

## Wastewater influences nitrogen dynamics in a coastal catchment during a prolonged drought

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### Abstract

Ecosystem function measurements can enhance our understanding of nitrogen (N) delivery in coastal catchments across river and estuary ecosystems. Here, we contrast patterns of N cycling and export in two rivers, one heavily influenced by wastewater treatment plants (WWTP), in a coastal catchment of south Texas. We measured N export from both rivers to the estuary over 2 yr that encompass a severe drought, along with detailed mechanisms of N cycling in river, tidal river, and two estuary sites during prolonged drought. WWTP nutrient inputs stimulated uptake of N, but denitrification resulting in permanent N removal accounted for only a small proportion of total uptake. During drought periods, WWTP N was the primary source of exported N to the estuary, minimizing the influence of episodic storm-derived nutrients from the WWTP-influenced river to the estuary. In the site without WWTP influence, the river exported very little N during drought, so storm-derived nutrient pulses were important for delivering N loads to the estuary. Overall, N is processed from river to estuary, but sustained WWTP-N loads and periodic floods alter the timing of N delivery and N processing. Research that incorporates empirical measurements of N fluxes from river to estuary can inform management needs in the face of multiple anthropogenic stressors such as demand for freshwater and eutrophication.

Nitrogen (N) cycling in river networks modulates downstream N availability (Peterson et al. 2001; Seitzinger et al. 2002; Thieu et al. 2009). The relative importance of N uptake and export within a river network depends on hydrology (e.g., water velocity and depth), and controls on biogeochemical transformations (e.g., temperature and nutrient concentrations; Saunders and Kalff 2001; Alexander et al. 2009; Aguilera et al. 2012). In coastal catchments, N processing in

freshwater and transitional estuarine environments controls delivery of N to marine ecosystems (D'Elia et al. 1986; Nixon et al. 1996; Arndt et al. 2011). Both the hydrological and the biogeochemical controls of N transport are impacted by the salinity gradient along a river-estuary continuum (Jordan et al. 2008; Santoro 2010; Cornwell et al. 2016). These factors interact to control N export to shallow marine environments, where N is both a critical base of production (Howarth and Marino 2006), and driver of coastal eutrophication and hypoxia (Rabalais 2002; Diaz and Rosenberg 2008).

Among the possible N transformations that occur from river to estuary, denitrification is the primary process removing N from the aquatic environment (Seitzinger et al. 2006). Denitrification is an anaerobic respiratory pathway that reduces nitrate ( $\text{NO}_3^-$ ) to nitrous oxide ( $\text{N}_2\text{O}$ ) or di-nitrogen gas ( $\text{N}_2$ ). Denitrification requires delivery of  $\text{NO}_3^-$  to microbes under anoxic conditions with labile organic carbon (C). Controls on denitrification (i.e.,  $\text{NO}_3^-$ , labile organic C, and redox conditions) vary from river to estuary, and the longitudinal pattern of denitrification is a critical factor driving downstream N availability (Alexander et al. 2009). Other processes, such as

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**Special Issue:** Headwaters to Oceans: Ecological and Biogeochemical Contrasts Across the Aquatic Continuum  
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assimilatory N uptake, also mediate downstream N availability but do not represent a permanent N sink (Arce et al. 2014).

Increased global temperatures affect the hydrological cycle by including more frequent droughts (Sheffield and Wood 2008; Dai 2011), which influence the timing and form of N delivery from rivers to marine environments. Under drought, river hydrographs are characterized by prolonged periods of baseflow and episodic storm events (Dahm and Molles 1992; Montagna and Kalke 1992; Mooney and McClelland 2012). Episodic storms are important mechanisms for delivery of nutrient pulses downstream (Paerl et al. 2010; Bruesewitz et al. 2013). However, the processing and export of N during droughts within lotic ecosystems can restrict N availability downstream, especially during prolonged periods of low baseflow. In arid climates, estuaries respond to drought conditions with signs of N limitation such as decreased primary productivity (D'Elia et al. 1986; Wetz et al. 2011), increased internal recycling of  $\text{NH}_4^+$ , and increased rates of N fixation (Bruesewitz et al. 2013). However, the fate of N is less understood as it travels from river to estuary during periods of prolonged drought.

Anthropogenic activities change the timing and transport of nutrients to coastal estuaries (Ryther and Dunstan 1971; Paerl et al. 2014). A key source of anthropogenic nutrients in many catchments is waste water treatment plant (WWTP) effluent, which increases the daily export of N and elevates the nutrient export baseline (Oelsner et al. 2007; Boynton et al. 2008; Carey and Migliaccio 2009), especially during drought or low-flow periods (Andersen et al. 2004; Passell et al. 2005). Depending on the degree of treatment, N can be released from WWTP as ammonium ( $\text{NH}_4^+$ ),  $\text{NO}_3^-$ , or organic N (Carey and Migliaccio 2009; Brion et al. 2015). The dominant form of N may subsequently change downstream (Garnier et al. 2001; Pennino et al. 2016). Anthropogenic point sources of N in coastal catchments increase with elevating human populations in coastal regions worldwide (Ache et al. 2013).

The goal of this paper is to compare N processing and export in two rivers in an arid south Texas coastal catchment, one influenced by high WWTP discharge, to evaluate the export of N to the estuary during a prolonged period of drought. We address the questions: (1) What are the N species and N export patterns from river to estuary during drought conditions relative to these patterns during periods of high flow? Because rivers are active sites of N processing, we hypothesize that: (a) organic N dominates N export during drought conditions, but inorganic forms contribute more to total N export during high flow (b) N export from river to estuary is low during drought periods. (2) Is N processing and export influenced by close proximity to upstream WWTP inputs? We hypothesize: (a) that rates of N uptake and removal are high in both rivers during drought, because water moves slowly and has prolonged contact and biogeochemical interactions with the benthic zone, and (b) despite high N

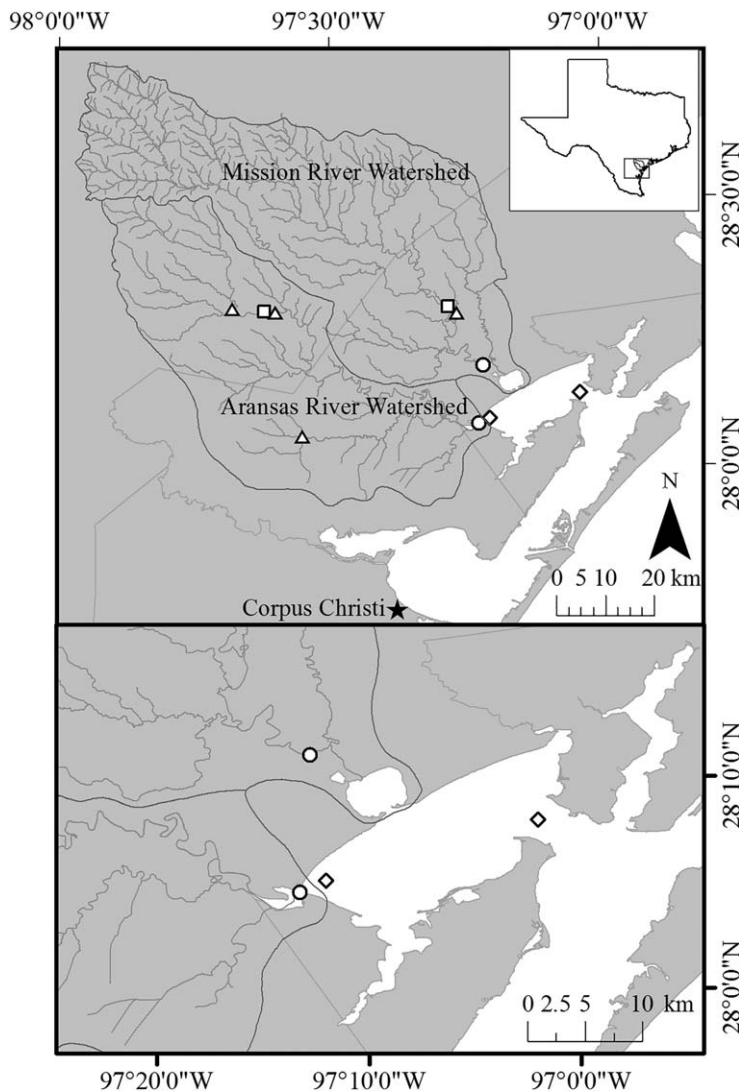
uptake and denitrification rates, more N is exported downstream out of the WWTP nutrient-influenced river than in the less WWTP-influenced river. (3) How do N uptake patterns vary from river to tidal river to estuaries during drought? We hypothesize that denitrification will be highest in tidal river sites as water residence time increases, but that there will be uptake and denitrification of N from river to estuary during the drought. To address these questions and hypotheses, we compare patterns of N species distributions and export at river, tidal river and estuarine sites, and we use direct measurements of N uptake processes at river, tidal river, and estuarine sites along the gradient from river to estuary during a period of prolonged drought. Our research objectives required using diverse field and laboratory methodology, developed for stream and coastal ecosystems, respectively, and we discuss the challenges of measuring N cycling across ecosystem types and disciplinary boundaries.

## Methods

### Site description

The river-estuary ecosystems of the Mission and Aransas rivers to Copano Bay, Texas are already described (Fig. 1; Mooney and McClelland 2012; Bruesewitz et al. 2015). These rivers are characterized by low base flow and episodic storm events. No rain fell during the period from April 2011 to April 2012 except for one event that elevated Mission River discharge on 20 March 2012. We considered a day to be a part of a flood if the discharge was at least 10 times the median discharge for that river (Bruesewitz et al. 2013). Mean river discharge was  $56 \pm 0.3 \text{ L s}^{-1}$  in the Mission River and  $126 \pm 2.5 \text{ L s}^{-1}$  in the Aransas River during this drought period. Low discharge during the drought period (baseflow is  $< 200 \text{ L s}^{-1}$ ) allowed use of stream sampling techniques in these rivers. Both rivers were sampled near the United States Geological Survey (USGS) real-time stream flow gages near Refugio (Mission River) and Skidmore (Aransas River), Texas. The flow gages are positioned upstream from the tidally influenced lower reaches, 36 km from the river mouth for the Mission River and 94 km from the river mouth for the Aransas River (Mooney and McClelland 2012) at 0.31 m and 22.06 m above sea level, respectively, for the Mission and Aransas Rivers. The mean annual hydraulic residence time in the non-tidal portions of the Aransas River is 3 d and it is 5 d in the Mission River (S. L. Johnson, unpubl.).

Although both river catchments contain permitted WWTP outfalls, the Aransas River is influenced more by WWTP than the Mission River (United States Environmental Protection Agency (EPA) 2008; Bruesewitz et al. 2015). The Aransas River watershed receives 14.4 million  $\text{L d}^{-1}$  from 10 WWTP, 79% of which is from one facility that discharges approximately 20 km upstream of our sampling site on Poesta Creek, a tributary to the Aransas River in Beeville, Texas (Fig. 1). This facility is permitted to discharge 11.4 million liters  $\text{d}^{-1}$  and has a maximum daily loading rate of 24.9 kg  $\text{N d}^{-1}$  (Bruesewitz et al. 2015). The Mission watershed



**Fig. 1.** Map of the Mission and Aransas river watersheds flowing into Copano Bay, Texas. Squares denote upper river samplings sites, also the location of the USGS gauging station for each river. Circles denote tidal river sampling sites and diamonds denote estuary sites (lower inset shows tidal river sites and estuary sites in more detail). Open triangles show all waste-water treatment plant discharge points in each catchment permitted to release  $>0.5$  million gallons  $d^{-1}$ .

contains three WWTP discharging a total of 1.9 million  $L d^{-1}$ , approximately 13% of the WWTP discharge received by the Aransas River (United States Environmental Protection Agency (EPA) 2008; Mooney and McClelland 2012). At median river discharge for the Aransas River, WWTP water comprises about 84% of river discharge, whereas the Mission River WWTP contributions are  $<1\%$  of river discharge at median discharge (Mooney and McClelland 2012). Isotopic data confirm the WWTP signature of N in the Aransas River (Mooney and McClelland 2012).

Both rivers have slow-flushing tidal reaches, that behave more like reservoirs than free-flowing rivers. Freshwater

residence times in the tidal rivers are variable, ranging from hours to days during periods of high river flow up to several months during periods low river flow. Modeled flow in the Aransas Tidal River is  $28.3 L s^{-1}$  at the 5<sup>th</sup> percentile of gaged flow upstream, and  $1076 L s^{-1}$  at median flow (S. L. Johnson, unpubl.). Modeled residence time for the Aransas Tidal River is 280 d at 5<sup>th</sup> percentile flow, and 52 d at median flow. Modeled flow in the Mission Tidal River is  $113.3 L s^{-1}$  at the 5<sup>th</sup> percentile of gaged flow upstream, and  $849.5 L s^{-1}$  at median flow (S. L. Johnson, unpubl.). Modeled residence time for the Mission Tidal River is 163 d at 5<sup>th</sup> percentile flow, and 22 d at median flow (S. L. Johnson, unpubl.). The average water residence time for Copano Bay is as long as 3 yr, although it is substantially reduced during periods of flooding.

The Aransas River flows into the west end of Copano Bay, and the Mission River flows into Copano Bay via the smaller Mission Bay (Fig. 1). Thus, the sampling site in western Copano Bay is influenced directly by Aransas River discharge, whereas the eastern Copano Bay site reflects estuarine conditions. Copano Bay, with an average depth of 2 m and an average tidal range of 0.15 m, is a part of the Mission-Aransas National Estuarine Research Reserve (NERR), and the western and eastern Copano Bay sites are part of the ongoing NERR monitoring program (<http://cdmo.baruch.sc.edu>).

#### Field sampling

Samples for water column nutrients and chlorophyll *a* (Chl *a*) were collected from the Aransas River and Mission River sites and the tidal river sites. All riverine water samples were collected from the thalweg, or the main thread of flow. Copano Bay nutrient and Chl *a* samples were collected at eastern and western Copano Bay sites at 0.5 m above the benthos as part of the NERR sampling program. Nutrient samples for calculation of export rates were collected monthly at all sites from August 2010 to August 2012, except for periods of  $\sim$  daily sampling during and after storm events as previously described (Bruesewitz et al. 2013, 2015). All nutrient samples were filtered in the field using a  $0.45 \mu m$  pore size syringe filter (Millipore) and frozen until analysis. Measurements of temperature, conductivity, salinity, and dissolved oxygen (DO) were collected with a YSI 6600 V2 sonde (Xylem, Yellow Springs, Ohio). Additional measurements of N transformations required a diversity of techniques (Table 2) and are described below and in the Supporting Information.

#### Daily N export

We collected nutrient samples from each river site for calculating watershed export from 01 August 2010 to 01 August 2012 monthly, and more frequent sampling during and after storms (Bruesewitz et al. 2013). We collected discharge data from the USGS real-time stream flow gauges collected from each river site (<http://waterdata.usgs.gov/tx/nwis/rt>). Nutrient exports were calculated from these sites because tributaries closer to the estuary were dry under baseflow conditions. Note that most Aransas River discharge results from



municipal inputs upstream from our sampling sites (Mooney and McClelland 2012). We used the LoadRunner application, which automates the USGS Load Estimator program (LOAD-EST) to calculate nutrient fluxes into the estuary (Runkel et al. 2004; Booth et al. 2007; Bruesewitz et al. 2013). The LOADEST model was calibrated as described in Mooney and McClelland (2012) and Bruesewitz et al. (2013). Baseflow export calculations were not scaled to the full watershed because scaling overestimates export during dry conditions (Mooney and McClelland 2012). Export calculations could not be completed for the tidal river sites because daily discharge data was lacking and the freshwater residence times is several months during periods of low flow in this region, as described above. Due to these differences in water residence time between high and low flow periods, calculated nutrient loads represent direct loading to Copano Bay during high flow periods, and loading to tidal river zones for mixing and dilution prior to delivery to the bay during low flow periods (Mooney and McClelland 2012).

### N uptake

We measured rates of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake in the Aransas and Mission Rivers using short term additions in 60–70 m reaches of each river, on 18 July 2011 and 10 July 2011. We added nutrients in two separate short-term additions using standard methods (Stream Solute Workshop 1990; Hoellein et al. 2011). The first addition was ammonium chloride ( $\text{NH}_4\text{Cl}$ ) and sodium chloride ( $\text{NaCl}$ ), (i.e., conservative tracer), and the second addition was sodium nitrate ( $\text{NaNO}_3$ ) and  $\text{NaCl}$ . The  $\text{NaCl}$  was measured as conductivity using a YSI model 30 conductivity meter (YSI, Yellow Springs, Ohio). We collected water samples every 10 m downstream of the addition site prior to starting the nutrient release to measure variation in ambient nutrient concentrations and conductivity. The solutes were added at  $200 \text{ mL min}^{-1}$  (Fluid Metering, Model RHB, Syosset, New York) to raise nutrient concentrations slightly above ambient concentrations ( $+10$ – $19 \mu\text{g NH}_4^+$ ,  $+12$ – $61 \mu\text{g NO}_3^-$ , and conductivity by  $+5$ – $42 \mu\text{S cm}^{-1}$ ). Once stable plateau conditions were reached based on conductivity, we collected and analyzed water samples ( $n = 3$ ) at each of downstream sampling site (see below). Nutrient and conductivity data were corrected for background concentrations, and nutrient uptake lengths ( $S_w$ ) were calculated as the inverse of the slope of nutrient concentrations divided by tracer concentrations at each downstream sampling site measured as distance from the addition site (Stream Solute Workshop 1990). Given the modest increase in nutrients, this uptake metric is between gross and potential uptake rate, (Mulholland 1992). The areal nutrient uptake rate ( $U$ ) and uptake velocity ( $V_f$ ), calculated by standard methods (Stream Solute Workshop 1990), allowed comparison of results among rivers with varying discharge.

We measured nutrient uptake and regeneration rates at the tidal river and estuary sites with intact sediment cores.

Collection of intact cores was possible at these sites due to low water velocity, deep water, and high sediment consolidation relative to upstream river sites. Additionally, the volume and long residence time of water at these sites prevented potential effectiveness of nutrient additions as described for the other river sites. We collected four in-tact sediment cores from each of the tidal river sites on 12 July 2011 using a pole corer with a one-way valve to minimize disturbance of the sediment (modified from Gardner et al. 2009). Each core (7.6 cm inner diameter and 20 cm long) was sealed and transported back to the lab in the dark. Two 20-L carboys of water were collected from each site for flow-through core incubations. Continuous flow-through core experiments were set up in the laboratory within 4 h of core collection as previously described (Bruesewitz et al. 2013; see Supporting Information). These experiments measured nutrient and gas fluxes, and denitrification rates via isotopic enrichment (An et al. 2001; Gardner and McCarthy 2009). Dissolved gas samples were collected by placing outflow tubing into the bottom of a 15 mL ground-glass stopper test tube and allowing it to overflow for several volumes. Fluxes of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , as well as dissolved gases  $\text{N}_2$  and  $\text{O}_2$ , were calculated from differences between the inflow and outflow concentrations, the flow rate, and the cross-sectional area of the cores (McCarthy et al. 2008; Bruesewitz et al. 2013).

### Denitrification and sediment oxygen demand

We quantified denitrification and sediment oxygen demand rates in the river sites, using microcosm incubations of river sediment and water (Reisinger et al. 2016). We collected sediment on 18 July 2011 and 10 July 2011 for the Aransas and Mission Rivers, respectively. Sediment from the top 10 cm of the river benthos represented different benthic habitats dominated by either fine benthic organic matter (FBOM) or sand across three random transects within the river reach used for the nutrient uptake experiments described above. These two substratum types represented 26% of the Mission River 46% of the Aransas River benthos based on transect surveys (Supporting Information), and the remainder of substrates, e.g., cobble and boulder or large wood, or other categories such as filamentous algae and glass, were difficult to include in our microcosms. We collected approximately 10 L of river water from the thalweg of each river site for the microcosms. We kept the water and sediment on ice during transit to the laboratory. Using composite sediment samples, rather than in-tact cores, alters sediment redox conditions and N delivery to the benthos, but they are often used in stream and shallow river habitats (Groffman et al. 2006; Arango and Tank 2008; Bruesewitz et al. 2008), because unconsolidated sediments and shallow water depths prevent collection of intact cores representative of the benthos (Turek and Hoellein 2015; Reisinger et al. 2016). The mesocosm approach also included diverse sediment types across the river reach and allowed increased replication

(Reisinger et al. 2016). However, these benefits are moderated by potential errors, e.g., by excluding some substratum types in our assays and scaling our data to the river reach.

In the laboratory, we constructed microcosms (after Reisinger et al. 2016). We homogenized sediment samples by stirring, and brought the sediment and river water to room temperature (22°C). We added 10 mL of homogenized sediment to 50 mL centrifuge tubes (Falcon, Corning, Corning, New York) ( $n = 5$  tubes per replicate,  $n = 80$  tubes per river). We added river water slowly via a syringe to fill each tube, and capped the tubes under river water to prevent headspace. If a bubble was observed, we topped off the tube with river water and re-capped it. We filled three tubes per river with river water to account for changes attributed to water column activity. We added  $^{15}\text{NO}_3^-$  ( $\sim 10 \mu\text{mol L}^{-1}$  final concentration) to one half of the tubes from each river and each substratum type, prior to capping for measurement of potential denitrification, and left one set of tubes unamended for control measurements. We placed centrifuge tubes on a shaker table at a gentle settling (i.e., to maintain gentle water movement within the tubes) at 24°C. We sampled respective microcosms and discarded them hourly for 4 h after starting the incubations. We collected dissolved gas samples for  $^{28}\text{N}_2$ ,  $^{29}\text{N}_2$ ,  $^{30}\text{N}_2$ ,  $\text{O}_2$ , and Ar at each time point by transferring water from the microcosm slowly to a 15 mL glass tube (Chemglass; 19.8 cm length  $\times$  1 cm inner diameter) with a syringe fitted with tubing (Turek and Hoellein 2015; Reisinger et al. 2016). We prevented atmospheric  $\text{N}_2$  contamination by filling tubes slowly from the bottom and overflowing the samples. We capped the samples with a ground glass stopper, stored at room temperature underwater and analyzed them for  $^{28}\text{N}_2$ ,  $^{29}\text{N}_2$ ,  $^{30}\text{N}_2$ ,  $\text{O}_2$ , and Ar on a membrane inlet mass spectrometer (MIMS; Kana et al. 1994; Bruesewitz et al. 2013; see below) within 1–15 min after collecting them. The oxygen effect on  $\text{N}_2$  : Ar associated with MIMS remains minimal on this MIMS and  $^{30}\text{N}_2$  production was not detected in control cores (Bruesewitz et al. 2013).

We calculated denitrification rates for each substratum type as the increase in  $\text{N}_2$  gas over the 4-h incubation period by measuring the linear slope of  $\text{N}_2$  gas production over incubation time (Reisinger et al. 2016; Supporting Information). We estimated  $^{28}\text{N}_2$  gas production, direct denitrification, and potential denitrification from net  $\text{N}_2$  flux,  $^{29} + ^{30}\text{N}_2$  from  $^{15}\text{NO}_3^-$  enriched microcosms, and  $^{28} + ^{29} + ^{30}\text{N}_2$  from  $^{15}\text{NO}_3^-$  enriched microcosms (Supporting Information; Bruesewitz et al. 2013). We converted volumetric microcosm denitrification rates to areal rates (Reisinger et al. 2016; Supporting Information). We multiplied the areal rates by the substratum areal coverage as measured by substratum transect surveys and summed across sampled substratum types to scale these substratum-specific rates to the river reach scale (see Supporting Information). We measured sediment oxygen demand (SOD) in each replicate as described using the decline in dissolved  $\text{O}_2$  over the 4-h incubation period,

and SOD was scaled as described for denitrification rates. These denitrification rates are conservative for river-reach scale denitrification because they exclude some benthic substratum types and disturb sediment redox conditions, but conversely the microcosms may overestimate some rates by enhancing delivery of N from the water to the benthos.

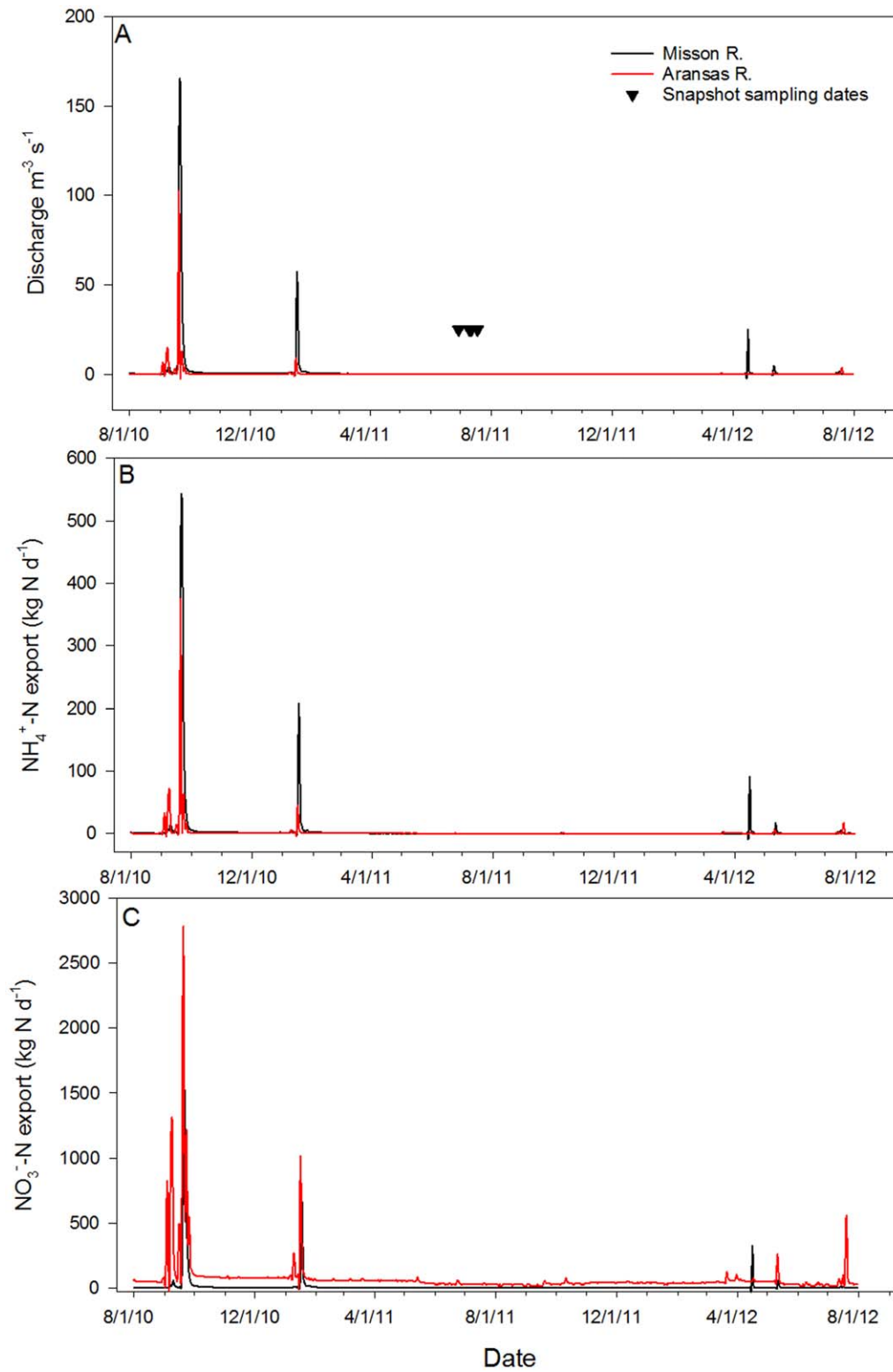
For denitrification measurements at the tidal river and estuary sites, two cores (described above and in Supporting Information) from each site received inflow water enriched with  $^{15}\text{NO}_3^-$  ( $\sim 10 \mu\text{mol L}^{-1}$  final concentration). We measured concentrations of  $^{28}\text{N}_2$ ,  $^{29}\text{N}_2$ , and  $^{30}\text{N}_2$  by membrane-inlet mass spectrometry (MIMS) to calculate simultaneous N fixation and denitrification (An et al. 2001; Bruesewitz et al. 2013). We measured gas samples immediately using the MIMS and methods described in An et al. (2001). Potential denitrification rates were calculated as the sum of  $^{28}\text{N}_2$ ,  $^{29}\text{N}_2$ , and  $^{30}\text{N}_2$  gas production after  $^{15}\text{NO}_3^-$  addition, but do not include incomplete denitrification to  $\text{N}_2\text{O}$ . We assessed potential rates of annamox or coupled nitrification-denitrification with  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$  production in  $^{15}\text{NH}_4^+$  enriched cores for a separate study (Bruesewitz et al. 2013) and values were small relative to denitrification.

### Ecosystem metabolism

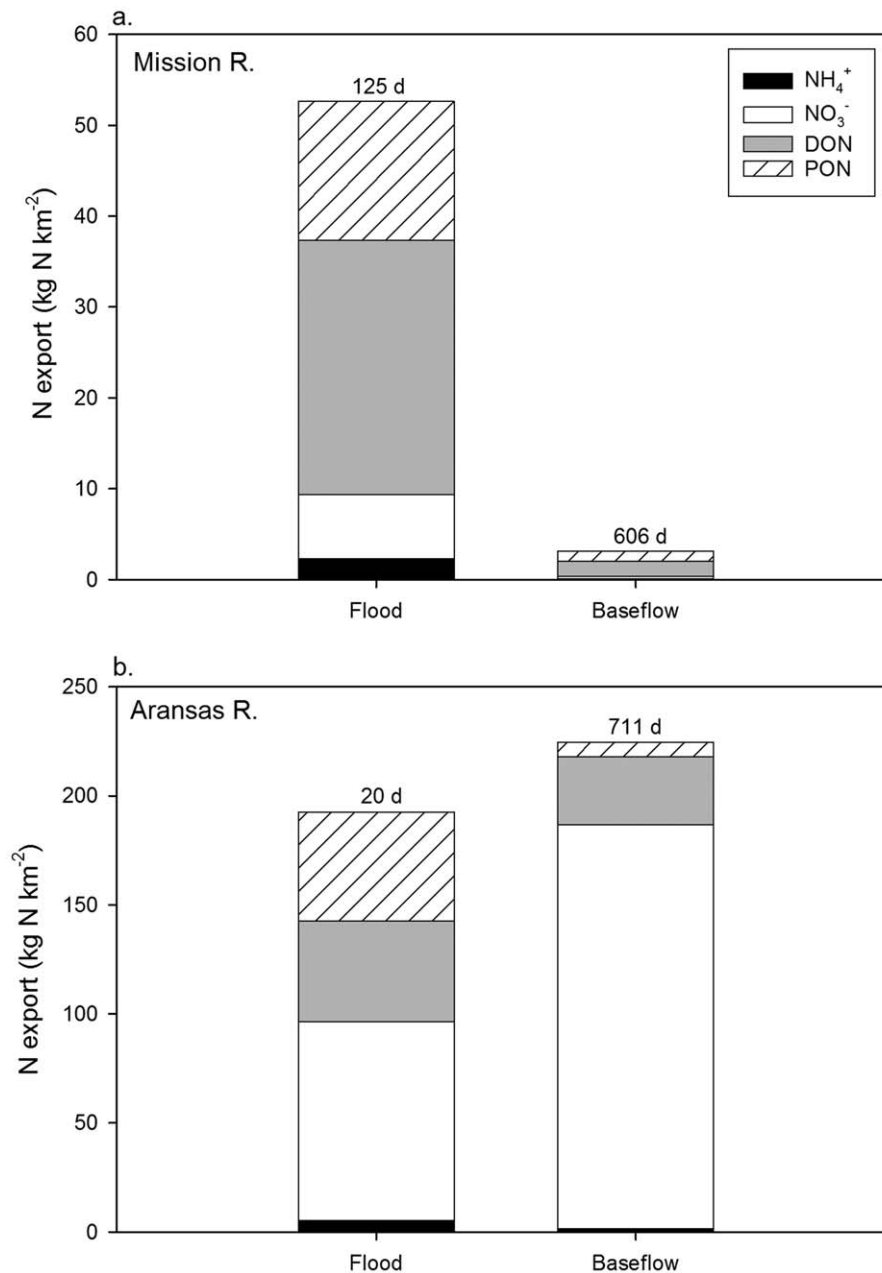
We measured river metabolism over 24 h concurrent with the short-term nutrient releases at each river site by deploying a Hydrolab Minisonde (Model 5a, Hach Corp.) with a luminescent DO probe to measure DO concentration, DO saturation, and temperature every 10 min, and logged PAR data from the riparian zone for the same period (Li-COR 190). We calculated reaeration (Atkinson et al. 2008) by regression, using Model Maker 4.0 (AP Benson, Wallingford, UK) to model respiration (R), gross primary production (GPP), with the balance between GPP and R as net ecosystem production (NEP) as described in Hoellein et al. (2011) and in the Supporting Information. We calculated metabolism metrics (GPP, R, and NEP) for estuary sites using free-water DO methods (Bruesewitz et al. 2013) using data from the Mission-Aransas NERR monitoring program (Caffrey 2004). We estimated GPP, R, and NEP metrics from daily calculated values for the week surrounding the July 2011 sampling date.

### Nutrients and Chl *a*

We measured water column nutrient and Chl *a* samples as part of the Mission-Aransas NERR monitoring program (Bruesewitz et al. 2013). We measured inorganic nutrients of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and SRP with a QuAatro nutrient autoanalyzer (Seal Analytical) using standard methods. We report all  $\text{NO}_3^-$  data reported as the sum of  $\text{NO}_3^-$  and nitrite ( $\text{NO}_2^-$ ), but  $\text{NO}_2^-$  is negligible in these samples, even below WWTP discharges (Mooney and McClelland 2012). We measured dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations on a Shmizadu TOC- $\text{V}_{\text{CSH}}$  with a TNM-1 Total Nitrogen detector, and estimated DON by



**Fig. 2.** Discharge (A),  $\text{NH}_4^+$  export (B), and  $\text{NO}_3^-$  export (C) based on routine sampling and LOADEST modeling for the period from 01 August 2010 to 01 August 2012. Discharge and nutrient concentration samples are collected from river sites.

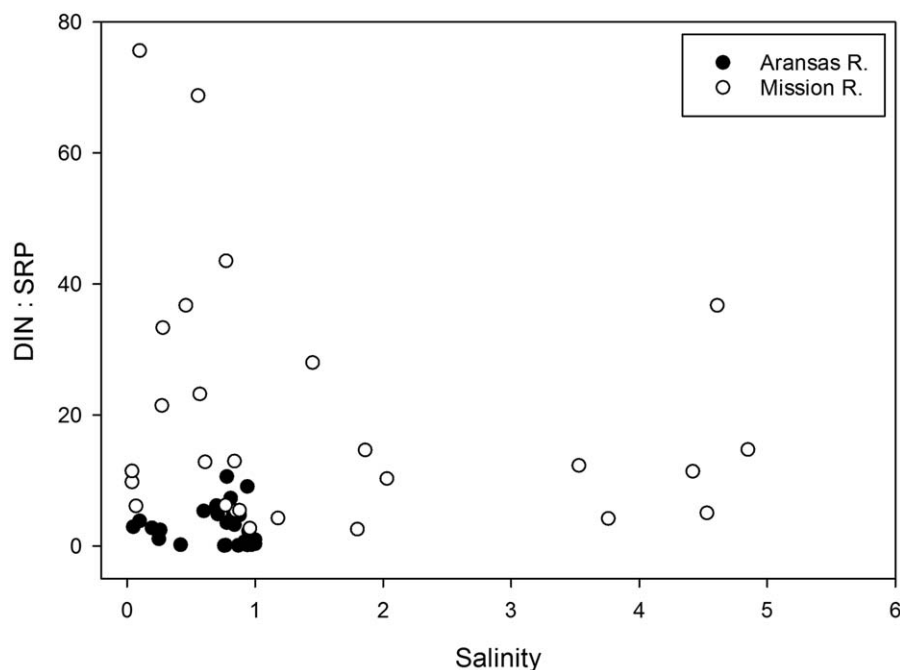


**Fig. 3.** Characterization of N export for the Mission (**a**) and Aransas (**b**) Rivers during periods of flood and baseflow from 01 August 2010 to 01 August 2012 based on sampling at the Mission and Aransas River sites. River sampling locations drain 67% and 30% of the Mission and Aransas River watersheds, respectively. During the drought, tributaries below the sampling points were dry, and for the Aransas River, WWTP inputs above the sampling site dominate flow.

subtracting measured inorganic N species. We measured particulate organic nitrogen (PON) concentrations with a Carlo Erba 2500 elemental analyzer to calculate PON export. PON filters were not acidified (Veradro et al. 1990; Mooney and McClelland 2012). We stored Chl *a* samples in amber bottles on ice and filtered on 0.7  $\mu\text{m}$  porosity filters (Whatman GF/F). We placed filters in glass scintillation vials with 10 mL of 90% acetone at  $-20^{\circ}\text{C}$  for 48 h. We determined Chl *a* concentrations with a fluorometer (Turner Designs Trilogy).

### Statistical analyses

We performed statistical analyses in the R statistical environment (R Core Development Team 2009). We tested all data for normality with a Kolmogorov–Smirnov–Liliefors test and transformed them to meet the assumption of normality if necessary. We measured differences between sites or site types (i.e., river, tidal river, estuary) using a mixed-design ANOVA followed by Tukey’s highly significant difference (HSD) test when appropriate. Many of our river-reach scale



**Fig. 4.** Molar dissolved inorganic nitrogen (DIN) to soluble reactive phosphorus (SRP) based on routine sampling in the Aransas and Mission Rivers from 01 August 2010 to 01 August 2012.

metrics, such as ecosystem metabolism and nutrient uptake were based on large data sets, but did not provide sufficient replication for statistical analyses.

## Results

### Storm vs. baseflow N export

Export of N from the Mission and Aransas rivers during periods of flood and drought from August 2010 to August 2012 (Fig. 2A) showed that storm exports generated pulses of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Fig. 2). The Mission River exported more  $\text{NH}_4^+$  than the Aransas River during floods, and both rivers exhibited minimal  $\text{NH}_4^+$  export during baseflow (Fig. 2B). However,  $\text{NO}_3^-$  export was higher in the Aransas River than in the Mission River during both flood and baseflow periods (Fig. 2C).

Over the 2-yr period, 606 d exhibited baseflow conditions and 125 d of flooded conditions in the Mission River. Flood events contributed  $52.6 \text{ kg N km}^{-2}$  to export (Fig. 3a), while baseflow N export was only  $3.2 \text{ kg N km}^{-2}$ . The majority of flood N exports from the Mission River were organic N, with  $28 \text{ kg N km}^{-2}$  as DON and  $15.3 \text{ kg N km}^{-2}$  as PON. Mission River N export was dominated by DON ( $1.6 \text{ kg N km}^{-2}$ ) and PON ( $1.1 \text{ kg N km}^{-2}$ ), relative to inorganic N ( $0.39 \text{ kg N km}^{-2}$ ) during baseflow.

Like the Mission River, the Aransas River had baseflow conditions most of the 2-yr period, with only 20 d of storm flow. In contrast to the Mission River, however, the Aransas River baseflow N exports of  $225 \text{ kg N km}^{-2}$  rivaled those of

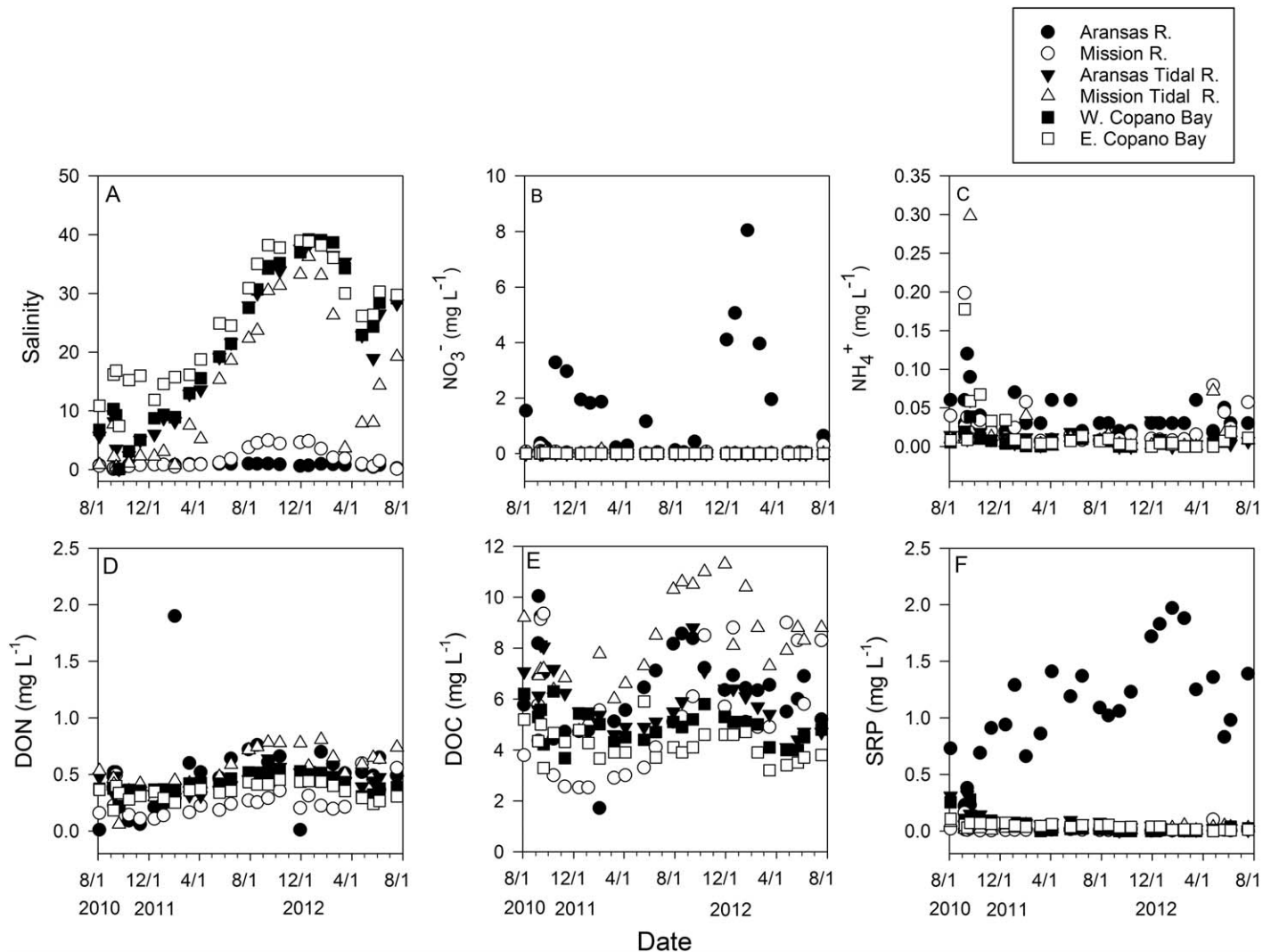
flood events of  $193 \text{ kg N km}^{-2}$ . The higher N export during baseflow was likely sustained by its close proximity to large WWTP inputs, which was evidenced further by the  $\text{NO}_3^-$  contributions to total N export, with 82% of baseflow export and 47% of flood export accounted for as  $\text{NO}_3^-$  (Fig. 3b). Both PON and DON also increased in the Aransas River during flooding, at  $50.1 \text{ kg N km}^{-2}$  and  $46.0 \text{ kg N km}^{-2}$ , respectively. DON also contributed to N export from the Aransas River during baseflow conditions, at  $31.1 \text{ kg N km}^{-2}$ . During drought conditions, salinity increased in both river sites, but the peak salinity (1 PSU) in the Aransas River was lower than in the Mission River (5 PSU). Water from the Beeville, Texas WWTP effluent may have diluted the salinity of the Aransas River relative to the drought salinity effects in the Mission River, which has much less WWTP effluent (Fig. 4).

The range of molar DIN ( $\text{NH}_4^+ + \text{NO}_3^-$ ) : SRP was low and consistent in the Aransas River as both N and P inputs from the WWTP effluent were stable (Fig. 4). In contrast, the Mission River DIN : SRP reached 75 : 1 during flood events, when DIN and SRP were delivered to the river from the catchment. Even during drought conditions, the DIN : SRP ranges were higher in the Mission River than the Aransas River. The DIN : SRP in the Aransas River ranged from 0.04 : 1 to 10.59 : 1, well below the Redfield ratio of ca. 16 : 1, as compared to 2.54 : 1 to 75.61 : 1 in the Mission River during the 2-yr period from August 2010 to August 2012 (Fig. 4).

### Patterns of nutrient concentrations from river to estuary

We examined 2 yr of salinity and nutrient concentration data across our six sites (i.e., river, tidal river, and estuary





**Fig. 5.** Salinity (A), nitrate ( $\text{NO}_3^-$ ) concentrations (B), ammonium ( $\text{NH}_4^+$ ) concentrations (C), dissolved organic nitrogen (DON) concentrations (D), dissolved organic carbon (DOC) concentrations (E) and soluble reactive phosphorus (SRP) concentrations (F) at all river, tidal river, and estuary sites from August 2010 to August 2012. Samples collected monthly except for more frequent intervals following storm events.

sites) over 2 yr, which included an extended drought from April 2011 to April 2012, to examine river-to-estuary patterns of nutrient concentrations (Fig. 5). Salinity in the tidal river and estuary sites steadily increased through the drought year. The elevated WWTP signature of the Aransas River is illustrated by the high concentrations of  $\text{NO}_3^-$ , SRP, and  $\text{NH}_4^+$  relative to those of the Mission River (Fig. 5). DOC and DON values were similar across both river sites (Fig. 5D,E). Longitudinal patterns for  $\text{NO}_3^-$  concentrations included dramatic declines of  $\text{NO}_3^-$  to low concentrations in both tidal river sites (Fig. 5B). Similarly,  $\text{NH}_4^+$  and SRP concentrations declined from river to tidal river to estuary sites, suggesting in-river nutrient processing (Fig. 5C,F). However, DON and DOC concentrations both increased in the tidal river sites relative to riverine and estuarine sites (Fig. 5D,E).

#### Snapshot of biogeochemical transformations from river to estuary during drought

We measured a snapshot of several components of N cycling in the Mission and Aransas rivers in late June and July 2011 (Table 2). The Mission River had a flood event on 11 February 2011, and the Aransas River had a flood on 17 January 2011, but both rivers remained at baseflow conditions for at least 5 months prior to our drought measurements. The Mission River discharge was very low, at  $7.25 \text{ L s}^{-1}$ , with little connectivity between riffles and pools, and increased water residence time (D. Bruesewitz pers. obs.). This lack of scouring events contributed to growth of filamentous algae in both rivers during the drought. We estimated benthic filamentous algae coverage as 12–14%. Mid-day temperatures and DO concentrations were similar among sites along the gradient (Table 1).

**Table 1.** Physiochemical parameters measured during the drought sampling.

Site	Depth (m)	Width (m)	Velocity (m s <sup>-1</sup> )	Temperature (°C; midday)	Conductivity (μS cm <sup>-1</sup> )	Salinity (PSU)	DO (mg L <sup>-1</sup> ; midday)	SRP (μg L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (μg L <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> (μg L <sup>-1</sup> )	DOC (mg L <sup>-1</sup> )	DON (mg L <sup>-1</sup> )	Chl <i>a</i> (μg L <sup>-1</sup> )	GPP (g O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> )	R (g O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> )	NEP (g O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> )
Mission R.	0.05	4.29	120	30.3	6.9	3.8	6.84	4.5	3.5	34.4	5.1	0.27	34.5 (0.6)	0.36	0.42	-0.06
Aransas R.	0.19	3.89	354	28.8	2.0	1.0	3.53	1587	464.5	37.7	8.2	0.72	1.43 (0.2)	0.28	0.51	-0.22
Mission Tidal R.	1.30	71	-	29.1	43.5	27.9	6.11	10.87	1.89	7.68	10.3	0.46	11.28 (1.1)	-	-	-
Aransas Tidal R.	0.70	416	-	30.0	43.6	27.9	6.56	78.23	1.14	9.46	5.5	0.71	4.38 (0.2)	-	-	-
W. Copano Bay	1.50	-	-	29.1	34.3	21.4	6.37	99.19	0	7.67	4.1	0.43	2.51 (0.04)	3.41 (0.1)	4.56 (0.5)	-1.14 (0.4)
E. Copano Bay	2.00	-	-	29.1	38.8	24.5	6.65	50.81	0.26	6.79	5.1	0.52	2.56 (0.3)	4.92 (0.4)	5.60 (0.7)	-0.67 (0.3)

The river sites were measured on 10 and 18 July 2011, tidal river sites on 12 July 2011, and the estuary sites on 29 June 2011. Temp = temperature, sp. cond = specific conductivity, DO = dissolved oxygen, DOC = dissolved organic carbon, DON = dissolved organic nitrogen, chl-*a* = chlorophyll-*a*, GPP = gross primary production, and NEP = net ecosystem production. metabolism data from the estuary site are presented as the mean of the week surrounding our sampling on 29 June 2011.

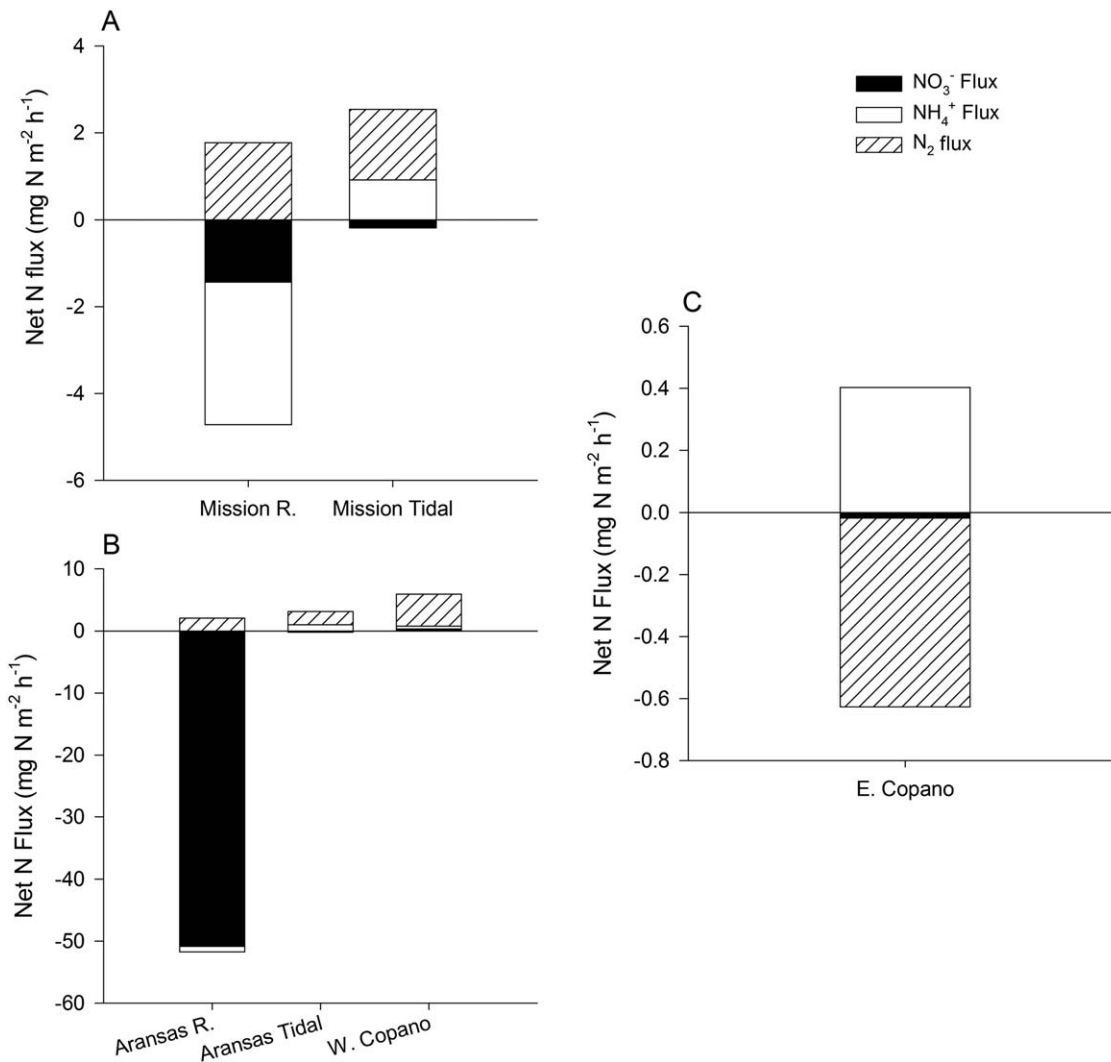
Reach-scale measurements of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> uptake and denitrification rates of N uptake were higher in the Aransas River than the Mission River (Fig. 6A). The Mission River N uptake processes were distributed quite evenly among different pathways, with a reach-scale NO<sub>3</sub><sup>-</sup> uptake rate of 1.44 mg N m<sup>-2</sup> h<sup>-1</sup>, NH<sub>4</sub><sup>+</sup> uptake rate of 3.28 mg N m<sup>-2</sup> h<sup>-1</sup>, and denitrification rate of 1.78 mg N m<sup>-2</sup> h<sup>-1</sup>. Note that reach-scale measurements of NO<sub>3</sub><sup>-</sup> uptake during short-term additions include denitrification and assimilatory NO<sub>3</sub><sup>-</sup> uptake, which occur simultaneously. In contrast to the Mission River, NO<sub>3</sub><sup>-</sup> uptake at 51 mg N m<sup>-2</sup> h<sup>-1</sup> dominated Aransas River dynamics. Denitrification rates (2.1 mg N m<sup>-2</sup> h<sup>-1</sup>) were similar to those in the Mission River, but was lower relative to NO<sub>3</sub><sup>-</sup> uptake, suggesting that denitrification accounted for a small fraction of total NO<sub>3</sub><sup>-</sup> demand. Ammonium uptake rates (0.88 mg N m<sup>-2</sup> h<sup>-1</sup>) were relatively low in the Aransas River, constituting about 25% of combined rates in the Mission River. Uptake velocities for NO<sub>3</sub><sup>-</sup> were comparable between the Mission and Aransas rivers (0.045 mm s<sup>-1</sup> and 0.030 mm s<sup>-1</sup>, respectively), but NH<sub>4</sub><sup>+</sup> V<sub>f</sub> was lower in the Aransas River relative to the Mission River (0.007 mm s<sup>-1</sup> and 0.027 mm s<sup>-1</sup>, respectively). The proportion of N denitrified, relative to overall N uptake processes, was 27% in the Mission River and 4% in the Aransas River.

Both tidal river sites have substantial net denitrification, with a positive net N<sub>2</sub> flux out of the sediment of 1.62 ± 0.90 and 2.12 ± 1.12 mg N m<sup>-2</sup> h<sup>-1</sup> in the lower Mission and Aransas rivers, respectively (Fig. 6B). Both sites had net positive NH<sub>4</sub><sup>+</sup> fluxes out of the sediment, 0.92 ± 0.19 mg N m<sup>-2</sup> h<sup>-1</sup> in lower Mission and 1.00 ± 0.17 mg N m<sup>-2</sup> h<sup>-1</sup> in the lower Aransas, indicating that overall NH<sub>4</sub><sup>+</sup> sinks (i.e., processes such as assimilatory uptake or nitrification) are not major fates of NH<sub>4</sub><sup>+</sup> in the tidal river sites. However, net NO<sub>3</sub><sup>-</sup> uptake occurred, as indicated by net NO<sub>3</sub><sup>-</sup> retention at both sites of 0.19 ± 0.13 mg N m<sup>-2</sup> h<sup>-1</sup> and 0.18 ± 0.18 mg N m<sup>-2</sup> h<sup>-1</sup> in the lower Mission and Aransas rivers, respectively. This corroborates with the positive net N<sub>2</sub> flux, as the NO<sub>3</sub><sup>-</sup> uptake could fuel denitrification in the tidal river sites (Fig. 6B). Unlike patterns of N fluxes in the river sites, the rates and direction of N fluxes were similar between Mission and Aransas Tidal River sites (Fig. 6B). The proportion of N denitrified increased to 49% in the Mission Tidal River and 55% in the Aransas Tidal River.

Patterns of N fluxes at the estuary sites differed from results at upstream stations, and between the two sampling locations in each river. The western Copano Bay site, near the mouth of the Aransas River, exhibited net denitrification with a positive net N<sub>2</sub> flux of 5.16 ± 0.23 mg N m<sup>-2</sup> h<sup>-1</sup>, with small net positive fluxes of NH<sub>4</sub><sup>+</sup> (0.43 ± 0.06 mg N m<sup>-2</sup> h<sup>-1</sup>) and NO<sub>3</sub><sup>-</sup> (0.35 ± 0.15 mg N m<sup>-2</sup> h<sup>-1</sup>). However, in eastern Copano Bay, N limitation was evident, with net N fixation (0.61 ± 1.71 mg N m<sup>-2</sup> h<sup>-1</sup>), low NH<sub>4</sub><sup>+</sup> flux out of the sediment (0.40 ± 0.06 mg N m<sup>-2</sup> h<sup>-1</sup>; Fig. 6C), and no detected net flux of NO<sub>3</sub><sup>-</sup>. Nitrogen fixation was the dominant process in eastern Copano Bay during this period of drought.

**Table 2.** A summary of methods used to measure ecosystem function across the diversity of sites. The river sites include the Aransas and Mission Rivers, tidal river sites are the Aransas Tidal and Mission Tidal Rivers, and estuary sites are western and eastern Copano Bay.

Measurement	River	Tidal river	Estuary
Ecosystem metabolism	Free-water; 24-h sonde deployment	-	Free-water; ongoing sonde deployment
Denitrification	Slurry with $^{15}\text{NO}_3^-$ tracer	Flow-through cores with $^{15}\text{NO}_3^-$ tracer	Flow-through cores with $^{15}\text{NO}_3^-$ tracer
Sediment oxygen demand	Oxygen decline in slurry	Flow-through cores	Flow-through cores
Whole system N uptake	Reach-scale nutrient releases	Flow through cores; net fluxes	Flow through cores; net fluxes
Sampling dates	10 Jul 2011 and 18 Jul 2011	12 Jul 2011	29 Jun 2011

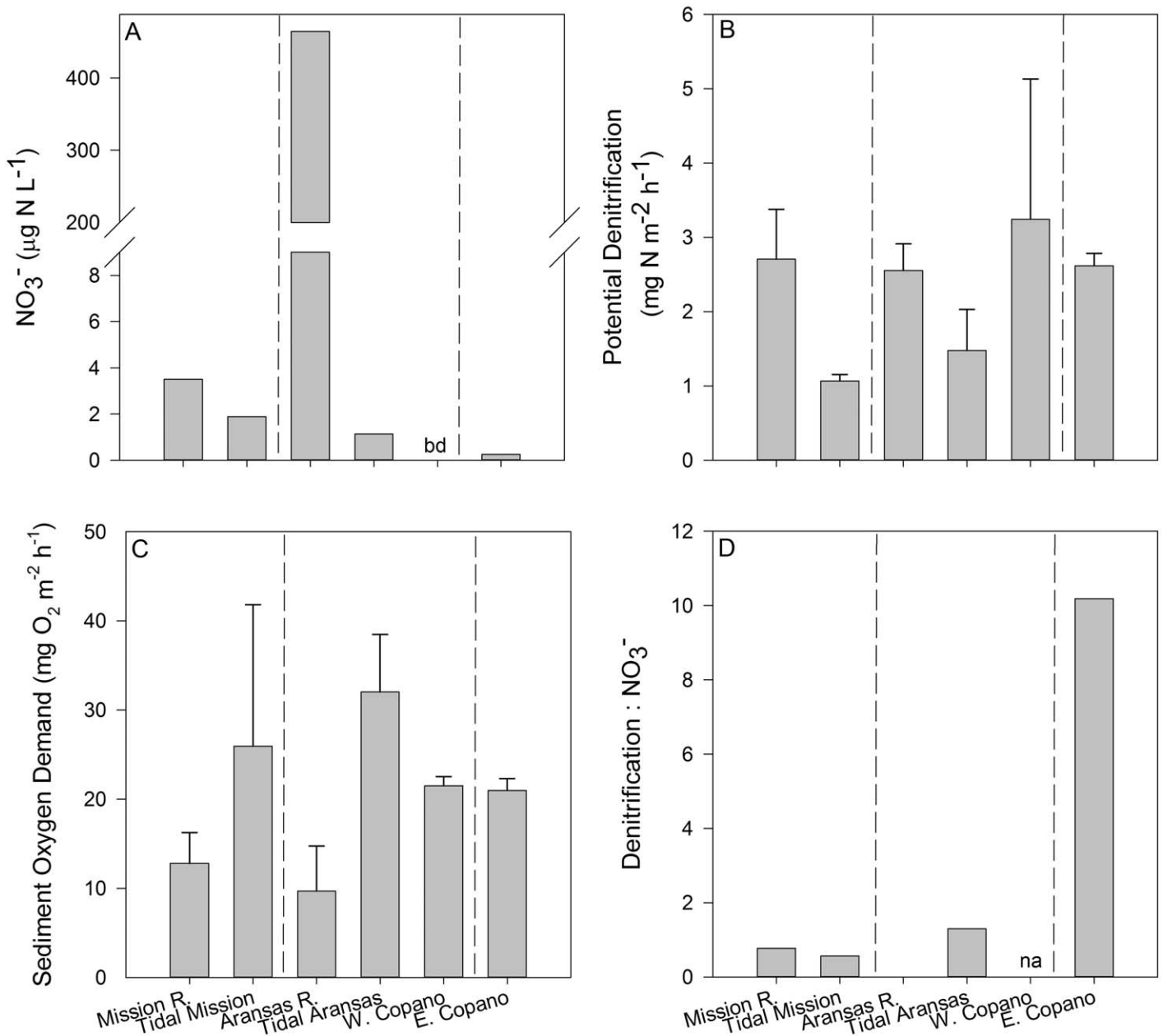


**Fig. 6.** Fates of N in the Mission river system (A), Aransas river system (B), and central estuary site (C). Positive fluxes of N are out of the sediment, negative fluxes are into the sediment. River sites were measured on 10 July 2011 and 18 July 2011, tidal river sites on 12 July 2011, and estuary sites on 29 June 2011.

**Comparisons from river to estuary**

Denitrification was measured directly at each site during the drought, using methods modified for each ecosystem from river to estuary (Table 2). Despite differences in NO<sub>3</sub><sup>-</sup>

concentrations across the sites (Fig. 7A), no differences among habitats (i.e., river, tidal river, and estuary) were observed for denitrification rates (mixed effects ANOVA  $p = 0.502$ ;) or potential denitrification rates (mixed effects



**Fig. 7.** Nitrate (NO<sub>3</sub><sup>-</sup>) concentrations (A), potential denitrification rates (B), sediment oxygen demand (C), and denitrification to NO<sub>3</sub><sup>-</sup> ratio (D) across the river-estuary sites. River sites were measured on 10 July 2011 and 18 July 2011, tidal river sites on 12 July 2011, and estuary sites on 29 June 2011. NO<sub>3</sub><sup>-</sup> was below detection in the western Copano Bay site, therefore the denitrification: NO<sub>3</sub><sup>-</sup> could not be calculated for this site. Dashed vertical lines denote the Mission and Aransas river systems.

ANOVA  $p = 0.187$ ; Fig. 7B). Despite NO<sub>3</sub><sup>-</sup> concentrations of nearly 0.5 mg L<sup>-1</sup>, the potential denitrification rates in the Aransas River resembled those in the Mission River at  $2.56 \pm 0.36$  and  $2.71 \pm 0.67$  mg N m<sup>-2</sup> h<sup>-1</sup>, respectively. Potential denitrification rates were low ( $< 2$  mg N m<sup>-2</sup> h<sup>-1</sup>; Fig. 7) at the tidal river sites. Denitrification rates varied most at the western Copano Bay site, where sampled NO<sub>3</sub><sup>-</sup> concentrations remained below detection at the time of

sampling. The ratio of denitrification rates to NO<sub>3</sub><sup>-</sup> concentrations illustrates the potential for water column NO<sub>3</sub><sup>-</sup> to be removed via denitrification at each site (Fig. 7C). This ratio was the lowest in the Aransas River, with high NO<sub>3</sub><sup>-</sup> concentrations, and highest in eastern Copano Bay, with low NO<sub>3</sub><sup>-</sup> concentrations (Fig. 7). Additionally, a high ratio of denitrification rate to water column NO<sub>3</sub><sup>-</sup> may suggest that water column NO<sub>3</sub><sup>-</sup> may not be the primary source of NO<sub>3</sub><sup>-</sup> for



denitrification. Instead, coupled nitrification-denitrification may be an important source of  $\text{NO}_3^-$  for denitrification in the estuary sites.

Broad indicators of microbial activity including sediment oxygen demand and ecosystem metabolism (Table 1) suggest active microbial communities and indicate their relative ability to process and transform nutrients from river to estuary. Sediment oxygen demand, measured concurrently with denitrification at each site, differed among the three habitat types from river to estuary (mixed effects ANOVA  $p < 0.01$ ) and across all six sampling sites (ANOVA  $p < 0.01$ ). In general, river sites exhibited the lowest SOD rates, and the tidal river sites exhibited the highest SOD rates. Along the Mission River, SOD was lower in the river compared to western (Tukey's HSD;  $p = 0.024$ ) and eastern (Tukey's HSD;  $p = 0.027$ ) Copano Bay. Along the Aransas River, the river site had lower SOD than the tidal river site, western and eastern Copano Bay (Tukey's HSD; each at  $p < 0.01$ ). Despite the larger WWTP effluent input to the Aransas River, SOD of  $9.69 \text{ mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$  was lower in the Aransas River than in the Aransas Tidal River (Tukey's HSD  $p < 0.01$ ) or Copano Bay (Tukey's HSD  $p < 0.01$ ).

Although ecosystem metabolism was not measured in the tidal river sites, comparisons between the river and estuary sites suggest that GPP and R were an order of magnitude lower in the river compared to estuary sites, but remained net heterotrophic throughout (Table 1). The Aransas River GPP, at  $0.284 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , was comparable to GPP in the Mission River at  $0.355 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ . Thus, the larger WWTP presence in the Aransas River catchment did not alter ecosystem metabolism relative to the Mission River (Table 1). The Mission River site was closest to balance between GPP and R (NEP =  $-0.063 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ; Table 1). Finally, the western Copano Bay site was the most net heterotrophic, at  $-1.14 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ .

## Discussion

### Do N cycling processes in rivers mediate N export to estuaries during drought?

A snapshot measurement of riverine N processing revealed that assimilatory uptake and denitrification occurred along the path from river to estuary during the drought (Figs. 6, 7; Vanderborgh et al. 2007; Pennino et al. 2016). Rivers are more hydrologically disconnected from the terrestrial landscape, groundwater, and between riffle and pool habitats within the river reach during the drought (Bernal et al. 2013). Additionally, shallower water and slower water velocities enhance contact time between the water and the benthic zones (Valett et al. 1996). As a result, in-stream N processing is more important at the catchment scale during periods of low hydrological connectivity (Bernal et al. 2012). Our patterns of declining N concentrations from river to estuary suggests N consumption from river to estuary during

the drought (Fig. 5). Our reach-scale empirical assessments of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake also showed active N processing (Fig. 6) and increased DON concentrations in the Mission Tidal River site (Fig. 5D) also suggest active N processing. Water column  $\text{NH}_4^+$  cycling data also show consistent rates from river to estuary during the drought and reinforce the importance of internal N cycling (Bruesewitz et al. 2015). Altered hydrological conditions during drought create favorable conditions for both assimilation and denitrification of N along the path from river to estuary. Nitrogen process rate data from other periods are limited or not available for comparison to drought conditions, so we cannot compare these patterns to rates under non-drought conditions. However, our nutrient loading data comparing high flow and low flow conditions (Figs. 2, 3) indicate that more N is exported to Copano Bay during floods.

Denitrification rates remained quite consistent from the rivers to the central estuary site (Fig. 7). They resemble results from streams across a salinity gradient in the semi-arid climate of the Mediterranean (Arce et al. 2014) and in coastal North Carolina (Fear et al. 2005) where denitrification rates also did not vary with salinity. In each case, denitrification was not a major contribution to the overall  $\text{NO}_3^-$  uptake in saline rivers (Fear et al. 2005; Arce et al. 2014). Likewise, only 4% of N was denitrified in the Aransas River. N limitation of denitrification is unlikely, given the high  $\text{NO}_3^-$  concentrations downstream of the WWTP in the Aransas River. We suspect that denitrification rates were limited by labile carbon availability in the benthos of the Aransas River. Sediment organic matter content was low, at  $< 1\%$  organic matter (data not shown; DeSimone and Howes 1996; Bernot and Dodds 2005) and autochthonous DOC may degrade rapidly during periods of drought (Casas-Ruiz et al. 2016). Overall, our data show consistent denitrification rates across river, tidal river, and estuarine sites (Fig. 7), despite the capacity for a diversity of environmental drivers within and among sites. Models of the Scheldt river-estuary system also illustrate similar N transformation rates can be similar across river to estuary gradients, despite large differences in surface area and volumes represented by riverine and estuarine habitats (Vanderborgh et al. 2007).

Water residence time is a key factor in regulating denitrification in river mouths and estuaries (Seitzinger et al. 2006; Silvennoinen et al. 2008). The water residence time in the Mission and Aransas Tidal Rivers is comparatively long during drought (S. L. Johnson, unpubl.), and denitrification accounted for more overall net N uptake in the tidal river and estuary sites than in the river sites (Fig. 6). Assimilation often dominates water column  $\text{NO}_3^-$  removal, whereas denitrification dominates benthic  $\text{NO}_3^-$  removal (Kemp et al. 1990; Cornwell et al. 2014). Our data suggest that both processes are important at tidal river sites during drought. Biological demand for N in the water column increased as severe drought conditions persisted (Bruesewitz et al. 2015).

### Influence of WWTP inputs during drought

The large presence of WWTP upstream in the Aransas River changed the character and quantity of N export relative to the Mission River (Fig. 3) despite the presence of fewer WWTP in the Mission River catchment, which released a smaller quantity of wastewater and were further away from our river sites than in the Aransas River (Fig. 1). The steady input of WWTP water in the Aransas River increased the baseflow export of N, switched the dominant export from organic to inorganic N, and minimized variation in both riverine salinity and N : P (Fig. 4). The input of wastewater can increase N export and water quantity (Bowen and Valiela 2001; Carey and Migliaccio 2009; Iverson et al. 2015). Additionally, the effects of WWTP inputs to rivers are enhanced during drought (Andersen et al. 2004; Passell et al. 2005) or seasonal low flow (Pennino et al. 2016), because the effluent proportion of the river flow increases. High  $\text{NO}_3^-$  uptake rates followed elevated  $\text{NO}_3^-$  concentrations in the Aransas River (Fig. 6), suggesting that increased assimilatory WWTP N uptake was the primary response of river organisms to this WWTP input (Pennino et al. 2016). However, the low uptake velocities of both  $\text{NO}_3^-$  ( $0.030 \text{ mm s}^{-1}$ ) and  $\text{NH}_4^+$  ( $0.007 \text{ mm s}^{-1}$ ) in the Aransas River, coupled with less relative denitrification, suggest that the N inputs of the WWTP overwhelmed the capacity of the river ecosystem to process N, reflected by sustained DIN export during the drought (Martí et al. 2004).

DIN : SRP never exceeded 11 : 1 in the Aransas River; but varied more in the Mission River (Fig. 4). The consistent inputs of WWTP in the Aransas River stabilized DIN : SRP ratios places them in the range of N limitation (Fisher et al. 1999; Jordan et al. 2008), whereas the Mission River DIN : SRP ratios suggest that periods of P limitation also occurred, particularly at low salinities (Fig. 4). DIN : SRP was below 16 : 1 at high salinities (river salinity > 2) at both river sites during all but one sampling occasion, suggesting that N limitation dominated both rivers during drought conditions. Strong N limitation of ecosystem productivity could drive N removal from river to river-mouth (Jordan et al. 2008), making these rivers “transformers” rather than “transporters” of N during drought conditions (Kaushal and Belt 2012).

Nitrate uptake was higher in the Aransas River than in the Mission River, but  $\text{NH}_4^+$  uptake and denitrification rates were similar between sites, suggesting that the latter two processes were not stimulated by the increased nutrient-rich WWTP outputs on the Aransas River (Fig. 6). Similarly, our snapshot of riverine GPP and R were not enhanced by the elevated WWTP nutrients (Table 1). Although some receiving rivers have high capacities for N assimilation and removal downstream of WWTP (Ribot et al. 2012; Pennino et al. 2016), river ecosystems often cannot process the WWTP nutrient inputs efficiently, e.g., in Mediterranean streams (Martí et al. 2004; Merseburger et al. 2011; Arnon et al. 2015), tropical streams (Figueroa-Nieves et al. 2016) and Arkansas, U.S.A. streams (Haggard et al. 2001) downstream

of WWTPs. The WWTP influence on the tidal river sites N processing was minimal, however, as both Mission and Aransas tidal river sites had similar N fluxes (Fig. 6). Our river site was 20 km downstream of the largest WWTP in the Aransas River catchment, and the tidal river site was an addition 55 km farther downstream. The WWTP-derived nutrients were likely processed before reaching the tidal river sampling sites (Vanderborgh et al. 2007; Pennino et al. 2016).

### Challenges and uncertainties of measuring N transformation rates across aquatic habitats

Measurement of ecosystem function metrics are crucial to understanding rapidly changing systems (Palmer and Febria 2012). Identifying differences in aquatic ecosystem function helps explain ecosystems functions, but effective tools are needed for such comparisons. The suite of metrics for nutrient cycling processes used in streams (Stream Solute Workshop 1990; Earl et al. 2007; Trentman et al. 2015) and estuaries (Cornwell et al. 1999; Joye and Anderson 2008) vary, and cannot be implemented directly across riverine and estuary sites. Effective stream measurements of N cycling rates must strongly consider the unidirectional movement of water across a diverse benthic habitat at the reach scale, whereas estuarine methods typically focus on processes that occur at the sediment-water interface in static cores or cores with minimal flow. Often, core studies require that different estuarine habitats be considered separately (Piehler and Smyth 2011), whereas reach-scale stream measurements often incorporate across habitat types within a stream reach (Fellows et al. 2006; Hoellein et al. 2009). Additionally, the structural differences along a river-estuary continuum make  $^{15}\text{N}$  tracer methods more accessible in estuarine sediment core studies (Gardner et al. 2006; Hardison et al. 2011; Smyth et al. 2015), whereas whole-stream  $^{15}\text{N}$  releases are expensive (but see Tank et al. 2000; Mulholland et al. 2008; Hall et al. 2009). Because we could not use  $^{15}\text{N}$  in our whole-stream nutrient uptake measurements, we slightly enriched N concentrations and examined the change in concentration with distance downstream, as values returns to near ambient concentrations. However, metrics of flow-through cores, have only a slight  $^{15}\text{N}$  enrichment, and the flux is determined from values which may increase or decrease relative to the ambient concentrations. While these differences in methods are acknowledged, combining approaches provides a crucial step to compare processes of N uptake and removal across these diverse biogeochemical sites.

Given these challenges, it is important but difficult to exchange ideas across freshwater and estuarine systems (Bierschenk et al. 2012). Continuing method syntheses is needed to generate and compare direct measurements of N cycling rates and pathways from river to estuary. In this study, methodology used to measure N cycling processes at each site within a short time frame attempted to balance physical

differences along the river-estuary continuum along with logistic considerations to provide legitimate comparisons (Table 2). We could only measure N uptake and removal processes during drought conditions, and so we limit our data discussions in this paper to drought conditions. Many of the methods, particularly at river sites, cannot be implemented during high flow conditions, when mass transport is relatively more important.

Despite decades of measurements, denitrification measurements remain challenging (Groffman et al. 2006), especially when research questions span aquatic habitats preventing use of same methods across habitats. Microcosm slurries for denitrification measurements at river sites allowed cost-effective use of  $^{15}\text{NO}_3^-$  tracer and high replication for the diverse sediment substrata within the river reach. Additionally, the shallow water and unconsolidated sediments of the river were assessed more accurately with our microcosm method than feasible with intact cores because they allowed for more exchange between the water and the sediment than would occur in a static core environment. Scaling of microcosm data to the river-reach scale may overestimate denitrification rates, but our riverine denitrification rates were comparable to other stream and river measurements using a variety of methods (McCarthy et al. 2007; Mulholland et al. 2008; Arce et al. 2014). Dissolved  $\text{N}_2$  gas methods, used for recent stream-reach denitrification measurements (Laursen and Seitzinger 2002; Pribyl et al. 2005; Reisinger et al. 2016), refine denitrification comparisons from river to estuary.

### Management implications

Direct comparisons of critical ecosystem processes from river to estuary are lacking even though anthropogenic land use undoubtedly alters ecosystem function in rivers and estuaries (Bierschenk et al. 2012; but see Garnier et al. 2001; Pennino et al. 2016). Unraveling these concepts is a non-trivial research challenge. Coastal ecosystem managers must consider eutrophication along the continuum that includes both freshwater and marine ecosystem dynamics (Smith et al. 2006), and management of both N and P export to aquatic ecosystems (Paerl et al. 2014, 2016). Ecosystem management must deliver freshwater to coastal ecosystems in semi-arid climates (Montagna et al. 2002; Russell et al. 2006). Our data suggest that drought periods may decrease anthropogenic N inputs to coastal systems, as N can be assimilated or removed along the route to estuaries. As coastal populations and freshwater demand increase, river regulations to meet anthropogenic freshwater needs are required to decrease downstream N export significantly (von Schiller et al. 2015). Our data suggest that this pattern may result from increased N processing in upstream environments, and diversion of freshwater from estuaries. Management decisions should include consideration of how these coastal ecosystems respond to flooding events

after prolonged periods of drought in management of downstream eutrophication.

### Conclusion

Our study of N dynamics across a coastal catchment during a period of prolonged drought highlights the unique behavior of a river influenced strongly by WWTP under low baseflow conditions. In such systems WWTP are a major source of N exported from river to estuary. They minimize differences in the amount of N export between periods of flood and drought. By contrast, rivers in semi-arid climates without major WWTP export very little N (mostly DON). The path from river to estuary is an active “waterscape” of N uptake and denitrification. Although denitrification rates are quite uniform, this process is not a quantitatively significant process removing N along the river-estuary continuum. WWTP  $\text{NO}_3^-$  was primarily assimilated in the river, representing a temporary storage of this anthropogenic N. Although rare, studies of ecosystem function from river to estuary are critical to improving understanding of N cycling mechanisms, and developing appropriate catchment-scale management strategies to prevent eutrophication while maintaining freshwater inflows to estuaries.

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### Conflict of Interest

None declared.

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