



Riverbed Sediments Control the Spatiotemporal Variability of *E. coli* in a Highly Managed, Arid River

James Fluke, Ricardo González-Pinzón* and Bruce Thomson

Department of Civil, Construction & Environmental Engineering, University of New Mexico, Albuquerque, NM, United States

High concentrations of fecal indicator bacteria (FIB) can result in exceedance of surface water quality standards, particularly in urban areas which receive stormwater runoff. Although FIB are considered to be an indicator of mammalian waste, there is increasing evidence of regrowth in sediments of warm streams. However, the role that sediment-water interactions play on the spatiotemporal distribution of FIB is not well-understood, particularly on scales relevant to water quality management. We examined *E. coli* loads in river water and concentrations in sediments in a 60 km reach of the Rio Grande near Albuquerque, USA to determine seasonal and spatial trends of the coupling between FIB in the water column and those in sediments. Our study reach progresses from a narrow, coarse-bottom channel (0–9.4 km) with insignificant anthropogenic FIB sources to a wide, sand-braided channel with prevalent mid-channel bars and islands (25–61.5 km) that flows through a city with 1 million inhabitants. At upstream sites, we observed low *E. coli* loads during low discharges and low sediment *E. coli* concentrations. Conversely, downstream sites had high *E. coli* loads and high sediment concentrations year-round. Using a simple framework to quantify sediment-water interactions and *E. coli* transferability, we found that the fine riverbed sediments present at downstream sites served as a source of *E. coli* to the overlying water and that they promoted the survival of *E. coli* along this subreach, contributing to frequent violations of the stream standard. Therefore, we propose that sediment-water interactions should be explicitly considered in water quality management, protection, and improvement efforts.

Keywords: *E. coli* dynamics, sediment-water interactions, rivers, non-point source pollution, point source pollution, environmental regulations

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*Correspondence:

Ricardo González-Pinzón
gonzaric@unm.edu

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INTRODUCTION

Surface water impairment due to fecal contamination is a worldwide concern. In the US, evidence of pathogens accounts for the greatest number of impaired miles of rivers and streams (178,000), of which 160,000 miles are considered impaired for indicators of fecal contamination such as *Escherichia coli* (*E. coli*) and other fecal indicator bacteria (FIB) [US Environmental Protection Agency (USEPA), 2018]. This has led regulatory agencies to require implementation of watershed management programs to reduce FIB concentrations in surface waters. However, numerous efforts have shown that identifying point and non-point sources of these organisms is challenging (Nix et al., 1993; Pandey et al., 2014; Goodwin et al., 2017), particularly because natural growth and persistence of FIB in sediments may constitute a significant source of organisms (Crabill et al., 1999; Cinotto, 2005; Pachepsky and Shelton, 2011; Rothenheber and Jones, 2018). Therefore, the

resulting lack of ability to reduce FIB concentrations and loads hinders the development, implementation and enforcement of strategies to prevent, mitigate and recover from episodic and/or chronic fecal contamination, and to meet water quality standards [He et al., 2007; Rowny and Stewart, 2012; New Mexico Environment Department (NMED), 2018].

Recent research in lakes and short stream reaches (<20 km) has revealed that key aspects affecting the sources, transport and fate of FIB in surface waters vary so widely that they generate conflicting relationships between FIB and common water quality parameters (Crabill et al., 1999; Cinotto, 2005), and between water and sediment FIB concentrations (Davies et al., 1995; An et al., 2002; Evanson and Ambrose, 2006). Numerous studies in freshwater lakes, marine beaches and estuaries, laboratory microcosms, and stream reaches have documented growth and survival of FIB including *E. coli* in surface waters and sediments (Davies et al., 1995; Desmarais et al., 2002; Boehm et al., 2009; Badgley et al., 2011; Pachepsky and Shelton, 2011), as well as natural occurrence and regrowth of FIB (Ishii et al., 2006; Vital et al., 2010), which results in high spatial and temporal variations in FIB concentrations (Pachepsky and Shelton, 2011; Harmel et al., 2016). Temporal variability in FIB levels has been attributed to variations in water mixing, sediment-water interactions, inactivation by sunlight, seasonal growth/die-off, and community redistribution (Boehm et al., 2009), while spatial variability in FIB levels has largely been attributed to natural and anthropogenic differences in geomorphologic controls and external inputs (Whitman et al., 2004; Pachepsky and Shelton, 2011; St Laurent and Mazumder, 2014; Oliver and Page, 2016).

While FIB are generally considered effective indicators of health risk from fecal contamination (Wade et al., 2006; Wiedenmann et al., 2006; US Environmental Protection Agency, Office of Water, 2012), the correlation between FIB concentrations and environmental conditions in rivers remains challenging. To date, the dynamic effects of sediment-water interactions on observed *E. coli* concentrations are particularly not well-understood. Simply put, we cannot predict the relative contribution of stream sediments to FIB concentrations in a stream and how local source/sink behaviors propagate down the fluvial network. The objective of this study was to investigate the seasonal and spatial variability of *E. coli* sediment-water transfer from upstream to downstream of a major city on a scale commensurate with human-related activities. We studied a ~60 km reach of the Rio Grande near Albuquerque, New Mexico, which is impaired due to high concentrations of *E. coli*. Frequent exceedances of the stream standard have occurred in this reach despite source reduction strategies focused on urban storm water management. These included development and implementation of a total maximum daily load (TMDL) criterion which has cost local governments ~20 million US dollars [New Mexico Environment Department (NMED), 2018]. High *E. coli* concentrations occur during both wet and dry weather conditions and at locations upstream from all anthropogenic sources. Our study reach traversed a range of urbanization levels, i.e., the watershed of the upstream reach is undeveloped and provides no human inputs of wastewater or urban stormwater runoff while the lower reach passes through

an urban area inhabited by nearly 1 million people and receives treated and disinfected municipal wastewater as well as urban stormwater runoff. Therefore, this study allows investigation of spatiotemporal dynamics of FIB over a longer river reach (>20 km) than previous studies and improves our understanding of the role of human activities and natural phenomena on the dynamics of *E. coli* concentrations and loads in the arid Southwest USA.

METHODS

Study Reach

We studied a 61.5 km reach of the Rio Grande near Albuquerque (Figure 1), which receives most of its discharge from upstream mountain snowmelt and is used for agriculture and drinking water supply for the City of Albuquerque. The study reach is 45 km downstream from a large flood control reservoir (Cochiti Lake) that removes virtually all sediment from the river and has changed the river characteristics from a wide, slow moving stream with a sand and silty channel bottom to a narrow, higher energy stream with a gravel bottom downstream of the reservoir (Musssetter Engineering, Inc., 2002; Makar et al., 2006). Our study reach thus progresses from a narrow coarse-bottom channel (0–9.4 km) to a wide, sand-braided channel with prevalent mid-channel bars and islands (25–61.5 km) (Massong et al., 2006). The watershed draining to the study reach is ~28% developed land, 4% land cultivated for crops, 49% grassland and shrubland, and 14% forest [US Geological Survey (USGS), 2018a]. Diversion structures at both ends of the study reach are used during the growing season to route river water into irrigation canals. The US Army Corps of Engineers (USACE) and Middle Rio Grande Conservancy District (MRGCD) release water from upstream reservoirs and operate diversion structures to manage river water for agricultural irrigation, municipal use, environmental flows, and delivery to downstream users. The United States Geological Survey (USGS) operates several stream gages in this reach, including gages USGS 08329918 at Alameda Bridge and USGS 08330000 at Central Bridge, which provided flow data for this study [US Geological Survey (USGS), 2018a,b]. The US Bureau of Reclamation (USBR) operates several *in-situ* water quality monitoring stations in this reach, which also provided data for this study [US Bureau of Reclamation (USBR), 2018]. Peak flows from snowmelt occur typically in May, while short duration high flows from monsoonal precipitation events occur between July and November (Passell et al., 2004). Water quality indicators (WQI) up- and downstream of the urbanized area are shown in Table 1. Typically, discharge and DO decrease along the reach, while turbidity, water temperature, and electrical conductivity (EC) increase (Figure 2).

The designated uses for the river in the urban area are irrigation, marginal warmwater aquatic life, livestock watering, public water supply, wildlife habitat, and primary contact recreation. Based on these uses, the stream standard for *E. coli* is a monthly geometric mean of 126 colony-forming units (cfu)/100 mL and a single sample maximum concentration of 410 cfu/100 mL. The Isleta Pueblo, a federally recognized Native American tribe, is located immediately downstream from

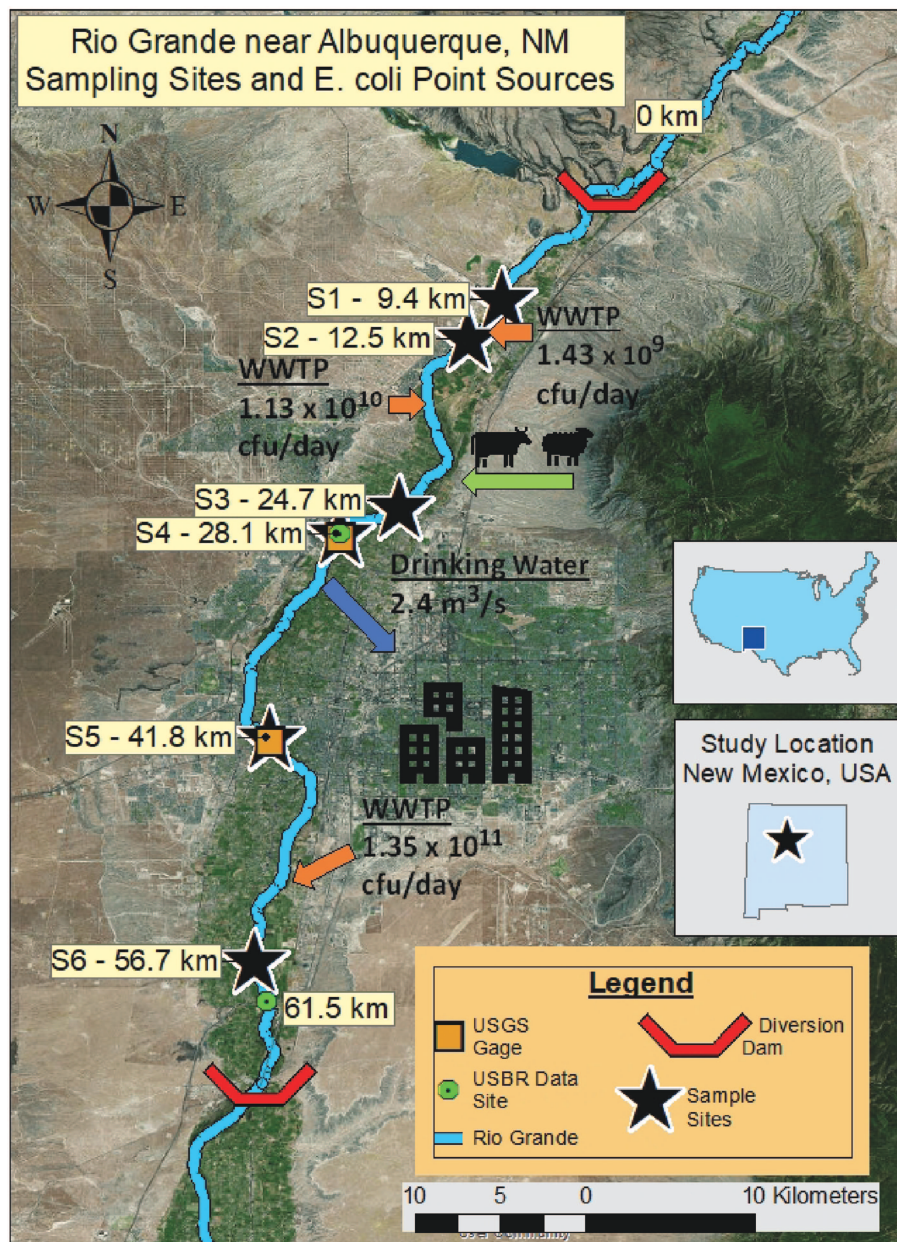


FIGURE 1 | Study reach map showing locations of stream gages (discharges in m^3/s), known *E. coli* sources (colony-forming units of *E. coli* per day; orange arrows), City of Albuquerque's water intake (blue arrow) and unquantified non-point sources (green arrow).

Albuquerque and has adopted their own federally approved water quality standards that include primary contact ceremonial use as a designated use of the river. The *E. coli* standard for this use is a monthly geometric mean concentration of 47 cfu/100 mL and a single maximum concentration of 88 cfu/100 mL.

Exceedances of stream standards for *E. coli* in the lower reach of the river have been frequent, triggering the implementation of a TMDL [New Mexico Environment Department (NMED), 2018]. The TMDL limits depend on river flow and location, ranging from an *E. coli* load of 2.96×10^9 cfu/day for low

flow conditions to 5.54×10^{12} cfu/day for high flow conditions. The monitoring of a major stormwater channel that drains approximately one-third of urban Albuquerque has found high concentrations of *E. coli* in stormwater, occasionally exceeding 50,000 MPN/100 mL (Storms et al., 2015). However, stormwater flows are infrequent in this arid environment and on average only contribute $\sim 1.4\%$ of the total annual Rio Grande flow; therefore, urban runoff is not believed to be a major contributor to the total *E. coli* load to the river. Aquatic water fowl, seasonal temperature variation, stormwater runoff and tributary

flows, wastewater effluents, and persistence of FIB in sand and sediments have been cited as likely causes for the elevated Summer levels and increase with downstream distance (Parson's Water and Infrastructure, Inc., 2005; CDM Smith, 2015). Finally, monitoring has shown that *E. coli* levels have not changed since 2001 despite an investment of ~\$20 million in non-point source control programs in the Albuquerque urbanized area, and the downstream section of the reach was still considered impaired as of 2018 [New Mexico Environment Department (NMED), 2018].

Sampling Site Description

We conducted synoptic sampling campaigns every 3 weeks, over a year, to measure *E. coli* concentrations from river sediment and surface water grab samples. During each sampling campaign, we collected samples at each of the 6 sampling sites on the same day (Figure 1). Water quality measurements on the first sampling event were compromised, so only 16 data points are included. Sediment sample collection and analyses were successful for all 17 sampling events.

The sampling sites were selected to capture the effects of major features of the system including urbanized areas, wastewater treatment plant (WWTP) discharges, and large stormwater outfalls. Site#1 (US550) is upstream of any WWTP effluents and urban stormwater discharges and is considered to be unaffected by urbanization. Historically, there have been low

E. coli levels between Cochiti Dam (~45 km upstream) and Site #1 [New Mexico Environment Department (NMED), 2018]; of the six small communities located in this river section, only two discharge stormwater to the Rio Grande and none discharge wastewater. Site #6 is the last section of the reach and is located downstream of the City of Albuquerque's WWTP. Table 2 summarizes point sources to the river and drinking water withdrawal between each sample site.

Sampling and Analytical Methods

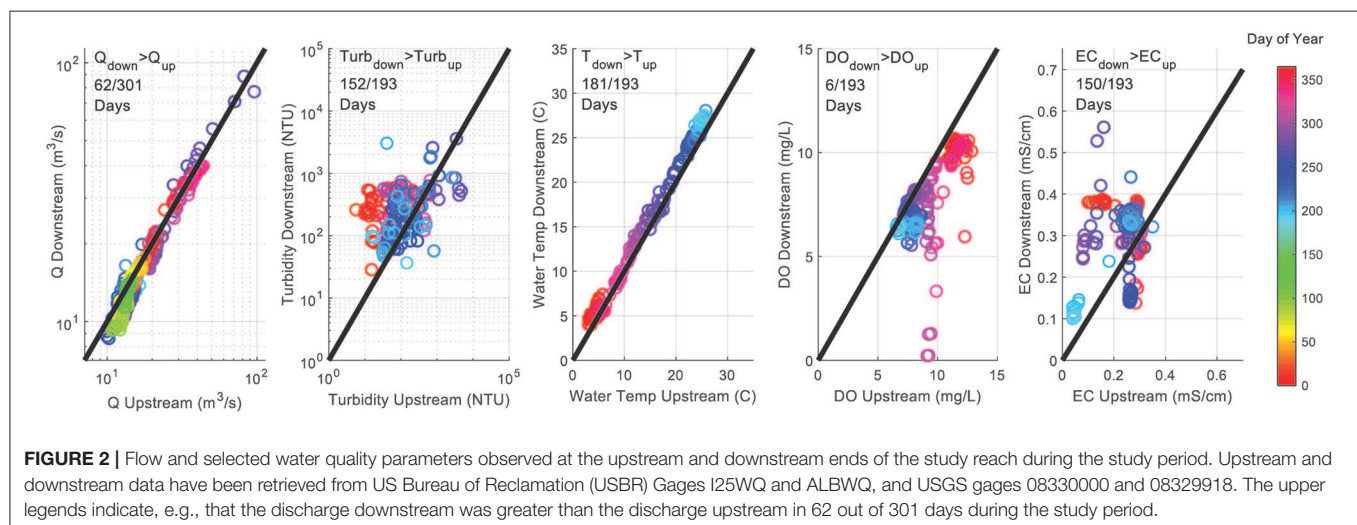
At each sampling site, 2 grab samples of sediment were taken from the sediment-water interface and 1 water sample was collected from the water surface. Sediment grab samples were taken from the thalweg of the river when possible using a Ponar sediment sampler and from near a river bank with shallow depth using a stainless-steel scoop. Surface water grab samples were taken from bridges (US550, Alameda, Central) or from the river bank using pre-sealed, 100 mL coliform sampling

TABLE 1 | Variability of water quality parameters along the study reach during the study period.

Rio Grande discharge and water quality indicators	Range during study period
Discharge (m ³ /s)	8–85
Turbidity (NTU)	10 ⁰ –10 ⁴
Temperature (°C)	2–28
Dissolved oxygen (mg/L)	5–13
Electrical conductivity (mS/cm)	0.1–1.3

TABLE 2 | *E. coli* inputs and water withdrawals along the study reach.

Sub-reach	Point sources and withdrawals
Site 1–Site 2	Waste water treatment plant (WWTP) with design capacity $Q = 0.035 \text{ m}^3/\text{s}$ and <i>E. coli</i> waste load allocation (WLA) = $1.43 \times 10^9 \text{ cfu/day}$
Site 2–Site 3	2 WWTPs (total design capacity $Q = 0.322 \text{ m}^3/\text{s}$, WLA = $1.13 \times 10^{10} \text{ cfu/day}$) 1 small agricultural return
Site 3–Site 4	Stormwater outfall (drains approximately one-third of urban area)
Site 4–Site 5	- Stormwater outfall - Small agricultural return flows - Drinking water diversion (average daily $Q = 2.4 \text{ m}^3/\text{s}$) - Recreation
Site 5–Site 6	WWTP (design capacity $Q = 3.33 \text{ m}^3/\text{s}$, WLA = $1.35 \times 10^{11} \text{ cfu/day}$)



bottles containing 0.1 g sodium thiosulfate (US Environmental Protection Agency, 2002).

Sediment samples were placed in a stainless-steel washbowl, and excess water was removed by decanting. A portion of the sediment sample without large rocks or plant matter was placed in a one-quart zip-top bag and labeled with the sample site, date and time collected. Samples were placed on ice and transported to the analytical laboratory and analyzed within 24 h of collection. Surface water samples were analyzed within 8 h of collection in accordance with EPA holding time requirements for bacteriological samples (US Environmental Protection Agency, 2002).

Sediment samples were analyzed using a variation of the Multiple Tube Fermentation (Standard Methods 9221 C-F) method by the Albuquerque Bernalillo County Water Utility Authority's analytical lab. In this method, a phosphate buffer solution was mixed with the sediment sample for 1–2 min in a blender to mix the sediment and buffer solution. The solid matter was allowed to settle, and the supernatant was analyzed for *E. coli* concentration. The MPN method was used to enumerate the *E. coli* concentration in the supernatant (LaLiberte and Grimes, 1982; Johnson et al., 1984; An et al., 2002) instead of the membrane filtration technique to avoid interference from sediments suspended in the solution (Davies et al., 1995; Crabill et al., 1999; Badgley et al., 2011; Pandey and Soupir, 2013, 2014).

The mass of dry sediment was determined by drying the sample at 110°C for 5 h and the amount of water in the sample was found as the difference between initial and final mass. The concentration of *E. coli* in the sediment was calculated from the concentration in the supernatant, normalized by the product of the mass of the wet sediment and the fraction of solids by mass. The concentrations of *E. coli* in sediment obtained through this method are reported as Most Probable Number (MPN) of colony forming units per 100 g of dry sediment (MPN/100 g). Although the units MPN and colony-forming unit (cfu) indicate measurement of the same analyte, the reported units are different because the MPN method is a probabilistic method while the membrane filtration method involves a direct colony count. These methods of measuring concentration are not statistically different on the log scale (Johnson et al., 1984) and were considered equivalent for the purposes of this study.

E. coli concentrations in surface water were determined using the Membrane Filtration Chromogenic Method (US Environmental Protection Agency, 2002). This method has estimated false-positive and false-negative rates for *E. coli* of 4.3%, and specificities for *E. coli* and total coliforms of 95.7 and 93.1%, respectively. We note that Harmel et al. (2016) present a detailed discussion of uncertainty measurement associated with *E. coli* concentrations in surface water. Following EPA recommendations, aliquots of 0.1, 1, and 5 mL were collected to bracket expected *E. coli* concentrations. Sample aliquots were diluted with DI water and vacuum-filtered through a 0.45 µm membrane filter. The membrane filters were placed on a petri dish containing the chromogenic nutrient solution and incubated for 72 h at room temperature. Blue colonies associated with *E. coli* organisms were enumerated automatically using a publicly available image analysis code modified to identify and count blue

colonies (Image Analyst, 2015). The final *E. coli* concentration was reported as the average of the 2 closest concentration values for each of the 3 plates. Digital images of the colonies were automatically generated, displayed, and recorded to verify the automated count. Surface water *E. coli* concentrations were multiplied by flow data at the time of sample collection to calculate the *E. coli* loads in units of cfu/day. For this, the USGS daily flow data from July 2017 to May 2018 from the USGS Rio Grande at Alameda Bridge (USGS 08329918) was used for Sites #1–#4 and the USGS Rio Grande at Central Bridge (USGS 08330000) was used for Sites #5 and #6.

To perform statistical calculations on these datasets, results of non-detection for either type of sample were assigned values of the detection limit based on the volume of material analyzed (10 non-detects in water samples, 26 non-detects in sediment samples). A detection limit of 20 cfu/100 mL (corresponding to detection of 1 cfu per 5 mL of water sample) was used for the chromogenic method and 1 MPN/20 g for the sediment samples because ~20 g of sediment was used in the analysis. A log-10 transformation of sediment and water *E. coli* concentration data, and *E. coli* load data resulted in an approximately normal distribution ($-1 < \text{skewness} < 1$, magnitude of kurtosis ~ 3) and the sample size is relatively large (*E. coli* load and water concentration $n = 96$, *E. coli* concentration in sediment $n = 204$). Since the distributions of the datasets are approximately symmetric, well-represented by the mean, and made up of >40 points, inferences using parametric t-procedures are considered appropriate in accordance with the Central Limit Theorem (Navidi, 2010). Confidence intervals for subsets of the population were generated using Students' t-distribution. One-way Analysis of Variance (ANOVA) tests were used to determine whether mean sediment and water concentrations, and loads were statistically different when grouped by site and by season. Tukey's Honest Significant Difference (HSD) range test was used to evaluate significant differences between estimated means of individual groupings *post-hoc*. The Pearson Correlation test was used to assess the data for spatial trends when grouped by season. All reported values are back-transformed from logarithmic units to arithmetic units of cfu/100 mL, cfu/day, or MPN/100 g.

RESULTS

A One-Way ANOVA test showed seasonal and longitudinal variations in *E. coli* river water concentrations, loads and sediment concentrations, and found differences between the groupings by site and season (ANOVA $p < 0.05$). The mean *E. coli* concentrations, loads and *E. coli* sediment concentration showed a decrease from Summer 2017 to Spring 2018 (Figures 3A,B), and an increase with distance downstream (Figures 3C,D).

By season, the Summer mean water concentration and load estimates were different from those in the Spring, and the Summer and Fall mean sediment concentrations were different from Winter and Spring values (Tukey's HSD $p < 0.05$). The estimated mean *E. coli* loads and river water concentrations were different between sites along the river (ANOVA $p < 0.05$), and the estimated mean concentrations of *E. coli* in

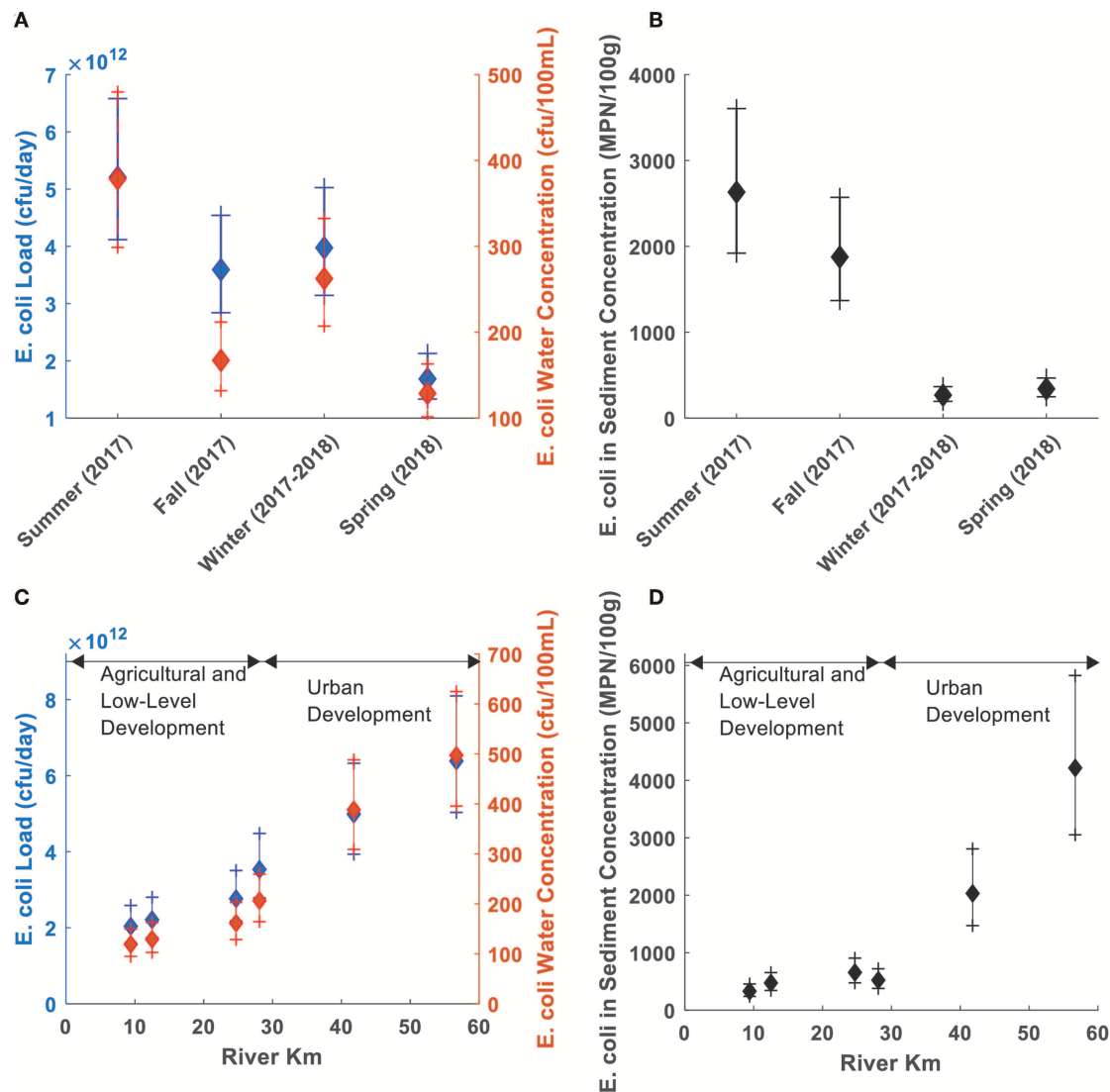


FIGURE 3 | *E. coli* loads and concentrations in the study reach. Error bars show confidence intervals at 95% (CI's) for mean *E. coli* loads (blue), water concentrations (red), and sediment concentrations (black) by season (top) and sample site (bottom). (A) *E. coli* water concentration and load CIs by season. (B) *E. coli* sediments concentration CIs by season. (C) *E. coli* water concentration and load CIs by site. (D) *E. coli* sediments concentration CIs by site.

sediments at Sites #5 and #6 were different from Site #1 and Sites #1–#4, respectively (Tukey's HSD $p < 0.05$). *E. coli* water concentration data groups were different when comparing Site #1 to the Sites # 5 and #6, and comparing Site #2 to Site #6. *E. coli* concentrations in water concentrations and sediment, and *E. coli* loads were increased with downstream distance (Pearson's Correlation $p < 0.05$).

Figure 4 presents a more disaggregated analysis of the data by season, showing that *E. coli* loads, and concentrations in water and sediment during Fall, Winter, and Spring increase with downstream distance (Pearson's Correlation $p < 0.05$), while these levels remain nearly constant for all sites during the Summer (Pearson's Correlation $p > 0.05$). **Figure 4** also shows that *E. coli* loads and sediment concentrations are

coupled, and *E. coli* concentrations in river water appear to be correlated with those in the sediments. Since discharge varied less than an order of magnitude over the sampling period, *E. coli* water concentrations and loads are highly correlated ($R = 0.94$).

To evaluate the sediment-water coupling, mean log-transformed loads and sediment concentration values for the data, grouped by season and sub-grouped by site (6 sites for 4 seasons hence $n = 24$) were tested for correlation. The estimated means by site and season were linearly correlated ($p < 0.05$) with a Pearson's correlation coefficient (R) of 0.71 (**Figure 5**). Similarly, *E. coli* water and sediment concentrations grouped by season and sub-grouped by site were linearly correlated ($R = 0.67$; **Figure 5**).

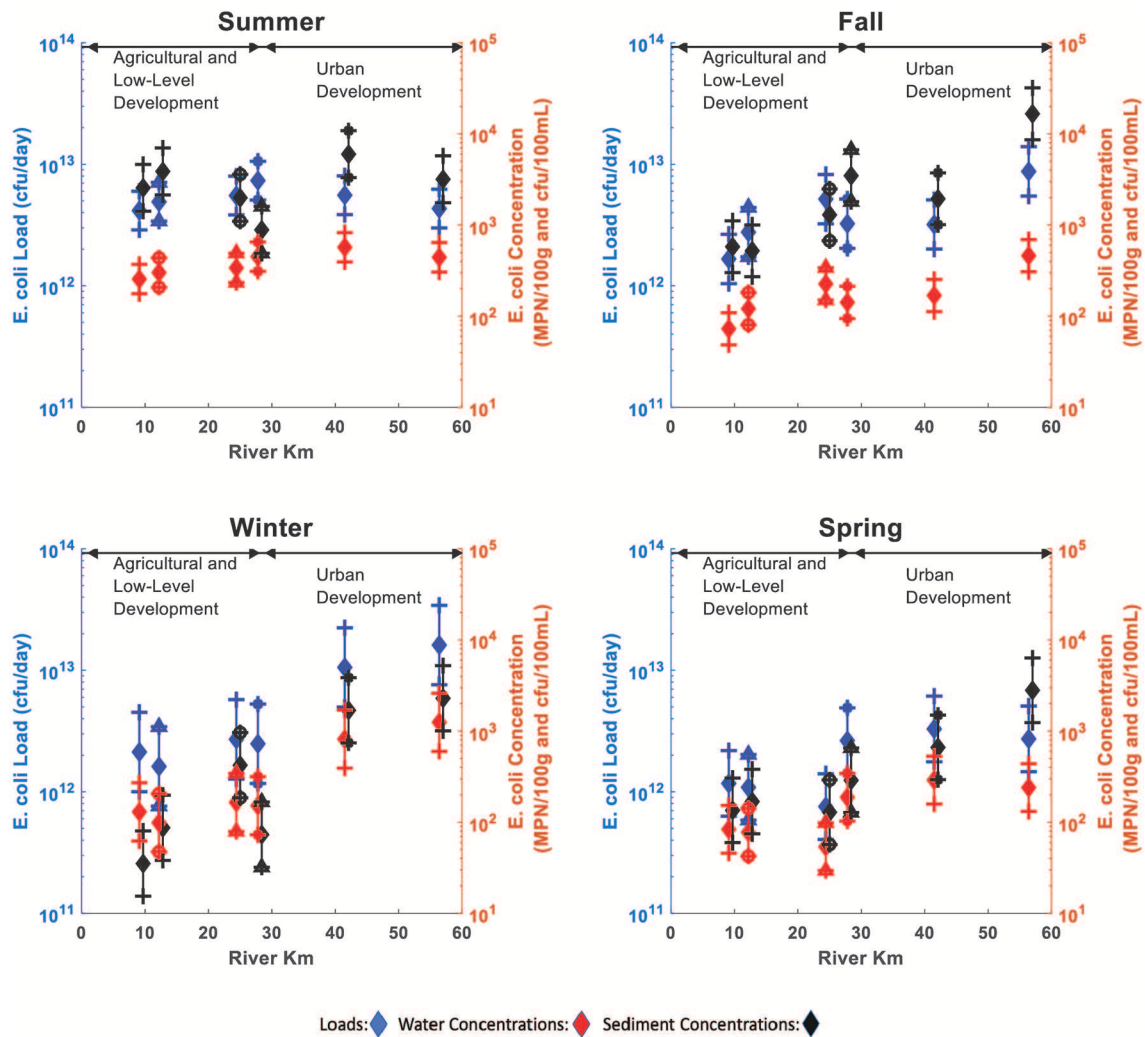


FIGURE 4 | Mean *E. coli* loads (blue), water concentrations (red) and sediment concentrations (black) by sample site and season. Error bars show confidence intervals at 95% (CI's) for mean *E. coli* loads (blue), water concentrations (red), and sediment concentrations (black) by sample site.

DISCUSSION

Average Seasonal and Longitudinal *E. coli* Behavior

The concentration of *E. coli* in water and sediment, and the *E. coli* load in the river varied seasonally and increased with downstream distance (Figures 3, 4). While the mean load from Summer months was the highest, it was not statistically higher than mean Winter or Fall loads (Tukey's HSD $p > 0.05$), as data from previous studies suggested (Parson's Water and Infrastructure, Inc., 2005; CDM Smith, 2015; Storms et al., 2015). Mean *E. coli* loads in this study reach increased approximately linearly with downstream distance (Pearson's $R = 0.99$; Figure 3), except during the Summer (Figure 4; see next section). A linear increase in loads along the length of the reach suggests that non-point sources dominate overall *E. coli* loads, instead of point sources such as WWTP effluents or illicit wastewater

discharges, agricultural return flows, or stormwater flows, which would cause clear deviations from the overall trends. Contrary to our findings of increasing *E. coli* concentrations and loads with downstream distance, Harmel et al. (2010) found that *E. coli* concentrations consistently decreased as watershed scale increased from field to small watershed to river basin scale in the more humid Leon River watershed in central Texas. We propose that the differences between our findings in the Rio Grande, where the average annual precipitation is 230 mm, and those by Harmel et al. (2010) in central Texas, where the average annual precipitation is 800 mm, can be explained by differences in dilution processes. While in our study reach the Rio Grande has only a few lateral runoff inputs, most of them being urban or agricultural point-sources, the Leon River watershed has contributions from multiple non-point sources that carry lower concentrations of *E. coli*, effectively decreasing concentrations with increasing spatial scale.

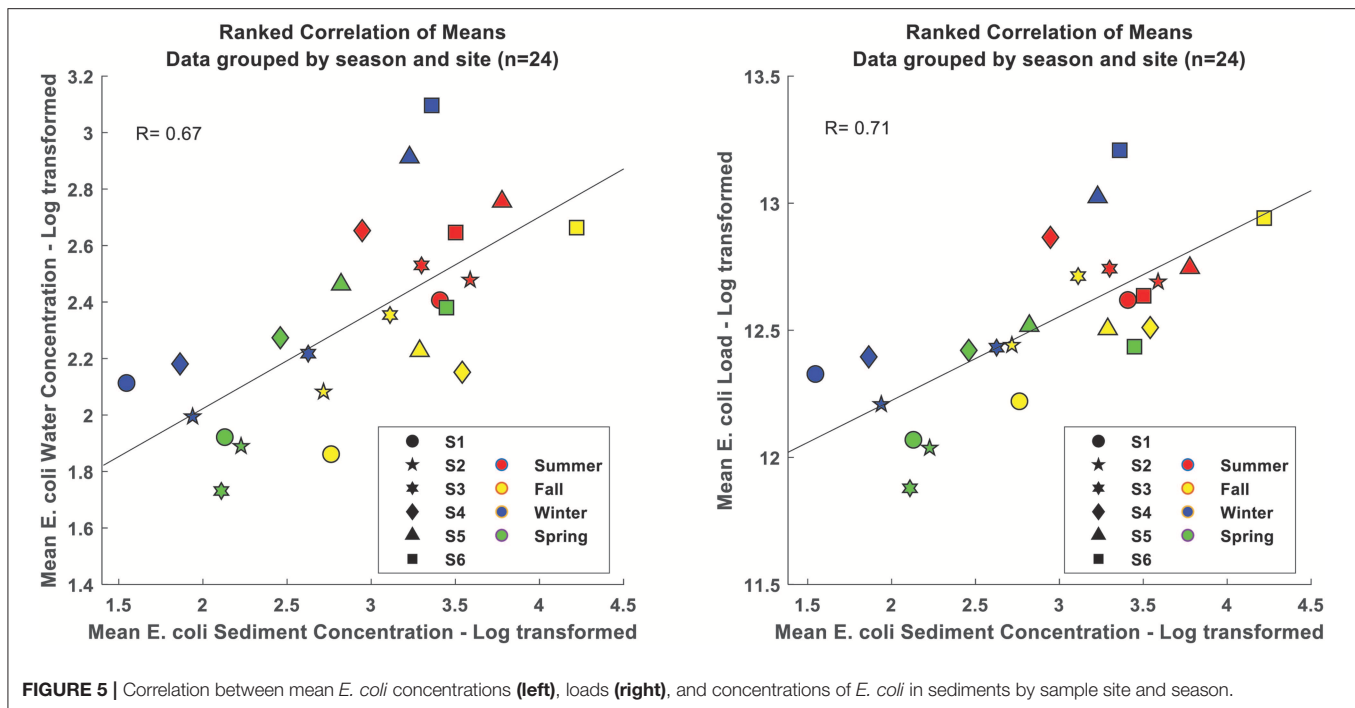


FIGURE 5 | Correlation between mean *E. coli* concentrations (left), loads (right), and concentrations of *E. coli* in sediments by sample site and season.

In contrast to the linear increase of *E. coli* loads and concentrations in the river water across the non-urban and urban subreaches, *E. coli* sediment concentrations observed in the sampling sites in the urban reaches (Sites #5 and #6; **Figure 3**) presented means and trends that were different from the upstream, non-urban sites (Tukey's HSD $p < 0.05$). Possible explanations for this behavior include urban sources of *E. coli* (WWTP discharges, agricultural return flows, surface runoff, etc.), and increased regrowth and persistence of *E. coli* in riverbed sediments due to more favorable conditions. In particular, lower reaches of the river are shallower, wider and have lower velocities, which increased water temperatures, and increased turbidity levels, which may shield *E. coli* from photodecay (cf. **Figure 2**). Perhaps most importantly, the lower reaches of the river have conditions suitable for *E. coli* survival and regrowth, especially during warm Summer months. Mathematically, the relationship between *E. coli* sediment concentration vs. distance is better fit by an exponential increase ($R^2 = 0.99$) than by a linear model ($R^2 = 0.85$). However, the differences in land use/land cover between non-urban and urban sites suggests that urban-related sources become relevant in establishing the differences in slope between the *E. coli* spatial trends in sediments (i.e., one linear trendline does not fit all non-urban and urban sites), suggesting that the mathematical description of a homogeneous, first-order growth of *E. coli* (exponential function) in the study reach is less realistic.

Spatial *E. coli* Dynamics by Season

Escherichia coli concentrations in sediments were related to *E. coli* concentrations and loads in the river water (**Figure 4**). *E. coli* concentrations in sediments along the urban reach of the river (Sites #5 and #6) had the highest values and smallest coefficients of variation (CV) of all sites, confirming the impact of the

urbanized, downstream reach on *E. coli* sourcing and persistence (CVs at Sites #5 and #6 are 0.21–0.29, compared to CVs at Sites #1–#4 of 0.37–0.45). However, this reach also had the warmest temperatures and bottom sediment conditions which have been shown by others to support survival and regrowth of *E. coli*. Literature on environmental FIB report that sandy and fine soils (characteristic of Sites #5 and #6) are more likely to contain higher FIB concentrations, possibly due to their high surface area and lower shear stresses from flowing water, compared to cobble bed channels (Crabill et al., 1999; Pachepsky and Shelton, 2011; Pandey and Soupir, 2013, 2014). The shift in morphology in the Rio Grande from a narrower, cobble-lined channel at the outlet of Cochiti Dam (~45 km upstream of Site #1), to a wider channel with silt and sand bottom sediments in the lower reach, contributes to higher water temperatures and more favorable habitat for regrowth (cf. **Figure 2**). Other differences in the lower reach which could contribute to elevated *E. coli* concentrations at Sites #5 and #6 include: (1) five small agricultural return and drainage return channels (4 return channels between Sites #4 and #6, 1 return channel between Sites #2 and #3), (2) Albuquerque WWTP effluent containing nutrients and UV deactivated FIB, (3) effects from urban and recreational use such as shedding from bathers and swimmers (Stevenson, 1953; Whitman et al., 2004) and increased *E. coli* sources from pets, horses and waterfowl, and (4) stormwater discharge.

Interestingly, during the Summer months *E. coli* sediment concentrations and *E. coli* loads and concentrations in the river water were nearly constant along the reach (**Figure 4**). This is inconsistent with a distributed non-point source of *E. coli* along the reach, which would result in a linear increase in loads and concentrations with distance. Besides the existence of favorable conditions for *E. coli* survival regrowth during the Summer, the constant *E. coli* concentrations and loads along the river

might be due to sediment bacterial populations approaching maximum concentrations based on available nutrients and environmental conditions. Another explanation could be that high flows during the Spring snowmelt flush finer, *E. coli* laden sediments from depositional zones of the riverbed and floodplain, resulting in a net decrease of autochthonous *E. coli* levels available for redistribution and less favorable organic and fine substrate material in the system to support *E. coli* survival. Our data suggest that saturation levels in sediments were not reached as higher concentrations were observed in other seasons and thus the second explanation seems more plausible and is supported by unusually high flows observed in 2017, before the Summer. Further explanations of the “equalizer” effect could be (1) a dynamic balance between sources of *E. coli* such as regrowth and external loadings and sinks such as predation and inactivation by UV light, and (2) a dynamic equilibrium where regrowth occurs in the sediments during warm months, but *E. coli* are periodically flushed into the water column by transient high flows from Summer thunderstorms. During the summer monsoon season, stormwater discharge from Albuquerque contains higher *E. coli* concentrations than we found in surface waters (Figure 3), and is enriched in nutrients, including labile organic carbon (Storms et al., 2015; Wise et al., 2019). Changing nutrient limitation conditions, along with increased temperatures favorable to regrowth, may simultaneously increase *E. coli* growth and predation, and provides initial support for either of the “equalizer” mechanisms.

Implications for Assessing and Monitoring Environmental FIB

The dynamic and difficult-to-quantify nature of river FIB challenges modeling efforts aimed at relating observed concentrations and loads to their sources. We used a simple mixing model framework which compares values of water column and sediment *E. coli* concentrations to characterize the observed variability of sediment-water *E. coli* interactions. We assumed that mixing between shallow sediment *E. coli* and surface water *E. coli* approaches a dynamic equilibrium and, thus, the concentration gradient at the sediment-water interface lets us estimate the direction of *E. coli* transfer for different flow regimes, seasons, and locations in our study reach. We used the ratio of *E. coli* concentration in the river water to estimated sediment pore-water *E. coli* concentrations (i.e., C_W/C_{PW}) to represent the concentration gradient over the sediment-water interface. Pore-water *E. coli* concentrations were estimated from the *E. coli* concentration by dry mass of sediment, i.e., $C_{PW} = C_S \bullet \beta / \Phi$, where C_{PW} is the *E. coli* concentration in the pore water of the sediments (MPN/100 mL), C_S is the *E. coli* concentration per mass of dry sediment (MPN/100 g), β is the density of the sediment solids (assumed 2.65 g/cm³), and Φ is the ratio of pore water volume to solids volume for the saturated sediments (assumed 0.2 for sandy sediments).

When $C_W/C_{PW} > 1$, there is a net transfer of *E. coli* from the river water to the bed sediments (sediment sink); when $C_W/C_{PW} < 1$, the opposite occurs (sediment source) (Figure 6). This assumes that *E. coli* behave conservatively and that diffusion controls the transport between the water column and pore water in the bed sediments. Since *E. coli* in this low velocity turbid,

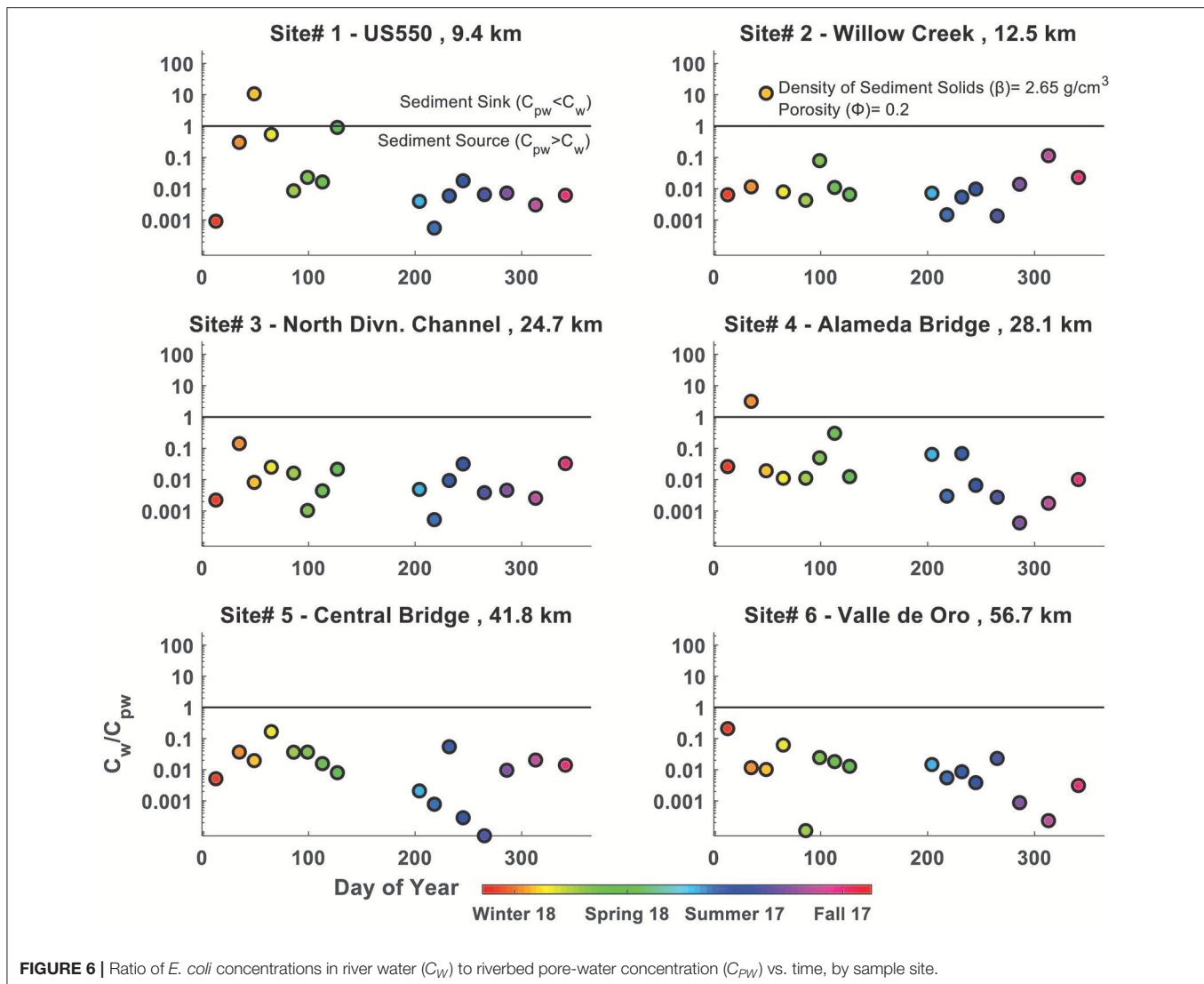
lowland river tend to be either dispersed or attached to fine particles rather than coarser sediments and water flora, this is a reasonable assumption in a turbid stream with little decay from UV light and limited predation by other organisms. We found that $C_W/C_{PW} < 1$ (i.e., *E. coli* concentration in sediments was greater than in river water; sediment source behavior) dominated throughout the year, at all sites (Figure 6). A value of $C_W/C_{PW} > 1$ (i.e., sediment sink behavior) was observed only during Winter and Spring months, and at upstream sites. This pattern is consistent with the decreased Winter and Spring mean *E. coli* concentrations in sediments. Also, the magnitudes of C_W/C_{PW} are consistent with those previously reported in other fluvial systems, suggesting that bed sediments typically constitute a source of *E. coli* to river water (Davies et al., 1995; Crabill et al., 1999; Garzio-Hadzick et al., 2010; Badgley et al., 2011; Pachepsky and Shelton, 2011; Pandey and Soupir, 2013, 2014). The magnitude of the contribution of *E. coli* in river bed sediments to the total *E. coli* load in the river was not determined in this investigation as it would require an understanding of the mixing zone in the channel bottom. Nevertheless, these results suggest that elevated concentrations of *E. coli* during storm events are due at least in part to suspension of bed material, including *E. coli*, by increased flows and associated turbulence.

This analysis provides information about how *E. coli* concentrations in water and sediments relate across time and space. Furthermore, the ratio C_W/C_{PW} establishes a simple indicator of easily measured parameters that provides a semi-quantitative explanation of spatial and temporal elevated concentrations of *E. coli* in the Rio Grande; bacterial sourcing from sediments vs. fecal contamination from human and natural sources. Further application of this framework could yield information about relative contributions of different sources across time and space and make these shifting relations easier to interpret in the context of unpredictable, difficult-to-observe FIB dynamics.

CONCLUSIONS

Our results show that riverbed sediments in the Rio Grande are an important contributor of *E. coli* to the river (Figure 6). This source is lowest during the Winter and at upstream locations, and increases as the weather warms and with downstream distance. Agreeing with past studies, *E. coli* concentrations in riverbed sediments were typically 10–100 times greater than those in river water throughout the year, with occasional exceptions during the Winter at upstream sampling sites.

Escherichia coli levels showed seasonal variability along the study reach. The highest *E. coli* concentrations in both the river water and the sediments occurred at the downstream extent, where water temperatures and turbidity are highest, and dissolved oxygen and water velocities are lowest. This reach of the river flows through a large urban area and thus receives treated wastewater, agricultural return flows, and urban stormwater runoff that are usually considered the major source of *E. coli* in the river and are subsequently the target of efforts to reduce their concentration. Although available monitoring data suggest that these point sources do not control *E. coli* water concentrations, their co-location with favorable geomorphologic



(slow warm river with a silt and sand bottom) conditions likely promote survival and regrowth of *E. coli* in river bed sediments. This enhanced persistence makes bed sediments a semi-continuous source of *E. coli* to the river water, augmenting loadings from upstream and contributing to frequent violations of the stream standard.

Our results suggest that the current TMDL for this reach will have limited effectiveness in reducing *E. coli* concentrations because it does not account for *E. coli* storage, regrowth, and persistence in river sediments. This conclusion is supported by the observation that traditional source reduction strategies implemented between 2001 and 2010 at a cost of ~\$20 M has produced no observable decrease in *E. coli* levels [New Mexico Environment Department (NMED), 2018]. Since the contribution of natural sources of *E. coli* to the river is not accounted for by the current regulatory framework, more specific spatiotemporal analysis of relative contaminant levels between different sources and the regulated water body are needed for

more appropriate regulatory efforts. Further investigation is thus needed to determine the contribution of sediment-derived *E. coli* to total loads in rivers. Specifically, information on the sediment mixing depth and resuspension of bed sediments during dry period and storm flows is needed to improve the modeling of the *E. coli* dynamics. Finally, the complexity of FIB source/sink dynamics and pathways in large river systems justify future investigations toward identifying dominant sources and sinks of FIB, and how their relative importance changes in time and space.

The presence of *E. coli* is assumed to be an indicator of contamination from human waste that might contain pathogenic organisms. Thus, a stream standard based on this organism is intended to protect human health from infectious agents that might be present in the water. However, the presence of a significant natural source of *E. coli* calls into question the validity of the parameter as a measure of surface water quality upon which to base an enforceable standard. This study has shown that *E. coli* concentrations are strongly related to elevated water

temperature, slow water velocities, and the presence of thick, silty bottom sediments, all conditions that exist in the lower segments of the reach of the Rio Grande studied in this investigation. Our data show that there is a positive concentration gradient favoring transport of these bacteria from the sediment pore water to the overlying water column. At present, it is not possible to calculate the magnitude of the *E. coli* load that originates in the bottom sediments; that remains a topic for future research. Nevertheless, it is clear that measurement of *E. coli* concentrations to quantify river water quality in a slow moving seasonally warm stream is not an appropriate regulatory parameter.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

JF and RG-P secured funding for the studies, designed and conducted the experiments, and wrote the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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