture, oil spill clean-up operations, automotive additives, and manufacturing (Glibert et al. 2006). However, commercially produced urea has become problematic through increased runoff into freshwater systems, largely due to plane de-icing solutions, bioremediation, and pesticide and fertilizer applications (Glibert et al. 2006; Thorén 2007). The global use of urea as a fertilizer has increased 100-fold in the last four decades, and in Canada over 70% of urea use is in the PPR (Glibert et al. 2006). Urea is applied to supplement plant and soil growth and accounts for 50-70% of all N applications in the prairies of Western Canada (Glibert et al. 2006; Finlay et al. 2010). To slow the transformation of applied urea to NH_3, and reduce the risk of N loss through volatilization (Glibert et al. 2006), many producers use urease inhibitors. However, this can decrease the amount of urea transformed and utilized by the plant, which may increase N export by 5–40% (Glibert et al. 2006). The additional influx of urea compounds to freshwaters can become problematic as it increases urea availability beyond the naturally occurring pool of urea supplied by mineralization processes of heterotrophic bacteria, excretion by macro- and micro-plankton, and release from phytoplankton (Solomon et al. 2010).

Excess N degrades water quality, promotes eutrophication of surface water, introduces health hazards for humans and animals, reduces biodiversity in aquatic ecosystems, and ultimately alters nutrient cycling (Saunders and Kalff 2001; Galloway et al. 2003). The increase of N loading due to anthropogenic activity and use of fertilizers, along with the high spring surface runoff events (Fang et al. 2007) and continued drainage of pothole wetlands, has led to an increasing need to understand the behaviour, processes, and cycling of N compounds in potholes.

1.2.3.2 Nitrogen retention

Nutrient loss from terrestrial ecosystems through runoff is a form of non-point source pollution and can aggravate eutrophication and the impairment of nearby waterbodies (Cade-Menun et al. 2013). Polluted runoff can also impair economic reward if the nutrients originate from applied fertilizers (Cade-Menun et al. 2013). Within the PPR, the spotlight on N pollution

is not only due to the harmful impacts, but also because of the increased usage during spring and thus a high susceptibility of runoff in spring melt and rainfall. Cade-Menun et al. (2013) found the transport potential of N compounds in spring melt to be significant and found concentrations to exceed water quality guidelines and limits. While the capacity for potholes to transform and process nutrients is substantial, thresholds do exist. For example, recent work found cyanobacteria blooms cause more harm to wetland communities (macroinvertebrates, Odonata) than pesticides, and the impact is exacerbated by anthropogenic stressors, such as mixtures of pesticides and agricultural activity (Wade 2021). With the growing demands on food production and agrochemical additions, it is imperative to define these limitations to ensure the health of wetland ecosystems are maintained. Determining how to sustainably use and retain, N on the landscape, is beneficial to producers and land managers and may ultimately reduce the degradation of surrounding ecosystems, such as wetlands, lakes and rivers, of the PPR.

Nutrient retention refers to the difference between the nutrient input and output of a pothole (Saunders and Kalff 2001) and is accomplished through biogeochemical processes that can transform constituents into biologically usable forms, promote sediment sorption, or return compounds to the environment in altered forms (e.g. release of N_2 gas to the atmosphere). High retention potential is linked to high water residence time in potholes, which promotes trapping and transformation of nutrients, agrochemicals, and sediments (Saunders and Kalff 2001). Understanding the nutrient retention capacity of the PPR is especially important considering the usage of fertilizers, a landscape of variable runoff potentials, a high volume of spring melt, and more extreme weather events as a product of climate change. A study by Donald et al. (2011) found N-polluted runoff significantly intensifies eutrophication and may increase the production of cyanobacterial toxins in freshwater (Downing et al. 2005; Finlay et al. 2010). By intercepting, trapping, and transforming N, potholes can protect the quality, health, and functioning of adjacent freshwater systems.

To help understand landscape-scale nutrient dynamics, wetlands are often characterized by the capacity at which N and other constituents are retained within the system. Wetlands which have a high potential to remove reactive N from the system, such as wetlands which support N release via denitrification, are considered a "sink", while wetlands which produce reactive N, or have a net increase of reactive N within the system, such as wetlands with high mineralization

and nitrification, are considered a "source" (Groffman 2000; Robertson and Groffman 2015; Lane et al. 2018). However, wetland biogeochemistry is often more complex; the collection, transport, and transformation of nutrients, pollutants, and sediments occur across a continuum and is mediated by various hydrologic and biogeochemical processes (Marton et al. 2015), which can ultimately prevent or alleviate export from the wetland (Hansson et al. 2005). Considering the high potential to process and/or retain nutrients, pollutants, and sediments, wetlands may be best described as "biogeochemical reactors" (Hansson et al. 2005), or "transformers" on the landscape, although as noted, they are also ecosystems vulnerable to the effects of eutrophication.

1.2.3.3 Nitrogen biogeochemistry

Nitrogen biogeochemistry in potholes is complicated due to multiple biologic, aquatic, and physical processes (Mills and Forney 1988; Seelig and DeKeyser 2006), a range of oxidation states, transfers between nutrient pools, and different chemical forms (Tamm 1991; Robertson and Groffman 2015). The benthic zone, along the bottom of the pothole, is dominated by sediment processes such as nutrient uptake through macrophyte roots, microbial processes, adsorption to sediment, particulate deposition, and mineralization. Some of these processes depend on the redox state, productivity, and vegetative composition of the pothole (Mitsch and Gosselink 1993). On the other hand, the open-water zone, within the water column, may be dominated by processes acting on N in free-living biomass and dissolved forms, with wind, waves, or current being important actors (Mitsch 2005). Although these separate zones house unique processes and conditions, the interaction is crucial for N biogeochemistry (Baustian et al. 2009).

Some of the most bioavailable N compounds found in potholes are NH_4^+, NO_3^-, and urea $(CO(NH_2)_2)$. These forms of N move through the environment very differently. Nitrate moves freely through the soil solution towards the plant root due to its negative charge, leading to higher susceptibility to leaching (McKeague et al. 2005). Nitrate can be an important nutrient source within freshwaters. For example, studies have shown a correlation with fast growing diatoms and high inputs of NO_3^- (Collos et al. 1992), but at elevated concentrations, NO_3^- can stimulate harmful algal blooms, create toxic conditions, and become detrimental for aquatic organisms and biodiversity (Gobler et al. 2012; Ma et al. 2014; Erratt et al. 2018), as also found

with urea concentrations (Glibert et al. 2006). For other animals, including humans, consuming NO_3^--contaminated water can cause methemoglobinemia, or Blue Baby Syndrome (Avery 2000; Giles 2005), and bladder cancer (Weyer et al. 2001); although, such high concentrations are unlikely in most lentic surface waters of the region. However, NO_3^- pollution potentially threatens groundwater (Burkart and Stoner 2008), raising concerns for the 95% of rural inhabitants and livestock populations which consume local groundwater sources (Follett 1999). Ammonium holds a positive charge and is therefore easily bound to clay particles, which feature negative charge on their surface (McKeague et al. 2005). The concentration of naturally occurring NH_4^+ in the soil is usually below 1 mg kg^{-1}, unless supplemented by human activities such as the effluent from urban sewage, manure, coal processing facilities, and wastewater treatment plants (Schuurkes and Mosello 1988) or NH_4^+ fertilizer applications such as anhydrous ammonia, ammonium sulfate, or urea (McKeague et al. 2005). Urea is also released into agricultural systems as urine from livestock, or regenerated in nucleotides of plant, animal, and microbial cells (Thorén 2007). This urea can be mineralized in bacteria and eukaryotes through hydrolysis into NH_3 and carbonic acid (Thorén 2007). Although some studies found urea uptake to be greater than inorganic uptake in some environments (McCarthy 1972; Bronk et al. 1998; Berman and Bronk 2003), the variability in type of site, community composition, and urease activity, can create uncertainty in urea transformations.

The biogeochemical processes which transform N in potholes may include the following: N fixation, nitrification, assimilation, anammox, dissimilatory nitrate reduction to ammonium (DNRA), denitrification, and decomposition-mineralization (Figure 1.1). Although prior research has placed emphasis on denitrification as the main N retaining process in wetlands (Saunders and Kalff 2001), these efforts neglect other crucial processes that play a role in N cycling and transformation, such as DNRA and planktonic uptake. DNRA is unique in that it leads to the recycling of NO_3^- into NH_4^+, while denitrification leads to the permanent removal of fixed N. DNRA may be as important, or more important, than denitrification in environmental conditions with ample electron donors (Nogaro and Burgin 2014; van den Berg et al. 2016, 2017), such as the high C and high sulfur soils seen across the PPR, but there is limited research exploring DNRA rates within the region. Likewise, planktonic uptake controls/reduces the bioavailable N sources by incorporating it into biomass and using it for energy. Though very important, the

speed and capacity at which this happens in potholes remains largely unknown. As such, planktonic uptake, DNRA, and denitrification warrant further consideration.

1.2.4 Nitrogen retention pathways of interest

1.2.4.1 Open-water zone

1.2.4.1.1 Planktonic uptake

Within areas of open water, phytoplankton, or free-floating photosynthetic microorganisms (Sigee 2005), and some bacteria, can rapidly take up inorganic N and urea, while in littoral areas, riparian plant, periphyton, and macrophyte uptake can be an important N transformation (Mitsch et al. 2005). N compounds taken up by phytoplankton are biochemically transformed into biomass or usable forms, such as amino acids (Schuchard et al. 2017). The production of biomass involves three steps: i) transport of the exogenous N into the cell, ii) conversion of N into organic compounds, iii) biosynthesis of proteins and nucleic acids (Vincent 1992). The initial step of transportation across the cell membrane is loosely referred to as uptake, while the subsequent N conversion steps (ii and iii) are known as assimilation (Vincent 1992). My research will focus on the (initial) uptake of inorganic and organic compounds.

The initial uptake rate of these N compounds by microorganisms (Healey 1977) alters availability to other organisms or processes, such as denitrification and DNRA — which can be controlled by substrate (benthic) N concentrations. Additionally, through biological uptake, N is cycled in the ecosystem, reducing the potential or concentration available for export into adjacent water bodies. Biota can have a substantial influence on harnessing N and reducing export (Goodale et al. 2000). However, studies have found organic N can account for a large proportion of N in runoff (Hood et al. 2003). To better understand N retention in potholes, one must explore the planktonic uptake rates of different N species (NO_3^- vs NH_4^+ vs urea).

There are various forms of inorganic (NH_3, NH_4^+, NO_3^-, NO_2^-) or organic (e.g., urea and other simple and more complex molecules) N that may support microorganisms (Glibert et al. 1991; Vymazal 2007; Presing et al. 2009). Ammonia tends to be the preferred N compound for phototrophic growth (Vincent 1992) because it is more reduced and therefore less energetically expensive to assimilate (Collos 2003; Vymazal 2007). It can also be readily incorporated into amino acids by microbial heterotrophs and autotrophs (Vymazal 2007). Nitrate is preferred by diatoms under some conditions (Collos et al. 1997) and may become more important in water

rich with the nutrient (Vymazal 2007). Urea, in a dissolved form, can also be used by some algae and bacteria. In some cases, urea uptake is nearly 4 times greater than NH_4^+ and 10–50-fold greater than that of NO_3^- (Bradley 2010), as it can be utilized to meet both C and N growth demands (Moneta 2014).

The mode of uptake for each N substrate varies. Active transport of NH_4^+ and NO_3^- across the cell membrane is performed by respective transporter proteins; NH_4^+ transporters and NO_3^- transporters (Vincent 1992; Glibert et al. 2016), though NO_3^- is largely dependent on light for transport activity (Flores and Herrero 2005; Sigee 2005). Depending on the negative electrical potential difference inside the cell, NH_4^+ may be taken up by diffusive influx (Glibert et al. 2016). Furthermore, if the pH rises above 9 (more alkaline) and temperatures are warm, which may be the case in many potholes in summer, NH_3 can occur in greater concentrations than NH_4^+; NH_3 moves across the cell membrane by passive diffusion (Vincent 1992) and can be directly transported into the plastid for conversion into amino acids (Sigee 2005). Urea can either be passively (Mobley and Hausinger 1989) or actively transported into the cell by high-affinity active membrane transporters (Solomon et al. 2010). Urease is an extracellular enzyme that catalyzes the hydrolysis of urea to yield NH_3 and carbamate (Mobley and Hausinger 1989). At physiological pH, a net increase of pH can occur as carbonic acid proton dissociates, and NH_3 equilibrates with water to become protonated.
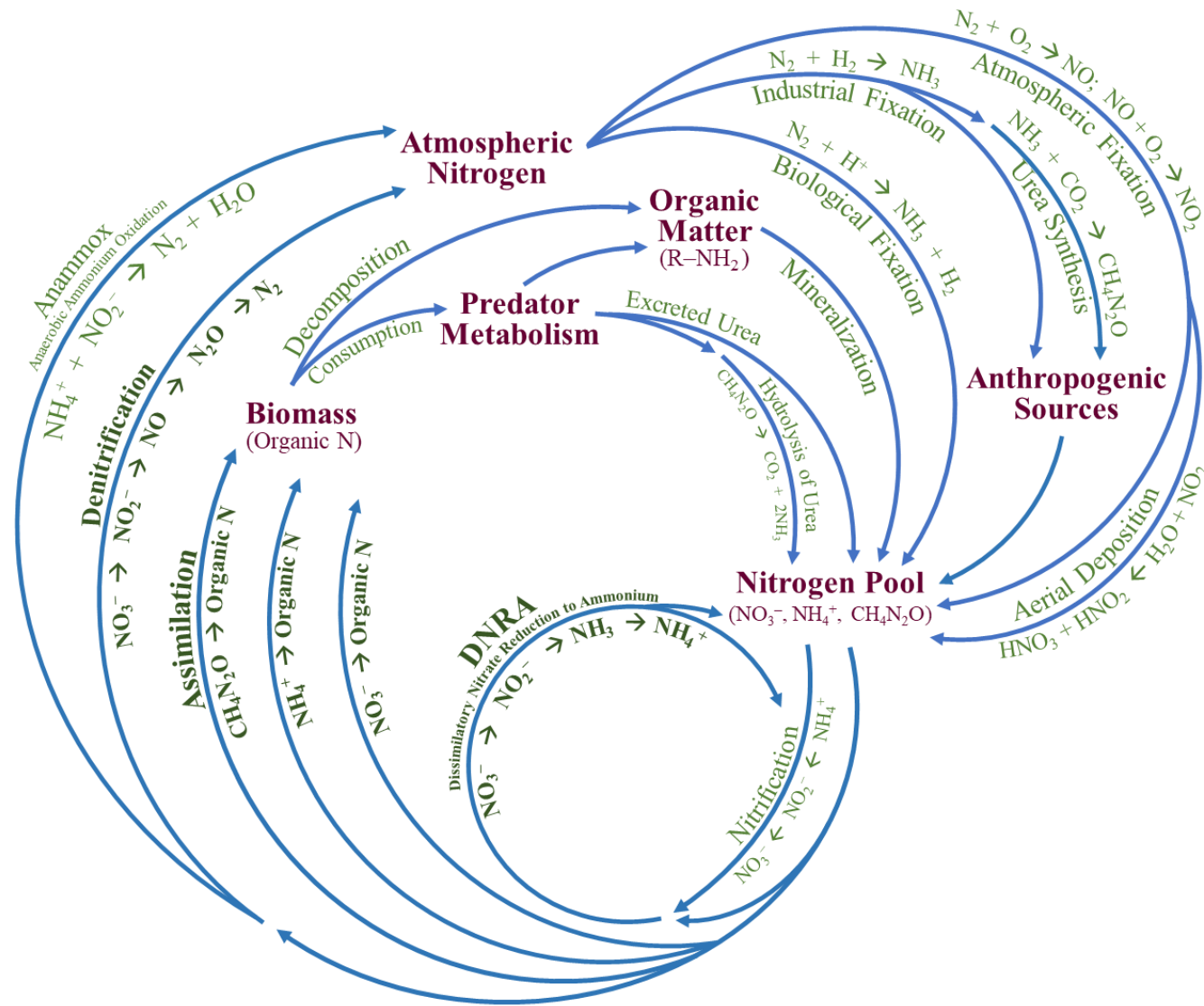
Figure 1.1 A schematic diagram illustrating the major N pathways within potholes, modified from Robertson and Groffman (2015). N pathways of focus are indicated by bold dark green text, and maroon text indicates N pools. The assimilation of N may be performed by plankton, bacteria, macrophytes, periphyton, riparian vegetation, among other biotic sinks. Likewise, the N pool may include additional N compounds not listed here. It is noted that the simplified chemical equations are shown, which are not stoichiometrically balanced.

The uptake and incorporation of N compounds is largely dependent on the external availability of N. Chlorophyll *a* (chl. *a*), a pigment responsible for the absorption of solar energy and a common measure of phytoplankton biomass, was found to have a significant correlation with N concentrations (NH_4^+, NO_3^-, NO_2^-) in an Iranian freshwater wetland (Bbalali 2013). Smith (1999) found total N to influence chl. *a* concentration even when the lake was presumed to be N limited and suggests increased attention to the role of N in regulating photosynthesis. The production of chl. *a* and uptake of N is also influenced by exposure to light (Vincent 1992). This variability is linked to the unique requirements for C and available light energy, which are required to drive photosynthetic C fixation and growth (Reynolds 2006). It is generally thought that inorganic N uptake is related to inorganic C uptake, such that the carbohydrates produced in the daytime can be stored and used for growth even at night (Miyazaki et al. 1987). Nitrate uptake is seemingly more dependent on light than NH_4^+ due to reductants only being produced during photosynthetic activity (Miyazaki et al. 1987; Vincent 1992), though there is little literature to support this finding. Urea uptake patterns have also received little attention, though rates of urea uptake have been found to be greater than that of inorganic N (McCarthy 1972; Healey 1977; Glibert et al. 1991; Bronk et al. 1998; Berman and Bronk 2003; Solomon et al. 2010), and been found to stimulate the production of toxic cyanobacteria (Gobler et al. 2002; Collos and Berges 2003; Finlay et al. 2010; Solomon et al. 2010). Although rates of urea uptake are greatest in light conditions (Healey 1977), growing research has acknowledged the contribution of bacteria to total planktonic uptake, accounting for up to 80% of urea uptake (Middelburg and Nieuwenhuize 2000; Solomon et al. 2010).

Some parameters that may influence the uptake of these compounds include the variation of cell size and NO_3^- reduction capability across algal species (Sigee 2005), and the external nutritional status regarding the availability of nutrients surrounding the cell (Vincent 1992). The surrounding nutrients play a large role in uptake efficiencies. As such, NO_3^- uptake can be completely suppressed at concentrations of NH_4^+ over 1 µg N L^{-1} (Dortch 1980), as well as partial suppression by urea at concentrations over 10 µg N L^{-1} (Cochlan and Harrison 1991). Ammonium uptake, along with NO_3^- uptake, can be inhibited by potassium concentrations as low as 4 mM, by reducing assimilatory enzymatic activity (Shukla and Rai 2007).

At elevated concentrations, it is possible for organisms to encounter a "saturation" point whereby uptake of the nutrient into the cell is no longer achieved. Commonly, the half-saturation

constant is used as a measure of the relative ability for an organism to use a nutrient and indicates nutrient limitation in natural communities (Reuter and Axler 1992). The Michaelis-Menten equation applies the half-saturation constant for enzymes to reflect half the maximum uptake rate of an organism and describes a rectangular hyperbola (Reuter and Axler 1992), while assuming a steady-state (Schnell 2014). Developing a Michaelis-Menten curve and finding the saturation point can help determine the uptake capacity of aquatic organisms and the impacts excessive nutrient pollution may cause, ranging from the stimulation of planktonic growth or the inhibition of nutrient uptake.

There is little research regarding the uptake of N in prairie potholes, but a review of the literature suggests effects of different environmental factors on occurrence and rates of uptake (Table 1.1). For all N forms (urea, NO_3^-, NH_4^+), there is a strong positive relationship with temperature, though NO_3^- is highly affected in lower temperatures due to a lower affinity for NO_3^- transport (Reay et al. 1999). Although NH_4^+ is the most energy efficient form for uptake, a variety of factors including community composition, light availability, nutrient concentration, and hydrology vary across season and can influence rates of N uptake (Solomon et al. 2010). Considering the highly seasonal changes and variation in environmental conditions throughout the pothole region, these factors are all thought to play a large role in N uptake rates in prairie potholes.

Table 1.1 Summary of the parameters influencing the uptake of different N species by freshwater phytoplankton.

Factor	Urea	Ammonium (NH_4^+)	Nitrate (NO_3^-)
Temperature	Positive relationship, spp. dependent (Solomon et al. 2010)	Strong positive relationship (Whalen and Alexander 1984)	Positive relationship (Whalen and Alexander 1984; Mobley and Hausinger 1989)
Light	Light stimulated (Healey 1977), but can occur in the dark (Kristiansen and Lund 1989)	Moderately light dependent (Turpin 1991; Skopec and Evelsizer 2017)	Light dependent (although dark uptake can occur) (Whalen and Alexander 1984; Flores and Herrero 2005; Sigee 2005)
pH	No known trend	At pH>9 converted to NH_3 and can diffuse through cell membrane, uptake increases as pH increase (Healey 1977; Vincent 1992)	No trend observed (Halterman and Toetz 1984)
Uptake process	Active or passive diffusion (Mobley and Hausinger 1989; Solomon et al. 2010)	Active or passive diffusion (Toetz 1981; Whalen and Alexander 1984; Vincent 1992; Glibert et al. 2016)	Light dependent active transport (Miyazaki et al. 1987)
Chemical interactions	Repressive effect on NH_4^+ uptake (Healey 1977)	Uptake suppressed by urea and potassium (Healey 1977; Shukla and Rai 2007)	Uptake suppressed by NH_4^+ and urea (Glibert et al. 2016)
Preferred by	Algae, bacteria (Solomon et al. 2010)	Green algae, diatoms (Lomas and Glibert 1999; Healey 1977)	Diatoms (Collos et al. 1997; Lomas and Glibert 1999; Glibert et al. 2006)

1.2.4.2 Benthic zone

1.2.4.2.1 Dissimilatory nitrate reduction to ammonium (DNRA)

At and below the sediment surface, there is an abundance of organisms which governs the processes and pathways that are crucial for N transformations. DNRA is a microbially mediated reduction of NO_3^- to NH_4^+ (Rütting et al. 2011). This NO_3^- transformation pathway is commonly overlooked, as DNRA is rarely measured. Only in the last few decades has DNRA been researched (Song et al. 2013). Although inadequately recognized, it can be equal to or more important than denitrification in aquatic systems for the reduction of NO_3^- concentrations (Song et al. 2013; van den Berg et al. 2017). DNRA is a type of anaerobic respiration performed by a variety of chemotrophic microbes, which may be facultative or obligate (Silver et al. 2001), that use NO_3^- as an electron acceptor (Lam and Kuypers 2011; van den Berg et al. 2017). Some eukaryotic microbes, such as diatoms, are able to perform DNRA (Kamp et al. 2015). DNRA

conserves N in the system (Nogaro and Burgin 2014) by transforming NO_3^- into a more reduced form, NH_4^+, which has a lower transport potential and may contribute to N retention via NH_4^+ adsorption in sediments (Kent and Yang 2017; van den Berg et al. 2016). Ammonium produced via DNRA is also available for other processes and organisms (An and Gardner 2002), including uptake by primary producers (Song et al. 2013; Van Den Berg et al. 2015; Shan et al. 2016).

DNRA is usually performed by obligate fermentative anaerobes in permanently water logged inundated wetland sediments (Burgin and Hamilton 2007; Reddy and Delaune 2008). This process can also be performed by a diverse group of proteobacteria (Kraft et al. 2011; Lam and Kuypers 2011) which may include NO_3^- storing bacteria and diatoms (Song et al. 2013). This process requires a high electron pressure and low redox potential (less than 0 mV) to transfer eight electrons from NO_3^-, at +5, to NH_3, at –3 (Reddy and Delaune 2008). The environment must have a high ratio of electron donors (labile C) to electron acceptors (NO_3^-, occasionally O_2) (Burgin and Hamilton 2007), and usually corresponds with environments like wetlands, anaerobic digesters, rumen, and lake sediments, but NO_3^- availability often limits DNRA (Reddy and Delaune 2008). Organic matter availability is one of the most influential factors for DNRA, along with temperature, sulfide concentration, and the availability of reduced iron (Deng et al. 2015; Shan et al. 2016). DNRA can also be carried out by sulfur oxidizing bacteria, which use sulfide or hydrogen sulfide as an electron donor to reduce NO_3^- (Burgin and Hamilton 2008; Russ et al. 2014). The resultant NH_4^+ and sulfate, or elemental sulfur in anaerobic environments, promote SO_4^{2-} driven anammox and thereby the complete removal of N (via N_2) (Jones et al. 2017; Grubba and Majtacz 2020; Dominika et al. 2021).

Recent studies have investigated the simultaneous competition for NO_3^- by DNRA and denitrification (Shan et al. 2016; Roland et al. 2018). The dominant process is dependent on the environment (Roland et al. 2018), such that DNRA is likely to outcompete denitrification in environments with a high organic matter to electron acceptor ratio when NO_3^- is limiting (Kelso et al. 1997), while denitrification dominates in higher NO_3^- and low C supply conditions (Kelso et al. 1997; Lind et al. 2013). A number of studies suggest a higher production of NH_4^+ occurring mostly in the upper portion of high C content soils with a lower NO_3^- load (Lind et al. 2013; Van Den Berg et al. 2015). Furthermore, in highly anaerobic habitats DNRA has a higher energy yield, accepting three additional electrons as compared with denitrification, and is therefore the favored process (Tiedje et al. 1982). Additionally, at high temperatures, DNRA is favored over

denitrification (Giblin et al. 2013), although Ribas et al. (2013) found the seasonal drying and freezing of the soils to stimulate DNRA. Furthermore, the soils of the PPR commonly contain high concentrations of organic matter and NH_4^+, along with sulfur that may contribute to the inhibition of nitrification-denitrification. The variable soil conditions across the PPR may allude to an under emphasized importance for DNRA in regulating NO_3^- in prairie potholes. Table 1.2 summarizes factors which may influence DNRA within potholes.

Many methods for measuring NH_4^+-N have been developed; however, many methods require relatively high NH_4^+ concentrations (Holmes et al. 1998). The diffusion disk procedure collects NH_4^+ by converting NH_4^+ to NH_3, which is then captured onto an acidified filter enclosed in polytetrafluoroethylene tape (Sørensen and Jensen 1991; Holmes et al. 1998). Although this method requires longer incubation periods (4 days or longer), there is increased precision and recovery of low concentration samples, while still applicable to a wide range of concentrations (Adamsen and Reeder 1983; Holmes et al. 1998). Due to the variable NH_4^+ concentrations across the PPR, and our interest in DNRA rates at ambient concentrations, a modified diffusion disk method as presented by Holmes et al. (1998) will be used.

1.2.4.2.2 Denitrification

There are few natural processes that are capable of counteracting the impacts of anthropogenic nutrient enrichment, but through the permanent removal of fixed N, denitrification is one of the most important ecological processes (Eyre et al. 2002). Denitrification is the anaerobic dissimilative pathway which reduces NO_3^- to N gas compounds (Sirivedhin and Gray 2005; Rütting et al. 2011) through the respiration of facultative bacteria (Seitzinger et al. 2006; Spanning et al. 2007). This involves stepwise reduction of NO_3^- or NO_2^- to intermediate products nitric oxide and nitrous oxide, which are further reduced with the formation of N_2 gas (Knowles 1982; Groffman et al. 2006; Lind et al. 2013). In some cases, the process is incomplete and nitrous oxide or nitric oxide are a product of this reduction pathway, rather than N_2.

Table 1.2 Summary of influencing factors and parameters on benthic processes; denitrification and DNRA.

Influencing Factor	Denitrification	DNRA
Location	Top mm of sediment (Seitzinger 1988; Crumpton and Goldsborough 1998), peak at 3 mm (Laverman et al. 2007)	Top of sediment with high organic matter to N content (Reddy and Delaune 2008)
Typical electron donor	Organic matter (Saunders and Kalff 2001)	Organic Matter (Reddy and Delaune 2008), H_2S and $S_2O_3^{2-}$ (Brunet and Garcia-Gil 1996; Burgin and Hamilton 2008)
Temperature	Temperature dependent (Brodrick et al. 1988; Reddy et al. 1989; Sirivedhin and Gray 2005), may cease below 4 °C (Limmer and Steele 1982; Pfenning and McMahon 1996)	Temperature dependent (Giblin et al. 2013), negative relationship (Rahman et al. 2019), Stimulated by drying and freezing soil conditions (Ribas et al. 2013)
Salinity	Negative relationship (Giblin et al. 2013)	Positive relationship (Gardner et al. 2006; Giblin et al. 2013; Deng et al. 2015)
Oxygen availability	Anaerobic (Vymazal 2007) or anoxic (Crumpton and Goldsborough 1998; Seitzinger et al. 2006)	Anaerobic (Tiedje et al. 1982)
Favorable environment	Correlated with organic carbon availability, requires high NO_3^- concentration (Tiedje et al. 1982; Sirivedhin and Gray 2005; Russell et al. 2018)	High donors: electron acceptors (high C: NO_3^- ratio) (Fazzolari et al. 1998; Rütting et al. 2011; Salk et al. 2017)
Dominant community	Bacteria (Seitzinger et al. 2006; Reddy and Delaune 2008; White and Reddy 2009)	Bacteria (Tiedje et al. 1989; Reddy and Delaune 2008), occasionally diatoms (Finlay et al. 1983; Kamp et al. 2015)
pH	Optimal between 6 and 8 (Paul and Clark 1996)	Favors alkaline environments (Nommik 1956; Gamble et al. 1977; Fazzolari and Germon 1991; Stevens et al. 1998),
Eh conditions	Negative trend with Eh (Brodrick et al. 1988), occurs below +350 mV (Vymazal 2007), moderately reduced conditions (Matheson et al. 2002)	Negative trend with Eh, must be less than 0 mV (Reddy and Delaune 2008), highly reduced (Matheson et al. 2002; Scott et al. 2008; Rütting et al. 2011)
Sulfide	Inhibits nitric oxide and nitrous oxide reductases and nitrification (Brunet and Garcia-Gil 1996; Lind et al. 2013; Cojean et al. 2020)	Stimulates DNRA via alternative electron donor, outcompete denitrification (Brunet and Garcia-Gil 1996; An and Gardner 2002; Rahman et al. 2019), can be coupled with H_2S oxidation (Burgin and Hamilton 2008)

The N oxides act as terminal electron acceptors in place of oxygen (Knowles 1982), but because oxygen is more efficient than NO_3^-, denitrification only occurs when oxygen is unavailable (Robertson and Groffman 2015) but NO_3^- is available. This typically occurs at the oxic-anoxic interface within the first few millimeters of sediment (Crumpton and Goldsborough 1998; Seitzinger et al. 2006) where the microorganisms which perform denitrification thrive. The microbes are commonly heterotrophic and use organic matter as an electron donor and NO_2^- or NO_3^- as the electron acceptor (Saunders and Kalff 2001; Seitzinger et al. 2006; Spanning et al. 2007). Alternatively, denitrification can be performed by chemolithotrophs, which use ferrous iron, sulfides, manganese, methane, or hydrogen as electron donors instead (Roland et al. 2018).

Due to the prominent roles both NO_3^- and organic matter play (Saunders and Kalff 2001; Bastviken et al. 2003; Seitzinger et al. 2006; Song et al. 2013), their availability and ratio to one another, is highly influential on the rate of denitrification (Hansen 2016). France and Mankin (1977) found microbial activity to decrease only after NO_3^- becomes limiting, unless the concentration is extremely elevated (> 6000 mg NO_3^- L^{-1}), in which the microbes may become completely inhibited. Lind et al (2013) and Blackmer and Bremner (1978) found high NO_3^- availability to inhibit the activity of the reductase responsible for the last step of denitrification, such that nitrous oxide is released rather than N_2. Hansen (2016) found the highest denitrification rates at a 1:1 ratio of dissolved organic C to NO_3^-. Davidsson and Stahl (2000) found denitrification to be highest in peaty soils rather than sandy loam, while Gale et al. (1993) found the highest rate with mineral rather than organic soils. Moreover, the availability of organic materials on the sediment surface, along with the sediment composition (Joye and Hollibaugh 1995), soil particle size (Xia et al. 2017), and the redox potential (Eh) of the sediment is more influential than the composition of the community performing the process (Bastviken et al. 2003).

The soils of the PPR are rich in minerals and organic matter (Richardson et al. 1994) and thus may have high denitrification rates, but this will depend on a variety of other factors including: NO_3^- loading, *in situ* nitrification, bioavailability of organic C, and temperature. A study performed by Reddy and Patrick (1984) found a two-fold increase in denitrification rate with each ten-degree increase in temperature. However, Uusheimo et al. (2018) found cold temperatures only slightly influence the N removal efficiency of wetlands, while Pfenning and McMahon (1996) found a complete halt below 4 °C. Due to the dramatic variation of

temperatures (–40 °C to +40 °C) and the elevated N loading, it is important to understand the rate, capacity, and factors at which pothole wetlands can transform NO_3^- of anthropogenic origins.

Among other variables important for denitrification are the aquatic vegetation composition (Song et al. 2010) and sulfide availability (Joye and Hollibaugh 1995). Sulfide can limit denitrification efficiency through the inhibition of nitrification, and thus interrupt the coupling of nitrification-denitrification, which is largely responsible for the regulation of N in sediments with low concentrations of free NO_3^- (Joye and Hollibaugh 1995). Interrupting the coupling of nitrification-denitrification could act as a positive feedback loop, enhancing primary production and eutrophication (Joye and Hollibaugh 1995). This is concerning as pothole sediments of the PPR can feature high sulfide content (McAdams et al. 2016). Another factor, which is critical in the PPR is the hydrology and morphometry of the pothole, considering factors such as retention time, depth, loading, and hydroperiods (Braskerud 2002), as this dictates the time NO_3^- is available for denitrifiers. Table 1.2 summarizes factors which may influence denitrification activity.

Although the broad importance and physiology of denitrification is well known, the rates of this process in prairie potholes are not well characterized. Estimates of denitrification rates in Canadian prairie potholes in the literature are scarce, but a N retention efficiency of over 60% in freshwater wetlands across the United States, Denmark, and Canada, suggest that it could be relatively high (Saunders and Kalff 2001). Additional literature found Prairie wetlands in Iowa can reduce up to 80% of externally loaded NO_3^- (Crumpton and Goldsborough 1998, Isenhart 1992). Similarly, wetlands within agriculturally dominated catchments in Denmark, much like those in the PPR, were responsible for the removal of most NO_3^- within the wetland (Lind et al. 2013).

Denitrification was first discovered in 1886 by Gayon and Dupetit, but the methods for robust exploration and quantification took years to develop (Galloway et al. 2013). The most recent technique for estimating denitrification is membrane inlet mass spectrometry (MIMS), developed by Kana et al. (1994). The precision of the MIMS is less than 0.05% for N_2:Ar and O_2:Ar (Kana et al 1994), providing a higher degree of accuracy and precision than previously used methods, such as gas chromatography (Barbaree and Payne 1967) and the acetylene block

method (Balderston et al. 1976). With use of isotope pairing techniques, MIMS provides highly sensitive methods for measuring denitrification activity (Kana et al. 1994; Lunstrum and Aoki 2016). However, some limitations do exist, such as the requirement for the extraction of sample from a previously subsampled water sample (Kana et al. 1994), the requirement for lower N fixation rates than those of denitrification (Eyre et al. 2002), and possible atmospheric interference during analysis (Eyre et al. 2002; Lunstrum and Aoki 2016). Regardless, Eyre et al. (2002) and Lunstrum and Aoki (2016) suggest isotope pairing techniques give comparable results to other methods, and effects of oxygen interference can be avoided using a heated inline copper reduction column to remove oxygen before analysis. Considering the precision and accessibility to equipment, and the variable concentrations of NO_3^- across the PPR, denitrification will be explored similar to methods outlined by Kana et al. (1994).

1.2.4.3 Synergistic potential for nitrogen retention

Planktonic uptake, DNRA, and denitrification are each unique, but also subject to a variety of physicochemical conditions. There is a great synergistic potential for N retention at the intersection of the benthic and open-water zones. These three processes display the potential potholes may have on mediating N pollution in water. Through the DNRA pathway, NO_3^- is recycled into NH_4^+, providing plankton reduced substrate to metabolize into biomass. Through this conversion, excess N is trapped and stabilized in organic matter or sediments and is likely only released through mineralization. Furthermore, denitrifying bacteria transform excess NO_3^- into the less harmful N_2 gas. By exploring the synergistic potential these processes have in prairie potholes, we can better understand the role of pothole wetlands for N retention on the landscape, and secure the health of our water resources.

2.0 Research overview

2.1 Research gaps and rationale

While prairie potholes are ubiquitous across much of the Prairies ecozone, they are largely a black box on the landscape for N transformations, despite the potential role they play in regulating export of N to downstream aquatic systems. There has been little research regarding how multiple processes interact and contribute to N retention and cycling in potholes. Biogeochemical measurements in the prairie potholes have included pesticide concentrations (Karpuzcu and Stringfellow 2012; Malaj et al. 2020b), greenhouse gas release (Bansal et al. 2016; Tangen and Bansal 2019), and SO_4^{2-} reduction rates (Dalcin-Martins et al. 2018) amongst others, but few kinetic measurements have been explored for N transformation and retention in potholes specifically, including the processes of denitrification, DNRA, and planktonic uptake. While they are not the only processes contributing to N retention, existing research suggests that denitrification and planktonic uptake have potential to be the most important processes in wetlands (Saunders and Kalff 2001), but DNRA could interact to compete with these processes. Determining what happens to N as it enters the system, whether it is taken up by plankton, transformed into another form of inorganic or organic N, or permanently removed, will provide insight into the role of pothole ponds in governing water quality across the Prairie landscape.

2.2 Research significance

Prairie potholes are subject to ever-changing landscapes and hydrological dynamics, creating unique ecosystems and processes therein. However, increase of anthropogenic nutrient inputs and modifications can alter rates of transformations, and retention capacity. By considering three nitrogen cycling pathways, planktonic uptake, DNRA, and denitrification, a better estimation of N dynamics and retention can be made in the PPR

Research of this nature can be used to improve nutrient budgets across the Prairies, support and guide the prediction of N behavior in prairie potholes, and supply information for future modeling exercises, enhancing the capacity of producers, managers, and agencies to make more informed nutrient-management and prairie pothole wetland management decisions. By developing an integrative understanding of multiple N processes, we can begin to

understand the capacity at which potholes may recycle, release, or retain N. Ultimately, this research can enhance our ability to better characterize ecosystem services provided by prairie potholes.

2.3 Research objectives

The overarching goal of this research is to explore how N is transformed by key N processes within prairie potholes, across select environmental gradients (e.g., pH, conductivity, temperature, and light) typically encountered at local to regional scales. Understanding the rates and controls of the transformation of inorganic N and urea into particulate organic N through *planktonic uptake,* the recycling of NO_3^- into NH_4^+ through *DNRA,* and the reduction of NO_3^- into N gases through *denitrification,* will be explored through three main objectives:

1) Measure open-water N uptake and assess controls on uptake, including planktonic biomass, seasonality, light availability, and key thresholds of nutrient availability.
2) Quantify the rate of benthic NO_3^- recycling via DNRA across gradients of pH and conductivity.
3) Quantify the rate of benthic NO_3^- removal through the denitrification pathway across gradients of pH and conductivity.

Open-water N uptake is hypothesized to be greater in light than in dark conditions, have greater rates at elevated concentrations than those at low concentrations, and follow Michaelis-Menten kinetics. Within the benthic zone, NO_3^- reduction is anticipated to be of great potential, with emphasis towards DNRA, rather than denitrification, due to the favorable characteristics of prairie potholes. Overall, N cycling within prairie potholes is expected to be significant, and validate their role as transformers on the landscape.

3.0 Methods

3.1 Site description

The St. Denis National Wildlife Area (SDNWA), within the Prairies Ecozone of Canada, near the Moist Mixed Grassland and the Aspen Parkland boundaries is an area devoted to research on waterfowl habitats and wetland ecology (Henderson 2013). The historic and current land use in the area can inform the impacts of land use practices across the prairies. At establishment (1968), 60% of the property was subject to plowed and cultivated crops, but has since transitioned to only 30% of active annual cropland (Henderson 2013). The 383 ha site features a matrix of grassland and cropland in hummocky moraine terrain with low hills. At the site, over 200 wetlands of varying size, permanence, and salinity (Henderson 2013) are surrounded by native or brome grassland, willow trees, and some cropland. The range in pothole wetland type within the SDNWA is attributed to the retreating glacier which deposited and carved the landscape into irregular knolls and knobs (Pennock 2005), underlain with soil of the Dark Brown Chernozem (Saskatchewan Centre for Soil Research 1989).

The regional climate of SDNWA is cool and subhumid (Environment and Climate Change Canada 2008; Henderson 2013), where precipitation averages 360 mm annually and evapotranspiration exceeds precipitation (van der Kamp and Hayashi 2009), limiting the development of surface flow through distinguished drainage systems (Richardson et al. 1994; Biswas et al. 2012). Although only one third of annual precipitation in the PPR occurs as snowfall, it accumulates in pothole depressions and produces 80% or more of annual surface runoff (Gray and Landine 1988; Fang and Pomeroy 2009). The snowmelt period, when frozen soils limit infiltration, is the major driver of pothole replenishment and connectivity (Gray and Landine, 1988; Waiser, 2001). During this period, spill-and-fill events distribute water (Spence 2003), as well as constituents (salts, nutrients, etc.) across the landscape (Seelig and DeKeyser 2006).

Pothole pond connectivity, or lack thereof, largely influences specific conductivity of individual ponds, with higher conductivity in the more stagnant, discharge ponds where salts can accumulate. Pothole ponds within SDNWA range from under 300 µS cm^{-1} (fresh) to over

137,000 µS cm^{-1} (saline) (Armstrong, 2018; Pham et al. 2008), while also being temporally dynamic, dependent on seasonal temperatures, hydroperiods, and pond characteristics. Furthermore, pond permanence and depth may range from deeper (> 5 m) permanent ponds, which are present year-round, to shallow ephemeral ponds, where surface water is present for less than a week immediately following snowmelt.

In this study, sites were selected to capture the variation in water chemistry (N concentrations, pH, and conductivity) and permanence classes seen throughout SDNWA. Additionally, data collected from a prairie-wide pothole survey in spring and summer of 2018 (n = 152, n = 234, respectively) and 2019 (n = 150, n = 141, respectively), further guided site selection and provided insight into the broad-scale variability of potholes. Study sites focussed on wetland classes I–V (ephemeral to permanent), as these systems are under the greatest threat from anthropogenic stressors. Pond permanence was determined using Stewart and Kantrud (1971). Additionally, given the history of SDNWA, some select sites had pre-existing long-term data that could benefit future studies.

3.2 Experimental approach

Though pothole ponds are relatively small (<1 ha), they can develop well defined ecological zones at which different processes take place, organisms reside, and physical features occur (vegetation, etc.). Due to these differences, this research has been grouped into two sets of experiments according to location of key processes within the pothole ponds. Planktonic uptake experiments were conducted using water collected from the open-water zone of a pond, where organisms can freely swim or float, and where most phytoplankton dwell. Bacteria that are responsible for denitrification and DNRA are predominantly found within the sediments and near the sediment-water interface, namely, the benthic zone. Both open-water and benthic process experiments were investigated in the lab using pond water and/or sediments as substrate for these experiments. To ensure experimental temperatures matched those of *in situ* temperatures, temperature loggers (HOBO MX2201) were placed in one pond at varying depths beneath the water surface (5 cm, 15 cm, 30 cm), and above the surface (air), to identify the temperature at the desired sampling depth. Though details are not presented here, at 0.15 m, the mean measured pond temperature (pond 88) in spring (May) was $10.7 \pm 3.1°C$, while in summer (August) the mean temperature was $17.6 \pm 3.8°C$, and in fall (October) the mean temperature was $5.2 \pm 1.4°C$.

Open-water experiments included ephemeral (n = 2), temporary (n = 2), seasonal (n = 9), semi-permanent (n = 2), and permanent (n = 1) ponds; benthic experiments used a subset of these ponds (with one exception) and included seasonal (n = 2), semi-permanent (n = 2), and permanent (n = 1) ponds (Table 3.1). Pond depth ranged from 10 cm to 318 cm and were primarily surrounded by grassland, with sedge grasses, cattails, and wooded vegetation along the perimeters. A summary of ponds used per each experiment (ambient/elevated, Michaelis-Menten, denitrification, DNRA) can be found in the appendix (Table A.1).

Table 3.1 Number of ponds sampled by permanence class during the study for each experiment type and season. Benthic experiments (summer 2019) used a subsample of ponds that were used for pelagic experiments (2018), with one exception. As expected, some ponds were dry prior to summer sampling; however, excessive heat and low rainfall expedited dry conditions in lower class ponds. Sample replicates and methods are detailed in Section 3.3.3, 3.4.3, and 3.4.4. Site characteristics can be found in Section 4.0 and the appendix.

Season	Month Sampled	Exp.	Ephemeral	Temporary	Seasonal	Semi-permanent	Permanent	Total
Spring	May	Ambient/ elevated	1	1	7	2	1	12
Spring	May	Michaelis-Menton	1	1	2	-	-	4
Summer	August	Ambient/ elevated, Michaelis-Menton	0 (dry)	0 (dry)	3	2	1	6
Fall	October	Ambient/ elevated, Michaelis-Menton	0 (dry)	0 (dry)	2	2	1	5
Summer	June, August	Benthic	-	-	2	2	1	5

To measure the above processes — DNRA, denitrification, and N uptake — we can strategically use different N isotopes. The radioactive isotopes of N range from ^{10}N to ^{25}N, but only two stable isotopes of N exist: ^{14}N and ^{15}N (He et al. 2009). Isotopes have nearly identical characteristics and behaviours, but differ in mass (Delaune et al. 2013). This allows for the use of ^{15}N as a "tracer", because its natural presence in the environment is 0.366 % of the concentration of ^{14}N (Chang et al. 2004). However, some methological limitations and assumptions exist with the use of isotope pairing analysis. There is an assumption of homogenous and uniform mixing with endogenous sources of ^{14}N, that each isotope is randomly paired and it does not affect the rate of the process (also assumed to be constant), that isotopic fractionation is negligible, and the diffusion of each molecule is similar (Nielsen 1992; Middelburg et al. 1996). Additionally, other phenomena that could affect isotope mixing are diffusion barriers, bioturbation, microoxic zones,

and reflux of ^{15}N (cell lysis, sediment mineralization, binding to humic substances) (Braun et al. 2018). Acknowledging these limitations and assumptions is important for understanding the robustness and accuracy of these analyses.

3.3 Open-water zone

3.3.1 Sample collection

To explore uptake kinetics across a range of seasonal temperatures, field collections were conducted at multiple sites in May (spring; n = 12), August (summer; n = 6), and October (fall; n = 5) of 2018 (Table 4.1). In spring, an additional 4 unique sites were used for Michaelis-Menton experiments (Table A.4). At each site, a multiparameter probe (YSI 600 XLM, Yellow Springs Instruments), was used to measure water temperature (°C), conductivity (μS cm^{-1}), dissolved oxygen (mg L^{-1}), and pH. Water samples were collected by dip sampling (10–20 cm below surface) where water depth was 20–30 cm and where sediments were not previously disturbed by wading. Site water was collected in acid-washed and site-rinsed HDPE bottles or 10 L carboys. Given shallow depths of the ponds, water samples are assumed to be representative of a well-mixed system. Samples were placed in a cooler on ice to slow activity and stabilize temperature for transport to the laboratory.

3.3.2 Lab analyses

Once at the laboratory, located at the Global Institute for Water Security, University of Saskatchewan, water samples were subsampled and processed for immediate analysis or held in refrigerated storage prior to analysis. Sample processing and analytical methods are described in Table 3.2. Briefly, a SmartChem™170 discrete analyzer (WESTCO Scientific Instruments, Inc.) was used to analyze NH_4^+-N, NO_3^--N, urea, SO_4^{2-}, total phosphorus (TP), total N (TN), and total dissolved phosphorus (TDP). Chl. *a* was analyzed using spectrophotometry (UV–1601PC, Mandel Scientific). External analysis for DOC occurred at University of Waterloo, using a Shimadzu TOC-L analyzer which combusts samples at >680 °C to convert CO_2, where it is then analyzed with non-dispersive infrared (NDIR) detectors (ASTM International 2018; Method D7573-18).

3.3.3 Planktonic uptake experimental procedures

Pelagic plankton N uptake rates were characterized during major growth seasons (spring, summer, fall) using isotope-enriched nutrient additions to site water at near-ambient

concentrations. Bacteria have been recognized to comprise a substantial proportion of the pelagic community (Kirchman et al. 1994; Løvdal et al. 2008; Yuan et al. 2012; Donald et al. 2013), and while we focus on phytoplankton (generalized as photosynthetic algae) throughout this thesis, "planktonic uptake" includes photosynthetic algae, non-photosynthetic algae, and bacteria residing in the water column. It is assumed N assimilation also occurs, but we do not explicitly quantify this process, as negligible excretion of the assimilated N compounds (regenerated as organic N) occurs (Dugdale and Goering 1967; Chan and Campbell 1978; Dugdale and Wilkerson 1986). N additions fell in the range of 10%–100% of *in situ* concentrations. The first experiment compared the rate of N (as urea, NO_3^-, or NH_4^+) uptake at ambient and elevated concentrations in light and dark treatments using ^{15}N as a tracer. Ambient concentrations were a nutrient spike of 10% of the *in situ* urea, NO_3^--N, or NH_4^+-N concentration. Dugdale and Goering (1967) suggest 10% of *in situ* levels will not further stimulate the rates of uptake or compromise the metabolism and biomass production. Elevated concentrations (spike of 80–100% of *in situ* urea, NO_3^--N, or NH_4^+-N concentration) were used to explore the extent of planktonic stimulation with supplementary nutrient availability, mimicking potential dosing after a heavy rain event or during spring melt. Two exposure treatments, light and dark, were also in place to explore the influence of light on uptake dynamics. To explore uptake kinetics and establish a rate and saturation point of each nutrient, a Michaelis-Menten experiment was also performed. A wider range of nutrient doses (10%, 30%, 60%, 80% and 100% of *in situ* concentrations) were used (light treatment) in this experiment. Maximum and minimum doses are summarized in Table A.2.

Immediately upon return to the lab, 50 mL of site water was added to 50 mL PET conical centrifuge tubes (Corning™ Falcon, Thermo Fisher Scientific), with two replicates of each treatment (ambient, elevated, suite of Michaelis-Menten treatments) for each analyte (NO_3^-, NH_4^+, or urea), and light condition. Additional samples were used as controls and for natural abundance analysis (n = 2, per site, per treatment). The tubes were placed in a water bath (light or dark) in a walk-in fridge with temperatures set to match field conditions (Spring: 10 °C; Summer: 20 °C; Fall: 5 °C). All tubes floated horizontally in the water bath to maximize exposure of each tube to available light and promote uniform depth for all tubes. Broad spectrum lights were deployed above the 'light' water bath and monitored using a quantum sensor (LiCor) for comparisons to natural conditions. Dark treatment tubes were wrapped in tin foil, covered by

a black garbage bag, then placed into a separate holding tank. A one-hour stabilization period acclimated samples to current conditions. Tubes were subsequently spiked with the appropriate N form and concentration using a 90% ^{14}N, 10% ^{15}N (98% purity) stock solution (100 mg L^{-1} K$^{14/15}$NO$_3^-$-N, 200 mg L^{-1} $^{14/15}$NH$_4$Cl-N, or 100 mg L^{-1} urea-N ((^{15}NH$_2$)$_2$CO)), then subject to an additional one-hour incubation period.

After the incubation, samples were vacuum filtered onto pre-combusted glass fiber filters (GF/F; 25 mm diameter, 0.70 µm pore size), suitable to capture phytoplankton, bacteria, and zooplankton (Teece and Fogel 2004). Both replicates were filtered onto one filter to ensure sufficient biomass. Filters were packed in tin boats for analysis at an external lab (UC Davis, Stable Isotope Facility). Briefly, sample ^{15}N (and ^{13}C) is measured using an elemental analyzer (PDZ Europa ANCA-GSL) interfaced to a continuous flow ratio mass spectrometer (PDZ Europa 20-20, Sercon Ltd., Cheshire, UK). In a reactor containing chromium oxide and silvered copper oxide, the sample is combusted at 1000 °C, followed by oxide removal through a reduction reactor (reduced copper) at 650 °C. After a helium carrier flows through a water trap, the N$_2$ and CO$_2$ are separated on a Carbosieve GC column (65 °C, 65 mL min^{-1}) prior to entering the mass spectrometer. Laboratory reference materials are interspersed during analysis and used to provisionally measure the relative isotope ratio in each sample. Using the reference material values from the batch analysis, values are corrected and finalized. The standard deviation from the reference materials is 0.3 permil. Because both sample replicates were processed onto one filter, sample variability cannot be reported.

Volumetric uptake was calculated according to Equation 1, modified from Kanda et al. (2003), Kumar et al. (2008), and Bhavya et al. (2016):

$$\text{Uptake (µg N L}^{-1}\text{ hr}^{-1}) = \frac{\left[\dfrac{P \times \Delta I_p}{T \times \left(\left(\dfrac{I_o \times S_a + I_r \times S_t}{S_a + S_t} \right) - I_o \right)} \right]}{V_F} \qquad 1$$

where P is particulate N post incubation (µg), ΔI_p is the difference in ^{15}N at-% (^{15}N at-% Lab – ^{15}N at-% natural abundance), S_a is the concentration of ambient N from all species + spike addition ^{14}N (µg L^{-1}), S_t is the added ^{15}N concentration (µg L^{-1}), I_r is the ^{15}N at-% added tracer (98% = 0.98), I_o is the ^{15}N at-% natural abundance of the sample, T is the incubation time (hr),

and V_F is the volume filtered through the filter (L). To account for variation due to pond biomass, rates were also normalized using *in situ* chl. *a* content.

The Michaelis-Menten equation (Equation 2), was used to quantify the uptake velocity (*v*) for different N species at select sites:

$$v = \frac{V_{max} \times [S]}{K_m + [S]} \qquad\qquad 2$$

where V_{max} represents the maximum velocity (μg N hr^{-1}) achieved by the system. K_m, the Michaelis constant, is the substrate concentration (*[S]*; μg L^{-1}) at which the reaction velocity is ½ V_{max}. Calculations were performed through the 'drc' package in R Studio, using a two parameter Michaelis-Menten model (drm) (Ritz et al. 2015; Ritz 2016)

Table 3.2 Summary of water chemistry sample processing, storage, and analysis. Methods as per SmartChem™170 discrete analyzer (WESTCO Scientific Instruments, Inc.), excluding chl. *a*.

Analyte	Processing Procedure	Storage	Colorimetric Analyses	Method Abbrev.	Reference Method Name
NO_3^--N	Filtered through 0.45 µm GF/F	−40 °C	Measures NO_2^- by reducing NO_3^- in a copperized cadmium redactor, diazotized with sulfanilamide, coupled with N-ethylenediamine dihydrochloride to form colored dye, measured at 520 or 550 nm, reports as $NO_2^- + NO_3^-$	WNO3	EPA 353.2, NO3-001-A
Urea	Filtered through 0.45 µm GF/F	−40 °C	Urea reacts with phenol to form indophenol yellow, measured at 480 nm	UREA	Navel Ship Research and Development Laboratory, 1967
SO_4^{2-}	As above	−40 °C	Converted to $BaSO_4$ suspension, stabilized by glycerin and sodium chloride to compare turbidity with standard curve at 420 nm	SO4Z	EPA 426C, SUL-001-A
TP	As above	Dark, room temp.	Ammonium molybdate and antimony potassium tartrate react to form antimony-phospho-molybdate complex, then reduced using ascorbic acid to form a blue-colored complex, measured at 880 or 660 nm	WP3D	EPA365.1, PHO-001-B
NH_4^+-N	As above, then preserved with 300 µL 10% H_2SO^4 (per 30 mL sample)	−40 °C	Reacts with alkaline phenol and then hypochlorite, forms indophenol blue, color is intensified through addition of sodium nitroprusside, measured 630 nm. Reports both NH_3 and NH_4^+	NH3K	EPA 350.1, AMM-001-A
TN	Unfiltered	−40 °C	All forms of N are converted into NO_3^- using potassium persulfate and NaOH digestion then analyzed for $NO_2^- + NO_3^-$ content through copperized cadmium column, then measured at 520 or 550 nm	TNAK	EPA 4500-N(org)
TDP	Unfiltered	Dark, room temp.	Manual persulfate digestion, whereby only organic P compounds form blue color, measured at 880 or 660 nm	WP3D	EPA 365.1, PHO-001-B
Chl. *a*	Subsurface collection in acid washed HDPE bottle, filtered through 0.7 µm GF/F	Light isolated petri dish, −40 °C	Filter residue extracted with 10 mL 95% ethanol, spectrophotometrically measures absorbance at 750, 665, and 649 nm against a 'blank' of ethanol	N/A	Wintermans and DeMots (1965)
NH_4^+-N Extraction using potassium chloride	filtered through 0.7 µm GF/F, analysed immediately	4 °C	Sample is buffered at a pH of 9.5 with borate buffer then distilled into a 2% solution of boric acid, NH_3 reacts with alkaline phenol then hypochlorite to form indophenol blue, measured colorimetrically at 630 nm	WNH3	EPA 350.1, AMM-001-A

3.4 Benthic zone

3.4.1 Sample collection

Benthic sampling (denitrification, n ponds = 5; DNRA, n ponds = 5) was limited to the summer months (July and August) of 2019. Due to their dynamic nature, coupled with record low spring rainfall and a warm summer (2019), some lower-class wetlands (ephemeral, temporary, seasonal) lost water more rapidly than expected prior to benthic experimentation dates and were not sampled. Sampled ponds were limited to seasonal (n = 2), semi-permanent (n = 2), and permanent (n = 1) classes. Water samples, sediment grabs, and physicochemical parameters were collected via wading into the pond 1–2 m from shore. Pond physicochemical parameters (water temperature (°C), conductivity ($\mu S\ cm^{-1}$), dissolved oxygen ($mg\ L^{-1}$), and pH) were collected *in situ* using a multiparameter probe (YSI 600 XLM, Yellowstone Scientific Instruments). Site water was collected in acid-washed site water–rinsed 10 L carboys by dip sampling 1–2 m from shore, at 10–30 cm below the surface, then laid along site shore to preserve *in situ* temperatures. Sediment grab samples were collected near the water collection site, but in areas free from any disturbance from previous wading. Two samples were collected using a short-handled flat headed shovel, with the upper 5–10 cm of sediment placed into clean sealable plastic bags. Water and sediment samples were placed on ice during transport to the laboratory to stabilize microbial activity and temperatures. Given shallow depths of the ponds, water samples are assumed to be representative of a well-mixed system.

3.4.2 Lab analyses

As described above for open-water samples, upon returning to the laboratory, sub-samples were processed for immediate analysis or processed then held in refrigerated storage prior to analysis for NO_3^--N, NH_4^+-N, urea, SO_4^{2-}, TP, TDP, and TN. Chl. *a* was processed and analyzed immediately. Analyses are summarized in Table 3.2.

Sediment samples were analyzed for organic matter (OM) content, sediment pH, and sediment conductivity. To improve storage conditions and for additional analyses, sediment was air dried, sieved to 2 mm, subsampled, and further oven-dried at 105 °C to vaporize residual water content. OM content was determined by loss-on-ignition (LOI; EMASC-001; Schumacher 2002); samples were weighed, combusted at 400 °C for 16 hours to remove organic material, then reweighed to determine OM content. Using the air-dried sediment, pH and conductivity

were also determined. Briefly, 20 mL deionized water was added to 10 g of sediment, stirring the suspension periodically for 30 minutes, followed by a 1-hr rest period, after which pH was measured using a hand-held electrode (YSI 600 XLM, Yellow Springs Instruments) (Ziadi and Sen Tran 2006). Sediment conductivity measurements were also obtained by the same hand-held electrode, following modified methods from Kalra and Maynard (1991). After creating a 1:5 soil suspension with 10 g dried sediment and 50 mL deionized water in an Erlenmeyer flask, mechanical shaking followed for 2.5 hours at 30 rpm to dissolve soluble salts.

Sediment used in DNRA experiments was also subject to an additional salt based NH_4^+ extraction to identify remaining NH_4^+-N using methods modified from Whitfield et al. (2006). In short, 40 mL of 2.0 M potassium chloride solution was added to 5.0 g fresh sediment in a 50 mL falcon tube and shaken end-to-end at 100 rpm for 15 minutes. After which, tubes were centrifuged at 3200 rpm for 15 minutes, and the supernatant poured off and filtered through a 0.45 µm GF/F, then analyzed on a SmartChem™ 170 discrete analyzer (WESTCO Scientific Instruments, Inc.; method EPA 350.1, AMM-001-A in Table 3.2).

3.4.3 DNRA experimental procedures

Immediately upon return to the lab, slurries were constructed by measuring and transferring 25 mL of sediment into a pre-weighed and combusted media bottle (Fisherbrand™, Thermo Fisher Scientific), then filled with ~135 mL site water (~1:5 vol. ratio). To eliminate any residual headspace, water was placed in the cap then swiftly sealed in one fluid motion, creating the reaction vessel. To eliminate sample loss during chemical addition in later steps, a 7 mL N_2 headspace was introduced through the septa, using a hypodermic needle, immediately after capping the vessel. The primary phase included an initial incubation of 24 hours, which allowed vessels to develop hypoxic conditions prior to experimental procedures. During this time, vessels were placed on a shaker table at room temperature and 100 rpm to promote oxygen release from the sediments. One replicate was used to ensure a hypoxic state of <2 mg L^{-1} dissolved oxygen was achieved within the initial 24-hour incubation (measured using a multiparameter probe [YSI 600 XLM, Yellowstone Scientific Instruments]). While oxygen could diffuse into the sample upon opening the vessel for insertion of the multiparameter probe, any oxygen replenishment during this short time window was negligible as these tests revealed the samples to be hypoxic.

Following the primary phase to create anoxic conditions, vessels were subject to a secondary (treatment) phase. A $K^{14/15}NO_3^- $-N (90% ^{14}N, 10% ^{15}N; 98% purity) spike was introduced to 9 replicates per site (6 replicates for a 4-hr incubation and 3 for 1-hr incubation) to mimic natural conditions and to enhance measurability of activity, while 6 replicates remained unspiked (Table 3.3). Preliminary experiments revealed considerable variation in enriched samples after an extended incubation, therefore, additional replicates were used for the 4-hr incubation. Treatments were administered by submerging vessels in water (to ensure atmospheric isolation), followed by needle insertion through the septa to inject 562.5 µL of a 3.6 mg L^{-1} $K^{15}NO_3^-$-N solution, for a final concentration of 0.015 mg L^{-1} $K^{15}NO_3^-$-N (0.130 mg L^{-1} $K^{14/15}NO_3^-$-N). Vessels were lightly hand shaken to homogenize treatment, then placed onto the shaker table for the designated incubation time. Two time point incubations, 1-hr and 4-hr, were used to increase the likelihood of observing measurable rates. Unspiked bottles, used for natural isotopic abundance analysis, as well as ambient nutrient dynamics were treated identically, with the exception of the spike treatment.

Following the secondary incubation phase (1- or 4-hr), the slurry was terminated with the addition of 0.15 g (2.0 M) sodium chloride; which was added to the reaction vessel to release any NH_4^+ bound to cation exchange sites on the sediment, and shaking continued for one hour. Potassium chloride is commonly used in sediment extractions; however, Sahrawat (1979) found a strong sodium chloride solution was as equally efficient as a potassium chloride solution. The sodium chloride also supplements the movement of water via osmosis and prevents the filter from bursting (Holmes et al. 1998) in later steps. A rest period following the incubation promoted sediment settling, allowing the overlaying water to be poured off and filtered through 0.70 µm pore glass fiber filters to halt microbial processes. Next, 50 mL of filtrate was transferred into a secondary reaction vessel (pre-combusted 100 mL media bottle). Immediately after filtration, 0.15 g of magnesium oxide (final concentration of 0.074 M) and a filter packet were added simultaneously to the media bottle, and the vessel sealed. Adding magnesium oxide (MgO) creates basic conditions and transforms the $^{15}NH_4^+$ produced by microbes into $^{15}NH_3$, attracting it to an acidified filter treated with 25 µL of 2.5 M potassium bisulfate. The filter is encapsulated by polytetrafluoroethylene tape (Teflon), which is hydrophobic, but permeable to gaseous NH_3. Within the filter packet, $^{15}NH_3$ reacts with potassium bisulfate, becoming trapped on the filter (Holmes et al. 1998). The reaction vessels were incubated at room temperature on a

shaker table at ~100 RPM for 3 weeks, after which the filter packs were removed from solution, rinsed with 10% HCl (by volume) and MilliQ, and placed into scintillation vials until packed appropriately for isotope analysis. Prior to shipping for analysis by a coupled elemental analyzer-isotope ratio mass spectrometer (UC Davis, Isotope Laboratory, California), the filters were removed from the Teflon and packed into tin boats. The ^{15}N on the filter represents the ^{15}N-labelled NO_3^- converted to ^{15}N-labelled NH_4^+. Rates were then calculated using the following equations according to Porubsky et al. (2008), Deng et al. (2015), and Shan et al. (2016).

The production of $^{15}NH_4^+$-N, $^{15}_pNH_4^+$, was determined through Equation 3:

$$^{15}_pNH_4^+ \; (\mu g \; ^{15}N \; g^{-1}hr^{-1}) = \frac{\left([^{15}NH_4^+]_f - [^{15}NH_4^+]_i\right) \times vol}{T \times mass} \qquad 3$$

where $[^{15}NH_4^+]_i$ is the natural abundance concentration of $^{15}NH_4^+$-N ($\mu g \; L^{-1}$), $[^{15}NH_4^+]_f$ is the final concentration of $^{15}NH_4^+$-N ($\mu g \; L^{-1}$) of the sample, vol is the sample solution volume (L), $mass$ is the dry sediment weight (g), and T is the incubation time (hr).

DNRA rates were then calculated using the mean $_p^{15}NH_4^+$ of site replicates:

$$DNRA \; (\mu g \; N \; g^{-1}hr^{-1}) = \frac{\left(^{15}_pNH_4^+{}_{spk} - ^{15}_pNH_4^+{}_{unspk}\right)}{\dfrac{[^{15}NO_3^-]}{[NO_3^-{}_{total}]}} \qquad 4$$

Where $_p^{15}NH4^+{}_{spk}$ is the production of $^{15}NH_4^+$-N of spiked samples, $_p^{15}NH_4^+{}_{unspk}$ is the production of $^{15}NH_4^+$-N of the unspiked samples, thereby accounting for potential mineralization, $^{15}NO_3^-$ is the background plus the added $^{15}NO_3^-$-N concentration, and $NO_3^-{}_{total}$ is the total $^{14/15}NO_3^-$-N concentration (spike additions plus *in situ* NO_3^--N).

Table 3.3 Replicate allocation for denitrification and DNRA experiments. Treatment additions were aimed to enhance and stimulate activity.

Secondary phase incubation period	Spike treatment	Number of replicates
4 h	spike	6
1 h	spike	3
4 h	unspiked	3
1 h	unspiked	3

3.4.4 Denitrification experimental procedures

Incubation periods, spike additions, and site selection were identical and simultaneous to DNRA experiments to allow for direct comparisons. Slurry samples used for denitrification were also constructed identically to that of DNRA, excluding the added headspace. Briefly, a 1:5 slurry was created and subject to an initial 24-hour incubation to establish hypoxic conditions. Following initial incubation, samples were subject to the secondary phase, much like that of DNRA. Identical to DNRA, 562.5 µL of a 3.6 mg L^{-1} $K^{15}NO_3^-$-N solution, for a final concentration of 0.015 mg L^{-1} $K^{15}NO_3^-$-N (0.130 mg L^{-1} $K^{14/15}NO_3^-$-N) was injected into treatment samples while the vessel was submerged. Samples were lightly hand shaken, and placed onto the shaker table at 100 rpm for the designated incubation period (1- or 4-hr). The remaining samples, used for ambient dynamics and natural isotopic abundance analysis, did not receive a nutrient addition but were handled identically (Table 3.3).

The secondary phase incubations were terminated at the respective time interval and the overlaying water from the replicate was subsampled into five evacuated 12 mL Exetainers® (Lab Co.) through a syringe and hose (to reduce atmospheric interaction). The hose was inserted to the bottom of the Exetainer, and slowly raised with the increasing water level, remaining just below the surface. Next, a 500 µL of 20% zinc acetate (ZnAc) was injected via hypodermic needle, to terminate all biological processes (Roland et al. 2018), and immediately capped tight, carefully avoiding bubbles. To ensure isolation of dissolved gas in the exetainers, they were stored underwater at 4 °C to prevent degassing, until analysis. Samples were analyzed for $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ within 24 hours of termination via membrane inlet mass spectrometry (MIMS), with use of a copper reduction coil and furnace to minimize oxygen interference, as found by Eyre et al. (2002, 2013), Lunstrum and Aoki (2016).

The MIMS signal for ^{29}N and Argon (Ar) were background corrected and adjusted for drift by manually fitting polynomial curves to the data. The difference in ^{29}N to Ar ratios between spiked and unspiked samples was determined and used with calculated Ar solubilities (Hamme and Emerson 2004) for each site to determine $^{29}N_2$ production. Sites with very noisy signals or for which a drift curve was not robust were excluded (n = 1). Total N_2 production was then quantified assuming ^{14}N and ^{15}N bind randomly to form N_2 gas (An et al. 2001). Lastly, denitrification rates (µg g^{-1} hr^{-1}) were determined using N_2 production quantified by MIMS,

volumes of water in the reaction vessels, dry sediment mass used in the incubation, and duration of the incubation.

3.5 Statistical analyses

All statistical analyses were performed in RStudio 1.3: A language and Environment for Statistical Computing and Graphics (R Core Team 2016). Sample sizes were small, and the data, as well as the residuals, collected in all three experiments of this study were determined to be non-normal via a Shapiro-Wilk test ($p < 0.01$), therefore, non-parametric statistics were used. All significance testing was determined at $\alpha = 0.05$ (95% confidence), and α values were adjusted (Holm correction) where multiple comparisons were made.

3.5.1 Open-water zone

Wilcoxon signed rank tests are the nonparametric analogue to a paired t-test (Kabacoff 2011), exploring the difference in median rates among paired samples. This test was used to test for difference in uptake rates between ambient and elevated concentrations of N. This method was also used to test for significant differences in light and dark conditions on uptake rate. For analyses where multiple observations were available for a single site (e.g. comparing uptake rates between different N forms), observations for different seasons were averaged for each site prior to analysis.

Due to the power and flexibility of permutation ANOVAs, they can be used on nonparametric data with non-normally distributed errors, outliers, and small sample sizes (Kabacoff 2011). Essentially, this method calculates a test statistic using random permutations of the data, creating a theoretical distribution to be compared to that of the observed sample data for which a p-value is calculated. A two-way permutation ANOVA was used to investigate whether there were differences in uptake across seasons and analytes. A pairwise permutation test with Holm correction was used to explore interactions and identify pairwise differences.

Environmental parameters such as conductivity, pH, and chl. *a* content may influence the rate of nitrogen uptake. Though acknowledging the possibility of underlying interactions among the variables, developing robust models to predict and analyze trends is outside the scope of this project. Instead, Kendall's τ rank correlation was used to explore relationships with water chemistry. This nonparametric test measures the strength of dependence between two variables (Zaid 2015), and is relatively robust to outliers and small sample sizes (Mangiafico 2016). The

correlation coefficient values are reported as *Tau* (τ) and range from +1 to –1 (0 represents no correlation) (Mangiafico 2016). Data used in these tests were limited to ambient concentrations and light treatments to mimic natural conditions.

3.5.2 Benthic zone

Due to low sample sizes, statistical analyses on benthic data were limited. Denitrification activity at site 88 could not be corrected for instrument noise and was removed from analysis. Therefore, no formal statistics were conducted on denitrification data (n = 4 ponds). A Kendall's τ rank correlation with Holm adjustment was used to test for correlation of chemical parameters with DNRA, though sample sizes were low. Meaningful differences between denitrification and DNRA data were assessed through propagation of error on observed rates for each site. Propagation of error was performed using the standard deviation of the average rate for each site and treatment (Fantner 2013).

4.0 Results

4.1 Open-water zone: Planktonic uptake

4.1.1 Site characteristics

Study sites at SDWNA exhibited a broad range of characteristics, including water chemistry, and pond permanence (Table 4.1). Sampling across multiple seasons led to a wide fluctuation in conditions at individual ponds. Permanent ponds ranged in depth (50–150 cm) at sampling, and were typically deeper than seasonal, temporary, and ephemeral ponds in spring, many of which had maximum depths less than 50 cm and dried out over the summer. Pond conductivity ranged from 492–3360 μS cm^{-1} and individual ponds fluctuated by 200–1400 μS cm^{-1} across seasons, reaching maximum conductivity in fall. There were also seasonal fluctuations in pH for all ponds. All ponds were circumneutral to basic (7.45–9.65 pH units). At individual ponds the range in pH was 0.45–1.5 units across seasons. Concentrations of chl. *a* fluctuated from 0.03–2600 μg L^{-1} (across all ponds and seasons). Inorganic N (NH_4^+, NO_3^-) concentrations were low (below detection limits–0.20 mg N L^{-1}), while urea ranged from 0.08–0.53 mg L^{-1} (Figure 4.1, A.1).
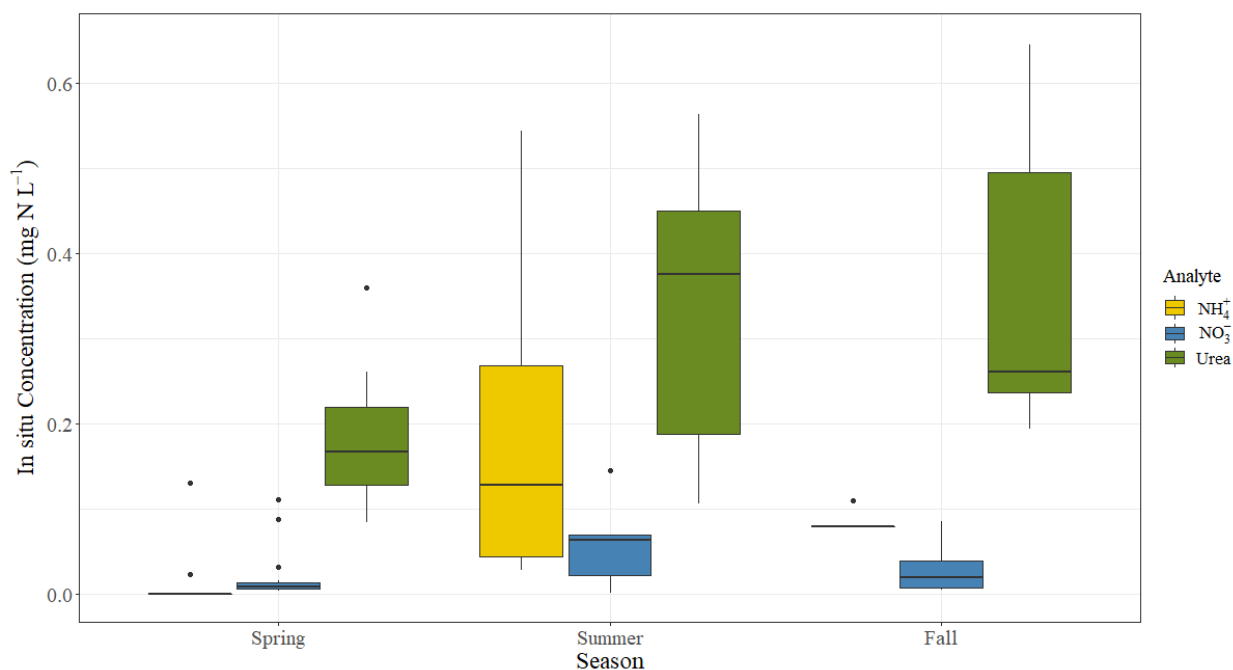
Figure 4.1 *In situ* concentrations of NH_4^+-N (yellow), NO_3^--N (blue), and urea (green) measured within study ponds at St. Denis National Wildlife Area across three seasons: spring (n = 48), summer (n = 18), fall (n = 15).

Table 4.1 Characteristics of five ponds used to measure uptake in all three seasons at St. Denis National Wildlife Area, including permanence class, water depth, and water chemistry. Details regarding ponds that dried prior to summer and fall sampling can be found in Table A.3. Additional ponds used for Michaelis-Menten experiments are detailed in Table A.4. Analyses below the method detection limit are indicated. "-" indicates value not available.

Pond	Seasons sampled	Permanence	Approx. max depth cm	Conductivity $\mu S\ cm^{-1}$	pH pH units	NO_3^--N mg L^{-1}	NH_4^+-N mg L^{-1}	Urea mg L^{-1}	TN mg L^{-1}	SO_4^{2-} mg L^{-1}	Chl. a µg L^{-1}	TP mg L^{-1}
	Spring			1890	8.04	0.016	<0.01	0.088	1.83	1000	32.3	0.150
1	Summer	Permanent	318	2780	8.73	<0.02	0.029	0.107	2.60	2290	56.5	0.202
	Fall			2890	8.50	0.086	0.080	0.262	2.30	-	81.6	0.242
	Spring			1650	8.32	<0.02	<0.01	0.084	1.76	-	0.03	0.108
20	Summer	Semi-perm.	70	2650	9.49	0.071	0.149	0.450	2.81	1990	24.7	0.169
	Fall			2760	9.65	<0.02	0.080	0.194	2.03	-	11.5	0.108
	Spring			492	7.66	<0.02	<0.01	0.127	0.90	120	0.91	1.59
88	Summer	Semi-perm.	60	563	8.88	<0.02	0.029	0.150	3.00	64.2	16.0	1.05
	Fall			700	7.45	<0.02	0.110	0.496	3.40	100	359	1.66
	Spring			622	7.84	<0.02	0.132	0.180	2.02	234	3.46	0.853
103	Summer	Seasonal	50	12.1	7.85	0.145	0.089	0.564	3.67	305	652	2.79
	Fall			1260	7.84	0.039	0.080	0.646	4.95	269	2640	2.66
	Spring			782	7.54	0.088	<0.01	0.122	1.54	291	17.0	0.440
109	Summer	Seasonal	80	1240	7.70	0.058	0.544	0.303	3.17	448	4.76	1.06
	Fall			1180	7.80	0.020	0.080	0.237	2.72	592	49.9	0.315

4.1.2 Nitrogen availability

Nitrogen uptake rates varied by several orders of magnitude across the seasons, form (NH_4^+, NO_3^-, urea), and light conditions (light vs. dark) investigated for both ambient and elevated N concentrations (Figure 4.2). At ambient N concentration, uptake rates ranged from 0.0015 to 16,100 µg N L^{-1} hr^{-1}, while at elevated N concentrations uptake rates ranged from 0.016 to 19,600 µg N L^{-1} hr^{-1}. Uptake rates (across all N species, ponds, and seasons) at elevated concentrations were not significantly higher than that at ambient concentrations (Wilcoxon signed rank test with Holm correction; $p = 0.92$). Furthermore, there was no significant difference between uptake rates at elevated and those at ambient concentrations for all analytes; NH_4^+ ($p = 0.63$), NO_3^- ($p = 0.31$), urea ($p = 0.98$), as determined by averaging across season and light treatment per site. Mean rates at elevated concentrations were 9% lower, 41% greater, and 52% lower than mean rates at ambient concentrations for NH_4^+, NO_3^-, and urea, respectively (Figure 4.2). These findings suggest elevated N concentrations may not statistically influence uptake rates in these study ponds. Moreover, in some cases, the spike amount (which was proportional to *in situ* concentrations) was small (in absolute terms); thus, differences may not be easily measurable. Because ambient concentrations better represent *in situ* conditions at the time of sampling, the following sections will focus on ambient N concentrations only. Uptake per unit biomass was also considered, though not statistically explored (Figure A.2). For full display of concentrations, light conditions, and seasons, see Figure A.3.
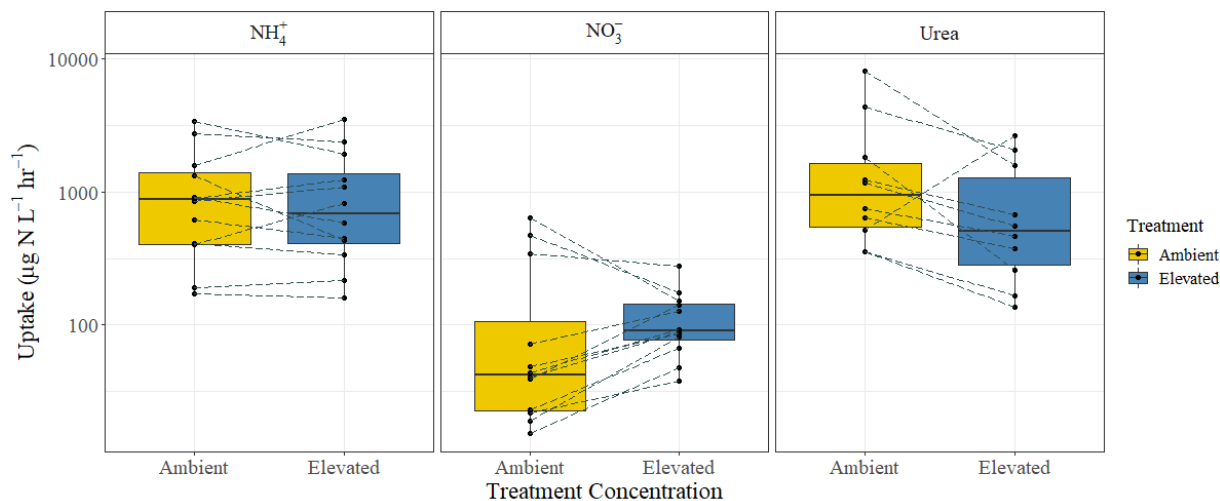
Figure 4.2 Uptake rates (µg N L−1 hr−1) observed across N analytes at ambient (10% of in situ, yellow) and elevated (80%−100% of in situ, blue) concentrations. Dashed lines connect sites, and observations were averaged across seasons for each pond (n = 12). Urea treatments were not applied to two ponds in spring (pond 62b and pond 13b).

4.1.3 Light availability

Uptake rates (across all N species and ponds) at ambient N concentrations ranged from 0.083 to 15,600 µg N L^{-1} hr^{-1} in light conditions. In dark conditions, ambient uptake rates ranged from 0.0015 to 16,100 µg N L^{-1} hr^{-1}. To compare uptake rates at light and dark conditions, uptake rates were averaged across season and analyte per site. At ambient concentrations, light uptake was significantly higher than that in dark conditions ($p = 0.03$). Uptake rates of NH_4^+ and urea were highly variable for both light and dark conditions, while NO_3^- uptake was more constrained (Figure 4.3). Averaging by season, uptake under light conditions (ambient concentrations) was significantly higher than under dark conditions for NH_4^+ ($p = 0.006$), but (marginally) insignificant for NO_3^- ($p = 0.08$) and urea ($p = 0.08$). Regardless, meaningful differences were evident between light and dark uptake rates; mean light uptake was 54%, 45%, and 47% greater than mean dark uptake for NH_4^+, NO_3^-, and urea, respectively. Because light can be important for uptake, analyses in the sections that follow focus on light conditions (and ambient concentrations, as described above). See Figure A.3 for a full display of concentrations, light conditions, and seasons.
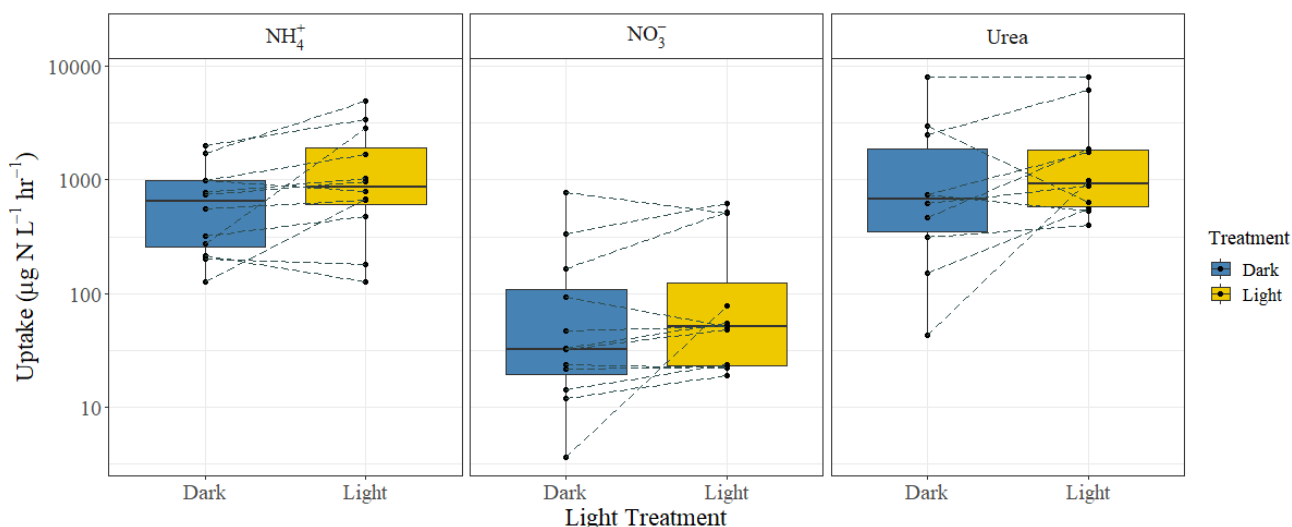
Figure 4.3 Ammonium, NO_3^-, and urea uptake rates (µg N L^{-1} hr^{-1}) at ambient N (N addition was maintained at 10% of *in situ*) concentrations, under light and dark conditions. Dashed lines connect sites. Observations across season were averaged for each site (represented by a black circle: n =12, with exception of urea: n=10).

4.1.4 Season and analyte preference

Temperature may have been a driver in uptake rates, as rates occurring in summer were generally greater than those in both spring and fall. The maximum rate (at ambient concentrations) observed in summer (20 °C) was 15,600 μg N L^{-1} hr^{-1}, while the maximum rate in spring (10 °C) and fall (5 °C) was 2650 μg N L^{-1} hr^{-1} and 11,900 μg N L^{-1} hr^{-1}, respectively. A pairwise permutation ANOVA with Holm correction for multiple testing revealed both the form of N ($p = 0.002$) and season ($p = 0.003$) had a statistically important effect on uptake rates at ambient conditions (in light), though the interaction of N form and season was not significant. Uptake rates were significantly higher under conditions with warmer water temperatures: uptake in summer (20 °C; $p = 0.006$) was significantly higher than spring (10 °C), but was not significantly different than fall (5 °C) (Figure 4.4). While urea and NH_4^+ uptake rates were not significantly different from one another ($p = 0.54$), NO_3^- uptake was significantly lower than both NH_4^+ ($p = 0.03$) and urea ($p = 0.03$). These results suggest NO_3^- uptake is consistently low across all seasons and will be excluded from further statistical analysis.
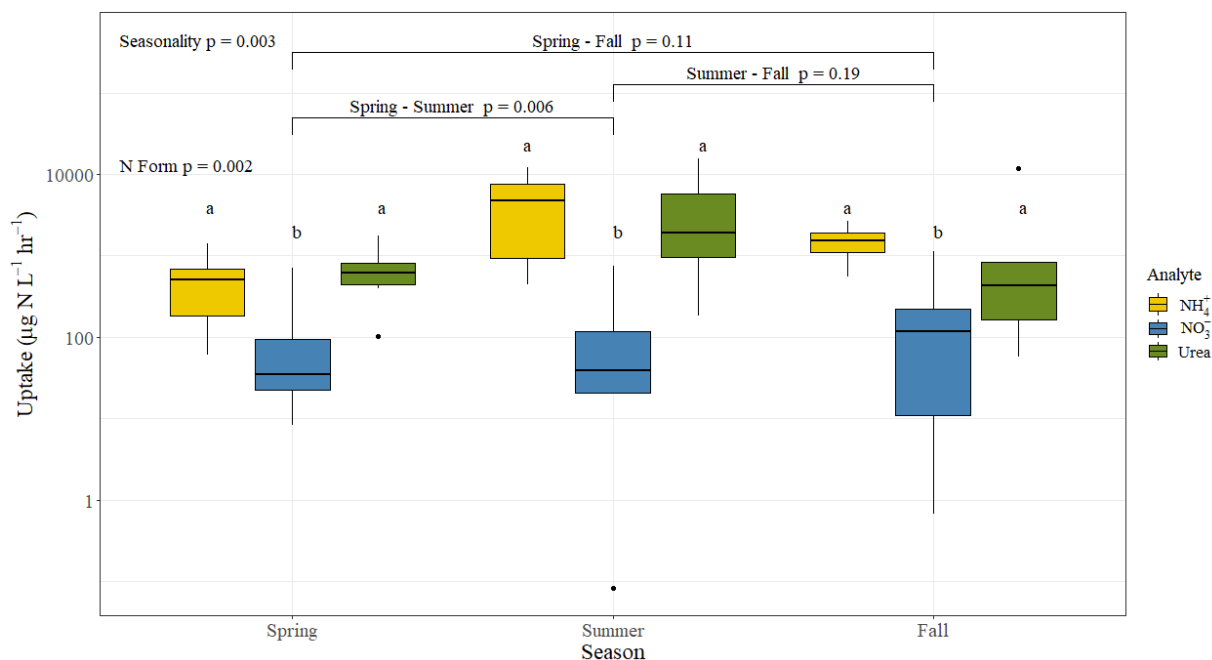
Figure 4.4 Uptake rates (μg N L^{-1} hr^{-1}) at ambient concentrations of N analytes (N addition was maintained at 10% of *in situ*) across seasons under light conditions. The horizontal brackets indicate the statistical differences between seasonal uptake rates. The significant difference (p < 0.05) between analyte-specific uptake rates are denoted by different lowercase letters.

4.1.5 Pond permanence and season

Ponds from five permanence classes (ephemeral, temporary, seasonal, semi-permanent, and permanent) were included, and season consisted of three levels: spring (10 °C, n = 12), summer (20 °C, n = 6), fall (5 °C, n = 5). Due to the nature of low permanence ponds, namely ephemeral and temporary ponds, the number of ponds sampled across seasons was not consistent. In addition to various drivers which may also be acting on ponds, the unequal and low seasonal sample sizes are problematic for accurate statistical testing required to explore the influence of pond permanence on seasonal uptake rates and were not performed. Considering that NO_3^- uptake was not found to vary across season, no significant differences were found between NH_4^+ and urea uptake, and NH_4^+ uptake is more widely understood, uptake rates of NH_4^+ were used to illustrate the potential influence of seasonality and pond permanence on NH_4^+ uptake (Figure 4.5).
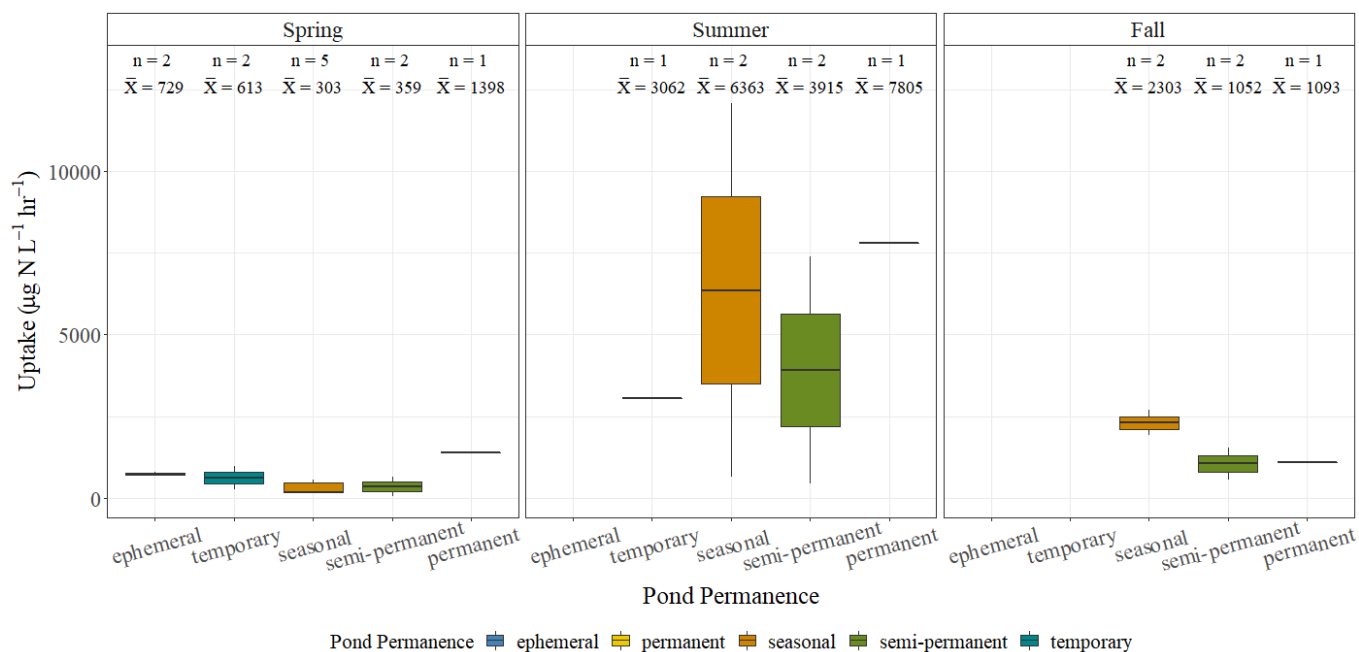
Figure 4.5 Uptake rates of NH_4^+ at ambient concentrations under light conditions across pond classification (permanence) and by season. Less permanent ponds dried up across season and could not be sampled in summer and fall. Sample size (n) and average uptake rate (μg N L^{-1} hr^{-1}) are displayed.

4.1.6 Environmental parameters

The relationships between various environmental parameters and uptake rates (at ambient concentration and light conditions) were explored using Kendall's τ correlation with Holm correction. Tests were performed on NO_3^- uptake rates, as they were significantly different than that of NH_4^+ and urea, in addition to NH_4^+ uptake rates, as they were not significantly different from urea. It is important to note the low sample sizes associated with this grouping may have consequences on the strength of analysis. Nitrate uptake rates in spring and summer had the strongest relationship with chl. a ($\tau = 0.61$, $p = 0.04$; $\tau = 0.76$, $p = 0.08$, respectively), while the strongest, though insignificant, relationship in fall was with pH ($\tau = -0.35$, $p = 0.57$). The strongest relationship between NH_4^+ uptake and environmental parameters was chl. a across all seasons. Ammonium uptake in spring was significantly correlated to chl. a ($\tau = 0.686$, $p = 0.01$), while summer and fall uptake did not have a significant relationship ($\tau = 0.76$, $p = 0.08$; $\tau = 0.804$, $p = 0.10$, respectively). All resulting τ and p values between environmental parameters and NO_3^- or NH_4^+ uptake rates are reported in Table 4.2 and Table 4.3, respectively.

Table 4.2 Test results of Kendall correlation with Holm correction between NO_3^- uptake rates and environmental parameters of interest.

Environmental parameter	Spring n = 12		Summer n = 6		Fall n = 6	
	τ	p value	τ	p value	τ	p value
Conductivity	−0.050	0.88	0.125	0.81	−0.337	0.58
pH	0.084	0.80	−0.050	0.93	−0.348	0.57
Chl. a	0.608	0.04	0.760	0.08	−0.128	0.84

Table 4.3 Test results of Kendall correlation with Holm correction between NH_4^+ uptake rates and environmental parameters of interest.

Environmental parameter	Spring n = 12		Summer n = 6		Fall n = 6	
	τ	p value	τ	p value	τ	p value
Conductivity	0.235	0.46	0.125	0.81	−0.705	0.18
pH	0.205	0.52	−0.049	0.93	−0.755	0.14
Chl. a	0.686	0.01	0.760	0.08	0.804	0.10

4.1.7 Michaelis-Menten uptake kinetics

Michaelis-Menten kinetics were used to explore N uptake across seasons and analytes. In addition to sites identified in Table 4.1, which were used for summer and fall Michaelis-Menten experiments, sites in Table A.4 in the appendix, were used in spring experiments. In many cases, attempts to fit a Michaelis-Menten curve revealed that this relationship was either poorly described, or a plateau was not reached under the conditions tested. In spring, NH_4^+ and NO_3^- saturation was not achieved, and estimated K_m values were above *in situ* concentrations of both, while in summer, NH_4^+ and NO_3^- uptake was poorly described by a Michaelis-Menten curve. Urea uptake across all seasons, as well as NO_3^- and NH_4^+ uptake in fall, could not be described by Michaelis-Menten kinetics. All K_m and V_{max} values are reported in Table A.5.

4.2 Benthic zone

4.2.1 Site characteristics

Five of the sites used for uptake experiments were also used to explore benthic N cycling. The five sites were investigated for benthic processes during summer 2019. At the time of sampling, sites had maximum water depths of 60–318 cm and exhibited a range in water chemistry and sediment characteristics (Table 4.4). Pond conductivity across all sites ranged from 557–6990 μS cm^{-1}, pH ranged from 8.33–9.68, SO_4^{2-} ranged from 103–5100 mg L^{-1}, while NO_3^--N varied from 0.019–0.045 mg N L^{-1}. Sediment conductivity ranged from 599–3750 μS cm^{-1}, sediment pH ranged from 6.02–8.06 units, and OM ranged from 5.8–28.7%.

Table 4.4 Depth, water chemistry and sediment characteristics for sites sampled in summer 2019 (June–September) for benthic experiments. Organic C was approximated using the van Bemmelen factor. Values below the method detection limit are indicated via '<'.

Site	Pond permanence	Approx. max depth	NO_3^--N	DO	*In situ* pH	*In situ* conductivity	SO_4^{2-}	OM	Organic C	Sediment pH	Sediment conductivity
		cm	mg L^{-1}	mg L^{-1}	pH units	μS cm^{-1}	mg L^{-1}	%	%	pH units	μS cm^{-1}
1	Permanent	318	0.045	7.05	8.33	3360	1970	5.8	3.36	7.62	1500
20	Semi-permanent	70	0.028	5.67	9.68	2790	1990	15.8	9.16	7.99	1330
63	Seasonal	75	0.022	4.69	8.78	6990	5100	28.6	16.6	8.06	3750
88	Semi-permanent	60	0.039	4.07	8.51	557	103	20.2	11.7	6.02	1220
109	Seasonal	80	<0.02	2.37	9.04	1180	543	10.9	6.32	7.1	599

4.2.2 DNRA

The maximum DNRA rate observed was 0.756 µg N g^{-1} hr^{-1} after a 1–hr incubation, while the minimum was 0.009 µg N g^{-1} hr^{-1} (Table 4.5). DNRA rates after extended incubation periods (4-hr) were characteristically lower than those after 1-hr incubations, suggesting DNRA activity is influenced by NO_3^-–N availability and highlights the rapidity of the process. Rates after 1-hr incubations will be of focus, as at extended incubation periods slurry conditions are more susceptible to unnatural changes during incubations and the potential for substrate limitation. See Table A.6 for DNRA rates after 4-hr incubation periods.

Table 4.5 Summary of DNRA rates (mean ± error (µg N g^{-1} hr^{-1})) after a 1-hr incubation period, across sites. Uncertainty was calculated by propagation of error.

Site	DNRA µg N g^{-1} hr^{-1}
1	0.113 ± 0.008
20	0.009 ± 0.033
63	0.434 ± 0.059
88	0.256 ± 0.107
109	0.756 ± 0.040

Kendall correlations with Holm adjustment were used to investigate the relationship between DNRA and environmental parameters. DNRA occurring after short incubation periods may be most influenced by *in situ* dissolved oxygen concentration; however, the relationship was not significant ($\tau = -0.855$, $p = 0.065$) and the strength of the correlation analysis was limited by the number of sites available. Figure A.4 illustrates DNRA relationships with select environmental parameters.

Table 4.6 Kendall's τ rank correlation values for DNRA activity (µg N g^{-1} hr^{-1}) and pond parameters of interest for samples which were subject to a 1-hr incubation.

Environmental Parameter	DNRA µg N g^{-1} hr^{-1}	
	τ	p value
DO mg L^{-1}	−0.855	0.07
SO_4^{2-}	−0.093	0.88
LOI	0.108	0.86
Sediment pH	−0.200	0.75
Sediment Cond.	−0.042	0.95

4.2.3 Denitrification

Denitrification was highly variable, and ranged by more than an order of magnitude across the four sites for which rates were determined. The highest denitrification rate (mean per site) observed after 1-hr incubations was 0.014 µg N g^{-1} hr^{-1}, while the minimum rate was 0.001 µg N g^{-1} hr^{-1}. Denitrification rates observed after extended incubation periods (4-hr) exhibited considerable variation, alluding to a change in conditions and probable substrate limitation (Table A.6). Denitrification rates were not quantified for all sites due to unstable instrument drift during some runs, for which observations were removed from the analysis completely. The remaining sites (n=4) were not subject to statistical analysis (i.e. difference between incubation length) or correlations with environmental parameters, though Figure A.5 illustrates denitrification rates across select parameters.

Table 4.7 Denitrification rate (mean ± error (µg N g^{-1} hr^{-1})) after 1-hr incubation period, across sites. Due to calculation methods, uncertainty was calculated by propagation of error.

Site	Denitrification µg N g^{-1} hr^{-1}
1	0.001 ± 0.005
20	0.010 ± 0.010
63	0.014 ± 0.034
109	0.005 ± 0.008

4.2.4 DNRA vs. denitrification activity

DNRA and denitrification rates in sediments were both explored through low concentration $K^{14/15}NO_3^--N$ additions. Our results show DNRA is substantially higher than that of denitrification and may be the dominant pathway for NO_3^- processing within prairie potholes. Measurements after a 1-hr incubation indicate that DNRA rates are often more than an order of magnitude higher than denitrification rates, with only one site exhibiting comparable rates (Figure 4.6).
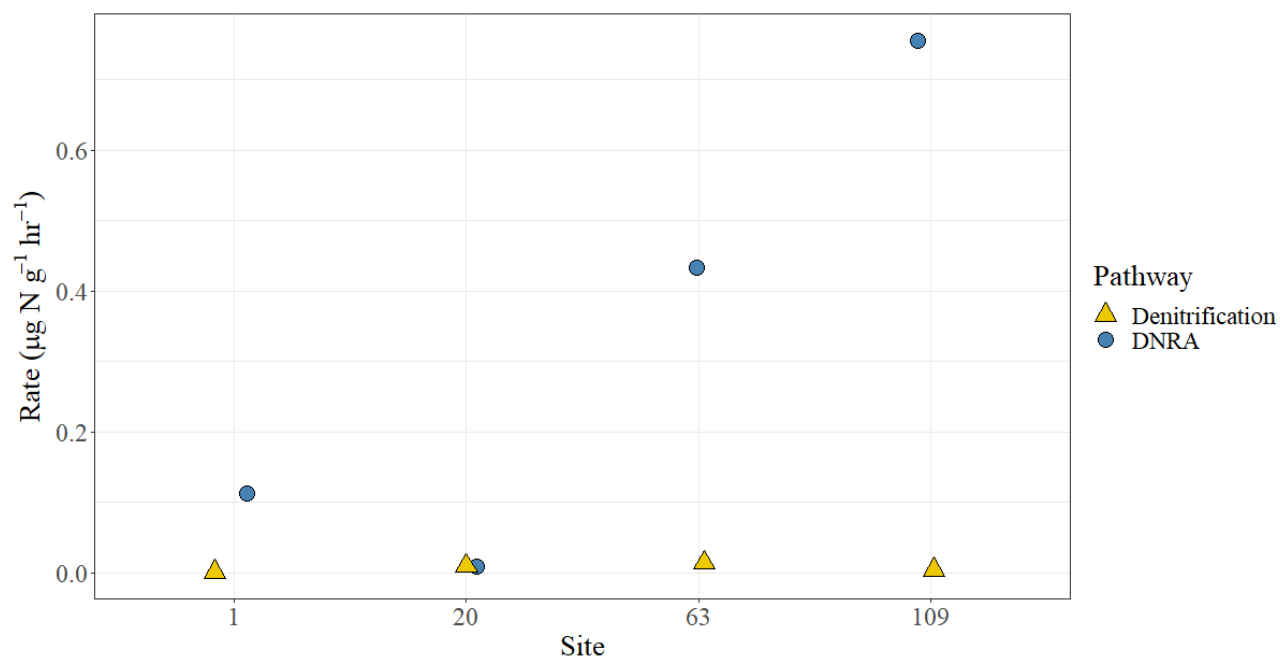
Figure 4.6 Rate (μg N g^{-1} hr^{-1}) of DNRA (blue circle) and denitrification (yellow triangle) observed in each pond, following a 1-hr incubation.

5.0 Discussion

The diverse use of land across the PPR, from conventional crop agriculture and livestock operations to untouched grasslands, along with variable hydroperiods and regional topography, have influence on the type, quantity, timing, and mode by which N compounds are spread across the landscape and/or enter nearby waterbodies. Results show the rapidity of cycling within pothole wetlands is likely to have an overarching effect on aquatic N availability. In this study, we see the potential for extremely high N uptake within planktonic communities, suggesting that available pools can be depleted in minutes throughout the open water season. While denitrification is typically thought to be an important N loss process in wetlands, preliminary work shown here, and supported by other studies in the region (Cavaliere 2018), suggest relatively low loss via denitrification due to low NO_3^- availability; instead, DNRA may be a more important pathway for NO_3^- transformation than denitrification within potholes. Ultimately, this work suggests that potholes are very important N transformers on the landscape, but more work is required to understand their potential role as N sinks or sources.

5.1 Open-water zone

5.1.1 Rapidity

Herein, we see that potholes had an extremely high capacity to process N. The maximum uptake rates across all analytes and seasons revealed turnover times ranging from seconds to minutes (Table A.7). Relative to other ecosystems, the associated uptake rates in potholes are considerably greater (Table 5.1). The high capacity for uptake and rapid drawdown of available pools is likely related to the high biomass (with maximum chl. *a* concentration reaching 2640 µg L^{-1}, and a mean of 92 µg L^{-1}) in these eutrophic to hypereutrophic ecosystems. In accounting for the uptake of N per unit biomass, we were able to identify the relative productivity of potholes across season, N concentration, and environmental parameters. Uptake per unit biomass reached a maximum of 28200 µg N (µg chl. *a*)$^{-1}$ hr^{-1} with a mean of 959 µg N (µg chl. *a*)$^{-1}$ hr^{-1} and peaked in spring (Figure A.2), likely due to rapidly growing communities competing for a limited N source.

Table 5.1. A (non-exhaustive) summary of N uptake rates (µg N L^{-1} hr^{-1}) across various ecosystems and locations. Areal rates, kinetic estimates, and alternative metrics were excluded to ensure comparability between methods and rates. The sampling season(s), spike addition(s), and incubation duration is noted under the "Conditions" column. "nd" indicates the rate was not detectable.

Ecosystem	Location	Urea	NH$_4^+$-N	NO$_3^-$-N	Conditions	Reference
Prairie Pothole	*St. Denis, Canada*	*57.0–15700*	*61.3–12000*	*0.083–1140*	*Spring, summer, fall, 10% of in situ, 1-hr*	*this study*
Prairie Pothole	St. Denis, Canada		0.13–0.68		Winter, 10% of *in situ*, 4- and 24-hr	(Cavaliere 2018)
Prairie Pothole	St. Denis, Canada		1.4–50.3	0.12–1.3	Melt, 10% of *in situ*, 4- and 24-hr	(Cavaliere 2018)
Estuary	Neuse River, USA	0.001–0.052	0.003–0.095	0.001–0.063	Spring, summer, fall, 0.021 µg N L^{-1} addition, 4-hr	(Twomey et al. 2005)
Estuary	Southampton, England	nd–9340	0.042–72.3	0.014–55.7	Spring, summer, 1.40 µg N L^{-1} addition, 4-hr	(Torres-Valdés and Purdie 2006)
Estuary	Swan River, Australia	0.1–12	0.4–9	0.02–7	Spring, summer, fall, 28.0 µg ^{15}N L^{-1} addition, 4-hr	(Rosser and Thompson 2001)
Estuary	Chesapeake Bay, USA		0.426–3.33	6.7	Spring, 0.5 hr incubations, 0.420 µg N L^{-1}, 30% of *in situ*, 21% of *in situ*, 5% of *in situ* (NO3)	(Bronk et al. 1994b)
Coastal	Southern California Bight		0.611–0.745	0.077–0.099	Spring, summer, fall, 1.40 µg N L^{-1} addition, 3-hr incubation	(Bronk et al. 1994b)
Lake	Northern Great Plains, Canada		2.6–4.3		Summer, 10% of *in situ* was lowest addition concentration	(Murphy and Brownlee 1981)
Lake	Lake Ontario, Canada		0.03–2.50	<0.001–0.420	Spring, summer, fall, saturating conditions (5–100 µg N L^{-1} NH$_4^+$, 100–500 µg N L^{-1} NO$_3^-$), 0-16 hr	(Murphy 1980)
Lake	Lake Balaton, Hungary	0.12–3.17	0.11–10.3	0.02–0.52	Spring, summer, fall, winter, 10–480 µg ^{15}N L^{-1} addition, 20–90 min. incubation	(Présing et al. 2001)
Tropical reservoir	São Paulo, Brazil		116	18.2	Spring, Summer, Autumn, 2–10% of *in situ*, 1-hr	(Cunha et al. 2017)
Oceanic	Caribbean Sea		0.363–0.430		May, 0.03 µg atoms of N per light of ^{15}N-NH$_4^+$, 1-hr	(Bronk et al. 1994b)
Oceanic	Southern California Bight		0.312–0.438	0.053–0.106	Spring, 1.40 µg N L^{-1} addition, 3-hr incubation	(Bronk et al. 1994b)
Sea	Wadden Sea, The Netherlands	1.41	8.82	2.40	Spring, summer, fall, 10% of *in situ*, 2-hr	(Moneta et al. 2014)

Across all sampling periods, 16 sites were considered N limited (molar TN:TP<10), while only 3 sites were likely to be P limited (molar TN:TP>20) according to Guildford and Hecky's (2000) classification. Nitrogen limited systems are not unique to the prairies (Leavitt et al. 2006), and some suggest small lakes and wetlands are primarily N or co-limited, rather than P limited (Scott et al. 2019; Maberly et al. 2020). This could be due to the high surface area to water ratios in small, shallow systems, which can enhance the importance of sedimentary processes, including the release of phosphorus (Miller et al. 1974; Elser et al. 1990; Scott et al. 2019), contributing to higher P concentrations in these systems. Higher P availability can push a wetland to a higher trophic state and lead to an increased demand for N by photosynthetic organisms (Fischer et al. 2017). Furthermore, at high productivity levels, C may ultimately become the limiting factor of N and P uptake by phytoplankton (Jansson et al. 1996; Hammer et al. 2019), and could consequently limit nutrient uptake, though C is not expected to be a limiting factor across the PPR. The complexity of potholes, along with the adaptability of phytoplankton to changing nutrient regimes, creates challenges in estimating N limitation and N cycling dynamics (Kanda et al. 1985; Weithoff and Walz 1999), despite the rapid N uptake.

5.1.2 Analyte preference

It was evident that N was rapidly utilized in these systems; however, there was a differentiation between uptake rates of reduced and oxidized N sources. While bacteria and phytoplankton growth can be supported by any of these substrates, here, and elsewhere, higher uptake of reduced substrates is commonly observed (Goldman and Glibert 1982; McCarthy et al. 1982; Lipschultz 1995). Microbial preference for NH_4^+ over NO_3^- is well characterized (Gu et al. 1997; Glibert et al. 2016) and research on inorganic N often indicates that NH_4^+ accounts for the majority of N assimilation (Toetz 1976; Gu et al. 1997; Rosser and Thompson 2001). The more rapid uptake of NH_4^+ is largely due to the higher availability of NH_4^+ in potholes and the less energy required to assimilate NH_4^+ compared to NO_3^-, which needs to be reduced before being incorporated into amino acids/biomass (Lachmann et al. 2018; Trommer et al. 2020). However, urea may ultimately be preferred to inorganic N sources (Gu et al. 1997), as two molecules of NH_4^+ are assimilated per one molecule of urea (Flores and Herrero 2005; Donald et al. 2013). In addition, urea can also provide a C source and has been linked to cyanobacteria blooms (Krausfeldt et al. 2019). In some cases, urea uptake is nearly four times greater than NH_4^+ and

10–50-fold greater than that of NO_3^- (Bradley et al. 2010; Moschonas et al. 2017), though urea and NH_4^+ uptake were not significantly different from one another in the current study.

The differential uptake of N substrates could also arise from suppression dynamics, species-specific requirements for various N forms, or nutrient limitations and/or ratios within the system. For example, NO_3^- uptake can be suppressed by NH_4^+ concentrations in the range of 0.002 mg L^{-1} to 0.180 mg L^{-1} (Eppley and Coatsworth 1968; Wheeler and Kokkinakis 1990; L'Helguen et al. 2008; Glibert et al. 2016), though the extent ultimately depends on the species present and environmental conditions of the system (Glibert et al. 2016). There are relatively high concentrations of NH_4^+-N within potholes, reaching a maximum of 4.23 mg L^{-1} and a mean of 0.146 mg L^{-1} across a prairie-wide survey (unpublished data); however, we were unable to confirm if suppression dynamics were a factor in this study. Additionally, the availability of multiple N forms may affect planktonic community composition and analyte preference. Because the study ponds were higher in NH_4^+ and urea, relative to NO_3^-, this could disadvantage taxa that are efficient at NO_3^- assimilation, such as diatoms, while other taxa that thrive on reduced species, such as cyanobacteria, cryptophytes, and dinoflagellates (Bronk et al. 1994a; Berg et al. 2003; Domingues et al. 2011; Moschonas et al. 2017) are at an advantage, which can ultimately shape the populations, communities, and the biogeochemistry of the system. Within prairie potholes, the concentration of NH_4^+ and urea tend to be greater than that of NO_3^-, as was reflected in the preference and rapidity of uptake for each analyte and may ultimately influence the community composition and utilization of nutrients within the open-water zone, and indirectly affect which compounds are available for benthic microorganisms.

5.1.3 Light and dark uptake

It is often assumed autotrophic N uptake is light dependent (Nelson and Conway, 1979); however, evidence suggests some phytoplankton are capable of N uptake in the dark (Glibert et al. 2004; Gobler et al. 2002; Joint et al. 2002), and complex planktonic communities with abundant heterotrophs may not have light-sensitive uptake. Here, we found uptake in the light was only slightly to moderately greater than uptake in the dark. These findings are consistent with accumulating evidence that suggests light dependence for N uptake has been overemphasized within phytoplankton (Glibert et al., 2004; Gobler et al., 2002; Joint et al., 2002), and we note these results are consistent with many other studies (Healey 1977; Lapointe

and Tenore 1981; Turpin 1991; Needoba and Harrison 2004; Yuan et al. 2012; Chamberlin et al. 2019). Importantly, within natural ecosystems, planktonic communities often have very large bacterial biomass. Yuan et al. (2012) found bacteria accounted for up to 60% of phytoplankton N uptake and Kirchman et al. (1994) found over 70% of total N uptake was by bacteria. The light independent transporters and inessential intracellular reduction of bacteria is advantageous and may outcompete algae for nutrients (Løvdal et al. 2008; Donald et al. 2013). The productivity and metabolism of pelagic bacteria in potholes has been acknowledged (Waiser and Robarts 2004); however, few studies have further explored bacterial composition and contribution to N uptake.

It is also important to consider the likely N limited conditions of the potholes. For example, as nutrients become more limiting, diel patterns in nutrients (affected by uptake) become more muted (Vincent 1992; Chamberlin et al. 2019), presumably related to the potential for unmet nutrient demand, and therefore extend nutrient uptake/transport through periods of darkness (Appling and Heffernan, 2014). As noted, growing evidence is emphasizing the importance of dark uptake and this appears true of all N substrates studied here. Nitrate is primarily taken up by active transport (Falkowski and Stone 1975); however, dark assimilation can account for up to half of the utilized NO_3^- (Needoba and Harrison 2004), presumably supported by available energy stores accumulated by the phytoplankton during light conditions (Miyazaki et al. 1987; Turpin 1991; Clark et al. 2002). Urea is taken up via passive diffusion or secondary transport (Syrett and Bekheet 1977; Williams and Hodson 1977; Bekheet and Syrett 1979; Mulholland and Lomas 2008) and is not known to be light sensitive (Vincent 1992; Korth and Deutsch 2012). Lastly, the transport of NH_4^+ is achieved through diffusion (Raven 1980; Stewart 1980; Donald et al. 2013; Glibert and Kana 2016), or secondary active transport (Pelley and Bannister 1979; Wheeler 1980a; b; Wheeler and Hellebust 1981), limiting the need for sunlight (Turpin 1991). Although there has been limited research, rates of dark NH_4^+ uptake exceed light assimilation rates in some cases (Kanda et al. 1985; Lipschultz et al. 1986; Turpin 1991; Hampel et al. 2018; Chen et al. 2020). Additional studies providing evidence for light independent N uptake suggest assimilation conditions, such as the availability and form of N (Cochlan and Harrison 1991; Clark et al. 2002; McCarthy et al. 2013; Chamberlin et al. 2019), light intensity (Kanda et al. 1985), and the communities present (Conway and Whitledge 1979), can influence light-dark changes in uptake. The key conclusion here, however, is that potholes as

major sites of N transformation cannot be assumed to be quiescent at night — instead, continued high uptake rates are apparent throughout light and dark cycles, although higher NH_4^+ and urea uptake was observed under light conditions.

5.1.4 Nitrogen utilization and environmental parameters

By using chl. *a* content as a proxy of algal biomass, we can assess how N utilization in and across potholes varies with algal biomass. Biomass, in this study, is representative of all organisms captured on the GF/F ($>0.70\,\mu m$), which may also include non-photosynthetic organisms. Regardless, this study, similar to others (Kanda et al. 1985; Bbalali 2013; Paparazzo et al. 2017; Trommer et al. 2020), found chl. *a* was strongly linked to planktonic N uptake, especially in the spring and fall. A pulse in biomass in spring could be due to a spring bloom, associated with increased light and temperature, and potential for nutrient transport into the systems with snowmelt, while peaks in biomass during the fall and late summer have been reported in highly productive systems (Crumpton 1989; Waiser and Robarts 2004). Biomass (as chl. *a*) did not exhibit a significant relationship with the uptake of N (all forms) in summer. This could be due to changing nutrient ratios, micro and macro nutrient limitation, herbivory, or turbidity that could be affecting the quantity of biomass, and in turn chlorophyll pigments, produced by the phytoplankton (Bbalali 2013). It could also point to the increased N utilization/activity of other microbes that are responsive to increased temperatures. Regardless, these results support evidence that N availability may influence chl. *a* production (Bbalali 2013; Paparazzo et al. 2017) and could be used as a tool to assess pothole productivity and phytoplankton community. However, in doing so, one must also consider the different nutrient physiology and requirements of phytoplankton (Smith 1982) and other pelagic species, which may be influenced by nutrient inputs, regional ecology, and climatic conditions.

The pH of water can directly affect phytoplankton metabolism as it influences protein and enzyme conformations, nutrient availability, intracellular conditions, and the generation of cellular energy (Casey et al. 2010), which is subsequently used for the active transport of nutrients into the cell (Vincent 1992). Some studies have found an increase in NH_4^+ and urea uptake with increasing pH (Healey 1977), possibly due to cell physiology and the occurrence of NH_3 diffusion across the cell membrane (Vincent 1992). Others, such as Toetz (1976) found a negligible effect on the uptake of NO_3^- or NH_4^+ by freshwater phytoplankton. Additionally,

photosynthetic activity consumes CO_2 (as carbonic acid), and thus influences pond pH (Brönmark and Hansson 2017). There was a fluctuation in pond pH observed across seasons, increasing from spring (with a mean of 7.42 pH units) to summer (mean of 8.60 pH units) and slightly declining in fall (mean of 8.25 pH units), and may allude to the increase in N uptake and biomass. While water column pH within prairie pothole wetlands is influenced by a suite of factors, including aquatic productivity, our results did not produce a strong relationship between N uptake rates (volumetric or per unit chl. *a*) and pH.

This lack of relationship with N uptake is also true for salinity, for which conductivity served as a proxy in this study. Pond salinity commonly increases throughout summer; the warming temperatures cause an increase in evapotranspiration and consequently increase the concentration of ions. The fluctuation of salinity can have a range of affects to biota, depending on the concentration of ions and communities present (Flöder et al. 2010), as each species has a different salinity tolerance which allows the management of osmotic stress, respiration activity, and cellular ionic ratio (Guillard 1962). Chl. *a* normalized rates did tend to increase with an increase in conductivity, comparable to results found by Drerup and Vadeboncoeur (2016) and Melack and Kilham (1974). This could be due to the increased cation concentration, mainly calcium and magnesium, stimulating nutrient transport across the cell membrane and enhancing productivity and biomass (Falkner et al. 1980; Kumar Singh et al. 2007; Drerup and Vadeboncoeur 2016), or due to differences in phytoplankton communities with salinity, which could lead to differences in both uptake rates and chlorophyll to biomass ratios. While we have explored these relationships, it should be acknowledged that investigation of the relationships between these processes and physicochemical parameters would be strengthened through inclusion of a larger numbers of sites, and perhaps better representation of environmental gradients.

5.1.5 Michaelis-Menten kinetics

An additional experiment was performed to understand N uptake using Michaelis-Menten enzyme kinetics, which relate the velocity of uptake with substrate concentration. Our results suggest potholes are N limited in spring, as few ponds reached a saturation plateau under the concentrations we examined (Table A.5) and had K_m values greater than *in situ* concentrations. N uptake in summer and fall displayed a decrease in uptake rate with increasing N concentration

with some K_m values greater than *in situ* concentrations, indicating the saturation point was exceeded. Similar studies found inconsistency with N uptake and Michaelis-Menten kinetics, likely due to low *in situ* concentrations of the N analyte of focus (Toetz et al. 1973; Murphy et al. 2003), as observed in some ponds in this study. Additional distortions in Michaelis-Menten uptake kinetics can occur when Michaelis-Menten assumptions are violated, such as non-steady state uptake (Conway et al. 1976; Collos 1983; Harrison et al. 1989; Collos et al. 1997) or an inconsistent affinity for nutrient uptake (Bonachela et al. 2011). However, mounting literature suggests Michaelis-Menten kinetics do not appropriately describe phytoplankton uptake (Deunff et al. 2019), which is further complicated in oligotrophic and eutrophic systems (Aksnes and Cao 2011; Bonachela et al. 2011).

Exploring the factors which may diverge from traditional enzyme-substrate kinetics is not new (Appling and Heffernan 2014; Collos et al. 1997; Cotner and Biddanda 2002; Dortch et al. 1984; Lakes and Murphy 1980; Nelson et al. 1981; Toetz et al., 1973). But, only recently have researchers advanced non-Michaelis-Menten kinetics, which facilitates the representation of a complex systems through more complicated mathematics (Srinivasan 2020), to suit the magnitude and flexibility of factors needed to appropriately describe phytoplankton uptake kinetics. For example, enzyme-substrate kinetics cannot distinguish biological controls such as ion diffusion properties, cell size, and community composition, or environmental factors such as temperature and light intensity, which may affect V_{max} and K_s values (Aksnes and Cao 2011; Fiksen et al. 2013; Deunff et al. 2019). Additionally, instances of nutrient patchiness (Schapira and Seuront 2020), complex, multiple, or inhibitory enzyme systems (Srinivasan 2020), or inter-dependence of rate-limiting resources (Li and Wang 2019) require extensive mathematics, exceeding those applied in Michaelis-Menten kinetics. The complexities of deciphering N transport kinetics within potholes may arise due to the multiple N uptake mechanisms, the diverse community of microorganisms, and the fluctuating nutrient regimes seen in the prairies, among other factors; however, disentangling the N kinetics within potholes will significantly contribute to a better understanding of N cycling and increase the accuracy of nutrient budgets and models.

5.2 Benthic zone

5.2.1 DNRA

Despite the early investigation of DNRA (Woods 1938), only recently has there been a focus on understanding major controls on the pathway, and recognizing its potential importance (Burgin and Hamilton 2007). Where DNRA is high, it can foster retention of N on the landscape through the recycling of NO_3^- into NH_4^+, which is then available for plants, algae, and microorganisms, or is bound to the sediments or soil (Pandey et al. 2020). This contrasts with denitrification, which, when favored, removes reactive nitrogen from the environment by returning dinitrogen gas to the atmosphere. Exploring rates of DNRA across ecosystems, we found our measured DNRA rates in the Prairie Potholes exceed many reported rates (Table 5.2). The high rates for prairie pothole wetlands are comparable to those occurring in humid soils of Puerto Rico (Pett-Ridge et al. 2006).

Our methodological assessments indicate that the approach to measuring DNRA will impact results. We tested two incubation periods; the 1-hr incubation period produced rates substantially greater than those at a 4-hr incubation period. In addition to substrate depletion potentially limiting rates, longer incubation periods may be affecting microbial processes via changes in physical and chemical conditions. Mineralization may affect DNRA rates and isotope ratios at extended incubation durations, or where OM content is notable. Mineralization will affect isotope ratios, due to production of unlabelled organic N to NH_4^+ within the sample, which thus dilutes the ^{15}N pool (Booth et al. 2005; Reddy and Delaune 2008; Braun et al. 2018). Correction for this phenomenon is achievable through accurate estimation of both ^{14}N and ^{15}N initial pool sizes prior to determining gross mineralization and DNRA (Davidson et al. 1991; Braun et al. 2018). Additionally, as used in this study, differentiation between NH_4^+ production and DNRA activity can account for the effects of mineralization. Mineralization rates are largely regulated by NH_4^+ accumulation in soil, microbial biomass, C:N ratio (<100), redox potential, soil pH, and soil type (Booth et al. 2005; Reddy and Delaune 2008). Although mineralization rates in potholes are not frequently studied (Bedard-Haughn et al. 2006; Brown et al. 2017), given the favorable conditions of potholes, mineralization rates could be an important aspect to consider in further investigations. For our purposes, we assume the 1-hr incubation period produces the most robust results, because conditions may be more natural, and changes in substrates will be lesser. We focus our discussion on these shorter-term results.

There were few statistically significant relationships with environmental parameters and DNRA rates, likely due at least in part to the low sample size. Additionally, although not shown here, the factors affecting DNRA are expected to be complex, and interacting. For example, DNRA has been found to favor alkaline environments (Nommik 1956; Gamble et al. 1977; Fazzolari and Germon 1991; Stevens et al. 1998), this could, however, be due to the increased availability of C with an increase in pH (Rütting et al. 2011). While there is ample research linking DNRA with carbon-rich systems (Fazzolari et al. 1998; Deng et al. 2015; Salk et al. 2017), our analysis, limited to five observations, did not detect a relationship between DNRA and OM. An expanded analysis featuring more sites, could be used to explore this, and whether other reported drivers of DNRA rates, including high $C:NO_3^-$ (Fazzolari et al. 1998; Rütting et al. 2011; Salk et al. 2017), salinity (Gardner et al. 2006; Giblin et al. 2010; Deng et al. 2015), and alkalinity (Nommik 1956; Gamble et al. 1977; Fazzolari and Germon 1991; Stevens et al. 1998), are important for pothole ponds. Reduced sulfur compounds, such as H_2S and thiosulfate, can also be used as an electron donor by chemolithoautotrophic bacteria, thereby increasing DNRA activity (Brunet and Garcia-Gil 1996; Burgin and Hamilton 2008). Ultimately, the effect of pH and/or conductivity on DNRA rates is likely to be complex. Both parameters influence multiple processes, and conductivity reflects multiple solutes; hence, the effects of these parameters may not be apparent across the relatively small range of conditions and measurements present in this study.

While rates of DNRA were high compared to the literature, we highlight our rates may be conservative (i.e., an underestimation), stemming from the inability to quantify the initial NH_4^+ pool bound to the sediments, along with incomplete recovery of $^{15}NH_4^+$ on the diffusion disk. While recovery rates of the method can be high (e.g., Kelley et al. (1991) report a recovery of 92–100% using the diffusion disk method), the initial amount of N added can be important for recovery. Lory and Russelle (1994) found low mass N samples directly reduce the amount recovered on the disk. Our NO_3^- addition was relatively low, and recovery efficiency was also lower than reported in the aforementioned studies (median = 76%). Even despite these factors likely contributing to underestimation, measured DNRA rates suggest this is an important process, and may influence the often-higher ratio of $NH_4^+:NO_3^-$ seen in prairie potholes (~2:1 to 10:1, unpublished data) as compared to other ecosystems.

5.2.2 Denitrification

There is often focus on denitrification in aquatic ecosystems because of its importance to N retention and removal. Interestingly, denitrification in prairie potholes was not the dominant NO_3^- reducing pathway. Denitrification rates in this study were low in the context of many ecosystems (Table 5.2) but are comparable to recent measurements in the local region using different methodology (Cavaliere 2018). Similar to what we found for DNRA, short incubations to measure denitrification produced higher rates than those in extended incubations, potentially due to the development of substrate limitation (Cooper 1990; White and Reddy 2009) and subject to influences of other processes, such as the competition with DNRA. Again, we focus on the shortest incubations for our discussion. Denitrification was measurable and variable.

Denitrification rates did not increase with increasing NO_3^- as expected (Pandey et al. 2020), though we highlight the very low NO_3^- conditions present in these potholes. We suggest that NO_3^- concentrations may be uniformly limiting denitrification rates across these ponds. For example, work in the region suggests that thresholds for NO_3^- limitation of denitrification in stream pools and reservoirs (0.68 mg NO_3^--N L^{-1}; Gooding and Baulch 2017) are higher than measured NO_3^- concentrations in our study sites (below detection limits to 0.145 mg NO_3^--N L^{-1}). Organic C is also a key substrate for denitrification (Seitzinger 1994), and was suggested by the positive trend between LOI and denitrification rate illustrated in Figure A.5. Lastly, a negative relationships with SO_4^{2-} due to the direct inhibition of nitric and nitrous oxide reductases (Brunet and Garcia-Gil 1996; Senga et al. 2006; Aelion and Warttinger 2009; Giblin et al. 2010; Fan et al. 2018; Murphy et al. 2020), and denaturing of proteins (Moosa and Harrison 2006; Chen et al. 2008), is commonly reported. However, this was not supported by the data, despite inclusion of sites spanning a relatively wide range of SO_4^{2-} concentrations (albeit for few observations/sites).

Challenges in measuring denitrification are well documented (Groffman et al. 2006). Here, the low rates of denitrification occurring in these potholes produced challenges. In particular, signal drift was not always uniform and so it was not possible to calculate rates for all sites (1 site excluded). In addition, measurements across more PPR wetlands would be of great benefit to understand the generality of our observations, and whether differences in denitrification for ponds of different characteristics are expected. Nonetheless, low NO_3^-

availability across many prairie pothole ponds (mean: 0.05 mg L^{-1} NO$_3^-$-N, unpublished data), does suggest that denitrification may be frequently limited by NO$_3^-$ availability, and denitrification rates may be low across many ponds of the region.

Table 5.2 DNRA (μg N g^{-1} hr^{-1}) and denitrification (μg N g^{-1} hr^{-1}) rates across various ecosystems and locations, as gathered by a (non-exhaustive) scan of the literature. Areal rates and alternative metrics were excluded to ensure comparability between methods and rates. The season sampled and the method used to measure DNRA and denitrification, respectively, are summarized in "Conditions". Additional references and details regarding terrestrial ecosystems are found in (Rütting et al. 2011). '*' indicates rate units of μg ^{15}N g^{-1} hr^{-1}.

Ecosystem	Location	DNRA rate μg N g^{-1} hr^{-1}	Denitrification rate μg N g^{-1} hr^{-1}	Conditions	Reference
Aquatic systems					
Prairie Pothole	*Canada*	*0.113–0.756*	*0.0002–0.014*	*Summer, diffusion disk, MIMS*	*This study*
Prairie Pothole	Canada	-	1.50 x 10^{-5}–0.067	Winter, chloramphenicol-amended acetylene block method	(Cavaliere 2018)
Prairie Pothole	Canada	-	6.80 x 10^{-5}–0.010	Summer, chloramphenicol-amended acetylene block method	(Cavaliere 2018)
Salt Marsh	China	0.003–0.023*	0.119–0.264*	Spring, OX/MIMS method, no details	(Yin et al. 2014)
Rice Paddy Soil	China	0.0004–0.0076	0.116	Summer, OX/MIMS, MIMS	(Shan et al. 2016)
Rice Paddy Soil	Australia	0.018–0.022	0.024–0.033	Spring, GC-IRMS, GC-IRMS	(Pandey et al. 2018)
Estuary	China	0.0004–0.013	0.008–0.063	Summer, Winter, OX/MIMS, MIMS	(Deng et al. 2015)
Terrestrial systems					
Temperate forest (pine, Douglas fir)	The Netherlands	0.0004–0.010	-	-	(Tietema and Van Dam 1996)
Temperate forest (spruce)	Sweden	0.003	-	-	(Bengtsson and Bergwall 2000)
Temperate forest (oak)	Belgium	0.0005	-	-	(Staelens et al. 2011)
Temperate forest (pine)	Belgium	0.0002	-	-	(Staelens et al. 2011)
Tropical (humid)	Puerto Rico	0.120	-	-	(Pett-Ridge et al. 2006)
Temperate grassland (meadow)	Germany	0.011	-	-	(Muller et al. 2009)
Temperate grassland (pasture)	New Zealand	0.001	-	-	(Rütting et al. 2010)

5.2.3 Dominant nitrate reducing pathway in Prairie Potholes

Denitrification is commonly thought to be the principal pathway by which N is removed from wetlands and anaerobic sediments (Cornwell et al., 1999; Saunders and Kalff, 2001; Seitzinger, 1988). This narrative has led to valuable insights on the importance of wetlands in the permanent removal of NO_3^- by reduction to N_2. However, other important pathways have been overlooked (Burgin and Hamilton 2007; Rütting et al. 2011). Recent literature has broadened our understanding of NO_3^- reducing pathways and suggests DNRA is of higher importance than historically perceived (Burgin and Hamilton 2007; Pandey et al. 2020). Findings in this study support that conclusion. Prairie potholes are rapidly reducing NO_3^- via DNRA, while only very low rates of denitrification are occurring. Ultimately, competition for NO_3^- across multiple processes, including planktonic uptake, create the low NO_3^- environment seen in these ecosystems.

Multiple factors may favour DNRA over denitrification within prairie potholes. One of the most influential factors in the competition between DNRA and denitrification is the soil redox potential (Matheson et al. 2002). Denitrification occurs in moderately anoxic soils and is favorable to environments with a low oxidisation level, while DNRA requires anaerobic, highly reduced environments (low oxygen and NO_3^-) (Matheson et al. 2002). While this study did not include redox measurements, low redox potentials in sediments, and steep redox potentials in the sediment profile are commonly seen in prairie potholes (Bansal et al. 2016; Dalcin-Martins et al. 2017; Tangen and Bansal 2019), which may influence the abundance and spatial distribution of benthic communities. Additionally, DNRA is favored in environments with high amounts of organic C (Tiedje 1988; Yin et al. 1998) that are common in the region (Ziegelgruber et al. 2013; Phillips et al. 2016; Dalcin-Martins et al. 2017), and low concentrations of NO_3^-, also common for PPR wetland ponds (unpublished data). In contrast, denitrification is often dominant in environments that have low C:N ratios (Tiedje 1988; Silver et al. 2001; Lind et al. 2013; Salk et al. 2017). This study found DNRA was dominant in most ponds, with the highest DNRA rates observed in ponds with relatively high sediment organic C and low *in situ* NO_3^--N, with C:NO_3^--N ratios of 7.5 and 6.4 (pond 63, pond 109, respectively). However, denitrification rates did exceed those of DNRA in one pond, which had higher *in situ* NO_3^--N and moderate organic C, with a ratio of 3.3 (pond 20). The distinction of C:N in ponds can dramatically influence the dominant NO_3^- reduction pathway, such that Salk et al. (2017) found DNRA was responsible for

up to 74% of total NO_3^- reduction in high C:N systems. Additionally, Yin et al. (1998) found DNRA to only occur above a C:NO_3^--N ratio of 12 and Fazzolari et al. (1998) found DNRA to be preferred over a sediment C:NO_3^--N ratio of 4, while Bowman and Focht (1974) found denitrification to prevail at a C:NO_3^--N ratio over 2. The high C content and low NO_3^- concentrations in the region are likely to produce C:N ratios favourable to DNRA.

Other factors that may lead to this dominance of DNRA, are the elevated concentrations of iron (Roberts et al. 2014; Robertson et al. 2016; Cojean et al. 2020; Pandey et al. 2020), SO_4^{2-} (Zeng et al. 2013), sulfide (McAdams et al. 2016), among other sulfur species (e.g., polysulfides, bisulfide) (Zeng et al. 2012), seen in prairie potholes (Goldhaber et al. 2016). Sulfate ranged from 102 to 5100 mg L^{-1} in the study ponds used for benthic experiments. DNRA is favored in systems with elevated iron and sulfide, as a variety of chemolithoautotrophic bacteria can utilize these molecules to reduce NO_3^- (Crane et al. 1995; Simon and Kroneck 2013; Cojean et al. 2020; Pandey et al. 2020). Conversely, these molecules impede denitrification at elevated concentrations (Brunet and Garcia-Gil 1996; Lind et al. 2013; Cojean et al. 2020). These factors, as with those mentioned previously, are the defining features in deciphering the competition and/or likelihood of the pathway of NO_3^- reduction in prairie potholes.

6.0 Conclusions

6.1 Nitrogen retention in potholes (sinks, sources, transformers)

There has been a longstanding discussion across the science community in attempts to classify wetlands and potholes as either a N source, sink, or transformer on the landscape (Groffman 2000; Bedard-Haughn et al. 2003; Gala and Young 2015; Robertson and Groffman 2015; Lane et al. 2018). One could argue the recycling of N via DNRA or the uptake of N via plankton can contribute to these systems exporting N during fill and spill events, while the release of N_2 via denitrification results in a net loss of fixed N and characterizes potholes as N sinks. This characterization, however, may be oversimplified. While this research has reinforced the complexity of biogeochemical processes in these pothole ponds and illustrated the N sink via denitrification may be smaller than anticipated, given the hydrological complexity of these systems, understanding nutrient retention ultimately requires an understanding of process mechanisms and hydrochemical dynamics. For example, this research revealed a high potential for DNRA to recycle N within the system, generating a less mobile form of N, which can be bound to sediments, transformed across other pathways, or utilized by organisms and plants, such as pelagic phytoplankton and bacteria, while retaining N in the ecosystem. Moreover, Li et al. (2020) found DNRA was an important factor in regulating the severity of eutrophication and cyanobacterial blooms through sediment sorption. As eutrophication and cyanobacterial blooms become more severe (Hayes et al. 2020), especially in agriculture-dominated regions (Smucker et al. 2021), it is imperative that we understand and recognize transformations which may be fundamental in mitigating the impact of nutrient transport.

The goal of this study was to quantify the major N cycling pathways in prairie potholes, to address gaps in the current understanding of the behaviour of these systems as biogeochemical reactors. Our study confirmed the high potential for rapid N transformations within these systems, exceeding rates found in tropical, humid soils. More importantly, however, are the biota responsible for cycling N within these systems. Historically, emphasis was placed on algae and sunlight-dependent N uptake (Wheeler and Kirchman 1986; Cochlan and Harrison 1991; Kirchman et al. 1994; Clark et al. 2002; McCarthy et al. 2013); however, growing amounts of research consider the role of bacteria in N uptake rates (Kirchman et al. 1994; Bradley et al. 2010; Yuan et al. 2012). This study found active N uptake in the pelagic zone in the dark,

comparable to rates during light conditions, suggesting a wide diversity of organisms responsible for facilitating N uptake in the water column. This suggests an importance in considering the rates of uptake under both light and dark conditions when quantifying and assessing the conversion of mineral N to organic forms. Additionally, there was an obvious preference for NH_4^+ and urea in the study ponds, likely due to the favorable energy yield and low availability of NO_3^- within potholes.

Similarly, emphasis has been disproportionately placed on denitrification as the principle NO_3^- reduction pathway. Historically, alternative pathways, such as DNRA and ANNAMOX, were overlooked (Burgin and Hamilton 2007), as these processes were thought to play a limited role in N cycling. This study found that benthic NO_3^- reduction in potholes is dominated by DNRA, while comparatively small amounts of denitrification are occurring. The underestimation of alternative pathways can influence the estimated amount of N bound to sediments, retained in wetlands, and released to the atmosphere. This research highlights the importance of broadening perspectives and exploring alternative pathways, biotic communities, and factors to gain a multi-dimensional understanding of the potential that prairie potholes may play in nutrient management and recycling across the landscape. Importantly, if DNRA rates far exceed rates of denitrification in the same systems, it is possible to overestimate the role of pothole ponds as N sinks.

6.2 Progress and remaining research gaps

This study was the first to quantify and compare DNRA and denitrification in prairie potholes, and adds to our knowledge of N uptake and cycling across diverse ecosystems, helping to lay the groundwork for a more comprehensive understanding of pothole N biogeochemistry. Continued research regarding DNRA and denitrification activity is needed to identify which physicochemical properties such as C content, water chemistry, sediment characteristics, vegetation, retention time, among others, are important factors controlling these rates. As well, for pelagic N uptake, the taxonomic classification of pelagic and benthic microbial communities could be used to identify how this diversity contributes to differing uptake behaviour. Additionally, exploring the role riparian vegetation, macrophytes, and periphyton have in N utilization would greatly contribute to a more comprehensive understanding of N demands, limitation, and cycling within potholes. Further, integrating our understanding of sediment-water

interactions, sediment-plant interactions, and pelagic processes, as they relate to pond characteristics (including depth, and permanence) will help better understand nutrient cycling across ponds. In doing so, it can help guide wetland and drainage policy and action to consider the diversity of ecosystems and their broad functions, integrating both these nutrient cycling functions, but also include key functions hydrologically and ecologically. Lastly, while laboratory experiments were valuable in documenting with precision these N processes, extrapolating measurements to ecosystem-level scales is challenging and does not capture the natural environment or dynamic conditions of the system, such as the ephemerality of lower permanence ponds. Thereby, *in situ* experiments (e.g., mesocosms, open-water enclosure, whole-pond assessments) or more detailed spatial assessments of key processes could be a useful follow-up to capture the unique geographical, hydrological, and ecological characteristics of each pothole.

Importantly, the enhanced threat of climate change, agricultural expansion, urban encroachment, and an ever-increasing population, are collectively diminishing the integrity of potholes across the landscape. With the increase of anthropogenic stressors, and a decrease of potholes, there is a level of urgency in determining how to maximize the potential of the remaining potholes for nutrient mitigation, water retention, climate regulation, and biodiversity protection. With continued research, we can unpack the complexities of these systems and harness the potential that prairie potholes have in nutrient management and mitigation and other key ecosystem services, aid in better decisions by land managers, and develop more accurate nutrient budgets and models. This work could ultimately lead to improved valuation of wetlands, and the ecosystem services they provide.

6.3 Closing Remarks

Since the early 1900s, N fertilizers have supplemented plant growth, increased crop yields, and fed millions of people; despite their value, N fertilizers are sometimes mismanaged. Farmers in Africa, Asia, and Latin America are deprived of commercial fertilizers (Vitousek et al. 2009; Austin et al. 2013; Houlton et al. 2019), while waterways in developed nations have become polluted due to excessive or untimely fertilizer applications. The demand for N fertilizers (as NH_3) in developed countries is predicted to increase by 1.5% annually (FAO 2017), reaching over 111.6 million tonnes by 2022 (FAO 2019). Despite the intense demand for synthetic

fertilizers, effective nutrient management strategies have not been at the forefront. With the looming threat of climate change on food availability, extreme weather events, and ultimately, water quality and quantity, it is imperative that we identify how to best manage nutrients to remain on the landscape and protect our water resources. Part of this solution will be to conserve wetlands, particularly in the PPR, where in some jurisdictions drainage of pothole ponds that are key to processing inorganic N inputs continues largely unabated.

7.0 References

Adamsen, F. J., and J. D. Reeder. 1983. A comparison of diffusion and distillation methods for preparing samples for 15N analyses. Soil Sci. Soc. Am. J. **47**: 612–613. doi:10.2136/sssaj1983.03615995004700030047x

Aelion, C. M., and U. Warttinger. 2009. Low sulfide concentrations affect nitrate transformations in freshwater and saline coastal retention pond sediments. undefined **41**: 735–741. doi:10.1016/J.SOILBIO.2009.01.015

Aksnes, D. L., and F. J. Cao. 2011. Inherent and apparent traits in microbial nutrient uptake. Mar. Ecol. Prog. Ser. **440**: 41–51. doi:10.3354/meps09355

An, S., and W. S. Gardner. 2002. Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas). Mar. Ecol. Prog. Ser. **237**: 41–50. doi:10.3354/meps237041

Appling, A. P., and J. B. Heffernan. 2014. Nutrient limitation and physiology mediate the fine-scale (de)coupling of biogeochemical cycles. Am. Nat. **184**: 384–406. doi:10.1086/677282

Armstrong, M. 2018. Water quality impacts of wetland drainage and climate change in the prairie pothole region.

Arya, S. R., and E. K. Syriac. 2018. Wetlands: The living waters-A review. Agric. Rev. **39**: 122–129. doi:10.18805/ag.R-1717

Austin, A. T., M. M. C. Bustamante, G. B. Nardoto, and others. 2013. Latin America's nitrogen challenge. Science (80-.). **340**: 149. doi:10.1126/SCIENCE.1231679

Avery, A. 2000. Blue baby syndrome and nitrates: New research casts doubt on an old regualtion. Water Resour. IMPACT **2**: 11–13.

Baldassarre, G., and E. Bolden. 2006. Waterfowl ecology and managment, 2nd ed. Krieger Publishing Company.

Balderston, W., B. Sherr, and W. Payne. 1976. Blockage by acetylene of nitrous oxide reduction

in Pseudomonas perfectomarinus. Appl. Environ. Microbiol. **31**: 504–508. doi:10.1128/aem.31.4.504-508.1976

Bansal, S., B. Tangen, and R. Finocchiaro. 2016. Temperature and hydrology affect methane emissions from prairie pothole wetlands. Wetlands **36**: 371–381. doi:10.1007/s13157-016-0826-8

Barbaree, J. M., and W. J. Payne. 1967. Products of denitrification by a marine bacterium as revealed by gas chromatography. Mar. Biol. **1**: 136–139. doi:10.1007/BF00386519

Bastviken, S. K., P. G. Eriksson, I. Martins, J. M. Neto, L. Leonardson, and K. Tonderski. 2003. Potential nitrification and denitrification on different surfaces in a constructed treatment wetland. J. Environ. Qual. **32**: 2414. doi:10.2134/jeq2003.2414

Batt, B., M. Anderson, C. Anderson, and F. Caswell. 1989. The use of prairie potholes by North American ducks, *In* Northern prairie wetlands. Iowa State University Press.

Baustian, M., K. Craig, and N. Rabalais. 2009. Effects of summer 2003 hypoxia on macrobenthos and Atlantic croaker foraging selectivity in the northern Gulf of Mexico. J. Exp. Mar. Bio. Ecol. **381**: S31–S37. doi:10.1016/j.jembe.2009.07.007

Bbalali, S. 2013. Relationships between nutrients and chlorophyll a concentration in the international Alma Gol wetland, Iran. J. Aquac. Res. Dev. **04**. doi:10.4172/2155-9546.1000173

Bedard-Haughn, A., J. W. Van Groenigen, and C. Van Kessel. 2003. Tracing ^{15}N through landscapes: Potential uses and precautions. J. Hydrol. **272**: 175–190. doi:10.1016/S0022-1694(02)00263-9

Bedard-Haughn, A., A. L. Matson, and D. J. Pennock. 2006. Land use effects on gross nitrogen mineralization, nitrification, and N_2O emissions in ephemeral wetlands. Soil Biol. Biochem. **38**: 3398–3406. doi:10.1016/j.soilbio.2006.05.010

Bekheet, I., and P. Syrett. 1979. The uptake of urea by Chlorella. New Phytol. **82**: 179–186. doi:10.1111/j.1469-8137.1979.tb07573.x

Bengtsson, G., and C. Bergwall. 2000. Fate of ^{15}N labelled nitrate and ammonium in a fertilized forest soil. Soil Biol. Biochem. **32**: 545–557.

van den Berg, E. M., M. Boleij, J. G. Kuenen, R. Kleerebezem, and M. C. M. van Loosdrecht. 2016. DNRA and denitrification coexist over a broad range of acetate/N-NO$_3^-$ ratios, in a chemostat enrichment culture. Front. Microbiol. **7**: 1–12. doi:10.3389/fmicb.2016.01842

Van Den Berg, E. M., U. Van Dongen, B. Abbas, and M. C. M. Van Loosdrecht. 2015. Enrichment of DNRA bacteria in a continuous culture. ISME J. **9**: 2153–2161. doi:10.1038/ismej.2015.26

van den Berg, E. M., M. P. Elisário, J. G. Kuenen, R. Kleerebezem, and M. C. M. van Loosdrecht. 2017a. Fermentative bacteria influence the competition between denitrifiers and DNRA bacteria. Front. Microbiol. **8**: 1–13. doi:10.3389/fmicb.2017.01684

van den Berg, E. M., J. L. Rombouts, J. G. Kuenen, R. Kleerebezem, and M. C. M. van Loosdrecht. 2017b. Role of nitrite in the competition between denitrification and DNRA in a chemostat enrichment culture. AMB Express **7**. doi:10.1186/s13568-017-0398-x

Berg, G. M., M. Balode, I. Purina, S. Bekere, C. Béchemin, and S. Y. Maestrini. 2003. Plankton community composition in relation to availability and uptake of oxidized and reduced nitrogen. Aquat. Microb. Ecol. **30**: 263–274. doi:10.3354/ame030263

Berman, T., and D. A. Bronk. 2003. Dissolved organic nitrogen: A dynamic participant in aquatic ecosystems. Aquat. Microb. Ecol. **31**: 279–305. doi:10.3354/ame031279

Bhavya, P. S., S. Kumar, G. V. M. Gupta, V. Sudheesh, K. V. Sudharma, D. S. Varrier, K. R. Dhanya, and N. Saravanane. 2016. Nitrogen Uptake Dynamics in a Tropical Eutrophic Estuary (Cochin, India) and Adjacent Coastal Waters. Estuaries and Coasts **39**: 54–67. doi:10.1007/s12237-015-9982-y

Biswas, A., H. W. Chau, A. K. Bedard-Haughn, and B. C. Si. 2012. Factors controlling soil water storage in the hummocky landscape of the Prairie Pothole Region of North America. Can. J. Soil Sci. **92**: 649–663. doi:10.4141/CJSS2011-045

Blackmer, A. M., and J. M. Bremner. 1978. Inhibitory effect of nitrate on reduction of N_2O to N_2 by soil microorganisms. Soil Biol. Biochem. **10**: 187–191. doi:10.1016/0038-0717(78)90095-0

Blackwell, M., D. Hogan, G. Pinay, and E. Maltby. 2009. The Role of Buffer Zones for Agricultural Runoff, p. 417–439. *In* E. Maltby and T. Barker [eds.], The Wetlands Handbook. Blackwell Publishing Ltd.

Bonachela, J. A., M. Raghib, and S. A. Levin. 2011. Dynamic model of flexible phytoplankton nutrient uptake. Proc. Natl. Acad. Sci. **108**: 20633–20638. doi:10.1073/pnas.1118012108

Booth, M. S., J. M. Stark, and E. Rastetter. 2005. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. Ecol. Monogr. **75**: 139–157. doi:10.1890/04-0988

Bowman, R. A., and D. D. Focht. 1974. The influence of glucose and nitrate concentrations upon denitrification rates in sandy soils. Soil Biol. Biochem. **6**: 297–301. doi:10.1016/0038-0717(74)90034-0

Bradley, P. B., M. P. Sanderson, M. E. Frischer, J. Brofft, M. G. Booth, L. J. Kerkhof, and D. A. Bronk. 2010. Inorganic and organic nitrogen uptake by phytoplankton and heterotrophic bacteria in the stratified Mid-Atlantic Bight. Estuar. Coast. Shelf Sci. **88**: 429–441. doi:10.1016/j.ecss.2010.02.001

Braskerud, B. C. C. 2002. Factors affecting nitrogen retention in small constructed wetlands treating agricultural non-point source pollution. Ecol. Eng. **18**: 351–370. doi:10.1016/S0925-8574(01)00099-4

Braun, J., M. Mooshammer, W. Wanek, J. Prommer, T. W. N. Walker, T. Rütting, and A. Richter. 2018. Full ^{15}N tracer accounting to revisit major assumptions of ^{15}N isotope pool dilution approaches for gross nitrogen mineralization. Soil Biol. Biochem. **117**: 16–26. doi:10.1016/j.soilbio.2017.11.005

Brodrick, S. J., P. Cullen, and W. Maher. 1988. Denitrification in a natrual wetland receiving secondary treated effulent. Water Res. **22**: 431–439. doi:10.1016/0043-1354(88)90037-1

Bronk, D. A., P. M. Gilbert, T. C. Malone, S. Banahan, and E. Sahlsten. 1998. Inorganic and organic nitrogen cycling in Chesapeake Bay: Autotrophic versus heterotrophic processes and relationships to carbon flux. Aquat. Microb. Ecol. **15**: 177–189. doi:10.3354/ame015177

Bronk, D. A., P. M. Gilbert, and B. B. Ward. 1994a. Nitrogen uptake, dissolved organic nitrogen release, and new production. Science (80-.). **265**: 1843–1846. doi:10.1126/science.265.5180.1843

Brönmark, C., and L. Hansson. 2017. The biology of Lakes, 3rd ed. Oxford University Press.

Brown, R., Z. Zhang, L. P. Comeau, and A. Bedard-Haughn. 2017. Effects of drainage duration on mineral wetland soils in a Prairie Pothole agroecosystem. Soil Tillage Res. **168**: 187–197. doi:10.1016/j.still.2016.12.015

Brunet, R. C., and L. J. Garcia-Gil. 1996. Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. FEMS Microbiol. Ecol. **21**: 131–138. doi:10.1016/0168-6496(96)00051-7

Bunn, T. 2012. The nitrogen cycle. Microbiol. Today **39**: 112–119.

Burgin, A. J., and S. K. Hamilton. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? Front. Ecol. Environ. **5**: 1–9. doi:10.1890/1540-9295(2007)5[89:HWOTRO]2.0.CO;2

Burgin, A. J., and S. K. Hamilton. 2008. NO_3^--driven SO_4^{2-} production in freshwater ecosystems: Implications for N and S cycling. Ecosystems **11**: 908–922. doi:10.1007/s10021-008-9169-5

Burkart, M. R., and J. D. Stoner. 2008. Nitrogen in groundwater associated with agricultural systems, p. 177–202. *In* R.F. Follett and J.L. Hatfield [eds.], Nitrogen in the Environment: Sources, Problems, and Management. Elsevier B.V.

Cade-Menun, B. J., G. Bell, S. Baker-Ismail, Y. Fouli, K. Hodder, D. W. McMartin, C. Perez-Valdivia, and K. Wu. 2013. Nutrient loss from Saskatchewan cropland and pasture in spring

snowmelt runoff. Can. J. Soil Sci. **93**: 445–458. doi:10.4141/cjss2012-042

Canada, E. 2008. Ecoregions of Saskatchewan.

Casey, J. R., S. Grinstein, and J. Orlowski. 2010. Sensors and regulators of intracellular pH. Nat. Rev. Mol. Cell Biol. **11**: 50–61. doi:10.1038/nrm2820

Cassidy, E. S., P. C. West, J. S. Gerber, and J. A. Foley. 2013. Redefining agricultural yields: From tonnes to people nourished per hectare. Environ. Res. Lett. **8**. doi:10.1088/1748-9326/8/3/034015

Cavaliere, E. 2018. Biogeochemistry Under Ice. University of Saskatchewan.

Chamberlin, C. A., E. S. Bernhardt, E. J. Rosi, and J. B. Heffernan. 2019. Stoichiometry and daily rhythms: experimental evidence shows nutrient limitation decouples N uptake from photosynthesis. Ecology **100**: 1–7. doi:10.1002/ecy.2822

Chan, H. K., and N. E. Campbell. 1978. Phytoplankton uptake and excretion of assimilated nitrate in a small Canadian shield lake. Appl. Environ. Microbiol. **35**: 1052–1060. doi:10.1128/aem.35.6.1052-1060.1978

Chang, C., S. Silva, C. Kendall, G. Michalski, K. Casciotti, and S. Wankel. 2004. Preparation and analysis of nitrogen-bearing compounds in water for stable isotope ratio measurement, p. 305–347. *In* P. Groot [ed.], Handbook of Stable Isotope Analytical Techniques, Volume 1. Elsevier B.V.

Chen, L., X. Zhang, B. He, and others. 2020. Dark Ammonium Transformations in the Pearl River Estuary During Summer. J. Geophys. Res. Biogeosciences **125**: e2019JG005596. doi:10.1029/2019JG005596

Chen, Y., J. J. Cheng, and K. S. Creamer. 2008. Inhibition of anaerobic digestion process: A review. Bioresour. Technol. **99**: 4044–4064. doi:10.1016/J.BIORTECH.2007.01.057

Clark, D., K. Flynn, and N. Owens. 2002. The large capacity for dark nitrate-assimilation in diatoms may overcome nitrate limitation of growth. New Phytol. **155**: 101–108.

doi:10.1046/j.1469-8137.2002.00435.x

Cochlan, W. P., and P. J. Harrison. 1991. Kinetics of nitrogen (nitrate, ammonium and urea) uptake by the picoflagellate Micromonas pusilla (Prasinophyceae). J. Exp. Mar. Bio. Ecol. **153**: 129–141. doi:10.1016/0022-0981(91)90220-Q

Cojean, A. N. Y., M. F. Lehmann, E. K. Robertson, B. Thamdrup, and J. Zopfi. 2020. Controls of H_2S, Fe^{2+}, and Mn^{2+} on Microbial NO_3^--Reducing Processes in Sediments of an Eutrophic Lake. Front. Microbiol. **11**: 1–17. doi:10.3389/fmicb.2020.01158

Cole, A. J., R. De Nys, and N. A. Paul. 2014. Removing constraints on the biomass production of freshwater macroalgae by manipulating water exchange to manage nutrient flux. PLoS One **9**: 1–9. doi:10.1371/journal.pone.0101284

Coles, A. E., and J. J. McDonnell. 2018. Fill and spill drives runoff connectivity over frozen ground. J. Hydrol. **558**: 115–128. doi:10.1016/j.jhydrol.2018.01.016

Collos, Y. 1983. Transient situations in nitrate assimilation by marine diatoms. IV Non-linear phenomena and the estimation of the maximum uptake rate. J. Plankton Res. **5**: 677–691. doi:10.1093/plankt/5.5.677

Collos, Y., and J. Berges. 2003. Nitrogen Metabolism in Phytoplankton. Mar. Ecol. 262–280.

Collos, Y., M. Y. Siddiqi, M. Y. Wang, A. D. M. Glass, and P. J. Harrison. 1992. Nitrate uptake kinetics by two marine diatoms using the radioactive tracer13N. J. Exp. Mar. Bio. Ecol. **163**: 251–260. doi:10.1016/0022-0981(92)90053-D

Collos, Y., A. Vaquer, B. Bibent, G. Slawyk, N. Garcia, and P. Souchu. 1997. Variability in nitrate uptake kinetics of phytoplankton communities in a mediterranean coastal lagoon. Estuar. Coast. Shelf Sci. **44**: 369–375. doi:10.1006/ecss.1996.0121

Conway, H., P. Harrison, and C. Davis. 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of Skeletonema costatum to a single addition of the limiting nutrient. Mar. Biol. **35**: 187–199. doi:10.1007/BF00390940

Conway, H., and T. Whitledge. 1979. Distribution, fluxes and biological utilization of inorganic nitrogen during a spring bloom in New York Bight. J. Mar. Res. **37**: 657–668.

Cooper, A. B. 1990. Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. Hydrobiologia **202**: 13–26. doi:10.1007/BF02208124

Costanza, R., R. D'Arge, R. de Groot, and others. 1997. The value of the world's ecosystem services and natural capital. Nat. TA - TT - **387**: 253–260. doi:10.1038/387253a0

Cotner, J. B., and B. A. Biddanda. 2002. Small players, large role: Microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems **5**: 105–121. doi:10.1007/s10021-001-0059-3

Coupland, R. 1983. A reconsideration of grassland classification in the northern great plains of North America. J. Ecol. **49**: 135–167. doi:10.2307/2257431

Crane, B. R., L. M. Siegel, and E. D. Getzoff. 1995. Sulfite Reductase Structure at 1.6 A: Evolution and Catalysis for Reduction of Inorganic Anions. Science (80-.). **270**: 59–67. doi:10.1126/science.270.5233.59

Crumpton, W. 1989. Algae in northern prairie wetlands, p. 188–203. *In* A. Van der Valk [ed.], Northern prairie wetlands. Iowa State University Press.

Crumpton, W. G., and L. G. Goldsborough. 1998. Nitrogen transformation and fate in prairie wetlands. Gt. Plains Res. **8**: 57–72.

Cunha, F., V. Fernandes de Melo Lima, A. Menegante Neri, and others. 2017. Uptake rates of ammonium and nitrate by phytoplankton communities in two eutrophic tropical reservoirs. Int. Rev. Hydrobiol. **102**: 125–134. doi:10.1002/iroh.201701900

Dahl, T. 2011. Status and trends of wetlands in the conteminous United States 2004 to 2009.

Dalcin-Martins, P., R. E. Danczak, S. Roux, J. Frank, M. A. Borton, R. A. Wolfe, M. N. Burris, and M. J. Wilkins. 2018. Viral and metabolic controls on high rates of microbial sulfur and carbon cycling in wetland ecosystems. Microbiome **6**: 1–17. doi:10.1186/s40168-018-0522-

4

Dalcin-Martins, P., D. Hoyt, S. Bansal, and others. 2017. Abundant carbon substrates drive extremely high sulfate reduction rates and methane fluxes in Prairie Pothole Wetlands. Glob. Chang. Biol. **23**: 3107–3120. doi:10.1111/gcb.13633

Davidson, E., S. Hart, C. Shanks, and M. Firestone. 1991. Measuring gross nitrogen mineralization, and nitrification by 15 N isotopic pool dilution in intact soil cores. J. Soil Sci. **42**: 335–349. doi:10.1111/j.1365-2389.1991.tb00413.x

Davidsson, T., and M. Stahl. 2000. The influence of organic carbon on nitrogen transformations in five wetland soils. Soil Soc. Am. **64**: 1129–1136.

Deghi, G. S., and K. C. Ewel. 1984. Simulated effect of wastewater application on phosphorus distribution in Cypress domes, p. 102–111. *In* K.C. Ewel and H.T. Odum [eds.], Cypress Swamps. University Presses of Florida.

Delaune, R., K. R. Reddy, C. Richardson, and J. Megonigal. 2013. Methods in Biogeochemistry of Wetlands, 10th ed. S. Logsdon [ed.]. Soil Science Society of America.

Deng, F., L. Hou, M. Liu, and others. 2015. Dissimilatory nitrate reduction processes and associated contribution to nitrogen removal in sediments of the Yangtze Estuary. J. Geophys. Res. Biogeosciences **120**: 1521–1531. doi:10.1002/2015JG003007.Received

Detenbeck, N. E. 2002. Methods for Evaluating Wetland Condition #7: Wetlands Classification. United States Environ. Prot. Agency, Off. Water. **EPA-822-R-**: 43.

Deunff, E., P. Malagoli, and M. Decau. 2019. Modelling nitrogen uptake in plants and phytoplankton: advantages of integrating flexibility into the spatial and temporal dynamics of nitrate absorption. Agronomy **9**: 116. doi:10.3390/agronomy9030116

Dierberg, F. E., and P. L. Brezonikt. 2017. Nitrogen and Phosphorus Mass Balances in Natural and Sewage-Enriched Cypress Domes. J. Appl. Ecol. **20**: 323–337. doi:https://doi.org/10.2307/2403395

Doherty, K., A. Ryba, C. Stemler, N. Niemuth, and W. Meeks. 2013. Conservation planning in an era of change: state of the U.S. Prairie Pothole Region. Wildl. Soc. Bull. **37**: 546–563. doi:10.1002/wsb.284

Domingues, R. B., A. B. Barbosa, U. Sommer, and H. M. Galvão. 2011. Ammonium, nitrate and phytoplankton interactions in a freshwater tidal estuarine zone: Potential effects of cultural eutrophication. Aquat. Sci. **73**: 331–343. doi:10.1007/s00027-011-0180-0

Dominika, G., M. Joanna, and M. Jacek. 2021. Sulfate reducing ammonium oxidation (SULFAMMOX) process under anaerobic conditions. Environ. Technol. Innov. **22**: 101416. doi:10.1016/j.eti.2021.101416

Donald, D. B., M. J. Bogard, K. Finlay, L. Bunting, and P. R. Leavitt. 2013. Phytoplankton-Specific Response to Enrichment of Phosphorus-Rich Surface Waters with Ammonium, Nitrate, and Urea. PLoS One **8**. doi:10.1371/journal.pone.0053277

Donald, D. B., M. J. Bogard, K. Finlay, and P. R. Leavitt. 2011. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. Limnol. Oceanogr. **56**: 2161–2175. doi:10.4319/lo.2011.56.6.2161

Dortch, Q. 1980. The interaction between ammonium and nitrate uptake in phytoplankton. Mar. Ecol. Prog. Ser. **61**: 183–201. doi:10.3354/meps061183

Dortch, Q., J. R. Clayton, S. S. Thoresen, and S. I. Ahmed. 1984. Species differences in accumulation of nitrogen pools in phytoplankton. Mar. Biol. **81**: 237–250. doi:10.1007/BF00393218

Downing, T., C. Meyer, M. Gehringer, and M. van de Venter. 2005. Microcystin content of Microcystis aeruginosa is modulated by nitrogen uptake rate relative to specific growth rate of carbon fixation rate. Wiley Period. Inc. doi:10.1002/tox.20106

Drerup, S. A., and Y. Vadeboncoeur. 2016. Elevated specific conductance enhances productivity and biomass of periphytic cyanobacteria from Lake Tahoe and Lake Tanganyika. Phycologia **55**: 295–298. doi:10.2216/15-100.1

Dugdale, R. C., and J. J. Goering. 1967. Uptake of new and regenreated forms of ntirogen in primary productivity. Limnol. Oceanogr. **12**: 196–206. doi:10.4319/lo.1967.12.2.0196

Dugdale, R. C., and F. P. Wilkerson. 1986. The use of ^{15}N to measure nitrogen uptake in eutrophic experimental considerations1,2. Limnol. Oceanogr. **31**: 673–689. doi:10.4319/lo.1986.31.4.0673

Dyke, S. 2004. An outline of North American deglaciation with emphasis on central and northern Canada, p. 373–403. *In* J. Ehlers and P. Gibbard [eds.], Quaternary Glaciations - Extent and Chronology, Part II. Elsevier B.V.

Eisenlohr, W. S. J. 1972. Hydrologic investigations of prairie potholes in North Dakota, 1959-68. Geol. Surv. Prof. Pap. **585**-**A**. doi:10.3133/pp585A

Elser, J., E. Marzolf, and C. Goldman. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Can. J. Fish. Aquat. Sci. **47**: 1468–1477. doi:10.1139/f90-165

Eppley, R. W., and J. L. Coatsworth. 1968. Uptake of nitrate and nitrite by Ditylum brightwellii—Kinetics and mechanisms. J. Phycol. **4**: 151–156. doi:10.1111/j.1529-8817.1968.tb04689.x

Erisman, J. W., M. A. Sutton, J. Galloway, Z. Klimont, and W. Winiwarter. 2008. How a century of ammonia synthesis changed the world. Nat. Geosci. **1**: 636–639. doi:10.1038/ngeo325

Erratt, K. J., I. F. Creed, and C. G. Trick. 2018. Comparative effects of ammonium, nitrate and urea on growth and photosynthetic efficiency of three bloom-forming cyanobacteria. Freshw. Biol. **63**: 626–638. doi:10.1111/fwb.13099

Euliss, N. H. J., D. Mushet, and D. Johnson. 2002. Using aquatic invertebrates to delineate seasonal and temporary wetlands in the prairie pothole region of North America. Wetlands **22**: 256–262. doi:10.1672/0277-5212(2002)022[0256:UAITDS]2.0.CO;2

Euliss, N. H., and D. M. Mushet. 1999. Influence of agriculture on aquatic invertebrate communities of temporary wetlands in the Prairie Pothole Region of North Dakota, USA.

Wetlands **19**: 578–583. doi:10.1007/BF03161695

Evenson, G. R., H. E. Golden, C. R. Lane, and others. 2018. Depressional Wetlands Affect
  Watershed Hydrological, Biogeochemical, and Ecological Functions. Ecol. Appl. **28**: 953–
  966. doi:10.1002/eap.01701

Eyre, B. D., D. T. Maher, and P. Squire. 2013. Quantity and quality of organic matter (detritus)
  drives N2 effluxes (net denitrification) across seasons, benthic habitats, and estuaries.
  Global Biogeochem. Cycles **27**: 1083–1095. doi:10.1002/2013GB004631

Eyre, B. D., S. Rysgaard, T. Dalsgaard, and P. B. Christensen. 2002. Comparison of isotope
  pairing and N2:Ar methods for measuring sediment denitrification - Assumptions,
  modifications, and implications. Estuaries **25**: 1077–1087. doi:10.1007/bf02692205

Falkner, G., F. Horner, and W. Simonis. 1980. The regulation of the energy-dependent phosphate
  uptake by the blue-green Alga anacystis nidulans. Planta **149**: 138–143.
  doi:10.1007/BF00380874

Falkowski, P. G., and D. P. Stone. 1975. Nitrate uptake in marine phytoplankton: Energy sources
  and the interaction with carbon fixation. Mar. Biol. **32**: 77–84. doi:10.1007/BF00395161

Fan, L., M. T. Brett, B. Li, and M. Song. 2018. The bioavailability of different dissolved organic
  nitrogen compounds for the freshwater algae Raphidocelis subcapitata. Sci. Total Environ.
  618: 479–486. doi:10.1016/j.scitotenv.2017.11.096

Fang, X., A. Minke, J. W. Pomeroy, T. Brown, C. Westbrook, X. Guo, and S. Guangul. 2007. A
  review of Canadian prairie hydrology: Principles, modelling and response to land use and
  drainage change.

Fang, X., and J. Pomeroy. 2009. Modelling blowing snow redistribution to prairie wetlands.
  Hydrol. Process. **23**: 2557–2569. doi:10.1002/hyp

Fantner, G. 2013. A brief introduction to error analysis and propagation. 1–13.

FAO. 2017. World fertilizer trends and outlook to 2020: Summary report. Food Agric. Organ.

United Nations 38.

FAO. 2019. World fertilizer trends and outlook to 2022.

Fazzolari, E., and J. Germon. 1991. Dissimilative nitrate reduction to ammonium in different soils in waterlogged conditions, p. 295–308. *In* J. Berthelin [ed.], Developments in Geochemistry. Elsevier.

Fazzolari, E., B. Nicolardot, and J. C. Germon. 1998. Simultaneous effects of increasing levels of glucose and oxygen partial pressures on denitrification and dissimilatory reduction to ammonium in repacked soil cores. Eur. J. Soil Biol. **34**: 47–52. doi:10.1016/S1164-5563(99)80006-5

Ficken, J. 1967. Winter loss and spring recovery of dissovled solids, p. 228–235. *In* Geological Survey Research.

Fiksen, Ø., M. J. Follows, and D. L. Aksnes. 2013. Trait-based models of nutrient uptake in microbes extend the Michaelis-Menten framework. Limnol. Oceanogr. **58**: 193–202. doi:10.4319/lo.2013.58.1.0193

Finlay, B., A. Span, and J. Harman. 1983. Nitrate respiration in primitive eukaryotes. Nature **303**: 333–336. doi:10.1038/303333a0

Finlay, K., A. Patoine, D. B. Donald, M. J. Bogard, and P. R. Leavitt. 2010. Experimental evidence that pollution with urea can degrade water quality in phosphorus-rich lakes of the Northern Great Plains. Limnol. Oceanogr. **55**: 1213–1230. doi:10.4319/lo.2010.55.3.1213

Fischer, R., H. A. Giebel, and R. Ptacnik. 2017. Identity of the limiting nutrient (N vs. P) affects the competitive success of mixotrophs. Mar. Ecol. Prog. Ser. **563**: 51–63. doi:10.3354/meps11968

Flöder, S., S. Jaschinski, G. Wells, and C. W. Burns. 2010. Dominance and compensatory growth in phytoplankton communities under salinity stress. J. Exp. Mar. Bio. Ecol. **395**: 223–231. doi:10.1016/j.jembe.2010.09.006

Flores, E., and A. Herrero. 2005. Nitrogen assimilation and nitrogen control in cyanobacteria. Biochem. Soc. Trans. **33**: 164–167. doi:10.1042/BST0330164

Follett, R. F. 1999. Nitrogen Transformation and Transport Processes, Elsevier B.V.

France, C., and J. Mankin. 1977. High nitrate denitrification in continuous flow-stirred reactors. Water Res. **11**: 289–294. doi:10.1016/0043-1354(77)90061-6

Gala, T. S., and D. Young. 2015. Geographically isolated depressional wetlands – Hydrodynamics, ecosystem functions and conditions. Appl. Ecol. Environ. Sci. **3**: 108–116. doi:10.12691/aees-3-4-3

Galbraith, H., P. Amerasinghe, and A. Huber-Lee. 2005. The effects of agricultural irrigation on wetland ecosystems in developing countries: A literature review.

Gale, P., K. R. Reddy, and D. A. Graetz. 1993. Nitrogen removal from reclaimed water applied to constructed wetland microcosms. Water Environ. Resour. **65**: 162–168. doi:10.2175/WER.65.2.9

Galloway, J. N., J. D. Aber, J. W. Erisman, S. P. Seitzinger, R. W. Howarth, E. B. Cowling, and J. B. Cosby. 2003. The Nitrogen Cascade. Bioscience **53**: 341. doi:10.1641/0006-3568(2003)053[0341:TNC]2.0.CO;2

Galloway, J. N., and E. B. Cowling. 2002. Reactive nitrogen and the world: 200 years of change. AMBIO A J. Hum. Environ. **31**: 64–71. doi:10.1579/0044-7447-31.2.64

Galloway, J. N., A. M. Leach, A. Bleeker, and J. W. Erisman. 2013. A chronology of human understanding of the nitrogen cycle. Philos. Trans. R. Soc. B Biol. Sci. **368**. doi:10.1098/rstb.2013.0120

Gamble, T. N., M. R. Betlach, and J. M. Tiedje. 1977. Numerically dominant denitrifying bacteria from world soils. Appl. Environ. Microbiol. **33**: 926–939. doi:10.1128/aem.33.4.926-939.1977

Gardner, W., M. McCarthy, S. An, D. Sobolev, and S. Karen. 2006. Nitrogen fixation and

dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries. Limnol. Oceanogr. **51**: 558–568. doi:10.4319/lo.2006.51.1_part_2.0558

Gayon, V., and G. Dupetit. 1886. Recherches sur la reduction des nitrates par les infiniments, petits. Soc. des Sci. Phys. Nat. Bordeaux, Fr. **3**: 201–307.

Ghermandi, A., J. Van den Bergh, L. Brander, H. de Groot, and P. Nunes. 2010. The values of natural and constructed wetlands: A meta-analysis. Water Resour. Res. **46**: 1–12. doi:10.2139/ssrn.1474751

Giblin, A. E., C. R. Tobias, B. Song, N. Weston, G. T. Banta, and V. H. Rivera-Monroy. 2013. The importance of dissimilatory nitrate reduction to ammonium (DNRA) in the nitrogen cycle of coastal ecosystems. Oceanography **26**: 124–131. doi:10.5670/oceanog.2013.54

Giblin, A. E., N. B. Weston, G. T. Banta, J. Tucker, and C. S. Hopkinson. 2010. The effects of salinity on nitrogen losses from an oligohaline estuarine sediment. Estuaries and Coasts **33**: 1054–1068. doi:10.1007/s12237-010-9280-7

Giles, J. 2005. Nitrogen study fertilizes fears of pollution. Nature **433**: 791–791. doi:10.1038/433791a

Glibert, P., J. Harrison, C. Heil, and S. Seitzinger. 2006. Escalating worldwide use of urea - a global change contributing to coastal eutrophication. Int. J. Clin. Exp. Med. **77**: 441–463. doi:10.1007/s10533-005-3070-5

Glibert, P., C. Heil, D. Hollander, M. Revilla, A. Hoare, J. Alexander, and S. Murasko. 2004. Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. Mar. Ecol. Prog. Ser. **280**: 73–83. doi:10.3354/meps280073

Glibert, P., and T. Kana. 2016. Aquatic microbial ecology and biogeochemistry: A dual perspective, Spring.

Glibert, P. M., C. Garside, J. A. Fuhrman, and M. R. Roman. 1991. Dependent coupling of inorganic and organic nitrogen uptake and regeneration in the plume of the Chesapeake Bay estuary and its regulation by large heterotrophs. Limnol. Oceanogr. **36**: 895–909.

doi:10.4319/lo.1991.36.5.0895

Glibert, P. M., C. Garside, J. A. Fuhrman, and M. R. Roman. 2011. Time-dependent coupling of inorganic and organic nitrogen uptake and regeneration in the plume of the Chesapeake Bay estuary and its regulation by large heterotrophs time-dependent coupling of inorganic and organic nitrogen in the plume of the Chesapeake. Limnol. Oceanogr. **36**: 895–909. doi:10.4319/lo.1991.36.5.0895

Glibert, P., F. Wilkerson, R. Dugdale, and others. 2016. Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. Limnol. Oceanogr. **61**: 165–197. doi:10.1002/lno.10203

Gobler, C. J., A. Burson, F. Koch, Y. Tang, and M. R. Mulholland. 2012. The role of nitrogenous nutrients in the occurrence of harmful algal blooms caused by Cochlodinium polykrikoides in New York estuaries (USA). Harmful Algae **17**: 64–74. doi:10.1016/j.hal.2012.03.001

Gobler, C. J., M. J. Renaghan, and N. J. Buck. 2002. Impacts of nutrients and grazing mortality on the abundance of Aureococcus anophagefferens during a New York brown tide bloom. Limnol. Oceanogr. **47**: 129–141. doi:10.4319/lo.2002.47.1.0129

Goldhaber, M. B., C. T. Mills, J. M. Morrison, C. A. Stricker, D. M. Mushet, and J. W. LaBaugh. 2014. Hydrogeochemistry of prairie pothole region wetlands: Role of long-term critical zone processes. Chem. Geol. **387**: 170–183. doi:10.1016/j.chemgeo.2014.08.023

Goldhaber, M. B., C. T. Mills, D. M. Mushet, B. B. McCleskey, and J. Rover. 2016. Controls on the Geochemical Evolution of Prairie Pothole Region Lakes and Wetlands Over Decadal Time Scales. Wetlands **36**: 255–272. doi:10.1007/s13157-016-0854-4

Goldman, J., and P. Glibert. 1982. Comparative rapid ammonium uptake by four species of marine phytoplankton. Limnol. Oceanogr. **27**: 814–827. doi:10.4319/lo.1982.27.5.0814

Goodale, C., J. Aber, and W. McDowell. 2000. The long-term effects of disturbance on organic and inorganic nitrogen export in the White Mountains, New Hampshire. Ecosystems **3**:

433–450. doi:10.1007/s100210000039

Gray, D. M., and P. G. Landine. 1988. An energy-budget snowmelt model for the Canadian
Prairies. Can. J. Earth Sci. **25**: 1292–1303. doi:10.1139/e88-124

Groffman, P. 2000. Nitrogen in the environment, p. 190–200. *In* M. Sumner [ed.], Handbook of
Soil Science. CRC Press.

Groffman, P. M., M. a. Altabet, J. K. Bohlke, and others. 2006. Methods for Measuring
Denitrification : Ecol. Appl. **16**: 2091–2122. doi:10.1890/1051-
0761(2006)016[2091:MFMDDA]2.0.CO;2

Grubba, D., and J. Majtacz. 2020. The influence of sulfate on anaerobic ammonium oxidation in
a sequencing batch reactor. Water (Switzerland) **12**. doi:10.3390/w12113004

Gu, B., K. E. Havens, C. L. Schelske, and B. H. Rosen. 1997. Uptake of dissolved nitrogen by
phytoplankton in a eutrophic subtropical lake. J. Plankton Res. **19**: 759–770.
doi:10.1093/plankt/19.6.759

Guildford, S. J., and R. E. Hecky. 2000. Total nitrogen, total phosphorus, and nutrient limitation
in lakes and oceans: Is there a common relationship? Limnol. Oceanogr. **45**: 1213–1223.
doi:10.4319/lo.2000.45.6.1213

Guillard, R. 1962. Salt and osmotic balance, p. 529–540. *In* R. Lewin [ed.], Physiology and
biochemistry of algae. Academic Press.

Halterman, S., and D. Toetz. 1984. Kinetics of nitrate uptake by freshwater algae. Hydrobiologia
114: 209–214. doi:10.1007/BF00031872

Hamme, R. C., and S. R. Emerson. 2004. The solubility of neon, nitrogen and argon in distilled
water and seawater. Deep. Res. Part I Oceanogr. Res. Pap. **51**: 1517–1528.
doi:10.1016/j.dsr.2004.06.009

Hammer, K. J., T. Kragh, and K. Sand-Jensen. 2019. Inorganic carbon promotes photosynthesis,
growth, and maximum biomass of phytoplankton in eutrophic water bodies. Freshw. Biol.

64: 1956–1970. doi:10.1111/fwb.13385

Hampel, J. J., M. J. McCarthy, W. S. Gardner, L. Zhang, H. Xu, G. Zhu, and S. E. Newell. 2018. Nitrification and ammonium dynamics in Taihu Lake, China: Seasonal competition for ammonium between nitrifiers and cyanobacteria. Biogeosciences **15**: 733–748. doi:10.5194/bg-15-733-2018

Hansson, L. A., C. Brönmark, P. A. Nilsson, and K. Åbjörnsson. 2005. Conflicting demands on wetland ecosystem services: Nutrient retention, biodiversity or both? Freshw. Biol. **50**: 705–714. doi:10.1111/j.1365-2427.2005.01352.x

Harrison, P., J. Parslow, and H. Conway. 1989. Determination of nutrient uptake kinetic parameters: a comparison of methods. Mar. Ecol. Prog. Ser. **52**: 301–312. doi:10.3354/meps052301

Hayes, N. M., H. A. Haig, G. L. Simpson, and P. R. Leavitt. 2020. Effects of lake warming on the seasonal risk of toxic cyanobacteria exposure. Limnol. Oceanogr. Lett. **5**: 393–402. doi:10.1002/lol2.10164

He, X., M. Xu, G. Y. Qiu, and J. Zhou. 2009. Use of ^{15}N stable isotope to quantify nitrogen transfer between mycorrhizal plants. **2**: 107–118. doi:10.1093/jpe/rtp015

Heagle, D., M. Hayashi, and G. Van Der Kamp. 2013. Surface-subsurface salinity distribution and exchange in a closed-basin prairie wetland. J. Hydrol. **478**: 1–14. doi:10.1016/j.jhydrol.2012.05.054

Heagle, D. J., M. Hayashi, and G. Kamp. 2007. Use of solute mass balance to quantify geochemical processes in a prairie recharge wetland. Wetlands **27**: 806–818. doi:10.1672/0277-5212(2007)27[806:UOSMBT]2.0.CO;2

Healey, F. P. 1977. Ammonium and urea uptake by some freshwater algae. Can. J. Bot. **55**: 61–69. doi:10.1139/b77-013

Henderson, D. 2013. St. Denis National Wildlife Area Management Plan,.

Higgins, K. F., D. E. Naugle, and K. J. Forman. 2002. A case study of changing land use practices in the Northern Great Plains , U.S.A.: An uncertain future for waterbird conservation. Waterbirds Int. J. waterbird Biol. **25**: 42–50.

Holmes, R. M., J. W. McClelland, D. M. Sigman, B. Fry, and B. J. Peterson. 1998. Measuring ^{15}N-NH$_4^+$ in marine, estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations. Mar. Chem. **60**: 235–243. doi:10.1016/S0304-4203(97)00099-6

Hood, E., M. Williams, and N. Caine. 2003. Landscape controls on organic and inorganic nitrogen leaching across an alpine/subalpine ecotone, Green Lakes Valley, Colorado Front Range. Ecosystems **6**: 31–45. doi:10.1007/s10021-002-0175-8

Houlton, B. Z., M. Almaraz, V. Aneja, and others. 2019. A World of Cobenefits: Solving the Global Nitrogen Challenge. Earth's Futur. **7**: 865–872. doi:10.1029/2019EF001222

Huel, D. 2000. Managing Saskatchewan wetlands - A landowner's guide.

Jansson, M., P. Blomqvist, A. Jonsson, and A. Bergstrom. 1996. Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton, and heterotrophic nanoflagellates in Lake Örträsket. Limnol. Oceanogr. **41**: 1552–1559. doi:10.4319/lo.1996.41.7.1552

Johnston, C. A. 1991. Sediment and nutrient retention by freshwater wetlands: Effects on surface water quality. Crit. Rev. Environ. Control **21**: 491–565. doi:10.1080/10643389109388425

Joint, I., P. Henriksen, G. Fonnes, D. Bourne, T. Thingstad, and B. Riemann. 2002. Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. Aquat. Microb. Ecol. **29**: 145–159. doi:10.3354/ame029145

Jones, B., and D. Deocampo. 2003. Geochemistry of saline lakes, p. 393–424. *In* J. Drever [ed.], Treatise on Geochemistry. Elsevier.

Jones, Z., J. Jasper, D. Sedlak, and J. Sharp. 2017. Sulfide-induced dissimilatory nitrate reduction to ammonium supports anaerobic ammonium oxidation (anammox) in an open-

water unit process wetland. Appl. Environ. Microbiol. **83**. doi:10.1128/AEM.00782-17

Joye, S. B., and J. T. Hollibaugh. 1995. Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. Science (80-.). **270**: 623–625. doi:10.1126/science.270.5236.623

Kabacoff, R. I. 2011. R in action - Data analysis and graphics with R, 1st ed. S. Stirling [ed.]. Manning Publications Co.

Kalkhoff, S. J., L. E. Hubbard, M. D. Tomer, and D. E. James. 2016. Effect of variable annual precipitation and nutrient input on nitrogen and phosphorus transport from two Midwestern agricultural watersheds. Sci. Total Environ. **559**: 53–62. doi:10.1016/j.scitotenv.2016.03.127

Kalra, Y. P., and D. G. Maynard. 1991. Methods Manual for Forest Soil and Plant Analysis. 116.

Kamp, A., S. Høgslund, N. Risgaard-Petersen, and P. Stief. 2015. Nitrate storage and dissimilatory nitrate reduction by eukaryotic microbes. Front. Microbiol. **6**: 1–15. doi:10.3389/fmicb.2015.01492

van der Kamp, G., and M. Hayashi. 2009. Groundwater-wetland ecosystem interaction in the semiarid glaciated plains of North America. Hydrogeol. J. **17**: 203–214. doi:10.1007/s10040-008-0367-1

Van der Kamp, G., and M. Hayashi. 1998. The groundwater recharge function of small wetlands in the semi-arid northern prairies. Gt. Plains Res. **8**: 39–56. doi:10.2307/24156333

van der Kamp, G., M. Hayashi, A. Bedard-Haughn, and D. Pennock. 2016. Prairie Pothole wetlands – Suggestions for practical and objective definitions and terminology. Wetlands **36**: 229–235. doi:10.1007/s13157-016-0809-9

Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennett, and J. C. Cornwell. 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples. Anal. Chem. **66**: 4166–4170. doi:10.1021/ac00095a009

Kanda, J., T. Itoh, D. Ishikawa, and Y. Watanabe. 2003. Environmental control of nitrate uptake

in the East China Sea. Deep. Res. Part II Top. Stud. Oceanogr. **50**: 403–422. doi:10.1016/S0967-0645(02)00464-2

Kanda, J., T. Saino, and A. Hattori. 1985. Nitrogen uptake by natural populations of phytoplankton and primary production in the Pacific Ocean: Regional variability of uptake capacity. Limnol. Oceanogr. **30**: 987–999. doi:10.4319/lo.1985.30.5.0987

Kantrud, H., G. Krapu, and G. Swanson. 1989. Prairie basin wetlnads of the Dakotas: A community profile.

Karpuzcu, M. E., and W. T. Stringfellow. 2012. Kinetics of nitrate removal in wetlands receiving agricultural drainage. Ecol. Eng. **42**: 295–303. doi:10.1016/j.ecoleng.2012.02.015

Kelley, K. R., D. C. Ditsch, and M. M. Alley. 1991. Diffusion and automated nitrogen-15 analysis of low-mass ammonium samples. Soil Sci. Soc. Am. J. **55**: 1016–1020. doi:10.2136/sssaj1991.03615995005500040021x

Kelso, B., R. Smith, R. Laughlin, and D. Lennox. 1997. Dissimilatory nitrate reduction in anaerobic sediments leading to river nitrite accumualtion. Appl. Environ. Microbiol. **63**: 4679–4685.

Kiel, W. M., A. S. Hawkinds, and N. G. Perret. 1972. Waterfowl habitat trends in the aspen parkland of Manitoba. Can. Wildl. Serv. Environ. Canada **18**.

Kirchman, D. L., H. W. Ducklow, J. J. McCarthy, and C. Garside. 1994. Biomass and nitrogen uptake by heterotrophic bacteria during the spring phytoplankton bloom in the North Atlantic Ocean. Deep. Res. Part I **41**: 879–895. doi:10.1016/0967-0637(94)90081-7

Knowles, R. 1982. Denitrification. Microbiol. Rev. **46**: 43–70. doi:10.1128/mr.46.1.43-70.1982

Korth, F., and B. Deutsch. 2012. Uptake of dissolved organic nitrogen by size-fractionated plankton along a salinity gradient from the North sea to the Baltic Sea. Biogeochemistry 347–360. doi:10.1007/s10533-011-9656-1

Kraft, B., M. Strous, and H. E. Tegetmeyer. 2011. Microbial nitrate respiration - Genes, enzymes

and environmental distribution. J. Biotechnol. **155**: 104–117. doi:10.1016/j.jbiotec.2010.12.025

Krausfeldt, L. E., A. T. Farmer, H. F. Castro Gonzalez, B. N. Zepernick, S. R. Campagna, and S. W. Wilhelm. 2019. Urea is both a carbon and nitrogen source for Microcystis aeruginosa: Tracking 13C incorporation at bloom pH conditions. Front. Microbiol. **10**: 1–12. doi:10.3389/fmicb.2019.01064

Kristiansen, S., and B. Lund. 1989. Nitrogen cycling in the Barents Sea-I. Uptake of nitrogen in the water column. Deep Sea Res. Part A. Oceanogr. Res. Pap. **36**: 255–268. doi:10.1016/0198-0149(89)90137-4

Kumar, S., R. W. Sterner, and J. C. J. C. Finlay. 2008. Nitrogen and carbon uptake dynamics in Lake Superior. J. Geophys. Res. Biogeosciences **113**: 1–15. doi:10.1029/2008JG000720

Kumar Singh, K., V. Pandey, and K. Pandey. 2007. Phosphate uptake kinetics and its regulation in N2- fixing cyanobacterium Anabaena oryzae Fritsch under salt stress. J. Biotechnol. **6**: 2363–2368. doi:10.5897/AJB2007.000-2371

L'Helguen, S., J. Maguer, and J. Caradec. 2008. Inhibition Kinetics of nitrate uptake by ammonium in size fractioned oceanic phytoplankton communities: Implications for new production and f-ratio estimates. J. Plankton Res. **10**: 1179–1188. doi:10.1093/plankt/fbn072

Labaugh, J., and G. Swanson. 1992. Changes in chemical characteristics of water in selected wetlands in the Cottonwood Lake Area, North Dakota, U.S.A. *Aquatic Ecosystems in Semi-Arid Regions: Implications for resource management*. 149–162.

Labaugh, J. W., T. C. Winter, V. A. Adomaitis, and G. A. Swanson. 1987. Hydrology and chemistry of selected prairie wetlands in the Cottonwood Lake area, Stutsman County, North Dakota, 1979-82. US Geol. Surv. Prof. Pap. **1431**. doi:10.3133/pp1431

Lachmann, S. C., T. Mettler-Altmann, A. Wacker, and E. Spijkerman. 2018. Nitrate or ammonium: Influences of nitrogen source on the physiology of a green alga. Ecol. Evol. **9**: 1070–1082. doi:10.1002/ece3.4790

Lam, P., and M. M. M. Kuypers. 2011. Microbial nitrogen cycling processes in oxygen
minimum zones. Ann. Rev. Mar. Sci. **3**: 317–345. doi:10.1146/annurev-marine-120709-
142814

Lane, C. R., S. G. Leibowitz, B. C. Autrey, S. D. LeDuc, and L. C. Alexander. 2018.
Hydrological, physical, and chemical functions and connectivity of non-floodplain wetlands
to downstream waters: A review. J. Am. Water Resour. Assoc. **54**: 346–371.
doi:10.1111/1752-1688.12633

Lapointe, B. E., and K. R. Tenore. 1981. Experimental outdoor studies with Ulva fasciata Delile.
I. Interaction of light and nitrogen on nutrient uptake, growth, and biochemical composition.
J. Exp. Mar. Bio. Ecol. **53**: 135–152. doi:10.1016/0022-0981(81)90015-0

Laverman, A. M., C. Meile, P. Van Cappellen, and E. B. A. Wieringa. 2007. Vertical distribution
of denitrification in an estuarine sediment: Integrating sediment flowthrough reactor
experiments and microprofiling via reactive transport modeling. Appl. Environ. Microbiol.
73: 40–47. doi:10.1128/AEM.01442-06

Leavitt, P. R., C. S. Brock, C. Ebel, A. Patoine, P. R. Leavitt, C. S. Brock, C. Ebel, and A.
Patoine. 2006. Landscape-scale effects of urban nitrogen on a chain of freshwater lakes in
central North America. Limnol. Oceanogr. **51**: 2262–2277. doi:10.4319/lo.2006.51.5.2262

Leibowitz, S. G., and T. L. Nadeau. 2003. Isolated wetlands: State-of-the-science and future
directions. Wetlands **23**: 663–684. doi:10.1672/0277-
5212(2003)023[0663:IWSAFD]2.0.CO;2

Li, L., and G. Wang. 2019. Enzymatic origin and various curvatures of metabolic scaling in
microbes. Sci. Rep. **9**: 4082. doi:10.1038/s41598-019-40712-5

Li, X., C. Song, Z. Zhou, J. Xiao, S. Wang, L. Yang, X. Cao, and Y. Zhou. 2020. Comparison of
community and function of dissimilatory nitrate reduction to ammonium (DNRA) bacteria
in Chinese Shallow Lakes with different eutrophication degrees. Water (Switzerland) **12**: 1–
15. doi:10.3390/w12010174

Limmer, A. W., and K. W. Steele. 1982. Denitrification potentials: Measurement of seasonal

variation using a short-term anaerobic incubation technique. Soil Biol. Biochem. **14**: 179–184. doi:10.1016/0038-0717(82)90020-7

Lind, L. P. D. D., J. Audet, K. Tonderski, and C. C. Hoffmann. 2013. Nitrate removal capacity and nitrous oxide production in soil profiles of nitrogen loaded riparian wetlands inferred by laboratory microcosms. Soil Biol. Biochem. **60**: 156–164. doi:10.1016/j.soilbio.2013.01.021

Lipschultz, F. 1995. Nitrogen-specific uptake rates of marine phytoplankton isolated from natural populations of particles by flow cytometry. Mar. Ecol. Prog. Ser. **123**: 245–258. doi:10.3354/meps123245

Lipschultz, F., S. Wofsy, and L. Fox. 1986. Nitrogen metabolism of the eutrophic Delaware River ecosystem. Limnol. Oceanogr. **31**: 701–716. doi:10.4319/lo.1986.31.4.0701

Lomas, M., and P. Glibert. 1999. Interactions between NH_4^+ and NO_3^- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. Mar. Biol. **133**: 541–551. doi:10.1007/s002270050494

Lomas, M. W. 2000. Ammonium release by nitrogen sufficient diatoms in response to rapid increases in irradiance. J. Plankton Res. **22**: 2351–2366. doi:10.1093/plankt/22.12.2351

Lory, J. A., and M. P. Russelle. 1994. Evaluation of a diffusion method for preparing low-nitrogen samples for nitrogen-15 analysis. Soil Sci. Soc. Am. J. **58**: 1400–1404. doi:10.2136/sssaj1994.03615995005800050017x

Løvdal, T., C. Eichner, H. P. Grossart, V. Carbonnel, L. Chou, V. Martin-Jézéquel, and T. F. Thingstad. 2008. Competition for inorganic and organic forms of nitrogen and phosphorous between phytoplankton and bacteria during an Emiliania huxleyi spring bloom. Biogeosciences **5**: 371–383. doi:10.5194/bg-5-371-2008

Lunstrum, A., and L. R. Aoki. 2016. Oxygen interference with membrane inlet mass spectrometry may overestimate denitrification rates calculated with the isotope pairing technique. Limnol. Oceanogr. Methods **14**: 425–431. doi:10.1002/lom3.10101

Ma, J., J. D. Brookes, B. Qin, and others. 2014. Environmental factors controlling colony

formation in blooms of the cyanobacteria Microcystis spp. in Lake Taihu, China. Harmful Algae **31**: 136–142. doi:10.1016/j.hal.2013.10.016

Maberly, S. C., J. A. Pitt, P. S. Davies, and L. Carvalho. 2020. Nitrogen and phosphorus limitation and the management of small productive lakes. Inl. Waters **10**: 159–172. doi:10.1080/20442041.2020.1714384

Malaj, E., L. Freistadt, and C. A. Morrissey. 2020a. Spatio-Temporal Patterns of Crops and Agrochemicals in Canada Over 35 Years. Front. Environ. Sci. **8**: 1–12. doi:10.3389/fenvs.2020.556452

Malaj, E., K. Liber, and C. A. Morrissey. 2020b. Spatial distribution of agricultural pesticide use and predicted wetland exposure in the Canadian Prairie Pothole Region. Sci. Total Environ. **718**: 134765. doi:10.1016/j.scitotenv.2019.134765

Mangiafico, S. 2016. Summary and analysis of extension program evaluation in R, 1.18.1. Rutgers Cooperative Extension.

Marton, J. M., I. F. Creed, D. B. Lewis, C. R. Lane, N. B. Basu, M. J. Cohen, and C. B. Craft. 2015. Geographically isolated wetlands are important biogeochemical reactors on the landscape. Bioscience **65**: 408–418. doi:10.1093/biosci/biv009

Matheson, F. E., M. L. Nguyen, A. B. Cooper, T. P. Burt, and D. C. Bull. 2002. Fate of ^{15}N-nitrate in unplanted, planted and harvested riparian wetland soil microcosms. Ecol. Eng. **19**: 249–264. doi:10.1016/S0925-8574(02)00093-9

McAdams, B. C., R. M. Adams, W. A. Arnold, and Y. P. Chin. 2016. Novel insights into the distribution of reduced sulfur species in prairie pothole wetland pore waters provided by bismuth film electrodes. Environ. Sci. Technol. Lett. **3**: 104–109. doi:10.1021/acs.estlett.6b00020

McCarthy, J. 1972. Uptake of urea by natural populations. Limnol. Oceanogr. **17**: 738–748. doi:10.4319/lo.1972.17.5.0738

McCarthy, J., D. Wynne, and T. Berman. 1982. The uptake of dissolved nitrogenous nutrients by

Lake Kinneret (Israel) microplankton. Limnol. Oceanogr. **27**: 673–680. doi:10.4319/lo.1982.27.4.0673

McCarthy, M. J., W. S. Gardner, M. F. Lehmann, and D. F. Bird. 2013. Implications of water column ammonium uptake and regeneration for the nitrogen budget in temperate, eutrophic Missisquoi Bay, Lake Champlain (Canada/USA). Hydrobiologia **718**: 173–188. doi:10.1007/s10750-013-1614-6

McMcKague, K., K. Reid, and H. Simpson. 2005. Environmental impacts of nitrogen use in agriculture.

Mekonnen, M. A., H. S. Wheater, A. M. Ireson, C. Spence, B. Davison, and A. Pietroniro. 2014. Towards an improved land surface scheme for prairie landscapes. J. Hydrol. **511**: 105–116. doi:10.1016/j.jhydrol.2014.01.020

Melack, J. M., and P. Kilham. 1974. Photosynthetic rates of phytoplankton in East African alkaline, saline lakes. Limnol. Oceanogr. **19**: 743–755. doi:10.4319/lo.1974.19.5.0743

Middelburg, J. J., and J. Nieuwenhuize. 2000. Nitrogen uptake by heterotrophic bacteria and phytoplankton in the nitrate-rich Thames estuary. Mar. Ecol. Prog. Ser. **203**: 13–21. doi:10.3354/meps203013

Middelburg, J., K. Soetaert, and P. Herman. 1996. Evaluation of the nitrogen isotope-pairing method for measuring benthic denitrification: A simulation analysis. Limnol. Oceanogr. **41**: 1839–1844. doi:10.4319/lo.1996.41.8.1839

Millar, J. B. 1976. Wetland classification in Western Canada.

Miller, W. E., T. E. Maloney, and J. C. Greene. 1974. Algal productivity in 49 lake waters as determined by algal assays. Water Res. **8**: 667–679. doi:10.1016/0043-1354(74)90126-2

Mills, E., and J. Forney. 1988. Trophic dynamics and development of freshwater pelagic food webs, *In* S. Carpenter [ed.], Complex Interactions in Lake Communities. Springer-Verlag New York.

Minke, A. G., C. J. Westbrook, and G. Van Der Kamp. 2010. Simplified volume-area-depth method for estimating water storage of prairie potholes. Wetlands **30**: 541–551. doi:10.1007/s13157-010-0044-8

Mitsch, W., and J. Gosselink. 1993. Wetlands 2nd Ed., John Wiley & Sons, Inc.

Mitsch, W. J. 2005. Wetland creation, restoration, and conservation: The state of science, Elsevier Science.

Mitsch, W. J., J. W. Day, L. Zhang, and R. R. Lane. 2005. Nitrate-nitrogen retention in wetlands in the Mississippi River Basin. Ecol. Eng. **24**: 267–278. doi:10.1016/j.ecoleng.2005.02.005

Miyazaki, T., S. Hideki, and H. Uotani. 1987. Diel changes of uptake of inorganic carbon and nitrogen by phytoplankton, and the relationship between inorganic carbon and nitrogen uptake in Lake Nakanuma, Japan. J. Plankton Res. **9**: 513–524. doi:10.1093/plankt/9.3.513

Mobley, H. L. T., and R. P. Hausinger. 1989. Microbial ureases: Significance, regulation, and molecular characterization. Microbiol. Rev. **53**: 85–108. doi:10.1128/mmbr.53.1.85-108.1989

Moneta, A., B. Veuger, P. van Rijswijk, F. Meysman, K. Soetaert, and J. J. Middelburg. 2014. Dissolved inorganic and organic nitrogen uptake in the coastal North Sea: A seasonal study. Estuar. Coast. Shelf Sci. **147**: 78–86. doi:10.1016/j.ecss.2014.05.022

Moosa, S., and S. T. L. Harrison. 2006. Product inhibition by sulphide species on biological sulphate reduction for the treatment of acid mine drainage. Hydrometallurgy **83**: 214–222. doi:10.1016/J.HYDROMET.2006.03.026

Moschonas, G., R. Gowen, R. Paterson, E. Mitchell, B. Stewart, S. McNeill, P. Glibert, and K. Davidson. 2017. Nitrogen dynamics and phytoplankton community structure: the role of organic nutrients. Biogeochemistry **134**: 125–145. doi:10.1007/s10533-017-0351-8

Mulholland, M., and M. Lomas. 2008. Nitrogen uptake and assimilation, p. 303–384. *In* D. Capone, D. Bronk, M. Mulholland, and E. Carpenter [eds.], Nitrogen in the marine environment. Elsevier.

Muller, C., T. Rütting, M. Abbasi, and others. 2009. Effect of elevated CO2 on soil N dynamics in a temperate grassland soil. Soil Biol. Biochem. **41**: 1996–2001. doi:10.1016/j.soilbio.2009.07.003

Murkin, H. R. 1998. Freshwater function and values of prairie wetlands. Gt. Plains Res. **8**: 3–15.

Murphy, A. E., A. N. Bulseco, R. Ackerman, J. H. Vineis, and J. L. Bowen. 2020. Sulphide addition favours respiratory ammonification (DNRA) over complete denitrification and alters the active microbial community in salt marsh sediments. Environ. Microbiol. **22**: 2124–2139. doi:10.1111/1462-2920.14969

Murphy, D. V., S. Recous, E. A. Stockdale, I. R. P. Fillery, L. S. Jensen, D. J. Hatch, and K. W. T. Goulding. 2003. Gross nitrogen fluxes in soil : theory, measurement and application of 15N pool dilution techniques. Adv. Agron. **79**: 69–118. doi:10.1016/S0065-2113(02)79002-0

Murphy, T., and B. Brownlee. 1981. Ammonia volatilization in a hypertrophic prairie lake. Can. J. Fish. Aquat. Sci. **38**: 1035–1039. doi:10.1139/f81-142

Murphy, T. P. 1980. Ammonia and Nitrate Uptake in the Lower Great Lakes. Can. J. Fish. Aquat. Sci. **37**: 1365–1372. doi:10.1139/f80-175

Nachshon, U., A. Ireson, G. Van Der Kamp, S. R. Davies, and H. S. Wheater. 2014. Impacts of climate variability on wetland salinization in the North American prairies. Hydrol. Earth Syst. Sci. **18**: 1251–1263. doi:10.5194/hess-18-1251-2014

National Wetlands Working Group. 1988. Wetlands of Canada. Sustainabile Development Branch, Environment Canada and Polyscience Publications Inc.

National Wetlands Working Group. 1997. The Canadian wetland classification system, 2nd ed. B.G. Warner and C.D.. Rubec [eds.].

Naugle, D., R. Johnson, M. Estey, and K. Higgins. 2001. A landscape approach to conserving wetland and bird habitat in the prairie pothole region of eastern south dakota. Wetlands **21**: 1–17. doi:10.1672/0277-5212(2001)021[0001:ALATCW]2.0.CO;2

Needoba, J. A., and P. J. Harrison. 2004. Influence of low light and a light: Dark cycle on NO_3^- uptake, intracellular NO3-, and nitrogen isotope fractionation by marine phytoplankton. J. Phycol. **40**: 505–516. doi:10.1111/j.1529-8817.2004.03171.x

Nelson, D., and H. Conway. 1979. Effects of the light regime on nutrient assimilation by phytoplankton in the Baja California and northwest Africa upwelling systems. J. Mar. Res. **37**: 301–318.

Nelson, S., B. Smith, and B. Best. 1981. Kinetics of nitrate and ammonium uptake by the tropical freshwater macrophyte Pistia stratiotes L. Aquaculture **24**: 11–19. doi:10.1016/0044-8486(81)90039-9

Nielsen, L. 1992. Denitrification in sediment determined from nitrogen isotope pairing technique. FEMS Microbiol. Lett. **86**: 357–362. doi:10.1111/j.1574-6968.1992.tb04828.x

Nogaro, G., and A. J. Burgin. 2014. Influence of bioturbation on denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in freshwater sediments. Biogeochemistry **120**: 279–294. doi:10.1007/s10533-014-9995-9

Nommik, H. 1956. Investigations on denitrification in soil. Acta Agric. Scand. **6**: 195–228. doi:10.1080/00015125609433269

Pandey, A., H. Suter, J. Z. He, H. W. Hu, and D. Chen. 2018. Nitrogen addition decreases dissimilatory nitrate reduction to ammonium in rice paddies. Appl. Environ. Microbiol. **84**: 1–14. doi:10.1128/AEM.00870-18

Pandey, C. B., U. Kumar, M. Kaviraj, K. J. Minick, A. K. Mishra, and J. S. Singh. 2020. DNRA: A short-circuit in biological N-cycling to conserve nitrogen in terrestrial ecosystems. Sci. Total Environ. **738**: 139710. doi:10.1016/j.scitotenv.2020.139710

Paparazzo, F. E., G. N. Williams, J. P. Pisoni, M. Solís, J. L. Esteves, and D. E. Varela. 2017. Linking phytoplankton nitrogen uptake, macronutrients and chlorophyll-a in SW Atlantic waters: The case of the Gulf of San Jorge, Argentina. J. Mar. Syst. **172**: 43–50. doi:10.1016/j.jmarsys.2017.02.007

Parsons, D. F., M. Hayashi, and G. van der Kamp. 2004. Infiltration and solute transfort under a seasonal wetland: Bromide tracer experiments in Saskatoon, Canada. Hydrol. Process. **18**: 2011–2027. doi:10.1002/hyp.1345

Paul, E., and F. Clark. 1996. Soil microbiology and biochemsitry, 2nd ed. Academic Press.

Pelley, J., and T. Bannister. 1979. Methylamine uptake in the green alga Chlorella pyrenoidosa. J. Phycol. **15**: 110–112. doi:10.1111/j.0022-3646.1979.00110.x

Pett-Ridge, J., W. Silver, and M. Firestone. 2006. Redox fluctuations frame microbial community impacts on N-cycling rates in humid tropical forest soil. Biogeochemistry **81**: 95–110.

Pfenning, K., and P. McMahon. 1996. Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. J. Hydrol. **187**: 283–295. doi:10.1016/S0022-1694(96)03052-1

Pham, S. V., P. R. Leavitt, S. McGowan, and P. Peres-Neto. 2008. Spatial variability of climate and land-use effects on lakes of the northern Great Plains. Limnol. Oceanogr. **53**: 728–742. doi:10.4319/lo.2008.53.2.0728

Phillips, R. L., C. Ficken, M. Eken, J. Hendrickson, and O. Beeri. 2016. Wetland soil carbon in a watershed context for the Prairie Pothole Region. J. Environ. Qual. **45**: 368–375. doi:10.2134/jeq2015.06.0310

Phipps, R. G., and W. G. Crumpton. 1994. Factors affecting nitrogen loss in experimental wetlands with different hydrologic loads. Ecol. Eng. **3**: 399–408. doi:10.1016/0925-8574(94)00009-3

Porubsky, W., L. Velasquez, and S. Joye. 2008. Nutrient-replete benthic microalgae as a source of dissolved organic carbon to coastal waters. Estuaries and Coasts **31**: 860–876. doi:10.1007/s12237-008-9077-0

Presing, M., S. Herodek, T. Preston, and L. Voros. 2009. Nitrogen uptake and the importance of internal nitrogen loading in Lake Balaton. Freshw. Biol. **46**: 125–139. doi:10.1111/j.1365-

2427.2001.00622.x

Présing, M., S. Herodek, T. Preston, and L. Vörös. 2001. Nitrogen uptake and the importance of internal nitrogen loading in Lake Balaton. Freshw. Biol. **46**: 125–139. doi:10.1046/j.1365-2427.2001.00622.x

Rahman, M. M., K. L. Roberts, F. Warry, M. R. Grace, and P. L. M. M. Cook. 2019. Factors controlling dissimilatory nitrate reduction processes in constructed stormwater urban wetlands. Biogeochemistry **142**: 375–393. doi:10.1007/s10533-019-00541-0

Ramsar Convention. 2007. Why conserve wetlands?

Rashford, B. S., J. A. Walker, and C. T. Bastian. 2011. Economía de la Conversión de Pastizales a Tierras Agrícolas en la Región Prairie Pothole. Conserv. Biol. **25**: 276–284. doi:10.1111/j.1523-1739.2010.01618.x

Raven, J. 1980. Nutrient transport in microalgae. Adv. Microb. Physiol. **21**: 47–226. doi:10.1016/s0065-2911(08)60356-2.

Reay, D. S., D. B. Nedwell, J. Priddle, and J. C. Ellis-Evans. 1999. Temperature dependence of inorganic nitrogen uptake: Reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. Appl. Environ. Microbiol. **65**: 2577–2584. doi:10.1016/j.dsr2.2010.10.039

Reddy, K. R., and R. Delaune. 2008. Biogeochemsitry of Wetlands, CRC Press.

Reddy, K. R., W. H. Patrick, and C. W. Lindau. 1989. Nitrification-denitrification at the plant root-sediment interface in wetlands. Limnol. Ocean. **34**: 1004–1013. doi:10.4319/lo.1989.34.6.1004

Reuter, J., and R. Axler. 1992. Physiological charactersitics of inorganic nitrogen utpake by spatially seperate algal communities in a nitrogen deficient lake. Freshw. Biol. **27**: 227–236. doi:10.1111/j.1365-2427.1992.tb00535.x

Reynolds, C. 2006. Ecology of phytoplankton, Cambridge University Press.

Ribas, D., M. Calderer, V. Marti, and M. Rovira. 2013. Effect of different seasonal conditions on the potential of wetland soils for groundwater denitrifcation. Desalin. Water Treat. **53**: 994–1000. doi:https://doi.org/10.1080/19443994.2013.871344

Richardson, J. L. L., J. L. Amdt, J. Freeland, J. L. Arndt, and J. Freeland. 1994. Wetland Soils of the Prairie Potholes. Adv. Agron. **52**: 121–171. doi:10.1016/S0065-2113(08)60623-9

Ritz, C. 2016. Analysis of dose-response curves.

Ritz, C., F. Baty, J. C. Streibig, and D. Gerhard. 2015. Dose-response analysis using R. PLoS One **10**: 1–13. doi:10.1371/journal.pone.0146021

Roberts, K. L., A. J. Kessler, M. R. Grace, and P. L. M. Cook. 2014. Increased rates of dissimilatory nitrate reduction to ammonium (DNRA) under oxic conditions in a periodically hypoxic estuary. Geochim. Cosmochim. Acta **133**: 313–324. doi:10.1016/j.gca.2014.02.042

Robertson, E. K., K. L. Roberts, L. D. W. Burdorf, P. Cook, and B. Thamdrup. 2016. Dissimilatory nitrate reduction to ammonium coupled to Fe(II) oxidation in sediments of a periodically hypoxic estuary. Limnol. Oceanogr. **61**: 365–381. doi:10.1002/LNO.10220

Robertson, G. P., and P. M. Groffman. 2015. Nitrogen Transformations, p. 421–446. *In* Soil Microbiology, Ecology and Biochemistry. Elsevier Inc.

Roland, F. A. E. E., F. Darchambeau, A. V. Borges, C. Morana, L. De Brabandere, B. Thamdrup, and S. A. Crowe. 2018. Denitrification, anaerobic ammonium oxidation, and dissimilatory nitrate reduction to ammonium in an East African Great Lake (Lake Kivu). Limnol. Oceanogr. **63**: 687–701. doi:10.1002/lno.10660

Rosenberry, D., and T. C. Winter. 1997. Dynamics of water-table fluctuations in an upland between two prairie-pothole wetlands in North Dakota. J. Hydrol. **191**: 266–289. doi:10.1016/S0022-1694(96)03050-8

Rosser, S. M. J. H., and P. A. Thompson. 2001. Phytoplankton of the Swan - Canning Estuary: A comparison of nitrogen uptake by different bloom assemblages. Hydrol. Process. **15**: 2579–

2594. doi:10.1002/hyp.288

Rover, J., and D. Mushet. 2015. Mapping Wetlands and Surface Water in the Prairie Pothole Region of North America R. Tiner, M. Lang, and V. Klemas [eds.]. Remote Sens. Wetl. 347–368. doi:10.1201/b18210-20

Rubec, C. 1994. The evolution of wetland policy in Canada.

Russ, L., D. R. Speth, M. S. M. Jetten, H. J. M. Op den Camp, and B. Kartal. 2014. Interactions between anaerobic ammonium and sulfur-oxidizing bacteria in a laboratory scale model system. Environ. Microbiol. **16**: 3487–3498. doi:10.1111/1462-2920.12487

Russell, M., R. Fulford, K. Murphy, and others. 2018. Relative importance of landscape versus local wetland characteristics for estimating wetland denitrification potential. Wetlands 1–11. doi:10.1007/s13157-018-1078-6

Rütting, T., P. Boeckx, C. Müller, and L. Klemedtsson. 2011. Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. Biogeosciences **8**: 1779–1791. doi:10.5194/bg-8-1779-2011

Rütting, T., T. Clough, C. Muller, M. Lieffering, P. Newton, and ; 2010. Ten years of elevated atmospheric CO2 alters soil N transformations in a sheep-grazed pasture. Glob. Chang. Biol. **16**: 2530–2542. doi:10.1111/j.1365-2486.2009.02089.x

Sahrawat, K. L. 1979. Evaluation of some chemical extractants for determination of exchangeable ammonium in tropical rice soils. **10**: 1005–1013. doi:10.1080/00103627909366957

Salk, K. R., D. V. Erler, B. D. Eyre, N. Carlson-Perret, and N. E. Ostrom. 2017. Unexpectedly high degree of anammox and DNRA in seagrass sediments: Description and application of a revised isotope pairing technique. Geochim. Cosmochim. Acta **211**: 64–78. doi:10.1016/j.gca.2017.05.012

Saskatchewan Centre for Soil Research. 1989. Rural municipality of grant number 372: preliminary soil map and report.

Saunders, D. L., and J. Kalff. 2001. Nitrogen rentention in wetlands, lakes and rivers. Hydrobiologia **443**: 205–212. doi:10.1023/A:1017506914063

Scarth, J. 1998. Wetland policy in Canada: A research agenda for policy reform. Gt. Plains Res. **8**: 169–182.

Schapira, M., and L. Seuront. 2020. Nutrient patchiness, phytoplankton surge-uptake, and turbulent history: A theoretical approach and its experimental validation. Fluids **5**. doi:10.3390/fluids5020080

Schnell, S. 2014. Validity of the Michaelis-Menten equation - Steady-state or reactant stationary assumption: That is the question. FEBS J. **281**: 464–472. doi:10.1111/febs.12564

Schuchard, K., U. Sicking, and S. Donaldson. 2017. Modeling nitrate uptake in freshwater phytoplankton nitrate reductase nutrient and enzyme kinetic behavior laboratory and field methods. Proc. Natl. Conf. Undergrad. Res. 584–593.

Schuurkes, J. A. A. R., and R. Mosello. 1988. The role of external ammonium inputs in freshwater acidification. Swiss J. Hydrol. **50**: 71–86. doi:10.1007/BF02538372

Scott, J. T., M. J. McCarthy, W. S. Gardner, and R. D. Doyle. 2008. Denitrification, dissimilatory nitrate reduction to ammonium, and nitrogen fixation along a nitrate concentration gradient in a created freshwater wetland. Biogeochemistry **87**: 99–111. doi:10.1007/s10533-007-9171-6

Scott, J. T., M. J. McCarthy, and H. W. Paerl. 2019. Nitrogen transformations differentially affect nutrient-limited primary production in lakes of varying trophic state. Limnol. Oceanogr. Lett. **4**: 96–104. doi:10.1002/lol2.10109

Seelig, B., and S. DeKeyser. 2006. Water quality and wetland function in the Northern Prairie Pothole Region.

Seitzinger, S. 1994. Linkages Between Organic-Matter Mineralization and Denitrification in 8 Riparian Wetlands. Biogeochemistry **25**: 19–39. doi:10.1007/BF00000510

Seitzinger, S., J. Harrison, J. Bohlke, A. Bouwman, R. Lowrance, B. Peterson, C. Tobias, and G. Van Drecht. 2006. Denitrification across landscaes and waterscapes: a synthesis. Ecol. Appl. **16**: 2064–2090. doi:10.1890/1051-0761(2006)016[2064:DALAWA]2.0.CO;2

Seitzinger, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. Limnol. Oceanogr. **33**: 702–724. doi:10.4319/lo.1988.33.4part2.0702

Senga, Y., K. Mochida, R. Fukumori, N. Okamoto, and Y. Seike. 2006. N_2O accumulation in estuarine and coastal sediments: The influence of H_2S on dissimilatory nitrate reduction. Estuar. Coast. Shelf Sci. **67**: 231–238. doi:10.1016/J.ECSS.2005.11.021

Shan, J., X. Zhao, R. Sheng, and others. 2016. Dissimilatory nitrate reduction processes in typical chinese paddy soils: Rates, relative contributions, and influencing factors. Environ. Sci. Technol. **50**: 9972–9980. doi:10.1021/acs.est.6b01765

Shaw, D. A., G. Vanderkamp, F. M. Conly, A. Pietroniro, and L. Martz. 2012. The Fill-Spill Hydrology of Prairie Wetland Complexes during Drought and Deluge. Hydrol. Process. **26**: 3147–3156. doi:10.1002/hyp.8390

Shook, K., J. W. Pomeroy, C. Spence, and L. Boychuk. 2013. Storage dynamics simulations in prairie wetland hydrology models: Evaluation and parameterization. Hydrol. Process. **27**: 1875–1889. doi:10.1002/hyp.9867

Shukla, B., and L. C. Rai. 2007. Potassium-induced inhibition of nitrogen and phosphorus metabolism as a strategy of controlling Microcystis blooms. World J. Microbiol. Biotechnol. **23**: 317–322. doi:10.1007/s11274-006-9226-1

Sigee, D. 2005. Freshwater Microbiology, John Wiley & Sons, Inc.

Silver, W. L., D. J. Herman, and M. K. Firestone. 2001. Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. Ecology **82**: 2410–2416. doi:10.1890/0012-9658(2001)082[2410:DNRTAI]2.0.CO;2

Simon, J., and P. M. H. Kroneck. 2013. Microbial sulfite respiration, p. 45–117. *In* Advances in

Microbial Physiology. Elsevier Ltd.

Sirivedhin, T., and K. Gray. 2005. Factors affecting denitrification rates in experimental wetlands: Field and laboratory studies. Ecol. Eng. **26**: 167–181. doi:10.1016/j.ecoleng.2005.09.001

Skopec, M., and V. Evelsizer. 2017. Spring Nutrient Levels in Drained Wetlands of IOWA's Prairie Pothole Region. Wetlands 1–13. doi:10.1007/s13157-017-0962-9

Smith, V. H. 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: An empirical and theoretical analysis. Limnol. Oceanogr. **27**: 1101–1111. doi:10.4319/lo.1982.27.6.1101

Smucker, N., J. Beaulieu, C. Nietch, and J. Young. 2021. Increasingly severe cyanobacterial blooms and deep water hypoxia coincide with warming water temperatures in reservoirs. Glob. Chang. Biol. **27**: 2507–2519. doi:10.1111/gcb.15618

Solomon, C., J. Collier, G. Berg, and P. Glibert. 2010. Role of urea in microbial metabolism in aquatic systems: A biochemical and molecular review. Aquat. Microb. Ecol. **59**: 67–88. doi:10.3354/ame01390

Song, G. D., S. M. Liu, H. Marchant, M. M. M. Kuypers, and G. Lavik. 2013. Anammox, denitrification and dissimilatory nitrate reduction to ammonium in the East China Sea sediment. Biogeosciences **10**: 6851–6864. doi:10.5194/bg-10-6851-2013

Song, K., S. H. Lee, W. J. Mitsch, and H. Kang. 2010. Different responses of denitrification rates and denitrifying bacterial communities to hydrologic pulsing in created wetlands. Soil Biol. Biochem. **42**: 1721–1727. doi:10.1016/j.soilbio.2010.06.007

Sørensen, P., and E. S. Jensen. 1991. Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for 15N determination. Anal. Chim. Acta **252**: 201–203. doi:10.1016/0003-2670(91)87215-S

Spanning, R., D. Richardson, and S. Ferguson. 2007. Introduction to the biochemistry and molecular biology of denitrification, p. 3–20. *In* H. Bothe, S.J. Ferguson, and W.E. Newton

[eds.], Biology of the Nitrogen Cycle. Elsevier.

Spence, C. 2003. Hydrology of subarctic Canadian shield: soil-filled valleys . J. Hydrol. **279**: 151–166. doi:10.1016/S0022-1694(03)00175-6

Srinivasan, B. 2020. Explicit treatment of non-michaelis-menten and atypical kinetics in early drug discovery. ChemMedChem **16**: 899–918. doi:10.20944/preprints202010.0179.v1

Staelens, J., T. Rütting, D. Huygens, A. De Schrijver, C. Muller, K. Verheyen, and P. Boeckx. 2011. In situ gross nitrogen transformations differ between temperate deciduous and coniferous forest soils. Biogeochemistry **108**: 259–277. doi:10.1007/s10533-011-9598-7

Van Stempvoort, D. R., M. J. Hendry, J. J. Shoenau, and H. R. Krouse. 1994. Sources and dynamics of sulfur in weathered till, Western. Chem. Geol. **111**: 35–56. doi:10.1016/0009-2541(94)90081-7

Stevens, R. J., R. J. Laughlin, and J. P. Malone. 1998. Soil pH affects the processes reducing nitrate to nitrous oxide and di-nitrogen. Soil Biol. Biochem. **30**: 1119–1126. doi:10.1016/S0038-0717(97)00227-7

Stewart, R. E., and H. A. Kantrud. 1971. Classification of natural ponds and lakes in the glaciated prairie region. Bur. Sport Fish. andWildlife Resour. Publ. 92 **554**: 64.

Stewart, W. 1980. Some aspects of structure and function in N_2-fixation cyanobacteria. Annu. Rev. Microbiol. **34**: 497–536. doi:10.1146/annurev.mi.34.100180.002433

Syrett, P., and I. Bekheet. 1977. The uptake of thiourea by Chlorella. New Phytol. **79**: 291–297. doi:10.1111/J.1469-8137.1977.TB02208.X

Tamm, C. 1991. Nitrogen in terrestrial ecosystems, Ecological. Springer Berlin Heidelberg.

Tangen, B. A., and S. Bansal. 2019. Soil properties and greenhouse gas fluxes of Prairie Pothole Region wetlands. U.S. Geol. Surv. data release.

Team, R. C. 2016. R: a language and environment for statistical computing.

Teece, M. A., and M. L. Fogel. 2004. Preparation of ecological and biochemical samples for isotope analysis, p. 177–202. *In* P. Groot [ed.], Handbook of Stable Isotope Analytical Techniques. Elsevier B.V.

Thompson, J., and D. A. Young. 2013. The optimal use of Prairie Pothole wetlands: An economic perspective. **1784**: 365–372. doi:10.4296/cwrj1704365

Thorén, A. K. 2007. Urea transformation of wetland microbial communities. Microb. Ecol. **53**: 221–232. doi:10.1007/s00248-006-9098-9

Tiedje, J. M. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. Environ. Microbiol. Anaerobes 179–244.

Tiedje, J. M., S. Simkins, and P. M. Groffman. 1989. Perspectives on measuremnet of denitrification in the field including recommended protocols for acetylene based methods. Plant Soil **115**: 261–284. doi:10.1007/BF02202594

Tiedje, J., A. Sexstone, D. Myrold, and J. Robinson. 1982. Denitrification: ecological niches, competition and survival. Antonie Van Leeuwenhoek **48**: 569–583. doi:10.1007/BF00399542

Tietema, A., and D. Van Dam. 1996. Calculating microbial carbon and nitrogen transformations in acid forest litter with 15N enrichment and dynamic simulation modelling. Soil Biol. Biochem. **28**: 953–965. doi:10.1016/0038-0717(95)00177-8

Toetz, D. 1976. Diel periodicity in uptake of nitrate and nitrite by reservoir phytoplankton. Hydrobiologia **49**: 49–52. doi:10.1007/BF00016167

Toetz, D. 1981. Effects of pH, phosphate and ammonia on the rate of uptake of nitrate and ammonia by freshwater phytoplankton. Hydrobiologia **76**: 23–26. doi:10.1007/BF00014029

Toetz, D., L. Varga, and E. Loughran. 1973. Half-saturation constants for uptake of nitrate and ammonia by reservoir plankton. Ecol. Soc. Am. **54**: 903–908. doi:10.2307/1935687

Torres-Valdés, S., and D. A. Purdie. 2006. Nitrogen removal by phytoplankton uptake through a

temperate non-turbid estuary. Estuar. Coast. Shelf Sci. **70**: 473–486. doi:10.1016/j.ecss.2006.06.028

Trommer, G., M. Poxleitner, and H. Stibor. 2020. Responses of lake phytoplankton communities to changing inorganic nitrogen supply forms. Aquat. Sci. **82**: 1–13. doi:10.1007/s00027-020-0696-2

Turpin, D. 1991. Effects of inorganic N availability on algal photosynthesis and carbon metabolism. 1Journal Phycol. **27**: 14–20. doi:10.1111/j.0022-3646.1991.00014.x

Twomey, L. J., M. F. Piehler, and H. W. Paerl. 2005. Phytoplankton uptake of ammonium, nitrate and urea in the Neuse River Estuary, NC, USA. Hydrobiologia **533**: 123–134. doi:10.1007/s10750-004-2403-z

Uusheimo, S., J. Huotari, T. Tulonen, S. Aalto, A. Rissanen, and L. Arvola. 2018. High nitrogen removal in a constructed wetland receiving treated wastewater in a cold climate. Environ. Sci. Technol. acs.est.8b03032. doi:10.1021/acs.est.8b03032

Vanderhoof, M., J. Christensen, and L. Alexander. 2017. Patterns and driversd for wetland connections in the Prairie Pothole Region, United States. Wetl. Ecol. Manag. **25**: 275–297. doi:10.1007/s11273-016-9516-9

Vincent, W. 1992. The daily pattern of nitrogen uptake by phytoplankton in dynamic mixed layer environments. Hydrobiologia **238**: 37–52. doi:10.1007/BF00048772

Vitousek, P. M., R. Naylor, T. Crews, and others. 2009. Nutrient Imbalances in Agricultural Development. Science (80-.). **324**: 1519–1520. doi:10.1126/SCIENCE.1170261

Vymazal, J. 2007. Removal of nutrients in various types of constructed wetlands. Sci. Total Environ. **380**: 48–65. doi:10.1016/j.scitotenv.2006.09.014

Wade, A. 2021. Role of vegetated buffer zones for mitigating wetland pesticide contamination and protecting aquatic invertebrate communities in northern prairie wetlands. University of Saskatchewan.

Waiser, M. J. 2001. Nutrient limitation of pelagic bacteria and phytoplankton in four prairie wetlands. Arch. für Hydrobiol. **150**: 435-455 ST-Nutrient limitation of pelagic bacte. doi:10.1127/archiv-hydrobiol/150/2001/435

Waiser, M. J., and R. D. Robarts. 2004. Photodegradation of DOC in a shallow prairie wetland: Evidence from seasonal changes in DOC optical properties and chemical characteristics. Biogeochemistry **69**: 263–284. doi:10.1023/B:BIOG.0000031048.20050.4e

Weithoff, G., and N. Walz. 1999. Problems in estimating phytoplankton nitrogen limitation in Shallow Eutrophic Lakes. Hydrobiologia **408/409**: 367–373. doi:10.1023/A:1017038914638

Weyer, P., J. Cerhan, B. Kross, and others. 2001. Municipal drinking water nitrate level and cancer risk in older women: The Iowa women's health study. Epidemiology **12**: 327–338. doi:10.1097/00001648-200105000-00013

Whalen, S. C., and V. Alexander. 1984. Influence of temperature and light on rates of inorganic nitrogen transport by alge in an Arctic lake. Can. J. Fish. Aquat. Sci. **41**: 1310–1318. doi:10.1007/s10608-010-9338-7

Wheeler, P. 1980a. Uptake of methylamine by the marine diatom Cyclotella cryptica., p. 633–634. *In* R. Spanswick, W. Lucas, and J. Dainty [eds.], Plant Membrane Transport: Current Conceptual Issues. Elsevier/North Holland Biomedical Press.

Wheeler, P. 1980b. Use of methylamine as an ammonium analogue in transport and assimilation studies with Cyclotella cryptica (Bacillariophyceae). J. Phycol. **16**: 328–334. doi:10.1111/j.0022-3646.1980.00328.x

Wheeler, P., and J. Hellebust. 1981. Uptake and concentration of alkylamines by a marine diatom: effects of H^+ and K+ and implications for the transport and accumulation of weak bases. Plant Physiol. **67**: 367–372. doi:10.1104/pp.67.2.367

Wheeler, P., and D. Kirchman. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnol. Oceanogr. **31**: 998–1009. doi:10.4319/lo.1986.31.5.0998

Wheeler, P., and S. Kokkinakis. 1990. Ammonium recylcing limits nitrate use in oceanic subarctic Pacific. Limnol. Oceanogr. **35**: 1267–1278. doi:10.4319/ lo.1990.35.6.1267

White, J. R., and K. R. Reddy. 2009. Biogeochemical Dynamics I: Nitrogen Cycling in Wetlands E. Maltby and T. Barker [eds.]. Wetl. Handb. 213–227. doi:10.1002/9781444315813.ch9

Whittaker, R., and G. Likens. 1973. Primary production: The biosphere and man. Hum. Ecol. **1**. doi:10.1007/BF01536732

Williams, S. K., and R. C. Hodson. 1977. Transport of urea at low concentrations in Chlamydomonas reinhardi. J. Bacteriol. **130**: 266–273. doi:10.1128/JB.130.1.266-273.1977

Winter, T. C. 2003. Hydrological, chemical, and biological characteristics of a prairie pothole wetland complex under highly variable climate conditions: the Cottonwood Lake area, east-central North Dakota,.

Woods, D. D. 1938. The reduction of ammonia by Clostridium welchii. Biochem. **32**: 2000–2012. doi:10.1042/bj0322000

Xia, X., Z. Jia, T. Liu, S. Zhang, and L. Zhang. 2017. Coupled nitrification-denitrification caused by suspended sediment (SPS) in rivers: Importance of SPS size and composition. Environ. Sci. Technol. **51**: 212–221. doi:10.1021/acs.est.6b03886

Yin, G., L. Hou, M. Liu, Z. Liu, and W. S. Gardner. 2014. A novel membrane inlet mass spectrometer method to measure $^{15}NH_4^+$ for isotope-enrichment experiments in aquatic ecosystems. Environ. Sci. Technol. **48**: 9555–9562. doi:10.1021/es501261s

Yin, S., Q. Shen, Y. Tang, and L. Cheng. 1998. Reduction of nitrate to ammonium in selected paddy soils in China. Pedosphere **8**: 221–228.

Yuan, X., P. M. Glibert, J. Xu, H. Liu, M. Chen, H. Liu, K. Yin, and P. J. Harrison. 2012. Inorganic and organic nitrogen uptake by phytoplankton and bacteria in Hong Kong waters. Estuaries and Coasts **35**: 325–334. doi:10.1007/s12237-011-9433-3

Zaid, M. 2015. Correlation and regression analysis, The Statistical, Economic and Sosical

Research Training Center for Islamic Countries (SESRIC).

Zedler, J. B., and S. Kercher. 2005. Wetland resources: status, trends, ecosystem services, and restorability. Annu. Rev. Environ. Resour. **30**: 39–74. doi:10.1146/annurev.energy.30.050504.144248

Zeng, T., W. A. Arnold, and B. M. Toner. 2013. Microscale characterization of sulfur speciation in lake sediments. Environ. Sci. Technol. **47**: 1287–1296. doi:10.1021/es303914q

Zeng, T., Y. P. Chin, and W. A. Arnold. 2012. Potential for abiotic reduction of pesticides in prairie pothole porewaters. Environ. Sci. Technol. **46**: 3177–3187. doi:10.1021/es203584d

Ziadi, N., and T. Sen Tran. 2006. Lime Requiement: Measurement of soil pH in water, p. 155–160. *In* E.G.G. M.R. Carter [ed.], Soil Sampling and Methods of Analysis. Taylor & Francis Group, LLC.

Ziegelgruber, K. L., T. Zeng, W. A. Arnold, and Y. P. Chin. 2013. Sources and composition of sediment pore-water dissolved organic matter in prairie pothole lakes. Limnol. Oceanogr. **58**: 1136–1146. doi:10.4319/lo.2013.58.3.1136

Appendix
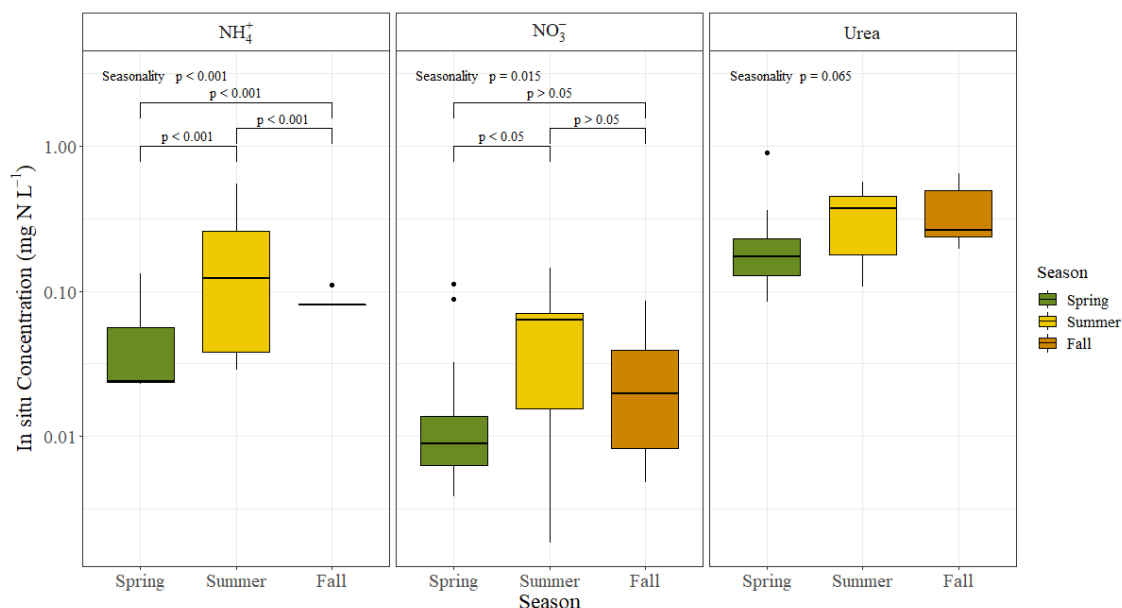
Figure A.1: *In situ* concentrations of NH_4^+-N, NO_3^--N, and urea measured within study ponds at St. Denis National Wildlife Area across spring (green), summer (yellow), and fall (orange). A pairwise permutation ANOVA with Holm correction for multiple testing revealed season had a significant effect on *in situ* concentrations of NH_4^+ ($p < 0.001$) and NO_3^- ($p = 0.015$), but not urea ($p = 0.065$). Seasonal NH_4^+ concentrations were significantly different from one another ($p < 0.001$). *In situ* NO_3^- concentrations were significantly different in spring than summer ($p = 0.015$), but was not significantly different between fall and spring ($p = 0.083$), or fall and summer ($p = 0.083$).
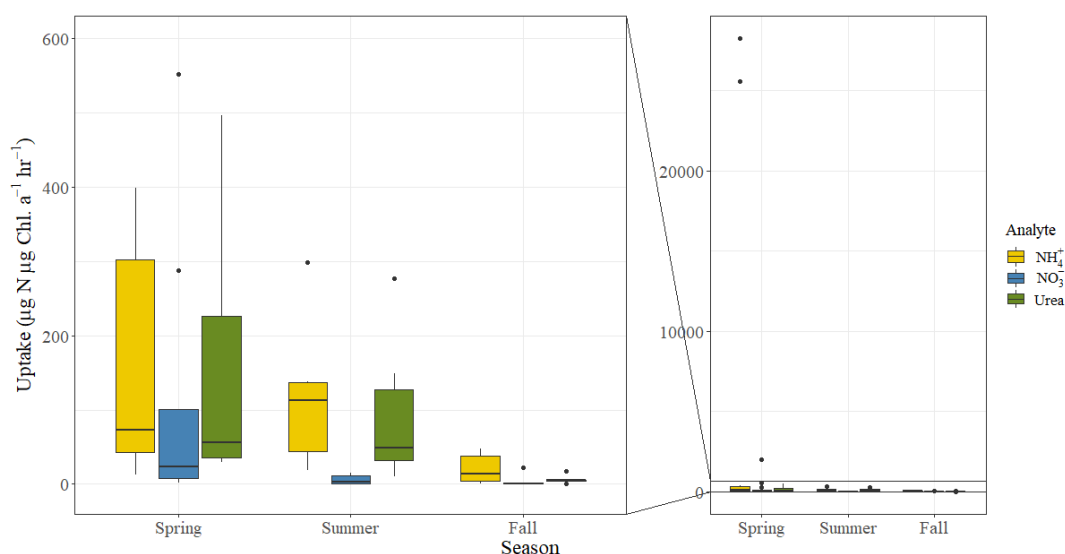
Figure A.2 Nitrogen (NH_4^+ = yellow, NO_3^- = blue, urea = green) uptake rate per unit biomass ($\mu g\ N\ \mu g\ Chl.\ a^{-1}\ hr^{-1}$), at ambient concentrations under light conditions, in spring, summer, and fall (2018, St. Denis National Wildlife Area).
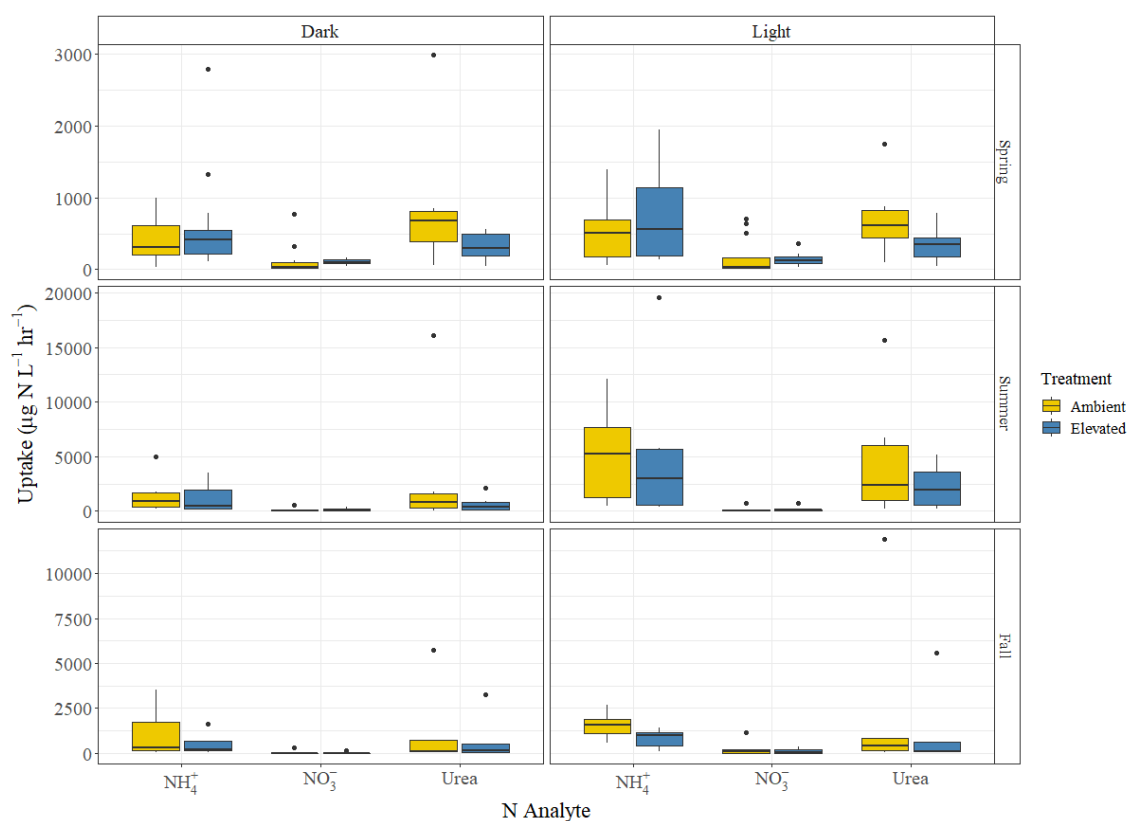
Figure A.3 Rate of planktonic uptake (μg N hr^{-1} L^{-1}) of NH$_4^+$, NO$_3^-$, and urea, in different light conditions, seasons, and each spike treatment; ambient (N addition maintained 10% of *in situ* concentrations) and elevated (N addition maintained 80–100% of *in situ* concentrations).
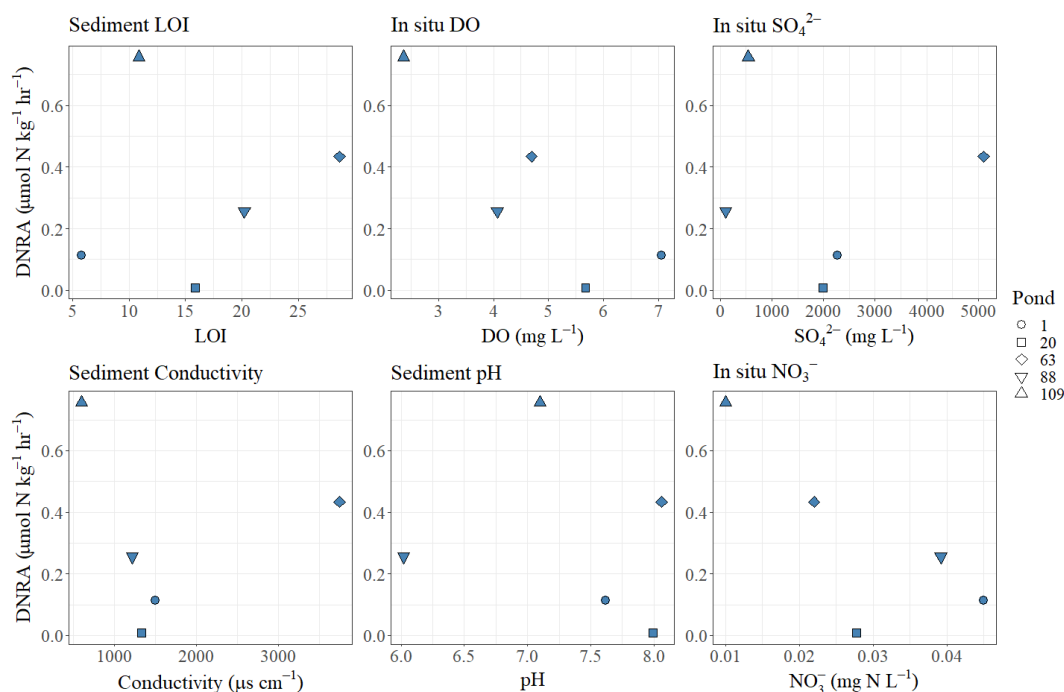
Figure A.4 Scatter plots displaying the mean DNRA rate (μg N hr^{-1} L^{-1}) after 1-hr incubation per pond across various environmental parameters.
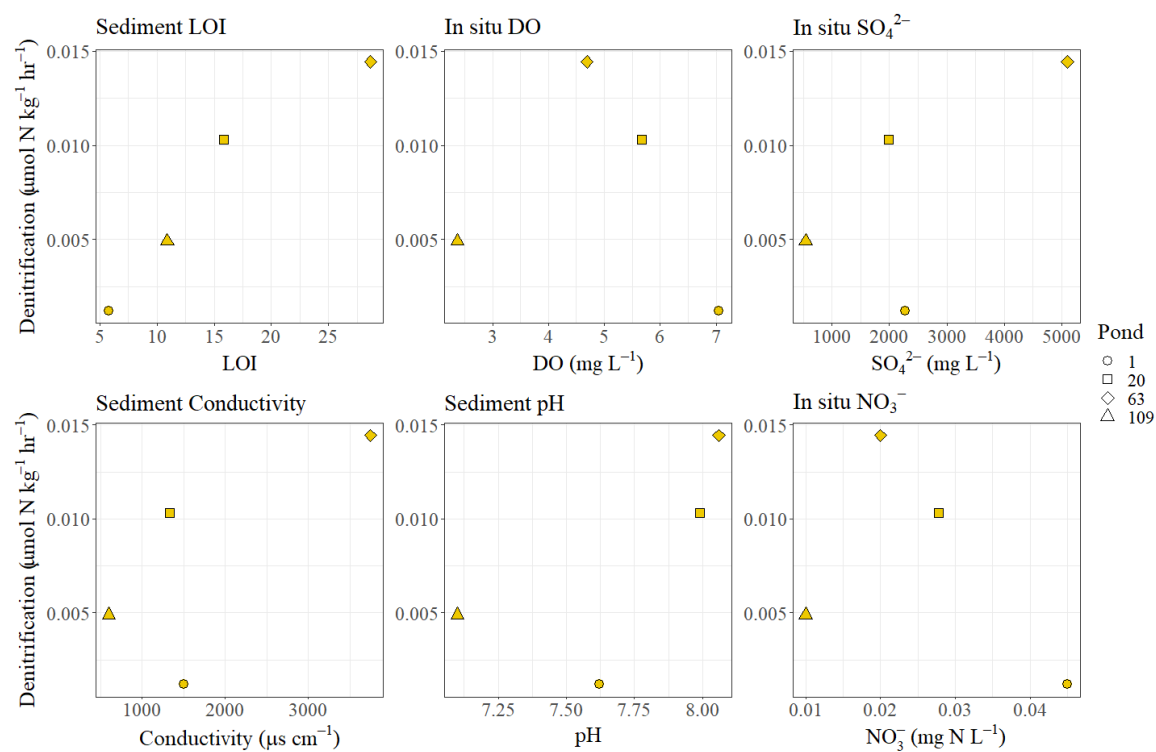
Figure A.5 Scatter plot displaying denitrification rates (μg N hr^{-1} L^{-1}) after 1-hr incubation per pond across various environmental parameters.

Table A.1 Summary of ponds at St. Denis National Wildlife Area used per experiment and season. "Benthic" indicates both denitrification and DNRA experiments were performed, "AE" indicates ambient/elevated uptake experiments, "MM" indicates Michaelis-Menten experiments. Sites listed below the dashed line were dry prior to subsequent sampling.

| Site	Experiment	Date sampled	Season	Class	Permanence	Notes
1	AE	17-May-18	Spring	5	Permanent	
	MM/AE	20-Aug-18	Summer	5	Permanent	Largest pond at SDNWA, devoid of emergent vegetation (veg.), perimeter mostly tall grasses and sedges
	MM/AE	09-Oct-18	Fall	5	Permanent	
	Benthic	21-Aug-19	Summer	5	Permanent	
20	AE	17-May-18	Spring	4	Semi-permanent	
	MM/AE	22-Aug-18	Summer	4	Semi-permanent	Adjacent to road, mucky substrate, dense mixed wetland veg. perimeter mostly tall grasses and sedges
	MM/AE	09-Oct-18	Fall	4	Semi-permanent	
	Benthic	06-Aug-19	Summer	4	Semi-permanent	
88	AE	17-May-18	Spring	4	Semi-permanent	
	MM/AE	22-Aug-18	Summer	4	Semi-permanent	Adjacent to road, surrounded by cropland, sedges, and cattail perimeter
	MM/AE	09-Oct-18	Fall	4	Semi-permanent	
	Benthic	20-Jun-19	Summer	4	Semi-permanent	
103	AE	18-May-18	Spring	3	Seasonal	
	MM/AE	21-Aug-18	Summer	3	Seasonal	Mucky substrate, abundant duckweed, perimeter of mostly woody veg., surrounded by grassland
	MM/AE	11-Oct-18	Fall	3	Seasonal	
109	AE	18-May-18	Spring	3	Seasonal	
	MM/AE	21-Aug-18	Summer	3	Seasonal	Soft bottom, dense submerged veg., some emergent veg., perimeter mostly woody veg.
	MM/AE	11-Oct-18	Fall	3	Seasonal	
	Benthic	29-Aug-19	Summer	3	Seasonal	
63	Benthic	15-Aug-19	Summer	3	Seasonal	Mucky, black substrate, smelly, perimeter of shrubs and short grasses, minimal visible, wetland veg.; only subject to benthic experiments
117	AE	18-May-18	Spring	3	Seasonal	Mixed wetland veg., perimeter of shrubs and sedges, many cattails throughout
13b	AE	17-May-18	Spring	1	Ephemeral	Small, shallow, primarily grasses
39a	MM	19-May-18	Spring	1	Ephemeral	Small, shallow, primarily grasses; spring MM only
62b	AE	17-May-18	Spring	1	Ephemeral	Small, mucky substrate, perimeter of short grasses
38	MM	19-May-18	Spring	3	Seasonal	Mixed wetland veg., perimeter of woody veg. and cattails; spring MM only
41	MM	19-May-18	Spring	3	Seasonal	devoid of emergent veg., perimeter of mostly woody veg. and short grasses, some cattails, surrounded by summer fallow; spring MM only
46	MM	19-May-18	Spring	2	Temporary	Devoid of emergent veg., perimeter of woody veg., cattails, and sedges, surrounded by summer fallow, spring MM only
105	AE	18-May-18	Spring	2	Temporary	Small ponds, wetland veg., perimeter of grasses and cattails, surrounded by grassland
107	AE	18-May-18	Spring	3	Seasonal	Small ponds, wetland veg. perimeter of grasses and few cattails, surrounded by grassland
108	AE	18-May-18	Spring	3	Seasonal	Small ponds, wetland veg, perimeter of grasses and cattails, surrounded by grassland
2	AE	17-May-18	Spring	3	Seasonal	
	MM/AE	20-Aug-18	Summer	3	Seasonal	Soft substrate, mostly submerged veg., surrounded by grassland
	MM/AE	11-Oct-18	Fall	3	Seasonal	

Table A.2 Summary of the concentration range added to samples in Michaelis-Menten experiments across seasons. Each sample was subject to doses 10%, 30%, 60%, 80% and 100% of *in situ* concentrations of the respective analyte.

Season	Analyte	Min. concentration added mg L^{-1}	Max. concentration added mg L^{-1}
	NH_4^+	0.020	1.91
Spring	NO_3^-	0.010	0.960
	Urea	0.010	0.960
	NH_4^+	0.012	2.64
Summer	NO_3^-	0.012	2.36
	Urea	0.013	2.70
	NO_3^-	0.030	1.91
Fall	NH_4^+	0.020	2.74
	Urea	0.088	2.74

Table A.3 *In situ* parameters and nutrient data of ponds at St. Denis National Wildlife Area that were sampled only in spring 2018 (dried prior to summer and fall sampling). Sites below the method detection limit are indicated, while '-' indicates the parameter was not measured.

Pond	Season	Class	Permanence	Depth at sampling cm	Conductivity µS cm^{-1}	pH pH units	NO_3^--N mg L^{-1}	NH_4^+-N mg L^{-1}	Urea mg L^{-1}	TN mg L^{-1}	SO_4^{2-} mg L^{-1}	Chl. *a* µg L^{-1}	TP mg L^{-1}
117	Spring	3	Seasonal	80	179	6.78	<0.02	<0.01	0.147	1.36	5.9	10.44	1.25
13b	Spring	1	Ephemeral	25	218	6.9	<0.02	<0.01	0.261	2.83	-	2	1.78
62b	Spring	2	Ephemeral	10	7003	7.96	<0.02	0.023	0.128	0.09	-	0.02	4.51
105	Spring	3	Temporary	49	280	7.03	<0.02	<0.01	0.222	2.27	-	23.93	2.86
107	Spring	3	Seasonal	45	292	6.82	<0.02	<0.01	0.36	2.33	-	2.3	2.11
108	Spring	3	Seasonal	50	295	7.01	0.111	<0.01	0.224	2.03	-	1.78	1.60
3	Spring	3	Seasonal	25	1672	8.02	<0.02	0.024	0.128	1.89	-	1.63	0.22
3	Summer	3	Seasonal	10	4094	8.92	0.070	0.149	0.45	3.06	-	32.9	0.31

Table A.4 *In situ* parameters and nutrient data of ponds at St. Denis National Wildlife Area that were used in Michaelis-Menten experiments in spring (May) 2018. Sites below the method detection limit are indicated, while '-' indicates the parameter was not measured.

Pond	Class	Permanence	Depth at sampling cm	Conductivity µS cm^{-1}	pH pH units	NO_3^--N mg L^{-1}	NH_4^+-N mg L^{-1}	Urea mg L^{-1}	TN mg L^{-1}	SO_4^{2-} mg L^{-1}	Chl. *a* µg L^{-1}	TP mg L^{-1}
39a	1	Ephemeral	50	1065	7.49	<0.02	<0.01	0.210	2.10	-	0.050	0.43
38	3	Seasonal	40	431	7.37	<0.02	<0.01	0.133	1.52	-	0.619	0.69
41	3	Seasonal	90	515	6.72	0.032	<0.01	0.129	1.48	-	1.16	1.16
46	3	Temporary	110	285	6.92	<0.02	<0.01	0.219	1.82	-	1.96	1.60

Table A.5 Estimated K_m and V_{max} values across seasons and analytes. Instances in which uptake of N could not be described by a rectangular hyperbola are indicated.

Season	Analyte	Site	*In situ* $\mu g\ L^{-1}$	V_{max} $\mu g\ hr^{-1}$	K_m $\mu g\ L^{-1}$	V_{max} p-value	K_m p-value	Notes
Spring	NO_3^-	41	32.0	21.8	538	0.025	0.069	No plateau
		46	6.00	32.7	1070	0.428	0.532	No plateau
		39a	4.00	17.7	436	0.004	0.016	No plateau
		38	13.0	50.2	1650	0.680	0.728	No plateau
	NH_4^+	41	<10	6.4	32.5	0.007	0.298	
		46	<10	27.8	12.2	0.009	0.493	Poor fit
		39a	<10	8.36	21.9	0.001	0.144	
		38	<10	9.84	2.19	0.001	0.352	Poor fit
	Urea	all sites	-	did not show Michaelis-Menten kinetics				
Summer	NO_3^-	1	<20	did not show Michaelis-Menten kinetics				
		2	70.0	4.72	5.01	0.103	0.937	
		20	71.0	13.3	58.7	0.022	0.416	
		88	10.0	17.8	454	0.007	0.048	No plateau
		103	145	9.22	39.8	0.464	0.923	Poor fit
		109	58.0	82.6	1420	0.631	0.718	No plateau
	NH_4^+	all sites	-	did not show Michaelis-Menten kinetics				
	Urea	all sites	-	did not show Michaelis-Menten kinetics				
Fall	NO_3^-	1, 20, 88, 109	-	did not show Michaelis-Menten kinetics				
		103	39.0	0.701	41.2	0.136	0.630	Poor fit
	NH_4^+	1, 20, 103, 109	-	did not show Michaelis-Menten kinetics				
		88	110	481	7830	0.867	0.875	Poor fit
	Urea	1, 88, 103, 109	-	did not show Michaelis-Menten kinetics				
		20	495	5.12	332	0.00932	0.151	Poor fit

Table A.6 Observed rates of DNRA and denitrification (mean ± error of propagation ($\mu g\ N\ g^{-1}\ hr^{-1}$)) after 4-hr incubations. Ponds at St. Denis National Wildlife Area were sampled in summer 2019. The DNRA rate for pond 20 could not be calculated due to sampling error. The denitrification rate in pond 88 could not be reported with confidence due to instrument noise.

Pond	DNRA $\mu g\ N\ g^{-1}\ hr^{-1}$	Denitrification $\mu g\ N\ g^{-1}\ hr^{-1}$
1	0.107 ± 0.016	0.004 ± 0.003
20	–	-0.003 ± 0.003
63	0.138 ± 0.027	0.020 ± 0.012
88	0.208 ± 0.030	–
109	–0.020 ± 0.012	0.001 ± 0.002

Table A.7 Summary of substrate turnover time[1] for different N species within study ponds, across treatment and season. The mean rate, mean available pool, and mean turnover time represent the average behaviour across potholes. To explore the quickest substrate turnover, we highlight the (single) maximum rate observed per treatment, analyte, and season.

Season	Analyte	Treatment	Mean			Maximum		
			Rate $\mu g\,N\,L^{-1}\,hr^{-1}$	Available pool $\mu g\,N\,L^{-1}$	Turnover time min	Rate $\mu g\,N\,L^{-1}\,hr^{-1}$	Available pool $\mu g\,N\,L^{-1}$	Turnover time min
Spring	NH_4^+	Ambient	526	28.1	8.46	1400	12.0	0.51
		Elevated	703	156	36.2	1950	111	3.39
	NO_3^-	Ambient	178	37.4	54.8	710	26.8	2.26
		Elevated	136	155	127	357	126	21.1
	Urea	Ambient	726	229	38.2	1740	257	8.84
		Elevated	356	334	138	788	325	24.7
Summer	NH_4^+	Ambient	5240	204	11.2	12,100	97.0	0.48
		Elevated	5360	309	30.3	19,600	179	0.55
	NO_3^-	Ambient	166	65.4	17,000	747	5.20	0.42
		Elevated	183	153	138	722	37.6	3.13
	Urea	Ambient	4700	348	26.1	15,700	111	0.43
		Elevated	2260	461	48.4	5160	468	5.44
Fall	NH_4^+	Ambient	1560	99.1	5.08	2700	91.0	2.03
		Elevated	821	186	52.9	1430	131	5.48
	NO_3^-	Ambient	298	37.7	300	1140	24.0	1.26
		Elevated	127	63.4	401	370	44.0	7.12
	Urea	Ambient	2680	404	103	11,900	686	3.46
		Elevated	1300	586	264	5600	792	8.49

[1] Turnover time is calculated as the concentration of substrate ($\mu g\,L^{-1}$) divided by the volumetric uptake rate ($\mu g\,N\,L^{-1}\,hr^{-1}$) for measurements of pelagic uptake. These calculations were done within each pothole, then averaged across season, analyte, and treatment. Therefore, dividing the average available pool by the average rate does not yield the turnover time reported here.