2009-01-01

Nutrient Dynamics in a Created Desert Wetland: Implications for the Rio Bosque Wetlands Park

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NUTRIENT DYNAMICS IN A CREATED DESERT WETLAND: IMPLICATIONS FOR THE RIO BOSQUE WETLANDS PARK

By

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THESIS

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

Department of Biological Sciences

THE UNIVERSITY OF TEXAS AT EL PASO

August 2009

Acknowledgements

For all her continued support and guidance through this study I would like to thank my advisor and mentor Vanessa L. Lougheed. I have learned what it takes to be a scientist and I am grateful and appreciate everything that I have learned through my years in the Aquatic Ecology Laboratory. This study was made possible through support of the World Wildlife Fund (WWF) and the efforts of Jennifer Montoya. I would also like to thank John Sproul, Manager Rio Bosque Wetland Park, for allowing us to conduct my research at Rio Bosque. For all their help in the field and in laboratory experiments, I would like to thank Christian Andresen, Fernanda De La Cerda, Sarah Renteria, Claudia Ortega, Ursula Sherrill, and Gilda Victorino. Finally I would like to thank my family for all their support throughout my career as a student. None of this would be possible without any of them. Thank You!

Abstract

Rio Bosque Wetland Park is an arid wetland currently under restoration, which primarily receives water inputs from the Bustamante Waste Water Treatment Plant (WWTP) during the non-irrigation season. Understanding the ability of a desert wetland to remove nutrients from the water column through uptake by primary producers or sediment transformations is key to justifying the protection and creation of other similar wetland sites. Nitrate concentrations tended to be reduced, relative to the inflow, near the outflow of the wetland.To further understand these trends, sediment nutrient release experiments and algal nutrient limitation experiments were completed. Sediment phosphorus release experiments indicated that Soluble Reactive Phosphorus (SRP) release rates at the sites were positively correlated with average well color development ($r=0.90$, $p=0.0347$), indicating greater phosphorus release at sites with higher levels of bacterial activity. Nutrient limiting experiments completed in the last 2 years of the study indicated that algae tended towards limitation by nitrogen at the outflow, with no nutrient limitation at the inflow. The presence of water year round, which is currently lacking from the Rio Bosque, and the increased growth of wetland plants it would promote, could have a substantial effect on the removal rates of nutrients from the wetland through not only the direct uptake of nutrients by plants but also by alterations in redox potential, denitrification, microbial activity, and sediment phosphorus dynamics.

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Introduction

Created wetlands can serve many of the same functions as naturally occurring wetlands. Wetlands function as a means of improving water quality through nutrient retention, sediment attenuation, increasing biodiversity, as well as flood abatement (Zedler 2003). A functioning wetland can reduce the levels of many pollutants, including nutrients, bacteria, and heavy metals (e.g. Quinonez-Diaz et al. 2001; Mitsch et al. 2005; Verhoeven et al. 2006; Yang et al. 2006). While created wetlands may be most successful at achieving these functions when constructed in areas where wetlands formerly existed, some notable successes have occurred in the absence of wetland soils. For example, the Oletangy River Wetland Research Park (Ohio State University, Columbus, OH) was constructed in non-wetland soils, produced hydric soils after 3 years, and exhibited an increase in water quality and plant diversity over the 10 initial years of study (Mitsch 2005).

Wetlands reduce nitrogen levels mainly by the process of denitrification and phosphorus by the process of sedimentation. Denitrification is the process in which bacteria convert nitrates into atmospheric nitrogen under anoxic wetland conditions; wetland denitrification is one of the primary ways in which nitrogen is returned to the atmosphere (Mitsch and Gosselink, 2007). Sedimentation, on the other hand, is a process in which suspended particles settle out of the water column due to reduced rate of flow and gravity. Because phosphorus attaches readily to organic matter and inorganic sediments, phosphorus tends to be reduced by sedimentation. Through this process, phosphorus retention is one of the most important attributes of wetlands (Mitsch and Gosselink, 2007). In addition to these two processes, nutrients are also taken up by plants and soil microbes. Mitsch et al. (2005) found that in a created wetland in Ohio, nitrate

concentrations were reduced by 35% on average after flowing through the wetland cells, while orthophosphorus declined by 70%. However, they found no effect of the wetland on total phosphorus, which seemed to be exported from the wetlands attached to suspended sediments.

Two of the main factors attributed to cleaning water and retaining nutrients in created wetlands are the plant composition and hydrology of the wetland (Thullen 2005). The role of plants in the reduction of nutrients is critical and has been recorded in several studies. The maximum potential rate of removal by plants is 1000 to 3000 Kg N/ha/yr and 60 to 100 Kg P/ha/yr (Verhoven et al, 2006) depending on several factors such as plant species, nutrient load amounts, and soil type. In particular, different species of plants are more efficient at the uptake of nutrients than others (Kao et al., 2003). In temperate regions, however, the absence of plants during the winter months may reduce the nutrient removal potential of wetlands (Landry et al. 2009).

Hydrologically, flow rate, depth and wetland size play an important role in nutrient retention. For instance, one study showed that while nitrate was efficiently absorbed in shallow waters, phosphorus was more readily absorbed in deep waters (Hansson et al, 2005). In terms of a wetland's surface area, several studies have arrived to the conclusion that the correct size of a wetland with the purpose of improving water quality and providing flood control has to be anywhere from 2% to 7% of the catchments' size (Verhoeven et al. 2006, Mistch & Gosselink 2000).

Although macrophytic plants and hydrology are two primary means of improving water quality, algae also play an important role in the uptake and removal of nutrients, especially phosphorus,

from aquatic systems. The two main algal primary producers in wetlands are phytoplankton, or free-floating algae, and periphyton, or attached algae. In wetlands, periphyton has shown to be efficient in the uptake of nutrient from sewage effluent; however, rates of uptake are variable and can be influenced by periphyton biomass and metabolic activity (Dodds 2003).

Studies on the effectiveness of created wetlands in arid regions are rare. While primary production in wetlands and other freshwater environments are usually nutrient limited by phosphorus (e.g. Vaithiyanathan and Richardson 1997), studies have shown that arid ecosystems, including wetlands in arid regions, can be limited by nitrogen (Grimm & Fisher 1986, Scott et al 2005). Desert aquatic ecosystems are also unique in that they are more likely to be seasonally inundated, and experience extended periods of drying.

Restoring or creating wetlands requires the restoration of healthy wetland functions, such a nutrient cycling. Soil microbial communities can aid in the process of denitrification (Nurk 2005), phosphorus exchange, nutrient retention, (Kelton 2004, Ahn et al 2007, Jackson 2009), and the decomposition of organic matter and plant litter (Anh et al 2007). Soil microbial community structure has been shown to be sensitive to environmental degradation of wetlands (Merkley 2004), has been linked to internal loading of nutrients in wetlands (Kelton et al. 2004) and varies depending on the vegetation type present (Sherrill 2007). Understanding the soil microbial community is key to elucidating nutrient dynamics. Scott et al (2005) showed that with flow through the wetland, nutrients are usually depleted and microbial community decreases with availability of nutrients.

During the process of canalization of the Rio Grande between El Paso (TX, USA) and Ciudad Juarez (CHI, Mexico) in 1938, many natural occurring riparian wetlands were destroyed (Watts 2002). Furthermore, increased population and industrialization in the El Paso-Ciudad Juarez region has contributed to impaired water quality in the Rio Grande due to inadequate sewage treatment, industrial pollution and agricultural inputs (e.g. Rios-Arana et al. 2003; Owens and Niemeyer 2004; IBWC 2008). With the loss of most of the valuable wetland habitats, and their associated water filtration and floodwater control abilities, it has become imperative to understand our regional wetlands, protect those that remain and potentially enhance our regional wetland pool. Protection and enhancement of functioning wetland has the potential to improve water quality and aid in the attainment of state-established water quality standards mandated by the US Clean Water Act (Table 1). In particular, given the relative scarcity and value of riparian systems in the Chihuahuan Desert, restoration and creation of wetlands, mostly notably in areas where historically once they occurred, is necessary to re-establish important ecosystem functions to the region.

Table 1. Selected water quality criteria and screening levels that must be met for Segment 2308 (Rio Grande below International dam to Riverside diversion) (IBWC, 2008).

	Water Quality Criteria
TDS (mg/L)	1400
Cl (mg/L)	250
Fecal coliforms (CFU/100mL)	2000
DO (mg/L)	3
Ammonia (mg/L)	0.17
Nitrate (mg/L)	2.76
Orthophosphorus (mg/L)	0.5
Total Phosphorus (mg/L)	0.8

Study Sites

Before the canalization of the Rio Grande in the 1930s, the river flowed through what is the present day site of the Rio Bosque Wetland Park; however, since that time, the park area has been without a perennial water source. In 1997, the park landscape changed dramatically as large areas were cleared and grated to build a wetland complex and water-delivery system as part of the mitigation for the American Canal extension. Currently, the park is 372 acres and is enclosed by irrigation canals and drains on three sides; approximately one-quarter of the park is dedicated to re-establishing riparian and wetland communities. Since 2001, secondary treated wastewater has been delivered to the wetland cells of the Rio Bosque from the Roberto Bustamante Wastewater Treatment Plant during the months of October to February, when it is not being used for irrigated agriculture. Water entering the park at the inflow (Site A) is delivered through water delivery gates 2, 3 and 4 into wetlands Cells 1 and 2 (Figure 1). Water exits Cell 2 at Gate 6, whereas Cell 1 has no outflow and water evaporates over time. The University of Texas at El Paso (UTEP) and its partners are working to guide and shape the recovery of the wetland park to promote native river-valley plant communities (Watts et al. 2002).

The Bustamante WWTP provides secondary treatment to wastewater before it is discharged into the Rio Bosque (non-irrigation season) or to the Riverside Canal (during the irrigation season). For irrigation, water is diverted from the Rio Grande into Canals that take the water to the fields; water percolates and gets into subsurface drain systems that take the water to a main drain which returns the flow to the river. The construction of more than 465 miles of drains, 457 miles of laterals and 139 miles of canals in the region (USBR) has increased the total length of all waterways in the region by more than four-fold. We visited 8 Canal, 11 Drain and 6 Rio Grande

sites, ranging from the Texas-New Mexico border through Hudspeth County south of Fort Quitman. We utilized these drains and canals, as well as multiple locations along the Rio Grande, to illustrate how a wetland could be used to improve water quality regionally.

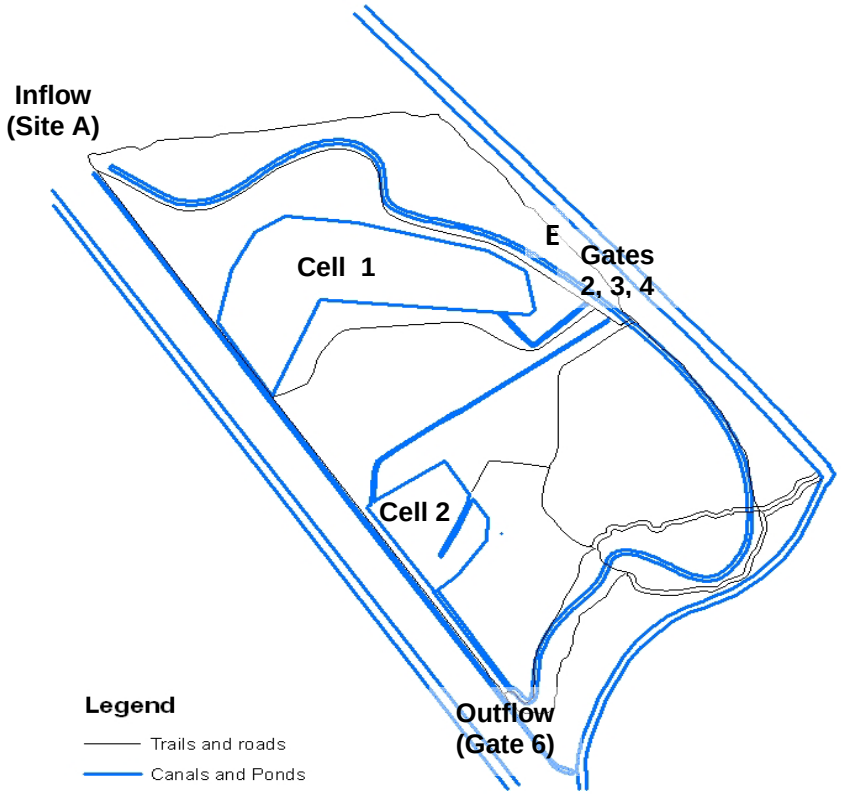


Figure 1. Map of Rio Bosque Wetland showing sample locations.

Figure 2. Map of sample locations of drains, canals, and Rio Grande in El Paso and Hudspeth Counties.

Although the Rio Bosque Wetland Park is more than 10 years old, there has yet to be a detailed analysis of spatial and temporal variability in water nutrient chemistry, and the relationship between primary production and nutrient levels. Understanding the ability of a desert wetland to remove nutrient from the water column through uptake by primary producers or sediment transformations is key to justifying the protection and creation of other similar wetland sites. In this chapter I will test the following hypotheses:

1) Water column nutrient levels will decline as water travels through the Rio Bosque wetland.
2) Nutrient-limitation of algal communities will increase nearer the outflow of the wetland, where nutrient availability will be lower.
3) Compared to the water delivery channels, wetland cells will have unique soil microbial communities that may act to modify nutrient cycling.

Methods

During the months of October to February from 2005-2009, the water delivery channels and wetlands cells of the Rio Bosque were sampled for water column (phytoplankton) and attached algae (periphyton), as well as the factors that may limit algal growth such as nutrients and light availability . Four sites were sampled: the first water control gate consisting of inflow from the Bustamante Wastewater Treatment Plant (WWTP, site A), in wetland cell 1, in wetland cell 2, and the water outflow gate from Cell 2 (gate 6). For the first two years of the study, all sites were sampled every 2-4 weeks. In the winter 2007-2008, the Rio Bosque was sampled bi-weekly until mid-December when water deliveries ended. In the winter of 2008-2009, sampling of the water continued on a monthly basis. To determine regional trends in water quality, we visited 8 Canal, 11 Drain and 6 Rio Grande sites 3 times sites in Spring, Summer and Fall 2007 and collected sampled for nutrient chemistry only (as described below). Sites ranged from the Texas-New Mexico Border through Hudspeth County south of Fort Quitman.

For determination of nutrient chemistry, water was collected from an open water area in 125mL acid-washed bottles. Total phosphorus (TP) was determined using the ascorbic acid method

following persulfate digestion (APHA 1998); nitrate (NO_3^-), ammonia (NH_3) and chloride (Cl^-) were analyzed according to Hach protocols and standard methods (APHA 1998). All analyses were performed on a Genesys 10 UV Spectrophotometer. Conductivity, temperature, pH, salinity and dissolved oxygen were measured in the field using an YSI® 556 multiprobe (YSI Incorporated, Yellow Springs, OH, USA), which was calibrated prior to each trip. Light was recorded at the surface and 30 cm depth using a LI-COR®LI-250A light meter and used to calculate light extinction coefficients. Turbidity was measured in triplicate using a HACH 2100 portable turbidimeter.

Chlorophyll-a concentration was used as a surrogate for algal biomass. For determination of phytoplankton chlorophyll, one liter of water was collected in opaque 1-L bottles and filtered immediately on Whatman GF/C filters, wrapped in foil and frozen until analysis. Sediment periphyton was collected from 5 separate locations at each sampling site using an inverted Petri dish and spatula. All 5 periphyton samples from the site were combined into one composite sample. Algal chlorophyll was separated from the sediment by vigorously mixing the sediment with distilled water and pouring off the surface liquid. This was repeated 10 times, until the water was relatively clear, to get the final periphyton sample. The liquid sample was stored in a test tube, wrapped in tin-foil and frozen until analysis. Chlorophyll was extracted from both phytoplankton and periphyton using 90% acetone and a 1-hour (phytoplankton) to 24-hour (periphyton) extraction period in the freezer. Results were corrected for phaeopigments by acidification (Wetzel and Likens 2000). Absorbances were determined on a Genesys 10 UV Spectrophotometer.

In order to determine the rate of phosphorus release from pond sediments in the Rio Bosque, sediment samples were collected from the inflow channel, outflow channel and three sites within cells 1 and 2 in Fall 2007 and refrigerated for two months until experimentation as described in Chow-Fraser et al. (1996) and Kelton et al. (2004). Wet sediment weighing 100mg, was spread at the base of an acid washed 250mL flask and covered with 150mL of deionized water. Flasks were covered with foil incubated at 25°C in the dark for eight days. Three flasks from each site were removed from the incubator on days 2, 4, 6, and 8 and 50mL of water from each flask was filtered and frozen. At the end of the experiment, all filtered water samples were thawed and tested for soluble reactive phosphorus (SRP) using the ascorbic acid method (APHA 1998). The rate of phosphorus release (mg m^{-2} day^{-1}) was determined from the slope of a linear regression relating SRP concentration (mg m^{-2}) to time.

In summer 2008, sediment from sites A, site E, and two locations from each of Cells 1 and 2 was also collected to determine the soil microbial activity at each of the sites. Because the ponds and channels were dry at the time of the experiment, soil from each site was placed into a plastic container and water from Riverside Canal, which receives input from the Bustamante WWTP, was added to each of the containers. The Riverside Canal water was autoclaved prior to the experiment to remove any water-associated microorganisms. The containers were left to stand in a greenhouse for a week, to saturate the sediment and approximate the conditions that may occur when Bustamante WWTP waters flood the Rio Bosque ponds and channels. The sediment was then extracted for analysis on Biolog EcoPlates. Soil samples (1g) were diluted in 99mL of 0.85% physiological saline-agar solution. The solution was covered and then stirred for 10 minutes on a Thermolyne 13000 multi-stir plate (Sybron /Thermolyne, Iowa, USA). The

saturated soil was diluted a further 10-fold, with 1 mL of the solution added to 99 mL of 0.85% physiological saline agar solution, for a final dilution of 10^{-2}. Biolog EcoPlates™ (Biolog, Inc. Hayward, CA) were injected with 150 µl of each dilution and incubated at 37°C. EcoPlates hold three replicates of 31 common carbon substrates used by the soil community**.** As soil microbes grow and metabolize the carbon source, a tetrazolium dye in the well is oxidized and develops a purple color. The EcoPlates were read initially at 12 hours, followed by 6 hours intervals using a SpectroMax190 spectrophotometer TM (Molecular Devices Corp, Sunnyvale, CA) until the average well color development (AWCD) of each plate was ~0.6. The diversity of carbon substrates used by the soil microbial community was expressed using Principal Components Analysis (see below). Substrate diversity was also expressed by counting the number of positive wells (absorbance > 0.10) at a similar time period (34 hours). Finally, soil microbial activity was measured by determining the AWCD after 34 hours.

During the winters of 2007-2008 and 2008-2009, nutrient diffusing substrates (NDS) were set out to determine which nutrient was limiting to algal growth at the Rio Bosque. Because of the unpredictability in water deliveries during these 2 years, the experiment was run only twice in 2007-2008 and twice in 2008-2009. Data from two of these dates were excluded because water deliveries were halted unexpectedly and the NDS were dry for 2-3 days before water deliveries re-started. NDS were placed at the inflow (site A) and outflow (site 6), and if water was present, in cells 1 and 2. Four NDS were constructed using Plexiglas, PVC pipe and 80 mL HDPE bottles. Plexiglas was cut into 50 x 40cm sheets and 12 holes (3.8 cm diameter) were drilled into the glass every 8 cm to allow for attachment of the bottles. A 1.9 cm hole was drilled into the lid of each bottle, which was then glued into the holes in the Plexiglas using non-toxic glue. The

Plexiglas was attached to a flotation frame made from PVC pipe using galvanized steel and plastic fasteners; the frame was constructed so as not to shade the nutrient treatments. A 0.2 μm membrane filter (Pall Corporation) was placed into the holes in the bottle lids as the substrate upon which algae would grow. Each NDS consisted of 3 replicates of each of 4 treatments: a control (ctrl), phosphorus enrichment (P), nitrogen enrichment (N), and a combination of phosphorus and nitrogen (N+P). All the treatments consisted of a 2% agar solution; phosphorus and nitrogen were added at a concentration of 0.5M using potassium phosphate (KH_2PO_4) and sodium nitrate ($NaNO_3$), respectively (Fairchild et al. 1985). At the end of 2-4 weeks (depending on water availability), each filter was removed, sorted by treatment into test tubes, covered with foil, and frozen. Chlorophyll-a was extracted and analyzed as described previously; concentration was expressed as $\mu g\ cm^{-2}\ day^{-1}$.

Because of the temporally repeated sampling design of this study, we used paired t-tests to compare whether there were differences among Rio Bosque sites sampled on the same dates. Levels of significance were Bonferonni corrected. Simple linear regressions were used to relate algal biomass to the measured environmental variables. Nutrient limitation experimental results were compared using t-tests. Data were log-transformed as required to normalize the data. Principal components analysis (PCA) was used to create linear combinations of the environmental data to describe the underlying environmental gradients in the data, and to identify EcoPlate C-sources explaining the greatest variation among sites. Prior to PCA environmental data were log-transformed to approximate normal distributions and standardized to zero mean and unit variance. Differences in water quality among drains, canals and the Rio

Grande were calculated using an ANOVA, followed by Tukey HSD test. All statistical analyses were performed using JMP software (Version 4.0, SAS Institute Inc., Cary, N.C.).

Results

Table 2 shows the mean nutrient and physical parameters at the four primary sampling sites in Rio Bosque Wetlands Park. Water quality varied throughout park; however, very few significant differences were observed in water quality after the water flowed through the wetland cells. Nitrates were significantly higher (paired t-test; $p=0.03$) in the inflow compared to Cell 2. The inflow also tended to have higher nitrates than the outflow, although this was not significant ($p=0.06$). Conversely, sodium chloride concentrations increased from the inflow (639.06 mg/L) to the outflow (743.06 mg/L); however, this was not significant. The water at the inflow was significantly warmer than all others sites ($p<0.001$).

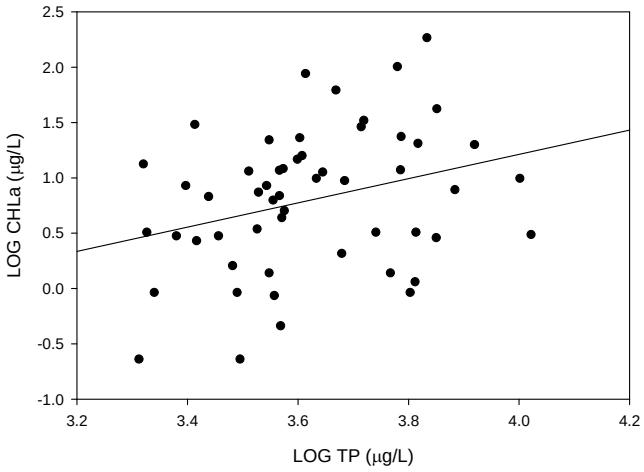


Figure 3. Regression of log transformed total phosphorus and phytoplankton chlorophyll a, ($y=-3.172716+1.096$, $r^2=0.09$, $p=0.026$).

A weak positive relationship was observed between total phosphorus and phytoplankton chlorophyll a (Figure 3). As TP increased, so did the amount of algae growth. No other

significant relationships were found between the individual water quality variables and chlorophyll biomass.

Table 2. Mean water quality and physical variables at 4 sampling sites in Rio Bosque Wetlands Park over four winters (2005-2009).

Site	*n*	TP (mg/L)	NO_3-N (mg/L)	NH_3-N (mg/L)	NaCl (mg/L)	Phyto CHLa (µg/L)	Peri CHLa (µg/cm^2)	Conductivity (mS·cm^{-1})	DO (mg/L)	Temperature (°C)
Inflow	18	4.37 (+/- 0.49)	10.29 (+/- 1.12)	6.40 (+/- 1.31)	639.06 (+/- 45.84)	2.237 (+/- 0.98)	3.08 (+/- 0.92)	1.80	6.196	23.55
Outflow	16	4.44 (+/- 0.46)	7.52 (+/- 1.17)	6.30 (+/- 1.76)	743.06 (+/- 50.82)	12.91 (+/- 2.06)	7.37 (+/- 2.70)	1.83	8.732	15.11
Cell 1	15	4.58 (+/- 0.64)	9.82 (+/- 2.16)	8.12 (+/- 1.71)	658.2 (+/- 58.46)	24.04 (+/- 14.62)	6.35 (+/- 1.98)	1.83	9.523	17.45
Cell 2	18	4.46 (+/- 0.58)	7.84 (+/- 1.26)	4.70 (+/- 0.93)	651.15 (+/- 48.19)	24.28 (+/- 8.78)	7.55 (+/- 2.70)	1.86	9.907	15.78

Principal Components Analysis (PCA) was used to determine which environmental variables explained the greatest amount of variation in the environmental dataset and to ascertain whether algal biomass can be explained by a multivariate environmental axis (Table 3, Figure 4). The environmental variables used in the Principal Components Analysis were nutrients (total phosphorus (TP), ammonia (NH_3-N), nitrate (NO_3-N), and sodium chloride (NaCl)), pH, conductivity, and temperature. The first three PC axes together explained 69% of the variation in the environmental data (Table 3) where the first PC axis explained 34% of the variance, 21% of the variation is explained by the second PC axis and 14% of the variance by the third axis. The first PC axis was positively correlated with NH_3-N, pH, and conductivity, and negatively correlated with TP, NO_3-N, and NaCl. The second PC axis was positively correlated with the temperature, conductivity, and NO_3-N, and negatively correlated with pH. The third PC axis was positively correlated with NaCl, pH, and conductivity.

PCA scores of Cell 1, Cell 2, inflow (site A) and outflow (gate 6) are depicted in Figure 4. Most of the inflow sites were found at higher levels of PC axis 2, and were thus associated with high conductivity, temperature, and nitrate. Conversely, the outflow sites tended to be found towards the negative end of PC axis 2 and were mostly associated with higher pH. The PCA scores of Cell 1 and 2 vary throughout; this could be due to seasonal variation. For example, along PCA1, sites sampled in November and December tend to be found on the positive end of the axis, while sites sampled in January and February cluster on the left hand side of the axis (Tukey HSD, $p<0.0001$).

Table 3. Correlation coefficients ($p<0.05$) between PC axes 1, 2, and 3 scores and environmental variables.

	Variance explained (%)	**Environmental variable**	**r**
PC axis 1	34%	NH_3-N	0.76
		pH	0.53
		Conductivity	0.46
		TP	-0.71
		NO_3-N	-0.61
		NaCl	-0.53
PC axis 2	21%	Temperature	0.77
		Conductivity	0.57
		NO_3-N	0.46
		pH	-0.49
PC axis 3	14%	NaCl	0.69
		pH	0.47
		Conductivity	0.43

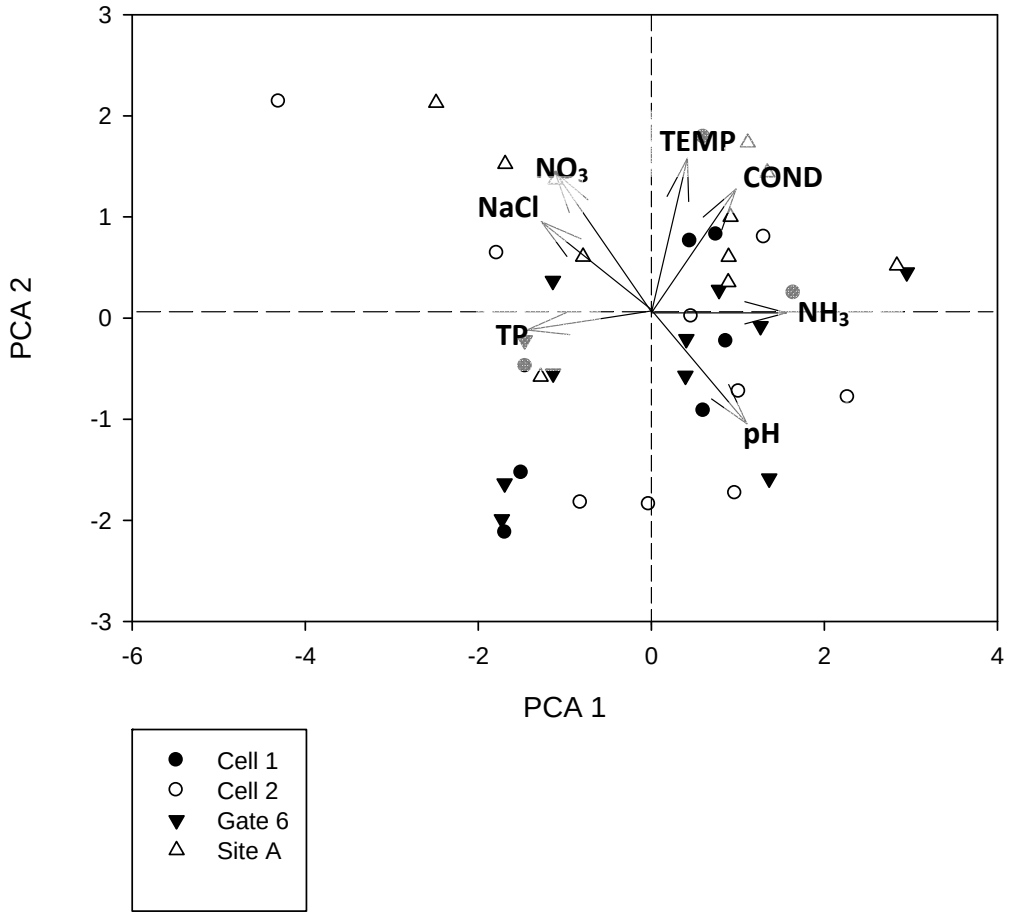
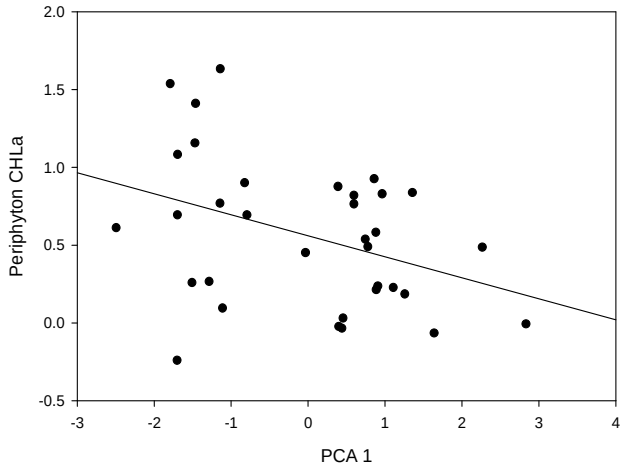
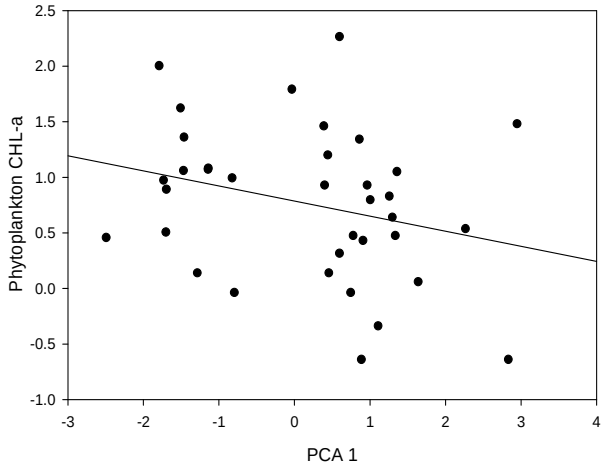


Figure 4. Plot of PC axis 1 and PC axis 2 scores. Sites are indicated by different symbols in the plots. Environmental vectors are correlation co-efficients (r) multiplied by 2.





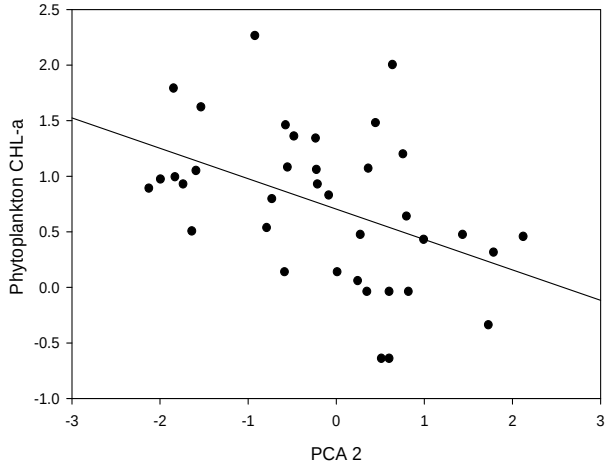


Figure 5. Regressions between PCA scores and log-transformed algal concentrations: TOP: periphyton chlorophyll-a versus PCA1 (y=0.559-0.135x, r^2=0.1452, p=0.0262); MIDDLE: phytoplankton chlorophyll-a versus PCA1 (y=0.7685-0.143x, r^2=0.083, p=0.0837); BOTTOM: phytoplankton chlorophyll-a versus PCA2 (y=0.7039-0.27349x, r^2=0.202, p=0.0053).

Both periphyton (y=0.559-0.135x, r^2=0.1452, p=0.0262) and phytoplankton (y=0.7685-0.143x, r^2=0.083, p=0.0837) biomass declined along PC Axis 1 (Figure 5), which indicates higher algal biomass at higher levels of TP, nitrate and chloride A stronger relationship was seen between phytoplankton versus PCA 2 scores (y=0.7039-0.27349x, r^2=0.202, p=0.0053), indicating that phytoplankton biomass was lowest at higher levels of temperature, conductivity, and nitrate. This last relationship is likely driven by data from the inflow (site A) where warmer temperatures and higher nitrates coincided with low phytoplankton biomass.

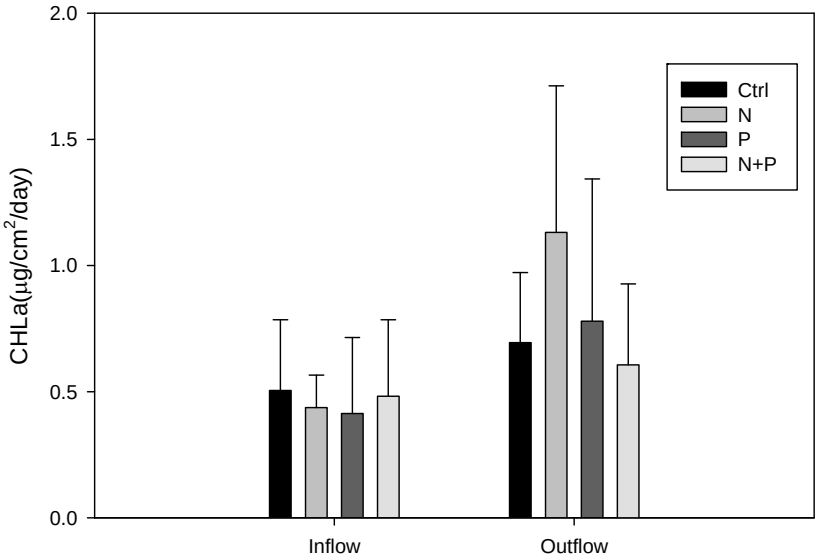


Figure 6. Graph showing the mean amount of periphyton (CHLa) growth collected from nutrient diffusion substrates from inflow and outflow, during January 2008 and February 2008. Standard error bars are included.

Nutrient limiting experiments completed in the last 2 years of the study indicated that algae tended towards limitation by nitrogen at the outflow, with no nutrient limitation at the inflow. However, these results were not significant whether the dates were kept separate (not shown), or

if both dates were combined (Figure 6; t-test; p=0.153), and the completion of additional experiments was hampered by the intermittent and unpredictable nature of water inflows at the Park. Future experiments will be performed with more than three replicates per treatment.

Table 4. Correlation coefficients between PC axis 1 scores and carbon sources from BIOLOG plates.

	Variance explained (%)	**Carbon Source**	**Carbon Code**	**PCA 1**
PCA 1	41%	**Carbohydrates**		
		D-Cellobiose	DCELL	-0.88
		B-Methyl-D-Glucoside	BMDG	-0.82
		Polymers		
		Tween 80	TWEE	-0.87
		Phosphorylated chemicals		
		D, L, -α-Glycerol phosphate	DGLP	0.81
		Amino Acids	LTHRE	-0.95
		L-Threonine		
		Carboxylic Acids	DAGYL	0.92
		D-Galactonic acid γ-Lactone	DGLA	0.89
		D-Glucosaminic acid		

Principal components analysis (PCA) was used to identify EcoPlate C-sources explaining the greatest variation among sites. PC axis one explained 41% of the variance in the carbon sources utilization; the additional axes explained less than 10% variation in the dataset (not shown). Carbon sources associated with PCA 1 scores were carbohydrates (DCELL and BMDG), polymers (TWEEN 80), carboxylic acids (DAGYL, DGLA, and FHBA), phosphorated

chemicals (DGLP), and amino acids (LTHRE) (Table 4). The Biolog EcoPlate data indicated that inflow sites had unique, relatively diverse and active soil microbial communities as compared to the wetland cells. Because only the first axis was important, the PCA scores are listed in Table 4, instead of a figure. The channels sites tended to have higher PCA 1 values, than the sites located in the wetland cells, especially those locations in the cells that were more regularly flooded (Cell 1 north, Cell 2 south) (Table 5). PCA 1 values were positively correlated with AWCD ($r=0.97$, $p=0.0061$) and the number of positive wells ($r=0.96$, $p=0.0098$) (Table 5).

Table 5. Soluble reactive phosphorus (SRP) release rates and soil microbial community indices. Average Well Color Development (AWCD) at 34 hours.

Site	SRP release rate (mg m^{-2} day^{-1})	AWCD	Mean # Positive Tests	PCA 1
Cell 1 north	0.32	0.073976	24	-3.604217
Cell 1 south	0.285	0.344	60	1.83
Cell 2 south	0.30	0.077588	17	-2.989003
Outflow	3.26	-	-	-
Inflow	0.99	0.627372	58	3.52657451
Site E	1.44	0.713233	62	4.18315293

Phosphorus release from the sediment from related to the Biolog data to determine if the microbial community can affect internal loading of phosphorus. SRP release rates from sediment were calculated based on the regression of SRP concentrations through time (Figure 7). The slope of the line indicated the rate of release (Table 5) and was lowest in the pond sites (mean of 0.3 mg m^{-2} day^{-1}), and highest in the channels, most notably at the pond outflow (3.26 mg m^{-2} day^{-1}) (Table 5). SRP release rates were positively correlated with average well color development ($r=0.90$, $p=0.0347$), indicating greater phosphorus release at sites with higher levels

of bacterial activity. No other correlations were significant. There was no relationship between SRP release rate and water column TP.

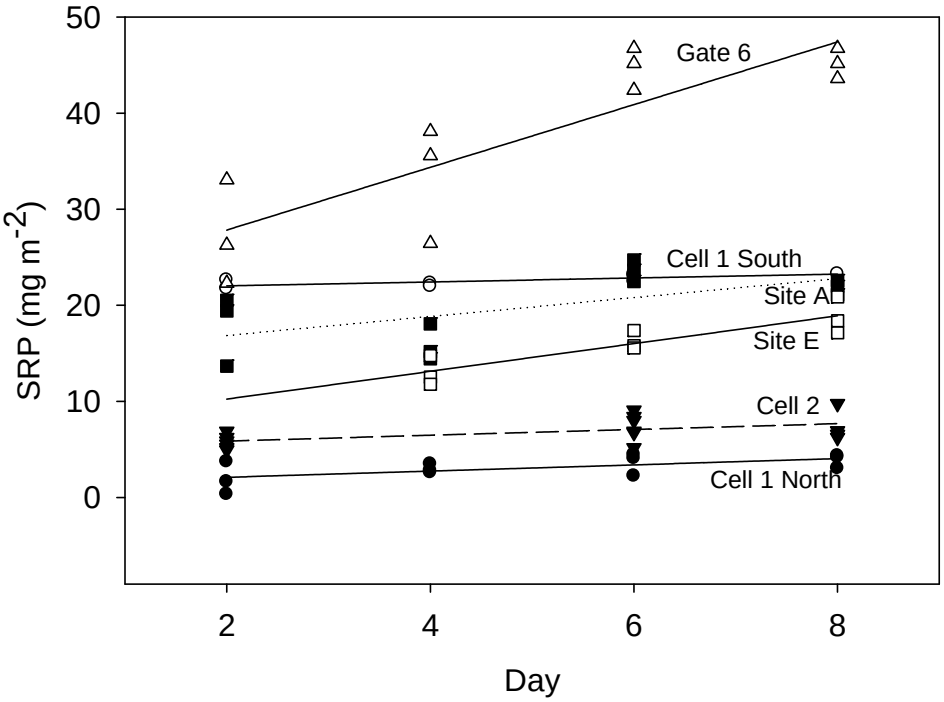


Figure 7. Soluble reactive phosphorus (SRP) release rates regressions relating SRP (mg m^{-2}) through time at all sites. See Table 6 for regression equations.

Table 6. Regression equations relating SRP (mg m^{-2}) to time (see Figure 6).

Site	Regression Equations
Cell 1 north	$y = 1.45 + 0.32x$; $r^2=0.37$, $p=0.0352$
Cell 1 south	$y = 21.62 + 0.203\ x$; $r^2=0.57$, $p=0.0311$
Cell 2 south	$y = 5.27 + 0.302\ x$; $r^2=0.29$, $p=0.0315$
Outflow (Gate 6)	$y = 21.3 + 3.26\ x$; $r^2=0.75$, $p=0.0003$
Inflow (Site A)	$y = 14.88 + 0.987x$; $r^2=0.39$, $p=0.0312$
Site E	$y = 7.35 + 1.44x$; $r^2=0.78$, $p=0.0016$

In many cases, nutrient levels in the Rio Bosque were an order of magnitude higher than those observed in other regional water bodies (Table 2; Figure 8). The eight water delivery canals visited in this study tended to have the highest concentration of nutrients, including TP ($p=0.0069$) and NO_3-N ($p=0.0225$). The agricultural return flow drains had the highest concentrations of Cl ($p=0.004$). On average, state nutrient criteria for NH_3-N and Cl were exceeded at several site types. In addition, total phosphorus criteria levels were exceeded on 6 occasions (site-date combinations), and nitrate criteria were exceeded on 3 occasions (not shown). Half of these occasional exceedances occurred in the Riverside Canal, downstream of the Bustamante WWTP discharge.

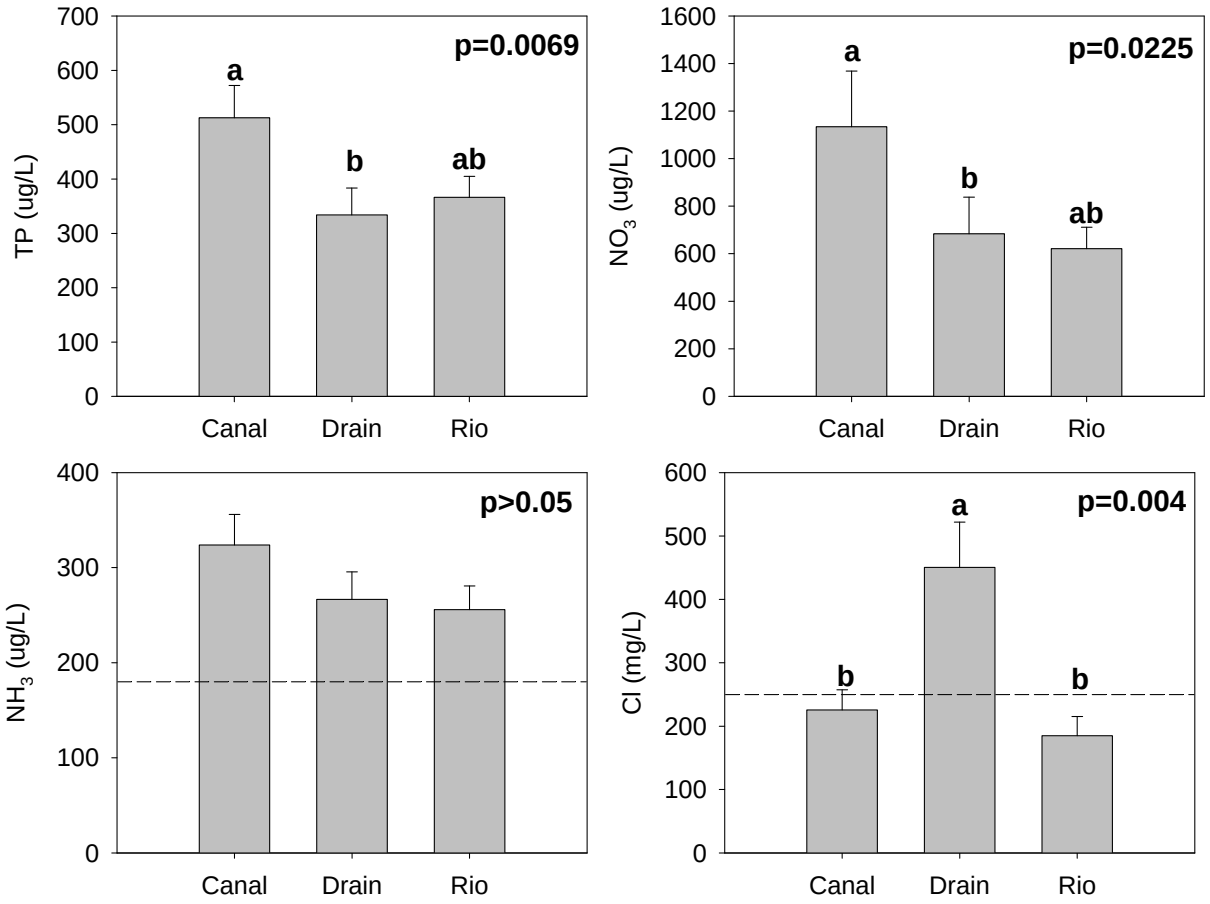


Figure 8. Comparison of mean nutrient concentrations in drains, canals and the Rio Grande in summer 2007. Dashed lines indicate state water quality criteria levels. Standard error bars are indicated.

Discussion

Rio Bosque Wetlands water comes directly from the Bustamante Wastewater Treatment Plant (WWTP). Water quality throughout the Rio Bosque Wetlands varies with flow and location within wetland. Total phosphorus (TP) levels do not vary throughout the wetland, where they are lowest at the inflow (4.37 mg/L) and outflow (4.44 mg/L) and not significantly higher in cell 1 (4.58 mg/L) and cell 2 (4.46 mg/L). Overall, the inflowing amount of TP is comparable to other constructed wetlands receiving effluent from sewage and WWTP's, (0.79 mg L^{-1}- 4.41 mg L^{-1}), (Vymazal 2002). However, other studies reported as much as 70% decline in orthophosphorus as water flows through a wetland (Mitsch 2005).

In functioning wetlands, the two primary mechanisms of phosphorus retention are via plant uptake and sedimentation (Fisher 2004, Gu and Dreshel 2008). Phosphorus is taken up by plants during the growing season; however, in the Rio Bosque, there were no plants growing during the study period, as water is only delivered to the park during the non-growing season. Phosphorus can also be easily adsorbed by soils. In particular, clay sediment has been shown to increase the amount of phosphorus being retained in a wetland (Vymazal 2007) and high organic matter soils also increase phosphorus retention capacity (Reddy 1999). While soils in the wetlands cells tend to be more organic than soils in more upland areas of the park, these levels are still relatively low (<6% organic matter; Sherrill 2007). Release rates of phosphorus from the sediment were relatively low compared to published values, which range from 0.250 to 51.50 mg m^{-2} day^{-1}; Nurnberg 1988).

The laboratory experiment to demonstrate internal loading of phosphorus from sediment in the Rio Bosque showed that the greatest rate of phosphorus release occurred from the channel sediments, most notably at the outflow (3.26 mg m^{-2} day^{-1}). High nutrient release from sediment in the region of the outflow may have masked any effect of the wetland cells in reducing phosphorus concentrations in the water. The lowest sediment nutrient release occurred in the wetland cells. The presence of vegetation throughout the wetland cells in the summer months, despite the absence of water, may have acted to uptake nutrients from the sediment and reduce the total quantity of phosphorus stored in wetland soils. Indeed, the two most wetland-like sites (Cell 1 north, Cell 2 south) tended to have lower total phosphorus release as compared to the other sites (Figure 8). Further experiments need to be conducted to confirm SRP release rates on sediment collected at different times of the year, and to ascertain the total concentration of phosphorus available for release from sediment at each site.

Phosphorus release and retention is also affected by the amount of oxygen in the sediment and water (Carlton and Wetzel 1988, Qualls 2001, Dodds 2003). Phosphorus tends to be released from soils under anoxic situations (Carlton and Wetzel 1988, Nurnberg 1988, Dodds 2003). Dissolved oxygen (DO) levels were relatively high during the daylight hours at the Rio Bosque; however, it is possible that lower nighttime DO may have encouraged phosphorus release. In particular, the greater amount of decomposing plant litter in the wetland cells could have influenced the redox potential in the soil, and in turn influence the microbial community and nutrient levels in the water and sediment (Qualls 2001).

Microbial activity also plays a role in phosphorus release rates from sediment (D'Angelo 2005, Aldous 2007, Gu and Dreshel 2008). Indeed, in our study, SRP release rates were highly correlated with average well color development, an estimate of microbial activity and microbial carbon substrate use in the soils. The Biolog Ecoplates with the highest average well color development (AWCD) were found in the inflow channel (Site E and the Inflow), while the lowest amount of AWCD was found in the wetland cells, primarily at the most regularly flooded sites, Cell 1 north and Cell 2 south. These levels in microbial activity were also correlated to a unique community of microbes, associated with different carbon sources, as indicated by the Principal Components Analysis.

Algae may also play an important role in the uptake of nutrients from a wetland (Dodds, 2003). In this study, univariate and multivariate analyses indicated that sites with increased TP, NO_3 and Cl concentrations coincided with higher algal growth. While this did not appear to be sufficient to reduce the phosphorus levels exiting the wetland at the outflow, nitrate levels were reduced as water flowed through the wetland.

Ammonia is a byproduct of fecal matter, so high amounts of nitrate and ammonia (NO_3-N: 10.29 mg/L and NH_3-N: 6.40 mg/L) are not unexpected near WWTP outfalls, and these levels were comparable to those observed in other studies (NH_3-N: 5.98 mg/L (Vymazal 2002)). Nitrate levels were significantly reduced at the outflow (NO_3-N: 7.52 mg/L), while ammonia remained unchanged (NH_3-N: 6.30 mg/L). Wetlands have proven to be efficient in the removal of nitrogen from water (Hansson 2005). The main way that nitrogen is removed from water in wetlands is through denitrification (Mitsch and Gosselink, 2007, Landry et al 2009), but it can also be taken

up by plants (Kadlec 2005). Uptake of nitrogen by plants is a slow process (Grimm and Fisher 1998, Kadlec 2005), and most of the nitrogen uptake is done by microbial communities, which need the ammonia for growth (Vymazal 2007). Denitrification is the process in which bacteria convert nitrates into atmospheric nitrogen under anoxic wetland conditions; wetland denitrification is one of the primary ways in which nitrogen is returned to the atmosphere (Mitsch and Gosselink, 2007). Seasonal variations, especially lower temperatures, limit the amount of denitrification that takes place (Toet 2005). Nonetheless, even though the Rio Bosque receives effluent only in the colder winter months, nitrate levels were reduced as water passed through the wetland suggesting the occurrence of denitrification. Denitrification tends to occur between the oxidation-reduction potential (ORP) values of -50 to +50 mV. On average, ORP in the Rio Bosque was 209 +/- 15 mV and rarely fell low enough (during daytime sampling) to promote denitrification. Lower dissolved oxygen levels during the night time may have promoted denitrification (Kuschk 2003); however, diurnal oxygen or ORP measurements were not taken.

In order to determine exactly which nutrient was limiting to algal growth in the wetland, a nutrient limitation experiment was conducted during the winter seasons of 2007-2009. This experiment was challenged by the unpredictability of water deliveries at the Rio Bosque, where two of four experiments were damaged when water delivery stopped unexpectedly. Although concentrations of nutrients at the inflow did not appear to limit algal growth, at the outflow, limitation tended towards nitrogen. This result was not significant, however, and future experiments in will attempt to refine these results. Limitation of algal growth by nitrogen at the outflow would support the observation that nitrate levels are significantly reduced towards the

outflow of the wetland. In desert streams in the US southwest, nitrogen has been found to be limiting, depending on the season (Grimm & Fisher 1998, Scott et al 2005).

Hydrophytic vegetation is key to providing the nutrient retaining capacities of wetlands, either through the uptake of nutrients directly (Kuusemets et al 2005, Thullen 2005, Gu and Dreshel 2009, Landry 2009), or by indirectly affecting nutrient concentrations through alteration of the redox potential, microbial community, and soil characteristics (Williams 1985, Brisson and Chazarenc 2009). Sherrill (2007) found that the ponds in the Rio Bosque have developed some characteristics of a wetland plant community since the park was established 10 years ago; however, the absence of water during the growing season has substantially slowed this process. Improved nutrient retention capacities in the Rio Bosque Wetlands, and resulting improvements to the out-flowing water, will only occur if water flows through the ponds in the summer months and hydrophytic vegetation is established.

Finally, salinity is a major concern of water bodies in the desert. Salinization is often associated with irrigation and occurs when salts are drawn to the soil surface as water evaporates. With a low amount of precipitation in the desert and the alternating periods of water delivery, Rio Bosque does not have sufficient water to flush salts from the system, especially in Pond 1 where water exits the pond through evaporation not a surface outflow. The arid conditions in the desert increase the rates of evaporation occurring at the park, so most of the salt is left in the soil. High salinity rates can increase the rates of decomposition (Bailey 2001), increase the amount of bacterial activity (Jackson and Vallarie 2009), decrease the abundance and diversity of aquatic microbial community (Bailey 2001), and reduce the emergence of zooplankton and aquatic

plants (Nielsen et al. 2003). We found no significant relationship between chloride levels and bacterial activity of the water column; however, salt content of the soil was not determined in this study and this may have played a larger role in structuring the microbial community.

Water quality in the Rio Grande and agricultural drains and canals exceeded state water quality standards for ammonia and chloride levels, and can exceed total phosphorus and nitrate levels on occasion, most notably downstream of the Bustamante WWTP. Nutrient concentrations were likely higher in canals in part because canals receive input directly from WWTPs, whereas lower nutrient levels in the drains may indicate that nutrients have been captured by the plants and microorganisms inhabiting the agricultural fields through which the water has recently passed. A functioning wetland adjacent to these higher nutrient canals could act to clean the water that flows into the Rio Grande and ultimately aid in the attainment of nutrient criteria in the region. The Rio Bosque is conveniently situated beside the Riverside Canal, receiving effluent from the Bustamante WWTP during the non-growing season. If water were diverted into the park during the summer, thereby promoting hydrophytic plant growth and other wetland functions, this could be a first step in improving regional water quality.

Conclusions and Management Implications

Rio Bosque Wetlands Park receives water only seasonally, during the winter months. This wetland is only one of two urban wetlands located in El Paso, TX, but is the only wetland in the region receiving effluent from a waste water treatment plant and, thus, with the potential to improve water quality in the Rio Grande. Our study indicates thatthe wetland is only functioning to remove moderate amounts of nitrate from the water column, but has no impact on phosphorus

or ammonia levels. Although, given the water delivery schedule, we were not able to study the wetland during the growing season, I predict that the presence of water year round, and the increased growth of wetland plants it would promote, may have a substantial effect on the removal rates of nutrients from the wetland through not only the direct uptake of nutrients by plants but also by alterations in redox potential, denitrification, microbial activity, and sediment phosphorus dynamics. Rio Bosque has the potential to be a highly functional wetland, but the lack of water during the growing season is a key factor missing from the Rio Bosque.

Further investigation is needed to understand the following questions regarding the dynamics of Rio Bosque Wetlands Park:

- Based on the experiments done using Biolog Ecoplates, it would be interesting to determine what carbon sources are available to the soil microbial community, what carbon sources are limiting microbial activity at Rio Bosque Wetlands Park, and what is the role of Rio Bosque in regional carbon dynamics?
- Does the distance from the inflow affect nutrient concentrations, and what implications does this have for channel or wetland cell restoration?
- Further study on phosphorus release from sediments in the Rio Bosque Wetlands is required. Experiments on the role of algal crusts on phosphorus release and retention could be performed (e.g. Thomas et al. 2006). In addition, the relative amount of available phosphorus and the availability of phosphorus seasonally could be determined? If phosphorus is being retained, why?

- What are the sediment characteristics (e.g. organic content, particle size) in the cells and channels at Rio Bosque Wetlands Park? What effect is this having on nutrient release and total nutrient content in the sediment?
- What is the role of groundwater on nutrient dynamics at Rio Bosque Wetlands Park? Is groundwater contributing to the nutrient load at Rio Bosque Wetlands Park?
- A groundwater pump was recently installed near the inflow (Site A) and can be used sporadically during the summer months to deliver water to the channel. Studies on the ideal frequency, magnitude and seasonal schedule of water delivery would aid in management decisions at the park to insure inputs of groundwater are being utilized to their maximum potential?

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Curriculum Vitae

Ruth Rodriguez was born in El Paso, TX. She is one of five children of Jesus and Sylvia Rodriguez, also of El Paso, TX. Ruth graduated from high school in 1997 from Faith Christian Academy in El Paso (UTEP). She pursued her collegiate education from the University of Texas at El Paso, and attained her Bachelors of Science Degree in Biology, with a focus on Ecology and Evolution, in 2006. She started her Master's Degree at UTEP in the fall of 2006, where she began working in the Aquatic Ecology Laboratory, under the direction of Dr. Vanessa L. Lougheed. She worked on a feasibility study for a bi-national working wetland with the World Wildlife Fund (WWF). She was a participant in International Polar Year-Research Opportunities in Antarctica for Minorities (IPY-ROAM), which took students to Antarctica during the winter of 2007-08. She has participated in numerous conferences presenting research collected in Antarctica, focusing mainly on the effect penguins have on offshore nutrients.