BACTERIALLY-MEDIATED CARBON DYNAMICS IN A HIGHLY IMPACTED RIVER NETWORK

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BACTERIALLY-MEDIATED CARBON DYNAMICS IN A HIGHLY IMPACTED RIVER NETWORK

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For my family, both the biological and the chosen.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

BACTERIALLY-MEDIATED CARBON DYNAMICS IN A HIGHLY IMPACTED RIVER NETWORK

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Inland freshwater ecosystems, though comprising a small portion of the earth's surface, are thought to be important in the global carbon (C) cycle. Carbon processing by heterotrophic microbes (bacteria) is a critical process, contributing considerably to overall ecosystem production and processing of dissolved organic carbon (DOC). This study assesses spatial variation in C processing by heterotrophic bacterioplankton in a semi-arid river network: the Rio Grande/Rio Bravo del Norte in Texas, USA. I examined how bacterial metabolism and C processing varied with spatial differences in physicochemical conditions and patterns in DOC lability in this highly impacted riverine network. Physicochemical and biological data were collected at 14 sites from March - December of 2010. I additionally analyzed phytoplankton biomass, bacterial density, bacterial community metabolic rates [bacterial respiration (BR), bacterial productivity (BP), and bacterial growth efficiency (BGE)], and C quality parameters at a subset of nine sites within this drainage. Across the drainage, hydrology and landscape position

(i.e., biogeoclimatic conditions, presence of reservoirs, and groundwater contribution to flow) substantially influenced in-stream physicochemical conditions, leading to spatial patterns in bacterial density, phytoplankton biomass, and bacterial metabolism. Bacterial C metabolism was influenced by both physicochemical and C quality – quantity gradients present within the drainage. Bacterial production and BR responded to different environmental gradients, with BP being driven by C quality and inorganic nutrients. This resulted in a negative correlation between BGE and the bacterial respiration of refractory C. Results from this study indicate that natural variation and anthropogenic impacts influence the physicochemical and biotic conditions across the Rio Grande/Rio Bravo del Norte drainage and these effects have implications for C sequestration, transformation, and transport, as well as for organic matter (OM) delivery to the Gulf of Mexico.

CHAPTER I

BACTERIALLY-MEDIATED CARBON DYNAMICS IN A HIGHLY IMPACTED RIVER NETWORK

INTRODUCTION

On a global scale, it is estimated that inland waters are responsible for processing, transporting, and sequestrating approximately 2.9 Pg of terrestrial carbon (C) annually (Tranvik et al. 2009). By comparison, fossil fuel combustion by humans releases 6.4 Pg C per year and 2.6 Pg C are sequestered in terrestrial C sinks, primarily by forests (Bergermeister 2007). At landscape and global scales, inland waters, including rivers and streams, are relatively active biogeochemically and process more C than is expected based solely on their area within the greater landscape (McClain et al. 2003; Cole et al. 2007). Cumulatively, freshwater environments, while constituting a small portion of the earth's surface, play an important role in global C transformations and processing (Dean and Gorham 1998; Downing et al. 2008; Tranvik et al. 2009). Carbon cycling in lotic ecosystems involves the complex interplay of numerous factors, including land use, geomorphology, quality and quantity of C sources, origin of C sources, autotrophic and heterotrophic microbial activity rates, and the composition and diversity of microbial communities (Moran et al. 2000; Cole et al. 2007; Williams et al. 2010). Given the

diverse number of factors and the potential number of interactions between them, it is difficult to determine how, and at what scale, these factors impact C cycling in fluvial networks (Cole et al. 2007).

Carbon processing by heterotrophic microbes is a critical process in freshwater systems, contributing significantly to overall ecosystem production (Dodds and Cole 2007; Cole et al.. 2007). In particular, C metabolism by microbial heterotrophs is the primary C sink of dissolved organic matter (DOM) or dissolved organic carbon (DOC) pools in aquatic systems (Weiss and Simon 1999). Although, many factors affect rates of microbial DOC processing, the relationship between the concentration, sources, and composition of the DOC pool and bacterial metabolism is highly debated. Bacterial production (BP), the rate at which C is incorporated into bacterial biomass, and respiration (BR), the rate at which bacteria use environmental C for cell maintenance rather than growth, are expected to exhibit a positive relationship with DOC concentration (Lennon and Pfaff 2005). However, the size of the DOC pool may not necessarily be closely related to BP and BR, particularly if the bulk DOC pool is composed of mostly refractory C sources (Findlay 2003; del Giorgio and Davis 2003). Indeed, it is hypothesized that increasing proportions of refractory C within the DOC pool will affect bacterial metabolic function and lead to greater BR per unit BP, indicating decreased bacterial growth efficiency (BGE).

Although the size and lability of the DOC pool is important in determining bacterial metabolism, other factors also influence bacterial C processing. The source of the DOC pool is important in determining bacterial metabolism in that the fraction of the labile C pool is thought to be largely produced *in situ* by primary producers (Wetzel

1983; Sondergaard and Middelboe 1995; del Giorgio and Davis 2003; del Giorgio and Pace 2008). A myriad of physicochemical processes other than bacterial processing can also act upon DOC (e.g., ultraviolet radiation exposure, flocculation with particulates), but the relative importance of these processes can be difficult to distinguish from the effects of bacteria (del Giorgio and Pace 2008). Additionally, increased availability of inorganic nutrients (especially P and N) can catalyze bacterial incorporation of C (i.e. increase BP) and, thus, can stimulate higher C processing rates (Granéli et al. 2004; Pace and Cole 2008; Scott et al. 2012).

Much of the research on landscape-level variation in large river bacterioplankton C processing has occurred in temperate climate zones (Maranger et al. 2005; del Giorgio and Pace 2008; Williams et al. 2010; Guillemette and del Giorgio 2011), although notable studies have included research into boreal (Holmes et al. 2008; Comte and del Giorgio 2009), sub-tropical (Hadwen et al. 2010), and tropical (Amon and Benner 1996) rivers. Arid and semi-arid ecosystems are distributed world-wide, comprising somewhere between 33 and 50% of the earth's land surface (Agnew and Anderson 1992; Middleton and Thomas 1997). Due to the critical need for water in these regions, the majority of rivers in arid landscapes are under increasing anthropogenic pressure (Kingsford 2000). . Previous studies have investigated aspects of arid river ecosystem processing and function (Williams 1999; Kingsford 2000; Feng et al. 2001; Hauer and Lorang 2004; Larned et al. 2008); few, if any, specifically address the contribution of bacterial metabolism to C dynamics.

One of the critical questions in aquatic ecosystem studies is the relative importance of allochthonous versus autochthonous subsidies to aquatic food webs. In

river systems with relatively limited productivity in adjacent terrestrial areas, such as arid river systems, *in situ* primary productivity is expected to be the main C source for heterotrophs (Finlay 2001). Gross primary productivity in arid rivers is greater than that of temperate rivers and this is likely due to a combination of high light availability, lower flows, higher water temperatures, and efficient nutrient cycling (Lamberti and Steinman 1997; Velasco 2003). Thus, it would be predicted that arid systems are more reliant upon autochthonously-derived OM and dissolved organic C (DOC) from *in situ* primary producers and less reliant upon allochthonously-derived C sources. In addition, autochthonously-generated DOC is generally considered to be more labile than allochthonous sources (Sondergaard and Middelboe 1995; del Giorgio and Davis 2002). Therefore, it can be predicted that arid river systems should exhibit relatively higher DOC lability and greater bacterial growth efficiency (BGE) than rivers in more mesic landscapes.

In the present study, I assessed spatial variation in C processing by heterotrophic bacterioplankton in a semi-arid river network: the Rio Grande/Rio Bravo del Norte in Texas, USA. I examined how bacterial metabolism and C processing vary with spatial differences physicochemical conditions and patterns in organic carbon (OC) lability in this highly impacted riverine network. Despite high economic, social, and ecological value, the Rio Grande is one of the world's most at-risk rivers (Wong et al. 2007). I hypothesized that there would be spatial variation in physicochemical conditions in the Rio Grande/Rio Bravo del Norte drainage and that this variation would lead to spatial variation in bacterial metabolism. I also hypothesized that both physicochemical and C

quality – quantity conditions would impact patterns in bacterial metabolism, but that C lability would be the largest influence on potential differences in bacterial C processing.

METHODS

Study area - The Rio Grande drainage originates in the state of Colorado and flows through New Mexico before forming the international border of Texas and Mexico (Fig. 1). Approximately 2010 river km (rkm) of the Rio Grande run along the Texas-Mexico border; the Pecos and Devils Rivers are the major tributaries on the Texas side of the drainage, with the Rio Conchos being the major tributary from Mexican along the section of the drainage examined by this study. The Pecos River originates in New Mexico and approximately 560 rkm flows through Texas before joining the Rio Grande in Amistad International Reservoir, one of the two major reservoirs located along this portion of the drainage. The Devils River originates within Texas and flows approximately 160 rkm before joining the Rio Grande at Amistad International Reservoir. Along the upstream - downstream gradient of the Texas portion of the Rio Grande drainage (inclusive of the Pecos and Devils River drainages), the surrounding landscape represents several ecotypes, transitioning from the arid Chihuahuan Desert and Trans-Pecos of the upper Rio Grande and Pecos Rivers, to the semi-arid Edwards Plateau in the region of the Devils River, to the subtropical South Texas brush country in the lower portion of the drainage.

I selected a group of 14 sites along the Texas portion of the Lower Rio Grande drainage (Fig. 1). Sites examined by this study were selected to assess potential differences in physicochemical and bacterial dynamics across the Rio Grande drainage

and its major tributaries associated with changing land cover types and changing biogeoclimatic gradients. Nine sampling sites were located along the mainstem of the Rio Grande: three sites were located above Amistad International Reservoir (designated URG sites 1-3), four sites were distributed between Amistad International Reservoir and Falcon Reservoir (LRG sites 1-4), and two sites were located below Falcon International Reservoir (LRG sites 5-6) (Fig. 1). Four sites were located along the Pecos River (PR sites 1-4) and one site was located on the Devils River (DR; Fig. 1). All of the URG sites are located in the Chihuahuan Desert/Trans-Pecos ecoregion, with one site (URG 2) being located immediately below the confluence of the Rio Grande and Rio Conchos. The LRG sites are located in the subtropical South Texas Brush ecoregion in the Lower Rio Grande River Valley; the landscape is characterized by a convergence of semitropical mesic and xeric plant species. Flows in this portion of the Rio Grande tend to be more predictable, of higher magnitude, and more consistent due to downstream controlled agricultural releases from Amistad and Falcon International Reservoirs. The PR sites are located in the scrub-brush-dominated arid Trans-Pecos ecoregion; the upper two sites in the drainage (PR1 and PR2) exhibit relatively low flows and higher salinities, but the lower two sites (PR3 and PR4) are strongly influenced by inputs of groundwater along the drainage (Texas AgriLife Extension Program 2010). The landscape surrounding the DR site is characterized by a convergence of the Texas Hill Country, the Chihuahuan Desert/Trans-Pecos, and the South Texas Brush Country ecotypes. The Devils River is considered a predominantly pristine river dominated by groundwater inputs from the western portion of the Edwards Aquifer.

Study design and field sampling regime - A suite of biological, environmental and water quality data were collected at all 14 sites on a seasonal basis (Spring, Summer, and Fall) from March - December of 2010. Here, I define "Spring" as March-May, "Summer" as July-August, and "Fall" as October-December. Seasons for this study were largely defined by the agricultural season in that flows are managed to provide water for irrigation during the late Spring-Summer growing season. Data collected at all sites included field-based physiochemical parameters, total, dissolved and sestonic nutrients, and algal biomass. I additionally analyzed bacterial density, bacterial community metabolic rates (i.e., BR and BP), and C processing and quality parameters at a subset of nine sites within the lower Rio Grande drainage. This subset consisted of URG1, URG2, LRG1, LRG4, LRG5, LRG7, PR1, PR4, and DR. These sites were selected to maximize representation of the variability within the study area and to serve as proxies for the system at each distinct ecotype/spatial type.

Field Methods – At each sampling site, field measurements of physicochemical data and water samples were collected from the thalweg of the river channel at the mid-point depth of the water column. Field measurements of physicochemical parameters included dissolved oxygen (DO; mg/L), specific conductivity (SpC; mS/cm), pH, and water temperature (^{o}C) were collected with a YSI 556 handheld multiparameter instrument (YSI Inc., Yellow Springs, Ohio, USA). Water was collected as duplicate grab samples and stored in pre-cleaned 2-L opaque or brown Nalgene™ high-density polyethylene (HDPE) bottles that had been rinsed with water from the field site prior to sample collection. At the sub-set of sites selected for bacterial metabolism estimates, an additional 7.5 L of water were collected in pre-cleaned opaque HDPE Nalgene™ carboys

that were rinsed in the field. Water samples were stored in coolers on ice from time of sampling until the return to Texas State University-San Marcos, where they were immediately placed in an incubator at ambient river temperature. Water samples were processed in the lab typically within 48 h of collection in the field. I also estimated the hydrologic conditions at each site at the time of sampling by calculating the mean discharge (Q_9) for the 9 day period prior to the sampling date using from established United States Geological Survey (USGS) or International Boundary Waters Commission (IBWC) gauging stations located near each of the sampling sites (Table 1; data sources: http://www.ibwc.gov/Water_Data/histflo1.htm and http://waterdata.usgs.gov/tx/nwis/rt). For sites in which data was not available, as in the lower Pecos region during flooding in the summer of 2010, mean flow across seasons and drainages was used.

Laboratory Methods- Water chemistry and phytoplankton biomass were evaluated in duplicate for total nitrogen (TN), total phosphorus (TP), nitrate (NO_3^-), ammonium (NH_4^+), soluble reactive phosphorus (SRP), non-volatile suspended solids (NVSS), dissolved organic carbon (DOC), and particulate (seston) nutrients (C, N and P) in the <1 μm size (bacteria-sized) fraction, and suspended phytoplankton biomass. Total nitrogen and TP were determined from unfiltered water samples and TN was digested with alkaline potassium persulfate and analyzed as nitrate on using second-derivative UV spectroscopy (Crumpton et al. 1992). Total phosphorus was measured as SRP following digestion with potassium persulfate and analysis with the molybdenum blue method (Wetzel and Likens 2000). Nitrate, NH_4^+, and SRP concentrations were determined in water samples filtered through pre-ashed Pall A/E filters (nominal pore size=1 μm).

Nitrate concentrations were determined with second-derivative UV spectroscopy (Crumpton et al. 1992) and SRP concentrations were measured as PO_4^{3-} with the molybdenum blue method (Wetzel and Likens 2000). Ammonium concentrations were determined using the phenate method (Wetzel and Likens 2000). Samples for dissolved organic carbon (DOC) consisted of filtrate which was passed through a pre-ashed Whatman GF/F filter (nominal pore size=0.7 μm); filtrate was analyzed on a Shimadzu TOC-V_{CSH} total organic carbon analyzer. Water color of the remaining DOC filtrate, was assessed spectrophotometrically as absorbance at 440 nm and color was calculated per Cuthbert and del Giorgio (1992). All spectrophotometry was performed on a Varian Cary 50 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, California, USA).

Concentration of NVSS was determined by filtering water onto pre-combusted and pre-weighed Pall A/E filters. After filtration, filters were dried at ~50℃ for 48 h and were re-weighed to determine total suspended solids (TSS). Filters were subsequently combusted at 500℃ for 4 h and re-weighed to determine NVSS. The C, N and P content of the bacterial size fraction of seston (<1 μm) were also assessed. Bacterial C, N and P were determined by first filtering samples through pre-ashed Pall A/E filters and this filtrate was filtered onto pre-ashed Whatman GF/F filters and dried at 50℃ for 48 h. The <1 μm C and N concentrations were measured on a CE Elantech Flash EA 1112 CN analyzer (CE Elantech Inc., Lakewood, New Jersey, USA). The <1μm particulate P samples were filtered onto Whatman GF/F filters, digested with HCl at 100℃ and measured as phosphate with the molybdenum blue method (Wetzel and Likens 2000). Suspended phytoplankton biomass was determined by chlorophyll *a* (Chl*a*)

concentration. Water was filtered onto pre-ashed Pall A/E filters and stored at -20°C until extraction with acetone for 8 h in the dark and measurement on a Turner Designs Trilogy fluorometer (Turner Designs, Inc., Sunnyvale, California, USA).

Bacterial cell density from the subset of sites was determined by preserving 60 mL of whole water in clean glass bottles with 4% filtered (<0.2 μm) formalin. Duplicate samples were kept in the dark at 4°C until analysis. Samples were filtered onto black Nucleopore membrane filters (pore size = 0.2 μm), stained with 4',6-diamidino-2-phenylindole (DAPI), and cells were counted at 1000x magnification. For counting, slides were mounted with Citifluor™ AF1 solution and examined with a Nikon Eclipse 80i microscope, fitted for epifluorescence microscopy with a mercury lamp (Nikon; X-Cite™ 120) and UV-2E/C filter for DAPI detection (Nikon Instruments, Inc., Melville, New York, USA). For each site on each sampling date, twenty fields of view (grid area = 1.0×10^{-4} mm^2) were counted for both duplicate samples.

Bacterial production (BP) was measured using the microcentrifuge ^{3}H-leucine method (Smith and Azam 1992; Pace et al. 2004; Caston et al. 2009). On each sampling date, river water from the well-mixed 7.5-L carboy was allocated into four "live" and two "killed" tubes. ^{3}H-leucine was added to all 6 tubes; the "killed' tubes received cold 50% trichloroacetic acid (TCA) prior to ^{3}H-leucine addition. Tubes were incubated for 45-60 minutes in the dark at site-specific water temperature, after which activity in "live" tubes was stopped with the addition of cold 50% TCA and all tubes were centrifuged for 10 min at 14,000 rpm. The supernatant was aspirated, cold 5% TCA was added, and tubes were re-centrifuged at 14,000 rpm. Supernatant was removed from each tube and

Scintiverse BD was added to each tube and ^{3}H activity was measured on a Beckman LS 60001C scintillation counter. Bacterial production was expressed as mg C L^{-1} h^{-1}.

Bacterial respiration (BR) was estimated through the use of biological oxygen demand (BOD) incubations. Biological oxygen demand assays are commonly used as a method of estimating bacterial community respiration and bacterial C demand (Roland et al. 1999; Williams and del Giorgio 2005). To estimate BR, I conducted relatively short-term (48-h) BOD experiments. Incubations were performed in cleaned and acid-washed 60-mL Whatman BOD bottles with ground glass stoppers. Five replicate bottles were filled with whole water (unfiltered) and five replicates were filled with water filtered through pre-ashed Pall A/E glass filters (thus, containing bacteria-sized particles <1 μm). Size fractionation of the water allowed me to assess patterns in respiration of the heterotrophic planktonic community and that of the bacterial sized fraction. Initial DO concentrations were measured on Day 0 in duplicate whole water and <1 μm fraction samples. Dissolved oxygen concentrations were measured using a modified spectrophotometric Winkler method (Roland et al. 1999) in which Winkler reagents, followed by 18 M sulfuric acid, were added to bottles. The remaining three replicate bottles for each site (three whole water bottles and three <1 mm bottles) were incubated in the dark at *in situ* river temperature. After 48 h, bottles were removed and DO was determined. Two day O_2 consumption (mg O_2 L^{-1} h^{-1}) was determined by calculating the difference between initial DO and DO at Day 2. Oxygen consumption values were converted to C respired (mg C L^{-1} h^{-1}) based upon a respiratory quotient of one. Measurements of short-term BR were coupled with concurrently measured BP estimates

in order to estimate bacterial growth efficiency (BGE). Bacterial growth efficiency was calculated as BGE = BP / (BP+BR).

The lability of C sources can be evaluated through use of relatively long-term BOD incubations (Ostapenia et al. 2009; Sullivan et al. 2010; Guillemette and del Giorgio 2011). The concentration of labile DOC (OC_L) can be estimated by interpreting plateaus in oxygen consumption over long-term BOD incubation time interval (Coffin et al. 1993). A majority of studies using this method utilize a first-order decomposition kinetics model (Equation 1):

$$BOD_t = BOD_{ult}(1-e^{-kt}) \quad (1)$$

where BOD_t is the biological oxygen demand at time t, BOD_{ult} is the total BOD possible for that bacterioplankton community (asymptotic at infinity, ∞), and k is the reaction constant of aerobic decomposition (Ostapenia et al. 2009). By conducting relatively long-term BOD assays (e.g., >10 days) an estimate can be calculated for the rate of breakdown of labile C (k), and, using the estimate of k, BOD_{ult} is inferred, providing an indication of the size of the labile DOC pool (Ostapenia et al. 2009; Sullivan et al. 2010).

In order to assess DOC lability and C processing rates by bacterioplankton across the lower Rio Grande drainage long-term BOD assays (20-day incubations) were performed using <1 μm size-fractionated water. Samples were incubated in the dark in cleaned and acid-washed 120 mL Whatman glass BOD bottles with ground glass toppers at *in situ* river temperatures. Because short-term and long-term incubations for a site were initiated at the same time, the initial (Day 0) DO concentrations from the short-term BOD assays were used as the starting point DO concentration the for long-term BOD assays. Dissolved oxygen concentrations over the course of the long-term incubations

were measured on days 2, 4, 8, 16, and 20 from triplicate <1 μm fractionated water samples with the modified Winkler method described above.

Biological oxygen demand kinetics and labile organic carbon concentration (OC_L) were calculated with Microsoft Excel Solver Tool, which employs Generalized Reduced Gradient (GRG2) nonlinear optimization code and assumes first-order kinetics. Use of first-order kinetics calculates a decay rate based on the concentration of a single reactant (in this case, OC_L) (Ostapenia et al. 2009; Sullivan et al. 2010). Estimates for BOD_{ult} and k were calculated by using O_2 consumption curves and solving for the minimized residual sum of squares using Equation 1 (Coffin et al. 1993; Ostapenia et al. 2009). Estimates of OC_L were obtained by multiplying the 20-day O_2 consumption (derived from the BOD assays) by a factor of 0.3 (per Winberg 1960; Ostapenia et al. 2009).

Data Analysis – In order to explore spatial patterns in water quality and physicochemical characteristics across the lower Rio Grande/Rio Bravo del Norte drainage, I initially utilized Principal Component Analysis (PCA) to investigate potential relationships among physicochemical and nutrient variables at all fourteen site locations in the drainage. In order to avoid inverting the matrix by including all the variables measured during field sampling, I used a subset of the range of variables. Particulate nutrients (<1 P, C, and N) were discarded because pelagic bacteria are expected to be more responsive to dissolved nutrients (Stelzer et al. 2003). Specific conductivity was discarded because Salinity was a more informative measure of riverine conditions and pH was discarded because it was consistent across the drainage. This subset of the physiochemical and nutrient variables collected at each site (Q_9, water temperature, DO, NO_3^-, NH_4^+, SRP, TP, DOC, NVSS, Salinity, Abs_{440}, and Season; Table 1) were used as

environmental variables were *z*-score transformed prior to analysis. When it was not possible to calculate Q_9 (i.e., PR3 and PR4 sites during the Summer sampling event), the mean Q_9 for all sites and all seasons was substituted for these two missing values.

Using the results of the initial PCA, groupings of sites of similar physicochemical and nutrient characteristics were formed. In order to explore how these site groups differed in bacterial metabolism, carbon cycling, and productivity, I performed one-way analysis of variance (ANOVA) using the PCA-defined site groups as the independent variable (factor) and the biotic variable (Chl*a*, bacterial cell density, BR, BP, BGE, *k*, OC_L, and OC_L:DOC) as the dependent variables. These analyses were performed only on the subset of sites where bacterial metabolic parameters were measured ($n = 9$ sites). Because season did not exhibit a strong influence in the initial PCA, seasonal variation was not assessed in this analysis and all observations, regardless of season, were combined. In order to accommodate uneven sample sizes between spatial groups and repeated measures within spatial groups, I performed a series of Type III Sum of Squares ANOVAs. Data were $\log_{10}$-transformed before analysis to meet assumptions of normality and homoscedasticity. Because I performed multiple comparisons of site groups for a variety of response variables, a sequential Bonferroni procedure was used to adjust α. Where significant spatial group effects were detected, a Dunnett-Tukey-Kramer (DTK) pairwise multiple comparison test was performed. The DTK permits the user to determine homogenous groups within a significant ($p \leq 0.05$) ANOVA analysis and is adjusted for unequal sample sizes.

In order to examine how biological variables (i.e, bacterial metabolism, bacterial density, and phytoplankton biomass) varied with both physicochemical and C quantity

and quality predictors, redundancy analysis (RDA) was performed. The RDAs were performed using the nine sites where bacterial metabolic parameters were measured. Unlike PCA, RDA allows for the examination of biological responses to environmental predictors (Legendre and Legendre 1998). Additionally, combining sites into groups that are similar in physicochemical conditions (i.e. the ANOVA framework) is not an explicit examination of which individual variables or combination of variables most influence biological responses. For RDA analyses, I examined the relationship between biological responses (BR, BP, BGE, Chl*a*, bacterial density, and *k*) and two groups of predictor variables. I first explored how biological responses varied with physicochemical variables (Q_9, water temperature, DO, NO_3^-, NH_4^+, SRP, TP, NVSS, Salinity, and Season). Secondly, I performed an RDA examining how biological responses were affected by a suite of C quality-quantity predictors (DOC, OC_L, Abs_{440}, POM, bacterial C:N, C:P, and N:P, as well as Season). All data were *z*-transformed prior to analysis. Because I created two RDA models and used the same response variables in both RDAs, I examined how physicochemical and C quality-quantity predictors co-vary and performed a Pearson correlation matrix ($\alpha \leq 0.05$ for all significant correlations) on predictor variable sets. All statistical analyses were performed using R 2.10.1 (R Development Core Team 2009) using the R statistical packages "vegan", "car", and "psych" (Fox and Weisberg 2011; Oksanen et al. 2011; Revelle 2012).

RESULTS

Spatial variation in physicochemical gradients

Physicochemical conditions in the Rio Grande drainage exhibited substantial spatial variation (Table 1; Figure 2). When variation in physicochemical conditions across all 14 sampling sites was examined, the first two principle components (PC I and PC II) explained 54.2% of the total variation (Figure 2). Principal component I explained 29.0% of the variation among sites and represented a gradient of sites with higher salinity, DO, and NH_4^+ concentrations to sites with relatively higher water temperature and Q_9 values. In general, this gradient described an upstream-downstream pattern wherein the upstream sites in the drainage (sites URG1 through URG3, PR1, and PR2) had higher salinities and DO and NH_4^+ concentrations, while the more downstream sites (LRG1 though LRG7) were characterized by higher flows and warmer water temperatures. Principal component II explained a further 25.1% of the variation among sites and described a gradient of sites largely influenced by surface water runoff (all URG sites, PR-1, PR2, and all LRG sites) to sites dominated by groundwater inputs (PR-3, PR-4, and DR). The surface water runoff influenced sites were characterized by higher NVSS, TP, DOC, and SRP concentrations and greater water color (Abs_{440}), whereas the groundwater-dominated sites were characterized by lower water color, greater Q_9 values, and higher DO and NO_3^- concentrations. The most upstream Rio Grande site, URG1, had extremely high NVSS, TP, and DOC concentrations during summer (Table 1, Fig 2) and this site appeared to strongly influence PC II; however, when URG1 data from summer was removed from the analysis, the influence of the various physicochemical variables along PC I and II did not change and the distribution of sites in multivariate space did not change substantially.

Based upon the PCA on the physicochemical analysis, there were two general gradients in physicochemical conditions within the Rio Grande drainage, an upstream-downstream gradient on PC I and a surface-water-to-groundwater gradient on PC II. These gradients revealed a separation of the sites into three general groups. The first group was composed of sites in the upper portion of the drainage (hereafter called the UD group of sites), which included the upper Rio Grande sites (URG1through 3) as well as the two upper Pecos sites (PR1and 2). The URG sites exhibited greater variation from each other than the two upper PR sites; however, all of the sites within the UD group generally exhibited similar physicochemical conditions. The second group of sites was made up of sites from mainstem of the Rio Grande in the lower portion of the drainage (hereafter the LD site group) below Amistad International Reservoir and Falcon International Reservoir (LRG1-5 and LRG7). The LD sites exhibited less inter-site variation in physiochemical conditions than sites within the upper portion of the drainage (the UD group). The last group was composed of the groundwater-influenced sites (hereafter the GR site group) and included the two lower Pecos sites (PR3 and 4) and the Devils River site (DR). The GR group exhibited little variation in physicochemical conditions among sites in the group and within a single site across seasons (i.e., temporal variation).

Spatial variation in bacterial community responses

When sites were separated into similar physicochemically-defined groups, phytoplankton biomass (Chl*a*) differed significantly between the three site groups ($F_{2,24} = 17.78$, $p < 0.001$) (Fig. 3a). Post-hoc tests determined that GR sites had significantly

lower phytoplankton biomass than UD and LD sites. Bacterial density was also significantly different between site groups ($F_{2,24} = 12.4$, $p < 0.001$) and, again, GR sites had significantly lower bacterial cell densities than the UD and LD sites (Fig. 3b). However, when sites were assembled into groups, there was little evidence of spatial variation in bacterial metabolism and C lability within the Rio Grande/Rio Bravo del Norte. Bacterial production, BR, BGE, OC_L, the proportion of the DOC pool composed of OC_L (OC_L/DOC) and *k* did not significantly different between site groups (Fig. 3c-h). Bacterial productivity and OC_L concentration were marginally non-significant when *p*-values were constrained by sequential Bonferroni adjusted α values (Table 3). Although these differences were marginally non-significant when constrained ($p > \alpha$ for both), GR sites exhibited lower BP and OC_L concentration than UD or LD sites; mean OCL concentrations for the LD sites was about half that of OC_L in the UD sites while BP did was not considerably different between the UD and the LD.

Bacterial response to physicochemical and C quantity and quality gradients

When two separate RDAs were performed to investigate how biological and bacterial metabolic parameters responded to both physiochemical (Fig. 4a) and C quality - quantity predictor sets (Fig. 4b), the first two axes of the RDA exploring biological responses to physicochemical predictors explained 25.7% of the variation in the data, with the overall model $R^2_{adj} = 33.0\%$. Permutation tests indicated that the model explained a significant amount of variation ($n = 999$, $p < 0.01$). The first axis (RDA 1) explained 15.1% of the variation and was significant ($n = 999$, $p < 0.01$), and described a gradient of sites with higher NVSS, TP, SRP, and NH_4^+ concentrations (predominantly

located within the UD region) to sites with greater NO_3^- concentrations (the GR sites); sites within the LD portion of the drainage exhibited minimal variation along this axis. The second axis (RDA 2) explained an additional 10.7% of the variation in the data, but was not significant (n=999, p = 0.111). The second axis largely described variation that largely occurred within data from LD sites; the axis represented a gradient of samples collected in the Spring sampling season with generally higher NVSS to samples collected in the Fall with greater Q_9 values. In terms of the biological responses to these gradients, the RDA indicated that BP, Chl*a*, and bacterial density were positively correlated with TP, SRP, and NH_4^+ concentrations, and salinity. In contrast, BR was negatively correlated with Q_9 and positively correlated with Spring and bacterial growth efficiency was positively correlated with Q_9 and Fall. The decomposition rate of C (k) was positively correlated with DO concentration. In general, the first RDA axis described the variation across sites, while the second axis describes variation largely describes within sites.

When biological and bacterial metabolic responses to C quality - quantity predictors was examined, the first two axes explained 27.2% of the variation and the R^2_{adj} for the overall model was 33.6% (n = 999, p < 0.01). The first RDA axis was significant (n = 999, p < 0.01) and described a gradient of sites with higher DOC and POM concentrations (mostly UD sites) to sites with low DOC and POM concentrations (GR sites). As observed in the physicochemical RDA, the LD sites were grouped in the center of this axis, indicating they were intermediate to UD and GR sites along this gradient. The second RDA axis was marginally non-significant (n = 9999, p = 0.073) and in general described a gradient that existed among the LD sites in which data collected in

the Fall generally had higher bacterial N:P and data collected in the Spring had greater water color and higher bacterial C:N. Bacterial metabolic parameters responded to C quality and quantity predictors in a qualitatively similar fashion to the physiochemical RDA in that BP, Chl*a*, and bacterial density positively correlated to one another. Bacterial production, Chla and bacterial density increased with DOC and OC_L concentrations, but the rate of BR responded orthogonally to BP and was positively correlated with water color, and bacterial C:N, and the Spring sampling period. Again, BGE was inversely related to BR and was also positively correlated with bacterial N:P.

Examination of the two RDA analyses indicates that there was likely a large amount of covariation among the physicochemical and C quantity and quality predictors. Thus, in order to investigate relationships between the two sets of predictors, a Pearson correlation matrix was performed (Table 4). Particulate organic matter, NVSS, DOC, and Abs440 were positively correlated with each other ($p \leq 0.01$ for all correlations) and TP was correlated with POM and Abs440 ($p \leq 0.01$ for both correlations). Other significant correlations included the correlation between Summer sampling season and water temperature ($p \leq 0.01$) and between bacterial C:N and the Fall sampling season ($p \leq 0.01$). These analyses indicate that many of the physicochemical RDA predictors were correlated with predictors in the C quality-quantity RDA and that biological and bacterial metabolism variables responded similarly in both analyses.

Table 1. Physicochemical variables, all study sites, Spring, Summer and Fall sampling. NO_3^-, nitrate; NH_4^+, ammonium; TP, total phosphorus; SRP, soluble reactive phosphorus; DOC, dissolved organic carbon; Abs_{440}, DOC absorbance measured at 440 nm; Temp, water temperature; DO, dissolved oxygen; Sal, Salinity; Q_9, mean discharge for the 9 day period prior to the sampling date; NVSS, non-volatile suspended solids. DO, Temp, and Sal were measured in the field. All nutrients, DOC, and NVSS values are means based on duplicate measurements.

Drainage	Site	Latitude and Longitude	Sampling Dates	NO_3^- ($\mu g\ L^{-1}$)	NH_4^+ ($\mu g\ L^{-1}$)	TP ($\mu g\ L^{-1}$)	SRP ($\mu g\ L^{-1}$)	DOC ($mg\ L^{-1}$)	Abs_{440} (m^{-1})*	Temp (°C)	DO ($mg\ L^{-1}$)	Sal ppt	Q_9 ($m^3\ s^{-1}$)	NVSS ($mg\ L^{-1}$)
Rio Grande	Candelaria (URG1)	30.138558N, -104.688935W	Sp 10	190.00	525.91	89.36	44.05	6.38	2.26	13.67	8.86	2.03	3.93	44.20
			Su 10	450.61	196.96	590.95	31.24	5.96	23.40	27.80	5.27	0.80	3.31	4883.38
			Fa 10	2774.27	651.91	361.45	580.03	6.69	1.44	10.00	13.12	1.80	3.29	18.62
	Redford (URG2)	29.432412N, -104.18925W	Sp 10	84.51	154.25	36.60	3.24	4.82	1.94	16.10	11.61	1.56	10.00	27.98
			Su 10	564.32	221.48	225.26	23.12	4.91	2.35	26.90	5.11	0.60	11.66	273.12
			Fa 10	215.68	241.20	87.98	3.16	3.94	0.70	16.70	14.82	1.40	8.18	23.94
	Contrabando (URG3)	29.280036N, -103.843138W	Sp 10	76.34	176.47	41.38	5.23	4.40	1.01	18.00	12.28	1.30	12.61	42.00
			Su 10	567.85	388.85	281.24	26.13	4.16	1.65	26.90	5.45	0.90	9.35	680.04
			Fa 10	367.03	302.09	64.57	5.74	4.09	0.77	15.10	6.94	1.40	6.08	11.94
	Quemado (LRG1)	28.929538N, -100.639422W	Sp 10	305.58	138.57	35.97	2.04	3.20	0.58	23.50	11.64	0.40	27.54	12.21
			Su 10	708.13	85.48	112.84	3.52	3.10	1.42	26.20	7.65	0.30	245.00	29.99
			Fa 10	395.21	66.63	162.84	7.01	3.43	0.79	23.20	9.35	0.30	144.78	27.28
	Eagle Pass (LRG2)	28.60794N, -100.443385W	Sp 10	356.25	425.69	35.80	32.16	3.10	0.25	24.60	6.47	0.40	63.14	12.30
			Su 10	685.86	115.97	11.98	13.04	3.04	2.38	26.70	7.15	0.30	274.67	24.54
			Fa 10	470.34	113.28	54.61	30.56	3.22	0.93	23.60	7.06	0.30	141.97	15.96
	Laredo (LRG3)	27.452351N, -99.493368W	Sp 10	417.17	475.12	56.28	83.87	3.61	0.60	28.20	6.37	0.40	52.81	20.84
			Su 10	751.47	104.52	25.62	12.31	2.98	0.92	29.30	6.64	0.30	355.11	66.15
			Fa 10	651.05	142.90	85.85	63.70	3.18	0.37	24.20	6.60	0.30	152.00	49.69
	San Ygnacio (LRG4)	27.015014N, -99.439058W	Sp 10	574.58	224.37	43.74	97.66	3.54	0.21	30.30	5.75	0.40	52.81	17.29
			Su 10	36.87	236.39	76.65	8.44	4.45	1.03	32.20	3.30	0.40	355.11	0.03
			Fa 10	362.73	204.46	35.17	36.03	3.71	0.23	25.20	4.85	0.30	152.00	14.38
	Fronton (LRG5)	26.399097N, -99.08498W	Sp 10	23.05	80.38	19.00	1.71	4.64	0.41	27.30	5.90	0.40	366.67	5.68
			Su 10	231.22	129.92	71.93	2.55	4.02	0.61	30.90	6.53	0.30	946.22	11.46
			Fa 10	163.86	86.73	90.57	2.04	4.02	0.34	26.20	6.71	0.30	616.00	5.39
	Hidalgo (LRG7)	26.095166N, -98.271589W	Sp 10	109.73	127.70	73.02	19.14	4.84	0.78	27.10	7.32	0.50	94.50	91.59
			Su 10	282.47	233.60	38.16	3.83	4.81	0.83	30.40	7.99	0.60	800.67	31.00
			Fa 10	371.19	85.00	268.82	10.36	4.51	0.80	26.50	3.35	0.40	601.00	52.83
Pecos River	Grand Falls (PR1)	31.305102N, -102.877865W	Sp 10	76.34	760.41	14.05	4.46	5.45	0.73	17.87	16.95	10.27	2.79	21.04
			Su 10	80.30	283.24	63.93	3.85	7.18	0.81	27.00	8.11	5.40	8.13	13.18
			Fa 10	69.13	593.14	51.68	1.78	6.11	1.52	12.80	11.80	9.10	7.08	38.58
	Iraan (PR2)	30.90576N, -101.880512W	Sp 10	654.12	373.46	2.49	1.63	1.51	1.30	15.27	7.49	12.40	7.42	6.79
			Su 10	98.75	558.42	27.80	1.55	7.17	0.79	24.60	15.75	4.80	14.87	6.04
			Fa 10	124.39	678.56	71.47	2.12	6.14	1.52	12.80	11.20	9.70	8.20	22.28
	Pandale (PR3)	30.190467N, -101.550665W	Sp 10	86.04	335.66	24.87	0.69	5.03	0.97	16.35	10.40	3.03	30.07	10.09
			Su 10	993.28	174.16	9.16	0.00	3.02	1.65	26.70	9.72	1.50	142.87	14.26
			Fa 10	532.80	320.56	22.11	6.78	1.73	1.15	15.20	10.07	2.20	33.16	3.25
	Oso Canyon (PR4)	30.024776N, -101.528778W	Sp 10	881.11	294.20	52.02	0.39	1.38	0.94	20.68	10.15	2.09	30.07	1.33
			Su 10	853.19	264.58	5.13	1.34	3.55	1.22	27.80	10.73	1.10	142.87	3.07
			Fa 10	700.97	233.60	39.02	3.50	1.46	0.88	16.70	10.85	1.60	33.16	0.63
Devils River	Dolan Falls (DR)	29.903963N, -101.005704W	Sp 10	976.77	93.75	5.59	0.87	2.98	2.77	24.00	6.71	0.20	9.17	0.00
			Su 10	755.48	76.82	53.35	0.68	2.35	3.27	31.20	7.98	0.20	14.46	0.00
			Fa 10	1361.64	56.05	3.75	5.57	2.80	1.61	14.60	11.04	0.20	5.76	0.00

Table 2. Biotic, bacterial metabolism, and C quality variables for the subset of 9 sites. Bact Dens, bacterial cell density; Chl*a*, chlorophyll a; BR, bacterial respiration; BP, bacterial productivity; BGE, bacterial growth efficiency; *k*, the aerobic decomposition rate of C; OC_L, labile organic carbon; OC_L/DOC, the ratio of labile organic carbon to the bulk DOC pool; <1 C:N, bacterial C:N; <1 C:P, bacterial C:P; <1 N:P, bacterial N:P; POM, particulate organic matter. BGE, OC_L/DOC, and bacterial stoichiometry are ratios and are unitless. BR, OC_L, and *k* values are the mean of triplicate measurements, BP values are the mean of quadruplicate measurements, and all other values are the mean of duplicate measurements.

Drainage	Site	Sampling Dates	Bact Dens ($\times 10^6$ cells mL^{-1})	Chl*a* ($\mu g\ L^{-1}$)	BR (ug C/L/h)	BP (ug C/L/h)	BGE	*k* (day^{-1})	OC_L ($mg\ L^{-1}$)	OC_L / DOC	< 1 C:N	< 1 C:P	< 1 N:P	POM ($mg\ L^{-1}$)
Rio Grande	Candelaria (URG1)	Sp 10	4.98	76.09	41.86	7.39	0.15	0.0549	1.80	0.282	4.732	98.206	20.751	145.34
		Su 10	3.56	11.45	114.29	0.78	0.01	0.0877	1.16	0.829	4.234	73.600	17.385	24.15
		Fa 10	4.53	47.61	9.27	3.09	0.25	0.1020	0.57	0.289	3.969	50.844	12.809	5.40
	Redford (URG2)	Sp 10	1.68	38.43	16.56	2.57	0.13	0.0727	4.00	0.482	3.961	61.485	15.523	206.79
		Su 10	5.73	5.38	7.37	3.04	0.29	0.1224	1.73	0.150	5.334	57.880	10.851	26.11
		Fa 10	3.99	59.73	10.83	3.03	0.22	0.1065	0.40	0.221	4.865	71.929	14.785	7.68
	Quemado (LRG1)	Sp 10	2.43	6.40	16.88	1.29	0.07	0.0963	0.92	0.550	4.215	99.500	23.608	44.21
		Su 10	4.58	1.23	9.39	1.36	0.13	0.1106	0.46	0.369	5.157	117.137	22.715	9.40
		Fa 10	2.53	3.06	3.90	1.26	0.24	0.0964	0.60	0.205	3.229	73.644	22.810	17.13
	San Ygnacio (LRG4)	Sp 10	5.73	1.75	16.93	2.45	0.13	0.1627	1.71	0.195	7.120	52.182	7.329	9.00
		Su 10	2.50	19.06	4.25	3.89	0.48	0.1182	1.20	0.352	4.376	127.941	29.234	1.46
		Fa 10	3.64	5.40	1.17	2.41	0.67	0.0002	3.00	0.149	3.409	94.650	27.765	4.69
	Fronton (LRG5)	Sp 10	5.37	11.35	13.37	1.32	0.09	0.1419	0.70	0.270	4.250	84.529	19.887	5.36
		Su 10	2.57	17.59	3.49	2.26	0.39	0.0947	0.84	0.209	4.316	67.311	15.595	46.48
		Fa 10	6.33	16.27	2.57	1.26	0.33	0.0271	1.04	0.204	3.707	77.614	20.937	3.45
	Hidalgo (LRG7)	Sp 10	3.86	7.44	46.40	1.93	0.04	0.5993	1.07	0.196	5.150	68.289	13.260	5.02
		Su 10	6.91	25.22	0.81	3.05	0.79	0.1119	0.98	0.214	4.717	86.474	18.331	29.33
		Fa 10	4.71	22.13	9.64	2.74	0.22	0.1408	0.69	0.260	4.244	69.086	16.280	1.70
Pecos River	Grand Falls (PR1)	Sp 10	2.51	12.15	15.19	3.38	0.18	0.0003	5.00	0.085	6.000	91.814	15.302	4.94
		Su 10	4.85	5.58	1.40	1.82	0.57	0.0349	1.41	0.101	4.748	231.873	48.840	2.30
		Fa 10	3.63	19.37	4.23	1.58	0.27	0.0007	1.21	0.176	4.279	77.169	18.035	19.03
	Oso Canyon (PR4)	Sp 10	0.46	1.28	2.51	1.08	0.30	0.1040	0.51	0.809	3.638	67.080	18.439	3.25
		Su 10	2.00	1.31	2.25	0.44	0.16	0.4391	0.76	0.260	5.063	43.315	8.555	98.36
		Fa 10	2.42	1.26	0.77	0.40	0.34	0.0003	0.68	0.152	3.490	36.099	10.343	5.99
Devils River	Dolan Falls (DR)	Sp 10	1.50	0.50	7.27	1.07	0.13	0.1542	0.61	0.198	6.916	267.064	38.617	5.25
		Su 10	1.48	0.55	7.56	1.21	0.14	0.1669	0.61	0.466	3.741	110.312	29.484	2.05
		Fa 10	1.59	0.30	3.06	2.97	0.49	0.4955	0.32	0.115	3.097	53.663	17.326	4.81

Table 3. Results of Type III Sum of Squares (ANOVA) examining variation in biotic and bacterial responses in the spatial groups identified by the PCA. The Upper Drainage group is composed of sites URG1-2, and PR1. The Lower Drainage group includes LRG1,4-5, and 7. The Groundwater-Influenced sites are PR4 and DR. Values are mean ± SE. ** indicates highly significant at sequential Bonferroni adjusted α.

Spatial Category	Bacterial Density ($\times 10^6$ cells)	Chl*a* ($\mu g\ L^{-1}$)	BR (μg C/L/h)	BP (μg C/L/h)	BGE	k (day^{-1})	OC_L ($mg\ L^{-1}$)	OC_L/DOC
Upper Drainage (UD)	3.94 ± 0.423	30.6 ± 8.63	24.6 ± 11.9	2.79 ± 0.623	0.230 ± 0.0501	0.060 ± 0.0150	1.92 ± .0518	0.290 ± 0.0783
Lower Drainage (LD)	4.26 ± 0.459	11.4 ± 2.41	10.7 ± 3.65	2.10 ± 0.246	0.300 ± 0.0703	0.140 ± 0.0437	1.10 ± 0.196	0.260 ± 0.0321
Groundwater Influenced (GR)	1.57 ± 0.268	0.870 ± 0.190	3.90 ± 1.15	1.20 ± 0.382	0.260 ± 0.0589	0.230 ± 0.0801	0.580 ± 0.0620	0.330 ± 0.108
$F_{3,24}$	12.4	17.8	1.37	4.66	0.266	2.81	4.25	0.221
Sequential Bonferroni corrected α	0.007	0.0006	0.0167	8.33×10^{-3}	0.0250	0.0125	0.0100	0.0500
***p*-value**	< 0.001**	< 0.001**	0.274	0.0194	0.769	0.0803	0.0263	0.804

Table 4. Correlation matrix for physicochemical and C quality-quantity predictors. The values are Pearson coefficients. * indicates $p < 0.05$ and ** indicates $p < 0.01$.

	SRP	DOC	Abs_{440}	Temp	DO	Sal	Q_9	NVSS	POM	OC_L	< 1 N:P	Spring	Summer	Fall
NO_3^-		-0.45*								-0.44*				
NH_4^+		0.41*		-0.47*	0.38*	0.78**	-0.47*			0.45*				
TP	0.5**		0.6**					0.65**	0.67**					
SRP		0.46*	0.43*					0.45*	0.42*					
DOC			0.55**			0.38*		0.59**	0.63**	0.42*				
Abs_{440}					-0.43*			0.81**	0.89**					
Temp					-0.68**	-0.52**	0.62**						0.58**	-0.49*
DO						0.56**	-0.56**							
Sal							-0.54**			0.43*				
Q_9														
NVSS									0.96**					
POM														
OC_L														
< 1 C:N												0.41*		-0.54**
< 1 C:P											0.89**			
< 1 N:P														
Spring													-0.5**	-0.5**
Summer														-0.5**

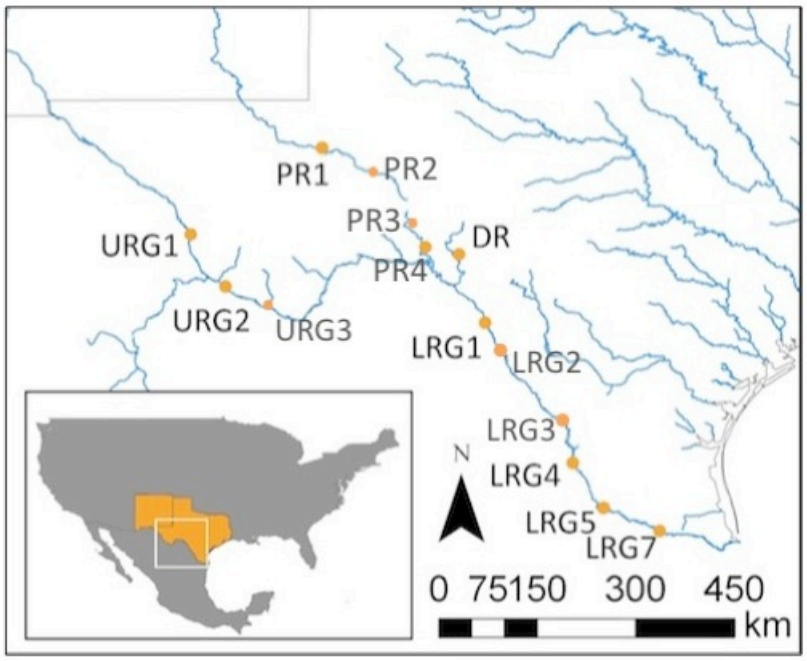

Figure 1. Map of study sites in the lower Rio Grande/Rio Bravo del Norte drainage. The designation "URG" indicates Rio Grande sites above Amistad International Reservoir. Sites designated LRG1-4 are between Amistad International Reservoir and Falcon International Reservoir and LRG5 and 7 are below Falcon Reservoir. Sites designated "PR" are on the Pecos River and "DR" represents the site on the Devils River.

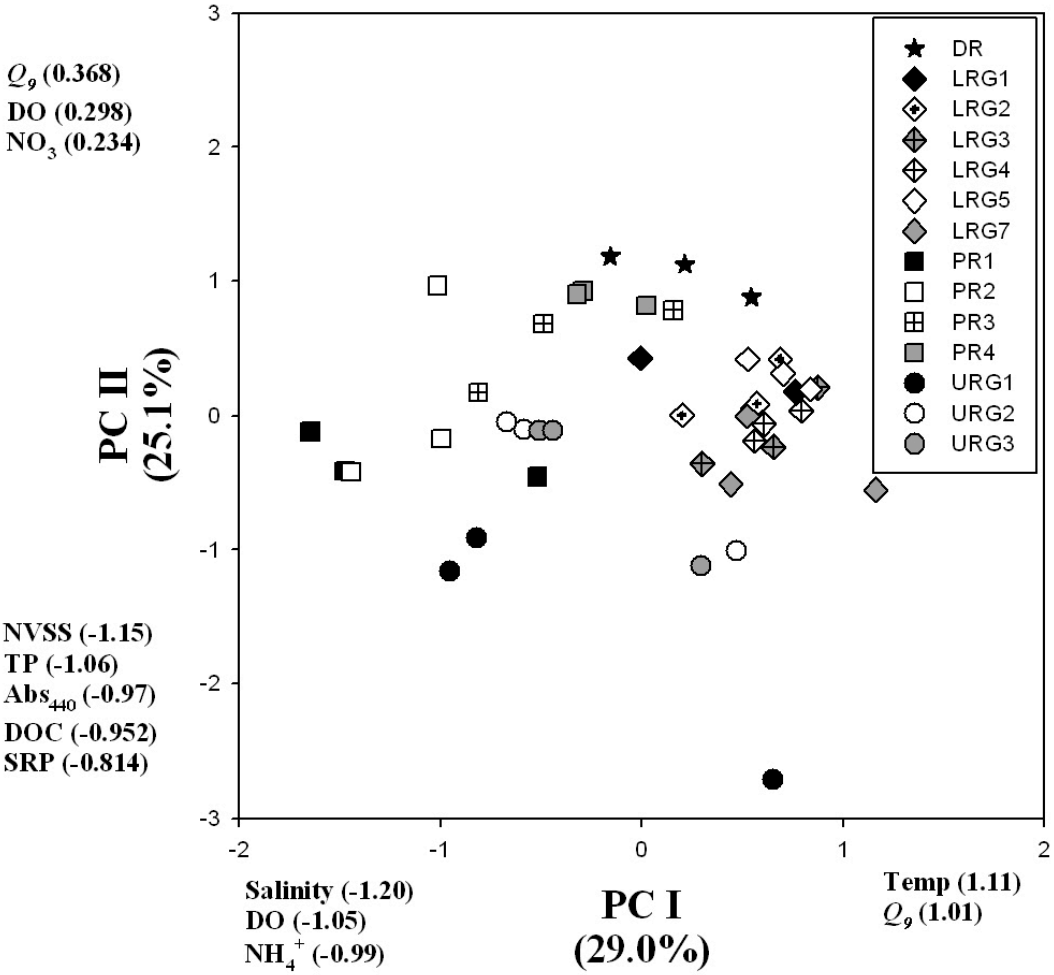

Figure 2. Results from the PCA examining spatial variation in physicochemical gradients. The amount of explained variation contained in each axis is provided as well as the loadings for the physicochemical variables. Physicochemical predictors in this analysis included are consistent with those in Table 1: NO_3^-, nitrate; NH_4^+, ammonium; TP, total phosphorus; SRP, soluble reactive phosphorus; DOC, dissolved organic carbon; Abs_{440}, DOC absorbance measured at 440 nm; Temp, water temperature; DO, dissolved oxygen; Sal, Salinity; Q_9, mean discharge for the 9 day period prior to the sampling date; NVSS, non-volatile suspended solids; and Season (Spring, Summer, and Fall). The response variables were all 14 study sites. Loadings shown on this graph are the most influential predictors on the two axes.

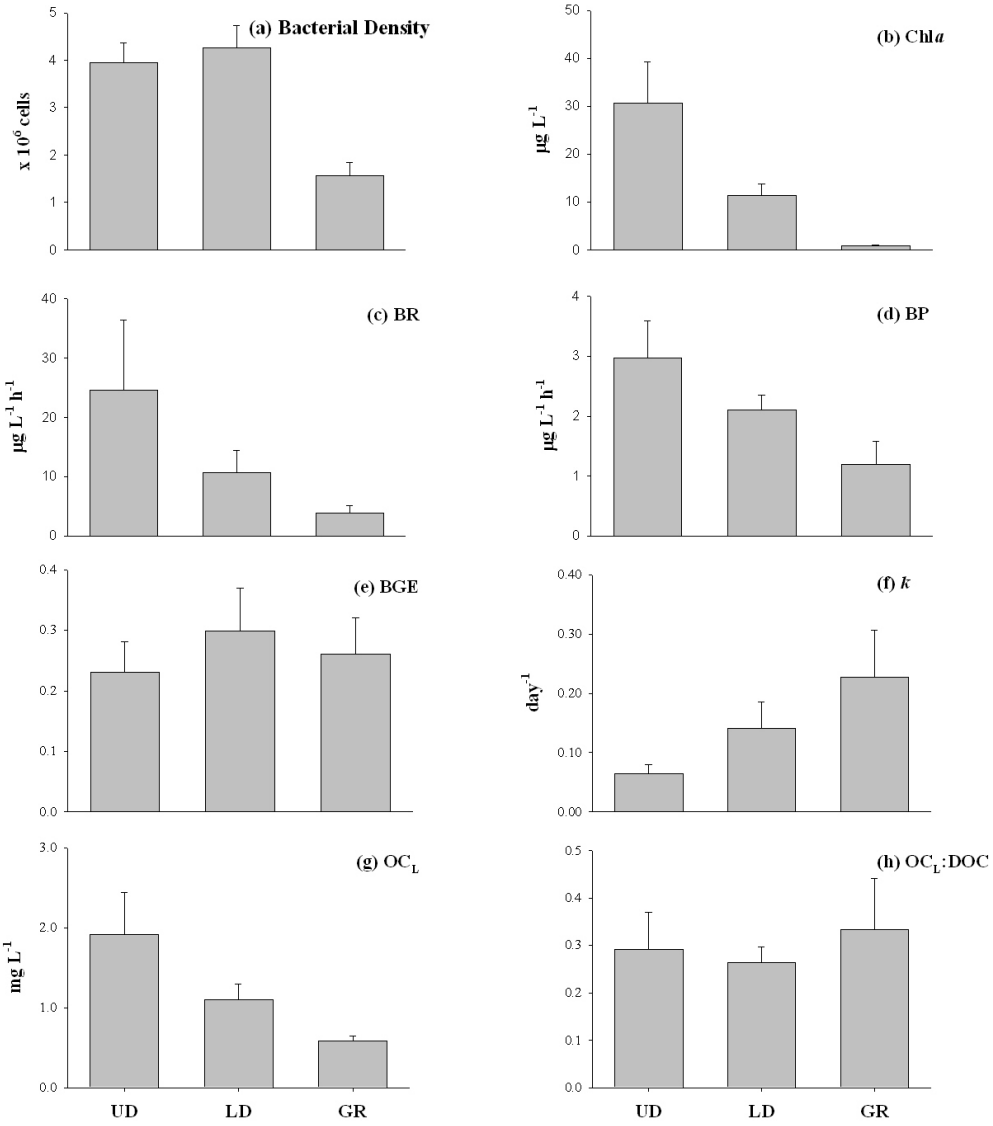

Figure 3. Mean biotic, bacterial metabolism, and C quality – quantity responses, grouped according to the spatial groups determined by the PCA. (a) Bacterial cell density (x 10^6 cells mL^{-1}; (b) Phytoplankton biomass concentration (Chla in µg L^{-1}); (c) BR rate (µg C/L/h); (d) BP rate (µg C/L/h); (e) BGE ratio (unitless); (f) *k*, the aerobic decomposition constant of C (day^{-1}); (g) OC_L concentration (mg L^{-1}); and (h) OC_L / DOC ratio (unitless). Again, the Upper Drainage group is composed of sites URG1-2, and PR1. The Lower Drainage group includes LRG1,4-5, and 7. The Groundwater-Influenced sites are PR4 and DR.

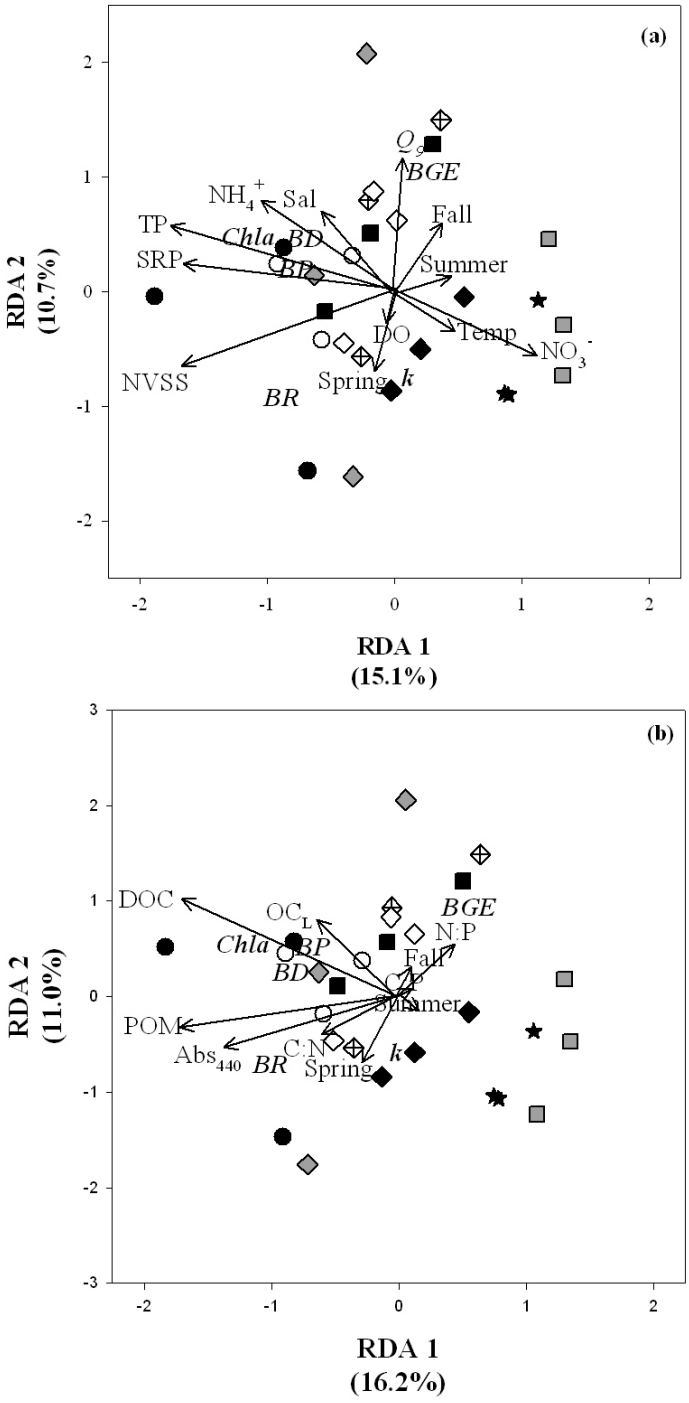

Figure 4. RDA analysis for (a) physicochemical predictors of biotic and bacterial metabolism responses and (b) C quality – quantity predictors for the same biotic and bacterial metabolism responses. Site symbols are consistent with those in Figure 1. Biotic and bacterial metabolism responses are in bold italics. Abbreviations for predictors and responses are consistent with those in Tables 1 and 2, with the addition that ***BD*** = bacterial cell density. (a) The physicochemical RDA explained 25.7% of the variation

(Figure 4 cont'd) among biotic predictors and sites, with an R^2_{adj} = 33.0%. The model was significant ($p < 0.01$) and RDA 1 was significant ($p < 0.01$) while RDA 2 was not ($p = 0.111$). (b) The C quality – quantity RDA explained 27.2% of the variation among biotic predictors and sites, with an R^2_{adj} = 33.6%. The model was significant ($p < 0.01$) and RDA 1 was significant ($p < 0.01$) while RDA 2 was marginally non-significant ($p = 0.073$).

DISCUSSION

Spatial patterns of physicochemical and biological characteristics

Results from the present study indicated that there was substantial spatial variation in physicochemical characteristics across the Grande/Rio Bravo del Norte drainage and that there were two main physicochemical gradients across the sampling sites in this study. The most profound variation in physicochemical conditions was represented by an upstream-to-downstream gradient wherein upstream sites were more saline and had higher DO and NH_4^+ concentrations, and downstream sites had greater flows and higher water temperatures. The secondary physicochemical gradient represented the differences between groundwater and surface water sites, where groundwater-influenced sites exhibited higher NO_3^- concentrations, but lower concentrations of all other nutrients, DOC and suspended matter than the surface water sites. Based upon the analysis of physicochemical characteristics, I initially segregated sites across the lower Rio Grande/Rio Bravo del Norte drainage into three broad groupings: (1) the UD sites in the upper portions of the Rio Grande and Pecos Rivers, (2) the LD sites along the lower portion of the mainstem of the Rio Grande between Amistad International Reservoir and its discharge point to the Gulf of Mexico, and (3) the groundwater-influenced sites in the lower reaches of the Pecos River and the Devils River.

Variation among the groups of sites (UD, LD, and GR) in the Rio Grande drainage in physicochemical characteristics was primarily driven by differences in hydrology and landscape position. Within the lower Rio Grande/Rio Bravo del Norte drainage, the presence of reservoirs appeared to strongly influence physicochemical

characteristics. The presence of impoundments within a drainage typically leads to increased water clarity and changes in dominant nutrient forms immediately below the reservoir (Wetzel and Likens 2000) with nutrient concentrations and turbidity increasing along the downstream continuum (Hoeinghaus et al. 2007). In the Rio Grande, sites in the LD group are located below two large reservoirs (Amistad and Falcon International Reservoirs) and nutrient and suspended solid concentrations are relatively lower in LD sites (when compared to the UD sites), indicating that these impoundments are a primary driver of physicochemical differences observed along the upstream – downstream gradient within the lower Rio Grande drainage. In addition, in the present study, the effect of the presence of impoundments is apparent when examining variation in physicochemical conditions within the individual site groups. Within the UG group, the Rio Conchos discharges into the Rio Grande below site URG1 and accounts for up to 40% of the flow below site URG1 (Douglas 2009). The Rio Conchos originates in the Sierra Madre Occidental in Mexico and the Conchos drainage contains seven reservoirs; below the confluence of the Rio Conchos and the Rio Grande (between sites URG1 and URG2), NVSS concentrations are greatly reduced in the Spring and Summer (mean reduction of 65%). However, despite the inflows of the Rio Conchos, NVSS and TP concentrations at the URG2 site are still relatively elevated when compared to other group sites (Table 1). It is critical to note that the upstream sections of the Pecos River and Rio Grande above my furthest upstream study sites also contain impoundments (i.e., Red Bluff Reservoir at the Texas-New Mexico border on the Pecos and Elephant Butte and Caballo Reservoirs in New Mexico on the Rio Grande). However, these reservoirs are relatively small when compared to Amistad and Falcon Reservoirs (Caballo and Red

Bluff are < 4,600 ha and Elephant Butte is < 15,000 ha in surface area while Amistad is > 26,000 ha and Falcon is > 33,000 ha in surface area) and are distant from the sample sites. These factors may, to some extent, mediate the effect of the upstream reservoirs on the sampling sites.

In addition to the presence of impoundments, groundwater inputs also strongly influence physicochemical patterns observed within the lower Rio Grande/Rio Bravo del Norte drainage. The lower two sampling sites in the lower Pecos River (PR3 and PR4) and Devils River are highly influenced by groundwater inputs. Groundwater inputs are supplied by the Edwards-Trinity Aquifer (Ashworth and Hopkins 1995), which is characterized by high water clarity and greater NO_3^- concentrations (Groeger et al. 1997). In addition, groundwater inputs serve to substantially augment and dilute flows, leading to declines in P, DOC, and NH_4^+; groundwater inputs along the Pecos River lead to a general reduction in these parameters between PR1 and PR4 (mean differences include a 19% reduction in SRP, a 67% reduction in DOC, and a 42% reduction in NH_4^+ along the downstream gradient). Thus, groundwater inputs play an important role in influencing variation in physicochemical characteristics within the lower Rio Grande/Rio Bravo del Norte drainage.

Differences in hydrology and landscape position and subsequent physicochemical conditions led to spatial differences in biological variables within the Rio Grande/Rio Bravo del Norte drainage. Both suspended phytoplankton biomass (Chl*a*) and bacterial density were significantly lower in the groundwater-influenced sites. The relatively elevated phytoplankton biomass and bacterial densities in the UD and LD portions of the drainage are likely a consequence of algal response to relatively higher concentrations of

P and NH_4^+ (Dodds 2006; Holmes et al. 2008; Guenet et al. 2010) and relatively high P and DOC may lead to greater bacterial densities (Sondergaard and Middelboe 1995; Murrel 2003). In contrast to biomass and density responses, estimates of bacterial metabolism and size of the labile OC pool did not significantly differ among the site groups within the drainage. Bacterial production and the size of the labile OC pool progressively declined from UD to LD to GR groups, but these trends were not significant when sequential Bonferroni procedures were applied and α was constrained. Although not significant, a majority of bacterial metabolism and C lability responses exhibited trends along the UD-LD-GR gradient: rates of BP, BR, and the concentration of OC_L all declined, while the decomposition constant of organic C (k) increased along this gradient. Thus, when sites were grouped into physicochemically similar groups based upon the results of the PCA, I observed a tendency for bacterial metabolic and C lability responses to respond to differences in the upstream – downstream and surface water – groundwater physicochemical conditions.

In the present study, I observed that BGE estimates did not significantly differ among site groups and there was no underlying spatial trend in the data. Bacterial growth efficiency ranged from 0.01 – 0.79 ($\bar{x}$ ±1 SE = 0.27±0.037), with the majority of values falling within the range of riverine BGE values reported by del Giorgio and Cole (1998) (reported range = 0.03-0.46). However, BGE estimates depend upon both BR and BP for calculations (see equation above) and BR estimates can be notoriously variable (del Giorgio and Cole 1998; Roland and Cole 1999; Lennon and Cottingham 2008). In the present study, hourly BR rates ranged from 0.77-114.29 μg C L^{-1} h^{-1} ($\bar{x}$ ±1 SE = 16.29±5.57 μg C L^{-1} h^{-1}), while BP rates ranged from 0.40 – 7.39 μg C L^{-1} h^{-1} ($\bar{x}$ ±1 SE =

2.19±0.27). Examination of the influence of both BR and BP on the estimates of BGE indicated that BGE was a significant negative function of BR ($F_{1,25} = 34.04$, $R^2 = 0.577$, $p < 0.001$), but BGE was not significantly related to BP ($F_{1,25} = 0.74$, $R^2 = 0.029$, $p = 0.398$). Indeed, BR measurements were much more variable across sites, varying by as much as three orders of magnitude, while BP measurements only varied by one order of magnitude. Thus, BGE values were primarily driven by variation in BR and not BP. These data also indicate that BR was more sensitive than BP to changes in environmental conditions and landscape position within the Rio Grande/Rio Bravo del Norte drainage. Maranger et al.. (2005) similarly found that bacterial metabolism (BP and BR) within a riverine system (the Hudson River, New York, USA) exhibited substantial spatial variation in response to spatial variation in environmental conditions in the drainage. However, in contrast to the results of the present study, Maranger et al.. (2005) found that BP was more responsive than BR to spatial variation in environmental conditions.

In the present study, the rate of OC processing (k) and the size of the OC_L pool did not differ significantly among site groups; however, there was a general trend of GR sites exhibiting the fastest OC processing rates and the lowest OC_L concentrations. Across all of the sites in the Rio Grande/Rio Bravo del Norte drainage, k ranged from 0.0002-0.599 day^{-1} ($\bar{x}$ ±1 SE = 0.13±0.028). This range spans four orders of magnitude and the mean is considerably higher than the mean k reported for rivers in southern Québec, Canada (~ 0.05 d^{-1}) (Guillemette and del Giorgio 2011), but is similar to k values reported for the Klamath River, Oregon, USA (Sullivan et al. 2010). Labile OC concentration across all sites within the Rio Grande/Rio Bravo del Norte drainage ranged from 0.32-5.00 mg L^{-1} ($\bar{x}$ ±1 SE = 1.39 ± 0.26) but, when OC_L was expressed as the

proportion of the DOC pool (OC_L/DOC), this ratio did not differ significantly across the site groups within the lower Rio Grande/Rio Bravo del Norte drainage. Across study sites, OC_L comprised 8 – 83% of the bulk DOC pool ($\bar{x}$±1 SE = 31±5%). Previous studies across a diversity of aquatic habitats indicate that the labile fraction typically composes 5 - 30% of the bulk DOM pool (Sondergaard and Middelboe 1995; Amon and Benner 1996). In the present study, the mean percent of the bulk DOC pool composed of OC_L is at the higher end of this literature-defined range, but a majority of observations (22/27) fall within the reported range of values. Sondergaard and Middelboe (1995) proposed that riverine systems contain relatively high amounts of labile DOC, but del Giorgio and Davis (2002) contend that riverine DOC tends to contain a smaller fraction of labile C than lacustrine systems. In the present study, OC_L/DOC values in the Rio Grande drainage are more similar to OC_L/DOC values from lakes (del Giorgio and Davis 2002). Mean DOC concentration in the Rio Grande drainage was 4.23 ± 0.28 mg/L, which is approximately half that of the lakes and rivers examined by del Giorgio and Davis (2002). Thus, DOC concentrations in the lower Rio Grande/Rio Bravo del Norte are relatively low, but the fraction of the bulk DOC pool composed of labile DOC in the lower Rio Grande drainage is relatively high. In addition, OC_L is not a function of the DOC concentration in the lower Rio Grande/Rio Bravo del Norte drainage ($F_{1,25}$=1.85, R^2_{adj} = 0.032, p = 0.19). These results corroborate a cross-system analysis which concluded that the DOC pool does not reliably predict the labile proportion of that pool (del Giorgio and Davis 2002).

Bacterial community responses to physicochemical and C quality and quantity gradients

In the present study, when I examined how suspended algal biomass, bacterial density, and bacterial metabolism responded to the environmental gradients present across sites in the lower Rio Grande/Rio Bravo del Norte drainage, rather than when sites were placed into groups of sites with similar hydrology and landscape position, I found that biological and metabolic responses were correlated with several physicochemical and C quantity - quality variables. The RDA models examining biological and metabolic responses to physicochemical and C quality - quantity predictors found that both models explained approximately the same amount of variation in response variables (25.7% versus 27.2%). When the physicochemical predictors were examined, Chl*a*, bacterial abundance, and BP exhibited a strong positive relationship with concentrations of P and NH_4^+. In contrast, BR was positively correlated with NVSS and lower riverine discharge values in the Spring sampling season. In the C quality- quantity RDA, Chl*a*, bacterial cell density, and BP were positively associated with increasing DOC and OC_L concentrations, whereas BR was more closely related to water color, bacterial C:N, and POM concentrations. For both of the RDA analyses, site groupings based upon hydrology and landscape position were still apparent and the spatial arrangement of individual data points was similar between both RDA plots. Indeed, many of the individual predictors from the physicochemical and C quantity – quality RDAs that displayed relationships with biological and metabolic responses exhibited substantial covariation. For example, DOC concentration was positively correlated with SRP (r=0.46, p=0.02), POM and NVSS were positively (r=0.96, $p < 0.01$), and TP increased significantly with Abs_{440} (r=0.50, $p < 0.01$). In addition, both RDA analyses indicated

that BR (and subsequently BGE) responded to different predictors than BP, as indicated by the approximately orthogonal relationship between BP and BR in both RDA plots (Fig. 4a and b).

In aquatic ecosystems, BP can respond to multiple biotic and abiotic factors, making it difficult to predict the relative importance of individual drivers in influencing BP in systems (del Giorgio and Cole 1998; Bergstrom and Jansson 2000). In the current study, BP was positively related to concentrations of DOC, OC_L, TP and SRP. However, given the covariation between the sizes of the DOC and P pools in the data set, it is difficult to determine whether BP was primarily responding to availability of DOC or P. There is evidence that BP can be P- or DOC-limited depending on *in situ* conditions (Amon and Benner 1996; Pace and Cole 1996; Makino et al. 2003; Lennon and Pfaff 2005; Lennon and Cottingham 2008; Cotner et al. 2010; Franklin et al. 2011; Hall et al. 2011). Research across a diversity of natural and experimental systems indicates that BP responses to DOC are equivocal; some studies find that there is no relationship between BP and DOC concentrations (Coffin et al. 1993; Findlay et al. 1996; Sherr et al. 2001; del Giorgio and Davis 2002; Judd et al. 2006), while others find that greater BP is associated with increased DOC supply (Amon and Benner 1996; Lennon and Pfaff 2005; Lennon and Cottingham 2008). Bulk DOC and OC_L pools are complex and are composed of C sources of varying quality and bulk OC can be conceptualized as a series of pools with progressively decreasing decomposition rates (Sondergaard and Middelboe 1995). In addition, studies have indicated that BP is responsive to the timing and nature of external DOC supply (i.e., pulsed versus continuous DOC supply) (Findlay 2002; Lennon and Cottingham 2008). Thus, given the often complex composition of the DOC pool,

variation in the timing of DOC supply, and the various DOC sources (e.g., autochthonous versus allochthonous sources), it is difficult to determine how and when BP will respond to changes in DOC supply.

In addition to the supply and composition of DOC affecting bacterial production, the supply of inorganic nutrients, in particular P, is often cited as a determinant of bacterial growth rates (Pace and Cole 1996; Makino et al. 2003; Lennon and Pfaff 2005; Cotner et al. 2010; Franklin et al. 2011; Hall et al. 2011). There are a limited number of studies which have assessed P-limitation in pelagic riverine bacteria, but data indicate that changes in seasonal hydrology can affect the severity of bacterial P-limitation (Rejas et al.. 2005). In the present study, higher BP rates occurred in areas with higher P and DOC concentrations and this response may be a consequence of several possible mechanisms. First, it is possible that BP is driven primarily by either P or OC across all parts of the drainage. This scenario is unlikely because the availability and supply of DOC and P varies substantially across the drainage; the ratio of labile DOC to P (OC_L (mM):SRP (mM)) varied from 2.52 - 3411.55, indicating high variability in external OC and P sources for bacteria. A second potential reason for increased BP at sites with higher OC and P is that bacterial growth rates are positively responding to the combination of both factors. A third potential mechanism is that BP is responding to increased supply of OC or P in different portions of the drainage throughout different portions of the study period. Although is it difficult to elucidate the exact nature of the interaction between BP and OC and P in the lower Rio Grande/Rio Bravo del Norte drainage, this study indicates that BP is likely responding to a defined subset of factors across the entire drainage.

Respiration rates of bacterial communities can be affected by abiotic factors such as temperature and pH (del Giorgio and Cole 1998), by biogeochemical factors such as DOC quantity and quality (Lennon and Pfaff 2005), DOC sources (allochthonous- versus autochthonous-derived sources) (Carpenter et al. 2005; Kritzberg et al. 2005), DOC substrate stoichiometry (del Giorgio and Cole 1998), bacterial biomass (Hamdan and Jonas 2006), and bacterial community composition (Judd et al. 2006). In the present study, higher BR rates were most closely correlated with water color, POM, and C:N of bacteria. Water color has been used as an indicator of allochthonously-derived OC in aquatic systems and colored DOC is generally considered to be more refractory than uncolored DOC (Carpenter et al. 2005; Kritzberg 2006; Jones et al. 2009). Respiration rates of bacterial communities can respond positively to increased concentrations of allochthonously-derived and refractory DOC, regardless of the availability of OC_L (del Giorgio and Davis 2002; Carpenter et al. 2005; Berggren et al. 2007). In the study presented here, BR was greatest in the upper portion of the Rio Grande/Rio Bravo del Norte drainage, was more closely related to water color and POM, and was not strongly correlated with concentrations of DOC and OC_L. This indicates that, during the study period, BR was likely responding to localized terrestrial inputs, and therefore, allochthonous C sources and not to the total DOC pool.

Differential responses of BP and BR to the supply and lability of OC serves as one of the mechanisms for the widespread occurrence of net heterotrophy in many aquatic systems; in order for ecosystem respiration to exceed net primary production, bacterial communities must respire C beyond the concentrations produced through autochthonous production (Cole and Caraco 2001; Dodds 2006; Cole et al. 2007). The

decoupling of BR and BP in ecosystems has been attributed to a number of different factors including a lack of BR responsiveness to C quality (Lennon and Cottingham 2008) and the presence of localized "hot spots" of increased bacterial metabolism in areas where aquatic and terrestrial ecosystems interface (del Giorgio et al. 2006). In the study presented here, BP and BR responded to differential environmental factors: BP increased with increased with concentrations of labile and total DOC, P, and NH_4^+ and was correlated with Chl*a*, while BR increased with concentrations of suspended matter and external sources of colored DOC. These findings suggest that different aspects of bacterial metabolism (BR versus BP) in the lower Rio Grande/Rio Bravo del Norte drainage are responding to different C sources: BP may primarily responding to autochthonous C supply and inorganic nutrients, while BR is responding primarily to localized supply of allochthonous particulate OM and DOC.

In the present study, the decoupled responses of BR and BP inevitably affected calculations of BGE (del Giorgio et al. 2006); the high variability in BR measurements relative to BP measurements disproportionately influences BGE estimates (recall, BGE = BP / (BP + BR)). As a result, BGE was inversely related to BR and thereby negatively correlated with POM concentration and Abs_{440}, and BGE exhibited no relationship with BP, DOC, OC_L, and the availability of inorganic nutrients. Lennon and Cottingham (2008) found that BGE did not respond to variation in DOC quality or quantity. In contrast, other studies have found that BGE is not influenced by DOC quantity, but is sensitive to changes in DOC quality (Middelboe and Sondergaard 1993; Eiler et al. 2003; Berggren et al. 2007). In the lower Rio Grande/Rio Bravo del Norte, results from the present study suggest that BGE is more responsive to changes in DOC quality and not to

DOC quantity. Bacterial growth efficiency responded negatively to Abs_{440} was and was unrelated to OC_L concentration because BR was highly sensitive to changes in water color and did not respond to OC_L concentrations. Bacterial production responded to changes in OC_L concentration, but the magnitude of responses were relatively constrained when compared to those observed in BR rates.

Conclusions and Implications

In the lower Rio Grande/Rio Bravo del Norte drainage, hydrology and landscape position (i.e., biogeoclimatic conditions, the presence of reservoirs, and groundwater inputs) substantially influenced instream physicochemical conditions. Spatial variation in physicochemical metabolism across the lower Rio Grande/Rio Bravo del Norte influenced spatial patterns in bacterial density, phytoplankton biomass, and bacterial metabolism in this drainage. Subsequently, bacterial C metabolism was influenced by both physicochemical and C quality – quantity gradients in the drainage. Rates of BR and BP exhibited differential responses to C quality and quantity and the availability of inorganic nutrients. In particular, the response of BR to the amount of refractory DOC, as indicated by water color, resulted in patterns of lower BGE and potentially lower ecosystem processing of C. Results from this study indicate that anthropogenic alteration of hydrology and inorganic nutrient loading affects C processing by bacteria across a large, complex drainage. Furthermore, this study suggests that anthropogenic alteration of landscapes affects ecosystem processes in this system, which has large-scale implications for C sequestration, transformation, and transport within the drainage, as well as for C delivery to the Gulf of Mexico.

Arid river ecosystems are thought to be more dependent on autochthonous C production (Grimm 1987; Jones et al. 1997; Bunn et al. 2003), but the results of this study show that allochthonous C had a strong influence on bacterial C metabolism in the lower Rio Grande/Rio Bravo del Norte. Traditional riverine conceptual models aim to predict how the hydrology and landscape factors of riverine systems affect biogeochemistry, primary productivity, diversity, and ecosystem function (Vannote et al. 1980; Ward and Stanford 1983; Junk et al. 1989; Thorp and Delong 2002). Although, bacteria are the greatest single contributor to total ecosystem respiration in many aquatic systems (del Giorgio and Cole 1998), widely-used riverine conceptual models do not explicitly incorporate bacterial C processing into their frameworks. The present study joins the growing body of work which demonstrates the importance of aquatic bacterial communities in the sequestration and transformation of both allochthonous and autochthonous C and the crucial role that bacterial community metabolism plays in ecosystem function at the landscape scale and beyond.

LITERATURE CITED

A Watershed Protection Plan for the Pecos River in Texas: An Overview. Texas AgriLife Extension Program. 2010.

Agnew C, Anderson E. 1992. Water resources in the arid realm. London: Routledge. 329 p.

Amon RM, Benner R. 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnology and Oceanography 41:41-51.

Ashworth JB, Hopkins J. 1995. Aquifers of Texas. Texas Water Development Board. Report 345.

Bergermeister, J. 2007. Missing carbon mystery: Case solved? Nature 3:36-37.

Berggren N, Laudon H, Jansson M. 2007. Landscape regulation of bacterial growth efficiency in boreal freshwaters. Global Biogeochemical Cycles 21:GB4002, 1-9. GB4002, doi:10.1029/2006GB002844.

Bergstrom AK, Jansson M. 2000. Bacterioplankton production in humic Lake Ortrasket in relation to input of bacterial cells and input of allocthonous organic carbon. Microbial Ecology 39:101-115.

Bunn SE, Davies PM, Winning M. 2003. Sources of organic carbon supporting the food web of an arid flood-plain river. Freshwater Biology 48:619-635.

Carpenter SR, Cole JJ, Pace ML, Van de Bogert M, Bade DL, Bastviken D, Gille C, and others. 2005. Ecosystem subsidies: terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. Ecology 86:2737-2750.

Caston CB, Nowlin WH, Gaulke A, Vanni MJ. 2009. The relative importance of heterotrophic bacteria to ecosystem dynamics varies with reservoir trophic state. Limnology and Oceanography 54:2143-2152.

Coffin RB, Connolly JP, Harris PS. 1993. Availability of dissolved organic carbon to backterioplankton examined by oxygen utilization. Marine Ecology Progress Series 101:9-22.

Cole JJ, Caraco NF. 2001. Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. Marine and Freshwater Research 52:101-10.

Cole JJ, Findlay S, Pace M. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Marine Ecology Progress Series 43:1-10.

Cole JJ, Prairie WT, Caraco NF, McDowell WH, Tranvik LJ, Striegle RG. 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10:171-184.

Comte J, del Giorgio PA. 2009. Links between resources, C metabolism and the major components of bacterioplankton community structure across a range of freshwater ecosystems. Environmental Microbiology 11:1704-1716.

Cotner JB, Hall EK, Scott JT, Heldal M. 2010. Freshwater bacteria are stoichiometrically flexible with a nutrient composition similar to seston. Frontiers in Microbiology 1:1-11.

Crumpton WG, Isenhart TM, Mitchell PD. 1992. Nitrate and organic N analyses with 2nd-derivative spectroscopy. Limnology and Oceanography 37:907-913.

Cuthbert ID, del Giorgio PA. 1992. Toward a standard method of measuring color in freshwater. Limnology and Oceanography 37:1319-1326.

Dean WE, Gorham E. 1998. Magnitude and significance of carbon burial in lakes, reservoirs, and peatlands. Geology 26:535–538.

del Giorgio PA, Cole JJ. 1998. Bacterial growth efficiency in aquatic systems. Annual Review of Ecology and Systematics. 29:503–501.

del Giorgio PA, Davis J. 2003. Patterns in dissolved organic matter lability and consumption across aquatic ecosystems. Findlay SEG, Sinsabaugh RL, editors. Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. San Diego (CA): Academic Press. p399-421.

del Giorgio PA, Pace ML, Fischer D. 2006. Relationship of bacterial growth efficiency to spatial variation in bacterial activity in the Hudson River. Aquatic Microbial Ecology 45:55-67.

del Giorgio PA, Pace ML. 2008. Relative independence of dissolved organic carbon transport and processing in a large temperate river: the Hudson River as both pipe and reactor. Limnology and Oceanography 53:185-197.

del Giorgio PA, Williams PJleB. 2005. Respiration in aquatic ecosystems: From single cells to the biosphere. del Giorgio PA, Williams PJleB, editors. Respiration in aquatic ecosystems. Oxford (England): Oxford University Press. p267-303.

Dodds W, Cole JJ. 2007. Eutrophication and trophic state in rivers and streams. Aquatic Sciences 69:427-439.

Dodds W. 2006. Eutrophication and trophic state in rivers and streams. Limnology and Oceanography 51:671-680.

Douglas A. 2009. Social, political, and institutional setting: water management problems of the Rio Grande. Journal of Water Resources Planning and Management 6:493-501.

Downing JA, Cole JJ, Middelburg J, Striegl RG, Duarte CM, Kortelainen P, Prairie YT, Laube KA. 2008. Sediment carbon burial in agriculturally eutrophic impoundments over the last century. Global Biogeochem. Cy. 22:GB1018, doi:1010.1029/2006GB002854.

Eiler A, Langenheder S, Bertilsson S, Tranvik LJ. 2003. Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. Applied Environmental Microbiology 69:3701–3709.

Feng Q, Endo KN, Cheng GD. Towards sustainable development of the environmentally degraded arid rivers of China- a case study from Tarim River. Environmental Geology 74:229-238.

Findlay S, Pace M, Fischer D. 1996. Spatial and temporal variability in the lower food web of the tidal freshwater Hudson River. Estuaries 19:866–73.

Findlay S. 2003. Bacterial Response to Variation in Organic Matter. Findlay SEG, Sinsabaugh RL, editors. Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. San Diego (CA): Academic Press. p363-377.

Finlay JC. 2001. Stable carbon isotope ratios of river biota: implications for energy flow in lotic food webs. Ecology 84:1052-1064.

Fox J, Weisberg S, editors. 2011. An {R} companion to applied regression. Thousand Oaks CA: Sage.

Franklin O, Hall EK, Kaiser C, Battin TJ, Richter A. 2011. Optimization of biomass composition explains microbial growth-stoichiometry relationships. American Naturalist 177:29-42.

Granéli W, Bertilsson S, Philibert A. 2004. Phosphorus limitation of bacterial growth in high arctic lakes and ponds. Aquatic Sciences 66:430-439.

Grimm NB. 1987. Nitrogen dynamics during succession in a desert stream. Ecology 68:1157-1170.

Groeger AW, Brown PF, Tietjen TE, Kelsey TC. 1997. Water quality of the San Marcos River. Texas Journal of Science 49:279-294.

Guenet B, Danger M, Abbadie L, Lacroix G. 2010. Priming effect: bridging the gap between terrestrial and aquatic ecology. Ecology 91:2850-2861.

Guillemette F, del Giorgio PA. 2011. Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. Limnology and Oceanography 56:734-748.

Hadwen WD, Fellows CS, Westhorpe DP, Rees GN, Mitrovic SM, Taylor B, Baldwin DS, Silvester E, Croome R. 2010. Longitudinal trends in river functioning: patterns of jutrient and carbon processing in three Australian rivers. River Research and Applications 26:1129-1152.

Hall EK, Maixner F, Franklin O, Daims H, Richter A, Battin T. 2011. Linking microbial and ecosystem ecology using ecological stoichiometry: a synthesis of conceptual and empirical approaches. Ecosystems 14:261-273.

Hauer FR, Lorang MS. 2004. River regulation, decline of ecological resources, and potential for restoration in a semi-arid lands river in the western USA. Aquatic Sciences 66:388-401.

Hoeinghaus DJ, Winemiller KO, Agostinho AA. 2007. Landscape-scale hydrologic characteristics differentiate patterns of carbon flow in large-river food webs. Ecosystems 10:1019–1033.

Holmes RM, McClelland JW, Raymond PA, Frazer BB, Peterson BJ, Stieglitz M. 2008. Lability of DOC transported by Alaskan rivers to the Arctic Ocean. Geophysical Research Letters 35:L03402, doi:10.1029/2007GL032837.

Jones Jr JB, Schade JD, Fisher SG, Grimm NB. 1997. Organic matter dynamics in Sycamore Creek, a desert stream in Arizona, USA. Journal of the North American Benthological Society 16:78-82.

Jones SE, Newton RJ, McMahon KD. 2009. Evidence for structuring of bacterial community composition by organic carbon source in temperate lakes. Environmental Microbiology 11:2463-2472.

Judd KE, Crump BC, Kling GW. 2006. Variation in dissolved organic matter controls bacterial production and community composition. Ecology 87:2068-2079.

Kingsford RT. 2000. Protecting rivers in arid regions or pumping them dry? Hydrobiologia 427:1-11.

Kritzberg ES, Cole JJ, Pace MM, Granéli W. 2005. Does autochthonous primary production drive variability in bacterial metabolism and growth efficiency in lakes dominated by terrestrial C inputs? Aquatic Microbial Ecology 38:103-111.

Kritzberg ES, Cole JJ, Pace MM, Granéli W. 2006. Bacterial growth on allocthonous carbon in humic and nutrient-enriched lakes: results from whole-lake ^{13}C addition experiments. Ecosystems 9:489-499.

Lamberti GA, Steinman AD. 1997. A comparison of primary production in stream ecosystems. Webster JR, Meyer JL, editors. Stream organic matter budgets. Journal of the North American Benthological Society. p95–104.

Larned ST, Hicks DM, Schmidt J, Davey AJH, K. Dey K, M. Scarsbrook M, Arscott DB, Woods RA. 2008. The Selwyn River of New Zealand: a benchmark system for alluvial plain rivers. River Research and Applications 24:1-21.

Lennon JT, Cottingham KL. 2008. Microbial productivity in variable resource environments. Ecology 89:1001-1014.

Lennon JT, Pfaff LE. 2005. Source and supply of terrestrial organic matter affects aquatic microbial metabolism. Aquatic Microbial Ecology 39:107-119.

Makino W, Cotner JB, Sterner RW, Elser JJ. 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. Functional Ecology 17:121–130.

Maranger RJ, Pace ML, del Giorgio PA, Caraco NF, Cole JJ. 2005. Longitudinal spatial patterns of bacterial production and respiration in a large river-estuary: implications for ecosystem carbon consumption. Ecosystems 3:318-330.

McClaine ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, Hart SC, Harvey JW, Johnston CA, Mayorga E, McDowell WH, Pinay G. 2003. Biogeochemical hotspots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301-12.

Middleton NJ, Thomas DSG. 1997. World Atlas of Desertification (2nd Edition). London: United Nations Environment Programme, Edward Arnold Murrel. 184p.

Moran MA, Sheldon WM, Zepp RG. 2000. Carbon loss and optical property changes during long-term photochemical and biological degration of estuarine dissolved organic matter. Limnology and Oceanography 45:1254-1264.

Murrell MC. 2003. Bacterioplankton, dynamics in a subtropical estuary: Evidence for substrate limitation. Aquatic Microbial Ecology 32:239–250.

Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2011. vegan: Community Ecology Package. R package version 2.0-1. http://CRAN.R-project.org/package=vegan

Ostapenia AP, Parparov A, Berman T. 2009. Lability of organic carbon in lakes of different trophic status. Freshwater Biology 54:1312-1323.

Pace ML, Cole JJ, Carpenter SR, Kitchell JF, Hodgson JR, Van de Bogert MC, Bade DL, Kritzberg ES, Bastviken D. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature 427:240-243.

Pace ML, Cole JJ. 1996. Regulation of bacteria by resources and predation tested in whole-lake experiments. Limnology and Oceanography 41:1448–1460.

R Development Core Team. 2009. R 2.10.1. R Foundation for Statistical Computing, Vienna, Austria. http://cran.r-project.org/bin/I

Reinthaler T, Herndl GJ. 2005. Seasonal dynamics of bacterial growth efficiencies in relation to phytoplankton in the southern North Sea. Aquatic Microbial Ecology 39:7-16.

Rejas D, Muylaert K, De Meester L. 2005. Nutrient limitation of bacteria and sources of nutrients supporting nutrient-limited bacterial growth in an Amazonian floodplain lake. Aquatic Microbial Ecology 39:57-67.

Revelle W. 2012. psych: Procedures for Personality and Psychological Research Northwestern University, Evanston, http://personality-project.org/r/psych.manual.pdf, 1.2.1.

Roland F, Caraco NF, Cole JJ. 1999. Rapid and precise determination of dissolved oxygen by spectrophotometry: evaluation of interference from color and turbidity. Limnology and Oceanography 44:1148–1154.

Sherr EB, Sherr BF, Cowles TJ. 2001. Mesoscale variability in bacterial activity in the Northeast Pacific Ocean off Oregon, USA. Aquatic Microbial Ecology 25:21-30.

Smith DC, Azam F. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. Marine Microbial Food Webs 6:107-14.

Sondergaard M, Middelboe M. 1995. A cross-system analysis of labile dissolved organic carbon. Marine Ecology Progress Series 118:283-294.

Stelzer RS, Heffernan J, Likens GE. 2003. The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. Freshwater Biology 48:1925-1937.

Sullivan AB, Snyder DM, Rounds SA. 2010. Controls on biochemical oxygen demand in the upper Klamath River, Oregon. Chemical Geology 269:12-21.

Thorp JH, Delong MD. 1994. The riverine productivity model: an heuristic view of carbon sources and organic processing in large river ecosystems. Oikos 70:305-308.

Thorp JH, Delong MD. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. Oikos 96:543-550.

Thorp JH, Thoms MC, Delong MD. 2006. The riverine ecosystem synthesis: Biocomplexity in river networks across space and time. River Research and Applications 22:123-147.

Tranvik LJ, Downing JA, Cotner JB, Loiselle SA, Striegl RG, Ballatore TJ, Dillon P, Finlay K, Fortino K, Knoll LB, Kortelainen PL, Kutser T, Larsen S, Laurion I, Leech DM, McCallister SL, McKnight DM, Melack JM, Overholt E, Porter JA, Prairie Y, Renwick WH, Roland F, Sherman BS, Schindler DW, Sobeck S, Tremblay A, Vannie MJ, Verschoor AM, von Wachenfeldt E, Weyhenmeyer GA. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography 54:2298-2314.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130-137.

Velasco J, Millan A, Vidal-Abarca MR, Suarez ML, Guerrero C, Ortega M, M. 2003. Macrophytic, epipelic and epilithic primary production in a semiarid Mediterranean stream. Freshwater Biology 48:1408-1420.

Ward JV, Stanford JA. 1983. The serial discontinuity concept of lotic ecosystems. In Dynamics of Lotic Ecosystems, TD Fontaine III, Bartell SM, editors. Ann Arbor Science: Ann Arbor, Michigan, USA.

Weiss M, Simon M. 1999. Consumption of labile dissolved organic matter by limnetic bacterioplankton: the relative significance of amino acids and carbohydrates. Aquatic Microbial Ecology 17:1–12.

Wetzel RG, Likens GE. 2000. Limnological methods, 3rd ed. New York City (NY):Springer-Verlag. 429p.

Wetzel RG. 1983. Limnology. Philadelphia (PA): Saunders. 767p.

Williams CJ, Yamashita Y, Wilson HF, Jaffe RJ, Xenopoulos MA. 2010. Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems. Limnology and Oceanography 55:1159-1171.

Williams PJleB, del Giorgio PA. 2005. Respiration in aquatic ecosystems: History and background. Respiration in Aquatic Ecosystems. del Giorgio PA, Williams PJleB, eds, Oxford University Press, New York. p1-17.

Williams WD. 1999. Salinisation: a major threat to water resources in the arid and semi-arid regions of the world. Lakes and Reservoirs: Research and Management 4:85-91.

Winberg GG.1960. Primary production of water bodies. Minsk: Academy of Sciences of BSSR (in Russian). 328p.

Wong CM, Williams CE, Pittock J, Collier U, Schelle P. 2007. World's top ten rivers at risk. Gland (Switzerland): WWF International. 53p.

VITA

Kelly Jean Rodibaugh has preferred to be called "Rodi" since 1999. Born to Scott and Cindy Rodibaugh in 1980, Rodi shared an idyllic childhood with her beloved sister, Rebecca, before leaving home to seek her fortune. She attended the University of Texas, Austin Community College, and the University of Arizona before settling in San Marcos to complete an undergraduate degree in Biology at Texas State University-San Marcos. During these turbulent years, Rodi was employed as an ice cream scooper, a Special Education teaching assistant at a juvenile detention center, and a professional hippie at a cooperative grocery store. In 2009, Rodi entered the Master's Program in Aquatic Resources at Texas State under the direction of Dr. Weston Nowlin. She has received numerous awards, including the Richan Aquatic Biology, Celebrity Classics, Schultze, and the Associated Student Government Scholarships. Rodi also won Best Poster Presentation at the 2011 Student Colloquium at Texas State. She has presented her research at international conferences such as the Association for the Sciences of Limnology and Oceanography and the Ecological Society of America.

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