Nitrogen Dynamics in the Jordan River and Great Salt Lake Wetlands

Department of
Civil & Environmental Engineering
THE UNIVERSITY OF UTAH

Executive summary: Jordan River receives discharges from various point and nonpoint sources. Due to rising concerns about the presence of nutrients in Jordan River and their transport to receiving wetlands in the Great Salt Lake ecosystem, this study was initiated to estimate the fate of nitrogen at selected sites in Jordan River and selected wetlands. Two sites were sampled in the lower Jordan River, one in the State Canal, and two sites in the Farmington Bay wetlands. Sediment oxygen demand and nutrient flux experiments were conducted twice through the summer of 2015. Sediments were observed as both sink and source for ammonia, whereas they acted as a sink for nitrate—reflecting the combined effects of ammonification, nitrification, and denitrification. Results from potential denitrification experiments supported high denitrification activity in the sediments as well. Variation in nitrification and denitrification rates was supported by molecular analysis of *key functional genes*. Comparing the potential denitrification and nitrification rates with the in-situ nitrogen flux, SOD, and bio-molecular sediment characteristics provided a useful insight to the nitrogen dynamics in the Jordan River and GSL wetlands, which can serve as essential additions to the continuing efforts of improvising the Jordan River TMDL.

Ramesh Goel, Shaikha Abedin

May, 2016

From
Dr. Ramesh Goel
Associate Professor
Civil & Environmental Engineering
University of Utah

To: Dr. Theron Miller

Subject: Research status report for the project titled "***Nitrogen dynamics in the Jordan River and Great Salt Lake Wetlands."***

Dear Dr. Miller,

I am pleased to submit this final report on nitrogen dynamics in the Jordan River and at selected sites in the Farmington Bay wetlands for the titled project. This project had two distinct objectives.

Objective 1: *Evaluate nitrogen fluxes and nitrification and denitrification rates using in-situ chambers at* ***5 sites*** *in the Jordan River (early and late summer)*
Objective 2*: Evaluate nitrification and denitrification rates (using serum bottles) at the selected critical sites sampled in Task 1 for late summer samples.*

Please note that sediment oxygen demand and fine scale molecular analysis were not a part of this project but we determined these parameters and are reporting them in this report. We have included detailed methodology, results, and discussion. In addition, we have conducted a complete analysis of nitrifying and denitrifying communities for all sites to further strengthen the claim that sampled sites are indeed active in nitrification and denitrification.

1.0 Introduction

Dissolved oxygen (DO) is an important index to the health of aquatic ecosystems (Chen et al., 2012; Yogendra & Puttaiah, 2008). Numerous studies have documented the detrimental effects of low DO concentrations in aquatic ecosystems (Connolly et al., 2004; Dai et al., 2006; Medeiros et al., 2009). Management decisions that might be responsible for producing low DO 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 (Chen et al., 2012). Sediment oxygen demand (SOD) (Miskewitz et al., 2010; Miskewitz & Uchrin, 2013) and nutrient dynamics (Esten & 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 sediment oxygen demand (SOD), how this rate varies spatially and temporally, and whether this demand is influenced by decomposing algal detritus (Chen et al., 2012; Liu & Chen, 2012; Esten & Wagner, 2010). The SOD operates on a longer timescale 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) (Chen et al., 2012; Wood, 2001). 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 (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 (Lillebø et al., 2007). Different internal biogeochemical processes controlled by microbial species play a central role in the change of nitrogen forms in waterbodies (Zhu et al., 2010), and establishes a linkage between the water column and sediment bed (Mulholland & Webster, 2010).

For a comprehensive surface water quality study, the knowledge of sediment biological activity and nutrient transformation/dynamics at the sediment–water interface in a waterbody is essential. The objective of this study was to improve the understanding of nitrogen dynamics in river and wetland. The following tasks were completed to achieve the goal:

2.0 Methodology

2.1 Sampling Locations: In order to fulfill the research objectives, 1300 South, Legacy Nature Preserve (LNP) from the Lower Jordan River, Unit 1 and Unit 2 from the Farmington Bay Wetland Management Area, and State Canal were selected as sampling sites. These sites were selected considering the different hydraulic reaches, tributaries, stormwater outfalls, proximity to wastewater treatment plant (WWTP) point discharges, and Utah Division of Water Quality (UDWQ) monitoring stations. Figure 1 shows the location of these sampling sites.

Figure 1: *Study Area showing sampling sites.*

2.2 Sediment Oxygen Demand: 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. Both the Control chamber and Testing chambers had a working volume of 44 liters. When deployed, the Testing chambers encapsulated a sediment area of 0.16 m^2.

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 their 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 UDWQ. The deployment of the chambers and calculations for SOD were completed following Hogsett (2015). Figure 2 provides a picture of the SOD chambers deployed at the sampling location.

Figure 2: *SOD chambers deployed at site.*

2.3 Nutrient Flux: To conduct the nutrient flux experiments, 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 (see Figure 3). The sediment chamber had both an 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 a closed bottom to measure nutrient dynamics in the water column only. The experiments were conducted under both ambient conditions and nutrient-spiked conditions to investigate the sediment's reaction to a nutrient pulse. The first four hours of the study were conducted under ambient conditions, while the last four hours involved spiking the chambers to a concentration of 0.5 mg/L NH_3-N, 0.5 mg/L NO_3-N, and 0.1 mg/L PO_4-P. The rate of change of dissolved nutrients was calculated using the slope of the concentration versus time plot.

2.4 Potential Denitrification and Nitrification: To quantify the potential denitrification rate, the rates of $^{30}N_2$ production were measured and calculated using a modification of the method of Long et al. (2013). For nitrification experiments, the top 5 cm sediment subcore was homogenized aseptically with a

laboratory-scale spatula. A predetermined amount of the homogenized slurry was placed in a 600 ml sterile beaker as shown in the left panel in Figure 1. The mixture was aerated with a low airflow rate and stirred continuously. Ammonia-nitrogen stock solution was added to start the nitrification process to accomplish a predetermined final ammonium nitrogen concentration. For each experiment, a sterile pipette was used to remove 25 ml of the slurry, with duplicates, and the ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen concentrations were measured. Total solids and volatile solids were also measured according to standard methods.

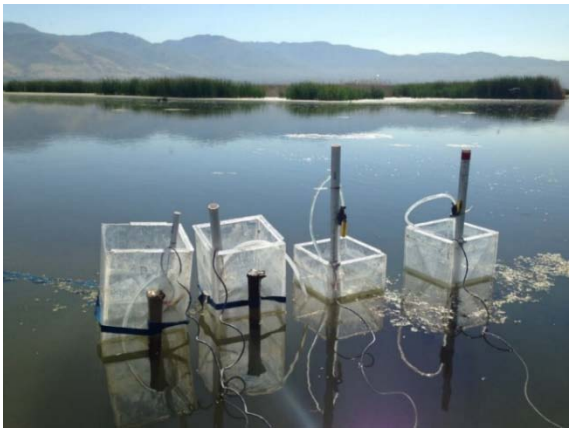


Figure 3: *Nutrient flux chambers deployed at site.*

2.5 Bio-molecular Analysis: Both ammonia oxidizers and denitrifiers were targeted in this research. Table 1 presents details on biomarkers that were employed to profile these bacteria. DNA was extracted from 0.25–0.40 g sediments using a MO Bio Power Soil extraction kit, and concentrations were measured in ng/µl using a NanoDrop ND-1000 UV-Vis Spectrophotometer.

Quantitative PCR (q-PCR) was performed to quantify amoA and nirS genes. Each qPCR reaction contained 10 µL of 2×SYBR green master mix (Life Technologies), 1 µM of forward primer and 1 µM of reverse primer, 1 µL of BSA (0.1 mg/mL) and 1 µL of cDNA template (10 to 100 ng). The primer sequences and annealing temperatures used in qPCR are summarized in Table 1

Table 1: q-PCR Primer and Size for Selected Genes

Enzyme	Primer	Size, bp	Reference
Ammonium monooxygenase (AMO, α subunit) for **ammonia oxidizers**	F:GGGTTTCTACTGGTGGT R:CCCCTCKGSAAAGCCTTCTTC	491	Rotthauwe et al., 1997
Heme containing nitrite reductase (NirS or cd_1NIR) for **denitrifiers**	Cd3a: AACGYSAAGGARACSGG R3cd: GASTTCGGRTGSGTCTTSAYGAA	425	Throbäck et al., 2004

Finally, the Terminal Restriction Fragment Length Polymorphism (TRFLP) technique (Osborn et al., 2000) was used to analyze the nitrifying bacterial communities present in the sediment.

3.0 Results

3.1 Ambient Water Characteristics: Two rounds of sampling were conducted. The first one was conducted in July and the second in September of 2015 to record nutrient dynamics and sediment oxygen demand in early and late summer, respectively. Table 2 shows the ambient concentrations of nutrients and other parameters under consideration at the selected five sites. Nutrient concentrations during some sampling events were under detection limit (UDL) and thus were not measurable. Detection limits for phosphate-phosphorous, nitrite-nitrogen/nitrate-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. For the wetland sites, Unit 1 had higher concentrations of phosphate and ammonium than Unit 2. The dissolved oxygen (DO), in general, was lower in the river sites than wetland sites. Temperatures were lower during late summer.

Table 2: 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	Early Summer	1.23	4.94	4.07	0.880	0.545
(mg/L)	Late Summer	3.76	4.88	3.18	0.089	3.220
NO_2-N	Early Summer	0.027	0.113	UDL	UDL	UDL
(mg/L)	Late Summer	0.093	UDL	UDL	0.144	UDL
PO_4-P	Early Summer	0.388	0.611	0.507	0.679	0.57
(mg/L)	Late Summer	0.283	0.530	0.698	0.095	UDL
NH_4-N	Early Summer	0.270	0.150	1.17	1.810	0.779
(mg/L)	Late Summer	0.408	0.244	1.38	0.947	0.127
DO	Early Summer	4.20	3.59	3.57	7.20	5.49
(mg/L)	Late Summer	5.93	5.44	7.26	7.65	7.30
Temperature	Early Summer	23.16	24.35	23.89	22.28	23.74
(^oC)	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

Higher nitrate and phosphate concentrations are perhaps the result of external inputs from various point and nonpoint sources. Unit 1 was observed to be the most active in terms of nutrient concentrations, pH, and dissolved oxygen. Nitrite was mostly nondetectable at all but the Unit I (late summer) and 1300 S sites.

3.2 Sediment Oxygen Demand (SOD): Sediment oxygen demand accounts for the depletion of oxygen due to various biogeochemical activities at the sediment water interface (Chen et al., 2012; Hogsett & Goel, 2013; Wood, 2001). 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 illustrates the dissolved oxygen depletion profiles measured in the SOD and WC_{dark} chambers at Unit 1 site during late summer.

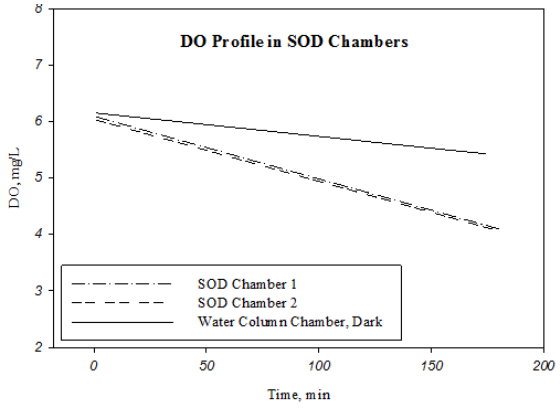


Figure 4: *DO Profiles in three SOD chambers at Unit 1 (late summer).*

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 & 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 5 shows the sediment oxygen demand (SOD) results for all sampling events in early and late summer. All SOD results were normalized to temperature at 25^{o}C 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 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 & Goel, 2013). It also emphasizes that SOD values predicted using temperature correction equations may not reflect actual SOD values and stream metabolism (Hogsett & Goel, 2013), which is vital to stream and wetland management decisions.

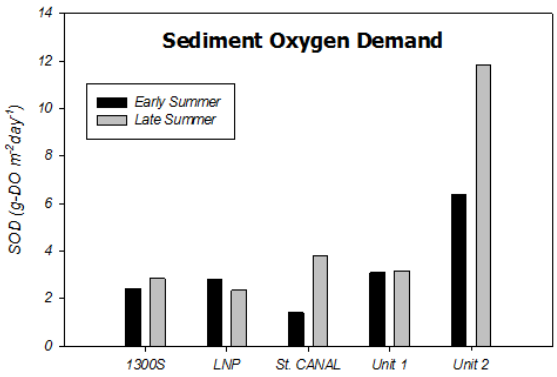


Figure 5: *Sediment Oxygen Demand (SOD) results for all sampling events.*

Butts and Evans (1978) categorized the benthic sediment condition based on the SOD_{T25C} values. 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 3. The least polluted site was 1300 South in the Jordan River, which can be categorized as 'moderately polluted.' The average SOD flux of the 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.

Table 3: Average SOD Results from 5 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

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 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 the water column, which means oxygen demand in the water column was higher than that of the sediment.

3.3 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 (Hantush et al., 2013). Sediment nutrient flux experiments for selected sites in the Jordan River and the Great Salt Lake wetlands were conducted in July and September considering both ambient conditions and the nutrient pulsed state. After conducting the first three hours of the experiment at ambient conditions, the chambers were spiked with 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.

3.3.1 Jordan River Sites: Figures 6 depicts the ammonium, nitrate, and phosphate flux of the Jordan River sites under unspiked and spiked conditions during early (July) and late (September) summer. A

negative bar in these plots indicates sediment as a sink, while a positive bar represents a source of nutrients. Under environmental conditions, these positive and negative nutrient fluxes can be the result of various biogeochemical reactions occurring at the water–sediment interface (Boulton et al., 1998; Friedrich et al., 2002; Lefebvre et al., 2006). For example, a positive ammonium flux indicates the possibility of ammonification (Lillebø et al., 2007; Strauss & Lamberti, 2002), 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 & Hill, 1984). For nitrate, a negative flux typically represents denitrification (Bartkow, 2004; Beaulieu et al., 2011; Holmes et al., 1996). A positive nitrate flux is indicative of ammonia oxidation (Strauss & Lamberti, 2000).

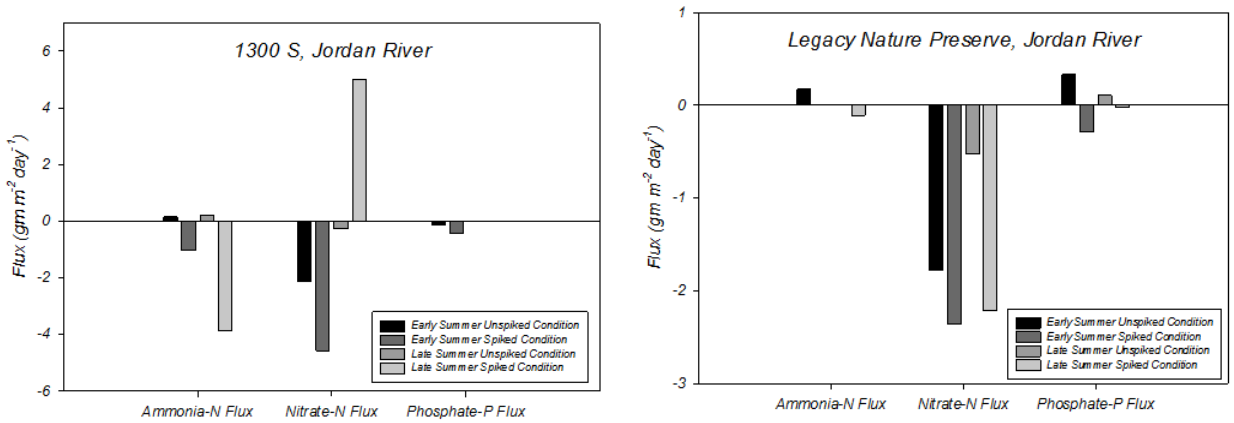


Figure 6: *Nutrient fluxes for Jordan River sites (i.e., 1300S and LNP).*

In case of phosphate-phosphorous, a positive flux typically occurs due to decomposition/mineralization of organic matter and/or its release and resuspension from the sediment bed (Macrae et al., 2003). However, simultaneous negative fluxes for both nitrogen (ammonia and nitrate) and phosphate may also be caused by algal uptake (Ho et al., 2003).

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

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

For the Legacy Nature Preserve site, nitrate flux was negative for both unspiked and spiked conditions during early and late summer. These fluxes were found to be greater for the spiked condition compared to the 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).

In summary, in general, both sites in the Jordan River were found to be sinks of nutrients, especially nitrate, and the LNP site was more active than the 1300S site in terms of nutrient fluxes.

3.3.2 Wetland sites: Figure 7 depicts the ammonium, nitrate and phosphate flux at Unit 1 and 2 in Farmington Bay wetland

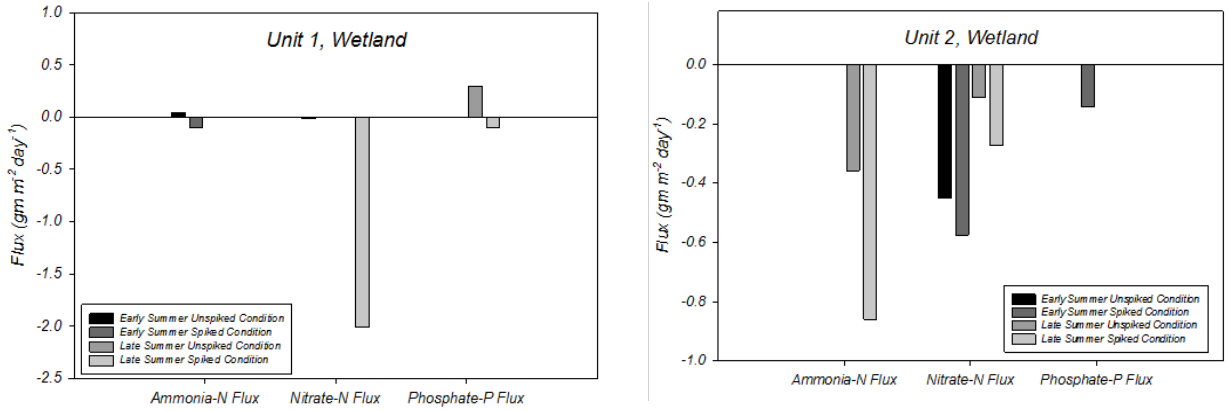
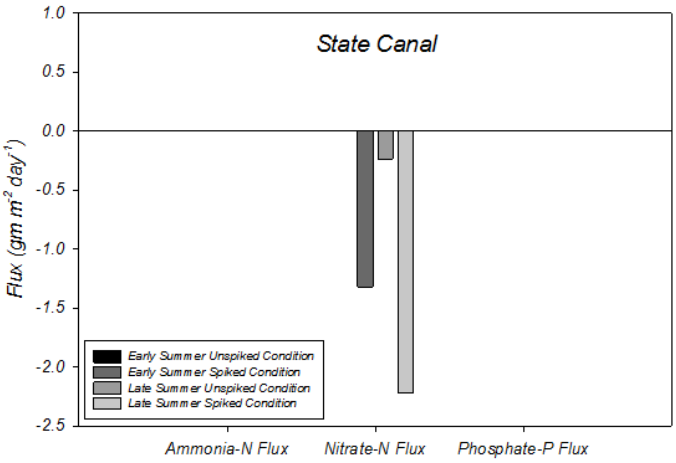


Figure 7: *Nutrient fluxes at two wetland sites.*

Compared to the Jordan River Sites, nutrient fluxes were observed to be lower at the wetland sites. Negligible fluxes of ammonia, nitrate, and phosphate were recorded at the Unit 1 site during early summer under spiked and unspiked conditions. The nitrate flux was negative at Unit 1 under spiked conditions indicating active loss of nitrate through denitrification. The phosphate flux was positive under unspiked but negative under spiked condition during the late summer sampling. Unit 2 displayed negative fluxes under spiked and unspiked conditions, indicating nitrification and denitrifictaion for nitrogen loss and primary production and other mechanisms (precipitation or binding to suspended

particles) for phosphorus loss (Strauss and Lamberti, 2000, 2002).



3.3.3 State Canal: Figure 8 depicts the ammonium, nitrate, and phosphate flux at the State Canal location—fluxes were not observed for ammonium and phosphate. The nitrate flux followed the typical pattern found for river and wetland sites.

Figure 8: *Nutrient fluxes for the State Canal site.*

Overall, the range of fluxes observed in this study for the selected Jordan River sites and the 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. The range of fluxes for the 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 (Reddy, 2008; 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.

4.0 Nitrification and denitrification rates

4.1 Nitrification rates: The 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 the 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. 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}, which is fairly close to the results from this study.

Table 4 shows the potential nitrification rates obtained for each site during early and late summer. The absence of nitrifying activity at the 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 the wetland sites, in general, indicated an absence of nitrifying activity, although gel electrophoresis and *g*ene copy number experiments showed the 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 the 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 was supported by the results for the functional gene responsible for nitrification (discussed later) 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 the 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 sites, respectively, which are comparable with the results from this study.

Table 4: 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

4.2 Potential Denitrification Rates: The denitrification experiments were 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 soil 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 9 shows the potential denitrification rates obtained for each sites 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 late summer except for the LNP site. These results are 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 & Reddy, 2003), fairly comparable to the results of this study.

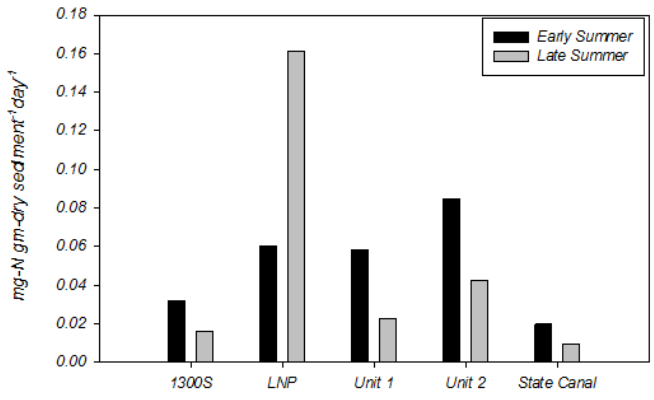


Figure 9: *Measured denitrification rates at selected sites.*

5.0 Molecular identification of ammonia oxidizers and denitrifiers

5.1 Presence of Denitrifying Genes*:* Denitrification is a four step process in which nitrate is reduced to nitrite in the first step and nitrite is reduced to nitric oxide in the second step; eventually nitrogen gas is produced through two other intermediate steps. The second step of denitrification (nitrite reduction to nitric oxide) is mediated by the nitrite reductase gene (*nir*). Denitrifying bacteria usually contain either the *nirK* or *nirS* enzyme (Bothe et al., 2007). The polymerase chain reaction (PCR) was performed using biomarkers specific for *nirS* and *nirK* genes. To verify the presence of denitrifying genes in the sediment samples from selected sites, the PCR product was run on gel electrophoresis and the pictures of the gel are shown in figure 10. As seen from Figure 10, bright bands were found for all the sites, except for State Canal and Unit 2 (early summer). In case of the *nirK* gene, only the Legacy Nature Preserve and Unit 2 sites showed a bright band in the late summer samples indicating the presence of the *nirK* gene.

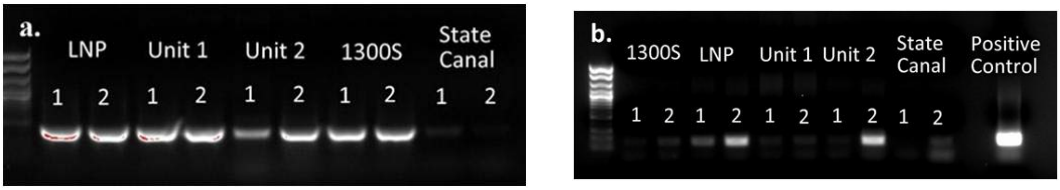


Figure 10: *Gel electrophoresis for a) nirS, b) nirK (1: early summer, 2: late summer)*

A polymerase chain reaction using specific biomarkers provides only qualitative information about the presence or absence of genes. In our next step, we employed quantitative PCR to quantify the number of functional genes present at each site. From previous studies (Bothe et al., 2007; Henry et al., 2004; Philippot, 2002), it is known that each denitrifying bacterial genome contains either a *nirS* or *nirK* gene

copy. Because *nirS* was present at all sites, this denitrifying functional gene was quantified for all sites. Figure 11 shows the *nirS* gene copy number for selected sites during both early and late summer. As seen from the figure, the *nirS* gene was found to be abundant at the Jordan River and wetland sites, ranging from 5.5 x 10^9 to 4.9 x 10^{10} copies per gram dry sediment. In comparison, the State Canal site had fewer *nirS* gene copy numbers (2.9 x 10^6 – 3.8 x 10^7), supporting the faded band obtained during gel electrophoresis and the lower denitrification rates. The gene copy numbers for all sites from both sampling times were very similar. The slight decrease in late summer can be attributed to the decrease in water temperature from July to September. The gene copy number of *nirK* was not detected from the qPCR experiment.

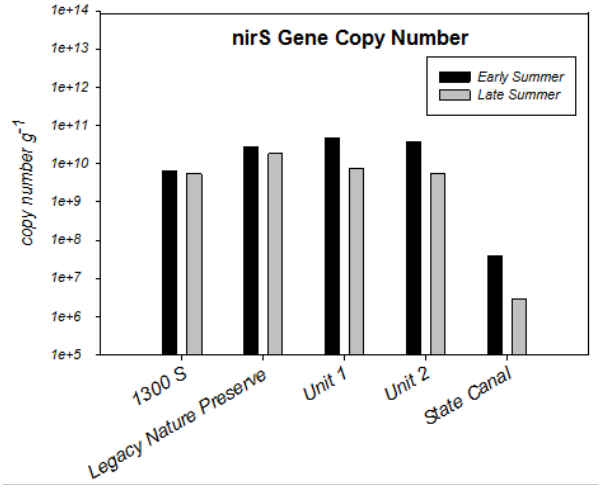


Figure 11: *nirS gene copy numbers.*

The *nirS* and *nirK* gene copy numbers 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 (Bowen et al., 2011; Chon et al., 2011; Kim et al., 2008), 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 a similar *nirS* gene copy number is removing significant nitrogen (Wang et al., 2014). The similarity of the river and wetland *nirS* gene copy numbers obtained from this study to the *nirS* gene copy numbers of an engineered ecosystem indicates that there is high potential for nitrogen removal in the river and wetland sites under favorable conditions.

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.

$$Bulk\ Density\ g/cm^3 = 1.776 - 0.363\ log_e\ OC\ (R^2 = 0.70) \qquad (5.1)$$

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), ranged from 0.84 – 12.45 g-N m^{-2} day^{-1}. Comparing these rates with the 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 the Unit 2 wetland site based on flux data was about 7–8% of the potential denitrification rate calculated using N15 spiked serum bottle tests. This indicates that there is a greater potential for denitrification at this site depending on favorable environmental conditions. Comparison of the potential denitrification rates with the in-situ nitrate fluxes supports that conclusion, and also strengthens the findings from the 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 (Bastviken et al., 2005; deBusk et al., 2001; Gale et al., 1993; Qiuying et al., 2012; Risgaard-Petersen, 2003). The results of this study are, in general, higher than the values reported in literature.

5.2 Nitrifying bacterial identification: A polymerase chain reaction was conducted for the ammonia monooxygenase (*amoA*) gene. Gel electrophoresis was performed using the PCR product to detect the presence of this nitrifying gene in the sediment samples. The results of gel electrophoresis is shown in Figure 12. The bright singular band at the 491 base pair in the gel identifies the presence of the *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.

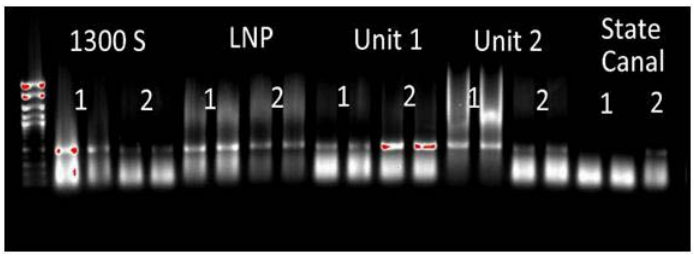


Figure 12: *Gel electrophoresis for amoA gene (1: early summer, 2: late summer).*

After confirming the presence of nitrifying genes from PCR and gel electrophoresis, quantitative PCR was performed on the ammonia monooxygenase α-subunit (*amoA)* gene to find out its abundance. From previous studies (Dionisi et al., 2002; Hommes et al., 1998), it is known that each ammonia-oxidizing bacterial cell contains two copies of the *amoA* gene. Figure 12 shows the *amoA* gene copy number for selected sites during both early and late summer. As seen in Figure 13, the *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 1300 South location where *amoA* presence was not

detected. Gel electrophoresis also indicated lower abundance of the *amoA* gene at 1300 South for this sampling event.

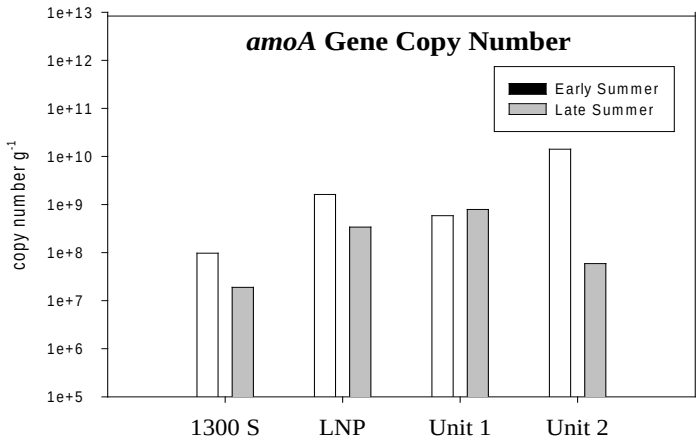


Figure 13: *amoA gene copy numbers.*

Overall, the *amoA* gene copy number ranged from 1.9 x 10^7 to 1.4 x 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.

The gene copy numbers from the two sampling time were very similar at different sites. The decrease of gene copy numbers in late summer at the Legacy Nature Preserve and Unit 2 locations can be attributed to the decrease in water temperature from July to September. The number of copies of the *amoA* gene found in other studies ranged from 10^3 to 10^7 copies per gram of 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 x 10^3 to 3.6 x 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 numbers obtained in this study to the *amoA* gene copy numbers of an engineered ecosystem indicates that these natural ecosystems have high potential for ammonia oxidation under favorable conditions.

To identify the ammonia oxidizing bacteria (AOB) present in the sediments, a TRFLP experiment was conducted using the amplified *amoA* gene. Figure 14 illustrates the presence of AOBs found in the sediments for each site. Samples showed T-RF peaks at 283/206 and 491/488, which corresponds to *Nitrospira*-like AOB and the *Nitrosomonas europaea/eutropha* lineage, respectively (Gilomen, 2008; Park & Noguera, 2004; Whang et al., 2009). Both *Nitrosomonas europaea* and *Nitrospira*-like AOB dominated at the Jordan River sites, while the wetland sites and State Canal location were dominated by *Nitrosomonas europaea* only.

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). The *Nitrospira* lineage, on the other hand, is a *Nitrosospira*-like

AOB (Gilomen, 2008; Park & Noguera, 2004). These bacteria are considered the dominant nitrifiers in wastewater treatment plants (Park & Noguera, 2004; Siripong & Rittmann, 2007; Whang et al., 2009). Therefore, the presence of *Nitrosomonas europaea* and the *Nitrospira* lineage in Jordan River and wetland sediments, as found in 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).

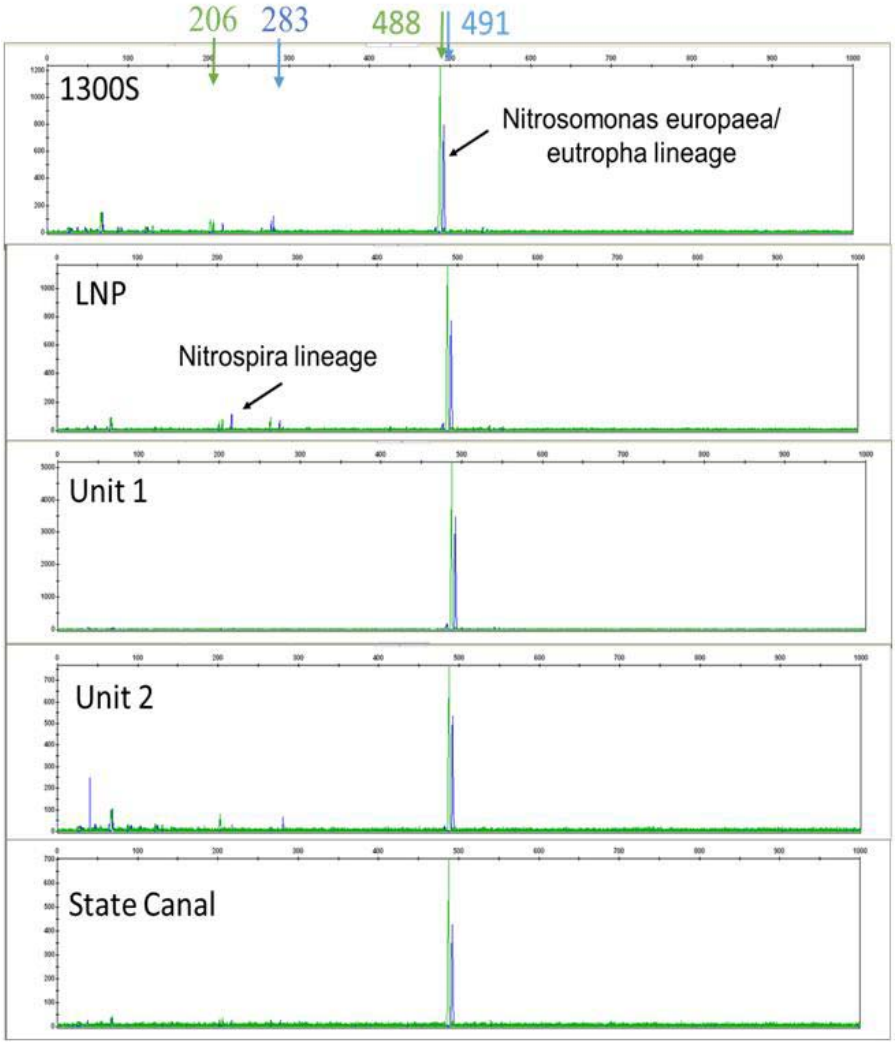


Figure 14: *Electropherograms of amoA gene TRFLP specific to AOB.*

6.0 Conclusions

The SOD for the Jordan River sites ranged from 2.4 to 2.9 g-DO m^{-2} day^{-1}, whereas the wetland sites had SOD values as high as 11.8 g-DO m^{-2} day^{-1}, which categorized the river and wetland sediments as between 'moderately polluted' and '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 loads significant organic matter to the waterbody. The SOD during late summer was perhaps higher than early summer due to the decomposition of this additional organic matter load.

Results of the sediment nitrogen flux experiments helped understand the combined effect of biochemical reactions like ammonification, nitrification, and denitrification at the sediment surface. Results confirmed the increase in denitrification and nitrification activity upon the availability of bioavailable nutrients.

Characterizing the sediment microbial features using bio-molecular tools confirmed the presence of denitrifying (*nirS* and *nirK*) and nitrifying (*amoA*) genes. *Nitrosomonas europaea* and *Nitrospira*-like AOB dominated the Jordan River sites, while the wetland and State Canal locations were dominated by *Nitrosomonas europaea* only. The similarity of the Jordan River and wetland sites' *nirS* and *amoA* gene copy numbers to that of an engineered ecosystem indicates that these natural ecosystems have a high potential for nitrogen removal and ammonia oxidation under favorable conditions.

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 further supported the findings of the biomolecular experiments for characterizing the sediment microbiology. Results suggested the dominance of denitrification over nitrification at river sites.

Comparing the potential rates of denitrification and nitrification with the in-situ nitrogen fluxes at the Jordan River and GSL Wetland sites provided a useful insight to the nitrogen dynamics at these locations. A good understanding of the nutrient concentration in the inflows and outflows of the Jordan River has been achieved through the extensive monitoring of UDWQ. However, the findings on the nitrogen transformation in the river and wetlands due to sediment biological activities can be valuable for the ongoing Jordan River TMDL study.

7.0 References

- Avnimelech, Y., G. Ritvo, L.E. Meijer, and M. Kochba, 2001). Water content, organic carbon and dry bulk density in flooded sediments. *Aquacultural Engineering 25*(1): 25-33.
- Bai, Y., Q. Sun, D. Wen, and X. Tang, 2012. Abundance of ammonia-oxidizing bacteria and archaea in industrial and domestic wastewater treatment systems. *FEMS Microbiology Ecology 80*(2): 323-330.
- Beaulieu, J.J., J.L. Tank, S.K. Hamilton, W.M. Wollheim, R.O. Hall, P.J. Mulholland, et al., 2011. Nitrous oxide emission from denitrification in stream and river networks. *Proceedings of the National Academy of Sciences 108*(1): 214-219.
- Bothe, H., S. Ferguson, and W.E. Newton (Eds.), 2007. *Biology of the nitrogen cycle*. Elsevier. ISBN: 978-0-444-52857-5.
- Butts, T. A., and Evans, R. L. (1978). "*Sediment oxygen demand studies of selected northeastern Illinois streams*." Illinois State Water Survey, Circular 129, NTIS Publication No. PB-285-788. Springfield, VA: NTIS.
- Cébron, A., J. Garnier and G. Billen, 2005. Nitrous oxide production and nitrification kinetics by natural bacterial communities of the lower Seine River (France). *Aquatic Microbial Ecology 41*(1): 25-38.
- Chain, P., J. Lamerdin, F. Larimer, W. Regala, V. Lao, M. Land, et al., 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph Nitrosomonas europaea. *Journal of Bacteriology 185*(9): 2759-2773.
- Chen, W.B., W.C. Liu and L.T. Huang, 2012. Measurement of sediment oxygen demand for modeling the dissolved oxygen distribution in a subalpine lake. *International Journal of Physical Sciences 7*(27): 5036-5048.
- Connolly, N.M., M.R. Crossland and R.G. Pearson, 2004. Effect of low dissolved oxygen on survival, emergence, and drift of tropical stream macroinvertebrates. *Journal of the North American Benthological Society 23*(2): 251-270.
- Dai, M., X. Guo, W. Zhai, L. Yuan, B. Wang, L.Wang, P. Cai, T. Tang, and W.J. Cai, 2006. Oxygen depletion in the upper reach of the Pearl River estuary during a winter drought. *Marine Chemistry 102*(1): 159-169.

- de Bie, M.J., M. Starink, H.T. Boschker, J.J. Peene and H.J. Laanbroek, 2002. Nitrification in the Schelde estuary: methodological aspects and factors influencing its activity. *FEMS Microbiology Ecology 42*(1): 99-107.
- Esten, M. E., & Wagner, K. J. (2010). "Investigation of benthic phosphorus flux controls in Lake Waco, Texas." *Lake and Reservoir Management*, *26*(2), 114-122.
- Garibyan, L. and N. Avashia, 2013. Polymerase chain reaction. *Journal of Investigative Dermatology 133*(3), e6.
- Hantush, M. M., Kalin, L., Isik, S., and Yucekaya, A. (2012). "Nutrient dynamics in flooded wetlands. I: model development." *Journal of Hydrologic Engineering*, *18*(12), 1709-1723
- Hogsett, M. C. (2015). "*Water Quality and Sediment Biogeochemistry in the Urban Jordan River, UT*." Doctor of Philosophy Thesis, Department of Civil and Environmental Engineering. The University of Utah.
- Hogsett, M., and Goel, R. (2013). "Dissolved Oxygen Dynamics at the Sediment–Water Column Interface in an Urbanized Stream." *Environmental Engineering Science*, *30*(10), 594-605.
- Kadlec, R. H., and Wallace, S. (2009). *Treatment wetlands*, 2nd edition. CRC press.
- Kemp, M. J., and Dodds, W. K. (2002). "The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated with prairie stream substrata." *Limnology and Oceanography*, *47*(5), 1380-1393.
- Levi, P.S., J.L. Tank, S.D. Tiegs, D.T. Chaloner and G.A Lamberti, 2013. Biogeochemical transformation of nutrient subsidy: salmon, streams and nitrification. *Biogeochemistry 113*(1-3): 643-655.
- Lillebø, A.I., M. Morais, P. Guilherme, R. Fonseca, A. Serafim and R. Neves, 2007. Nutrient dynamics in Mediterranean temporary streams: a case study in Pardiela catchment (Degebe River, Portugal). *Limnologica - Ecology and Management of Inland Waters 37*(4): 337-348.
- Liu, W. C., & Chen, W. B. (2012). "Monitoring sediment oxygen demand for assessment of dissolved oxygen distribution in river." *Environmental monitoring and assessment*, *184*(9), 5589-5599.
- Long, A., J. Heitman, C. Tobias, R. Philips and B. Song, 2013. Co-occurring anammox, denitrification, and codenitrification in agricultural soils. *Applied and environmental microbiology 79*(1): 168-176.
- Macrae, M. L., English, M. C., Schiff, S. L., and Stone, M. A. (2003). "Phosphate retention in an agricultural stream using experimental additions of phosphate." *Hydrological processes*, *17*(18), 3649-3663.

- Malecki, L. M., White, J. R., and Reddy, K. R. (2004). "Nitrogen and phosphorus flux rates from sediment in the lower St. Johns River estuary." *Journal of environmental quality*, *33*(4), 1545-1555.
- Medeiros, A.O., C. Pascoal and M.A.S. Graça, 2009. Diversity and activity of aquatic fungi under low oxygen conditions. *Freshwater Biology 54*(1): 142-149.
- Miskewitz, R. J., Francisco, K. L., and Uchrin, C. G. (2010). "Comparison of a novel profile method to standard chamber methods for measurement of sediment oxygen demand." *Journal of Environmental Science and Health Part A*, *45*(7), 795-802.
- Miskewitz, R., & Uchrin, C. (2013). "In-Stream Dissolved Oxygen Impacts and Sediment Oxygen Demand Resulting from Combined Sewer Overflow Discharges." *Journal of Environmental Engineering*, *139*(10), 1307-1313.
- Mulholland, P. J., Valett, H. M., Webster, J. R., Thomas, S. A., Cooper, L. W., Hamilton, S. K., and Peterson, B. J. (2004). "Stream denitrification and total nitrate uptake rates measured using a field 15N tracer addition approach." *Limnology and Oceanography*, 49(3), 809-820.
- Mulholland, P.J. and J.R. Webster, 2010. Nutrient dynamics in streams and the role of J-NABS. *Journal of the North American Benthological Society 29*(1): 100-117.
- Osborn, A.M., E.R. Moore and K.N. Timmis, 2000. An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. *Environmental microbiology 2*(1): 39-50.
- Park, H.D. and D.R. Noguera, 2004. Evaluating the effect of dissolved oxygen on ammonia-oxidizing bacterial communities in activated sludge. *Water Research 38*(14): 3275-3286.
- Pauer, J. J., and Auer, M. T. (2000). "Nitrification in the water column and sediment of a hypereutrophic lake and adjoining river system." *Water Research*, *34*(4), 1247-1254.
- Reddy, K. R., and DeLaune, R. D. (2008). *Biogeochemistry of Wetlands*. CRC Press.
- Saiki, R.K., S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich and N. Arnheim, 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science 230*(4732): 1350-1354.
- Shrestha, N.K., S. Hadano, T. Kamachi and I. Okura, 2001. Conversion of ammonia to dinitrogen in wastewater by Nitrosomonas europaea. *Applied biochemistry and biotechnology 90*(3): 221-232.
- Starry, O. S., Valett, H. M., and Schreiber, M. E. (2005). "Nitrification rates in a headwater stream: influences of seasonal variation in C and N supply." *Journal of the North American Benthological Society*, 24(4), 753-768.
- Strauss, E. A., and Lamberti, G. A. (2002). "Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments." *Freshwater Biology*, 47(1), 65-74.

- Teeters, D.S., 2015. *Nutrients in the Great Salt Lake Wetlands: The Importance of Sediment Fluxes and Biogeochemistry*. Master's Thesis, The University of Utah.
- Utley, B. C., Vellidis, G., Lowrance, R., and Smith, M. C. (2008). "Factors affecting sediment oxygen demand dynamics in blackwater streams of Georgia's coastal plain." *JAWRA Journal of the American Water Resources Association*, 44(3), 742–753.
- VanZomeren, C. M., White, J. R., & DeLaune, R. D. (2013). "Ammonification and denitrification rates in coastal louisiana bayou sediment and marsh soil: Implications for Mississippi River diversion management." *Ecological Engineering, 54*, 77-81.
- Wang, Z., X.X. Zhang, X. Lu, B. Liu, Y. Li, C. Long and A. Li, 2014. Abundance and diversity of bacterial nitrifiers and denitrifiers and their functional genes in tannery wastewater treatment plants revealed by high-throughput sequencing. *PloS one* 9(11): e113603.
- Wood, T. M., 2001. *Sediment oxygen demand in Upper Klamath and Agency Lakes, Oregon, 1999*. U.S. Geological Survey Water-Resources Investigations Report 01–4080, 13 p.
- Yogendra, K. and E.T. Puttaiah, 2008. Determination of Water Quality Index and Suitability of an Urban Waterbody in Shimoga Town, Karnataka. In Proceedings of *Taal 2007: The 12th World Lake Conference*, 342-346. Sengupta, M., and Dalwani, R. (Editors).
- Zhu, G., M.S. Jetten, P. Kuschk, K.F. Ettwig and C. Yin, 2010. Potential roles of anaerobic ammonium and methane oxidation in the nitrogen cycle of wetland ecosystems. *Applied microbiology and biotechnology*
- Ziadat, A. H., and Berdanier, B. W. (2004). "Stream depth significance during in-situ sediment oxygen demand measurements in shallow streams." *JAWRA Journal of the American Water Resources Association*, 40(3), 631–638.