Estuarine, Coastal and Shelf Science 231 (2019) 106477

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: http://www.elsevier.com/locate/ecss

The bioavailability of riverine dissolved organic matter in coastal marine waters of southern Texas

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ARTICLE INFO

Keywords:
South Texas
Riverine dissolved organic matter
Bioavailability
Dissolved organic nitrogen
Total hydrolyzable amino acids
Bacterial community

ABSTRACT

To examine the bioavailability of dissolved organic carbon (DOC) and nitrogen (DON) in riverine dissolved organic matter (DOM) discharged to the coastal ocean, we conducted a series of month-long (24 days) incubation experiments with filtered samples collected from five southern Texas rivers (Lavaca, San Antonio, Mission, Aransas, and Nueces) inoculated using the same natural coastal microbial assemblages during summer (June) and winter (January) in 2016. The bioavailable fractions of DOC and DON (BDOC% and BDON%) varied substantially in different rivers and seasons, ranging respectively from 6–11% and 15–38% during winter, and 0–6% and 9–15% during summer. Relatively higher BDOC% and BDON% occurred in the San Antonio and Aransas Rivers, which are impacted more by human activities through discharge from wastewater treatment plants. Seasonally, the riverine DOM was more bioavailable in winter than in summer when DOM may have been extensively degraded *in situ* due to the low base flow (or long residence time) and the elevated temperature in river water in summer. The principal component analysis on amino acid composition further confirmed that DOM was less degraded in winter than in summer. Functional gene abundance data revealed that winter riverine DOM was relatively labile as evidenced by an increase in N-metabolism pathways and functional genes during the winter incubation, whereas the opposite pattern was observed in summer. The findings of the varying bioavailability of DOM among rivers and seasons have important implications about the fate of riverine DOM and their potential contributions to nutrient supplies as southern Texas bays and estuaries are often nitrogen limited.

1. Introduction

Dissolved organic matter (DOM) presents an important pool of carbon and nitrogen in aquatic systems (Seitzinger and Sanders, 1997; Mattsson et al., 2005). In rivers and estuaries, dissolved organic carbon (DOC) is a major organic carbon pool, and dissolved organic nitrogen (DON) can account for up to 90% of total dissolved nitrogen (Seitzinger and Sanders, 1997; Berman and Bronk, 2003). Riverine DOM supplies substantial energy and nutrients to the microbes and phytoplankton in estuaries, and may contribute to coastal eutrophication and hypoxia (Seitzinger and Sanders, 1997; See et al., 2006; Wiegner et al., 2006; Glibert et al., 2006; Conley et al., 2009). Such contribution is primarily determined by the bioavailability of DOM in the recipient environment.

The bioavailability of riverine DOM in estuaries is controlled by multiple factors including its chemical composition, often related to its sources, ecosystem dynamics (e.g., bacterial community, grazing, and viral lysis) and environmental conditions such as temperature and inorganic nutrients (Kaartokallio et al., 2016 and references therein). The DOM sources include autochthonous DOM derived from organisms in the river, such as phytoplankton exudations, and allochthonous DOM originated from numerous natural and anthropogenic processes. Riverine DOM derived from autochthonous production, sub-urban/urban runoff and agricultural soils typically has low C:N ratios and is more bioavailable in estuaries (Bronk and Glibert, 1993; Seitzinger and Sanders, 1999; Glibert et al., 2001; Seitzinger et al., 2002). However, DOM originated from forests, peatlands, and pristine wetland are often less bioavailable to estuarine bacteria (Stepanauskas et al., 1999; Wiegner and Seitzinger, 2004; Asmala et al., 2013).

The quantity and quality of riverine DOM can also be modified by processes such as photochemical oxidation, biological degradation, and flocculation in estuaries. Vannote et al. (1980) proposed the river continuum concept, emphasizing the effect of microbial degradation on the

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https://doi.org/10.1016/j.ecss.2019.106477
Received 17 June 2019; Received in revised form 28 October 2019; Accepted 6 November 2019
Available online 8 November 2019

bioavailable DOM along the river flow path (Stepanauskas et al., 2002). Exposure of DOM to sunlight can either promote or inhibit the microbial utilization of DOM by producing or removing bioavailable DOM (Pisani et al., 2017 and references therein). However, since much of the DOM pool remains chemically uncharacterized, the relative contributions of these processes in altering the chemical composition and bioavailability of riverine DOM are not well understood.

As a quick and simple approach, bioassays or incubation experiments can help quantify the bioavailable fraction of riverine DOM to the coastal bacterial community by monitoring decreases in bulk DOC and/or DON concentrations with incubation time (Seitzinger and Sanders, 1997; Moran et al., 1999; Stepanauskas et al., 2002; Wiegner et al., 2006; Asmala et al., 2013). This approach, however, does not offer insights into why certain fractions of DOM are metabolized easily and what types of bacteria and genetic pathways are involved in DOM utilization (Gan et al., 2016). Addressing these questions is needed to understand and predict the bioavailability of riverine DOM exported to bays and estuaries. Considering the challenges of analyzing the whole DOM in a molecular level, total dissolved amino acids (TDAA), a relatively bioavailable component of DOM, can provide qualitative and quantitative insights into bioavailability and degree of diagenetic alteration of riverine DOM (Benner, 2002; Davis and Benner, 2007; Duan and Bianchi, 2007; Peter et al., 2012; Shen et al., 2015; Li et al., 2018). Bioavailable DOM can also be evaluated from biological perspectives, including the changes of bacterial community, the functional genes involved in the utilization of bioavailable DOM, and bacterial growth efficiency (BGE) during the incubation (Rochelle-Newall et al., 2004). For example, the BGE and community structures change considerably in the mixing zone of riverine and saline waters, and up to 75% of the variability of BGE can be explained by the variation in DOM quality in estuaries (Kroer, 1993; Hopkinson et al., 1998; Troussellier et al., 2002). Hence, evaluating the bioavailable DOM from not only the bulk measurement approach, but also chemical composition and microbial community analyses are needed to gain mechanistic insights into factors controlling the bioavailability of riverine DOM in estuaries and bays.

Rivers and bays in southern Texas are ideal systems to evaluate the bioavailability of riverine DOM in estuaries and the associated biogeochemical processes because of their unique characteristics. The bays receive freshwater from rivers in an episodic mode with extremely low yearlong base flow interspersed with storms, which often supply over 90% of the annual discharge; these bays are shallow (<1 m) and have limited water exchange with the Gulf of Mexico (Mooney and McClelland, 2012). These characteristics make estuaries in this region respond rapidly to the freshwater inflows, which affect the biogeochemical cycling and net ecosystem metabolism (Russell and Montagna, 2007; Shank et al., 2009; Bruesewitz et al., 2013; Reyna et al., 2017). For example, storm events decouple N and C cycles in the Copano Bay of southern Texas due to the input of bioavailable riverine organic matter (Bruesewitz et al., 2013). However, little is known about the bioavailability of riverine DOM to the bacterial community in southern Texas coastal seawater. Quick remineralization of DOM may be important to supplying inorganic nutrients to primary producers in these bays and estuaries, which are often severely limited by nitrogen (Pennock et al., 1999; Brock, 2001; Gardner et al., 2006), yet currently there is no data about bioavailable DOM. Through incubation experiments as well as chemical (THAAs) and bacterial community analyses, our objectives of this study are to: (1) quantify the bioavailability of riverine DOC and DON from south Texas rivers to coastal bacteria in adjacent bays, and (2) evaluate the influence of sources and seasons on the riverine DOM bioavailability and its linkage with bacterial community and functional genes.

2. Materials and methods

2.1. Study sites

DOM samples were collected in the up-river mouth region (0 salinity) of five rivers along the coast of southern Texas (Fig. 1), including: Lavaca, San Antonio, Mission, Aransas, and Nueces Rivers, during winter (January 2016) and summer (July 2016). These five rivers discharge into Lavaca Bay, San Antonio Bay, Corpus Christi Bay, and Copano Bay, respectively. The watersheds of these rivers differ in size, land use, and land cover (Table S1). The Aransas and Nueces catchments include primarily cultivated crops and multiple wastewater treatment plants, while the Mission river is relatively pristine, dominated by shrubs less than 5 m tall (Bruesewitz et al., 2013; Reyna et al., 2017). The catchment of Lavaca River has mixed land-cover distribution with the cultivated crops and forests (Mooney and McClelland, 2012). The San Antonio River catchment is dominated by the shrub/scrub, forest, and agricultural lands (Arismendez et al., 2009). The San Antonio River flows through the city of San Antonio, which is the second largest city in Texas, and is highly influenced by human activities (Kreuter et al., 2001; Sahoo and Smith, 2009).

2.2. Sample collection

All plastic bottles used in this work were acid-cleaned. Glassware and GF/F filters (Whatman, pore size 0.7 μm) were pre-combusted at 500 °C to render them carbon-free. River water was collected using a Van Dorn sampler, and the sample was transferred to high-density polyethylene bottles (1 or 4 L) that were first rinsed several times with the sample water. Salinity, pH, dissolved oxygen, conductivity, and temperature were measured *in situ* at each site using a YSI Sonde. River samples for the chemical analyses, including NH_4^+, NO_3^- + NO_2^-, soluble reactive phosphorus (SRP), TDN (total dissolved nitrogen), DOC, and TDAA, were syringe-filtered (0.2 μm polycarbonate) on site. All water samples were stored on ice and transported to the laboratory the same day, where they were filtered through 0.2 μm cartridge filters for the incubation experiments. Coastal bacterial inoculums were collected using 2 L polyethylene bottles at the Port Aransas ship channel (27.84°N, 97.05°W, S6 in Fig. 1), which is connected to the open Gulf of Mexico,

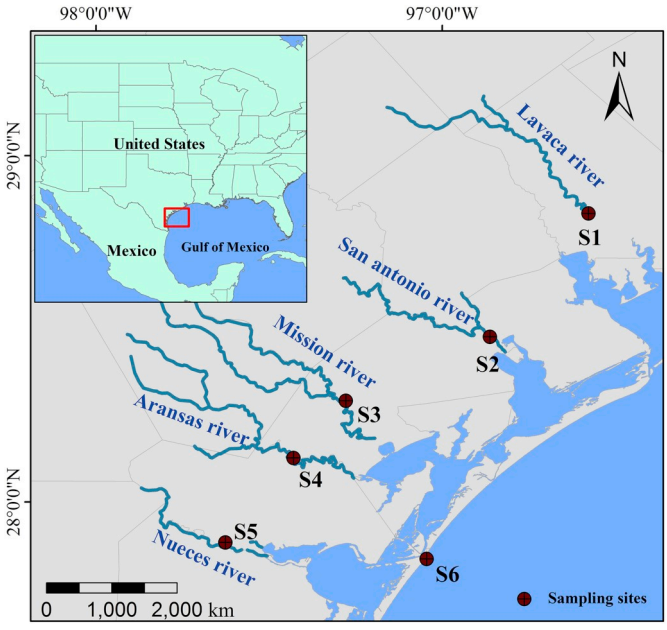

Fig. 1. Map of south Texas showing the sampling sites (S1, S2, S3, S4 and S5) located in the lower reaches of five south Texas rivers (S6 denotes the sampling station for the coastal inoculum near the south Texas bays).

and filtered through 0.8 μm nylon filters to remove larger organisms and particles.

2.3. Incubation experiments

Given the fact that the degradation of DOM is highly specific to microbial organisms, the most important assessment in DOM bioavailability would be those in the recipient environment, such as the estuarine environment to which the riverine DOM is discharged in this study. We used the natural coastal microbial assemblage collected from the Port Aransas ship channel to assess the bioavailability of riverine DOM in the coastal ocean (Site S6 in Fig. 1). Bacterial consortium from the ship channel served as a composite inoculum of brackish bay water and saline seawater. Salinity generally drops to less than 20‰ around 10 a.m. and increases to over 30‰ in the early afternoon (System-Wide Monitoring Program, SWMP, https://missionaransas.org/science/research), indicating exchanges of bay water and Gulf water on a daily basis. Salinity of the coastal seawater collected from the ship channel was 30 and 32 during summer and winter, respectively, which was slightly higher than those (25.8–29.6) in the bays measured during the bay cruise at the same month, further confirming the mixing of bay water and Gulf water at the ship channel. Similar designs are also reported in previous studies (Seitzinger and Sanders, 1997; Wiegner and Seitzinger, 2004; Wiegner et al., 2006). A same bacterial consortium for all the winter or summer incubations also helped reduce the number of variables and focus on the comparison of DOM bioavailability from different rivers. Coastal bacterial consortium was added to the filtered river waters, and then the changes of DOC/DON and bacterial community structure were monitored over time.

We used river water directly without modifying the salinity of the incubation medium because (1) the introduced salts could potentially contaminate the solution if they contain organic matter, and (2) this setup represents the adjacent estuaries where the salinity often drops to zero during the high flow periods (Mooney and McClelland, 2012; Reyna et al., 2017). The relative stable bacterial community structure during the incubation further demonstrated the adaptability of the coastal bacteria to salinity changes (see Section 3.3).

The filtered (0.8 μm pore size) inoculating coastal seawater (50 mL) was added to 1 L of each filtered (0.2 μm pore size) river water and control waters (deionized water). A pore size of 0.8 μm is able to remove most, if not all, of the eukaryote predators efficiently (Massana, 2011). The mixed ratio (1/20) in this work was similar to other incubation experiments (Rochelle-Newall et al., 2004; Petrone et al., 2009). The incubation experiments lasted about 3 weeks at close to *in situ* temperature (14 °C in winter, 31 °C in summer) under dark. The bioavailable DOM is expressed as percent of consumed DOM at the end of the incubation (DOM consumed/initial DOM concentration × 100%). The added inoculating coastal seawater might contain bioavailable DOM, but the coastal seawater only represented 5% of the total water volume, thus the contribution of inoculated DOM to the bioavailable DOM should be negligible. This conclusion is further confirmed by the insignificant changes of bacterial abundance and DOC in the control waters (deionized water) during the incubations.

At each time point (day 0, 1, 2, 3, 5, 7, 9, 11, 13, 24), duplicate aliquots from the bottles were collected and filtered through 0.2 μm syringe filters and then stored under −20 °C for later chemical analyses (NH_4^+, $NO_3^- + NO_2^-$, SRP, TDN, DOC, and TDAA). An aliquot (1 mL) from each incubation bottle was also collected and preserved in 3% formaldehyde at 4 °C for bacterial enumeration. An aliquot (15 mL) was filtered through 0.2 μm polycarbonate filter, and the filters were preserved at −80 °C for bacterial community structure analysis. The impact of water sampling on the incubation was minimized by retaining more than 50% of water in the bottles at the end of the incubation.

2.4. Chemical measurements

Dissolved combined amino acids (DCAA) were hydrolyzed into individual amino acids using 6 M HCl under nitrogen at 110 °C for 20 h (Kuznetsova and Lee, 2002). Then, TDAA, including DCAA and dissolved free amino acids (DFAA), were measured using HPLC with fluorescence detection after pre-column *o*-phthaldialdehyde derivatization (Lee et al., 2000; Liu et al., 2013). The relative standard deviations of TDAA in replicate samples were within 20%. DOC and TDN were measured using a Shimadzu total organic carbon/TDN analyzer (TOC-V/TDM-1), and the precision of results between duplicate samples agreed within 6%. Nitrate and nitrite were measured using the cadmium reduction method; SRP was measured using ascorbic acid method and ammonium using indophenol method through a UV–Vis spectrophotometer (Strickland and Parsons, 1968). DON concentrations were estimated by subtracting NO_3^-, NO_2^- and NH_4^+ concentrations from TDN.

2.5. Bacterial abundance, community structure and functional gene analyses

Bacterial cells were enumerated using an Accuri C6 Flow Cytometer (FCM) system with a laser emitting at 488 nm (Marie et al., 1997). Details of the method are provided in Liu et al. (2013). Briefly, a 5 μL SYBR Green II (Molecular probes) working solution (1:100 v/v) was added to 500 μL samples, which were stored in the dark for at least 15 min before analysis. Validation beads (Polysciences) served as a calibration reference daily. Bacterial cell counts were detected on a two-dimensional dot plot (size scatter versus fluorescence signal). Counting error of bacterial abundance was within 11% for duplicate samples.

DNA on filter samples were extracted with PowerLyzer®PowerSoil® DNA Isolation Kit (Catalog Number 12855-100; Mo Bio). The DNA extracts were sent to Research and Testing Laboratory (Lubbock, TX) for 16S rRNA Illumina sequencing. Subsequent community structure analysis was performed using MOTHUR (version 1.40.5; Schloss et al., 2009). A further prediction of functional genes was conducted based on 16s rRNA marker gene with the bioinformatic tool Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt; Langille et al., 2013). The input ".biom" file for PICRUSt was created with the MOTHUR command "make.biom". PICRUSt predictions were conducted on closed OTUs at the 97% similarity level following a previous study (Liu et al., 2017). Metabolic pathways and functional genes abundance on copy-number-normalized OTUs were made online using the Galaxy server (Goecks et al., 2010) at http://huttenhower.sph.harvard.edu/galaxy/.

2.6. Statistical analysis

The amino acid compositional and bacterial community data were analyzed by principal component analysis (PCA) using Matlab (version 2012b). The mole percentages of individual amino acids were applied as statistical variables. The optimal weights were derived by the principle of least squares, similar to linear regression. The significant difference between data in this paper were tested by independent-samples-t-test (SPSS 16.0).

3. Results

3.1. Chemical characteristics of the initial river water

DOC concentrations of the five rivers were in the ranges of 222–779 μM and 208–568 μM during winter and summer, respectively, with the highest value in the Nueces River and lowest in the San Antonio River (Table 1). DON concentrations were in the ranges of 18.4–40.8 μM and 26.9–44.9 μM during winter and summer, respectively, with relatively higher values in the Nueces River and lower values in the Lavaca River. Seasonally, DOC concentrations were significantly higher during

Table 1
Chemical characteristics of southern Texas river waters in winter (January) and summer (July) 2016. The error bars represent standard deviations of triplicate samples.

Rivers	Date 2016	DOC (μM)	DON (μM)	NH_4^+ (μM)	NO_3^- + NO_2^- (μM)	SRP (μM)	C/N in DOM	TDAA (μM)	TDAA-C/DOC (%)	DON/(DON + DIN)
Lavaca	Jan. 15	449.3 ± 4.6	18.4 ± 1.8	1.9 ± 0.2	12.1 ± 0.8	2.8 ± 0.2	24.4 ± 2.4	2.1 ± 0.1	1.6 ± 0.1	0.56 ± 0.06
San Antonio	Jan. 15	221.7 ± 2.6	31.4 ± 2.3	1.2 ± 0.1	113.7 ± 1.1	6.2 ± 0.2	7.1 ± 0.5	1.3 ± 0.05	2.2 ± 0.1	0.22 ± 0.02
Mission	Jan. 15	561.1 ± 4.9	24.6 ± 1.9	0.9 ± 0.2	1.8 ± 0.5	0.07 ± 0.03	22.8 ± 1.8	2.5 ± 0.1	1.8 ± 0.2	0.90 ± 0.09
Aransas	Jan. 15	396.2 ± 4.7	23.5 ± 2.5	1.1 ± 0.3	24.2 ± 0.8	13.0 ± 0.7	16.8 ± 1.8	5.0 ± 0.2	5.1 ± 0.4	0.48 ± 0.06
Nueces	Jan. 15	778.8 ± 5.4	40.8 ± 2.4	6.8 ± 0.5	8.1 ± 0.8	2.7 ± 0.3	19.1 ± 1.1	6.5 ± 0.3	3.2 ± 0.4	0.73 ± 0.05
Lavaca	Jul. 16	463.9 ± 2.7	26.9 ± 1.4	2.6 ± 0.2	7.6 ± 0.03	2.4 ± 0.2	17.2 ± 0.7	3.6 ± 0.1	2.9 ± 0.2	0.72 ± 0.04
San Antonio	Jul. 16	208.5 ± 0.7	27.5 ± 2.3	0.6 ± 0.3	68.5 ± 0.5	2.1 ± 0.3	7.6 ± 0.9	2.3 ± 0.2	4.0 ± 0.3	0.30 ± 0.04
Mission	Jul. 16	427.9 ± 5.4	27.1 ± 1.6	0.3 ± 0.1	0.4 ± 0.02	0.3 ± 0.05	15.8 ± 0.9	3.9 ± 0.3	3.3 ± 0.5	0.96 ± 0.08
Aransas	Jul. 16	414.0 ± 4.6	30.5 ± 1.8	0.5 ± 0.1	0.3 ± 0.08	17.9 ± 0.3	13.6 ± 0.7	4.8 ± 0.3	4.3 ± 0.4	0.98 ± 0.07
Nueces	Jul. 16	567.6 ± 5.6	44.9 ± 3.2	7.1 ± 0.4	6.5 ± 0.7	4.8 ± 0.1	12.6 ± 0.9	6.0 ± 0.2	3.7 ± 0.3	0.77 ± 0.07

winter than in summer ($P < 0.05$) in the Mission and Nueces Rivers, while they remained relatively constant in other rivers. DON concentrations were generally higher in summer than in winter for each river, but not statistically different (paired *t*-test; $P = 0.502$).

Dissolved inorganic nitrogen (DIN) concentrations ranged from 2.7–115 μM and 0.7–69 μM during winter and summer, respectively (Table 1), with the lowest value in the Mission River. The highest DIN concentration (115 μM, dominated by nitrate and nitrite) was observed in the San Antonio River, suggesting that it was highly influenced by the wastewater treatments and human activities. SRP concentrations ranged from 0.07 to 13.0 μM and 0.3–17.9 μM during winter and summer, respectively, with highest concentrations in the Aransas River and the lowest in the Mission River. The low DIN and SRP concentrations in the Mission River indicated its relatively pristine nature. The wide concentration ranges of inorganic nutrients across the south Texas rivers suggested that the influence of human activities on the catchments of these rivers differed greatly.

The C/N ratios of DOM in the rivers ranged from 7.1–24.4 and 7.6–17.2 during winter and summer, respectively. San Antonio River had particularly low ratios in both seasons (Table 1). TDAA concentrations ranged from 1.3–6.5 μM and 2.3–6.0 μM during winter and summer, respectively, with high values in the Aransas and Nueces Rivers and low values in the San Antonio River. The initial C and N in TDAA (TDAA-C, TDAA-N) in all the rivers accounted for 1.6–5.1% and 2.9–4.3%, 4.7–25.8% and 8.6–16.4% of DOC and DON, respectively, with highest values in the Aransas River. Although the average TDAA-C/DOC% was relatively high in summer (3.6%) compared to winter (2.8%), no statistical difference was observed ($P = 0.247$). The average TDAA-C/DOC % and TDAA-N/DON% of these rivers were comparable with previous reports in lakes and marine environments (Jørgensen et al., 1999).

Glycine (Gly) was the most abundant amino acid (19–38%) of the TDAA in the initial river water, followed by alanine (Ala, 8–14%), aspartic acid (Asp, 5–13%), glutamic acid (Glu, 6–12%), serine (Ser, 4–12%) and threonine (Thr, 3–10%). Compared to the mole fractions of amino acids in winter, Gly% and β-aminobutyric acid (GABA)% exhibited an increase from 24.5 ± 4.4% to 31.8 ± 4.1%, and 1.8 ± 0.7% to 3.4 ± 0.4% in summer, respectively. Asp and Met were relatively more abundant in winter than in summer. We further applied PCA on amino acid composition (mol%) data to explore the diagenetic states of DOM in the rivers (Fig. 2). Principle component 1 (PC1) and principle component 2 (PC2) explained 37% and 27% of the data variance, respectively, with winter samples spread along the positive direction of PC2, whereas summer samples along the negative direction but more compacted (Fig. 2b), demonstrating compositional difference between winter and summer DOM samples. This seasonal separation could be explained by the relatively higher mol% of Gly, Leu and GABA in the summer samples, and the relatively higher mol% of Arg, Phe and Met in the winter samples (Fig. 2a).

3.2. Changes of nutrients, DOM, BDOC and BDON during incubation

Concentrations of different inorganic nutrients (SRP, NH_4^+, and NO_x), except for slight decreases in only a few cases (e.g., NH_4^+ in AR in summer, NH_4^+ in NR in winter, and NO_x in LR in winter, etc.), generally increased throughout both the summer and winter incubations (Table S2). This change is more evident in winter incubations than that in summer. The fact that nutrients was not quickly depleted suggested that inorganic nutrients were sufficient and not a limiting factor for the

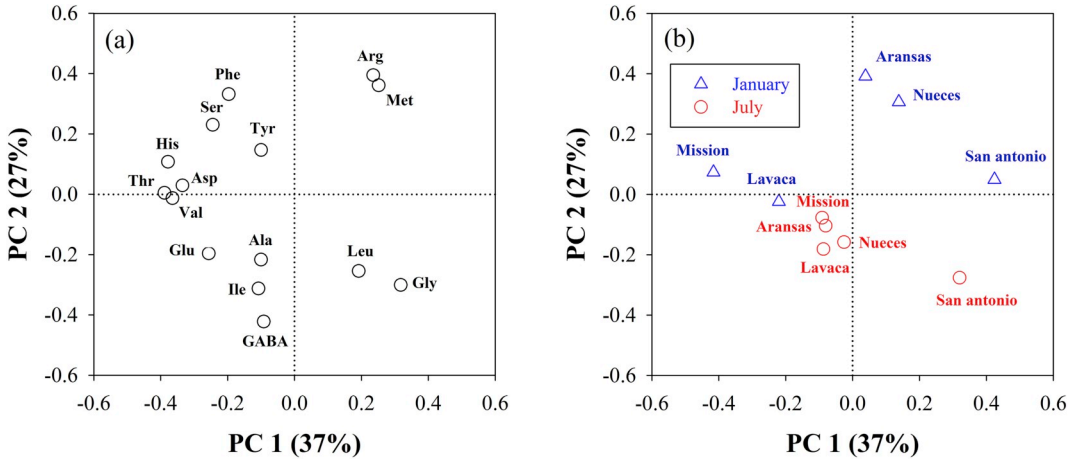

Fig. 2. (a) Amino acids loadings plot and (b) sample scatter plot of the PCA derived from the mol% of the individual amino acids of the south Texas rivers in January and July 2016. (The blue triangles denote the winter, while the red cycles denote the summer). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

bacterial growth during the degradation of riverine DOM.

During the incubation, there was substantial loss of DOC (24.4–58.9 μM) and DON (3.2–9.0 μM) for all the rivers during winter, which were higher than those (0–20 μM and 2.8–4.7 μM) in summer (Fig. 3a, b, c, d). The net removal of DOC and DON were highest in the Nueces and Aransas Rivers during winter, but comparable among the rivers during summer (Fig. 4a and b). Small net increases of DIN concentration with time were observed for both winter and summer incubations, indicating the remineralization of riverine DON by the bacterial consortia (Fig. 3e and f). Meanwhile, large decreases in TDAA concentrations were observed, with respective ranges of 17–36% and 12–73% utilized during the winter and summer incubations (Fig. 3g and h), suggesting a more bioavailable nature of TDAA relative to DOM.

Furthermore, we estimated that BDOC and BDON accounted for 6–11% and 0–6%, and 15–38% and 9–15% of DOC and DON in the rivers during winter and summer, respectively (Fig. 4c and d). These values were comparable with the results from the rivers of the eastern United States (23% of DON and 4% of DOC, Moran et al., 1999; Wiegner et al., 2006). BDOC% and BDON% showed spatial and temporal variations across the five Texas rivers (Fig. 4c and d). BDOC concentrations were relatively low in the San Antonio River, but accounted for the highest fraction of DOC for both seasons (11% and 6%, Fig. 4c), while the BDOC % was lowest in the Mission River for both seasons (6% and 0%, Fig. 4c). However, BDON% for different rivers in different seasons were similar within 9–19%, except a high value (38%) observed in the Aransas River during winter (Fig. 4d). Seasonally, both BDOC% and BDON% were significantly higher in winter (8 ± 2% and 21% ± 10%) than those in summer (3 ± 2% and 12 ± 3%) ($P = 0.008$; Fig. 4c and d), indicating that riverine DOM was more bioavailable in winter. BDON% (15–38% and 9–15% in winter and summer) was higher than BDOC% (6–11% and 0–6% in winter and summer), indicating a more labile nature of nitrogen-containing molecules. This pattern is consistent with previous studies reporting that nitrogen-rich components of riverine DOM were utilized preferentially (Wiegner et al., 2006; Petrone et al., 2009).

3.3. Changes of bacterial community structure and functional genes during incubations

Bacterial abundance increased initially in all river samples, but showed different patterns between winter and summer (Fig. 5). In winter, bacterial abundance in Nueces, Lavaca, Mission, and Aransas Rivers increased rapidly within the first 5–10 days, and then decreased to near the initial levels by the end of incubation. The bacterial abundance in San Antonio River increased slowly until day 12 and then remained relatively constant through the end of the incubation. In contrast, bacterial abundances in summer incubations increased rapidly and slowed down after day 5. At the end of the incubation, bacterial abundances were 1–2 times higher in summer than in winter except for the San Antonio River, where the bacterial abundances were similar between the seasons.

The initial bacterial communities differed between summer and winter, but shared a similar diversity on Phylum level (averaged Chao1: 10.75 in summer vs. 10.10 in winter). While bacterial communities in summer were dominated by *Proteobacteria* (>50%) at Phylum level (day 0 for Aransas River was not sequenced due to sample loss), those in winter included *Cyanobacteria* (>3%) and *Bacteroidetes* (>20%) besides *Proteobacteria* (Fig. S1). In the summer incubation, *Planctomycetes*, *Bacteroidetes*, *Actinobacteria* and *Firmicutes* developed, but overall *Proteobacteria* remained the dominant phylum, despite the changes on Genus level (Fig. S2). In winter, *Bacteroidetes* and *Cyanobacteria* decreased as the incubation proceeded, while *Planctomycetes* developed from nearly undetectable to >10% (except for the Nueces River incubation). This change of bacterial community structure can be visualized better by PCA, following the published work (Liu and Liu, 2016). Almost

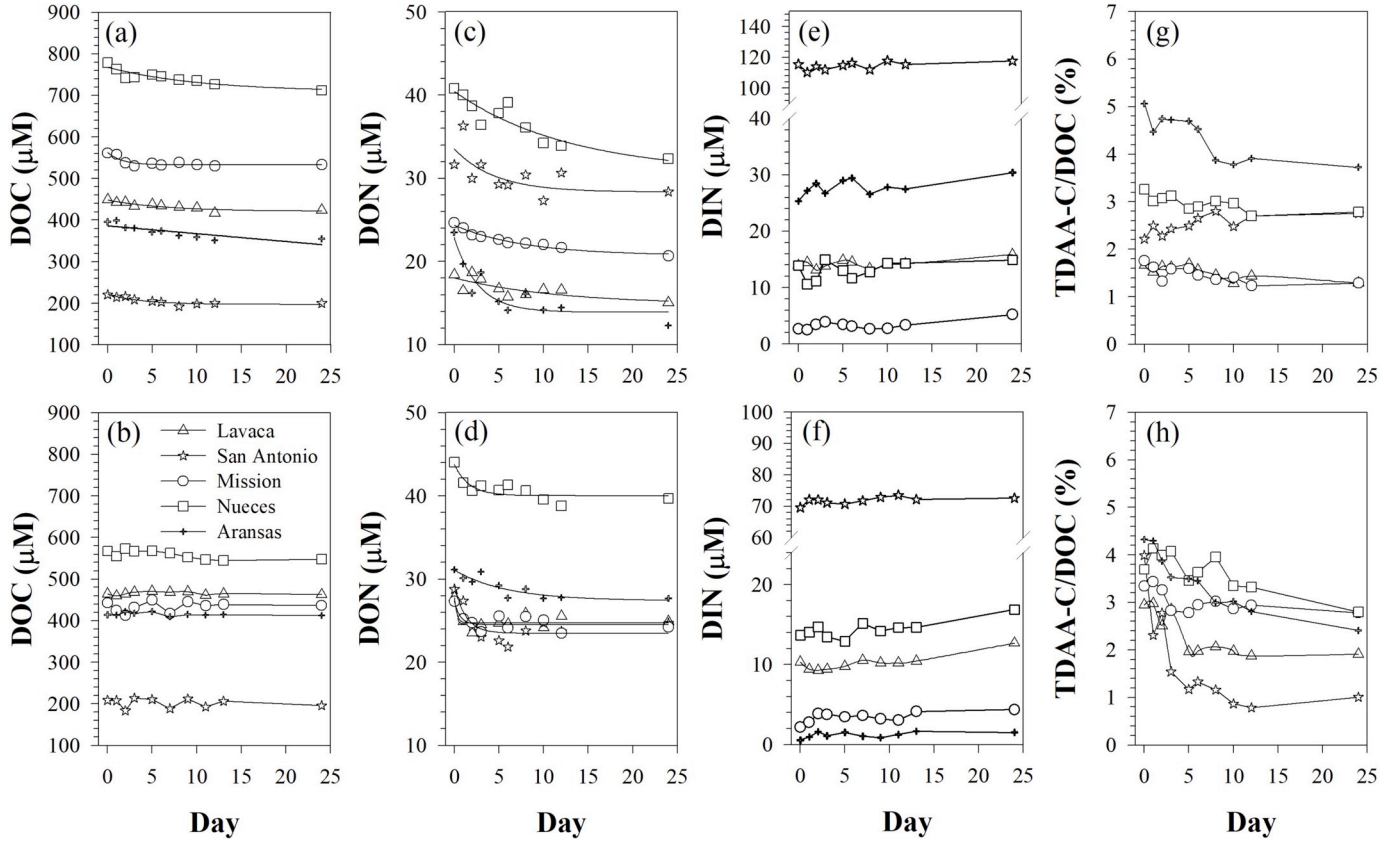

Fig. 3. Changes in DOC (μM) (a, b), DON (μM) (c, d), DIN (μM) (e, f), and TDAA-C/DOC (%) (g, h) during the incubation experiments in winter (14 °C) (a, c, e, g) and summer (31 °C) (b, d, f, h) 2016.

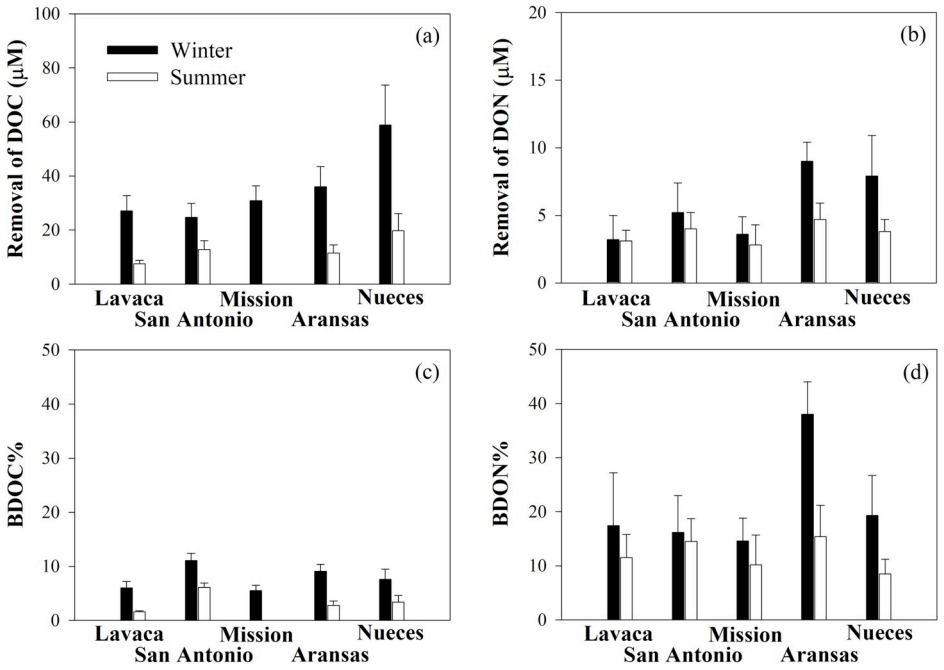

Fig. 4. Concentration of bioavailable DOC (a) and bioavailable DON (b); percentage of BDOC (c) and BDON (d).

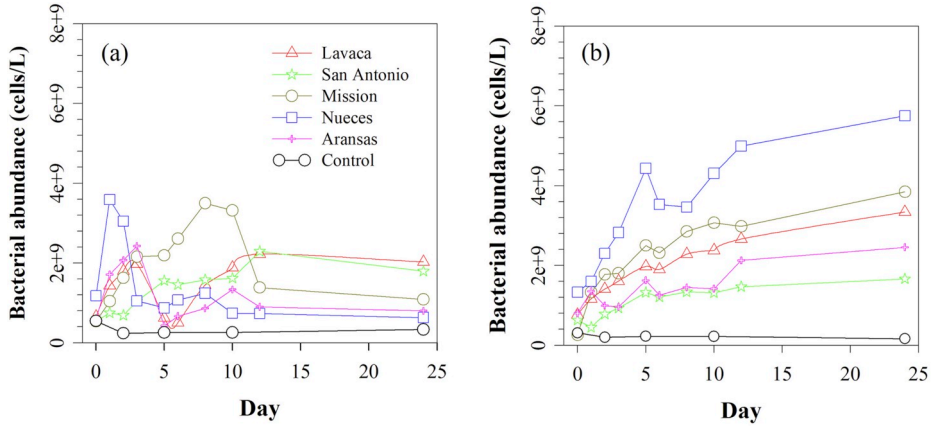

Fig. 5. Changes in bacterial abundances during the incubation experiments in winter (a) and summer (b) 2016 for the five south Texas rivers.

all summer samples were clustered together regardless of sampling time, whereas all winter samples shifted to *Planctomycetes* and *Verrucomicrobia* (Fig. 6). By the end of the incubation (day 24), the taxonomic richness, estimated by the Chao1 index, was higher in winter than that in summer on both Genus (averaged: 131 vs. 78; $P = 0.02$) and Phylum levels (averaged: 13 vs. 8; $P = 0.05$). Overall, the bacterial community structures across different rivers remained similar during the 24-day incubation within the same season, but summer samples were grouped more tightly and less diverse than winter samples (Fig. 6).

Predictions based on 16S rRNA community data via PICRUSt indicated that the abundance of nitrogen metabolism related genes generally decreased in the summer incubation but increased during winter (Supporting Information Section 1). PCA based on selected predicted functional gene abundance showed that summer samples were clustered closely, while winter samples were more spread (Fig. S5).

4. Discussion

4.1. Factors affecting the riverine DOM bioavailability

The potential bioavailability of DOC and DON in different seasons can be indicated by TDAA composition. The percentage of Gly (Gly%) relative to other amino acids increases as degradation proceeds (Lee et al., 2000; Benner and Kaiser, 2011). The percentage of GABA, as decay product of glutamic acid, also increases due to microbial activity (Lee and Cronin, 1984). In addition, Phe and Met are enriched in labile organic matter such as algae (Yamashita and Tanoue, 2003). Thus, the different amino acid compositions of DOM between winter and summer on PC1 axis (Fig. 2; summer enriched with GABA and Gly and winter with Phe and Met), suggests that DOM in the rivers was altered less diagenetically during winter than in summer. Consistently, the incubation results showed the relatively high BDOC% and BDON% during winter (Fig. 4).

Given that all incubation experiments of each season were conducted under the same conditions (darkness, temperature, incubation time, and initial bacterial community), DOM composition and other inorganic

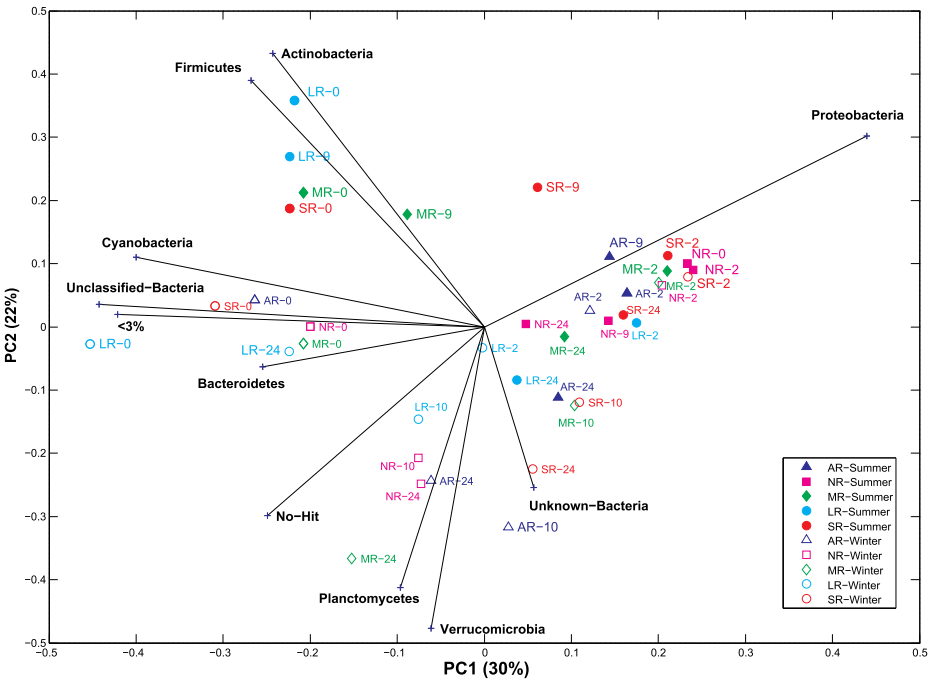

Fig. 6. PCA on bacterial community on Phylum level. MR: Mission River, AR: Aransas River, LR: Lavaca River, SR: San Antonio River, NR: Nueces River. The numbers after the dash represent the incubation dates.

parameters such as nutrient levels are assumed to be the only variables determining the riverine DOM bioavailability. We correlated the BDOC % and BDON% with the bulk DOC and DON concentrations, and other indicators (i.e., C/N ratios and TDAA yields). Significant positive correlations were found between BDOC and DOC concentrations ($R^2 = 0.72$, $P = 0.038$) in winter (Fig. 7a), implying that a relatively constant proportion of the DOM was bioavailable in winter. Significant negative correlations between BDOC% and C/N ratios were also found in winter (Fig. 7c). Although minor changes of DOC were observed during the summer incubation, the negative trends between BDOC% and C/N ratios were detectable during summer. These results suggested that DOM concentration and C/N ratio, reflecting the quality of DOM, both play a role in controlling the amount of bioavailable DOM in these Texas rivers.

However, no significant relationships were observed between BDON and DON in either season, suggesting that DON concentration is not the determining factor of BDON. The relation between BDON% and C/N ratios was not significant (Fig. 7d), further confirming the decoupling of bioavailable DOC and DON. However, significant positive relations between BDON% and TDAA-N% were found during winter ($R^2 = 0.85$, $P = 0.026$) and summer ($R^2 = 0.75$, $P = 0.037$), indicating the significant contributions of TDAA to the bioavailable DON pool (Fig. 7f). The slope of the linear regression of BDON% vs TDAA-N% was significantly higher in winter (1.08) than that in summer (0.38), suggesting the higher contribution of TDAA to bioavailable DON in winter.

The plot of BDOC vs. BDON further shows that the C/N ratio of bioavailable DOM is distinct from that of bulk DOM (Fig. 7g). The C/N ratios of the bioavailable DOM were mainly constrained in the range of 3–9 (Fig. 7g), which were significantly lower than the initial C/N ratios of DOM (7.1–22.4). This pattern showed that the DOM components with low C/N ratios were preferentially used by the coastal bacteria. The C/N ratios of bioavailable DOM were close to the Redfield ratio (106:16), pointing to the contributions of *in situ* biological production to the bioavailable DOM pools. Indeed, substantial amounts of bioavailable DON in the Baltic rivers are autochthonous (Stepanauskas et al., 2002). Similar to our results from sampling sites in the lower reaches of south Texas rivers, high DOM bioavailability was also reported in the lower parts of large rivers such as Mississippi River due to the contribution of bioavailable DOM by primary production (Hedges et al., 1994; Benner et al., 1995). Compared with the Mississippi River (TDAA-C/DOC % = 0.85–1.4%) (Shen et al., 2012), the southern Texas rivers had higher TDAA-C/DOC% (1.6–5.1% and 2.9–4.3%), suggesting that DOM in this region may be more bioavailable.

In addition to DOM chemical composition, the availability of nutrients and micronutrients can affect DOM bioavailability. No significant correlations were found between the initial NH_4^+ and $NO_3^- + NO_2^-$ concentrations and BDOC/N% ($P > 0.05$), suggesting that DIN did not limit the bioavailability of riverine DOM. However, the initial SRP concentrations and BDOC/N% were correlated ($P < 0.05$) during our winter experiments, suggesting that SRP availability could stimulate the utilization of riverine DOM by coastal bacteria. Similar results were observed in the wetland and coastal waters, where SRP may limit the growth of bacteria and their utilization of labile DOM (Wiegner and Seitzinger, 2004; Liu and Liu, 2016).

Overall, the quantity and sources of DOM controlled the bioavailability of DOC in the rivers, but the DON bioavailability was controlled by the bioavailable N components such as dissolved amino acids. Despite the comparable quantity of DOM in the different Texas rivers between winter and summer, riverine DOM was more bioavailable in winter than that in summer, which was likely associated with the quality of DOM and the ability of bacterial consortium. High temperature may enhance DOM processing due to the increased bacterial production and respiration (Berggren et al., 2010; Asmala et al., 2013). However, we found higher bioavailable fractions of DOM in the winter than the summer incubation despite the fact that temperature in summer (averaged 31 °C) was much higher than that in winter (14 °C). This pattern suggested that the high labile nature of winter DOM may have dominated the temperature effect.

4.2. *Response of bacterial community to riverine DOM*

Another goal of this study was to examine how the coastal bacterial community respond to the environmental variables, including riverine DOM quality, nutrients, and temperature. During the incubations, the net increase of bacterial abundance at the end of the incubation in summer was much higher than that in winter. However, the net

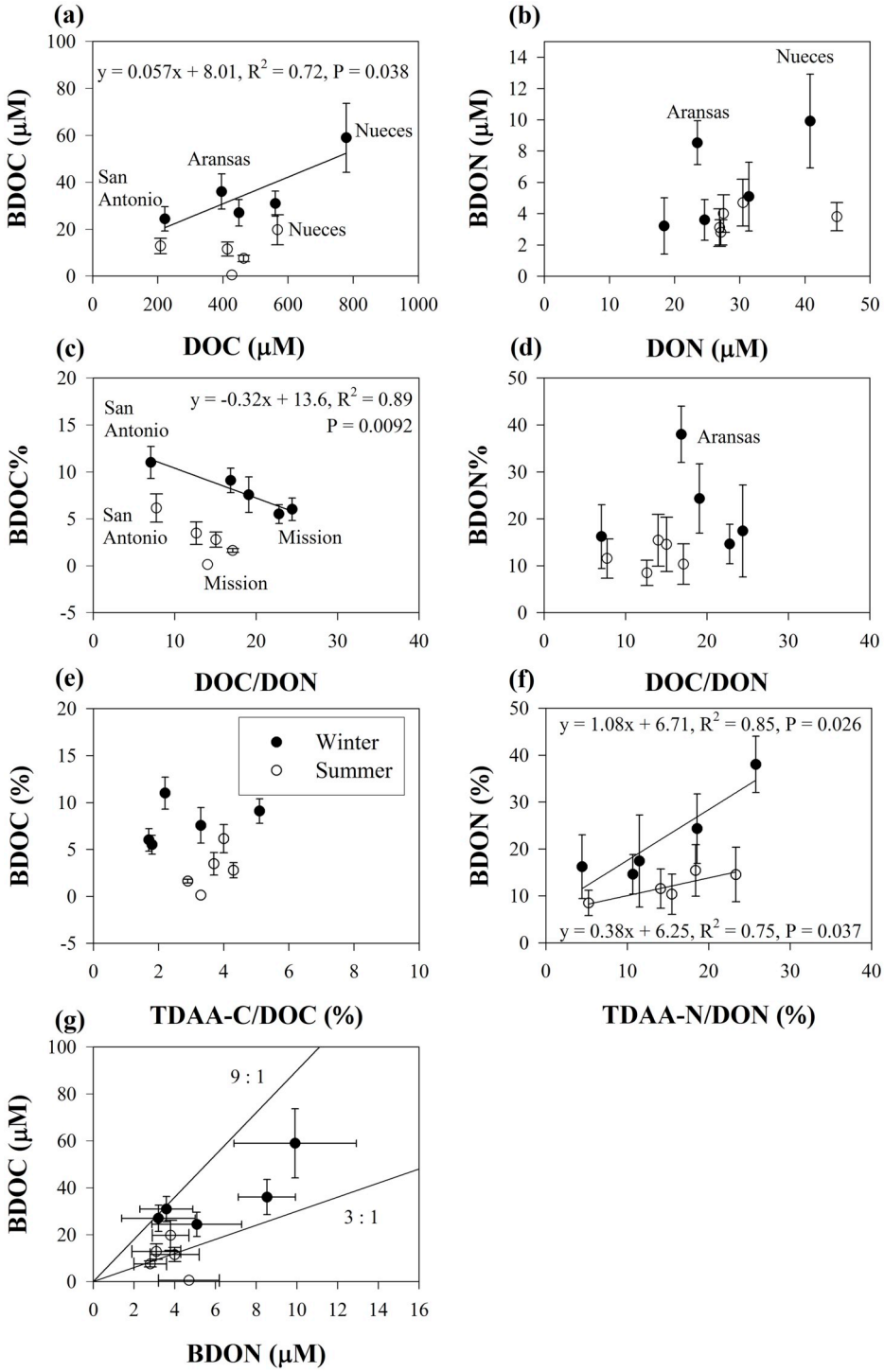

Fig. 7. Relationships between bioavailable DOC/N and its initial DOC/N (a, b), BDOC% and BDON% and DOC/DON ratios (c, d), BDOC% and BDON% and TDAA-C/DOC% and TDAA-N/DON% (e, f), and BDOC and BDON (g). (All values are average amounts, and bars represent the standard errors. The dark dots denote the winter, while the white dots represent the summer. The linear regressions used in the (a) and (c) only include the winter data.

removals of DOC by the bacteria in winter (24.4–58.9 μM) were significantly higher than those (0–20 μM) in summer. A rough calculation showed that bacterial growth efficiency (BGE) was higher in summer (averaged 22.3%) than that in winter (averaged 10.5%; Supporting Information Section 2). Previous studies showed that the consumption of molecules with low C/N ratios resulted in more efficient growth of bacteria (Sun et al., 1997; Hopkinson et al., 1998). Therefore, bioavailable DOM components with relatively low C/N ratios (Fig. 7g) in summer might be more favorable for bacterial growth. Alternatively, high temperature may support increased bacterial growth in summer (Raymond and Bauer, 2000; Hoppe et al., 2002). Combined with the net DOC consumptions and the trends of bacterial abundance during the incubations, we inferred that BGE varied in the Texas rivers (Table S3), which could be attributed to the riverine DOM quality and temperature. Consequently, the functional response of coastal bacteria to riverine DOM would potentially influence the food webs and the cycling of carbon and nitrogen in the bays.

It remains controversial how the labile DOM shifts the bacterial

community structure. Previous studies suggested the addition of labile DOM could lead to either a more diverse bacterial community (e.g., Pérez and Sommaruga, 2006; Blanchet et al., 2017; Jessen et al., 2017), or a more convergent community (e.g., Findlay et al., 2003). In this work, statistical analysis showed that bacterial community in the summer incubation (less labile DOM) were less diverse than those in the winter incubation (more labile DOM). Taxonomic richness Chao1 index was higher in winter on both Genus level (averaged: 131 in winter vs. 78 in summer; $P = 0.02$) and Phylum level (averaged: 13 in winter vs. 8 in summer; $P = 0.05$). PCA analysis based on bacterial community structure (Fig. 6), in which summer samples are grouped more closely than winter samples. Furthermore, the functional genes in the summer incubation were less diverse than those in the winter incubation (Fig. S5). We speculated that this observation may result from higher BDOC and BDON (in terms of both concentration and percentage) in winter season (Pérez and Sommaruga, 2006; Blanchet et al., 2017; Jessen et al., 2017), whereas limited resources may have constrained the development of the bacterial community in summer. Note that all DOM samples in this study were natural substrates, different from those in typical addition experiments using single labile substrates. Future work examining the molecular level information (i.e., molecular-level formula, functional groups, and structures) of the DOM community of these rivers across different seasons will help shed light on mechanisms underlying this intriguing phenomenon.

In winter incubations, the change in bacterial community, reflected by functional gene abundance, was related to the sampling time points, but not to sampling locations (Fig. S5). This observation indicates that riverine DOM shares a similar stimulation effect in bacterial growth, in spite of different chemical properties among the rivers (i.e., DOC concentration, DON concentration, DIN concentration, SRP concentration, C:N ratio, and etc.; Table 1). It is possible that certain groups of organic compounds may have played a key role in bacterial development. More empirical data is needed to test this hypothesis.

Further correlation analysis of data from the summer incubation, shows that the concentration of SRP was correlated negatively with the activity of the N metabolism pathway, as well as with several N metabolism-related genes including ammonia oxidation genes and amino acid decarboxylase (Fig. S6a). This pattern is consistent with the positive relationship observed between BDON and SRP. In the winter incubation, correlations between chemical properties (DOC, DON, DIN and SRP) and biological data were weak (Fig. S6b). The difference in correlations across different seasons may result from contrasting bacterial responses. Riverine bioavailable DOM supported more bacterial growth in summer, during when nutrients are assimilated causing negative correlations. Bioavailable DOM supported much more bacterial respiration in winter than in summer. With more organic matter being respired to inorganic nutrient, the correlation between chemical parameters and bacterial metabolisms was not as evident as that in summer.

4.3. Biogeochemical implications of bioavailable riverine DOM to the coastal ecosystems

In addition to inorganic nutrients, an understanding of the organic fraction of C and N is crucial in elucidating biogeochemical processes in coastal ecosystems (i.e., Seitzinger and Sanders, 1997; Seitzinger et al., 2002; Berman and Bronk, 2003; Cira et al., 2016; Wetz et al., 2017; Montagna et al., 2018). River input and biological production are the two major sources of DOC in estuaries. Although DOC concentrations in the south Texas rivers were comparable between winter and summer, the relatively low bioavailability of riverine DOC to coastal bacteria observed in summer may imply that more riverine DOC would remain in the bays during summer, or be respired during winter. In other words, the relative contribution of river input to the accumulation of DOM in the south Texas bays may vary, which is related to the spatial and seasonal variation of DOM, and its fate and transport in the bays. River discharge may also be a factor affecting DOM bioavailability, as high-flow events can mobilize a new pool of DOM that is not accessible in base-flow conditions (Lu and Liu, 2019). However, in this work the average flow rates in January (9.9–459.5 ft^3/s) and July (4.2–693.5 ft^3/s) 2016 were not significantly different (paired *t*-test; $P = 0.9$, Table S1), thus results of this work represent base flow conditions. Further work is needed to evaluate the bioavailability of DOM under different flow conditions.

Note that our incubation experiments did not include other biological and physical processes (e.g., photochemical reaction, particle adsorption, and the priming effect) that may control the fates of riverine DOC in these bays, as well as the long residence time of the bay water (1–3 years) which greatly exceeds our incubation time. For example, Shank et al. (2009) estimated that CDOM photobleaching processes could support up to 20% of the daily microbial respiratory C demand in Lavaca-Matagorda Bay. Our results, however, elucidated the role of biological factors in controlling the bioavailability of riverine DOM and the seasonal differences in estuaries and bays of south Texas, at least from a relative perspective. For example, the higher proportion of bioavailable DOM in winter may mean more regenerated nutrients, which may support more primary production in spring in adjacent bays and estuaries.

The magnitude of DIN export by rivers, and its impacts on the nitrogen budget in the estuaries are recognized. Freshwater inflow maintains the inorganic nitrogen in these estuaries based on a long term (1987–2012) dataset of nutrients (Paudel and Montagna, 2014). However, a quantitative understanding of how much DON is bioavailable from the rivers is needed to predict the effects of N loading on the coastal ecosystems in these estuaries. In this context, proportions of DIN and BDON in the bioavailable N of the rivers during winter and summer were calculated (Fig. S7). Assuming that all inorganic forms of N are bioavailable, DIN and BDON together can be defined as the bioavailable N, which is an important parameter in understanding the ecosystem (Jørgensen et al., 2014). Bioavailable N was significantly higher in the San Antonio and Aransas Rivers (73.6–119.5 μM and 25.7–34.3 μM), than in the Mission River (3.5–6.3 μM). Furthermore, BDON dominated in the bioavailable N (57–80%) in the relatively pristine Mission River. DIN dominated in the bioavailable N pool (64–95%) in the other rivers, which were impacted by the wastewater treatments or agricultural lands to different extents (Fig. S7). This result illustrates that the catchments of rivers could affect the relative amounts of DIN and BDON exported to the south Texas coastal systems. Overall, 14.6–38.0% and 8.6–15.1% of riverine DON in winter and summer is bioavailable to coastal bacteria in winter and summer, respectively. The BDON accounted for 28.4% ± 19.8% and 29.7% ± 29.0% of bioavailable N during winter and summer, respectively, suggesting that DON from these Texas rivers may provide considerable bioavailable nitrogen to the coastal ecosystems. Furthermore, the difference in the form of bioavailable N may be a key driver of the differences observed in primary producer community composition (Cira et al., 2016; Montagna et al., 2018). Given the long residence time of seawater in the south Texas bays (~3 years) and the priming effect (Solis and Powell, 1999), the magnitudes of riverine DON that can be used within the bays are expected to be higher, and hence represent a key nutrient source to coastal ecosystems. These quantified percentages of riverine BDON also provide a solid foundation for future modelling work in the bays.

5. Conclusion

Our incubation results showed that the ranges of BDOC% and BDON % in the five southern Texas rivers were higher during winter (5.5–11.1% for BDOC and 14.6–38.0% for BDON) than during summer (0–6.1% and 8.6–15.1%). TDAA compositional data confirmed that winter DOM, enriched with Phe and Met, had higher nutritional quality than the summer DOM that was enriched with GABA and Gly. The summer DOM may have already been processed extensively before

reaching the river mouth due to the low base flow, or long residence time, and high summer temperatures. Correlation analyses indicated BDON% was positively related to SRP concentrations, a trend that may be driven by the fact that high levels of SRP can stimulate bacterial growth. Relatively higher BDOC% and BDON% were observed in the San Antonio and Aransas Rivers, which may be related to the influence by human activities. We also investigated the response of coastal bacteria during incubations of riverine DOM. The seasonal and spatial variations of bacterial growth efficiency were associated with the quality of riverine DOM and other environmental factors (such as temperature). Additionally, bacterial community structure tended to converge in both seasons after incubation. This tendency was stronger in summer than in winter, reflecting the more recalcitrant nature of riverine DOM in summer. This conclusion is confirmed by the functional gene abundance data, in which N-metabolism pathways and functional genes increased during winter incubation but decreased in summer. Our data provide new insights about the bioavailable fractions of riverine DOM from the southern Texas rivers to the coastal bacterial community. As the input of riverine DOM is closely related to the ecosystem metabolisms and the cycling of carbon and nitrogen in the bays, our results will help modelling and budgeting efforts in these coastal ecosystems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We thank Jiqing Liu for assistance in the field and laboratory. We also thank Shuting Liu for help with the measurements of amino acids. Comments from two anonymous reviewers improved the manuscript. China Scholarship Council supported Wu's stay at UTMSI as a visiting student. This research was funded by National Oceanic and Atmospheric Administration (NA15NOS4780185), by an Institutional Grant (NA14OAR4170102) to the Texas Sea Grant College Program from the National Sea Grant Office, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and by the National Science Foundation Chemical Oceanography Program (OCE 1763167).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2019.106477.

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