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Condition and function of Red River Valley streams receiving nutrients from human activities in Manitoba, Canada

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Abstract

Small tributary streams are conduits between the landscape and larger waterbodies and provide important ecosystem services including assimilation of nutrients from human activities which may be protective of downstream waterbodies. In the Red River Valley (RRV), Manitoba, numerous nutrient sources including agricultural runoff, livestock manure, and municipal wastewater effluent, have been attributed to the eutrophication of Lake Winnipeg and thus require targeted management to prevent loss of nutrients from land to lake. However, linkages between land-based nutrient-producing human activities in the RRV and the ecological status of the tributaries remain poorly understood. Furthermore, the proportional contribution of each source of nutrients to the tributaries is not known and the capacity of streams to take up these nutrients thereby preventing downstream losses is also unknown. My doctoral research improved understanding of the interaction between human activities in the RRV and the streams that serve to assimilate nutrients through the following three projects: 1) an evidence-weighting assessment identifying nutrient-producing human activities in the RRV with evidence of stream ecological effects; 2) an assessment determining the sources and seasonal variation of nitrogen to 14 RRV tributaries using established (stable isotope ratios) and novel (artificial sweeteners) tracers; and, 3) an examination of the fate of nutrients from municipal wastewater lagoon effluent releases into an RRV stream by measuring nutrient transformation, uptake, and retention. Together, these three projects identified human and livestock wastes as important drivers of current ecological condition of RRV tributaries, while underlining the need for better biomonitoring strategies in the region. Furthermore, my research highlighted the key role of livestock manure as a contributor to snowmelt nutrient runoff and improved knowledge about seasonal differences in the transfer of nutrient sources from RRV catchments to streams. Finally, I showed that the large pulsed delivery of nutrients during wastewater lagoon discharge may overwhelm stream capacity for uptake; a finding that has important implications for the protection of downstream waterbodies. The outcomes of my thesis research will enable managers to more effectively target nutrient mitigation efforts and improve management of stream ecosystem services that reduce transport of nutrients to Lake Winnipeg.

Keywords

Streams, ecosystem services, anthropogenic nutrients, ecological causal assessment, stable isotopes, artificial sweeteners, nutrient cycling, wastewater

Summary for Lay Audience

Nutrients are required by aquatic organisms; however, when nutrients are present in excess, they are detrimental to aquatic life. Humans release nutrients to the environment through activities such as agriculture and wastewater treatment. Streams receive nutrients from human activities via landscape runoff or through direct release to waterways. Within streams nutrients are transformed, retained, or removed through chemical and biological reactions facilitated by stream organisms. However, when the amount of nutrients is high and/or conditions are not favorable for stream organisms, nutrients are lost downstream to larger lakes and rivers where they may cause harm to aquatic life. Streams in the Red River Valley (RRV), Manitoba are at risk due to the detrimental effects of human activities related to agriculture and growing communities. Furthermore, RRV streams flow downstream to Lake Winnipeg, which is already vulnerable due to the high amount of nutrients entering the lake over the past three decades. The objectives of the projects presented in this thesis were to 1) identify which human activities are most likely having an effect on RRV streams; 2) identify when specific sources of nutrients are most likely to enter streams; and, 3) examine what happens to the nutrients once they enter a stream via release from a wastewater treatment lagoon. I identified human wastewater as the source of nutrients most likely to impact streams. Nutrients from livestock manure entered streams in the early spring when cold weather likely slows chemical and biological reactions and prevents transformation and retention. In contrast, wastewater from lagoons entered streams in later spring and summer when stream organisms were more active. I found that stream organisms do transform and retain some of the nutrients from wastewater treatment lagoons, but the large amount of nutrients released is likely more than organisms need; therefore, the excess is transported downstream. The outcomes of these projects will help managers to effectively plan nutrient reduction strategies in the RRV and provide insight about the amount of work a stream can do to prevent nutrient loss downstream. Overall, these outcomes will aid in the protection of RRV streams and downstream Lake Winnipeg.

Co-Authorship Statement

A version of Chapter 2 has been accepted for publication in the *Journal of Great Lakes Research* with co-authors Robert B. Brua, Patricia A. Chambers, Joseph M. Culp, Chris Chesworth, Sophie N. Cormier, Christopher D. Tyrell, and Adam G. Yates. KJP was the lead author of the manuscript and performed all data analyses. RBB, PAC, JMC, and AGY contributed to the design and synthesis of the research and provided review of the manuscript. RBB, PAC, JMC, CC, SNC, CDT, and AGY contributed data for use in the manuscript. Funding was provided via RBB through the Environment and Climate Change Canada Lake Winnipeg Basin Initiative and through Natural Science and Engineering Research Council (NSERC) Discovery Grants to AGY and JMC.

A version of Chapter 3 has been accepted for publication to *Science of the Total Environment* with co-authors Robert B. Brua, Geoff Koehler, John Spoelstra, and Adam G. Yates. KJP was the lead author of the manuscript and performed all data analyses. KJP, RBB, and AGY designed the study. KJP performed field sampling with the assistance of RBB. GK and JS provided expertise and facilitated laboratory analysis. RBB and AGY provided consultation and review of statistical analysis. RBB, GK, JS, and AGY reviewed the manuscript. Research funding was provided via RBB through the Environment and Climate Change Canada Lake Winnipeg Basin Initiative and through a NSERC Discovery Grant to AGY.

A version of Chapter 4 has been submitted for publication to *Environmental Science: Processes and Impacts* with co-authors Robert B. Brua, John Spoelstra, Geoff Koehler, and Adam G. Yates. KJP was the lead author of the manuscript and performed all data analyses. KJP, RBB, and AGY designed the study. KJP performed field sampling with the assistance of RBB. GK and JS provided expertise and facilitated laboratory analysis. AGY provided consultation and review of statistical analysis. RBB, GK, JS, and AGY provided review of the manuscript. Research funding was provided via RBB through the Environment and Climate Change Canada Lake Winnipeg Basin Initiative and through a NSERC Discovery Grant to AGY.

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List of Abbreviations

ACE	Acesulfame
BMI	Benthic macroinvertebrates
BMP	Beneficial management practice
DIN	Dissolved inorganic nitrogen
ER	Ecosystem respiration
GHG	Greenhouse gas
GLM	General linear model
GPP	Gross primary productivity
LWB	Lake Winnipeg Basin
N	Nitrogen
NH ₄ ⁺	Ammonium
NMDS	Non-metric multidimensional scaling
NO ₃ ⁻	Nitrate
O	Oxygen
P	Phosphorus
PPCPs	Pharmaceuticals and personal care products
RRV	Red River Valley
SAC	Saccharin

SRP	Soluble reactive phosphorus
SUC	Sucralose
<i>Sw-net</i>	Net nutrient uptake length
TN	Total Nitrogen
TP	Total Phosphorus
WWT	Wastewater treatment

Chapter 1

1 Introduction

1.1 Overview

Rivers are extensively used by humans for the services they provide (e.g., water supply and purification, economic activities, recreation), yet their structure and function are often degraded due to the effects of widespread human land use (Vörösmarty et al., 2010; Yates et al., 2019). Indeed, in intensively utilized landscapes, the physical structure of rivers is often heavily modified to enhance drainage (e.g., via channelization), to alleviate flooding or for water storage (e.g., via damming/reservoir creation), activities that detrimentally affect aquatic habitat structure and biodiversity (Foley et al., 2005; Dudgeon et al., 2006). Furthermore, rivers often receive discharged effluent from urban wastewater treatment facilities and overland runoff or subsurface drainage from agricultural land. Such anthropogenic inputs are rich in nutrients and have the potential to impair the ecological condition of rivers and contribute to the eutrophication of downstream waterbodies (Smith, 2003).

Well-managed rivers may have the functional capacity to both provide key services and to protect downstream systems (Yates et al., 2019). For example, streams and rivers can retain, transform, or remove nutrients through biogeochemical processes, thus providing water purification services to water users (Bernot & Dodds, 2005; Withers & Jarvie, 2008). Therefore, instead of viewing rivers and streams simply as conduits that move water and materials through the landscape, they should be viewed as modulators. However, the ecological functions of streams and rivers, especially in intensively utilized regions, are often overlooked. There is thus a need for knowledge of linkages between the ecological condition and function of rivers and their stressors to better balance river ecosystem health with the need for river ecosystem services. This thesis examines the linkages between stressors related to human activities (e.g., crop production, livestock, urban wastewater) and stream condition and identifies how anthropogenic nutrients, key

proximate drivers of stream ecosystem health, vary spatially and temporally at both the watershed and individual stream scales.

1.2 Background

1.2.1 Nutrients from human activities

Nitrogen (N) and phosphorus (P) are important macronutrients required for aquatic life. The bioavailable forms of these nutrients, soluble reactive P (SRP), nitrate (NO_3^-), and ammonium (NH_4^+), are readily utilized by aquatic organisms. These nutrients are transported into aquatic ecosystems from both natural and anthropogenic sources. However, anthropogenic sources of N and P, such as agriculture (e.g., synthetic fertilizers, N-fixing crop plants, livestock manure) and urbanization (e.g., human wastewater, fossil-fuel emissions), now eclipse those of all natural sources combined (Tilman et al., 2001). As the human population continues to grow and land use activity intensifies, continuing release of these nutrients into surface waters has become a global problem (Foley et al., 2005). Consequently, cultural eutrophication, excessive nutrient enrichment linked to human activity, affects lakes, rivers, wetlands, estuaries, and marine coastal ecosystems worldwide (Smith, 2003; Smith & Schindler, 2009). Eutrophication has been linked to degraded ecological condition of water bodies due to overgrowth of algae and aquatic plants, harmful algal blooms, degraded water quality, oxygen depletion, and loss of biodiversity (Smith, 2003; Foley et al., 2005; Dudgeon et al., 2006). Subsequently, the loss of economic and recreational opportunities, and increased expenditures for water treatment and protection of sensitive aquatic species have resulted in significant economic losses (Dodds et al., 2009).

Nutrients are released to rivers through point sources (i.e. “end of pipe”) and non-point (diffuse) sources (Carpenter et al., 1998). Point sources, such as municipal wastewater treatment plants or urban storm sewer outfalls, are localized and relatively easy to study and manage, whereas non-point sources, such as agricultural runoff or septic leakage, are difficult to monitor because they may be intermittent (i.e. delivered with precipitation events) and are transported over large areas by surface and subsurface flows, and atmospheric deposition (Carpenter et al., 1998). Thus, although nutrient pulses

to rivers are often a natural phenomenon (Yang et al., 2008), human activities have exacerbated their effects as landscapes have been altered for agricultural and urban drainage (Kaushal et al., 2010). Indeed, over the past 40 years, global fertilizer application has increased by approximately 700% (Matson et al., 1997; Tilman et al., 2001; Foley et al., 2005), resulting in large pulses of land-based nutrients entering waterbodies after rain events (Kaushal et al., 2010) or during snowmelt (Corriveau et al., 2011). Furthermore, climate change models predict increases in the strength and frequency of storms, suggesting that pulsed delivery of nutrients to aquatic systems will also become more frequent (Kaushal et al., 2014). As such, rivers in agricultural landscapes are especially vulnerable to high nutrient loads from land-based sources.

Agriculture is one the largest contributors of N and P to aquatic ecosystems globally (Tilman, 1999; Withers et al., 2014). Crop production relies heavily on the direct application of synthetic N and P fertilizers to agricultural fields to improve crop yields and livestock animals such as cattle, hogs, and chickens produce manure rich in bioavailable nutrients, which may be applied to land as fertilizer or runoff from large livestock holding areas (Tillman, 1999; Follett & Hatfield 2001). Streams in agricultural areas are often modified to enhance drainage, either through ditching and channelization or the installment of subsurface drains, thus increasing the amount of land-based nutrient runoff they receive and transport (Baulch et al., 2019). As a result, nutrient concentrations in streams draining agricultural land are often high and transient storage is low relative to less disturbed systems (Bernot et al., 2006). Consequently, excessive nutrient loading related to agriculture has been implicated in the degradation of many aquatic systems; for example, the eutrophication of Lake Erie (Michalak et al., 2013) and Lake Winnipeg (Schindler et al., 2012).

In addition to fertilizer and livestock sources of nutrients, small municipalities throughout agricultural areas use waterways for waste assimilation through the release of sewage effluent into streams. Municipal wastewater is one of the largest point-source contributors, by volume, to pollution of Canada's surface waters (Chambers, 2001; Holeton et al., 2011). While many large Canadian cities that produce high volumes of wastewater are serviced by wastewater treatment plants with advanced nutrient-removal

technology (i.e. tertiary treatment), many of the small municipalities rely on wastewater lagoons that regularly release nutrient-rich effluent into streams and rivers and lack these advanced technologies (Holeton et al., 2011). Lagoons typically provide facultative treatment that allows for some nutrient removal; however, more than one third of N and P may not be removed and thus released to waterways (Chambers, 2001). Furthermore, it is difficult for small municipalities to meet stringent nutrient requirements and thus are often not required to meet nutrient targets imposed on larger facilities. As urban populations continue to grow and the demand for wastewater treatment increases, some municipalities, even those with larger facilities, have outgrown their existing infrastructure and are thus permitted to release effluent on an emergency basis resulting in higher nutrient loads than would be encountered during normal releases.

1.2.2 Deriving ecosystem services from rivers

The benefits that humans derive from ecosystems are known as ecosystem services (Millennium Ecosystem Assessment, 2005). Rivers provide many ecosystem services to humans including provisioning services (e.g., supply of water and food), regulating services (e.g., water transport and purification), cultural services (e.g., recreation, natural beauty), and spiritual services (Vörösmarty et al., 2005). In agricultural landscapes, where the threat of excessive nutrient loading to waterways is high, the water purification service provided by streams and rivers may be especially valuable (Yates et al., 2019). However, there is a need to balance human benefits with ecosystem health to maintain ecosystem function, while still meeting wider societal goals (e.g., agricultural production to supply food and economic benefits; Withers et al., 2014; Yates et al., 2019). There is thus a need for improved understanding of the ecosystem functions inherent to rivers that retain, transform, or remove nutrients (e.g., biogeochemical processes, assimilative uptake by organisms) from wide-spread anthropogenic activities, thereby providing water purification and attenuating downstream losses (Bernot et al., 2006; Withers et al., 2014).

Rivers have the functional capacity to attenuate both N and P from human activities (Figure 1.1). N is primarily attenuated by streams and rivers through biosynthetic assimilation, nitrification, and denitrification (Bernot & Dodds, 2005;

Kendall et al., 2007). Both autotrophic and heterotrophic organisms can assimilate N into their tissues thereby removing it from the water column until biotic tissues break down and are mineralized. Nitrification is the oxidation of NH_4^+ to NO_3^- and denitrification is the dissimilatory reduction of NO_3^- to N_2 , N_2O and NO gases, usually under anaerobic conditions. Both nitrification and denitrification are mediated by microbial communities living on stream substrates or in the sediments (Battin et al., 2003; Ribot et al., 2012). P retention typically occurs via assimilation into biotic tissues (e.g., Stutter et al., 2010) or through physical reactions such as sorption or precipitation (Withers & Jarvie, 2008). P can also be released back to the overlying water column through desorption or dissolution, especially under low oxygen conditions, as well as from the decomposition of organic matter.

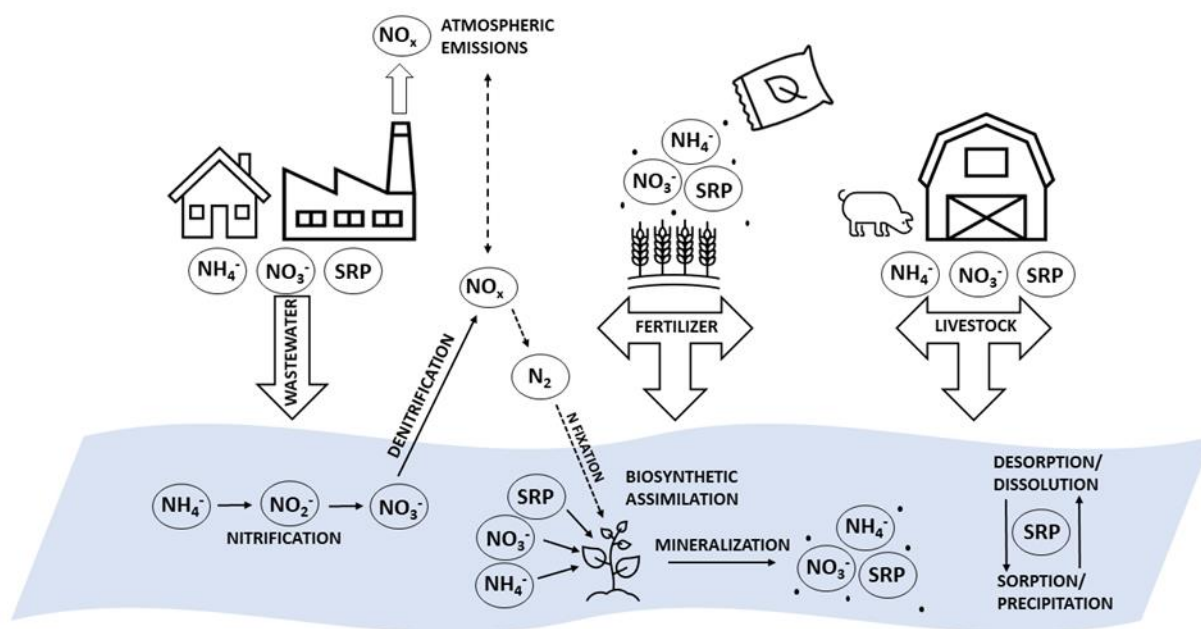


Figure 1.1 Simplified schematic diagram showing the major anthropogenic point (wastewater) and diffuse (fertilizer, livestock) sources of bioavailable nitrogen, ammonium (NH_4^+) and nitrate (NO_3^-), and phosphorus (soluble reactive phosphorus; SRP), and the processes that influence their assimilation in streams and rivers

N and P retention in streams is influenced by ambient stream conditions such as flow rates and temperature. For example, faster flows reduce residence time and result in increased downstream transport (Withers & Jarvie, 2008). Uptake length, the distance that nutrients travel before they are immobilized through uptake by biotic or abiotic processes (Stream Solute Workshop, 1990), is heavily influenced by stream flow such that uptake length increases with velocity due to the reduced interaction time between nutrients and assimilators (Peterson et al., 2001; Allan & Castillo, 2007). Furthermore, streams that undergo frequent high flow events, such as the major precipitation events associated with nutrient pulses, can lose nutrient assimilating biomass through scouring (Dodds et al., 1996). In addition to flow rate, the activity of stream organisms is influenced by temperature. For example, measures of stream metabolism (ecosystem respiration and gross primary production) have been shown to increase with increasing water temperature (Demars et al., 2011). In addition, cooler temperatures may inhibit the microbial activity required to mediate nitrification and denitrification activity. Indeed, Ribot et al. (2012) found that biogeochemical cycling of N from a wastewater treatment plant outfall was more intense during the warmer summer season compared with the cooler winter season. Therefore, seasonal variation in stream condition is an important determinant of N and P retention and processing.

Furthermore, supply and demand for nutrients is an important determinant of assimilation because P and/or N commonly limit biotic uptake in aquatic systems (Redfield, 1958). Moreover, during large nutrient pulses, such as those associated with agricultural runoff or wastewater lagoon releases, supply of nutrients may exceed demand. Indeed, nitrate saturation has been reported in stream pools and reservoirs in an agricultural watershed in Manitoba (e.g., Gooding & Baulch, 2017), indicating that nitrate availability exceeded the demand by denitrifying microbes, limiting denitrification potential. Similarly, O'Brien et al. (2007) found that the nitrate retention by stream biota in nine Kansas streams decreased along an increasing gradient of ambient stream nitrate concentrations. Price and Carrick (2016) found that P uptake rates by stream biofilms declined with increased experimental P loading and biofilms had significantly higher uptake rates in lower productivity streams than in high productivity streams. Moreover, rivers receiving large nutrient loads from wastewater treatment plants have become P

saturated to the point that their sediments become P sources (e.g., Jarvie et al., 2012, Waiser et al., 2011). As such, understanding the role stream condition plays in the attenuation of anthropogenic N and P is needed to maximize the use of stream function because assimilative capacity may be impaired when catchment nutrient loading is high.

1.2.3 Prairie streams at risk

Water security in Canada's prairie region is at risk due to the cumulative effects of land use, changing climate and increasing demand for water resources (Schindler & Donahue, 2006). Because of their placement in often heavily utilized landscapes, prairie streams are prone to modification due to human activity resulting in hydrological (e.g. channelization or diversion, increased siltation) and biological (e.g. alteration of riparian vegetation, reduced habitat for stream organisms) changes (Dodds et al., 2004). Moreover, increasing fertilizer use and increased discharge of municipal and livestock waste are causing excessive nutrient loading within prairie catchments (Schindler & Donahue 2006, Yates et al., 2012). As such, the functional capacity of prairie streams to assimilate nutrients may be particularly important because they provide many ecosystem services yet are highly vulnerable to excessive nutrient loading related to human activities and climatic variation (Dodds et al., 2004). Furthermore, biotic activity in prairie streams is high due to an ample supply of nutrients and light from agricultural catchments with little shade from the natural grass riparian vegetation (Bernot et al., 2006). Thus, these systems may have substantial potential for mitigating nutrient loadings associated with agriculture and wastewater lagoon releases.

1.2.3.1 The Lake Winnipeg Basin

Most streams and rivers in the Canadian prairies are encompassed within the vast Lake Winnipeg Basin (LWB). The LWB stretches from the Rocky Mountains in the west to the Precambrian Shield in the east across four provinces and south into four states (Figure 1.2). Lake Winnipeg has the largest commercial freshwater fishery in western Canada, serves as a source of drinking water to communities along its shores, and is heavily used for recreation and tourism (Environment Canada and Manitoba Water Stewardship, 2011). During the mid-1990s, Lake Winnipeg underwent a period of rapid

eutrophication (Schindler et al., 2012). Because the watershed is very large (approx. one million km²) compared to the surface area of the lake (approx. 24,500 km²), and is dominated by agricultural land use, potential for nutrient loading into the lake is substantial (LWSB, 2006; Environment Canada and Manitoba Water Stewardship, 2011). There is thus a great need to protect the lake from further degradation by reducing and attenuating nutrient sources within its basin.

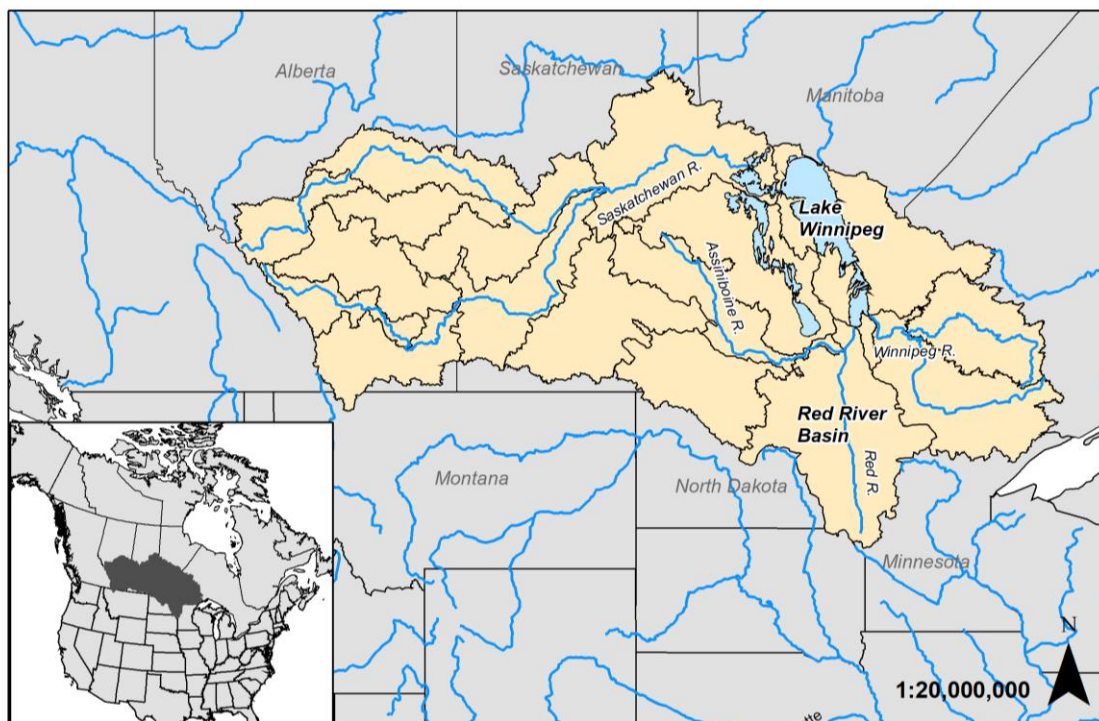


Figure 1.2 Map of the extent of the Lake Winnipeg Basin showing the major rivers draining to Lake Winnipeg and the boundaries of major sub-basins including the Red River Basin.

The majority of the inputs to Lake Winnipeg come from three major river systems: the Saskatchewan River to the west, the Winnipeg River to the east, and the Red River to the south. In addition to agriculture and livestock, increasing intensity and frequency of spring flood events in the LWB have been identified as a dominant source of nutrient loading to Lake Winnipeg (Schindler et al., 2012; Benoy et al., 2016). While lakes and reservoirs in the Saskatchewan and Winnipeg River basins have been shown to

sequester much of the nutrient load coming from their respective catchments (Donald et al., 2015), a large proportion of the annual nutrient loading comes from the Red River Basin (McCullough et al., 2012; Benoy et al., 2016).

Despite climate models predicting drier conditions on the prairies overall due to evapotranspiration exceeding precipitation (Schindler and Donahue 2006), the Red River Basin has undergone a multi-decadal wet spell (Ehsanzadeh et al., 2012; McCullough et al., 2012) and precipitation is expected to increase in the future (Dibike et al., 2012; Asong et al., 2016). It has been shown that run-off from flood events (i.e., nutrient pulses) driven by wet years in the Red River sub-basin of Lake Winnipeg are responsible for the sharp increase in total P (TP) loading to the lake since the 1990s (McCullough et al., 2012). Indeed, in years when watershed hydrologic connectivity is greatest major algal blooms have occurred in the lake (Ali and English 2019). Modelled estimates of the annual TP and total N (TN) loads to Lake Winnipeg indicate that approximately 70% of the total annual loading comes from the Red River Basin (Environment Canada and Manitoba Water Stewardship, 2011; Benoy et al., 2016). Because the Red River Basin contributes a disproportionate amount of nutrients to Lake Winnipeg, understanding the processes affecting nutrient uptake in the tributaries in this area is important because streams have the potential to provide a first line of defense against nutrient loading to the lake in addition to the services they can provide to land users in the area.

Recent and future hydrologic changes (e.g., multi-decadal wet spell, future effects of climate change) highlight the need to link stream function to temporal variation in the LWB. Delivery of nutrients in cold region prairie streams is heavily influenced by seasonal hydrologic trends (Shrestha et al., 2012; Cade-Menun et al., 2013). The majority of runoff occurs during snowmelt, whereas sporadic precipitation events during the ice-free months account for the remainder (Dibike et al., 2012; Cade-Menun et al., 2013). As such, streams are intermittently connected to the landscape, which has important implications for nutrient delivery. Previous studies have shown variation in nutrient loading throughout the year generally follows hydrologic patterns (e.g., Rattan et al., 2017). Moreover, Soto et al. (2019) found that synthetic nitrate fertilizers contributed a greater proportion of N to nitrate in the Red River during wet conditions, whereas nitrate

from wastewater effluent was more constant, likely because point sources are not driven by landscape hydrology. Thus, such variation in nutrient sources to the Red River warrants further study of nutrient sources and their effects on stream function in Red River tributaries.

1.2.3.2 The Red River Valley

The Red River Valley (RRV) is the portion of the Red River Basin immediately adjacent to Lake Winnipeg. Like prairie streams elsewhere, streams in the RRV face imperilment due to intense land use in their catchments. The RRV has undergone significant change from pre-settlement times with much (>70%) of the original wetland and tallgrass prairie landscape transformed for crop and livestock production by the 20th century (Bossenmaier & Vogel 1974). As such, human activities in the RRV have undoubtedly altered stream condition. However, a lack of consistent, long-term biomonitoring studies in the region makes clarifying the linkages between human activities and stream condition difficult.

Sediment cores from Lake Winnipeg have recorded the historical change in the region. For example, Bunting et al. (2016) inferred the gradual eutrophication of the lake from 1900 to 1990 from nutrients and algal fossil pigments in cores, which increased in line with human activities and landscape modification in the RRV. Therefore, though hydrology may have driven much of the recent (i.e. post-1990s) increase in nutrient loading to the lake, modification of the landscape and likely subsequent impairment of stream structure and function has probably played a key role in the loading of nutrients to Lake Winnipeg over the past century. Recent studies have shown links between land use (crop production, livestock, municipal wastewater) and ecological metrics such as stream metabolism, benthic communities, and natural abundance stable isotope ratios (Yates et al., 2014; Yates et al., 2018) in the RRV. However, much is still unknown about how individual land use stressors vary in their impacts and contributions to streams in the region. Furthermore, despite being the final gatekeepers of water and nutrients to the Red River and Lake Winnipeg (Ali & English, 2019), the role of RRV streams as modulators of nutrients (e.g., via functional processes) has not been well established.

1.2.4 Measuring sources and assimilation of nutrients in streams

Improved understanding of the impacts of nutrient-producing human activities in regions such as the RRV requires the identification of anthropogenic nutrient sources. Natural abundance stable isotope ratios (e.g., $^{15}\text{N}/^{14}\text{N}$, hereafter $\delta^{15}\text{N}$; $^{18}\text{O}/^{16}\text{O}$, hereafter $\delta^{18}\text{O}$) of DIN and stream organisms can be used to identify sources of N from human activities because biological processing and chemical reactions favour lighter isotopes (e.g., ^{14}N or ^{16}O) resulting in the predictable enrichment or depletion of heavier isotopes (e.g., ^{15}N or ^{18}O) (Mariotti et al., 1981; Kendall et al., 2007). Anthropogenic sources of N to streams, such as fertilizer and wastewater, can be identified using the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate (e.g., Kaushal et al., 2011; Soto et al., 2019) and the $\delta^{15}\text{N}$ value of ammonium (e.g., Hood et al., 2014; Kendall et al., 2015). Likewise, the $\delta^{15}\text{N}$ of biota, such as algae, that acquire N from water column DIN, can also be used to infer source (e.g., Cole et al., 2004) and may provide a better indication of exposure to N sources over time because of longer tissue turnover time relative to water. For example, N and O arising from synthetic fertilizers are typically isotopically similar to the atmosphere ($\delta^{15}\text{N}$ near 0‰, $\delta^{18}\text{O}$ near +23‰) due to the Haber-Bosch process used in their manufacture (Bateman & Kelly, 2007; Michalski et al., 2015). In contrast, N and O in human and livestock wastes tend to be relatively enriched in ^{15}N and depleted in ^{18}O due to wastewater treatment processes and volatilization (Kendall et al., 2007).

Stable isotopes can also be used to infer the biogeochemical processing of N that occurs longitudinally in streams. The relationships between the isotopic composition of DIN species and their concentrations can be used to infer nitrification and denitrification because their mediating biogeochemical reactions result in the enrichment of ^{15}N in the substrate (e.g., water column DIN) relative to the product (e.g., N transformed or lost to the atmosphere, Kendall et al. 2007). Nitrification results in the concomitant decrease of ammonium concentration and increase of nitrate concentration, and the increase of $\delta^{15}\text{N}$ - NH_4^+ over the length of the stream (e.g., Gammons et al., 2011). In contrast, denitrification results in the decrease of nitrate concentration and increase in $\delta^{15}\text{N}$ - NO_3^- (e.g., Lofton et al., 2007). Assimilation by stream organisms, such as algae or

macrophytes, should result in a correlation between the $\delta^{15}\text{N}$ of biota and $\delta^{15}\text{N}$ of their DIN source (e.g., Ribot et al., 2012).

Additional tools may be required to trace sources of N in regions where source isotopic signatures overlap. In particular, human wastewater and livestock manure are indistinguishable using stable isotopes alone (Kendall et al., 2007), which makes it difficult to develop targeted nutrient reduction strategies in regions where both occur, as is the case in the RRV. Artificial sweeteners, such as acesulfame (ACE), sucralose (SUC), and saccharin (SAC), have shown promise as highly specific tracers of human wastewater because of their ubiquitous nature in consumer products (e.g., diet drinks and foods, toothpastes) and the fact they are not removed by wastewater treatment (Spoelstra et al., 2017; Spoelstra et al., 2020). Indeed, it has been suggested that ACE, SUC, and SAC may assist in the differentiation of human wastewater from livestock manure (Spoelstra et al., 2020) as ACE and SUC are ubiquitous in human wastewater, and not found in animal waste. ACE is resistant to degradation and thus is a good overall indicator of the presence of human wastewater whereas SUC appears to be more readily degraded in the environment and thus likely indicative of recent wastewater inputs (Spoelstra et al., 2013; Robertson et al., 2016). In contrast, SAC is found in human waste, but is also the only artificial sweetener approved for use in Canadian livestock feed (Spoelstra et al. 2020) and is commonly used as a feed additive for piglets (Buerge et al., 2011; Ma et al., 2017). The combination of enriched ^{15}N (e.g., in DIN or algae) and dominance of saccharin over other sweeteners at a given site may therefore indicate livestock manure is the primary N source.

Highly specific tracers such as ACE may also be useful for measuring net nutrient uptake downstream from wastewater point sources. For example, the depletion of nutrient concentration relative to a conservative tracer is used to calculate the distance travelled by a given nutrient molecule before uptake (nutrient uptake length; Stream Solute Workshop, 1990). Many studies use chloride as a tracer of point source wastewater effluent (e.g., Martí et al., 2004; Ribot et al., 2012). However, chloride is also a major constituent of road salt and certain fertilizers (e.g., potassium chloride), both of which are commonly used in many watersheds (Kelly et al., 2010). Therefore, a highly wastewater-

specific and recalcitrant tracer such as ACE may help improve the accuracy of uptake length calculations because ACE will not be confounded by other sources (e.g., during unforeseen stormflow events). Such calculations can then be used to infer the retention or the release of nutrients (i.e., net nutrient retention efficiency, Martí et al., 2004) in streams receiving point source nutrient inputs such as those which receive wastewater effluent from municipal lagoons.

1.3 Objectives

Streams are an important link between landscapes such as the RRV and larger downstream waterbodies like Lake Winnipeg. Yet, when stressors associated with land use are considered, streams often do not receive the same research or management attention as their downstream counterparts. For example, the rapid eutrophication of Lake Winnipeg spurred action and legislation to “Save the Lake” (LWSB, 2006; The Save Lake Winnipeg Act, 2011), yet the functional capacity of streams to provide water resources to land users and to ameliorate land use impacts on the lake did not receive comparative attention. More recent studies have underscored the importance of small tributaries in modulating the flow of water and nutrients to Lake Winnipeg (Ali & English, 2019), and ecological indicators suggest the structure and function of RRV streams are impacted by land use (Yates et al., 2014). However, prioritizing management of streams requires the identification of key stressors (e.g., crop production, livestock, urban wastewater) that may be impairing ecological condition and demonstrating clear causal linkages between stressors and stream condition. Moreover, little is known about the functional capacity of streams in the RRV to attenuate nutrients from human activities, thus research is needed to demonstrate the potential of streams to assimilate or remove nutrients at the stream scale.

In this thesis, I undertake the following three research objectives to provide knowledge about stream condition and assimilative function in the RRV:

Objective 1) Provide a thorough review of RRV stream condition. Then, synthesize currently available data linking nutrient producing human activities to

stream condition in the RRV and conduct a meta-analysis to identify linkages between human activities and ecological effects.

- Objective 2) Identify important sources of N from nutrient producing human activities to 14 streams across the RRV and assess how sources vary seasonally
- Objective 3) Quantify the retention and instream processing of nutrients from the episodic release of municipal wastewater lagoon effluent into an RRV stream

1.4 Thesis Structure

This thesis is presented in an integrated article format. This introduction chapter (Chapter 1) provides background information about the consequences of nutrient loading to streams, the ecological functions of streams that allow for the attenuation of nutrients, and the important role of streams across the prairies and in the Red River Valley. Three original research chapters (Chapters 2 to 4) correspond to the objectives stated above:

1.4.1 Description of Chapter 2

Agriculture, urbanization, and climatic change are known drivers of degraded ecological condition of streams and rivers. The RRV represents a nexus of these stressors. However, linking stream condition to the cumulative stressors related to land use presents a complex problem due to the difficulty of isolating linkages between individual stressors and ecological effects. In Chapter 2, I first provide a review of current condition of the RRV. I then synthesize available ecological data and perform an ecological causal assessment, a qualitative, evidence-weighting method of meta-analysis, of tributaries draining the RRV to establish links between individual stressors and stream condition. The outcomes of this research will improve knowledge about drivers of stream condition in the RRV while highlighting knowledge gaps where linkages between existing drivers and ecological effects are unknown. Such findings will help inform effective management strategies as well as aid in the development of informed research questions and study designs in the RRV.

1.4.2 Description of Chapter 3

Human activities across the RRV produce large amounts of bioavailable N, a key driver of eutrophication. Nutrient delivery to streams in the cold prairie region is known to be driven by seasonal hydrologic trends (e.g., Corriveau et al., 2011; Cade-Menun, 2013). However, in the RRV little is known about the relative contribution of different sources of anthropogenic nutrients to streams and how those contributions vary among seasons. In Chapter 3, I use natural abundance stable isotope ratios and artificial sweeteners to identify sources of N to streams in the RRV and examine how they change throughout the ice-free season. The outcomes of this research will improve understanding of which human activities are the greatest contributors of N to streams in the RRV and how they differ throughout the year. Furthermore, these findings will inform targeted nutrient management strategies, a key objective of ongoing efforts to decrease nutrient loading from the Lake Winnipeg watershed (e.g., Environment and Climate Change Canada, 2017).

1.4.3 Description of Chapter 4

Municipal wastewater lagoons that discharge effluent directly to streams are widely used by communities across the prairies and the RRV. Stream organisms are known to assimilate and transform nutrients thus providing water purification services. However, the functional capacity of streams to process nutrients from lagoon effluent is not known. Moreover, lagoons release effluent episodically and thus conditions during releases may vary. In Chapter 4, I compare the longitudinal patterns of bioavailable N and P concentrations and assimilative processes between three wastewater lagoon discharge events occurring under different conditions. The outcomes of this research will provide new knowledge about the functional capacity of a prairie stream to attenuate nutrients from municipal lagoon effluent. Furthermore, this study will provide information about how discharge timing influences the attenuation of nutrients from lagoon discharge events. Such information is important if managers are to maximize the water purification ecosystem service of streams to aid in nutrient mitigation strategies.

The above three research chapters are presented as individual manuscripts. The final chapter of this thesis (Chapter 5) synthesizes and summarizes the collective findings of the research chapters. Chapter 5 provides discussion about the significance of my research findings and their contribution to the overall knowledge about stream condition and stream function.

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Chapter 2

2 An ecological causal assessment of tributaries draining the Red River Valley, Manitoba

2.1 Introduction

Rivers and streams have the capacity to provide ecosystem services needed to both maintain human land use activities and ameliorate their impact (Yates et al., 2019). For example, in agricultural watersheds, rivers provide services that improve crop yields, such as drainage and irrigation, while indirectly assimilating excess nutrients (e.g., Bernot et al., 2006). In urban areas, rivers provide disposal of storm water and wastewater effluent, but also assimilate the nutrients and contaminants therein (e.g., Waiser et al., 2011a,b). Moreover, rivers act as water gatekeepers, modulating hydrology, which controls the timing and quantity of materials transported from the land to downstream ecosystems (Ali & English, 2019). However, global water resources are increasingly imperiled because of environmental stresses related to human settlement (Vitousek et al., 1997), intensifying land use (e.g., agriculture) (Foley et al., 2005) and impacts from climatic variables (Paerl & Paul, 2012). Informed management is thus essential in order to preserve ecological structure and function of rivers in heavily utilized landscapes and thereby maintain the ecosystem services that directly benefit watershed users and indirectly provide protection to downstream ecosystems.

Ecological impairment of rivers and streams typically arises from the synergistic effects of multiple stressors, resulting in a complicated or ‘wicked’ problem – one that cannot be readily solved, is highly complex and often disputed, and must be solved repeatedly (Davies et al., 2015; Grundmaan, 2016). Ecological causal assessment is well suited to investigating the complexities of multiple stressor systems because it can derive and weight all available lines of evidence to determine candidate causes of degraded ecological condition, using standardized and transparent methods of inference (Norton et al., 2015; US EPA, 2017). The result is that causal linkages between sources and ecological effects are identified and, equally importantly, so are the gaps for which linkages are unknown. Such a framework thus allows for incremental progression and

continual adaptation to new information (Norton et al., 2015). In using this framework, the objective is not to wait to synthesize and weight evidence until comprehensive analyses of all possible causes have been carried out. Rather, the goal is to investigate the known lines of evidence now so the most plausible causal linkages can be acted upon, and knowledge gaps identified, thus providing direction for future research and management activities.

The Manitoba portion of the Red River Basin, the Red River Valley (RRV), is an appropriate region for the application of an ecological causal assessment framework because it represents a nexus of human settlement, agricultural intensification, and climatic variability (McCullough et al., 2012; Schindler et al., 2012). Historically, the small streams crossing the RRV have been treated as conduits to be utilized for drainage and assimilation of runoff and wastewater from farmland and communities, transferring water, nutrients, and contaminants from the landscape into the Red River. In the RRV, streams function as conduits during the hydrologically dynamic snowmelt period when runoff is greatest and cold temperatures inhibit stream productivity (Corriveau et al., 2013; Rattan et al., 2017). However, during the ice-free seasons from spring to autumn when streams are most ecologically productive, they provide key ecosystem services by assimilating the nutrients in agricultural runoff and wastewater effluent (Voora & Venema, 2008; Yates et al., 2012; Yates et al., 2019). Yet, for the ecosystem services of streams to be maintained and enhanced to confront intensified or changing stressors, a thorough understanding of their ecological condition and function is needed. While the ecology of RRV streams has received considerable research effort over the past decade, a full synthesis of these data and associated knowledge remains lacking.

Thus, we aimed to summarize and assess the available information to improve understanding of the ecological condition and associated ecosystem services provided by tributaries in the RRV. Our first objective was to establish current ecosystem status of tributaries in the major Red River sub-basins that form the RRV. We achieved this objective by reviewing and comparing contemporary and historical climatic, hydrologic, land use, and water quality conditions within the RRV (Section 2.2). Our second objective was to apply an ecological causal assessment framework to determine the most

likely causes of current ecological condition of RRV tributaries by analyzing data from the RRV and similar anthropogenically modified systems around the globe, and linking human activities to ecological effects (Section 2.3). RRV tributaries have the potential to regulate the delivery of water, nutrients, and ecosystem services within their watersheds and to downstream habitats. By undertaking this review and producing a targeted, regional-specific assessment, we were able to highlight the causal linkages and knowledge gaps unique to the tributaries of the RRV. As such, the outcomes of this assessment will assist in the preservation of the ecological condition of RRV streams and the key ecosystem services they provide. Furthermore, our assessment will aid in the direction of research, policy, and management needed to protect the water gatekeepers of Lake Winnipeg.

2.2 Review of contemporary and historical conditions

2.2.1 Description of the Red River Valley

The RRV is located in southern Manitoba, Canada in the northeastern portion of the North American Great Plains. For this study, we defined the RRV as the Canadian portions of the following seven sub-basins: Upper Red, Roseau, Rat/Tourond, Morris, La Salle, Seine, and the Lower Red river basins (Figure 2.1). These sub-basins are located within the confines of the Manitoba escarpment to the west and the boundary of the Red River Basin to the east and have been the focus of much of the recent collective research effort (e.g., Yates et al., 2012; Corriveau et al., 2013; Mahmood et al., 2017; Rattan et al., 2017; Yates et al., 2018). While the Assiniboine River is a major tributary of the Red River, its sub-basin extends far beyond the bounds of the RRV and Manitoba escarpment and has received comparatively less ecological research focus; thus, it was excluded from this review.

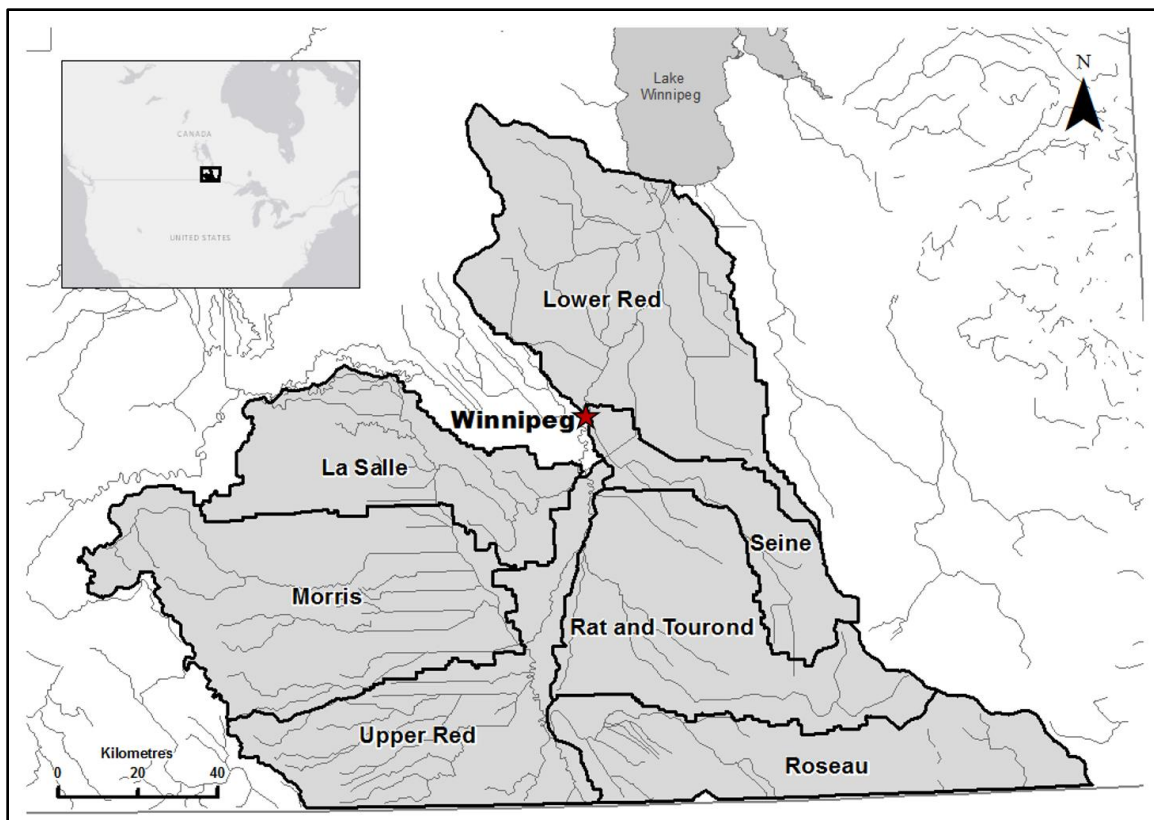


Figure 2.1 The spatial extent of the seven major sub-basins of the defined Red River Valley (RRV), Manitoba, Canada; Manitoba's capital city, Winnipeg, is denoted by the coloured star

Historically, the RRV was dominated by tallgrass prairie and wetlands (Bossenmaier & Vogel, 1974). Much of the RRV is now modified to improve drainage for agricultural development, resulting in loss of wetlands and alteration of stream and river channels to improve crop production and protect infrastructure. There are approximately 500,000 people (excl. portion of Winnipeg in the Assiniboine sub-basin) living in the RRV, approximately 40% of which reside in the City of Winnipeg and its metropolitan area. The remaining 60% of the population in the RRV reside in small rural communities and on farms. Winnipeg is serviced by mechanical wastewater treatment plants with effluent discharged continuously to the Red River, whereas the smaller communities typically use wastewater lagoons that are permitted to periodically discharge into Red River tributaries during summer and early autumn. Combined effects of agriculture, landscape modification, and wastewater in the RRV have caused an

increase in nutrient transport from land-based sources to Lake Winnipeg over the past century, culminating with a sharp, sustained increase in lake surface water nutrient concentrations in the mid-1990s (McCullough et al., 2012). As such, wide-scale efforts to “Save the Lake” have been ongoing by governments, researchers, and policy makers since the mid-2000s, with much of that effort focused in the RRV (LWSB, 2006; Schindler et al., 2012).

2.2.2 Land use in the Red River Valley

Land cover in the RRV has undergone a dramatic transformation from 17th century pre-settlement conditions (Figure 2.2A) to present (Figure 2.2B). The RRV area was once largely composed of a vast tract of permanent and semi-permanent wetlands and wet prairie (~45%), deciduous parkland (~20%), forest (~20%), and grassland (~10%) (Bossenmaier and Vogel, 1974). Between initial European settlement in Manitoba in the early 1800s and agricultural intensification of the 20th century, greater than 85% of the RRV was transformed from wetlands to cropland. Over this period, almost 90% of wetland cover was lost such that land cover in the RRV is now dominated by cropland (>70%) (Natural Resources Canada, 2017, available from:

<https://open.canada.ca/data/en/dataset/c688b87f-e85f-4842-b0e1-a8f79ebf1133>).

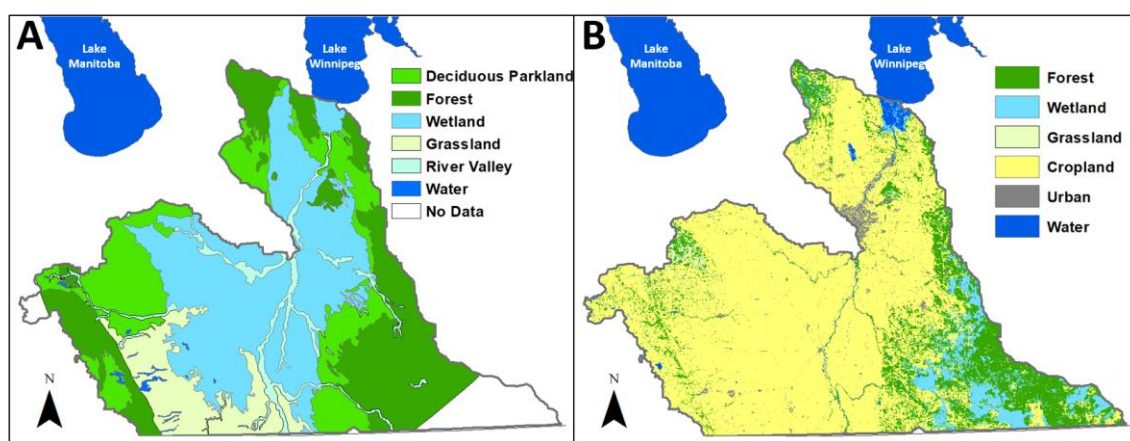


Figure 2.2 Landcover change in the Red River Valley, Manitoba, Canada from A) 17th century pre-settlement conditions (adapted from Bossenmaier & Vogel 1974) to B) present day (Natural Resources Canada, 2017). Much of the wetland (permanent

and semi-permanent wetlands and wet prairie) and grassland has been converted to agricultural land.

Large-scale transformation of wetlands to cropland during the 20th century made way for rapid expansion of agriculture and human settlements. Data compiled from Canadian agriculture censuses (Statistics Canada, 2019, available from: <https://www150.statcan.gc.ca/n1/en/subjects/Agriculture>) over five time periods (1931, 1951, 1976, 1996, and 2016) showed that the proportion of farmland reported to be cultivated for crops, as opposed to pasture, summer fallow, or unimproved land (natural pasture or prairie, woodland, and wetland), has steadily increased from 48% in 1931 to 83% by 2016 (Figure 2.3A). This is paralleled by a decrease in unimproved land from 39% in 1931 to 15% in 2016 (Figure 2.3C) suggesting that natural areas of grassland, woodland and wetland continued to be converted to cropland through 1976. The share of nitrogen producing crops including varieties of peas, beans, and N-producing hay crops, such as alfalfa and sweet clover, has tripled from 1931 to 2016 (Figure 2.3B). Fertilizer application statistics were not included in earlier versions of the agriculture census. However, in 1996 and 2016 the proportion of cropland with fertilizer applied in the RRV was 85% and 79%, respectively. Reported manure application was smaller, with manure applied to only 5% and 6% of crop area in 1996 and 2016, respectively. Cattle numbers in the RRV have been stable since the 1970s (Figure 2.3D). However, pigs increased rapidly from approximately 260,000 in 1976 to nearly 1,000,000 in 1996, and then to over 2,300,000 in 2016 (Figure 2.3E). In addition to agricultural changes, the human population in the RRV has increased steadily from about 265,000 people in 1931 to over 500,000 (value excludes Winnipeg population in the Assiniboine basin) in 2016 (Figure 2.3F).

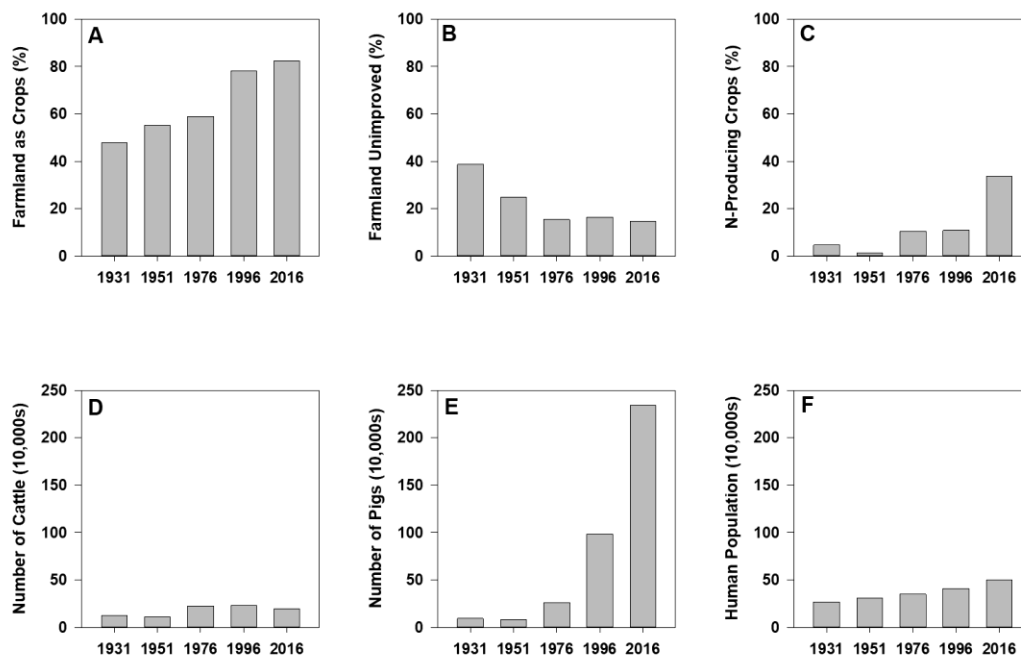


Figure 2.3 Changing land use patterns in the Red River Valley, Manitoba, Canada based on Statistics Canada agriculture census data (Statistics Canada, 2019) from five assessment years between 1931 and 2016: **A)** percentage of reporting farmland under crops, **B)** percentage of reporting farmland considered unimproved (natural pasture or prairie, woodland, and wetland), **C)** percentage of reported crops as N-producing crops (varieties of peas, beans, and alfalfa/clover), **D)** number of cattle (10,000s), **E)** number of pigs (10,000s), and **F)** human population (10,000s)

Spatial patterns of contemporary land use derived from the 2016 agriculture census indicate that a large proportion of the livestock in the RRV is concentrated in the Rat/Tourond sub-basin, particularly pigs (Figure 2.4A, B). Yates et al. (2012) also reported that swine operations in several sub-catchments in the Rat/Tourond basin accounted for elevated annual areal mass nutrient production (5 times the median reported in that study). Much of the human population in the RRV falls within the Seine and Lower Red basins, largely due to portions of the City of Winnipeg within these basins (Figure 2.4C). Of the total area with commercial fertilizer applied, the highest

proportions are in the Upper Red (21%) and Morris (27%) sub-basins on the southwestern side of the RRV (Figure 2.4D). Fertilizer application patterns are proportional to the amount of croppable land cover: greater on the west side of the RRV with the Upper Red, Morris, and La Salle sub-basins each under 92%, 90%, and 85% cropland and receiving 21%, 27% and 15%, respectively, of the total fertilizer applied. Much of the remaining forest cover (66%) is on the east side of the RRV in the Roseau, Rat/Tourond, and Seine sub-basins, which have the lowest reported fertilizer application (4%, 11%, and 4% of total applied fertilizer, respectively).

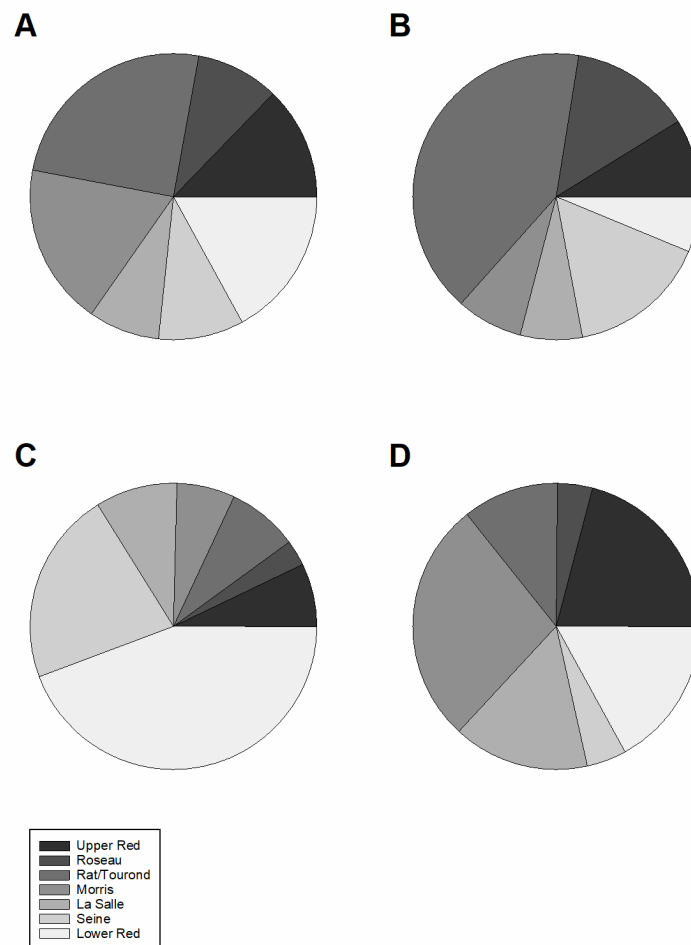


Figure 2.4 Proportion of total in the major sub-basins of the Red River Valley, Manitoba, Canada: A) cattle, B) pigs, C) human population, and D) applied commercial fertilizer (based on 2016 census data from Statistics Canada)

2.2.3 Climate and Hydrology of the Red River Valley

The RRV experiences a cold, continental climate typical of the Canadian Prairies. Average annual temperature in the RRV is 4 °C and the region receives approximately 500 mm of precipitation annually, with greater precipitation occurring above the Manitoba escarpment on its western edge, and drier conditions below (Mahmood et al., 2017). Most precipitation in the region falls as rain from April to October (~75%) with lesser amounts occurring as snow (~25%) in the winter months (Dibike et al., 2012; Mahmood et al., 2017). A multi-decadal wet spell since the early 1990s has seen summer precipitation in the RRV increase 20% (Dibike et al. 2012; Ehsanzadeh et al., 2012). Temperatures and temperature extremes, especially in winter, have also increased (Asong et al., 2016; Dibike et al., 2012). Future climate scenarios for the RRV region, predict temperature and precipitation will continue to increase over the next century (Asong et al., 2016; Dibike et al., 2012). However, the magnitude of that increase depends on greenhouse gas (GHG) concentrations in the atmosphere with stronger responses predicted with greater radiative forcing (Asong et al., 2016). Regardless of GHG scenario, summer maximum and winter minimum temperatures are predicted to continue to warm under all scenarios while winter precipitation is expected to decrease and summer precipitation will increase (Asong et al., 2016; Dibike et al., 2012). Asong et al. (2016) also predicted an increase in consecutive wet days and mean wet spell lengths under all GHG scenarios. This finding combined with the prediction that annual precipitation will increase suggests that precipitation events in the RRV will become more intense in the future.

The hydrologic regime of the RRV is dominated by spring snowmelt, with more than 80% of annual surface runoff associated with snowmelt despite snowfall accounting for approximately 25% of total annual precipitation (Dibike et al., 2012; Shrestha et al., 2012; Mahmood et al., 2017). Using the Soil and Water Assessment Tool, Shrestha et al. (2012) simulated future climate induced hydrologic changes for the Morris sub-basin and concluded that future changes in snowmelt driven runoff will be characterized by earlier onset of spring snowmelt and peak discharges accompanied by overall increases in runoff volume. Mahmood et al. (2017) found that a larger proportion of heavy rainfall days in

summer resulted in extreme rainfall runoff volumes, even following a dry winter. The findings of these studies combined with the projection of drier winters, wetter summers, and more intense precipitation events over the next century suggest a possible shift towards summer flooding in the RRV, potentially offsetting declining snowpack volumes.

Historically, the RRV has always been prone to flooding because of its low topographic relief and poor infiltration capacity owing to its clay soils (Brooks, 2017). Much of the RRV falls within the Prairie Pothole Region, an area naturally rich in embedded wetlands. Embedded “potholes” provide a great deal of hydrological storage, and when not full, act as isolated and disconnected storage units. However, when hydrologic storage capacity is reached, potholes “spill”, essentially increasing the contributing area of the basin (Shaw et al., 2012; Werner et al., 2013). This expansion of watershed contributing area is known to amplify runoff production such that even moderate changes in precipitation can trigger depression storage thresholds and the generation of disproportionate runoff (Ehsanzadeh et al., 2012; Shook et al. 2013). More recently, Ali and English (2019) determined that temporal variability in the hydrologic connectivity of headwater regions in the Lake Winnipeg watershed and the RRV were key drivers of algal bloom development on Lake Winnipeg. These findings indicate that depression storage thresholds and intermittent hydrologic connectivity likely play a key role in governing hydrologic dynamics in the RRV (Ehsanzadeh et al., 2012; Schindler et al., 2012). Moreover, reduction in depression storage due to historical landscape modification has likely reduced hydrologic storage capacity in the RRV. Combined with observed trends of past and increasing precipitation in the RRV (e.g., Asong et al., 2016), climatic variables and landscape modification are likely triggers of the disproportionately high runoff observed in the Red River basin over that past several decades.

2.2.4 Water Chemistry

2.2.4.1 Nutrients

Over the past several decades, nutrients (N and P) in RRV tributaries have increased. Trend analysis of flow-adjusted concentrations revealed significant increases

in total N (TN) in four and increases in total P (TP) in six of seven RRV tributaries from the mid-1970's to 1999 (Jones & Armstrong, 2001). Most notable were increases in the La Salle (146% TN and 194% TP), Seine (75% TN and 188% TP), Marsh (Rat/Tourond sub-basin, 114% TN, 66% TP), and Roseau (45% for both nutrients) rivers. Significant increases in TP, but not TN, were also observed in the Boyne (Morris sub-basin) and Rat rivers. While the increase in P concentrations over time appears to be pervasive across the RRV, in-stream nutrient concentrations are generally always greater on the west side of the RRV (e.g., Corriveau et al., 2013; Yates et al., 2018), likely reflecting the influence of greater forest cover and granitic Canadian Shield in the headwaters of the eastern tributaries.

Major nutrient sources to RRV tributaries include diffuse sources, such as inputs from croplands (synthetic fertilizer, crop residue) and livestock operations, and point sources, such as wastewater effluent (Bourne et al., 2002; Pip, 2005; Yates et al., 2013). Analysis of stream nutrient loads for major tributaries within the Manitoba Red River Basin indicated that 59% of the TN and 73% of the TP load was derived from diffuse sources (Bourne et al., 2002; Jones & Armstrong, 2001). Moreover, of these diffuse inputs, 70% of the TN and 86% of the TP diffuse loads were derived from cropland (Bourne et al., 2002). In the RRV, delivery of land-based nutrients to tributaries is strongly connected to the snowmelt driven hydrologic regime (Corriveau et al., 2013; Rattan et al., 2017). For example, 62% of the annual TP and 67% of the annual TN loads were found to be delivered during snowmelt for 11 tributaries in the La Salle, Morris, and Upper Red sub-basins (Rattan et al., 2017). Similarly, Corriveau et al. (2013) reported 29-92% of the annual TP and 42-81% of the annual TN loads were delivered during snowmelt in tributaries in the La Salle, Morris and Seine sub-basins. Application of a load apportionment model to eight RRV watersheds showed that diffuse sources accounted for 70-100% of the annual P load. However, point sources may contribute more P in summer when wastewater lagoons are allowed to discharge (Rattan et al., in press).

Much of the nutrient load entering tributaries is in the dissolved form (Schindler et al., 2012; Corriveau et al., 2013). For example, Corriveau et al. (2013) found the

dissolved fraction of P was high in tributaries in the agriculturally rich La Salle and Morris sub-basins (> 78% and 71%, respectively) and less, but still high, in the more forested Seine sub-basin (< 62%). Corriveau et al. (2013) also reported that dissolved N comprised greater than 88% of TN in the Morris tributary. These findings indicate that bioavailable dissolved forms of both P and N are being delivered to RRV tributaries in a large pulse during snowmelt, with greater fractions coming from the heavily cropped west side of the RRV. Common erosion control practices, such as conservation tillage (e.g., Tiessen et al., 2010) and cover crops (e.g., Liu et al., 2014), are not effective in reducing soluble P export in the region and in some cases have been observed to increase the dissolved fraction of P exported (Flaten, 2016). Schindler et al. (2012) suggested that flood water remaining in contact with heavily fertilized agricultural soils for extended time periods during high spring runoff events is a likely source of dissolved nutrients to the Red River. Indeed, laboratory testing of southern Manitoba soils subject to induced flooding conditions resulted in increases in dissolved reactive P in overlying water in both unamended and manure-amended treatments (Amarawansa et al., 2015)

Point sources represent a smaller proportion of the TN and TP loads in tributaries draining the Manitoba Red River Basin, comprising 41% of the TN and 27% of the TP load (Jones & Armstrong, 2001; Bourne et al., 2002). However, point source contributions of P have the potential to contribute a large proportion of the P in individual watersheds: for example Rattan et al. (in press) found that a fugitive point-source, likely septic or manure storage leakage, contributed 74% of the 2014 snowmelt TP load in a sub-watershed of the La Salle River. The most prevalent point source of nutrients to tributaries in the RRV is wastewater lagoons. Approximately 200 small communities and religious colonies in southern Manitoba are serviced by public or private wastewater lagoons (LWSB, 2006), more than 80 of which are known to discharge directly into the Red River or its tributaries (Bourne et al., 2002).

Wastewater is rich in dissolved inorganic N and P (Carey & Migliaccio, 2009). Although lagoons reduce nutrient concentrations in proportions similar to secondary

sewage treatment, more than one third of influent N and P may not be removed and thus released to tributaries (Chambers et al., 2001). Nutrient loads from RRV lagoons are difficult to quantify because those servicing smaller communities (pop. <2000) are not required to monitor N and P (Manitoba Water Stewardship, 2011), and they discharge for only short periods of time (usually \leq two weeks per discharge event) during the ice-free season (Bourne et al., 2002; LWSB, 2006). Rattan et al. (2017) observed summer peaks of >2.0 mg/L TP and >5.0 mg/L TN associated with wastewater effluent discharge into Deadhorse Creek, a tributary in the Upper Red sub-basin receiving wastewater effluent from three communities. Concentrations of such magnitude, especially TP, sometimes exceed those measured during snowmelt, the period of highest loading in the RRV. Moreover, application of Partial Least Squares regression showed that for 11 sub-watersheds in the RRV to the west of the Red River, human activity variables (notably sewage discharge but also livestock density and P fertilizer) influenced TN and, to a less extent, TP concentrations (Rattan et al., 2017). From analysis of nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ data, Soto et al. (2019) concluded that nitrate in the Red River originates in nearly equal proportions from inorganic fertilizers and waste sources (i.e., manure and wastewater discharge). Collectively, these findings suggest that under certain situations (e.g., wet vs. dry year, higher human population densities), wastewater treatment (WWT) inputs may represent a sizable contribution to stream nutrient loads in the RRV during the open water period.

2.2.4.2 Sediment

Much of the detailed research on stream sediments in the RRV has focused on South Tobacco Creek in the Morris sub-basin. Sediment fingerprinting conducted using radionuclide and geochemical tracers showed that suspended sediment in South Tobacco Creek was derived from erosion of topsoil from agricultural fields in the case of the upstream reaches compared to streambank erosion in mid- and lower reaches (Koiter et al., 2013). Liu et al. (2015) estimated that these two sources accounted for 40 and 60%, respectively, of the sediment yield in the South Tobacco Creek watershed. Model simulations indicated that sediment yield at the outlet of South Tobacco Creek has a strong linear relationship with runoff suggesting that climate is a strong driver of

sediment transport at the watershed scale (Liu et al., 2015). This relationship did not, however, exist at the local scale, suggesting that multiple factors contribute to sediment transport in the RRV at the reach scale (Liu et al., 2015). Although evidence in the literature is lacking, there is anecdotal evidence of bank instability and erosion due to livestock trampling, unbuffered cropland, and man-made structures. For example, a conservation district report for the 1140 km² Boyne River watershed in the Morris sub-basin identified more than 120 sites in need of riparian rehabilitation largely due to either lack of a protective buffer or bank erosion due to livestock access to streams (Graveline & Larter, 2007).

2.2.4.3 Contaminants

Streams are the most vulnerable waterbody type to anthropogenic contamination in Manitoba (Pip, 2005). Classes of contaminants found in RRV tributaries include agrochemicals (e.g., pesticides), pharmaceuticals and personal care products (PPCPs) associated with human wastewater effluent, and pharmaceuticals associated with livestock production (Carlson et al., 2013; Izral, 2016). Carlson et al. (2013) observed elevated concentrations of human-use pharmaceuticals in Deadhorse Creek (in the Upper Red sub-basin) at sites downstream of the communities of Morden and Winkler (populations approx. 8,000 and 12,000 respectively) following lagoon discharge events. By comparison, agrochemicals were detected at similar concentrations both upstream and downstream of the lagoon outfalls. Notably, the agricultural pesticide diazinon was present at levels exceeding guidelines for aquatic life in the stream, but also in the lagoon, suggesting chemical drift during agricultural application or residential use. Diazinon, as well as the human pharmaceuticals erythromycin and clarithromycin, had hazard quotients greater than one, but in the case of the human pharmaceuticals, these values decreased following effluent discharge. A similar study reported 15 PPCP compounds in Deadhorse Creek during wastewater lagoon discharge compared to only one in an agriculturally dominated stream (erythromycin) and no PPCPs (of 58 tested) in a reference stream located on the outer periphery of the RRV (Izral, 2016). Although most compounds decreased in concentration after effluent release, the anti-convulsant carbamazepine (a known persistent compound; Ebele et al., 2017) remained constant

while two agricultural compounds, carbadox (a swine growth promoter/antibiotic) and thiabendazole (a fungicide), increased post-discharge. Metabolomic changes to gill and tail tissue were also observed in crayfish collected from Deadhorse Creek as part of a reciprocal transfer experiment among the three stream sites (Izral, 2016). Moreover, metabolites in crayfish tail muscle (e.g., amino acids) persisted even after transfer of crayfish from Deadhorse Creek to the reference site. These findings suggest that while human-use PPCPs are delivered to RRV streams in short pulses and likely return to lower concentrations post-lagoon discharge, their effects may persist in biota. Agrochemicals and livestock pharmaceuticals associated with manure may be delivered to streams over longer periods and/or more frequently.

2.2.5 Ecological status of the Red River Valley tributaries

2.2.5.1 Ecological communities

Aquatic macrophytes were collected at 28 sites across the RRV in 2010 and 2011 (Tyrell, 2015). Of the 43 genera identified, the most common were hardy, nutrient-tolerant, generalists including the arrowhead *Sagittaria* (93% of sites) and the sedge *Schoenoplectus* (74% of sites). Other common genera include *Eleocharis*, *Lemna*, *Potamogeton*, and *Sparganium*, which were observed at more than 60% of sites, and *Alisma*, *Bolboscheonis*, and *Typha*, which occurred at more than 50% of sites. We conducted a non-metric multidimensional scaling (NMDS) ordination analysis using the R package *vegan* (Oksanen et al., 2019; R Core Team, 2018) on this macrophyte assemblage data (NMDS not shown, stress >0.2), which showed that macrophyte communities lack the variability required for grouping in ordination space.

Benthic macroinvertebrate data were obtained for 43 reaches from 20 RRV streams sampled during mid-summer between 2010 and 2014 (Yates et al., 2014; Cormier, 2017; Yates et al., 2018). Meta-analysis of data from these 43 sites identified a total of 105 different taxonomic groups of benthic invertebrates, largely described at the family or sub-family level. Chironomids, which commonly dominate in systems characterized by fine sediments (Molineri et al., 2020), such as those found in the RRV, were the predominant taxa with the sub-families Chironominae, Tanypodinae, and

Orthocladiinae occurring at 100, 93 and 86%, respectively, of all sites. Other common taxa present at more than 80% of sites were moderately to highly nutrient tolerant species including mayflies from families Baetidae and Caenidae, riffle beetles (Elmidae), and Oligochaete worms. Other commonly occurring taxa include amphipods (Hyalellidae, 74%), snails (Physidae, 67%), fingernail clams (Sphaeridae, 67%), water boatmen (Corixidae, 61%), and Heptageniid mayflies (54%). These common taxa spanned habitat preferences and included those more typical of wetland environments (e.g. Hyalellidae, Corixidae) as well as lotic taxa (e.g. Baetidae, Elmidae) (Yates et al. 2014; Yates et al. 2018).

An NMDS ordination analysis performed on the invertebrate data (stress=0.17) showed no spatial pattern in benthic macroinvertebrate (BMI) assemblages across the RRV (Figure 2.5). The lack of association among sites within sub-basins or between the East and West sides of the RRV suggests that groupings in ordination space are likely due to site-specific features, rather than land use patterns. For example, at 10 of the sites included in our ordination, Yates et al. (2018) found distinct differences in BMI assemblages between hydrogeomorphic zones in Tobacco Creek (Morris sub-basin), indicating that site-specific differences are more important than those at the basin level. This finding is consistent with the observed dominance of species from multiple habitat types as listed above.

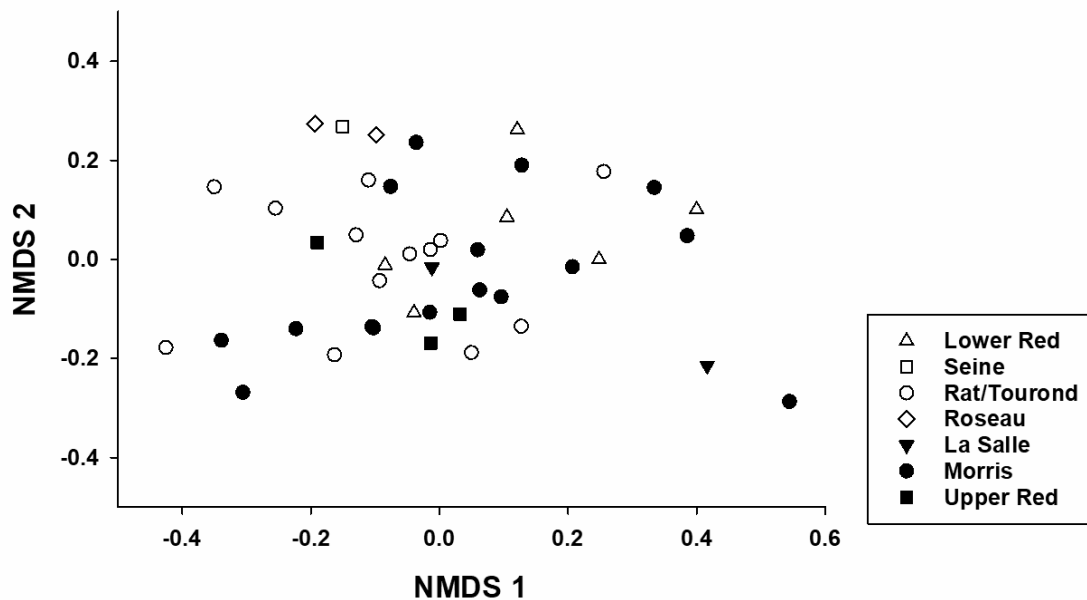


Figure 2.5 Non-metric multidimensional scaling (NMDS) ordination analysis of benthic macroinvertebrate assemblages collected at 43 sites across the Red River Valley (RRV), Manitoba, Canada (stress value = 0.17). Open symbols indicate sites from basins on the East side of the RRV and closed symbols indicate sites from the West side of the Red River Valley.

An inventory of the fish community and assessment of habitat in streams and constructed drains of southern Manitoba was undertaken from 2002-2006 (Milani, 2013). A total of 37 species were reported at approximately 320 sites sampled across the RRV, however seven tolerant species accounted for 85% of fish collected: fathead minnow (*Pimephales promelas*; 45%), brook stickleback (*Culaea inconstans*; 19.4%), white sucker (*Catostomus commersonii*; 8.9%), black bullhead (*Ameiurus melas*; 5%), central mudminnow (*Umbra limi*; 3.7%), northern redbelly dace (*Chrosomus eos*; 3.2%), and common carp (*Cyprinus carpio*; 3.1%). By sub-basin, total richness was greatest in the Seine (30 species), followed by 25 species in the Morris, 18 in the Upper Red, 17 in the Rat/Tourond, 15 in the La Salle, 13 in the Lower Red, and 12 in the Roseau. There was little variation in the fish community across the sub-basins with the above-listed tolerant species comprising at least 75% of total fish collected in each sub-basin, apart from the more species-rich Seine where these species made up 55%. Average habitat assessment

scores (0-60 = poor, 61-120 = marginal, 121-180 = sub-optimal, and 181-240 = optimal; from Milani, 2013) for the sub-basins of the RRV also exhibit limited variation and range from marginal (La Salle = 102, Lower Red = 109, Upper Red = 117, Roseau = 118, Morris = 119) to sub-optimal (Rat/Tourond = 123, Seine = 125). Some conservation districts (Seine and La Salle) have reported greater numbers of fish species, including some species of concern (bigmouth buffalo [*Ictiobus cyprinellus*], silver chub [*Macrhybopsis storeriana*], chestnut lamprey [*Ichthyomyzon castaneus*]), during high flow years when migration can occur into the lower reaches of rivers from the Red River (LaSalle Redboine Conservation District, 2007; Seine-Rat River Conservation District, 2019). However, most of the streams and drains that make up the smaller tributaries across the RRV sub-basins are classified as “simple” habitat: a linear channel with a trapezoidal cross section, fine uniform substrate and grassed banks (Milani, 2013). These channels are subject to low flows as the ice-free season progresses; therefore, most species are limited to those that can survive such poor habitat conditions.

Relatively few aquatic invasive species have been collected in Manitoba (n = 15). Of these, zebra (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*), spiny water flea (*Bythotrephes longimanus*), rusty crayfish (*Faxonius rusticus*), and common carp are of particular concern. Much of the focus on prevention and control is aimed at the recent zebra mussel invasion. Zebra mussels have the potential to severely impact aquatic ecosystems and man-made infrastructure and have resisted eradication efforts in Lake Winnipeg (Gingera et al., 2017). Zebra mussels are established in both the south and north basins of Lake Winnipeg (Depew et al., in press) and the Red River (Manitoba Sustainable Development, 2019a). However, zebra mussel advancement throughout RRV waterways is likely limited due to the fine substrate common in RRV streams. Carp, which have potential to impair water quality and benthic habitat through foraging behaviour (Millar & Crowl, 2006), have been reported in the upper reaches of the La Salle sub-basin (LaSalle Redboine Conservation District, 2007) and the Morris sub-basin (Manitoba Sustainable Development, 2019b) and are likely present throughout the RRV (Invasive Species Council of Manitoba, 2019).

2.2.5.2 Stream ecological function

Stream metabolism, the collective metabolic activity of organisms within a stream reach, is a measure of the synthesis of organic matter (i.e., gross primary production, GPP) versus the consumption of organic matter (i.e., ecosystem respiration, ER). Stream metabolism is associated with the cycling of energy and nutrients through stream ecosystems (Mulholland et al., 2001) and is therefore a useful indicator of stream ecosystem condition (Von Schiller et al., 2017). Stream metabolism responds to human impacts in watersheds (Clapcott et al., 2016) and increased GPP and ER values have been linked to WWT discharge (Gücker et al., 2006) and increasing intensity of human activities in stream catchments (Yates et al., 2013). Stream metabolism was measured in 23 tributaries throughout the RRV (Figure 2.6) during late summer 2010 (Yates et al., 2013; Yates et al., 2018) and 2014 (Chesworth, 2016). Stream metabolism was greater in the western compared to the eastern sub-basins of the RRV (Fig. 6), with the largest mean GPP and ER values occurring in the La Salle (GPP=8.20, ER=10.50), Upper Red (GP=7.49, ER=9.22) and Morris (GPP=5.89, ER=8.99) sub-basins. On the East side, GPP and ER are greatest in the Lower Red sub-basin (GPP=4.56, ER=5.13), followed by the Rat/Tourond (GPP=3.81, ER=6.63) and Roseau (GPP=2.33, ER=4.87) sub-basins.

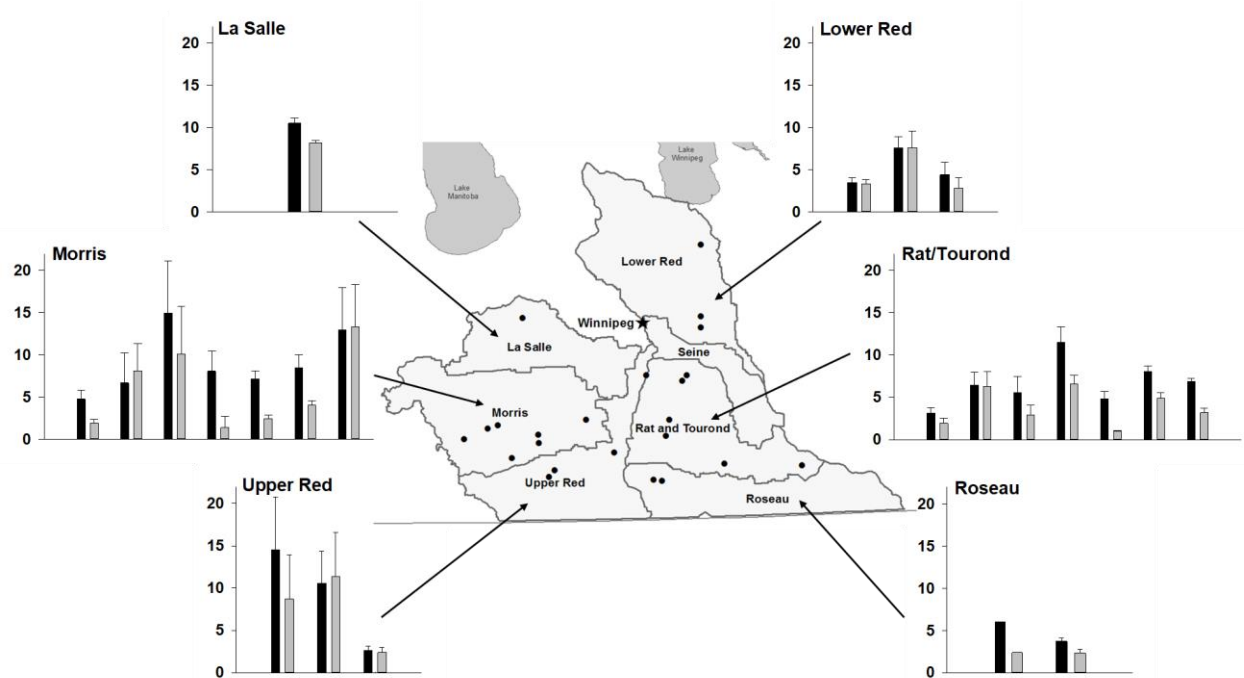


Figure 2.6 Ecosystem respiration (black bars) and gross primary production (grey bars) values ($\text{g O}_2/\text{m}^3/\text{day}$) calculated for streams throughout the Red River Valley, Manitoba, Canada (Yates et al., 2013; Chesworth, 2016; Yates et al., 2018). Site locations within each basin are shown on the map (black points).

Denitrification, the reduction of bioavailable nitrate to nitrogen gas, can serve as an important mechanism for nitrate removal in aquatic systems. Moreover, nitrate saturation (i.e., where nitrate concentrations exceed the denitrification capacity of microbes) can be an indicator of ecological status whereby frequent saturation is associated with poor ecological status (Bernot & Dodds, 2005; Gooding & Baulch, 2017). Analysis of denitrification activity in the South Tobacco Creek watershed (Morris sub-basin) showed higher denitrification activity in man-made reservoirs than natural stream pools (Gooding & Baulch, 2017). Nitrate saturation in stream pools was especially apparent in July when water temperatures were greatest and stream metabolic activity was likely at its height. Across the eight reservoirs and seven stream pools included in their study, Gooding & Baulch (2017) reported a concentration of $0.68 \text{ mg/L NO}_3+\text{NO}_2\text{-N}$ as the threshold above which denitrification activity becomes saturated. Applying this value to other RRV streams with nitrate concentration data reveals that nitrate saturation

is likely to have occurred during episodes of lagoon effluent discharge. For example, during lagoon discharges in summer 2014, $\text{NO}_3+\text{NO}_2\text{-N}$ concentrations of 1.05 and 3.42 mg/L were reported for Devil's Creek (Lower Red sub-basin; Chesworth, 2016) and Dead Horse Creek (Upper Red sub-basin; Izral, 2016), respectively. $\text{NO}_3+\text{NO}_2\text{-N}$ concentrations exceeding the threshold value were also observed in streams with low WWT in their watersheds during the snowmelt period (max 3.68 mg/L) of 2013 and 2014 (Rattan et al., 2019), an important finding considering denitrification activity is likely saturated at even lower concentrations during early spring when cool temperatures limit microbial activity.

2.3 Ecological Causal Assessment

2.3.1 Description of the Ecological Causal Assessment framework

Our review of the status of RRV watersheds indicates that ecological effects associated with agriculture and human settlements are likely occurring in the region. However, the potential causes of these effects are many, and those requiring the most urgent management interventions are unclear. An ecological causal assessment framework (Cormier et al., 2010; Norton et al., 2015; US EPA, 2017) can be used to evaluate and better link the observed environmental status of a region to potential ecological effects. Causal assessment involves the identification of the causes or proximate causes of ecological effects that are best supported by available scientific evidence (Cormier et al., 2010). Following the terms defined in Cormier et al. (2010), 'ecological effects' are changes in entities of interest and 'proximate causes' are causal agents that interact directly with ecological entities to cause change. For example, causal agents such as nutrients interact directly with ecological entities like algae or invertebrates to cause change evidenced by increased algal growth or change in a biological diversity. In addition to establishing causal linkages, equally important are the identification of knowledge gaps from causal assessment outcomes, which can inform future research priorities and provide useful direction to managers.

The key component of causal assessment is the derivation and weighting of the evidence or knowledge that informs causation (Cormier et al., 2010). Because causal

assessment is qualitative, evidence weighting must be explicit and transparent, and justification is required for the inclusion and evaluation of each piece of evidence. By being explicit in defining the bounds and components of this process, assessor subjectivity and bias is reduced (Norton et al., 2015). First, a minimum standard of evidence relevance (e.g., organism or ecosystem specificity) and quality (e.g., standard of data presented) must be defined for inclusion in the causal assessment. Then, each piece of information is critically evaluated and scored based on evidence of causal characteristics. These causal characteristics, often referred to as evidence types, are described in detail in Cormier et al. (2010), Norton et al. (2015), and the US EPA (2017). A supporting body of evidence from outside the case geographic domain may also be included as an evidence type because supporting evidence from outside the case can add weight to the causal assessment when within-case evidence is limited (Norton et al., 2015); this approach is commonly used by causal assessment practitioners (e.g., Haake et al., 2010). The strength of each evidence type linking causes to ecological effects is then scored using a system of symbols per Norton et al. (2015) and US EPA (2017) (Table 2.1). The use of symbols rather than numbers avoids the implication that weights for different types of evidence should be counted or added.

Table 2.1 Evidence scoring rubric based on Norton et al. (2015) and US EPA (2017) used to describe the strength of evidence between candidate causes and ecological effects in our ecological causal assessment of tributaries in the Red River Valley, Manitoba, Canada

++	Strongly supports	Supporting evidence in ≥ 3 cases from within the region
+	Somewhat supports	Supporting evidence in ≥ 1 case within the region or ≥ 3 cases from outside the region
-	Somewhat weakens	Contrary evidence from ≥ 1 case within the region or ≥ 3 cases outside the region
--	Strongly weakens	Contrary evidence in ≥ 3 cases from within region
NE	No evidence	

2.3.2 Ecological Causal Assessment of Red River Valley tributaries

2.3.2.1 Methods

The overall process of this ecological causal assessment is summarized in Figure 2.7. Because of the regional nature of our causal assessment, for evidence to be relevant we determined that it must be specific to tributaries in the RRV region. However, we also considered evidence from similar systems outside the RRV, but only as supportive evidence (described further below). To be considered of sufficient quality, evidence must have been peer-reviewed or from other high-quality studies (i.e., from a government agency or similar) with clearly defined protocols and known quality assurance/quality control measures, such as from trusted laboratories or professional taxonomists. All evidence considered for the ecological causal assessment of RRV tributaries is listed in detail in Table A1 in Appendix A. It should be noted that studies included as evidence for the causal assessment were limited to those providing evidence of ecological effects and

did not include studies or reports that were more descriptive in nature (e.g., surveys). Thus, it should not be assumed that evidence presented in the causal assessment is the same as that described throughout our preceding review of current or historical tributary conditions (Section 2.2).

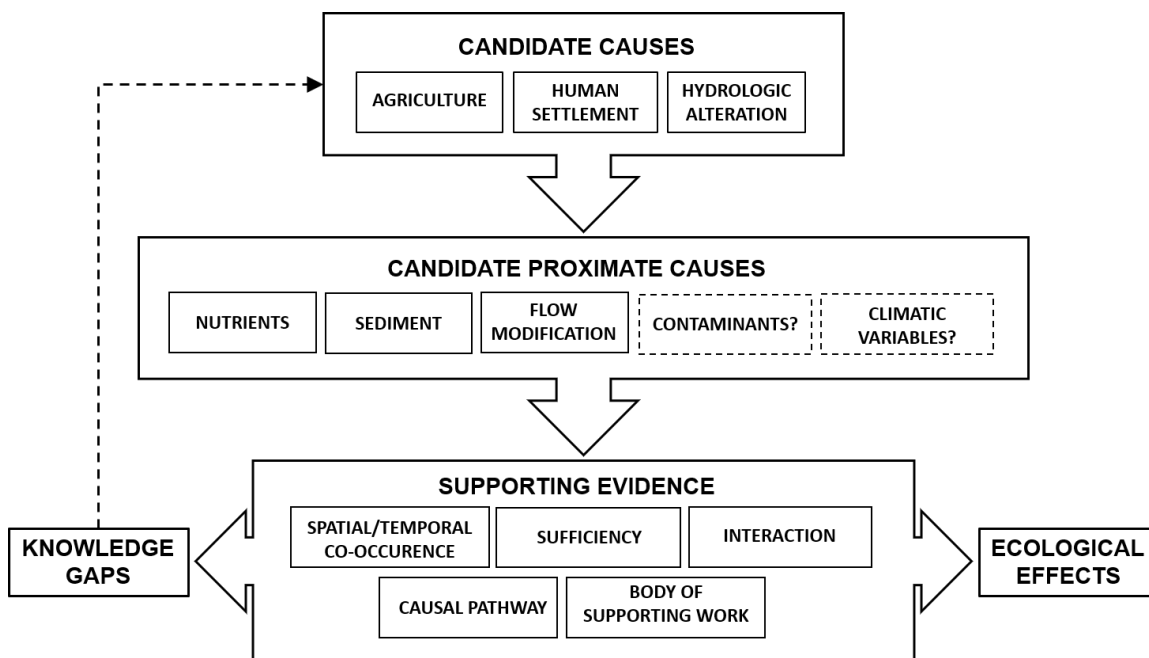


Figure 2.7 The ecological causal assessment process: First, we identified candidate causes of ecological effects from human activities (agriculture, human settlement, and hydrologic alteration) followed by the identification of possible candidate proximate causes (nutrients, sediment, flow modification, contaminants, climatic variables). Those candidate proximate causes for which we could not find applicable evidence are shown in boxes with dashed outlines. We then considered all pieces of relevant evidence (specific and high-quality) for weighting based on set criteria (evidence of co-occurrence, sufficiency, interaction, causal pathway, body of supporting evidence). The key outcomes are identification of evidence-based linkages between causes and ecological effects and the identification of knowledge gaps which inform the need for more evidence.

Using the assessed evidence, candidate causes were then evaluated for evidence of linkage to ecological effects. In the case of candidate causes associated with

agriculture and human settlements, we assessed agricultural land cover (% land cover) and crop production in catchment (from census data), livestock in catchment (livestock densities), urban land use in catchment (% land cover), and municipal WWT (in catchment or direct discharge to waterway, based on human population densities). Altered hydrologic regime is a candidate cause driven by both agricultural and human settlement activities; therefore, it was categorized separately. The two candidate causes for which regionally specific evidence was available for altered hydrologic regime are reservoirs and channelization. Because only candidate causes for which there was evidence of linkage to ecological effects were included in this assessment, we could not assess other hydrologic variables (e.g., subsurface drainage, flow control structures), climatic variables or contaminants.

We selected five evidence types on which to score the strength of the linkages between candidate causes and ecological effects in the RRV region. Four regionally specific evidence types (see Table 2.2) from within the RRV were included: 1) spatial or temporal co-occurrence of cause and effect; 2) sufficiency to cause effect; 3) interaction between cause and entity in which effects are produced; and 4) causal pathway (i.e., a connection can be made between the cause, an environmental condition, and the effect). The fifth evidence type included was the existence of a body of supporting evidence from similar cases outside the RRV region. To be included, this outside evidence was required to be from similar ecosystems (i.e., prairie streams) or scenarios (e.g., agricultural watersheds or downstream from WWT). Because our assessment was regional in nature and was not triggered by a specific event, we did not include preceding causation and time order as evidence types as has been done in other studies (*sensu* Norton et al., 2015).

Table 2.2 Definition of terms used to describe within-region evidence types used in our ecological causal assessment of the Red River Valley, Manitoba, Canada.

Definitions are adapted from US EPA (2017) and Cormier et al. (2010).

Term	Definition	Example
Spatial/temporal co-occurrence	The cause co-occurs in space or time with the entity	Where nutrient concentrations are elevated, algal growth is also observed to increase and fish community abundance is observed to decline
Sufficiency	Intensity, frequency, and/or duration of the cause are adequate to produce the observed effect	Nutrient concentrations are elevated for a sufficient time period and/or concentration to cause algal growth to increase
Interaction	The cause physically interacts with the entity in such a way that induces the effect	There is evidence of uptake of nutrients into algae; fish exhibit metabolic stress typical of low oxygen conditions
Causal pathway	Evidence of steps in the pathway(s) linking a cause and effect	Elevated nutrient concentrations lead to algal overgrowth which in turn causes low dissolved oxygen conditions which impair fish communities

For the purpose of this regional causal assessment, we first scored each piece of evidence separately (see Table A1 for individual scores). Evidence in support of a causal relationship between a candidate cause and effect was scored with a “+” while evidence that did not support a causal linkage was scored with a “-“ or, in the case of no evidence, an “NE”. Stronger causal linkages were given greater weight (denoted by “+ +” or “- -“). Then, individual cases were synthesized and categorized by ecological effect for final scoring (Table 2.1). For example, if there were three pieces of evidence supporting a causal relationship between a candidate proximate cause and an effect, each of the three pieces of evidence received an initial score. If these pieces of supporting evidence were of the same type (i.e., spatial/temporal co-occurrence), their synthesized score would be stronger, a “+ +”, for that single, shared evidence type category. If these supporting pieces of evidence were of three different evidence types, each would be scored with a single “+” under their respective category and would not contribute to a stronger weight.

In the case of supporting evidence from outside the RRV, because evidence was not regionally specific, we required there to be at least three supporting studies to denote a single “+” score.

2.3.2.2 Results

Causal assessment to determine candidate causes of degraded ecological condition of tributaries in the RRV, Manitoba, Canada identified linkages between human activity and ecological effects (Table 2.3). The most common ecological effects linked to human activity and its proximate drivers were alterations to benthic community composition, increased primary production (i.e., elevated GPP and algal abundance), and enrichment of ^{15}N in multiple endpoints (macrophytes, particulate organic matter, invertebrates). All effects spatially or temporally co-occurred with agriculture, human settlements, and hydrologic alteration and most were supported by evidence from outside of the RRV, but direct evidence (e.g., uptake, sufficiency) of the interaction between entities and their proximate causes along whole or partial causal pathways from within the RRV was lacking.

Table 2.3 Summary table of the ecological causal assessment of Red River Valley, Manitoba, Canada tributaries showing linkages between three candidate causes (and their related candidate proximate causes) and ecological effects as scored (see Table 2.2) according to five evidence types

	Agriculture				Human Settlement					Altered Hydrologic Regime		
	% Ag/Crop in Catchment		Livestock		% Urban in Catchment		Municipal Wastewater Treatment			Reservoirs	Channelized Zones	
	↓%EPT, ↑FBI	↑GPP	↑ $\delta^{15}\text{N}$ arrowhead	↑algal abundance	↑GPP, ER	↑ $\delta^{15}\text{N}$ willow	↑GPP, ER	↑ $\delta^{15}\text{N}$ macrophytes, POM, invertebrates	altered crayfish metabolome	↑denitrification	↑GPP	benthic community shift
Spatial/temporal Co-occurrence	+	+	+	+	+	+	++	+	+	+	+	+
Sufficiency	NE	NE	NE	NE	NE	NE	NE	NE	+	+	NE	NE
Interaction	NE	NE	+	NE	NE	+	NE	+	+	NE	NE	NE
Causal Pathway	NE	NE	NE	NE	NE	NE	+	NE	NE	NE	NE	NE
Body of Evidence	+	+	+	-	+	+	+	+	NE	+	+	+

The candidate cause most strongly supported by evidence in our causal assessment was WWT (Table 2.3). Each of the ecological effects linked to WWT were supported by three evidence types. Increased GPP and ER were supported strongly by evidence of spatial/temporal co-occurrence (Yates et al., 2013; Chesworth, 2016), and somewhat supported by evidence of causal pathway (Chesworth, 2016) and a body of outside evidence (e.g., Gücker et al., 2006; Aristi et al., 2015). Furthermore, the relationship between WWT and increased GPP and ER was the only linkage with three or more supporting cases of a single evidence type (spatial/temporal co-occurrence) making it the only candidate cause scored as “+ +”. Enrichment of ^{15}N was somewhat supported by spatial/temporal co-occurrence, evidence of interaction (Cormier, 2017) and a body of outside evidence (e.g., Anderson & Cabana, 2006; Morrissey et al., 2013). Altered crayfish metabolome was somewhat supported by spatial/temporal co-occurrence, evidence of sufficiency, and evidence of interaction (Izral, 2016). Livestock in catchment and % urban land use in catchment were both linked to an ecological effect (enriched ^{15}N of arrowhead and willow, respectively) supported by three evidence types. However, because $\delta^{15}\text{N}$ from livestock is difficult to disentangle from other waste source signatures (Kendall et al., 2007), and urban land use is likely to co-vary with WWT (Yates et al., 2014), WWT may also be a contributing cause in these cases.

Most of the causal linkages were limited to evidence of spatial or temporal co-occurrence and supporting evidence from outside the region (Table 2.2). Other evidence types (e.g., sufficiency, interaction, causal pathway) from within the RRV were uncommon, especially evidence of complete causal pathways. These clear pathways between causes, their proximate drivers (i.e., candidate proximate causes), and ecological effects were in many cases either suspected, but lacked evidence or were altogether unclear. For example, elevated nutrients are a known driver of stream metabolism (Mulholland et al., 2001; Bernhardt et al., 2018), but we could not directly link nutrients (or other proximate drivers) to GPP and ER because of a lack of studies controlling for the confounding effects of other variables (e.g., light availability, flow regime).

Additionally, altered crayfish metabolome has been linked to WWT (Izral, 2016). However, the causal linkage (i.e., mechanism) describing how this change occurs could be due to any of the myriad stressors associated with WWT (e.g., nutrients, contaminants, oxidative stress, food stress). Likewise, benthic invertebrate communities are known to differ between channelized and meandering zones within RRV streams (Yates et al., 2018), yet the reason for the observed shifts in community structure are not known despite widespread construction of drainage channels throughout the region. Another example of an unclear proximate cause is the weak causal linkage between livestock and algal abundance. This linkage was supported by only one case of spatial co-occurrence in our causal assessment and evidence from outside the case is contradictory, showing a decrease in algal abundance associated with increased sedimentation and/or riparian erosion linked to livestock (Young & Huryn, 1999).

Our causal assessment identified knowledge gaps in which there was limited available evidence to establish linkages between candidate causes and ecological effects in the RRV. For example, the alteration of flow regimes via the construction of small reservoirs serves as a nutrient removal beneficial management practice (BMP) in the RRV (Tiessen et al., 2011). Greater denitrification in these small reservoirs as compared to natural pools (Gooding & Baulch, 2017) is supported by three evidence types, highlighting the potential utility of this BMP to sequester nitrogen. However, the wider ecological effects of reservoir creation are unknown and some studies have shown small reservoirs emit disproportionately large quantities of GHGs (e.g., Gorsky et al., 2019; Peacock et al., 2019). In some cases, potential causes of ecological effects were established, but a lack of regionally specific studies linking those causes to ecological effects prevented their inclusion in our causal assessment. For example, it is known that contaminants are present in RRV streams (Carlson et al., 2013; Challis et al., 2018) and that those contaminants may occur at concentrations that present a threat to aquatic life, particularly during wastewater releases (Carlson et al., 2013). Yet studies assessing the ecological effects of contaminants associated with WWT have not been conducted in the region and, thus, contaminants were excluded from evaluation as a cause in our assessment. Likewise, transport of sediment both from the landscape and through erosion of stream channels is a known source of nutrients and contaminants (Walling et al., 2003)

and can increase turbidity and alter stream metabolic activity (Young & Huyrn, 1999). Even though a significant portion of the suspended sediment in RRV streams is known to be derived from upland soil sources and stream bank erosion (Koiter et al., 2013; Liu et al., 2015), we found no studies establishing ecological effects associated with sediment loading to RRV streams. Underlying these and other potential candidate causes are the effects of a changing climate and hydrological regime (described in the status review above). While these examples are not a complete accounting of potential candidate causes of ecological effects within the RRV region, their listing here exemplifies the need for usable knowledge of ecosystem condition.

2.4 Discussion

Although agriculture, human settlements, and hydrologic alteration are pervasive in the RRV, the candidate proximate cause of ecological effects most strongly supported by available evidence from regional research studies was WWT. In Manitoba, and across the Canadian prairies, wastewater lagoons represent, by number, approximately 95% of the WWT systems (LWSB, 2006; Saskatchewan, 2014). Although these point sources individually contribute small volumes of effluent (because they usually serve populations of less than 15,000), wastewater lagoons number around 200 in the RRV alone (LWSB, 2006). Rattan et al. (2017) estimated that on an annual basis, 3% of the TP and TN loads in streams flowing to the Red River, Manitoba were derived from sewage lagoons, a value similar to the sewage load contributed by the City of Winnipeg. As such, RRV waterways are likely at risk of degraded water quality and impairment of ecological condition due to sewage impacts. For example, Carlson et al. (2013) found that stream nutrient concentrations often exceeded water quality guideline thresholds during lagoon discharge events in Deadhorse Creek, MB. Cormier et al. (in rev.) found that N associated with WWT sources was readily assimilated by biota in RRV streams and was in fact the dominant source of N to organisms in the streams most heavily impacted by WWT. The ecological consequences of WWT discharge is further exacerbated by the fact that streams in the RRV often experience low to no flow as the summer season progresses. Lagoon discharges, which occur later in the year, may release into nearly dry stream channels resulting in lagoon effluent making up much of the total stream volume

(K. J. Painter, personal observation). Alternatively, as observed elsewhere (Jarvie et al., 2012), high flows may result in the overestimation of nonpoint agricultural sources and underestimation of WWT sources.

Despite widespread agricultural activities across the RRV, we observed less evidence for agricultural sources of ecological effects than between WWT and ecological effects. The major sources of nutrient loads to RRV streams have been attributed to diffuse watershed sources, especially crop production (Bourne et al., 2002; Schindler et al., 2012), with most nutrient delivery occurring with spring snowmelt (Corriveau et al., 2013; Rattan et al., 2017). It is possible the lack of evidence connecting agriculture to ecological effects in RRV tributaries is due to the asynchronous timing of nutrient delivery and ecological activity. The major pulse of agriculturally derived nutrients is delivered during snowmelt, yet ecological studies occur in spring or summer when streams are warmer and exhibit increased ecological activity. This temporal incongruence in nutrient supply and stream ecology is not well incorporated in studies of the RRV, nor in studies of prairie streams worldwide. Furthermore, the effects of interannual variation on stream ecology is also lacking in studies of the RRV despite strong year-to-year variability in the hydrologic conditions that influence nutrient delivery to tributaries in cold regions (e.g., Rattan et al., 2019). Thus, more evidence connecting the ecological condition of streams to possible causes of impairment in wet versus dry years, and among seasons, is needed to ensure a complete understanding of how human activities, and agricultural land uses in particular, are influencing RRV streams.

Limited evidence for the link between agriculture and ecological effects could also be an artifact of the difficulty of detecting effects in such a uniform and pervasive agricultural landscape. The spatial homogeneity of the RRV landscape may reduce the suitability of many traditional ecological indicators. For example, a fish community and fish habitat inventory of Manitoba streams reported simplification of habitat throughout the RRV that may have limited many stream fish communities to tolerant species (Milani, 2013). On the other hand, given the intermittent nature of temperate prairie streams governed by snowmelt dominated hydrologic regimes, the fish community in the RRV may have always been dominated by tolerant species. Likewise, a survey of macrophyte

communities along human activity gradients found no spatial patterning of the macrophyte community, likely due to dominance by nutrient-tolerant, generalists (Tyrrell, 2015). As such, it may well be that communities in this region with its naturally fine, nutrient-rich sediments and minimal, if any, light limitation may have always been composed of tolerant species. Although informative of current ecosystem status, these ecological surveys occurred after alteration of regional land cover, hydrology, and climate, and as such, it is unknown how communities responded to historical alterations. The lack of undisturbed reference sites in the region inhibits the application of reference condition approach (*sensu* Stoddard et al., 2006) to address this knowledge gap. There is thus a need for development of a baseline condition against which to assess future change.

An option for enhanced biomonitoring that explicitly considers maintenance of desired ecosystem services in the setting of baseline conditions is the river services assessment framework (Yates et al., 2019). This framework focuses on the evaluation of rivers based on their delivery of ecosystem services (e.g., water purification and transport) and sets ecosystem health targets through an iterative process that considers both societal needs and wants as well as ecological integrity. This approach would be applicable to the RRV where historical baseline conditions are unavailable because the vast majority of rivers are already extensively developed. As such river management in the RRV may be more effectively focused on the maintenance of cultural and economic values and hence biomonitoring is likely to be most informative if it provides information on indicators associated with ecosystem services. Indicators that have shown potential by responding to ecological stressors in the RRV such as stream metabolism (GPP and ER), $\delta^{15}\text{N}$ (e.g., Yates et al., 2014), and denitrification (e.g. Gooding & Baulch, 2017) should be considered. Implementation of a services-based biomonitoring framework would balance the functional needs of both the ecology and the delivery of valued ecosystem services that residents of the RRV rely upon.

Ecological indicators that can better differentiate between agriculture and WWT should be included in a biomonitoring framework. Indeed, the findings of our causal assessment highlight the utility of functional indicators, such as $\delta^{15}\text{N}$ and stream

metabolism, for biomonitoring in the RRV. When source signatures are known, ^{15}N allows for differentiation of N sources to consumers because it becomes enriched in the tissues of organisms (Finlay & Kendall, 2007). N associated with sewage is known to be more enriched isotopically than synthetic fertilizer N, and thus can be used to differentiate agricultural and waste sources (Kendall et al., 2007) and also to trace uptake from these sources into ecosystem compartments (e.g., water, primary producers, consumers). For example, Morrissey et al. (2013) found that ^{15}N of benthic invertebrates was consistently enriched downstream from 11 wastewater treatment plants in the United Kingdom. Furthermore, these data were correlated with several macroinvertebrate community indices used in traditional biomonitoring (e.g., Shannon Diversity, %EPT) as well as effluent discharge volumes. $\delta^{15}\text{N}$ samples are easy to collect as a simple tissue sample is the only requirement. In our causal assessment, $\delta^{15}\text{N}$ features prominently as an indicator of human and livestock waste in the RRV and is particularly useful because enriched ^{15}N provides evidence of direct interaction between sources (livestock, urban land use, WWT) and ecological compartments (riparian vegetation, particulate organic matter, benthic invertebrates). Its use in conjunction with other known tracers of WWT, such as artificial sweeteners (Spoelstra et al., 2013), could be used to differentiate between waste N-sources in the RRV.

Stream metabolism (GPP and ER) is another prominent indicator in our causal assessment. Our findings suggest stream metabolism can differentiate agricultural drivers from WWT. Indeed, elevated GPP was observed in RRV systems dominated by agriculture effects (Yates et al., 2018), whereas both GPP and ER tend to be elevated in urban/WWT influenced systems (Yates et al., 2014; Chesworth, 2016). Elevated ER has been observed in several rivers receiving wastewater (e.g., Aristi et al., 2015; Bernhardt et al., 2018). The positive response of both GPP and ER in RRV streams is likely due to the lack of light limitation on GPP as a result of little or no canopy overhanging prairie streams (Dodds et al., 2004), particularly in the lower reaches where municipal lagoons are often located. For example, Yates et al. (2018) found that increased canopy cover in the upper reaches of two RRV tributaries, the Rat River (Rat/Tourond sub-basin) and Tobacco Creek (Morris sub-basin), was associated with a reduction in GPP compared to sites located in lower reaches, which were closer to WWT. However, a drawback of

using stream metabolism as an indicator in prairie streams is that obtaining high quality data may be limited due to inadequate flow as the season progresses. The fluctuation of stream velocity and water levels during reservoir and wastewater effluent discharges, particularly in drier years, could also potentially make stream metabolism difficult to measure due to its reliance on accurate velocity and depth measurements.

Spatial or temporal co-occurrence of causes and effects was a common finding in our causal assessment. However, direct evidence from within the RRV was mostly lacking for interaction between entities and their proximate causes (e.g., uptake, sufficiency) along whole or partial causal pathways. For example, Yates et al. (2018) conducted a longitudinal study of stream metabolism and benthic communities that indicated that ecological condition differs among stream hydrologic zones, but the proximate drivers of these differences are unknown. Similarly, adoption of agricultural BMPs, such as vegetated buffer strips and conservation tillage, has been encouraged throughout the RRV, but we found no studies linking their implementation to stream ecology, apart from increased denitrification activity in retention ponds (Gooding & Baulch, 2017). Comparative studies before and after BMP implementation are urgently needed to both assess the utility of these practices for nutrient mitigation (Baulch et al., 2019) and determine if there are any associated ecological effects or benefits associated with their implementation. The lack of controlled studies could in part be due to the lack of consistent ecological data collection in the RRV. To our knowledge, there have been no longer-term surveys of biological communities and habitat condition conducted at the regional scale within the RRV. As such, the ability to make connections between ecological status from season-to-season, year-to-year, or before and after implementation of management interventions (e.g., BMPs, WWT upgrades) is limited and has impeded initiation of such studies that could generate stronger causal linkages between proximate causes and ecological effects.

Several of the issues highlighted in our review of the status of the RRV could not be included in our causal assessment due to the lack of studies connecting these drivers to ecological effects. In particular, hydrological connectedness and the effects of channelization and agricultural drainage have received very limited attention. For

example, subsurface drainage (tile drainage) is becoming increasingly prevalent in the RRV and across the prairies despite potential for high nutrient export (Baulch et al., 2019), yet associated effects on tributary water quality and ecology are not known. Similarly, climatic changes affecting the hydrology of the region (McCullough et al., 2012) have thus far not been linked to ecological condition of the RRV. This lack of connection between climatic variables and ecological effects is an artifact of infrequent (or simply non-existent) ecological data collection. It is known that hydrologic changes from season-to-season affect nutrient delivery to streams (e.g., Rattan et al., 2019); thus variability in ecological response under differing climatic conditions is likely. A consistent long-term biomonitoring program would allow for the detection of temporal change and better inform management decisions in response to changing climatic conditions.

Our causal analysis points to specific management actions that could improve ecological condition of streams in the RRV. Specifically, wastewater lagoons could be managed to both improve ecological outcomes in the streams into which they discharge and reduce overall nutrient loads to Lake Winnipeg. For example, the most notable reduction in nutrients entering Lake Winnipeg since 2012 is due to decommissioning of a single lagoon in the small community (pop. ~ 4000) of Niverville, MB (ECCC, 2017). The sizes, locations and discharge points of municipal wastewater lagoons are well-documented because they require government permits to operate and those facilities discharging effluent from lagoons serving greater than 2000 people (or equivalent) are required to comply with testing of nutrients, bacteria, and biological oxygen demand. Thus, the availability of these data records makes them more easily and readily managed than diffuse sources related to other activities, such as agriculture. The technology to improve treatment of wastewater is also readily available for implementation; the factor limiting its adoption is typically economic and not technological. Canada and Manitoba recently invested in upgrades at 24 sewage treatment facilities through the Clean Water and Waste Water Fund (Canada, 2017). These upgrades represent an opportunity to improve knowledge about the ecological response of streams and rivers to effluent through the monitoring of discharges before and after improvements. This ecological

information could be used throughout the RRV to optimize the waste assimilation ecosystem service that streams provide in conjunction with effluent improvements.

2.4.1 Conclusion

The findings of the ecological causal assessment of RRV streams highlight, first, a need for management of those stressors for which we currently have evidence and are effectively able to address in the short-term (e.g., this causal assessment has identified WWT as a candidate proximate cause of ecological effects well-supported by evidence). Second, there is a need for consistent and targeted biomonitoring using region and stressor-specific indicators to better detect major stressors (i.e., agriculture) that are presently not well perceived. Third, more research is required to link hydrology, climate, and BMP implementation to the ecology of the RRV. Although diffuse nutrient sources, hydrology, and climate were prominent features of our status assessment, evidence linking these potential causes to ecological effects was lacking. As knowledge gaps are filled and management interventions are put into place, policy-makers can use the ecological causal assessment framework as an evidence-based policy tool to identify new gaps and create well-posed research questions and study designs. Adaptive management directed by evidence-based policy can protect the ecosystem services provided by the tributaries of the RRV from further ecological and economic degradation by triggering actionable short-term interventions (e.g., improve WWT), while simultaneously gathering data required to inform longer term management strategies (e.g., assessing the effectiveness of BMPs). Sustained, targeted research aimed at understanding ecological conditions and associated human drivers in the RRV has been underway for the better part of a decade. However, a targeted synthesis of this evidence was lacking until now. The findings presented here can be used to establish the concise and clearly targeted policy decisions needed to protect rivers in the RRV from the effects of human activities.

Protecting surface water quality and ecological condition in the RRV requires a holistic approach that considers not only the downstream receiving environment (e.g., Lake Winnipeg), but also the upstream ecosystem health and services. However, the concept of managing river health and services may be difficult to prioritize when the long-time goal within the region has been to “Save the Lake” and there is a mismatch

between periods of stream connectivity and degradation of lake water quality. Indeed, headwater streams in cold, prairie regions like the RRV are often intermittently connected to their own lower reaches (Dodds et al., 2004), let alone larger downstream systems. Furthermore, given the short-term nature of most ecological studies, observations in a given year or season might not be the same in the next when hydrological conditions change. However, when hydrologically connected, it has been shown that RRV streams act as gatekeepers, controlling delivery of nutrients from watershed sources to downstream waterbodies (i.e., Red River and Lake Winnipeg) and thereby dictating the timing and magnitude of downstream impacts, such as major algal bloom formation (Ali & English, 2019). Maintaining river health in the RRV tributaries is thus doubly critical as the services provided by ecological functions are not only essential to maintenance of economic and social well-being in the catchments of the RRV, but also are likely important to the protection of economically and culturally significant waterbodies downstream such as Lake Winnipeg. Indeed, the International Joint Commission recently proposed nutrient water quality objectives for the Red River at the Canada-USA border with the goal of restoring and protecting aquatic ecosystem health and water uses in the Red River watershed as well as Lake Winnipeg (IJC, 2019). Our findings thus support the increasing recognition by stakeholders within the RRV of the need to maintain stream health and ecosystem services for the benefit of both local communities and landowners (e.g., La Salle River Watershed Planning Authority, 2010) as well as protect the health of the downstream waterbody, Lake Winnipeg.

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Chapter 3

3 Contribution of nitrogen sources to streams in mixed-use catchments varies seasonally in a cold temperate region

3.1 Introduction

Human activities have contributed to increased nitrogen (N) transport from land to waterways worldwide (Vitousek et al., 1997; Galloway et al., 2008). The subsequent eutrophication of downstream waterbodies degrades water quality, disrupts food webs, threatens water supplies, and impairs economic and recreational use (Smith et al., 1999). Sources of N vary with the intensity of N-producing human activities in watersheds. For example, in agricultural areas mineral fertilizers and livestock manure are used widely as soil amendments for crop production but can contribute to the eutrophication of surface waters (Foley et al. 2005). Likewise, wastewater treatment (WWT) facilities in urbanized areas discharge nutrient-rich effluent to waterways increasing loads of bioavailable nutrients (Carey & Migliaccio, 2009). However, there is a high degree of source overlap on large spatial scales where many sources of N may contribute to waterway N loads (Chang et al., 2002). Thus, effective mitigation of eutrophication in mixed land use settings requires identification of the relative contributions of key sources of N to enable effective, targeted nutrient management strategies.

Sources of N to waterways, such as fertilizer and WWT, can be identified using the abundance of stable isotope ratios of N ($^{15}\text{N}/^{14}\text{N}$, reported as $\delta^{15}\text{N}$) and O ($^{18}\text{O}/^{16}\text{O}$, reported as $\delta^{18}\text{O}$) of dissolved inorganic nitrogen (DIN), specifically the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate (e.g., Burns et al., 2009; Kaushal et al., 2011; Soto et al., 2019) and the $\delta^{15}\text{N}$ value of ammonium (e.g., Hood et al., 2014; Kendall et al., 2015). Nitrogen isotope ratios can also be used to infer nutrient uptake by biota, such as algae, that acquire N from the water column (Peipoch et al., 2014). Biological processing and chemical reactions favour lighter isotopes (e.g., ^{14}N) resulting in predictable enrichment or depletion of heavier isotopes (e.g., ^{15}N) that can be used to infer source (Kendall et al., 2007). N and O from synthetic nitrate and N from ammonium fertilizers are typically

isotopically similar to the atmosphere (~ 0 ‰ for $\delta^{15}\text{N}$ and ~ 23 ‰ for $\delta^{18}\text{O}$) due to the Haber-Bosch process used in their manufacture (Bateman & Kelly, 2007; Michalski et al., 2015; Soto et al., 2019). In contrast, the nitrification of ammonium arising from the mineralization of soil organic matter and ammonium fertilizers can be more clearly distinguished using oxygen isotope ratios because $\delta^{18}\text{O}$ values arising from nitrification processes tends to be lower relative to those of synthetic nitrate (e.g., Soto et al., 2019). N in human and livestock wastes tend to be relatively enriched in ^{15}N (approx. +7 to $> +20$ ‰) due to wastewater treatment processes and volatilization (Kendall et al., 2007; Soto et al., 2019). Algal nitrogen isotopic compositions may provide a better indication of exposure to these N sources over time because algae tissue has a longer turnover time than water. However, the majority of biotic productivity is limited to the warm water seasons, thus study of multiple compartments (e.g., water in addition to biota) is needed to fully comprehend N source patterns seasonally to aquatic ecosystems.

Distinguishing human waste N from livestock manure N is not possible using $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values due to overlapping isotopic signatures. Artificial sweeteners have the potential to successfully identify and distinguish human and livestock waste N sources in conjunction with stable isotopes. Artificial sweeteners are commonly added to diet food, beverages, and consumer products, such as sodas and toothpaste, and because they are not completely removed through common wastewater treatment processes have become ubiquitous in human wastewater (Buerge et al., 2009; Scheurer et al., 2009; Spoelstra et al., 2017). The presence of artificial sweeteners may help confirm the presence of human sewage and allow for the differentiation of this source from livestock manure (Spoelstra et al., 2020). For example, acesulfame is an artificial sweetener that is ubiquitous in human sanitary wastewater, is not found in animal waste, and is resistant to degradation and thus is a good indicator of human waste sources (Spoelstra et al., 2013). Other common sweeteners, such as sucralose, are also human waste-specific, but appear to be more readily degraded in the environment and therefore their presence may be indicative of more recent WWT inputs (Robertson et al., 2016). In contrast, saccharin is found in human waste, but is also the only artificial sweetener approved for use in Canadian livestock feed (Spoelstra et al., 2020) and is commonly used as a feed additive for piglets (Buerge et al., 2011; Ma et al., 2017). The combination of enriched ^{15}N (e.g.,

in DIN or algae) and dominance of saccharin over other sweeteners at a given site may therefore be an indication that manure N inputs are the primary N source.

Lake Winnipeg, the world's tenth largest freshwater lake, has undergone rapid cultural eutrophication over the past several decades (Schindler et al., 2012). The cumulative effects of intensive agriculture, livestock production, and growing communities in the Lake Winnipeg Basin have contributed to excessive in-lake nutrient concentrations and increasingly frequent algal bloom formation (Bunting et al., 2016). Much of the nutrient load (68% phosphorus and 34% nitrogen) enters the lake via the Red River (EC & Manitoba Water Stewardship, 2011), thus N sources within the Red River watershed are of particular concern (Mayer & Wassesnaar, 2012). Sources of nitrate to the Red River and its major tributary, the Assiniboine River, have been broadly identified (Soto et al., 2019). However, sources of N to the tributaries of the Red River Valley (RRV), the portion of the Red River Basin directly adjacent to Lake Winnipeg, have not been well evaluated. The tributaries of the RRV are ideal for the identification of N sources because their small catchments cover a range of land use types and may provide insight into N source patterns not possible to distinguish in larger mixed-use watersheds (e.g., Burns et al., 2009).

Nutrient delivery in cold temperate regions, such as the RRV, is associated with the hydrologic regime (Shrestha et al., 2012; Cade-Menun et al., 2013) and the majority of nutrient runoff (up to 80%) in the RRV occurs during snowmelt (Glozier et al., 2006; Corriveau et al., 2013; Rattan et al., 2017). For example, Rattan et al. (2019) found that the key bioavailable forms of N, ammonium (NH_4^+) and nitrate (NO_3^-), varied significantly by season in the RRV with the largest loads occurring during snowmelt. However, the few stream studies in the region that captured intra-annual variation (Corriveau et al., 2013; Rattan et al., 2017), particularly those that captured the snowmelt pulse, have not identified N sources to streams. Rather, it has been hypothesized that diffuse, land-based runoff from agriculture, the predominant nutrient-producing human activity in the region (Yates et al., 2012), is the most important contributor of N during the snowmelt period (Schindler et al., 2012). In contrast, other sources, such as effluent from municipal WWT lagoons, discharged directly to streams may be more important

later in the season when catchment hydrologic connectivity is lowest (Rattan et al., 2017). However, the relative contributions of agricultural and WWT sources of N to tributaries and how they change seasonally have not been assessed.

Our study identified sources of N from nutrient-producing human activities to streams in the RRV and the Red River and tested the hypothesis that key N sources shift seasonally from diffuse agricultural sources to point source WWT. We achieved our objectives by first assessing seasonal change in the sources of N to RRV tributaries by examining a) whether $\delta^{15}\text{N}$ -DIN in water differs seasonally, and b) if algal $\delta^{15}\text{N}$ values differ where WWT lagoons are present or absent in each season. Second, we sought to distinguish source inputs of agricultural fertilizers, manure, and WWT using stable isotope and artificial sweetener data to estimate the proportional contributions of key N sources to stream water and benthic algae. Third, we aimed to establish downstream linkages from the RRV to the Red River by relating the isotopic compositions of DIN and estimating N source contributions from the tributaries to those in the Red River. Our findings will inform more effective targeting and timing of nutrient mitigation activities in the RRV. Moreover, by establishing how N sources to the water compare to sources of N assimilated by algae, we will improve understanding of ecosystem function of RRV streams. Our results will also aid in connecting N sources from the landscape through the small tributaries of the RRV to Lake Winnipeg's key nutrient source, the Red River. More broadly, we aim to highlight the importance of considering both DIN species (nitrate and ammonium) when using stable isotopes to assess sources of N from human activities. Furthermore, the results of our study aim to provide evidence that the analysis of stable isotopes of DIN in conjunction with artificial sweeteners can be used to distinguish human and livestock N sources.

3.2 Materials and Methods

3.2.1 Study Area

Our study was conducted in the Red River Valley of southern Manitoba, Canada. Study sites were located on 14 tributaries within the RRV and at three locations on the Red River (Figure 3.1). As part of the former bed of post-glacial Lake Agassiz, the RRV

is characterized by fine glaciolacustrine sediments and its low-relief topography, changing only 70 m in elevation over the approximately 500 km distance from the headwaters of the Red River to Lake Winnipeg (Brooks, 2017). The valley is bounded to the west by the Manitoba Escarpment and to the east by the Precambrian shield. The low relief topography and impermeability of the fine clay soils make the RRV prone to flooding, thus wetlands and streams in the region have been heavily modified to enhance drainage of land for agriculture (Yates et al., 2012; Yates et al., 2014a). The dominant N producing human activities in the RRV include cultivation of crops, livestock production, and municipal wastewater discharge (Yates et al., 2012; Rattan et al., 2017).

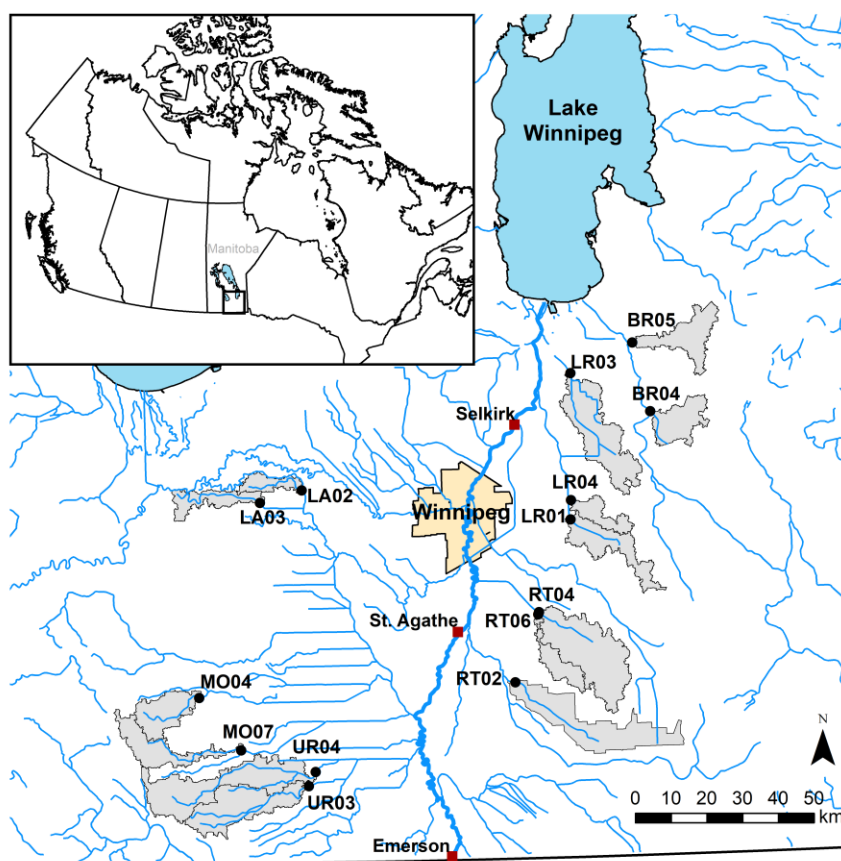


Figure 3.1 Map of study area showing 14 tributaries and their watersheds (shaded gray areas with black circular points), sampling sites in the Red River (red square

points; Emerson, St. Agathe, and Selkirk) and the City of Winnipeg in Manitoba, Canada. Watershed delineations were adapted from Cormier et al. (2017).

Land use in the catchments of the 14 study tributaries spans a range of amounts and types of N-producing human activities (Table 3.1). Information about nutrient unit equivalents can be found in Yates et al. (2012) and Cormier et al. (2017). Catchments on the west side of the RRV are more heavily cropped, whereas eastern catchments tend to have more forest cover and livestock production of pigs, cattle, and poultry. Rural communities in the RRV rely on municipal wastewater lagoons for sewage disposal and treatment. Lagoons discharge effluent periodically to streams over one- to two-week periods during the ice-free months from May to October. Lagoons serving smaller communities (e.g., <1000 pop.) may discharge only once or twice per year while larger communities may discharge more frequently and/or for longer periods of time. Municipal lagoons are present in seven of the 14 catchments, three on the west side and four on the east. Descriptions of crop cover, production of major livestock types, and human populations served by municipal lagoons were calculated using ArcGIS v. 10.7 (Environmental Systems Research Institute, Redlands, CA, USA) and data from the 2016 Canadian Census of Agriculture (Statistics Canada, 2016).

The Red River study sites were located near three small communities at Emerson, St. Agathe, and Selkirk, Manitoba (Figure 3.1). The Emerson study site is located immediately North of the Canada-USA border and is representative of N inputs from the USA portion of the Red River Basin. St. Agathe is located at the center of the RRV upstream from the City of Winnipeg and was chosen to be representative of tributary inputs from within the RRV. The Selkirk sampling site is located downstream from Winnipeg's mechanical wastewater treatment plants, which are the largest point source contributors of N to the Canadian portion of the Red River (EC and Manitoba Water Stewardship, 2011).

Table 3.1 Description of common nitrogen producing human activities in 14 study watersheds in the Red River Valley (RRV), Manitoba, Canada. Crop (%) is derived from the Agriculture and Agri-Food Canada (AAFC) Annual Crop Inventory (2018). Numbers of the most common livestock animals in the RRV (pigs, cattle, and chickens) are derived from the Canadian Census of Agriculture (2016) and are expressed as individuals per kilometer squared of total agricultural land cover (AAFC, 2018) in each watershed. Populations served by wastewater treatment (WWT) indicates the total number of individuals served by a municipal lagoon discharging to the tributary.

Site ID	Stream Name	Latitude	Longitude	Watershed Area (km²)	Crop (%)	Pigs (ind./km²)	Cattle (ind./km²)	Chickens (ind./km²)	Pop. Served by WWT (ind.)
LA02	W. Branch La Salle Drain	49.930662	-97.774963	64.9	84.9	71.9	9.0	237.9	0
LA03	Elm River	49.90095	-97.938353	105.3	75.3	56.3	13.8	166.5	621
MO04	S. Tobacco Creek	49.41194	-98.183248	149.4	67.1	62.4	11.6	91.1	434
MO07	N. Shannon Creek	49.278703	-98.024131	157.3	70.9	51.7	13.8	152.7	0
UR03	Hespeler Drain	49.186287	-97.765623	255.7	79.1	53.6	9.5	68.9	0
UR04	Deadhorse Creek	49.222022	-97.738643	360.0	77.4	50.7	11.1	104.5	22163
BR04	Bears Creek	50.10757	-96.406425	136.8	22.8	19.2	9.9	107.0	0
BR05	Unnamed Drain	50.281579	-96.467793	163.4	49.9	10.1	4.2	50.0	0
LR01	Cooks Creek	49.841808	-96.730688	176.5	39.8	687.1	37.8	1491.8	0
LR03	Devils Creek	50.209652	-96.714113	305.5	48.7	15.6	10.8	35.3	647
LR04	Edie Creek	49.890319	-96.72689	80.4	17.8	33.5	27.6	91.6	0
RT02	Joubert Creek	49.436585	-96.962504	348.3	17.8	1374.8	67.3	2984.4	1680
RT04	Manning Canal	49.611398	-96.863448	375.3	41.6	887.1	43.1	1921.9	15829
RT06	Unnamed Drain	49.605429	-96.867008	88.7	41.0	817.8	39.7	1771.8	500

3.2.2 Field Sampling

We sampled the 14 tributaries once during each of snowmelt, spring, summer, and autumn of 2019. Field sampling campaigns were completed in early April (snowmelt), mid-June (spring), late July (summer), and late September (autumn). One site during each of snowmelt (BR05) and spring (LA03) was not sampled due to frozen and dry conditions, respectively. Instantaneous stream water pH measurements were taken at the tributary study sites using a YSI ProDSS handheld instrument (YSI Incorporated, Yellow Springs OH, USA) and the median pH over the course of the study was 7.71 (range 6.95 to 8.86). Water samples were also collected in spring (mid-June), summer (mid-August) and autumn (late September/early October) at the three locations in the Red River. Water samples for DIN (ammonium and nitrate), stable isotopes of DIN, and artificial sweeteners were collected with an extendable sampling pole or by hand while wading. DIN samples were immediately refrigerated, and ammonium samples were preserved with sulfuric acid before being shipped to analytical laboratories within 24 to 48 hours of collection. Samples for DIN stable isotopes were filtered the same day of collection through a 142 mm Supor® polyethersulfone 0.45 µm (Pall Corporation, Ann Arbor MI, USA) filter and immediately frozen. Samples for analysis of sweetener concentrations were collected in the 14 tributaries, but not the Red River. After discarding the first approx. 5 mL of filtrate, artificial sweetener samples were filtered through a 28 mm 0.45 µm cellulose-acetate syringe filter (Corning Incorporated, Corning NY, USA) in the field and frozen until time of analysis. In spring and summer, benthic algae samples were collected by hand while wading at each site. Bulk biomass samples of attached algae were collected from three locations representative of the stream reach and were rinsed in local stream water at each site before they were frozen until time of laboratory processing. Benthic algae samples were collected for biomass only and were not taxonomically identified. Algae samples for stable isotope analysis could not be collected due to a lack of available algae at all sites during snowmelt and autumn. Algae samples were not collected at the Red River sites.

3.2.3 Laboratory Analysis

Tributary nitrate (NO_3^- ; measured as $\text{NO}_2^- + \text{NO}_3^-$) was measured at the University of Alberta Biogeochemical Analytical Service Laboratory (BASL), Edmonton, Canada via flow injection analysis using a Lachat QuikChem 8500 FIA automated ion analyser. Red River NO_3^- was measured via cadmium reduction and high-resolution colorimeter continuous flow analysis at the National Laboratory for Environmental Testing (NLET), Saskatoon, Canada. Ammonium (NH_4^+) from the tributaries and the Red River was measured via high-resolution colorimeter continuous flow analysis at the National Laboratory for Environmental Testing, Saskatoon, Canada. Detection limits for NO_3^- and NH_4^+ were 0.002 mg N/L (BASL), 0.010 (NLET) and 0.005 mg N/L, respectively.

Concentrations of artificial sweeteners acesulfame, saccharin, and sucralose were measured at the Canada Centre for Inland Waters in Burlington, Canada using a suppressed ion chromatography (IC), tandem mass spectrometry (MS) method (Van Stempvoort et al. 2011; Spoelstra et al. 2017). Method detection limits were 2, 2, and 20 ng/L for acesulfame, saccharin, and sucralose, respectively. Samples were analysed via ion chromatography using a Thermo Fisher Dionex ICS 5000 system and ion suppression using a Dionex AERS 500 2 mm. The IC was interfaced to an AB Sciex 5500 QTrap MS/MS and operated in the negative electrospray ionization mode.

Isotope ratios were measured by stable isotope ratio mass spectrometry and expressed as delta (δ) values. The ratio of the raw isotopic sample (R_{sample} , e.g. $^{15}\text{N}/^{14}\text{N}$) was compared to a known standard value (R_{standard}) according to:

$$\delta X(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1)$$

Where X is the heavy isotope (e.g., ^{15}N). Delta values are reported relative to international isotope standards of atmospheric nitrogen (AIR) for $\delta^{15}\text{N}$ and Vienna standard mean ocean water (VSMOW) for $\delta^{18}\text{O}$. Delta values are multiplied by 1000 and thus reported as per mil (‰).

Benthic algae samples were freeze-dried and subsampled into glass vials and then acidified with 10% HCl using the drop-by-drop technique (Jacob et al., 2005) under a

fume hood to remove excess carbonate. Algae samples were then weighed into tin boats and shipped to the UC Davis Stable Isotope Facility for solid $^{15}\text{N}/^{14}\text{N}$ stable isotope ratio analysis using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 continuous flow isotope ratio mass spectrometer (CF-IRMS) (Sercon Ltd. Cheshire, UK). Accuracy and precision were measured relative to laboratory standards calibrated against international reference materials and were compositionally similar to the sample material. The standard deviation for within run reference material was 0.05‰.

$\delta^{15}\text{N}$ of ammonium ($\delta^{15}\text{N-NH}_4^+$) was analysed using the Holmes et al. (1998) micro-diffusion method in which sample NH_4^+ is converted to NH_3 and trapped on an acidified glass fibre filter. Fractionation standards were prepared during every sample collection event using ultra-pure water and a laboratory standard material (reagent grade $(\text{NH}_4)_2\text{SO}_4$) with a known $\delta^{15}\text{N}$ value ($\delta^{15}\text{N} = +1.0 \pm 0.2\text{‰}$) to control for possible fractionation after sample collection and during conversion to NH_3 . Correction for fractionation during collection and processing was minimal ($< 0.6\text{‰}$). Samples were analyzed using Carlo Erba NA1500 elemental analyser interfaced to an IsoPrime CF-IRMS (Elementar, Langensfeld, Germany) at the National Hydrology Research Centre (NHRC) Stable Isotope Laboratory, Saskatoon, Saskatchewan, Canada. The standard deviations for within run reference materials (bowhead whale baleen keratin ($\delta^{15}\text{N} = +14.3\text{‰}$), porcine gelatin ($\delta^{15}\text{N} = +5.1\text{‰}$), laboratory standard ($(\text{NH}_4)_2\text{SO}_4$) were $\leq 0.1\text{‰}$.

$\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate ($\delta^{15}\text{N-NO}_3^-$, $\delta^{18}\text{O-NO}_3^-$) were measured by using Ti(III) to reduce sample nitrate to N_2O (Altabet et al. 2019) and then analysed using a trace gas system (Elementar, Langensfeld, Germany) and IsoPrime CF-IRMS at the NHRC Stable Isotope Laboratory. Standard deviations for within-run dissolved NO_3^- reference materials (IAEA NO3 ($\delta^{15}\text{N} = +4.7\text{‰}$, $\delta^{18}\text{O} = +25.6\text{‰}$), USGS34 ($\delta^{15}\text{N} = -1.8\text{‰}$, $\delta^{18}\text{O} = -27.9\text{‰}$), USGS35 ($\delta^{15}\text{N} = +2.7\text{‰}$, $\delta^{18}\text{O} = +57.5\text{‰}$)) were $\leq 0.5\text{‰}$ for $\delta^{15}\text{N}$ and $\leq 1.5\text{‰}$ for $\delta^{18}\text{O}$.

3.2.4 Data Analysis

Nitrate concentrations were often below detection limits at multiple sites during spring, summer, and autumn. Therefore, we combined the $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$ (when available) into a single DIN concentration-weighted average $\delta^{15}\text{N-DIN}$ value for the following statistical analyses. Logarithmic transformation of $\delta^{15}\text{N}$ data did not improve normality therefore analyses were conducted using untransformed $\delta^{15}\text{N}$ values. However, one outlier was four times larger than next closest value and was removed from the $\delta^{15}\text{N-DIN}$ dataset (MO04 spring).

A general linear model (GLM) was used to test if $\delta^{15}\text{N-DIN}$ differed among seasons. We selected a significance level of $\alpha = 0.10$ to better balance the likelihood of Type I and Type II errors given our small sample size ($n = 13-14$) and limited knowledge of the uncontrolled variability across our study sites. When $p \leq 0.10$, we performed Tukey-adjusted post hoc pairwise comparisons of least square means. A GLM was also used to test our prediction that $\delta^{15}\text{N-algae}$ differs where municipal WWT lagoons are present or absent. Season (spring and summer) was included in the $\delta^{15}\text{N-algae}$ model to account for differences associated with algal turnover between sampling events, as was a season-lagoon interaction term. GLMs were performed in R v. 3.6.1 (R Core Team, 2019).

Isotopic signatures were used to estimate the relative contribution of potential N sources to ammonium (using $\delta^{15}\text{N}$ only) and nitrate (using both $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) in water for each site using Bayesian-based stable isotope mixing models. Bayesian-based mixing models allow for the incorporation of uncertainty (e.g., source variation) as well as a greater number of sources than traditional mixing models which were otherwise underdetermined when the number of possible sources exceeded the number of isotopes plus one (Phillips et al. 2014). Mixing models were carried out using R package *simmr* v. 0.4.1 in R (Parnell, 2019), which employs a Markov chain Monte Carlo algorithm to produce a posterior distribution used to estimate the proportional contribution of each source (expressed as mean \pm SD). The isotopic composition and associated uncertainties of the three dominant N sources in the RRV were assigned based on the study of N sources to the Assiniboine and Red Rivers by Soto et al. (2019). Sources and their

associated $\delta^{15}\text{N}$ (used for ammonium and nitrate) and $\delta^{18}\text{O}$ (used for mixing models with nitrate only) values include synthetic N fertilizer (e.g., produced from atmospheric N; $\delta^{15}\text{N} = 0 \pm 3\text{‰}$, $\delta^{18}\text{O} = +22 \pm 3\text{‰}$), nitrification of/and ammonium occurring in soils (e.g., nitrification of NH_4^+ fertilizers and mineralization of organic matter; $\delta^{15}\text{N} = -5 \pm 5\text{‰}$, $\delta^{18}\text{O} = -10 \pm 10\text{‰}$), and waste from human WWT and manure ($\delta^{15}\text{N} = +15 \pm 5\text{‰}$, $\delta^{18}\text{O} = -10 \pm 10$). Sources of N to nitrate could not be estimated at all sites for all seasons because nitrate concentrations at many sites during spring, summer and fall were below detection. Thus, only proportional contributions of N sources to snowmelt nitrate were estimated. The above $\delta^{15}\text{N}$ values were also used to estimate the relative contribution of N sources to algae in spring and summer (Cormier et al., in press). The utilization of nitrogen by algae is typically accompanied by a negative isotopic discrimination due the preferential uptake of the lighter ^{14}N (Mayer & Wassenaar, 2012). Thus, a conservative overall fractionation of -4.0‰ (Kendall et al., 2015) was incorporated into mixing models for algae. The default burn-in (1000) and iteration values (10,000) in *simmr* produced satisfactory convergence of all models (Gelman diagnostic < 1.1).

3.3 Results

3.3.1 Concentrations and $\delta^{15}\text{N}$ of DIN in tributaries

DIN concentrations varied by season and were greatest during snowmelt (Figure 3.2). Snowmelt concentrations ranged from 0.20 to 0.80 mg N/L for NH_4^+ and 0.73 to 3.14 mg N/L for NO_3^- . In spring, DIN concentrations decreased by at least an order of magnitude from snowmelt at all sites except MO04. Spring NH_4^+ ranged from 0.01 to 0.04 mg N/L at all but one site, MO04 (2.43 mg N/L), that had recently received WWT effluent. NO_3^- was detectable at only two sites, MO04 (0.38 mg N/L) and UR03 (0.03 mg N/L), in spring. In summer, NH_4^+ ranged from 0.02 to 0.10 mg N/L, whereas NO_3^- was only detectable at five sites, where concentrations ranged from 0.03 to 0.16 mg N/L. In autumn, NH_4^+ ranged from 0.02 to 0.13 mg N/L across all sites and NO_3^- ranged from 0.02 to 2.28 mg N/L at the 8 sites with detectable concentrations.

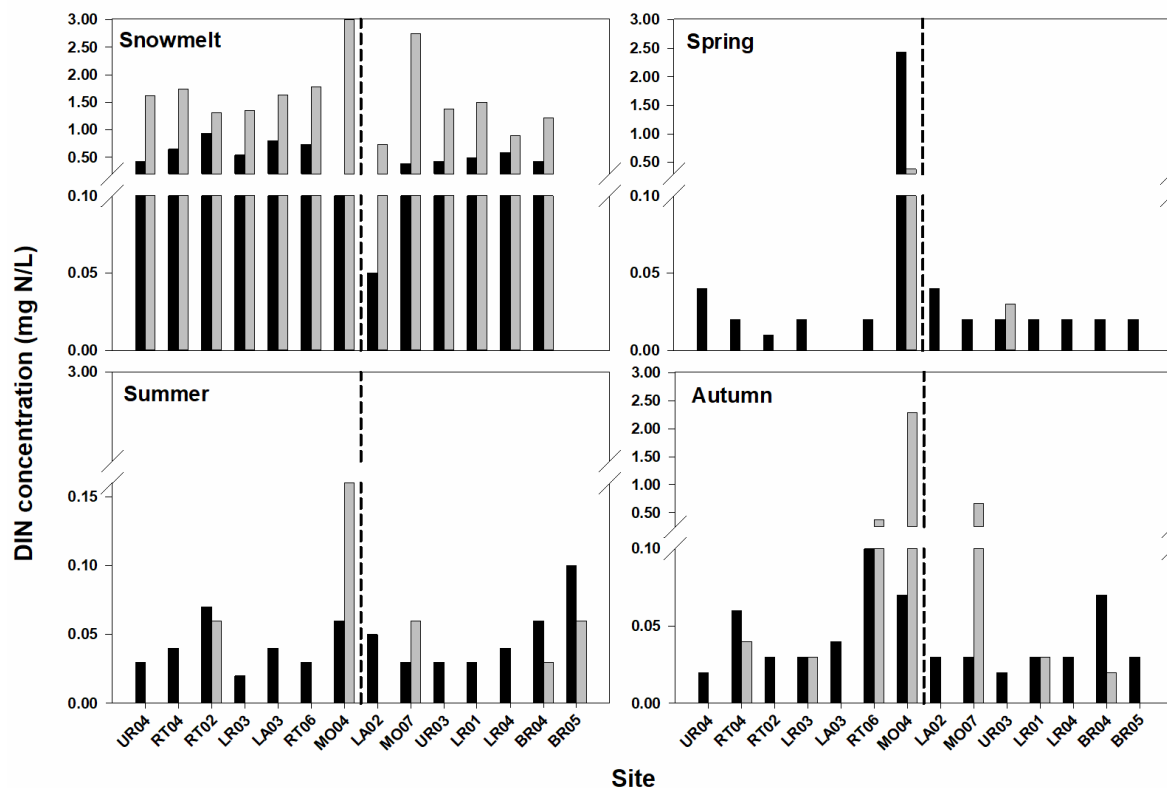


Figure 3.2 Dissolved inorganic nitrogen (DIN) concentrations of ammonium (black bars) and nitrate (gray bars) in water at each site during snowmelt, spring, summer, and autumn. Sites are organized from the highest (left) population served by a wastewater treatment (WWT) lagoon to the lowest (right). Site with zero people served by WWT appear to the right of the dashed line.

Average $\delta^{15}\text{N}$ -DIN (mean \pm 1 SD) was highest during snowmelt ($\delta^{15}\text{N} = +7.5 \pm 4.6\text{‰}$) and lowest during spring ($\delta^{15}\text{N} = +3.1 \pm 5.3\text{‰}$) and summer ($\delta^{15}\text{N} = +3.1 \pm 3.5\text{‰}$) (Figure 3.3A). In autumn, average $\delta^{15}\text{N}$ -DIN increased ($\delta^{15}\text{N} = +6.0 \pm 4.6\text{‰}$), relative to spring and summer. We found $\delta^{15}\text{N}$ -DIN to be different among seasons ($F = 2.97$, $p = 0.04$). The seasonal effect was driven by differences between snowmelt and spring ($p = 0.07$) and snowmelt and summer ($p = 0.06$), based on post-hoc pairwise comparisons of least square means. Average $\delta^{15}\text{N}$ -algae did not differ ($F = 1.12$, $p = 0.30$) between spring ($\delta^{15}\text{N} = +5.0 \pm 4.7\text{‰}$) and summer ($\delta^{15}\text{N} = +6.9 \pm 4.5\text{‰}$) (Figure 3.3B). However, $\delta^{15}\text{N}$ -algae from sites with lagoons present (spring $\delta^{15}\text{N} = +7.4 \pm 5.9\text{‰}$, summer $\delta^{15}\text{N} = +8.7 \pm 4.6\text{‰}$) was higher ($F = 6.27$, $p = 0.02$) than $\delta^{15}\text{N}$ -algae from sites without lagoons (spring

$\delta^{15}\text{N} = +2.9 \pm 1.8\text{‰}$, summer $\delta^{15}\text{N} = +5.1 \pm 4.0\text{‰}$). There was no interaction between season and presence/absence of lagoons ($p = 0.93$).

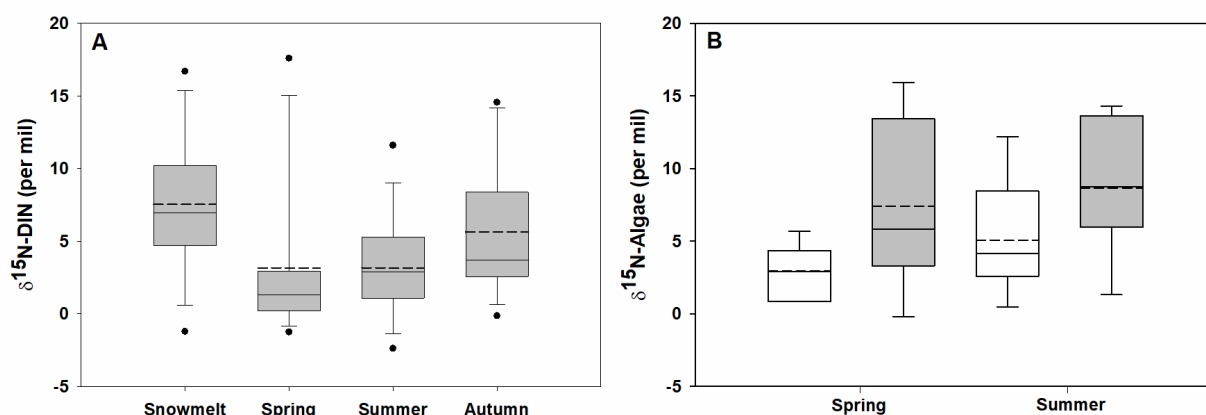


Figure 3.3 Boxplot showing seasonal difference in concentration weighted average dissolved inorganic nitrogen (DIN) $\delta^{15}\text{N}$ values from 14 southern Manitoba, Canada streams, and; B) Boxplot showing difference of algae $\delta^{15}\text{N}$ values from 14 southern Manitoba streams in spring and summer where lagoons are absent (white boxes) versus present (gray boxes). For both panel A and panel B lines indicate the median (solid line) and mean (dashed line), outer bounds of boxes indicate 25th and 75th percentiles (boxes), and whiskers indicate 10th and 90th percentiles. Filled black circles depict values falling outside the 10th and 90th percentiles and do not include one outlier (MO04 in spring) removed prior to analysis.

During snowmelt, $\delta^{15}\text{N-NH}_4^+$ values ranged from +9.3‰ to +18.0‰ (Figure 3.4). Mixing models indicated that N from waste sources was the dominant source proportion to the DIN ammonium fraction at all sites during snowmelt (range $58 \pm 15\%$ to $81 \pm 8.5\%$). In contrast, DIN contributions from synthetic N fertilizer (range $10 \pm 7.0\%$ to $22 \pm 14\%$) and soil sources of ammonium (range $9.2 \pm 6.1\%$ to $19 \pm 13\%$) during snowmelt were smaller. The average estimated contribution of waste sources to snowmelt ammonium was the same at sites with lagoons ($n = 7$; $74 \pm 7.7\%$) and without ($n = 6$, $74 \pm 7.9\%$) lagoons.

In spring, $\delta^{15}\text{N-NH}_4^+$ values ranged from -1.3‰ to +87.7‰ (Figure 3.4). Municipal wastewater effluent was being discharged to the stream at the ultra-enriched site (MO04) and it was the outlier removed from the seasonal comparison. The $\delta^{15}\text{N-NH}_4^+$ value at MO04 (+87.7‰) was almost five times more enriched than the second greatest spring $\delta^{15}\text{N-NH}_4^+$ value (17.6‰ at UR03). The range of estimated waste N proportions was $17 \pm 11\%$ to $99 \pm 0.6\%$ though most sites ($n = 10$) had waste proportions of less than 30%. On average, the proportion of waste N was greater at sites with lagoons ($n = 6$, $43 \pm 31\%$) than sites without lagoons ($n = 7$, $30 \pm 23\%$) in spring. The range of contributions of synthetic fertilizer (range $0.7 \pm 0.4\%$ to $42 \pm 23\%$) and soil sources (range $0.6 \pm 0.4\%$ to $44 \pm 22\%$) to ammonium in spring were greater than those observed during snowmelt though most sites ($n = 9$) ranged between 32 and 44% for both N sources.

Summer $\delta^{15}\text{N-NH}_4^+$ values ranged from -11.9‰ to +14.9‰ (Figure 3.4). The $\delta^{15}\text{N-NH}_4^+$ value on the low end of the range (MO07) was nearly 10‰ lower relative to the second lowest value (-2.4‰ at LR01). Like spring, summer waste sources to ammonium also ranged widely from $7 \pm 5\%$ to $75 \pm 11\%$ and were greater on average at sites with lagoons ($n = 7$, $37 \pm 19\%$) than sites without lagoons ($n = 7$, $25 \pm 13\%$). During summer, synthetic fertilizer contributed $11 \pm 8\%$ to $42 \pm 24\%$ to ammonium N but at most sites ($n = 12$) contributed an average of 30 to 42%. Soil sources of N ranged from $12 \pm 8\%$ to $82 \pm 9\%$ with contributions at most sites ($n = 11$) falling between 25 and 40%.

The range of $\delta^{15}\text{N-NH}_4^+$ values in autumn was -6.9‰ to $+13.5\text{‰}$ (Figure 3.4). In autumn, estimated contributions of waste sources to ammonium ranged from $10 \pm 6.8\%$ to $72 \pm 11\%$. As in spring and summer the average contribution of waste N sources was estimated to be greater at lagoon sites ($n = 7$, $46 \pm 20\%$), than sites without lagoons ($n = 7$, $29 \pm 15\%$). The contributions of synthetic fertilizers to ammonium in autumn ranged from $15 \pm 10\%$ to $41 \pm 24\%$, and the contribution of soil N ranged from $15 \pm 10\%$ to $69 \pm 17\%$, although the average soil N contribution was less than 40% at all but one site (MO07).

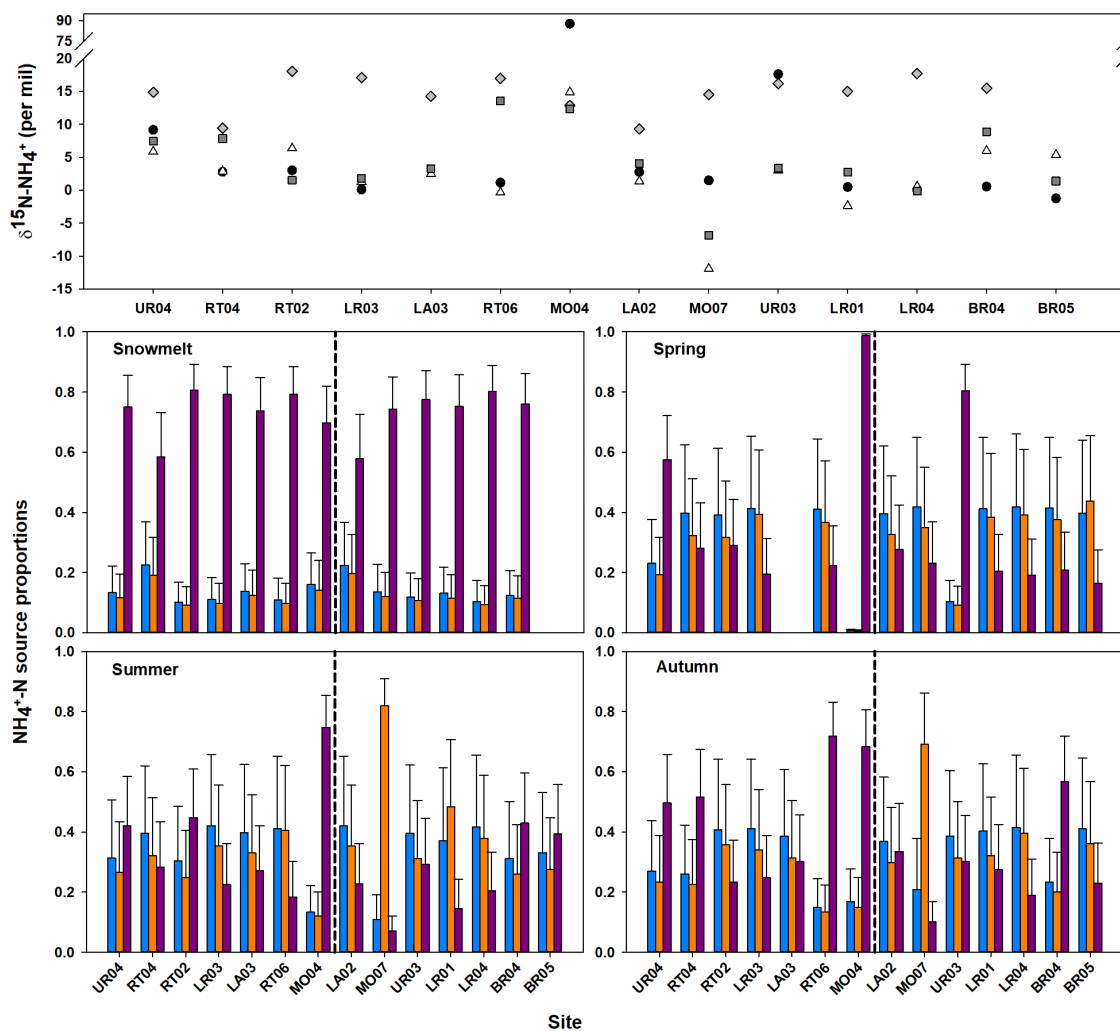


Figure 3.4 Plot (top) of $\delta^{15}\text{N}$ of ammonium ($\delta^{15}\text{N-NH}_4^+$) in water measured at each site during snowmelt (light gray diamonds), spring (black circles), summer (white

triangles), and autumn (dark gray squares), and; bar charts showing estimated mean and standard deviation (error bars) proportional contribution of synthetic fertilizer (blue bars), soil ammonium (orange bars), and waste (purple bars) to ammonium ($\text{NH}_4^+\text{-N}$) in streams in the Red River Valley, Manitoba. Sites are organized from the highest (left) population served by a wastewater treatment (WWT) lagoon to the lowest (right). Site with zero people served by WWT appear to the right of the dashed line.

During snowmelt, the isotopic composition of nitrate ranged from -3.4 to $+16.6\%$ and -5.6 to $+11.7\%$ for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, respectively (Figure 3.5A). The proportional contribution of waste sources of N to nitrate during snowmelt ranged from $15 \pm 9\%$ to $77 \pm 10\%$ (Figure 3.5B). The greatest proportion of waste N occurred at sites with the largest catchment livestock values and/or lagoons present (RT04, RT02, RT06). The average proportional contribution of waste N at lagoon sites ($n = 7$, $42 \pm 20\%$) was greater and more variable than at sites without lagoons ($n = 6$, $34 \pm 11\%$). Contribution of N from synthetic fertilizer ranged from $15 \pm 10\%$ to $52 \pm 15\%$ and the contribution of nitrification processes occurring in soils ranged from $8 \pm 6\%$ to $61 \pm 14\%$.

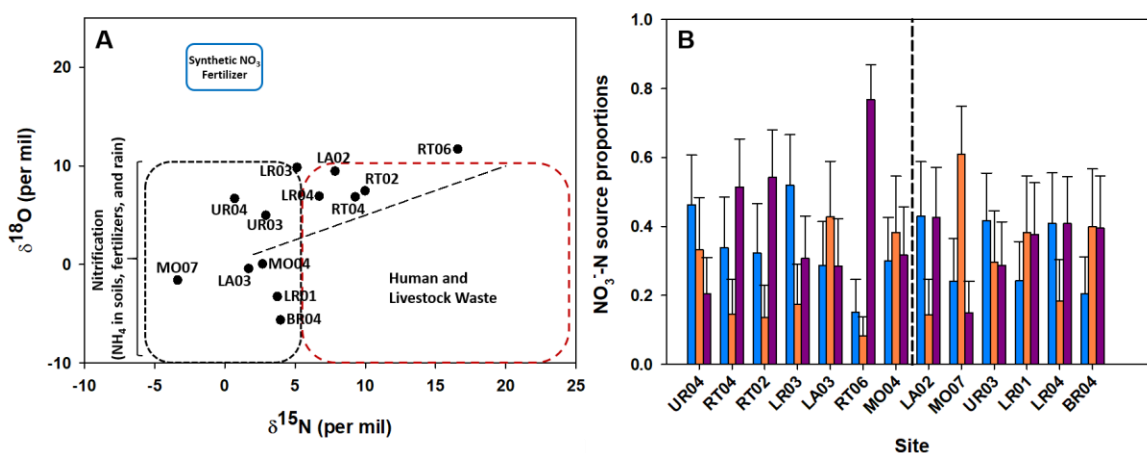


Figure 3.5 A) $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate (NO_3^- -N) in Red River Valley, Manitoba, Canada streams during snowmelt compared to the known isotopic composition of nitrogen sources from synthetic nitrate fertilizers (blue box), arising from the nitrification of ammonium from fertilizer, rain and soil (dashed black box),

and waste sources (red box). Medium dashed line indicates the 2:1 $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ relationship expected due to denitrification (adapted from Kendall et al., 2007 and Soto et al., 2019), and; B) the estimated mean and standard deviation (error bars) proportional contribution of synthetic nitrate fertilizer (blue bars), nitrification sources (orange bars), and waste (purple bars) to NO_3^- -N in the same streams during snowmelt. Sites in panel B are organized from the highest (left) population served by a wastewater treatment (WWT) lagoon to the lowest (right). Site with zero people served by WWT appear to the right of the dashed line.

The estimated proportional contribution of N sources to benthic algae in spring ranged from $8.8 \pm 6.0\%$ to $36 \pm 21\%$ for synthetic fertilizer, $8 \pm 5.5\%$ to $31 \pm 19\%$ for N from nitrification processes and $32 \pm 16\%$ to $83 \pm 7.8\%$ for waste N (Figure 3.6). At eight sites, the estimated contribution of waste sources to algae was greater than 50%. Similar to spring DIN waste estimates, the average contribution from waste for algae at lagoon sites ($n = 6$) was larger ($61 \pm 19\%$) than for algae from sites without lagoons ($n=7$, $47 \pm 8.5\%$). In summer, the range of contributions of synthetic fertilizer ($10 \pm 7\%$ to $36 \pm 21\%$), nitrification processes ($9.1 \pm 6.3\%$ to $29 \pm 18\%$), and waste ($35 \pm 16\%$ to $81 \pm 8.6\%$) to algae were similar to spring. The average proportional contribution of waste sources to algae at lagoon sites ($n = 7$, $66 \pm 15\%$) was higher than sites without lagoons ($n = 7$, $55 \pm 15\%$). However, in summer the estimated contribution of waste sources to algae was greater than 50% at 10 sites and the overall average waste proportion was greater at all sites in summer ($n = 14$, $61 \pm 15\%$) than in spring ($n = 13$, $53 \pm 15\%$).

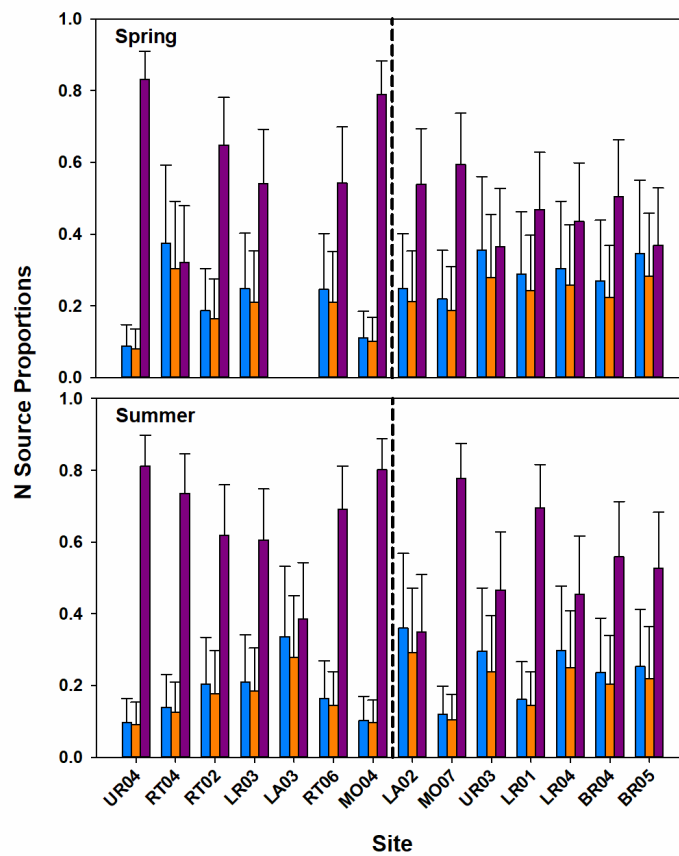


Figure 3.6 Estimated mean and standard deviation (error bars) proportional contribution of synthetic fertilizer (blue bars), nitrification/soils (orange bars), and waste (purple bars) to algae from streams in the Red River Valley, Manitoba, Canada. Sites are organized from the highest (left) population served by a wastewater treatment (WWT) lagoon to the lowest (right). Site with zero people served by WWT appear to the right of the dashed line.

3.3.2 Concentrations of artificial sweeteners in tributaries

Acesulfame (ACE) was either not detectable ($n = 2$) or present at concentrations less than 30 ng/L ($n = 11$) for all sites during snowmelt (Figure 3.7). Saccharin (SAC) was present at concentrations less than 50 ng/L at most sites ($n = 9$), but was greater than 100 ng/L at four sites (LA03 = 532 ng/L, UR03 = 151 ng/L, RT06 = 565 ng/L, and RT04 = 207 ng/L) during snowmelt. Sucralose (SUC) was not detected at any site during snowmelt. During spring, both ACE and SAC were detected at approximately 80% of sites but were largely present at concentrations below 35 ng/L for ACE and below 70

ng/L SAC. Exceptions were MO07 in spring (SAC = 147 ng/L) and two sites (MO04 and UR04) known to have recently received municipal lagoon WWT effluent where ACE (> 3000 ng/L), SAC (> 380 ng/L), and SUC (> 11,000 ng/L) were all detected. In summer, ACE, SAC, and SUC co-occurred at five sites (LA03, MO04, UR04, RT02, LR03) known to have recently received lagoon discharge, but at concentrations less than those observed at recent discharge sites in spring (max. summer concentrations for ACE = 785 ng/L, SAC = 145 ng/L, SUC = 1962 ng/L). In autumn, artificial sweeteners were present at concentrations below 25 ng/L for ACE and below 40 ng/L for SAC at all but three sites. Two streams were known to have recently received WWT discharge (UR04, RT04) and had concentrations like those observed in summer, and one (RT06) had high SAC (523 ng/L) only.

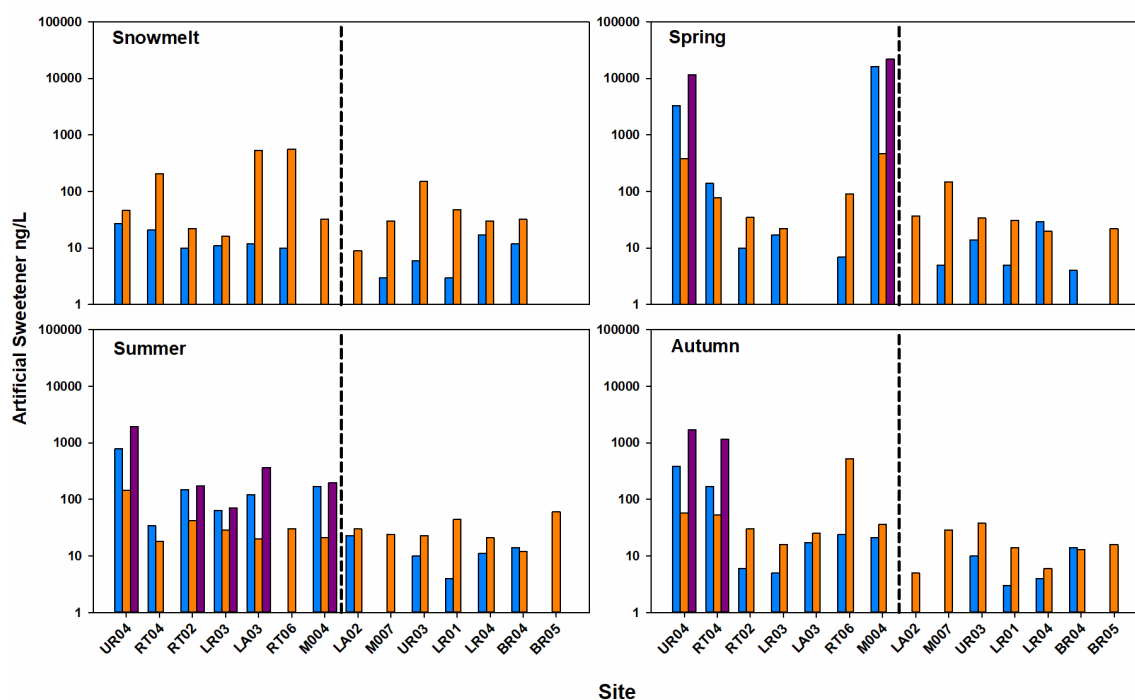


Figure 3.7 Concentrations (ng/L) of artificial sweeteners acesulfame (blue bars), saccharin (orange bars), and sucralose (purple bars) observed at sites in the Red River Valley, Manitoba, Canada during snowmelt, spring, summer, and autumn. Sites are organized from the highest (left) population served by a wastewater treatment (WWT) lagoon to the lowest (right). Sites with zero people served by WWT appear to the right of the dashed line.

3.3.3 Concentrations and $\delta^{15}\text{N}$ of DIN in the Red River

Unlike the tributaries of the RRV, nitrate contributed a greater average fraction of the total DIN concentration (~80%) than ammonium (~20%) in the Red River in spring, summer and autumn (Figure 3.8A,B), especially at sites upstream of the City of Winnipeg (Emerson and St. Agathe). Concentrations of nitrate and ammonium were consistently higher at Selkirk downstream from Winnipeg compared to the two upstream locations. In spring, ammonium concentrations ranged from 0.054 mg/L at Emerson to 0.163 mg/L at Selkirk, whereas spring nitrate concentrations were 0.222 mg/L and 0.546 mg/L at Emerson and Selkirk, respectively. In summer, at Emerson, ammonium was 0.013 mg/L and nitrate was 0.314 mg/L. However, the summer DIN sample from Selkirk had similar percentages from ammonium (49% of DIN, 0.414 mg/L) and nitrate (51% of DIN, 0.429 mg/L). In autumn, concentrations of both DIN species were lower than in spring and summer ranging from 0.024 mg/L to 0.092 mg/L for ammonium and 0.165 to 0.300 mg/L for nitrate. Like DIN concentrations, concentration weighted $\delta^{15}\text{N}$ -DIN in all seasons increased from Emerson (range +6.7‰ in spring to +8.0‰ in autumn) to Selkirk (range +8.8‰ in spring to +10.2‰ in summer) (Figure 3.8C). In summer and autumn, we also observed an increase in $\delta^{15}\text{N}$ -DIN between Emerson and St. Agathe (+2.1‰ in summer and +0.8‰ in autumn) but did not observe an increase in total DIN concentrations between these sites in spring.

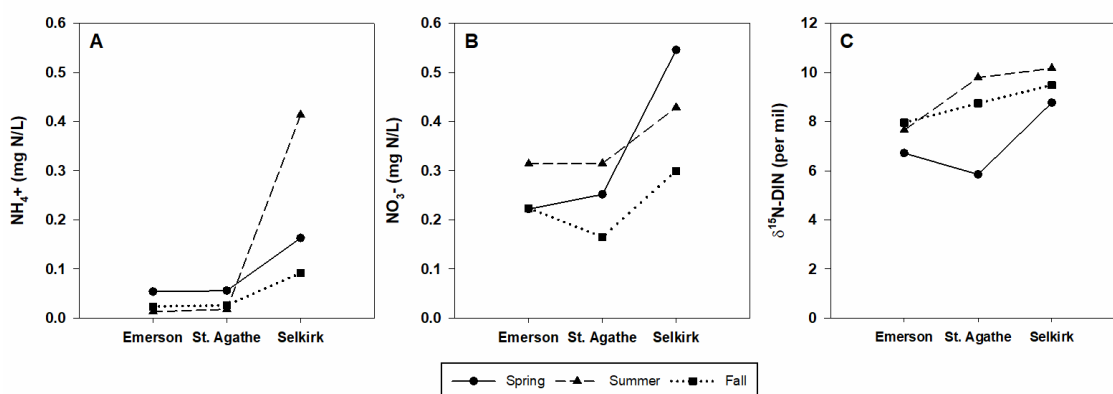


Figure 3.8 Concentrations of ammonium (A), nitrate (B) and concentration weighted average $\delta^{15}\text{N}$ of dissolved inorganic nitrogen (C) in the Red River, Manitoba at Emerson, St. Agathe, and Selkirk (downstream of Winnipeg) during

the spring (solid line w/ circle), summer (dashed line w/ triangle) and autumn (dotted line w/ square) of 2019.

Spring $\delta^{15}\text{N-NH}_4^+$ was +10.3‰ at Emerson and increased to +12.9‰ at St. Agathe before decreasing to +10.4‰ at Selkirk. Estimated average N source contributions to ammonium in the Red River revealed that in spring, ammonium N was mostly derived from waste sources ($65 \pm 5\%$) relative to synthetic fertilizer ($19 \pm 3\%$) and soil ammonium sources ($16 \pm 2\%$) (Figure 3.9). Waste contributed the greatest proportion of N to ammonium ($70 \pm 12\%$) at St. Agathe. Spring $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate were $\delta^{15}\text{N} = +5.9\%$ and $\delta^{18}\text{O} = -3.2\%$ at Emerson, $\delta^{15}\text{N} = +4.3\%$ and $\delta^{18}\text{O} = -3.57\%$ at St. Agathe, and $\delta^{15}\text{N} = +9.0\%$ and $\delta^{18}\text{O} = +9.0\%$ at Selkirk. Spring nitrate N sources were estimated to be $29 \pm 10\%$ from synthetic fertilizer, $27 \pm 11\%$ from nitrification of soil sources, and $44 \pm 4\%$ waste N.

In summer $\delta^{15}\text{N-NH}_4^+$ was +4.6‰ at Emerson, +4.0‰ at St. Agathe, and increased to +12.8‰ at Selkirk. Waste sources of N to ammonium were dominant only at Selkirk ($70 \pm 12\%$; Figure 3.9). In contrast, the proportional contributions of the three sources to ammonium at Emerson and St. Agathe were more evenly distributed (range 29% to 36%). Summer $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate were $\delta^{15}\text{N} = +7.8\%$ and $\delta^{18}\text{O} = -13.0\%$ at Emerson, $\delta^{15}\text{N} = +10.1\%$ and $\delta^{18}\text{O} = -12.2\%$ at St. Agathe, and $\delta^{15}\text{N} = +7.6\%$ and $\delta^{18}\text{O} = -16.8\%$ at Selkirk. In contrast to ammonium, the estimated waste proportion of nitrate increased to an average of $61 \pm 4\%$ in summer at all sites and was highest at St. Agathe ($66 \pm 13\%$).

In autumn, $\delta^{15}\text{N-NH}_4^+$ was +4.8‰ at Emerson, +5.2‰ at St. Agathe, and increased to 12.7‰ at Selkirk, and estimated contributions of each N source to ammonium were nearly identical to summer (Figure 3.9). $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate in autumn were $\delta^{15}\text{N} = +8.3\%$ and $\delta^{18}\text{O} = -11.7\%$ at Emerson, $\delta^{15}\text{N} = +9.3\%$ and $\delta^{18}\text{O} = -14.3\%$ at St. Agathe, and $\delta^{15}\text{N} = +8.5\%$ and $\delta^{18}\text{O} = -6.8\%$ at Selkirk. Like summer, waste N was estimated to be greatest contributor to nitrate in the Red River in autumn (average $61 \pm 3\%$), with the highest proportion again observed at St. Agathe ($65 \pm 14\%$).

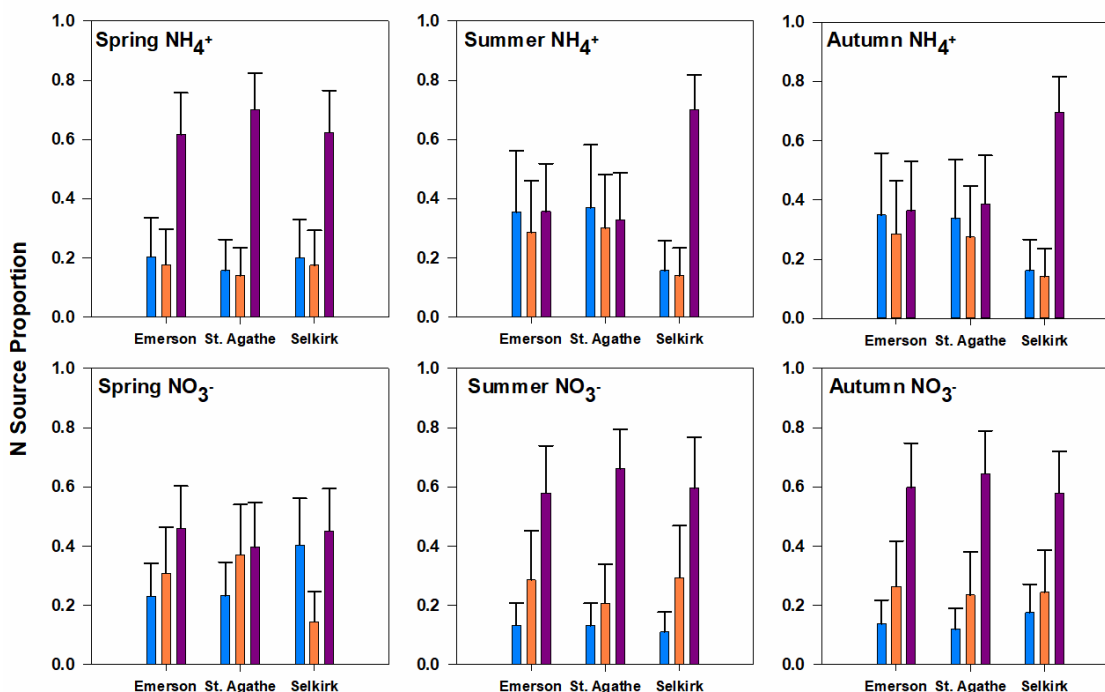


Figure 3.9 Estimated mean and standard deviation (error bars) proportional contribution of synthetic nitrate fertilizer (blue bars), nitrification of/ammonium in soils (orange bars), and waste (purple bars) to ammonium (NH₄⁺) and nitrate (NO₃⁻) in the Red River at Emerson, St. Agathe, and Selkirk (downstream of Winnipeg), Manitoba, Canada in spring, summer, and autumn.

3.4 Discussion

The findings of our study show that the dominant source of N delivered to RRV streams varies seasonally. The seasonal variation we observed was largely the result of livestock and human waste sources as evidenced by $\delta^{15}\text{N}$ values and artificial sweetener concentrations. Moreover, our results indicate that stream N is primarily from livestock sources during snowmelt, whereas WWT sources become more important in spring and summer. In contrast, the proportional contribution of N from fertilizers and soils appeared to be more consistent outside of snowmelt (e.g., each account for approx. 20 to 40% of N from spring to autumn) and less variable than N from waste sources.

The observed seasonal patterns in N sources are likely in response to changing hydrologic connectedness. Like most cold-regions, hydrologic connectivity in the RRV is greatest during snowmelt and decreases with the progression of the ice-free season (Shrestha et al., 2012). For example, Yates et al. (2014b) found that areas of influence (i.e., areas most strongly associated with in-stream nutrient levels) varied seasonally in response to hydrologic conditions in the RRV with less connectedness occurring outside of snowmelt and precipitation events. Indeed, we observed that in spring and summer, the overall contribution of waste to NH_4^+ -N decreased, while nitrate was largely not detected, indicating sources from landscape runoff (e.g., livestock manure) are primarily delivered during snowmelt when hydrologic connectivity is greatest. When runoff is minimal, site specific variation in N source delivery appears to become more important. For example, during spring and summer when streams were likely disconnected from their watersheds and lagoons had begun seasonal effluent discharges, we observed that N delivery from point source WWT lagoons became more evident.

Our results indicate a substantial contribution of livestock waste sources to the N delivered to streams during snowmelt. Indeed, livestock waste was particularly important in the ammonium fraction and ^{15}N -enriched NH_4^+ was observed at all sites during snowmelt regardless of the presence of WWT lagoons. Furthermore, our results suggest that waste sources in snowmelt are largely associated with runoff of livestock manure because elevated $\delta^{15}\text{N}$ values co-occurred with low concentrations of acesulfame (e.g., less than 30 ng/L) and no sucralose indicative of the discharge or leakage of small, household septic systems rather than major WWT outfalls (e.g., Richards et al., 2017; Spoelstra et al., 2017). Moreover, saccharin was present at greater concentrations than acesulfame at all sites during snowmelt, and concentrations of saccharin greater than 100 ng/L were observed at some sites with intensive hog production occurring in their watersheds (e.g., RT04, RT06), consistent with the inclusion of saccharin in piglet feed. However, because saccharin is typically used as an additive to hog feed and its presence in the feed of other livestock animals in Canada is unknown (Buerge et al., 2011; Spoelstra et al., 2020), it is not possible to identify manure inputs other than from hogs consuming artificial sweeteners. The importance of the ammonium fraction to N source determination suggests previous studies examining sources of N based on nitrate alone

may underestimate the importance of the waste contribution from watersheds, especially given our observation that manure was primarily associated with the ammonium fraction.

Waste N was estimated to be the dominant source of N to algae at approximately 60 to 70% of sites in both seasons. Given that we did not measure fractionation of ^{15}N between water and algae at each site and during each sampling event, our incorporation of site-wide fractionation of -4‰ into our algae mixing models likely influences the calculated source proportions given that fractionation between ^{15}N in DIN and that of primary producers can vary (e.g., Hood et al., 2014). However, the $\delta^{15}\text{N}$ of algae was highest at sites where WWT lagoons were present, suggesting algae takes up, and retains, N from the lagoon discharge events. We hypothesize that dominance in uptake of waste N by benthic algae in the RRV could be related to the preferential uptake of ammonium over nitrate (Ribot et al., 2015). Indeed, the ammonium content of livestock manure can be significant, especially for liquid pig manure (66% $\text{NH}_4\text{-N}$, OMAFRA 2009), and likely serves as key ammonium source given the large number of pigs in the RRV (2.3 million, Statistics Canada 2016). Likewise, effluent from WWT systems, such as municipal lagoons, that lack advanced treatment is an important source of ammonium (Holeton et al., 2011) and it is likely that the ammonium discharged from municipal lagoons during the spring and summer months in the RRV is rapidly taken up by biota. Biotic uptake of waste derived ammonia is consistent with a study by Cormier et al. (2017), who reported ^{15}N -enriched particulate organic matter and benthic invertebrates was associated with WWT at many of the same sites sampled for our study. Similarly, in their study of the La Tordera River in Spain, Ribot et al. (2012) observed rapid longitudinal depletion of WWT ammonium compared to nitrate during the summer season and hypothesized that preferential ammonium uptake by epilithic biofilm may play a key role in river DIN processing. We suggest waste sources likely serve as a key source of ammonium to organisms throughout the Red River drainage and thus streams should be managed to maximize their assimilatory capacity and prevent downstream losses of both ammonium and nitrate.

The influence of waste sources of N from the tributaries was visible in the Red River, where we found an increase in $\delta^{15}\text{N}$ -DIN values between the Emerson and St.

Agathe. Waste sources of N at St. Agathe were estimated to account for a greater proportion of ammonium in spring and a greater proportion of nitrate in summer and autumn than at the other Red River sites. Given there are no major point source nutrient inputs between the USA-Canada border at Emerson and the City of Winnipeg, the contributions of N observed at St. Agathe appear to be from the RRV tributaries. Despite our observation of lower or non-detectable nitrate in tributaries relative to the nitrate concentrations in the Red River, the finding of greater waste source contributions at St. Agathe suggests that waste N from the tributaries adds to the overall contribution of waste N to the Red River. We hypothesize that the increased contribution of waste sources to nitrate at St. Agathe in summer and autumn may reflect nitrification of the waste-derived ammonium in the tributaries at some point during transport to the Red River. Indeed, study of streams receiving WWT effluent has shown that longitudinal decreases of ammonium are often accompanied by increases in nitrate (Martí et al., 2004), suggesting nitrification and downstream transport may exceed retention of ammonium N by biota. Our finding that waste contributes greater than 60% of the nitrate to the Red River in summer and autumn of 2019 differs from Soto et al. (2019) who observed proportions closer to 40%. Soto et al. (2019) concluded that hydrologic conditions played an important role in delivery of fertilizer sources of N when conditions were wetter than average while WWT associated N delivery was relatively constant. As such it is possible that lower flows during 2019 relative to the Soto et al. (2019) study resulted in a greater proportion of waste N because of less water volume in the Red River. Nevertheless, there was little or no increase in the contribution of waste N to nitrate from St. Agathe to Selkirk suggesting tributary N is either utilized or diluted by contributions of other sources before Selkirk where the known influence of Winnipeg's wastewater treatment facilities likely account for the large increases in waste N contributions to ammonium.

3.4.1 Management implications

Hydrologic conditions in the RRV and watersheds worldwide are variable and because our findings are a snapshot of stream nutrient conditions in each season in only one year, they may not be consistent across years. Thus, our study may not reflect

conditions in years with different hydrologic conditions and when management practices vary. Indeed, the prevailing hydrologic conditions in the RRV in 2019 likely contributed to the seasonal variability in N source importance we observed and are likely to change from year to year. For example, lower than normal precipitation in the RRV in spring of 2019 was accompanied by minimal stream discharge ($0.0 \text{ m}^3/\text{s}$, observed at Deadhorse Creek in June, wateroffice.ec.gc.ca). This period of low hydrologic connectivity coincided with our observations of low nitrate concentrations, decreased influence of livestock sources, and increased relative contribution of N from WWT lagoons. However, autumn was wetter than normal and received nearly four times the expected precipitation which generated more streamflow ($2.07 \text{ m}^3/\text{s}$ observed at Deadhorse Creek in September, wateroffice.ec.gc.ca). During wetter conditions source differentiation was less clear and it is likely that the waste N contribution to streams was the mixed influence of land-based runoff and point source N from discharging lagoons. Such findings suggest that short-term events, such as lagoon discharges and precipitation events, have the potential to influence the outcomes of N source studies like ours. Furthermore, hydrologic conditions likely dictate the ability of land users to apply N. For example, it is possible that wet autumn conditions would influence agronomic practices, such as post-harvest manure or fertilizer application, which normally take place under drier circumstances, and thus influence the contribution of these N sources during the following snowmelt. Despite the snapshot nature of our study, our findings suggest that both intra-annual variability and interannual variability are important drivers of N source patterns in the RRV. Our conclusion is consistent with those of Ali and English (2019) who posited that selective hydrologic connectedness driven by variation in hydrologic conditions within the Lake Winnipeg watershed is likely a key driver of nutrient transport linking land sources to streams. Our finding that the relative importance of waste N sources varies temporally highlights the need for management strategies that can accommodate variation in the delivery of N to streams.

In addition to hydrologic variability, management strategies should consider the potential for N source patterns to vary because of evolving human land use practices that will likely change nutrient delivery pathways over time. For example, subsurface drainage (e.g., tile drain systems) has recently expanded in the RRV and drain outflows

have been shown to efficiently deliver N directly to streams, especially during periods of high precipitation (Baulch et al. 2019, Kokulan et al. 2019). Systems that promote efficient drainage to improve crop yields may also reduce the residence time of N in soils allowing for less in-soil processing and more bioavailable N to reach streams (Randall and Goss 2008). Organic farming is also increasing in Manitoba (Statistics Canada 2016) and thus the use of manure in place of synthetic fertilizers will likely increase as well. Lastly, improvements of WWT systems in the RRV may result in reduced nutrient loads associated with municipal lagoons; for example, the decommissioning of the Niverville, MB municipal lagoon is believed to have resulted in reduced phosphorus loading to Lake Winnipeg (ECCC 2017). Thus, consistent monitoring of N sources is needed to fully understand the consequences of changes to agronomic activities and municipal upgrades.

The use of both $\delta^{15}\text{N}$ of ammonium and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate in our study allowed us to more comprehensively identify sources of N to RRV streams. For example, mixing model estimates for the ammonium fraction indicated manure was dominant at all sites during snowmelt, whereas estimates for the nitrate fraction indicated manure was dominant at only three sites. However, studies examining the contribution of DIN sources to waterways have typically relied solely on $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate, despite ammonium being an important source of bioavailable N to aquatic biota (Peipoch et al. 2012) and widespread evidence that ammonium from human activities is discharged to rivers (Ribot et al. 2012; Hood et al. 2014). Given sources of ammonium-rich N, such as livestock manure (Bouwman et al. 2013) and wastewater (Van Drecht et al. 2009) are increasing worldwide, we recommend managers and researchers consider the isotopic composition of both species of DIN when attempting to identify N sources to freshwater ecosystems.

The addition of artificial sweeteners as waste-specific tracers in our study aided our ability to identify the roles of livestock manure during snowmelt and human wastewater in spring and summer. The difficulty in distinguishing human and animal waste sources has been a challenge for researchers and managers attempting to identify and trace N sources in mixed-use watersheds like the RRV because their isotopic signatures overlap. Furthermore, uncertainty arising from underdetermined mixing where there are more known sources than the number of isotopes plus one limits the precision of

isotopic mixing models (Parnell et al. 2010, Phillips et al. 2014), for example when $\delta^{15}\text{N}$ is analyzed alone such as for ammonium. We suggest that the addition of artificial sweeteners may reduce some of the uncertainty associated with using stable isotopes in such situations by providing another line of evidence for N source identification. However, some uncertainty remains because the rates and drivers of degradation of artificial sweeteners in stream environments are still relatively unknown. Although saccharin has been shown to degrade quickly (half life 3-12 days) in soils, it is also known to be relatively stable in manure and has been shown to resist degradation in manure stored in manure pits for more than two months (Buerge et al. 2011). Thus, it is possible that saccharin present in manure applied post-harvest in autumn could be preserved in frozen soils until snowmelt resulting in the elevated snowmelt saccharin concentrations we observed from sites with large numbers of pigs in their catchments. Saccharin is also known to be a breakdown product of some sulfonylurea herbicides (Berger and Wolf 1996). However, given the typical timing of herbicide application either near seeding time or post-emergence (Manitoba 2019), it is more likely that the concentrations of saccharin exceeding 100 ng/L in our study are manure related. On the other hand, we found artificial sweeteners were a strong indicator of discharging WWT lagoons and sites where acesulfame, saccharin, and sucralose co-occurred were all confirmed to have recently received effluent discharge by lagoon operators. Therefore, our findings indicate that artificial sweeteners are a useful tool for managers and researchers attempting to elucidate the contributions of land-based livestock operations and urban wastewater to nutrient inputs to waterways.

3.4.2 Summary

We have built upon past studies in the RRV by identifying sources of N to streams using stable isotopes and artificial sweeteners. Furthermore, we have shown that the sources of N to the tributaries of the Red River vary seasonally, likely with the hydrologic patterns of the region. Previous studies have established that a large proportion of the N in the RRV is delivered via snowmelt (Rattan et al. 2017; Rattan et al. 2019). Our study builds on this work by providing evidence that livestock manure N is likely a major contributor to the snowmelt runoff pulse in the RRV. In contrast, human

waste sources became important when municipal lagoons began discharging in the spring and summer when land-based runoff was comparatively less prevalent. Previous studies (Rattan et al. 2017; Rattan et al. 2019; Soto et al. 2019) did not identify specific sources of N to the tributaries nor did they consider N sources associated with ammonium despite the evidence that both nitrate and ammonium occur at high concentrations during snowmelt. Here, we have shown that the estimation of a source's contribution of N to streams should consider both ammonium and nitrate given our finding that ammonium from livestock manure is a key N source in the region.

Our finding that algae readily incorporate N from waste sources, perhaps due to ammonium preference, shows that prairie streams have the potential to function as nutrient assimilators rather than simply as conduits for drainage of farmland runoff. Researchers and managers attempting to manage these sources should be cognizant that N delivery to streams is likely subject to intra- and interannual variation due to changing hydrologic conditions between seasons and years, evolving land use practices, and upgrades to municipal WWT systems. Therefore, we recommend routine monitoring of N sources to detect temporal change in source delivery and inform more effective targeting and timing of nutrient mitigation activities. Stable isotope analysis in particular may be an accessible, cost-effective means of tracking N sources in the environment. However, the combined use of stable isotopes and artificial sweeteners may help to more clearly interpret the contribution of different N sources in mixed use watersheds. We suggest that management of N sources and preservation of stream function at the catchment level may mitigate the amount of bioavailable forms of N from reaching downstream waterbodies such as the Red River and Lake Winnipeg.

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Chapter 4

4 Fate of bioavailable nutrients released to a stream during episodic effluent releases from a municipal wastewater treatment lagoon

4.1 Introduction

Humans rely on rivers for the treatment and transport of wastewater, and consequently, sewage has become one of the largest point-source contributors of nutrients to surface waters worldwide (e.g., Holeton et al., 2011). Globally, human sewage contributes an estimated 6.4 Tg of nitrogen (N) and 1.3 Tg of phosphorus (P) to surface waters annually, with loads expected to triple by 2050 (Van Drecht et al., 2009). Rivers provide water treatment via the transformation and assimilation of nutrients from wastewater, thus removing or retaining N and P and thereby attenuating downstream transport (Martí et al., 2004). However, because N and P in human wastewater effluent is often highly labile, the increasing volume of wastewater entering rivers contributes to eutrophication and associated ecological degradation of aquatic ecosystems (Smith, 2003). Thus, excess nutrient loading to streams and rivers may impair a river's functional capacity to provide wastewater treatment services (e.g., Aristi et al., 2015). Understanding the fate of nutrients in wastewater effluent-receiving streams and rivers is important if humans are to better utilize and manage the treatment service that streams provide.

The fate of bioavailable N and P inputs to streams is mediated through biogeochemical pathways that modulate downstream nutrient retention or removal (Bernot & Dodds, 2005; Withers & Jarvie, 2008). Bioavailable forms of N, nitrate (NO_3^-) and ammonium (NH_4^+), are mainly processed via biosynthetic assimilation (uptake and incorporation into biotic tissues), nitrification (oxidation of NH_4^+ to NO_3^-), and denitrification (dissimilatory reduction of NO_3^- to N_2 , N_2O , or NO gases; Bernot & Dodds, 2005; Kendall et al., 2007). Bioavailable P is retained via assimilation as well as through physical interaction with sediments (e.g., sorption and precipitation reactions; Withers & Jarvie, 2008). The high concentrations of nutrients from wastewater may

exceed the capacity for instream processing (e.g., Haggard et al., 2005; Waiser et al., 2011) leading to export of nutrients to sensitive downstream ecosystems such as lakes and estuaries. Knowledge of the fate of N and P downstream from wastewater outflows is thus needed to improve understanding of the functional capacity of streams to aid in wastewater treatment and to better protect aquatic systems.

Net nutrient uptake lengths (S_{w-net}) and natural abundance stable isotope ratios (e.g., $^{15}\text{N}/^{14}\text{N}$; reported as $\delta^{15}\text{N}$ values) are commonly used to describe nutrient processing in streams receiving point-source nutrient inputs. S_{w-net} describes the average distance travelled by a nutrient molecule before it is either retained (via transformation or assimilation) or released (e.g., from sediments or through remineralization), commonly referred to as net nutrient retention efficiency (Martí et al., 2004; Haggard et al., 2005; Ribot et al., 2012). Previous studies have found that net nutrient uptake lengths below wastewater treatment plant (WWTP) outflows are longer than those typically observed in more pristine systems, indicating decreased nutrient retention efficiency (e.g., Martí et al., 2004; Waiser et al., 2011). $\delta^{15}\text{N}$ values are commonly used to trace the biogeochemical transformation of N. Such studies have been able to detect nitrification (e.g., Gammons et al., 2011; Ribot et al., 2012) and denitrification (e.g., Lofton et al., 2007) below WWTP outflows. Others have shown that primary producers in rivers readily assimilate wastewater N and that uptake of N from continuously discharging WWTPs by algae and macrophytes may occur over many meters (e.g., Ribot et al., 2012) to many kilometers downstream (e.g., Wassenaar et al., 2010; Hood et al., 2014; Kendall et al., 2015).

Unlike previously studied river systems that receive nutrients continuously from WWTPs (e.g., Ribot et al., 2012; Hood et al., 2014), many streams across North America receive wastewater effluent in intermittent pulses from municipal wastewater treatment lagoons (Holeton et al., 2011; US EPA, 2011). Municipal lagoons lack the advanced nutrient-removal technology (i.e., tertiary treatment) common to the WWTPs in large cities (Holeton et al., 2011; US EPA, 2011) and thus more than one third of N and P may not be removed during treatment and therefore is ultimately released into waterways (Chambers, 2001). Lagoons that service rural communities commonly release wastewater

effluent periodically throughout the ice-free months from spring to autumn. Effluent releases may occur in mid-summer when streams are at the height of ecological productivity, or in the early spring or late autumn under cool conditions when stream productivity, and thus capacity for uptake, may be diminished. Indeed, Ribot et al. (2012) determined that the intensity of N cycling below a continuously discharging WWTP outflow varied seasonally between summer and winter with nitrification and denitrification being most active in the warm summer period. However, the fate of nutrients from episodic municipal lagoon effluent release is largely unknown. Thus, improved understanding of the assimilative function of streams could help managers to more effectively utilize ecosystem services to mitigate nutrient impacts from lagoons thus maximizing the wastewater treatment service streams provide.

The objective of this study was to assess the fate of bioavailable nutrients from the episodic release of municipal wastewater lagoon effluent. We examined the longitudinal pattern of concentrations of dissolved inorganic N (DIN) species (NH_4^+ and NO_3^-) and soluble reactive P (SRP) and biogeochemical processing of DIN (assimilation, nitrification, denitrification) downstream from a municipal wastewater lagoon in Deadhorse Creek, Manitoba, Canada. We assessed three separate lagoon effluent releases (summer 2018, summer 2019, autumn 2019) to determine how temporal variation in stream conditions may impact nutrient demand (i.e., nutrient ratios), nutrient retention efficiency (i.e., net nutrient uptake lengths) and biogeochemical processing (i.e., using $\delta^{15}\text{N}$ of DIN). We also assessed the longitudinal patterns of primary producer $\delta^{15}\text{N}$ values to infer assimilation of N from the lagoon during the summer releases. The outcomes of this study will improve knowledge about the transmission and processing of nutrients released from wastewater lagoons to stream ecosystems. Furthermore, our findings highlight the implications of temporal variability (e.g., hydrology, temperature) in stream condition for lagoon releases and will aid managers in understanding the potential of streams to provide wastewater treatment services.

4.2 Materials and Methods

4.2.1 Study Area

Our study was conducted in Deadhorse Creek, a fourth order stream in southern Manitoba, Canada. Deadhorse Creek is located approximately 100 km south-west of Winnipeg (Figure 4.1A). The Deadhorse Creek catchment features fine clay soils, low relief topography, and landcover that has been intensively modified from wetlands and tallgrass prairie prior to the 20th century to approximately 80% agriculture (Annual Crop Inventory 2018, open.canada.ca). Flow in Deadhorse Creek is maintained by an upstream reservoir.

Our study focused on the segment of Deadhorse Creek between the communities of Morden and Winkler (Figure 4.1B). Here, the creek receives effluent from the Morden lagoon, which provides facultative wastewater treatment for approximately 8,000 people and consists of one primary cell and five secondary cells. As a facility serving less than 10,000 people, Morden's lagoon is required to limit the P content of treated effluent to less than 1 mg/L but is not required to limit DIN concentrations (Manitoba Water Stewardship, 2011). Thus, the lagoon is treated using alum precipitation to reduce P as required. Morden's effluent is typically accumulated over the winter months and released episodically to Deadhorse Creek in two (or more) week periods approximately two to five times per year throughout the ice-free season from June to October.

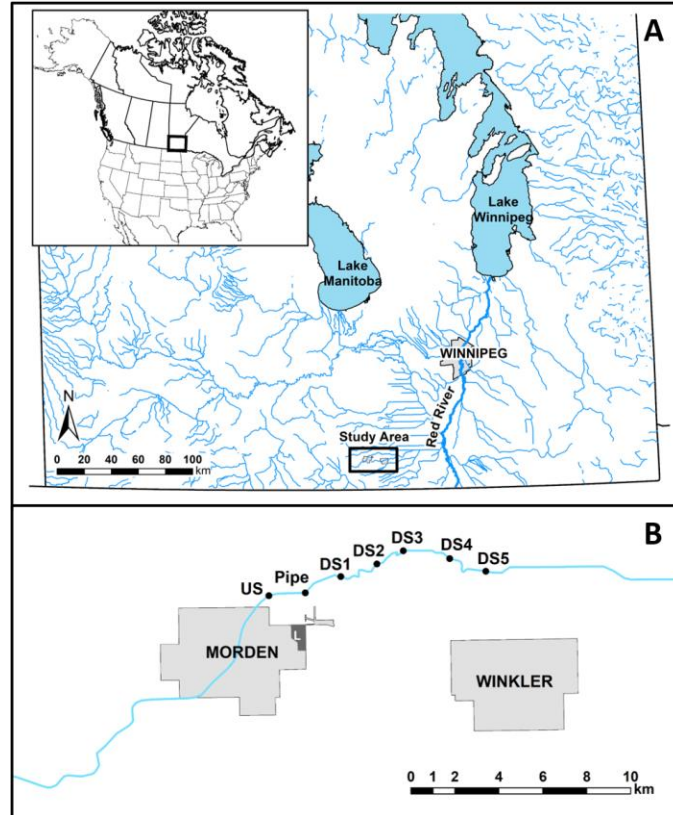


Figure 4.1 A) Map of the study location in Manitoba, Canada, B) The studied segment of Deadhorse Creek is located between the communities of Morden and Winkler and receives wastewater discharge from the Morden wastewater treatment lagoon (dark gray outline, labelled “L”). Study sites are located 1.6 km upstream (US) from the lagoon outflow, at the outflow (Pipe), and at five progressively farther downstream sites (DS1=2.4 km, DS2=4.5 km, DS3=6.5 km, DS4=8.9 km, and DS5=11.3 km).

We sampled seven study sites along Deadhorse Creek (see Table B.1 in Appendix B for site coordinates). First, a control site (US) approximately 1.6 km upstream from the lagoon outflow. Next, an end-of-pipe site at the Morden wastewater treatment lagoon outflow (Pipe, 0 km), and, lastly, a series of five progressively farther downstream sites (DS1 = 2.4 km, DS2 = 4.5 km, DS3 = 6.5 km, DS4 = 8.9 km, and DS5 = 11.3 km) located near successive road crossings. The lagoon outflow pipe sampling location was in a small side channel with a cobble substrate which occasionally dries between lagoon effluent releases and is not sustained by the upstream reservoir. All other sites were

located on the main channel and have fine sediment substrate, some of which have small riparian trees and shrubs (e.g., *Salix* sp.) along their banks (US, DS2, DS4), whereas others (Pipe, DS1, DS3, DS5) have been channelized and larger riparian vegetation cleared. No tributaries or other known point sources of effluent or water (i.e. tile drain outflows) occur along the study area other than roadside ditches that are typically dry outside of precipitation events.

4.2.2 Field Sampling

We sampled the seven study sites during lagoon effluent releases in summer 2018 (late July 2018), summer 2019 (late July 2019), and autumn 2019 (early October 2019). Each release lasted approximately two weeks. Stream discharge measured on the day of sampling in summer 2018 at the nearest active hydrologic gauging station (05OC016, wateroffice.ec.gc.ca) was $0.12 \text{ m}^3/\text{s}$. Instantaneous stream water temperature and pH measurements were taken at the study sites using a YSI ProDSS handheld instrument (YSI Incorporated, Yellow Springs OH, USA) and are presented in Appendix B (Table B2). The average (mean \pm 1 SD) water temperature in summer 2018 was $20.8 \pm 0.9 \text{ }^\circ\text{C}$ and pH was 7.37 ± 0.19 . In summer 2019, stream discharge at the gauging station measuring $0.50 \text{ m}^3/\text{s}$ with average water temperature of $21.04 \pm 0.76 \text{ }^\circ\text{C}$ and pH of 7.81 ± 0.33 . Autumn 2019 was unusually wet and received four times the average expected precipitation (based on 1980 to 2010 normals for Morden, MB; weatheroffice.ec.gc.ca), and stream discharge measured at the gauging station was $5.26 \text{ m}^3/\text{s}$. Average water temperature in autumn at the study sites was $5.2 \pm 0.2 \text{ }^\circ\text{C}$ and pH was 8.05 ± 0.13 .

Water samples were collected at least 72 hours after the initiation of effluent release to ensure effluent had travelled the extent of the study area. Samples for nutrients (total P, total N, DIN, soluble reactive P), stable isotopes of DIN, and acesulfame, an artificial sweetener, were collected with an extendable sampling pole or by hand while wading. All nutrient samples were refrigerated immediately post-sampling and NH_4^+ samples were preserved with sulfuric acid. Nutrient samples were shipped to analytical laboratories within 24 to 48 hours of collection. Samples for DIN stable isotopes were filtered the same day of collection through a rinsed 142 mm Supor® polyethersulfone $0.45 \text{ } \mu\text{m}$ (Pall Corporation, Ann Arbor MI, USA) filter and immediately frozen.

Acesulfame samples were filtered through a rinsed 28 mm 0.45 μm cellulose-acetate syringe filter (Corning Incorporated, Corning NY, USA) in the field and frozen until time of analysis.

Primary producer biota was collected in summer 2018, summer 2019, and autumn 2019 for stable isotope analysis. In summer 2018 and summer 2019, benthic algae samples were collected approximately 24 hours after effluent release had ceased to allow for exposure to the entire two-week release. Benthic algae was collected by hand at each site from three locations in the stream reach and were rinsed in local stream water at each site before they were frozen until time of processing. Algae could not be collected due to a lack of available algae at all downstream sites (DS3 and DS5) due to high water levels in summer 2019 so the free-floating macrophyte duckweed (*Lemna* sp.) was collected as an alternative primary producer when possible. In autumn 2019, primary producer biota was collected approximately 10 days into the effluent release period. In autumn, benthic algae was collected at the upstream site and the lagoon outflow. High water levels and fast flows prohibited wading and benthic algae could not be collected at the five downstream sites. Duckweed was collected in place of algae at DS1, DS3, and DS5, but primary producers could not be collected from sites DS2 and DS4 due to a lack of available organisms.

4.2.3 Laboratory Analysis

Total N (TN), total P (TP), nitrate (NO_3^- ; measured as $\text{NO}_2^- + \text{NO}_3^-$), ammonium (NH_4^+) and soluble reactive P (SRP) concentrations were measured at the National Laboratory for Environmental Testing (NLET), Saskatoon, Canada. Total N was calculated from the addition of total soluble N (measured via alkaline digestion and hydrazine reduction with flow injection analysis) and particulate N (measured via combustion). NO_3^- was measured via cadmium reduction and high-resolution colorimeter continuous flow analysis (CFA). NH_4^+ was converted to ammonia salicylate and measured via high-resolution colorimeter CFA. TP and SRP were measured using stannous chloride and high-resolution colorimeter CFA. Detection limits for TN, NO_3^- , and NH_4^+ were 0.015, 0.010, 0.005 mg N/L, respectively. Detection limit for TP and SRP was 0.002 mg P/L. Results below detection limits are reported as 0.

Acesulfame concentrations were measured at the Canada Centre for Inland Waters in Burlington, Canada, using a suppressed ion chromatography, tandem mass spectrometry method (Van Stempvoort et al., 2011; Spoelstra et al., 2017). Samples were analysed via ion chromatography (IC) using a Thermo Fisher Dionex ICS 5000 system and ion suppression using a Dionex AERS 500 2 mm. The IC was interfaced to an AB Sciex 5500 QTrap MS/MS and operated in the negative electrospray ionization mode. The method detection limit for acesulfame was 2 ng/L.

Isotope ratios were measured by stable isotope ratio mass spectrometry and expressed as delta (δ) values. The ratio of the raw isotopic sample (R_{sample} , e.g. $^{15}\text{N}/^{14}\text{N}$) was compared to a known standard value (R_{standard}) according to:

$$\text{Eq. 1) } \delta X (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1)$$

Where X is the heavy isotope (e.g., ^{15}N). Delta values are reported relative to international isotope standards of atmospheric nitrogen (AIR) for $\delta^{15}\text{N}$ and Vienna standard mean ocean water (VSMOW) for $\delta^{18}\text{O}$. Delta values are multiplied by 1000 and thus reported as per mil (‰).

Primary producer biota (algae and duckweed) samples were freeze-dried, weighed into tin boats, and shipped to the UC Davis Stable Isotope Facility for solid $^{15}\text{N}/^{14}\text{N}$ stable isotope ratio analysis using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 continuous flow isotope ratio mass spectrometer (CF-IRMS) (Sercon Ltd. Cheshire, UK). Accuracy and precision were measured relative to laboratory standards calibrated against international reference materials and were compositionally similar to the sample material. The standard deviation for within run $\delta^{15}\text{N}$ reference material was 0.05‰.

$\delta^{15}\text{N}$ values of ammonium ($\delta^{15}\text{N}\text{-NH}_4^+$) were determined using the Holmes et al. (1998) micro-diffusion method in which sample NH_4^+ is converted to NH_3 and trapped on an acidified glass fibre filter. Fractionation standards were prepared during every sample collection event using ultra-pure water and a laboratory standard material (reagent grade $(\text{NH}_4)_2\text{SO}_4$) with a known $\delta^{15}\text{N}$ value ($\delta^{15}\text{N} = +1.0 \pm 0.2\text{‰}$) to control for possible

fractionation after sample collection and during conversion to NH_3 . Correction for fractionation during collection and processing was minimal ($< 0.6\text{‰}$). Samples were analyzed using Carlo Erba NA1500 elemental analyser interfaced to an IsoPrime CF-IRMS (Elementar, Langensfeld, Germany) at the National Hydrology Research Centre (NHRC) Stable Isotope Laboratory in Saskatoon, Saskatchewan, Canada. The standard deviations for within run reference materials (bowhead whale baleen keratin ($\delta^{15}\text{N} = +14.3\text{‰}$), porcine gelatin ($\delta^{15}\text{N} = +5.1\text{‰}$), laboratory standard $(\text{NH}_4)_2\text{SO}_4$) were $\leq 0.1\text{‰}$.

$\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate ($\delta^{15}\text{N}\text{-NO}_3^-$, $\delta^{18}\text{O}\text{-NO}_3^-$) were determined using Ti(III) to reduce sample NO_3^- to N_2O (Altabet et al., 2019) and then analysed using a trace gas system (Elementar, Langensfeld, Germany) and IsoPrime CF-IRMS at the NHRC Stable Isotope Laboratory. Standard deviations for within-run dissolved NO_3^- reference materials (IAEA NO3 ($\delta^{15}\text{N} = +4.7\text{‰}$, $\delta^{18}\text{O} = +25.6\text{‰}$), USGS34 ($\delta^{15}\text{N} = -1.8\text{‰}$, $\delta^{18}\text{O} = -27.9\text{‰}$), USGS35 ($\delta^{15}\text{N} = +2.7\text{‰}$, $\delta^{18}\text{O} = +57.5\text{‰}$)) were $\leq 0.5\text{‰}$ for $\delta^{15}\text{N}$ and $\leq 1.5\text{‰}$ for $\delta^{18}\text{O}$.

4.2.4 Data Analysis

4.2.4.1 Calculation of nutrient ratios and net nutrient uptake lengths

To demonstrate demand for N and P uptake, we calculated molar TN to TP (TN:TP) and molar DIN to SRP (DIN:SRP) ratios at each sampling location along the length of Deadhorse Creek during each of the three effluent releases to identify the limiting nutrient. Following the Redfield ratio (16:1; Redfield, 1958), when TN:TP was greater than 16, P was considered limiting, and when TN:TP was less than 16, N was limiting. DIN:SRP is considered less reliable as an indicator of nutrient limitation (Dodds, 2003), however, generally when DIN:SRP is greater than 16-20, P is considered limiting and when DIN:SRP is less than 10, N is considered limiting (e.g., Tank & Dodds, 2003).

Net nutrient retention efficiency in Deadhorse Creek was estimated using net nutrient uptake lengths (S_{w-net}). S_{w-net} differs from traditional nutrient uptake length (S_w ; Stream Solute Workshop, 1990) because S_{w-net} is typically used where nutrient

concentrations may saturate uptake, thus providing a measure of net nutrient retention (i.e., the stream is either sink or source; Martí et al., 2004). Estimates of net nutrient uptake lengths over the length of Deadhorse Creek downstream from the lagoon outflow were calculated for NH_4^+ , NO_3^- , and SRP during the three lagoon effluent releases in summer 2018, summer 2019, and autumn 2019.

Calculation of nutrient uptake length requires the use of a simultaneously injected conservative tracer to correct for longitudinal dilution (Stream Solute Workshop, 1990). Previous studies of nutrient uptake below WWTPs have used chloride as a conservative tracer of wastewater discharge (e.g., Martí et al., 2004; Ribot et al., 2012). However, other sources of chloride such as synthetic fertilizers and the application of road salt can complicate the use of chloride as a wastewater tracer. Following publication of the initial studies of artificial sweeteners in the environment (e.g. Buerge et al., 2009; Scheurer et al., 2009), these compounds have increasingly been used as tracers of human sanitary wastewater in groundwater, rivers, and lakes. Acesulfame (ACE) in particular, has several advantages compared to other wastewater tracers, including: 1) it is ubiquitous in sanitary wastewater, 2) it is typically present in effluent at concentrations many orders of magnitude above detection limits, 3) it is very resistant to degradation in the environment, and 4) there are few other sources of ACE to the environment (see Van Stempvoort et al., in review). ACE is the most commonly detected artificial sweetener in groundwater and streams impacted by wastewater (e.g., Wu et al., 2014; Schaidler et al., 2016; Spoelstra et al., 2017, 2020; Van Stempvoort et al., in press), mainly due to its prevalence in consumer products (e.g., diet drinks) and resistance to degradation in the environment. Therefore, ACE is highly suitable as a conservative tracer for calculating nutrient uptake lengths below wastewater outflows. ACE concentrations (Figure B1, Appendix B) were used to correct for physical dilution of effluent due to lateral or vertical inflow (e.g., subsurface-to-surface diffusion) or dispersion (e.g., transient storage) during longitudinal transport following Martí et al. (2004).

S_{w-net} was calculated as described by Haggard et al. (2005) as the negative inverse of the slope of the linear relationship between the natural log of the dilution-corrected nutrient concentration and distance using the following equation:

$$\text{Eq. 2) } C_x = C_0 e^{kx}$$

Where C_x = the dilution-corrected nutrient concentration at distance x (e.g., km), C_0 is the nutrient concentration at the outflow (e.g., distance 0), k is nutrient change coefficient (e.g., 1/km), and S_{w-net} is equal to $-1/k$. Positive values of S_{w-net} indicate nutrient retention (i.e., the stream is a nutrient sink), whereas negative values of S_{w-net} indicate nutrient release (i.e., the stream is a nutrient source). Stream flow during lagoon release was dominated by effluent as evidenced by ACE (Figure B1, Appendix B), thus because site-specific flow data was lacking, we did not correct for upstream nutrient concentrations prior to calculation of S_{w-net} .

4.2.4.2 Measurement of instream DIN processes using $\delta^{15}\text{N}$

The relationships between the isotopic composition of NH_4^+ and NO_3^- and their concentrations were used to infer nitrification, denitrification and biosynthetic assimilation because their mediating biogeochemical reactions favour reaction of molecules containing the lighter isotope (e.g., ^{14}N or ^{16}O) resulting in enrichment of the heavier isotopes (^{15}N , ^{18}O) in the residual substrate relative to the product (Mariotti et al., 1981; Kendall et al., 2007). For example, nitrification should result in the concomitant decrease of NH_4^+ and increase of NO_3^- , and the increase of $\delta^{15}\text{N-NH}_4^+$ values over the length of the stream (e.g., Gammons et al., 2011; Hood et al., 2014). Likewise, denitrification should result in the decrease of NO_3^- and increase in $\delta^{15}\text{N-NO}_3^-$ values (e.g., Lofton et al., 2007). Furthermore, denitrification causes $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate to increase in an approximate 2:1 ratio (Kendall et al., 2007).

Biotic assimilation of N from wastewater can be estimated using $\delta^{15}\text{N}$ values of biota because wastewater N is typically enriched in ^{15}N relative to other common anthropogenic N sources due to the action of biogeochemical processes that occur in lagoons, such as denitrification and volatilization (Kendall et al., 2007). However, because primary producers preferentially assimilate the lighter isotope, they will typically have $\delta^{15}\text{N}$ values that are depleted by some fractionation factor relative to their source, unlike trophic enrichment of $\delta^{15}\text{N}$ observed for higher order consumers (Kendall et al., 2015). Thus, assimilation should result in a correlation between the $\delta^{15}\text{N}$ of primary

producers and $\delta^{15}\text{N}$ of their DIN source (e.g., Ribot et al., 2012), albeit $\delta^{15}\text{N}$ of primary producers will be lower than $\delta^{15}\text{N}$ of their DIN source (e.g., Kendall et al., 2015).

Spearman's rank correlations (expressed using Spearman's correlation coefficient, r) were used to test the strength of associations between DIN species and their $\delta^{15}\text{N}$ values (e.g., Ribot et al., 2012). Strong negative correlations between ammonium and $\delta^{15}\text{N-NH}_4^+$ were considered indicative of nitrification being the dominant process. Similarly, negative correlations between nitrate and $\delta^{15}\text{N-NO}_3^-$ were considered to be indicative of denitrification being the dominant process. Additionally, we examined the relationship between $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ for which a significant positive correlation could be considered indicative of denitrification (Kendall et al., 2007). Correlations between $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$ and the $\delta^{15}\text{N}$ values of primary producer biota were also examined to assess DIN uptake by primary producers. Longitudinal patterns of primary producer $\delta^{15}\text{N}$ values were examined for all three discharge events, but correlations between $\delta^{15}\text{N}$ values of primary producer biota and those of ammonium and nitrate were only examined for the summer 2018 and summer 2019 sampling events when benthic algae or duckweed could be collected at all sites. Given our sample size ($n=6$ downstream sites including the pipe), we adopted a significance level of $\alpha = 0.10$ for all statistical tests used to assess instream DIN processing to better balance the likelihoods of Type I and Type II errors. All analyses were conducted using R v. 3.6.1 (R Core Team, 2019).

In addition to examining correlations between primary producer $\delta^{15}\text{N}$ and $\delta^{15}\text{N-DIN}$ to infer assimilation of N from wastewater, we used $\delta^{15}\text{N}$ values of primary producers to estimate the relative contribution of lagoon N to primary producer biota (benthic algae or duckweed) at each site using the Bayesian-based stable isotope mixing models package *simmr* v. 0.4.1 in R (Parnell, 2019). *Simmr* employs a Markov chain Monte Carlo algorithm to produce a posterior distribution used to estimate the proportional contribution of each N source expressed as mean \pm SD. The isotopic composition and associated uncertainties of wastewater and other known N sources in southern Manitoba were assumed based on a past study of N sources to the Manitoba portion of the Red River (Soto et al. 2019), to which Deadhorse Creek is a tributary.

Sources included in the models and their associated $\delta^{15}\text{N}$ values include untransformed synthetic N fertilizer ($\delta^{15}\text{N} = 0 \pm 3\text{‰}$), nitrification of/and ammonium occurring in soils (e.g., nitrified fertilizers or mineralization of organic matter; $\delta^{15}\text{N} = -5 \pm 5\text{‰}$), and wastewater from the lagoon or manure ($\delta^{15}\text{N} = +15 \pm 5\text{‰}$). Manure is typically similarly enriched in ^{15}N compared to human waste (Kendall et al., 2007) and was considered a plausible source upstream from the lagoon. Because utilization of nitrogen by algae is typically accompanied by a negative isotopic discrimination due the preferential uptake of ^{14}N , a conservative discrimination of -4.0‰ (Kendall et al., 2015) was incorporated into mixing models. The default burn-in (1000) and iteration values (10,000) in simmr produced satisfactory convergence of all models (Gelman diagnostic <1.1).

4.3 Results

4.3.1 Longitudinal patterns of nutrient concentrations

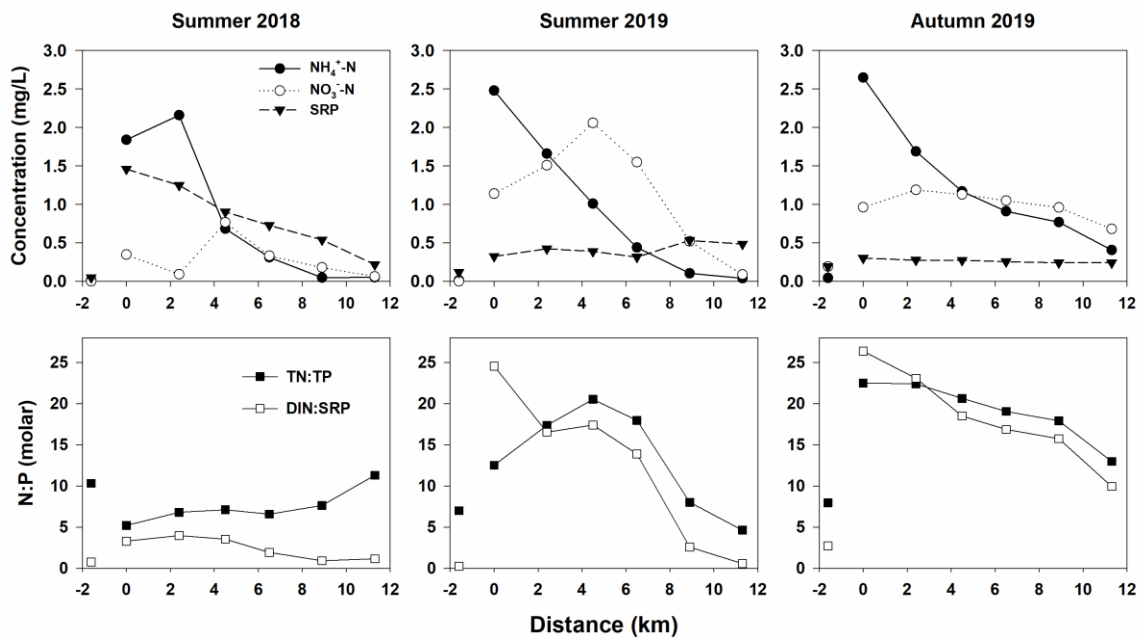


Figure 4.2 Top: Concentrations of ammonium (NH_4^+ , filled circles), nitrate (NO_3^- , open circles), and soluble reactive phosphorus (SRP, filled triangles); and, bottom: total nitrogen (TN) to total phosphorus (TP; filled squares) and dissolved inorganic nitrogen (DIN) to SRP (open squares) molar ratios measured upstream (-1.6 km), at the lagoon outflow (0 km), and five progressively farther (2.4 to 11.3 km)

downstream sites during three wastewater lagoon effluent releases in Deadhorse Creek, Manitoba, Canada in summer 2018, summer 2019, and autumn 2019.

In summer 2018, NH_4^+ at the upstream site (-1.6 km) was 0.018 mg N/L and increased approximately 100 times to 1.84 mg N/L at the lagoon outflow pipe (0 km), reached its maximum 2.4 km downstream (2.16 mg N/L), then decreased to approximately 0.05 mg N/L over the remaining distance (Figure 4.2). Summer 2019 NH_4^+ increased from 0.014 mg N/L upstream to 2.48 mg N/L at the pipe before decreasing to 0.04 mg N/L at the farthest site (11.3 km). In autumn 2019, NH_4^+ similarly increased from 0.045 mg N/L upstream to 2.65 mg N/L at the pipe. However, depletion of NH_4^+ was slower and the concentration at the final site was 0.41 mg N/L.

NO_3^- was below detection limits at the upstream site during both summer sampling events (Figure 4.2). In summer 2018, NO_3^- decreased from the lagoon outflow pipe (0.35 mg N/L) to 2.4 km downstream (0.09 mg N/L), before increasing to a maximum concentration of 0.77 mg N/L, 4.5 km downstream. Over the remaining sites, NO_3^- approximately halved in concentration over each 2 km interval to 0.06 mg N/L at the final site, 11.3 km downstream. In summer 2019, downstream NO_3^- was greater at all sites than the previous summer. NO_3^- increased from 1.14 mg N/L at the lagoon outflow to 2.06 mg N/L at the site 4.5 km downstream from the pipe, but decreased quickly (>1.0 mg N/L change) over the remaining sites to 0.09 mg N/L at 11.3 km. In autumn 2019, NO_3^- was above detection at the upstream site (0.19 mg N/L) and increased to 0.96 mg N/L at the lagoon outflow pipe. Autumn NO_3^- increased to 1.19 mg N/L at the first site (2.4 km) downstream, decreased an average of 0.07 mg/L per 2 km interval to 0.96 mg N/L by 8.9 km, then decreased to 0.68 mg N/L at 11.3 km.

In summer 2018, SRP increased approximately 30 times from 0.05 mg P/L upstream to the 1.46 mg P/L at the lagoon outflow pipe (Figure 4.2). Below the outflow, SRP concentrations decreased by an average of 0.25 mg/L per 2 km interval to 0.22 mg P/L at 11.3 km. In contrast, in summer 2019 and autumn 2019, SRP increased less than three times (≤ 0.2 mg P/L) between the upstream site and the lagoon outflow. In summer 2019, SRP concentration was 0.12 mg P/L upstream and, rather than decreasing

longitudinally, varied between 0.32 and 0.53 mg P/L along the length of the stream segment. In autumn 2019, SRP concentrations increased only 0.11 mg P/L, from 0.19 mg P/L upstream to 0.30 mg P/L at the lagoon outflow. Autumn SRP concentrations changed only 0.06 mg P/L between the lagoon outflow and the farthest site 11.3 km downstream.

4.3.2 Nutrient ratios

Molar TN:TP during the summer 2018 effluent release was 10.32 upstream and ranged from 5.22 at the lagoon outflow pipe to 11.30 at the farthest downstream (11.3 km) site (Figure 4.2). Molar DIN:SRP during the same release was 0.80 at the upstream site and was greatest (3.99) at the first downstream site (2.4 km) and decreased to 0.94 at 8.9 km. In summer 2019, the upstream TN:TP was 7.01 and was greatest at the site 4.5 km downstream from the pipe (20.51) and smallest (4.64) at the farthest (11.3 km) site. DIN:SRP in summer 2019 was 0.26 at the upstream site and, unlike TN:TP, was greatest at the pipe (24.56), but decreased to 0.59 by 11.3 km downstream. In autumn 2019, TN:TP was 7.99 at the upstream site and was greatest at the lagoon outflow pipe (22.49) and the site 2.4 km downstream from the pipe (20.41) and declined to 12.98 at the farthest site 11.3 km from the lagoon outflow. DIN:SRP during the autumn release was 2.73 at the upstream site and downstream ratios were similar to those for TN:TP and were greatest at the pipe (26.37) and first downstream site (23.07) declining to 9.95 by 11.3 km downstream.

4.3.3 Net nutrient uptake lengths

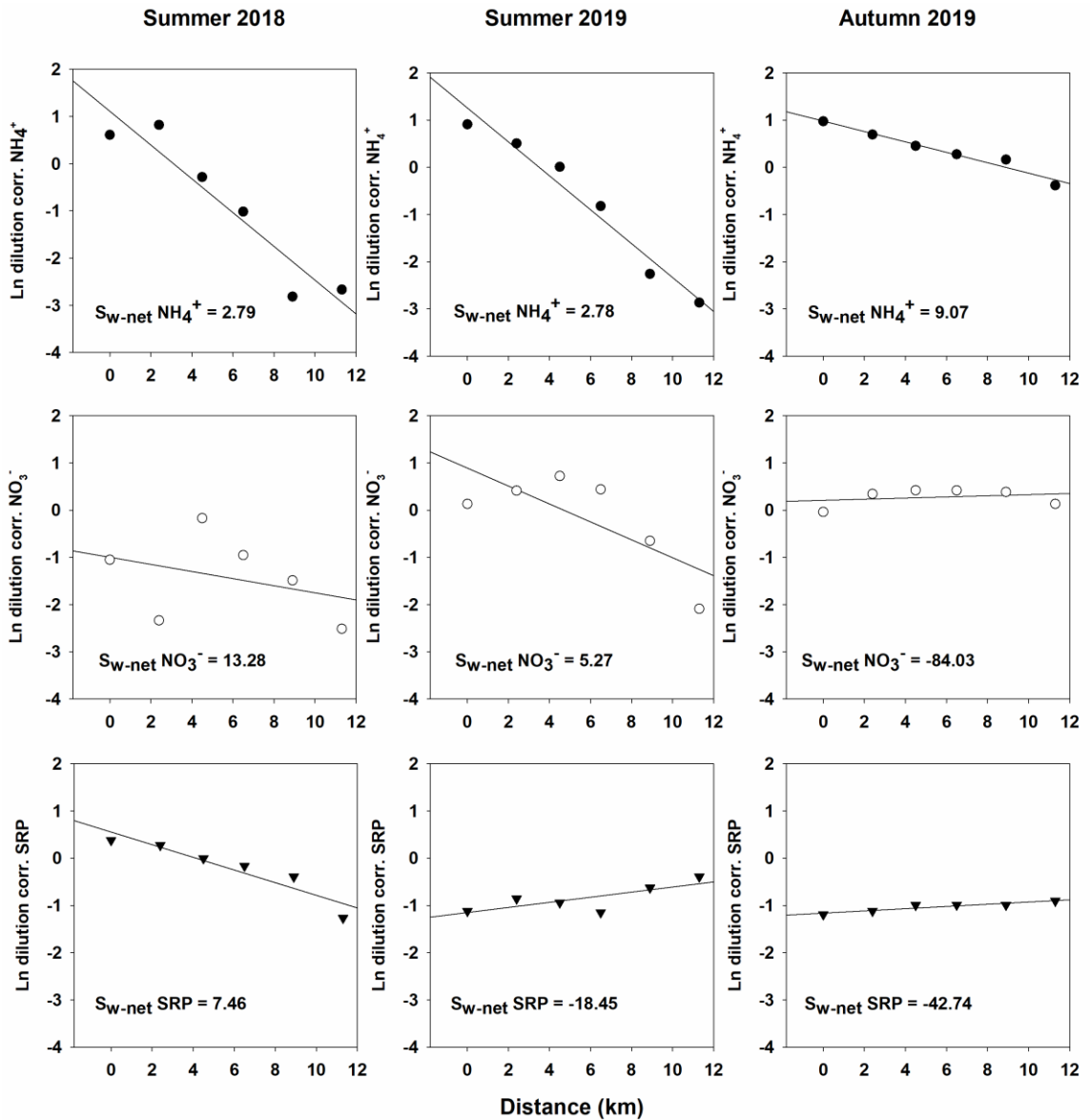


Figure 4.3 Relationship (indicated by trendline) between the natural log of the acesulfame dilution-corrected nutrient concentration and distance for ammonium (NH_4^+ , filled circles), nitrate (NO_3^- , open circles), and soluble reactive phosphorus (SRP, filled triangles) used to calculate net nutrient uptake lengths downstream of the lagoon outflow (0 km) and five progressively farther (2.4 to 11.3 km) downstream sites during three wastewater lagoon effluent releases in Deadhorse Creek, Manitoba, Canada in summer 2018 (left), summer 2019 (centre), and

autumn 2019 (right). Net nutrient uptake lengths (S_{w-net} , km) for NH_4^+ , NO_3^- , and SRP during each release are displayed in the lower corner of each panel.

In summer 2018, based on acesulfame concentrations, dilution of the effluent over the length of the study segment ranged from 5% at 2.4 km downstream to 28% at farthest downstream site (11.3 km). S_{w-net} values calculated for the entire length of the downstream segment of Deadhorse Creek were 2.79 km for NH_4^+ , 13.28 km for nitrate, and 9.07 km for SRP (Figure 4.3). S_{w-net} of NH_4^+ was negative ($S_{w-net} = -11.33$ km) between the lagoon outflow pipe and the first downstream site (2.4 km) but became positive over the remainder of the stream segment. The downstream pattern for NO_3^- was more hump-shaped than for NH_4^+ and SRP. S_{w-net} for NO_3^- at the 4.5 km and 6.5 km sites was negative ($S_{w-net} = -5.10$ and -65.34 km, respectively) before becoming positive again from 8.9 to 11.3 km downstream. SRP had positive S_{w-net} values over the length of the stream segment in line with the consistent decrease in SRP concentration.

In summer 2019, dilution was minimal (less than 1%) over the length of the study segment until the last downstream site where dilution was 38%. S_{w-net} over the length of the downstream segment for NH_4^+ was similar to summer 2018 ($S_{w-net} = 2.78$ km), but shorter for NO_3^- ($S_{w-net} = 5.27$ km; Figure 4.3). For NO_3^- , the relationship was again hump-shaped, and S_{w-net} was negative for the first three sites downstream from 2.4 to 6.5 km before becoming positive for the last two (8.9 to 11.3 km). S_{w-net} was -18.45 km for SRP and was negative over the entire reach except at third downstream site (6.5 km) where it was positive but large ($S_{w-net} = 232.1$ km).

Dilution was greatest in autumn 2019, ranging from 18% at the first downstream site (2.4 km) and increasing by an average of 12% per 2 km interval to 67% at the site 11.3 km downstream. S_{w-net} values for NH_4^+ during the autumn 2019 release were positive consistent with the decrease in concentration over the stream segment (Figure 4.3). However, the overall S_{w-net} for NH_4^+ was longer ($S_{w-net} = 9.07$ km) than during the summer discharges. S_{w-net} was -84.03 km for NO_3^- and again followed a hump-shaped rather than linear pattern. However, unlike the summer events, S_{w-net} values for NO_3^- were

negative the entire length of the downstream segment. S_{w-net} for SRP in autumn 2019 was -42.74 km.

4.3.4 Longitudinal patterns of $\delta^{15}\text{N}$

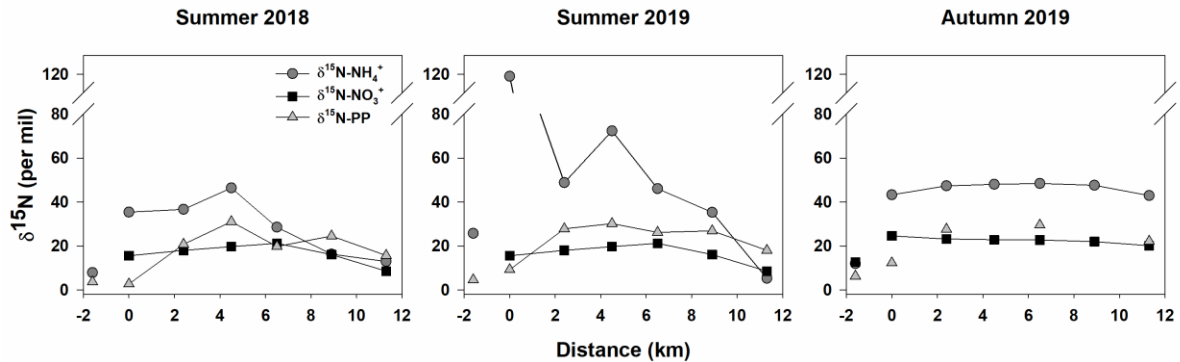


Figure 4.4 $\delta^{15}\text{N}$ of dissolved inorganic nitrogen species ammonium ($\delta^{15}\text{N-NH}_4^+$) and nitrate ($\delta^{15}\text{N-NO}_3^-$), and primary producers (benthic algae or duckweed, $\delta^{15}\text{N-PP}$) measured upstream (-1.6 km) and at increasing distance downstream from the lagoon outflow (0 to 11.3 km) during three lagoon effluent releases in summer 2018, summer 2019, and autumn 2019 in Deadhorse Creek, Manitoba, Canada.

In summer 2018, $\delta^{15}\text{N-NH}_4^+$ initially increased along the stream segment from +7.9‰ at the upstream site to +35.4‰ at the lagoon outflow pipe, before further increasing to a maximum of +46.4‰ at 4.5 km and then decreasing over the last three sites to +13.0‰ at 11.3 km (Figure 4.4). In summer 2019, $\delta^{15}\text{N-NH}_4^+$ was +25.8‰ upstream, extremely enriched at the outflow pipe (+119‰), then decreased to +48.8‰ at the site 2.4 km downstream. $\delta^{15}\text{N-NH}_4^+$ then increased by more than 20‰ to +72.3‰ at the site 4.5 km downstream before decreasing nearly 70‰ to +5.3‰ at the 11.3 km site. The longitudinal pattern of $\delta^{15}\text{N-NH}_4^+$ in autumn 2019 was less variable with an approximately 30‰ increase from +12.0‰ upstream to +43.3‰ at the lagoon outflow. Moreover, $\delta^{15}\text{N-NH}_4^+$ increased only ~5‰ from the outflow to the next downstream site (2.4 km) and remained approximately +48‰ for the remainder of the stream segment before decreasing to +42.9‰ at the farthest site 11.3 km downstream.

$\delta^{15}\text{N-NO}_3^-$ could not be determined upstream during either summer sampling event, because NO_3^- was below detection. In summer 2018, $\delta^{15}\text{N-NO}_3^-$ was +4.1‰ at the lagoon outflow and increased to approximately +23‰ between 2.4 km and 4.5 km before decreasing to +8.1‰ at 11.3 km (Figure 4.4). In summer 2019, $\delta^{15}\text{N-NO}_3^-$ increased by ~2‰ per 2 km from +15.6‰ at the pipe to +21.2‰ at 6.5 km before decreasing to +8.6‰ at the farthest (11.3 km) site. In autumn 2019, $\delta^{15}\text{N-NO}_3^-$ was measured upstream (+12.7‰) and increased to +24.6‰ at the pipe, but did not increase longitudinally over the first few sites as it did during the summer releases. Conversely, in autumn 2019 $\delta^{15}\text{N-NO}_3^-$ decreased by 4.5‰ over the 11.3 km length of the stream segment to +20.1‰.

4.3.4.1 $\delta^{15}\text{N}$ of primary producers

In summer 2018, $\delta^{15}\text{N}$ of primary producers was +3.7‰ at the upstream site and increased over the first two sites from +2.8‰ at the pipe to +31.1‰ at 4.5 km before decreasing to +15.7‰ at the farthest site 11.3 km downstream (Figure 4.4). In summer 2019, $\delta^{15}\text{N}$ of primary producer biota was +4.7‰ upstream, and again increased from +9.3‰ at the pipe to +30.2‰ at 4.5 km before decreasing to +18.0‰ at 11.3 km. The same pattern appeared again in autumn 2019, though some sites were missing samples (DS2 at 4.5 km and DS4 at 8.9 km). The upstream $\delta^{15}\text{N}$ of primary producer biota was +6.3‰ and increased to +27.6‰ at the first downstream site (2.4 km) and eventually decreasing to +22.2‰ at the farthest downstream site (11.3 km).

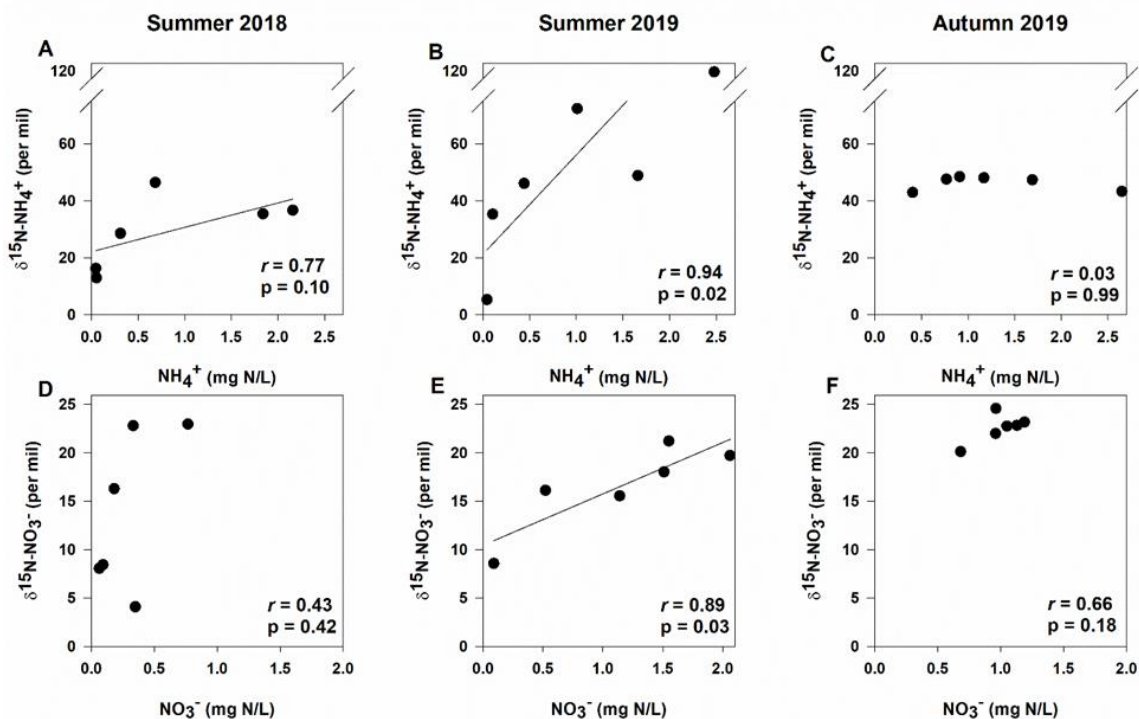


Figure 4.5 Relationships between the concentrations of ammonium (NH₄⁺; top) and nitrate (NO₃⁻; bottom) and their respective δ¹⁵N values downstream from the lagoon outflow during three wastewater lagoon effluent releases in Deadhorse Creek, Manitoba, Canada during A, D) summer 2018, B, E) summer 2019 and C, F) autumn 2019. Spearman's correlation coefficient (r) and p-values and shown are for Spearman's rank correlations.

Spearman's rank correlations for the relationships between NH₄⁺ and δ¹⁵N-NH₄⁺ had $p \leq 0.10$ in summer 2018 ($r = 0.77$, $p = 0.10$; Figure 4.5A) and summer 2019 ($r = 0.94$, $p = 0.02$; Figure 4.5B). However, there was no relationship in autumn 2019 ($r = 0.03$, $p = 0.99$; Figure 4.5C). The relationship between NO₃⁻ and δ¹⁵N-NO₃⁻ had a Spearman's correlation coefficient of 0.89 and p-value of 0.03 and in summer 2019 (Figure 4.5E). However, there was no correlation between NO₃⁻ and δ¹⁵N-NO₃⁻ in either summer 2018 ($r = 0.43$, $p = 0.42$; Figure 4.5D) or autumn 2019 ($r = 0.66$, $p = 0.18$; Figure 4.5F). No relationship between δ¹⁵N-NO₃⁻ and δ¹⁸O-NO₃⁻ was observed for any of the three lagoon effluent releases (Figure B2, Appendix B).

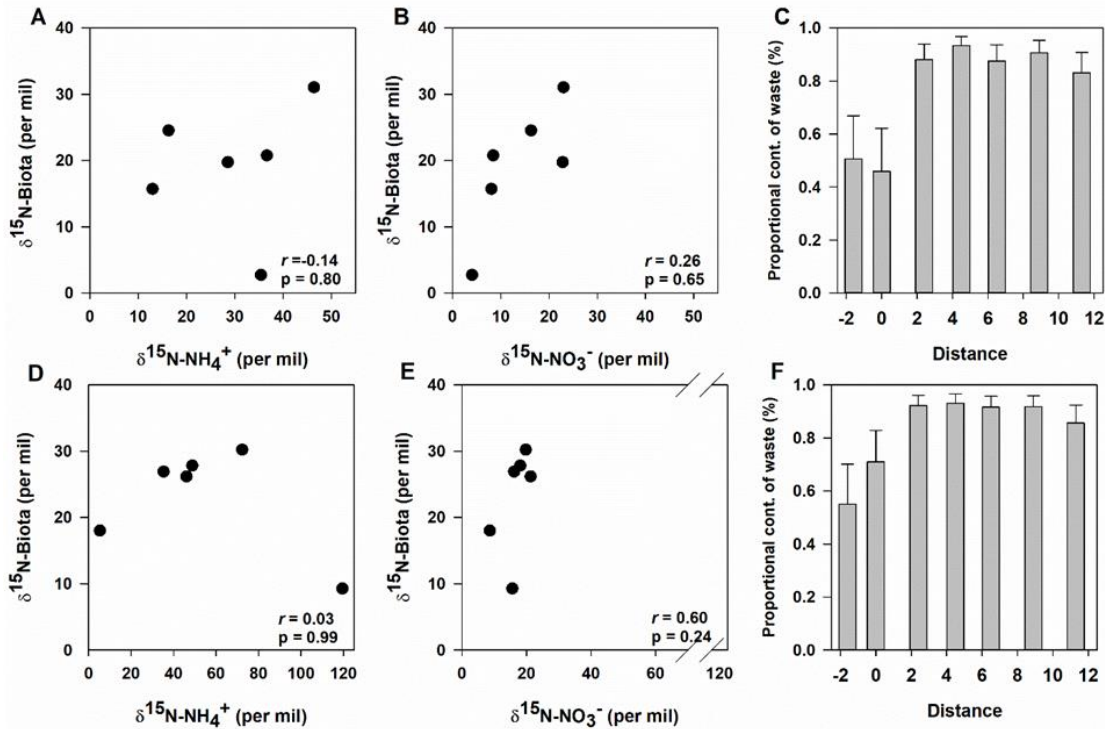


Figure 4.6 Relationships between ammonium $\delta^{15}\text{N}$ ($\delta^{15}\text{N}$ - NH_4^+ ; A, D) or nitrate ($\delta^{15}\text{N}$ - NO_3^- ; B, E) and primary producer (benthic algae and duckweed) $\delta^{15}\text{N}$, and the estimated % proportional contribution of waste (lagoon + manure; cont. of waste) nitrogen to primary producers (C, F) during lagoon effluent releases in summer 2018 (top) and summer 2019 (bottom). Spearman's correlation coefficient (r) and p -values and shown are for Spearman's rank correlations.

There was no relationship between $\delta^{15}\text{N}$ - NH_4^+ and $\delta^{15}\text{N}$ of downstream primary producers in the summers of 2018 ($r = 0.14$, $p = 0.80$; Figure 4.6A) or 2019 ($r = 0.03$, $p = 0.99$; Fig. 4.6D). There was also no relationship between $\delta^{15}\text{N}$ - NO_3^- and $\delta^{15}\text{N}$ of downstream primary producers in summer 2018 ($r = 0.26$, $p = 0.65$; Fig. 4.6B) or summer 2019 ($r = 0.60$, $p = 0.24$; Figure 4.6E). Despite the lack of relationships between $\delta^{15}\text{N}$ of the DIN species, mixing models estimated that waste N accounted for greater than 80% of primary producer tissue N at all sites downstream from the lagoon outflow. In summer 2018, N from waste sources (manure or sewage) contributed an estimated $50 \pm 16\%$ at the upstream site, $46 \pm 16\%$ to algae at the pipe and ranged from $83 \pm 7.7\%$ to $93 \pm 3.4\%$ at the downstream sites (Figure 4.6C). In summer 2019, N from waste sources

contributed $55 \pm 15\%$ at the upstream site, $71 \pm 12\%$ to algae sampled at the pipe and ranged from $86 \pm 6.8\%$ to $93 \pm 3.5\%$ in primary producer biota at the downstream sites (Figure 4.6F).

4.4 Discussion

Our results demonstrate that processing and retention of nutrients from episodic release of municipal wastewater lagoon effluent varies among the measured nutrient fractions. Our findings also show that timing of release is important because nutrient processing and retention appears to depend on hydrologic and thermal conditions. Furthermore, our results indicate that although biota take up N from wastewater, biotic assimilation does not appear to substantially reduce nutrient concentrations in Deadhorse Creek during effluent releases. From our findings we hypothesize that a combination of N limitation and high upstream P concentrations likely influences the extent to which biota can assimilate nutrients from wastewater effluent.

The fate of DIN in Deadhorse Creek appears to depend on the hydrologic and thermal conditions at the time of lagoon discharge. DIN processing was most efficient during the summer discharges, which is not unexpected given the biogeochemical reactions that transform DIN are microbially mediated and thus temperature dependant (e.g., Boulétreau et al., 2012). For example, we observed longer uptake lengths for NH_4^+ in autumn ($S_{w\text{-net}}$ approx. 9 km) versus summer ($S_{w\text{-net}}$ approx. 2.8 km) and NO_3^- retention in autumn was negligible ($S_{w\text{-net}}$ approx. -84 km). Furthermore, there was no correlation between NH_4^+ and $\delta^{15}\text{N}\text{-NH}_4^+$ or NO_3^- and $\delta^{15}\text{N}\text{-NO}_3^-$ in autumn when stream temperatures were cool (approximately 5 °C) and flows were more than 10 times higher than in summer. Ribot et al. (2012) observed similarly lower rates of nitrification and denitrification in a wastewater-recipient stream during winter and suggested cool temperatures inhibited microbial activity. However, our findings are confounded by the co-occurrence of lower temperatures with high flows observed in autumn 2019. As such, it is unclear if reduced nutrient processing in Deadhorse Creek can be entirely attributed to changes in water temperature or if the interacting effect of high flows reduced uptake capacity due to decreased transit time of nutrients (e.g., Triska et al., 1989). Furthermore, greater dilution of effluent during autumn relative to summer suggests additional

nutrients may have entered the stream channel from outside sources (e.g., runoff), which would have likely affected nutrient retention efficiency.

The longitudinal pattern of SRP concentrations was more variable among discharge events than observed for DIN concentrations. For example, S_{w-net} for SRP was positive in summer 2018 and lagoon outflow SRP concentrations were reduced 85% by the final site. However, in summer 2019, S_{w-net} values for SRP were negative and the concentration at the final site was nearly 150% greater than it was at the lagoon outflow. We hypothesize that the differences in P retention between sampling events are driven by biotic and/or abiotic supply and demand for P. Indeed, the upstream nutrient ratios (TN:TP, DIN:SRP) we observed, as well as those from previous studies in Deadhorse Creek (Carlson et al., 2013), suggest N is limiting. Moreover, background SRP concentrations in Deadhorse Creek measured at the upstream site were high, thus low demand for P may have influenced uptake. For example, the SRP concentrations we observed at the upstream site ranged from 0.05 mg P/L in summer 2018 to 0.19 mg/L in autumn 2019. Given that previous studies suggest stream algae SRP saturation occurs at 0.025 mg/L (Hill et al., 2009), the biotic demand for P in Deadhorse Creek may have been met before P from lagoon wastewater entered the stream.

Our findings indicate that Deadhorse Creek may act as either a sink (e.g., summer 2018) or a source (e.g., summer 2019, autumn 2019) for SRP. For example, in summer 2019, we observed negative S_{w-net} values over the entire length of the stream segment and dilution was less than 1% at all but the farthest downstream site, thus it is likely that Deadhorse Creek's sediment was the source of P. In their study of a prairie stream receiving tertiary treated sewage effluent, Waiser et al. (2011) found uptake lengths for SRP to be in the order of tens to hundreds of kilometers and were often negative indicating net nutrient release rather than retention. Indeed, the Waiser et al. (2011) study, and others (e.g. Jarvie et al., 2012), have shown that sediments rich in P from continual exposure to nutrient-rich effluent have the potential to become a P source to the overlying water. There was at least one previous discharge into Deadhorse Creek from the Morden lagoon in early spring 2019 prior to the summer 2019 sampling event, but we are not aware of a discharge occurring prior to sampling in 2018. Thus, it is possible that

previous effluent exposure in combination with high background SRP had satisfied the relatively low demand for P in Deadhorse Creek well before the lagoon effluent release in summer 2019.

NH_4^+ was most efficiently processed as evidenced by short, positive uptake lengths relative to NO_3^- and SRP. NH_4^+ also exhibited the greatest proportional reduction in concentration from the pipe to the last downstream site (approx. 85 to 98%). However, the dominant process behind the longitudinally decreasing pattern we observed for NH_4^+ is unclear. We observed a positive correlation between NH_4^+ and $\delta^{15}\text{N-NH}_4^+$ during both summer discharges, making nitrification, which should produce negative correlations, unlikely to be the dominant process. The lack of correlations between $\delta^{15}\text{N}$ of primary producers and $\delta^{15}\text{N-NH}_4^+$ suggests biotic assimilation also does not play a significant role in N uptake. Adsorption of NH_4^+ to sediment is another mechanism known to occur in fluvial ecosystems (Bernot & Dodds, 2005). Adsorption to clay particles is known to favour the retention of ^{15}N in the adsorbed NH_4^+ (Karamanos & Rennie, 1978) thereby depleting the pool of $^{15}\text{N-NH}_4^+$ in the water column. As such, adsorption has been suggested as the mechanism responsible for the longitudinal decrease in $\delta^{15}\text{N-NH}_4^+$ accompanying decreasing NH_4^+ in regions with clay soils (Nilsson & Widerlund, 2017). Given the clay-rich soils and sediment inherent to our study area, we suggest adsorption of ammonium may be the process responsible for the observed pattern of decreasing $\delta^{15}\text{N-NH}_4^+$ in Deadhorse Creek.

Nevertheless, the decrease in NH_4^+ was accompanied by an increase in nitrate concentrations over the first several downstream sites (4.5 to 6.5 km) during the summer effluent releases. Indeed, similar findings of short NH_4^+ uptake lengths and concomitant increases in NO_3^- (e.g., Martí et al., 2004; Haggard et al., 2005) have been attributed to nitrification. Therefore, it is possible that nitrification played a secondary role in N transformation during effluent release because nitrification has been shown to play a key role in summer and/or low flow N processing in many wastewater-recipient streams (Wasier et al., 2011; Ribot et al., 2012; Bernal et al. 2020). We suggest future research in Deadhorse Creek should include an examination of nutrient fate over the course of an

entire lagoon effluent release event to account for variation in effluent composition and better capture the true extent of the NH_4^+ attenuation processes occurring.

The longitudinal pattern for NO_3^- was hump-shaped, suggesting a shift from nitrification (i.e. producing NO_3^-) to denitrification (i.e. removing NO_3^-) mid-way along the stream segment, a pattern that has been observed in other wastewater-recipient streams (e.g., Ribot et al., 2012). The lack of correlations between $\delta^{15}\text{N}$ of primary producers and $\delta^{15}\text{N}\text{-NO}_3^-$ supports the hypothesis that processing of NO_3^- occurred via denitrification rather than biotic assimilation. However, the correlation between NO_3^- and $\delta^{15}\text{N}\text{-NO}_3^-$ was positive in summer 2019, contrary to the expected negative correlation if denitrification were the dominant process. Furthermore, the lack of correlations between $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ do not support denitrification as a key N removal process in Deadhorse Creek. The correlation between NO_3^- and $\delta^{15}\text{N}\text{-NO}_3^-$ was significant only in summer 2019; however, both summer 2018 and 2019 nitrate $S_{\text{w-net}}$ values were positive. Furthermore, NO_3^- was reduced 82 to 92% at the final downstream site during the summer effluent releases. Yet, the lack of correlations between $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ do not support denitrification as a key N removal process in Deadhorse Creek. It is possible that denitrification was occurring in the sediment (e.g., Gooding and Baulch, 2017; Inwood et al., 2007), but was not captured by our sampling of the overlying stream water. Indeed, it is possible for benthic denitrification to be a major process in aquatic ecosystems without resulting in a noticeable ^{15}N and ^{18}O enrichment of NO_3^- in the overlying water column (Kendall et al., 2007). Therefore, future research is needed to conclusively identify the mechanism responsible for nitrate attenuation in Deadhorse Creek, and examination of N cycling in the sediment may be of particular interest.

The results of our stable isotope mixing models indicated that waste N was the dominant source of N to primary producers at the downstream sites, even with a conservative fractionation factor. However, the results at the lagoon outflow pipe were less conclusive, possibly reflecting the influence of municipal stormwater or atmospheric N depleted in ^{15}N (Kendall et al., 2007) on algae growing in the side channel hosting the effluent pipe. Along the main channel of Deadhorse Creek, primary producer $\delta^{15}\text{N}$ exceeded $\delta^{15}\text{N}\text{-NO}_3^-$ and the longitudinal pattern of primary producer $\delta^{15}\text{N}$ appeared to

generally follow that of $\delta^{15}\text{N-NH}_4^+$ suggesting primary producers are assimilating effluent NH_4^+ . Indeed, preferential uptake of NH_4^+ by primary producers in streams receiving wastewater has been shown elsewhere (Hood et al., 2014; Ribot et al., 2012). Such findings suggest biotic uptake of lagoon N was occurring. However, we observed no correlations between the $\delta^{15}\text{N}$ of primary producers and the $\delta^{15}\text{N}$ of either potential DIN source. The age of the collected primary producers is unknown, therefore sources of N from upstream may be contributing to primary producer N content, though it is unlikely this contribution is significant given the low upstream DIN concentrations and likelihood that N is limiting. It is thus possible that the supply of N during release of effluent exceeds the capacity of primary producers to assimilate it at a rate that would influence overall N retention.

4.4.1 Conclusion

The findings of our study indicate that effluent releases during the warmer months when flows are low may be most effective for nutrients to be transformed and removed. However, such events likely have greater ecological impact on the stream and may impair ecological function (e.g., Aristi et al., 2015). For example, the low dilution during the summer effluent releases suggests stream flow in Deadhorse Creek is entirely effluent dominated in summer. Effluent-dominated streams are vulnerable due to contaminant inputs, flow modification, and rapid changes in dissolved oxygen in addition to the high nutrient concentrations reported here (Brooks et al., 2006). Indeed, Bernal et al. (2020) also reported greater biogeochemical cycling during low flow periods in a semi-arid Spanish stream receiving WWTP effluent, however these processes were limited in their effect on DIN and SRP. In contrast, effluent releases in the cooler months or during high flows may have little impact on ecological function (e.g., Ribot et al., 2012), but are likely to have a greater effect on the downstream environment. Depending on management goals, protection of vulnerable downstream ecosystems may be prioritized over stream condition. For example, in southern Manitoba, P from human activities has been implicated in the eutrophication of Lake Winnipeg (Schindler et al., 2012), thus it is likely that water resource managers here may weigh protection of the lake more heavily than that of stream ecosystems.

The findings of our study also indicate that the large pulses of nutrients delivered during episodic wastewater effluent releases have the potential to overwhelm stream capacity for removal and retention even in summer and consequently provide limited downstream protection. Furthermore, the high nutrient concentrations in effluent may become toxic to aquatic life. For example, Waiser et al. (2011) reported toxic levels of ammonium (e.g., exceeding 1.24 mg/L) in a prairie stream receiving WWTP effluent, concentrations that were also exceeded at the first two sites below the lagoon outflow in our study regardless of season. We suggest it may be more effective for lagoons to release wastewater effluent in smaller amounts continuously rather than in large pulses to better maximize the ability of streams to transform and assimilate nutrients from wastewater. Indeed, the community of Morden has proposed to upgrade the existing wastewater lagoon to discharge continuously to Deadhorse Creek in the future (City of Morden, 2017). However, our findings also suggest it is unlikely that stream ecosystem functions will be effective in reducing downstream P from Morden's wastewater effluent if supply from upstream is not also reduced. Ultimately, a holistic approach that considers both wastewater effluent quality and upstream land use is needed for water treatment to be a viable ecosystem service in effluent-recipient streams like Deadhorse Creek, regardless of whether the goal is to preserve stream condition or to protect downstream waterbodies.

4.5 References

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Chapter 5

5 General Discussion

There has been increasing recognition of the need to protect the functional capabilities of streams to modulate the flow of energy and materials through landscapes in order to maintain river ecosystem services (Johnson et al., 2019; Yates et al., 2019). However, many rivers are located in highly modified “anthromes” (Ellis et al., 2010), wherein changes to the landscape due to anthropogenic activities have caused rivers to lose much of their original functionality (Johnson et al., 2019). For example, rivers that have been heavily modified to enhance water transport via widening and channelization can attenuate large flow events, but have lost much of the ecological function of natural channel forms that support biodiversity and nutrient cycling (Castro & Thorne, 2019, Johnson et al., 2019). Furthermore, modifications that benefit land users, such as improving water transport, may be detrimental to downstream ecosystems due to increased movement of nutrients and other chemicals (Ali & English, 2019). Aquatic ecosystems thus require protection if they are to deliver the benefits humans rely upon (Vörösmarty et al., 2010).

Water resource managers need to strike a balance between river condition and ecosystem services (Yates et al., 2019), but to do so first requires the identification of causes (i.e., human activities) of ecological impairment and their related proximate drivers (i.e., nutrients). Furthermore, identification of key sources of nutrients to streams and subsequent knowledge of nutrient fate is critical to aid in the development of effective, targeted management strategies. However, such information remains lacking in vulnerable regions, such as the Manitoba Red River Valley (RRV), where cumulative effects of stressors associated with human activities have imperiled stream condition. Accordingly, the research presented in this thesis aimed to assess drivers of stream ecological condition on regional and local scales by first establishing causal linkages between human activities and stream condition, second, identifying the relative contributions of anthropogenic nutrients to streams, and third, examining the fate of nutrients influenced by stream functions.

5.1 Summary of Research Findings

5.1.1 Causal linkages between human activities and stream ecological effects

In intensively utilized landscapes like the RRV stream ecological condition can be impaired by the myriad stressors. However, clear linkages between causes of impairment and stream condition are often not known due to the complex nature of such regions (Ormerod et al. 2010). The research presented in Chapter 2 of this thesis used ecological causal assessment, an inference-based evidence weighting method of assessment (Norton et al., 2015), to demonstrate causal linkages between specific nutrient-producing human activities and stream ecological effects in the RRV. I found that the presence of wastewater treatment in stream catchments was the cause of stream ecological effects best supported by empirical evidence. However, clear linkages between causes associated with agriculture and stream ecological effects were absent despite widespread agriculture in the region.

These findings were contrary to watershed agriculture being the long-hypothesized driver of impaired water quality in Lake Winnipeg (e.g., Schindler et al., 2012). The reasons for such disparate findings for streams versus the lake are likely two-fold; the first of which is data limitation. Available biomonitoring data for the region lacks a temporal component and as such the effects of landscape modification over time have not been well-captured. Furthermore, the intensification of agriculture over the past two centuries in the RRV has created a largely homogeneous landscape with modified stream channels dominated by hardy generalist species (e.g., Milani, 2013; Tyrell, 2015). Therefore, existing studies in the region have likely been unable to detect ecological effects associated with agriculture due to the lack of both spatial and temporal variability in landscape structure.

The second reason that I found wastewater to be more clearly linked to stream condition than agriculture is likely because ecological sampling occurs during the warm ice-free months when stream productivity is high. However, much of the nutrient load

from the landscape is delivered to Lake Winnipeg during snowmelt and hydrologic connectivity between land and lake typically decreases as the ice-free season progresses (Shrestha et al., 2012; Corriveau et al., 2013). Moreover, the typical timing of stream sampling (spring and summer) temporally co-occurs with municipal wastewater lagoon release into streams in the RRV. There is thus a mismatch between the timing and sources of nutrient delivery to the streams as opposed to the lake. Therefore, management of nutrient-producing human activities in the RRV to protect stream condition requires a different approach than the management strategies for Lake Winnipeg. However, many potential causes of impairment of stream condition have yet to be studied in the RRV such as the effects of contaminants and climate change. Furthermore, the role of other nutrient fractions such as dissolved organic nitrogen (DON) have not been studied in RRV streams. Though an examination of DON was beyond the scope of this thesis, others have found that DON has the potential to contribute to the bioavailable N pool (Kaushal & Lewis Jr., 2005) and thus DON from both agricultural activities (e.g., application of urea fertilizers) and in wastewater may play a role in N cycling in the RRV. Thus, the outcomes of the research presented in Chapter 2 identified wastewater as a priority management area needed to protect tributaries but also highlighted the need for more research to inform an integrative strategy to protect both streams and lake.

5.1.2 Temporal change in nutrient sources to streams in the RRV

The findings presented in Chapter 2 highlighted the need for better understanding of intra-annual variation in nutrient delivery to streams in the RRV. Previous studies in the RRV have identified the spatial extent of different nutrient-producing human activities (e.g., crop production, livestock, human wastewater) in the region (Yates et al., 2012), and there is evidence of their effects on stream condition (Yates et al., 2014; Yates et al., 2018, Painter et al., 2020). Furthermore, it is known that nutrient delivery to streams in the region generally follows seasonal hydrologic patterns (Corriveau et al., 2013; Rattan et al., 2017). However, the actual contribution of individual nutrient-producing human activities to RRV streams has not been clear, nor has there been confirmation of seasonal source variation. The research presented in Chapter 3 helps to

fill this knowledge gap by identifying sources of anthropogenic nitrogen (N) to RRV streams and how the importance of those sources varies seasonally.

I found that anthropogenic sources of N to RRV streams shifted seasonally from primarily diffuse sources to point sources as the seasons progressed. Specifically, my findings demonstrated the importance of livestock manure during the snowmelt nutrient pulse when hydrologic connectivity was highest, whereas site-specific sources of N such as point-source wastewater effluent were more important during spring and summer when hydrologic connectivity was low. These findings provide further evidence of the importance of wastewater to RRV streams and is consistent with my hypothesis that wastewater is an important driver of stream condition because timing of delivery coincides with stream ecological activity.

The findings presented in Chapter 3 also have important implications for Lake Winnipeg. First, because low ecological productivity and high flows are typical during snowmelt, it is likely that much of the livestock manure N is lost downstream. Second, during the warmer months when hydrologic connectivity is low, nutrients from land-based human activities are unlikely to be transported to the lake. In the warmer months, it is likely that the biogeochemically driven stream functions (e.g., denitrification, biological assimilation) that transform and remove N from the water column are most active (Ribot et al., 2012). However, I presented evidence that N from wastewater lagoons released to tributaries contributed to the N in the Red River, which suggests that streams are not able to attenuate all of the N from point-source wastewater. Indeed, it has been shown that high concentrations of bioavailable N, such as from wastewater, decrease N removal efficiency (Mulholland et al., 2008). Thus, wastewater likely presents a risk to both stream (e.g., Chapter 2) and lake conditions.

5.1.3 Fate of nutrients from wastewater treatment lagoon effluent

Municipal wastewater lagoons are prevalent across the Lake Winnipeg Basin (LWSB 2006; Saskatchewan 2014). Lagoons release nutrient-rich effluent to many tributaries in the RRV in large pulses lasting one or more weeks during the ice-free months from May to October. The research presented in Chapters 2 and 3 highlighted the

importance of nutrients in municipal lagoon wastewater effluent to stream conditions in the RRV, both due to its potential to degrade stream condition (Chapter 2) and because evidence suggests RRV streams do not entirely attenuate downstream transport of nutrients from wastewater (Chapter 3). The research presented in Chapter 4 assessed the fate of bioavailable nutrients from lagoon wastewater effluent in an RRV stream during three episodic effluent releases during warm conditions when flows were low (summer 2018, 2019) and cool conditions when flows were high (autumn 2019).

My findings showed that the large nutrient pulse from the episodic release of municipal wastewater effluent has the potential to overwhelm the nutrient attenuation capacity of streams. I linked this finding to two conclusions. First, the upstream supply of nutrients to the stream was an important driver of downstream nutrient demand. Upstream concentrations of bioavailable phosphorus (P) were consistently higher than concentrations found to saturate uptake in other studies (e.g., Hill et al., 2009, Jarvie et al., 2018) and N appeared to be limiting in the study stream. Therefore, though I found evidence of the transformation and assimilation of bioavailable N during summer releases, there appeared to be great potential for P export to downstream ecosystems.

Second, I found that nutrients were not well-retained and thus likely exported when water temperature was cool and flows high. There are no regulations governing the condition of streams during municipal wastewater release and thus effluent releases either early (e.g., in May) or late (e.g., in September) in the year under cool and/or wet conditions are not uncommon. Furthermore, municipalities are granted exceptions to release effluent outside of the warmer months if lagoon capacity is reached before the permitted release period or if they cannot meet nutrient reduction limits within the permitted release period (Manitoba Environmental Assessment and Licensing, n.d.). Because stream ecological activity is low during such times, nutrients are not likely to have immediate effects on stream condition. However, less functional capacity for nutrient transformation and uptake likely results in most of the nutrient load being exported downstream to Lake Winnipeg.

5.2 Significance

The body of work presented in this thesis collectively shows that the nutrient-producing human activities on the RRV landscape that drive ecological effects in streams may not necessarily drive the same effects downstream in Lake Winnipeg. Indeed, there appears to be a dichotomy between drivers of stream condition and drivers of lake water quality. When most nutrients are delivered to the lake (e.g., snowmelt), the streams are less likely to be affected because stream productivity is limited by cold temperatures and high flows associated with snowmelt runoff. Whereas when nutrients are most likely to cause stream effects (e.g., spring and summer), they are less impactful to the lake because a) both nutrient loads and hydraulic discharge are typically smaller overall than those during snowmelt, and b) stream function may provide some degree of downstream protection. Thus, there is a temporal mismatch between nutrient delivery and nutrient-attenuating stream function.

The delivery of nutrients from diffuse human activities (e.g., fertilizer or manure runoff) to downstream ecosystems such as the Red River or Lake Winnipeg is governed by the regional hydrologic patterns of the RRV (Ehsanzadeh et al., 2012; Shrestha et al., 2012). Downstream nutrient transport is likely further compounded by the fact that many streams are modified to enhance rapid water transport off the land. Therefore, much of the diffuse nutrient load likely moves through stream channels early in the year and has little effect on stream ecological function. In contrast, the work presented here has shown that point source wastewater is an important driver of stream condition in the RRV. This may be especially true because large pulses of nutrients from municipal lagoons are released directly to streams during spring and summer when streams are most likely to experience the effects of nutrient-driven impairment such as algae and macrophyte overgrowth and decreased dissolved oxygen availability.

Nutrient-driven stream impairments lead to decreased stream function and loss of ecosystem services (Biggs et al., 2017). For example, evidence from the causal assessment presented in Chapter 2 suggests wastewater increases stream primary productivity and respiration indicating that wastewater likely promotes autotrophic and heterotrophic activity and biomass growth (Aristi et al., 2015). Stimulated ecological

communities may perform more stream functions (e.g., assimilate, transform, or remove nutrients). However, subsidy can become stress (e.g., Aristi et al., 2015; Sutton et al., 2011) whether due to the detrimental effects of other wastewater constituents (e.g., contaminants such as pharmaceuticals and personal care products, Carlson et al., 2013) or due to eutrophication effects like algae overgrowth and fish kills (Sutton et al., 2011). Therefore, management of anthropogenic inputs to streams is critical for the preservation of stream condition and ecosystem services.

The findings in Chapter 4 included nutrient ratios that suggested N was limiting in Deadhorse Creek during three sampling events in summer 2018, summer 2019 and autumn 2019. N limitation in Deadhorse Creek has been shown previously (Carlson et al., 2013) and it is likely that long-term N limitation is not unique to Deadhorse Creek given that other streams in the RRV are similar. For example, Rattan et al. (2017) measured total N (TN) and total P (TP) from 11 RRV streams during snowmelt, spring, summer, and autumn in 2010, 2013, and 2014. Molar nutrient ratios calculated from mean TN and TP data from the Rattan et al. (2017) study indicated N limitation during 10 out of the 12 sample events ($TN:TP \leq 16$, *sensu* Redfield, 1958). Indeed, nutrient data from the 14 streams sampled as part of the research presented in Chapter 3 indicates many streams are either N or N+P limited, especially outside of the snowmelt period (Figure 5.1). Furthermore, supply of dissolved P (mean >0.29 mg P/L) was well above limiting thresholds reported elsewhere (e.g., 0.05 mg P/L from Jarvie et al., 2018) in all seasons. Similarly, mean TP data from the Rattan et al. (2017) study exceeded 0.3 mg/L during all sampling events. Thus, the findings for Deadhorse Creek presented in Chapter 4 are most likely also true for other streams in the RRV.

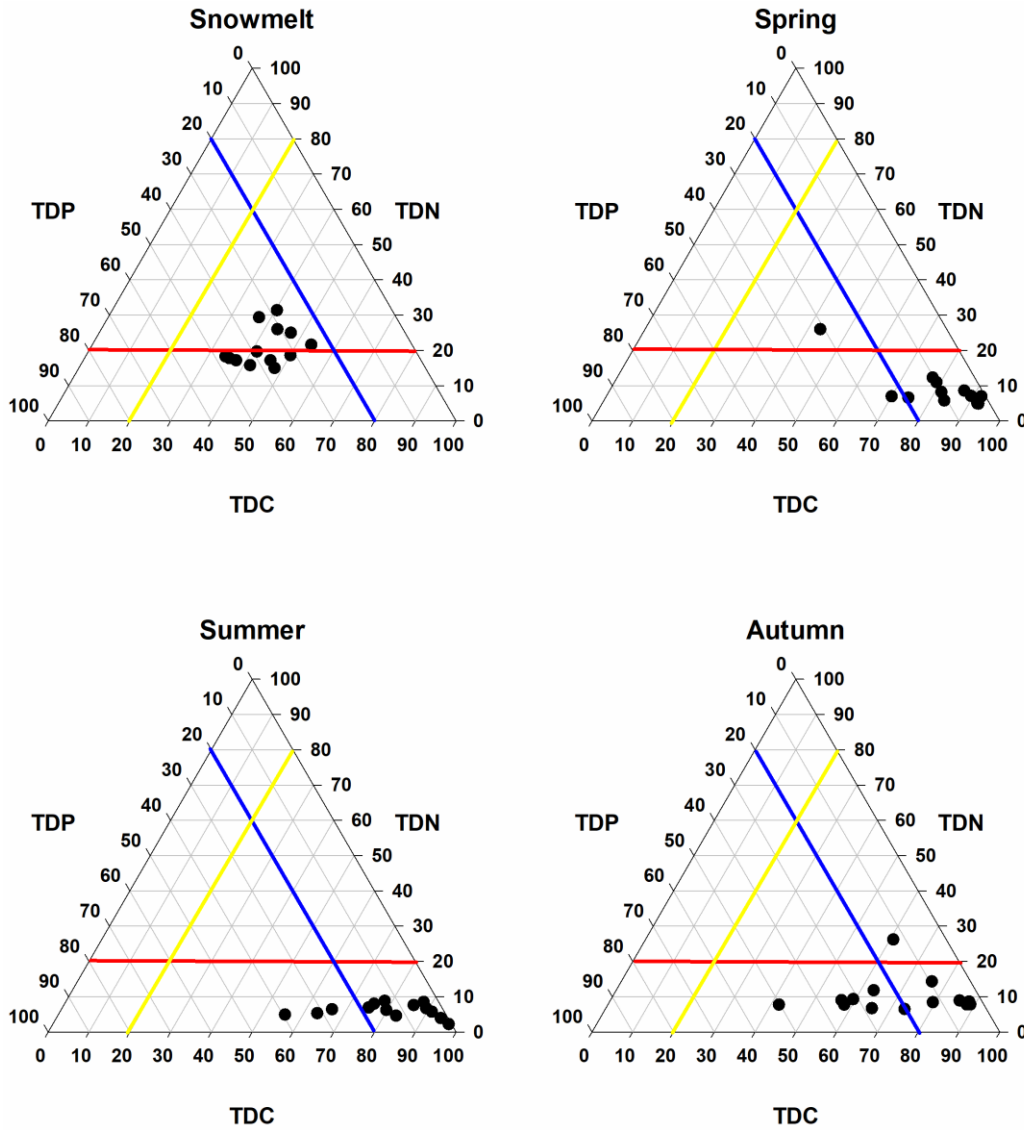


Figure 5.1 Ternary diagrams showing the stoichiometric ratios of total dissolved carbon (TDC), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) in 14 streams in the Red River Valley, Manitoba sampled during snowmelt, spring, summer, and autumn 2019. The center of the plot represents balanced Redfield 106C/16N/1P ratios. Values to right of the blue line are considered P depleted, values to the left of the yellow line are considered C depleted, and values below the red line are considered N depleted. Corners represent co-depletion (e.g., bottom right corner = N+P depletion). Ternary diagrams are based on Smith et al. 2017. The term depletion is used to refer to stoichiometric depletion relative to the Redfield ratio.

If N is limiting in streams across the RRV, large inputs of bioavailable N (e.g., ammonium from wastewater or manure) would be more impactful to streams than P inputs and may explain, in part, why streams don't respond to diffuse agricultural inputs as they do to point source inputs from wastewater. However, P has largely been implicated in the eutrophication of Lake Winnipeg (Schindler et al., 2012) and as such P reduction has been the main goal of nutrient reduction strategies in the region (Ulrich et al., 2016). Yet, the efficacy of nutrient management based solely on P reduction has been called into question because such management strategies often overlook the role the stream network plays in nutrient reduction and controlling downstream transport (Jarvie et al., 2013). Thus, it may be that the emphasis on P reduction in the RRV has come at the cost of preserving stream function.

The capacity of anthropogenic nutrients to overwhelm assimilative processes suggests there is a need for management strategies in the RRV to consider both nutrient reduction and stream function. Wastewater lagoons are likely the most easily managed source in the RRV because they require licenses and testing of effluent to operate (Manitoba Water Stewardship, 2011) and as point sources, can be readily monitored. Furthermore, better management of wastewater lagoons could be accomplished through investments in the upgrading of infrastructure, something that has already been proposed by some of the RRV's largest municipalities (e.g., City of Winkler, 2014; City of Morden, 2017). For example, the community of Morden has proposed upgrading their current episodically releasing lagoon to a more advanced facility that will release effluent continuously to Deadhorse Creek during the ice-free season (City of Morden, 2017). Such upgrades would allow for continuous release of smaller amounts of effluent rather than large pulses. Smaller volumes of effluent would allow for stream functions to play a larger role in nutrient attenuation as opposed to overwhelming capacity for uptake as was observed in Deadhorse Creek in Chapter 4.

The findings presented in Chapter 4 also showed that elevated nutrient supply from the watershed, likely diffuse agricultural sources, may overwhelm instream nutrient

processing. Management of diffuse agricultural sources of nutrients is more difficult because it is more challenging to identify diffuse nutrient sources spread over landscapes than point-sources (Carpenter, 1998), and while the work presented in Chapter 3 has improved this knowledge for the RRV, reductions require the participatory action of many stakeholders (Ulrich et al., 2016). The use of agricultural beneficial management practices (BMPs) have been widely touted in the RRV, but it remains unclear which BMPs are truly effective in cold regions with clay soils like the RRV (Baulch et al., 2019). Furthermore, an important knowledge gap identified in Chapter 2 was the lack of studies assessing impact of BMPs on stream ecological condition. However, the use of small dams to create reservoirs to reduce runoff and encourage nutrient processing have shown promise in the region (e.g., Tiessen et al., 2011; Gooding & Baulch, 2017) and may return some ecological function to heavily modified waterways. Nevertheless, it is unknown whether BMPs can offset nutrient delivery to streams and Lake Winnipeg in the absence of reduced nutrient application to the land.

Overall, management goals may differ depending on the ecosystem services most valued in each catchment or region and a one-size-fits-all management strategy for the entire RRV, while possibly effective for the lake (i.e., reduce P), is not necessarily effective for each community or conservation district. For example, Deadhorse Creek is primarily used for waste assimilation and in addition to inputs from the Morden lagoon described in Chapter 4, there are two more wastewater lagoon outflows located downstream. Here, it may be more imperative to improve nutrient attenuation capacity than to improve recreational opportunities. On the other hand, promoting stream functions to improve nutrient assimilation in a river that is valued for its contribution to community greenspace may be beneficial to the lake, but would not benefit the local stakeholders. Thus, there is a need for the development of management strategies that balance local and regional benefits.

5.3 Recommendations and future research

The overarching goal of the research in this thesis was to provide new knowledge about the interactions between nutrients from human activities and streams in the RRV. However, my findings also identified important knowledge gaps. For example, in

addition to the lack of linkages between agriculture and stream condition, the ecological causal assessment presented in Chapter 2 identified a lack of studies connecting hydrologic modification and climatic variability to stream condition in the RRV. Climate and hydrology have been well-linked to the degradation of water quality and the onset of algal bloom formation in Lake Winnipeg (Kling et al., 2011, Schindler et al., 2012, Ali & English, 2019). However, there are no studies to date linking these drivers to stream ecology in the RRV despite predictions that climatic change will cause changes to stream flow regimes, loss of endemic biodiversity, and incursion of invasive species (Brooks, 2009, Whitehead et al., 2009). Furthermore, climate change is likely to increase the occurrence of large nutrient pulses to streams as flow regimes become flashier (Kaushal et al., 2014) which, as shown in Chapter 4, may overwhelm stream capacity for nutrient uptake.

The importance of hydrologic variation appeared throughout the research presented here and has important implications for the interpretation of this thesis. Particularly, the findings presented in Chapters 3 and 4 are only snapshot examples of nutrient source and fate in the RRV because intra- and interannual hydrologic variability could result in different outcomes. For example, runoff generation and contributing area may change depending on regional climatic conditions (Mahmood et al., 2017). A stream that may not be hydrologically connected to an area of intense land use one year could be connected the next, which would influence the outcomes of nutrient source identification (Chapter 3). Furthermore, such connections may also control upstream supply of nutrients to streams, influencing the likelihood of downstream transport (Chapter 4). For example, Ali and English (2019) found that selective hydrologic connectivity and associated nutrient transport within the watershed was an important driver of large algal bloom formation on Lake Winnipeg. Therefore, future studies in the RRV are needed that compare source contributions in different years (e.g., wet year versus dry year) in addition to different seasons.

The identification of the above knowledge gaps thus supports the need for development of an adaptive monitoring strategy for the RRV which includes multiple spatial (e.g., site and region) and temporal (e.g., season, year) scales to capture the

variation inherent to the region. Such strategies are designed to build upon current knowledge by asking well-posed scientific questions, carrying out data collection and analysis, and using the findings to highlight new knowledge gaps (Lindenmayer & Likens, 2010). Lindenmayer and Likens (2010) describe the need to first develop a conceptual model explaining the current understanding of a given system before continuing to build an adaptive monitoring program. This thesis has filled important gaps in the RRV's conceptual model of drivers of stream condition (Figure 5.2), particularly around wastewater, but also highlights future research needs to better understand how ultimate physical drivers, such as climate and hydrologic regime, impact stream condition and to examine the interplay of such drivers with known anthropogenic stressors in the region. Furthermore, local-scale drivers, such as sediment and contaminants, that enter streams with landscape runoff (Pip, 2005; Liu et al., 2015) and human wastewater (Carlson et al., 2013) have thus far been under-studied in the RRV.

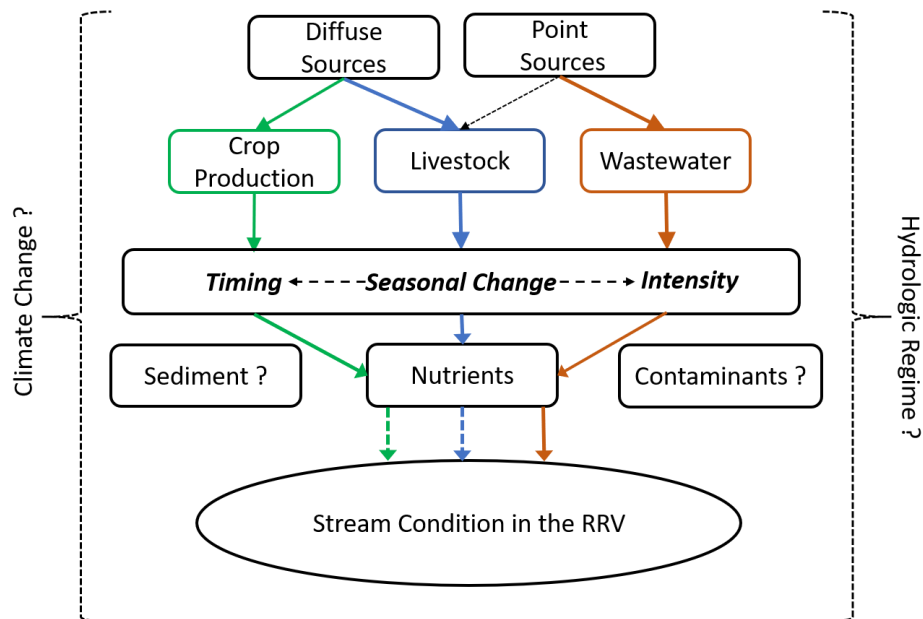


Figure 5.2 Conceptual diagram illustrating linkages between major diffuse and point sources of anthropogenic impact (crop production, livestock, wastewater) on stream condition in the RRV. This thesis established clear linkages between wastewater (orange arrows) and stream condition (Chapters 2, 3, and 4). This thesis also identified delivery of nutrients from livestock manure (blue arrows) and

agricultural fertilizer (green arrows) to streams (Chapter 3). However, this thesis did not establish strong linkages (dashed coloured arrows) between these drivers and stream condition (Chapter 2) though they have been shown to have some interaction with stream biota (Chapter 3). A key overarching finding of this thesis is that seasonal variation appears to temper the effects of anthropogenic drivers on stream condition. Other drivers shown are not well-linked to stream condition (question marks) and require further research.

5.4 Conclusion

Streams in heavily utilized landscapes like the RRV have a key role to play in the delivery of ecosystems services to land users, for example by providing water transport (e.g., for flood regulation), water supply (e.g., for irrigation) or water purification (e.g., to assimilate wastes). However, small streams also act as modulators, controlling the retention and transport of nutrients to vulnerable downstream ecosystems like Lake Winnipeg (Ali & English, 2019). Much of the monitoring and management attention is often focused on these downstream ecosystems. However, this thesis and other research from the RRV (e.g., Yates et al., 2014; Chesworth, 2016; Rattan et al., 2017) suggests there is a need to shift some of that focus upstream. Small streams collectively drive much of the water and nutrient transport through large watersheds (Alexander et al., 2007), thus the development of stream-based management strategies would ensure better water security for local water users, as well as aid in the protection of downstream rivers and lakes.

5.5 References

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Appendices

Appendix A: Evidence scoring for ecological causal assessment

Table A.1 Individual scoring of strength of causal linkages between candidate causes and individual pieces of evidence for the ecological casual assessment of Red River Valley, Manitoba tributaries (prior to synthesis into Table 2.3). Arrows indicate direction of effect (increase/decrease). See Table 2.1 for a summary of terms and Table 2.2 for the scoring rubric. Acronyms that appear in this table are described in the footnotes below.

Candidate Cause	Candidate Proximate Cause	Ecological Effect	Evidence Description	Reference	Evidence of Effect in RRV				Body of Supporting Evidence	
					Co-Occurrence	Sufficiency	Interaction	Causal Pathway	References	Score
¹ WWT	nutrients	↑ ² GPP	Human WWT is strongly positively associated with GPP in RRV streams	Yates et al. (2013) <i>Freshwater Biol</i>	+	³ NE	NE	NE	Aristi et al. (2015); Bernot et al. (2010); Gücker et al. (2006)	+
Urban	unclear	↑ GPP, ⁴ ER	GPP($r^2=0.48$) and ER ($r^2=0.80$) significantly positively associated with % urban land use in RRV stream catchments	Yates et al. (2014) <i>Ecol Indic</i>	+	NE	NE	NE	Bernot et al. (2010); Clapcott et al. (2010); Clapcott et al. (2016)	+

<p>Mixed Waste (human and livestock)</p>	<p>nutrients</p>	<p>↑ δ¹⁵N</p>	<p>Significant positive relationship between δ¹⁵N enrichment of willow (r²=0.50) and arrowhead (r²=0.22) with WWT; significant positive relationship between δ¹⁵N enrichment of willow with % urban land use (r²=0.64) and δ¹⁵N enrichment of arrowhead with livestock (r²=0.38) in catchment of RRV streams</p>	<p>Yates et al. (2014) <i>Ecol Indic</i></p>	<p>+ NE + NE</p>	<p>Cole et al. (2004), (2005); Hood et al. (2014); Peipoch et al. (2014); Schade et al. (2004)</p>
<p>Agriculture</p>	<p>unclear</p>	<p>↓ %⁵EPT, ↑ %FBI</p>	<p>%EPT negatively associated with crop production (r²=0.32) and % agricultural land use (r²=0.24); FBI significantly positively associated with crop production (r²=0.62) and % agriculture land use (r²=0.57) in RRV stream catchments</p>	<p>Yates et al. (2014) <i>Ecol Indic</i></p>	<p>+ NE NE NE</p>	<p>Brisbois et al. (2008); Harding et al. (1998); Yates et al (2007)</p>

Livestock	unclear	↑ algal abundance	Sestonic chl-a in RRV streams is significantly positively related to livestock production ($r^2=0.42$)	Yates et al. (2014) <i>Ecol Indic</i>	+	NE	NE	NE	-	
WWT	nutrients	↑ GPP, ER	Significant increase in GPP and ER observed downstream of municipal lagoon during discharge into an RRV stream, with greatest increase observed for ER. Regression analysis shows in-stream nutrient concentrations are best predictors of GPP and ER.	Chesworth (2016) <i>MSc Thesis, University of Western Ontario</i>	+	+	NE	+	Aristi et al. (2015); Gücker et al. (2006); Marti et al. (2004); Stutter et al. (2010)	+
WWT	unclear	↑ GPP, ER	Greater GPP and ER in 4 effluent-exposed RRV stream reaches compared to 6 unexposed RRV stream reaches during lagoon discharge periods	Chesworth (2016) <i>MSc Thesis, University of Western Ontario</i>	+	NE	NE	NE	Aristi et al. (2015); Gücker et al. (2006); Stutter et al. (2010)	+

WWT	nutrients	↑ $\delta^{15}\text{N}$	$\delta^{15}\text{N}$ enrichment of primary consumers and POM associated with intensity of wastewater in catchment (n=20) in RRV in both spring and summer	Cormier et al. (in press) <i>J Great Lakes Res</i>	+	NE	+	NE	Anderson and Cabana (2006); deBruyn and Rasmussen (2002); Morrissey et al. (2013)	+
WWT	unclear	altered metabolome	Metabolomic changes in crayfish gill/tail tissue during reciprocal transfer experiment between WWT and reference sites	Izral (2016). <i>MSc Thesis, University of Western Ontario</i>	+	+	+	NE	Limited, evidence in other species (e.g., Skelton et al. 2014 fathead minnow study)	-
Altered Hydrologic Regime	nutrients, sediment	↑ nitrate saturation	Frequent saturation of denitrification in stream pools compared to man-made reservoirs in an RRV watershed, especially during mid-summer when production is high	Gooding and Baulch (2017) <i>J Environ Qual</i>	+	+	NE	NE	Bernot and Dodds (2005); David et al. (2006); Mullholland et al. (2009)	+

Altered Hydrologic Regime	flow modification	↑ GPP	Significant increase in GPP where hydrogeomorphic discontinuities present (channelized zones)	Yates et al. (2018) <i>Can J Fish Aquat Sci</i>	+	NE	NE	NE	Aristi et al. (2014); Elosegi and Sabater (2013); Roley et al. (2014)	+
Agriculture	unclear	↑ GPP	Increasing % agriculture is associated with longitudinal patterns of GPP in two RRV rivers	Yates et al. (2018) <i>Can J Fish Aquat Sci</i>	+	NE	NE	NE	Bernot et al. (2010); Clapcott et al. (2010); Hagen et al. (2010)	+
Altered Hydrologic Regime	flow modification	benthic community shift	Shifts in BMI composition observed between channelized zones and meandering/upland zones; shifts in BMI composition observed seasonally in stream with disrupted flow/more flow modification vs. stream with perennial flow	Yates et al. (2018) <i>Can J Fish Aquat Sci</i>	+	NE	NE	NE	Bogan and Lytle (2007); Delong and Busven (1998); Sponseller et al. (2008)	+

¹WWT = wastewater treatment

²GPP= gross primary production (g O²/m³/day)

³NE = no evidence

⁴ER = ecosystem respiration (g O₂/m³/day)

⁵EPT = Ephemeroptera, Plecoptera, and Trichoptera biotic index (descriptor of benthic macroinvertebrate assemblage used for assessment of stream condition)

⁶FBI = Family Biotic Index (descriptor of benthic macroinvertebrate assemblage used for assessment of stream condition)

Studies included in Table A.1 were selected using a combination of literature review (e.g., using search terms) and through the use of internal databases. Preliminary lists of possible candidate studies for inclusion as evidence in the causal analysis were reviewed by co-authors before inclusion in the final analysis. All included studies were required to examine cause-effect relationships between the candidate causes and stream ecological effects, therefore descriptive evidence such as surveys and non-ecological studies were not included in the analysis and instead listed as part of the review in Section 2.2.

Appendix B: Supplemental Information for Chapter 4

Table B.1 Location (in decimal units) of sites along Deadhorse Creek, Manitoba, Canada sampled during three lagoon effluent release events.

Site	Distance (km)	Latitude	Longitude
US	-1.6	49.219139	-98.091874
Pipe	0.0	49.220127	-98.069125
DS1	2.4	49.226539	-98.046807
DS2	4.5	49.231512	-98.02415
DS3	6.5	49.236742	-98.00755
DS4	8.9	49.233302	-97.97862
DS5	11.3	49.227928	-97.956163

Table B.2 Instantaneous stream water temperature and pH measurements taken upstream, at the lagoon outflow pipe, and five progressively farther downstream sites along Deadhorse Creek, Manitoba, Canada during three lagoon effluent release events in summer 2018, summer 2019, and autumn 2019

Date	Site	Distance (km)	Temperature (°C)	pH
Summer 2018	US	-1.6	19.2	7.27
	Pipe	0.0	21.5	7.73
	DS1	2.4	21.6	7.41
	DS2	4.5	20.3	7.20
	DS3	6.5	20.3	7.20
	DS4	8.9	21.4	7.45
	DS5	11.3	21.5	7.30
Summer 2019	US	-1.6	19.7	7.25
	Pipe	0.0	20.9	8.22
	DS1	2.4	21.9	7.94
	DS2	4.5	21.4	7.62
	DS3	6.5	21.8	7.82
	DS4	8.9	20.6	7.70
	DS5	11.3	21	8.15
Autumn 2019	US	-1.6	4.8	7.87
	Pipe	0.0	5.4	8.31
	DS1	2.4	5.3	8.02
	DS2	4.5	5.2	8.09
	DS3	6.5	5.2	8.05
	DS4	8.9	5.2	8.06
	DS5	11.3	5.3	7.98

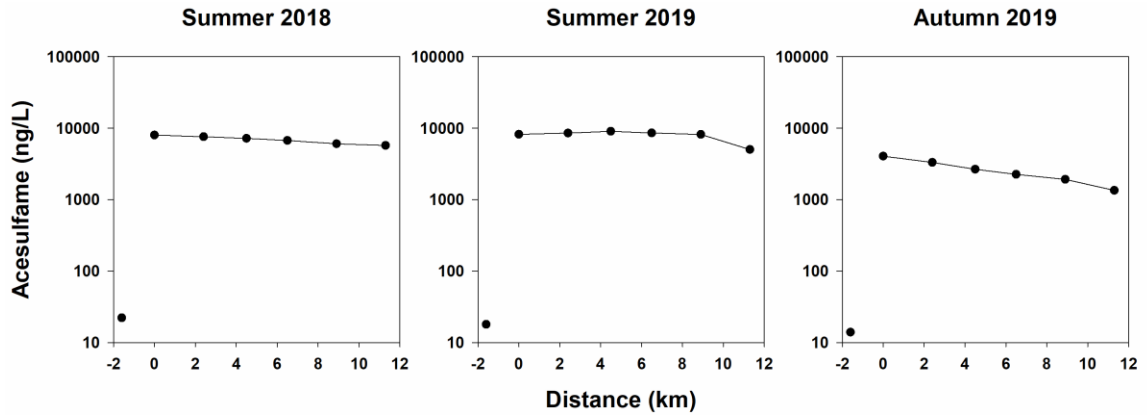


Figure B.1 Acesulfame concentrations (ng/L) measured upstream (-1.6 km) and at increasing distance downstream from the lagoon outflow (0 to 11.3 km) during three lagoon effluent releases in summer 2018, summer 2019, and autumn 2019 in Deadhorse Creek, Manitoba, Canada. The sampling site at 0 km is the lagoon effluent prior to mixing with Deadhorse Creek.

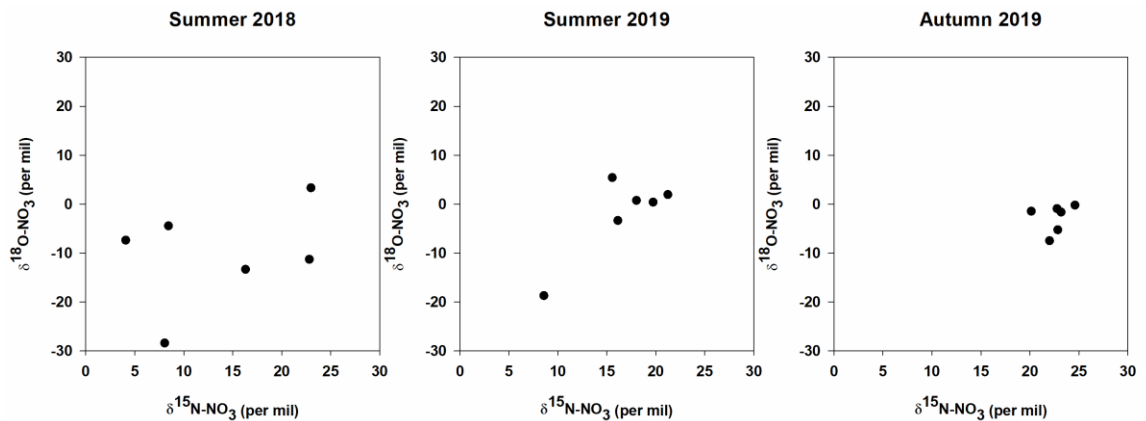


Figure B.2 Spearman rank correlations indicate no relationship ($p \geq 0.5$, $\rho \leq 0.3$) between $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ downstream from the lagoon outflow during three wastewater lagoon effluent releases in Deadhorse Creek, Manitoba, Canada during summer 2018, summer 2019 and, autumn 2019.

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