NUTRIENT DYNAMICS IN THE JORDAN RIVER AND GREAT SALT LAKE WETLANDS

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

In an era of growing urbanization, anthropological changes like hydraulic modification and industrial pollutant discharge have caused a variety of ailments to urban rivers, which include organic matter and nutrient enrichment, loss of biodiversity, and chronically low dissolved oxygen concentrations. Utah's Jordan River is no exception, with nitrogen contamination, persistently low oxygen concentration and high organic matter being among the major current issues. The purpose of this research was to look into the nitrogen and oxygen dynamics at selected sites along the Jordan River and wetlands associated with Great Salt Lake (GSL). To demonstrate these dynamics, sediment oxygen demand (SOD) and nutrient flux experiments were conducted twice through the summer, 2015.

The SOD ranged from 2.4 to 2.9 g-DO m^{-2} day^{-1} in Jordan River sediments, whereas at wetland sites, the SOD was as high as 11.8 g-DO m^{-2} day^{-1}. Sediments were observed as both a sink and source for ammonia, whereas for nitrate it was mostly a sink, reflecting a combined effect of bio-chemical reactions like ammonification, nitrification, and denitrification. Ammonium flux at ambient conditions at the 1300 South location was observed to be positive. Interestingly, in the presence of additional bioavailable nutrients, a negative flux was observed as a result of higher nitrification rate instigated by the nutrient pulse, which presumably dominated ammonification. The results from potential denitrification experiments using ^{15}N supported the high denitrification activity in the

sediments. Variation in nitrification and denitrification rates was also supported by molecular analysis on *amoA*, *nirS*, and *nirK* genes. Comparing the potential rates of denitrification and nitrification with the in-situ nitrogen flux, SOD, and bio-molecular sediment characteristics provided a useful insight of the nutrient dynamics along the Jordan River and GSL wetland, which can serve as essential additions to the continuing efforts of improving the Jordan River Total Maximum Daily Load (TMDL).

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CHAPTER 1

INTRODUCTION

1.1 Problem Description

Dissolved oxygen is an important index to the health of aquatic ecosystems (Caldwell and Doyle, 1995; Rounds and Doyle, 1997; Yogendra and Puttaiah, 2008; Chen et al., 2012). Numerous studies have documented the detrimental effects of low dissolved oxygen concentrations in streams and wetlands, such as limited activity of aquatic hyphomycetes (decomposers), increased mortality and suppressed emergence of macroinvertebrates, increased fish kills, decreased natural stream purification, altered biochemical processes and distribution pattern of carbonate species, etc. (Connolly et al., 2004; Xu et al., 2004; Dai et al., 2006; Yogendra and Puttaiah, 2008; Medeiros et al., 2009). Management decisions that might be responsible for producing low dissolved oxygen concentrations in streams and wetlands are of particular interest (Wood, 2001). This warrants the need for a comprehensive understanding of the oxygen budget in streams and wetlands (Caldwell and Doyle, 1995; Rounds and Doyle, 1997; Chen et al., 2012; Liu and Chen, 2012). Sediment oxygen demand (SOD) (Rounds and Doyle, 1997; Wood, 2001; Miskewitz et al., 2010; Chen et al., 2012; Miskewitz and Uchrin, 2013) and nutrient dynamics (Price et al., 1994; Allan, 1995; Howes et al., 1998; Lillebø et al., 2007; Esten and Wagner, 2010) are both important components of the oxygen budget.

In order to successfully manage healthy dissolved oxygen levels in rivers and wetlands, it is essential for stakeholders to identify the magnitude of SOD (Wood, 2001; Liu and Chen, 2012), how this rate varies spatially and temporally (Hatcher, 1987; Chen et al., 2012), and whether this demand is influenced by decomposing algal detritus (Rounds and Doyle, 1997; Esten and Wagner, 2010). The SOD operates on a longer time scale than the highly dynamic processes of algal photosynthesis and respiration, thereby providing a "background" oxygen demand over the demands of algal respiration and carbonaceous biochemical oxygen demand (CBOD) (Hatcher, 1986; Wood, 2001; Chen et al., 2012). In the absence of primary production, particularly during periods of high water temperature and low flows, SOD can significantly deplete the dissolved oxygen in the water column (Hatcher, 1986; Wood, 2001).

Regeneration or release of nutrients, such as inorganic nitrogen, is related to organic matter degradation at the sediment surface, which enhances the oxygen depletion of bottom waters (Howes et al., 1998; Esten and Wagner, 2010). Different internal biogeochemical processes controlled by microbial species (Grimm, 1988; Johnston, 1991; Zhu et al., 2010) play a central role in the change of nitrogen forms in waterbodies, and contribute toward nitrogen cycling stability in rivers and wetlands (Spieles and Mitsch, 2000; Lillebø et al., 2007; Mulholland and Webster, 2010). Nitrogen dynamics in rivers and wetlands depend not only on transport of nitrogen loads from the catchment to the water column, but also on the aquatic vegetation and the nutrient's chemical transformation processes linking the water column to the sediment bed (Allan, 1995; Lillebø et al., 2007) and the background concentration of nitrogen and dissolved oxygen (Kemp and Dodds, 2002).

Sediment oxygen demand (SOD) and nitrogen cycling are therefore cornerstone processes impacting ecosystem functions (Howes et al., 1998), the inclusive quantitative measurements of which is essential to support predictions of potential bottom water hypoxia in rivers and wetlands (Howes et al., 1998). Besides, excessive loading of nutrients can overwhelm these processes leading to further degradation of water quality (Rittmann and McCarty, 2001; McCormick and Laing, 2003; Mulholland and Webster, 2010).

For a comprehensive surface water quality study, the knowledge of sediment biological activity and nutrient transformation and dynamics at the sediment water interface of a water body is essential. This warrants the necessity of understanding nitrogen dynamics in the water column and sediment-water interface, together with sediment oxygen demand.

1.2 Objectives

The overall goal of this study was to improve the understanding of sediment oxygen demand and nitrogen dynamics in a coupled manner. To help achieve the goal, the following specific objectives will be completed:

1. Measure sediment oxygen demand at river and wetland sites;
2. Evaluate the flux and fate of nutrients as they interact with sediments and the water column;
3. Determine sediment microbial characteristics using bio-molecular tools;
4. Determine potential nitrification and denitrification rates at river and wetland sites.

These objectives were achieved by conducting sediment oxygen demand (SOD) and nitrogen flux experiments at Jordan River and Farmington Bay Wetland locations. The influence of the biogeochemical processes of nitrogen cycling on sediment oxygen demand was the rationale for conducting nitrogen flux experiments along with field SOD experiments. Field experiments were conducted during both early and late summer for comparison. Quantification of nitrification and denitrification rates, analysis of nutrient concentrations, and identification of microbial species were performed in the laboratory using the water and sediment samples collected from selected sites.

Chapter 1 of this study provides an introduction to the work and states the objectives. Chapter 2 and Chapter 3 offer a detailed literature review and methodology of the work, respectively. The results of the field and laboratory experiments are presented in Chapter 4. Chapter 5 provides a discussion on the results and the conclusions of this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Study Area

2.1.1 Jordan River

Utah's Jordan River is a fourth (4^{th}) order river that stretches 52 miles from Utah Lake north to the Great Salt Lake (GSL). It travels through the Salt Lake Valley and enters a series of managed wetlands before discharging into the Great Salt Lake (GSL). The Jordan River has been classified as impaired by the Utah Division of Water Quality (UDWQ). This river experiences both 'chronic' and 'acute' dissolved oxygen (DO) deficits. A Total Maximum Daily Load (TMDL) study is being conducted to address the Jordan River's water quality issues and estimate its ability to assimilate pollutants without impairing ecosystem functions.

The Jordan River passes through Salt Lake County, Utah County, and Davis County and receives wastewater discharges from four municipal wastewater treatment plants (WWTP) – South Davis-South WWTP, Central Valley Water Reclamation Facility (WRF), South Valley WRF, and Jordan Valley Water Treatment Plant. The WWTPs discharging into Utah Lake indirectly add nutrients and organic matter to the downstream Jordan River. Several diversions and dams are located along the path of the Jordan River.

City Creek, Red Butte Creek, Emigration Creek, and Parleys Creek are the major tributaries of the lower parts of the Jordan River (Jensen and Rees, 2005). All of these tributaries are merged to stormwater conduits/pipes below the ground, as a result of which the stream loses its natural functions before discharging into the Lower Jordan River. Other tributaries that feed into the upper part of the river as it flows north to the Great Salt Lake include Little Cottonwood Creek, Big Cottonwood Creek, and Mill Creek (Jensen and Rees, 2005). The population density in the Salt Lake Valley has been growing at a considerably faster rate – 900 people per square mile in 1990 to 1,218 people per square mile in 2000 (SLCO, 2005). The rate of growth through the year 2020 is expected to be 1.9 % annually (0.5 % and 2.8 %) on average throughout the period, with a projected population of 1,300,100 by the year 2020 (Jensen and Rees, 2005). The increasing population and urbanization in the surrounding areas of the Jordan River is being reflected in the additional untreated runoff, and higher sediment and pollutant inputs and the subsequent acute dissolved oxygen (DO) levels. Figure 2.1, Figure 2.2, Figure 2.3 show the locations of the WWTPs along the Jordan River and upstream of Utah Lake, the dams and weirs located on the Jordan River and the complex canal network utilizing Jordan River and Utah Lake water, and the primary tributaries to the Jordan River.

The Surplus Canal diversion located at 2100 South was built to mitigate flooding in Salt Lake City during spring runoff and during large storm events. Roughly 72% of flow in the Jordan River is diverted to the west towards the Great Salt Lake via the Surplus Canal. This diversion point marks the division of the Jordan River into lower and upper reaches. The downstream portion of the Jordan River is called the Lower Jordan

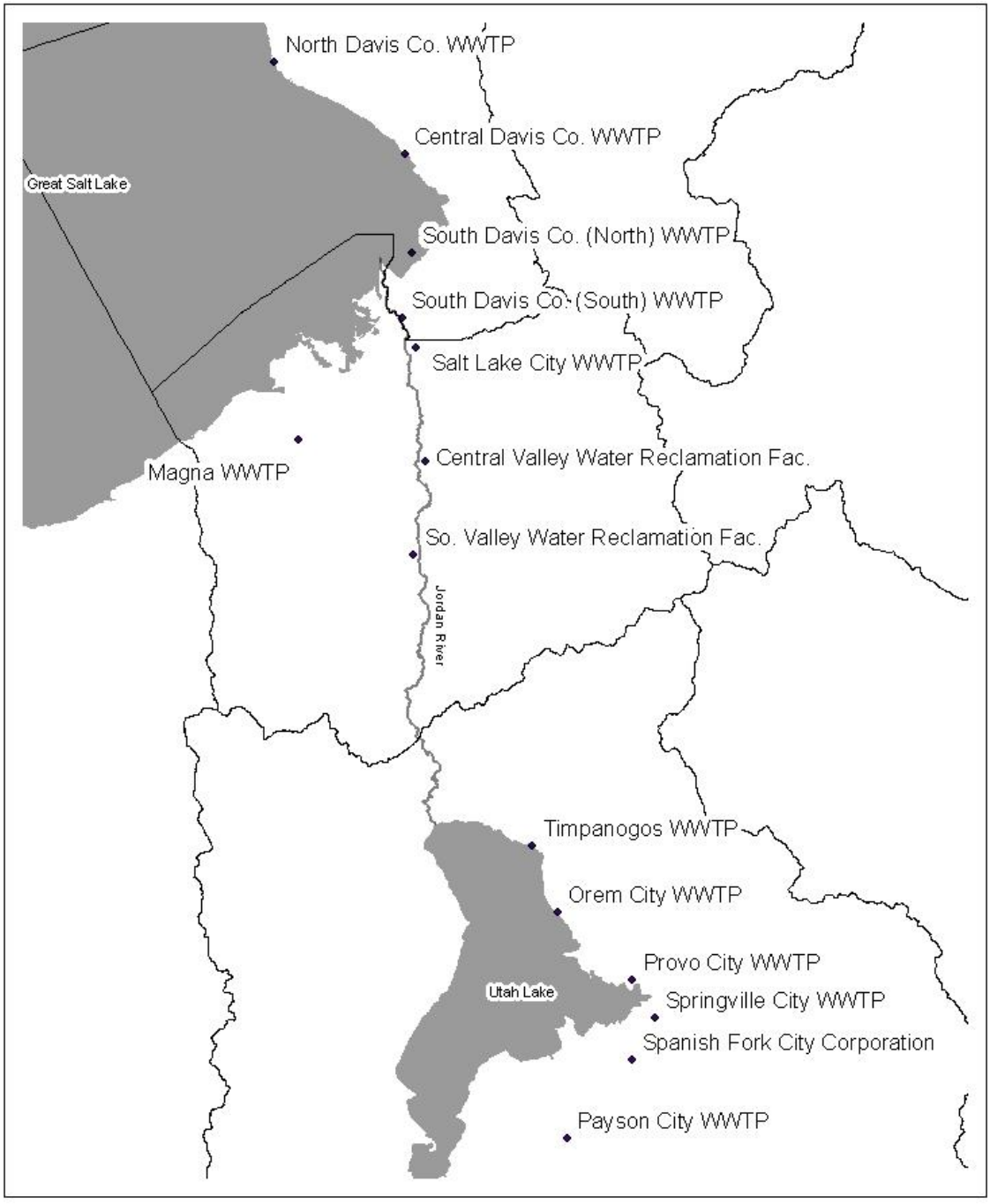

Figure 2.1, WWTPs Discharging to Utah Lake, Jordan River, and Great Salt Lake (Hogsett, 2015)

Figure 2.2, Major Diversions, Canals, and Flow Control Structures (Hogsett, 2015)

Figure 2.3, Primary Tributaries to the Jordan River (Hogsett, 2015)

River (LJR), and is the main focus area of this study. As a result of the Surplus Canal diversion, the Lower Jordan River hardly experiences variation in annual flow. Receiving only 30% of the flows after diversion, Lower Jordan River (LJR) experiences acute pollution conditions throughout the year, which is the key focus of this study and also the rationale for selecting sampling sites in the LJR.

The annual mean daily flow rates observed during 2007-2012 for the Upper Jordan River, Surplus Canal, and Lower Jordan River were 704 cfs, 576 cfs, and 128 cfs respectively. The maximum mean daily flow rate observed in the Lower Jordan River over this time period was 303 cfs. The highest flows appear in the month of January and the lowest flows typically occur in March (Jensen and Rees, 2005).

Annual precipitation totals in the Jordan River Watershed vary dramatically due to the large differences in elevation. The average annual precipitation ranges from 12 inches in the lower valleys to 50+ inches in the highest mountain areas (Jensen and Rees, 2005). Snow accumulation and melt is an important feature of the annual hydrologic cycle for this watershed (Jensen and Rees, 2005). Mean air temperature in the Jordan River area varies between 17.94°C to 20.34°C. Water temperatures in the Jordan River range from 0.5°C to 26.0°C (data sampled at 9400 South, 5800 South, and 1700 South locations). Nitrate concentration varies between 1.0-7.4 mg/L at 9400 South, 1.2-3.0 mg/L at 5800 South, and 0.03-0.439 mg/L at 1700 South sample locations. Variability of nitrate concentration generally decreases as the river moves downstream (Jensen and Rees, 2005). Mean total phosphorus levels varies between 0.11 mg/L and 1.09 mg/L during June to August in the river. The phosphorus indicator criterion in the Jordan River (0.05 mg/L) is exceeded at both 5800 South and 1700 South (Jensen and Rees, 2005).

The significant pollutant sources to the Jordan River include tributaries, dischargers (Central Valley WRF, South Valley WRF, South Davis WWTP), stormwater from Salt Lake and Utah counties, direct surface runoff, and groundwater inflows.

Mean biochemical oxygen demand (BOD) levels in the Jordan River range between 1.72 and 4.42 mg/L for the months of June, July and August, with higher BOD concentrations at the downstream sites. Due to high BOD levels in the river (especially below 2100 South), dissolved oxygen (DO) concentration suffers throughout the river reach (ranges from 4.4-6.4 mg/L) (Jensen and Rees, 2005). A geographic trend of decreasing DO levels at downstream sites is observed, which is consistent with the increase in BOD at downstream sites. The Lower Jordan River is currently experiencing minimal daily dissolved oxygen conditions during summer months between 2100 South and 400 South locations, a matter of concern for water managers.

Total suspended solid levels in the Jordan River generally increase downstream. TDS standards along the Jordan River (1200 mg/L) are violated at several locations. In general, TDS levels appear to decrease as the river progress downstream (Jensen and Rees, 2005). The coliform levels at different sites of the Jordan River, such as 1300 South and 700 South, have also been found to violate the standard for coliform forming units (CFUs) of 5,000 CFU/100 mL (Jensen and Rees, 2005).

For the purpose of assessment, the Jordan River has been divided into eight hydraulic reaches. The designated reaches of the Jordan River are shown in Figure 2.4. Several of these reaches have been classified as impaired for the designated uses of secondary recreational contact (2B), cold and warm water fisheries (3A, 3B), and agriculture (4) due to the violation of E. coli, temperature, dissolved oxygen (DO), and

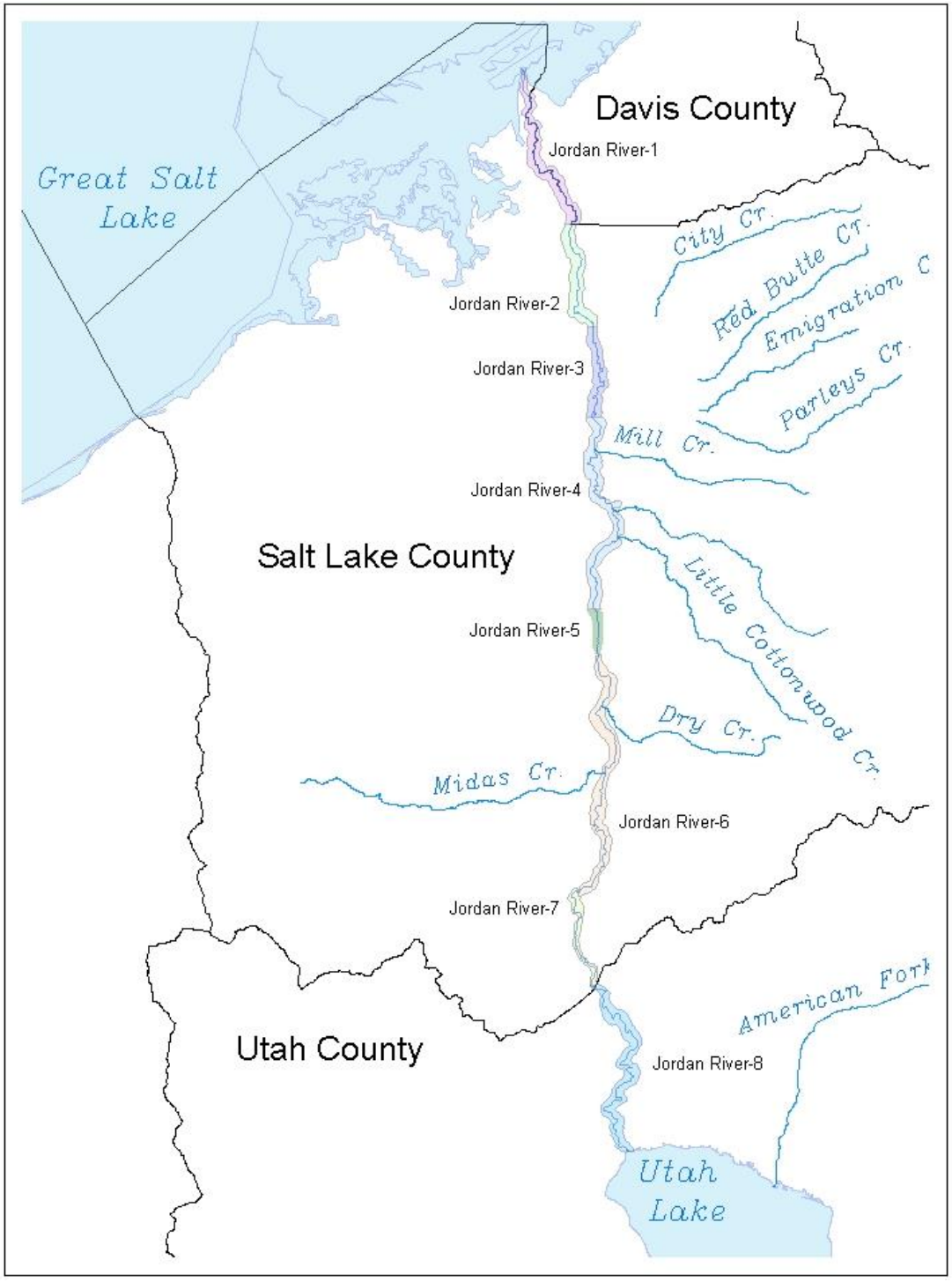

Figure 2.4, Jordan River Hydraulic Reaches (Hogsett, 2015)

total dissolved solids (TDS) standards (Jordan River TMDL, 2009). Table 2.1, adapted from the Jordan River TMDL, Work Element 2 (2009), contains more details on the impaired reaches and the associated designations of impairment. Figure 2.5 indicates the Jordan River reaches and the associated water quality impaired parameters (Jordan River TMDL, 2009).

2.1.2 Wetlands Associated with Great Salt Lake

The GSL, located in the northern part of Utah, is the largest salt water lake in the Western Hemisphere and covers an area of approximately 1,699 square miles. It is the

Table 2.1, Jordan River Hydraulic Reach Descriptions and Impairments

Reach #	Description	Impairment
1	Burton dam to Davis County line (Cudahy Ln.)	3B
2	Cudahy Ln. to North Temple St. (City Creek tributary)	2B, 3B
3	North Temple St. to 2100 S (Surplus Canal)	2B, 3B
4	2100 S to 6400 S (Mill, Big and Little Cottonwood Cr.)	4
5	6400 S to 7800 S (Midvale Slag Superfund site)	2B, 3A, 4
6	7800 S to Bluffdale Rd. (14600 S)	3A
7	Bluffdale Rd. to Salt Lake County line (Traverse Mtns.)	3A, 4
8	Salt Lake County line to Utah Lake	3A, 4

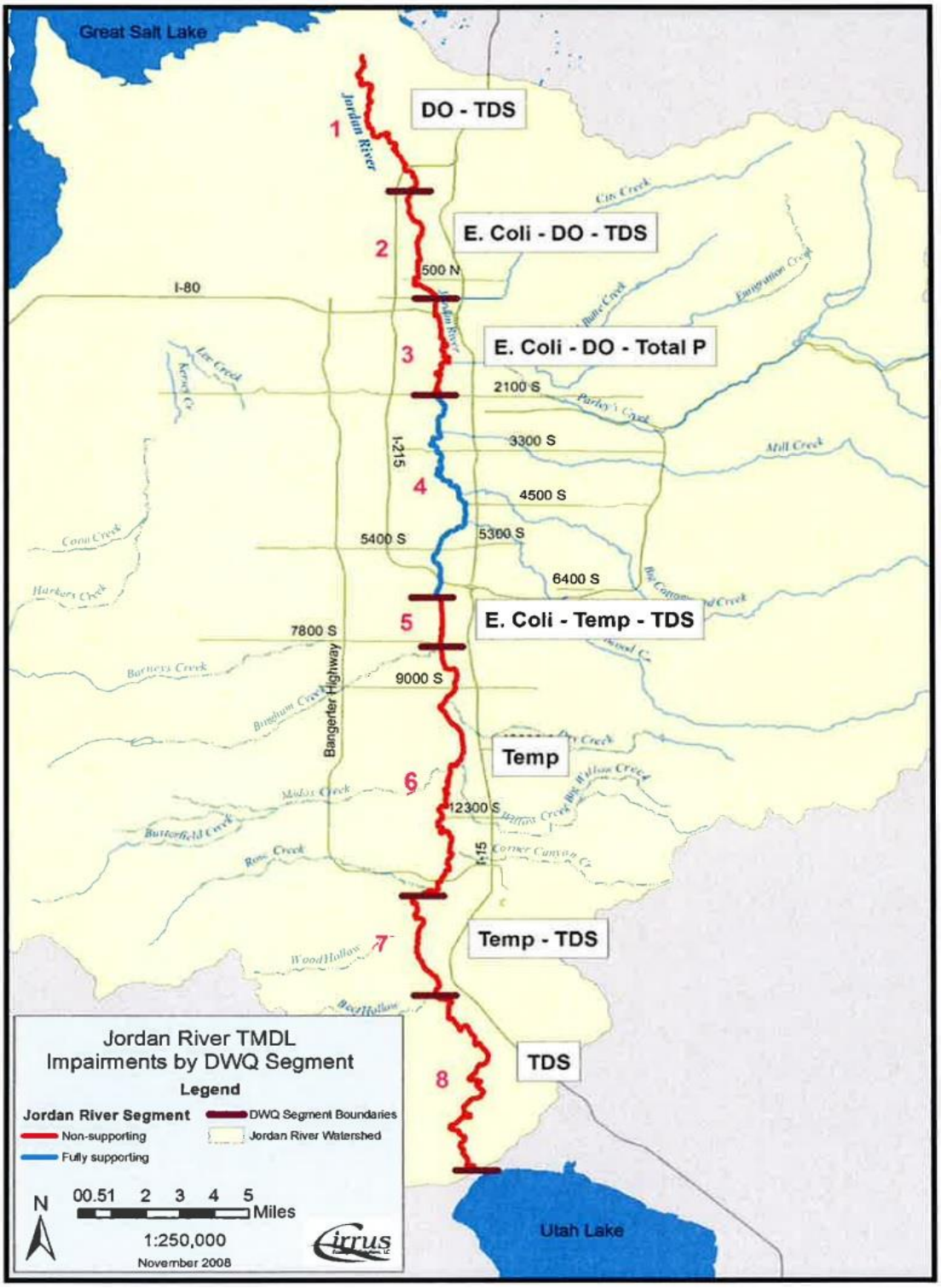

Figure 2.5, DWQ Segments and Water Quality Impairments on the Jordan River

largest remnant of Lake Bonneville, a prehistoric pluvial lake that once covered much of western Utah. The GSL is fed by three major rivers: the Jordan, Weber, and Bear Rivers. As the lake is endorheic (has no outlet besides evaporation), it has very high salinity (USGS, 2001). The wetlands associated with the GSL are a vast ecosystem consisting of approximately 400,000 acres of wetland habitat (DEQ, 2009). These wetlands are an integral part of a larger system that provides habitat for migratory shorebirds, waterfowl, and water birds from both the Central and Pacific flyways of North America. This highly valued resource is currently at risk from urban development and the water resources requirement to provide for the growing population within the watershed. High nutrient concentrations are contributing to the formation of excessive surface mat growths and the spreading of invasive species such as phragmites (Carling et al., 2013).

Great Salt Lake wetlands represent 75% of Utah's wetlands (DEQ, 2009). 100,000 acres of these wetlands are classified as impounded and managed by protection agencies and regional hunting clubs, while the rest of the wetlands are considered as sheet flow wetlands (Miller and Hoven, 2007; UDWQ, 2014). The impoundments in the wetlands (mostly dikes, berms, ditches, and culverts) dampen the impacts of the dynamic fluctuations of the lake and help control or constrict the inflow or outflow of water from the wetlands (DEQ, 2009; UDWQ, 2014). The residence time in these impounded wetlands ranges from a few days to weeks in length (DEQ, 2009). As water moves through successive impoundments toward Great Salt Lake, salinity levels in the system increase (DEQ, 2009). Outlet water from these wetlands flows through sheetflow wetlands and mudflats until it reaches the open waters of Great Salt Lake (Miller and Hoven, 2007). The wetlands vary in size from just a few acres to up to 500 acres (Miller

and Hoven, 2007). There are currently seven wildlife management areas (WMAs), one federal bird refuge, and numerous private duck clubs that maintain impounded wetlands along Great Salt Lake.

There are many factors that contribute to the characteristics of the wetlands of Great Salt Lake. However, salinity in the water and sediments of the shoreline are the primary factor that determines the nature, location, and extent of wetlands around the lake (DEQ, 2009). The level of salinity in these waters and sediments varies widely depending on the availability of freshwater and the water level of the lake (Aldrich and Paul, 2002). The wetlands receive a large portion of fresh water via Jordan River, Bear River, Weber River, creeks, and canals, and therefore also receive a large portion of the nutrients having a considerable salinity (Wurtsbaugh and Marcarelli, 2004; Wurtsbaugh and Marcarelli, 2006; Goel and Myers, 2009).

Farmington Bay, which received attention in recent years due to degraded water quality issues, hosts an array of wetland habitats including fresh water ponds, marshes, expansive flats, and open salt water. These wetlands cover roughly one third of the Great Salt Lake wetlands (Hoven, 2010). Farmington Bay wetlands receive the majority of its water from the Jordan River and State Canal. Projected population increase of Salt Lake City and surrounding areas suggests that more nutrients will end up in the Farmington Bay Wetlands via the Jordan River and other non-point sources. Recent findings from Miller and Hoven (2007) indicated possible water quality stressor gradients related to nutrients, salinity, pH, DO, and total suspended solids (TSS) in the Farmington Bay. Evaluation of recent water quality data (CH2M HILL, 2009) shows that total phosphorous and total nitrogen in the wetland ponds ranged from 0.02-6.4 mg/L and 0.5-

52.0 mg/L, respectively (DEQ, 2009). DO ranged from 0.04-23.0 mg/L, while TSS ranged from 4-4458 mg/L (DEQ, 2009). The wetlands of concern in this study are Unit 1 and Unit 2, both of which are part of Farmington Bay (Figure 2.6). Unit 1 and Unit 2 are both impounded type wetlands. Unit 2 is a site with high nutrient concentrations, while Unit 1 has mid-range concentrations. The reason behind high nutrient concentration at Unit 2 is the discharge of effluent from the South Davis WWTP to the State Canal, which makes its way into Unit 2. Multiple drainage canals and creeks from Bountiful and the Wasatch Front feed into Unit 1 causing high nutrient concentrations in the Unit 1. In addition, Unit 1's proximity to a landfill contributes to the high nutrient concentrations.

Figure 2.6, Farmington Bay Wetland Study Area

2.2 Laboratory and Field Techniques

2.2.1 Sediment Oxygen Demand (SOD)

Sediment oxygen demand (SOD) is the rate at which dissolved oxygen is depleted from the water column during the decomposition of organic matter in streambed or lakebed sediments (Doyle and Lynch, 2005; Todd et al., 2009; Hogsett and Goel, 2013). The SOD also accounts for the reduction of DO due to the respiration of benthic flora and fauna, and the biotic and abiotic oxidation of reduced inorganic chemical species diffusing from the sediments (Utley et al., 2008; Todd et al., 2009; Hogsett and Goel, 2013). Most of the SOD at the surface of the sediment is due to the biological decomposition of organic material and the microbial facilitated nitrification of ammonia (Rounds and Doyle, 1997), while SOD several centimeters into the sediment is often dominated by the chemical oxidation (Price et al., 1994; Rounds and Doyle, 1997; MacPherson, 2003). Biological consumption may control SOD in summer and fall, while chemical consumption may be dominant in winter and spring (Seiki et al., 1994; MacPherson, 2003). The SOD is typically measured as g O_2 $m^{-2}d^{-1}$ (Slama, 2010). In the absence of primary production, SOD could deplete the water column oxygen in a few days (Wood, 2001). Assessment of the magnitude and variability of SOD in rivers and wetlands, and its change from season to season, is significant in terms of water quality management (Wood, 2001).

Sources of organic matter contributing to SOD include the sedimentation of suspended solids originating from point dischargers, settled suspended solids associated with diffused runoff, settled periphyton and phytoplankton biomass, eroded organic rich sediments, and microbial growth (Goonetilleke et al., 2005; Hogsett, 2015). Factors like

quality of organic matter present, microbial community responsible for organic matter degradation, ecosystem metabolism, and hospitality of the general environment to support the microbial and macroinvertebrates community all have an influence on the SOD (Webster & Benfield, 1986; MacPherson, 2003; Young et al., 2008). As the sediment-water interface is responsible for majority of the heterotrophic activity in stream ecosystems (Pusch et al., 1998; MacPherson, 2003), SOD can be responsible for a significant portion of the ambient oxygen deficit (Matlock et al., 2003; Chen et al., 2012; Hogsett and Goel, 2013).

The important physical parameters affecting SOD in rivers are water temperature, water velocity, and the depth of the water column (Price et al., 1994; MacPherson, 2003; Ziadat and Berdanier, 2004; Utley et al., 2008; Chen et al., 2012). These parameters can cause seasonal variation in SOD patterns. SOD rates are assumed to decrease with lower temperatures as a result of the decreased metabolic rate of microbes (Otubu et al., 2006; Utley et al., 2008; Hogsett, 2015).

Deeper depths are associated with slow moving waters, which can cause less mixing and therefore decreased fluxes of DO to the benthic zone (MacPherson, 2003; Hogsett, 2015). As velocities increase, SOD increases to a point where the dissolved oxygen consuming activities occurring within the sediments become the limiting factor and SOD rates reach a maximum (Nakamura and Stefan, 1994; Utley et al., 2008). Mackenthun and Stefan (1998) found a linear relationship between SOD and flow velocity in the range 0-10 cm/sec.

Sediment oxygen demand can be measured in the laboratory using sediment cores as well as in-situ using chamber methods (Price et al., 1994; Utley et al., 2008). However,

in-situ measurements are preferred over laboratory-scale experiments to avoid uncertainties associated with disturbing the sediments during collection, transportation, and testing (Price et al., 1994; Chen et al., 2012; Hogsett and Goel, 2013; Hogsett, 2015). In-situ chambers measure either the drop in DO concentration over time (batch method) or the difference in DO concentration in the inflow and outflow (continuous method) (Lee et al., 2000; Utley et al., 2008).

Sediment oxygen demand can contribute to significant DO depletion in streams and wetlands (MacPherson, 2003) and can contribute to stream impairment. Researchers have reported SOD can account for more than half of the total oxygen demand and can play a primary role in the water quality (Rounds and Doyle, 1997; Matlock et al., 2003; Slama, 2010; Chen et al., 2012). Sediments with SOD_{T20} rates >1.6 $gm/m^2/day$ are considered moderately polluted, while rates >2.4 $gm/m^2/day$ are considered polluted in terms of organic enrichment (Butts and Evans, 1978). The relative contribution of SOD in stream and wetland impairment makes its study and quantification imperative for long-term prediction of environmental quality (Howes et al., 1998), proper TMDL practices and stream management (Hogsett and Goel, 2013).

A large body of literature on SOD experiments and quantification exists. Sediment oxygen demand measurements in streams, rivers, and lakes is available in a number of publications (Caldwell and Doyle, 1995; Rounds and Doyle, 1997; Borsuk et al., 2001; Wood, 2001; Matlock et al., 2003; Ziadat and Berdanier, 2004; Crompton et al., 2005; MacPherson et al., 2007; Utley et al., 2008; Liu et al., 2009; Miskewitz et al., 2010; Chen et al., 2012). Table 2.2 lists the SOD rates of different shallow water ecosystems (rivers, streams, and lakes) from literature review.

Table 2.2, Literature Values of Sediment Oxygen Demand Rates

Site/Location	SOD, gm O_2 m^{-2} day^{-1}	Reference
Suwannee River Basin, GA	0.1-2.3	Utley et al. (2008)
Lower Willamette River, OR	1.3-4.1	Caldwell and Doyle (1995)
Arroyo Colorado River, TX	0.62 – 1.2	Matlock et al. (2003)
Arkansas	0.15-1.36	Matlock et al. (2003)
Missouri	1.2-2.0	Borsuk et al. (2001)
Lower Rapid Creek, SD	3.80-6.98	Ziadat and Berdanier (2004)
Cayuga Lake, NY	0.3 – 1.0	Newbold and Liggett (1974)
Lake Sammamish, WA	1.0	Bella (1970)
Lake Lyndon B. Johnson, TX	1.7 – 5.8	Schnoor and Fruh (1979)
Saginaw River, MI	0.1 – 5.3	Chiaro & Burke (1980)
Tualatin River basin, OR	0.4 – 4.4	Rounds and Doyle (1997)
Blackwater Stream, GA	1.1e2.6	Crompton et al. (2005)
Upper Wisconsin River, WI	0.022 – 0.92	Sullivan et. al. (1978)
Northern Illinois River, IL	0.27 – 9.80	Butts and Evans (1978)

2.2.2 Nitrogen and Its Importance in Nutrient Cycle

All aquatic bacteria and vegetation require nutrients to survive and are often the limiting growth factors for most autotrophs (Elser et al., 2007). These nutrients are cycled through the food web while organic matter is being produced or degraded. Nitrogen, an important macronutrient, receives a great deal of attention from stream and wetland scientists and resource managers because nitrogen enrichment of stream and wetland zone plays a major role in anthropogenic eutrophication. Excess quantities of nitrogen

can cause unregulated growth (Elser et al., 2007), causing significantly low dissolved oxygen (DO) conditions. Microbes such as bacteria are the primary mediators of nitrogen transformations, converting inorganic nitrogen into a variety of other inorganic or organic species.

2.2.3 Nitrogen Cycle

The major constituents comprising the nitrogen cycle are detrital organic nitrogen (org-N), ammonia-nitrogen (NH_3-N), and nitrate nitrogen (NO_3-N), with gaseous nitrogen being important when organisms are present having nitrogen fixation capabilities (Zilson et al., 1978). It is an interconnected process between land organisms, bacteria in sediment, chemical reactions, and weather contributions (Brown et al., 1991). Sources of organic nitrogen which contribute to the nitrogen concentration in aquatic systems are generally considered to be respiration of algae and zooplankton, natural death of zooplankton, and external sources of organic nitrogen (such as wastewater discharges) (Zilson et al., 1978). Approximately 70% of the nitrogen respired by zooplankton is assumed to enter the water in an organic form, while the remaining is considered as ammonia-nitrogen (Zilson et al., 1978). Other processes that produce organic nitrogen include agricultural activity and nitrogen fixation by plants (Brown et al., 1991). Ammonia-nitrogen is formed in the aquatic environment from other nitrogen species through nitrogen fixation (bacteria and blue-green algae converting gaseous nitrogen to inorganic nitrogen), ammonification or mineralization (organic nitrogen converted to ammonia by certain organism), and denitrification (Zilson et al., 1978; Burger et al., 2003). The main focus of this study was on processes within the inorganic nitrogen pool.

Depending on the stream and wetland, the majority of the nitrogen in a system could enter through influent flow. Influent inflows typically consist of streams/tributaries, runoff from surrounding land, and groundwater inputs. Nitrate dominates the nitrogen entering a system due to its mobile nature (Webster et al., 2003). Ammonia, being more labile, is rapidly immobilized by various biological and physical processes (Webster et al., 2003). Figure 2.7 shows a schematic of the nitrogen cycle.

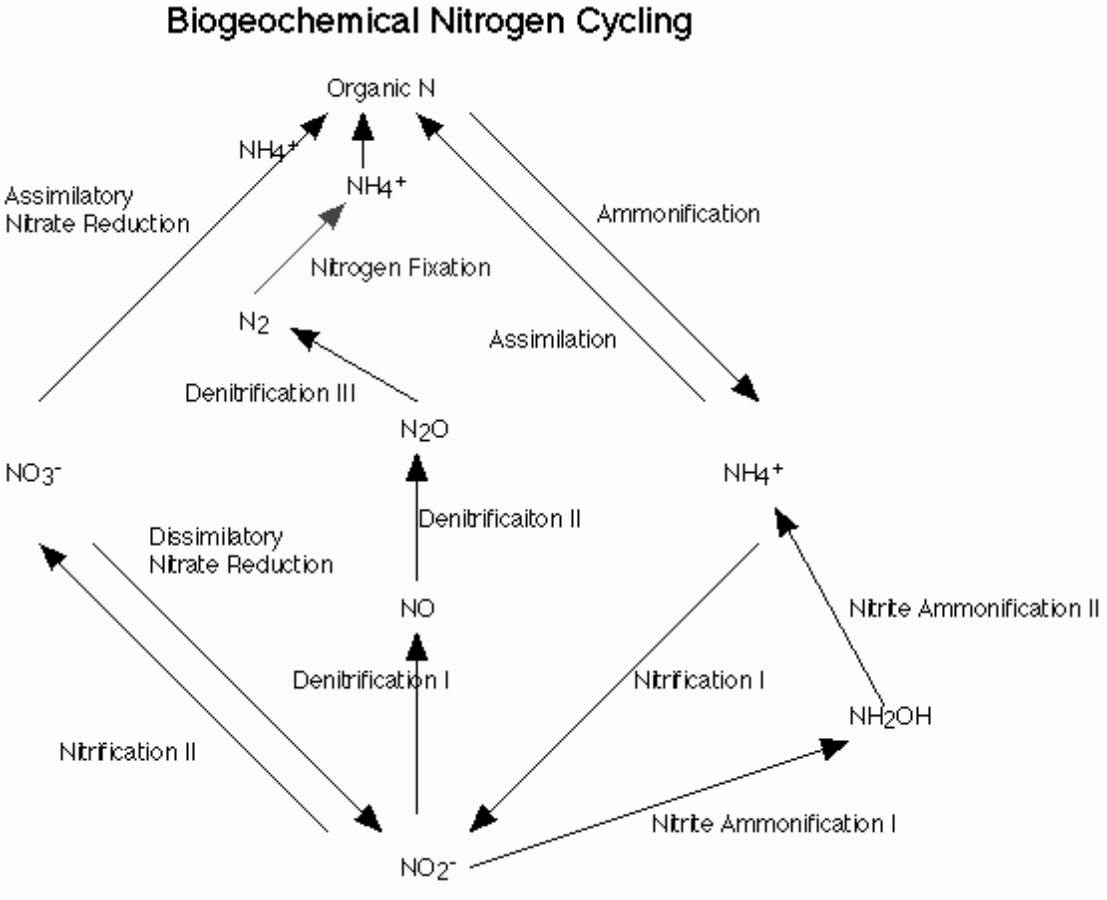

Figure 2.7, Nitrogen Cycle

2.2.3.1 Nitrification

The major processes regulating the nitrogen cycle are nitrification and denitrification. These processes are controlled by separate bacterial communities, namely nitrifiers and denitrifiers, as well as by physical processes. Nitrification, which consists of one of the major sinks of ammonia, refers to the sequential oxidation of ammonia to nitrite and finally to nitrate. Autotrophic bacteria responsible for nitrification are *Nitrosomonas* (ammonia oxidation, nitritation) and *Nitrobacter* (nitrite oxidation, nitratation) (Zilson et al., 1978; Metcalf and Eddy, 2003). The oxygen demand required for nitrification can add an additional 30% to the oxygen demand associated with only organic carbon. Ammonium produced during the decomposition of organic material within the sediments requires 4.57 g-O_2/g-N to complete the two-step biological nitrification process according to the following stoichiometric equations (Metcalf & Eddy, 2003; Reddy et al., 2008).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \text{ (Nitritation)}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \text{ (Nitratation)}$$

$$NH_4^- + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \text{ (Combined Nitrification)}$$

The first metabolism, nitritation, is carried out by autotrophic nitroso-bacteria, also known as ammonia oxidizing bacteria (AOB) utilizing 3.43 g-O_2/g-NH_4^+-N to produce nitrite. AOB uses the enzyme *amoA*, the α-subunit of ammonia monooxygenase, and hydroxylamine oxidoreductase (HAO) to catalyze these transformation reactions (Kowalchuk et al., 2001). In general, two copies of *amoA* exist per nitrifier (Hommes et

al., 1998). Therefore, quantifying the *amoA* genes can be used indirectly to obtain the number of AOBs present.

Nitrite, produced from nitritation, is toxic in the aquatic environment and does not accumulate in healthy lotic systems due to the rapid oxidation to nitrate by autotrophic nitro-bacteria, or nitrite oxidizing bacteria (NOB). The oxidation of nitrite, or nitratation, requires 1.14 g-O_2/g-NO_2^--N. This reaction can be carried out both heterotrophically by the genus *Nitrobacter* or autotrophically by nitrite oxidizers such as *Nitrospina*, *Nitrococcus*, and *Nitrospira* (Burrell et al., 1998). The dominance of the NOB genus depends on the location. *Nitrobacter* has been found to be a ubiquitous bacteria, found in both sewage and marine environments, dry environments, and with a wide range of preferences for pH (Spieck et al., 2005). However, *Nitrospira* tends to outnumber *Nitrobacter* when both communities are in competition (Spieck et al., 2005). Nitrate, the end product of nitrification, is eventually reduced or bioassimilated by phototrophs and bacteria during cell growth and can be utilized as an electron acceptor under low DO conditions during microbial denitrification.

The rate of nitrification is primarily dependent on temperature and pH (Zilson et al., 1978). The optimum pH for nitrification is 7.5 to 8.0 (Metcalf and Eddy, 2003). Nitrification requires alkalinity as an inorganic carbon source as well as oxygen as an electron acceptor (Kowalchuk et al., 2001). Alkalinity is consumed throughout the entire process. Generally, nitrification occurs in the top layers of sediment as this region has more exposure to oxygen than deeper layers and contains the highest concentration of ammonia from decomposition (Kadlec et al., 2009). Sediments with higher volatile solid concentrations can contain more ammonia, which can stimulate nitrification (Frazier et

al., 1996).

2.2.3.2 Denitrification

Denitrification is the heterotrophic process by which highly oxidized forms of nitrogen (such as nitrate) are converted to more reduced forms (such as ammonia). Denitrification is typically the principal pathway for nitrate removal from streams and wetlands (DeBusk et al., 2001). This process requires organic carbon and occurs mostly under anaerobic/anoxic conditions (such as in muds, either below or at surface). Two commonly proposed mechanisms for denitrification are:

$$HNO_3 \rightarrow HNO_2 \rightarrow H_2N_2O_2 \rightarrow NH_2OH \rightarrow NH_3; \text{ and}$$

$$2NO_3^- \rightarrow N_2 + H_2O + 5/2\ O_2 \text{ (Zilson et al., 1978)}$$

The important difference between these two possible mechanisms is that in the first expression, nitrate is reduced to soluble and readily oxidizable ammonia, while in the second, it is reduced to gaseous nitrogen. Zilson et al. (1978) stated that it is safe to assume that the gaseous nitrogen end product dominates from the quantitative point of view. DeBusk et al. (2001) discusses further about the nitrate reduction processes in aquatic systems – assimilatory nitrate reduction and dissimilatory nitrate reduction. In assimilatory nitrate reduction process, nitrate is reduced into ammonia before incorporation into the biomass of the organism (DeBusk et al., 2001). On the other hand, dissimilatory nitrate reductase simply uses nitrate as an electron source through two reactions; dissimilatory nitrate reduction to ammonia (DNRA) which reduces nitrate into ammonia, and denitrification which reduces nitrate into nitrogen gas (DeBusk et al.,

2001). Denitrification requires organic carbon as an electron donor to reduce nitrate to nitrogen gas, and hence, the rate of denitrification is controlled by the amount of carbon present.

Each step in the denitrification process ($NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$) is catalyzed by a different enzyme: nitrate reductase (*nar*) converts nitrate to nitrite; nitrite reductase (*nir*) transforms nitrite to nitric oxide; nitric oxide reductase (*nor*) supports the conversion to nitrous oxide; and nitrous oxide reductase (*nos*) completes the conversion of nitrous oxide into nitrogen gas (Bothe et al., 2007). Nitrite reductase is the key enzyme of denitrification in catalyzing the first committed step that leads to a gaseous intermediate. Nitrite reductase exists in two different forms coded by the genes *nirK* and *nirS* (Bothe et al., 2007). The *nirK* specifies the enzyme which reduces nitrite using a copper subunit. On the other hand, *nirS* corresponds to an enzyme which reduces nitrite using a cytochrome cd_1 catalyst (Bothe et al., 2007). Typically, *nirS* is more common in marine and estuary environments, while *nirK* genes dominate terrestrial environments (Jones et al., 2010). Denitrifying bacteria usually contain either the *nirK* or *nirS* enzyme (Bothe et al., 2007). This helps in correlating the amount of denitrifying bacteria to the number of *nirK* genes present.

2.2.3.3 Denitrification and Nitrification Rates

In general, the denitrification rates are higher than nitrification rates due to the presence of a broader diversity of microorganisms that can facilitate denitrification (DeBusk et al., 2001). Moreover, denitrification takes place heterotrophically. Denitrification rates in river and stream sediments typically range from 0 to 345 µmol N

m^{-2} h^{-1} (Seitzinger, 1988a). The higher rates are from systems that receive substantial amounts of anthropogenic nutrient input. The major source of nitrate for denitrification in most river sediments underlying an aerobic water column is the nitrate produced in the sediments rather than the nitrate diffusing into the sediments from the overlying water (Seitzinger, 1988a). Table 2.3 and Table 2.4 present the nitrification and denitrification rates obtained from literature.

Table 2.3, Nitrification Rates Obtained from Literature

Site	**Nitrification rate**	**Reference**
Onondaga Lake, Seneca River, NY	0.21 – 0.67 g N/m^2/day	Pauer and Auer (2000)
Chattahoochee River, GA	0.26 day^{-1}	McCutcheon (1987)
Delaware River	0.09 – 0.54 day^{-1}	Bansal (1976)
West Fork Trinity River, TX	0.50 day^{-1}	McCutcheon (1987)
Truckee River, NV	0.09 – 1.30 day^{-1}	Bansal (1976)
Ohio River	0.25 day^{-1}	Bansal (1976)
Clinton River, MI	0.4 – 4.0 day^{-1}	Wezernak and Gannon (1968)
Big Blue River, NB	0.17 – 0.25 day^{-1}	Bansal (1976)
Flint River, MI	0.10 – 2.50 day^{-1}	Bansal (1976)
Mohawk River, NY	0.23 – 0.40 day^{-1}	Bansal (1976)
Grand River, IL	0.80 day^{-1}	Bansal (1976)

Table 2.4, Denitrification Rates Obtained from Literature

Site	Denitrification rate	Reference
Millstone River, NJ	0.27 ± 1.21 mmol N m^{-2} h^{-1}	Laursen and Seitzinger (2002)
Sugar Creek, IN/IL	15.81 ± 2.51 mmol N m^{-2} h^{-1}	Laursen and Seitzinger (2002)
Swale-Ouse River System, England	20 – 659 µmol N m^{-2} h^{-1}	Pattinson et al. (1998)
San Francisquito Creek, CA	54 µmol N m^{-2} h^{-1}	Duff et al. (1984)
Little Lost Man Creek	0 µmol N m^{-2} h^{-1}	Duff et al. (1984)
Delaware River	166 – 345 µmol N m^{-2} h^{-1}	Seitzinger (1988b)
Potomac River	210-235 µmol N m^{-2} h^{-1}	Seitzinger (1987)
Lake Michigan	12 – 51 µmol N m^{-2} h^{-1}	Gardner et al. (1987)
Swift Brook, Ontario	121 – 302 µmol N m^{-2} h^{-1}	Robinson et al. (1979)

2.2.4 Leaf Leachate

Leaf litter processing is one major pathway of the global organic carbon cycle. Fresh leaf litter loses solutes when immersed, but gradually throughout the breakdown process rather than instantly upon wetting. Leaching is considered to be the characteristic mechanism initiating leaf breakdown in aquatic environments (Gessner et al., 1999) and is thought to lead to a substantial mass loss (up to 30%) within 24 hours after immersion of leaves (Petersen and Cummins, 1974; Benfield, 1996). According to existing views (Webster and Benfield, 1986; Allan, 1995), leaf breakdown in streams proceeds in three distinct phases separated on a temporal scale: leaching, conditioning, and fragmentation (Gessner et al., 1999) – shown in Figure 2.8. Drying at the ambient temperatures kills the leaf tissue, resulting in a lack of structural integrity and the rapid leaching of soluble

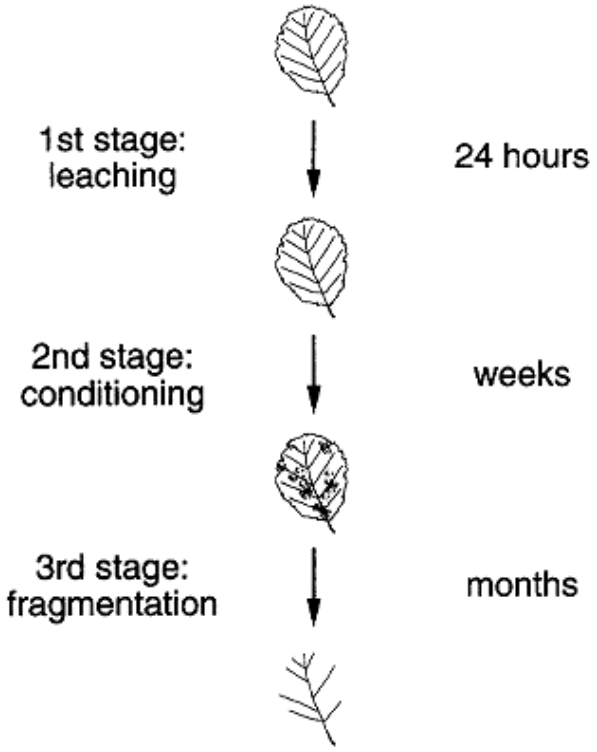

Figure 2.8, Current Conceptual Model of Leaf Litter Breakdown in Streams

constituents which is usually observed. During this process, a range of small reactive organic and inorganic compounds (Tukey and Morgan, 1964) are released and transported to the aquatic environment, which may directly impact aquatic organisms (Hofmann et al., 2012). Different forest stockings produce different leachate qualities, which in turn stress the aquatic communities (Hofmann et al., 2012). While leaves of some species that die naturally on the trees may leach solutes instantly during rain events even before abscission (Gessner et al., 1999), leaves of other species may become initially trapped in the canopy (Campbell and Fuchshuber, 1994) or on the ground (Mayack et al., 1989), where they may undergo partial breakdown and lose their solutes before they enter the aquatic environment (Gessner et al., 1999).

Leaf litter age, chemical quality, and photodegradation control the fate of dissolved organic matter in the leaf leachate. Previous studies demonstrated that sunlight can moderate the degradation of plant litter in terrestrial environments through photo-mediated shifts in dissolved organic matter (DOM) composition and its bioavailability in streams (Fellman et al., 2013). Leaching from piles of leaves carries high levels of nutrients in urban runoff (Cowen and Lee, 1973), which has been reported to have an adverse effect on the vegetation in streams by influencing the dynamics of the dissolved organic matter pool and turbidity in the water column (Bärlocher et al., 1989). More turbid leachate has higher DOC and phenolic concentrations (McArthur and Richardson, 2002).

On a global scale, considerable variation in leaching behavior occurs in relation to riparian tree species composition, climate, and a variety of other factors (such as timing of leaf fall, prevailing weather conditions, stream channel, and bank and valley morphology) (Gessner et al., 1999). Different species of leaf litter decompose at different rates (Pérez-Harguindeguy et al., 2000) and are colonized by different macroinvertebrates in streams (Braatne et al., 2007). The rate of decomposition is significantly affected by the amount of water-soluble and leachable substances, nitrogen content, and polyphenol content of the fresh litter (Singh and Gupta, 1977). In summer, streams frequently experience drought resulting in isolated pools. These pools become frequently saturated with leaf litter, where the associated leaf leachates may generate toxic and hypoxic conditions (Canhoto et al., 2013).

Water-soluble or leachable substance of leaf litter provides a readily available energy source for decomposers (Singh and Gupta, 1977) and is therefore most influential

in initial stages of decomposition. During decomposition, carbon is used as an energy source by decomposers, while nitrogen is assimilated into cell proteins and other compounds (Singh and Gupta, 1977). Hence, a higher nitrogen content in the original leaf material promotes decomposition, particularly in the earlier stages of decomposition (Satchell and Lowe, 1967). In later stages, there is little net change in nitrogen content, and the carry-over organic nitrogen becomes more resistant to decomposition (Singh and Gupta, 1977). Temperature and moisture are the two important abiotic factors controlling the rate of leaf litter decomposition under natural conditions (Singh and Gupta, 1977). Moreover, soil aeration and soil structure indirectly play an important role in decomposition on leaf litter. Some authors considered leaf litter chemical quality (C:N ratio and polyphenolics content) as an indicator of leaf litter mass losses and DOC released into stream water through leaching (Bastianoni et al., 2012).

2.3 Tools for Bio-molecular Analysis

2.3.1 DNA Extraction

The first step to study the microbial characteristics of sediment is extraction of DNA from a sample. The procedure of DNA extraction starts with breaking the cells open (commonly referred to as cell lysis) using chemical and physical methods (MO BIO, DNA Isolation Kit Manual). The next steps involve removing membrane lipids, proteins, and RNA from the cell. The final important step is to purify the DNA from detergents, proteins, salts and reagents used during cell lysis step (MO BIO, DNA Isolation Kit Manual). The commonly used procedures to purify the DNA are ethanol precipitation, phenol–chloroform extraction, or minicolumn purification.

The purity of DNA is assessed by the ratio of absorbance at 260 nm and 280 nm (A260/A280). The recommended value for A260/A280 is approximately 1.8, which indicates pure DNA without any contamination of protein, phenol, or other contaminants that absorb strongly at or near 280 nm (William et al., 1997). A secondary measure of nucleic acid purity used is the absorbance ratio of 260mm and 230mm. Expected A260/A230 values are commonly in the range of 2.0-2.2 indicating no contamination (William et al., 1997).

Several methods for DNA extraction from soil samples have been proposed (Ogram et al., 1987; Tsai and Olsen, 1991; Smalla et al., 1993; Hurt et al., 2001). These procedures are not always suitable for processing large number of samples. This limitation is overcome through the use of commercially available extraction kits, which are cheaper and less time consuming (Mahmoudi et al., 2011). Commercial DNA extraction kits such as Power Max and Power Soil kits can provide clean and pure DNA with optimum A260/A230 and 260/230 ratios.

2.3.2 Polymerase Chain Reactions (PCR)

Polymerase Chain Reaction (PCR), a revolutionary method developed by Kary Mullis (Saiki et al., 1985) in the 1980s, is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand (Mullis, 1990).

Each PCR assay requires template DNA, primers, nucleotides, and DNA polymerase. The DNA polymerase is the key enzyme that links individual nucleotides together to form the PCR product – the DNA polymerase adds the first nucleotide to the

primer (Garibyan and Avashia, 2013). This makes PCR possible to delineate a specific region of template sequence that is needed to be amplified in approximately 2 hours (Mullis, 1990). The automated process bypasses the need to use bacteria for amplifying DNA (Bustin, 2004). At the end of the PCR reaction, the specific sequence is accumulated in billions of copies (amplicons).

Polymerase Chain Reaction (PCR) follow a cycle of DNA denaturation, primer annealing, and primer extension (elongation) (Mullis, 1990). This means that the double-stranded DNA becomes single-stranded, which facilitates a primer to attach to gene-specific binding sites on the DNA (Dorak, 2007). The DNA polymerase then extends the DNA strand until the reverse primer is encountered on the DNA (Dorak, 2007). Multiple cycles of this process result in much higher DNA concentrations of the target gene.

To amplify a segment of DNA, the sample is first heated to 94-96^{o}C, so the DNA target denatures (separates into two pieces of single-stranded DNA) (Muhlrad, 2003). The mixture is then lowered to 50-65^{o}C so that the primers anneal (bind) to the DNA template (complementary sequence) (Muhlrad, 2003).

The primers are designed to bracket the DNA region to be amplified (Saiki et al., 1988). The temperature is then raised to 72^{o}C. At this point, the DNA polymerase begins to synthesize new strands of DNA starting from the primers, and extend a new DNA strand (Muhlrad, 2003). At the end of this cycle, each double-stranded DNA molecule consists of one new and one old DNA strand. The cycle of changing temperatures is then repeated, and Polymerase Chain Reaction (PCR) continues with similar additional sequences producing billions of copies in a geometric pattern. Figure 2.9 shows a schematic of the PCR process.

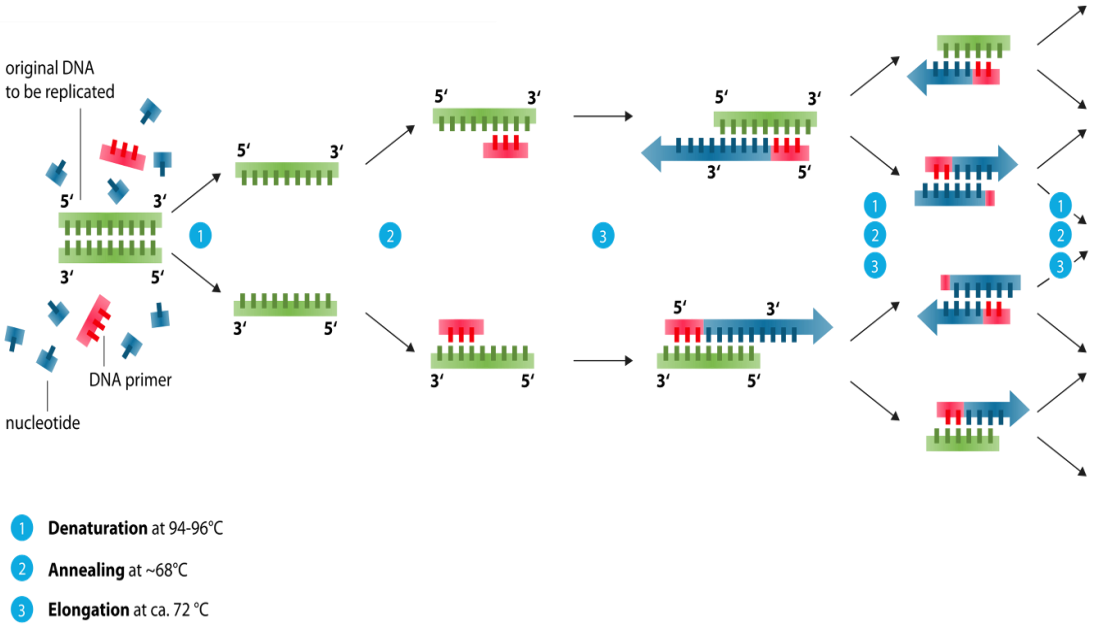

Figure 2.9, Schematic of the PCR Process

Polymerase Chain Reaction is a highly sensitive process that can be used only to identify the presence or absence of a known pathogen or gene. As a result, there are scopes of contamination of the sample ensuing in misleading results (Vogel et al., 2012; Smith and Osborn, 2009). The primers used for PCR can anneal nonspecifically to sequences that are similar, but not completely identical, to target DNA (Garibyan and Avashia, 2013).

2.3.3 Quantitative PCR (qPCR)

Quantitative PCR or qPCR provides information beyond the mere detection of DNA. It specifies the amount of a specific DNA or gene is present in the sample

(Garibyan and Avashia, 2013). The method can detect and quantify the PCR product in real time, while it is being synthesized (VanGuilder et al., 2008). As a result, quantitative PCR has the advantage of quantification of the desired gene during the exponential amplification, avoiding the problems that are associated with end-point PCR (analyzed after completion of the final PCR cycle) (Smith and Osborn, 2009; Garibyan and Avashia, 2013).

In the quantitative PCR (qPCR) process, a fluorescent stain for double stranded (ds) DNA is added to the reaction, which enables monitoring the number of gene copies (replicates) as the cycle progresses (Dorak, 2007). With each amplification cycle, the fluorescence intensity increases proportionally with the increase in amplicon concentration (Smith and Osborn, 2009). The increase in fluorescence is plotted against the cycle number to generate the amplification curve, from which a quantification cycle value can be determined, which helps in monitoring the progress of the amplification reaction (Postollec et al., 2011). The use of fluorescence-based detection in quantitative PCR offers greater sensitivity and enables discrimination of gene numbers across a wider dynamic range (Smith and Osborn, 2009). Figure 2.10 shows a schematic of quantitative PCR process.

Quantitative PCR (qPCR) can be used to analyze single cells and quantify any combination of DNA, messenger RNA (mRNAs), and proteins (Stahlberg et al., 2012). The process is robust, highly reproducible, and sensitive that enables tracking phylogenetic and functional gene changes across temporal and spatial scales under varying environmental or experimental conditions (Smith and Osborn, 2009; Garibyan and Avashia, 2013).

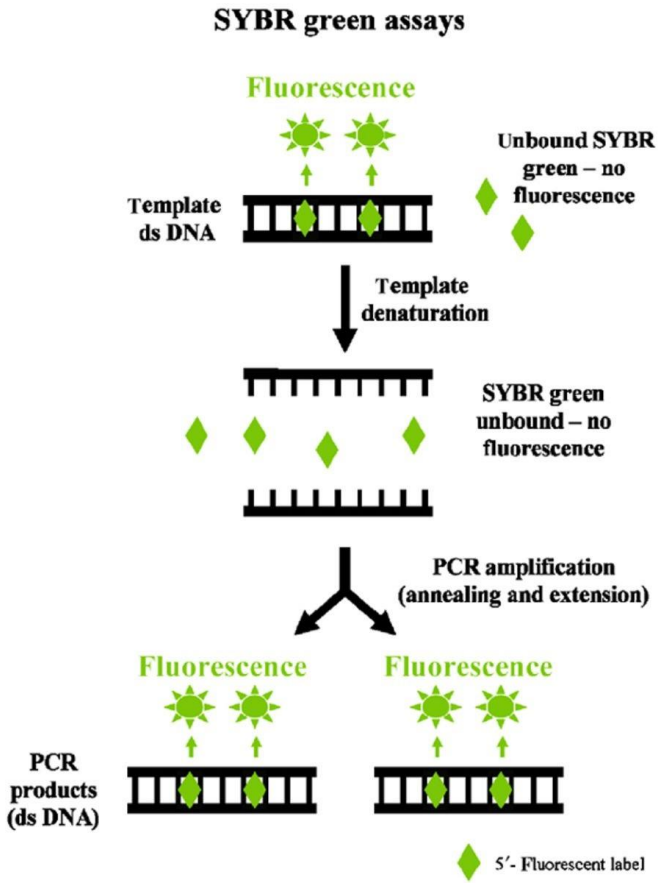

Figure 2.10, qPCR Schematic

2.3.4 TRFLP

Terminal Restriction Fragment Length Polymorphism (TRFLP) is a molecular biology technique for profiling of microbial communities based on the position of a restriction site closest to the labelled end of an amplified gene (Osborn et al., 2000). TRFLP analysis can be used to examine the microbial community dynamics in response to changes in different environmental parameters or to study the bacterial populations in natural habitats (Derakshani et al., 2001). This technique is a culture independent, rapid,

sensitive, and reproducible method of assessing diversity of complex communities without the need for any genomic sequence information (Osborn et al., 2000).

The method is based on digesting a mixture of target PCR product containing fluorescently-labeled genes with one or more restriction enzymes and detecting the size of each of the individual resulting terminal fragments using a DNA sequencer (Liu et al., 1997). The digestion used is mixed with a DNA size standard and sent through capillary electrophoresis for laser detection of the fluorescent DNA fragments (Osborn et al., 2000). The identity of the bacteria present in the sample is then determined based on the resulting electropherogram (Osborn et al., 2000). Electropherogram is a graph image where the X axis represents the sizes of the fragment and the Y axis marks the fluorescence intensity of each fragment. In a Terminal Restriction Fragment Length Polymorphism profile, each peak corresponds to one genetic variant in the original sample, while its height or area corresponds to its relative abundance in the specific community (Blackwood et al., 2003).

The steps involved in a typical Terminal Restriction Fragment Length Polymorphism analysis are DNA isolation and purification, PCR amplification and restriction enzyme digestion, separation and detection of the digested products via electrophoresis, analysis of data to generate the fragment profile for each sample, and clustering analysis based on the profile of samples (Osborn et al., 2000; Blackwood et al., 2003; Zhang et al., 2008). Because TRFLP depends on DNA extraction method and PCR, the biases integral to both the steps will affect the results of the TRFLP analysis (Egert and Friedrich, 2003; Sharifian, 2010; Brooks et al., 2015). Figure 2.11 shows a schematic of the TRFLP process.

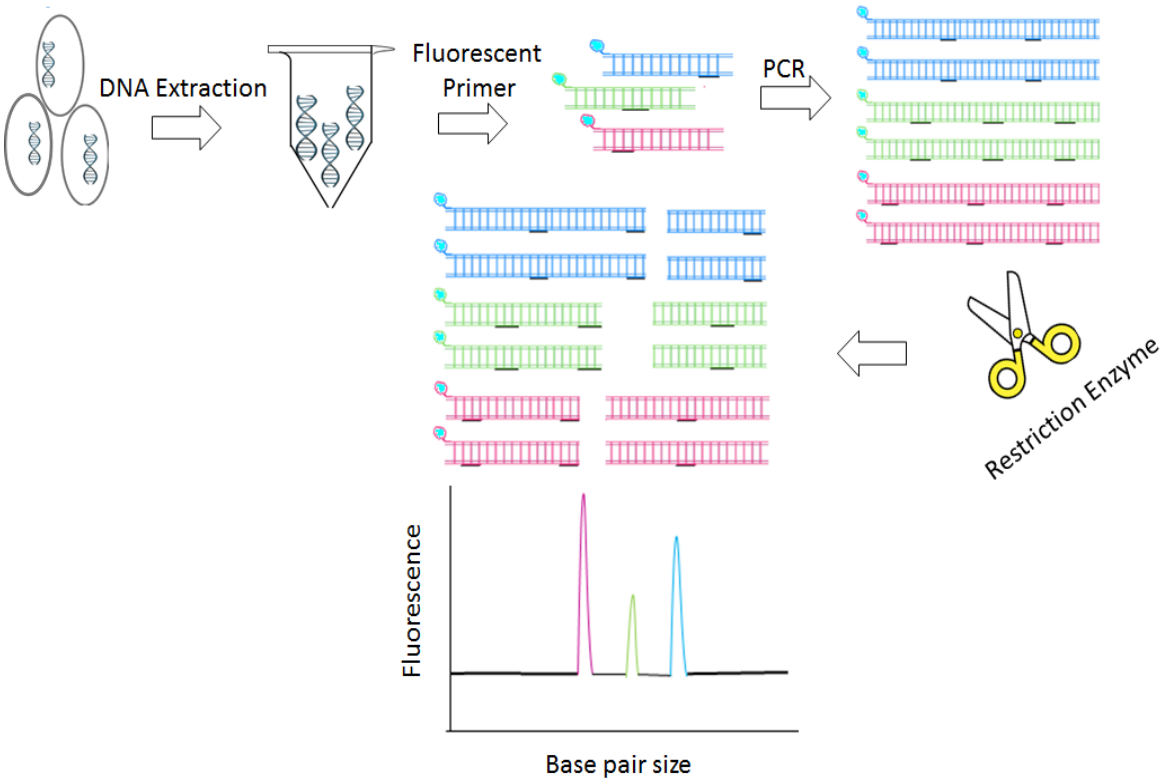

Figure 2.11, TRFLP Schematic

Profiling AOB populations via the *amoA* gene can be accomplished using Terminal Restriction Fragment Length Polymorphism sequencing technique (Osborn et al., 2000). The AOB species that can be identified using TRFLP technique include *Nitrosomonas europaea/eutropha, Nitrosomonas cryotolerans, Nitrosomonas marina, Nitrosomonas oligotropha, Nitrosomonas communis,* and the genus *Nitrospira* (Siripong et al., 2007). These species of AOB share the same functional class (Koops et al., 1991). Table 2.5, adapted from Koops et al. (1991), contains details on these species, including the expected TRFLP peaks (Park and Noguera, 2004; Siripong et al., 2007; Gilomen, 2008; Whang et al., 2009).

Table 2.5, Expected TF Sizes and Their Corresponding AOB Groups Based on TRFLP

Species	**Growth at 0°C**	**Salt requirement**	**Maximum ammonia tolerance***	**TRFLP Electropherogram peak**
***N. eutropaea/ eutropha* lineage**	No	No	High	219/270, 491/491
N. oligotropha	No	No	Low	48/135, 354/135
N. cryotolerans	Yes	Yes	Mid	48/441, 354/48
N. marina	No	Yes	Mid	48/441, 48/135
N. communis	No	No	Mid	491/491
***Nitrosospira* lineage**	-	-	-	283/206

* High: > 400 mM ammonia; Mid: 100-400 mM ammonia; Low: <100 mM ammonia

CHAPTER 3

METHODOLOGY

3.1 Sampling Locations

In order to fulfill the research objectives, 1300 South, Legacy Nature Preserve (LNP) from Lower Jordan River, Unit 1 and Unit 2 from Farmington Bay Wetland Management Area, and State Canal were selected as sampling sites within the study area. The locations for these sites were selected keeping in mind the different hydraulic reaches, tributaries, stormwater outfalls, and the proximity to wastewater treatment plant (WWTP) point discharges and UDWQ monitoring stations.

The sampling location for 1300 South was selected at the downstream point of the combined discharges from City Creek, Red Butte Creek, Emigration Creek, and Parleys Creek and stormwater conduits. Legacy Nature Preserve (LNP) was selected at the downstream point of South Davis South wastewater treatment plant effluent discharge where the Utah Division of Water Quality (UDWQ) monitoring station is located. Significant nutrient load enters this location from a cow ranch located adjacent to the river.

State Canal diverts off from the Jordan River at the west of Legacy Parkway to feed the southeast side of the Farmington Bay of Great Salt Lake (GSL). The sampling

location for State Canal was selected downstream of the South Davis County North wastewater treatment plant (WWTP) discharge and Bountiful Pond ‘tributary’.

Sites selected from the Farmington Bay Wetland Management Area were Unit 1 and Unit 2. After the discharge of South Davis County North wastewater treatment plant (WWTP) effluent and Bountiful Pond ‘tributary’, State canal discharges into the Farmington Bay South wetland first. The canal then feeds Unit 2. After Unit 2, State Canal discharges directly into the Farmington Bay. Unit 1 receives water from a variety of different sources, including Unit 2, NE Pond, Farmington Canyon, and various creeks. These sites are also shown on a map in Figure 3.1. Details of the selected sampling sites are provided in Table 3.1.

Figure 3.1, Selected Location for Sampling Sites

Table 3.1, Location of Sampling Sites

Site	Latitude	Longitude	Type
1300S	40°44'37.68"N	111°55'7.82"W	Jordan River
Legacy Nature Preserve	40°50'43.41"N	111°57'12.56"W	Jordan River
State Canal	40°54'29.47"N	111°55'50.58"W	Canal
Unit 1	40°56'36.98"N	111°56'3.86"W	Impounded Wetland
Unit 2	40°55'8.48"N	111°56'49.17"W	Impounded Wetland

3.2 Sediment Oxygen Demand (SOD)

3.2.1 SOD Chamber Details

Three SOD chambers, one Control (transparent acrylic) and two Testing (made of aluminum), were used in the Jordan River SOD study. The chamber tops had arrangements for mounting a submersible pump to circulate water inside the chamber. The flow rate and average flow velocity used were 11 L/min and of 8 cm/sec, respectively. Influent and effluent ends of the plumbing were located inside the chamber and were connected to a polyvinyl chloride (PVC) water distribution system. The distribution pipe contained small holes to evenly distribute the re-circulated flow within the chamber. Both the Control chamber and testing chambers have a working volume of 44 liters. When deployed, the Testing chambers encapsulated a sediment area of 0.16 m^2. Figure 3.2 shows a picture of an SOD chamber with the main components.

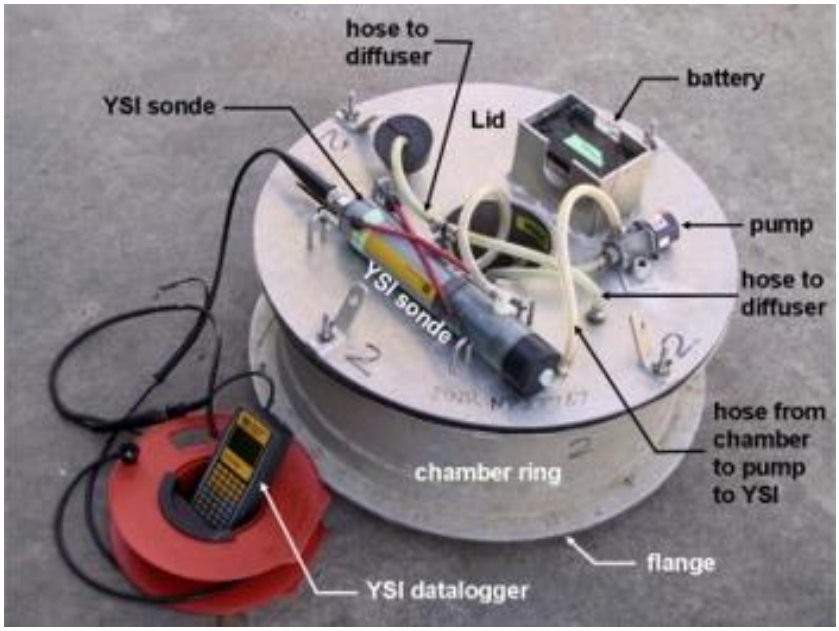

Figure 3.2, SOD Chamber Showing the Main Components of the System

Both the Control and Testing SOD chamber configurations were identical in construction and operation except for the bottom sections. The bottom of the Control chamber was sealed to measure oxygen consumption associated with the water column only, whereas the bottom of the Testing SOD chamber was open. Hence, the river water contained in the chamber was in constant contact with the river sediments during the experimental period, which facilitates the measurement of DO consumption associated with the sediments as well as in the water column. The chambers were tested for water tightness and the pumps were tested to ensure its circulation functioning before performing the on-site experiments.

Water quality probes (sondes, In-Situ Inc. model Troll 9500) required to perform the experiments were provided by the Utah Division of Water Quality (UDWQ). The probes were capable of measuring DO, temperature, conductivity, pH, and barometric

pressure. However, only DO and temperature sensors were used directly while calculating oxygen demands. The probes were checked for quality control and calibration before all sampling events. Figure 3.3 provides a picture of the SOD chambers deployed at the sampling location.

3.2.2 SOD Chamber Deployment

Sampling locations for deploying SOD chambers were carefully selected considering straightness of river sections, representative sediment substrate characteristics, and obstructions and potential safety issues (such as rebar, barbed wire, construction debris, submerged logs).

Figure 3.3, SOD Chambers Deployed at Site

Once the suitable location of SOD chamber deployment was determined, the chambers were deployed with water quality probes turned on for data collection. It was done carefully following the chamber deployment protocol for avoiding sediment disturbances. The Control chamber was placed first due to the additional time it requires to reach a stable DO reading. If possible, the Control chamber was filled sideways in a deeper section of the river immediately upstream or off to the side to minimize sediment disturbances. After filling the Control chamber with river water, the chamber was flipped upside down while keeping the chamber completely submerged. Any trapped air inside the chamber was let out using the pump. Any air left in the system will contain oxygen that will slowly dissolve into the chamber water, leading to misleading results. The Control chamber was then placed carefully on top of the sediments without disturbing the surrounding area. Two large black plastic bags were used to wrap the Control chamber to prevent any daylight activity from the exposure of sunlight. The chamber was attached to a wooden stake hammered into the sediments to prevent downstream drifting. The water quality probe was then screwed into the probe housing on the Control chamber lid. Next, the water circulation pump was turned on and was kept on for the remainder of the testing period.

Similar to the Control chamber, the two Testing chambers were filled with river water and flipped upside down (keeping the chambers submerged), while removing any trapped air inside the chambers (in the same manner as done for the Control chamber). The Testing chambers were deployed upstream of the Control chamber to ensure undisturbed sediments. The chambers were set by inserting the 1½ inch bottom ridge of the chamber into the sediment surface while the coupling flange of the chambers were

parallel to the surrounding sediments. Proper placement of the testing chambers into the sediments was ensured by carefully checking if the coupling flange was in contact with the surrounding sediment surface. After seating the two Testing chambers, the water quality probes were installed and the pumps were turned on.

3.2.3 Calculation of SOD

The sediment oxygen demand (SOD) fluxes and dark water column respiration (WC_{dark}) rates were calculated using the following equations (Butts, 1978; Chiaro and Burke, 1980; Murphy and Hicks, 1986). Sediment area within the chamber is designated by A, while V represents the volume of SOD and water column chambers.

$$SOD = 1.44\ (V/A)\ (b_{SOD} - b_{WC}) \tag{3.1}$$

Where,

SOD = Sediment Oxygen Demand (g/m^2 day)

1.44 = unit conversion (mg / L min) → g / L day)

V = volume of SOD and WC chambers (38 L)

A = sediment area within the chamber (0.16 m^2)

b_{SOD} = bulk DO depletion rate in SOD chamber (mg / L min)

b_{WC} = DO depletion rate in WC chamber (mg / L min)

$$WC_{dark} = 1440(b_{WC}) \tag{3.2}$$

Where,

WC_{dark}=DO depletion rate in WC chamber (g / m^3 day)

1440=unit conversion (mg / L min → g / m^3 day)

WC_{dark}, representing the dark respiration associated with the water column, is the volumetric oxygen consumption rate measured in the Control chamber. WC_{dark} is comparable to one-day biochemical oxygen demand (BOD) test having no nitrification inhibitor. Subtracting the oxygen demand required by the water column makes SOD a two-dimensional flux associated with the sediments and benthos. The working volumes and sediment areas were kept constant since the Testing chambers were placed to a uniform depth of 1½ inch. The SOD fluxes calculated for both Testing chambers were averaged for further analysis and oxygen mass balances.

SOD values found in literature are typically normalized to 20°C (SOD_{20}) using the modified van't Hoff form of the Arrhenius equation based on ambient water temperature (Berthelson et al., 1996; Chapra, 2008):

$$SOD_{20} = SOD / \theta^{t-20} \quad (3.3)$$

Where,

SOD_{20} = SOD normalized to 20 ^oC

t = observed temperature (^oC)

θ = temperature normalization coefficient

θ = 1.047 (WC BOD decomposition) (Chapra, 2008)

The ambient DO deficit is a result of various biogeochemical activities occurring in the water column and at the sediment-water interface. Through the use of chambers, these parameters are decoupled and the percent of the ambient oxygen demand associated with the sediments ($\%_{SOD}$) can be calculated using Equation 3.4.

$$\%_{SOD} = SOD / (SOD + WC_{dark} * d) * 100 \quad (3.4)$$

Where,

d = mean river depth at the sampled site (m)

3.3 Sediment Core Collection and Depth Partitioning

Sediment samples were collected using a 3 foot long 2 inch inner diameter acrylic open-barrel core, or open-drive sampler. The core sampler was pushed into the sediments and a stopper was inserted into the top of the coring unit, which facilitated the removal of an intact sediment core. Another stopper was inserted into the bottom of the core tube during transportation.

Sediment core samples were taken out of the sampler onsite using a plunger inserted into the bottom of the coring unit and pushed upwards. This allowed sediment samples to be collected at specific depths within the sediment column.

These depth-specific core samples were collected in containers and stored on ice until laboratory analysis. Figure 3.4 shows a picture at the time of sediment core collection at a site in the Jordan River.

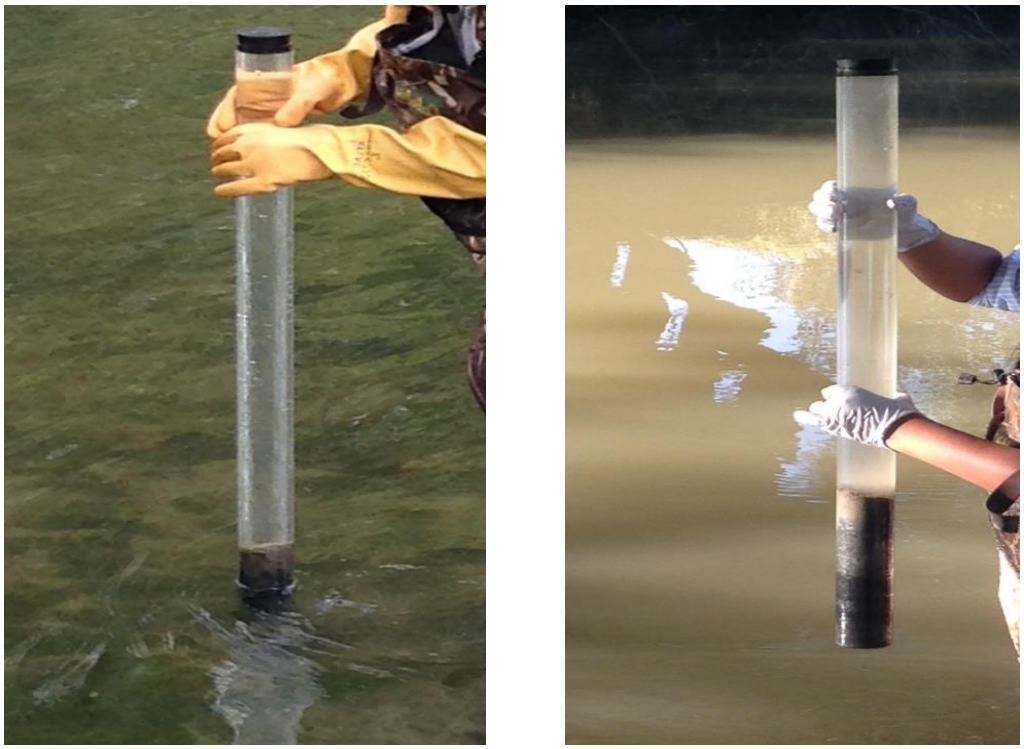

Figure 3.4, Sediment Core Collection

3.4 Nutrient Flux Chamber

Transparent acrylic flux chambers of dimension 10in × 10in × 36in were used for the nutrient flux study. Chambers were tested in the lab for water tightness and quality controls. Two sediment chambers and two water column chambers were deployed at each site to measure the daytime nutrient dynamics at the sediment-water interface and within the water column, respectively. The sediment chamber had open top and open bottom that facilitated the measurement of nutrient dynamics in the water column while interacting with sediments. Meanwhile, the water column chambers had an open top, but closed bottom to measure nutrient dynamics in the water column only. An open top in the chambers accounted for sunlight exposure (photosynthesis) allowed gases to escape the

chamber, and allowed easy access to the chamber for mixing and sample collection. The nutrient flux experiments were conducted under both ambient conditions and nutrient spiked conditions. Later sections include additional discussion of this article.

The sediment chambers were installed first, followed by the installation of the water column chambers. The sediment chambers were pushed 10-15 cm into the sediment to isolate the water column above the contained sediments. Care was taken to avoid sediment disturbance. However, small sediment disturbance at the chamber walls was unavoidable. Significant re-suspension of sediment may skew results due to the release of sediment pore water and artificial turbidity. Nonetheless, the clay layer underlying the surface sediments at Jordan River and wetland locations helped in easy penetration into the fine sediments with minimal disturbances, and also provided a foundation to support the chambers during winds.

After the chambers were installed, the water column control chamber was gradually filled with ambient water to the same level as the sediment chamber. The working volumes of the sediment and water column chambers mimicked ambient conditions by having a water height in the chambers equal to the depth of the ambient water column. Care was taken not to disturb any sediment while completing this procedure. The chambers were tied to stakes to make sure chambers did not tilt or move during sampling. Figure 3.5 shows a water column and sediment chambers deployed next to each other.

Each chamber had its own submersible pump. For the water column chambers, the pump was placed directly on the closed bottom. For the sediment chambers, the pump could not be placed directly on the sediments, as this would result in resuspension of the

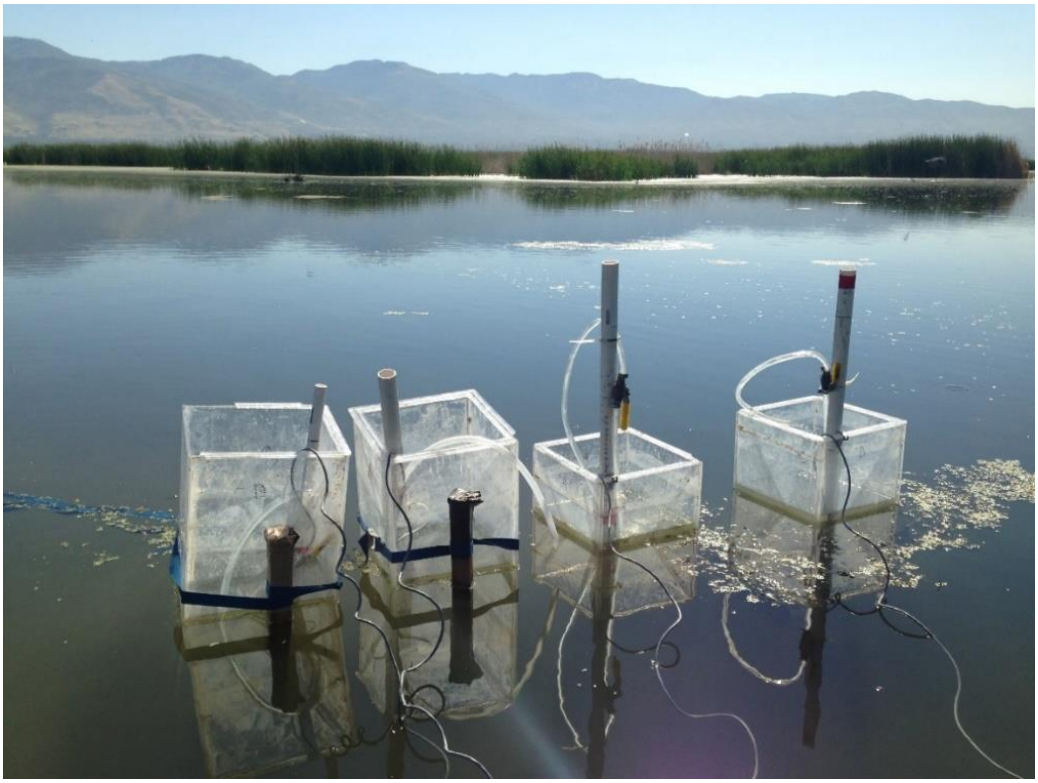

Figure 3.5, Nutrient Flux Chambers Deployed at Site

sediments leading to a failed experiment. To avoid this, the depth of the submersible pump in the sediment chambers was tested by hanging and adjusting the pump from outside of the chamber and confirming that the bottom of the pump was roughly 2-3 inches above the sediment surface. If the pump was found to enter the sediments outside of the chamber, the length was adjusted using a hanger and the pump was cleaned before installation in the chamber. The pump outlet tubing had a ball valve installed near the top of the PVC tube. The valve was closed halfway to avoid disturbing the sediments when the pumps were initially turned on. After all chambers and pumps were properly

installed, a visual observation of turbidity, green water, surface foaming, floating periphyton mats, the presence of carp, etc. was made, as these conditions can have an impact on results. Then, the pumps were all powered at the same time by manually connecting them to a deep cycle 12V battery.

A nutrient flux study was performed with nutrient spiked condition (as mentioned before) to investigate the sediment's reaction to a pulse of nutrients. The first 4 hours of the study were conducted under ambient conditions, while the last 4 hours involved spiking the chambers to a calculated concentration of 0.5 mg/L NH_3-N, 0.5 mg/L NO_3-N, and 0.1 mg/L PO_4-P. Five samples were taken during both ambient and spiked conditions at consistent time intervals. However, collecting a sample slightly earlier or later than planned would not have any influence on the final calculations. Longer chamber deployment times are preferred to capture sediment and water column nutrient dynamics of river or wetland sites having very low ambient nutrient concentrations.

The chambers were lightly mixed with the submersible pump before taking any water samples to account for potential stratification in the chambers. Constant mixing was not employed while the chambers were sitting in wetland sediments. For the wetland sites, the pumps were powered for 10 minutes to ensure complete and consistent mixing in the chambers before each sample collection. The reason behind not continually mixing in the chambers in the wetland sites was to represent the stagnant wetland condition. For the river sites, the pumps were kept on during the whole experiment to imitate the flowing river condition. While collecting samples, care was taken not to allow pumps to re-suspend any sediments. Samples were directly collected using the circulation pump tube. The outlet of the tubing above the water was carefully lifted to fill a water quality

sampling container. Following water quality sample collection, the container was immediately capped and stored on ice in a cooler. Nitrate, nitrite, and phosphate concentrations were analyzed with a Methrohm 883 plus Ion Chromatograph using EPA Method 300.0 for Determination of Inorganic Anions by Ion Chromatography. Ammonia concentrations were measured using a HACH TNT 830 ULR Ammonia Kit. All water samples were analyzed within 24 hours of collection.

3.4.1 Nutrient Flux Calculation

The water column rates and sediment fluxes are generally reported based on concentrations greater than analytical detection limits. The rate of change of dissolved nutrients in each chamber is calculated using the slope of the concentration (mg/L) versus time (day) plot.

All raw data and regressions are reported in the units of mg/L/day, and the final water column rates and sediment fluxes in terms of $g/m^3/day$ and $g/m^2/day$, respectively. The water column rate was primarily calculated, since the field observed rate describes the nutrient dynamics occurring in the water column.

$$WC = \frac{dC}{dt} \tag{3.5}$$

Where,

WC = WC nutrient rate during daytime conditions ($g/m^3/d$)

dC = Change of nutrient concentration in chamber (mg/L)

dt = length of sampling event (day)

Before the sediment flux was calculated, a linear regression of the nutrient concentration over time was investigated. When plotting time versus nutrient concentration, a linear relationship must be statistically significant before concluding that nutrient changes are actually taking place in the water column or sediment column. For this study, if the R^2 of this regression was > 0.65, it was considered a significant enough trend to continue with sediment flux calculation. The value of R^2 from a range of 0.6-0.79 is typically indicated as having "strong" correlation in a variety of fields (Evans, 1996).

The sediment nutrient flux was calculated next by subtracting out the activity in the water column and normalizing the chamber working volume to the area of sediments enclosed in the chamber. Since the entire depth of the water column is used, the normalization factor becomes equal to the depth of the water column in meters. Note that dC/dt and WC are in mg/L/day and g/m^3/day units, which are equivalent.

$$Sed = \left(\frac{dC}{dt} - WC\right) \times d \tag{3.6}$$

Where,

Sed = Sediment nutrient flux during daytime conditions ($g/m^2/d$)

WC = Rate of change of nutrient concentration in water column (g/m^3/day)

d = depth of ambient water column (m)

A negative rate or flux occurs when nutrients are being removed from the ambient water and a positive rate or flux occurs when nutrients are being added to ambient water. After calculation of *WC* and *Sed*, the two parameters can be compared directly by

normalizing one of the parameters to water depth. A water column aerial flux can be expressed by multiplying *WC* by the water depth. The *Sed* can be expressed as a rate influencing the ambient water by dividing by water depth.

3.5 Potential Denitrification Rate Experiment: Soil Slurry Incubation Using $^{15}NO_3^-$ Substrates

The rates of $^{30}N_2$ production were measured and calculated using a modification of the method of Long et al. (2013). Approximately 1 g (0.5gm for second sampling) of sediment was transferred to 12-ml Exetainer tubes (Labco, High Wycombe, United Kingdom) and sealed using gas-tight septa. Each tube was flushed with ultra-high pure (UHP) He gas for 8-10 minutes at 10psi and incubated overnight at room temperature to reduce the background concentrations of NO_3^- and NO_2^- (NO_x). After the initial overnight incubation, the tubes were flushed with Helium (He) gas to remove any produced N_2 gas.

Before staring the experiment, 1 mM $K^{15}NO_3$ (99.5 atom%; Cambridge Isotope Laboratory, Andover, MA) and 5M KOH solution were prepared and flushed with He in a gas tight serum bottle. $K^{15}NO_3$ was added to each tube using a 1 mL BD Luer-Lok™ disposable syringe (1/100 mL graduation). The syringes were flushed with He gas before every use. Time course incubation was carried out in duplicates (time points 0, 0.5 and 1 hour for first sampling and time points 0, 0.25 and 0.5 hour for second sampling) at room temperature. Figure 3.6 shows the experimental setup for denitrification rate experiment.

A 5M KOH solution was added at each time point after the incubation in order to stop microbial activity. After stopping microbial activity, the exetainers were vortexed briefly and centrifuged at 2000 rpm for 5 minutes. The exetainers were sent overnight to the Virginia Institute of Marine Science (VIMS) to measure the N_2 gas in the headspace

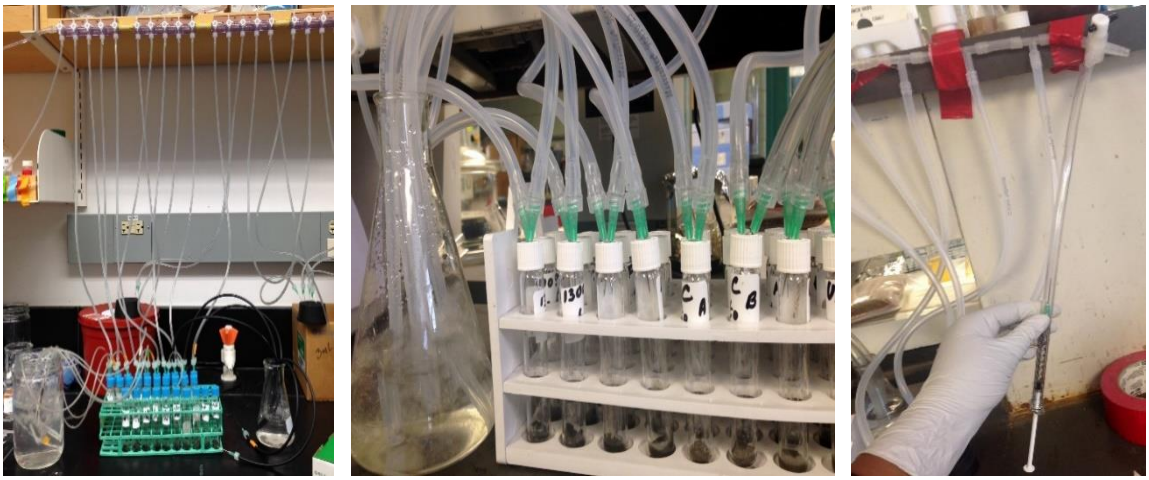

Figure 3.6, Experimental Setup for Denitrification Rate Using $^{15}NO3^-$ Substrates

of each sample. The N_2 gas was measured on a continuous-flow isotope ratio mass spectrometer (Thermo Finnigan Delta V; Thermo Scientific, Waltham, MA) in line with an automated gas bench interface (Thermo Gas Bench II). $^{30}N_2$ production was measured for all the samples on the same day.

3.6 Leaf Leachate and Serum Bottle Potential Denitrification with $^{14}NO_3^-$

Big Tooth Maple (*Acer grandidentatum*) leaf was selected for this section of the study due its greater local availability. To obtain leaf leachate, the leaves were first cleaned with deionized water to wash off the dust or other undesirable particles and then dried in an oven overnight (12-15 hours) at 60^{o}C. For each reactor, a weighed amount of leaves was added with 2 liters of deionized water. The stirrer was used at 45 rpm to create a disturbance to imitate the flowing river water. Water samples were collected every 24 hours to measure dissolved organic carbon (DOC). Figure 3.7 shows the reactor setup for

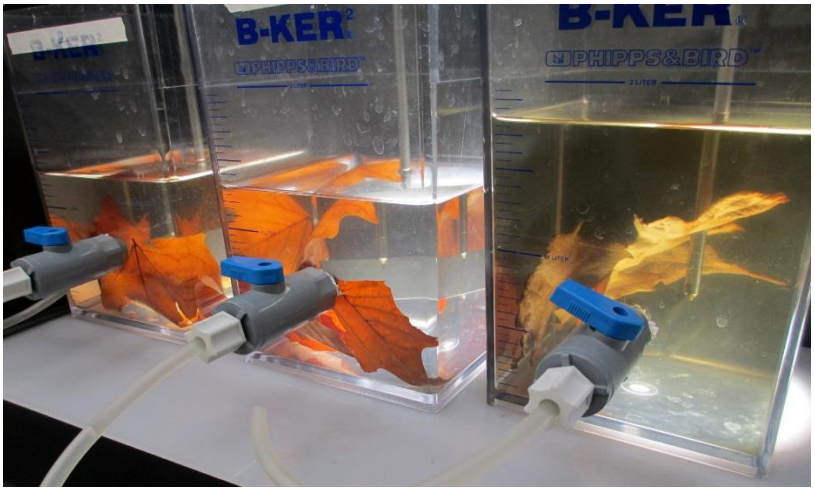

Figure 3.7, Experimental Batch Reactor Setup for Leaf Leachate Study

the leaf leachate experiment. The DOC was measured using a TOC-V instrument following the standard protocol provided from Schimadzu Corporation.

The soil sample from 0-10 cm depth was homogenized at first using a sterile spatula. Required amount of weighed samples was taken into each serum bottle. The same amount of sample was also used for total solid and total volatile solid analysis following EPA method 1684 (EPA, 2001). After adding the fixed amount of sediment, deionized water, leaf leachate, or acetate solution was added in to the serum bottles depending on the purpose.

The serum bottles were then crimped using grey septa, cap, and crimper, as shown in Figure 3.8. In order to make the system anoxic, dinitrogen gas was purged in to the serum bottle for about 15 to 20 minutes. As a source of nitrogen, sodium nitrate stock solution was added into the serum bottle after 10 minutes of purging of N_2 gas using a 5ml syringe. Purging was continued for 10 more minutes after nitrate addition. When purging was over, one of the bottles was opened and it was considered as time zero hour.

Figure 3.8, Preparation for Denitrification

The rest of the bottles were kept in a shaker and opened two at a time in a definite time interval for the rate experiment.

3.7 Potential Nitrification Rate Experiment

For nitrification experiments, the top 5-cm sediment sub-core was homogenized aseptically with a laboratory-scale spatula. A weighed amount of this homogenized sediment was then taken in to 600 ml sterile beakers in duplicate and mixed with deionized water to make a slurry. The mixtures were stirred continuously. Ammonia-nitrogen stock solution was added to start the nitrification process. Time points of 0 hour, 3 hours, 6 hours, and 10 hours were considered for the rate experiment. Mixed liquor (25 ml) was taken out after each time point using a sterile pipette and concentration of ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen was measured using Ion Chromatograph (IC 883) and Hach Nitrogen-Ammonia Reagent Set, TNT, AmVer (Salicylate), High Range. 25 ml of the mixed liquor was taken to analyze total solids and

volatile solids.

To evaluate the role of abiotic processes in ammonium fate, experiments were also conducted in the presence of a nitrification inhibitor (50 mg/L) allylthiourea. Allylthiourea was added with sediment slurry, and stirred for about 3 hours to inhibit the nitrification process. Equal target concentration of ammonia-nitrogen, as before, was added after 3 hours of mixing. Time point of zeroth and third, sixth, and tenth hour were considered after adding ammonia.

3.7 Identifying Bacteria Participating in Nitrification and Denitrification Using Advanced Bio-molecular Tools

Bio-molecular tools were used to investigate the nitrifying and denitrifying species in the sediment. Extracting DNA, running PCR, and gel electrophoresis were the primary steps to confirm the presence of specific genes in the sediment. To quantify these specific genes of interest, qPCR was performed. The nitrifying and denitrifying bacterial communities present in the sediment were then analyzed using TRFLP, and cloning and sequencing methods.

3.7.1 DNA Extraction

A small amount of collected sediment was homogenized and kept at -80^{o}C for the extraction of DNA. Duplicate DNA samples were extracted for each site using the PowerSoil (R) DNA Isolation Kit (12888-50, MoBio Laboratories Inc.). The protocol provided from the MoBio Laboratories was followed with some exceptions. In order to get a higher concentration of DNA, 400 mg of sediment sample was taken for each

extraction and 50µL of elution buffer was used in the last step of DNA extraction. Concentration of DNA for each site was measured using Nanodrop 2000 (Thermo, USA). Proper precautions were taken to avoid any contamination (such as cleaning the bench top with 70% alcohol before extraction, running milliQ water on Nanodrop before running the samples).

To ensure proper quality control, the ratios of absorbance between multiple wavelengths were observed. The purity of the DNA was accepted when the ratio of A260/A280 was around 1.8, indicating no contamination with protein while ratio of A260/A230 was 2.0 – 2.2 (William et al., 1997).

3.7.2 Functional Gene Identification: Polymerase Chain Reactions (PCR)

Polymerase Chain Reactions (PCR) were carried out using a Mastercycler gradient (Eppendorf, USA) for ammonia mono-oxygenase and nitrite reductase genes in order to identify ammonia oxidizing bacteria and denitrifying bacteria, respectively. Each PCR reaction mixture contained 12.5 µL of 2X GoTaq, 1.0 µL of each of 10 µM forward and reverse primers, 1.0 µL of 10mg/mL BSA, and 1-3 µL DNA template. Nuclease free ultrapure water was added to the mixture to scale up the volume to 25 µL. Table 3.2 shows the primers and PCR programs for *amoA* (AOB), and *nirS* and *nirK* target genes.

Gel electrophoresis was carried out using 1% agarose gel for the product obtained from PCR. Ethidium bromide was used along with 1X TAE buffer to prepare the gel. After the gel was solidified, it was submerged in TAE buffer and run at 80V for about an hour. A DNA ladder was used in every electrophoresis run to estimate the base pair size of the PCR product.

Table 3.2, Primers and PCR Programs for Target Genes

Metabolic Function	Gene	Primers Used	PCR Program	Reference
Nitrification	*amoA* (AOB)	amoA-1F (GGGGTTTCTACTGGTGGT) amoA-2R (CCCCTCKGSAAAGCCTTCTTC)	95°C, 5 minute (95°C, 60s; 56°C, 90s; 72°C, 90s) x 34; 72°C, 10 minute	Rotthauwe et al. (1997)
	nirS	cd3aF (GTCAACGTCAAGGAAACCGG) R3cd (GACTTCGGATGCGTCTTGA)	95°C, 2 minute (95°C, 15s; 60°C, 40s; 72°C, 30s) x 30; 72°C, 5 minute	Throback et al. (2004)
Denitrification	*nirK*	876F (ATYGGCGGVAYGGCGA) 1040R (GCCTCGATCAGRTTRTGGTT)	95°C, 2 minute (95°C, 15s; 63°C, 30s; 72°C, 30s) x 6 – touchdown 1°C each step until annealing temp = 51°C; (95°C, 15s; 60°C, 30s; 72°C, 30s) x 30; 72°C, 5 minute	Yu et al. (2014)

3.7.3 Quantifying Gene Copy Number: qPCR

To measure the gene copy number of *amoA* and *nirS* functional genes, a standard was prepared targeting each gene. In order to prepare the standards, clones carrying the particular functional gene were taken for incubation. After incubating the colonies overnight, plasmids were extracted and its concentration was measured using Nanodrop 2000 (Thermo, USA). The gene copy number of the standards was calculated from the plasmid DNA concentration using Equation 3.7.

$$\text{Number of copies (molecules)} = \frac{\boldsymbol{X}\ ng * 6.0221\ x\ 10^{23}\ molecules/mole}{\left(\boldsymbol{N} * 660\frac{g}{mole}\right) * 10^{9}\ ng/g} \qquad (3.7)$$

Where,

X = amount of amplicon (ng)

N = length of dsDNA amplicon

660 g/mole = average mass of 1 bp dsDNA

A calibration curve was prepared for each standard (*amoA* and *nirS*). The DNA of the sediment samples were run along with the three standards of known concentration. All the samples were run in triplicate to avoid possible error. The reagents used for running the qPCR analysis were 10 µL SYBR GREEN (light sensitive), 1 µL Forward Primer (10 µM), 1 µL Reverse Primer (10 µM), 1 µL BSA, 1-3 µL DNA Template, and ultrapure Nuclease free water. The Nuclease free water was added to scale up the volume to 20 µL. A mastermix was prepared using all the reagents except DNA, and added into a white skirted 96-well reaction plate. The melting curve for each sample was then compared to the melting curve of the standards to obtain the gene copy for each sample.

3.7.4 TRFLP for Nitrifying Functional Gene

TRFLP analysis was carried out for AOB in order to identify the species of nitrifying bacteria present. PCR and gel electrophoresis are run, and DNA is extracted from the gel to purify the DNA before running TRFLP. A labeled primer for *amoA* (1F 5'-GGGGTTTCTACTGGTGGT-3' labeled with blue dye, and 2R 5'-CCCCTCKGSAAAGCCTTCTTC-3' labeled with green dye) was used to run a 50 µL PCR. Before running the whole PCR product on gel electrophoresis, only 3 µL was first used to verify the product. If a single and bright band appeared, the rest of the PCR

product of samples was loaded on the gel for electrophoresis. A sterile blade was used to excise the bright gene band from the gel. The DNA were extracted from the gel using a QIAGEN Gel Extraction Kit (QIAGEN, USA) following the protocol provided by QIAGEN. All the steps were performed in a dark room as the primers were labeled with light sensitive dye. The concentration of DNA was measured using Nanodrop 2000 (Thermo, USA). After eluting the DNA, the sample was checked again on the Nanodrop to ensure a high enough DNA concentration required for further TRFLP analysis.

The extracted products were then digested with Taq 1 restriction enzyme, which creates fluorescently-labeled terminal restriction fragments. The size of each fragment varies for each species of AOB. Final samples were prepared using 2 uL of the digested product and 10 uL of Hi-Di formamide. These samples were then sent to the DNA Sequencing CORE facility at the University of Utah to run the TRFLP experiments. The resulting TRFLP electropherogram illustrated the fluorescence intensity at different base pair depending on the presence of AOB. Comparing the forward and reverse base pair size with literature, the AOB species was identified.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overview of the Chapter

Chapter 4 presents the results and discussion of the tasks that were completed to fulfill the objectives of the study. The discussion begins with a summary of the water characteristics at ambient conditions during different sampling and experimental events, followed by the results from sediment oxygen demand (SOD) experiments. Later sections discuss results from the nutrient flux experiments, and laboratory experiments to determine the potential denitrification and nitrification rates for selected river and wetland sites.

4.2 Ambient Water Characteristics

Two rounds of sampling were conducted, the first in July and the second in September of 2015, to record nutrient dynamics and sediment oxygen demand in early and late summer, respectively. Table 4.1 shows the ambient concentrations of nutrients and other parameters under consideration at selected five sites. Nutrient concentrations during some sampling events were under detection limit (UDL) and thus were not measurable. The detection limits for phosphate-phosphorous, nitrite-nitrogen/nitrate-

Table 4.1, Ambient Nutrient Concentrations in Water Column Measured at Each Site

Parameter	Sampling Event	1300 S	LNP	State Canal	Unit 1	Unit 2
NO_3-N (mg/L)	Early Summer	1.23	4.94	4.07	0.880	0.545
	Late Summer	3.76	4.88	3.18	0.089	3.220
NO_2-N (mg/L)	Early Summer	0.027	0.113	UDL	UDL	UDL
	Late Summer	0.093	UDL	UDL	0.144	UDL
PO_4-P (mg/L)	Early Summer	0.388	0.611	0.507	0.679	0.57
	Late Summer	0.283	0.530	0.698	0.095	UDL
NH_4-N (mg/L)	Early Summer	0.270	0.150	1.17	1.810	0.779
	Late Summer	0.408	0.244	1.38	0.947	0.127
DO (mg/L)	Early Summer	4.20	3.59	3.57	7.20	5.49
	Late Summer	5.93	5.44	7.26	7.65	7.30
Temperature (^oC)	Early Summer	23.16	24.35	23.89	22.28	23.74
	Late Summer	21.83	23.33	20.06	20.96	19.42
pH	Early Summer	7.78	7.79	7.68	9.16	8.96
	Late Summer	7.89	7.96	7.92	7.64	8.16

nitrogen, and ammonia-nitrogen were 0.006 mg/L, 0.006 mg/L, and 0.015 mg/L, respectively. Between the Jordan River sites, the Legacy Nature Preserve (LNP) site had higher nutrient concentrations when compared to 1300 South. Unit 1 had higher concentrations of phosphate and ammonium than Unit 2 wetland site. The dissolved oxygen (DO), in general, was lower in the river sites than wetland sites. Temperatures were lower during late summer.

4.3 Sediment Oxygen Demand (SOD)

Sediment oxygen demand accounts for the depletion of oxygen due to various biogeochemical activities at the sediment water interface (Caldwell and Doyle, 1995; Rounds and Doyle, 1997; Wood, 2001; Chen et al., 2012; Hogsett and Goel, 2013). SOD was measured at five selected sites in the Jordan River, Great Salt Lake wetlands, and State Canal during both early and late summer. Figure 4.1 illustrates the dissolved oxygen depletion profiles measured in the SOD and WC_{dark} chambers at Unit 1 site during late summer.

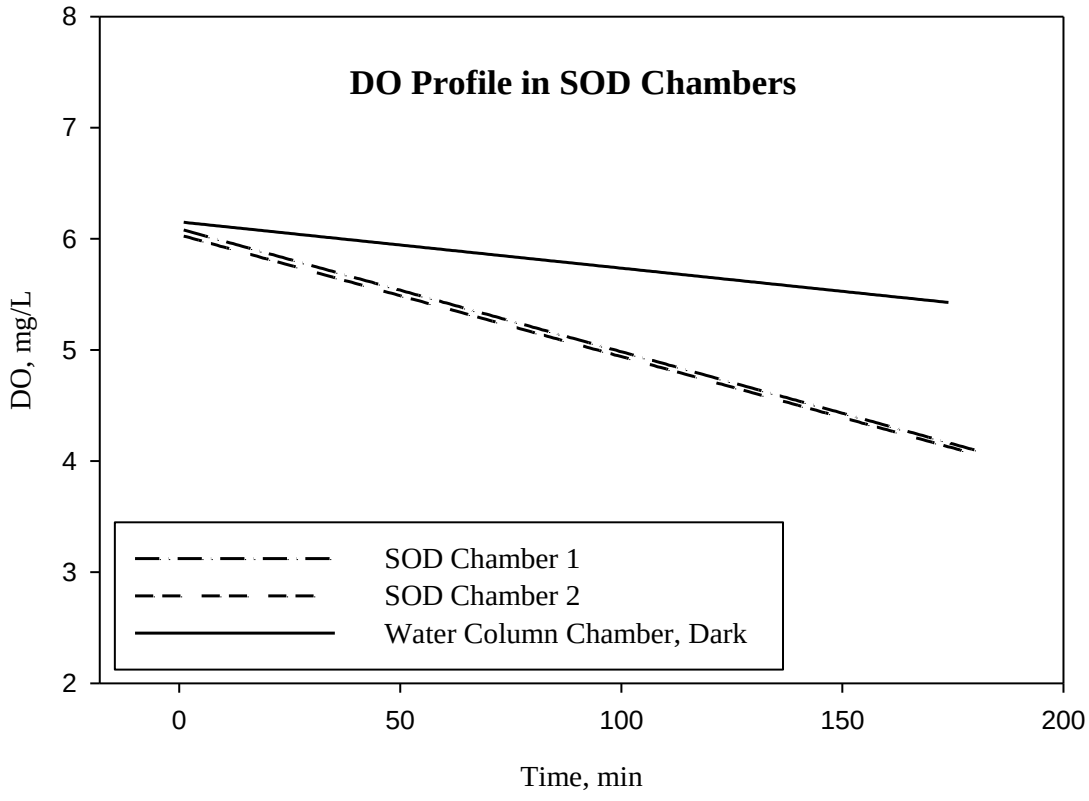

Figure 4.1, DO Profiles in Three SOD Chambers at Unit 1 (late summer)

In this sampling event, the DO profile for the WC_{dark} chamber is represented as the solid black line showing DO depletion. The dashed lines correspond to DO profiles for the two SOD chambers. The DO profile in both SOD chambers demonstrated a decreasing trend with time, which indicates that the DO was consumed in these chambers due to various biogeochemical activities (Hogsett and Goel, 2013). The slopes of the oxygen depletion profiles in both the SOD chambers were much higher than that of WC_{dark} chamber. These slopes were used to calculate oxygen consumption rates for each chamber.

Figure 4.2 shows the sediment oxygen demand (SOD) results for all sampling events in early and late summer. All the SOD results were normalized for temperature at 25ºC following Butts and Evans (1978). The SOD ranged from 2.4 to 2.9 g-DO m^{-2} day^{-1} in Jordan River sediments, whereas at wetland sites, the SOD was as high as 11.8 g-DO m^{-2} day^{-1}. The SOD rates obtained in this study were comparable to the values reported in Butts and Evans (1978), Schnoor and Fruh (1979), Caldwell and Doyle (1995), Rounds and Doyle (1997), Ziadat and Berdanier (2004), Utley et al. (2008), and Hogsett and Goel (2013). Higher SOD during late summer may be attributed to the fallen leaves adding organic matter loads to the river and wetlands and the subsequent increase in bacterial metabolism (Hogsett and Goel, 2013). It also emphasizes that predicted SOD values using temperature correction equations may not reflect actual SOD values and stream metabolism (Hogsett and Goel, 2013), which is vital to stream and wetland management decisions.

Butts and Evans (1978) categorized the benthic sediment condition based on the SOD_{T25C} values. Table 4.2 presents the classification of sediment based on SOD values

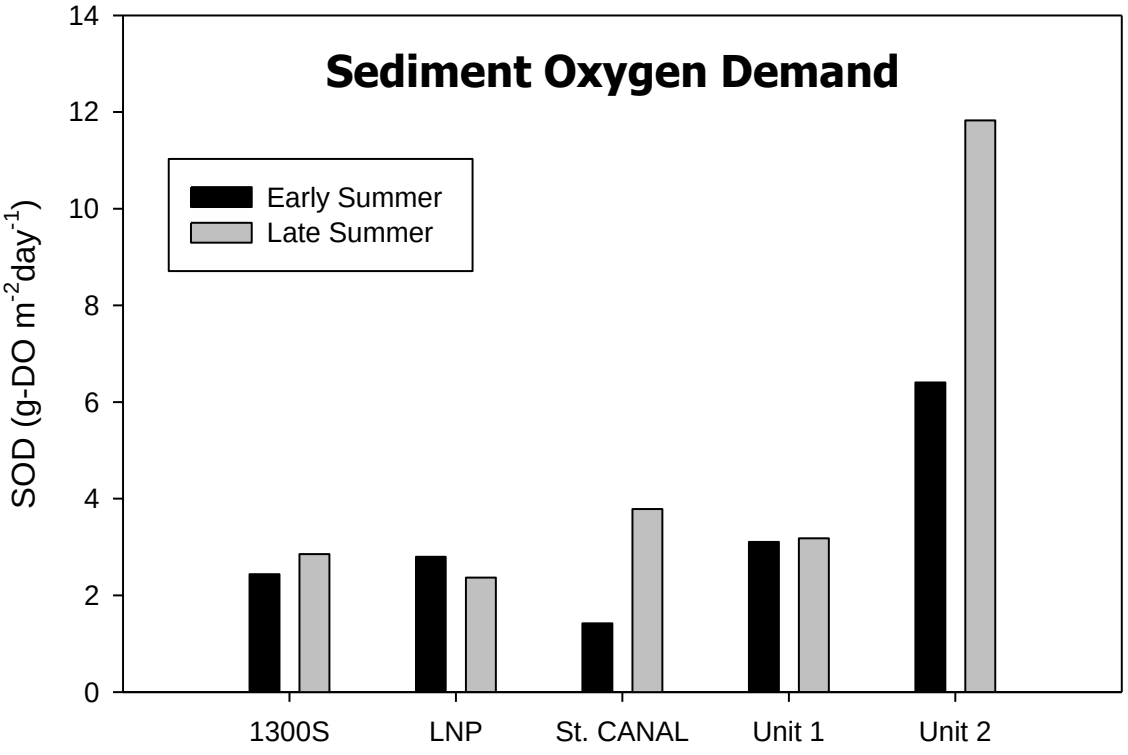

Figure 4.2, Sediment Oxygen Demand (SOD) Results for all Sampling Events

Table 4.2, Benthic Sediment Condition at Different SOD Ranges

SOD range g-DO/m^2/day	**Benthic Sediment Condition**
< 0.5	Clean
0.5 – 1.0	Moderately Clean
1.0 – 2.0	Slightly Degraded
2.0 – 3.0	Moderately Polluted
3.0 – 5.0	Polluted
5.0 – 10.0	Heavily Polluted
> 10	Sewage Like Sludge

(Butts and Evans, 1978; Hogsett and Goel, 2013). Based on Butts and Evans (1978) and Hogsett and Goel (2013), the classification of sediment of selected sites in this study is provided in Table 4.3. The least polluted site was 1300 South in the Jordan River which can be categorized as 'moderately polluted'. The average SOD flux of Unit 2 wetland characterizes its deteriorated condition and its classification as 'heavily polluted'. The higher percentage of volatile solids obtained at this site also suggests its high organic matter pollution.

The percentage of the ambient DO deficit associated with SOD was calculated for each sampling event. The range of results were 72-97% for 1300 South, 72-90% for Legacy Nature Preserve, 64-96% for State Canal, 33-43% for Unit 1, and 79-87% for Unit 2 site. These ranges agree with Hogsett and Goel (2013) who also calculated the

Table 4.3, Average SOD Results from Five Sites

Site Name	**SOD_{T25}**	**Benthic Sediment Condition**
1300 South	2.64 ± 0.31	Moderately Polluted
Legacy Nature Preserve	2.58 ± 0.52	Moderately Polluted to Polluted
State Canal	2.60 ± 1.54	Moderately Polluted to Polluted
Unit 1	3.14 ± 1.02	Polluted
Unit 2	8.21 ± 3.21	Heavily Polluted to Sewage Like Sludge

percentage of the ambient DO deficit due to SOD for Jordan River sites. The majority of the ambient DO deficit was found to be associated with SOD with more than 70% of ambient DO demand partitioned into sediments. Interestingly, 57-67% of the DO demand at Unit 1 is associated with water column, which means oxygen demand in water column was higher than that of sediment.

4.4 Nutrient Flux

Benthic nutrient fluxes determined with flux chambers help understand the combined effect of bio-chemical reactions like ammonification, nitrification, and denitrification at the sediment surface (Friedrich et al., 2002; Hantush et al., 2013). Sediment nutrient flux experiments for selected sites in Jordan River and Great Salt Lake wetland were conducted in July and September considering both ambient conditions and the nutrient pulsed state. After conducting first 3 hours of the experiment at ambient condition, the chambers were spiked to 0.5 mg/L ammonia-nitrogen, 0.5 mg/L nitrate-nitrogen, and 0.1 mg/L phosphate-phosphorous to observe the reactions to the nutrient pulse. The nutrient spike also made nutrient concentrations become noticeable which were below detection limit previously.

Figure 4.3, Figure 4.4, and Figure 4.5 illustrate the ammonium, nitrate, and phosphate flux of Jordan River sites, wetland sites, and State Canal location, respectively, at both unspiked and spiked condition during early (July) and late (September) summer. A negative bar in these plots indicates sediment as a sink, while positive bar represents source of nutrient. Under environmental condition, these positive and negative nutrients fluxes can be the results of various biogeochemical reactions occurring at water sediment

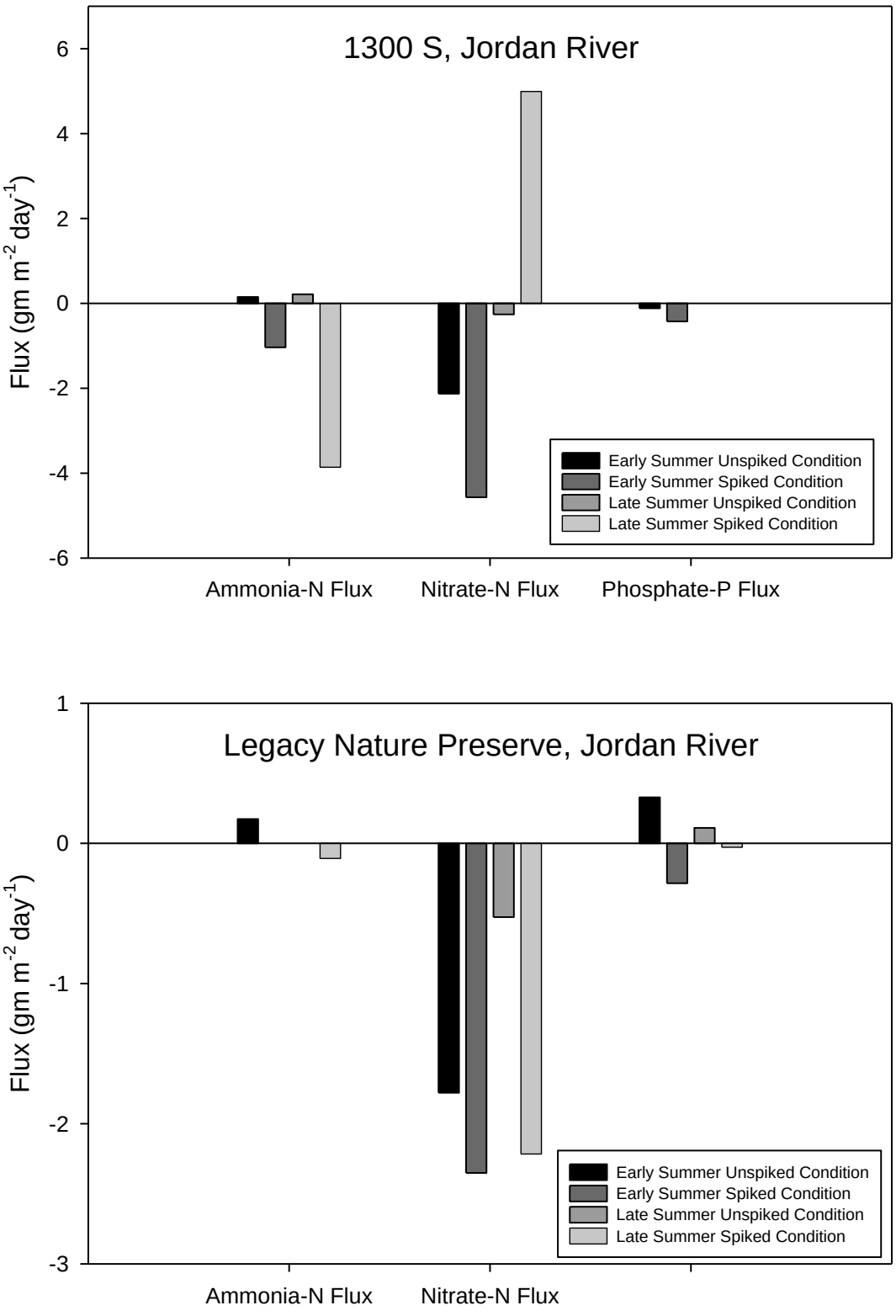

Figure 4.3, Nutrient Fluxes for Jordan River Sites

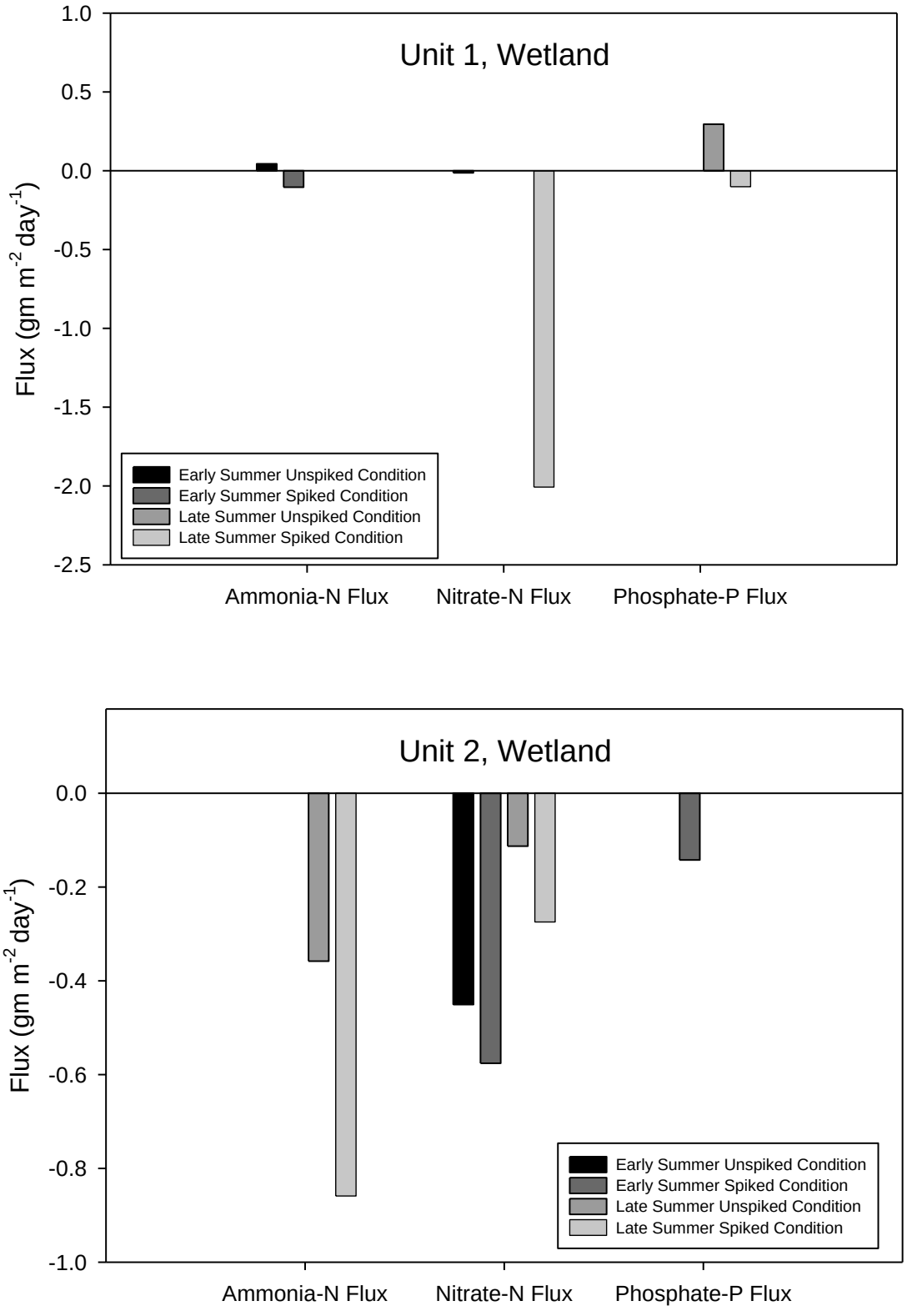

Figure 4.4, Nutrient Fluxes for Wetland Sites

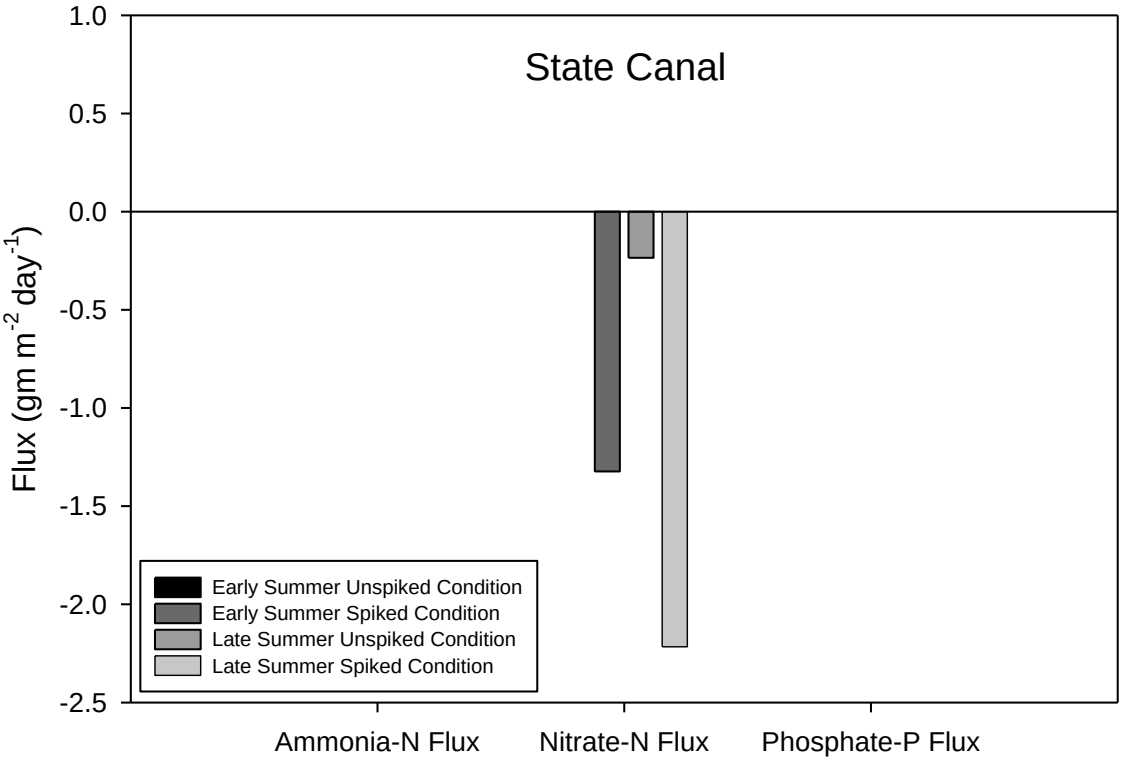

Figure 4.5, Nutrient Fluxes for State Canal Site

interface (Boulton et al., 1998; Friedrich et al., 2002; Lefebvre et al., 2006). For example, a positive ammonium flux indicates the possibility of ammonification (Strauss and Lamberti, 2002; Lillebø et al., 2007), while negative ammonium flux can be explained by nitrification (Schmidt, 1982; Strauss and Lamberti, 2000). However, nitrification and ammonification may occur simultaneously with nitrate reduction in stream sediments (Wyer and Hill, 1984). For nitrate, a negative flux typically represents denitrification (Holmes et al., 1996; Bartkow, 2004; Beaulieu et al., 2011). A positive nitrate flux is indicative of ammonia oxidation (Strauss and Lamberti, 2000).

In case of phosphate-phosphorous, a positive flux typically occurs due to

decomposition/mineralization of organic matter (Lowrance et al., 1984; McMillan et al., 2013) and/or its release and re-suspension from sediment bed (Froelich, 1988; Macrae et al., 2003). However, simultaneous negative fluxes for both nitrogen (ammonia and nitrate) and phosphate may also be caused from algal uptake (Humborg, 1997; Ho et al., 2003).

For the 1300 South location at Jordan River, ammonium flux increased during early summer for unspiked (ambient) condition, most likely due to ammonification (Strauss and Lamberti, 2002). Simultaneously, nitrate and phosphate fluxes were negative, which was probably the result of denitrification (Seitzinger et al., 2006; Beaulieu et al., 2011) and algal uptake (Humborg, 1997). After addition of the nutrient pulse, all these fluxes became negative. Additional bioavailable nutrients perhaps supported higher denitrification (Beaulieu et al., 2011), causing greater negative nitrate flux. Negative ammonium flux was the result of higher nitrification rate instigated by the nutrient pulse (Kemp and Dodds, 2002; Strauss et al., 2002; Starry et al., 2005), which presumably dominated ammonification (Kadlec et al., 2009).

Comparing to early summer, similar responses were observed for ammonium, nitrate, and phosphate fluxes for ambient condition during late summer. A greater negative ammonium flux during late summer was observed for spiked condition. Interestingly, at this condition, nitrate flux was found to be positive. This was most likely caused by greater nitrate production through ammonia oxidation than nitrate reduction from denitrification due to the presence of nutrient pulse (Kemp and Dodds, 2002; Levi et al., 2013).

For Legacy Nature Preserve site, nitrate flux was negative for both unspiked and

spiked condition during early and late summer. These fluxes were found to be greater for spiked condition compared to unspiked condition. When the nutrient pulse was introduced, phosphate flux became negative. This behavior can be explained by higher algal uptake due to the presence of bioavailable nutrients (Tantanasarit et al., 2013).

Compared to the Jordan River Sites, nutrient fluxes were observed to be lower at the wetland sites. However, the pattern of fluxes were similar, except for the fact that ammonium flux for Unit 2 was negative at unspiked condition, indicating nitrification at ambient condition (Strauss and Lamberti, 2000; Strauss and Lamberti, 2002). At State Canal location, fluxes were not observed for ammonium and phosphate. Nitrate flux followed the typical pattern found for river and wetland sites.

Overall, the range of fluxes observed in this study for the selected Jordan River sites and State Canal location were -3.9 – 0.2 g m^{-2} day^{-1} for ammonium, -4.6 – 5.0 g m^{-2} d^{-1} for nitrate, and -0.4 – 0.3 g m^{-2} d^{-1} for phosphate; and for wetland sites were -0.9 – 0.0 g m^{-2} d^{-1} for ammonium, -2.0 – 0.0 g m^{-2} day^{-1} for nitrate, and -0.1 – 0.3 g m^{-2} day^{-1} for phosphate.

Ammonification rates found in different studies for lake and river sediments (Höhener and Gächter, 1994; Fisher et al., 2005; Reddy, 2008; deBusk et al., 2001; VanZomeren et al., 2013) ranged from 0.004 – 0.357 g NH_4-N m^{-2} day^{-1}, which compares favorably with the positive ammonium flux results of this study. Malecki et al. (2004) also reported an average NH_4-N release rate of 0.018 g m^{-2} day^{-1} from the anaerobic river sediment.

Nitrification rates of river and lake sediments reported in other studies, ranging from 0.01 – 0.42 g N m^{-2} d^{-1} (Pauer and Auer, 2000; deBusk et al., 2001), also compare

well to the negative ammonium flux. Nitrate uptake rates for stream reported in literature, such as Mulholland et al. (2004), (0.027 – 0.138 g N m^{-2} day^{-1}), were lower than the sediment nitrate uptake rate obtained in this study.

Jenson and Anderson (1992), Reddy (1999), Malecki et al. (2004), and Fisher et al. (2005) found sediment flux rates of phosphorus to be 0.015 – 1.1 g PO_4-P m^{-2} day^{-1}; fairly close to the results from this study. Figures in the Appendix show the nitrate, phosphate, and ammonia fluxes separately for the selected sites in this study.

4.5 Potential of Denitrification

4.5.1 Presence of Denitrifying Genes

Denitrifying bacteria usually contain either the *nirK* or *nirS* enzyme (Bothe et al., 2007). The polymerase chain reaction (PCR) was performed for *nirS* and *nirK* genes. To detect the presence of denitrifying genes in the sediment samples from selected sites, gel electrophoresis was performed using the PCR product.

Figure 4.6 shows the presence of *nirS* and *nirK* gene in the sediment of each site obtained from gel electrophoresis. Bright singular band in the gel identifies the presence of genes. As seen from Figure 4.6, bright bands for *nirS* gene were found at all the sites, except for State Canal and Unit 2 (early summer). The brighter the band, the more possibility there is of the presence of higher gene copy number in the sediment.

In case of *nirK* gene, only Legacy Nature Preserve and Unit 2 sites showed bright band in late summer samples indicating presence of *nirK* gene. As mentioned above, denitrifying bacteria usually contain either the *nirK* or the *nirS* enzyme, is most likely the reason for lesser presence of the *nirK* enzyme in the sediment.

Figure 4.6, Gel Electrophoresis for a) *nirS,* b) *nirK* (1: Early summer, 2: Late summer)

4.5.2 Abundance of Denitrifying Genes

After confirming the presence of denitrifying genes from PCR and gel electrophoresis, quantitative PCR was performed on *nirS* and *nirK* gene to find out their abundance. From previous studies (Philippot, 2002; Henry et al., 2004; Bothe et al., 2007), it is known that each denitrifying bacterial genome contains one of *nirS* or *nirK* gene copy. Figure 4.7 shows the *nirS* gene copy number for selected sites during both early and late summer. As seen from the figure, *nirS* gene was found to be abundant at Jordan River and wetland sites, ranging from 5.5×10^9 to 4.9×10^{10} copies per gram dry sediment. In comparison, State Canal site had lower *nirS* gene copy number (2.9×10^6 – 3.8×10^7), supporting the faded band obtained during gel electrophoresis (Figure 4.6). The gene copy numbers for all sites from two sampling times were very similar. The slight decrease in late summer can be attributed to the decrease in water temperature from

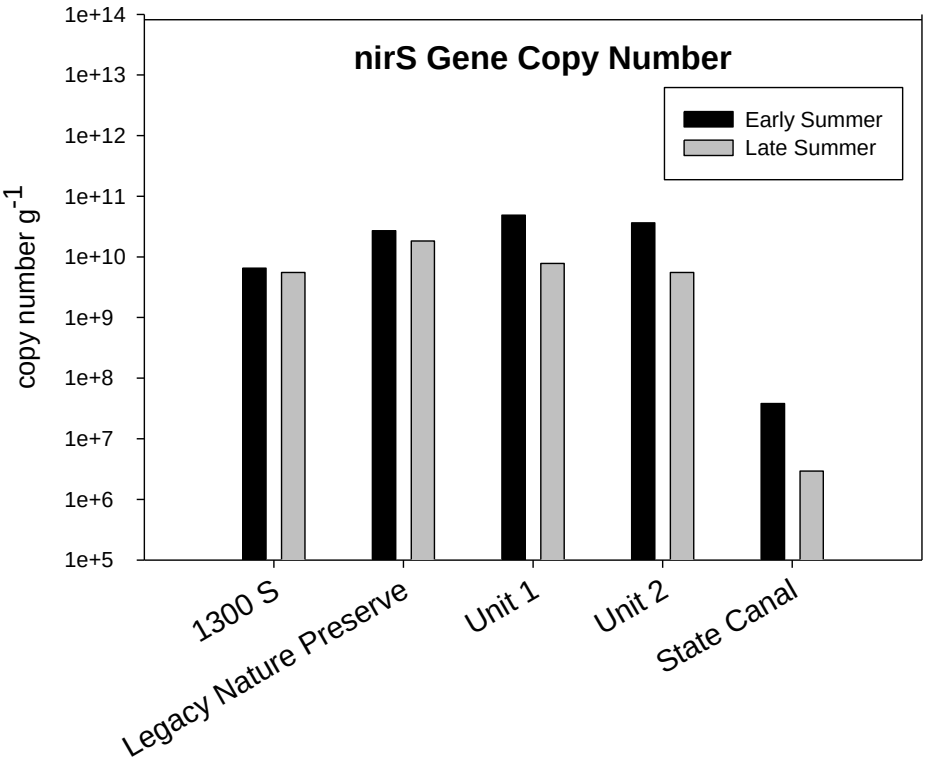

Figure 4.7, *nirS* Gene Copy Number

July to September. Gene copy number of *nirK* was not detected from the from the qPCR experiment.

The gene copy number of *nirS* and *nirK* found from other studies ranged from 10^7 – 10^{10} and 10^4 – 10^7 per gram sediment, respectively, in rivers (O'Connor, 2006; Veraart et al., 2014), and 10^4 – 10^9 copies *nirS* per gram dry sediment in wetlands and marshes (Kim et al., 2008; Bowen et al., 2011; Chon et al., 2011), which agrees well with the results of this study. In comparison, the abundance of *nirS* genes in wastewater was found to be 10^4 – 10^5 copies per gm DNA (Wang et al., 2014), whereas in this study, the range of *nirS* gene copies per gm DNA was found to be 10^3 – 10^6. Interestingly, a municipal WWTP with similar *nirS* gene copy number is removing significant nitrogen (Wang et

al., 2014). The similarity of the river and wetland *nirS* gene copy number obtained from this study to the *nirS* gene copy number of an engineered ecosystem indicates that there is high potential of nitrogen removal in the Jordan River sites and wetland sites in a favorable environment.

4.5.3 Potential Denitrification Rate

After studying the presence of denitrifiers in the sediment, a potential denitrification experiment was conducted using ^{15}N as the nitrogen source. The $^{30}N_2$ production from $^{15}NO_3$ tracer incubations is considered an indication of the presence of denitrification in the soils samples (Long et al., 2013). The $^{30}N_2$ production rates from tracer incubations were used to calculate the potential rates of denitrification. Denitrification potential is assumed to correspond to the maximum denitrification rate (Holmes et al., 1996).

Figure 4.8 shows the potential denitrification rates obtained for each site during early and late summer. The $^{30}N_2$ production rates from denitrification varied from 0.01 – 0.16 mg N_2-N g^{-1} day^{-1} for the river, wetland sites, and State Canal location.

The potential denitrification rates were in general lower during the late summer, which were consistent with the results of gene copy number obtained. The only exception is the rate obtained for Legacy Nature Preserve during late summer. Potential denitrification rates measured using $^{14}NO_3$ in serum bottle experiments were close to these results. Literature values of potential denitrification rates in wetlands range from 0.01 – 0.34 mg-N g^{-1} day^{-1} (White and Reddy, 2003), fairly comparable to the results of this study.

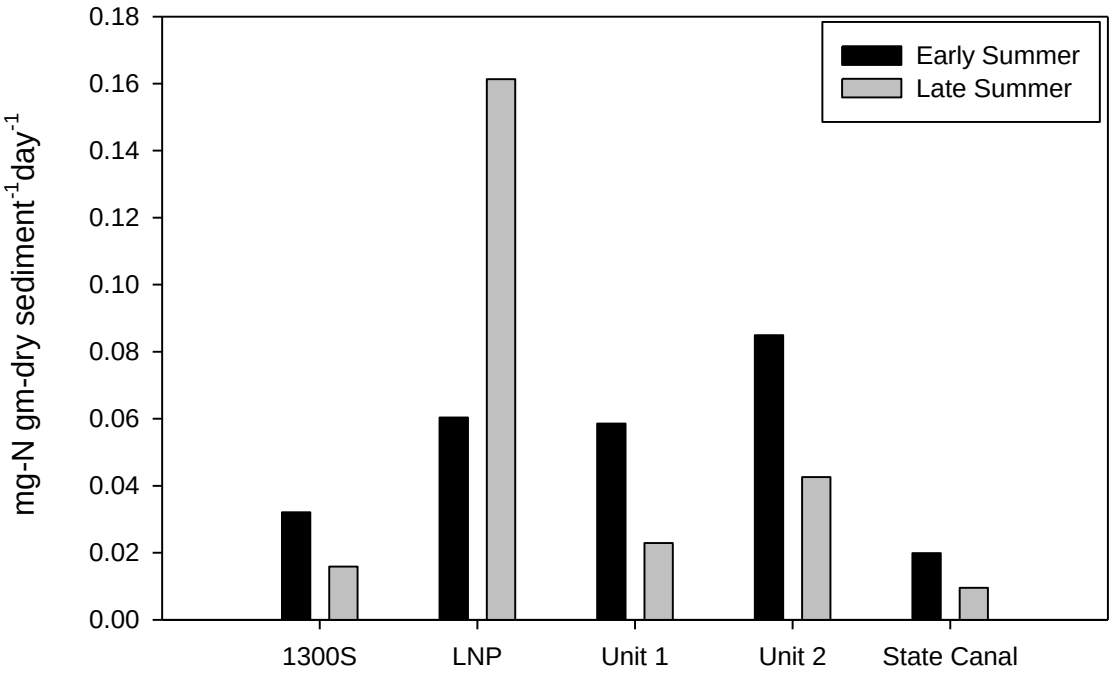

Figure 4.8, Potential Denitrification Rates at Selected Sites

Further analysis was done to compare the potential denitrification rates with in-situ nitrate flux. The sediment density was calculated following Avnimelech et al. (2001). Avnimelech et al. (2001) tested the correlation between bulk density and organic matter in six different systems including rivers, lake, sea floor, and pond sediments. Sediment bulk density was found to be inversely related to the organic carbon concentration, which followed the regression equation given below.

$$\text{Bulk Density g / cm}^3 = 1.776 - 0.363 \log_e \text{OC} \quad (R^2 = 0.70) \qquad (4.1)$$

Where, OC is the organic carbon concentration (mg/g).

The organic carbon (OC) was considered to be 50% of the volatile solids (VS) in the sediment, following Hogsett and Goel (2013) and Hogsett (2015). The calculated potential denitrification rates (per area), presented in Table 4.4, ranged from 0.84 – 12.45 g-N m^{-2} day^{-1}. Comparing these rates with in-situ nitrate flux can provide more insight into the nutrient flux experiments conducted for this study. For example, the in-situ nitrate reduction at Unit 2 wetland site was about 7-8% of the potential denitrification rate. This indicates that there is more potential for denitrification at this site depending on favorable environmental condition. Earlier, it was stated that the nitrate reduction at Unit 2 was perhaps due to denitrification.

Comparison of the potential denitrification rates with the in-situ nitrate fluxes supports that, and also strengthens the findings from nutrient flux experiments. On the other hand, at the 1300 South location during early summer, the potential denitrification rates were found to be smaller than the in-situ nitrate flux. This indicates that the nitrate decrease was possibly a combined effect of denitrification and algal uptake.

Previous studies reported potential denitrification rates (per area) of roughly 0.003 – 1.02 g-N m^{-2} day^{-1} in wetland sediments (Gale et al., 1993; deBusk et al., 2001; Risgaard-Petersen, 2003; Bastviken et al., 2005; Qiuying et al., 2012). The results of this study are, in general, higher than the values reported in literature.

4.5.4 Organic Carbon Source for Denitrification

Denitrification requires organic carbon to be used as an electron donor in order to complete reduction of nitrate to nitrogen gas (Bernet et al., 1996; Holmes et al., 1996; Aravena and Robertson, 1998; van Rijn et al., 2006). To observe the effect of naturally

Table 4.4, Potential Denitrification Rates (g N_2-N m^{-2} day^{-1}) Per Area

Site Name	Potential Denitrification Rates	
	Early Summer	Late Summer
1300 South	2.505	1.242
Legacy Nature Preserve	4.658	12.451
Unit 1	4.295	1.681
Unit 2	7.083	3.553
State Canal	1.753	0.841

available organic carbon sources for denitrifiers, serum bottle denitrification experiments were performed using leaf leachate. In addition, the denitrification rates were compared with the rate obtained using acetate as carbon source. Biomass was used from an in-house activated sludge reactor (ASP) for both the experiments. These results were also compared with serum bottle denitrification experiments using Jordan River sediment as biomass.

Figure 4.9 shows the dissolved organic carbon (DOC) leached from Big Tooth Maple (*Acer grandidentatum*) leaves over the experiment period. The curve illustrates DOC leached in mg/L, while the bars indicate DOC in mg per gram of dry leaves per day. Big Tooth Maple leached 10.8 mg-DOC/gm of dry leaves in 24 hours.

Figure 4.10 provides the results from denitrification experiment with leaf leachate and ASP biomass. It can be seen from the figure that both nitrate-nitrogen and COD

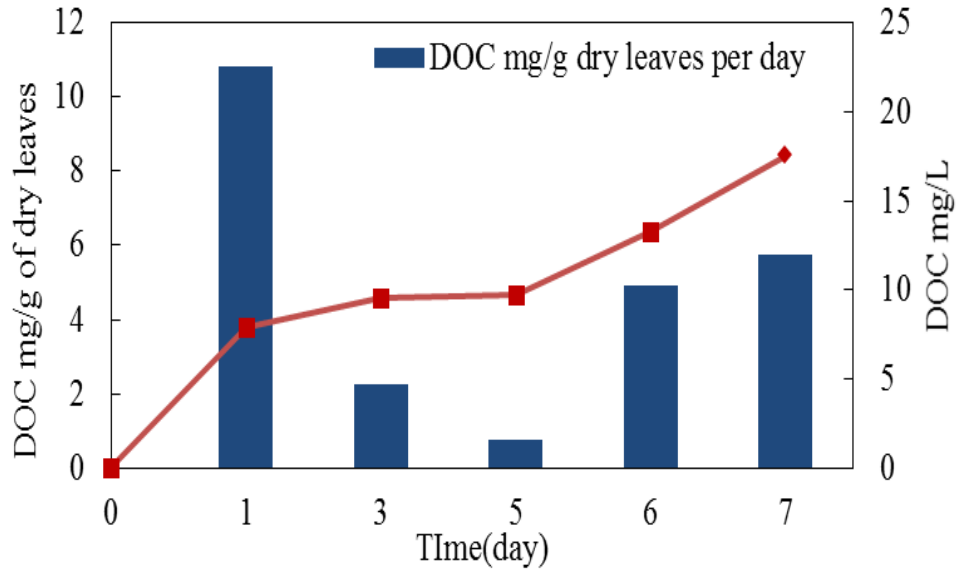

Figure 4.9, Dissolved Organic Carbon (DOC) Leached from Big Tooth Maple Leaves

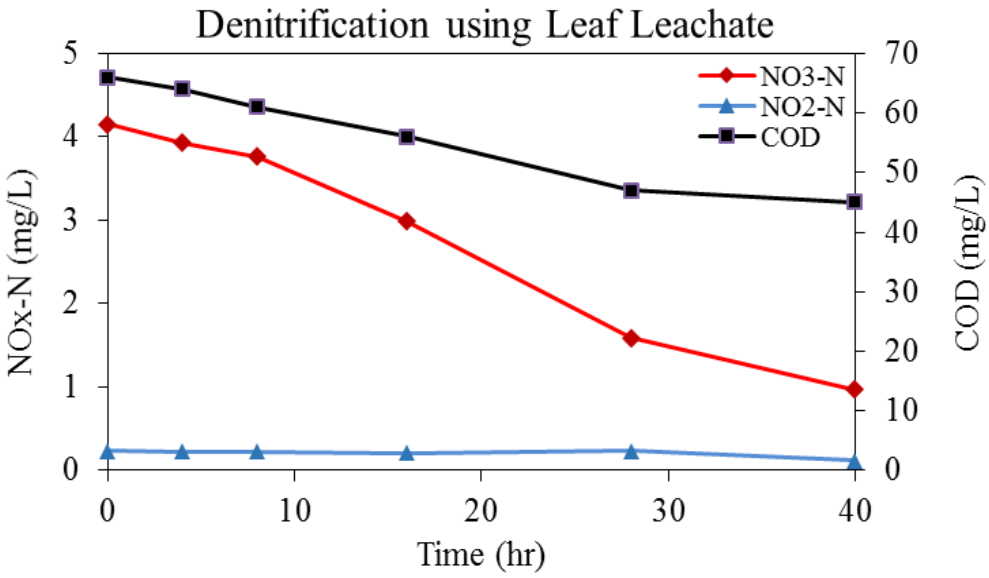

Figure 4.10, Denitrification Results Using Leaf Leachate as Carbon Source

decreased with time, indicating denitrification. The reaction of nitrite converting to gaseous nitrogen occurred very fast because of its unstable nature (Biswas and Nandy, 2015). Hence, a significant change in nitrite-nitrogen was not observed.

Table 4.5 summarizes the results obtained in denitrification experiments using different carbon sources. This experiment confirmed that biomass from ASP could use leaf leachate as a source of organic carbon to denitrify the available nitrate.

When Jordan River sediment was used as biomass, the denitrification rate using leaf leachate was found to be higher than the rate using no carbon source. Thus, the organic carbon leached from leaves is supporting the denitrification reaction in the ecosystem.

Table 4.5, Denitrification Rates Obtained with Different Carbon and Biomass Sources

Denitrification Rates (mg-N/gm VS/day)			
Biomass	Added Carbon Source		
	Acetate	Leaf Leachate	None
1300 S	2.47	1.85	0.713
Legacy Nature Preserve	2.88	1.78	1.09
In-house ASP	58.8	2.21	N/A

4.6 Potential of Nitrification

4.6.1 Presence of Nitrifying Genes

The polymerase chain reaction was conducted for ammonia monooxygenase (*amoA*) gene. Gel electrophoresis was performed using the PCR product to detect the presence of this nitrifying genes in the sediment samples. The results of gel electrophoresis is shown in Figure 4.11. The bright singular band at 491 base pair in the gel identifies the presence of *amoA* gene in the sediments of each site. Bright bands were found for all the sites, except for State Canal. The nonappearance of *amoA* bands for these sediment samples suggested the absence of ammonia-oxidizing bacteria.

4.6.2 Abundance of Nitrifying Genes

After confirming the presence of nitrifying genes from PCR and gel electrophoresis, quantitative PCR was performed on ammonia monooxygenase α-subunit (*amoA)* gene to find out its abundance. From previous studies (Hommes et al., 1998; Dionisi et al., 2002), it is known that each ammonia-oxidizing bacterial cell contains two

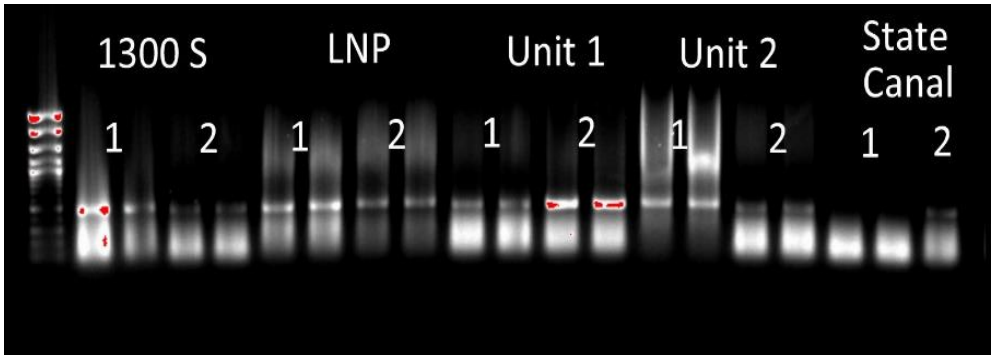

Figure 4.11, Gel Electrophoresis for *amoA* Gene (1: Early summer, 2: Late summer)

copies of the *amoA* gene. Figure 4.12 shows the *amoA* gene copy number for selected sites during both early and late summer.

As seen from Figure 4.12, *amoA* gene was found to be abundant at the Jordan River and wetland sites during early summer. The same was found during late summer, except for the 1300 South location where *amoA* presence was not detected. Gel electrophoresis also indicated less abundance of *amoA* gene at 1300 South for this sampling event.

Overall, the *amoA* gene copy number ranged from 1.9×10^7 to 1.4×10^{10} copies per gram dry sediment for the river and wetland sites. In comparison, *amoA* gene was found to be absent at State Canal site, as suggested from the gel electrophoresis outcome in Figure 4.11.

The gene copy numbers from the two sampling time were very similar at different sites. The decrease of gene copy number in late summer at Legacy Nature Preserve and Unit 2 locations can be attributed to the decrease in water temperature from July to September. The number gene copy of *amoA* found from other studies ranged from 10^3 to 10^7 copies per gram dry sediment for rivers and wetlands (Erguder et al., 2009; Sims et al., 2012), which was comparable to the results from this study.

In industrial and domestic wastewater treatment systems, the abundance of the *amoA* gene was found to be 7.2×10^3 to 3.6×10^9 copies per gm dry solid (activated sludge or biofilm) (Bai et al., 2012). The similarity of the river and wetland *amoA* gene copy number obtained in these study to the *amoA* gene copy number of engineered ecosystem indicates that these natural ecosystems have high potential of ammonia oxidation under favorable condition.

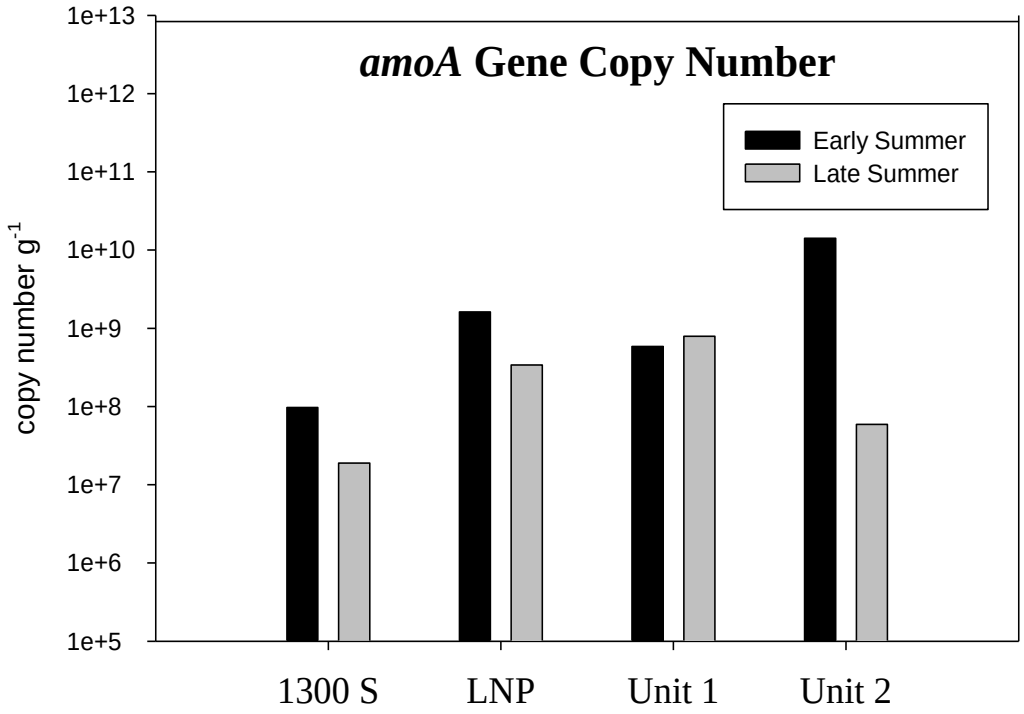

Figure 4.12, *amoA* Gene Copy Number

4.6.3 Identification of Nitrifying Species

To identify the ammonia oxidizing bacteria (AOB) present in the sediments, TRFLP experiment was conducted using amplified *amoA* gene. Figure 4.13 illustrates the presence of AOBs found in the sediments for each site. Samples showed T-RF peak at 283/206 and 491/488, which corresponds to *Nitrospira*-like AOB and *Nitrosomonas europaea/eutropha* lineage, respectively (Park and Noguera, 2004; Gilomen, 2008; Whang et al., 2009). Both *Nitrosomonas europaea* and *Nitrospira*-like AOB dominated at the Jordan River sites, while wetland sites and State Canal location was dominated by *Nitrosomonas europaea* only.

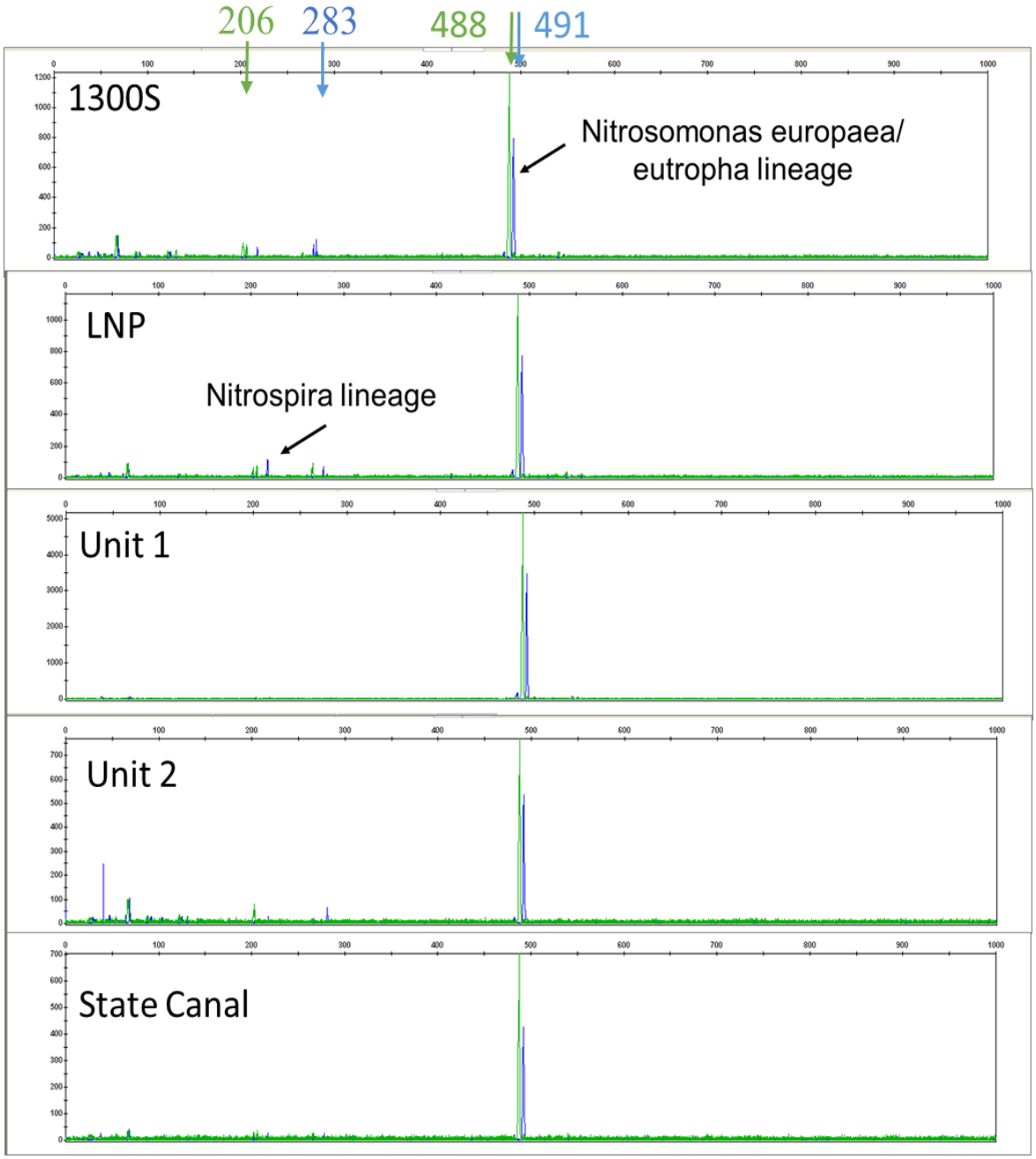

Figure 4.13, Electropherograms of *amoA* Gene TRFLP Specific to AOB

Typically found in the treatment of industrial and sewage waste (Chain et al., 2003), *Nitrosomonas europaea* is an ammonia-oxidizing bacterium (Chain et al., 2003) that lives in places rich in ammonia and inorganic salt (Shrestha et al., 2001). *Nitrospira* lineage, on the other hand, is a Nitrosospira-like AOB (Park and Noguera, 2004; Gilomen, 2008). These bacteria are considered the dominant nitrifiers in wastewater treatment plants (Park and Noguera, 2004; Siripong and Rittmann, 2007; Whang et al., 2009). Therefore, the presence of *Nitrosomonas europaea* and *Nitrospira* lineage in Jordan River and wetland sediments, as found from this study, point toward the degraded and polluted nature of the sediments due to the contamination of high levels of nitrogen compounds (Shrestha et al., 2001), particularly ammonia, as AOBs mostly thrive in areas of high ambient ammonia concentrations (Erguder et al., 2009; Sims et al., 2012). Moreover, ambient low dissolved oxygen (3.6-7.6 mg/L), ammonia concentration (0.13-1.38 mg/L), and water temperatures (19.4-23.9 ^{o}C) at the selected sites (Table 4.1) also were within the optimal growth range of the AOBs (Erguder et al., 2009).

4.6.4 Nitrification Rates

After studying the presence of *amoA* gene in the sediment, a potential nitrification experiment was conducted at the laboratory. The potential nitrification rate is the nitrification rate that occurs under ideal conditions in the presence of ample NH_4^+, well aerated soil, and without any restriction on NH_4^+ diffusion (Sujetovienė, 2010). It can be considered as a measure for the nitrifying biomass present at time of sampling (Bodelier et al., 1996; de Bie et al., 2002). High potential nitrification is often accompanied by strong oxygen depletion (Garnier et al. 2001, Cébron et al. 2003; Cébron et al. 2005).

Table 4.6 shows the potential nitrification rates obtained for each sites during early and late summer. Absence of nitrifying activity at State Canal site, as seen from the potential rate table, also supported the findings of gel electrophoresis and *amoA* gene copy number experiments. In comparison, nitrification rates for wetland sites, in general, indicated absence of nitrifying activity, although gel electrophoresis and *g*ene copy number experiments showed presence of *amoA* gene. This suggested that the nitrifying genes present at these sites were most likely inactive. Further evidence to this statement came from the negative nitrate flux results of the nutrient flux experiments at the wetland sites.

For Jordan River sites, the nitrification rates varied from 0.008 to 0.07 mg-N g^{-1} day^{-1}. The decrease of potential nitrification rate at 1300 South from early to late summer

Table 4.6, Potential Nitrification Rates

Sites	Nitrification rate (mg-N g^{-1} dry sediment day^{-1})	
	Early Summer	Late Summer
1300 South	0.06	0.008
Legacy Nature Preserve	0.04	0.07
State Canal	N/A	N/A
Unit 1	N/A	N/A
Unit 2	0.11	N/A

was supported by the results from PCR. Results of potential nitrification rates were, in general, coherent with the gene copy number. However, comparison of these results with nutrient flux results suggested the dominance of denitrification over nitrification at river sites. White and Reddy (2003) and Damashek et al. (2015) reported potential nitrification rates of 0.12 – 0.30 mg-N g^{-1} day^{-1} and 0.003 – 0.05 mg-N g^{-1} day^{-1} for wetland and river, respectively, which are comparable to the results from this study.

CHAPTER 5

CONCLUSION

The overall goal of this study was to improve the understanding of sediment oxygen demand and nitrogen dynamics at sediment-water interface. The specific objectives tied to the larger goal were to measure the sediment oxygen demand at river and wetlands sites, evaluate the flux and fate of nutrients as they interact with sediments and water column, determine sediment microbial characteristics using bio-molecular tools, and determine potential nitrification and denitrification rates at river and wetlands sites. Jordan River sites and Farmington Bay Wetland locations were selected to conduct these experiments.

From the results of the study, the following conclusions can be made –

1. The SOD for Jordan River sites ranged from 2.4 to 2.9 g-DO m^{-2} day^{-1}, whereas wetland sites had values of SOD was as high as 11.8 g-DO m^{-2} day^{-1}, which categorized the river and wetland sediments as ‘moderately polluted’ to ‘sewage like sludge’. The majority of the ambient DO deficit was found to be related to SOD with more than 70% of ambient DO demand partitioned into sediments. Leaf shedding in Utah typically starts in September, which adds significant organic matter loading to the waterbody. The SOD during late summer was perhaps higher than early summer due to the decomposition of these additional

organic matter loads.

2. Results of the sediment nutrient flux experiments helped understand the combined effect of bio-chemical reactions like ammonification, nitrification, and denitrification at the sediment surface. Results confirmed the increase in denitrification and nitrification activity upon availability of bioavailable nutrients. Overall, the nutrient fluxes found in this study for Jordan River and State Canal sites ranged from -3.9 – 0.2 g m^{-2} day^{-1} for ammonium, -4.6 – 5.0 g m^{-2} d^{-1} for nitrate, and -0.4 – 0.3 g m^{-2} d^{-1} for phosphate; and for wetland sites were -0.9 – 0.0 g m^{-2} d^{-1} for ammonium, -2.0 – 0.0 g m^{-2} day^{-1} for nitrate, and -0.1 – 0.3 g m^{-2} day^{-1} for phosphate. These flux values and nitrification-denitrification experiment results provided supporting evidence for each other.
3. Characterizing the sediment microbial features using bio-molecular tools indicated the presence of denitrifying (*nirS* and *nirK*) and nitrifying (*amoA*) genes. The copy number of *nirS* and *amoA* genes at selected Jordan River and Farmington Bay wetland sites ranged from 2.9 x 10^6 to 4.9 x 10^{10} and 1.9 x 10^7 to 1.4 x 10^{10} copies per gram dry sediment, respectively. *Nitrosomonas europaea* and *Nitrospira*-like AOB dominated the Jordan River sites, while the wetland and State Canal locations were dominated by *Nitrosomonas europaea* only. Organic carbon leached from leaf litter seemed to support the denitrification reaction in the natural ecosystem. Moreover, the similarity of the Jordan River and wetland site's *nirS* and *amoA* gene copy numbers to that of an engineered ecosystem indicated that these natural ecosystems have high potential of nitrogen removal and ammonia oxidation under favorable conditions.

4. The potential denitrification and nitrification rates at the Jordan River and Farmington Bay wetland sites ranged from 0.01 – 0.16 mg N_2-N g^{-1} day^{-1} and 0.008 to 0.07 mg-N g^{-1} day^{-1}, respectively. These rates supported the findings of the bio-molecular experiments for characterizing sediment microbiology.

Jordan River has been reported to have major pollution issues with relation to nitrogen contamination, persistently low oxygen concentration, and high organic matter. Comparing the potential rates of denitrification and nitrification with the in-situ nitrogen flux and sediment oxygen demand at different locations along the Jordan River and Great Salt Lake Wetland provided an insight to the nutrient and oxygen dynamics along the river and wetland. A good understanding of the nutrient concentration in the inflows and outflows of the Jordan River has been achieved through extensive monitoring of UDWQ. However, the knowledge of river and wetland's nutrient and oxygen changes from sediment biological activity is also important to incorporate in the ongoing Jordan River TMDL study.

APPENDIX

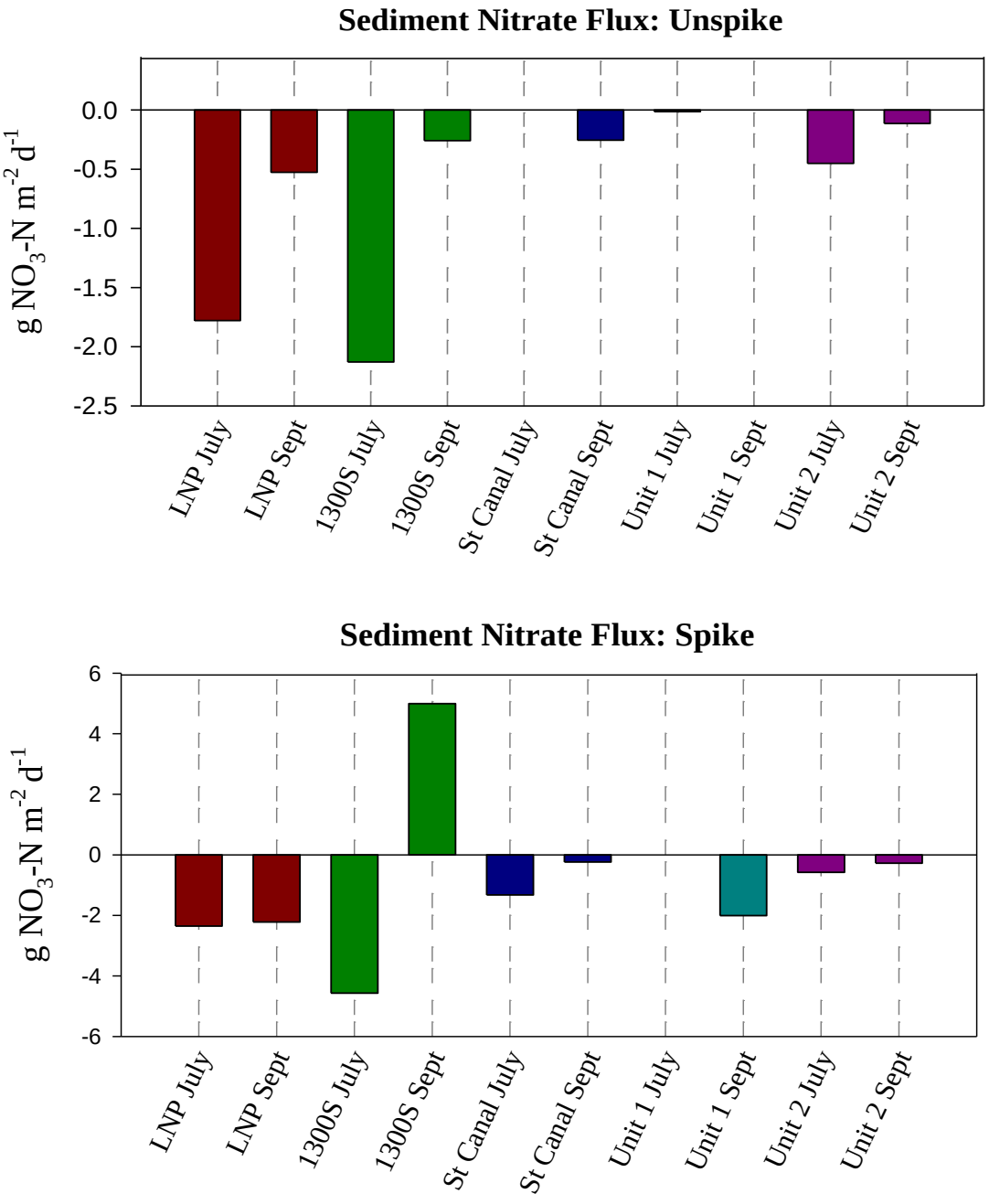

Figure A.1, Sediment Nitrate Flux in Early Summer (July) and Late Summer (Sept)

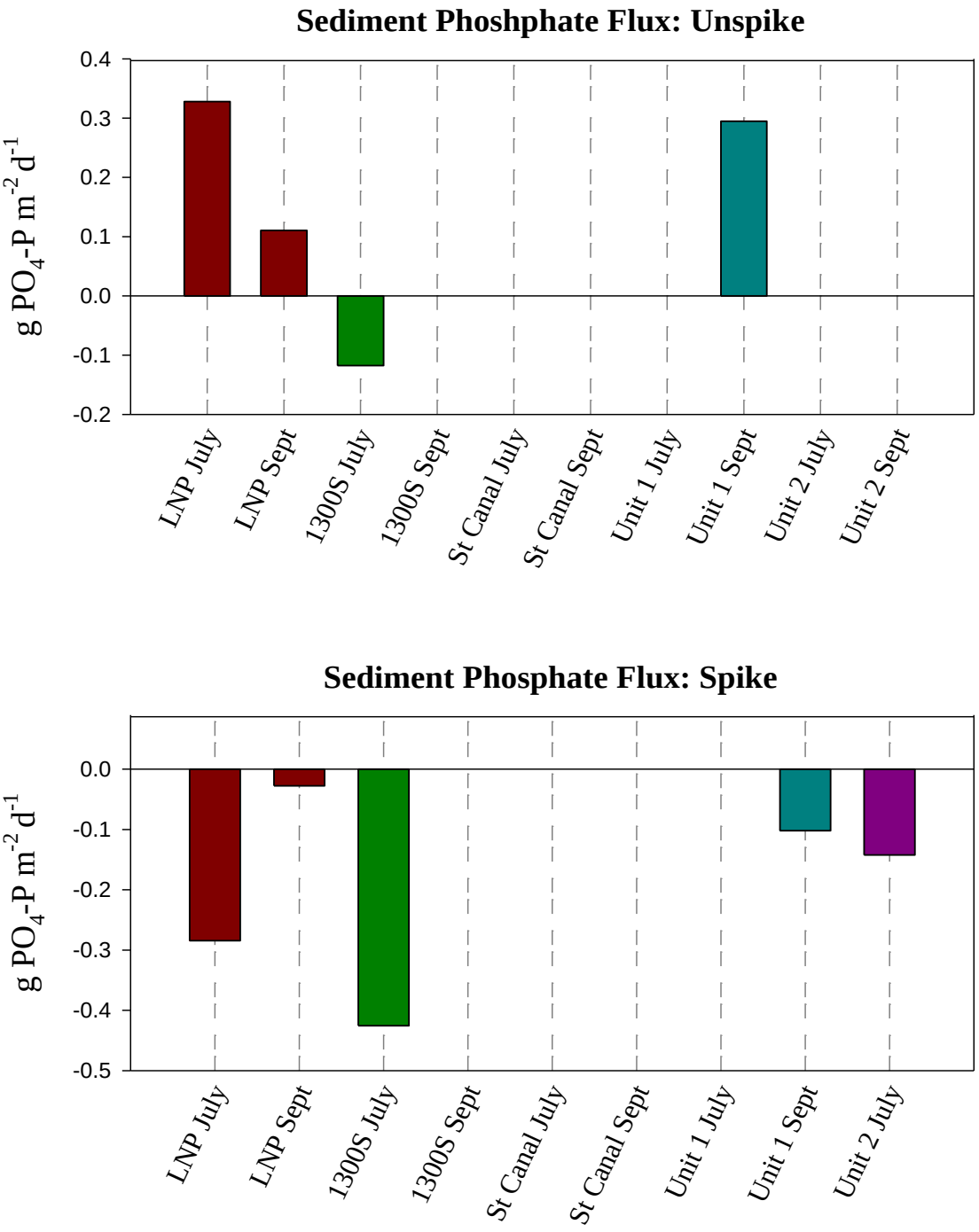

Figure A.2, Sediment Phosphate Flux in Early Summer (July) and Late Summer (Sept)

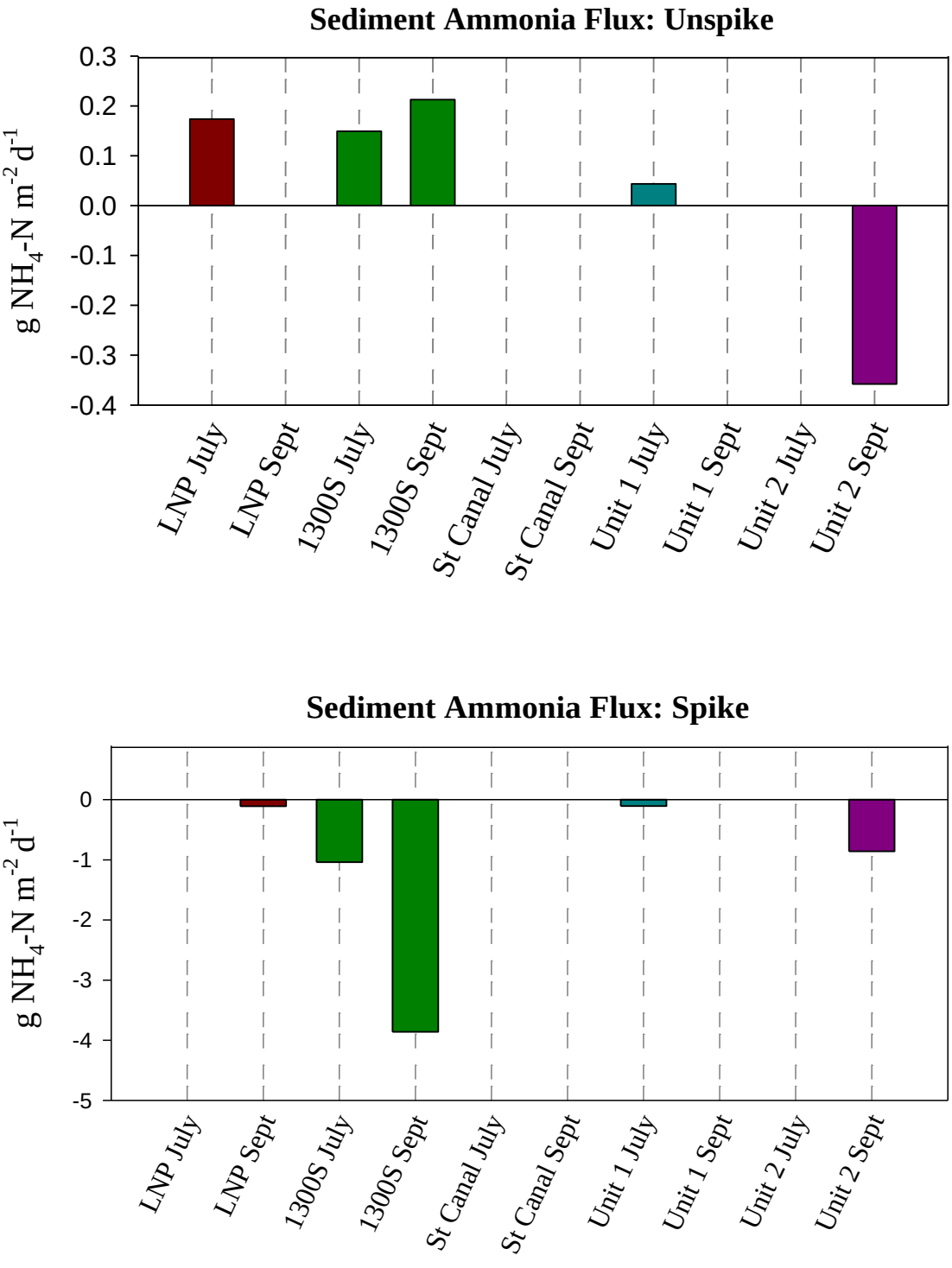

Figure A.3, Sediment Ammonia Flux in Early Summer (July) and Late Summer (Sept)

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