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Nitrogen biogeochemistry in Binghamton, NY, a medium-sized urban ecosystem

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NITROGEN BIOGEOCHEMISTRY IN BINGHAMTON, NY, A MEDIUM-SIZED URBAN ECOSYSTEM

BY

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ABSTRACT

Humans have altered nitrogen (N) cycling on a global scale, and elevated nitrogen levels are characteristic of urban ecosystems. The major reasons that N is higher in cities include imports of food, fuel and fertilizer. High N export from both point- and nonpoint-sources is common in large cities. While N cycling has been studied in large urban areas, less is known about its cycling in medium-sized cities, such as Binghamton, N.Y. We found that point-source N exported from the Binghamton-Johnson City Wastewater Treatment Plant (B-JC WWTP) was greater than nonpoint-source N exported from eight urban streams to the Susquehanna River, which runs through the Binghamton area. The point-source N fluxes we measured from the B-JC WWTP were high because its function was impaired during the study, causing major environmental impacts on the Susquehanna River. Nonpoint-source N exported from eight urban streams was low, and comparable to N exported from streams of forested watersheds in the region. In an experiment in a roadside ecosystem, experimental deposition of N and road salt (NaCl) did not affect N cycling in roadside soils. However, NaCl negatively impacted C mineralization and soil respiration in situ. In a final experiment, we found that the microbial community of urban stream sediment had the capacity to substantially reduce NO_3^- through denitrification.

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Table of Contents

List of Tables:

List of Figures

Chapter 1: Introduction

1.1 Anthropogenic Changes to the Global Nitrogen Cycle

Living things need nitrogen (N) to grow but its availability is relatively scarce in natural systems (Galloway et al. 2002, Vitousek et al. 1997). Nitrogen is abundant in the atmosphere, which is 78% gaseous dinitrogen (N_2), but this is a stable form of N that can't be used directly by most organisms. Biological N fixation (BNF) has evolved in only a few specialized organisms of Bacteria and Archaea which can convert atmospheric N to forms of N that are available for use by plants and animals ("bioavailable N") (Chapin et al. 2002). They include ammonium (NH_4^+) and nitrate (NO_3^-). These compounds are scarce in most natural setting because relatively large quantities of energy are required to break the stable triple bond of N_2 and convert it to bioavailable N (Chapin et al. 2002). Lightning is another, less substantial contributor of N globally. Humans have substantially altered the global nitrogen (N) cycle, in direct and indirect ways. The Haber-Bosch process of N fixation and extensive use of fossil fuels have substantially increased NH_4^+ and NO_3^- inputs to the biosphere, and altered N cycling on global and local scales (Galloway et al. 2004).

The Haber-Bosch process was developed in the early 20th century, and revolutionized the agricultural system. The process uses fossil fuels to overcome energy limitations of stable atmospheric N_2, transforming it to ammonia (NH_3) (Galloway et al. 2004). The NH_3 produced by the Haber-Bosch process reacts with nitric acid (HNO_3), resulting in NH_4^+ and NO_3^- fertilizers that are used widely (Galloway et al. 2004). The

Haber-Bosch process makes it possible to industrially produce NH_4^+ and NO_3^- fertilizers, that are applied to crops, increasing agricultural yields and ultimately aiding the rapid population increase of the past century (Galloway et al. 2002). It is estimated that industrially produced fertilizers support more than 40% of the world's population (Galloway et al. 2002).

Another major anthropogenic contributor of N to the biosphere is fossil fuel combustion (Galloway et al. 2004). The primary source of fossil fuel-derived N is from the atmosphere; gaseous N_2 is oxidized to form nitrogen oxide (NOx) gases under the high temperature and high pressure conditions in the internal combustion engines of automobiles (Abel-Raman 1998, Bettez et al. 2013). Atmospheric NO_X may be converted to NO_3^-, and NO_X concentrations resulting from combustion are correlated with higher NO_3^- concentrations in precipitation (Butler et al. 2003). Three-way catalytic converters remove some NOx by reducing it to N_2. During this process "over-reduction" also occurs, resulting in the release of NH_3 (Heeb et al. 2006). The NH_3 in the atmosphere is converted to NH_4^+, which becomes an important component of wet deposition (Asman et al. 1998). A smaller source is N in that is in fossil fuels (that was fixed by plants hundreds of millions of years ago) which is released when fuel is burned (Galloway et al. 2002).

Rampant anthropogenic uses of fertilizer and fuel have greatly increased bioavailable N to the biosphere. In pre-industrial times, human-mediated BNF and fuel contributed 15 Tg yr $^{-1}$ and 0.3 Tg yr $^{-1}$, respectively. Natural BNF and lightning contributed 120 Tg yr $^{-1}$ and 5.4 Tg yr $^{-1}$ of N to the biosphere, respectively (Galloway et al. 2004). By the early 1990's, food and energy production added an estimated 156 Tg yr

$^{-1}$ to the biosphere globally, which is a more than 10 fold increase of anthropogenic N compared to pre-industrial contributions. Changes to land use had lowered natural BNF to 107 Tg yr $^{-1}$ while lightning still contributed 5.4 Tg yr $^{-1}$ (Galloway et al. 2004). The increase in N availability and changes to land use (such as deforestation and increases in impervious surface cover) have directly and indirectly caused various population and ecosystem changes worldwide (Vitousek et al. 1997).

1.2 Nitrogen in Urban Ecosystems

A large and growing proportion of the human population live in urban areas, which can be grouped into urban ecosystems based on political and biophysical delineations. Urban ecosystems are defined as ecosystems where people live at high densities and where built infrastructure covers a large portion of the land surface (Pickett et al. 2001). Migration to cities has been rapid: in 1800, 1950 and 2014, 3%, 30%, and 54% of the global population was urban, respectively (United Nations 2014). This percentage is predicted to climb to 66% by 2050 (United Nations 2014). High density city populations demand food, fossil fuels and fertilizers in large quantities (Fissore et al. 2011, Kaye et al. 2006). These are rich sources of bioavailable N and come from diverse and often diffuse sources. Of these N imports, food is often the largest source of N (Fissore et al. 2011). Food must be imported to sustain city populations and consequently, humans and their pets produce waste that is high in N. Additionally, cities import fossil fuels for energy purposes, and they release N (often NOx) as by-products of combustion (Fissore et al. 2011). Finally, cities and their suburbs use fertilizer on green spaces including parks and lawns, which is a smaller contributor of N than food and fuel (Fissore et al. 2011, Raciti et al. 2008). In addition to increasing inputs of N, humans change factors that drive the biogeochemical cycling, or the transport and transformation, of N (Kaye et al. 2006). This changes the fate of N within cities compared to natural areas, usually resulting in higher outputs of N from cities (Kaye et al. 2006). As such, urban ecosystems have more “open” cycling of N, meaning that inputs and outputs are a larger percentage than N that is cycled internally, while natural forests have “closed” cycles, where internal cycling is a larger proportion of N. The reduction of vegetation and soil microbes to use NH_4^+ and NO_3^- contributes to the openness of urban N cycling; so does the lack of infiltration precipitation and its resulting runoff. In cities, elevated inputs and changes to the drivers of biogeochemistry may lead to N saturation, inorganic N in stream water, changes in soil processes of nitrogen mineralization and nitrification, and increases in primary productivity in cities (Magill et al. 1997, Pickett et al. 2011, Pouyat and Turechek 2001, Zhu et al. 2006).

1.2.1 Nitrogen in Urban Streams and Rivers

Historically, cities have been formed along rivers and deltas because of water accessibility, and consequently many waterways have been modified by activities in cities (Grimm et al. 2008). Water in cities is used domestically and is also linked to sanitation and industry (Grimm et al. 2008). Urbanization greatly affects streams and rivers by altering hydrology, and degrading water quality, and biodiversity (Paul and Meyer 2001, Pickett et al. 2011).

Streams are typically modified to accommodate the infrastructure of surrounding cities (Grimm et al. 2008). In addition to this direct alteration, the hydrology of streams and rivers is impacted by the characteristic increase in impervious surface cover in urban watersheds, which takes the form of buildings, roads, sidewalks and parking lots (Kaye et al. 2006, Paul and Meyer 2001). Land transformed from natural ecosystems (mostly forest in Upstate New York) to impervious surface cover results in lower percentages of vegetated areas and less water infiltration. This leads to diminished groundwater recharge and lower base flows. Less infiltration also creates more surface runoff during precipitation events and spring snow melt (Kaye et al. 2006, Paul and Meyer 2001). As impervious surface cover increases by just 10-20%, runoff increases twofold over runoff from forested watersheds (Paul and Meyer 2001). Reduced tree cover also increases the rate of runoff (Pickett et al. 2011). Increases in surface water runoff causes streams to be flashier, with a decrease in lag time between a storm event and the beginning of runoff, causing more frequent flooding (Pickett et al. 2011). Another common modification to urban streams is stream channelization, which leads to stream incision and contributes to increases in discharges during storm events (Paul and Meyer 2001). Consequently, the "urban stream syndrome" is common and characterized by lower base flows, larger storm flows and altered hydrographs, and stream incision in addition to increased loading of nutrients and pollutants (Paul and Meyer 2001). Furthermore "sewersheds" alter the hydrological connections of cities. Sewersheds and other changes to land cover greatly affect the transport of nutrients from cities to waterways (Paul and Meyer 2001, Vrebos et al. 2015).

Urban streams and rivers tend to have higher concentrations of dissolved inorganic nitrogen (NH_4^+ and NO_3^-) than their natural counterparts due to food, fuel and fertilizer that is imported by the human populations (Baker et al. 2001, Groffman et al. 2004, Shields et al. 2008). Dissolved organic N in urban streams and rivers can also be considerably higher in cities (Lewis and Grimm 2007). Nitrogen that is a by-product of human dietary needs is discharged to waterways via wastewater treatment plants (WWTP) and combined sewage overflows (CSO), which are point- sources of nutrient pollution. Human waste is usually treated at WWTPs to remove N in developed countries. Even after treatment of WWTP effluent, higher N than baseline concentrations often remains (Carey and Miglaccio 2009). Combined sewage overflows are often a feature of older cities. They discharge untreated sewage to waterways when sewer infrastructure cannot accommodate the combined volume of sewage and precipitation during storm events (Carey and Miglaccio 2009, Paul and Meyer 2001).

In cities, other sources of bioavailable N are deposited intentionally to fertilize green spaces, and unintentionally as a by-product of fossil fuel combustion (Bettez et al. 2013, Raciti et al. 2008). Greater impervious surface cover and less vegetative cover present fewer opportunities for deposited N to be utilized by plants and microbes, and result in less uptake of N by plant and soil processes (Kaye et al. 2006, Zhu et al. 2006). Greater surface runoff mobilizes N that has been deposited on the ground, moving it to aquatic ecosystems in and downstream of cities. Nitrogen that is dissolved in precipitation can run off into nearby waterways or leach into groundwater. As concentrations of N increase, it becomes a pollutant and degrades water quality (Groffman et al. 2004, Pickett et al. 2011, Shields et al. 2008). Elevated N, and also phosphorus, in urban waterways contribute to problems with eutrophication (Grimm et al. 2008).

Additionally, urban planning often eradicates riparian systems, and reduces the connectivity between stream water and adjacent floodplain areas, both of which are dynamic areas of nutrient uptake and cycling (Groffman et al. 2002, Kaushal et al.

2008b). These alterations to urban streams often lead to less nutrient retention within stream systems, and greater exports of nutrients downstream. Strategies for increasing nutrient retention in urban watersheds, and decreasing nutrient export from urban streams often focus on stream restoration (Craig et al. 2008, Newcomer Johnson 2016).

1.2.2 Decreasing Nutrient Loads from Urban Streams

Stream restoration has been shown to reduce nutrient loads downstream (Craig et al. 2008, Newcomer Johnson 2016). Urban stream restoration has often focused on reducing stream NO_3^- levels, which tend to be higher in urban streams (Groffman et al. 2004, Shields et al. 2008, Kaushal et al. 2008b, Newcomer Johnson et al. 2014). Restoration of riparian areas is an obvious direction to follow, but there has been evidence that urban riparian areas can become so degraded and disconnected from groundwater that they are net NO_3^- producers, rather than hotspots of denitrification as they often are in their natural existence (Groffman et al. 2002). However, riparian areas can be resurrected if certain criteria are met. More recent research has shown that nutrient retention and denitrification are promoted when riparian areas and floodplains are hydrologically reconnected to urban streams, allowing for NO_3^-- laden water to contact organic carbon- rich saturated soils, providing excellent conditions for denitrification (Kaushal et al. 2008b, Newcomer Johnson et al. 2014). Another important feature of stream restoration that promotes nutrient cycling is slow flowing water, which provides enough time for the N and other nutrients to be cycled (Groffman et al. 2002, Newcomer Johnson et al. 2014). If these conditions are met, then urban riparian areas, floodplains and even stormwater control structures can contribute to nutrient uptake and denitrification (Kaushal et al. 2008b, Newcomer Johnson et al. 2014).

The impacts of climate change can complicate the issue of retaining N and other nutrients in urban ecosystems. Climate change is expected to—and may already have started—increasing precipitation in the Northeastern U.S., particularly during winter months (Hayhoe et al. 2007). A number of studies have shown that increases in precipitation and stream flow have concomitant increases in N export (Kaushal et al. 2008a, Chiwa et al. 2010). In a study of stream N export over 14 years from urban streams in Baltimore, Bettez et al. (2015) reported that total N loads from streams were 3x higher during wet years compared to dry years. Therefore, stream restoration efforts should become part of a larger goal to make cities more sustainable in regard to water issues in the future (Kaushal et al. 2015).

1.2.3 Nitrogen in Urban Soils

Soils are fundamental to many ecological processes, including the cycling of N and other elements (Picket et al. 2011). Increases in N deposition may increase the pools of bioavailable N in urban soils, and may change the rates of N transformation in urban soils. Nitrogen mineralization is the conversion of organic N to NH_4^+, and nitrification is the conversion of NH_4^+ to NO_3^- (Chapin et al. 2002). Nitrogen mineralization and nitrification often increase in response to N deposition (Phoenix et al. 2012). Pouyat and Turechek (2001) provide evidence that urban soils have higher rates of N mineralization and nitrification than rural soils. These observations may result from higher N pools in urban soils, and other factors like the quality of organic matter and presence of exotic earthworms (Pouyat and Turechek 2001). The processes of nitrification and denitrification release NO, N_2O and N_2, so increases of N deposition can increase fluxes of these gases from urban soils (Matson et al. 2002). Nitrate that is not lost via denitrification is extremely soluble, and is likely to be leached from soils if it is not used by plants and soil fauna (Matson et al. 2002). If NO_3^- accumulates in groundwater, it can become an important component of N in urban streams. Urban soils can vary widely in character, and since not all are tremendously disturbed by humans it can be difficult to predict exactly what impact urbanization will have on soil N transformations (Pickett et al. 2008). Whether urban soils actually show increases in rates of N transformation can depend on the presence of other urban pollutants (like road salt and heavy metals), soil structure, soil organic matter content, and pH (Pickett et al. 2008). For example, Green and Cresser (2008) observed decreases in N processing where soil NaCl was high in Scottish roadsides.

1.3 Other Elements in Urban Ecosystems

Changes to other biogeochemical cycles are among the many impacts of humans on urban environments. Characteristic of urban ecosystems are elevated inputs of carbon (C), phosphorus (P) and road salt (often NaCl). In turn, changes to the drivers of urban biogeochemistry change the cycling of these nutrients in urban ecosystems. This has become a concern, as the expansion of urban areas outpaces attempts at pollution control (Grimm et al. 2008).

Carbon inputs and outputs are high due to energy consumption for transportation, in addition to residential, commercial and industrial energy consumption (Bin and Dowlatabadi 2005, Fissore et al. 2011). More specifically, urban C inputs and outputs include travel (ground and air), energy use, human diet, pet diet, landscape, solid waste (paper and plastic) and wastewater (Fissore et al. 2011). Energy use is a major contributor of C to cities. Lifestyle decisions account for an estimated 85% of energy use in the

United States (Bin and Dowlatabadi 2005). This directly and indirectly impacts CO_2 emissions, and is particularly apparent in cities, which collectively emit an estimated 80% of CO_2 emissions (Bin and Dowlatabadi 2005, Grubler 1994 and O'Meara 1999 as cited by Churkina 2008). Cities have the capacity to absorb a portion of CO_2 emissions, but cities are ultimately sources of CO_2 (Fissore et al. 2011, Kaye et al. 2006). This contributes to changes in nutrient cycling and primary productivity in adjacent landscapes and water bodies, and global climate change (Grimm et al. 2008). Cities provide sinks of C in urban vegetation and soils, but given the current trends of energy use such processes will not substantially offset CO_2 emissions (Pataki et al. 2006). As such cities are sources of CO_2, which as a greenhouse gas, contributes to climate changes issues (Grimm et al. 2008).

Phosphorus in cities is higher due to human and pet dietary demands, and the use of detergents and fertilizers (Fissore et al. 2011). Global inputs of P are estimated to be ~18.5 Tg yr^{-1} and ~15-20 Tg yr^{-1} with mining and weathering, respectively (Bennett et al. 2001), while outputs are around 23 Tg yr^{-1}. Preindustrial estimates of inputs and outputs are 10-15 Tg yr^{-1} and 9 Tg yr^{-1}, respectively. Mining and weathering from mechanical industrial operations contribute a large portion of P to the biosphere annually. Retention of P in agricultural landscapes appears to be high (Bennett et al. 2001). While cities can sequester a portion of P, the effluent of WWTPs can be a source of P (Carey and Migliaccio 2008, Fissore et al. 2011). Soluble orthophosphate (PO_4-P) can be a major component of WWTP effluent, and can be directly assimilated by autotrophs (Carey and Migliaccio 2008). High P inputs lead to eutrophication problems; high N inputs exacerbate issues associated with eutrophication (Bennett et al. 2001).

Road salt inputs and outputs are high in cities that experience snow and ice during the winter months. Typically in the form of sodium chloride (NaCl), it enters roadside ecosystems dissolved in runoff. From there NaCl can run into surface waters, and leach into ground water if it is not retained by the ecosystem. Recently, research suggests that a large portion of NaCl is retained by ecosystems, which is against the commonly held belief that the majority of salt applied to roads runs off to streams and rivers (Cunningham et al. 2008, Kelly et al. 2008). Kelly et al. (2008) showed that in Wappinger Creek, NY, NaCl concentrations in the stream increased over two decades without an appreciable increase in salt inputs over the same period, implying some sort of NaCl retention mechanism in the watershed. This new evidence runs contrary to the once widely-held belief that NaCl is quickly removed from soils because it is readily dissolves in water. Given that deicing salt may not quickly flush out of soils, NaCl concentrations can be high even during summer months when biological activities are elevated. Furthermore, these concentrations may not have reached their greatest possible level (Findlay et al. 2011).

1.4 Biogeochemistry of Small to Medium- Sized Cities

Much of the research on urban biogeochemistry has been located in larger cities, such as Baltimore, Phoenix, and the Minneapolis-St. Paul region. A smaller city like Binghamton, New York, has several fundamental differences from large urban ecosystems. Smaller cities encompass less area, with less development and less impervious surface cover than larger cities. There are fewer people living in less dense settlements. With a smaller population, there are fewer imports of food, fertilizer and fuel to support the inhabitants and metabolism of the city. With less food, fertilizer fuel, and less area, there are smaller nutrient inputs of N, C, P and road salt. With fewer inputs, we might expect less intense changes to the biogeochemistry of small to medium-sized cities, including less N in urban streams compared to large cities.

1.5 Conclusions and Research

Urban ecosystems are a relatively small proportion of the earth's surface (approximately 3%), but the impacts of urbanization can be large, extending beyond political or biophysical boundaries (Folke et al. 1997, Grimm et al. 2008, Pickett et al. 2001). Since the impacts of urbanization are widespread and likely growing, better understanding of the ecology of cities will improve efforts aimed at minimizing and mitigating environmental problems (Grimm et al. 2000, Pickett et al. 2001). Long Term Ecological Research (LTER) sites located in the cities of Baltimore, MD and Phoenix, AZ have provided important information on ecological change in large urban metropolises since 1998 and 1997, respectively; less is known about the ecological change in smaller cities. The United Nations estimates that in 2015, 50% of urban dwellers—the largest percentage—live in cities that have fewer than 500,000 inhabitants. The city of Binghamton, New York falls within this category. Roughly 47,400 people live within the City of Binghamton, which covers 11.14 mi^2. More than 260,000 people reside in the Greater Binghamton area which includes the City and Town of Binghamton, Johnson City, Endicott, Endwell, Vestal, Maine, Conklin, Union, Kirkwood, and Port Dickinson (www.cityofbinghamton.com).

Nitrogen and other biogeochemical cycling has been a major research focus at the Baltimore and Phoenix LTERs and in other cities (Kaye et al. 2006, Fissore et al. 2011). The biogeochemistry of larger cities has been the subject research in the Baltimore and

Phoenix LTERs but less is known about biogeochemical changes of smaller cities such as Binghamton. Because of its smaller size, population density, and impervious surface cover, inputs of C, N, P, and road salt are lower than in large cities. Do these features of the Binghamton area result in a lower export of bioavailable N? Binghamton is an ideal place to study urban ecology, particularly because it has a major river (the Susquehanna River) running through it, and small watersheds with streams that discharge directly to the river. The watershed approach has been central to the study of ecosystem ecology, and there is an opportunity in Binghamton to utilize this approach and investigate the human- impacted dynamics of nitrogen biogeochemistry. A better understanding of N/pollutant cycling in and export from urban ecosystems and the drivers of these processes will contribute to N management and retention efforts. My research of N dynamics in the Binghamton urban ecosystem aimed to address the following questions:

1. Do road-associated pollutants of NO_3^- and NaCl affect N cycling and microbial activity in roadside soils?
2. Does nonpoint-source N from urbanization impact urban stream NO_3^- concentrations?
3. How do urban stream N concentrations and fluxes in Binghamton compare to the Baltimore and Phoenix LTERs, and to natural counterparts?
4. Is the impaired Binghamton-Johnson City Wastewater Treatment Plant a substantial point-source of N to the Susquehanna River?
5. Does urban stream sediment have the capacity to substantially decrease NO_3^- through denitrification?

Chapter 2: Impacts of Deicing Salt and Nitrogen Additions on Nitrogen and Carbon Cycling in a Roadside Ecosystem

2.1 Introduction

Biogeochemical cycles in urbanized areas are changed in part by alterations to land cover, such as roads, and changes to atmospheric chemistry, including chemicals released from motor vehicles and roadways (Kaye et al. 2006, Vitousek et al. 1997). Roadways and vehicles can impact the biogeochemistry of adjacent ecosystems. The impact of roadways and vehicles on biogeochemical cycling has been of particular interest, since roadway expansion has accompanied the growth of urban areas worldwide; in the U.S. alone, there are over 4 million kilometers of roads (USDOT 2013). Deicing salt, inorganic nitrogen (N) from vehicle fossil fuel combustion, and metals released from vehicles and roads are common roadway-associated chemicals that may enter ecosystems adjacent to roadways (Bettez et al. 2013, Findlay and Kelly 2011, Johansson et al. 2009). Over time they may accumulate in soils and can leach to water resources, becoming detrimental to ecosystem function and human health. The impact of heavy metals on roadside and urban soils has been well-documented, but research on the effects of deicing salt and inorganic nitrogen on biogeochemical cycling in roadside ecosystems is more limited

Deicing road salt is a common traffic-related pollutant in temperate climates, where it is critical to maintain road safety during winter months (Fay and Shi 2012). Deicing salt is usually sodium chloride (NaCl) because of its relatively low cost compared to other chloride salts (Fay and Shi 2012). Road salt use has increased dramatically, is highly soluble and usually enters ecosystems dissolved in runoff. Roadside soils have been found to have salt concentrations that positively correlate with the rate of salt application (Fay and Shi 2012, Findlay and Kelly 2011). In soils, high salt concentrations can impact roadside microbial communities that mediate carbon (C) and N cycling, thus altering biogeochemical cycling in areas adjacent to roadways (Fay and Shi 2012, Green and Cresser 2008, McCormick and Wolfe 1980). This can indirectly influence roadside plant growth, plant community structure, and animal habitat in addition to direct damages of salt absorbed by root systems and when it is sprayed onto plants by passing vehicles (Bryson and Barker 2002, Fay and Shi 2012, Green and Cresser 2008, Heintzman et al. 2015). Deicing road salt was widely thought to quickly "flush-out" of soils and groundwater, but this view has been challenged by a number of recent studies that suggest a large proportion of it is retained by watersheds (Cunningham et al. 2008, Kelly et al. 2008, Kincaid and Findlay 2009). For example, Kelly et al. (2008) showed a doubling in concentration of salt in the East Branch of the Wappinger Creek watershed. This increase was not accompanied by an increase in salt loads, road density or population, indicating salt is retained within the watershed in soils, groundwater or both (Kelly et al. 2008). Kaushal et al. (2005) found that impervious surface cover in Baltimore was strongly related to chloride (Cl^-) increases in urban and suburban streams. Elevated urban and suburban stream Cl^- concentrations were observed in winter months, and persisted in the spring, summer and fall (Kaushal et al. 2005). Year-round elevation of Cl^- suggested that salt contamination had spread to groundwater resources (Kaushal et al. 2005). Because road networks are widespread and road salt applications are yearly events, roadside ecosystem processes may be critically altered by deicing salt.

In addition to road salt, inorganic N is a major roadside pollutant. Fossil fuel combustion in motor vehicles releases inorganic N in the form of nitrogen oxides (NO_X) and ammonia (NH_3) to the atmosphere, and to road surfaces, where it may be dissolved in precipitation and run off to roadsides and streams. The high temperatures generated by combustion cause atmospheric N_2 and O_2 to split; N and O can then react to form NO and NO_2 (NO_X) (Abdel-Rahman 1998). Three-way catalytic converters remove some NOx by reduction to N_2—however “over-reduction” also occurs, resulting in the release of NH_3 (Heeb et al. 2006). As a result of these processes, automobile-sourced fluxes of NOx and NH_3, which are highly reactive forms of N, can be high near roadways (Cape et al. 2004, Redling et al. 2013). Atmospheric NO_X may be converted to nitrate (NO_3^-), and NO_x concentrations resulting from combustion are correlated with higher NO_3^- concentrations in precipitation (Butler et al. 2003). Atmospheric NH_3 is converted to ammonium (NH_4^+), which becomes an important component of wet deposition (Asman et al. 1998). Consequently, roadsides have elevated NH_4^+ and NO_3^- inputs, making these areas “hot spots” of N deposition (Bettez et al. 2013, Padgett et al. 1999).

Greater N deposition may increase the N that is available in soils, which can then affect the structure and function of plant and microbial communities (Compton et al. 2004, Magill et al. 1997). Nitrogen deposition is believed to be a contributing factor to higher rates of N mineralization and nitrification in urban soils (Pouyat and Turechek 2001, Zhu and Carreiro 2004). Magill et al. (1997) found greater rates of nitrification and N mineralization, along with greater fluxes of N_2O, in plots with experimental N deposition. Elevated inputs of inorganic N can also stimulate plant growth, and may change plant community structure (Angold et al. 1997, Bignal et al. 2007). Besides uptake and loss via denitrification, NO_3^- is very soluble and is readily leached from soil, especially during storm events.

Metals released from roadway activities have been recognized as a threat to ecosystem health, and research on roadside biogeochemistry has focused on metal contamination (Pouyat et al. 2010, Yesilonis et al. 2008). Less is known about the effects of road salt and N than heavy metals on roadside ecosystem processes. Since roadways are inescapable fixtures of urban and suburban landscapes, understanding the impacts of these pollutants is crucial to mitigate potential problems they may cause to roadside ecosystems. This experiment investigates the effects of common roadside pollutants on processes in an ecosystem adjacent to a major highway. Salt and N were experimentally applied to plots during the growing seasons of 2010, 2011, and 2012 to determine if they affected soil N transformation, C mineralization and soil respiration in roadside soils. We also compared soils adjacent to the highway that had been exposed to roadside pollutants for years with soils 50 meters from the road that had been less exposed. We hypothesized that (1) treatment with salt would result in soils with lower rates of nitrification, N mineralization, and C mineralization and (2) treatment with N would result in soils with higher rates of nitrification and N mineralization. Further, we predicted that (3) long-term exposure to pollutants at the transect adjacent to the road would cause soils to have lower rates of nitrification, N mineralization and C mineralization due to years of exposure to roadside pollutants. The data from the experiment in 2010 are reported by Scott et al. (2011). The data from 2011 and 2012 are presented here.

2.2 Materials and Methods

2.2.1 Study Site and Experimental Design

The study was conducted in Binghamton, New York (42.1'N, 75.92'W) at an experimental site alongside Interstate 81 (I-81). Interstate-81 stretches from Tennessee to the Canadian border, passing through Binghamton which has a metropolitan population of about 260,000. This portion of the interstate was built in the 1960’s and experiences an average daily traffic flow of approximately 70,000 automobiles (NYSDOT 2010). The study site is an open field located directly southeast of the northbound lane of I-81 that dominated by *Solidago canadensis* and *Lythrum salicaria*. The area has a temperate climate, receiving a yearly average of about 100 cm of rainfall. The experiment occurred from June 4, 2010 and continued through October 2012. In 2011, the average temperature was 8.83 degrees C, and the precipitation was 172.85 cm. The 2011 precipitation was higher than average, due to Tropical Storm Lee. In 2012, the average temperature was 9.67 degrees C and the precipitation was 99.92 cm, which is the typical annual precipitation for the area. The treatments were $NaNO_3$, NaCl and a water control, which were experimentally applied to plots established in a blocked ANOVA design. Six plots were positioned randomly on each transect and three 1 m^2 sub-plots were created at each of the six plots. The experimental plots were on two transects. One was at the base of the highway bank (0-m transect), and the other was 50 meters away from and parallel to the highway (50-m transect). The 0-m transect had been exposed to roadside pollutants over a long period of time, while the 50-m transect had been exposed to fewer pollutants. In mid-July 2012, expansion of I-81 resulted in the loss of the 0-m transect. At each plot, sub-plots received treatments of $NaNO_3$, NaCl, or water. The plots were experimentally treated on a bi-weekly (every other week) schedule for five months of the growing season in 2010 (June-October) and six months in 2011 and 2012 (May-October). At the time of the soil collection on July 26, 2011, $NaNO_3$- treated plots had received 8.17 g Na and 4.98 g N, and NaCl- treated plots received 323.18 g Na and 498.40 g Cl. The plots had been treated through the growing season of 2010, and May, June and July of 2011 by the July soil sampling. Soil was collected again on November 8, 2011 and at this collection $NaNO_3$-treated plots had 12.10 g Na and 7.37 g N, and NaCl-treated plots had 478.31 g Na and 738.42 g Cl. A final soil collection for this experiment occurred on June 25, 2012, a few days prior to the loss of the 0-m transect due to construction. The $NaNO_3$-treated plots had received a total of 13.96 g Na and 8.97 g N, and NaCl plots had received 582.57 g Na and 898.42 g Cl. Nitrogen deposition in the area is approximately 1 g m^{-2} $year^{-1}$. The N concentrations for this study were therefore elevated over background N deposition levels, reflective of deposition in larger metropolitan areas. The salt concentrations were similar to amounts reported in previous research on deicing salt deposition (Blomqvist and Johansson 1999, Lundmark and Olofsson 2007).

2.2.2 Soil Collections and Processing for Measuring Rates of N Mineralization, Nitrification and Soil Chemistry

Soil cores (15cm long and 5cm diameter) were collected in July 2011, November 2011 and June 2012. Cores were immediately transported to the lab in a cooler and stored in a cold room until processing, which occurred within 18-72 hours of collection. Cores were separated into depths of 0-5cm and 5-15cm, and were sieved through 4mm sieves, removing roots and large debris. Fifteen grams of sieved soils were immediately extracted using 1 M KCl and were shaken in a reciprocal shaker for 1 hour and the supernatant was filtered through Whatman 40 filter papers, acidified with 6N HCl and stored in cold room until analysis for NH_4^+ and NO_3^- concentrations.

Potential rates of N mineralization and nitrification were measured using 28-day lab incubations. The incubations were maintained at a constant temperature (22°C) and water was added weekly to maintain constant soil moisture. Concentrations of NH_4^+ and NO_3^- were obtained using a Lachat QuickChem 8000 Flow Injection Analyzer (Lachat Instruments, Milkwaukee W.I.). Soil samples were dried at 60°C for 72 hours to determine percent moisture. Percent organic matter was calculated using the loss-on-ignition method where samples were combusted in a muffle furnace at 550°C for two hours. Soil slurries of 10 g dried soil mixed with 20 mL Nanopure water were used to measure pH and conductivity of soils.

2.2.3 Soil Carbon Mineralization

Rates of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) flux from sieved soils were measured in the lab in 2011. In July and November, twenty grams of soil were weighed into acid-washed glass Wheaton bottles in the lab to incubate. On the days of sampling, a syringe was used to expel air from the bottles three times, and then they were allowed to aerate for one hour prior to sampling. Using plastic syringes with 3-way stoppers, a 20 mL (Time 0) gas sample was collected from each bottle, and then another 20mL gas sample was taken after incubation for four hours (Time 4). The difference between these two values was used to calculate the flux of gases from soils. A Shimadzu Gas Chromatograph GC 14-A was used for analysis; it was equipped with a thermal conductivity detector (TCD) for CO_2, flame ionization detector (FID) for CH_4, an electron capture detector (ECD) for N_2O. Gas samples were analyzed within 10 hours of collection. A pilot study determined that the plastic syringes retained the gas samples for this period of time.

2.2.4 In Situ Soil Respiration

In the summer of 2012, we measured in situ soil respiration. Collars of white PVC pipe were permanently installed in each of the sub-plots on 6 May 2012. On the day of sampling, gas caps of the same white PVC and fitted with tubes to obtain gas samples were transported to the site. The caps were placed on the collars and secured with 3" rubber bands. If vegetation had grown within the collars since the previous sampling, it was removed prior to that day's sampling. Initial gas samples were taken immediately after the caps were secured, and were followed with another sampling after 60 minutes to yield a 1-hour incubation. All gas samples were taken with syringes with 3-way stoppers, between 9:30 and 11:30AM. Gas samples were immediately transported back to the lab and analyzed using the Shimadzu GC described above.

2.2.5 Data analysis

The results of the experimental treatments at the 0-m and 50-m transects in years 2011 and 2012 were analyzed using a blocked 2-way ANOVA. Where there were differences between treatments, Tukey's HSD (Honest Significant Difference) test was used to determine which averages differed. Statistical analyses were computed using the R Statistical program. Statistically significant results were reported when $p<0.05$. Averages are presented ± one standard error (SE).

2.3 Results

2.3.1 Rates of N transformation and Soil Inorganic N Concentrations

Experimental treatments with salt and N did not discernably impact rates of nitrification or N mineralization during the study period, and the rates differed between transects only in June 2012 (Figure 2.1). In 0-5cm soils collected in July 2011, nitrification was 0.51±0.08 mg kg^{-1} day^{-1} and N mineralization was 0.53±0.08 mg kg^{-1} day^{-1} (Figure 2.1 a,c). In these soils, ANOVA's showed that treatment with salt and N did not significantly impact rates of nitrification (F value=0.56, p=0.58) or N mineralization (F value=1.50, p=0.24) (Appendix A, Tables A2.1 and A2.2). In 0-5cm soils from November 2011, nitrification was 0.42±0.07 mg kg^{-1} day^{-1} and N mineralization was 0.30±0.07 mg kg^{-1} day^{-1} (Figure 2.1 a,c). Impacts of treatment were not detectable in nitrification rates (F value=0.66, p=0.52) or N mineralization rates (F value=0.46, p=0.64) in November 2011 (Appendix A, Tables A2.3 and A 2.4). In June 2012, treatments did not impact nitrification (F value=1.03, p=0.37) or N mineralization (F value=0.91, p=0.42) (Appendix A, Tables A2.5 and A2.6). Rates of nitrification and N mineralization in 0-5cm soils did not significantly differ between the 0-m and 50-m transects in July 2011 (F value=0.38, p=0.54 and F value=1.36, p=0.25, respectively) or November 2011 (F value=0.15, p=0.70 and F value=0.04, p=0.83, respectively) (Appendix A, Tables A2.1-A2.4). However in June 2012, soils from the 0-m transect had significantly elevated rates of nitrification (F value=11.71, p=0.002) and N mineralization (F value=12.43, p=0.002) (Appendix A, Tables A2.5 and A2.6). Nitrification rates 0-5cm soils collected in June 2012 were 1.42±0.18 mg kg^{-1} day^{-1} at the 0-m transect and 0.44±0.23 mg kg^{-1} day^{-1} at the 50-m transect (Figure 2.1 a,c). Nitrogen mineralization was 1.28±0.18 mg kg^{-1} day^{-1} at the 0-m transect and was 0.30±0.21 mg kg^{-1} day^{-1} at the 50-m transect in 0-5cm soils collected in June 2012 (Figure 2.1 a,c).

In 5-15cm soils, nitrification and N mineralization did not differ between treatments or between transects. In 5-15cm soils from July 2011, treatment with salt and N did not affect rates of nitrification (F value=0.05, p=0.95) or N mineralization (F value=0.16, p=0.86) (Appendix A, Tables A2.7 and A2.8). Treatments did not impact November 2011 rates of nitrification (F value=1.06, p=0.36) or N mineralization (F value=0.74, p=0.48), nor did they affect June 2012 rates of nitrification (F value=2.76, p=0.08) or N mineralization (F value=2.26, p=0.13) (Appendix A, Tables A2.9-A2.12). Rates of nitrification in 5-15cm soils did not differ between 0-m and 50-m transects in July 2011 (F value=0.10, p=0.76), November 2011 (F value=1.34, p=0.28) or June 2012 (F value=0.58, p=0.45) (Appendix A, Tables A2.7, A2.9, A2.11). Nitrogen mineralization rates did not differ between transects in June 2011 (F value=0.53, p=0.47), November 2011 (F value=3.19, p=0.08) and was nearly significant in June 2012 (F value=4.32, p=0.05) (Appendix A, Tables A2.8, A2.10, A2.12).

Nitrification and N mineralization rates were lower in 5-15 cm soils compared to 0-5cm soils (Figure 2.1). In July 2011, nitrification in 5-15cm soils was 0.18±0.02 mg kg^{-1} day^{-1} (Figure 2.1b). This rate was 64% lower and significantly less than 0-5cm soils in July (t statistic=4.58, n=72, $p<0.00001$). Nitrogen mineralization was 0.15±0.02 mg kg^{-1} day^{-1}, which was 73% lower, and significantly less than in 0-5cm soils (t statistic= 5.26, n=72, $p<0.00001$) (Figure 2.1d). In November 2011, nitrification was 0.25±0.03 mg kg^{-1} day^{-1} which was 41% lower than shallow soils (Figure 2.1b). Nitrogen mineralization was 0.19±0.02 mg kg^{-1} day^{-1}, or 37% lower than in shallow soils (Figure 2.1d). The lower measurements in November 2011 in 5-15cm soils were significant for both nitrification (t statistic=2.79, n=72, p=0.004) and N mineralization (t statistic=1.70, n=72, p=0.04). In 5-15cm soils from June 2012, rates of nitrification were 0.26±0.02 mg kg^{-1} day^{-1}, and rates of N mineralization were 0.21±0.03 mg kg^{-1} day^{-1} (Figure 2.1 b,d). Compared to 0-5cm soils, 5-15cm soils from June 2012 had nitrification rates that were 82% lower than in the 0-m transect and 41% lower than the 50-m transect, while N mineralization rates were 84% lower than the 0-m transect and 30% lower than the 50-m transect. In 5-15cm soils from June 2012 there were significantly lower rates of nitrification (t statistic=6.37, n=60, p<0.00001) and N mineralization (t statistic=6.21, n=50, p<0.00001) at the 0-m transect while rates of nitrification (t statistic=0.72, n=60, p=0.24) and N mineralization (t statistic=0.18, n=60, p=0.43) did not significantly differ between depths at the 50-m transect.

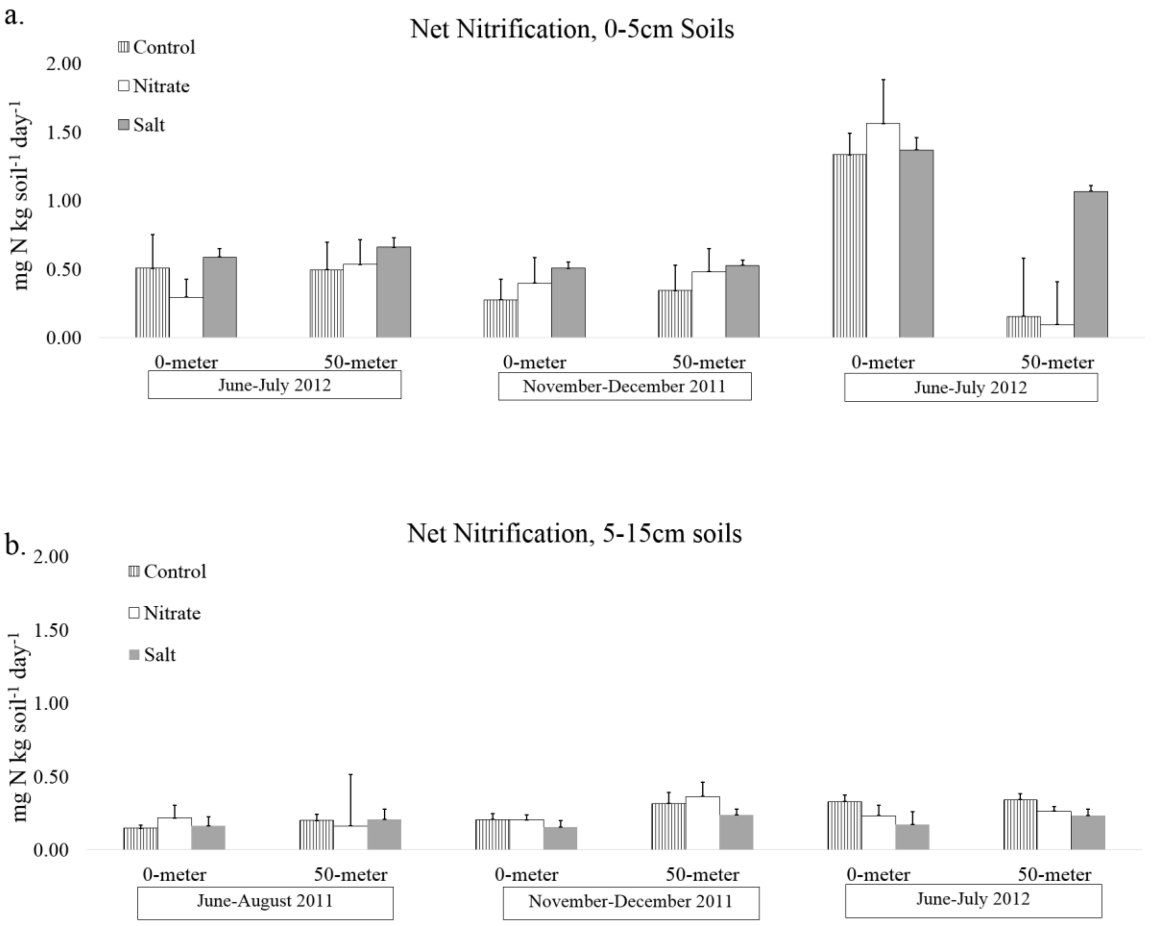
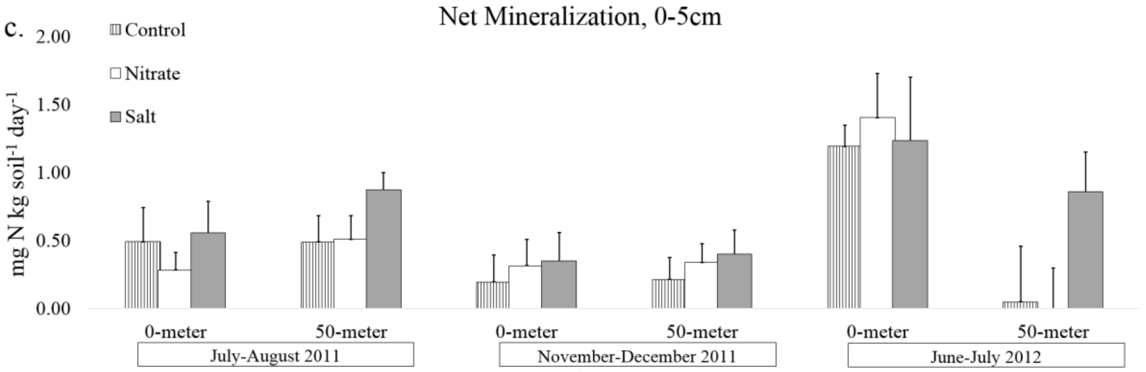


Figure 2.1: Soil nitrification rates (a, b) and net mineralization rates (c, d) in experimental plots, at 0- and 50-m transects in July 2011, November 2011, and June 2012.



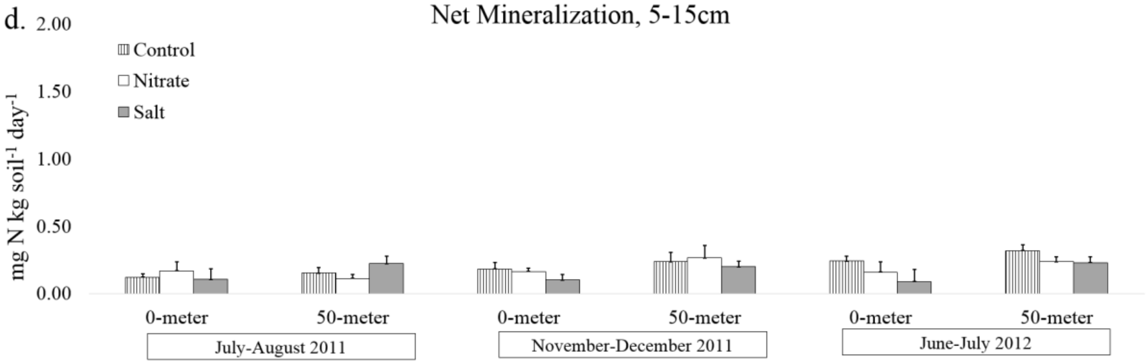


Figure 2.1, continued: Soil nitrification rates (a, b) and net mineralization rates (c, d) in experimental plots, at 0- and 50-m transects in July 2011, November 2011, and June 2012.

Soil NH_4^+ and NO_3^- concentrations were not impacted by salt and N treatments in this experiment. In 0-5cm soils from July 2011, inorganic N concentrations were 4.12±0.63 mg kg^{-1} NH_4^+ and 1.92±0.25 mg kg^{-1} NO_3^- (Figure 2.2a). In July 2011, ANOVA's showed that experimental treatments did not significantly impact concentrations of soil NH_4^+ (F value=3.11, p=0.06) or NO_3^- (F value=1.32, p=0.28) (Appendix A, Tables A2.13 and A2.14). November 2011 soils had NH_4^+ concentrations of 7.48±0.45 mg kg^{-1} and NO_3^- had concentrations of 2.05±0.30 mg kg^{-1} in the 0-5cm layer. In November 2011 ANOVA's showed treatments did not significantly affect extractable NH_4^+ (F value=0.02, p=0.98) or NO_3^- (F value=0.72, p=0.49) (Appendix A, Tables A2.15 and A2.16). In June 2012, NH_4^+ and NO_3^- concentrations did not differ between treatments (F value=0.90, p=0.42 and F value=1.42, p=0.26, respectively) (Appendix A, Tables A2.17 and A2.18).

Soil N concentrations did not differ between transects, except in June 2012 when NO_3^- was higher at the 0-m transect in 0-5cm soils. In June 2012, soil NO_3^- concentrations in 0-5cm samples were significantly higher at the 0-m transect (F value=4.44, p=0.04) while NH_4^+ did not differ between transects (F value=0.04, p=0.84), (Appendix A, Tables A2.17 and A2.18). June 2012 concentrations of NO_3^- in 0-5cm soils were 43.49±5.86 mg kg^{-1} at the 0-m transect and 29.40±3.47 mg kg^{-1} at the 50-m transect, while NH_4^+ concentrations averaged 5.94±0.51 mg kg^{-1}. Extractable N did not differ between the soils at the 0-m and 50-m transects in July 2011 for NH_4^+ (F value= 1.12, p=0.30) and NO_3^- concentrations (F value= 1.68, p=0.21) or in November 2011 for NH_4^+ (F value= 0.61, p=0.44) and NO_3^- concentrations (F value=0.42, p=0.52) (Appendix A, Tables A2.13-A2.16).

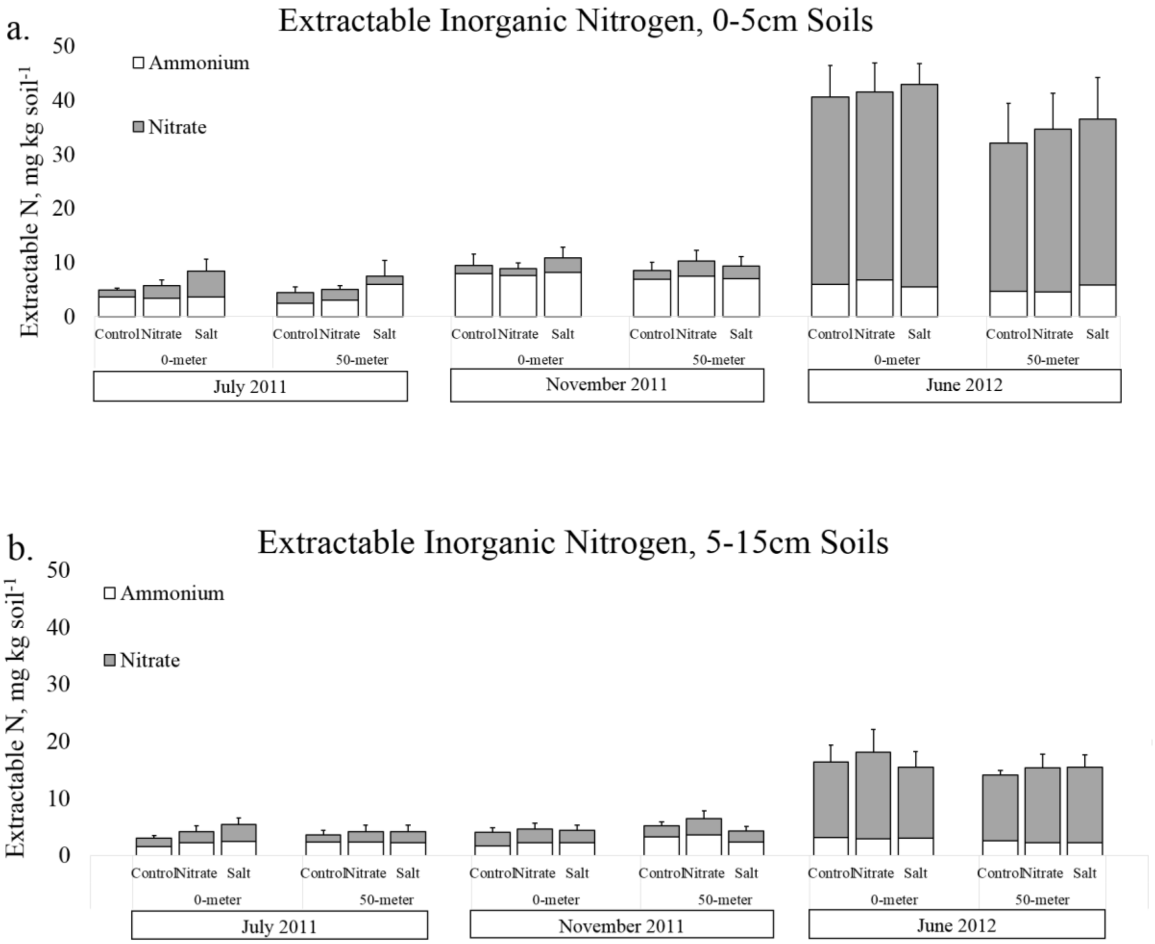


Figure 2.2: Extractable soil NH_4^+ and NO_3^- in experimental plots, at 0- and 50-m transects in July 2011, November 2011, and June 2012. Extractable inorganic N from the 0-5cm layer is illustrated in Figure 2.2a and 5-15cm soil extractable inorganic N in Figure 2.2b.

In the 5-15cm soil layer, treatment with salt and N did not impact July 2011 concentrations of NH_4^+ (F value=0.48, p=0.62) and NO_3^- (F value=1.39, p=0.27) (Appendix A, Tables 2.19 and 2.20). Treatments did not affect November 2011 concentrations of NH_4^+ (F value=1.28, p=0.29) or NO_3^- (F value=0.52, p=0.60) and did not impact the June 2012 concentrations of NH_4^+ (F value=0.29, p=0.75) and NO_3^- (F value=0.65, p=0.53) (Appendix A, Tables 2.21-2.24). Ammonium concentrations in 5-15cm soils did not differ between the 0-m and 50-m transects in July 2011 (F value=0.46, p=0.50), November 2011 (F value= 2.26, p=0.23) or June 2012 (F value=3.16, p=0.09) (Appendix A, Tables A2.19, A2.21, and A2.23). Concentrations of NO_3^- in 5-15cm soils did not differ between transects in July 2011 (F value=1.79, p=0.19), November 2011 (F value=0.05, p=0.83) or June 2012 (F value=0.28, p=0.60) (Appendix A, Tables A2.20, A2.22, A2.24). Compared to the 0-5cm soil layer, the 5-15cm layer had lower NH_4^+ concentrations, while NO_3^- concentrations were lower in June 2012 only. Ammonium was 2.17±0.19 mg kg^{-1} in July 2011, 2.54±0.19 mg kg^{-1} in November 2011, and 2.68±0.19 mg kg^{-1} in June 2012 in 5-15cm soils. The lower NH_4^+ concentrations in 5-15cm soils were significant in July 2011 (t statistic= 1.69, n=72, p=0.001), November 2011 (t statistic=11.87, n=72, p<0.00001), and June 2012 (t statistic=5.97, n=72, p<0.00001). Soils from the 5-15cm layer had NO_3^- concentrations of 1.87±0.22 mg kg^{-1} in July 2011, 2.27±0.23 mg kg^{-1} in November 2011, and 14.47± 1.58 mg kg^{-1} in June 2012. Nitrate did not differ between depths in July 2011 (t statistic=1.20, n=72, p=0.12) or November 2011 (t statistic=0.97, n=72, p=1.69). However in June 2012 NO_3^- concentrations were significantly higher in 0-5cm soils (t statistic =6.79, n=72, p<0.00001).

2.3.2 Carbon Mineralization and Soil Respiration

Potential C mineralization was measured from lab-incubated 0-5cm soils in July and November 2011. There was a salt-treatment effect on CO_2-C emissions, while they did not significantly differ between N-treated and control soils. Carbon emissions were significantly lower in salt-treated soils in both July 2011 (F value=19.30, p<0.00001, TukeyHSD p<0.00001) and November 2011 (F value=12.89, p<0.00001, TukeyHSD p<0.00001) (Appendix A, Tables A2.25 and A2.26) (Figure 2.3). Carbon mineralization was significantly higher at the 0-m transect in July 2011 (F value=4.51, p=0.03) and November 2011 (F value=4.03, p=0.04) (Appendix A, Tables A2.25 and A2.26). In July 2011 at the 0-m transect, C mineralization in salt-treated soils was 1.34±0.08 µg CO_2-C g^{-1} h^{-1}, and in N-treated and control soils it was 1.55±0.12 µg CO_2-C g^{-1} h^{-1}. At the 50-m transect in July, C mineralization in salt-treated soils was 0.83±0.08 µg CO_2-C g^{-1} h^{-1} and in N-treated and control soils was 1.57±0.12 µg CO_2-C g^{-1} h^{-1}. In November 2011 at the 0-m transect, salt-treated soils emitted 1.72±0.06 µg CO_2-C g^{-1} h^{-1} and N-treated and control soils emitted 2.03±0.09 µg CO_2-C g^{-1} h^{-1}. At the 50-m transect in November, C mineralization in salt-treated soils was 1.38±0.07 µg CO_2-C g^{-1} h^{-1} and in N-treated and control soils was 1.96±0.09 µg CO_2-C g^{-1} h^{-1}. Over 16-day incubations in July and November, cumulative CO_2-C emissions were lowest from salt-treated soils at the 50-m transect (Figure 2.3). They were approximately 43% lower in July and 30% lower in November 2011 when compared to cumulative emissions from N and control soils.

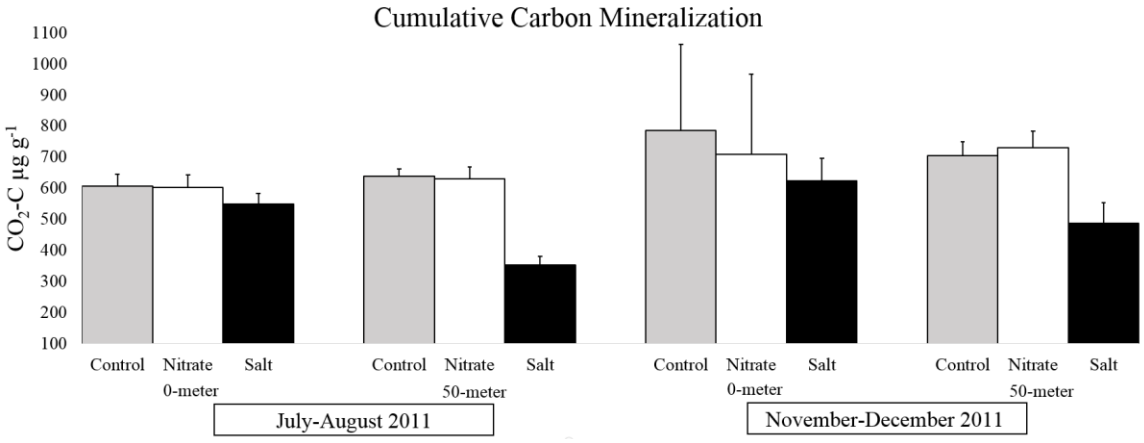


Figure 2.3: Cumulative carbon mineralization over 16 days of incubation from soil from July and November 2011.

In situ soil respiration was measured from June through October 2012. The soil respiration data, which include both heterotrophic microbial respiration and plant root respiration, are from the 50-m transect only because the 0-m transect was demolished for highway construction. Experimental salt plots had significantly lower respiration than N-treated or control plots (F value=8.44, p=0.0004) (Appendix A, Table A2.27), and soil respiration was higher when temperatures were warmer during June, July and August (Figure 2.4). The average respiration from salt-treated plots during the summer was 7943±648 μg CO$_2$-C day^{-1} while it was 12,079±3,306 μg CO$_2$-C day^{-1} from N-treated and control plots. In September and October, it was 4,004±806 μg CO$_2$-C day^{-1} in salt-treated plots and was 5,533±250 μg CO$_2$-C day^{-1} in N-treated and control plots.

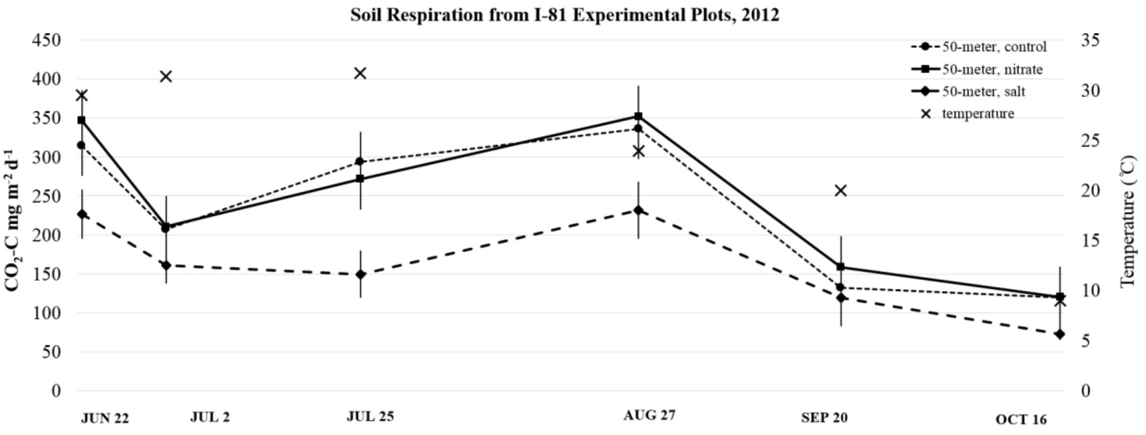


Figure 2.4: In situ soil respiration (CO$_2$-C mg mg^{-2} day^{-1}) and soil temperature (℃) from June-October 2012 in roadside experimental plots. Data presented are from the 50-m transect only, as the other transect was lost due to construction of I-81.

2.3.3 Other Soil Chemistry Characteristics

The electrical conductivities of soils were elevated in salt-treated soils, indicating impacts of salt exposure, while conductivities of soils treated with N did not differ from control soils (Table 2.1). There were significant differences between soil conductivities of the different treatments in 0-5cm soils in July 2011 (F value=274.64, $p<0.00001$), November 2011 (F value=14.26, $p<0.00001$) and June 2012 (F value=19.95, $p<0.00001$) (Appendix A, Tables A2.28-A2.30). Tukey HSD tests showed that in the 0-5cm layer, salt- treated soils had significantly greater conductivities than N-treated or control soils in July 2011 ($p<0.00001$), November 2011 ($p<0.0001$) and June 2012 ($p<0.001$). Soils from the 0-5cm layer of salt-treated plots had conductivities of 2,637±837 µS cm^{-1}, 719±53 µS cm^{-1}, and 1,133±186 µS cm^{-1} in July 2011, November 2011 and June 2012, respectively. Soils from N-treated and control plots had conductivities of 256±180 µS cm^{-1}, 317±68 µS cm^{-1}, 379±44 µS cm^{-1} in July 2011, November 2011 and June 2012, respectively. Soils at the 0-m transect had conductivities that were higher than soils at the 50-m transect (Table 2.1 a,b,c), and this was significant in June 2012 (F value=11.04, $p=0.003$) but not in July 2011 (F value=3.04, $p=0.09$) or November 2011 (F value=0.46, $p=0.50$) (Appendix A, Tables A2.28-A2.30). Conductivities of 5-15 cm soils differed significantly in July 2011 (F value=20.66, $p<0.00001$), November 2011 (F value=12.19, $p=0.0001$) and June 2012 (F value=4.36, $p=0.02$) (Appendix A, Tables A2.31-A2.33). Tukey HSD tests showed that in the 5-15cm layer, salt-treated soils had significantly larger conductivities than N-treated or control soils in June 2011 ($p<0.0001$), November 2011 ($p<0.001$), and June 2012 ($p<0.001$). Conductivities of salt-treated soils from the 5-15cm layer were 845±101 µS cm^{-1} in July 2011, 372±55 µS cm^{-1} in November 2011, and 268±33 µS cm^{-1} in June

2012. In the 5-15cm layer N-treated and control soils had averages of 236±49 µS cm^{-1} in July 2011, 153±16 µS cm^{-1} in November 2011, and 189±17 µS cm^{-1} in June 2012. Soils from the 5-15cm layer had higher conductivities at the 0-m transect, and this was significant in June 2012 (F value=13.19, p=0.001) but not July 2011 (F value=3.28, p=0.08) or November 2011 (F value=0.70, p=0.41) (Appendix A, Tables A2.31-A2.33). Conductivities were also higher in 0-5cm soils compared to 5-15cm (Table 2.1). This was significant in July 2011 (t statistic=4.02, p=0.009), November 2011 (t statistic=5.48, p<0.00001), and June 2012 (t statistic=5.40, p<0.00001).

Soil pH in in the 0-5cm layer averaged 6.74±0.13 in July 2011, 7.16±0.10 in November 2011, and 6.52±0.10 in June 2012. There were no discernable impacts of salt or N treatment on pH in 0-5cm soils from July 2011 (F value=1.05, p=0.36), November 2011 (F value=1.09, p=0.35) or June 2012 (F value=2.45, p=0.12) (Appendix A, Tables A2.34-A2.36). Soil pH was generally higher at the 0-m transect compared to the 50-m transect (Table 2.1 a,b,c), and this was significant in 0-5cm soils from June 2012 (F value= 64.72, p<0.00001), and 5-15cm soils from July 2011 (F value=64.06, p<0.00001), November 2011 (F value=65.08, p<0.00001) and June 2012 (F value=79.61, p<0.00001) (Appendix A, Table A2.36-A2.39). Soil pH was higher in 5-15cm soils than 0-5cm soils, where it was 6.90±0.11 in July 2011, 7.24±0.11 in November 2011, and 6.80±0.11 in June 2012. Soil pH was significantly higher in 5-15cm soils compared to 0-5cm soils in June 2012 (t statistic=8.00, p<0.00001).

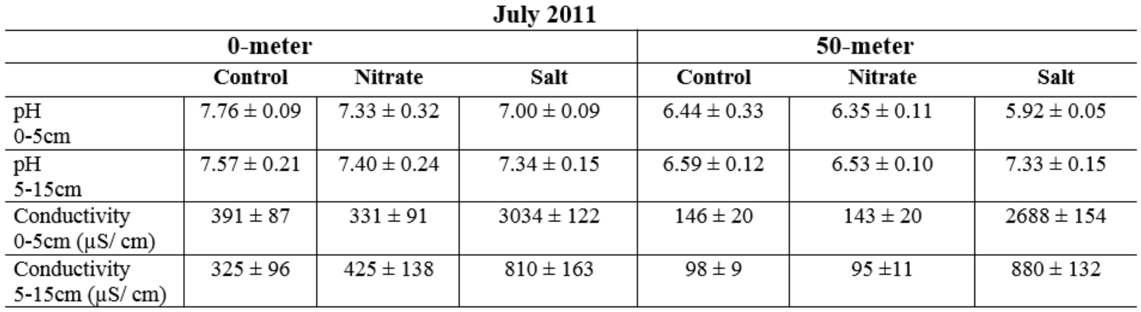
Soil moisture and organic matter were not impacted by treatment with salt and N but both were higher at the 0-m transect. Moistures of 0-5cm soils were not impacted by treatments in July 2011 (F value=2.02, p=0.15), November 2011 (F value=0.85, p=0.44), or June 2012 (F value=0.47, p=0.63) or in the 5-15cm layer in November 2011 (F value=0.20, p=0.66) and June 2012 (F value=1.57, p=0.22) (Appendix A, Tables A2.40-A2.42, A2.44 and A2.45). In July 2011, moisture of 5-15cm soils was significantly lower in salt-treated soils compared to N-treated or control soils (F value=10.08, p=0.0005) (Appendix A, Table A2.43) (Tukey HSD, p<0.01). Soil moisture in the 0-5cm layer was higher at the 0-m transect in July 2011 (F value=15.22, p=0.0005), November 2011 (F value=6.55, p=0.02), and June 2012 (F value=16.02, p=0.0005) (Appendix A, Tables A2.22-A2.24). Average moistures of 0-5cm soils were 31.04±1.27% at the 0-m and 25.45±1.06% at the 50-m transects in July 2011, 58.21±2.96% at the 0-m and 49.42±1.64% at the 50-m transects in November 2011, and 52.02±1.03% at the 0-m and 42.64±2.04% at the 50-m transects in June 2012. Soil moisture was not higher at the 0-m transect in 5-15cm soils from July 2011 (F value=1.90, p=0.18), November 2011 (F value=0.20, p=0.66), or June 2012 (F value=1.57, p=0.22) (Appendix A, Tables A2.43-A2.45). In the 5-15cm layer, soil moisture was 18.41±4.70% in July 2011, 32.04±4.07% in November 2011 and 30.67±0.71% in June 2012.

Soil organic matter did not show an impact of treatment in 0-5cm soils from July 2011 (F value=1.11, p=0.34), November 2011 (F value=1.42, p=0.26) or June 2012 (F value=2.95, p=0.07) (Appendix A, Tables A2.46-A2.48). Soil organic matter was significantly higher in 0-5cm soils at the 0-m transect in July 2011 (F value=14.81, p=0.0006), November 2011 (F value=7.61, p=0.009), and June 2012 (F value=21.13, p=0.0001) (Appendix A, Tables A2.46-A2.48). It was 9.04±0.20% at the 0-m and 8.10±0.14% at the 50-m transects in July 2011, 9.56±0.31% at the 0-m and 8.65±0.14% at the 50-m transects in November 2011, and was 9.46±0.23% at the 0-m and

8.10±0.21% at the 50-m transects in June 2012. Soil organic matter did not differ between treatments in 5-15cm soils in July 2011 (F value=0.88, p=0.43), November 2011 (F value=1.55, p=0.23) and June 2012 (F value=0.03, p=0.97) (Appendix A, Tables A2.49-A2.51). It did not differ between transects in 5-15cm soils from July 2011 (F value=1.25, p=0.27), November 2011 (F value=0.25, p=0.62) or June 2012 (F value=0.63, p=0.44) (Appendix A, Tables A2.49-A2.51). In 5-15cm soils, soil organic matter was 5.57±0.39% in July 2011, 5.49±0.10% in November 2011, and 5.35±0.11% in June 2012.

Table 2.1: Soil pH and soil conductivities for 0-5cm and 5-15cm soils of experimental plots on 0- and 50-m transects. Values from July 2011 are shown in (a), November 2011 are shown in (b) and June 2012 are shown in (c).

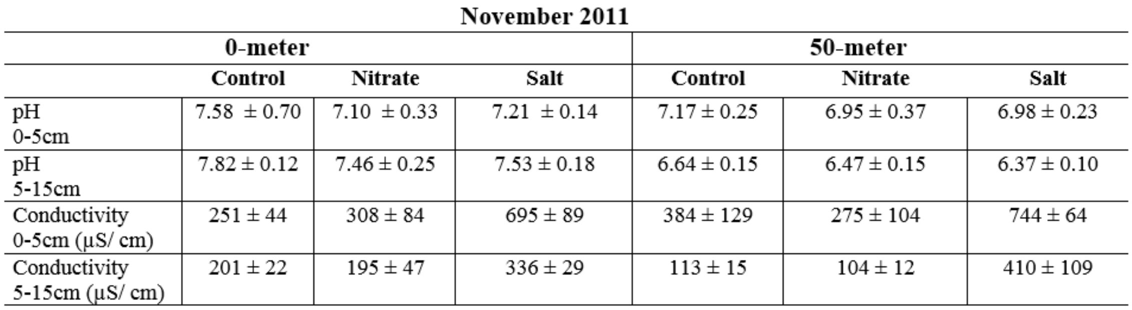
a.



July 2011

	0-meter			50-meter		
	Control	**Nitrate**	**Salt**	**Control**	**Nitrate**	**Salt**
pH 0-5cm	7.76 ± 0.09	7.33 ± 0.32	7.00 ± 0.09	6.44 ± 0.33	6.35 ± 0.11	5.92 ± 0.05
pH 5-15cm	7.57 ± 0.21	7.40 ± 0.24	7.34 ± 0.15	6.59 ± 0.12	6.53 ± 0.10	7.33 ± 0.15
Conductivity 0-5cm (µS/ cm)	391 ± 87	331 ± 91	3034 ± 122	146 ± 20	143 ± 20	2688 ± 154
Conductivity 5-15cm (µS/ cm)	325 ± 96	425 ± 138	810 ± 163	98 ± 9	95 ±11	880 ± 132

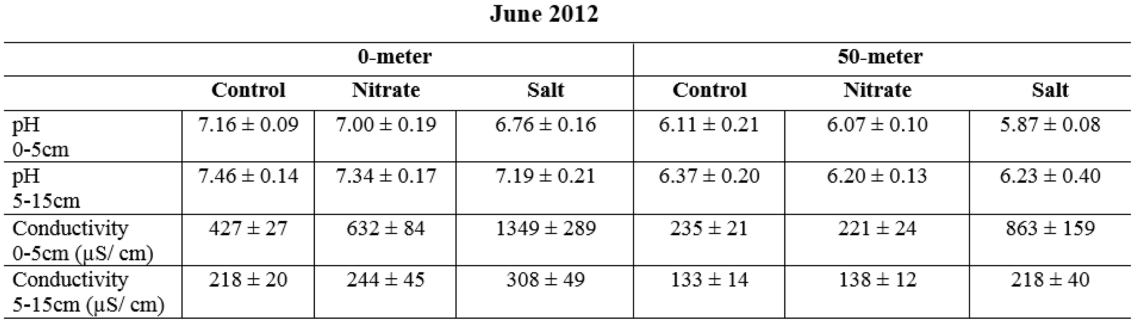
b.



November 2011

	0-meter			50-meter		
	Control	**Nitrate**	**Salt**	**Control**	**Nitrate**	**Salt**
pH 0-5cm	7.58 ± 0.70	7.10 ± 0.33	7.21 ± 0.14	7.17 ± 0.25	6.95 ± 0.37	6.98 ± 0.23
pH 5-15cm	7.82 ± 0.12	7.46 ± 0.25	7.53 ± 0.18	6.64 ± 0.15	6.47 ± 0.15	6.37 ± 0.10
Conductivity 0-5cm (µS/ cm)	251 ± 44	308 ± 84	695 ± 89	384 ± 129	275 ± 104	744 ± 64
Conductivity 5-15cm (µS/ cm)	201 ± 22	195 ± 47	336 ± 29	113 ± 15	104 ± 12	410 ± 109

c.



June 2012

	0-meter			50-meter		
	Control	**Nitrate**	**Salt**	**Control**	**Nitrate**	**Salt**
pH 0-5cm	7.16 ± 0.09	7.00 ± 0.19	6.76 ± 0.16	6.11 ± 0.21	6.07 ± 0.10	5.87 ± 0.08
pH 5-15cm	7.46 ± 0.14	7.34 ± 0.17	7.19 ± 0.21	6.37 ± 0.20	6.20 ± 0.13	6.23 ± 0.40
Conductivity 0-5cm (µS/ cm)	427 ± 27	632 ± 84	1349 ± 289	235 ± 21	221 ± 24	863 ± 159
Conductivity 5-15cm (µS/ cm)	218 ± 20	244 ± 45	308 ± 49	133 ± 14	138 ± 12	218 ± 40

2.4 Discussion

We measured rates of N and C cycling, and common chemistry characteristics of soils from a roadside ecosystem. In this experiment, plots were treated with roadside pollutants of N and salt to explore the impacts of exposure over a short-term time frame. Plots were treated on two transects. The 0-m transect was adjacent to I-81, and soils on this transect were exposed to roadside pollutants over a long-term period of time. The 50-m transect was 50 meters away from the interstate and parallel to the 0-m transect, and was assumed to be less acutely exposed to roadside pollutants. We did not find impacts of treatment or long-term exposure to roadside pollutants on rates of N cycling, or extractable N; however, there were measurable impacts of salt treatments on C mineralization, respiration and conductivities on soils in this roadside ecosystem.

2.4.1 Nitrification, Nitrogen Mineralization, and Soil N Concentrations Differed Seasonally but Not Between Treatments or Transects

We hypothesized that N treatments would increase rates of nitrification and N mineralization because it tends to be a limiting nutrient in terrestrial ecosystems, but did not observe this effect. Nitrogen applications can stimulate soil microbial communities to increase their rates of nitrification and N mineralization (Magill et al. 2000, Phoenix et al. 2012, Pouyat and Turechek 2001). Furthermore, N enriches the microbial community itself, and when microbes decompose they release N back into the soil. This further elevates the N that is available for processing. These effects have been shown to be larger with higher N inputs (Magill et al. 2000, Phoenix et al. 2012, Pouyat and Turechek 2001). For example in the Harvard Forest of Massachusetts, Magill et al. (2000) found that N additions of low (5mg yr^{-1}) and high (15mg yr^{-1}) concentrations increased rates of N mineralization, and high N (15mg yr^{-1}) increased rates of nitrification of soils in pine and hardwood stands. Another example is from Pouyat and Turechek (2001), who compared rates of nitrification and N mineralization in urban, suburban and rural soils. They showed that with more mineralizable N in the top 10 cm of suburban and urban soils, rates of both nitrification and N mineralization were higher than in reference rural sites (Pouyat and Turechek 2001). However in this experiment, we did not observe changes in rates of nitrification or N mineralization in N-treated plots, and our experimental treatments of N may have been too low to have a discernable impact on the rates of nitrification and N mineralization.

In temperate climates where the use of deicing salt is common, it can accumulate in soils alongside roads (Bryson and Barker 2002, Cunningham et al. 2008, Findlay and Kelly 2011, Kincaid and Findlay 2009). Exposure to deicing road salt alters the trajectories of roadside ecosystems by affecting plant growth and soil processes (Green and Cresser 2007, Green et al. 2008, Heintzman et al. 2015). The use of deicing salt has been shown to negatively impact plant growth in roadsides (Bryson and Barker 2002, Heintzman et al. 2015). Another experiment at this site showed reduced growth of plants that were grown in soil collected from the 0-m transect (Heintzman et al. 2015). The reduction of plant growth was linked to plant tissue Na^+ concentrations and soil Cl^- concentrations, indicating impacts of deicing salt (Heintzman et al. 2015). When salt accumulates in soils, it affects not only plants, but the microbial community. Deicing salt has been shown to inhibit or enhance rates of nitrification and N mineralization. For example, McCormick and Wolf (1980) showed that treatment of soils with salt inhibited rates of nitrification and N mineralization. On the other hand, Green and Cresser (2007) found that salt-impacted roadsides in Scotland had higher soil pH measurements, due to displacement of ions with Na^+, and higher rates of nitrification and N mineralization. They reasoned the higher pH may have stimulated rates of nitrification and N mineralization. In our experiment, salt treatments were expected to reduce rates of nitrification and N mineralization, but we did not measure such an affect (Figure 2.1 a-d). Therefore, we conclude that treatments with salt did not impact rates of nitrification or N mineralization during the experimental period.

We examined differences in rates of N cycling between the 0-m transect, which was adjacent and parallel to I-81, and the 50-m transect, which was 50 meters away from and parallel to I-81. In addition to higher N and deicing salt concentrations in roadside soils, they can accumulate metals from the automobiles. Brake linings emit cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), antimony (Sb) and zinc (Zn) (Hjortenkrans et al. 2006). Tire abrasion is another significant source of Zn (Councell et al. 2004, Yesilonis et al. 2008). Lead was an important component of automobile exhaust until 1986 and persists in many urban soils (Pouyat et al. 2010). Soil contamination with metals is greatest within 10m of the road (Hjortenkrans et al. 2008). Therefore, we assumed that the soils at the 0-m transect had been exposed to a range of roadway-derived pollutants over a long period of time, and soils at the 50-m transect would have been exposed to less, which could cause differences in soil processes.

Heavily trafficked roads have been reported to have high fluxes of N from automobiles, so the experimental plots at the 0-m transect were expected to have exposure to more N than the 50-m transect which could enhance nitrification and N mineralization (Bettez et al. 2013, Padgett et al. 1999). Deicing salt has also been shown to be high in roadsides, which could inhibit nitrification and N mineralization (Bryson and Barker 2002, Cunningham et al. 2008, Findlay and Kelly 2011, Kincaid and Findlay 2009). However there were no differences in nitrification and N mineralization between transects in July and November 2011, but the rates of nitrification and N mineralization were significantly elevated at the 0-m transect in June 2012. It is possible that fluxes of N from automobiles could have stimulated rates of nitrification and N mineralization at this time, but there are other important factors to consider. At the 0-m transect, we consistently measured higher soil moisture and soil organic matter in 2011 and 2012, which would enhance rates of nitrification and N mineralization. Warm June temperatures, combined with higher soil moisture and organic matter likely increased in soil microbial activity at the 0-m transect, which would correspond to the significantly higher nitrification and N mineralization rates.

Extractable soil inorganic N concentrations were not impacted by treatments of N or salt (Figure 2.2a, b). Experimental N applications could have been too low to discernibly impact microbial communities, and it could have been absorbed by plants, microbes or leached from soils. While the 0-m transect was likely exposed to more inorganic N due to atmospheric deposition derived from vehicles, we did not measure differences in extractable N between the 0-m and 50-m transects (Figure 2.2 a,b). Concentrations of NH_4^+ did not differ between transects and concentrations of NO_3^- differed between transects only in June 2012 when rates of nitrification were significantly higher at the 0-m transect (Figure 2.2 a, b).

We observed seasonal differences in extractable soil inorganic N (Figure 2.2a). Inorganic N was lowest in 2011, and was dominated by NH_4^+ which is more effectively retained by soils than NO_3^-. In 0-5cm soils, there was less soil NH_4^+ in July 2011 compared to November 2011, which was likely due to plant uptake and microbial immobilization during the growing season. Ammonium was significantly higher in 0-5cm soils compared to 5-15cm soils in 2011. In 2011, NO_3^- concentrations were lower than NH_4^+ concentrations, and did not significantly differ by soil depth, which could reflect NO_3^- uptake, leaching or both.

During the experimental period, June 2012 had the highest NO_3^- concentrations. In June 2012, extractable inorganic N from soils was dominated by NO_3^-, with concentrations that were 10-15x higher than concentrations that were measured in July and November 2011. The drastic increase in extractable NO_3^- in June 2012 compared to 2011 is probably a result of the time of the soil collection. The June 2012 sampling was earlier in the growing season than other soil collections due to compliance with unexpected road construction at the site. Vegetation was scant at this point in the growing season. At the time of the June 2012 soil sampling, the vegetative cover of plots ranged from 0-5%. This would have resulted in less plant uptake of N, which along with the high rates of nitrification and N mineralization, explain the high concentrations of soil NO_3^- (Figure 2.2a). By mid- July, vegetation cover had grown to 50-100%, which is probably why we measured lower inorganic N concentrations in soils from July 2011. Furthermore, both NO_3^- concentrations and nitrification were elevated at the 0-m transect in June 2012. As discussed above, the high rates of nitrification are probably attributable to higher soil moisture and soil organic matter at the 0-m transect. Additionally, June 2012 soil collection was the only time that NO_3^- was significantly higher in the 0-5cm soils, which is not surprising given the higher rates of nitrification and low vegetation cover. The June 2012 NH_4^+ concentrations were similar between the June 2012 and 2011 soil samplings.

2.4.2 Negative Impacts of Deicing Salt on Carbon Mineralization and Soil Respiration

Rates of C mineralization in the lab and soil respiration in situ were significantly lower in salt-treated soils at both transects, which supported the hypothesis that salt inputs would negatively impact the soil microbial communities. While C mineralization was negatively impacted by exposure to salt, rates of nitrification and N mineralization did not exhibit an analogous decrease, although this was reported in other research (McCormick and Wolfe 1980, Pathak and Rao 1998). The reduction in C mineralization was more evident at the 50-m transect compared to the 0-m transect. Salt-treated soils had 38% fewer CO_2-C emissions at the 50-m transect than the 0-m transect in July 2011, while there were 19% fewer CO_2-C emissions at the 50-m transect in November 2011. This impact could be due to the higher soil moisture and soil organic matter at the 0-m transect, which have been shown to increase soil microbial respiration (Bowden et al. 1998, Fierer et al. 2003). However, since CO_2-C fluxes did not differ between the 0-m and 50-m transects in N-treated and control soils, soil moisture and organic matter are probably not the major drivers of lower C mineralization in salt-treated soils at the 50-m transect. This evidence suggests that soil microbial communities near the road may have evolved to be more resilient to the impacts of road salt, inorganic N and metal deposition. Nitrogen additions have been shown to reduce rates of soil respiration (Bowden et al. 2004, Mo et al. 2007), but N additions were probably not high enough to induce this effect.

In situ CO_2-C fluxes were also significantly lower in salt-treated plots. At the 50-m transect, emissions were 33% fewer than emissions from N-treated and control soils. Only two days of gas sampling were completed before the 0-m transect was lost to demolition for expansion of I-81. From the two days of data we obtained, CO_2-C emissions were 22% lower from salt-treated soils than from N-treated and control soils. Additionally, CO_2-C fluxes were higher in situ during summer months when ambient temperatures were higher. This seasonal observation was expected since microbial activity tends to be elevated by warmer temperatures (Bowden et al. 1998, Fierer et al. 2003, Mo et al. 2007).

2.4.3 Salt Impacts on Soil Chemistry Characteristics

The conductivities of all salt-treated soils were significantly elevated above control and N -treated soils, and were higher at the 0-m transect (Table 2.1). Previous research on the fate of deicing salt has suggested that the majority of it ends up within 10m of the roadside (Lundmark and Olofsson 2007). Conductivities were also significantly higher in 0-5cm soils compared to 5-15cm soils, suggesting retention of road salt in the upper layer of soil even after precipitation and leaching. Conductivities of salt-treated soils were generally higher in July 2011 and June 2012 compared to November 2011, which may have been due to less precipitation and infiltration of salt prior to the soil collections in summer months.

Soil pH did not significantly differ between treatments, and soil pH was found to be generally higher at the 0-m transect compared to the 50-m transect (Table 2.1). Green and Cresser (2008) provide evidence that salt in roadside soil can raise pH due to Na^+ occupying more exchange sites in the soil. Although salt-treated plots did not have higher soil pH, the long-term impacts of repeated road-salting could have caused Na^+ to displace other cations in the soil, increasing the pH. However, we do not have data to support that conclusion. Soil pH was typically higher in 5-15cm soils than 0-5cm soils. The higher soil pH in deeper soils could have been due to plant and microbial uptake of cations in 0-5cm soils.

2.5 Conclusions

We did not find impacts of experimental treatments or long-term exposure to roadside pollutants on rates of N cycling, or extractable N. However, there were seasonal differences in rates of nitrification and N mineralization. Rates were higher during warm months, with the highest rates in June 2012 and the lowest in November 2012. The highest rates of nitrification and N mineralization were at the 0-m transect in June 2012, which corresponded to significantly higher soil NO_3^- concentrations at that time. Salt treatments induced measurable impacts on C mineralization, respiration and conductivities on soils in this roadside ecosystem. Salt treatments negatively impacted C mineralization and in situ soil respiration. This affect was less acute in soils from the 0-m transect, indicating that perhaps the soil microbial communities near the road have evolved to withstand the impacts of deicing salt. Deicing salt accumulation in soils was evident at the 0-m transect and at salt-treated plots, which could affect the long-term trajectory of the plant community and soil processes of this roadside ecosystem. The impacts of deicing salt have also been shown to extend beyond roadside ecosystems, including elevating the salt concentrations of freshwater resources (Kaushal et al. 2005). Best management practices (BMPs) are useful for the attenuation of the impacts of deicing salt on roadside environments, and should be implemented to maintain the health of roadside and urban ecosystems (Fay and Shi 2012).

Chapter 3: Inorganic Nitrogen Discharged to the Susquehanna River from Eight Urban Streams of Binghamton, NY, a Medium-Sized City

3.1 Introduction

Urbanization changes the ecology of landscapes in many ways, and recent urban ecological research has shown that cities have a distinct biogeochemistry (Kaye et al. 2006). Urban nitrogen (N) biogeochemistry has been of particular interest, since urban N availability is greatly elevated in comparison to undisturbed landscapes, where it tends to be a limiting nutrient (Chapin et al. 2002, Kaye et al. 2006). The major reasons for high urban N availability in cities are imports of food, fertilizer and fuel to feed inhabitants and maintain cities' metabolisms (Baker et al. 2001, Fissore et al. 2011). Additionally, N transfers in cities are less efficient, or "leakier," than in undisturbed ecosystems, where N is cycled in a tightly controlled manner between plants and soils (Baker et al. 2001). Inefficient transfers result in more N in urban soils, groundwater and surface waters (Baker et al. 2001, Zhu et al. 2006). Pollution with N and other contaminants is common in water resources adjacent to and downstream of cities, particularly since urbanization has long occurred near water bodies to provide inhabitants with water for consumption, agriculture and travel (Grimm et al. 2008). Increases in N and also phosphorus (P) loading into aquatic systems can cause eutrophication (Rabalais 2002). Eutrophication impairs water quality and ecosystem function and is a concern downstream of cities and agriculture, which discharge large nutrient loads to waterways (Nixon 1995, Rabalais 2002). In cities, N is discharged from both point-sources (wastewater treatment plants, combined sewage overflows) and nonpoint-sources like deposition from fossil-fuel combustion and fertilizers; to address issues of eutrophication, we require a better understanding of both sources of N (Baker et al. 2001). The focus of this chapter will be on nonpoint-source inorganic N in urban streams and other aspects of urban stream chemistry. Chapter 4 will address inorganic N fluxes from the point source of the local Binghamton-Johnson City wastewater treatment plant.

Control of point-source pollution is a large part of the Clean Water Act's (CWA) initiative to improve water quality (Clean Water Act of 1972). Besides point-source pollution, nonpoint-source pollution remains a critically important but under- addressed part of the CWA's goal to "restore and maintain the physical, chemical, and biological integrity of the Nation's waters" (Carey and Migliaccio 2009, Carpenter et al. 1998). Nonpoint-sources of N in cities include gaseous nitrogen oxides (NO_X) that are a by-product of fossil fuel combustion, greenspace fertilizers and leaking sewage infrastructure. Nitrogen oxides are formed when the high temperatures of fossil fuel combustion cause atmospheric N_2 and O_2 to react and form nitric oxide (NO) and nitrogen dioxide (NO_2), or collectively NO_X (Abdel-Raman 1998). Some NO_X is removed by three-way catalytic converters, but some is "over-reduced" to form NH_3 (Heeb et al. 2006). Atmospheric NO_X can be converted to nitrate (NO_3^-), and ammonia (NH_3) to ammonium (NH_4^+), which are deposited on city surfaces (Asman et al. 1998, Butler et al. 2003). Another nonpoint-source of urban N includes fertilizers that contain NH_4^+ and NO_3^-, which are used for parks and greenspaces. Once deposited on urban surfaces, especially impervious surfaces, NH_4^+ and NO_3^- are likely to wash directly in surface runoff to urban streams or leach into groundwater resources during precipitation events. Another nonpoint-source of N is from sewage infrastructure beneath cities where the sewer system and watershed directly interact. For example, sewage infrastructure beneath cities can leak, and may contaminate groundwater that contributes to streamflow (Groffman et al. 2004).

Nonpoint-source N is more difficult to quantify than point-source N due to its diffuse nature, but can be a major contributor to elevated N in urban streams and groundwater (Groffman et al. 2004, Shields et al. 2008). Urban stream N levels are higher than in streams of undisturbed landscapes, which has been shown in research at the Long Term Ecological Research Network (LTER) sites of the Baltimore Ecosystem Study (BES) and the Central Arizona-Phoenix (CAP) ecosystem, and elsewhere (Baker et al. 2001, Fissore et al. 2011, Groffman et al. 2004). Research at the BES has shown that dissolved inorganic nitrogen (DIN) concentrations are higher in urban areas compared to their natural counterparts. Nitrate dominates DIN in urban streams of the BES, partly because NO_3^- is very mobile in soils, making it likely to leach to groundwater, while NH_4^+ is more efficiently retained by soils; furthermore NH_4^+ that is in streams is likely to volatize to NH_3 at high stream water pH (Groffman et al. 2004, Shields et al. 2008). Groffman et al. (2004) reported that in Baltimore, NO_3^- concentrations were about 10 times higher in urban and suburban streams compared to reference forested streams (Groffman et al. 2004). In the same system, Shields et al. (2008) also found high stream NO_3^- concentrations. Both studies found that stream N concentrations were correlated with impervious surface cover. Moreover Shields et al. (2008) found that in low-density housing developments, septic systems released plumes of N to groundwater, causing a notable increase in N to streamflow.

The impacts of urbanization on water quality extend beyond N. Urban streams often have elevated electrical conductivity, road salt, pH, and sometimes phosphorus. Urban streams in temperate climates have higher electrical conductivity and chloride (Cl^-) concentrations that result from wintertime use of deicing salt (Cooper et al. 2014, Kaushal et al. 2005, Kelly et al. 2008). Additionally, accelerated chemical weathering of building materials have increased the alkalinity and pH in streams and rivers (Kaushal et al. 2013). Furthermore, levels of soluble reactive phosphorus (SRP) can be high in streams due to detergents, fertilizers and leaking sewage infrastructure (Sonoda et al. 2001).

While there has been a great deal of research on stream N in large metropolises, much less is known about its status in smaller cities like Binghamton, NY. The Greater Binghamton area has more than 260,000 inhabitants and is a medium-sized urban area, similar to many in upstate New York and Pennsylvania. The City of Binghamton, New York was established at the confluence of two rivers, where the Chenango River discharges to the Susquehanna River. The city is at the headwaters of the Chesapeake Bay watershed; the Susquehanna River flows south into Pennsylvania, and ultimately discharges into the Chesapeake Bay. Several small watersheds in the Greater Binghamton Area have streams that discharge directly to the Susquehanna River, which provides a unique and useful study system. At the Hubbard Brook Ecosystem Study site, Bormann and Likens demonstrated how the small watershed approach is a powerful technique to study biogeochemistry in the 1960’s and 1970’s (Likens et al. 1970). The small watershed approach to studying biogeochemistry was developed at Hubbard Brook, and continues to yield important information regarding biogeochemistry in undisturbed watersheds and watersheds that they have manipulated (Likens et al. 1970, Bernhardt et al. 2003). The Binghamton area has a number of small watersheds that are ideal for the study of how urbanization impacts stream N in a medium-sized city.

Questions remain about the impacts of smaller urban cities on urban watersheds and waterways. In this study, we used a small watershed approach along with urban and rural comparisons of inorganic N to investigate the impact of a medium-sized city on N losses to the Susquehanna River. Our driving questions were (1) whether concentrations and fluxes of DIN were higher at downstream/urban sites compared to upstream/rural sites and (2) if developed land cover was a predictor of stream N content. We also investigated stream conductivity, Cl^-, pH and SRP concentrations. Since these water quality parameters impact aquatic health, we wanted to know if and how they were affected by urbanization in a medium-sized city.

3.2 Methods

3.2.1 Study Locations and Sampling Period

Our study of stream water chemistry in Binghamton was initiated in June 2011 and concluded in May 2013. Binghamton (42°6′08″N 75°54′42″W) is a medium-sized city located in upstate New York. The boundaries of our small watershed study system are within the Greater Binghamton area, which includes the City of Binghamton, Villages of Johnson City and Endicott, Towns of Binghamton, Vestal, Kirkwood, Fenton, Union, Dickinson, Conklin, Maine and the Hamlet of Endwell, has more than 260,000 inhabitants. The average yearly temperature is approximately 8 ℃ and the average yearly precipitation is about 1000 mm. Precipitation totals were calculated for the sampling periods of June 2011-May 2012 and June 2012-May 2013. From 2011-2012 total precipitation was 1372mm, which was about 137% of the average precipitation in Binghamton. This period included Tropical Storm Lee in September 2011, which contributed 190mm of precipitation. Total precipitation from June 2012-May 2013 was 1008mm, which is more similar to the average yearly precipitation in Binghamton of 1000mm.

The study was conducted in eight first and second order streams of the Greater Binghamton area. The streams are Patterson, Willow Run, Fuller Hollow, Little Choconut, Pierce, Nanticoke, Choconut and Tracey. The streams discharge directly to the Susquehanna River (Figure 3.1). Each stream had two sampling sites. The downstream/urban sites were near where the streams discharge to the river, while the other sites were rural and upstream of development (Table 3.1). Water samples taken from downstream/urban sites reflect the impacts of non-point nutrients inputs from watersheds to streams and then the Susquehanna River.

3.2.2 Land Cover Analysis

Watershed delineations for the eight streams were Hydrological Unit Code (HUC) 11 watersheds obtained from the Upper Susquehanna Coalition (http://www.u-s-c.org/html/index.htm). All land area, stream length, and land cover analyses were performed using ArcMap 10.2.2 (https://www.esri.com). The watersheds of the eight streams were analyzed for land cover using data from the National Land Cover Database 2011 (NLCD 2011) (Homer et al. 2015). The pixel size for the NLCD data is 30x30 m.

Table 3.1: Coordinates of downstream and upstream stream sampling locations.

Stream	Downstream Location	Upstream location
Patterson	42.111 Latitude -76.020 Longitude	42.158 Latitude -76.011 Longitude
Willow Run	42.094 Latitude -76.020 Longitude	42.082 Latitude -76.004 Longitude
Fuller Hollow	42.097 Latitude -75.965 Longitude	42.077 Latitude -75.958 Longitude
Little Choconut	42.113 Latitude -75.977 Longitude	42.123 Latitude -75.572 Longitude
Pierce	42.100 Latitude -75.891 Longitude	42.066 Latitude -75.875 Longitude
Choconut	42.082 Latitude -76.064 Longitude	42.013 Latitude -76.007 Longitude
Nanticoke	42.092 Latitude -76.090 Longitude	42.149 Latitude -76.067 Longitude
Tracey	42.069 Latitude -76.103 Longitude	42.021 Latitude -76.082 Longitude

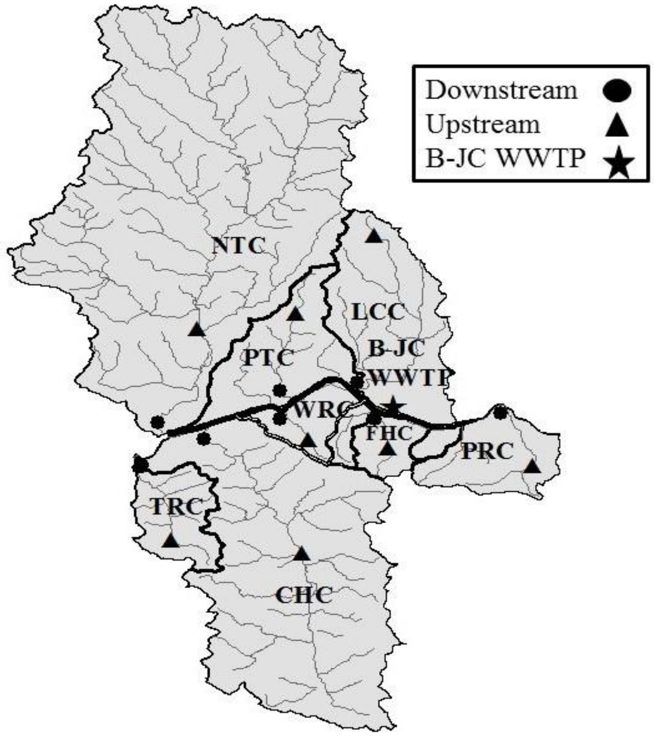


Figure 3.1: Watersheds of the Binghamton area: Patterson (PTC), Willow Run (WRC), Fuller Hollow (FHC), Little Choconut (LCC), Pierce (PRC), Choconut (CHC), Nanticoke (NTC), Tracey (TRC) and their streams. Downstream/urban sites are denoted by circles, upstream/rural by triangles, and the B-JC WWTP by a star.

3.2.3 Stream Water Sampling and Chemistry Analysis

From June 2011 – May 2013, water was collected at each site approximately twice per month, except during winter months when sampling was done once per month; samples were not collected in January or February 2012. Water samples were collected using acid-washed 250mL bottles, and were rinsed once *in situ* with stream water before the sample was collected. Duplicate samples were obtained at each site for the purpose of quality control. Measurements of pH, conductivity and temperature were obtained using a YSI Model 63 Handheld pH, Conductivity, Salinity and Temperature System. Stream cross-sections were used to obtain discharge measurements, using a Global Water Model FP111 flow meter, tape measure and ruler. On several occasions, streams were too deep and flow was too swift to take measurements at Nanticoke and Choconut creeks. For these higher-flow events, regressions were performed to compare the relationship between stream discharge measurements of other collections and Susquehanna River discharge, which is hourly data published on the USGS website (http://waterdata.usgs.gov/usa/nwis). These relationships were significant (r^2 values ranged from from 0.67 to 0.95), and resulting equations were used to estimate stream discharge for the days it could not be measured directly.

After field collection, water samples were transported to the laboratory on ice in coolers. They were stored overnight in a cold room and were processed within 24 hours. Samples were vacuum filtered individually using Whatman GF/A glass microfiber filters into acid-washed 60mL bottles, and treated with 0.2 mL of a 50% H_2SO_4 solution to inhibit microbial activity. Processed stream and effluent samples were stored in a cold room until analysis. Samples were analyzed for NH_4-N and NO_3-N concentrations using a Lachat QuickChem 8000 Flow Injection Analyzer (Lachat Instruments, Milkwaukee, Wisconsin). The determination of NH_4-N is based on the Berthelot reaction, where ammonia reacts with alkaline phenol, then with sodium hypochlorite. The resulting indophenol blue reacts with sodium nitroprusside to enhance sensitivity, and the reaction product is directly proportional to the original NH_4-N concentration. The NO_3-N concentration is determined on the Lachat by quantitatively reducing nitrate to nitrite by passing the sample through a copperized cadmium column. The Lachat was also used to analyze samples for Cl^- and soluble reactive phosphorus (SRP) concentrations for stream and effluent samples from June 2011-May 2012.

3.2.4 Nutrient flow-weighting and flux calculations

Nitrate concentrations were flow-weighted by season to account for differences between stream discharges and variations in seasonal hydrology. We used the Equation 3.1 to calculate the seasonally flow-weighted mean stream N concentrations.

Equation 3.1:

$$FW = \frac{\sum_{i=1}^{n} Ci\ x\ Fi}{\sum_{i=1}^{n} Fi}$$

Where C_i is the concentration of the sample in mg L^{-1}, and F_i is the discharge of the stream in L $second^{-1}$ when the sample was taken. We used Equation 3.2 to calculate the TIN flux (kg day^{-1}) from the eight streams.

Equation 3.2:

$$\begin{aligned} TIN\ flux\ (\text{kg per day}) \\ &= \sum_{i=1}^{8}\left(\left(\frac{mg\ \ NO3-N}{L} x\ \frac{L}{day}\ \div 1000000\right)\right. \\ &\left.+\left(\frac{mg\ NH4-N}{L} x\ \frac{L}{day}\ \div 1000000\right)\right) \end{aligned}$$

Equation 3.3 was used to calculate fluxes of TIN per watershed per year (kg N ha^{-1} yr^{-1}). Fluxes were calculated using seasonally flow-weighted N concentrations (Equation 3.1) and average seasonal discharge (L day^{-1}).

Equation 3.3:

$$Total\ Inorganic\ N\ discharged\ per\ year = \left(\sum_{i=1}^{4}\left(\left(\frac{flow-weighted\ mg\ \ NO3-N}{L}x\ \frac{average\ L}{day} \div 1000000\ x\ days\ in\ season\right) + \left(\frac{flow-weighted\ mg\ NH4-N}{L}x\ \frac{average\ L}{day} \div 1000000\ x\ days\ in\ season\right)\right)\right) \div area\ of\ watershed$$

3.2.5 Data Analyses

We ran an initial data quality analysis comparing the water chemistry between duplicate samples using a linear correlation along a 1:1 line. The average concentrations of the two duplicate samples were used for further analyses. The R statistical program was used to analyze data. Significance was defined as $p<0.05$. Comparisons between upstream and downstream concentrations were calculated using paired t-tests. We analyzed the data using analysis of variance (ANOVA) tests with a general linear model. We used ANOVAs to determine whether concentrations differed by between streams and between seasons. Significant results were analyzed further using Tukey's HSD test. Simple linear regressions were used to analyze the relationship between urban stream NO_3^- concentrations and watershed development. The regressions were run for each season using seasonally flow-weighted urban stream NO_3^- and watershed percent total development. To tests whether the assumptions of the ANOVA and regressions were met, residues were checked for normality and homoscedasticity. Means are given ± 1 standard error (SE).

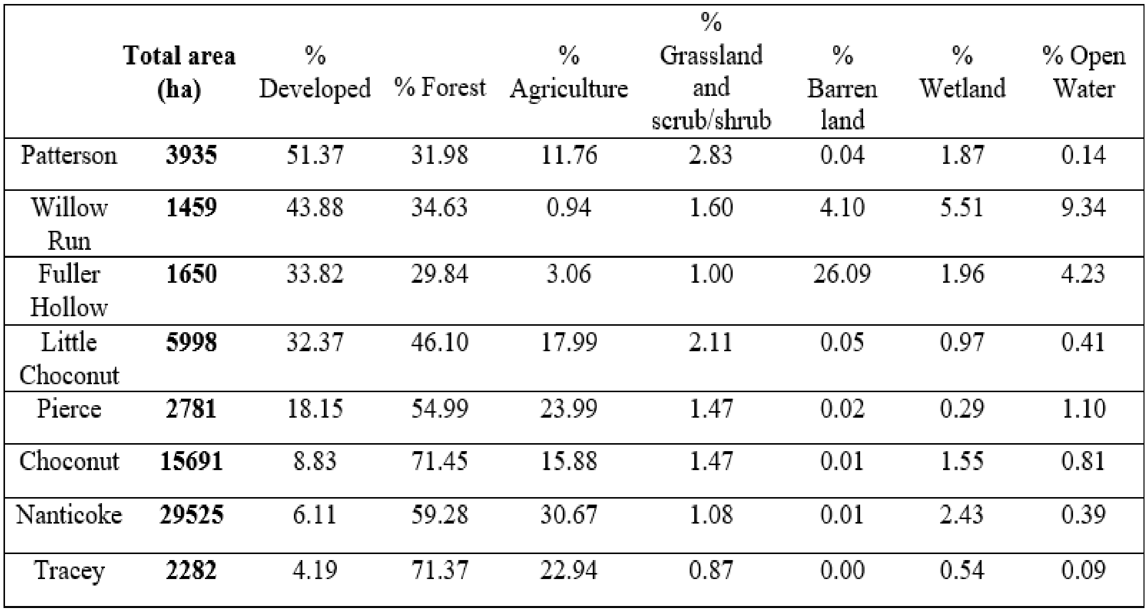
3.3 Results

3.3.1 Land Use Analyses

Watershed sizes varied considerably; the largest watersheds are Nanticoke and Choconut which are 29525 ha and 15691 ha, respectively. Little Choconut, Patterson, Pierce, Tracey, Fuller Hollow, and Willow Run are 5998 ha, 3935 ha, 2781 ha, 2282 ha, 1650 ha, and 1459 ha, respectively (Table 3.2). Watersheds were analyzed for percent land cover using data from the National Land Cover Database (NLCD) 2011 (Tables 3.2 and 3.3). Land cover information provided by the NLCD includes: development, agriculture (cultivated crops and pasture/hay), forest (deciduous forest, evergreen forest, and mixed forest), grassland, scrub/shrub, barren land, wetlands, and open water. The NLCD classifies "development" further into four sub- categories which are "developed, open space" (where impervious surfaces are <20% of total cover; mostly vegetation in the form of lawn grasses mixed with some constructed materials), "developed, low intensity" (where impervious surfaces are 20-49% of total cover; mixture of constructed materials and vegetation), "developed, medium intensity" (where impervious surfaces are 50-79% of total cover; mixture of constructed materials and vegetation), "development, high intensity" (where impervious surfaces are 80-100% of total cover; people reside or work in high numbers) (Homer et al. 2015).

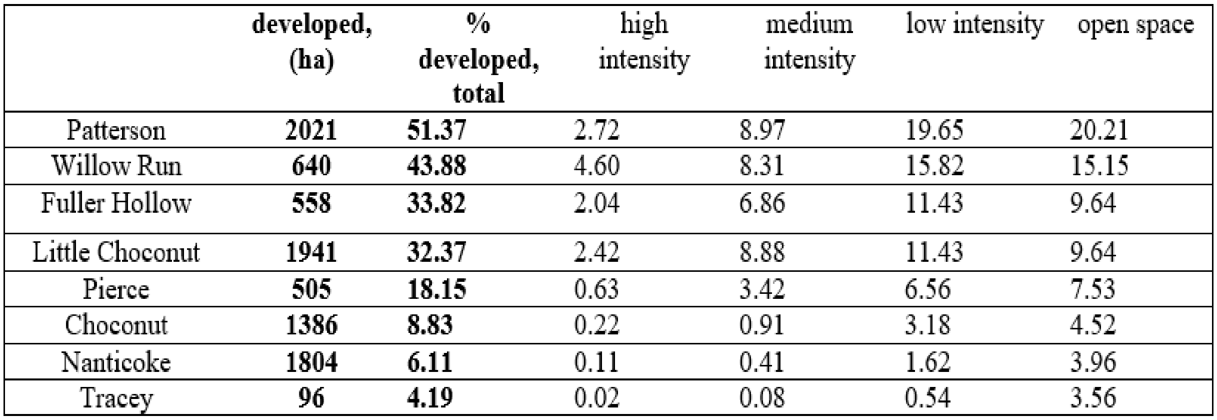
Developed land was the largest percentage of land cover in the Patterson, Willow Run, and Fuller Hollow watersheds, and forest cover dominated the watersheds of Little Choconut, Pierce, Choconut, Nanticoke and Tracey (Table 3.2). The four watersheds with the most- developed land cover are Patterson, Willow Run, Fuller Hollow and Little Choconut with 51%, 44%, 34%, and 32% total development of each watershed, respectively (Table 3.2). Development in Binghamton's watersheds is mostly suburban single-family housing units, parks and golf courses where impervious surface cover (ISC) is <50% of land cover (Table 3.3). Higher intensity development, where ISC ranges from 50-100% is a smaller proportion of total development in the Binghamton urban ecosystem (Table 3.3).

Table 3.2: Land Cover in Eight Watersheds of the Greater Binghamton Area.



	Total area (ha)	% Developed	% Forest	% Agriculture	% Grassland and scrub/shrub	% Barren land	% Wetland	% Open Water
Patterson	**3935**	51.37	31.98	11.76	2.83	0.04	1.87	0.14
Willow Run	**1459**	43.88	34.63	0.94	1.60	4.10	5.51	9.34
Fuller Hollow	**1650**	33.82	29.84	3.06	1.00	26.09	1.96	4.23
Little Choconut	**5998**	32.37	46.10	17.99	2.11	0.05	0.97	0.41
Pierce	**2781**	18.15	54.99	23.99	1.47	0.02	0.29	1.10
Choconut	**15691**	8.83	71.45	15.88	1.47	0.01	1.55	0.81
Nanticoke	**29525**	6.11	59.28	30.67	1.08	0.01	2.43	0.39
Tracey	**2282**	4.19	71.37	22.94	0.87	0.00	0.54	0.09

Table 3.3: Percent Developed High, Medium, Low Intensity and Open Space



	developed, (ha)	**% developed, total**	high intensity	medium intensity	low intensity	open space
Patterson	**2021**	**51.37**	2.72	8.97	19.65	20.21
Willow Run	**640**	**43.88**	4.60	8.31	15.82	15.15
Fuller Hollow	**558**	**33.82**	2.04	6.86	11.43	9.64
Little Choconut	**1941**	**32.37**	2.42	8.88	11.43	9.64
Pierce	**505**	**18.15**	0.63	3.42	6.56	7.53
Choconut	**1386**	**8.83**	0.22	0.91	3.18	4.52
Nanticoke	**1804**	**6.11**	0.11	0.41	1.62	3.96
Tracey	**96**	**4.19**	0.02	0.08	0.54	3.56

3.3.2 Dissolved Inorganic Nitrogen in Urban Streams

Nitrate (NO_3^-) was the dominant form of stream inorganic N (>95%) and it ranged from 0.00-1.08 mg L^{-1} NO_3-N at urban sites and 0.00-0.99 mg L^{-1} NO_3-N at rural sites. Ammonium (NH_4^+) was present in trace concentrations ranging from 0.00 to 0.12 mg L^{-1} NH_4-N. Nitrate concentrations were generally higher at urban sites compared to rural sites (Figure 3.2). Paired t-tests were used to determine whether this observation was significant within each stream (Appendix A, Table A3.1). The streams in watersheds with the most development had significantly higher concentrations of NO_3^- at urban sites, including Patterson ($p<0.0001$), Willow Run ($p=0.001$), Little Choconut ($p<0.0001$) and Fuller Hollow ($p<0.0001$). The less- developed watersheds of Pierce, Nanticoke and Tracey did not have significant differences between NO_3^- in urban or rural samples, but Choconut did have significantly higher urban NO_3^- concentrations ($p<0.0001$).

Urban and rural stream NO_3^- concentrations were flow-weighted by season to account for differences in the sizes of streams, and for differences in seasonal hydrology (see section 3.2.4 for the flow-weighting equation). Seasonally flow-weighted NO_3^- concentrations were calculated for summer and fall 2011, spring, summer, and fall 2012, and winter 2013 and spring 2013; the winter 2012 season had one sampling event (Figure 3.2). Seasonally flow-weighted NO_3^- ranged from 0.03-0.58 mg NO_3-N L^{-1} at urban sites and from 0.0-0.43 mg NO_3-N L^{-1} at rural sites. Figure 3.2 shows seasonally flow-weighted NO_3^- for each stream with urban and rural comparisons.

Mean urban stream NO_3^- concentrations were higher in watersheds that have more development, and there were seasonal differences in stream NO_3^- concentrations. One-way ANOVA's were used to analyze whether NO_3^- concentrations differed significantly between downstream/urban sites and between seasons (Appendix A, Tables A3.2 and A3.3). Nitrate concentrations were significantly different between streams and were highest in Patterson, Willow Run and Fuller Hollow during the two-year sampling period ($F=13.64$, $p<0.0001$) (Figure 3.3). Mean urban NO_3^- concentrations were also significantly different between seasons ($F=7.41$, $p<0.0001$). Summer 2011, spring 2012, summer 2012 and winter 2013 had the highest NO_3^- concentrations while fall 2011, fall 2012 and spring 2013 had lower concentrations (Figure 3.4).

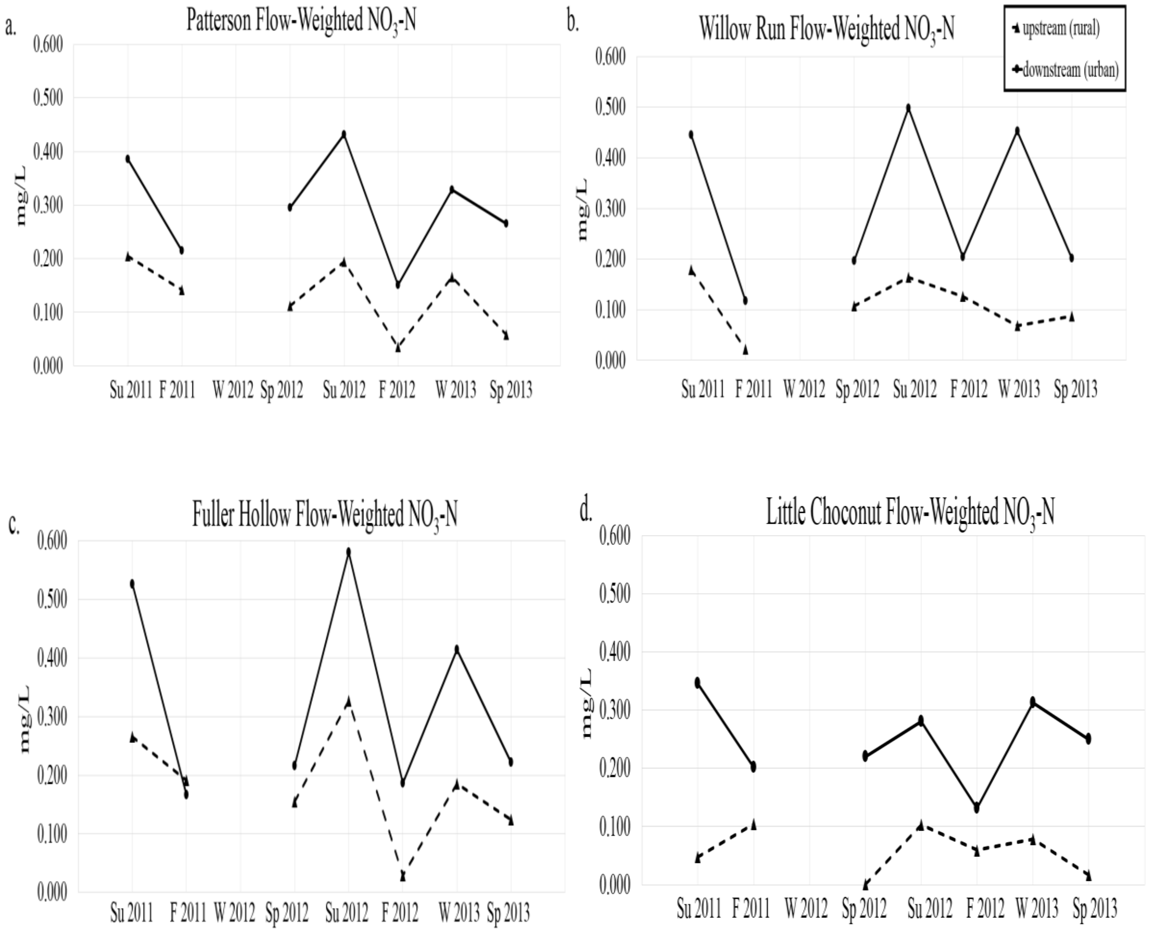


Figure 3.2: Flow-weighted mean seasonal concentrations of NO_3-N, upstream and downstream. Figures 3.2a-d are streams in watersheds with the most development, and Figures 3.2e-h are streams in the watersheds with less development.

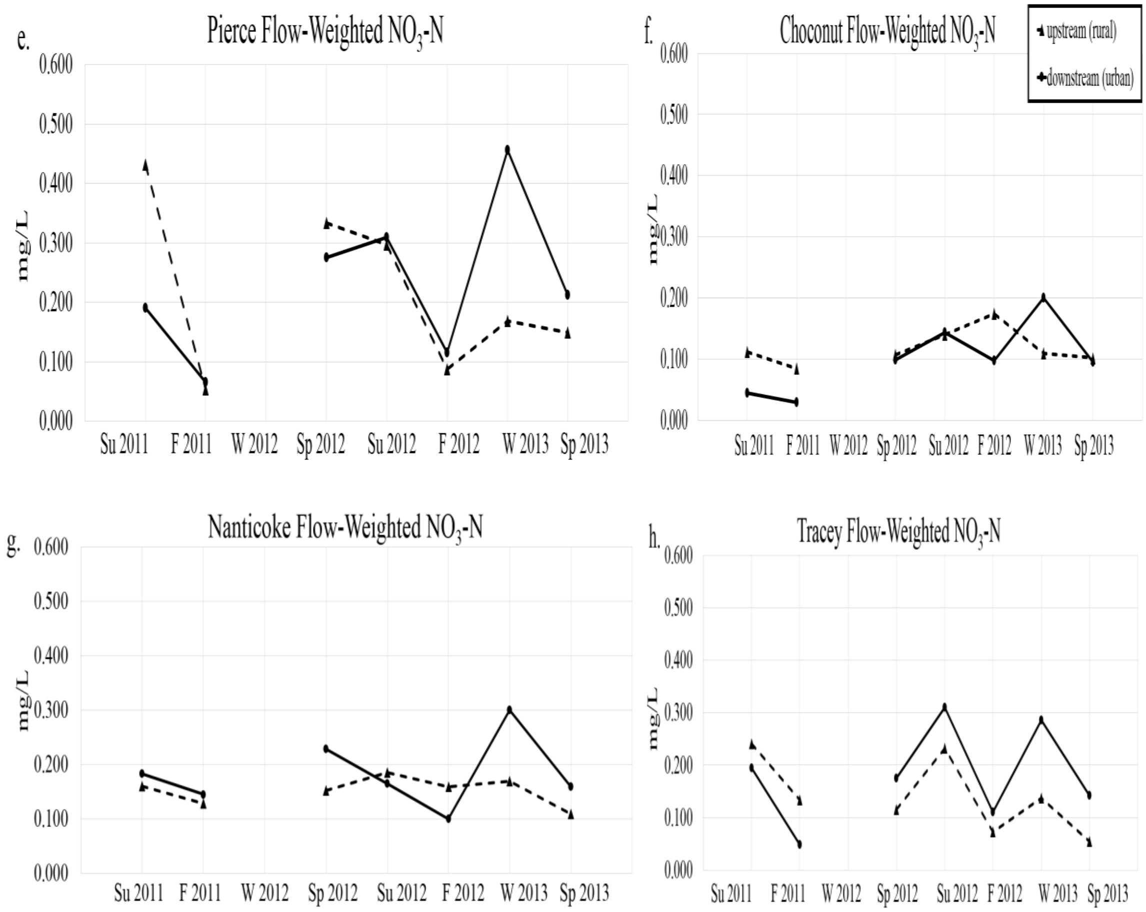


Figure 3.2, continued: Flow-weighted mean seasonal concentrations of NO_3-N, upstream and downstream. Figures 3.2a-d are streams in watersheds with the most development, and Figures 3.2e-h are streams in the watersheds with less development.

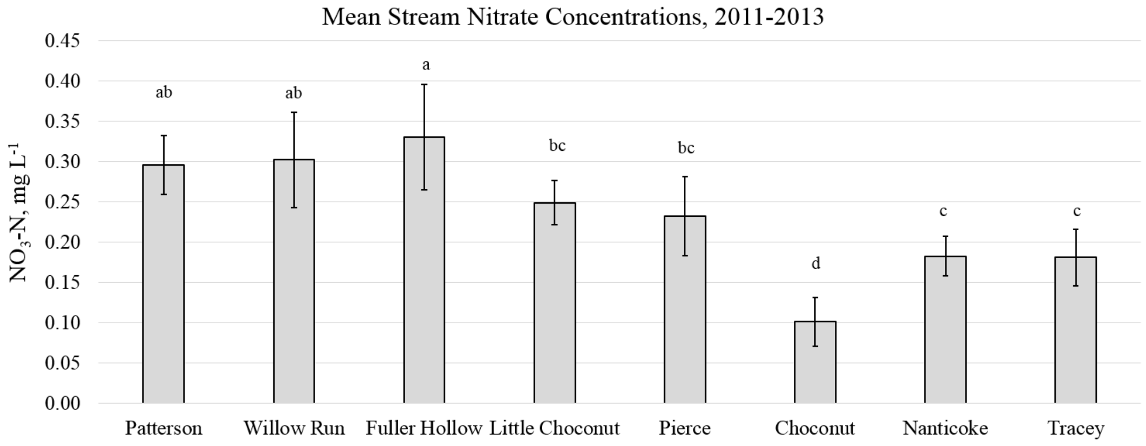


Figure 3.3: Mean NO_3-N concentrations from downstream/urban sites of the eight streams, ±1 SE. Means that do not share a common letter signify a significant difference at p=0.05, according to the Tukey means comparison.

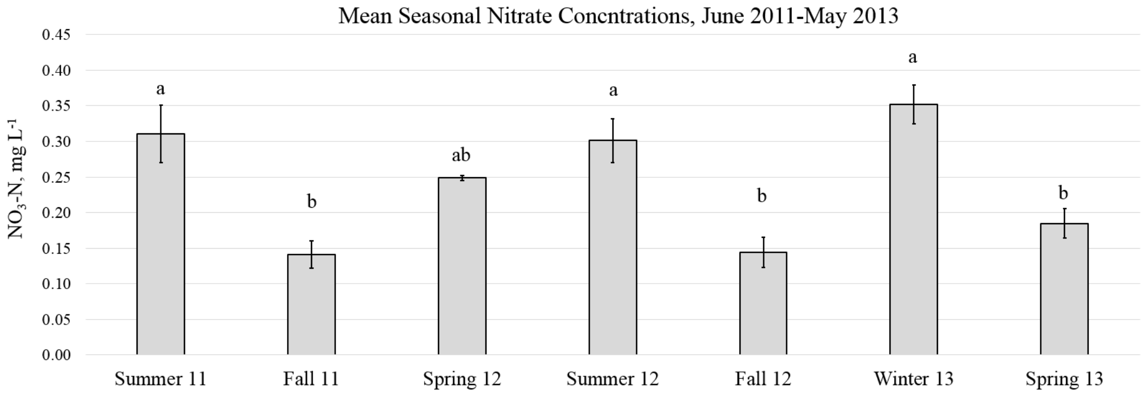


Figure 3.4: Mean seasonal NO_3-N concentrations from downstream/urban sites of the eight streams, ±1SE. Means that do not share a common letter signify a significant difference at p=0.05, according to the Tukey means comparison.

Regressions were run for each season using seasonally flow-weighted urban stream NO_3^- and watershed percent total development. The regression analyses showed strong and significant relationships in all seasons, except in spring 2012 and winter 2013.

In seasons where regressions between NO_3^- and percent development were significant, r^2 values ranged from 0.536 – 0.717 (Table 3.4).

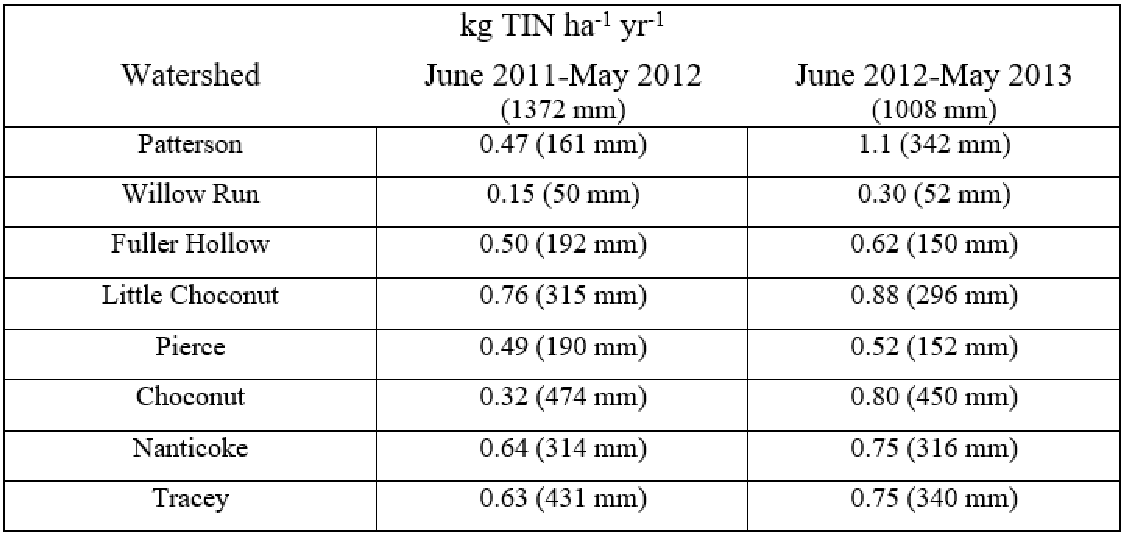
Table 3.4: Regressions results comparing the relationship between flow-weighted NO_3-N and percent watershed development.

Season	**Year**	**r^2**	**p-value**
Summer	2011	0.699	0.01
Fall	2011	0.536	0.04
Spring	2012	0.302	0.16
Summer	2012	0.613	0.02
Fall	2012	0.686	0.01
Winter	2013	0.297	0.16
Spring	2013	0.717	0.01

3.3.3 Dissolved Inorganic Nitrogen Fluxes from Downstream/Urban and Upstream/Rural Sites

Fluxes, or rates of N movement, were calculated using N concentrations and stream discharge for each site (see section 3.2.4 for the daily TIN flux equation). The total fluxes of inorganic N (TIN, or the sum of NO_3^- and NH_4^+) from streams were calculated for each day of downstream/urban and upstream/rural sampling. Total inorganic N fluxes were linked to stream discharge, with higher stream flows discharging more N. Total inorganic N fluxes were consistently higher at urban sites compared to rural sites (Figure 3.5a, b). From June 2011-May 2012, fluxes of TIN ranged from 7-214 kg day^{-1} at urban sites and from 4-170 kg day^{-1} at rural sites. From June 2012-May 2013, fluxes of TIN ranged from 1-466 kg day^{-1} at urban sites and from 1-76 kg day^{-1} at rural sites. The winter 2013 season had the highest average daily TIN flux, followed by the spring of 2013. The lowest TIN fluxes per day were in the summer 2011, summer 2012 and fall 2012 seasons (Figure 3.5c). The sum of yearly TIN output per watershed was divided by the watershed area to obtain an estimate of TIN output per hectare per year (see section 3.2.4 for the yearly TIN output equation). Total inorganic N outputs from watersheds per year ranged from 0.15 – 0.76 kg ha^{-1} yr^{-1} from June 2011-May 2011 and from 0.30 – 1.1 kg ha^{-1} yr^{-1} from June 2012-May 2013 (Table 3.5).

Table 3.5: TIN outputs (kg) per hectare per year and stream discharge (mm) per year of Binghamton area watersheds.



	kg TIN ha^{-1} yr^{-1}	
Watershed	June 2011-May 2012 (1372 mm)	June 2012-May 2013 (1008 mm)
Patterson	0.47 (161 mm)	1.1 (342 mm)
Willow Run	0.15 (50 mm)	0.30 (52 mm)
Fuller Hollow	0.50 (192 mm)	0.62 (150 mm)
Little Choconut	0.76 (315 mm)	0.88 (296 mm)
Pierce	0.49 (190 mm)	0.52 (152 mm)
Choconut	0.32 (474 mm)	0.80 (450 mm)
Nanticoke	0.64 (314 mm)	0.75 (316 mm)
Tracey	0.63 (431 mm)	0.75 (340 mm)

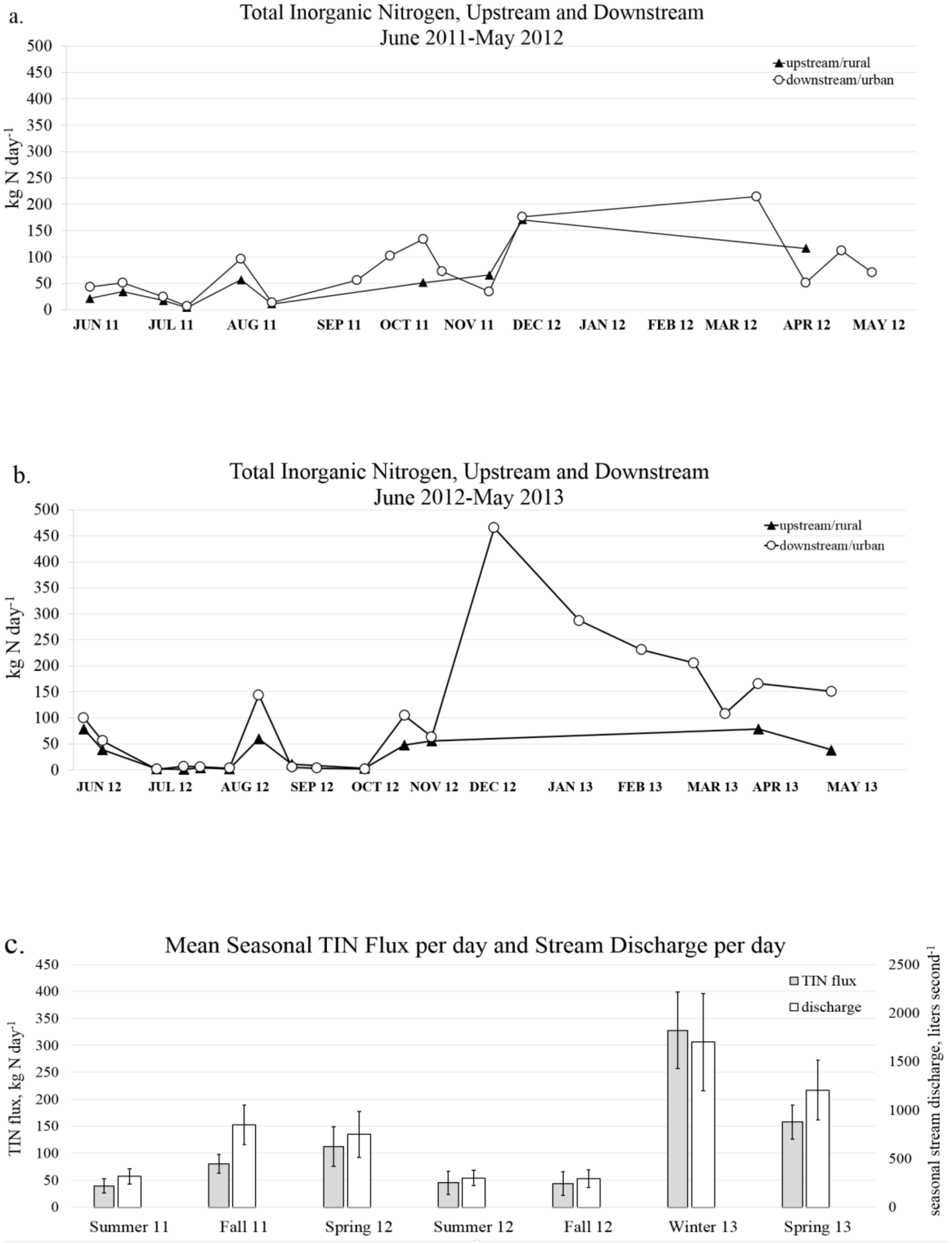


Figure 3.5: Daily total inorganic nitrogen fluxes (kg NO_3-N + kg NH_4-N) from downstream/urban and upstream/rural sites from June 2011-May 2012 (a) and from June 2012-May 2013 (b); (c) shows average seasonal TIN flux (kg day^{-1}) and discharge (L $second^{-1}$) from the eight streams.

3.3.4 Conductivity and pH of Stream Water

Stream water conductivity and pH were measured in all samples, from June 2011-May 2013. Conductivity is a measure of a water sample's capacity to conduct electricity, and more ions in solution elevate conductivity readings. Conductivity is often higher in urban areas due to the impacts of urbanization, such as the use of deicing road salt. Urban stream water conductivity ranged from 76-1202 µS cm^{-1} and rural conductivity ranged from 46-544 µS cm^{-1}. Conductivity of stream water were significantly higher in all urban stream sites compared to rural sites (Appendix A, Table A3.4). Urban conductivity measurements were higher in streams of watersheds that had larger percentages of development (Figure 3.6a). One-way ANOVAs showed that conductivity were significantly different between downstream/urban sites (F=10.47, p<0.0001) (Appendix A, Table A3.5) and between seasons (F=4.12, p=0.0005) (Appendix A, Table A3.6). Conductivity were highest in the winter of 2013, and did not significantly differ between other seasons (Figure 3.6b). Urban pH ranged from 6.9-10.2 and rural pH ranged from 6.7-9.4. Urban sites had significantly higher stream pH than rural sites (Appendix A, Table A3.4). Stream pH differed significantly between urban sites (F=10.26, p<0.0001) (Appendix A, Table A3.7) and between seasons (F=8.78, p<0.0001) (Appendix A, Table A3.8) (Figure 3.7a, b). Seasonal pH was highest in the summer of 2012, and lowest in the winter of 2013 (Figure 3.7b).

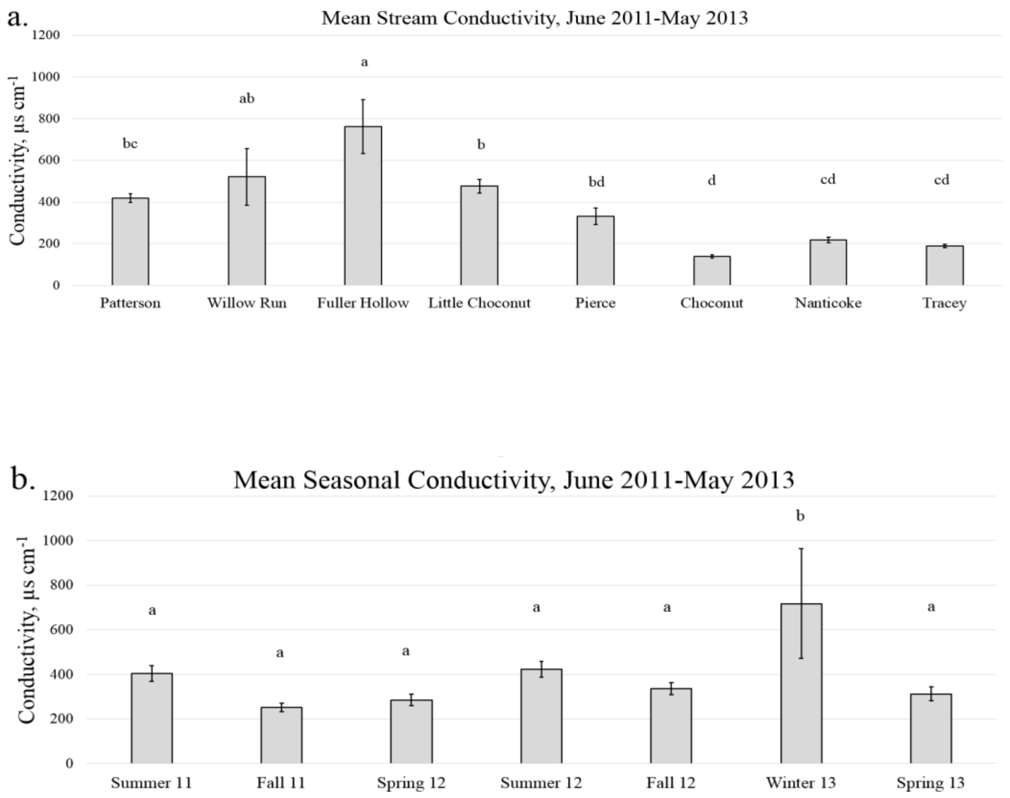


Figure 3.6: Conductivity measurements from downstream/urban sites of the eight streams, ±1SE. Means that do not share a common letter signify a significant difference at p=0.05, according to the Tukey means comparison.

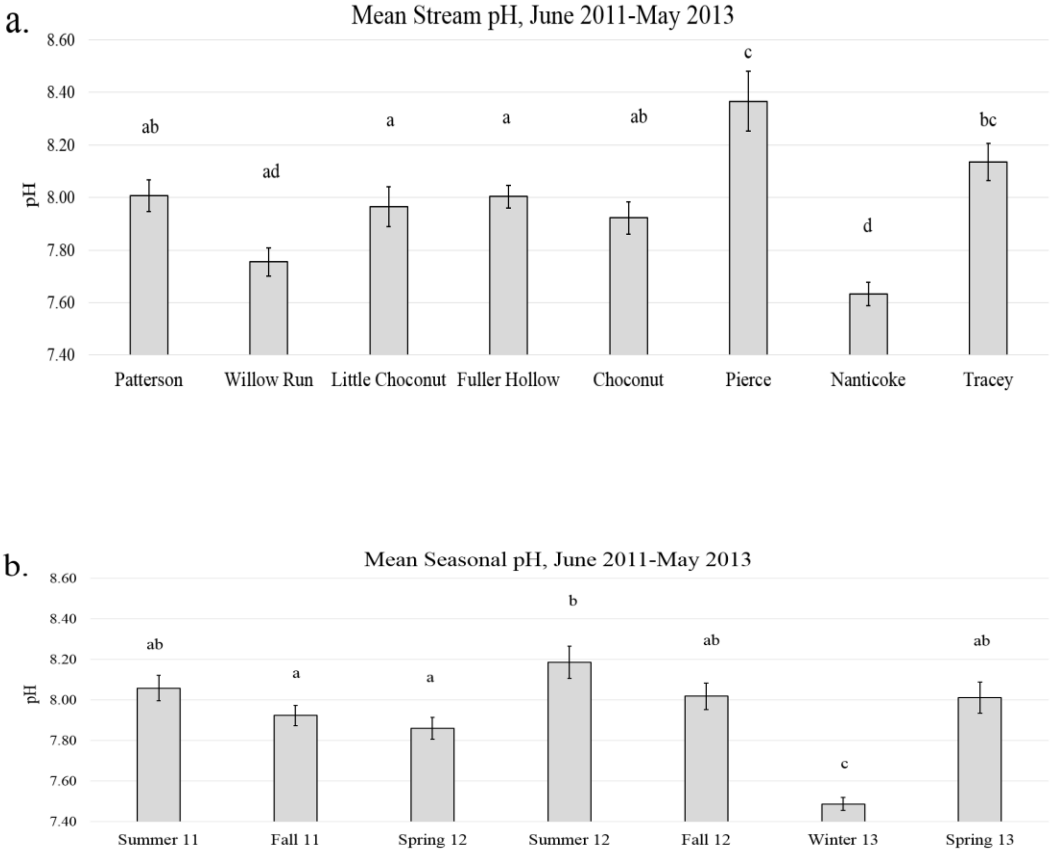


Figure 3.7: Measurements of pH from downstream/urban sites of the eight streams, ±1SE. Means that do not share a common letter signify a significant difference at p=0.05, according to the Tukey means comparison.

3.3.5 Chloride Concentrations in Streams

Stream water samples from June 2011 – May 2012 were analyzed for chloride (Cl^-) concentrations. Urban Cl^- ranged from 3.0 – 214.3 mg L^{-1} and rural Cl^- ranged from 2.9 – 82.6 mg L^{-1}. Paired two-sample t-tests showed Cl^- concentrations were significantly higher at urban sites compared to rural sites in all streams except for Tracey, which didn't have a significant difference between urban or rural Cl^- concentrations (Appendix A, Table A3.9). The results of ANOVAs examining the differences in Cl^- concentrations showed that they differed significantly between downstream/urban sites (F=12.25, p<0.0001) (Appendix A, Table A3.10) and between seasons (F=5.04, p=0.009) (Appendix A, Table A3.11). The streams in watersheds with more development had higher Cl^- concentrations, and there were lower Cl^- concentrations in streams of watersheds with less development (Figure 3.8). Average concentrations of Cl^- were significantly higher in the summer of 2011 (64.3±12.2) compared to the fall of 2011 (21.1±4.6), while the average spring concentration (39.0±6.1) did not significantly differ from the other seasons. Linear regressions did not show strong relationships between percent watershed development and seasonally flow-weighted Cl^- concentrations for the summer of 2011 (r^2=0.249, p=0.207), fall of 2011 (r^2=0.327, p=0.138) or spring of 2012 (r^2=0.495, p=0.051).

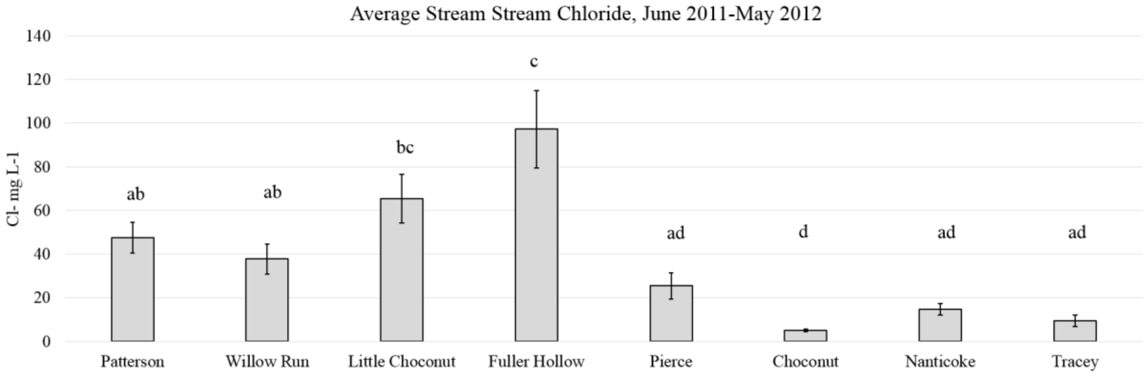


Figure 3.8: Mean stream Cl^- concentration from downstream/urban sites of the eight streams, ±1SE. Means that do not share a common letter signify a significant difference at $p=0.05$, according to the Tukey means comparison.

Chloride concentrations are often higher in urban streams due to the impacts of road salt, which in turn contributes to higher stream water conductivity. Since conductivity measurements can be used to approximate whether road salt has impacted water quality, we expected to find a relationship between stream Cl^- and conductivity data. We ran a regression examining the relationship between urban stream conductivity and urban Cl^- for samples from June 2011-May 2013, which is the period that we had data for both measurements (Figure 3.9). The regression shows a strong and significant relationship between stream conductivity and Cl^- concentrations ($r^2=0.744$, $p<0.0001$). We investigated this relationship further in the laboratory by making solutions of known concentrations of Cl^- and measuring their conductivity. The resulting conductivity and known Cl^- concentrations were analyzed using a regression, which showed a strong and significant relationship between conductivity and Cl^- concentrations ($r^2=0.9994$, $p<0.0001$). An analysis of covariance showed that the two regressions were significantly different ($p=0.0004$).

We then estimated the proportion of Cl^- that contributes to conductivity measurements in the streams. Conductivity of water samples are typically measured in microSiemens per centimeter ($\mu S\ cm^{-1}$), which is a measure of electrical conductance, but we had measurements of stream Cl^- concentrations. Conductivity in $\mu S\ cm^{-1}$ can be multiplied by 0.64 to convert the measurement to the approximate concentration of total dissolved solids (TDS) in parts per million (ppm) (Ali et al. 2012). This conversion results in an estimation of the concentration of ions in solution, which can include Cl^- and sodium (Na^+) from road salt, and other ions that are anthropogenically derived, such as heavy metals. Chloride was a larger proportion of TDS in the four watersheds with the most developed land cover. It ranged from 17-29% in the four more- developed watersheds, and from 7-14% in the watersheds developed land cover. We did not measured the stream water concentration of Na^+, but have estimated its concentration using a 1:1 molar ratio of Na:Cl. The Na^+ in stream water may account for as much as 11-19% and 4-9% of TDS in the four more-developed and less-developed watersheds, respectively. Using the measured Cl^- and estimated Na^+ concentrations, these ions could account for 29-47% of TDS in the four more-developed watersheds and 11-23% of TDS in the four less-developed watersheds.

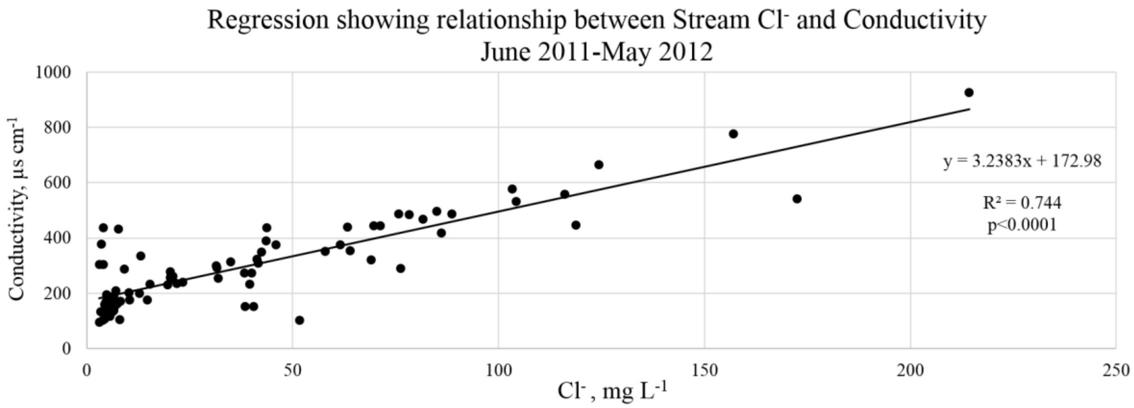


Figure 3.9: Regression showing the relationship between stream chloride concentrations and conductivity from eight urban streams that were collected from June 2011 – May 2012.

3.3.6 Soluble Reactive Phosphorus in Streams

Soluble reactive phosphorus (SRP) concentrations were analyzed for samples from June 2011-May 2012. Concentrations of SRP were low and ranged from 0.00-0.02 mg L^{-1}. They did not differ significantly between urban and rural sites in any of the streams (Figure 3.10) (Appendix A, Table A3.12). A one-way ANOVA showed that SRP did not differ between downstream/urban sites and its mean was 0.006±0.001 mg L^{-} PO$_4$-P (F=0.72, p=0.652) (Appendix A, TableA3.13). It did differ between seasons and was significantly higher in the summer (0.009±0.003) and fall (0.008±0.001) of 2011 compared to spring (0.003±0.0001) 2012 (F=4.70, p=0.01) (Appendix A, Table A3.14).

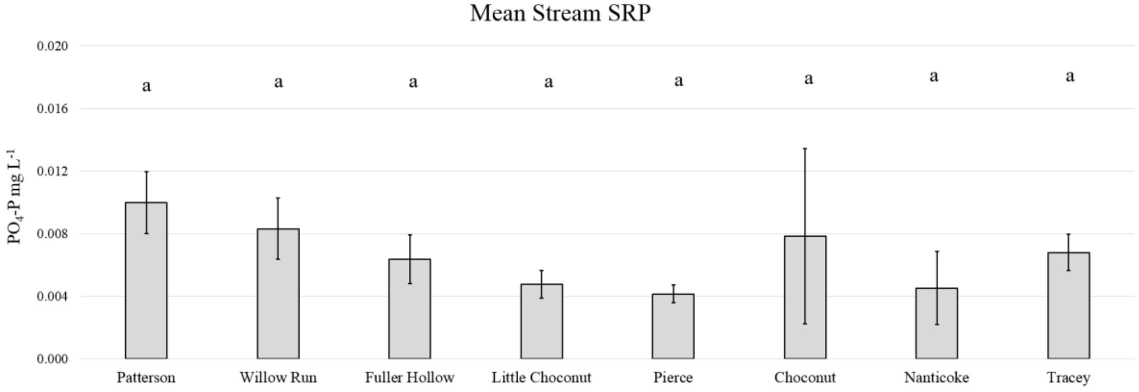


Figure 3.10: Mean stream SRP concentration from downstream/urban sites of the eight streams, ±1SE.

3.3.7 Hydrology of Binghamton area streams

The Binghamton area streams from which we sampled varied in size, and stream discharges were different between seasons (Figures 3.11 and 3.12). A simple linear regression showed that watershed size and average stream discharge were strongly positively correlated ($r^2 = 0.954$, $p<0.0001$). One-way ANOVAs showed that there were significant differences in discharge between downstream/urban sites ($F=30.82$, $p<0.0001$) (Appendix A, Table A3.15) and between seasons ($F=5.67$, $p<0.0001$) (Appendix A, Table A3.16). Nanticoke and Choconut were the largest and second-largest streams, respectively (Figure 3.11). Nanticoke had an average discharge of 2372±348 liters second^{-1} and Choconut had an average discharge of 1796±271 liters second^{-1}. They had significantly larger discharges than the other six streams ($p<0.0001$) (Figure 3.11). Average stream flow did not differ significantly between the six smaller streams, and their average discharges ranged from 17 – 449 liters second^{-1}. Total yearly discharges were calculated and divided by the watershed area in hectares to obtain runoff in mm ha^{-1}

yr^{-1} (Table 6). Downstream/urban discharges were generally greater than upstream/rural discharges, with the exception of Willow Run. Willow Run is a “losing stream” and often had a smaller downstream/urban discharge measurements compared to its upstream/rural counterpart, and sometimes the urban site was dry. Stream discharges were greatest in fall 2011, spring 2012, winter 2013, and spring 2013 (Figure 3.12). The summer of 2011 received more precipitation than usual, and the fall 2011 season received an unprecedented amount of precipitation due to Tropical Storm Lee, which caused extensive flooding in the Binghamton area. These factors may have contributed to the larger mean stream discharges of fall 2011 compared to fall 2012.

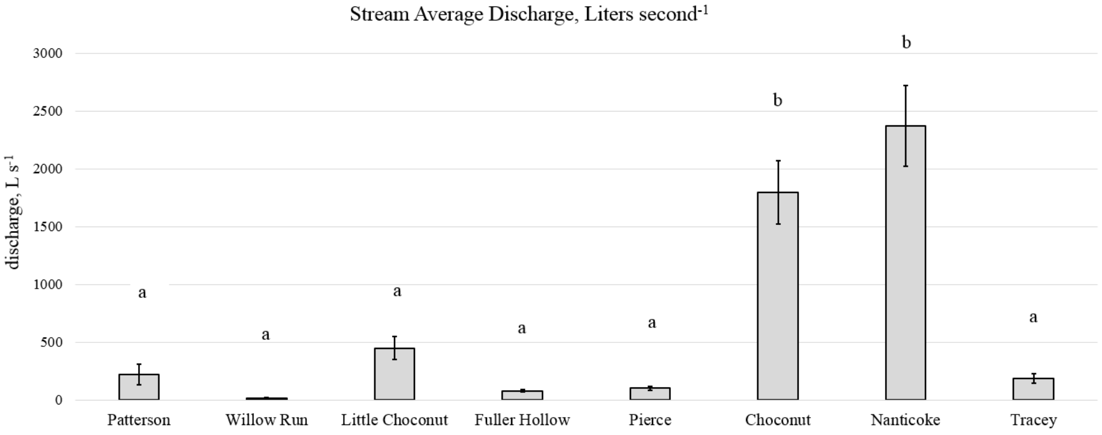


Figure 3.11: Mean discharge ±1SE of Binghamton area streams, June 2011-May 2013.

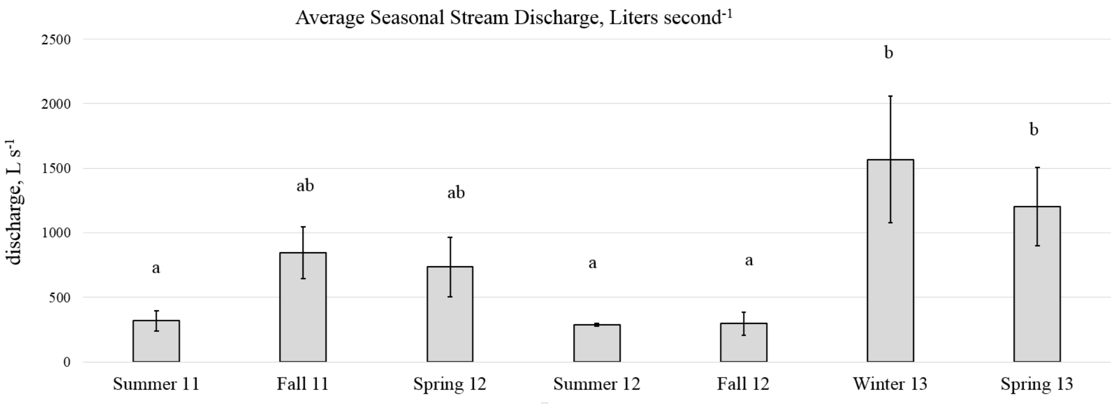


Figure 3.12: Mean seasonal stream discharge ±1SE of Binghamton area streams, June 2011-May 2013.

3.4 Discussion

3.4.1 Stream water NO_3-N concentrations

Dissolved inorganic N (DIN) in Binghamton's urban streams was dominated by NO_3^- at both downstream/urban and upstream/rural sites. This is consistent with findings that NO_3^- dominates stream water N from the Baltimore Ecosystem Study (BES) and also undisturbed forested watersheds at the Hubbard Brook Ecosystem Study (HBES) and the Catskill Mountains Region of the Northeastern U.S. (Groffman et al. 2004, Lawrence et al. 2000, Likens et al. 1970, Shields et al. 2008). Our urban and rural comparison showed higher NO_3^- concentrations in urban stream water. This trend was significant in Patterson, Willow Run, Fuller Hollow and Little Choconut Creeks, which are the four watersheds with the most developed land cover, and in Choconut Creek, which is in a less-developed watershed (Figure 3.2a-d; Appendix A, Table A3.1). Streams that had significant differences between urban and rural sites had urban NO_3^- concentrations that were generally about 2x higher than rural concentrations (Figure 3.2). Since the streams in this study do not receive discharges from wastewater treatment plants or combined sewage overflows, the higher urban stream NO_3^- concentrations that we have measured likely result from nonpoint-source pollution. Nitrogen deposition from fossil fuel combustion and fertilizer use are nonpoint-sources of N that are probably the major contributors to the increase in NO_3^- in the urban reaches of these streams. Nitrogen that is deposited can be dissolved in precipitation and runoff to streams. Runoff, especially from impervious surfaces, can be a major way that N gets into urban streams (Paul and Meyer 2001).

The four less-developed watersheds of Pierce, Choconut, Nanticoke, and Tracey had stream NO_3^- concentrations that were lower than the more-developed watersheds (Figure 3.2e-h). The concentrations of NO_3^- in Pierce, Nanticoke and Tracey were not consistently higher at the downstream/urban reaches (Appendix A, Table A3.1). Anthropogenic activities in the four watersheds with less development could have affected stream NO_3^- concentrations; however if that is the case we do not have evidence to show there were impacts on stream NO_3^- concentrations, leading us to believe that the impacts were minimal.

We measured higher urban stream NO_3^- concentrations in the four more-developed watersheds of the medium-sized city of Binghamton, but they are lower than N concentrations in Baltimore and Phoenix's urban, suburban and exurban stream systems (Grimm et al. 2005, Groffman et al. 2004, Shields et al. 2008). The seasonally flow-weighted concentrations we measured ranged from 0.03-0.58 mg L^{-1} NO_3-N, whereas Groffman et al. (2004) reported urban stream NO_3^- concentrations in Baltimore that are 2-8x higher than what we measured in Binghamton. Nitrogen in Phoenix's urban streams is dominated by organic N, but its stream NO_3^- concentrations are 3x higher than in

Binghamton (Grimm et al. 2005). The Binghamton area is smaller, less densely populated, and has fewer inputs of fuel and fertilizer than the larger metropolises of Baltimore and Phoenix, so it is not surprising that we have measured lower concentrations of stream N.

Flow-weighted NO_3^- concentrations were similar to concentrations Lawrence et al. (2000) and Lovett et al. (2000) reported in streams of forested watersheds in the Catskills mountains, which are about 158 km (98 miles) away from Binghamton. Lawrence et al. (2000) reported stream NO_3^- concentrations in a major watershed of the Catskill Mountains region that were comparable to ours. The researchers expected to find a link between higher atmospheric N deposition and higher stream NO_3^- export, but rather found higher stream NO_3^- concentrations that were related to high rates of nitrification in soils of the watershed (Lawrence et al. 2000). The relatively high NO_3^- concentrations in forested watersheds found by Lovett et al. (2000) were hypothesized to result from a combination of forest history, forest growth rate and species composition rather than N deposition. Our study compared urban and rural reaches of streams, and since we measured higher urban stream NO_3^- concentrations we believe they are due in large part to fossil fuel combustion and fertilizer applications.

We observed seasonal trends in stream NO_3^- concentrations. Nitrate concentrations were highest in the summer and winter (Figures 3.2 and 3.4). Flow-weighted urban stream NO_3^- ranged from 0.35-0.53 mg L^{-1} NO_3-N and from 0.28-0.58 mg L^{-1} NO_3-N in the summers of 2011 and 2012, respectively. High urbans stream NO_3^- concentrations during the summer could be because there are smaller stream discharges during this season, which would concentrate NO_3^-. High urban stream NO_3^-

concentrations during the growing season could have been because development and impervious surface cover have replaced vegetation to an extent, so in addition to having higher N inputs, there are fewer opportunities for N uptake by vegetation. Less N uptake by vegetation is probably only part of the story, because the watersheds have high percentages of forest and greenspace cover and opportunities for N retention (Table 2). Moreover, high urban stream NO_3^- concentrations in the growing season can be indicative of NO_3^- buildup in groundwater. If groundwater NO_3^- concentrations are high, this would be more apparent in the growing season when groundwater is a larger proportion of stream flow because of less precipitation and higher ambient temperatures. In this scenario, high stream NO_3^- concentrations from groundwater inputs would be diluted by greater precipitation and larger stream discharges in the fall and spring. We measured lower N concentrations and larger discharges in fall 2011, spring 2012 and 2013 (Figure 3.5c).

In the BES, urban and suburban stream NO_3^- concentrations remained relatively high year-round, and were between 2-3 mg L^{-1} NO_3-N in the summers (Groffman et al. 2004, Shields et al. 2008). The higher stream NO_3^- concentrations that we measured in the summer are more similar to reference forest stream concentrations, but they do not follow the same seasonal pattern as forested systems. At Hubbard Brook, stream NO_3^- concentrations in the summer averaged <0.01 mg L^{-1} and were the lowest of all seasons (Likens et al. 1970). In undisturbed watersheds of Hubbard Brook, stream NO_3^- export showed distinct seasonal patterns. Summer stream NO_3^- concentrations were low, began to increase in November, and by April stream NO_3^- was highest at 2 mg L^{-1}, and began to decline by May (Likens et al. 1970). The low stream NO_3^- concentrations that began in

May and persisted during the growing season were attributed to nutrient demands of vegetation and soil fauna (Likens et al. 1970).

Stream NO_3^- concentrations were also high during the winter months in our study. Flow-weighted urban stream NO_3^- in the winter ranged from 0.20-0.46 mg L^{-1} NO_3-N. In Baltimore, urban stream NO_3^- concentrations were highest in the winter, and were typically between 3-4 mg L^{-1} NO_3-N (Groffman et al. 2004, Shields et al. 2008). In relatively undisturbed systems, high urban stream NO_3^- concentrations during the dormant season when stream discharges were also high is indicative of inputs from outside the system. At Hubbard Brook, Likens et al. (1970) attributed high stream NO_3^- concentrations to inputs of N from precipitation (Likens et al. 1970). Winter stream NO_3^- concentrations could have been elevated because this is the dormant period in upstate New York and there is considerably less N uptake by plants.

3.4.2 Total inorganic N outputs from the Binghamton, NY area

Total inorganic N (NH_4^+ + NO_3^-) fluxes from Binghamton area urban streams were lower than TIN fluxes from larger cities and some undisturbed watersheds in the Northeastern U.S. Binghamton urban stream TIN outputs ranged from 0.15-0.76 kg ha^{-1} $year^{-1}$ during June 2011-May 2012 and ranged from 0.30-1.1 kg ha^{-1} $year^{-1}$ during June 2012-May 2013 (Table 6). Our calculations of TIN fluxes are much smaller than fluxes from urban and suburban streams of larger cities. In Baltimore, urban and suburban stream TN outputs ranged from 4.5-11.4 kg ha^{-1} $year^{-1}$ (Groffman et al. 2004). In the same system, Shields et al. (2008) reported TN outputs from urban and suburban watersheds that were 7-8 kg N ha^{-1} yr^{-1}, and the 70-90% of this was in the form of NO_3^-. They reported TN export from a reference forested watershed that was approximately 1 kg N ha^{-1} yr^{-1} (Shields et al. 2008). Another forested watershed had higher stream N fluxes of 6 kg N ha^{-1} yr^{-1}, which they attributed to exurban housing that had leaking septic systems (Shields et al. 2008). Lewis and Grimm (2007) reported outputs of TIN from Phoenix's streams that were even larger than Baltimore's stream N outputs. Total inorganic N from streams in Phoenix was about 20 kg ha^{-1} $year^{-1}$ from residential areas and 150 kg ha^{-1} $year^{-1}$ from commercial areas (Lewis and Grimm 2007).

Total inorganic N fluxes from streams in undisturbed forested watersheds can vary between seasons and years (Aber et al. 2003). In one review, Aber et al. (2003) reported that NO_3^- export from forested watersheds ranged from 0.3-5 kg NO_3-N ha^{-1} $year^{-1}$, with higher exports linked to higher N deposition. Goodale et al. (2009) found that in small watersheds near Ithaca, NY, NO_3^- export was 0.1-0.6 kg ha^{-1} yr^{-1}. Mitchell et al. (1996) found that in the HBES, stream NO_3^- export was dependent on seasonal conditions and climatic factors. They found stream NO_3^- export typically ranged from 0.1-0.7 kg NO_3-N kg ha^{-1} during the growing season. In the winter, stream NO3- export ranged from 0.3-0.7 kg NO_3-N kg ha^{-1}, and larger exports were linked to freeze-thaw cycles that freed immobilized N (Mitchell et al. 1996).

The TIN fluxes we calculated in Binghamton area urban streams are surprisingly low, and comparable to low TIN exports from forested watersheds in other parts of the Northeastern U.S. However, our calculations could be underestimations. We sampled from 26 low-flow events, and shortly after 9 storm events, and had fewer sampling events in the dormant season, which may have biased our results in the direction of lower N fluxes. Our TIN outputs were mostly <1 kg ha^{-1} $year^{-1}$, but Binghamton is a medium-sized city of about 260,000 individuals. At the time of Groffman and et al.'s study (1999-

2001), the metro population of Baltimore was 2,500,000 and stream TIN outputs were as high as about 11 kg ha^{-1} year^{-1}. Lewis and Grimm (2007) reported the highest stream TIN outputs as 150 kg ha^{-1} year^{-1}, and at the time of their writing, their study area encompassed 4.2 million people. Bigger cities have higher N inputs, and it's not surprising that they have larger N outputs, too.

3.4.3 Land cover signal and stream water NO_3-N

We found positive and significant relationships between percent watershed development and flow-weighted stream NO_3^- concentrations. We saw clear connections between percent development of the watersheds and their downstream/urban NO_3^- concentrations in almost all seasons (Table 5). This connection between development and urban stream N was made by using data from the National Land Cover Database (NLCD). Standardized classifications from the NLCD were developed to normalize mapping of land cover over large areas (Anderson 1976). The NLCD provides land cover information that is the "industry standard" for assessing percent development in urban watersheds (Groffman et al. 2004, Shields et al. 2008). Although the "industry standard" is to use large-scale land cover classifications from the NLCD, Cadenasso et al. (2007) and others have shown that landscape analysis in finer detail is preferred to gain a more thorough ecological understanding of cities, but fine-scale land cover assessments are not yet widely available. Nonetheless, the percent development data from the NLCD were useful and informative to this study.

3.4.4 Conductivity and Cl^- patterns in urban streams

Stream conductivity measurements were significantly higher at urban sites compared to rural sites and were linked to stream Cl^- concentrations, indicating a strong impact of urbanization on conductivity in Binghamton area streams (Figure 3.9) (Appendix A, Table 3.4). Conductivity ranged from 76-1202 µS cm^{-1} at urban sites and from 46-544 µS cm^{-1} at rural sites. Conductivity, which is a measurement of the electrical conductance of water, increases with increasing ion concentrations (Griffith 2014). In undisturbed areas, streams have baseline water conductivity measurements that result from ions from underlying geologic formations (e.g., Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-}) (Griffith 2014). However, urbanization increases conductivity of streams and rivers, and measurements over 100 µS cm^{-1} are generally indicative of human impacts (Cooper et al. 2014). Conductivity measurements are often used as indicators of deicing salt impacts, so we analyzed samples from June 2011-May 2012 for Cl^- concentrations (Cooper et al. 2014). Like conductivity measurements, stream Cl^- concentrations were significantly higher at urban sites compared to rural sites (Appendix A, Table A3.9). Stream Cl^- concentrations ranged from 3.0-214.3 mg L^{-1} at urban sites and ranged from 2.9-82.6 mg L^{-1} at rural sites. Conductivity measurements and Cl^- concentrations showed similar patterns among streams (Figure 3.6a, Figure 3.8). The more-developed watersheds had higher stream conductivity and Cl^- concentrations, ranging from 419±22 to 762±130 µS cm^{-1} and from 37.8±7.0 to 97.3±17.7 mg L^{-1} Cl^{-1}, respectively. Fuller Hollow had the highest conductivity and Cl^- measurements (Figure 3.6a, Figure 3.8). We found that there were significant relationships between conductivity measurements and Cl^- concentrations, suggesting strong impacts of deicing salt on stream conductivity (Figure 3.9). Our finding is supported by the work of Cooper and colleagues (2014), who reported that Cl^- and Na^+ were both contributors to specific conductance measurements in the urban stream of Minebank Run, Maryland.

For a long time, researchers believed that after road salt was applied during winter months, it was flushed out of systems during spring runoff and storm events. Recently, research has suggested otherwise (Daley et al. 2009, Kaushal et al. 2005, Kelly et al. 2008, Novotny et al. 2009). In at study of the Twin Cities metropolitan area of Minneapolis and St. Paul, Minnesota, Novotny et al. (2009) found that a large portion of deicing salt that entered the urban ecosystem was retained, rather than exported. They estimated that of the NaCl that entered the system as road salt, 77% was retained, while only 23% was exported to the Mississippi River (Novotny et al. 2009). Urban retention of road salt typically occurs in soils and groundwater (Cooper et al. 2014, Kincaid and Findlay 2009). Reservoirs of Cl^- in soils and groundwater have caused stream Cl^- concentrations to increase in recent decades, even during seasons where road salting does not occur (Daley et al. 2009, Kaushal et al. 2005, Kelly et al. 2008). Kaushal et al. (2005) found that in the Baltimore area, urban stream Cl^- concentrations were linked to impervious surface area and were up to 100x greater than concentrations in forested streams. They also found that while stream Cl^- concentrations were high in the winter (approximately 900-5,000 mg L^{-1} Cl^{-1}), they remained high through the other seasons of the year (44-336 mg L^{-1} Cl^{-1}), indicating that Cl^- had accumulated in groundwater (Kaushal et al. 2005). Daley et al. (2009) reported that in urban streams of central New Hampshire, Cl^- concentrations increased by 2-3x between 1991 and 2005. They studied two urban watersheds; over the 14 year period Cl^- concentrations climbed from 60 to 155 mg L^{-1} Cl^- in one of the watersheds and from 70 to 260 mg L^{-1} Cl^- in the other watershed. Roadways and other impervious surfaces like sidewalks are more dense in but not limited to urban areas, and rural streams have higher Cl^- concentrations from deicing salt, too (Kaushal et al. 2005). In a study at the rural Wappinger Creek, NY, Kelly and colleagues (2008) reported stream Cl^- concentrations that ranged from 25-45 mg L^{-1} in 2005, which was a 3 fold increase over the 19-year study period.

We measured higher stream conductivity and Cl^- concentrations at urban sites compared to rural sites, in more-developed watersheds and the high measurements persisted during all seasons. These data suggest it is likely that deicing salt has been accumulating in groundwater in the Binghamton area. Our highest measurement of Cl^- was 214 mg L^{-1}, which is lower than the EPA standard of 250 mg L^{-1} that is considered detrimental to biotic life (https://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals) (Cooper et al. 2014, Kaushal et al. 2005). Chloride concentrations will probably continue to increase in this area's streams and streams everywhere in the Northeast, if NaCl continues to be the preferred deicing method during winter months.

3.4.5 Differences between urban and rural stream pH

Urban stream pH was significantly higher at urban sites compared to rural sites (Appendix A, Table 3), showing impacts of urbanization on pH. Urban streams and rivers can have higher pH measurements because over time, building materials degrade, releasing ions that wash into water resources and increase pH (Kaushal et al. 2013). Urban pH ranged from 6.89-10.21 and rural pH ranged from 6.73-9.41. Both urban and rural stream pH measurements are high compared to undisturbed temperate forests, which are typically acidic. Measurements of pH of 4.5-5.5 are characteristic of undisturbed watersheds in the Hubbard Brook Ecosystem Study, and elsewhere in the Northeast U.S. (Likens et al. 1970). Pierce and Tracey Creeks had the highest average stream pH measurements in this study, and both were sampled downstream of where they discharged through several concrete culverts (Figure 3.7a). Major components of concrete are CaO or $Ca(OH)_2$; since Ca^{2+} increases pH, this is a possible explanation for why the urban sites at Pierce and Tracey Creeks had high stream pH.

3.4.6 Soluble Reactive Phosphorus (SRP) in Binghamton area streams

Common sources of SRP to urban streams include fertilizers and wastewaters, in addition to leaching from soils that have naturally high levels of P (Janke et al. 2014, Sonoda et al. 2001). However, we measured very low concentrations of stream SRP (Figure 3.11). It did not differ between urban and rural sites, and it did not differ between streams (Figure 3.11) (Appendix A, Tables A3.12, 3.12). Its mean was 0.006±0.001 mg L^{-} PO_4-P. In a study in Northwest Oregon, Sonoda et al. (2001) reported urban stream SRP concentrations that were 3-6x higher than our measurements of SRP. The researchers attributed higher P to runoff from fertilizers and less uptake by degraded riparian areas (Sonoda et al. 2001). Their nonurban reference sites had SRP concentrations that were very similar to ours (Sonoda et al. 2001). Fitzgerald et al. (2015) found that in an urban watershed in Canada, stream SRP was 4x higher than what we measured, which they attributed to either degrading organic material in groundwater, or the impacts of a landfill that has been leaching into groundwater. Our low SRP measurements indicate that this nutrient has not been augmented by urban and suburban activities, or the impacts of other land uses. However, we did not measure total P, which would include organic P and SRP. Since SRP can be low even in eutrophic systems, and we do not have information on the total P concentration of stream water, we cannot definitively conclude that P has not been impacted by human activities in this urban ecosystem.

3.4.7 Conclusions

We studied N concentrations in eight urban streams of Binghamton, N.Y., which is a medium-sized city. At the eight streams, NO_3^- concentrations were compared between downstream/urban and upstream/rural locations. Nitrate concentrations were highest in the streams of the four watersheds with the highest percentages of developed land. Concentrations of stream NO_3^- were also significantly higher at downstream/urban sites of the four most-developed watersheds. However our measurements of urban stream NO_3^- concentrations and N fluxes are lower than measurements of large cities such as Baltimore and Phoenix (Groffman et al. 2004, Lewis and Grimm 2007). Our measurements of N fluxes are surprisingly low and are more similar to fluxes from streams of forested watersheds of the Northeastern U.S. (Goodale et al 2009, Mitchell et al. 1996). We also found that stream conductivity, pH and Cl^- were significantly elevated at downstream/urban sites compared to upstream/rural counterparts. Downstream/urban conductivity measurements were strongly linked to stream Cl^- concentrations, and both measurements were highest in the four most-developed watersheds.

Chapter 4: Impacts of Point- Source Nitrogen to the Susquehanna River from Binghamton, NY

4.1 Introduction

For centuries, cities have formed beside rivers and other water resources for purposes of drinking water, food, agriculture and travel (Grimm et al. 2008). Ever since, the activities of people in cities have altered the quality of the water bodies along which they are built. Over the past 100 years, industrialization and the growth of urban areas has required massive imports of food, fertilizer and fuel that have contributed to high levels of nitrogen (N) in cities (Baker et al. 2001, Fissore et al. 2011). Some of the imported N is emitted to the atmosphere and released to waterways (Baker et al. 2001, Fissore et al. 2011). Due to the impacts of urbanization (and agriculture), many rivers have higher loads of N than they did before industrialization (Bouwman et al. 2005). A major way that cities contribute to higher river N is due to human waste. Imports of food and exports of N in human waste account for large portions of N fluxes in cities (Fissore et al. 2011). In an extensive study of nutrient fluxes in the Minneapolis-St. Paul metropolitan area in Minnesota, USA, researchers estimate that human diet accounted for 40% of N imported to the urban ecosystem and also accounted for 40% of N that was exported from the system in wastewater (Fissore et al. 2011). In developed countries, human waste is treated at wastewater treatment plants (WWTPs) to remove N and is often discharged to adjacent water bodies (Fissore et al. 2011). In WWTP effluent, ammonium (NH_4^+), nitrate (NO_3^-), and organic N are bioavailable forms of N that elevate river N loads which, in addition to phosphorus, can cause eutrophication of waterways and estuaries (Carey and Migliaccio 2009). Wastewater treatment plants have discrete discharge pipes, are tested regularly for contaminants, and are therefore point-sources of pollution (Carey and Migliaccio 2009). In contrast, fertilizer and fuel are diffuse, nonpoint-sources of urban N, and are usually elevated in urban waterways; they also contribute to eutrophication (Shields et al. 2008). The focus of this chapter is on how the Binghamton area, including the point-source of the Binghamton-Johnson City WWTP (B-JC WWTP), impacts water quality of the Susquehanna River; the focus of Chapter 3 is on nonpoint-source pollution in urban streams that discharge to the Susquehanna River. In this chapter, we focus on N but also address phosphorus (P), conductivity, chloride (Cl^-), and pH of the Susquehanna and Chenango Rivers and B-JC WWTP effluent.

Point-sources of pollution to water bodies, particularly from WWTPs, have been recognized as threats to human and ecological health. Wastewater treatment plants have been regulated in part by the Clean Water Act (CWA) of 1972 and its subsequent amendments (Carey and Migliaccio 2009). The CWA has focused on reducing WWTP effluent pollutant loads, including N (Carey and Migliaccio 2009). Wastewater treatment plants can release >50% of urban nutrients to streams and rivers and plants have several phases of treatment that are aimed at reducing N in effluent (Carey and Migliaccio 2009, Carpenter et al. 1998). The goal of preliminary treatment is to remove large solids that are in raw sewage. Then, primary treatment aims to remove organic and inorganic solids, typically by sedimentation, which is promoted by chemicals such as aluminum sulfate or ferric sulfate (Sonune and Ghate 2004, Chereminisoff 1995). Major goals of primary treatment are to remove >50% of total suspended solids, 25-50% of biochemical oxygen demand (BOD) and oil and grease. Sedimentation during primary treatment can also remove some organic N and organic P (Sonune and Ghate 2004). Next, secondary treatment aims to remove more BOD, suspended solids, and also reduce nutrient loads (Sonune and Ghate 2004). During secondary treatment NH_4^+, which is the product of the hydrolysis of urea, is oxidized to NO_3^-. Nitrification is the conversion of NH_4^+ to NO_3^-, and is a major step toward N removal. In developed countries, WWTPs are generally equipped with at least secondary treatment, but many WWTPs go further to remove more nutrients. Tertiary treatment is the final step of N removal from sewage, where denitrifying bacteria remove NO_3^- and convert it to N_2 and by doing so, release it to the N_2 “sink” in the atmosphere.

The efficiency of WWTPs ultimately determines how much and what form of N and other pollutants are released to adjacent waterways. Extreme precipitation events and improper construction can cause impairments and failures of WWTP function. As of early 2011, the B-JC WWTP had an advanced system of sewage treatment and N removal. After preliminary and primary treatment, sewage was sent to a series of biological aerated filters (BAFs). At the first building, the BAF was geared toward reducing BOD in sewage. Then the effluent was sent to have NH_4^+ oxidized via nitrification, and the resulting NO_3^- was denitrified to N_2 (Catherine Young, personal communication). In May of 2011, the BAF building devoted to reducing BOD had a wall collapse, which reduced the WWTP’s function. During the summer of 2011, sewage bypassed the impaired BAF and went straight to the nitrification BAF, retaining some of the plant’s function. Then in September 2011, Tropical Storm Lee caused major flooding in the Binghamton area. The B-JC WWTP was flooded, which halted the pumps of the plant, stopping sewage treatment beyond primary treatment, and devastating its advanced nutrient treatment system (Catherine Young pers. communication).

The B-JC WWTP isn’t the only sewage treatment plant to experience adverse impacts of extreme weather events. The recent influx of intense storms in the Northeastern United States has compromised the function of many WWTPs, which are commonly constructed in low-lying areas adjacent to rivers, lakes and oceans. Recently many have been flooded after intense storms (Schwirtz 2012, 2013). Other cities in New York, New Jersey and Pennsylvania have experienced major flooding events in the past decade; many of these floods have resulted in losses WWTP function, which affect water quality downstream (Schwirtz 2012, 2013). More flooding, WWTP impairment, and related water quality issues are likely to continue, since intense storm patterns have been predicted as an outcome of climate change (Kaushal et al. 2015).

The Susquehanna River in the Binghamton area receives inputs of N and other nutrients from the B-JC WWTP, combined sewage overflows, the Chenango River at their confluence, and many small streams. The Susquehanna discharges to the Chesapeake Bay in Baltimore, MD, which is the largest estuary in the Northeastern U.S. (Hagy et al. 2004). It is the largest tributary and largest source of N to the Chesapeake Bay, which has experienced ongoing problems with eutrophication and hypoxia (Hagy et al. 2004). This has decreased the value of its fisheries and general water quality, leading to economic and environmental problems (Hagy et al. 2004). We initiated this study to examine the impacts of Binghamton on N- loading to the Susquehanna River. In particular, we wanted to examine the impacts of the B-JC WWTP’s reduced function on N-loading to the river, and we wanted to know how that compared to nonpoint-source N loading from the eight urban streams detailed in Chapter 3. Our driving questions were (1) how the reduced B-JC WWTP function from flooding affected water chemistry and N loads to the Susquehanna River and (2) whether more N is discharged from the WWTP or the eight urban streams (Chapter 3) to the Susquehanna River.

4.2 Methods

4.2.1 Study Locations

We sampled wastewater effluent from the B-JC WWTP, which is located at 4480 Old Vestal Road, in Broome County. It is owned by the City of Binghamton and the Village of Johnson City, and also serves "outside users" from the Towns of Vestal, Union, Dickinson, Binghamton, Conklin, Kirkwood, and Fenton. From these areas approximately 174,000 people are served by the B-JC WWTP. The plant also serves the Binghamton University Vestal Campus, which has about 5,000 students living on campus during the academic year. The B-JC WWTP serves approximately 70% of the Greater Binghamton population. The B-JC WWTP effluent we sampled was during a special window of time during which the plant operated under minimal function. A wall collapse in May 2011 compromised the plant's nitrification efficiency. Months later in September 2011, the Binghamton area experienced extensive flooding that worsened the function of the plant. During this study period, the effluent was not optimally treated due to damage from a wall collapse in May 2011 and flooding from Tropical Storm Lee in September 2011. The effluent we sampled after the flood underwent preliminary and primary treatment, was treated with sodium hypochlorite to kill microbes, then with sodium thiosulfate (to neutralize the hypochlorite) before it was discharged to the Susquehanna River (Catherine Young pers. communication).

Water samples for the study were obtained from the Susquehanna and Chenango Rivers and the B-JC WWTP. A five-point river sampling plan determined N levels in the Susquehanna and Chenango Rivers upstream of the Binghamton area, at the confluence of the rivers in downtown Binghamton, and in the Susquehanna River downstream of the point-source of the B-JC WWTP (Table 4.1). The Susquehanna River upstream (SR Upstream) site was in Hallstead, PA, and the Chenango River upstream (CR Upstream) site was in Chenango Bridge, New York. From these sites, we obtained measurements of the rivers' upstream water quality, before they received point- and nonpoint-source N from the Greater Binghamton area. In downtown Binghamton, we sampled the Susquehanna and Chenango Rivers before they joined (SR Confluence and CR Confluence sites, respectively). The Susquehanna River downstream (SR Downstream) sampling site was located at a bridge joining Endicott and Vestal NY. The SR Downstream site had received B-JC WWTP effluent.

Table 4.1: River sampling sites and abbreviations.

River and WWTP Sampling Locations		
Abbreviation	Location	Coordinates
SR Up	Hallstead, PA	41.96 Latitude -75.74 Longitude
CR Up	Chenango Bridge, NY	42.17 Latitude -75.87 Longitude
SR Confluence	Binghamton, NY	42.09 Latitude -75.91 Longitude
CR Confluence	Binghamton, NY	42.09 Latitude -75.92 Longitude
SR Down	Vestal, NY	42.09 Latitude -76.06 Longitude
B-JC WWTP	Vestal, NY	42.10 Latitude -75.96 Longitude

4.2.2 Water Collection Protocol

River sampling began in May 2013 and continued through October 2013. River water was collected twice monthly and there were 12 collections. Water was sampled from bridges over the sampling locations in Table 4.1. From each bridge, we used a depth- integrated sampler to obtain vertically-integrated water samples. Duplicate samples of river water were taken from three locations on the bridges (left, middle and right) to assess quality control.

At the B-JC WWTP, we sampled effluent as it discharged to the Susquehanna River. We sampled B-JC WWTP effluent in two phases. During Phase 1, we sampled WWTP effluent with every sampling event of the eight streams that were discussed in Chapter 3. The duration of Phase 1 was from June 2011-May 2013. Extensive flooding in September 2011 essentially destroyed any treatment beyond preliminary and primary treatment at the WWTP. Due to accessibility and safety issues at the plant, we did not sample effluent again until October 2011 and were only able to use two effluent samples for the fall 2011 N calculations. Effluent was sampled in October, November and December 2011, and resumed in March 2012. Phase 1 of WWTP effluent sampling had 33 effluent samples. Phase 2 of effluent sampling was in conjunction with the river sampling described above. We sampled B-JC WWTP effluent with every 5-point river sampling event, from May 2013-October 2013. Phase 2 had 12 WWTP effluent samples. For all collections, acid-washed 250 mL collection bottles were rinsed 3 times with water from the sample location *in situ* before the sample was collected.

For river and effluent samples, measurements of pH, conductivity and temperature were obtained using a YSI Model 63 Handheld pH, Conductivity, Salinity and Temperature System at each river site (with one reading per each left, middle and right location on the bridge) and B-JC WWTP. After collecting water samples, they were placed in ice-filled coolers and were transported back to the laboratory.

4.2.3 Water Chemistry Analysis

Samples were processed immediately upon return to the lab. Samples were vacuum filtered individually using Whatman GF/A glass microfiber filters into acid-washed 60 mL bottles. The samples were treated with 0.2 mL of a 50% H_2SO_4 solution to suppress microbial activity, were stored in a cold room until analysis, and were analyzed for NH_4^+ and NO_3^- concentrations using a Lachat QuickChem 8000 Flow Injection Analyzer (Lachat Instruments, Milkwaukee W.I.). The determination of NH_4-N is based on the Berthelot reaction, where ammonia reacts with alkaline phenol, then with sodium hypochlorite. The resulting indophenol blue reacts with sodium nitroprusside to enhance sensitivity, and the reaction product is directly proportional to the original NH_4-N concentration. The NO_3-N concentration is determined on the Lachat by quantitatively reducing NO_3-N to nitrite (NO_2^-) by passing the sample through a copperized cadmium column. All duplicate samples were determined to be the same within each sampling site. The November 3, 2011 WWTP collection showed inconsistency between the duplicate samples and was removed from further analyses.

Total nitrogen and total phosphorus concentrations were obtained from unfiltered river water samples and unfiltered Phase 2 WWTP effluent samples. They were digested according to the Kjeldahl method. Samples of 25 mL of unfiltered water samples were measured into digestion tubes, and then we added 3mL of the $H_2SO_4 \bullet H_2O_2 \bullet Li \bullet Se$ digestion reagent and 2 Hengar boiling granules. The resulting solution was placed in a pre-heated digester at 180 ˚ C for 2 hours. Next, 1 mL of H_2O_2 was added to the solutions, and the digestion tubes were placed back in the digester for 2 hours at 380 ˚ C. The resulting solution was diluted with Nanopure water to 100 mL, and samples were stored in acid-washed 60-mL bottles at room temperature until analysis. The digested samples were analyzed on the Lachat Autoanalyzer for TKN and TKP concentrations. The TKN digestion converts organic N to NH_4^+; so to obtain total N concentrations, the NO_3^- concentrations were added to the TKN concentrations. Dissolved organic N concentrations were obtained by subtracting the previously measured NH_4^+ concentrations from each sample's measured TKN concentration. Total phosphorus is the TKP concentration measured by the Lachat Autoanalyzer.

4.2.4 River Discharge Measurements

River discharge measurements of the Susquehanna and Chenango Rivers were obtained from the United States Geological Survey. They were used to calculate the total inorganic N flux in the rivers (http://waterdata.usgs.gov/usa/nwis). The SR Upstream discharge is measured by a gauge in Hallstead, PA, upstream of the Binghamton area. The CR Upstream discharge is measured by a gauge near Chenango Bridge, New York, upstream of the Binghamton area. The SR Downstream discharge is measured by a gauge at Vestal, NY. Gauge stations are located near the SR and CR Upstream sampling points, and the SR Downstream sampling point. However, the confluence locations don't have nearby gauge stations and discharge measurements from the SR Upstream and CR Upstream were used for the SR Confluence and CR Confluence locations, respectively. Of the 12 river water and WWTP effluent collections, 7 were taken during baseflow conditions and 5 were taken during stormflow conditions. Storm discharge is defined as discharge that is preceded, within 72 hours, by a precipitation event that results in 0.25 cm or more of rainfall (www.epa.gov).

4.2.5 Calculations

Wastewater treatment plant N concentrations were flow-weighted by season to compare them to flow-weighted stream N from Chapter 3, which accounted for differences between stream discharges and variations in seasonal hydrology. We used the Equation 4.1 to calculate the seasonally flow-weighted mean B-JC WWTP effluent and stream N concentrations.

Equation 4.1:

$$FW = \frac{\sum_{i=1}^{n} Ci \ x \ Fi}{\sum_{i=1}^{n} Fi}$$

In Phase 1 of the WWTP sampling, there were 7 seasons of flow-weighted nutrient concentrations. In Phase 2 of the WWTP sampling and river sampling, there were 3 seasons of flow-weighted nutrient concentrations. We used Equation 4.2 to calculate the TIN flux (kg day^{-1}) from the five river locations and the B-JC WWTP.

Equation 4.2:

$$TIN = \sum\left(\left(\frac{mg \ \ NO3 - N}{L} x \ \frac{L}{day}\right) + \left(\frac{mg \ NH4 - N}{L} x \ \frac{L}{day}\right)\right) \div \ 1000000$$

4.2.6 Statistical Analysis

All statistical analyses were performed using the R Statistical Package. Analysis of variance (ANOVA) tests were used to determine if mean N concentrations varied by site and between baseflow and stormflow conditions. To test whether the assumptions of the ANOVA and regressions were met, residues were checked for normality and homoscedasticity. Data from the B-JC WWTP were not normally distributed, and were log-transformed; the transformed data met the ANOVA assumptions of normality and homoscedasticity. Comparisons between baseflow and stormflow N fluxes were determined using a t-test. Significant results were analyzed further using Tukey's HSD test. All means are given ±1 standard error (SE).

4.3. Results

4.3.1 Nitrogen in B-JC WWTP Effluent and in the Susquehanna and Chenango Rivers

4.3.1.a Nitrogen Concentrations

The dissolved N of B-JC WWTP effluent was dominated by NH_4^+ for Phase 1 (June 2011-May 2013) and Phase 2 (May 2013-October 2013) of the sampling period (Figure 4.1, Table 4.2). Phase 1 of effluent sampling included analysis for dissolved NH_4^+ and NO_3^- concentrations. Phase 1 of effluent sampling began in June 2011, which was after the BAF wall collapse. In the summer of 2011, flow-weighted inorganic N concentrations were 6.0 mg L^{-1} NH_4-N and 0.9 mg L^{-1} NO_3-N. The data we obtained in the summer of 2011 suggest reduced function of the nitrification system at the B-JC WWTP. In the fall of 2011, flow-weighted inorganic N concentrations were 6.4 mg L^{-1} NH_4-N and 0.5 mg L^{-1} NO_3-N. For the remainder of Phase 1 B-JC WWTP effluent sampling, flow-weighted NH_4^+ ranged from 12.7-19.1 mg L^{-1} NH_4-N and NO_3^- ranged from 0.03-0.5 mg L^{-1} NO_3-N (Figure 4.1). A one-way ANOVA showed that the WWTP inorganic N concentrations were significantly different between seasons (F value=5.6, $p=0.002$) (Appendix A, Table A4.1); the Tukey means comparison showed that the summer 2011 and fall 2011 DIN concentrations were significantly lower than later seasons ($p<0.05$). During Phase 1, the concentration of NH_4^+ during baseflow (n=24) conditions averaged 13.4±1.1 mg L^{-1} NH_4-N and was an average of 10.0±1.3 mg L^{-1} NH_4-N during stormflow (n=9) conditions. Nitrate concentrations averaged 0.3±0.1 mg L^{-1} NO_3-N during baseflow (n=24) and 0.4± 0.1 mg L^{-1} NO_3-N during stormflow (n=9) conditions. In the B-JC WWTP effluent from Phase 1 of sampling, there was a marginally significant difference between baseflow and stormflow NH_4^+ concentrations (t statistic= 2.07, p= 0.05) but not between baseflow and stormflow NO_3^- concentrations (t statistic=1.17, p=0.25).

For Phase 2 of WWTP effluent sampling, we obtained measurements of total dissolved N (TDN) which included NH_4^+, NO_3^- dissolved organic N (DON) concentrations. During Phase 2, NH_4^+ continued to dominate TDN, and its seasonally flow-weighted concentrations ranged from 10.2-16.5 mg L^{-1} NH_4-N. Phase 2 effluent NH_4^+ concentrations averaged 17.7±1.0 mg L^{-1} NH_4-N during baseflow (n=7) and 13.0±1.3 mg L^{-1} NH_4-N during stormflow (n=5), and these averages were significantly different (t statistic=3.57, p=0.01). Concentrations of DON in B-JC WWTP effluent were the second major component of WWTP effluent N, after NH_4^+ (Table 4.2). Dissolved organic N in effluent ranged from 2.4-11.9 mg L^{-1} N during the study period. Dissolved organic N concentrations did not differ between baseflow (n=7) and stormflow (n=5) conditions and its average was 6.01±1.0 mg L^{-1} N (t statistic= 0.68, p=0.52). Flow-weighted NO_3^- concentrations ranged from 0.1-0.3 mg L^{-1} NO_3-N and did not differ between baseflow (n=7) and stormflow (n=5) conditions (t statistic=1.05, p=0.33).

Total dissolved N concentrations in WWTP effluent, including both inorganic and organic N, ranged from 10.8-30.0 mg L^{-1} N during Phase 2. Table 4.2 shows NH_4^+, NO_3^- and DON as percentages of TDN for Phase 2 of effluent sampling. During this time, TDN from effluent was dominated by NH_4^+, which comprised 69% of N dissolved in effluent. Organic N accounted for 30%, and NO_3^- accounted for 1% of effluent TDN concentrations (Table 4.2).

In the Chenango and Susquehanna rivers, TDN was dominated by NO_3^- (Figure 4.2). River NO_3^- concentrations ranged from 0.24 to 0.63 mg L^{-1} NO_3-N. A two-way ANOVA examining differences in NO_3^- concentrations showed significant differences between sites (F value=3.21, p=0.02) but not between baseflow and stormflow conditions (F=1.81, p=0.19) (Appendix A, Table A4.2). Nitrate was significantly higher at the CR Upstream site compared to the SR Confluence location (Figure 4.2a).

River NH_4^+ concentrations ranged from 0.04 to 0.15 mg L^{-1} NH_4-N during the study period. A two-way ANOVA evaluating differences in NH_4^+ concentrations showed significant differences between sites (F value=8.38, p<0.00001) but not among baseflow or stormflow conditions (F=1.07, p=0.31) (Appendix A, Table A4.3). Concentrations of NH_4^+ did not significantly differ between SR and CR Upstream and Confluence sites, but were significantly higher at the SR Downstream site, which is downstream of the B-JC WWTP (Figure 4.2b).

Dissolved organic N in the rivers ranged from 0.001 to 3.4 mg L^{-1} N at the river sites (Figure 4.2c). A two-way ANOVA showed that organic N concentrations did not differ significantly among river sites (F value=0.78, p=0.54) or between baseflow and stormflow conditions (F value=0.25, p=0.62) (Appendix A, Table A4.4). River total N concentrations were usually dominated by NO_3^-, and organic N was the secondary component. Ammonium was always the lowest fraction of total N concentrations in the rivers, although it rose from 7-10% of TDN at upstream sites to 14% of TDN at the SR Downstream site, which is downstream of the B-JC WWTP (Table 4.2).

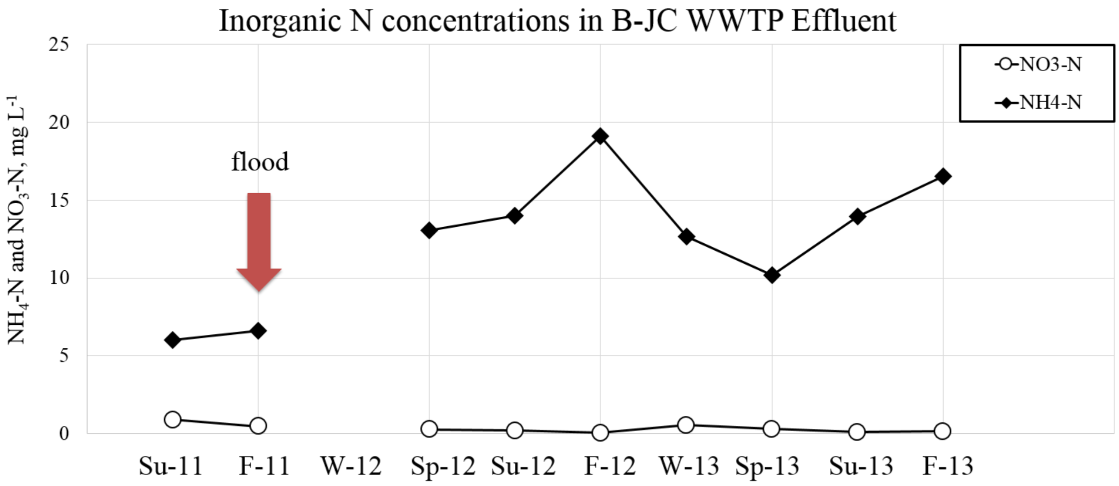


Figure 4.1: Seasonally flow-weighted mean NH$_4$-N and NO$_3$-N concentrations in B-JC WWTP effluent.

Table 4.2: Nitrate, NH$_4^+$ and organic N concentrations as percentages of total N concentrations are given for the five river sampling locations and Phase 2 of the B-JC WWTP effluent sampling.

N Concentrations as Percentages of Total N
Susquehanna and Chenango Rivers and B-JC WWTP
May-October 2013

	SR Upstream	CR Upstream	SR Confluence	CR Confluence	SR Downstream	B-JC WWTP
NO$_3$-N	49%	46%	50%	62%	51%	1%
NH$_4$-N	10%	7%	10%	10%	14%	69%
Organic N	41%	47%	40%	28%	35%	30%

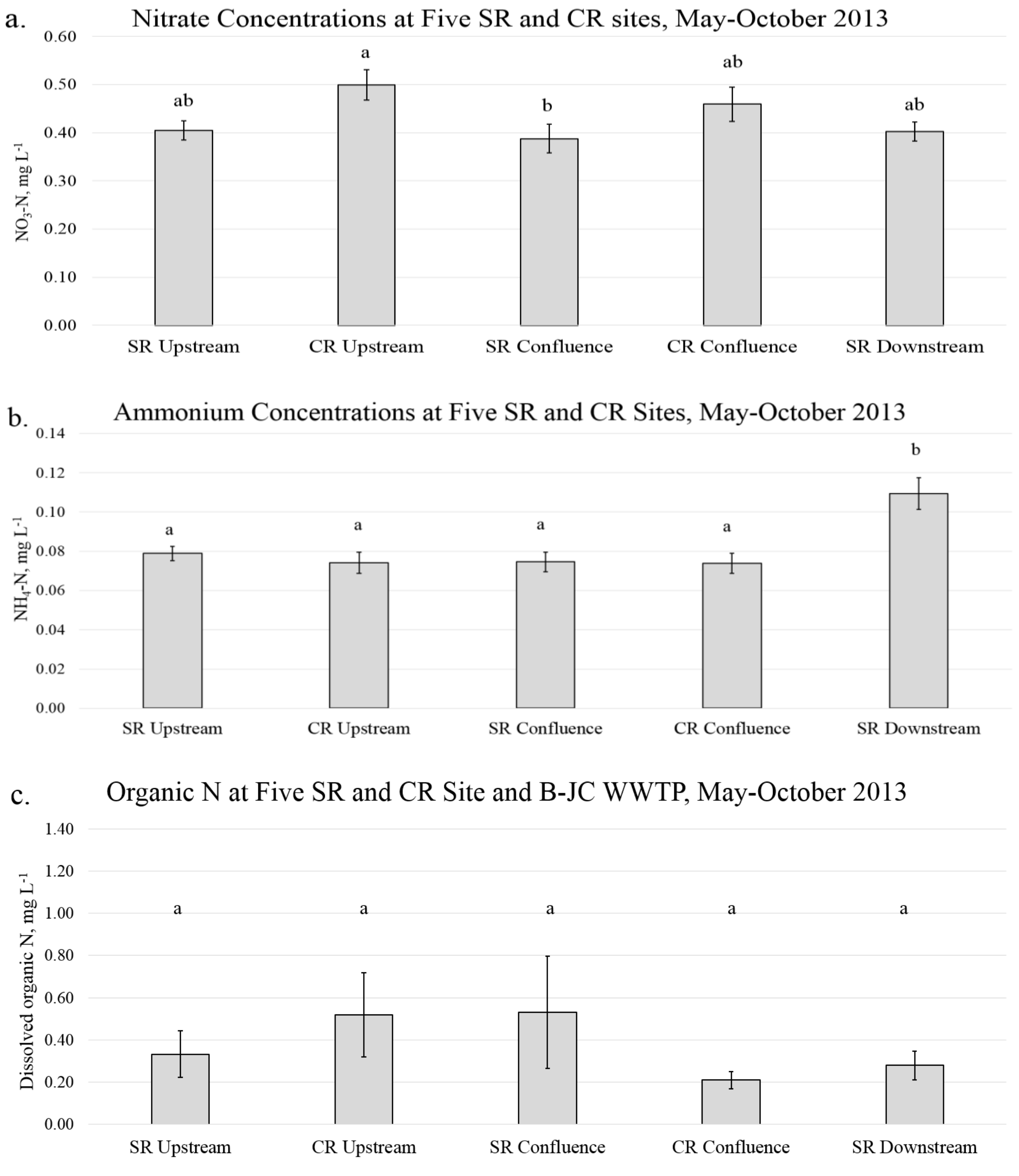


Figure 4.2. Mean (a) NO_3-N and (b) NH_4-N concentrations of each of the 5 river sampling locations, ± 1SE. Mean DON concentrations are given in graph (c) for rivers sites. Means that do not share a common letter signify a significant difference at $p=0.05$, according to the Tukey means comparison. Note differences in y-axes.

4.3.1.b Nitrogen Fluxes

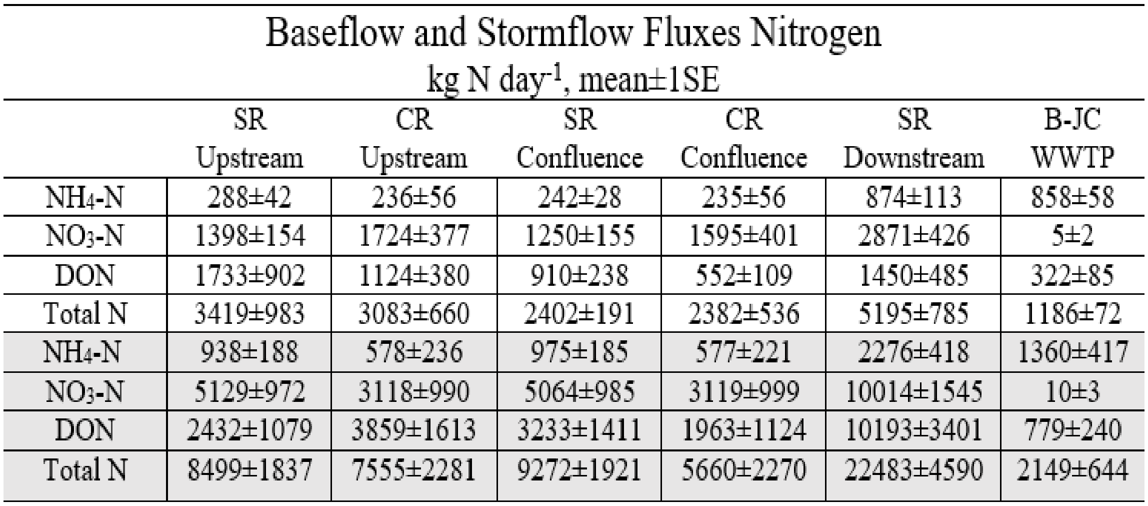
Fluxes N from the B-JC WWTP were lowest in the summer and fall of 2011, during Phase 1 of sampling. A one-way ANOVA showed that inorganic N fluxes were different among seasons (F value= 3.12, p=0.01) (Appendix A, Table A4.5). The Tukey HSD test showed that the summer and fall 2011 fluxes were lower than subsequent seasons ($p<0.05$). Nitrogen fluxes from the B-JC WWTP were higher during stormflow conditions (Table 4.3). During Phase 1 fluxes of NO_3^- from the WWTP were 24.3± 8.1 kg NO_3-N day^{-1} during baseflow (n=24) and 72.7±27.2 kg NO_3-N day^{-1} during stormflow (n=9) conditions, and this difference was significant (t statistic=3.91, p=0.0007). Fluxes of NH_4^+ were 823.5±63.8 kg NH_4-N day^{-1} during baseflow (n=24) conditions and 1177.1±559.7 kg NH_4-N day^{-1} during stormflow (n=9) conditions. However this difference between means was not significant (t statistic=1.60, p=0.12). Compared to the urban streams, seasonal means of inorganic N fluxes from the BJ-WWTP are 10x greater than the seasonal means of total inorganic N fluxes from the eight urban streams that were studied in Chapter 3 (Figure 4.3).

During Phase 2 of sampling from the B-JC WWTP effluent, we had measurements of NO_3^-, NH_4^+ and DON fluxes. Fluxes of NO_3^- were 5.4±1.6 kg NO_3-N day^{-1} during baseflow (n=7) and 10.2±3.2 kg NO_3-N day^{-1} during stormflow (n=5) conditions, and did not significantly differ (t statistic=1.50, p=0.19). Fluxes of NH_4^+ were 858.2±58.1 kg NH_4-N day^{-1} during baseflow (n=7) and 1360.3±416.6 kg NH_4-N day^{-1} during stormflow (n=5) conditions. The difference between the baseflow and stormflow NH_4^+ fluxes was not significant (t statistic=1.71, p=0.06). Fluxes of organic N were 322.0±84.5 kg N day^{-1} during baseflow (n=7) and 778.7± 240.8 kg N day^{-1} during stormflow (n=5) conditions, and fluxes were significantly higher during storms (t statistic=3.11, p=0.02).

Nitrogen fluxes in the rivers were dominated by NO_3^- during both baseflow and stormflow conditions (Table 4.3). The second-largest component was organic N, and NH_4^+ was a smaller proportion of river N fluxes (Table 4.3). A two-way ANOVA showed that NO_3^- fluxes were significantly different among sites (F value=9.06, p<0.00001) and between baseflow and stormflow conditions (F value=61.13, p<0.00001) (Appendix A, Table A4.6). The Tukey means comparison test showed that the SR Downstream site had significantly higher fluxes than all other river sites (p=0.01).

Fluxes of NH_4^+ were the smallest proportion of total N fluxes (Table 4.3). A two-way ANOVA showed that fluxes of NH_4^+ differed significantly between sites (F value=15.58, p<0.00001) and between baseflow and stormflow conditions (F value=44.50, p<0.00001) (Appendix A, Table A4.7). The Tukey means comparison showed that fluxes of NH_4^+ were significantly higher at the SR Downstream site compared to all upstream river sites (p= 0.001-0.00001). Fluxes of organic N were the second-largest component of total N fluxes in the rivers (Table 4.3). A two-way ANOVA showed that it did not significantly differ between sites (F value=0.76, p=0.54) or between baseflow and stormflow conditions (F value=0.05, p=0.83) (Appendix A, Table A4.8).

Table 4.3: Fluxes of NH_4^+, NO_3^- , DON and Total N at SR, CR sites and B-JC WWTP during baseflow and stormflow conditions. Means are given ±1SE. The rows that are not shaded are baseflow conditions, and the rows that are shaded are stormflow conditions.



Baseflow and Stormflow Fluxes Nitrogen

kg N day^{-1}, mean±1SE

	SR Upstream	CR Upstream	SR Confluence	CR Confluence	SR Downstream	B-JC WWTP
NH_4-N	288±42	236±56	242±28	235±56	874±113	858±58
NO_3-N	1398±154	1724±377	1250±155	1595±401	2871±426	5±2
DON	1733±902	1124±380	910±238	552±109	1450±485	322±85
Total N	3419±983	3083±660	2402±191	2382±536	5195±785	1186±72
NH_4-N	938±188	578±236	975±185	577±221	2276±418	1360±417
NO_3-N	5129±972	3118±990	5064±985	3119±999	10014±1545	10±3
DON	2432±1079	3859±1613	3233±1411	1963±1124	10193±3401	779±240
Total N	8499±1837	7555±2281	9272±1921	5660±2270	22483±4590	2149±644

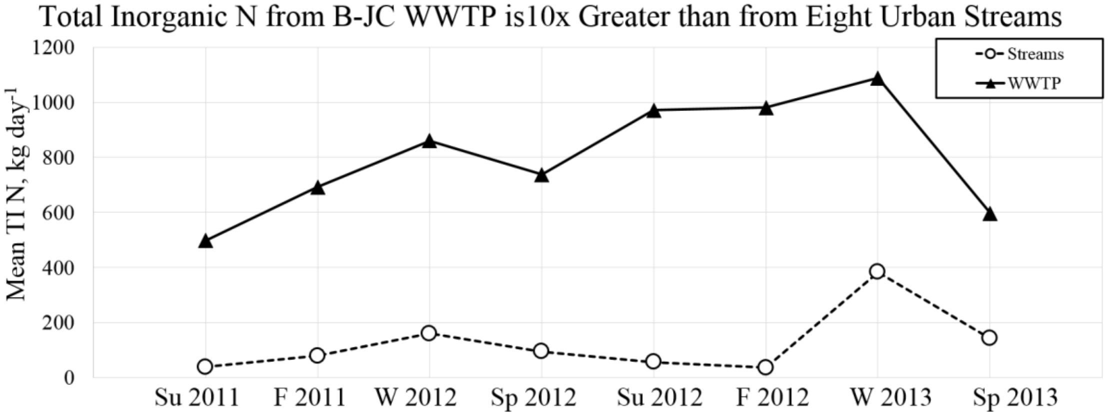


Figure 4.3: Total inorganic N fluxes (NH_4-N and NO_3-N) discharged from B-JC WWTP and eight urban streams, summer 2011-spring 2013.

Total N fluxes include NO_3^-, NH_4^+ and organic N. Fluxes of N were lower at river sites upstream of the B-JC WWTP (Figure 4.4, Table 4.3). A two-way ANOVA showed that total N fluxes were significantly different between sites (F value=8.15, p<0.00001) and between baseflow and stormflow conditions (F value=45.96, p<0.00001) (Appendix

A, Table A4.9). During baseflow conditions, NH_4^+ was about 71% of total N fluxes in B-JC WWTP effluent, and NH_4^+ fluxes at the SR Downstream site were 15% of total N fluxes, compared to about 10% at sites upstream of the plant (Table 4.4). During stormflow conditions, NH_4^+ was 66% of total N fluxes from the effluent, and SR Downstream fluxes of NH_4^+ were 10% which is similar to fluxes at sites upstream of the plant. Organic N in effluent from the WWTP was 29% of baseflow fluxes and increased to 33% of stormflow fluxes of total N. During stormflow, fluxes of organic N at the SR Downstream site were 45% of total N (Table 4.4).

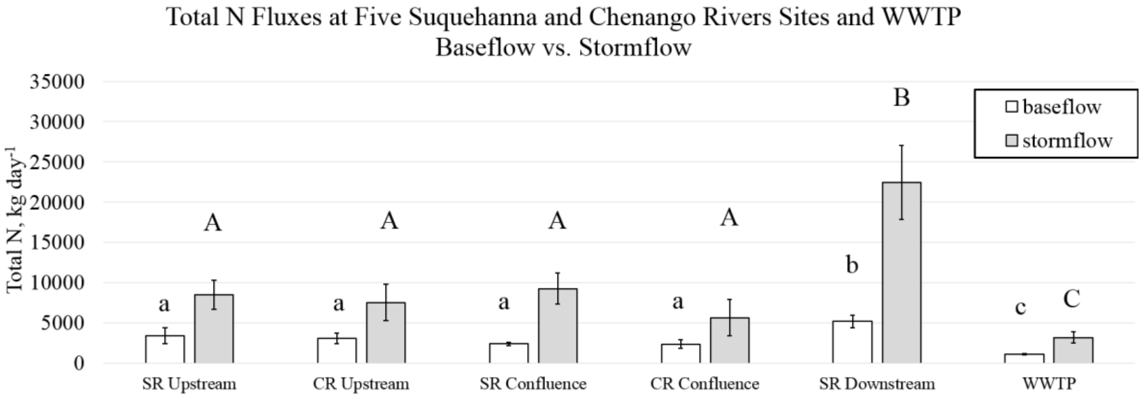


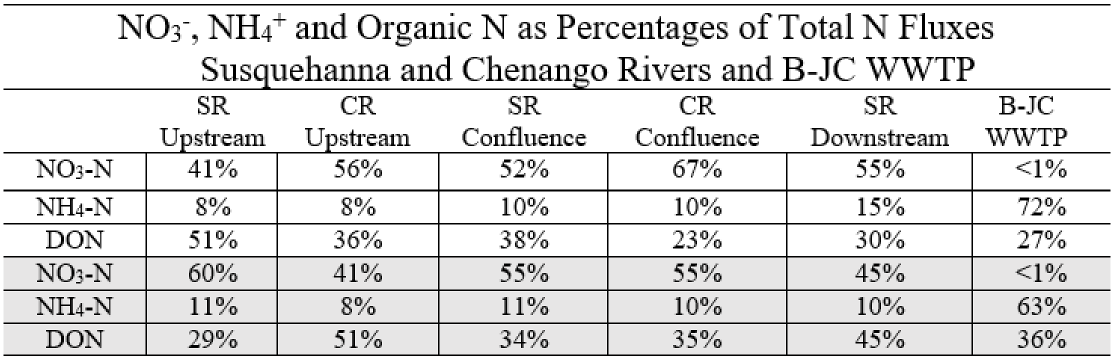
Figure 4.4: Total N fluxes from SR and CR sites, and WWTP during baseflow and stormflow conditions. Means are given ±1SE. Means that do not share a common letter signify a significant difference at p=0.05, according to the Tukey means comparison.

4.3.2 Phosphorus in B-JC WWTP Effluent and in the Susquehanna and Chenango Rivers

4.3.2.a Phosphorus Concentrations

Average concentrations of total P in B-JC WWTP effluent were 0.43±0.17 mg L^{-1} P. Concentrations of total P in the rivers were lower upstream of the B-JC WWTP than at the SR Downstream site (Figure 4.5a). However a two-way ANOVA showed that total P concentrations did not differ significantly between sites (F value=1.49, p=0.23), or between baseflow and stormflow conditions (F value=3.21, p=0.08) (Appendix A, Table A4.10). Concentrations of total P in the rivers ranged from 0.0 – 0.5 mg L^{-1} P during the sampling period. Soluble reactive phosphorus (SRP) was measured for baseflow sampling events only, and did not differ between the five river sites (F value=0.10, p=0.98) (Appendix A, Table A4.11). It ranged from 0.0-0.02 mg L^{-1} PO_4-P. Soluble reactive P in B-JC WWTP effluent averaged 0.18±0.08 mg PO_4-P L^{-1}. Soluble reactive P was about 7% of total P at the river sites, while SRP was 40% of total P in B-JC WWTP effluent.

Table 4.4: Nitrate, NH_4^+ and organic N fluxes as percentages of total N concentrations are given for the five river sampling locations and the B-JC WWTP.



NO_3^-, NH_4^+ and Organic N as Percentages of Total N Fluxes
Susquehanna and Chenango Rivers and B-JC WWTP

	SR Upstream	CR Upstream	SR Confluence	CR Confluence	SR Downstream	B-JC WWTP
NO_3-N	41%	56%	52%	67%	55%	<1%
NH_4-N	8%	8%	10%	10%	15%	72%
DON	51%	36%	38%	23%	30%	27%
NO_3-N	60%	41%	55%	55%	45%	<1%
NH_4-N	11%	8%	11%	10%	10%	63%
DON	29%	51%	34%	35%	45%	36%

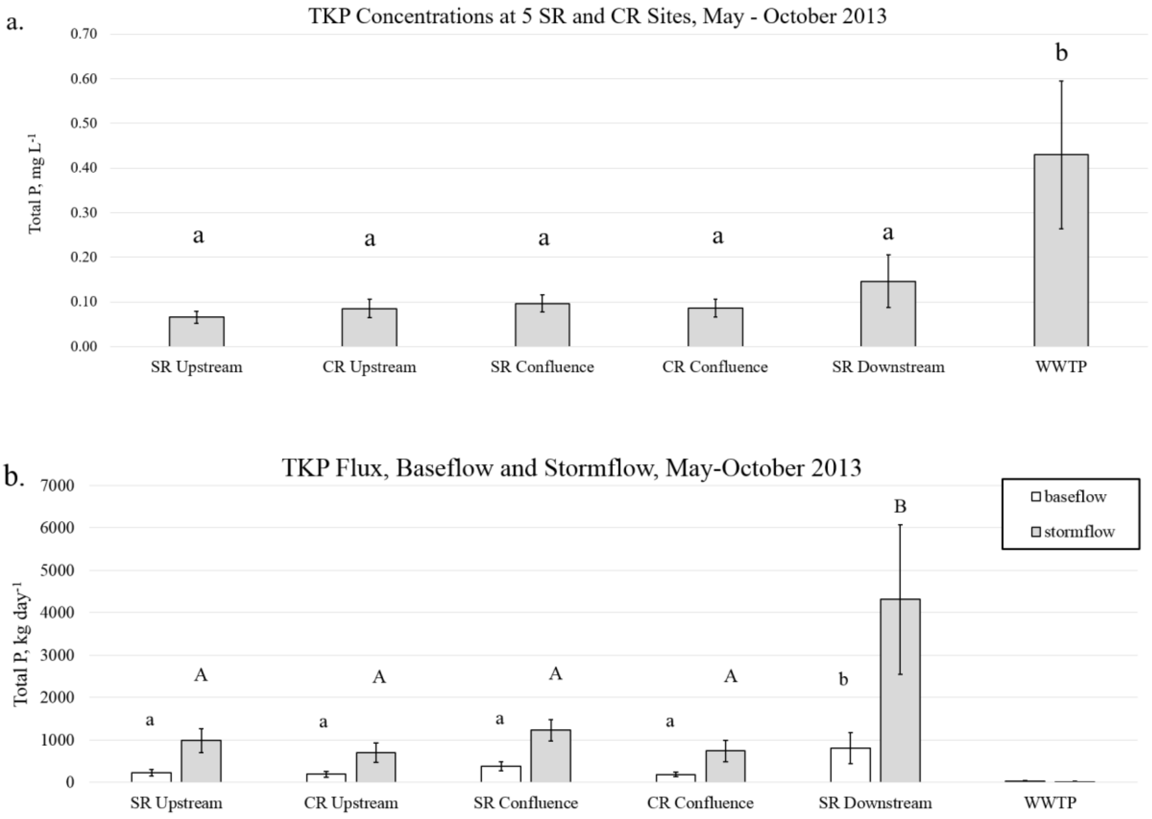


Figure 4.5: Graph (a) shows mean total phosphorus concentrations at each of each of the river 5 sampling locations, ± 1SE while (b) shows mean total phosphorus fluxes at each of each of the 5 river sampling locations and WWTP, ± 1SE. Means that do not share a common letter signify a significant difference at p=0.05, according to the Tukey means comparison.

4.3.2.b Phosphorus Fluxes

Fluxes of total P were lower upstream of the B-JC WWTP plant than at the SR Downstream site (Figure 4.5b). A two-way ANOVA showed that total P fluxes differed significantly between sites (F value=15.06, p=0.0003) and between baseflow and stormflow conditions (F value=5.07, p=0.002) (Appendix A, Table A4.12). During baseflow conditions, fluxes of P ranged from 182±53 – 376±113 kg P day^{-1} upstream of the WWTP and were 805±369 kg P day^{-1} at the SR Downstream site. During stormflow conditions, total P ranged from 701±229-1231±252 kg P day^{-1} and were 4311±1763 kg P day^{-1} downstream of the plant (Figure 4.5b). Total P in WWTP effluent was 31±9 kg P day^{-1} during baseflow and 17±9 kg P day^{-1} during stormflow events (Table 4.4). Soluble reactive P (SRP) data for the river sites and WWTP were available for baseflow conditions. Fluxes of SRP did not significantly differ between sites (F value=0.35, p=0.84) (Appendix A, Table A4.13). Fluxes of SRP ranged from 26±14-75±67 kg PO_4-P day^{-1} at the five river sites and were about 20% of total P fluxes.

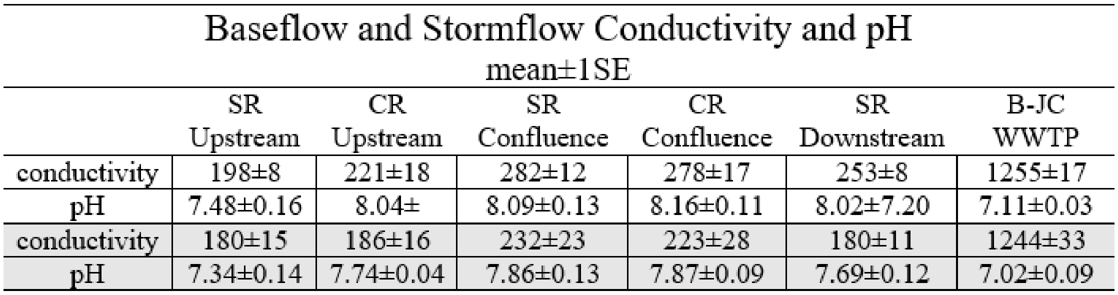
4.3.3 Conductivities, chloride and pH of B-JC WWTP Effluent and River Water

Measurements of conductivity and pH of B-JC WWTP effluent occurred during Phases 1 and 2 of sampling. Conductivities of WWTP effluent were high, ranging from 513-1314 µS cm^{-1}. During Phase 1, conductivities did not differ between baseflow and stormflow conditions (t statistic=0.79, n=33, p=0.25) and averaged 1153±51 µS cm^{-1}. During Phase 2, conductivities did not differ between baseflow and stormflow conditions (t statistic=0.28, n=12, p=0.39) and averaged 1251±17 µS cm^{-1}. The conductivity readings that we obtained from the rivers are elevated above background levels. A two-way ANOVA showed that there were differences in conductivities between sites (F=20.94, p<0.0001) and between baseflow and stormflow conditions (F=7.46, p<0.0001) (Appendix A, Table A4.14). Conductivities of rivers ranged from 145-325 µS cm^{-1} during the sampling period. Average river conductivities were about 200 µS cm^{-1} at the SR and CR Upstream sites, were 250 µS cm^{-1} at the SR and CR Confluence sites, and were about 225 µS cm^{-1} at the SR Downstream site (Table 4.5). Rivers' conductivities were higher during times of baseflow and lower during times of stormflow (Table 4.5).

Measurements of pH also occurred during Phase 1 and 2 of effluent sampling. During Phase 1, effluent pH did not differ between baseflow and stormflow conditions (t statistic=0.94, n=33, p=0.18) and averaged 7.4±0.03. During Phase 2 of effluent sampling, pH did not differ between baseflow and stormflow conditions (t statistic=0.98, n=12, p=0.19) and averaged 7.1±0.04. The pH of rivers ranged from 6.8-8.8 during the sampling period. A two-way ANOVA showed that there were differences in pH between river sites (F value= 15.24, p<0.0001) and between river baseflow and stormflow conditions (F value = 0.01, p=0.01) (Appendix A, Table A4.15). Average river pH were highest at the SR Confluence, CR Upstream and Confluence, and SR Downstream sites. The SR Upstream site and B-JC WWTP effluent had lower pH measurements. Rivers' pH measurements were higher during times of baseflow and lower during times of stormflow (Table 4.5).

In WWTP effluent, Cl^- concentrations ranged from 96-246 mg L^{-1} Cl^-, and fluxes were an average of 9551±967 kg Cl^- day^{-1}. A simple linear regression showed that in WWTP effluent, the relationship between conductivities and Cl^- concentrations was significant ($r^2=0.64$, p=0.0006). River Cl^- concentrations were low, and ranged from 3.7-20.8 mg L Cl^-. A one-way ANOVA examining differences in Cl^- concentrations between sites during baseflow only showed that concentrations did not differ (F value = 1.50, p=0.26) (Appendix A, Table A4.16). Average Cl^- concentrations were not higher downstream of the B-JC WWTP, and was highest in at the Chenango River (Table 4.6). Fluxes of Cl^- in the rivers during baseflow conditions were higher downstream of the B-JC WWTP, although this was not significant (F value=2.75, p=0.07) (Appendix A, Table A4.17) (Table 4.6).

Table 4.5: Conductivity and pH measurements from SR, CR sites and B-JC WWTP during baseflow and stormflow conditions. Means are given ±1SE. The rows that are not shaded are baseflow conditions, and the rows that are shaded are stormflow conditions.

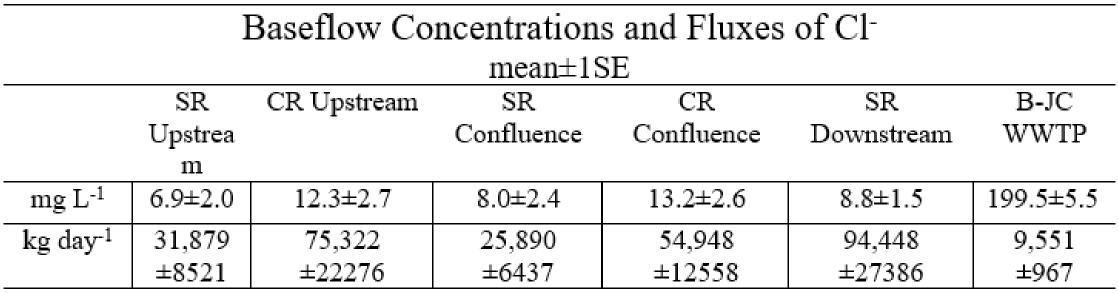


Baseflow and Stormflow Conductivity and pH

mean±1SE

	SR Upstream	CR Upstream	SR Confluence	CR Confluence	SR Downstream	B-JC WWTP
conductivity	198±8	221±18	282±12	278±17	253±8	1255±17
pH	7.48±0.16	8.04±	8.09±0.13	8.16±0.11	8.02±7.20	7.11±0.03
conductivity	180±15	186±16	232±23	223±28	180±11	1244±33
pH	7.34±0.14	7.74±0.04	7.86±0.13	7.87±0.09	7.69±0.12	7.02±0.09

Table 4.6: Chloride concentrations and fluxes at SR, CR sites and B-JC WWTP during baseflow conditions. Means are given ±1SE.



Baseflow Concentrations and Fluxes of Cl$^-$

mean±1SE

	SR Upstream	CR Upstream	SR Confluence	CR Confluence	SR Downstream	B-JC WWTP
mg L^{-1}	6.9±2.0	12.3±2.7	8.0±2.4	13.2±2.6	8.8±1.5	199.5±5.5
kg day^{-1}	31,879 ±8521	75,322 ±22276	25,890 ±6437	54,948 ±12558	94,448 ±27386	9,551 ±967

4.4 Discussion

4.4.1 Nitrogen

4.4.1.a Nitrogen concentrations

In this study, we were interested in the contributions of point- and nonpoint-source N from Binghamton, a medium-sized city, to the Susquehanna River. The Susquehanna River is the largest contributor of N to the Chesapeake Bay (Hagy et al. 2004, Howarth et al. 2008). Nitrogen loading from urban and agricultural areas to the river has been of particular concern, since increases in N loading in recent decades have contributed to problems with eutrophication and hypoxia in the Bay (Hagy et al. 2004, Howarth et al. 2008). Urban areas have high inputs of N due to imports of food, fertilizer and fuel (Baker et al. 2001, Fissore et al. 2011). Nitrogen inputs from food are usually discharged after sewage treatment from point-source WWTPs to waterways, while fertilizer and fuel are more diffuse nonpoint-sources of N. Urban N that is exported to waterways from both point- and nonpoint-sources includes dissolved NH_4^+, NO_3^-, and organic N. We found that N concentrations in the rivers were dominated by NO_3^-, organic N was the second-largest component, and NH_4^+ was a minor component of total N concentrations (Figure 4.2). Nitrate concentrations were 0.4-0.5 mg L^{-1} NO_3-N, which are fairly low measurements (Hagy et al. 2004, Howarth et al. 2008). They did not increase downstream of the Binghamton area (Figure 4.2a). Nicole Hantsch completed a similar study in Weixing Zhu's lab from 2005-2006, where she sampled the Susquehanna and Chenango Rivers from the same bridges; she did not sample from the B-JC WWTP. She also reported that rivers were dominated by NO_3^- , and her NO_3^- and organic N measurements are comparable to our measurements (Hantsch 2008, Figure 4.2a). Hantsch

(2008) correspondingly found that NO_3^- and organic N concentrations didn't increase at the SR Downstream site (Figure 4.2a). However Hantsch (2008) reported lower NH_4^+ concentrations (0.01-0.02 mg L^{-1} NH_4-N) at all river sites upstream of the B-JC WWTP compared to our measurements (0.07-0.08 mg L^{-1} NH_4-N) upstream of the B-JC WWTP. Furthermore her average SR Downstream NH_4^+ concentration was about 0.08 mg L^{-1} NH_4-N, which is about 70% of our average concentration of 0.11 mg L^{-1} NH_4-N at the same location. At the SR Downstream site, which is downstream of the B-JC WWTP, we found that NH_4^+ concentrations were elevated from around 10% upstream to 14% of total N concentrations downstream of the plant (Table 4.2). This comparison with Hantstch's (2008) data shows how higher river NH_4^+ concentrations increased in the 7 years in between the studies, providing evidence that the impaired B-JC WWTP has had impacts on river water chemistry downtream.

Other work has reported on Susquehanna River N concentrations and loading nearer to where the river discharges to the Chesapeake Bay. A study of NO_3^- concentrations in the Susquehanna River in its lower watershed near Harrisburg, P.A. detailed how the river has had significant increases in NO_3^- over a 52-year period (Hagy et al. 2004). In 1945, NO_3^- concentrations averaged 0.46 mg L^{-1} NO_3-N, and concentrations doubled to an average of 0.9 mg L^{-1} NO_3-N by the mid-2000's (Hagy et al. 2004). The long-term increase in Susquehanna River NO_3^- concentrations and loading over the period of this 52-year study were attributed to greater N inputs from nonpoint-sources of atmospheric deposition, fertilizers, and less N retention in the watershed (Hagy et al. 2004). Our measurements of river NO_3^- were about 0.4 mg L^{-1} NO_3-N at the SR Downstream site, which are lower than Hagy et al.'s (2004) measurements. However this is expected, since Binghamton is at the headwaters of the Susquehanna River; Hagy et al.'s (2004) study occurred 322 km (200 miles) to the south, and had received additional inputs from various point- and nonpoint-sources of N.

4.4.1.b Nitrogen Fluxes

The Twin Cities Household Ecosystem Project quantified N and P fluxes to and from households and the researchers concluded that urban N fluxes are dominated by human diets (Fissore et al. 2011). Human diets accounted for 40% of N fluxes in their system and the majority of this N was exported via WWTPs to nearby river systems (Fissore et al. 2011). Human diets are likely the major source of urban N in most cities, which use WWTPs to treat excreted N (Lauver and Baker 2000). The efficiency of nutrient removal by WWTPs is largely dependent on the treatment technologies that are used and there have been concerted efforts to have effective nutrient removal technologies in urban WWTPs (Carey and Migliaccio 2009, Hale et al. 2015). For example by the year 2000, WWTPs with secondary treatment served 40% of the population and WWTPs with tertiary treatment served another 45% of the population of the Northeastern U.S. (Hale et al. 2015). Nitrification-denitrification functions are crucial components of WWTPs, since it is important to remove as much organic N, NH_4^+ and NO_3^- as possible; effluent is typically discharged to nearby water bodies and these forms of N contribute to eutrophication (Lauver and Baker 2000, Baker et al. 2001). Wastewater treatment plants that have nitrification-denitrification functions can reduce total N in effluent to be ≤ 6 mg L^{-1} N (Lauver and Baker 2000).

In this study, the B-JC WWTP had nitrification-denitrification functions that were impaired by a BAF wall collapse before research started, and then were destroyed by flooding after an extreme weather event. In the summer of 2011 when the plant had reduced function, flow-weighted N concentrations were nearly 7 mg L^{-1} N, and were dominated by NH_4^+. This indicated that the nitrification function was not working properly. After the September 2011 flood, when preliminary and primary treatments were the only available sewage treatments, effluent N concentrations were as high as 30 mg L^{-1} N, and were still dominated by NH_4^+. We observed an impact of the NH_4^+ in effluent on river NH_4^+ concentrations that were significantly higher at the SR Downstream site, compared to sites upstream of the B-JC WWTP (Figure 4.2b). SR Downstream NH_4^+ concentrations had a 57% increase compared to concentrations upstream of the plant (Figure 4.2b). This was also evident in fluxes of NH_4^+ at the SR Downstream site, particularly during baseflow conditions (Tables 4.3 and 4.4). Fluxes of NH_4^+ were 15% of total N fluxes compared to about 10% of fluxes upstream, indicating that the nitrification failure at the B-JC WWTP elevates NH_4^+ fluxes downstream of the plant.

During stormflow conditions, effluent NH_4^+ fluxes doubled, and organic N fluxes were 2.5 times higher than baseflow (Tables 4.3 and 4.4). At the SR Downstream site, stormflow NH_4^+ fluxes were about 3x and organic N fluxes were 7x fluxes of baseflow conditions. Since inputs from the SR and CR Confluence sites alone did not account for the increases in N fluxes downstream, the impaired B-JC WWTP must have been a major contributor (Tables 4.3 and 4.4). During stormflow events NH_4^+ fluxes at the SR Downstream site were 10% of total N fluxes and were similar to fluxes upstream, suggesting that NH_4^+ fluxes from the plant were diluted by the higher river discharges (Table 4.4). However the elevated organic N in B-JC WWTP effluent during stormflow had clearer impacts on river organic N fluxes downstream (Tables 4.3 and 4.4). During stormflow conditions, combined sewage overflows (CSOs) could have contributed to more river organic N, but we did not sample from CSOs. Furthermore, we report higher NH_4^+ fluxes downstream of the B-JC WWTP than Hantsch (2008). She reported NH_4^+ fluxes of 82 and 1,100 kg NH_4-N day^{-1} during baseflow and stormflow, respectively. At the SR Downstream site, we calculated 874±113 and 2276±418 kg NH_4-N day^{-1} during baseflow and stormflow, respectively. This is 11x the NH_4^+ in baseflow and 2x the NH_4^+ in stormflow that Hantsch (2008) reported, which is likely due to the reduced B-JC WWTP function.

We have a comparison of 7 baseflow and 5 stormflow events, which show differences in concentrations and fluxes of N in the rivers and B-JC WWTP. Storm discharge from rivers has been defined as discharge that is preceded, within 72 hours, by precipitation amounting to 0.25cm or greater (www.epa.gov). This definition was designed for the watershed approach; but when the sewershed receives precipitation, it is channeled more quickly to WWTPs. Storm discharge from the B-JC WWTP takes about 1.5-3 hours to start after a precipitation event begins (Catherine Young, pers. communication). Using this information, we can be sure that we sampled storm discharge effluent at 3 events while the other 2 may have had too much of a lag time between the precipitation and our sampling to obtain a sample from effluent that was truly representative of stormflow. Considering just the 3 stormflow effluent samples, NH_4^+ and DON continue to dominate total N fluxes. Under this scenario fluxes of organic N become a larger proportion of total N fluxes and average 3202±690 kg N day^{-1} and be about 9x higher than baseflow fluxes. Using just the 3 stormflow events, fluxes of NH_4^+ continue to be high and average 2042±515 kg NH_4^+ day^{-1}, or about 2.5x baseflow fluxes.

We estimated that during the 5 stormflow WWTP fluxes, NH_4^+ was about 66% and DON was about 33% of total N fluxes to the Suquehanna River. If we consider only the 3 stormflow events, NH_4^+ would be about 56% and DON would be 42% of total N fluxes to the Susquehanna River, which suggests that organic N could be a large contributor of N to the river during intense storm events.

The baseflow and stormflow river NO_3^- fluxes that Hantstch (2008) report are similar to ours. She reported about 2,800 kg NO_3-N day^{-1} and 12,700 NO_3-N day^{-1} during baseflow and stormflow, respectively. The SR Downstream site baseflow and stormflow NO_3^- fluxes we calculated were 2871±426 kg NO_3-N day^{-1} and 10,014±1545 kg NO_3-N day^{-1}, respectively. During times of stormflow, the SR Downstream site was not the only site to experience higher fluxes of N. At river sites upstream of the B-JC WWTP, N fluxes were at least double those of baseflow N fluxes. Therefore N must be entering from elsewhere in the landscape. This study was done during the growing season, when warmer temperatures foster nitrification in soils. Since NO_3^- is quite mobile in soils, it could have leached during and shortly after storm events, increasing river NO_3^- (Chapin et al. 2002). Nitrate is also deposited on surfaces as a result of fossil fuel combustion, and it can build up during dry periods, and then flush from pervious and impervious surfaces during and shortly after precipitation events (Lewis and Grimm 2005). Lewis and Grimm (2005) found evidence for a “build and flush” hypothesis of N in urban streams in Phoenix. Nitrogen deposited on impervious surfaces wasn’t biotically processed, and was washed into urban streams during storm events (Lewis and Grimm 2005).

4.4.2 Phosphorus

As part of the Twin Cities Household Ecosystem Project, Fissore et al. (2011) found that the majority of P exported from urban systems is from human diets, and that is followed by detergents. Total P concentrations from the B-JC WWTP ranged from 0.07 - 1.2 mg P L^{-1}. The total P concentrations we measured in the rivers ranged between 0.06 and 0.08 mg P L^{-1} upstream of the WWTP and were about 0.15 mg P L^{-1} downstream of the plant (Figure 4.5). These concentrations are higher than Hantsch (2008) reported, as her measurements were an average of 0.04 mg P L^{-1} at all sites, including downstream of the B-JC WWTP. Our average concentration at the SR Downstream site is comparable to the low end of what Withers and Jarvie (2008) reported in a WWTP-impacted river.

Our fluxes of total P were higher than Hantsch (2008) reported. She reported Chenango River fluxes of 263 kg P day^{-1} during baseflow and 3200 kg P day^{-1} during stormflow, while the Susquehanna River had 404 kg P day^{-1} during baseflow and 3700 kg P day^{-1} during stormflow. While our calculated fluxes of total P in the rivers were higher than what Hantsch (2008) measured, our calculated total P fluxes were also significantly elevated during stormflow. Our measured fluxes of total P in rivers were about 4x higher during stormflow events at all sites (Figure 4.5). The contribution of total P from the B-JC WWTP effluent was low. Sedimentation during primary treatment at the plant probably removed a large percentage of the P in the incoming sewage. The fluxes of P from the WWTP do not explain the higher P fluxes during stormflow at the SR Downstream site. Since the increase in stormflow P fluxes was observed at all five river sites, this must be due to other factors. An explanation for high P fluxes during large river discharges is resuspension of river bed sediment, since during times of high flows, dissolved P that is in river bed sediment can be resuspended and enter the water column (Withers and Jarvie 2008).

4.4.3 Conductivities, chloride and pH

Conductivity was high in the B-JC WWTP effluent, probably due to the use of water softeners and other ions in effluent (Table 4.5). Wastewater treatment plant effluent conductivity and Cl^- were found to be positively related. Chloride is probably the major contributor to high conductivity of B-JC WWTP effluent. However the high effluent conductivity was diluted by river water; conductivity at the SR Downstream site was around 225 uS cm^{-1}. Our measurements of Cl^- concentrations, which averaged about 200 mg L^{-1}, are similar to concentrations in WWTP effluents of the Minneapolis/St. Paul M.N. area that were reported by Novotny et al. (2009). Our average daily fluxes of Cl^- from the B-J WWTP were around 9,500 kg Cl^- day^{-1}, and were similar to those reported by Novotny et al. (2009) as well. River Cl^- concentrations ranged from 4 - 21 mg L Cl^-. Rivers in forested watersheds often have Cl^- concentrations that are below 5 mg L^{-1} Cl^-, while rivers in watersheds with 5-10% development have river Cl^- concentrations that are 10-20 mg L^{-1} Cl^- (Corsi et al. 2015). Our measurements of Cl^- are in the range of concentrations of streams in both forested watersheds and watersheds with low-intensity development (Table 4.6) (Corsi et al. 2015). However, our fluxes of Cl^- from the SR Downstream site from May-October are comparable to those in the Mississippi River during the same time period (Novotny et al. 2009).

4.4.4 Implications

Substantial efforts have been made to reduce point- and nonpoint-source nutrients to waterways, but the effects of climate change will further complicate the issue of controlling nutrient loading (Bettez et al. 2015). In the Northeastern U.S., climate change is expected to harbor increasingly frequent and more intense precipitation events (Hayhoe et al. 2007). More intense storms will likely cause flooding of low-lying WWTPs, as has been observed in this study and elsewhere (Schwirtz 2012, 2013). Reduction of WWTP function could become and may already be a major issue regarding the control of N loading. Therefore, it would be advisable to rebuild damaged WWTPs to be more resilient to the effects of climate change, such as flooding (Kaushal et al. 2015). Constructed wetland systems may serve a dual purpose to this problem, since they can reduce nutrient loads and can absorb large inputs of water, making them more resilient to impacts of storms and flooding (Chang et al. 2013, Ciria et al. 2005, Huang et al. 2000). Studies of N removal by constructed wetlands have yielded promising results. In studies of N removal wetlands built for the purpose of wastewater treatment, research groups have reported up to 70-75% removal of inorganic N, and this is especially effective when effluent has longer residence times (Ciria et al. 2005, Huang et al. 2000, Maine et al. 2006). These and other studies provide evidence that constructed wetlands can not only be effective at N removal, but have similar removal capacities as conventional WWTPs (Ciria et al. 2005). Though it is unlikely that constructed wetland systems will overtake conventional WWTPs in the near future, policy makers should consider incorporating more resilient systems into at least parts of their wastewater treatment designs. By incorporating wetlands into wastewater treatment designs, WWTPs could become more resilient to the storms and flooding that result from climate change, allowing them to continue nutrient reduction in effluent even after extreme storms.

4.4.5 Conclusions

We found that the function of the B-JC WWTP was impaired by the collapse of a wall of a BAF, and later flooding from Tropical Storm Lee. The flooding of the plant constrained sewage treatment to preliminary and primary treatment only, resulting in a large point-source fluxes of N to the Susquehanna River. This was evident in our measurements of B-JC WWTP effluent N concentrations, which was dominated by NH_4^+ and also had large proportions of organic N. Impacts of N in effluent were detectable in river water N concentrations and fluxes at the SR Downstream site, which is downstream of the B-JC WWTP. SR Downstream NH_4^+ concentrations were 57% higher than concentrations upstream of the plant. Fluxes of NH_4^+ were 15% of total N fluxes downstream, compared to 10% upstream of the plant during baseflow conditions. During stormflow conditions, organic N became a larger proportion of total N in B-JC WWTP effluent, which contributed to SR Downstream fluxes of organic N that were 7x higher during stormflow conditions compared to baseflow. At the SR Downstream site higher NH_4^+ fluxes during baseflow, and higher organic N fluxes during stormflow indicated that the impaired WWTP impacts water quality of the Susquehanna River. In these river systems, NO_3^- concentrations dominated total N concentrations and fluxes in the rivers, both upstream and downstream of the B-JC WWTP. Nonpoint- sources of NO_3^- from urban streams (Chapter 3) and from B-JC WWTP effluent are minor components of N fluxes to the Susquehanna River, and NO_3^- did not increase downstream of Binghamton. Measurements of P, conductivity, Cl^- and pH did not increase downstream of Binghamton as a result of the impaired WWTP, and the damage to the plant was most evident in N export in effluent and in the rivers.

Chapter 5. Nitrate processing in the stream sediment of an urbanized watershed

5.1 Introduction

In urban and suburban areas, nitrate (NO_3^-) is usually the dominant form of inorganic N in stream water, and is elevated above concentrations in streams of "natural" watersheds (Groffman et al. 2004, Shields et al. 2008). Higher NO_3^- concentrations and loads are undesirable, because they contribute to problems of eutrophication (Nixon et al. 1995). However stream NO_3^- has potential avenues of removal by microbial processing and denitrification (Mulholland et al. 2008). During the process of denitrification, microbes consume NO_3^- and release N_2 to the N "sink" of the atmosphere (Chapin et al. 2002). Nitrous oxide (N_2O) is a common by-product of this process, and is a potent greenhouse gas (Chapin et al. 2002). Denitrification is dependent on NO_3^- and carbon (C) availability, low O_2, and is promoted by low-flow conditions in streams (Chapin et al. 2002, Kaushal and Lewis 2005, Pickett et al. 2011). It commonly occurs in urban wetlands, riparian areas, retention basins and in the hyporheic zone of stream beds (Arango and Tank 2008, Pickett et al. 2008, Zhu et al. 2004). Riparian areas in particular are considered "hot spots" of denitrification and can help remove excess NO_3^- in urban streams. However, work at the Baltimore Long Term Ecological Research site (LTER) has shown that this prediction is not necessarily true in urban riparian areas (Pickett et al. 2008). Reasons for this include 1.) the alteration of urban hydrology, where water moves as surface runoff in infrastructure rather than shallow groundwater, which feeds riparian areas and 2.) urban stream channels are often incised, leading to drier riparian soils with less denitrification and an increase in nitrification (Groffman et al. 2002, 2003; Pickett et al. 2008).

Another place of microbial denitrification in streams is the hyporheic zone at the stream bed, where surface water mixes with groundwater (Arango and Tank 2008, Boulton et al. 1998, Lawrence et al. 2013). Small urban headwater streams, like many in Binghamton, may be important places of nitrogen (N) processing and removal. Small headwater streams tend to have considerable allochthonous C inputs and lower water flows that facilitate NO_3^- removal by denitrifying bacteria, which releases N_2 and N_2O to the atmosphere (Kaushal and Lewis 2005, Mulholland et al. 2008, Pickett et al. 2011). In addition to NO_3^-, dissolved organic C (DOC) in streams is an important source of C to hyporheic microbes and predictor of denitrifying activities (Findlay et al. 1993, Arango and Tank 2008). While natural headwater streams will have allochthonous C inputs from leaves and woody material of adjacent trees and riparian areas, urban streams often lack such inputs due to the nature of urban design. However, gutters can collect leaves and soil, discharge them to urban streams, and enhance available organic C (Kaushal and Belt 2012).

Chapter 3 describes how in Binghamton, NO_3^- is higher at urban stream sites compared to rural sites, and because of this we wanted to investigate NO_3^- processing within the stream system. While riparian areas are not common in these streams, we hypothesized that NO_3^- processing and denitrification might happen in the sediment of the hyporheic zone. We chose to conduct the study on stream sediment from Fuller Hollow Creek, which is located within one of the most developed watersheds in the Binghamton area (Chapter 3). We ran experiments in a factorial design using NO_3^-, organic C and a combination of NO_3^- and C to determine if there was a sediment microbial community with denitrifying bacteria in the sediment, and if NO_3^- or C were limiting nutrients to the system. We hypothesized that (1) there would be less microbial activity from urban sediment compared to rural sediment, due to negative effects of urbanization, (2) the addition of C would stimulate CO_2 emissions from the sediment microbial community, (3) NO_3^- would stimulate N_2O emissions, (4) the addition of both NO_3^- and C would elevate N_2O emissions more than NO_3^- alone, and (5) concentrations of NO_3^- would be reduced after an incubation period compared to the initial NO_3^- concentrations. We used sediment from a downstream/urban reach within a more developed portion of the watershed and sediment from a less developed upstream/rural location. We reasoned that since NO_3^- fluxes are higher at the Fuller Hollow downstream/urban site, NO_3^- processing might be greater downstream than upstream due to NO_3^- availability. However, the upstream/rural site has less of an urban influence and more adjacent vegetation, perhaps adding to the available organic C pool, which could impact rates of NO_3^- processing.

5.2 Methods

5.2.1 Study Location

We collected sediment from Fuller Hollow Creek, which is located within a 1650ha watershed that has 34% development (Chapter 3). The stream is a tributary of the Susquehanna River, which flows into the Chesapeake Bay. The watershed includes the Binghamton University campus, and the stream receives runoff from the campus and adjacent developed areas. We collected sediment from a downstream/urban site that is adjacent to campus and suburban areas, and from an upstream/rural site in the Town of

Vestal's Stair Park, that is less impacted by development within the watershed (Chapter 3).

5.2.2 Sediment Collection

Sediment was collected in September 2014, and in December 2015 when the experiment was performed a second time. When samples were collected on September 23, 2014, the weather was partly sunny and the ambient temperature was 12℃. When samples were collected on December 14, 2015, the weather was partly sunny and the ambient temperature was 14℃. There had not been a significant precipitation event in the three days preceding the collections. Five samples were taken from both the downstream/urban and upstream/rural sites. The sediment collection locations were in the hyporheic zone of the stream bed that had low stream water flows (0.01-0.1 m/s) and shallow stream water depths (2-6 cm). Sediment was collected to a depth of 8-10 cm using a trowel and was passed through a mesh 4mm sieve into acid-washed 500 mL bottles. The downstream/urban site was sampled before the upstream/rural site. Duplicate water samples were collected from the sites before sediment collection. Sediment and water samples were transported back to the lab on ice.

5.2.3 Sediment Processing in September 2014

Upon return to the lab, 50 g of sediment were weighed into glass 125 mL Wheaton bottles. The treatments were nitrate only, carbon only, nitrate and carbon combined, and water only (control). Hereafter, the treatments will be referred to as Nitrate, Carbon, Nitrate + Carbon and Control. The sediment was covered with 23 mL of Nanopure water and pre-incubated overnight. In the morning, bottle caps were removed, air within the bottles was expelled three times with a syringe, and then bottles were left open to equilibrate with the atmosphere for one hour. Then, treatments were then added to their respective bottles. The Nitrate treatments received 1mL 125 mg L^{-1} NO_3-N and 1mL Nanopure water; the Carbon treatments received 1mL 1250 mg L^{-1} $C_6H_{12}O_6$-C in the form of dextrose and 1mL Nanopure water; the Nitrate + Carbon treatments received 1mL NO_3-N and 1mL $C_6H_{12}O_6$-C in the form of dextrose; the Control treatments received 2mL Nanopure water. The volume of water in each bottle was 25 mL at the start of the experiment. Gas samples were taken with 20mL syringes fitted with 3-way stoppers immediately after the sediment was treated to obtain initial gas concentrations within the bottles. Bottles incubated for 4 hours then gas was sampled again. Gas samples were immediately analyzed on a Shimadzu Gas Chromatograph 14-A. After the end of the incubation, sediment was extracted with 25mL 0.5 M K_2SO_4 by vigorously shaking for 3 minutes by hand, then allowing to settle in a refrigerator for 30 minutes. The short extraction time was aimed at obtaining the sediment NH_4^+/NO_3^- concentrations before further microbial activity. The supernatant was filtered through Whatman 40 filter papers and acidified with 0.2 mL 50% H_2SO_4. Samples were stored in a cold room until analysis for NH_4-N and NO_3-N concentrations using a Lachat QuickChem 8000 Flow Injection Analyzer (Lachat Instruments, Milkwaukee W.I.).

In addition to the treatments described above, we autoclaved sediment to show that sediment gas emissions resulted from biotic processes. These were the Kill Control and Kill Nitrate treatments, which were autoclaved sediment that received the same treatments as the Control and Nitrate treatments. Immediately after the sediment collection, sediment for the Kill Control and Kill Nitrate replicates from each collection site were weighed (50g), covered in 23mL Nanopure water, and autoclaved. The autoclaved sediment conditioned overnight with the rest of the treatments, air was expelled, and they equilibrated for one hour as described above. Then 2 mL Nanopure water were added to the Kill Control, and 1mL 125 mg L^{-1} NO_3-N and 1mL Nanopure water were added to the Kill Nitrate treatments. Gas samples were taken and sediment was extracted exactly as described above. A pre-incubation control (Initial Control) that had 50g sediment and 25mL Nanopure water conditioned overnight with the other incubations, and was extracted immediately the next morning as described above to obtain the sediment NO_3^- concentration before the experiment began.

Two sediment samples from each of the five collections at downstream/urban and upstream/rural sites were taken to determine the moisture content and organic carbon content of the sediment. Soil samples were dried at 60℃ for 72 hours to determine percent moisture. Percent organic matter was calculated using the loss-on-ignition method where samples were dried in a muffle furnace at 550˚C for two hours. Fifty grams of sediment were extracted immediately after returning to the lab for initial inorganic N concentrations. Measurements of pH were taking using a slurry of 10g sediment and 20mL Nanopure water.

5.2.4 Sediment Processing in December 2015

When the experiment was repeated in December 2015, sediment processing and experimental treatments were the same as in September 2014 with a few modifications to improve the experimental design. In December 2015 we added a pre-incubation NO_3^- treatment, which was 50g of sediment that incubated overnight in 23 mL Nanopure water; in the morning 1mL of NO_3^- and 1 mL Nanopure water were added and extracted immediately. The goal of this additional treatment was to determine what the actual N concentration of NO_3^- was in Nitrate and Nitrate + Carbon treatments at the start of the experiment, and is known as the Nitrate Control treatment. In December 2015 we extended the incubation time from 4 hours to 24 hours. After the gas had been sampled at the end of the 24 hour incubation, sediment was extracted, filtered, stored and analyzed exactly as described in section 5.2.3.

5.2.5 Gas Flux Calculations

The concentration of gas (µg L^{-1}) that was produced in each bottle was calculated as

$$\mathrm{Cm} = \frac{\mathrm{Cv\ x\ M\ x\ P}}{\mathrm{R\ x\ T}}$$

Where Cv is the measured concentration of gas produced in ppm, M is the molecular mass of the atom(s) of interest (i.e., 12 for CO_2-C and 28 for N_2O-N), P is the pressure, R is the universal gas constant, and T is the room temperature in kelvins.

The flux of gas (µg g^{-1} h^{-1}) from the sediment to the headspace in the bottle was calculated as

$$\mathrm{Flux} = \mathrm{Cm\ x}\ \frac{\mathrm{V}}{\mathrm{SDW\ x\ t}}$$

where SDW is the dry weight of the sediment, and t is the number of incubation hours. The term V is the volume of the headspace in the bottles, which was calculated as

$$\mathrm{V} = 159 - 25 - \frac{\mathrm{SDW}}{2.65}$$

where 159 is the total volume within each bottle in mL, and 25 is the solution in each bottle in mL. Soil dry weight divided by 2.65, which is the sediment particle density, yields the volume of sediment in the bottle.

5.2.6 Statistical Analyses

Statistical analyses were performed on transformed data using the R statistical program. Analyses of variance (ANOVAs) were performed and then residues were checked for normality. Bartlett's Test for Equal Variance was used to ascertain whether the groups of data shared equal variances. The data met the ANOVA assumption of normality but did not meet the ANOVA assumption of homoscedasticity (equal variances). Data were then log- transformed and ANOVAs were run again. With transformed data, the assumptions of normality and homoscedasticity were met. Transformed data were used to run the ANOVAs and t-tests that are reported in Sections 5.3 and 5.4. All means are reported ±1 standard error.

5.3 Results

5.3.1 Sediment gas measurements in 2014 after the 4- hour incubation

In 2014, sediment that incubated for 4 hours generated CO_2, indicating there was microbial activity in the stream sediment. Urban sediment emissions ranged from 0 – 0.08 µg CO_2-C g^{-1} h^{-1} and rural emissions ranged from 0 – 0.03 µg CO_2-C g^{-1} h^{-1}. Kill Control and Kill Nitrate sediment did not generate CO_2, showing that CO_2 emissions were solely due to biological processes. A two-way ANOVA examined differences between urban/rural sites and treatments (+Nitrate, +Carbon, +Nitrate and Carbon). Carbon dioxide emissions were significantly higher in urban sediment (F value=15.8, p=0.0004) (Appendix A, Table A5.1) (Figure 5.1). The mean urban emissions were

0.03±0.003 µg g^{-1} h^{-1} CO_2-C and mean rural emissions were 0.01±0.002 µg g^{-1} h^{-1} CO_2-C. Treatments did not significantly differ in CO_2 emissions (F value = 1.15, p = 0.343) (Appendix A, Table A5.1). Carbon dioxide was not generated at a rapid pace. Nitrous oxide was generated from only one sediment sample, in the Nitrate + Carbon treatment. It was a very low emission rate of 0.02 ng N_2O-N g^{-1} h^{-1}.

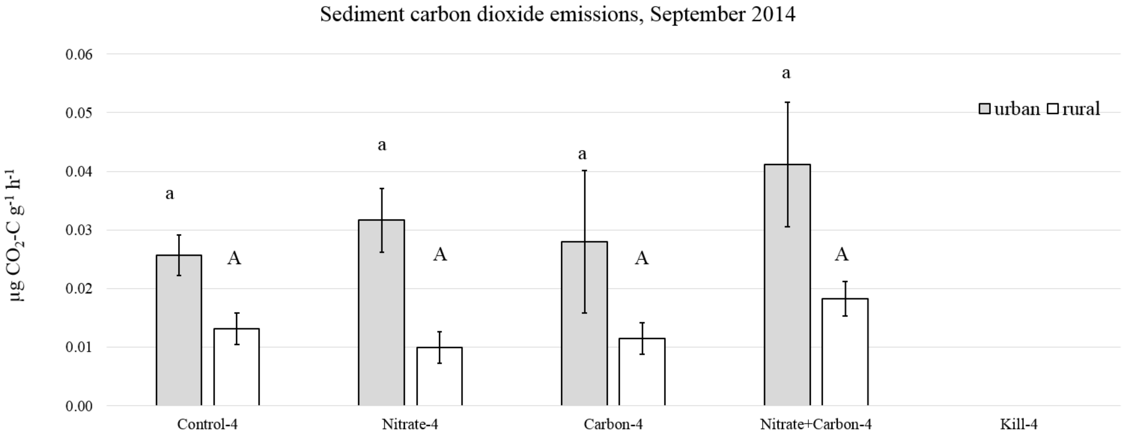


Figure 5.1: Mean CO_2-C emissions ± 1SE after the 4-hour incubation from downstream/urban and upstream/rural stream sediment: Control, Nitrate, Carbon, Nitrate + Carbon, Kill treatments. Means that do not share a common letter signify a significant difference at p=0.05.

5.3.2 Sediment gas measurements in 2015 after the 24- hour incubation

In 2015, urban sediment had higher CO_2 emissions than rural sediment, and treatments with added C had elevated CO_2 emissions. Carbon dioxide emissions were analyzed with a two-way ANOVA, which showed that urban sediment emissions were significantly higher (F value=8.640, p=0.006) and there were also significant differences in CO_2 emissions between treatments (F value=6.203, p=0.002) (Appendix A, Table A5.2) (Figure 5.2). Urban emissions were 0.05±0.01 µg g^{-1} h^{-1} CO_2-C and rural emissions were 0.02±0.003 µg g^{-1} h^{-1} CO_2-C. Urban sediment CO_2 emissions ranged from 0.02-0.25 ug g^{-1} h^{-1} CO_2-C after the 24-hour incubation. The urban Carbon treatment elevated CO_2

emissions to 0.06±0.01 µg g^{-1} h^{-1} CO_2-C, which was 174% of the urban Control (Figure 5.2). The addition of both NO_3^- and organic C to urban sediment caused CO_2 emissions of the Nitrate + Carbon treatments to be 0.11±0.04 µg g^{-1} h^{-1} CO_2-C, or 235% of urban Control. Rural sediment CO_2 ranged from 0.01-0.09 ug g $^{-1}$ h^{-1}. The rural Carbon treatments emitted 0.04±0.01 µg g^{-1} h^{-1} CO_2-C and had 174% of rural Control emissions. The rural Nitrate + Carbon-treated sediment had 0.06±0.01 µg g^{-1} h^{-1} CO_2-C, or 283% of rural Control CO_2 emissions (Figure 5.2). There were no CO_2 emissions from the Control Kill or Nitrate Kill treatments.

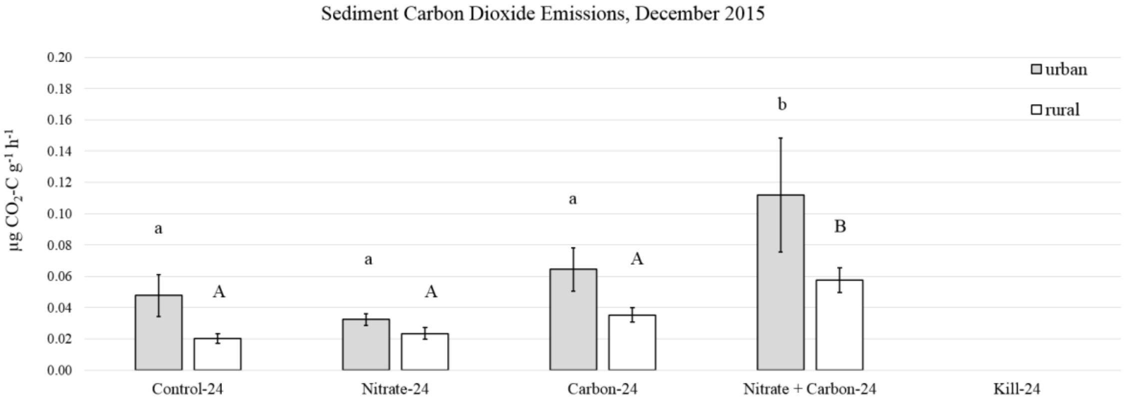


Figure 5.2: Mean CO_2-C emissions ± 1SE after the 24-hour incubation from downstream/urban and upstream/rural stream sediment: Control, Nitrate, Carbon, Nitrate+Carbon, Kill (autoclave) treatments. Means that do not share a common letter signify a significant difference at p=0.05.

In 2015, the addition of NO_3^- stimulated sediment N_2O emissions in all of the Nitrate-treated and Nitrate + Carbon-treated sediment (Figure 5.3). Only 20% of Control and Carbon replicates emitted N_2O, and the emission rates were very low (Figure 5.3). A two-way ANOVA showed that there were significant differences between treatments (F value=7.868, p=0.0004) but not between urban and rural sediment (F value= 0.123, p=0.729) (Appendix A, Table A5.3). Nitrate and Nitrate + Carbon treatments had significantly higher N_2O emissions than Control and Carbon treatments (Figure 5.3). Nitrous oxide emissions didn't differ between Nitrate and Nitrate + Carbon treatments (F value=1.498, p=0.253). Nitrous oxide emissions from NO_3^- - amended treatments ranged from 0.02-1.50 ng g^{-1} h^{-1} N_2O-N, while emissions from treatments without additional N ranged from 0.00-0.02 ng g^{-1} h^{-1} N_2O-N. Nitrate treatments emitted 0.11± 0.05 ng g^{-1} h^{-1} N_2O-N from urban sediment and 0.20 ± 0.20 ng g^{-1} h^{-1} N_2O-N from rural sediment. Nitrate + Carbon treatments of urban and rural sediment emitted 0.50±0.25 ng g^{-1} h^{-1} N_2O-N and 0.33±0.20 ng g^{-1} h^{-1} N_2O-N, respectively.

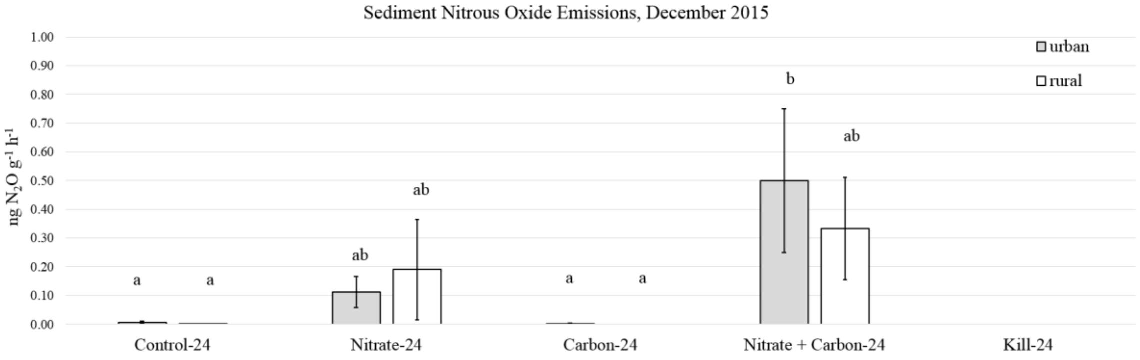


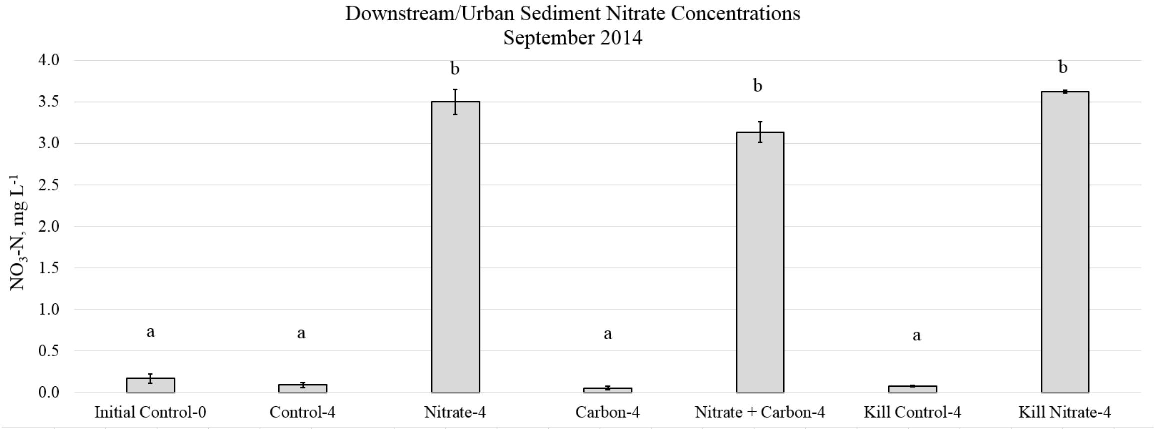
Figure 5.3: Mean N_2O-N ± 1SE emissions from downstream/urban and upstream/rural stream sediment after 24 hour incubation. Means that do not share a common letter signify a significant difference at p=0.05.

5.3.3 Sediment nitrate concentrations in 2014 after the 4-hour incubation

Nitrate was added to the Nitrate, Nitrate + Carbon, and Nitrate Kill treatments in 2014. The sediment was extracted for NO_3^- to see if its concentration was reduced after 4 hours of incubation. A two-way ANOVA showed significant differences between treatments (F value=2346.9, p<0.0001) and higher NO_3^- concentrations in rural sediment (F value= 4.576, p=0.04) (Appendix A, Table A5.4). The mean Kill Nitrate extractable

NO_3^- concentration was 3.62±0.02 mg L^{-1} NO_3-N, and was used as an estimate of the initial concentration due to no biological consumption of NO_3^-. In urban sediment, extractable NO_3^- from Nitrate treatments averaged 3.50±0.15 mg L^{-1} NO_3-N and the Nitrate + Carbon treatments had 3.13±0.13 mg L^{-1} NO_3-N. Extractable NO_3^- in the urban Nitrate, Nitrate + Carbon and Kill Nitrate treatments did not significantly differ (F value= 3.388, p=0.075) (Appendix A, Table A5.5) (Figure 5.4a). In the rural sediment, Nitrate treatment had a mean of 3.57±0.02 mg L^{-1} NO_3-N and the Nitrate + Carbon treatments had a mean of 3.42±0.06 mg L^{-1} NO_3-N. Extractable NO_3^- in the rural Nitrate, Nitrate + Carbon and Kill Nitrate treatments did not significantly differ (F value=0.687, p=0.526) (Appendix A, Table A5.6) (Figure 5.4b).

a.



b.

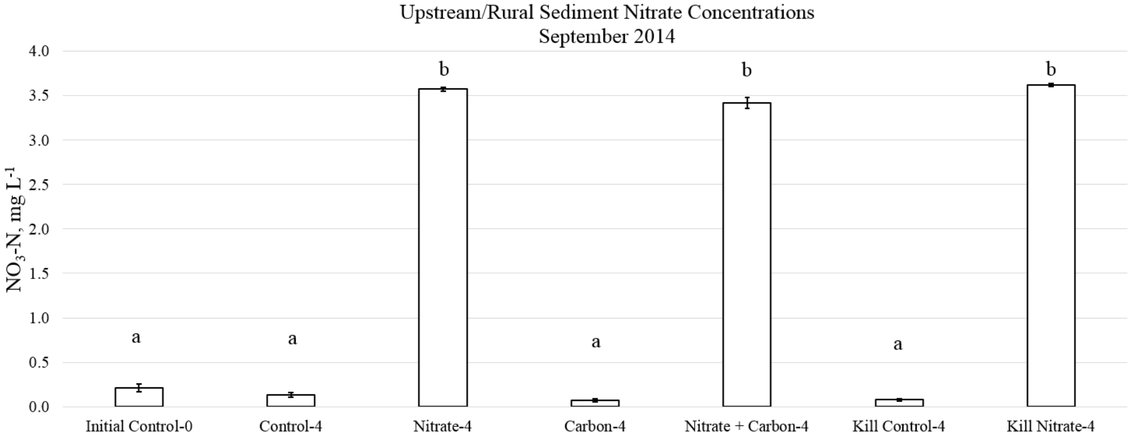


Figure 5.4: Mean extractable NO_3-N ± 1SE after the 4-hour incubation from downstream/urban sediment (a) and upstream/rural sediment (b). Means that do not share a common letter signify a significant difference at $p=0.05$.

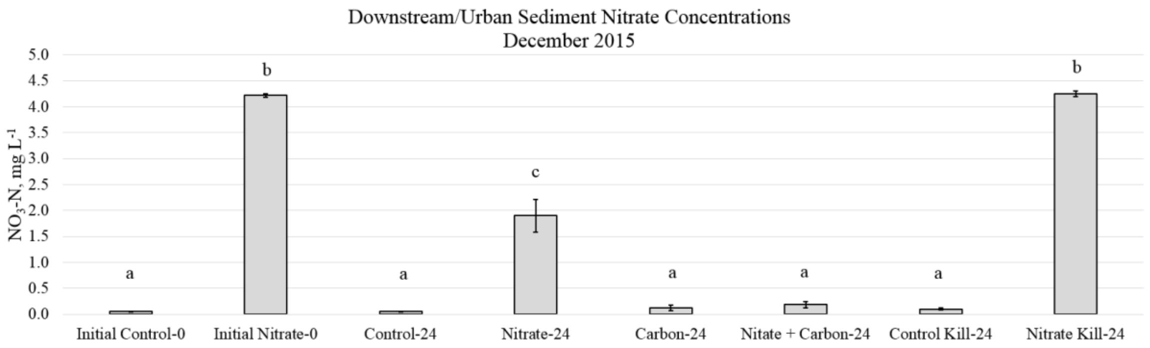
5.3.4 Sediment nitrogen concentrations in 2015 after the 24 hour incubation

Nitrate was considerably reduced in NO_3^- -amended treatments after 24 hours of incubation. Nitrate reduction was large in Nitrate treatments and more extreme in Nitrate + Carbon treatments. Microbial communities in the urban sediment consumed more NO_3^- compared to rural sediment. A two-way ANOVA indicated there were significant differences in NO_3^- concentrations between urban and rural sediment ($F=5.968$, $p=0.02$) and between treatments ($F=131.3$, $p<0.00001$) (Appendix A, Table A5.7).

The Nitrate Control was incorporated into the experimental design in 2015 to obtain a more accurate estimate of initial NO_3^- concentrations at the start of incubation. Losses of NO_3^- from Nitrate and Nitrate + Carbon treatments were compared to the concentrations in the Nitrate Control. The urban Nitrate Control concentration at Time 0 was 4.22±0.03 mg L^{-1} NO_3-N, and did not differ from the Kill Nitrate treatment average of 4.25±0.05 mg L^{-1} NO_3-N (Tukey means comparison, $p=1.00$), which indicates that NO_3^- losses resulted from biological processes. The Nitrate treatments had 1.91±0.31 mg L^{-1} NO_3-N after 24 hours of incubation, which was a 55% decrease from the initial NO_3^- concentration. Nitrate + Carbon treatments had more dramatic NO_3^- reductions after 24 hours of incubation to a mean of 0.18±0.06 mg L^{-1} NO_3-N, which was a 96 % reduction of available NO_3^- (Figure 5.5a). The extractable NO_3^- concentrations from the Nitrate + Carbon treatments at the end of the 24 hour incubation were not significantly different from the Initial Control, Control, Carbon, or Kill Control treatments, which did not receive additional NO_3^- (F=2.02, p=0.13) (Appendix A, Table A5.8) (Figure 5.5a).

The rural Nitrate Control concentration at Time 0 was 4.14±0.03 mg L^{-1} NO_3-N, and did not differ from the Kill Nitrate treatment average of 4.23±0.04 mg L^{-1} NO_3-N (Tukey means comparison, p=0.96), which showed that NO_3^- losses were biological. The extractable NO_3^- from the Nitrate treatments was 2.85±0.30 mg L^{-1} NO_3-N, which indicates consumption of 31% of NO_3^- over the 24-hour incubation. Amendment with both NO_3^- and organic C resulted in a more dramatic 91% reduction of NO_3^-, with 0.39±0.11 mg L^{-1} NO_3-N remaining (Figure 5.5b).

a.



b.

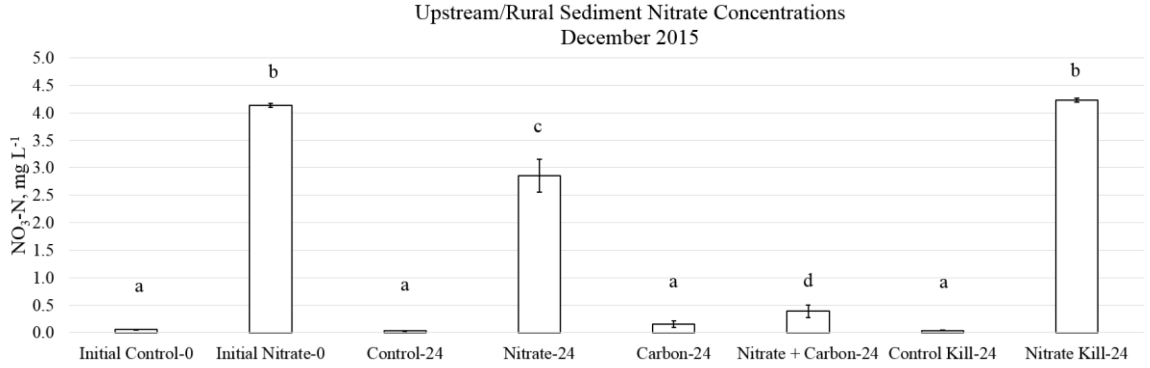


Figure 5.5: Mean extractable NO_3-N ± 1SE after the 24-hour incubation from downstream/urban sediment (a) and upstream/rural sediment (b). Means that do not share a common letter signify a significant difference at p=0.05, according to the Tukey means comparison.

*5.3.5 Sediment organic matte*r

Sediment organic matter (SOM) was low (Figure 5.6). In September 2014 urban SOM ranged from 2.00 to 2.15% and rural SOM ranged from 2.16 to 2.53%. In December 2015 urban SOM ranged from 1.46 to 1.94% and rural SOM ranged from 1.74 to 2.15%. Organic matter was significantly higher in upstream sediment samples in both September 2014 (t statistic=4.30, one-tailed p=0.01) and December 2015 (t statistic=4.50, one-tailed p<0.0001).

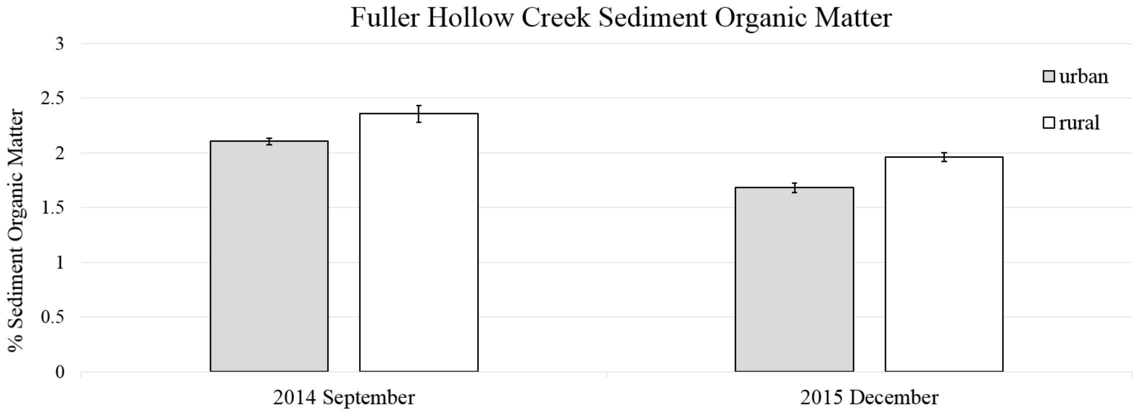


Figure 5.6: Mean percent sediment organic matter, ± SE. Paired t-tests showed that upstream sites had greater percent organic matter compared to downstream in 2014 (t statistic=4.30, one-tailed p=0.01) and 2015 (t statistic=4.50, one-tailed p<0.0001).

5.3.6 Carbon and Nitrogen Budgets

Sediment from each Nitrate, Carbon, Nitrate + Carbon and Control treatment respired CO_2 during the course of the 24-hour incubation. The sediment had organic carbon that was available to the microbial communities, and was estimated to be 98 µg in urban and 114 µg in rural sediments. The urban Control and Nitrate treatments respired 38.1±6.54 µg CO_2-C, which was ~37% of the sediment organic C. Rural Control and Nitrate treatments had emissions of 20.3±2.1 µg CO_2-C, or ~18% of sediment organic C.

Carbon and Nitrate + Carbon treatments received additional organic C, which increased CO_2 emissions in comparison to Control and Nitrate treatments, which relied solely on sediment organic C. Carbon and Nitrate + Carbon treatments were amended with 1250 µg $C_6H_{12}O_6$-C, which augmented the available organic C in the sediment. The total organic C that was available to Carbon and Nitrate + Carbon treatments was approximately 1348 µg C in urban and 1364 µg C in rural sediments. Urban Carbon treatments respired an average of 61.2 ± 13.1 µg CO_2-C, or 161% of Control and Nitrate CO_2 emissions (which weren't amended with C). Urban Nitrate + Carbon treatments, which received amendments of both organic C and NO_3^- had the highest emissions of 106.0 ± 34.0 µg CO_2-C. The urban Nitrate + Carbon treatment CO_2 emissions were 278% of Control and Nitrate emissions. Urban Carbon treatments respired ~5% and Nitrate + Carbon treatments respired ~8% of available organic C.

Rural Carbon treatments respired 32.8 ± 4.2 µg CO_2-C, which were 162% of Control and Nitrate emissions. Nitrate + Carbon treatments had higher emissions of 53.7 ± 7.25 µg CO_2-C, or 265% of respiration from Control and Nitrate treatments. Rural Carbon treatments respired ~2% and Nitrate + Carbon treatments respired ~4% of available organic C.

Because CO_2 respiration was higher in Carbon and Nitrate + Carbon treatments, this implies growth of the microbial community. We did not measure bacterial growth efficiency (BGE), which is the amount of new bacterial biomass produced per unit of organic C substrate assimilated (del Grigorio and Cole 1998). However del Grigorio and Cole (1998) describe that growth can be estimated by the equation

$$\mathrm{BGE} = \frac{\mathrm{BP}}{\mathrm{BP} + \mathrm{BR}}$$

Where BR is bacterial respiration and BP is new bacterial biomass. If we assume BGE is 50%, then the urban Carbon treatments would have assimilated approximately 60 µg CO_2-C and the Nitrate + Carbon treatments would have assimilated about 106 µg CO_2-C more in microbial biomass. Rural Carbon treatments would have assimilated approximately 33 µg CO_2-C and Nitrate + Carbon treatments would have assimilated approximately 51 µg CO_2-C into microbial biomass. The majority of additional organic C probably remained in the water column at the end of the experiment, because we added more than the microbial community needed to be sustained over the 24-hour incubation.

The supplement of 125 µg NO_3^- stimulated N_2O emissions in Nitrate and Nitrate + Carbon treatments, and N_2O emissions were coupled with reduction of NO_3^- in the water column. Available NO_3^- to Nitrate and Nitrate + Carbon treatments included the amendment of 125 µg NO_3^- and NO_3^- that was in the stream sediment. The urban Nitrate Control had approximately 152 µg NO_3^- and rural Nitrate Control had 149 µg NO_3^-, from available NO_3^- in stream sediment and the NO_3^- amendment. We used these as estimates of the available NO_3^- to Nitrate and Nitrate + Carbon treatments at the beginning of the experiment.

Urban Nitrate treatments had total N_2O emissions of 0.12±0.05 µg N_2O-N and 68 µg NO_3^- remaining after the 24-hour incubation. This was a loss of 55% of the available NO_3^-. Nitrate + Carbon treated urban sediment had emissions of 0.47±0.23 µg N_2O-N and 7 µg NO_3^- remaining after incubation, which was a 96% loss of NO_3^-. Urban N_2O emissions accounted for only 0.1% and 0.3% of available NO_3^- from Nitrate and Nitrate + Carbon treatments, respectively. Rural Nitrate treatments emitted 0.18±0.07 µg N_2O-N from Nitrate treatments and had 103 µg NO_3^- remaining after incubation for 24 hours. Rural Nitrate + Carbon treatments emitted 0.31±0.08 µg N_2O-N and had 14 µg NO_3^- remaining, which was a 91% decrease of available NO_3^-. Nitrous oxide emissions from rural sediment accounted for 0.1% and 0.2% of available NO_3^- from Nitrate and Nitrate + Carbon treatments, respectively.

We measured N_2O emissions directly, but not the final product of denitrification, which is N_2. Groffman et al. (2000) found that riparian denitrification resulted in a 50:1

ratio of N_2:N_2O, although this ratio can vary substantially (Groffman et al. 2000). If we apply this ratio to our system, then denitrification would account for 4% and 16% of the added NO_3^- to urban Nitrate and Nitrate + Carbon treatments, respectively. If we assume the same ratio for rural sediment, then we can estimate denitrification in Nitrate and Nitrate + Carbon treatments removed 6% and 10% of NO_3^-, respectively. Since we do not have direct measurements of microbial biomass N (MBN), we estimated the NO_3^- that was taken up by the microbial community. Microbial biomass C:N ratios vary but have a mean of about 10:1 (Chapin et al. 2002). We used the estimated microbial biomass C above, and the C:N ratio of 10:1 to estimate NO_3^- uptake by the microbial communities. Urban sediment Control, Nitrate, Carbon, and Nitrate + Carbon treatments had 3%, 2%, 7% and 7% uptake of available NO_3^- to MBN, respectively. Rural sediment Control, Nitrate, Carbon and Nitrate + Carbon treatments had 1%, 2%, 2%, and 3% uptake of available NO_3^- to MBN, respectively. Even though these are rough estimates, we still can't account for the majority of the NO_3^- that was added to Nitrate and Nitrate + Carbon treatments. We hypothesize that the 50:1 ratio of N_2:N_2O release via denitrification is a significant underestimation of N_2 release in this system. Therefore we hypothesize that the majority of the consumed NO_3^- amendments were released as N_2.

5.4 Discussion

5.4.1 Sediment Microbial Activity

We measured sediment microbial activity in the lab, which didn't account for diurnal differences in temperature, light, seasonal changes, and didn't simulate stream flow or mixing of surface and groundwater. Microbial respiration and denitrification rates increase with rising temperatures, and our gas measurements may have been elevated in the lab setting where the ambient room temperature is around 21 C (Atlas and Bartha, Pattinson et al. 1998). Our gas measurements may be higher than measurements in situ, since stream water and sediment temperatures were probably considerably lower. However our experimental treatments have elicited information that can help us better understand NO_3^- and C processing in urban and rural stream sediment.

Urban sediment microbial activity was higher than rural in both 2014 and 2015 (Figures 5.1 and 5.2), which is the opposite of what we expected. Higher urban microbial activity was surprising for two reasons. First, percent SOM was greater in rural sediment, so its microbial community could have been more robust at the start of the experiment. Second, we thought impacts of urbanization would negatively affect the urban microbial community. For example, deicing road salt (often NaCl) gets into urban groundwater and streams, and NaCl has been shown to negatively affect soil microbial communities (Chapter 2, Kelly et al. 2008, McCormack and Wolfe 1980). In the urban portion of Fuller Hollow Creek, near where we collected sediment, we have consistently measured higher Cl^- concentrations (Chapter 3). Since there is more microbial activity in the urban sediment, it is possible that the urban microbial communities have evolved resistance to potentially detrimental urban stream pollutants, like NaCl. Another reason for higher urban microbial activity could involve stream water dissolved organic carbon (DOC) concentrations. Higher DOC in urban stream water would help explain the higher baseline urban sediment microbial activity, but we do not have data on stream DOC concentrations.

Emissions of CO_2 from stream sediment were enhanced by amendment with labile C, which is consistent with our prediction that microbial communities would be C-limited. The addition of organic C to urban Carbon treatments resulted in 174% of the CO_2 emissions of urban Control sediments, and rural Carbon treatments also had 174% of the CO_2 emissions from rural Control sediments. Findlay et al. (1993) found similarly positive impacts of increased DOC availability on hyporheic bacteria in Wappinger Creek, which is a stream also located in Upstate New York. Findlay and colleagues (1993) measured greatest bacterial abundance when stream water DOC was highest, and concluded that DOC could be a more important factor than sediment organic C when predicting the size and function of hyporheic microbial communities. We did not measure microbial abundance directly, but we did see clear increases in microbial CO_2 emissions with additional organic C, which may have resulted in part from greater bacterial abundance.

We found that sediment microbial CO_2 emissions are ultimately limited by both NO_3^- and organic C availability. The Nitrate + Carbon treatments were amended with both nutrients which stimulated CO_2 emissions that were substantially greater than Carbon treatments, which only received organic C. Urban Nitrate + Carbon treatments had CO_2 emissions that were 235% of Control emissions and rural Nitrate + Carbon treatments had CO_2 emissions that were 283% of Control emissions. These results are consistent with our hypothesis that both NO_3^- and organic C would be limiting factors to microbial activity, since N and C can be limiting nutrients in headwater streams. Although we have consistently measured higher stream NO_3^- concentrations at the urban stream site, they are not as high as in larger urban areas and the stream system may still be N-limited (Chapter 3, Groffman et al. 2004, Shields et al. 2008).

5.4.2 Sediment Nitrogen Dynamics

The 2014 experiment had a 4-hour incubation, and amendment with NO_3^- only yielded one N_2O measurement. When we extended the incubation time to 24 hours in 2015, we measured N_2O emissions from each Nitrate and Nitrate + Carbon treatment. This result suggests that the sediment microbial community needed more than 4 hours to adjust to the lab conditions, treatment(s), or both.

Nitrate availability is the main limiting factor to N_2O emissions in this system. Nitrate and Nitrate + Carbon treatments were amended with NO_3^-, which stimulated N_2O emissions, while addition of organic C alone did not (Figure 5.3). The Carbon and Control sediments emitted N_2O only rarely and in smaller concentrations. The urban Nitrate treatments had 16x the N_2O emissions of the urban Controls, while the rural Nitrate N_2O emissions were almost 1000x the emissions of rural Control sediment. As we predicted, adding both NO_3^- and organic C resulted in even higher N_2O emissions, indicating that while denitrifying microorganisms in this system are primarily N-limited, the process is further enhanced by amendment with organic C. Urban and rural Nitrate + Carbon treatments had 4x and 2x the N_2O emissions of urban and rural Nitrate treatments, respectively. These results are not surprising, since the precursors to denitrification are NO_3^- and organic C (Chapin et al. 2002). There has been some concern regarding the contribution of N_2O from denitrification to the atmosphere, but a recent meta-analysis by Beaulieu et al. (2011) estimated that N_2O was less than 1% of denitrified N from streams and rivers in North America. The N_2O emissions we measured were a likely a very low proportion of denitrification, and accounted for less than 1% of the available NO_3^- at the start of the experiment. This finding is good, because N_2O is a potent greenhouse gas (Beaulieu et al. 2011, Groffman et al. 2000).

Our findings of N and C limitation in stream sediment are similar to published research on N_2O emissions and denitrification from stream sediment. Arango and Tank (2008) found that mean stream sediment denitrification rates were positively correlated with available NO_3^- and C in stream water. Harrison and Matson (2003) measured N_2O emissions from sediment in streams that drain from agricultural fields. They found that higher stream water NO_3^- from agricultural runoff resulted in high N_2O and N_2 emissions (Harrison and Matson 2003). When they measured sediment emissions in the lab, they found that adding organic C in the presence of high NO_3^- concentrations increased N_2O by an order of magnitude more than just adding NO_3^- (Harrison and Matson 2003).

Stream nutrient concentrations are not static, and can vary seasonally and after storm events. For example, Arango and Tank (2008) found strong seasonal controls on denitrification in hyporheic stream sediment. They found the highest rates of denitrification in the winter when stream water NO_3^- was highest (Arango and Tank 2008). We have measured seasonal changes in NO_3^- concentrations in Fuller Hollow Creek, with high concentrations in summer and winter (Chapter 3). While we didn't perform this experiment in situ or during all seasons, it is possible that denitrification rates could be higher in the summer and winter, when NO_3^- availability is higher in Fuller Hollow Creek (Chapter 3). Additionally pulses of nutrients to urban streams often occur during and shortly after storm events (Arango and Tank 2008, Hook and Yeakley 2005, Paul and Meyer 2001). Arango and Tank (2008) found that after storm events, pulses of stream NO_3^- were linked to peaks in denitrification in stream sediment. Urban streams can also receive pulses of C after storms, from leaf litter and grass clippings that discharge from storm water infrastructure to streams (Kaushal and Belt 2012). Our additions of high concentrations of NO_3^- and organic C are somewhat analogous to nutrient pulses to streams. So, there may be opportunities for enhanced microbial activity and denitrification in Fuller Hollow Creek sediment in different seasons, and after storm events.

The N_2O emissions we measured from Nitrate and Nitrate + Carbon treatments were coupled with lower extractable NO_3^- after the 24-hour incubation. Nitrate treatments in urban and rural sediments had 55% and 31% less available NO_3^-, respectively. In Nitrate + Carbon treatments, amendment with both NO_3^- and organic C resulted in greater N_2O emissions, and drastic reductions in extractable NO_3^-. Nitrate amendments were reduced by 96% in urban and by 91% in rural sediments in Nitrate + Carbon treatments. This made the final NO_3^- concentrations of the Nitrate + Carbon treatments comparable to the Control, Carbon and Control Kill treatments. In urban Nitrate and Nitrate + Carbon treatments, NO_3^- reduction was greater and coupled to larger N_2O emissions in comparison to the corresponding rural treatments, which further exemplifies the more robust urban sediment microbial community.

5.4.3 Implications for Urban Stream Restoration and Conclusions

As part of stream management and restoration efforts, a great deal of research and money has been applied to better understand N dynamics in urban areas and promote denitrification of elevated NO_3^- in urban streams, riparian areas, and wetlands. While our laboratory measurements of CO_2, N_2O emissions and NO_3^- processing may have been elevated above in situ measurements, we have interesting information on N processing in the hyporheic sediment of Fuller Hollow Creek. We have shown that both urban and rural sediment microbial communities processed amendments of NO_3^- and organic C, but there are other factors that impact denitrification from stream sediment. As previously discussed, stream NO_3^- concentrations vary between seasons and can be high after storm events. We have shown that the sediment microbial communities have the potential to remove a large portion of additional NO_3^- , particularly in the presence of organic C. Organic C availability can be promoted by maintaining leaf debris and debris dams to promote C availability. Increasing organic C availability could aid the process of NO_3^- removal by microbial denitrification, preventing it from traveling downstream. Management for more available organic C could be particularly important in the more urban portion of the watershed for three reasons. One reason is that our data show that the urban sediment community is more effective at removing NO_3^- from the water column; a second reason is that NO_3^- concentrations are consistently higher in the urban portion of the stream; and a third reason is that SOM is low (Chapter 3).

Urban stream and riparian restoration has had some success at reducing nutrient loads to waters downstream, especially when urban stream hydrological conditions are improved (Kaushal et al. 2008b, Sivirchi et al. 2011). Research on nutrient dynamics in restored streams suggests that the rate of stream flow is an important factor contributing to nutrient processing within a given stream (Kaushal et al. 2008b). For example, Kaushal et al. (2008) found that slower stream flows and longer residence times promote NO_3^- removal by denitrification in restored streams. However a common problem is urban stream incision, which increases discharges during storm events and decreases retention time (Paul and Meyer 2001). Streams that are less damaged by incision tend to have slower stream flows, providing time for NO_3^- removal (Groffman et al. 2002, Kaushal et al. 2008b). Urban portions of Fuller Hollow Creek have become incised as a result of urbanization and if this causes short stream water residence times, denitrification could be inhibited following storm events, even if stream NO_3^- and organic C are high. In the future the stream should be managed for higher available organic C, reduced incision and longer water residence times to promote NO_3^- removal by denitrification, to reduce NO_3^- loading to downstream water systems.

Appendix A: Statistical Tables

Statistical Tables for: Chapter 2—Impacts of Deicing Salt and Nitrogen Additions on Nitrogen and Carbon Cycling in a Roadside Ecosystem.

Table A2.1: Two-way ANOVA comparing differences in nitrification between treatments and transects in 0-5cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.263	0.132	0.557	0.579
Transect	1	0.091	0.091	0.383	0.541
Treatment x Transect	2	0.100	0.050	0.211	0.811
Residuals	30	7.099	0.237		

Table A2.2: Two-way ANOVA comparing differences in net mineralization between treatments and transects in 0-5cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.651	0.326	1.495	0.241
Transect	1	0.297	0.297	1.364	0.252
Treatment x Transect	2	0.164	0.082	0.377	0.689
Residuals	30	6.535	0.218		

Table A2.3: Two-way ANOVA comparing differences in nitrification between treatments and transects in 0-5cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.263	0.132	0.663	0.523
Transect	1	0.030	0.030	0.149	0.702
Treatment x Transect	2	0.007	0.003	0.016	0.984
Residuals	30	5.956	0.199		

Table A2.4: Two-way ANOVA comparing differences in net mineralization between treatments and transects in 0-5cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.183	0.091	0.458	0.637
Transect	1	0.009	0.009	0.044	0.834
Treatment x Transect	2	0.002	0.001	0.004	0.996
Residuals	30	5.992	0.200		

Table A2.5: Two-way ANOVA comparing differences in nitrification between treatments and transects in 0-5cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	1.273	0.636	1.027	0.373
Transect	1	7.257	7.257	11.711	0.002
Treatment x Transect	2	1.847	0.923	1.490	0.246
Residuals	30	14.872	0.620		

Table A2.6: Two-way ANOVA comparing differences in net mineralization between treatments and transects in 0-5cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	1.049	0.524	0.905	0.418
Transect	1	7.199	7.199	12.425	0.002
Treatment x Transect	2	1.467	0.733	1.266	0.300
Residuals	30	13.905	0.579		

Table A2.7: Two-way ANOVA comparing differences in nitrification between treatments and transects in 5-15cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.002	0.001	0.049	0.952
Transect	1	0.002	0.002	0.097	0.758
Treatment x Transect	2	0.022	0.011	0.542	0.587
Residuals	30				

Table A2.8: Two-way ANOVA comparing differences in net mineralization between treatments and transects in 5-15cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.005	0.003	0.155	0.857
Transect	1	0.009	0.009	0.529	0.473
Treatment x Transect	2	0.044	0.022	1.343	0.276
Residuals	30	0.494	0.017		

Table A2.9: Two-way ANOVA comparing differences in nitrification between treatments and transects in 5-15cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.049	0.024	1.057	0.360
Transect	1	0.024	0.024	1.344	0.278
Treatment x Transect	2	0.008	0.004	0.182	0.835
Residuals	30	0.694	0.023		

Table A2.10: Two-way ANOVA comparing differences in net mineralization between treatments and transects in 5-15cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.031	0.015	0.744	0.484
Transect	1	0.065	0.065	3.191	0.084
Treatment x Transect	2	0.004	0.002	0.102	0.903
Residuals	30	0.614	0.021		

Table A2.11: Two-way ANOVA comparing differences in nitrification between treatments and transects in 5-15cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.092	0.046	2.761	0.083
Transect	1	0.010	0.010	0.582	0.453
Treatment x Transect	2	0.003	0.002	0.095	0.910
Residuals	30	0.400	0.017		

Table A2.12: Two-way ANOVA comparing differences in net mineralization between treatments and transects in 5-15cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.076	0.0377	2.259	0.126
Transect	1	0.072	0.072	4.317	0.054
Treatment x Transect	2	0.006	0.003	0.192	0.827
Residuals	30	0.401	0.017		

Table A2.13: Two-way ANOVA comparing differences in NH_4^+ between treatments and transects in 0-5cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	71.0	35.49	3.109	0.059
Transect	1	12.6	12.65	1.108	0.301
Treatment x Transect	2	68.7	34.33	3.007	0.065
Residuals	30	342.5	11.42		

Table A2.14: Two-way ANOVA comparing differences in NO_3^- between treatments and transects in 0-5cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	14.14	7.072	1.324	0.281
Transect	1	8.98	8.984	1.682	0.205
Treatment x Transect	2	26.62	13.310	2.492	0.100
Residuals	30	160.26	5.342		

Table A2.15: Two-way ANOVA comparing differences in NH_4^+ between treatments and transects in 0-5cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.27	0.136	0.016	0.984
Transect	1	5.11	5.110	0.605	0.443
Treatment x Transect	2	1.56	0.782	0.093	0.912
Residuals	30	253.36	8.445		

Table A2.16: Two-way ANOVA comparing differences in NO_3^- between treatments and transects in 0-5cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	4.89	2.446	0.722	0.49
Transect	1	1.43	1.427	0.421	0.52
Treatment x Transect	2	6.57	3.283	0.969	0.39
Residuals	30	101.69	3.390		

Table A2.17: Two-way ANOVA comparing differences in NH_4^+ between treatments and transects in 0-5cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	13.15	6.574	0.904	0.418
Transect	1	0.30	0.303	0.042	0.840
Treatment x Transect	2	35.96	17.979	2.472	0.106
Residuals	24	174.59	7.274		

Table A2.18: Two-way ANOVA comparing differences in NO_3^- between treatments and transects in 0-5cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	950	475.1	1.416	0.262
Transect	1	1489	1489.1	4.439	0.044
Treatment x Transect	2	743	371.6	1.108	0.347
Residuals	24	8051	335.5		

Table A2.19: Two-way ANOVA comparing differences in NH_4^+ between treatments and transects in 5-15cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	1.30	0.652	0.484	0.621
Transect	1	0.62	0.619	0.460	0.503
Treatment x Transect	2	1.53	0.763	0.567	0.573
Residuals	30	40.38	1.346		

Table A2.20: Two-way ANOVA comparing differences in NO_3^- between treatments and transects in 5-15cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	4.66	2.330	1.387	0.265
Transect	1	3.01	3.009	1.790	0.191
Treatment x Transect	2	3.03	1.515	0.902	0.417
Residuals	30	50.41	1.680		

Table A2.21: Two-way ANOVA comparing differences in NH_4^+ between treatments and transects in 5-15cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	2.642	1.321	1.282	0.293
Transect	1	2.540	2.540	2.258	0.235
Treatment x Transect	2	4.014	2.007	1.947	0.160
Residuals	30	30.915	1.030		

Table A2.22: Two-way ANOVA comparing differences in NO_3^- between treatments and transects in 5-15cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	2.16	1.079	0.520	0.600
Transect	1	0.10	0.097	0.047	0.831
Treatment x Transect	2	0.86	0.428	0.206	0.815
Residuals	30	62.31	2.077		

Table A2.23: Two-way ANOVA comparing differences in NH_4^+ between treatments and transects in 5-15cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.632	0.316	0.290	0.751
Transect	1	3.450	3.450	3.161	0.088
Treatment x Transect	2	0.088	0.044	0.041	0.960
Residuals	24	26.18	1.091		

Table A2.24: Two-way ANOVA comparing differences in NO_3^- between treatments and transects in 5-15cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	101.1	50.53	0.651	0.531
Transect	1	21.7	21.74	0.280	0.602
Treatment x Transect	2	189.4	94.71	1.219	0.313
Residuals	24	1864.1	77.67		

Table A2.25: Two-way ANOVA comparing differences in C mineralization between treatments and transects in July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	10.04	5.021	19.296	<0.00001
Transect	1	1.17	1.173	4.506	0.03
Treatment x Transect	2	1.68	0.840	3.227	0.04
Residuals	174	45.28	0.260		

Table A2.26 Two-way ANOVA comparing differences in C mineralization between treatments and transects in November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	7.34	3.670	12.887	<0.00001
Transect	1	1.15	1.149	4.034	0.04
Treatment x Transect	2	1.31	0.657	2.305	0.10
Residuals	138	39.30	0.285		

Table A2.27: One-way ANOVA comparing differences in situ soil respiration between treatments in 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	2.698e+08	134875460	8.437	0.0004
Residuals	105	1.679e+09	15986082		

Table A2.28: Two-way ANOVA comparing differences in 0-5cm soil conductivities between treatments and transects in July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	50651925	25325963	274.635	<0.00001
Transect	1	280370	280370	3.040	0.0915
Treatment x Transect	2	9871	4936	0.054	0.9480
Residuals	30	2766506	92217		

Table A2.29: Two-way ANOVA comparing differences in 0-5cm soil conductivities between treatments and transects in November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	1381908	690954	14.259	<0.00001
Transect	1	22500	22500	0.464	0.501
Treatment x Transect	2	41508	20754	0.428	0.656
Residuals	30	1453762	48459		

Table A2.30: Two-way ANOVA comparing differences in 0-5cm soil conductivities between treatments and transects in June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	3571354	1785677	19.95	<0.00001
Transect	1	987905	987905	11.04	0.003
Treatment x Transect	2	116418	58209	0.65	0.531
Residuals	24	2148015	89501		

Table A2.31: Two-way ANOVA comparing differences in 5-15cm soil conductivities between treatments and transects in July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	2987758	1493879	20.655	<0.00001
Transect	1	237331	237331	3.281	0.080
Treatment x Transect	2	259698	129849	1.795	0.184
Residuals	30	2169792	72326		

Table A2.32: Two-way ANOVA comparing differences in 5-15cm soil conductivities between treatments and transects in November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	385225	192613	12.19	0.0001
Transect	1	11130	11130	0.704	0.408
Treatment x Transect	2	53151	26576	1.682	0.203
Residuals	30	474021	15801		

Table A2.33: Two-way ANOVA comparing differences in 5-15cm soil conductivities between treatments and transects in June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	43767	21884	4.359	0.024
Transect	1	66223	66223	13.190	0.001
Treatment x Transect	2	579	290	0.058	0.944
Residuals	24	120501	5021		

Table A2.34: Two-way ANOVA comparing differences in soil pH between treatments and transect in 0-5cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	1326	663.0	1.048	0.363
Transect	1	461	460.7	0.729	0.400
Treatment x Transect	2	1206	602.9	0.953	0.397
Residuals	30	18970	632.3		

Table A2.35: Two-way ANOVA comparing differences in soil pH between treatments and transect in 0-5cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.832	0.416	1.088	0.350
Transect	1	0.611	0.611	1.598	0.216
Treatment x Transect	2	0.102	0.051	0.133	0.876
Residuals	30	11.468	0.382		

Table A2.36: Two-way ANOVA comparing differences in soil pH between treatments and transect in 0-5cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.515	0.258	2.450	0.108
Transect	1	6.807	6.807	64.719	<0.00001
Treatment x Transect	2	0.042	0.021	0.199	0.821
Residuals	24	2.524	0.105		

Table A2.37: Two-way ANOVA comparing differences in soil pH between treatments and transect in 5-15cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	1.097	0.549	3.478	0.044
Transect	1	10.102	10.102	64.055	<0.00001
Treatment x Transect	2	0.339	0.169	1.075	0.354
Residuals	30	4.731	0.158		

Table A2.38: Two-way ANOVA comparing differences in soil pH between treatments and transect in 5-15cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.529	0.265	1.594	0.220
Transect	1	10.802	10.802	65.079	<0.00001
Treatment x Transect	2	0.051	0.025	0.153	0.859
Residuals	30	4.980	0.166		

Table A2.39: Two-way ANOVA comparing differences in soil pH between treatments and transect in 5-15cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.219	0.109	1.024	0.374
Transect	1	8.495	8.495	79.608	<0.00001
Treatment x Transect	2	0.044	0.022	0.208	0.814
Residuals	24	2.561	0.107		

Table A2.40: Two-way ANOVA comparing differences in soil moisture between treatments and transect in 0-5cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	56.2	28.09	2.017	0.151
Transect	1	280.8	280.78	15.215	0.001
Treatment x Transect	2	9.1	4.56	0.247	0.783
Residuals	30	553.6	18.45		

Table A2.41: Two-way ANOVA comparing differences in soil moisture between treatments and transect in 0-5cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	180	90.2	0.850	0.438
Transect	1	695	695.4	6.552	0.016
Treatment x Transect	2	138	69.1	0.651	0.529
Residuals	30	3184	106.1		

Table A2.42: Two-way ANOVA comparing differences in soil moisture between treatments and transect in 0-5cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	38.4	19.2	0.467	0.633
Transect	1	659.6	659.6	16.02	0.001
Treatment x Transect	2	68.2	34.1	0.829	0.449
Residuals	24	987.9	41.2		

Table A2.43: Two-way ANOVA comparing differences in soil moisture between treatments and transect in 5-15cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	280.8	140.38	10.082	0.0005
Transect	1	26.5	26.47	1.901	0.178
Treatment x Transect	2	56.2	28.09	2.017	0.151
Residuals	30	417.7	13.92		

Table A2.44: Two-way ANOVA comparing differences in soil moisture between treatments and transect in 5-15cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	63.3	31.64	1.870	0.172
Transect	1	3.3	3.33	0.197	0.660
Treatment x Transect	2	5.6	2.81	0.166	0.848
Residuals	30	507.6	16.92		

Table A2.45: Two-way ANOVA comparing differences in soil moisture between treatments and transect in 5-15cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	6.0	3.003	0.182	0.834
Transect	1	25.8	25.817	1.569	0.222
Treatment x Transect	2	12.7	6.373	0.387	0.683
Residuals	24	395.0	16.457		

Table A2.46: Two-way ANOVA comparing differences in soil organic matter between treatments and transect in 0-5cm soils from July 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	1.190	0.595	1.113	0.3418
Transect	1	7.918	7.918	14.809	0.0006
Treatment x Transect	2	0.861	0.430	0.805	0.457
Residuals	30	16.040	0.535		

Table A2.47: Two-way ANOVA comparing differences in soil organic matter between treatments and transect in 0-5cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	2.750	1.375	1.417	0.258
Transect	1	7.386	7.386	7.611	0.010
Treatment x Transect	2	3.616	1.808	1.863	0.173
Residuals	30	29.113	0.970		

Table A2.48: Two-way ANOVA comparing differences in soil organic matter between treatments and transect in 0-5cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	3.825	1.912	2.947	0.072
Transect	1	13.709	13.709	21.129	0.0001
Treatment x Transect	2	1.521	0.760	1.172	0.327
Residuals	24	15.572	0.649		

Table A2.49: Two-way ANOVA comparing differences in soil organic matter between treatments and transect in 5-15cm soils from July 2011.

	Df	*SS*	*MS*	*F*	*p*
Treatment	2	7.75	3.877	0.879	0.426
Transect	1	5.51	5.509	1.249	0.273
Treatment x Transect	2	14.38	7.192	1.631	0.213
Residuals	30	132.31	4.410		

Table A2.50: Two-way ANOVA comparing differences in soil organic matter between treatments and transect in 5-15cm soils from November 2011.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.952	0.4761	1.550	0.229
Transect	1	0.077	0.0769	0.250	0.621
Treatment x Transect	2	0.732	0.3659	1.191	0.318
Residuals	30	9.218	0.3073		

Table A2.51: Two-way ANOVA comparing differences in soil organic matter between treatments and transect in 5-15cm soils from June 2012.

	df	*SS*	*MS*	*F*	*p*
Treatment	2	0.024	0.012	0.029	0.971
Transect	1	0.254	0.254	0.628	0.436
Treatment x Transect	2	0.279	0.139	0.344	0.712
Residuals	24	9.711	0.405		

Statistical Tables for: Chapter 3— Inorganic Nitrogen Discharged to the Susquehanna River from Eight Urban Streams of Binghamton, NY, a Medium-Sized City

Table A3.1: Nitrate, Downstream and Upstream Analysis

Paired two-sample t-test results comparing downstream/urban and upstream/rural NO_3-N concentrations. In the cases of streams with an "n" of less than 24, there were fewer samples because of dry conditions during one or more of the summer samplings.

Nitrate

Stream	**t statistic**	**p value, one-tailed**	
Patterson	7.35	< 0.0001	(n=22)
Willow Run	3.78	0.001	(n=17)
Little Choconut	6.76	< 0.0001	(n=23)
Fuller Hollow	5.66	< 0.0001	(n=24)
Pierce	1.01	0.16	(n=24)
Nanticoke	1.28	0.11	(n=24)
Choconut	5.56	<0.0001	(n=24)
Tracey	1.28	0.12	(n=21)

Table A3.2: Downstream/urban Nitrate Analysis Between Streams

Results of a one-way ANOVA analyzing the differences in stream water NO_3-N concentrations between the eight streams.

	df	*SS*	*MS*	*F*	*p*
Stream	7	2.82	0.40	13.64	<0.00001
Residuals	261	7.55	0.03		

Table A3.3: Downstream/urban Nitrate Analysis Between Seasons

Results of a one-way ANOVA analyzing the differences in stream water NO_3-N concentrations between seasons.

	df	*SS*	*MS*	*F*	*p*
Stream	6	1.53	0.25	7.41	<0.00001
Residuals	262	8.84	0.03		

Table A3.4: Analysis of downstream/urban and upstream/rural conductivity and pH.

Paired two-sample t-test results comparing downstream and upstream conductivity (µS/cm) and pH.

Stream	**Conductivity**		**pH**		**n**
	t statistic	**p value, one-tailed**	**t statistic**	**p value, one-tailed**	
Patterson	10.44	< 0.001	6.90	< 0.001	22
Willow Run	11.58	< 0.001	2.97	0.005	17
Fuller Hollow	2.20	0.02	3.31	0.001	24
Little Choconut	7.30	< 0.001	3.76	< 0.001	23
Pierce	5.66	< 0.001	6.88	< 0.001	24
Choconut	7.81	< 0.001	7.04	< 0.001	24
Nanticoke	7.18	< 0.001	3.62	0.001	24
Tracey	9.63	< 0.001	5.33	< 0.001	21

Table A3.5: Downstream/urban Conductivity Analysis Between Streams

Results of a one-way ANOVA analyzing the differences in stream water conductivities of the eight streams.

	df	*SS*	*MS*	*F*	*p*
Stream	7	10240079	1462868	10.47	<0.00001
Residuals	261	36450537	139657		

Table A3.6: Downstream/urban Conductivity Analysis Between Seasons

Results of a one-way ANOVA analyzing the differences in stream water conductivities between seasons.

	df	*SS*	*MS*	*F*	*p*
Stream	6	4013269	668878	4.12	0.0005
Residuals	262	42677346	162891		

Table A3.7: Downstream/urban pH Analysis Between Streams

Results of a one-way ANOVA analyzing the differences in stream water pH between streams.

	df	*SS*	*MS*	*F*	*p*
Stream	7	11.79	1.68	10.26	<0.00001
Residuals	261	42.83	0.16		

Table A3.8: Downstream/urban pH Analysis Between Seasons

Results of a one-way ANOVA analyzing the differences in stream water pH between seasons.

	df	*SS*	*MS*	*F*	*p*
Stream	6	9.14	1.52	8.78	<0.00001
Residuals	262	45.47	0.17		

Table A3.9: Chloride, Downstream and Upstream Analysis

Paired two-sample t-test results comparing downstream/urban and upstream/rural NO_3-N concentrations. In the case of Willow Run with an "n" of less than 7, there were fewer samples because of dry conditions during one or more of the summer samplings.

Chloride		
Stream	**t statistic**	**p value, one-tailed**
Patterson	3.53	0.006 (n=7)
Willow Run	2.44	0.046 (n=4)
Fuller Hollow	3.91	0.006 (n=7)
Little Choconut	3.49	0.007 (n=7)
Pierce	2.02	0.045 (n=7)
Nanticoke	1.89	0.030 (n=7)
Choconut	2.41	0.050 (n=7)
Tracey	0.80	0.227 (n=7)

Table A3.10: Downstream/urban Cl$^-$ Analysis Between Streams

Results of a one-way ANOVA analyzing the differences in stream water Cl$^-$ concentrations between the eight streams.

	df	*SS*	*MS*	*F*	*p*
Stream	7	81396	11628	12.25	<0.00001
Residuals	72	68323	949		

Table A3.11: Downstream/urban Cl$^-$ Analysis Between Seasons

Results of a one-way ANOVA analyzing the differences in stream water Cl$^-$ concentrations between seasons.

	df	*SS*	*MS*	*F*	*p*
Stream	7	17325	8663	5.04	0.009
Residuals	77	132394	1719		

Table A3.12: Paired two-sample t-test results comparing downstream and upstream SRP.

Stream	**SRP**		**n**
	t statistic	**p value, two-tailed**	
Patterson	0.32	0.76	7
Willow Run	0.80	0.46	7
Little Choconut	0.64	0.55	7
Fuller Hollow	0.95	0.38	7
Pierce	1.02	0.35	7
Choconut	0.85	0.43	7
Nanticoke	1.59	0.164	7
Tracey	1.32	0.23	7

Table A3.13: Downstream/urban SRP Analysis Between Streams

Results of a one-way ANOVA analyzing the differences in downstream/urban SRP concentrations between streams.

	df	*SS*	*MS*	*F*	*p*
Stream	7	0.000319	4.558e-05	0.724	0.652
Residuals	68	0.004282	6.297e-05		

Table A3.14: Downstream/urban SRP Analysis Between Seasons

Results of a one-way ANOVA analyzing the differences in stream water SRP concentrations between seasons.

	df	*SS*	*MS*	*F*	*p*
Stream	2	0.000525	2.626e-04	4.70	0.012
Residuals	72	0.004076	5.583e-05		

Table A3.15:

Results of a one-way ANOVA analyzing the differences in stream water discharge (L second^{-1}) between the eight streams.

	df	*SS*	*MS*	*F*	*p*
Stream	7	195564588	27937798	30.82	<0.00001
Residuals	264	239342333	906600		

Table A3.16:

Results of a one-way ANOVA analyzing the differences in stream water discharge (L second^{-1}) between seasons.

	df	*SS*	*MS*	*F*	*p*
Stream	6	49490305	8248384	5.67	<0.00001
Residuals	265	385416616	1454402		

Statistical Tables for: Chapter 4— Impacts of Point- Source Nitrogen to the Susquehanna River from Binghamton, NY

Table A4.1: Results of a one-way ANOVA examining the differences in WWTP effluent DIN concentrations between seasons.

	df	*SS*	*MS*	*F*	*p*
Flow	8	0.7215	0.091	5.591	0.002
Residuals	32	0.5162	0.016		

Table A4.2: Results of a two-way ANOVA examining the differences in NO_3^- concentrations between the five river sites and baseflow/stormflow conditions.

	df	*SS*	*MS*	*F*	*p*
Flow	1	0.0163	0.016	1.805	0.185
Site	4	0.1155	0.029	3.205	0.020
Flow x Site	4	0.0369	0.009	1.025	0.404
Residuals	50	0.4503	0.009		

Table A4.3: Results of a two-way ANOVA examining the differences in NH_4^+ concentrations between the five river sites and baseflow/stormflow conditions.

	df	*SS*	*MS*	*F*	*p*
Flow	1	0.0004	0.0004	1.065	0.307
Site	4	0.011	0.003	8.384	<0.00001
Flow x Site	4	0.004	0.001	1.890	0.289
Residuals	50	0.017	0.0003		

Table A4.4: Results of a two-way ANOVA examining the differences in organic N concentrations between the five river sites and baseflow/stormflow conditions

	df	*SS*	*MS*	*F*	*p*
Flow	1	0.0036	0.004	0.248	0.620
Site	4	0.0452	0.011	0.782	0.542
Site x Flow	4	0.0500	0.013	0.866	0.491
Residuals	50	0.7226	0.015		

Table A4.5: Results of a one- way ANOVA looking at differences in WWTP fluxes between seasons.

	df	*SS*	*MS*	*F*	*p*
Flow	8	1172018	146502	3.118	0.01
Residuals	32	1503754	46992		

Table A4.6: Results of a two-way ANOVA examining the differences in NO_3^- fluxes between the five river sites and baseflow and stormflow conditions.

	df	*SS*	*MS*	*F*	*p*
Flow	1	180830170	180830170	61.133	<0.00001
Site	4	107208151	26802038	9.061	<0.00001
Site x Flow	4	63456484	15864121	5.363	0.001
Residuals	50	147899397	2957988		

Table A4.7: Results of a two-way ANOVA examining the differences in NH_4^+ fluxes between the five river sites and baseflow and stormflow conditions.

	df	*SS*	*MS*	*F*	*p*
Flow	1	7021730	7021730	44.495	<0.00001
Site	4	9834798	2458699	15.580	<0.00001
Site x Flow	4	2193545	548386	3.475	0.014
Residuals	50	7890424	157808		

Table A4.8: Results of a two-way ANOVA examining the differences in organic N fluxes between the five river sites and baseflow and stormflow conditions.

	df	*SS*	*MS*	*F*	*p*
Flow	1	0.016	0.016	0.049	0.826
Site	4	0.999	0.250	0.785	0.540
Site x Flow	4	1.187	0.297	0.933	0.453
Residuals	50	15.905	0.318		

Table A4.9: Results of a two-way ANOVA examining the differences in total N fluxes between the five river sites and baseflow and stormflow conditions.

	df	*SS*	*MS*	*F*	*p*
Flow	1	798066650	798066650	45.959	<0.00001
Site	4	565968072	141492018	8.148	<0.00001
Site x Flow	4	376239392	94059848	5.417	0.001
Residuals	50	868230608	17364612		

Table A4.10: Results of a two-way ANOVA examining the differences between total P concentrations between river sites and between baseflow and stormflow conditions.

	df	*SS*	*MS*	*F*	*p*
Flow	1	0.023	0.023	3.213	0.079
Site	4	0.042	0.011	1.492	0.219
Flow x Site	4	0.005	0.001	0.165	0.955
Residuals	50	0.353	0.007		

Table A4.11: Results of a one-way ANOVA examining the differences in SRP concentrations between the five river sites.

	df	*SS*	*MS*	*F*	*p*
Site	4	0.00001	4.120e-06	0.100	0.981
Residuals	15	0.0006	4.143e-05		

Table A4.12: Results of a two-way ANOVA examining differences between total P fluxes between river sites and between baseflow and stormflow conditions.

	df	*SS*	*MS*	*F*	*p*
Flow	1	22324037	22324037	15.062	0.0003
Site	4	30049172	7512293	5.069	0.002
Site x Flow	4	18984971	4746243	3.202	0.020
Residuals	50	74104978	1482100		

Table A4.13: Results of a one-way ANOVA examining differences between SRP fluxes between river sites.

	df	*SS*	*MS*	*F*	*p*
Site	4	15832	3958	0.347	0.842
Residuals	15	171092	11406		

Table A4.14: Results of a two-way ANOVA examining the differences in conductivities between the five river sites and baseflow/stormflow conditions.

	df	*SS*	*MS*	*F*	*p*
Flow	1	31359	31359	20.94	<0.00001
Site	4	44677	11169	7.459	<0.00001
Flow x Site	4	5166	1292	0.862	0.493
Residuals	50	74875	1498		

Table A4.15: Results of a two-way ANOVA examining the differences in pH between the five river sites and baseflow/stormflow conditions.

	df	*SS*	*MS*	*F*	*p*
Flow	1	0.582	0.582	7.103	0.01
Site	4	4.997	1.249	15.236	<0.00001
Flow x Site	4	0.477	0.119	1.453	0.230
Residuals	50	4.099	0.082		

Table A4.16: Results of a one-way ANOVA examining the differences in Cl^- concentrations between the five river sites.

	df	*SS*	*MS*	*F*	*p*
Site	4	122.3	30.57	1.473	0.26
Residuals	15	311.3	20.75		

Table A4.17: Results of a one-way ANOVA looking at Cl^- fluxes between river sites.

	df	*SS*	*MS*	*F*	*p*
Site	4	1.336e+10	3.340e+09	2.751	0.067
Residuals	15	1.822e+10	1.214e+09		

Statistical Tables for: Chapter 5—Nitrate processing in the stream sediment of an urbanized watershed.

Table A5.1: Results of a 2-way ANOVA looking at differences in 2014 CO_2 emissions between treatments and downstream/urban and upstream/rural sites.

	df	*SS*	*MS*	*F-value*	*p-value*
Treatment	3	0.0001	0.00004	1.152	0.343
Site (down/up)	1	0.001	0.0006	15.781	0.0004
Treatment x Site	3	0.00003	0.00001	0.242	0.866
Residuals	32	0.001	0.00004		

Table A5.2: Results of a 2-way ANOVA looking at differences in 2015 CO_2 emissions between treatments and downstream/urban and upstream/rural sites.

	df	*SS*	*MS*	*F-value*	*p-value*
Treatment	3	0.003	0.001	6.203	0.002
Site (down/up)	1	0.002	0.002	8.640	0.006
Treatment x Site	3	0.0004	0.0001	0.696	0.562
Residuals	32	0.005	0.0002		

Table A5.3: Results of a 2-way ANOVA looking at differences in 2015 N_2O emissions between treatments and downstream/urban and upstream/rural sites.

	df	*SS*	*MS*	*F-value*	*p-value*
Treatment	3	2.155e-07	7.184e-08	7.868	0.0004
Site (down/up)	1	1.120e-09	1.120e-09	0.123	0.729
Treatment x Site	3	1.497e-08	4.990e-09	0.547	0.654
Residuals	32	2.922e-07	9.130e-09		

Table A5.4: Results of a 2-way ANOVA looking at differences in 2014 NO_3^- emissions between treatments and downstream/urban and upstream/rural sites.

	df	*SS*	*MS*	*F-value*	*p-value*
Treatment	3	3.697	1.232	2346.938	<0.0001
Site (down/up)	1	0.002	0.002	4.576	0.040
Treatment x Site	3	0.001	0.0003	0.493	0.689
Residuals	32	0.017	0.0005		

Table A5.5: Results of a one-way ANOVA examining differences in NO_3^- of downstream/urban N-amended Treatments only from 2014.

	df	*SS*	*MS*	*F-value*	*p-value*
Treatment	2	0.006	0.003	3.388	0.075
Residuals	10	0.008	0.0008		

Table A5.6: Results of a one-way ANOVA examining differences in NO_3^- of upstream/rural N-amended Treatments only from 2014.

	df	*SS*	*MS*	*F-value*	*p-value*
Treatment	2	5.11e-05	2.554e-05	0.687	0.526
Residuals	10	3.72e-04	3.720e-05		

Table A5.7: Results of a 2-way ANOVA looking at differences in 2015 NO_3^- emissions between treatments and downstream/urban and upstream/rural sites.

	df	*SS*	*MS*	*F-value*	*p-value*
Treatment	3	1.601	0.534	131.319	<0.00001
Site (down/up)	1	0.024	0.024	5.968	0.020
Treatment x Site	3	0.028	0.009	2.312	0.095
Residuals	32	0.130	0.004		

Table A5.8: Results of a one-way ANOVA looking at differences in extractable NO_3^- from the Nitrate + Carbon, Initial Control, Control, Carbon and Kill Treatments after the 24 hour incubation in 2015.

	df	*SS*	*MS*	*F-value*	*p-value*
Treatment	4	0.062	0.015	2.022	0.13
Residuals	20	0.152	0.008		

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