



Physicochemical and carbon quantity–quality gradients equally influence bacterial carbon metabolism across an arid riverscape

Kelly J. Rodibaugh · Jesse C. Becker · Gabrielle Timmins · Weston H. Nowlin

Received: 2 December 2019 / Accepted: 26 March 2020 / Published online: 8 April 2020
© Springer Nature B.V. 2020

Abstract Although freshwater systems comprise a small portion of land surface, they play a pivotal role in landscape carbon (C) cycling. Carbon processing by heterotrophic bacteria is critical, contributing to ecosystem production and dissolved organic carbon (DOC) processing. Riverine bacterial C metabolism is related to a diversity of factors, including (1) physicochemical conditions and inorganic nutrient concentrations and (2) DOC quality and quantity; however, the relative importance of these factors in influencing C metabolism across arid fluvial networks remains equivocal. This study examined C metabolism by heterotrophic bacterioplankton in the Rio Grande drainage, an arid river network in Texas, USA. We examined spatial variation in physicochemical and inorganic nutrient conditions, bacterial C metabolism, and DOC lability across the drainage and assessed whether variation in bacterial C metabolism was more related to physicochemical–inorganic nutrient conditions or DOC quantity and lability. Across the drainage, hydrology and landscape position influenced physicochemical conditions, bacterial abundance, phytoplankton biomass, and bacterial C metabolism

and the proportion of variation in bacterial C metabolism explained by physicochemical/inorganic nutrient and DOC quality–quantity gradients was approximately equal. Bacterial abundance and production were associated with greater NH_4^+ and DOC concentrations and phytoplankton biomass, whereas bacterial respiration and growth efficiency were driven by DOC color and suspended particulate concentrations. Results indicate that aspects of bacterial C metabolism are influenced by different environmental factors related to autochthonous and allochthonous inputs to riverine systems, which has implications for C transformation, sequestration, and transport to the ocean.

Keywords Riverine network · Carbon metabolism · Bacterial production · Bacterial respiration · Nutrients · Rio Grande

Introduction

Globally, inland waters are responsible for processing, transporting, and sequestering ~ 2.9 Pg of terrestrial carbon (C) annually (Tranvik et al. 2009). At landscape and global scales, inland waters (including rivers and streams) constitute a small portion of the land surface but are biogeochemically active and process more C than is expected based solely on area

Handling Editor: Téséphore Sime-Ngando.

K. J. Rodibaugh · J. C. Becker · G. Timmins ·
W. H. Nowlin (✉)
Aquatic Station, Department of Biology, Texas State
University, San Marcos, TX 78666, USA
e-mail: wn11@txstate.edu

(Cole et al. 2007; Tranvik et al. 2009; Raymond et al. 2013). River ecosystems also have a large role in C export to oceans, contributing an estimated 1.06 Pg/year as particulate and dissolved forms (Battin et al. 2009; Aufdenkampe et al. 2011; Li et al. 2017). In lotic ecosystems, C cycling involves interplay of numerous factors, including land use, geomorphology, origin of C sources, C quality and quantity, microbial activity, and microbial community composition and diversity (Cole et al. 2007; Williams et al. 2010). Given the diverse factors and interactions between them, it is often difficult to determine how, and at what scale, these factors affect C cycling in fluvial networks (Amado et al. 2013).

Carbon metabolism by aquatic heterotrophic microbes is a critical process, contributing to overall ecosystem production, and representing a primary route of dissolved organic carbon (DOC) transformation (Cole et al. 2007). Multiple factors affect DOC processing rates, and the relationship between bacterial C metabolism and the concentration, sources, and composition of DOC is complex (Hitchcock and Mitrovic 2015; Figueroa et al. 2016). Bacterial production (BP), the rate which C is incorporated into biomass, and bacterial respiration (BR), the rate at which bacteria use C for cell maintenance, can exhibit a positive relationship with DOC concentration (Lennon and Pfaff 2005). However, BP and BR may not be strongly related to the size of the DOC pool, particularly if the DOC is refractory (Findlay 2003; del Giorgio and Davis 2003). Thus, increasing the proportion of refractory C within DOC affects bacterial metabolic function and leads to greater BR per unit BP, indicative of decreased bacterial growth efficiency (BGE; del Giorgio and Cole 1998). A critical question in aquatic ecosystem studies is the relative importance of allochthonous versus autochthonous C sources in aquatic food webs because autochthonous DOC is generally considered more labile than allochthonous (terrestrial) DOC and particulate organic matter (Sondergaard and Middelboe 1995; del Giorgio and Davis 2003; Raymond and Spencer 2015). Evidence indicates that autochthonous DOC sources are important in determining bacterial C metabolism because most labile C is generated by in situ primary producers (Wetzel 2001; Sondergaard and Middelboe 1995). However, DOC lability is also influenced by other factors such as DOC age (e.g., younger pools are more reactive), molecular structure (e.g., lignin content),

photo-degradation, and anthropogenic inputs (e.g., wastewater) (Raymond and Spencer 2015).

Although DOC concentration, sources, and lability are important determinants of bacterial C metabolism, physicochemical processes also act upon DOC (e.g., ultraviolet radiation exposure, flocculation), but the relative importance of these processes can be difficult to distinguish from the effects of bacterial processing (del Giorgio and Pace 2008). Increased availability of inorganic nutrients can catalyze bacterial C utilization and stimulate greater bacterial production (Granéli et al. 2004; Ochs et al. 2010). In freshwater systems, phosphorus (P) can limit bacteria (as opposed to inorganic nitrogen or DOC), influencing bacterial growth rates, metabolism, and community composition (Granéli et al. 2004; Nelson and Carlson 2011; Peura et al. 2012). To understand how microbial communities process C in riverine ecosystems, it is necessary to examine how the combination and co-occurrence of physicochemical characteristics, dissolved inorganic nutrients, and C quality and quantity influence bacterial C metabolism.

Most research on landscape-level variation in river bacterioplankton C processing has been in temperate systems (e.g., Maranger et al. 2005; del Giorgio and Pace 2008; Guillemette and del Giorgio 2011), but studies have been conducted in boreal (Holmes et al. 2008; Comte and del Giorgio 2009), subtropical (Hadwen et al. 2010), and tropical (Amon and Benner 1996; Farjella et al. 2009) rivers. Arid and semiarid ecosystems comprise ~ 40% of the earth's land surface (Middleton and Thomas 1997), but few studies address bacterial C metabolism to arid rivers. In situ gross primary production in arid rivers (via algae) is thought to be greater than that in temperate rivers due to higher light availability and water temperatures, lower flows, and more efficient nutrient cycling (Lamberti and Steinman 1997; Velasco et al. 2003). In arid rivers, adjacent terrestrial productivity is also relatively low; thus, in situ (autochthonous) algal primary production is expected to be the main C source for organisms, including bacteria (Finlay 2001). Consequently, it can be predicted that a greater relative amount of autochthonous C in arid river systems leads to greater DOC lability, more rapid bacterial C processing, and greater BGE when compared to more mesic rivers. Understanding the role of microbial C processing and the factors that influence rates of processing in arid river systems is relevant as

rivers in arid landscapes are under increasing anthropogenic pressure (Kingsford 2000).

We assessed spatiotemporal variation in heterotrophic bacterioplankton C metabolism in a large, complex arid and semiarid riverine network in the southwestern USA: the Rio Grande/Río Bravo del Norte (hereafter the Rio Grande drainage). Specifically, we examined how bacterial C metabolism was affected by spatiotemporal variation in physicochemical conditions, DOC concentration, and DOC lability. We investigated whether bacterial abundance and C metabolism in the basin were more influenced by large-scale physicochemical gradients, or whether they were more influenced by the quantity and quality of C sources and bacterial stoichiometry. We hypothesized that spatiotemporal variation in physicochemical conditions across the drainage would affect rates of bacterial C metabolism, but that variation in the quantity and quality of DOC would be the primary driver of the bacterial C processing. We also hypothesized that bacterial DOC processing in more arid portions of the drainage would have greater reliance on autochthonous and more labile C sources.

Methods

Study area and sampling design

Headwaters of the US portion of Rio Grande drainage originate in Colorado and New Mexico before entering Texas and forming the border between the USA and Mexico (Fig. 1). This study focused on major river catchments within the Texas portion of the drainage. The Rio Grande subcatchment originates in Colorado, with ~ 2000 river km (rkm) of the river running along the border. The Pecos and Devils Rivers are on the US side of the drainage and the Río Conchos is the major Mexican tributary in the study area. The Pecos River flows approximately 560 rkm from New Mexico and through west Texas before joining the Rio Grande at Amistad International Reservoir. The Devils River originates within Texas and flows ~ 160 rkm before joining the Rio Grande at Amistad International Reservoir. Along the upstream–downstream gradient of study area, the surrounding landscape represents several ecoregions, transitioning from the arid Chihuahuan Desert and Trans-Pecos of the upper Rio Grande and Pecos Rivers (mean annual

rainfall < 400 mm year⁻¹), to the semiarid Edwards Plateau in the region of the Devils River, to semiarid subtropical South Texas brush country in the lower portion of the drainage (mean annual rainfall ~ 700 mm year⁻¹).

Data were collected at 14 sites (Fig. 1) to assess differences in physicochemical and bacterial C dynamics along the drainage. All sites were in the mainstem of rivers; sampling sites were free flowing (i.e., not backwater areas or behind impoundments), not small order headwaters, and were wadeable. Nine sampling sites were on the Rio Grande mainstem: three sites were located above Amistad International Reservoir (designated RG1–3), four sites were distributed between Amistad International and Falcón International reservoirs (RG4–7), and two sites were located below Falcón International Reservoir (RG8 and 9). Four sites were located along the Pecos River (PR1–PR4), and one site was located on the Devils River (DR1). Sites RG1–RG3 are in the Chihuahuan Desert and Trans-Pecos ecoregions, with RG2 located immediately below the confluence of the Rio Grande and Rio Conchos. Sites RG4–9 are distributed through semiarid subtropical South Texas Brush ecoregion in the Lower Rio Grande River Valley. Flows in this portion of the Rio Grande tend to be of higher magnitude and more consistent due to releases from Amistad and Falcón International Reservoirs for agricultural needs and to fulfill international treaty requirements. Pecos River (PR) sites are found in the Trans-Pecos and along the edge of the Edwards Plateau; PR1 and PR2 exhibit relatively low flows and higher salinities (Trans-Pecos sites), but PR3 and PR4 (edge of Edwards Plateau) are strongly influenced by groundwater contribution (Edwards–Trinity aquifer) (Gregory and Hatler 2008). The landscape surrounding DR1 is characterized by a convergence of the three main ecoregions. The Devils River is remote and largely pristine (little anthropogenic impact), dominated by groundwater from the Edwards–Trinity aquifer.

Field sampling

Data were collected on a seasonal basis (Spring, Summer, and Fall) from March to December 2010. We define “Spring” as March to May, “Summer” as July to August, and “Fall” as October to December. Seasons were defined by the agricultural calendar

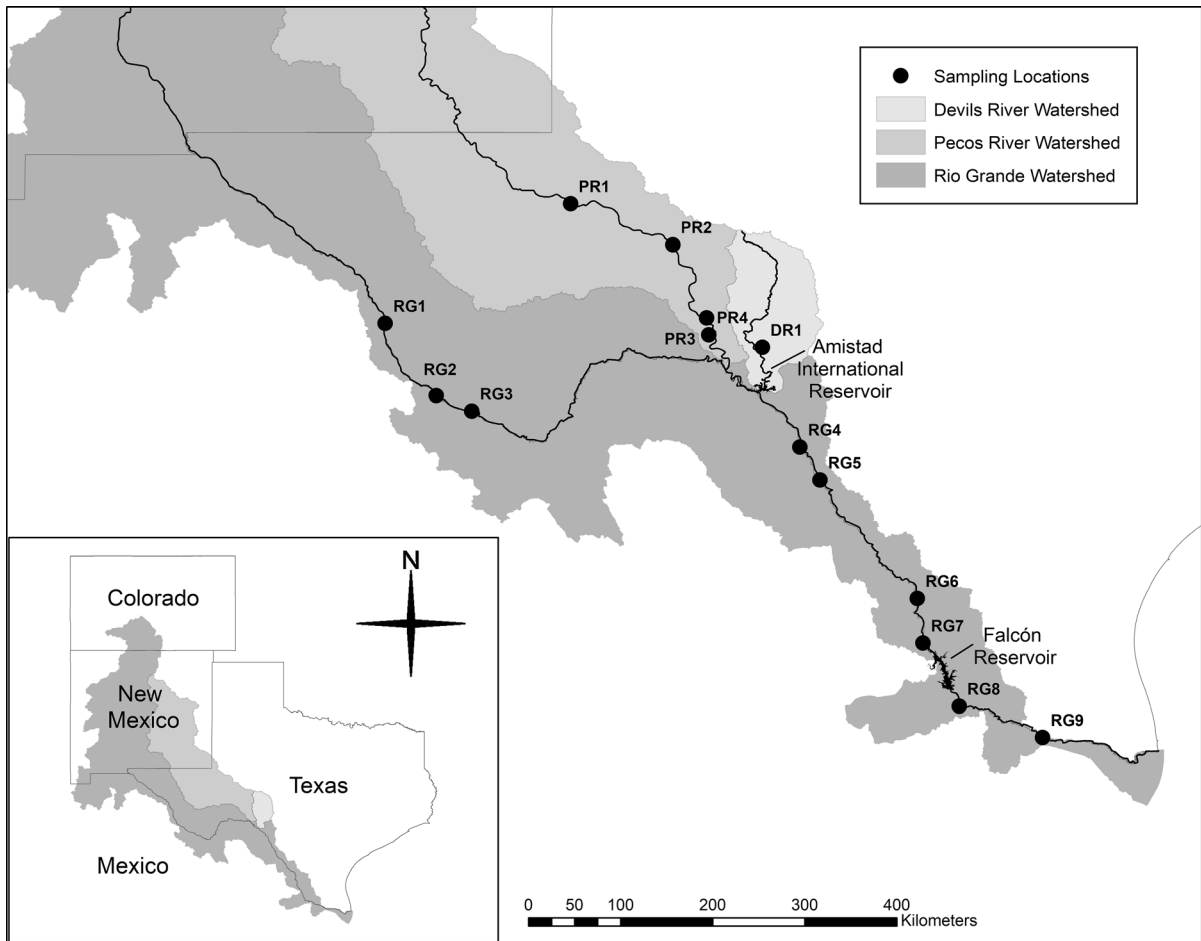


Fig. 1 Map of the Rio Grande, Pecos River, and Devils River catchments in Texas, USA. Points indicate study site locations, and the designations RG, PR, and DR for sites indicate sites in the Rio Grande, Pecos River, and Devils River watersheds,

respectively. Site designations and numbers correspond to sites in Tables 1 and 2. Locations of Amistad International Reservoir and Falcón Reservoir are also indicated

because flows are managed for irrigation. Data collected included physicochemical parameters, total, dissolved and sestonic nutrients, and phytoplankton biomass. We additionally assessed bacterial abundance, bacterial C metabolic rates (i.e., BR and BP), and DOC quality at a subset of nine sites consisting of RG1, RG2, RG4, RG7, RG8, RG9, PR1, PR4, and DR1. This subset was selected to maximize variation and to serve as proxies for conditions across the drainage.

At each site, physicochemical data and water samples were collected from the thalweg at the water column mid-point. Dissolved oxygen (DO; mg L^{-1}), specific conductivity (SpC; $\mu\text{S cm}^{-1}$), pH, salinity (ppt) and water temperature ($^{\circ}\text{C}$) were collected with a

YSI 556 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 NalgeneTM high-density polyethylene (HDPE) bottles that had been rinsed with site water prior to collection (Becker et al. 2017). At the subset of sites selected for bacterial C metabolism estimates, an additional 7.5 L of water was collected in pre-cleaned opaque HDPE NalgeneTM carboys that were field rinsed. Water samples were stored on ice in coolers until the return to Texas State University, where they were held at ambient river temperature. Samples were processed within 48 h of collection. We estimated hydrological conditions at each site prior to and at the time of sampling by calculating the mean daily

Table 1 Study site catchment, location, and nearest USGS or IBWC gauging station site for 14 sites distributed across the Rio Grande drainage in Texas, USA

Catchment	Site	Latitude, longitude (decimal °)	USGS or IBWC site ID	Season	Q_b ($m^3 s^{-1}$)	Temp (°C)	DO (mg L^{-1})	Salinity (ppt)	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 ₃₄₀ (m^{-1})	NVSS (mg L^{-1})
Rio Grande	Candelaria (RG1)	30.138538 N, -104.688935 W	IBWC RG above Rio Conchos	Spr	3.93	13.67	8.86	2.03	190.00	525.91	89.36	44.05	6.38	4.51	44.20
				Sum	3.31	27.80	5.27	0.80	450.61	196.96	590.95	31.24	5.96	23.40	4883.38
				Fal	3.29	10.00	13.12	1.80	2774.27	651.91	361.45	580.03	6.69	1.44	18.62
	Redford (RG2)	29.432412 N, -104.18925 W	USGS 08374200	Spr	10.00	16.10	11.61	1.56	84.51	154.25	36.60	3.24	4.82	3.87	27.98
				Sum	11.66	26.90	5.11	0.60	564.32	221.48	225.26	23.12	4.91	2.35	273.12
				Fal	8.18	16.70	14.82	1.40	215.68	241.20	87.98	3.16	3.94	0.70	23.94
	Contrabando (RG3)	29.280036 N, -103.843138 W	USGS 08374550	Spr	12.61	18.00	12.28	1.30	76.34	176.47	41.38	5.23	4.40	2.02	42.00
				Sum	9.35	26.90	5.45	0.90	567.85	388.85	281.24	26.13	4.16	1.65	680.04
				Fal	6.08	15.10	6.94	1.40	367.03	302.09	64.57	5.74	4.09	0.77	11.94
	Quemado (RG4)	28.929538 N, -100.639422 W	USGS 08451800	Spr	27.54	23.50	11.64	0.40	305.58	138.57	35.97	2.04	3.20	0.78	12.21
				Sum	245.00	26.20	7.65	0.30	708.13	85.48	112.84	3.52	3.10	0.83	29.99
				Fal	144.78	23.20	9.35	0.30	395.21	66.63	162.84	7.01	3.43	0.73	27.28
	Eagle Pass (RG5)	28.60794 N, -100.443385 W	USGS 08458000	Spr	63.14	24.60	6.47	0.40	356.25	425.69	35.80	32.16	3.10	0.73	12.30
				Sum	274.67	26.70	7.15	0.30	685.86	115.97	11.98	13.04	3.04	0.81	24.54
				Fal	141.97	23.60	7.06	0.30	470.34	113.28	54.61	30.56	3.22	0.64	15.96
	Laredo (RG6)	27.452351 N, -99.493368 W	USGS 8458800	Spr	52.81	28.20	6.37	0.40	417.17	475.12	56.28	83.87	3.61	1.30	20.84
				Sum	355.11	29.30	6.64	0.30	751.47	104.52	25.62	12.31	2.98	0.79	66.15
				Fal	152.00	24.20	6.60	0.30	651.05	142.90	85.85	63.70	3.18	0.82	49.69
	San Ygnacio (RG7)	27.015014 N, -99.439058 W	USGS 8459000	Spr	52.81	30.30	5.75	0.40	574.58	224.37	43.74	97.66	3.54	0.97	17.29
				Sum	355.11	32.20	3.30	0.40	36.87	236.39	76.65	8.44	4.45	1.65	0.03
				Fal	152.00	25.20	4.85	0.30	362.73	204.46	35.17	36.03	3.71	1.08	14.38
	Fronton (RG8)	26.399097 N, -99.08498 W	USGS 8461300	Spr	366.67	27.30	5.90	0.40	23.05	80.38	19.00	1.71	4.64	0.94	5.68
				Sum	946.22	30.90	6.53	0.30	231.22	129.92	71.93	2.55	4.02	1.22	11.46
				Fal	616.00	26.20	6.71	0.30	163.86	86.73	90.57	2.04	4.02	0.86	5.39
	Hidalgo (RG9)	26.095166 N, -98.271589 W	IBWC RG below Anzalduas Dam	Spr	94.50	27.10	7.32	0.50	109.73	127.70	73.02	19.14	4.84	2.77	91.59
				Sum	800.67	30.40	7.99	0.60	282.47	233.60	38.16	3.83	4.81	1.61	31.00
				Fal	601.00	26.50	3.35	0.40	371.19	85.00	268.82	10.36	4.51	3.23	52.83

Table 1 continued

Catchment Site	Latitude, longitude (decimal °)	USGS or IBWC site ID	Season	Q_9 ($m^3 s^{-1}$)	Temp (°C)	DO ($mg L^{-1}$)	Salinity (ppt)	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})	NVSS ($mg L^{-1}$)
Pecos River (PR1)	31.305102 N, -102.877865 W	USGS 08437710	Spr	2.79	17.87	16.95	10.27	76.34	760.41	14.05	4.46	5.45	1.17	21.04
			Sum	8.13	27.00	8.11	5.40	80.30	283.24	63.93	3.85	7.18	1.42	13.18
			Fal	7.08	12.80	11.80	9.10	69.13	593.14	51.68	1.78	6.11	0.79	38.58
Iraan (PR2)	30.90576 N, -101.880512 W	USGS 08446500	Spr	7.42	15.27	7.49	12.40	654.12	373.46	2.49	1.63	1.51	0.50	6.79
			Sum	14.87	24.60	15.75	4.80	98.75	558.42	27.80	1.55	7.17	2.38	6.04
			Fal	8.20	12.80	11.20	9.70	124.39	678.56	71.47	2.12	6.14	0.93	22.28
Pandale (PR3)	30.190467 N, -101.550665 W	USGS 08447300	Spr	30.07	16.35	10.40	3.03	86.04	335.66	24.87	0.69	5.03	1.20	10.09
			Sum	142.87	26.70	9.72	1.50	993.28	174.16	9.16	0.00	3.02	0.92	14.26
			Fal	33.16	15.20	10.07	2.20	532.80	320.56	22.11	6.78	1.73	0.37	3.25
Oso Canyon (PR4)	30.024776 N, -101.528778 W	IBWC Pecos near Langtry	Spr	30.07	20.68	10.15	2.09	881.11	294.20	52.02	0.39	1.38	0.43	1.33
			Sum	142.87	27.80	10.73	1.10	853.19	264.58	5.13	1.34	3.55	1.03	3.07
			Fal	33.16	16.70	10.85	1.60	700.97	233.60	39.02	3.50	1.46	0.23	0.63
Devils River (DR1)	29.903963 N, -101.005704 W	IBWC Devils at Pafford	Spr	9.17	24.00	6.71	0.20	976.77	93.75	5.59	0.87	2.98	0.41	0.00
			Sum	14.46	31.20	7.98	0.20	755.48	76.82	53.35	0.68	2.35	0.61	0.00
			Fal	5.76	14.60	11.04	0.20	1361.64	56.05	3.75	5.57	2.80	0.34	0.00

Mean daily discharge for the nine-day interval prior to sampling (Q_9), physicochemical conditions, nutrients, DOC, water color (Abs₄₄₀), and NVSS concentration are provided for each site at each sampling event. *Spr* Spring, *Sum* Summer, and *Fal* Fall

All nutrient, DOC, Abs₄₄₀, NVSS values are means of duplicate measurements

Table 2 Biological, bacterial metabolism, and OC variables for the subset of 9 sites across the Rio Grande drainage in Texas, USA

Catchment	Site	Season	BA ($\times 10^6$ cells mL ⁻¹)	Chla ($\mu\text{g L}^{-1}$)	BR ($\mu\text{g C/L/h}$)	BP ($\mu\text{g C/L/h}$)	BGE	k (day ⁻¹)	OC _L (mg L ⁻¹)	< 1 μm C:N (molar)	< 1 μm C:P (molar)	< 1 μm N:P (molar)	
Rio Grande	Candelaria (RG1)	Spr	4.98	76.09	41.86	7.39	0.15	0.05	1.80	4.73	98.21	20.75	
		Sum	3.56	11.45	114.29	0.78	0.01	0.09	1.16	1.16	4.23	73.60	17.38
	Redford (RG2)	Fal	4.53	47.61	9.27	3.09	0.25	0.10	0.57	0.57	3.97	50.84	12.81
		Spr	1.68	38.43	16.56	2.57	0.13	0.07	4.00	4.00	3.96	61.48	15.52
	Quemado (RG4)	Sum	5.73	5.38	7.37	3.04	0.29	0.12	1.73	1.73	5.33	57.88	10.85
		Fal	3.99	59.73	10.83	3.03	0.22	0.11	0.40	0.40	4.87	71.93	14.78
	San Ygnacio (RG7)	Spr	2.43	6.40	16.88	1.29	0.07	0.10	0.92	0.92	4.21	99.50	23.61
		Sum	4.58	1.23	9.39	1.36	0.13	0.11	0.46	0.46	5.16	117.14	22.71
	Fronton (RG8)	Fal	2.53	3.06	3.90	1.26	0.24	0.10	0.60	0.60	3.23	73.64	22.81
		Spr	5.73	1.75	16.93	2.45	0.13	0.16	1.71	1.71	7.12	52.18	7.33
	Hidalgo (RG9)	Sum	2.50	19.06	4.25	3.89	0.48	0.12	1.20	1.20	4.38	127.94	29.23
		Fal	3.64	5.40	1.17	2.41	0.67	0.00	3.00	3.00	3.41	94.65	27.77
Pecos River	Grand Falls (PR1)	Spr	5.37	11.35	13.37	1.32	0.09	0.14	0.70	4.25	84.53	19.89	
		Sum	2.57	17.59	3.49	2.26	0.39	0.09	0.84	4.32	67.31	15.59	
Oso Canyon (PR4)	Dolan Falls (DR1)	Fal	6.33	16.27	2.57	1.26	0.33	0.03	1.04	3.71	77.61	20.94	
		Spr	3.86	7.44	46.40	1.93	0.04	0.60	1.07	5.15	68.29	13.26	
Deviils River	Dolan Falls (DR1)	Sum	6.91	25.22	0.81	3.05	0.79	0.11	0.98	4.72	86.47	18.33	
		Fal	4.71	22.13	9.64	2.74	0.22	0.14	0.69	0.69	4.24	69.09	16.28
Deviils River	Dolan Falls (DR1)	Spr	2.51	12.15	15.19	3.38	0.18	0.00	5.00	6.00	91.81	15.30	
		Sum	4.85	5.58	1.40	1.82	0.57	0.03	1.41	4.75	231.87	48.84	
Deviils River	Dolan Falls (DR1)	Fal	3.63	19.37	4.23	1.58	0.27	0.00	1.21	4.28	77.17	18.04	
		Spr	0.46	1.28	2.51	1.08	0.30	0.10	0.51	3.64	67.08	18.44	
Deviils River	Dolan Falls (DR1)	Sum	2.00	1.31	2.25	0.44	0.16	0.44	0.76	5.06	43.31	8.55	
		Fal	2.42	1.26	0.77	0.40	0.34	0.00	0.68	3.49	36.10	10.34	
Deviils River	Dolan Falls (DR1)	Spr	1.50	0.50	7.27	1.07	0.13	0.15	0.61	6.92	267.06	38.62	
		Sum	1.48	0.55	7.56	1.21	0.14	0.17	0.61	3.74	110.31	29.48	
Deviils River	Dolan Falls (DR1)	Fal	1.59	0.30	3.06	2.97	0.49	0.50	0.32	3.10	53.66	17.33	

BA bacterial abundance, *Chla* suspended phytoplankton biomass, *BR* bacterial respiration; *BP* bacterial production; *BGE* bacterial growth efficiency; *k* aerobic OC decomposition rate, *OC_L* = labile OC concentration, and < 1 μm C:N, C:P, and N:P = bacterial nutrient molar ratios. *Spr* Spring, *Sum* Summer, and *Fal* Fall sampling events. *BR*, *OC_L*, and *k* values are the mean of at least triplicate measurements, *BP* values are the mean of quadruplicate measurements, and all other values are the mean of duplicate measurements

discharge (Q_9) for the 9-day period prior to the sampling date using data from United States Geological Survey (USGS) or International Boundary Waters Commission (IBWC) gauging stations located nearest each sampling site (Table 1).

Laboratory analyses

Total P (TP), nitrate (NO_3^-), ammonium (NH_4^+), soluble reactive P (SRP), non-volatile suspended solids (NVSS), dissolved organic carbon (DOC), and suspended particulate nutrients (C, N and P) in the $< 1 \mu\text{m}$ size (bacteria-sized) fraction, and suspended phytoplankton biomass were estimated in duplicate for each site on each sampling date. The amount of variation among duplicate samples for a site for each parameter was very small. TP was measured as PO_4^{3-} 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 = $1 \mu\text{m}$). 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 by the phenate method (Wetzel and Likens 2000). DOC samples were filtered through a pre-ashed Whatman GF/F filter and analyzed on a Shimadzu TOC- V_{CSH} total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, Maryland, USA). DOC color on the same samples was assessed as absorbance at 440 nm and calculated and reported as absorbance coefficients ($g; \text{m}^{-1}$) from Cuthbert and del Giorgio (1992). Spectrophotometry was performed on a Varian Cary 50 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, California, USA).

NVSS was determined by filtering water onto pre-combusted and pre-weighed Pall A/E filters. Filters were dried at $50 \text{ }^\circ\text{C}$ for 48 h and were re-weighed to determine total suspended solids (TSS) and subsequently combusted at $500 \text{ }^\circ\text{C}$ for 4 h and re-weighed to determine NVSS. C, N, and P content of the bacterial size fraction ($< 1 \mu\text{m}$) of the seston was determined by filtering samples through pre-ashed Pall A/E filters, and filtrate was subsequently filtered onto pre-ashed Whatman GF/F filters and dried at $50 \text{ }^\circ\text{C}$ for

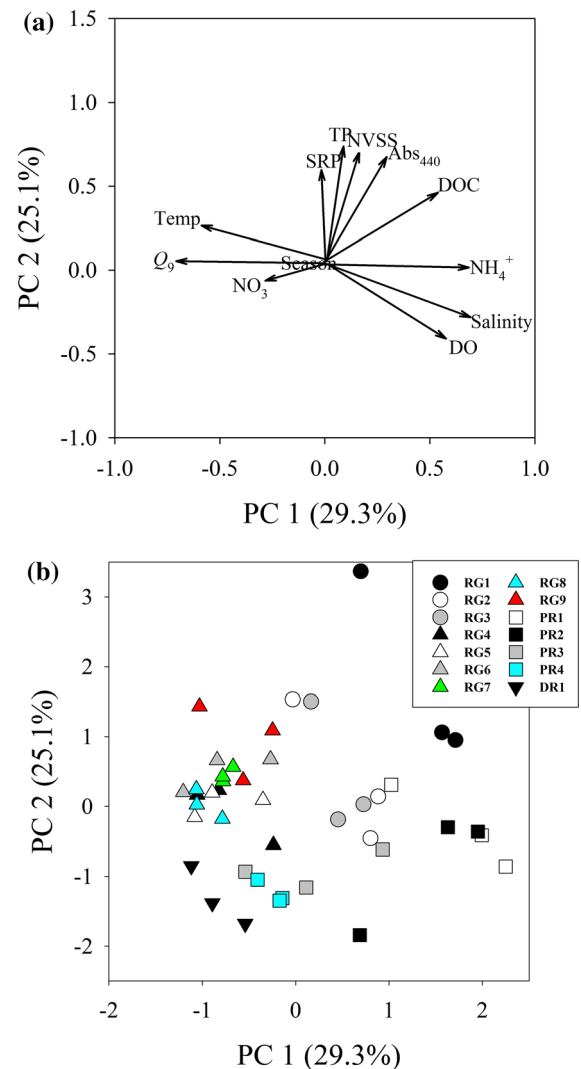


Fig. 2 Principle component analysis (PCA) of physicochemical and nutrient variables across $n = 14$ study sites over three sampling seasons in the Rio Grande, Pecos River and Devils River drainages. **a** Multivariate relationships among physiographic variables across all sites. **b** Ordination of sampling sites. Drainage designations (RG, PR, and DR) and site numbers correspond to those in Fig. 1, Tables 1 and 2

48 h. The $< 1 \mu\text{m}$ C and N concentrations were measured on a CE Elantech Flash EA 1112 CN analyzer (CE Elantech Inc., Lakewood, New Jersey, USA). For $< 1 \mu\text{m}$ particulate P samples, filtrate was filtered onto Whatman GF/F filters, digested with HCl at $100 \text{ }^\circ\text{C}$ and measured as PO_4^{3-} with the molybdenum blue method (Wetzel and Likens 2000). Suspended phytoplankton biomass was estimated using chlorophyll *a* (Chl_a) concentration. Water was filtered

onto Pall A/E filters and stored at $-20\text{ }^{\circ}\text{C}$ 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 abundance (BA) from the subset of sites was determined by preserving 60 mL of whole water clean glass bottles with 4% filtered ($< 0.2\text{ }\mu\text{m}$) formalin. Duplicate samples for each site on each sampling date were kept in the dark at $4\text{ }^{\circ}\text{C}$ until analysis. Samples (1–6 mL) were filtered onto black $0.2\text{-}\mu\text{m}$ Nucleopore membrane filters, stained with 4',6-diamidino-2-phenylindole. Immediately after staining, slides were mounted with CitifluorTM AF1 solution and examined with a Nikon Eclipse 80i microscope, fitted for epifluorescence microscopy with a mercury lamp (Nikon; X-CiteTM 120) and UV-2E/C filter (Nikon Instruments, Inc., Melville, New York, USA). Cells were counted at $1000\times$ magnification, and 20 fields of view (grid

area = $1.0 \times 10^{-4}\text{ mm}^2$) were counted for each duplicate sample.

At the subset of sites, BP was measured using the microcentrifuge ^3H -leucine method (Pace et al. 2004). On each sampling date, whole (unfiltered) water from the well-mixed 7.5-L carboy was allocated into four “live” and two “killed” 2-mL microcentrifuge tubes. ^3H -leucine ($\sim 60\text{ Ci/mmol}$ leucine) was added to all tubes, but the “killed” tubes received cold 50% trichloroacetic acid (TCA) prior to ^3H -leucine addition; each tube received $1.4\text{ }\mu\text{Ci}$ of ^3H -leucine (Smith and Azam 1992; Kirchman 1993). Tubes were incubated for 45–60 min 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. Supernatant was aspirated, cold 5% TCA was added, and tubes were re-centrifuged. Supernatant was removed, scintillation fluor was added, and ^3H activity was measured on a Beckman LS 6000IC scintillation

Table 3 Analysis of variance (ANOVA) results examining the effect of site group, season, and the site \times season interaction on biological and C quantity–quality response variables at the subset of nine sites across the Rio Grande drainage in Texas, USA

Response variable abbreviations are provided in Tables 1 and 2. Site degrees of freedom (*df*) for all response variables = 2.27; season *df* for all response variables = 2.27. Site \times season *df* for all response variables = 4.27. Values indicated in bold and an asterisk (*) indicate significance at the $\alpha < 0.05$

Response variable	Parameter	Site	Season	Site \times season
BA	<i>F</i>	7.42	0.653	0.398
	<i>p</i>	0.004*	0.532	0.807
Chla	<i>F</i>	24.06	0.439	1.59
	<i>p</i>	< 0.001*	0.651	0.221
BP	<i>F</i>	5.51	0.605	1.20
	<i>p</i>	0.014*	0.557	0.346
BR	<i>F</i>	3.24	3.39	0.575
	<i>p</i>	0.063	0.056	0.684
BGE	<i>F</i>	0.372	2.03	1.25
	<i>p</i>	0.694	0.161	0.325
DOC	<i>F</i>	23.79	0.679	0.137
	<i>p</i>	< 0.001*	0.520	0.967
OC _L	<i>F</i>	7.00	3.16	3.04
	<i>p</i>	0.006*	0.066	0.045*
<i>k</i>	<i>F</i>	0.679	1.812	0.514
	<i>p</i>	0.520	0.192	0.726
Abs ₄₄₀	<i>F</i>	2.55	1.54	1.20
	<i>p</i>	0.106	0.240	0.345
< 1 μm C:N	<i>F</i>	0.625	5.10	0.531
	<i>p</i>	0.546	0.018*	0.715
< 1 μm C:P	<i>F</i>	0.128	2.36	1.44
	<i>p</i>	0.881	0.123	0.262
< 1 μm N:P	<i>F</i>	0.062	0.305	1.19
	<i>p</i>	0.940	0.741	0.351

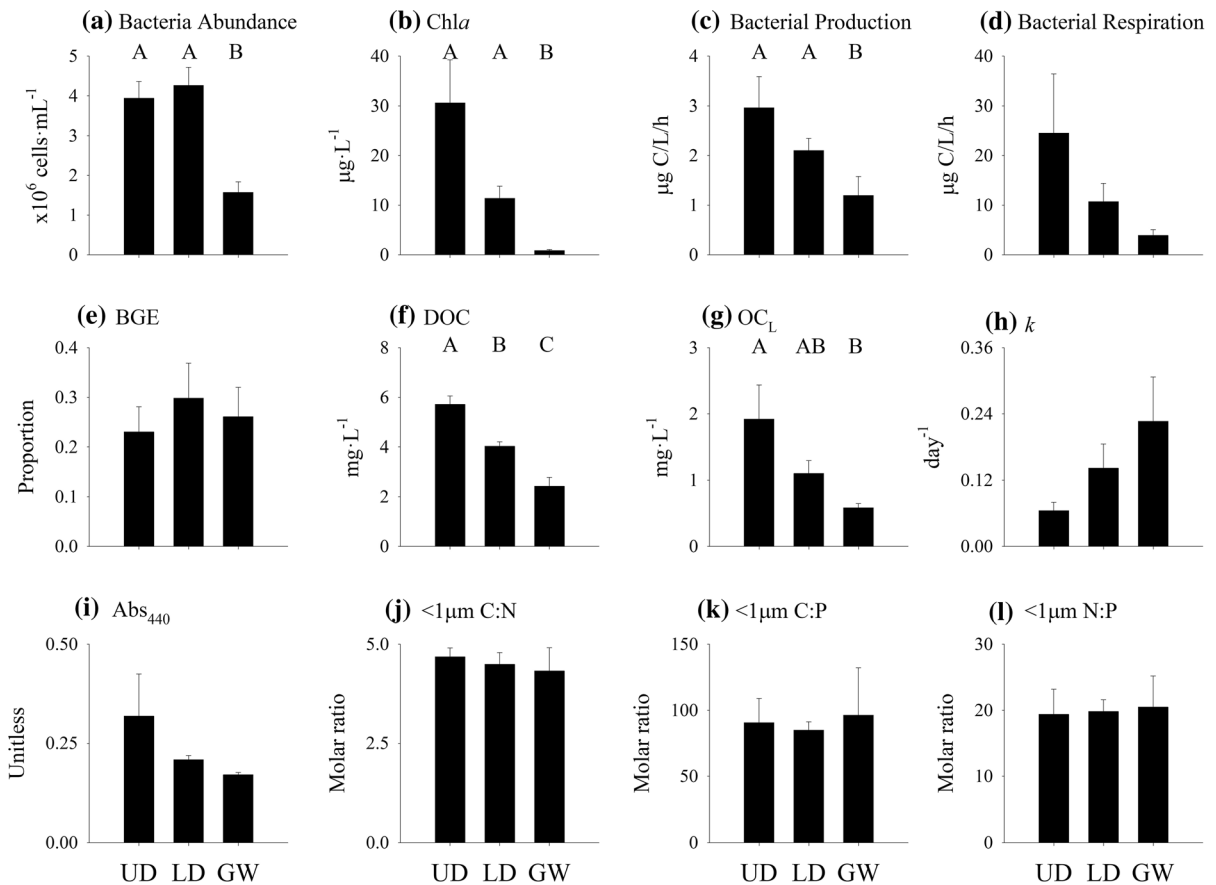


Fig. 3 Mean **a** bacterial abundance, **b** phytoplankton biomass (measured as Chl a), **c** rate of bacterial production, **d** rate of bacterial respiration, **e** BGE, **f** DOC concentration, **g** OC $_L$ concentration, **h** breakdown rate of OC $_L$ (k), **i** Abs $_{440}$, **j** $<1\mu\text{m C:N}$ (molar), **k** $<1\mu\text{m C:P}$, **l** $<1\mu\text{m N:P}$ at the three site groups as determined from principle component analysis. Site groups are composed of the subset of sites ($n = 9$) at which bacterial metabolism estimates were obtained and the

sites groupings were the upper drainage sites, lower drainage sites, and groundwater-dominated sites (indicated by UD, LD, and GW, respectively). Bars represent the mean of each variable for all sites in each site group across sampling dates, and errors represent ± 1 SE. Capital letter designations above site group bars indicate that there is a significant difference among site groups for a variable and capital letters indicate homogenous groups as indicated by post hoc Tukey tests

counter and bacterial production was expressed as $\mu\text{C L}^{-1} \text{h}^{-1}$. The same microcentrifuge tube type and brand were used throughout (Pace et al. 2004).

BR at the subset of sites was estimated via biological oxygen demand (BOD) (Roland et al. 1999; Williams and del Giorgio 2005). To estimate BR, we conducted 48-h BOD experiments; the 48-h incubation time was utilized because initial BOD incubations found that this incubation length was adequate to measure changes in DO in systems. Incubations were performed in clean and acid-washed 60-mL Whatman BOD bottles with stoppers. For each site on each sampling dates, five replicate bottles were filled with whole (unfiltered) water. Initial DO

concentrations were measured on Day 0 on duplicate bottles using a spectrophotometric Winkler method (Roland et al. 1999). Remaining replicate bottles were incubated in the dark at in situ site temperatures. After 48 h, bottles were removed and processed as above. Hourly O $_2$ consumption rate ($\text{mg O}_2 \text{L}^{-1} \text{h}^{-1}$) was calculated as the difference between initial and final DO, and O $_2$ consumption values were converted to C respired ($\mu\text{g C L}^{-1} \text{h}^{-1}$) using a respiratory quotient of 1 (del Giorgio et al. 2006). To be consistent with BP methods, we used whole water for BR estimates; use of whole water includes respiration by free-floating and particle attached bacteria, as well as larger planktonic organisms (del Giorgio et al. 2006; Vidal

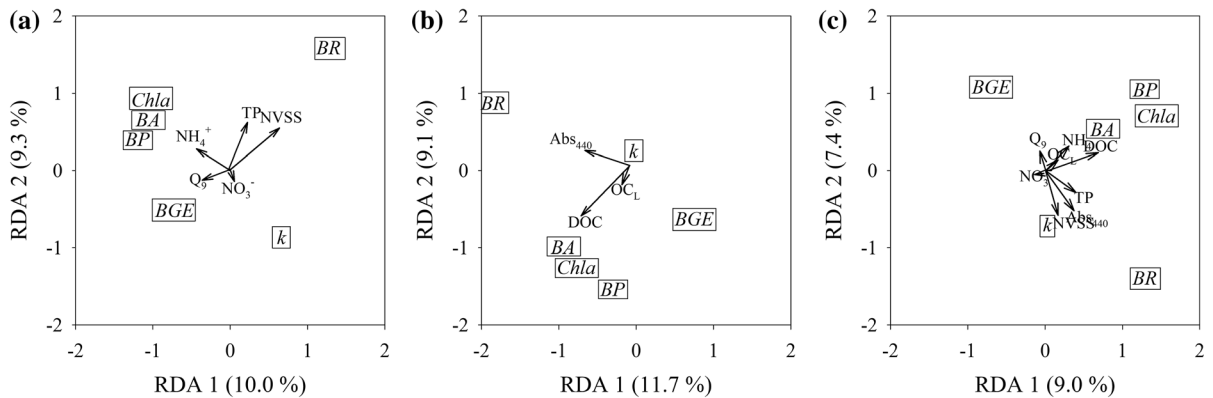


Fig. 4 Partial redundancy analysis (RDA) plots of relationships between predictor variable sets and biological and bacterial metabolism responses in the Rio Grande drainage. **a** Relationships between physicochemical and nutrient predictors and biological and bacterial metabolism response variables after accounting for the influence of DOC quantity–quality and bacterial stoichiometric predictors. **b** Relationships between DOC quantity–quality and bacterial stoichiometric predictors and response variables after accounting for the influence of physicochemical and nutrient predictors. **c** Relationships

between the global (complete) predictor variable sets (physicochemical and nutrient predictors, DOC quantity–quality and bacterial stoichiometric predictors) and response variables. Response variables are in italics and in boxes in all RDA plots. Bacterial (< 1 μm) C:N and N:P are not presented because they have little predictive power and were plotted near the origin. See Tables 1 and 2 for variable abbreviations. Percent of the variance in response variables explained by each RDA axis (RDA 1 and 2) is provided in parentheses in axis titles

et al. 2011). Abundance of larger planktonic organisms (e.g., zooplankton) is low in flowing river systems such as the Rio Grande (Basu and Pick 1996); thus, these organisms likely did not have substantial influence on respiration rates. Use of filtered (e.g., < 1 μm) water can lead to underestimates of BR (del Giorgio et al. 2006) because a substantial portion of the bacterial community is attached to particles in river systems (Ochs et al. 2010), and filtration removes particle-associated and some free-floating bacteria (del Giorgio and Pace 2008). Thus, BR measurements in this study are likely representative of the maximum potential BR rate of the microbial community (Becker et al. 2017). In addition, use of whole water BR estimates could lead to lower estimates of BGE than if we used filtered water for incubations. Measurements of short-term BR were coupled with the concurrently measured BP estimates in order to calculate BGE from del Giorgio and Cole (1998) as

$$BGE = BP / (BP + BR)$$

DOC lability at the subset of sites was evaluated through use of long-term BOD incubations on water that was first filtered through Pall A/E filters (< 1 μm); water was filtered in order to remove the effect of decomposition of particulate matter and to target free-

floating bacteria (Ostapenia et al. 2009; Sullivan et al. 2010). Concentration of labile DOC (OC_L) was estimated by interpreting plateaus in DO consumption over long-term BOD incubation time interval (Coffin et al. 1993). We used a first-order decomposition kinetics model:

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

where BOD_t is the biological oxygen demand at time *t*, BOD_{ult} is the total BOD possible for the bacterio-plankton community (asymptotic at infinity, ∞), and *k* is the reaction constant of aerobic decomposition of OC (Ostapenia et al. 2009). The breakdown rate of OC_L (*k*) can be estimated, and using this estimated *k*, BOD_{ult} can be inferred, providing the concentration of labile DOC (Ostapenia et al. 2009; Sullivan et al. 2010). We conducted 20-day incubations that were held in the dark in pre-cleaned and acid-washed 120-mL Whatman glass BOD bottles with glass stoppers at in situ site temperatures. DO concentrations were measured on days 0, 2, 4, 8, 16, and 20 from triplicate bottles with the modified Winkler method. Using Eq. 1 from Ostapenia et al. (2009), we calculated the breakdown rate of labile C (*k*), and using estimated *k*, BOD_{ult} is inferred, providing an indication of the size of the labile DOC pool. BOD kinetics and OC_L concentration were calculated with

Microsoft Excel Solver Tool, which employs generalized reduced gradient 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 Eq. 1 (Coffin et al. 1993; Ostapenia et al. 2009). Estimates of OC_L were obtained by multiplying the 20-day O_2 consumption by 0.30 (Ostapenia et al. 2009).

Data analysis

To assess spatiotemporal patterns in physicochemical characteristics across all sites in the drainage, we used principal component analysis (PCA). To avoid inclusion of too many variables in a single PCA (McCune et al. 2002), we performed data reduction procedures. Specific conductivity and salinity were highly correlated (Pearson $r = 0.992$, $p < 0.001$); thus, we only used salinity. We also excluded pH because it exhibited little variation across sites. Remaining physicochemical and nutrient variables (i.e., Q_9 , water temperature, DO, NO_3^- , NH_4^+ , SRP, TP, DOC, NVSS, salinity, Abs_{440} , and season; Table 1) were \log_{10} or $\log_{10}(x + 1)$ to meet assumptions of normality and z-score transformed prior to analysis. It was not possible to determine Q_9 for two sites on one sampling date (i.e., PR3 and PR4 sites during Summer sampling) because gauging stations were inoperable; thus, mean Q_9 across all sites and seasons was substituted (McCune et al. 2002).

Using PCA, we identified groups of sites that exhibited similar physicochemical and nutrient conditions. We examined how bacterial C metabolism, bacterial nutrient stoichiometry, BA, and *Chla* varied with among these site PCA-defined site groups with multivariate analysis of variance (MANOVA) on the subset of nine sites. In the MANOVA, site group and sampling season (Spring, Summer, and Fall) were used as fixed independent factors and BA, *Chla*, BR, BP, BGE, k , OC_L , Abs_{440} , $< 1 \mu\text{m C:N}$, $< 1 \mu\text{m C:P}$ and $< 1 \mu\text{m N:P}$ were used as dependent variables in the analysis. If a significant overall effect of a dependent factor was detected, we then performed individual ANOVAs with post hoc Tukey HSD tests. Data were \log_{10} -transformed before analysis to meet

assumptions of normality and homoskedasticity as needed, and we utilized Type III sum of squares because of unequal n among site groups. Significance was inferred at $\alpha \leq 0.05$, and analyses were performed in SPSS version 24.

To test the hypothesis that C quality–quantity would exert a greater influence than physicochemical conditions across the study drainage, we utilized redundancy analysis (RDA). RDA allows for examination of biological responses to environmental gradients and can be used to assess the relative importance of groups of predictor variables on response variables (e.g., proportion of variance explained; Legendre and Legendre 2012). RDAs were performed using the subset of nine sites where biological and bacterial C metabolic responses were measured. Specifically, we were interested in determining whether landscape-level variation in (1) physicochemical and nutrient conditions or (2) C quality–quantity and bacterial nutrient stoichiometry was more important in determining patterns of bacterial C metabolism across the drainage. Thus, we examined the relationship between biological and metabolic responses (BR, BP, BGE, *Chla*, BCD, and k) and variables we classified as either physicochemical (i.e., Q_9 , water temperature, DO, NO_3^- , NH_4^+ , SRP, TP, NVSS, and salinity) or (2) C quality–quantity and bacterial stoichiometry variables (i.e., DOC, OC_L , Abs_{440} , and $< 1 \mu\text{m C:N}$, $< 1 \mu\text{m C:P}$ and $< 1 \mu\text{m N:P}$). Season (Spring, Summer, and Fall) was initially included as a predictor in RDAs but was not significant and was therefore excluded. Initially, there were a large number of predictor variables relative to the number of samples, so we performed data reduction on predictor variables to avoid excessive variable-to-sample ratios (McCune et al. 2002). We used a Pearson correlation matrix among predictor variables in each group and excluded variables that were highly correlated ($|r| = 0.70$). After variable reduction, the physicochemical predictor data set contained NO_3^- , NH_4^+ , TP, NVSS, and Q_9 , and the C quality–quantity and bacterial stoichiometry data set contained DOC, OC_L , Abs_{440} , $< 1 \mu\text{m C:N}$, and $< 1 \mu\text{m N:P}$.

Using remaining predictors, we conducted partial RDAs, where the analysis is performed on one set of predictor variables while controlling for the effect of the other. We then performed a global analysis (both predictor sets combined) and used variance partitioning to evaluate the combined and pure effects of the

two predictor sets (Peres-Neto et al. 2006; Legendre and Legendre 2012). Variance partitioning provides the amount of variation explained by each set of predictors (physiochemical/nutrient vs. C quality–quantity/bacterial stoichiometry), as well as the amount of variation shared between the two groups of predictors. Permutation tests ($n = 999$, $\alpha = 0.05$) were used to assess significance of partial and global effects models (Legendre and Legendre 2012). For all RDA models, we present the first two axes corrected by the R_{adj}^2 (Peres-Neto et al. 2006; Legendre and Legendre 2012). All multivariate analyses were performed using R 3.3.2 (R Development Core Team 2013) using the R statistical packages “vegan,” “car,” and “psych” (Oksanen et al. 2012; Revelle 2019; Fox et al. 2016).

Results

Spatial variation in physicochemical gradients

Physicochemical conditions across the drainage exhibited substantial spatial variation (Table 1; Fig. 2a). The first two principle components (PC 1 and PC 2) explained 54.4% of the total variation. PC 1 (29.3% of variation explained) generally described an upstream–downstream gradient in which the more upstream sites in the drainage (sites RG1 through RG3, PR1, and PR2; Fig. 2b) exhibited higher salinities, DO and NH_4^+ concentrations, while the more downstream sites (RG4 through RG9) were characterized by higher mean discharge and warmer water temperatures. PC 2 (25.1% of variation explained) generally described a gradient of sites dependent on surface flows (all RG sites and PR1, PR2) to sites dominated by groundwater contributions (PR3, PR4, and DR). Sites influenced by surface water were characterized by higher NVSS, TP, DOC, and SRP concentrations and greater Abs_{440} values, whereas the groundwater-dominated sites were characterized by lower P and NVSS and higher water clarity and NO_3^- concentrations. Groundwater-dominated sites also exhibited limited seasonal variation in conditions as indicated by relatively tight clustering of measurements from a site in the ordination. In contrast, the furthest upstream site in the Rio Grande (RG1) showed the greatest seasonal variability, exhibiting

extremely high NVSS, TP, and DOC concentrations during Summer (Table 1; Fig. 2b). However, season had minimal overall influence on the data set (Fig. 2a). This site and this sampling date strongly influence PC 2; however, when this point was removed prior to analysis, the influence of the physicochemical variables along PC 1 and 2 did not change and the spatial distribution of sites in the ordination does not change substantially.

Overall, ordination of sites revealed a segregation of sites into three general groups based upon physicochemical and nutrient conditions. The first group was composed of sites in the upper portion of the drainage (hereafter called the UD group), which included the upper Rio Grande sites (RG1 through 3) as well as the two upper Pecos sites (PR1 and 2). A second group was made up of sites from the mainstem of the Rio Grande in the lower portion of the drainage (hereafter, LD group) below and between Amistad International Reservoir and Falcón International Reservoir (RG4 and RG9). The last group was composed of groundwater-influenced sites (hereafter, GR group) and included the two lower Pecos sites (PR3 and 4) and the Devils River (DR1). These physicochemically defined site groups were used to assess spatial patterns in biological and metabolic responses across the drainage.

Spatiotemporal variation in biological and bacterial C metabolism responses

BA, Chla, bacterial C metabolism and C processing responses varied among site groups (UD, LD, and GW; Pillai's Trace: $F_{18, 22} = 2.18$; $p = 0.049$) and with sampling season (Pillai's Trace: $F_{18, 22} = 4.37$; $p = 0.001$), and the interaction effect between these factors was significant (Pillai's Trace: $F_{44, 44} = 1.74$; $p = 0.035$). When the effect of site groups was assessed on individual response variables, BA, Chla, DOC concentration, BP, and OC_L concentration differed among site groups (Table 3), with GW sites exhibiting lower BA (Fig. 3a), Chla (Fig. 3b), and BP rates (Fig. 3c) than the UD and LD site groups. DOC concentration differed among the site groups (Fig. 3f), with URG sites having the highest and GW sites having the lowest concentrations. OC_L concentration was also lower in the GW group than the UD group (Fig. 3g). In contrast, seasonal effects on BA, Chla, and bacterial C metabolism were substantially more

limited. Bacterial C:N varied among seasons, with the C:N in the Spring (mean across sites = 5:1) being greater than bacterial C:N in the Fall (mean across sites = 4:1) (Table 3). In addition, OC_L concentration was the only variable to exhibit a site group \times season interaction (Table 3); OC_L concentrations in the URG and LRG groups were substantially higher than those in the GW group in the Spring, but OC_L similar among groups by Summer. Note that these season and interaction effect results are not presented in a figure due to the limited nature of these effects.

Drivers of biological and bacterial C metabolism responses

The RDA model investigating solely the effect of physicochemical and inorganic nutrient gradients on biological and bacterial metabolic responses was significant ($p = 0.006$) and explained 22.3% of the variation in the data (19.3% explained by the first two axes; Fig. 4a). NH_4^+ ($p = 0.016$), TP ($p = 0.024$), and Q_9 ($p = 0.038$) were significant individual predictors in the model. RDA 1 described a gradient of sites with higher NH_4^+ and Q_9 to sites with lower discharge and higher NVSS and TP. RDA 2 largely described a gradient of groundwater-dominated sites (higher NO_3^- , but lower TP and NVSS) to more turbid, nutrient-rich sites. BP rates, BGE, BA, and Chl a were positively correlated with higher Q_9 and NH_4^+ concentrations, while BR rates increased with NVSS. In addition, BR and BGE were negatively correlated with each other indicating that variation in BR was largely driving variation in BGE (Pearson r between $\log_{10}BR$ and $\log_{10}BGE = -0.842$); BP rate responses were orthogonal not related to BR and BGE (Pearson r between $\log_{10}BP$ and $\log_{10}BGE$ and $\log_{10}BGE = 0.295$ and 0.229 , respectively).

The RDA model examining biological and bacterial C metabolic responses to C quality–quantity predictors was significant ($p = 0.002$), explaining 25.6% of the variation in the data (first two axes explaining 20.8% of the variation; Fig. 4b). Abs_{440} ($p = 0.002$) and DOC concentration ($p = 0.013$) were the significant predictors in the model. In this model, BA, Chl a , and bacterial production were correlated with DOC and OC_L , whereas BR was correlated with Abs_{440} . Again, BR and BGE were negatively correlated with each other (with BP responses being orthogonal),

indicating that variation in BGE was being primarily driven by changes in BR.

The global RDA model which included both physicochemical/inorganic nutrient and C quality–quantity and bacterial stoichiometry predictors was significant ($p = 0.001$) and explained a substantial portion (39.5%) of the variation in biological and bacterial C metabolic responses across the Rio Grande drainage, with the first two axes explaining 16% of the variation (Fig. 4c). In the global model, NH_4^+ ($p = 0.008$), TP ($p = 0.004$), Q_9 ($p = 0.012$), NVSS ($p = 0.026$), and Abs_{440} ($p = 0.007$) were significant predictors. There was a substantial covariation among physicochemical and C quantity–quality predictors across the drainage. In the global model, BP rates, BA, and Chl a were greater at sites with higher NH_4^+ and DOC concentrations, whereas BR rates increased with NVSS, TP, and Abs_{440} . Variance partitioning indicated that C quality–quantity predictors accounted for slightly more (17.2%) of the variation than physicochemical variables (13.9%). Covariation among variables in the predictor variable data sets is portrayed by the amount of shared variation between the two groups of predictor variables and 8.0% shared variance was explained.

Discussion

The Rio Grande basin exhibited substantial spatial variation in physicochemical characteristics, divided into two main gradients across the drainage. The primary physicochemical gradient was an upstream-to-downstream gradient wherein the upstream sites in the more arid portion of the drainage had higher salinity and nutrient concentrations, and downstream sites had lower salinity and higher discharge and water temperatures. In many United States Gulf Coast drainages, nutrients and turbidity generally increase in an upstream-to-downstream direction with river order and river gradient changes (e.g., Becker et al. 2014, 2017), but the presence of two large reservoirs in the Rio Grande drainage appeared to strongly modify upstream–downstream physicochemical gradients. Impoundments typically lead to increased water clarity, changes in the dominant nutrient forms, and alteration of OM sources supporting food webs below dams (Wetzel 2001; Hoeninghaus et al. 2007; Proia et al. 2016). LD sites in the Rio Grande drainage were

located between and below Amistad and Falcón International Reservoirs and nutrient and suspended solid concentrations were substantially lower at LD sites, indicating that these impoundments serve as a primary driver of physicochemical and water quality differences observed along the upstream–downstream gradient in the drainage. In addition, the effect of impoundments is apparent when examining variation in physicochemical conditions within some of the individual site groupings in the drainage. Within the UG group, the Río Conchos from Mexico discharges into the Rio Grande below site RG1 and accounts for up to 40% of the flow below this site (Douglas 2009). The Río Conchos originates in the Sierra Madre Occidental and the drainage contains seven reservoirs; below the confluence of the Río Conchos and the Rio Grande (between sites RG1 and RG2), NVSS concentrations are reduced in the Spring and Summer (mean reduction $\sim 65\%$). However, despite inflow of the Río Conchos, NVSS and TP concentrations at the RG2 site are still elevated when compared to LD sites (Table 1). It is also noteworthy that upstream sections of the Pecos River and Rio Grande above the furthest upstream study sites also contain impoundments (i.e., Red Bluff Reservoir at the Texas–New Mexico border on the Pecos River and Elephant Butte and Caballo Reservoirs in New Mexico on the Rio Grande). However, these reservoirs are substantially smaller than Amistad and Falcón (Caballo = 4500 ha, Red Bluff = 4530 ha, Elephant Butte = 15,000 ha, Amistad = 26,000 ha, Falcon = 33,000 ha), are relatively distant from sampling sites, and thus likely had marginal influence on patterns observed in this study.

The second physicochemical gradient in the drainage was between groundwater-influenced and the surface water-dominated sites, where groundwater-influenced sites exhibited lower nutrient concentrations (except NO_3^-), DOC, and suspended matter. The two sampling sites in the downstream portion of the Pecos River (PR3 and PR4) and the Devils River site are highly influenced by groundwater contributions from the Edwards–Trinity aquifer, which is characterized by high water clarity, greater NO_3^- concentrations, and low P concentrations (Mace et al. 2004). The Devils River is almost entirely supported by groundwater contributions during base flow conditions and exhibits little temporal variation in physicochemical conditions (i.e., consistent high water clarity and low nutrients; see Fig. 2). However,

substantial groundwater contributions to rivers whose upstream sections are dominated by surface water can augment and dilute flows, leading to upstream–downstream declines in nutrients and turbidity. For example, groundwater contributions along the Pecos River lead to a notable reduction in nutrients and DOC between PR1 and PR4 (Table 1). Thus, groundwater contributions play an important role in influencing spatial variation in physicochemical characteristics within the Rio Grande drainage through dilution of more turbid and nutrient-rich surface waters and by providing more stable base flows. The Rio Grande drainage is in an arid and semiarid landscape with limited surface water, and groundwater overexploitation and extraction have the potential to destabilize base flows and decrease water quality in portions of the drainage.

Consistent with our first hypothesis, we found that spatial variation in hydrology and physicochemical conditions in the Rio Grande drainage led to differences in biological and bacterial C metabolism responses. In general, Chl*a*, BA, bacterial C metabolism varied with both the upstream–downstream and surface water–groundwater gradients in the drainage. Sites dominated by groundwater contributions had lower Chl*a*, BA, and BP rates. This spatial variation is likely in response to the nutrient and DOC gradients in the drainage; increased nutrients (in this case P and NH_4^+) and DOC supply can increase plankton production and biomass (Murrell 2003; Holmes et al. 2008; Guenet et al. 2010).

Labile OC concentration differed among site groups in the Rio Grande drainage, with the UD and GW sites having the highest and lowest concentrations, respectively. (LD sites were intermediate and did not differ from UD and GW sites.) DOC concentration varied among all site groups; however, the fraction of the DOC pool composed of OC_L (OC_L/DOC) did not differ across site groups. Across study sites, OC_L comprised 8–83% of the bulk DOC pool (mean = 31%) and this is in general agreement with previous studies across aquatic habitats (i.e., range 5–30% of the bulk DOC pool; Sondergaard and Middelboe 1995; Amon and Benner 1996). In the present study, the mean percent of bulk DOC composed of OC_L is at the higher end of this range, but most observations fall within the reported range of values. The proportion of the DOC pool composed of relatively labile C sources is equivocal and can depend

upon a variety of factors; Sondergaard and Middelboe (1995) state that riverine systems contain relatively high amounts of labile DOC, whereas others contend that riverine DOC contains a smaller fraction of labile C than lacustrine systems (del Giorgio and Davis 2003). Mean concentration of DOC in the Rio Grande drainage ($4.23 \pm 0.28 \text{ mg L}^{-1}$) was approximately half that of the lakes and rivers examined by del Giorgio and Davis (2003), but OC_L/DOC values are similar to OC_L/DOC values for lakes. Thus, in comparison with other studies, DOC concentrations in the Rio Grande are relatively low, but the fraction of the bulk DOC pool composed of labile DOC in the Rio Grande drainage is high. In addition, the concentration of OC_L was not related to DOC concentration across the Rio Grande drainage (Pearson $r = 0.33$, $p = 0.094$), further corroborating cross-system analyses that conclude that bulk DOC concentration does not reliably predict the labile fraction of the pool (del Giorgio and Davis 2003).

Environmental drivers of bacterial C metabolism responses

Bacterial C metabolism responded to environmental gradients across sites in the Rio Grande drainage and responses were correlated with both physicochemical/inorganic nutrient and C quantity–quality variables. The percent of variation in biological and metabolic responses across the drainage was approximately equivalent when physicochemical and C quality–quantity predictors were compared (13.9% vs. 17.2%, respectively). This finding did not support our hypothesis that variation in C quality–quantity would be the primary driver of bacterial C metabolism across the drainage. In addition, there was a substantial amount of covariation among many of the individual physicochemical and C quantity–quality predictors, leading to $\sim 8\%$ of the variation in biological and metabolic responses being shared between the two predictor sets. For example, DOC concentration was positively correlated with NH_4^+ (Pearson $r = 0.54$, $p = 0.004$) and TP concentration (Pearson $r = 0.40$, $p = 0.039$). In addition, Abs_{440} was positively correlated with TP ($r = 0.80$, $p < 0.001$) and NVSS concentration ($r = 0.80$, $p < 0.001$). Thus, it appeared that physicochemical/inorganic nutrient and C quality–quantity conditions were responding similarly to the overall gradients across the Rio Grande drainage.

In the current study, BA and BP were positively related to NH_4^+ and DOC concentrations across the Rio Grande drainage. BP responds to a variety of biotic and abiotic factors, making it difficult to predict the relative importance of individual drivers influencing rates (del Giorgio and Cole 1998; Bergstrom and Jansson 2000). Numerous studies have found that BP can be limited by inorganic nutrients or DOC, depending on in situ conditions (e.g., Makino et al. 2003; Lennon and Cottingham 2008). BP responses to increasing bulk DOC concentration are equivocal; some studies find no relationship between BP and DOC concentrations (e.g., del Giorgio and Davis 2003; Judd et al. 2006; Becker et al. 2017), while others found that BP increases with DOC supply (e.g., Lennon and Pfaff 2005; Lennon and Cottingham 2008). Bulk DOC pools in natural systems are complex and composed of C sources of different quality and decomposition rates (Sondergaard and Middelboe 1995) and BP response to DOC is also dependent upon the timing of DOC supply (e.g., pulsed vs. continuous; Findlay 2002; Lennon and Cottingham 2008). In addition, supply of inorganic nutrients, specifically P is often a determinant of bacterial growth (e.g., Makino et al. 2003; Lennon and Pfaff 2005). In the present study, BA and BP were not related to TP or SRP concentrations, indicating that external P supply likely did not strongly affect spatial patterns of BA and BP across the drainage. However, we also found that BP concomitantly increased with *Chla* across the Rio Grande drainage (Pearson $r = 0.73$, $p < 0.001$), suggesting that either (1) bacteria and phytoplankton populations were simultaneously responding to the same physicochemical/inorganic nutrient gradients, or (2) that suspended phytoplankton was positively associated with increased inorganic nutrient supply (i.e., NH_4^+) and bacteria were responding to changes in phytoplankton biomass through utilization of labile C algal cell exudates (e.g., Wolter 1982). BP rates were not related to OC_L concentrations across the Rio Grande drainage, indicating that specific mechanisms behind relationships between BP, DOC, inorganic nutrients, and phytoplankton remain to be elucidated.

In contrast to BP, BR rates responded to environmental drivers linked to specific in situ conditions across the Rio Grande drainage. BR rates can be affected by a diversity of factors, including temperature (del Giorgio and Cole 1998), DOC quantity and

quality (del Giorgio and Cole 1998; Lennon and Pfaff 2005; Kritzberg et al. 2005), bacterial biomass (Hamdan and Jonas 2006), and community composition (Judd et al. 2006). In the present study, higher BR rates were correlated with Abs₄₄₀ (water color), TP concentration, and NVSS concentration. Water color is often used as an indicator of DOC allochthony, and highly colored DOC is considered refractory (Kritzberg et al. 2006; Jones et al. 2009). Respiration rates of bacterial communities increase with concentrations of allochthonous and refractory DOC, regardless of the availability of OC_L in the overall DOC pool (del Giorgio and Davis 2003; Berggren et al. 2007). In addition, BR rates across the Rio Grande drainage were positively correlated with NVSS concentrations, suggesting that BR was likely responding to localized terrestrial runoff contributions, and therefore, allochthonous C sources and not necessarily to the bulk DOC pool.

Differential responses of BP and BR to OC supply and lability serve as one of the mechanisms for widespread occurrence of net heterotrophy in aquatic systems (Cole and Caraco 2001). Decoupling of BR and BP 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 C metabolism in areas where aquatic and terrestrial ecosystems interface (del Giorgio et al. 2006). In the Rio Grande drainage, BP and BR responded to different environmental factors: BP increased with increased concentrations of labile and total DOC and NH₄⁺ (which were also correlated with Chl_a), while BR increased with water color and NVSS. These findings suggest that different aspects of bacterial C metabolism (BR vs. BP) in the Rio Grande drainage are responding to different mechanisms of nutrient and OC supply that exist across the drainage: BP is likely primarily responding to autochthonous C supply and inorganic nutrients, while BR is responding to localized supplies of allochthonous particulate matter and DOC.

In the Rio Grande drainage, BGE did not differ among site groups and there was no obvious relationship with hydrological and physicochemical gradients. The range of observed BGE values in the Rio Grande drainage (range 0.01–0.79) generally falls within those of riverine BGE values reported by del Giorgio and Cole (1998) (range 0.03–0.46), but BGE values across

the drainage were more variable than those reported by del Giorgio and Cole (1998). In addition, relationships between BP, BR and BGE were different from those observed in several other studies. In other systems, BGE increases with BP (Maranger et al. 2005; Amado et al. 2013), but in the present study BGE was not related to BP (Pearson $r = 0.05$, $p = 0.785$), but instead declined with increasing BR (Pearson $r = -0.50$, $p = 0.008$). Calculation of BGE requires estimates of both BR and BP, but BR is often cited as being less responsive than BP to changes in environmental conditions (del Giorgio and Cole 1998). This was not the case in the Rio Grande: BR rates exhibited substantially more variation (range 0.77–114.29 $\mu\text{g C L}^{-1} \text{h}^{-1}$) than BP rates across the drainage (range 0.40–7.39 $\mu\text{g C L}^{-1} \text{h}^{-1}$). Other studies have similarly found that BGE varied directly with BR (and not BP) and suggested that variation in BGE across a subtropical riverine network was a more related to temperature responses of BR rather than DOC or inorganic nutrients (Becker et al. 2017). However, in the Rio Grande drainage neither BGE (Pearson $r = 0.11$, $p = 0.595$) nor BR (Pearson $r = 0.05$, $p = 0.819$) were related to water temperature. Thus, in the Rio Grande drainage BGE was driven by conditions that affected BR rates, namely DOC color and allochthonous sediment loading and BR was more sensitive than BP to changes in landscape position and environmental conditions.

Conclusions

Arid river systems are thought to be largely dependent on autochthonous C (Bunn et al. 2003), but the results of this study show that allochthonously derived C also influenced spatial variation in bacterial C metabolism across the Rio Grande drainage. Although bacteria are the greatest single contributor to ecosystem respiration in many aquatic systems, widely used riverine conceptual models do not incorporate bacterial DOC processing into frameworks (e.g., Vannote et al. 1980; Junk et al. 1989). The present study joins the growing body of work which demonstrates the importance of aquatic bacteria communities in the sequestration and transformation of both allochthonous and autochthonous C and the crucial role that bacterial C metabolism plays in ecosystem function at the landscape scale (e.g., Becker et al. 2017). Finally, this study suggests

that anthropogenic alteration of arid river hydrology (e.g., reservoir construction) and inputs of groundwater affect biogeochemical processes at the riverscape scale, which has implications for C and nutrient sequestration, transformation, and transport within the drainage, and ultimately delivery to the ocean.

Acknowledgements This study was funded by a Grant to the United States Department of Agriculture Sustainable Agricultural Water Conservation in the Rio Grande program. We thank The Nature Conservancy for access and use of Dolan Falls Preserve and the Oso Canyon site, and The Meadows Institute for Water and the Environment at Texas State University and Sul Ross University for their support. The authors would like to thank Chekka Lash, Pete Diaz, Alisa Abuzeineh, Alex Smith, and Megan Bean for field assistance and Dittmar Hahn for editorial help.

Author's contributions KJR and WHN designed the study. KJR, GT, JCB, and WHN conducted fieldwork and collected the data. KJR, JCB, and GT performed laboratory analyses. WHN, KJR, and JCB analyzed the data. KJR drafted the manuscript with assistance by JCB, GT, and WHN.

References

- Amado AM, Meirelles-Pereira F, Vidal LD, Sarmento H, Suhett AL, Farjalla VF, Cotner JB, Roland F (2013) Tropical freshwater ecosystems have lower bacterial growth efficiency than temperate ones. *Front Microbiol*. <https://doi.org/10.3389/fmicb.2013.00167>
- Amon RM, Benner R (1996) Bacterial utilization of different size classes of dissolved organic matter. *Limnol Oceanogr* 41:41–51
- Aufdenkampe AK, Mayorga E, Raymond PA, Melack JM, Doney SC, Alin SR, Aalto RE, Yoo K (2011) Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Front Ecol Environ* 201:53–60
- Basu BK, Pick FR (1996) Factors regulating phytoplankton and zooplankton biomass in temperate rivers. *Limnol Oceanogr* 41:1572–1577
- Battin TJ, Luysaert S, Kaplan LA, Aufdenkampe AK, Richter A, Tranvik LJ (2009) The boundless carbon cycle. *Nat Geosci* 2:598–600
- Becker JC, Rodibaugh KJ, Labay BJ, Bonner TH, Zhang Y, Nowlin WH (2014) Physiographic gradients determine nutrient concentrations more than land use in a Gulf Slope (USA) river system. *Freshw Sci* 33:731–744
- Becker JC, Rodibaugh KJ, Hahn D, Nowlin WH (2017) Bacterial community composition and carbon metabolism in a subtropical riverscape. *Hydrobiologia* 792:209–226
- Berggren N, Laudon H, Jansson M (2007) Landscape regulation of bacterial growth efficiency in boreal freshwaters. *Glob Biogeochem Cycles* 21:1–9
- Bergstrom AK, Jansson M (2000) Bacterioplankton production in humic Lake Ortrasket in relation to input of bacterial cells and input of allochthonous organic carbon. *Microb Ecol* 39:101–115
- Bunn SE, Davies PM, Winning M (2003) Sources of organic carbon supporting the food web of an arid flood-plain river. *Freshw Biol* 48:619–635
- Coffin RB, Connolly JP, Harris PS (1993) Availability of dissolved organic carbon to bacterioplankton examined by oxygen utilization. *Mar Ecol Prog Ser* 101:9–22
- Cole JJ, Caraco NF (2001) Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. *Mar Freshw Res* 52:101–110
- 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. *Environ Microbiol* 11:1704–1716
- Crumpton WG, Isenhardt TM, Mitchell PD (1992) Nitrate and organic N analyses with 2nd-derivative spectroscopy. *Limnol Oceanogr* 37:907–913
- Cuthbert ID, del Giorgio PA (1992) Toward a standard method of measuring color in freshwater. *Limnol Oceanogr* 37:1319–1326
- del Giorgio PA, Cole JJ (1998) Bacterial growth efficiency in aquatic systems. *Annual Rev Ecol Systematics* 29:503–541
- del Giorgio PA, Davis J (2003) Patterns in dissolved organic matter lability and consumption across aquatic ecosystems. In: Findlay SEG, Sinsabaugh RL (eds) *Aquatic ecosystems: interactivity of dissolved organic matter*. Academic Press, San Diego, pp 399–421
- 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. *Limnol Oceanogr* 53:185–197
- del Giorgio PA, Pace ML, Fischer D (2006) Relationship of bacterial growth efficiency to spatial variation in bacterial activity in the Hudson River. *Aquat Microb Ecol* 45:55–67
- Douglas A (2009) Social, political, and institutional setting: water management problems of the Rio Grande. *J Water Resour Plan Manag* 6:493–501
- Figueroa D, Rowe OF, Paczkowska J, Legrand C, Andersson A (2016) Allochthonous carbon—a major driver of bacterioplankton. *Microb Ecol* 71:789–801
- Findlay S (2003) Bacterial response to variation in organic matter. In: Findlay SEG, Sinsabaugh RL (eds) *Aquatic ecosystems: interactivity of dissolved organic matter*. Academic Press, San Diego, pp 363–377
- 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, Adler D, Bates D, Baud-Bovy G, Ellison S et al (2016) Package 'car'. R Foundation for Statistical Computing, Vienna
- Granéli W, Bertilsson S, Philibert A (2004) Phosphorus limitation of bacterial growth in high arctic lakes and ponds. *Aquat Sci* 66:430–439
- Gregory L, Hatler L (2008) A watershed protection plan for the Pecos River in Texas: an overview. Texas State Soil and

- Water Conservation Board and U.S. Environmental Protection Agency
- 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. *Limnol Oceanogr* 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 nutrient and carbon processing in three Australian rivers. *River Res Appl* 26:1129–1152
- Hamdan LJ, Jonas RB (2006) Seasonal and interannual dynamics of free-living bacterioplankton and microbially labile organic carbon along the salinity gradient of the Potomac River. *Estuar Coasts* 29:40–53
- Hitchcock J, Mitrovic S (2015) After the flood: changing dissolved organic carbon bioavailability and bacterial growth following inflows to estuaries. *Biogeochemistry* 124:219–233
- 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. *Geophys Res Lett* 35:L03402. <https://doi.org/10.1029/2007GL032837>
- Jones SE, Newton RJ, McMahon KD (2009) Evidence for structuring of bacterial community composition by organic carbon source in temperate lakes. *Environ Microbiol* 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
- Junk WJ, Bayley PB, Sparks RE (1989) The flood pulse concept in river-floodplain systems. In: Dodge DP (ed) Proceedings of the international large river symposium. Canadian special publication for fisheries and aquatic sciences, vol 106, pp 110–127
- Kingsford RT (2000) Protecting rivers in arid regions or pumping them dry? *Hydrobiologia* 427:1–11
- Kirchman DL (1993) Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, London, pp 509–512
- 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? *Aquat Microb Ecol* 38:103–111
- Kritzberg ES, Cole JJ, Pace MM, Granéli W (2006) Bacterial growth on allochthonous carbon in humic and nutrient-enriched lakes: results from whole-lake ¹³C addition experiments. *Ecosystems* 9:489–499
- Lamberti GA, Steinman AD (1997) A comparison of primary production in stream ecosystems. In: Webster JR, Meyer JL (eds) Stream organic matter budgets. *J North Am Benth Soc* 16:95–104
- Legendre P, Legendre L (2012) Numerical ecology, 3rd edn. Elsevier, Oxford
- 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. *Aquat Microb Ecol* 39:107–119
- Li M, Peng C, Wang M, Xue W, Zhang K, Wang K, Shi G, Zhu Q (2017) The carbon flux of global rivers: a re-evaluation of amount and spatial patterns. *Ecol Indic* 80:40–51
- Mace RE, Angle ES, Mullican III WF (eds) (2004) Aquifers of the Edwards Plateau, Texas. *Water Dev Board Rep* 360:345–366
- 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. *Funct Ecol* 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
- McCune B, Grace JB, Urban DL (2002) Analysis of ecological communities. MjM Software Design, Gleneden Beach
- Middleton NJ, Thomas DSG (1997) World Atlas of desertification, 2nd edn. United Nations Environment Programme, London
- Murrell MC (2003) Bacterioplankton, dynamics in a subtropical estuary: evidence for substrate limitation. *Aquat Microb Ecol* 32:239–250
- Nelson CE, Carlson CA (2011) Differential response of high-elevation planktonic bacterial community structure and metabolism to experimental nutrient enrichment. *PLoS ONE* 6:e18320
- Ochs CA, Capello HE, Pongruektham O (2010) Bacterial production in the lower Mississippi River: importance of suspended sediment and phytoplankton biomass. *Hydrobiologia* 637:19–31
- Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2012) Vegan: community ecology package. R package version 2.0-5
- Ostapenia AP, Parparov A, Berman T (2009) Lability of organic carbon in lakes of different trophic status. *Freshw Biol* 54:1312–1323
- Pace ML, del Giorgio P, Fischer D, Condon R, Malcom H (2004) Estimates of bacterial production using the leucine incorporation method are influenced by differences in protein retention of microcentrifuge tubes. *Limnol Oceanogr Methods* 2:55–61
- Peura S, Eiler A, Hiltunen M, Nykänen H, Tiirola M et al (2012) Bacterial and phytoplankton responses to nutrient amendments in a boreal lake differ according to season and to taxonomic resolution. *PLoS ONE* 7:e38552
- Proia L, von Schiller D, Gutierrez C, Casas-Ruiz JP, Gómez-Gener L, Marcé R, Obrador B, Acuña V, Sabater S (2016) Microbial carbon processing along a river discontinuum. *Freshw Sci* 35:1133–1147
- R Development Core Team (2013) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

- Raymond PA, Spencer RGM (2015) Riverine DOM. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter, 2nd edn. Academic Press, Philadelphia
- Raymond P, Hartmann J, Lauerwald R, Sobek S, McDonald C et al (2013) Global carbon dioxide emissions from inland waters. *Nature* 503:355–359
- Revelle W (2019) psych: Procedures for psychological, psychometric, and personality research. R package version 1.9.7
- Roland F, Caraco NF, Cole JJ (1999) Rapid and precise determination of dissolved oxygen by spectrophotometry: evaluation of interference from color and turbidity. *Limnol Oceanogr* 44:1148–1154
- Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^3H -leucine. *Mar Microb Food Webs* 6:107–114
- Sondergaard M, Middelboe M (1995) A cross-system analysis of labile dissolved organic carbon. *Mar Ecol Prog Ser* 118:283–294
- Sullivan AB, Snyder DM, Rounds SA (2010) Controls on biochemical oxygen demand in the upper Klamath River, Oregon. *Chem Geol* 269:12–21
- 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, Sobek S, Tremblay A, Vanni MJ, Verschoor AM, von Wachenfeldt E, Weyhenmeyer GA (2009) Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol Oceanogr* 54:2298–2314
- Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. *Can J Fish Aquat Sci* 37:130–137
- Velasco J, Millan A, Vidal-Abarca MR, Suarez ML, Guerrero C, Ortega M (2003) Macrophytic, epipellic and epilithic primary production in a semiarid Mediterranean stream. *Freshw Biol* 48:1408–1420
- Vidal LO, Granelli W, Daniel CB, Heiberg L, Roland F (2011) Carbon and phosphorus regulating bacterial metabolism in oligotrophic boreal lakes. *J Plank Res* 33:1747–1756
- Wetzel RG (2001) *Limnology*, 3rd edn. Academic Press, Philadelphia
- Wetzel RG, Likens GE (2000) *Limnological methods*, 3rd edn. Springer, New York
- Williams PJB, del Giorgio PA (2005) Respiration in aquatic ecosystems: history and background. In: del Giorgio PA, Williams PJB (eds) *Respiration in aquatic ecosystems*. Oxford University Press, New York
- 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. *Limnol Oceanogr* 55:1159–1171
- Wolter K (1982) Bacterial incorporation of organic substances released by natural phytoplankton populations. *Mar Ecol Prog Ser* 7:287–295

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.