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Benoit Lebreton, Jennifer A Pollack, Brittany A Blomberg, Terence A Palmer, Leslie A Adams, Gaël Guillou, Paul A Montagna

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**Origin, composition and quality of suspended particulate organic matter in relation to  
freshwater inflow in a South Texas estuary**

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**Abstract**

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SPOM: suspended particulate organic matter; Chl *a*: chlorophyll a; GoM: Gulf of Mexico; POC: Particulate organic matter.

1 South Texas has a semi-arid climate with a large interannual variability of freshwater inflows. This  
2 study sought to define how changes in freshwater inflow affect the composition, quantity and  
3 quality of suspended particulate organic matter (SPOM) in a South Texas estuary: the Mission-  
4 Aransas estuary. The study was implemented 1.5 months after a large rain event in September 2010  
5 and continued for 10 months of drought conditions. The composition of SPOM originating from  
6 rivers, the Gulf of Mexico and the estuary were determined using stable isotopes ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  
7  $\delta^{34}\text{S}$ ). The quantity and quality of SPOM were assessed using organic carbon content, chlorophyll *a*  
8 concentrations and C/chl *a* ratios. Our results demonstrated that autochthonous phytoplankton was  
9 the dominant component of SPOM in the Mission-Aransas estuary during droughts. Benthic organic  
10 matter from local primary producers (i.e., seagrass, salt marsh plants, benthic microalgae) did not  
11 influence SPOM composition, either as fresh material or as detritus. A comparison with a positive  
12 estuary (i.e., Sabine-Neches estuary, TX) indicates that decreases in freshwater inflow may lead to  
13 decreases of terrestrial organic matter inputs and to increase the ratio of autochthonous  
14 phytoplanktonic material in SPOM.  
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## 32 **Highlights**

- 33 - Phytoplankton dominate particulate organic matter in the Mission-Aransas estuary.
- 34 - Benthic organic matter does not influence particulate organic matter composition.
- 35 - Organic matter drained by rivers during minor rain events is of very poor quality.

## 36 **Keywords**

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## 1. Introduction

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Estuaries are among the most productive ecosystems on Earth, with production equal to that of tropical rain forests (McLusky, 1989). The quantity and timing of freshwater delivery to the mixing zone is essential to their functioning (Montagna et al., 2002). Freshwater inflows play key roles in carrying continental organic matter from the watershed to the estuary (Hedges et al., 1997), and in balancing effects of tidal inputs of saltwater and of evaporation, consequently affecting the structure of habitats. As a result, freshwater inflows affect inputs of continental and marine organic matter and autochthonous pelagic and benthic primary production (Longley, 1995). Increasing human populations will result in an increasing demand for freshwater, which will affect freshwater inflow into estuaries and have consequences on the functioning of estuarine ecosystems (Montagna et al., 2002).

One consequence is the potential change of composition of suspended particulate organic matter (SPOM), an integral component of estuarine food webs (Longley, 1994). Organic matter locally produced in estuaries or provided by rivers or the sea varies in terms of quantity and quality depending on its origin and its state of decay (Dunton et al., 2001; Raymond and Bauer, 2001). This diversity of primary productions can be used by many different consumers, making highly complex estuarine food webs. Consequently, estuaries provide many ecological functions, including breeding grounds, nurseries and feeding sites for a large diversity of pelagic and benthic species (Beck et al., 2001). For example, SPOM is generally the main food source of suspension feeders like oysters. As a result, a change of abundance or composition of SPOM may severely affect the oyster diet (Riera and Richard, 1997), and therefore the general functioning of oyster reefs, which provide many ecological functions (Beck et al., 2011; Grabowski et al., 2012).

The origins, quantity, and quality of SPOM can be determined by measuring several variables, including stable isotope ratios, chlorophyll *a* (chl *a*) content and C/chl *a* ratios. Stable isotope ratios of carbon and of sulfur provide information about the origin of organic matter. Stable isotopes of carbon allow for discrimination between the different estuarine primary producers (Fry and Sherr, 1984; Peterson and Fry, 1987) and are often used to determine composition of SPOM in estuaries

(Cifuentes et al., 1988; Riera and Richard, 1997; Savoye et al., 2003; 2012; Modéran et al., 2012).

Due to potential overlaps of carbon stable isotope composition between different primary producers and the effects of decay processes on isotopic composition (Benner et al., 1987), stable isotopes of sulfur can be used to discriminate between the different food sources. Primary producers from continental areas are generally much more  $^{34}\text{S}$ -depleted than primary producers from oceanic environments (Peterson et al., 1985; Peterson and Howarth, 1987; Thode, 1991). Stable isotope composition of nitrogen is generally used to determine the trophic levels of consumers (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 1999), but can also be an indicator of anthropogenic influence (i.e., release of wastewater from treatment plants) due to increase of  $\delta^{15}\text{N}$  values with bacterial activity (McClelland and Valiela, 1998; Mooney and McClelland, 2012). The measurement of these three stable isotopes enables discrimination between the different pools of organic matter in order to determine its origin. The measurement of total organic carbon, chl *a* and C/chl *a* ratios along with these stable isotope ratios help determine the quantity and the quality of SPOM along a salinity gradient. The aim of this study was to determine how the different primary producers and inputs of allochthonous organic matter (i.e., from continental and marine origins) affect the composition, quantity and quality of SPOM, and to determine the evolution of these parameters in relation to changes in freshwater inflows. This study was conducted in the Mission-Aransas estuary, Texas, a semi-arid estuary that is vulnerable to changes in natural and anthropogenic changes in freshwater inflow.

## **2. Material and methods**

### **2.1 Study area**

The Mission-Aransas estuary is one of seven major estuarine systems located along the Texas coast (Fig. 1). It is a shallow, bar-built estuary composed of Aransas bay, the primary bay located closest to the Gulf of Mexico inlet, and Copano Bay, the secondary bay located closest to the two main rivers: the Mission and Aransas rivers. The Mission-Aransas estuary is 2 m deep on average, its

1 surface is 463 km<sup>2</sup> and its volume is 0.93 km<sup>3</sup> (Armstrong, 1987). The Copano bay watershed is 4851  
2 km<sup>2</sup> (Borel et al., 2015); 49% of the freshwater comes from the Mission river and 15% from the  
3 Aransas river (Orlando et al., 1993).  
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7 The Mission-Aransas estuary is located in a sub-tropical humid climate. Rainfalls generally occur  
8 as brief and intense rain showers and as a result, freshwater inflows occur as episodic pulses.  
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10 Rainfall, and consequently freshwater inflows, varies from year to year with alternating dry and wet  
11 periods (Evans and Morehead Palmer, 2012). From 2001-2010, freshwater inflows from both rivers  
12 ranged from 17 to 430 million m<sup>3</sup> y<sup>-1</sup> (U.S. Geological Survey, 2012). On an annual basis, Mission-  
13 Aransas estuary is losing water due to higher evaporation (1513 mm y<sup>-1</sup>) than precipitation (886 mm  
14 y<sup>-1</sup>) (Armstrong, 1987). The estuary has low mixing efficiency (< 0.05) and long residence times (~360  
15 days, Solis and Powell, 1999). The salinity structure is driven by episodic freshwater pulses that  
16 depress salinities and then maintain low salinities for a prolonged period (Orlando et al., 1993).  
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28 The Mission-Aransas estuary hosts diverse primary producers and is fueled by different  
29 allochthonous sources i.e., continental organic matter from Mission and Aransas rivers in Copano bay,  
30 pelagic organic matter from the Gulf of Mexico in Aransas bay, organic matter from San Antonio bay  
31 transported southward across the Mesquite bay via the Intracoastal Waterway (Solis and Powell,  
32 1999).  
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## 42 **2.2 Sampling stations**

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44 Five stations were sampled along a salinity gradient in the Mission-Aransas estuary, with station 1  
45 (St. 1) the closest to the Aransas river mouth and station 5 (St. 5) the closest to the Gulf of Mexico  
46 (Fig. 1). Three other stations were sampled to collect freshwater from the two main rivers (Mission  
47 river (MIS) and Aransas river (ARA), and sea water from the Gulf of Mexico (GoM). Freshwater inputs  
48 into Copano bay were determined by summing flows of Mission and Aransas rivers using data from  
49 USGS gauge stations (Fig. 1) located at Refugio (Mission river, Site ID: 08189500) and Skidmore  
50 (Aransas river, Site ID: 08189700) (U.S. Geological Survey, 2012).  
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### 2.3 Collection of water samples and sample processing

At each station, three samples of surface water were collected on a monthly basis, from November 2010 (relatively high freshwater inflow) to August 2011 (relatively low freshwater inflow). All water samples were collected far enough from the shore to be representative of the river or bay water mass. Due to restricted access, it was impossible to sample water at the mouth of the Mission river.

Salinity was measured with an YSI 6920 multiprobe sonde, using practical salinity scale. Water samples were pre-filtered on a 250- $\mu\text{m}$  sieve to eliminate large zooplankton and detritus. For each water sample, SPOM was collected on four different filters (precombusted Whatman GF/F glass fiber filters, 0.7  $\mu\text{m}$  porosity) in order to measure stable isotope composition ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$ ) and chl *a* concentration. A volume ranging from 5 to 325 ml was filtered under moderate vacuum; filters were then frozen at  $-20^{\circ}\text{C}$  and freeze-dried. Carbonates were removed from filters for  $\delta^{13}\text{C}$  and %C analyses by contact with HCl fumes in a vacuum-enclosed system.

### 2.4 Collection of primary producers for stable isotope analyses

To determine the composition of SPOM, the main primary producers ( $\text{C}_3$  and  $\text{C}_4$  salt marsh plants, seagrass, benthic microalgae) were sampled at different locations along the salinity gradient and during different seasons to examine possible spatio-temporal changes in isotopic composition. Vascular plants (leaves) and seagrass (leaves and roots) were collected, placed in plastic bags and stored in ice until return at the lab. They were then rinsed with tap water to remove detritus and sediment, frozen at  $-20^{\circ}\text{C}$ , freeze-dried and ground to a fine and homogeneous powder using a ball mill.

Benthic microalgae were collected by scraping surface sediments in the field and then by extracting the microalgae in the laboratory following the method of Riera and Richard (1996), slightly modified by Herlory et al. (2007). Extracted samples were checked under a microscope for

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purity, then benthic microalgae were collected on three different filters (precombusted Whatman GF/F glass fiber filters) in order to measure stable isotope composition ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$ ). Samples were frozen at  $-20^\circ\text{C}$  then freeze-dried.

## 2.5 Chl *a* and stable isotope ratio measurements

Chl *a* was extracted overnight from filters and read on a Turner Trilogy fluorometer (Turner Designs, Sunnyvale, USA) using a non-acidification technique (Welschmeyer, 1994). Detection limit of the fluorometer was  $0.025 \mu\text{g.L}^{-1}$  with a linear range of  $0\text{-}300 \mu\text{g.L}^{-1}$ . Calibration was performed once a year using commercially available fluorometric chlorophyll calibration standards for chlorophyll *a* (Turner Designs).

Samples for stable isotope analyses and particulate organic carbon percentages were analyzed using an elemental analyzer (Flash EA 1112, Thermo Scientific, Milan, Italy) coupled with an isotope ratio mass spectrometer (Delta V Advantage with a ConFlo IV interface, Thermo Scientific, Bremen, Germany) at the LIENSs stable isotope facility of the University of La Rochelle, France. Results are expressed in the  $\delta$  notation as deviations from standards (Vienna Pee Dee Belemnite for  $\delta^{13}\text{C}$ ,  $\text{N}_2$  in air for  $\delta^{15}\text{N}$  and Canyon Diablo Troilite for  $\delta^{34}\text{S}$ ) following the formula:  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  or  $\delta^{34}\text{S} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$ , where  $R$  is  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$  or  $^{34}\text{S}/^{32}\text{S}$ , respectively. Calibration was done using reference materials (USGS-24, IAEA-CH6, -600 for carbon; IAEA-N2, -NO-3, -600 for nitrogen; IAEA-S-1, -S-2 for sulfur). Analytical precision based on the analyses of acetanilide (Thermo Scientific) used as laboratory internal standard was  $<0.06$  and  $<0.1\text{‰}$  for carbon and nitrogen, respectively. Analytical precision based on the analyses of sulfanilamide (Thermo Scientific) used as laboratory internal standard was  $<0.15\text{‰}$ . Vanadium pentoxide was added in each SPOM sample as a catalyzer during sample combustion for sulfur elemental analysis.

## 2.6 Data analyses and statistics



1 Mean daily freshwater discharges were determined from September 1<sup>st</sup>, 2010 to September, 30<sup>th</sup>  
2 2011, using data from USGS gauge stations. Loads of POC into the Copano bay from the Aransas and  
3 the Mission rivers were computed by multiplying the mean daily freshwater discharge with the  
4 concentrations of POC ( $\mu\text{g}\cdot\text{ml}^{-1}$ ). C/chl *a* ratios were computed using concentrations of particulate  
5 organic carbon ( $\mu\text{g l}^{-1}$ ) from elemental analyses and of chlorophyll *a* from fluorimetry measurements  
6 ( $\mu\text{g l}^{-1}$ ). Comparisons between stable isotope values of primary producers and SPOM samples were  
7 conducted using nonparametric procedures (i.e., Kruskal-Wallis rank sum test and Wilcoxon signed  
8 rank test), to determine any differences between locations, sampling dates and tissues (roots and  
9 leaves). Kruskal–Wallis tests were followed by multiple comparisons of means using the pgirmess  
10 package (Giraudoux, 2011) in R statistical software (R Development Core Team, 2010).  
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### 26 **3. Results and discussion**

#### 27 **3.1 River discharges and hydrological features of the Mission-Aransas estuary in 2010-2011**

28 The year 2011 was a very dry year—the second driest (after 2008) of the past 10 years (Mooney  
29 and McClelland, 2012). Inputs of freshwater from the Aransas and Mission rivers to Copano bay  
30 were very low, with 21 million  $\text{m}^3$ , compared to an average of 170 million  $\text{m}^3 \text{y}^{-1}$  over the past 10  
31 years (U.S. Geological Survey, 2012). Freshwater inflows in the Mission-Aransas estuary decreased  
32 by more than 2 orders of magnitude from September 2010 ( $2.30 \cdot 10^6 \text{ m}^3 \text{ d}^{-1}$ ) to September 2011  
33 ( $8.05 \cdot 10^3 \text{ m}^3 \text{ d}^{-1}$ ). A large pulse of freshwater occurred in September 2010—before the start of the  
34 experiment— with the highest input of  $1.92 \cdot 10^7 \text{ m}^3 \text{ d}^{-1}$  on September 20<sup>th</sup>, leading to a strong salinity  
35 gradient in the estuary (Fig. 2 ; Mooney and McClelland, 2012; Bruesewitz et al., 2013). A minor  
36 pulse occurred in January 2011, with the highest input of  $5.15 \cdot 10^6 \text{ m}^3 \text{ d}^{-1}$  on January 17<sup>th</sup>, 2011.  
37 During the January pulse (January 17<sup>th</sup> and 18<sup>th</sup>, 2011), 96 and 4 % of the freshwater inflow were  
38 issued from Mission and Aransas rivers, respectively.  
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56 Salinity increased along a gradient from Aransas river mouth (Station 1) to the Gulf of Mexico  
57 (GoM) (Fig. 2). This gradient was more evenly distributed along the length of the sampling transect  
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in November 2010 (salinity from 5.2 to 24.3) than in August 2011 (salinity from 30.5 to 37.3) due to the overall increase of salinities in Copano and Aransas bays. The salinities of the river station ARA were close to zero throughout the study period although the salinities at the MIS river station rose to 5 by August 2011. Salinities measured at Station 5 and GoM were similar throughout the sampling period.

On average, evaporation largely exceeds precipitation in the Mission-Aransas estuary and south east winds generally predominates (Armstrong, 1987), which leads to a flow of water from Aransas bay into Copano bay, this flow being twice more than the volume of the freshwater issued from rivers (Johnson, 2009). Minor rain events in the Mission-Aransas watershed, like the one observed in January 2011, do not have any effect on the general hydrology of the bay, as evidenced by the general increase of salinity in the bay throughout the study period (Bruesewitz et al., 2013). Nevertheless, a slight decrease in salinity was observed at Station 4 in January 2011, indicating a potential flow of freshwater originating from San Antonio bay, which is connected to Aransas bay via the Intracoastal Waterway in Mesquite bay (Solis and Powell, 1999). As a result, from November 2010 to August 2011, a combination of low freshwater inflow, high evaporation and flow of saline water from Aransas bay into Copano bay led to a general increase in salinity throughout the estuary and compression of the salinity gradient, which was observed in November 2010. Thus, from November 2010 to August 2011, the Mission-Aransas estuary went from a system dominated by precipitation (i.e., positive estuary) to a system dominated by evaporation (i.e., negative estuary) (Pritchard, 1952; Montagna et al., 2013).

During the study period, particulate organic carbon (POC) concentrations ranged from 0.48 (December 10) to 2.8  $\mu\text{g ml}^{-1}$  (August 11) in Aransas river and from 0.53 (March 11) to 2.12  $\mu\text{g ml}^{-1}$  (August 11) in Mission river. POC concentration data in Mission river in November and December are missing due to technical issues. Except in January, computed load of POC into the Mission-Aransas estuary ranged from 334 to 939  $\text{kg month}^{-1}$  and from 15 to 1241  $\text{kg month}^{-1}$  for Aransas and Mission rivers, respectively. In January, POC load into the Mission-Aransas estuary was much higher,

1 particularly for Mission river (13097 kg month<sup>-1</sup>) while Aransas river POC load was 3292 kg month<sup>-1</sup>  
2 (Fig. 2).  
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### 7 **3.2 Discrimination of the different benthic primary producers using stable isotopes**

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9 Salt marsh plants had typical isotopic compositions for carbon as C<sub>3</sub> and C<sub>4</sub> plants were relatively  
10 <sup>13</sup>C-depleted (-29.2 to -26.8‰) and <sup>13</sup>C-enriched (-15.5 to -12.5 ‰), respectively (Table 1, Fig. 3). This  
11 disparity is mostly related to different enzymatic processes occurring during photosynthesis and  
12 differential utilization of HCO<sub>3</sub> and CO<sub>2</sub> (Smith and Epstein, 1971; Fry and Sherr, 1984; O'Leary et al.,  
13 1992). Seagrass material was the most <sup>13</sup>C-enriched, with δ<sup>13</sup>C values ranging from -14.7 to -10.7‰,  
14 typical for seagrasses (Hemminga and Mateo, 1996) and slightly higher than C<sub>4</sub> vascular plants (Table  
15 1, Fig. 3). Detrital matter from seagrass had a very low δ<sup>13</sup>C value at station 3 (-18.1‰) whereas δ<sup>13</sup>C  
16 value at station 4 (-11.9‰) was in the range of fresh material. This large difference is probably  
17 related with the degradation process which leads to higher levels of lignin in detrital matter, which is  
18 <sup>13</sup>C-depleted (Benner et al., 1987). As a result, degradation of seagrass may lead to a larger overlap  
19 between δ<sup>13</sup>C values of seagrass detritus and those of C<sub>4</sub> salt marsh plants, either as fresh or as  
20 detritus. Benthic microalgae had a wide range of δ<sup>13</sup>C values, ranging from -21.7 to -12.0‰, largely  
21 overlapping the ranges of values observed for seagrass and C<sub>4</sub> vascular plants.  
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40 No pattern was observed for δ<sup>13</sup>C values of C<sub>3</sub> and C<sub>4</sub> plants between species, sampling dates and  
41 locations in the bay. Leaves and roots of seagrass were more <sup>13</sup>C-enriched in downstream sections  
42 than in upstream sections of the Mission-Aransas estuary (Table 1, Kruskal-Wallis rank sum test, p-  
43 values: *Ruppia maritima*: leaves: 0.013, roots: 0.016, *Halodule wrightii*: leaves: 0.001, roots: 0.004).  
44 A similar pattern was observed for microphytobenthos (Kruskal-Wallis rank sum test, p-value: 0.035)  
45 and was clearly related to salinity: microphytobenthos had lower δ<sup>13</sup>C values (from -21.7 to -16.4‰)  
46 at low salinities (Stations 1 and 3 in November, station 1 in March) than at high salinities (Fig. 4). This  
47 is probably due to the higher influence of dissolved inorganic carbonates from freshwater, which are  
48 <sup>13</sup>C-depleted (Fry and Sherr, 1984). It is thus possible to discriminate these benthic algae from the  
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1 other primary producers. The  $\delta^{13}\text{C}$  values of benthic algae collected in marine influenced  
2 environments, ranging from -14.2 to -12.0‰, were in the range of those from the literature (Currin  
3 et al., 1995; Riera and Richard, 1996; Riera et al., 1999; Kharlamenko et al., 2008. Lebreton et al.,  
4 2011).  
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9 Seagrass,  $\text{C}_3$  and  $\text{C}_4$  vascular plants had a large range of  $\delta^{15}\text{N}$  values, ranging from 2.0 to 9.2‰,  
10 from 4.5 to 10.0‰ and from -1.1 to 7.7‰, respectively. Very different  $\delta^{15}\text{N}$  values were observed  
11 between the two samples of seagrass detrital matter, with values of 7.5‰ at station 3 and 1.8‰ at  
12 station 4 in November 2010. *Halodule wrightii* and *Spartina alterniflora* clearly had higher  $\delta^{15}\text{N}$   
13 values in upstream parts of the estuary than in downstream parts (Table 1, Wilcoxon signed rank  
14 test, p-values: *Halodule wrightii*: leaves: 0.003, roots: 0.004, *Spartina alterniflora*: <0.001).  $\delta^{15}\text{N}$   
15 values of microphytobenthos ranged from -0.6 to 5.3‰, with slightly higher  $\delta^{15}\text{N}$  values at lower  
16 salinities.  
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28 Sulfur stable isotope composition of primary producers was highly variable, even at the species  
29 level (e.g., *Spartina alterniflora*, *Borrchia frutescens*, *Ruppia maritima*) (Table 1, Fig. 3). Among  
30 primary producers,  $\text{C}_3$  vascular plants were the most enriched in  $^{34}\text{S}$ , with  $\delta^{34}\text{S}$  values ranging from  
31 7.1 to 18.4‰.  $\text{C}_4$  vascular plants were less enriched in  $^{34}\text{S}$ , with values ranging from -5.8 to 7.1‰.  
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33 However, these values were higher than those previously observed during other studies (Peterson et  
34 al., 1986; Peterson and Howarth, 1987; Sullivan and Moncreiff, 1990; Currin et al., 1995). This large  
35 range of  $\delta^{34}\text{S}$  values is linked with differential assimilation of sediment sulfides, which are  $^{34}\text{S}$ -  
36 depleted, and of sulfates, which are  $^{34}\text{S}$ -enriched, depending on seasonal and spatial conditions  
37 (Carlson and Forrest, 1982; Fry et al., 1982; Currin et al., 1995; Stribling et al., 1998). The much  
38 higher  $\delta^{34}\text{S}$  values measured on  $\text{C}_3$  plants in comparison with those of  $\text{C}_4$  plants indicate that  $\text{C}_3$   
39 plants assimilate sulfates originating from precipitation (0 to 15‰) and seawater (21‰) (Peterson et  
40 al., 1986; Peterson and Howarth, 1987; Krouse et al., 1991; Connolly et al., 2004). No clear pattern  
41 was observed between  $\delta^{34}\text{S}$  values and salinity, location or sampling time for  $\text{C}_3$  and  $\text{C}_4$  vascular  
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1 Microphytobenthos had intermediate  $\delta^{34}\text{S}$  values, going from 4.1 to 11.8‰, in the range of those  
2 reviewed by Currin et al. (1995). No relation was observed between  $\delta^{34}\text{S}$  values of  
3 microphytobenthos and salinities, in contrast to the pattern observed in carbon isotopic  
4 composition, as these samples were collected at salinities (>5.2) for which isotopic composition of  
5 sulfates is already relatively high. In the Mississippi river estuary, Fry and Chumcall (2011)  
6 highlighted that the stable isotope composition of sulfates mostly varies at very low salinities (<0.6)  
7 and then remains relatively stable (21‰), close to the isotopic composition of sulfates in seawater  
8 (Nriagu et al., 1991).  
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19 Seagrass also had a wide range of  $\delta^{34}\text{S}$  values, with roots generally much more  $^{34}\text{S}$ -depleted than  
20 leaves: -11.1 to 7.8 ‰ vs. -1.5 to 6.1‰, respectively. The differences observed between leaves and  
21 roots are probably related with a differential uptake of sulfur by these two tissues. Roots mostly use  
22 sediment sulfur which contains high quantities of sulfides resulting from anaerobic bacterial activity,  
23 and are generally very  $^{34}\text{S}$ -depleted (-10 to -35 ‰), whereas leaves use dissolved sulfates from the  
24 water column (21‰) (Fry et al., 1982; Nriagu et al., 1991; Moncreiff and Sullivan, 2001). As a result,  
25 there is good discrimination between seagrass roots and benthic algae or  $\text{C}_4$  plants thanks to  $\delta^{34}\text{S}$   
26 values. However, the large range of  $\delta^{34}\text{S}$  values observed on seagrass leaves and  $\text{C}_4$  plants do not  
27 allow for discrimination between these two primary producers. The large variations described for  
28  $\delta^{34}\text{S}$  values, depending on species, tissues, locations and seasons (Fry et al., 1982; Moncreiff and  
29 Sullivan, 2001; Oakes and Connolly, 2004; Belicka et al., 2012), signify that information issued from  
30 sulfur stable isotope composition should be used with caution in large scale ecosystem studies  
31 (Connolly et al., 2004).  
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50 As a result,  $\text{C}_3$  vascular plants are very well differentiated from all other primary producers and  
51 some microphytobenthos samples are clearly separated from seagrass and  $\text{C}_4$  plants.  $\delta^{34}\text{S}$   
52 measurements allow for good discrimination between microphytobenthos and seagrass detritus  
53 (Table 1, Fig. 3). The combination of three different stable isotope markers ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ ) thus  
54 allowed the discrimination of three different groups of benthic primary producers from the Mission-  
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1 Aransas estuary: 1) C<sub>3</sub> salt marsh plants, 2) benthic microalgae, and 3) C<sub>4</sub> salt marsh plants and  
2 seagrass (Peterson and Howarth, 1987; Sullivan and Moncreiff, 1990; Currin et al., 1995; Connolly et  
3 al., 2004).  
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### 9 **3.3 Origin and quality of allocthonous inputs from the Aransas and Mission rivers and from the** 10 **Gulf of Mexico** 11

12 The Mission-Aransas estuary receives inputs of organic matter from its different watersheds,  
13 mostly the Aransas and Mission rivers, which drain the highest quantity of freshwater into this  
14 estuary. Some allocthonous inputs may also originate from the Gulf of Mexico, via the pass at Port  
15 Aransas, due to movements of water masses due to tides, winds and evaporation of water into  
16 Aransas bay (Solis and Powell, 1999). During this survey, the origin and quality of allocthonous inputs  
17 of organic matter may have been influenced by two patterns: 1) From November 2010 to August  
18 2011, a general decrease of freshwater inflow, and 2) A minor rain event in January 2011.  
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30  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values of SPOM at GoM station were stable throughout the year, ranging from  
31 to -23.2 (January 2011) to -20.8‰ (April 2011), from 6.5 (December 2010) to 8.6‰ (May 2010) and  
32 from 16.5 (May 2011) to 18.5‰ (July and January 2011), respectively (Fig. 5, Table 2, Appendix A).  
33 Chl *a* concentrations were relatively low all year long, with values ranging from 2.1±0.2 (May 2011)  
34 to 6.7±0.3 µg.l<sup>-1</sup> (February 2011) (Fig. 6). Combined to the low C/chl *a* values (ranging from 154 to  
35 484, Fig. 6), indicating that this organic matter was fresh (Cifuentes et al., 1988; Savoye et al., 2012),  
36 stable isotope values indicated that the composition of organic matter from the Gulf of Mexico  
37 varied little all year long, being mostly composed of fresh marine phytoplankton. Seawater SPOM  
38  $\delta^{13}\text{C}$  values range indeed typically from -18 to -24‰ (Fry and Sherr, 1984, Riera et al., 2000) and  $\delta^{34}\text{S}$   
39 values are close to 21‰ (Fry et al., 1982; Nriagu et al., 1991).  
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54 Composition of SPOM from Aransas and Mission rivers was generally stable and much more <sup>13</sup>C-  
55 depleted (i.e., ranging from -35.5 to -27.7‰) than SPOM at GoM station, except in January 2011,  
56 when  $\delta^{13}\text{C}$  values of SPOM from Aransas (-24.0‰) and Mission rivers (-25.1‰) were similar to that  
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1 from GoM (Fig. 5, Tables 2 and 3, Appendix A).  $\delta^{15}\text{N}$  values of SPOM from rivers were much more  
2 variable (Table 2, Fig. 5). SPOM from Mission river typically had  $\delta^{15}\text{N}$  values similar to those observed  
3 in the Mission-Aransas estuary, but low  $\delta^{15}\text{N}$  values in winter (3.6‰ in January 2011) (Fig. 5, Table  
4 3).  $\delta^{15}\text{N}$  values of SPOM from Aransas river were the most variable, with a large enrichment in  $^{15}\text{N}$   
5 between January and February 2011 (17.5‰) and then a gradual decrease in  $^{15}\text{N}$ , until reaching  $\delta^{15}\text{N}$   
6 values observed in the Mission-Aransas estuary in July 2011 (4.8‰) (Fig. 5). SPOM sulfur stable  
7 isotope composition was generally relatively depleted in the Aransas and Mission rivers (from 3.3 to  
8 9.6‰), except in November and December 2010 in the Mission river, when  $\delta^{34}\text{S}$  values of SPOM  
9 were slightly higher (12.1 and 10.7‰, respectively) (Fig. 5).  
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21 Due to sampling complications, chl *a* concentrations and C/chl *a* ratios are not available at station  
22 MIS in November and in December 2010. From November 2010 to August 2011, chl *a* concentrations  
23 were highly variable in Aransas and Mission rivers, ranging from 0.7 (MIS, January) to 43.2  $\mu\text{g}\cdot\text{l}^{-1}$   
24 (ARA, February) (Fig. 6, Table 2). Chl *a* concentrations were particularly high in Aransas river in  
25 February and March. From May to August, chl *a* concentrations were frequently higher in Aransas  
26 and Mission rivers than at station 5 or GoM (Table 3). C/chl *a* ratios were high in January in Mission  
27 (1638) and Aransas (1385) rivers (Fig. 6).  
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38 Except in January and February 2011, the composition and quality of organic matter issued from  
39 the Aransas and Mission rivers was pretty constant. The combination of C/chl *a* ratios and of  $\delta^{13}\text{C}$   
40 and  $\delta^{34}\text{S}$  values demonstrates indeed that the organic matter from the Aransas and Mission rivers  
41 was mostly composed of freshwater phytoplankton. The  $\delta^{13}\text{C}$  values of SPOM, lower than -27 ‰, are  
42 typical of freshwater phytoplankton (Fry and Sherr, 1984; Cifuentes et al., 1988; Boutton, 1991) and  
43  $\delta^{34}\text{S}$  values (from 3.3 to 12.2‰) indicated that these primary producers were relying on sulfates  
44 from riverine waters (Nriagu and Coker, 1978; Chukhrov et al., 1980; Nriagu et al., 1991). Carbon to  
45 chl *a* ratios, with most of the values lower than 200, confirmed that SPOM was mostly composed of  
46 fresh material (Cifuentes et al., 1988; Savoye et al., 2012). Blooms of freshwater phytoplankton were  
47 observed in June and July in the Mission river and in July and August in the Aransas river, when Chl *a*  
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1 concentrations were high ( $>15 \mu\text{g l}^{-1}$ ) and C/chl *a* ratios were lower than 100, demonstrating that  
2 SPOM was only composed of fresh phytoplankton.  
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4 In January 2011, a minor rain event strongly affected the composition of the riverine SPOM.  
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6 Much higher C/chl *a* values ( $> 1300$ ) in January demonstrated that SPOM was mostly composed by  
7 refractory material (i.e., detrital matter) (Cifuentes et al., 1988; Savoye et al., 2012). Higher  $\delta^{13}\text{C}$   
8 values ( $-24.0$  and  $-25.1\text{‰}$  in Aransas and Mission rivers, respectively) indicated a higher influence of  
9  $\text{C}_4$  plant material in the riverine SPOM composition than during previous months. The Mission-  
10 Aransas estuary watershed has a high number of  $\text{C}_4$  primary producers, since it is composed of 30%  
11 of grassland (Mooney and McClelland, 2012), with 68% of the grasses being  $\text{C}_4$  plants (Teeri and  
12 Stowe, 1976). Moreover, main crops in the Mission-Aransas estuary, particularly in the Aransas  
13 watershed are  $\text{C}_4$  plants (i.e., sorghum and corn) (Mooney and McClelland, 2012). The  $^{13}\text{C}$   
14 enrichment indicates rainfall drained high quantities of detrital matter from continental origin within  
15 the watershed into the rivers. In February 2011, the peak of  $\delta^{15}\text{N}$  values in the Aransas river is very  
16 likely related with the release of highly  $^{15}\text{N}$ -enriched nitrogen originating from wastewater treatment  
17 plants (McClelland and Valiela, 1998), probably due to the overwhelming of their treatment  
18 capacities during the January rain event (Mooney and McClelland, 2012). This higher release of  
19 enriched nitrogen only occurred in the Aransas river, which is subject to a much higher discharge of  
20 wastewater than the Mission river (Mooney and McClelland, 2012). The release of enriched  
21 nitrogen, in higher quantities than during base flow (Bruesewitz et al., 2013), stimulated the  
22 production of freshwater phytoplankton in February and March as illustrated by the higher chl *a*  
23 concentrations. The gradual decrease of  $\delta^{15}\text{N}$  values after this event indicates that there was a slow  
24 dilution of this enriched nitrogen during the following months, due to the very low flows of fresh  
25 water. This highlights that the different management and land use land cover of these two rivers—  
26 one with numerous discharge points of wastewater and dominated by a watershed dominated by  
27 agricultural fields, and the other one with low wastewater release and a watershed dominated by  
28 shrub—have a different effect on the riverine SPOM composition. Rain events also have a clear  
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1 effect on the composition of riverine organic matter: In periods of normal flow, riverine waters  
2 contain low concentrations of high quality SPOM whereas they contain high concentrations of low  
3 quality SPOM during rain events.  
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7 As evidenced by the decrease in salinity observed at St. 4 in January, there are also exchanges of  
8 organic matter between Copano and Aransas bays, San Antonio bay and Corpus Christi bay through  
9 the Intracoastal Waterway. Unfortunately, our sampling design did not take into account these  
10 potential flows of organic matter, and it is therefore not possible to determine the composition and  
11 the quantity of organic matter carried by the Intracoastal Waterway.  
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### 21 **3.4 Autochthonous phytoplankton dominates SPOM composition during droughts**

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23  $\delta^{13}\text{C}$  values of SPOM in the Mission Aransas estuary (i.e., stations 1 to 5) ranged from -26.9  
24 (January 2011, Station 2) to -20.8‰ (April 2011, station 4) and were relatively stable throughout the  
25 year. No clear temporal pattern was observed even though some  $\delta^{13}\text{C}$  values of SPOM were  
26 occasionally significantly lower during some fall and winter months than during some spring and  
27 summer months (Fig. 5, Table 2). No clear spatial difference was observed between  $\delta^{13}\text{C}$  values in  
28 the estuary, except in January 2011, when SPOM at station 2 was significantly more depleted than at  
29 station 5 (Fig. 5, Table 3).  $\delta^{15}\text{N}$  values of SPOM at stations 1 to 5 were also relatively stable and  
30 similar between stations, ranging from 4.7 (August 2011, station 1) to 8.8‰ (November 2010,  
31 station 2) (Fig. 5, Tables 2 and 3).  $\delta^{34}\text{S}$  values of SPOM were rather relatively stable and similar  
32 throughout the year from stations 1 to 5, ranging from 11.9 (January 2011, station 1) to 20.0‰ (April  
33 2011, station 4) (Fig. 5, Tables 2 and 3). Chl *a* concentrations were less variable in the Mission-  
34 Aransas estuary (St. 1 to St. 5) than in the rivers, ranging from 0.7 (July 2011, station 5) to 20.1  $\mu\text{g.l}^{-1}$   
35 (April 2011, station 1). Two peaks of chl *a* concentrations in November and April were observed at  
36 station 1 (Fig. 6, Table 2). C/chl *a* ratios observed in the Mission-Aransas estuary ranged from 114  
37 (January 2011, station 1) to 803 (February 2011, station 3) (Fig. 6).  
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1 As a result, the composition of SPOM was relatively constant in the Mission-Aransas estuary  
2 during the survey, even if some slight spatio-temporal differences were observed. On a yearly basis,  
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4  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values of estuarine SPOM were very different from the carbon and sulfur isotopic  
5  
6 compositions of benthic primary producers (Fig. 3), demonstrating that these primary producers had  
7  
8 a negligible influence on the composition of estuarine SPOM, either as fresh (i.e., resuspended  
9  
10 benthic microalgae) or as detrital matter (i.e., seagrass,  $\text{C}_3$  or  $\text{C}_4$  plant) (Qian et al., 1996). The carbon  
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12 and sulfur isotopic composition of estuarine SPOM demonstrated that this organic matter was, all  
13  
14 year long, mostly composed by locally produced phytoplankton. This indicates minimal benthic-  
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16 pelagic coupling in the Mission-Aransas estuary, which is relatively surprising because windy  
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18 conditions can increase turbidity (Beseres Pollack et al., 2012), allowing for resuspension of  
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20 sediment organic matter. Organic matter of benthic origin is thus either exported as large particles  
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22 (e.g., seagrass leaves) or buried in the sediment.  
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28 The carbon and sulfur isotopic compositions of estuarine SPOM were very different from those of  
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30 riverine SPOM (Fig. 3). From March to July, there was also no influence of the highly  $^{15}\text{N}$ -enriched  
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32 SPOM discharge from the Aransas river in Copano bay (Fig. 5). This indicates that organic matter  
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34 from rivers had a very low influence on the general functioning of the Mission-Aransas estuary  
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36 during droughts, probably because inputs of riverine SPOM were in very low quantity, due to the  
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38 very low freshwater inflows, whereas autochthonous phytoplanktonic production is high in the  
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40 Mission-Aransas estuary, like in other Texas estuaries (Pennock et al., 1999).  
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### 47 **3.5 Effect of freshwater inflow into composition of SPOM**

48 A slight influence of riverine SPOM was occasionally and very locally observed during the minor  
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50 rain event - which occurred in January - at stations close to the Aransas and Mission river mouths  
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52 and at station 4, probably due to an influence of riverine organic matter from San Antonio bay  
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54 drained through the Intracoastal Waterway. We used data from our survey (i.e. during drought) and  
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56 from the survey carried out by Mooney and McClelland (2012) (i.e. during drought and flood) for  
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1 Copano bay, the secondary bay of the estuary, to determine how freshwater inflows affect the  
2 composition and the quality of the SPOM. We determined that an exponential relationship exists  
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4 between SPOM  $\delta^{13}\text{C}$  values and monthly freshwater inflows (moving average of the present and the  
5  
6 two previous monthly freshwater inflows) from the Aransas and Mission rivers (Fig. 7).  
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9 We then considered two scenarios: droughts and rain events. In our study, we observed that the  
10 riverine SPOM is dominated by freshwater phytoplankton ( $\delta^{13}\text{C} = -31.4\text{‰}$ ) during droughts and by  
11  
12 detrital matter of  $\text{C}_3$ -plants ( $\delta^{13}\text{C} = -24.7\text{‰}$ ) during rain events. We considered that the SPOM  $\delta^{13}\text{C}$   
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14 value in Copano bay without any freshwater inflow (i.e., autochthonous production only) would be  
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16 equal to the  $\delta^{13}\text{C}$  value of SPOM from the Gulf of Mexico ( $-22.1 \text{‰}$ ). For both scenarios, we  
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18 computed the average  $\delta^{13}\text{C}$  value which would result from an equal mixture of SPOM from rivers and  
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20 SPOM from autochthonous production. We then determined the threshold of monthly freshwater  
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22 inflow between a system more influenced by riverine inputs vs. a system more influenced by  
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24 autochthonous production. In other words, a monthly freshwater inflow higher than the threshold  
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26 indicates that SPOM in the secondary bay is dominated by riverine inputs whereas a monthly  
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28 freshwater inflow lower than the threshold indicates that SPOM in the secondary bay is dominated  
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30 by autochthonous production.  
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38 The scenario based on drought conditions gave an inflow threshold of  $6.8 \text{ million m}^3 \text{ month}^{-1}$ . This  
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40 inflow of freshwater is much higher than the base flow conditions, and corresponds to a very high  
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42 discharge of freshwater, which only occurs during large rain events. As a result, this computation  
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44 demonstrates that the required inflow of freshwater for a significant effect of riverine inputs into the  
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46 SPOM composition of Copano bay corresponds to a large rise in river level (i.e., rain event, which  
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48 does not correspond to this scenario anymore). This means that riverine inputs during droughts  
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50 (composed by riverine fresh phytoplankton) cannot have any major influence in the SPOM  
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52 composition of this secondary bay. As a result, during droughts, SPOM will always be dominated by  
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54 locally produced phytoplankton.  
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1 The scenario based on rain event conditions gave an inflow threshold of 910,000 m<sup>3</sup> month<sup>-1</sup>.  
2 Consequently, when freshwater inflow is higher than this threshold the SPOM in Copano bay shifts  
3 gradually from fresh autochthonous phytoplankton to highly degraded terrestrial material. Indeed,  
4 the very high C/chl *a* ratios of riverine SPOM drained during rain events indicate that this material is  
5 highly degraded, and is probably of very low nutritive quality for potential consumers (e.g.,  
6 suspension feeders) (Ittekkot, 1988). The quality of the SPOM thus decreases into Copano bay,  
7 which may affect its fate and very likely the functioning of the estuarine food web. In 2007, the large  
8 floods, which occurred in the Mission Aransas estuary watershed affected oyster filtration rates, due  
9 to low salinities (Beseres Pollack et al., 2011). We suggest that the poor quality of the organic matter  
10 provided to the oysters during rain events may also affect the filtration rates, and thus the growth of  
11 oysters.  
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26 Minor rain events may also affect local production of phytoplankton, as demonstrated by the  
27 peak of chlorophyll *a* in January (Pennock et al., 1999; Mooney and McClelland, 2012).  
28 Phytoplankton production increase due to a higher discharge of nutrients and remineralization of  
29 terrestrial organic matter drained into the estuary (Mooney and McClelland, 2012; Bruesewitz et al.,  
30 2013). As a result, even if pulses of freshwater do not drain SPOM of sufficient nutritional quality for  
31 its assimilation by consumers, the release of nutrients allows for local increase of autochthonous  
32 phytoplankton production, which may locally support the functioning of food webs.  
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#### 45 **4. Conclusion: Functioning of a South Texas estuary**

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47 Overall, autochthonous phytoplankton was the dominant component of SPOM in the Mission-  
48 Aransas estuary, during droughts but also during the minor rain event. Benthic organic matter did  
49 not influence SPOM composition, either as fresh material or as detritus. The influence of riverine  
50 inputs was very low and restricted to areas close to river mouths, due to the high residence time in  
51 the Mission-Aransas estuary (Solis and Powell, 1999).  
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A comparison of the functioning of the Mission-Aransas estuary with the Sabine-Neches estuary (i.e., neutral vs. highly positive estuary) reveals that freshwater inflow clearly affects composition of SPOM in these two estuaries. On a global basis, the Sabine-Neches receives high loads of low quality organic matter which are relatively quickly flushed outside of the system due to the large freshwater inflow whereas the biomass of autochthonous phytoplankton is low due to the high turbidity (Cifuentes et al, 1999). The general functioning of the Mission-Aransas estuary is different, with high phytoplanktonic production and very low inputs of refractory continental organic matter. The Mission-Aransas estuary is thus dominated by autochthonous production. Because of predicted global change, which will result in a drier climate along Gulf of Mexico coastline (Nielsen-Gammon, 2011 ; IPCC, 2013) and the increasing demand for fresh water for human activities (Montagna et al., 2002), it is therefore probable that general functioning of Texas estuaries will shift towards systems less based on allochthonous inputs of SPOM and much more influenced by SPOM from autochthonous pelagic production. These changes in organic matter quality will probably have a large effect on the functioning of food webs in Texas estuaries.

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A potential shift from estuaries where SPOM is dominated by poor quality allochthonous inputs to estuaries dominated by high quality autochthonous production would also lead to issues related with nutrients, due to the importance of nutrients for phytoplankton production. A better understanding of the relationships between nutrient inputs and freshwater inflows, such as that carried out by Mooney and McClelland (2012) in Copano bay, may be necessary at a much larger regional scale. A monitoring of these changes along the Texas coastline will require a combination of studies based on SPOM composition and nutrient loads along the coastal shoreline.

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## Tables

Table 1: Stable isotope compositions ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$ , mean  $\pm$  standard deviation, ‰) of the different benthic primary producers in the Mission-Aransas estuary. Sample size is displayed in parentheses.

Species	Location	Date	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$	
<b>C3 vascular plants</b>						
<i>Borrchia frutescens</i>	Station 1	November 2010	$-26.8 \pm 1.5$ (3)	$7.0 \pm 0.8$ (3)	$7.1 \pm 4.6$ (3)	
		March 2011	$-29.1 \pm 0.2$ (3)	$6.6 \pm 1.0$ (3)	$7.5 \pm 0.4$ (3)	
		June 2011	$-28.9 \pm 0.9$ (3)	$6.5 \pm 0.1$ (3)	$17.0 \pm 0.5$ (3)	
<i>Phragmites australis</i>	Station 5	November 2010	$-26.8 \pm <0.1$ (3)	$5.4 \pm 0.5$ (3)	$14.2 \pm 0.7$ (3)	
	Station 1	November 2010	$-27.2 \pm 0.3$ (3)	$9.2 \pm 0.5$ (3)	-	
<i>Salicornia virginica</i>	Station 1	November 2010	$-27.7 \pm 1.0$ (3)	$6.7 \pm 0.9$ (3)	$10.3 \pm 0.9$ (3)	
		June 2011	$-29.2 \pm 0.2$ (3)	$4.8 \pm 2.3$ (3)	$13.3 \pm 0.9$ (3)	
<i>Suaeda linearis</i>	Station 5	November 2010	$-28.8 \pm 0.3$ (3)	$10.0 \pm 0.4$ (3)	$12.0 \pm 0.7$ (3)	
	Station 1	June 2011	$-27.3 \pm 0.8$ (3)	$7.9 \pm 0.5$ (3)	$18.4 \pm <0.01$ (3)	
<i>Suaeda virginica</i>	Station 5	November 2010	$-27.8 \pm 0.2$ (3)	$8.0 \pm 0.3$ (3)	$13.7 \pm 1.7$ (3)	
		March 2011	$-29.2 \pm 0.4$ (3)	$4.5 \pm 2.3$ (3)	$13.6 \pm 0.7$ (3)	
<b>C4 vascular plants</b>						
<i>Monanthochloe littoralis</i>	Station 5	November 2010	$-15.5 \pm 0.9$ (3)	$6.6 \pm 1.6$ (3)	-	
<i>Spartina alterniflora</i>	Station 1	November 2010	$-13.4 \pm 0.3$ (3)	$5.5 \pm 0.5$ (3)	$7.1 \pm 4.6$ (3)	
		March 2011	$-12.5 \pm 0.2$ (3)	$6.0 \pm 0.6$ (3)	$2.2 \pm 3.0$ (3)	
		June 2011	$-13.4 \pm 0.1$ (3)	$4.6 \pm 0.8$ (3)	-	
	Station 3	November 2010	$-13.6 \pm 0.3$ (3)	$3.5 \pm 1.3$ (3)	$-0.8 \pm 0.5$ (3)	
<i>Spartina patens</i>	Station 5	June 2011	$-13.5 \pm 0.4$ (3)	$3.6 \pm 0.3$ (3)	-	
		November 2010	$-13.1 \pm 0.1$ (3)	$-1.1 \pm 0.6$ (3)	$-5.8 \pm 1.6$ (3)	
		November 2010	$-13.3 \pm 0.1$ (3)	$2.8 \pm 1.0$ (3)	$-5.3 \pm 1.7$ (2)	
<i>Spartina spartinae</i>	Station 1	June 2011	$-13.6 \pm 0.3$ (3)	$7.7 \pm 0.5$ (3)	-	
<i>Spartina spartinae</i>	Station 1	November 2010	$-13.6 \pm 0.2$ (3)	$5.3 \pm 0.7$ (3)	-	
<b>Seagrass plants</b>						
<i>Halodule wrightii</i>	Leaves	Station 3	November 2010	$-13.4 \pm 0.6$ (3)	$9.2 \pm 0.6$ (3)	$2.7 \pm 1.5$ (3)
			March 2011	$-14.2 \pm 0.9$ (3)	$7.8 \pm 0.2$ (3)	$-1.5 \pm 1.5$ (3)
		Station 4	November 2010	$-11.4 \pm 0.1$ (3)	$2.0 \pm 0.5$ (3)	$2.3 \pm 1.7$ (3)
			June 2011	$-12.2 \pm 0.4$ (3)	$4.3 \pm 0.2$ (3)	$6.1 \pm 1.8$ (3)
			November 2010	$-11.3 \pm 0.1$ (4)	$4.4 \pm 0.2$ (4)	$-0.2 \pm 1.8$ (3)
			November 2010	$-11.3 \pm 0.4$ (3)	$8.3 \pm 0.6$ (3)	$-1.4 \pm 0.4$ (3)
	Station 5	November 2010	$-13.8 \pm 0.3$ (3)	$7.5 \pm 0.2$ (3)	$-8.9 \pm 1.2$ (3)	
		November 2010	$-11.8 \pm 0.1$ (3)	$2.3 \pm 0.8$ (3)	$-2.6 \pm 0.4$ (3)	
	Roots	Station 3	November 2010	$-13.3 \pm 0.2$ (2)	$4.1 \pm 0.7$ (2)	$7.8 \pm 0.5$ (2)
			March 2011	$-10.9 \pm 0.5$ (4)	$4.0 \pm 0.2$ (4)	$-11.1$ (1)
		Station 4	November 2010	$-10.9 \pm 0.5$ (4)	$4.0 \pm 0.2$ (4)	$-11.1$ (1)
			November 2010	$-18.1 \pm 1.9$ (3)	$7.5 \pm 0.6$ (3)	$-2.3 \pm 2.1$ (3)
Station 5		November 2010	$-11.9 \pm <0.1$ (3)	$1.8 \pm 0.3$ (3)	$0.3 \pm 2.5$ (3)	
		November 2010	$-11.9 \pm <0.1$ (3)	$1.8 \pm 0.3$ (3)	$0.3 \pm 2.5$ (3)	
Detrital matter	Station 3	November 2010	$-12.9 \pm 0.1$ (3)	$6.2 \pm 1.1$ (3)	$6.0 \pm 1.7$ (3)	
		June 2011	$-13.0 \pm 0.3$ (3)	$4.4 \pm 0.2$ (3)	$1.6 \pm 0.8$ (3)	
	Station 4	June 2011	$-10.7 \pm 0.4$ (3)	$6.0 \pm 0.8$ (3)	$3.8 \pm 0.7$ (3)	
		November 2010	$-10.9 \pm 0.3$ (3)	$4.1 \pm 0.3$ (3)	$4.6 \pm 2.7$ (3)	
<i>Ruppia maritima</i>						
Leaves	Station 1	March 2011	$-12.9 \pm 0.1$ (3)	$6.2 \pm 1.1$ (3)	$6.0 \pm 1.7$ (3)	
		June 2011	$-13.0 \pm 0.3$ (3)	$4.4 \pm 0.2$ (3)	$1.6 \pm 0.8$ (3)	
	Station 3	June 2011	$-10.7 \pm 0.4$ (3)	$6.0 \pm 0.8$ (3)	$3.8 \pm 0.7$ (3)	
		November 2010	$-10.9 \pm 0.3$ (3)	$4.1 \pm 0.3$ (3)	$4.6 \pm 2.7$ (3)	

	Roots	Station 1	March 2011	-14.7 ± 2.1 (3)	3.5 ± 0.4 (3)	2.5 ± 3.0 (3)	
1			June 2011	-13.4 ± 0.5 (3)	3.6 ± 0.6 (3)	-3.7 ± 1.8 (3)	
2		Station 3	June 2011	-11.1 ± 0.4 (3)	5.0 ± 0.5 (3)	-8.3 ± 0.9 (3)	
3		Station 4	November 2010	-11.1 ± 0.5 (3)	2.6 ± 0.7 (3)	-3.4 ± 0.7 (3)	
4	<hr/>						
5	<b>Benthic microalgae</b>						
6	Diatoms	Station 1	November 2010	-19.9 ± 0.5 (3)	5.3 ± 0.1 (3)	5.0 (1)	
7			March 2011	-14.2 ± 0.1 (3)	5.0 ± 0.1 (3)	8.2 (1)	
8			June 2011	-13.3 ± 0.4 (9)	4.2 ± 0.6 (9)	5.8 ± 1.3 (8)	
9		Station 3	November 2010	-18.6 ± 0.8 (10)	4.7 ± 0.2 (10)	8.8 ± 2.5 (4)	
10			March 2011	-13.1 ± 0.8 (6)	4.4 ± 0.2 (6)	8.2 (1)	
11			June 2011	-12.7 ± 0.6 (3)	3.8 ± 0.3 (3)	7.7 (1)	
12	Oscillatoriales 1	Station 1	November 2010	-18.2 ± 0.6 (4)	0.7 ± 0.2 (4)	-	
13	(Cyanobacteria)		March 2011	-16.4 ± 0.5 (9)	2.8 ± 1.3 (9)	11.8 ± 2.9 (2)	
14			June 2011	-13.6 ± 0.6 (3)	3.0 ± 0.1 (3)	5.1 ± 0.3 (3)	
15		Station 2	May 2011	-14.4 ± <0.1 (3)	-0.6 ± 0.2 (3)	-	
16		Station 3	June 2011	-12.0 ± 0.1 (3)	4.8 ± 0.1 (3)	8.6 (1)	
17	Oscillatoriales 2	Station 3	March 2011	-13.2 ± 0.1 (2)	3.5 ± 0.1 (2)	6.2 ± 1.6 (7)	
18	(Cyanobacteria)						
19	Merismopedia sp.	Station 1	November 2010	-21.7 ± 0.1 (4)	2.8 ± <0.1 (4)	4.1 (1)	
20	(Cyanobacteria)						
21		Station 3	June 2011	-13.4 ± 0.4 (3)	3.3 ± 0.2 (3)	6.2 ± 0.9 (3)	
22	Benthic algae	Station 3	March 2011	-13.6 ± 0.4 (6)	3.8 ± 0.5 (6)	-	
23	(mixture cyanobacteria and						
24	diatoms)						
25		Station 5	November 2010	-15.1 ± 0.4 (4)	2.4 ± 0.1 (4)	4.6 (1)	
26	<i>Spirogyra</i> sp.	Station 1	November 2010	-17.8 ± 0.6 (7)	4.2 ± 1.2 (7)	-	
27	(Charophyta)						
28		Station 5	November 2010	-13.6 ± 0.4 (2)	1.0 ± 0.1 (2)	-	
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Table 2: Summary of Kruskal-Wallis tests and comparisons of means for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$ , Chl  $a$  and C/chl  $a$  of SPOM between sampling months at each station. Stations: ARA: Aransas river, MIS: Mission river, GoM: Gulf of Mexico.

Station	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$		Chl $a$		C/chl $a$	
	p-values	Comparisons of means	p-values	Comparisons of means	p-values	Comparisons of means	p-values	Comparisons of means	p-values	Comparisons of means
ARA	0.001	Jan > Feb Feb < Aug	0.001	Feb > Jul Feb > Aug	0.025	Nov > Feb	0.001	Dec < Feb Jan < Feb Jan < Mar	0.008	Jan > Feb
MIS	0.001	Nov < Jan Dec < Jan	0.001	Nov > Jan	0.016	-	0.004	Jan < Jul Jan < Jun	0.004	Jan > Jun Jan > Jul
Station 1	0.001	Jan < Apr	0.004	Dec > Aug Jun > Aug	0.008	-	0.001	Nov > Dec Dec < Apr	0.003	Jan < Feb
Station 2	0.001	Nov < Aug Jan < Aug	0.004	Nov > Mar Nov > Jul	0.017	-	0.001	Jan < Mar Mar > May	0.002	Dec > Feb
Station 3	0.004	-	0.043	-	0.087	-	0.006	-	0.016	Jan < Feb
Station 4	0.002	Nov < Apr Jan < Apr	0.002	Nov > Jul Jan > Jul	0.060	-	0.034	Apr > Jul	0.003	Dec < Jul Jan < Jul
Station 5	0.006	-	0.007	-	0.049	-	0.002	Jan > Jul Apr > Jul	0.007	Nov > Apr
GoM	0.011	-	0.006	-	0.118	-	0.009	Feb > May Feb > Aug	0.014	-

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Table 3: Summary of Kruskal-Wallis tests and comparisons of means for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$ , Chl *a* and C/chl *a* of SPOM between sampling stations for each month. Stations: ARA: Aransas river, MIS: Mission river, St. 1 to 5: Stations 1 to 5, GoM: Gulf of Mexico.

Station	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$		Chl <i>a</i>		C/chl <i>a</i>	
	p-values	Comparisons of means	p-values	Comparisons of means	p-values	Comparisons of means	p-values	Comparisons of means	p-values	Comparisons of means
November 10	0.007	MIS < St. 5 MIS < GoM	0.005	ARA > St. 1	0.039	-	0.004	St. 1 > St. 5	0.004	ARA < St.5 St.2 < St. 5
December 10	0.003	MIS < St. 5 MIS < GoM	0.096	-	0.017	-	0.030	-	0.030	St. 2 > St. 4
January 11	0.003	St. 2 < St. 5 St. 2 < GoM	0.003	MIS < St. 4	0.013	-	0.003	MIS < St. 1	0.012	-
February 11	0.002	ARA < GoM	0.007	ARA < MIS	0.004	ARA < St. 5	0.002	ARA > MIS ARA > St. 5	0.002	ARA < St. 3
March 11	0.003	ARA < St. 5	0.006	ARA < MIS	0.009	MIS < GoM	0.003	ARA > MIS ARA > St. 5	0.004	ARA < St. 1 ARA < St. 2
April 11	0.004	ARA < St. 4	0.008	ARA > St. 1	0.013	-	0.003	MIS < St. 1	0.012	ARA < St. 1
May 11	0.002	ARA < St. 5 ARA < GoM	0.003	ARA > MIS	0.025	-	0.007	ARA > St. 5 ARA > GoM	0.074	ARA < St. 3
June 11	0.005	-	0.010	ARA > MIS	0.041	-	0.006	MIS > St. 5	0.015	-
July 11	0.030	-	0.006	ARA < MIS ARA < GoM	0.008	-	0.003	ARA > St. 5 MIS > St. 5	0.009	MIS < St. 5
August 11	0.007	-	0.002	ARA < St. 3 St.1 < St. 3	0.174	-	0.002	ARA > St. 5 ARA > GoM	0.070	-

## Figure captions

1  
2  
3 Figure 1: Location of the Mission-Aransas estuary and of the sampling stations.  
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8 Figure 2: Salinity (full and dotted lines), monthly inputs of particulate organic carbon from Aransas  
9 and Mission rivers and total freshwater inflow (i. e., Mission and Aransas rivers, shaded curve) in the  
10 Mission-Aransas estuary from September 2010 to December 2011. ARA: Aransas river, MIS: Mission  
11 river, St. 1 to 5: Stations 1 to 5, GoM: Gulf of Mexico. Stars represent missing data.  
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19 Figure 3:  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values of primary producers and SPOM in the Mission-Aransas estuary. SPOM  
20 values are means  $\pm$  standard deviations of samples collected from November 2010 to August 2011.  
21  
22 Primary producer values are means of all samples collected in the Mission-Aransas estuary from  
23 November 2010 to August 2011 (see table 1 for standard deviations). ARA: Aransas river, MIS:  
24 Mission river, St. 1 to 5: Stations 1 to 5, GoM: Gulf of Mexico.  
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34 Figure 4:  $\delta^{13}\text{C}$  values of benthic microalgae and salinities of sampling stations in the Mission-Aransas  
35 estuary at the sampling dates.  
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42 Figure 5:  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  values (full and dotted lines) and total freshwater inflow (i. e., Mission  
43 and Aransas rivers, shaded curve) in the Mission-Aransas estuary from September 2010 to December  
44 2011. ARA: Aransas river, MIS: Mission river, St. 1 to 5: Stations 1 to 5, GoM: Gulf of Mexico.  
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51 Figure 6: Chlorophyll *a* concentration ( $\mu\text{g.l}^{-1}$ ), C/chlorophyll *a* ratios (full and dotted lines) and total  
52 freshwater inflow (i. e., Mission and Aransas rivers, shaded curve) in the Mission-Aransas estuary  
53 from September 2010 to December 2011. ARA: Aransas river, MIS: Mission river, St. 1 to 5: Stations  
54 1 to 5, GoM: Gulf of Mexico.  
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Figure 7: Relationship between freshwater inflow and stable isotope composition of the SPOM in the secondary bay of the Mission-Aransas estuary (i.e., Copano bay). Two scenarios are considered (i.e., drought, rain event) in order to compute the averaged stable isotope composition of the SPOM and the threshold of freshwater inflow for both scenarios. Data used in this figure are issued from our survey and from the survey carried out by Mooney and McClelland (2012).

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Figure 1

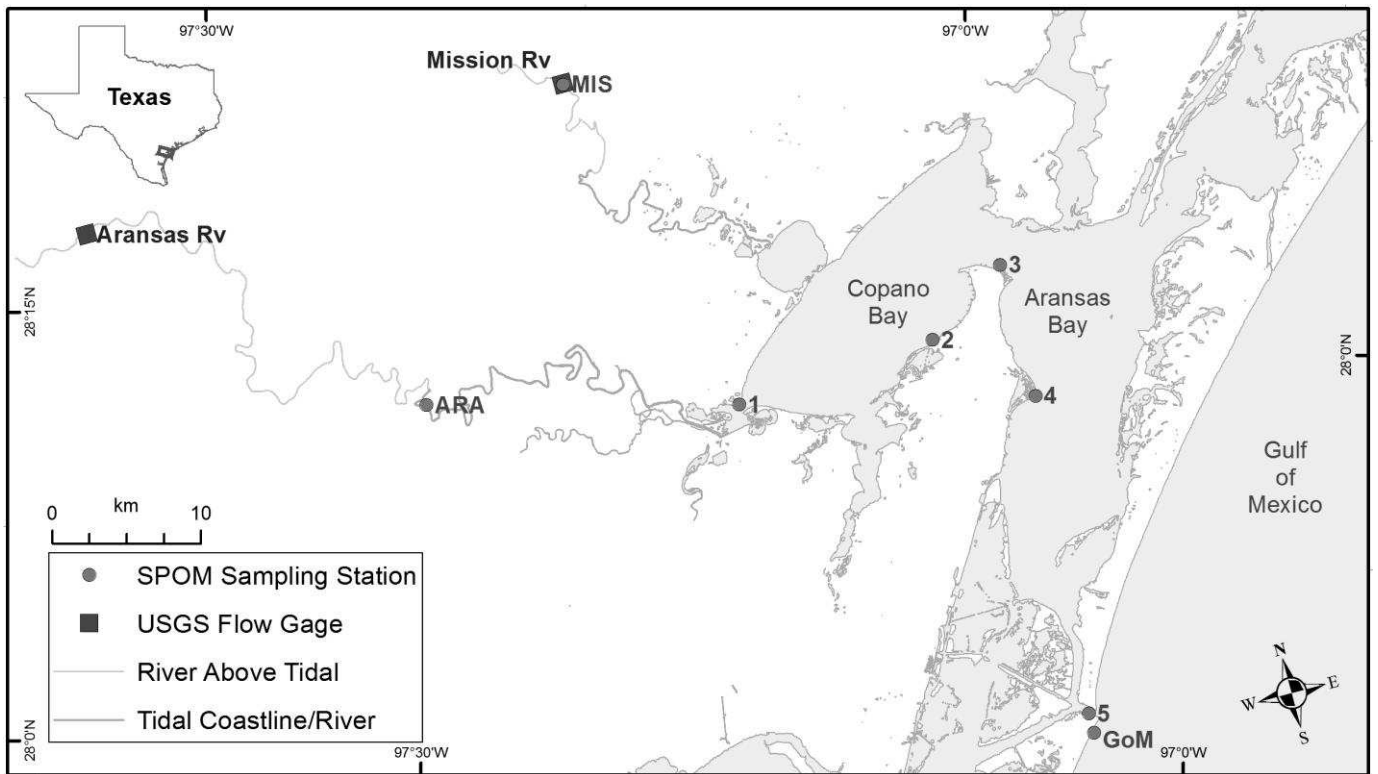


Figure 2

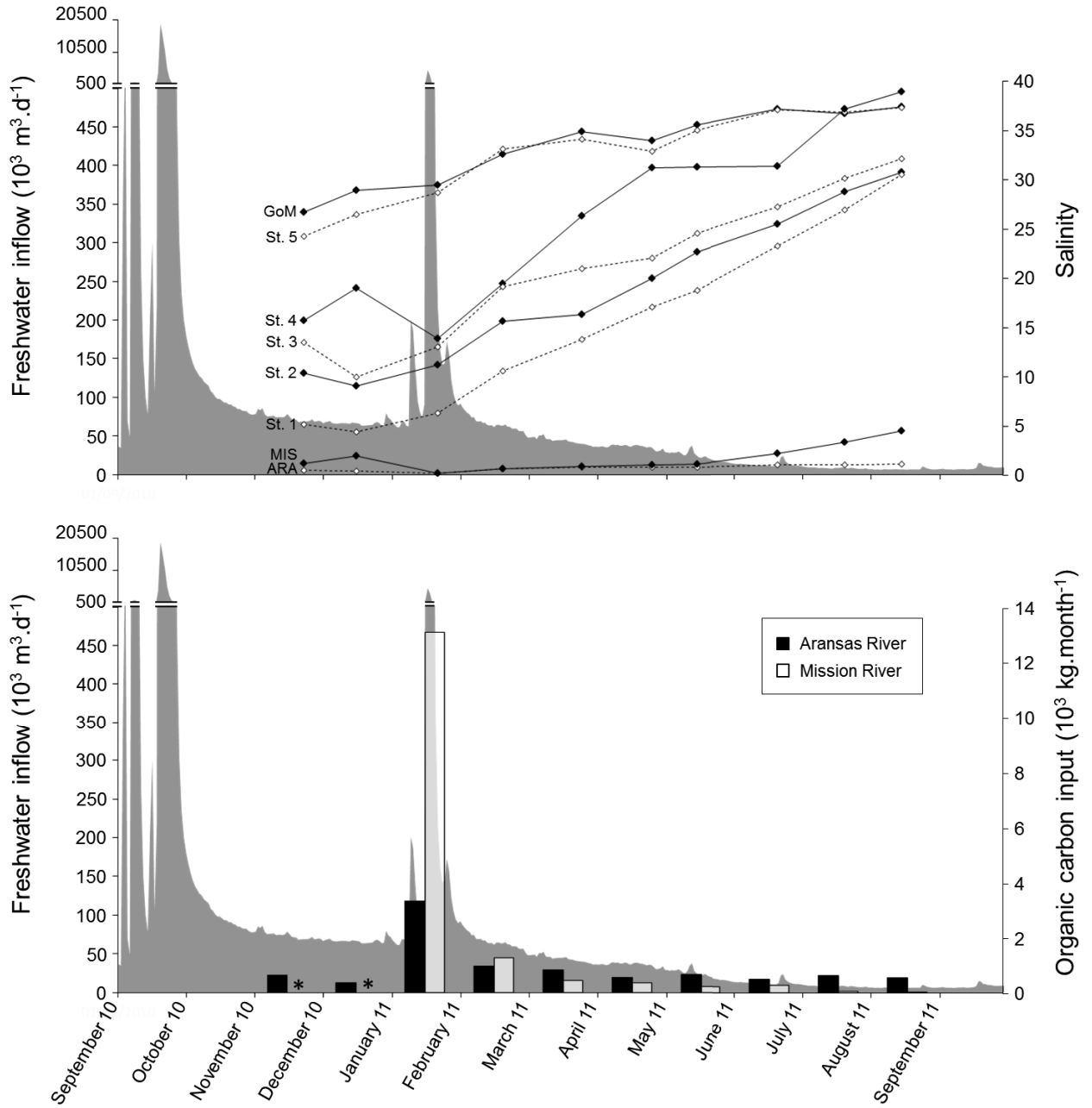


Figure 3

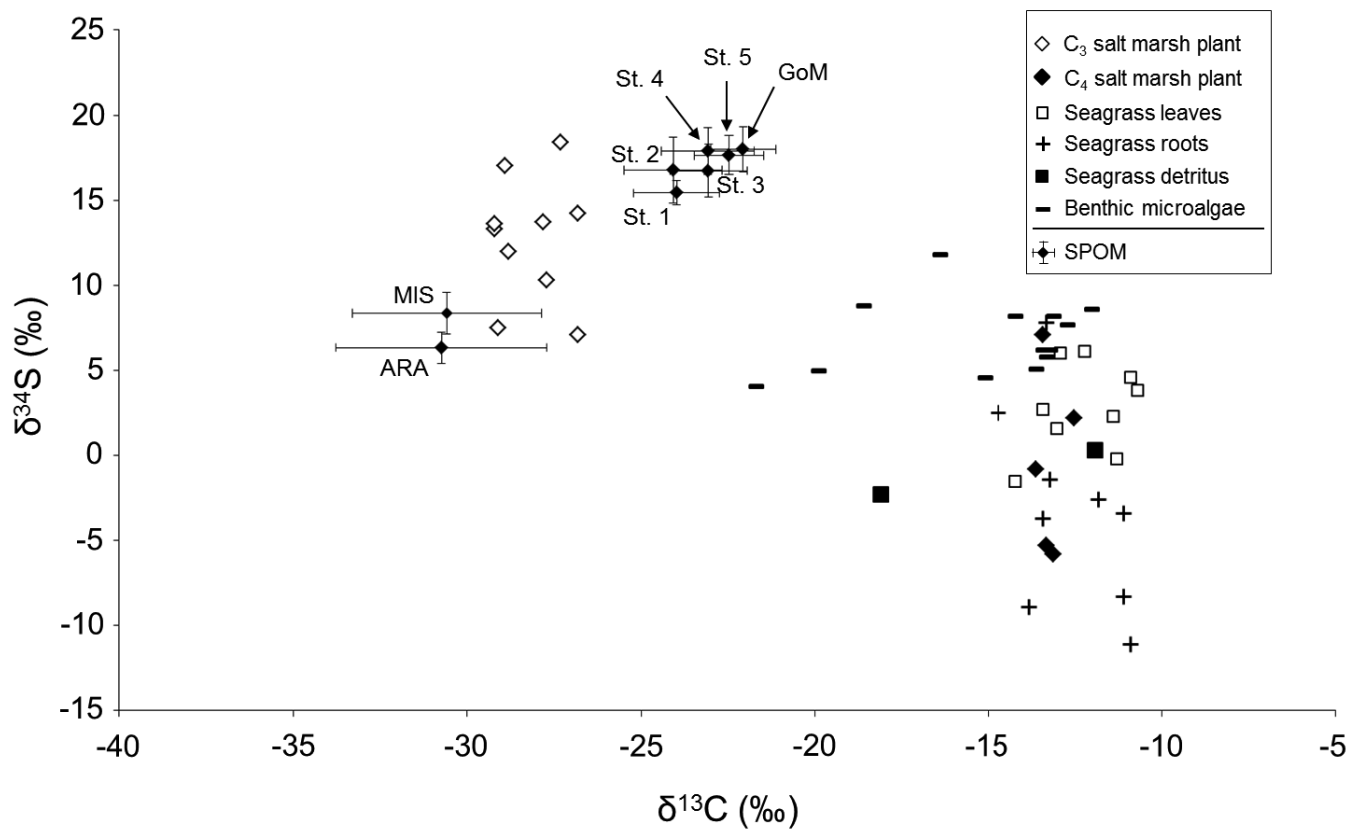


Figure 4

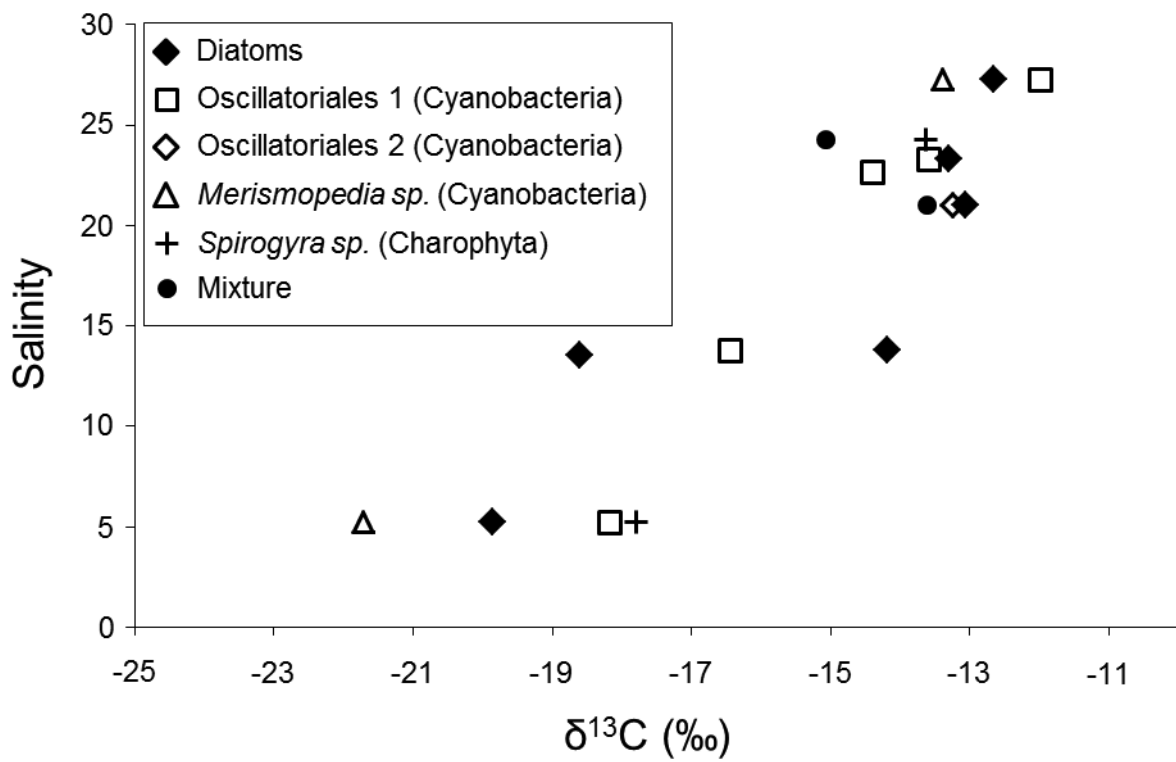


Figure 5

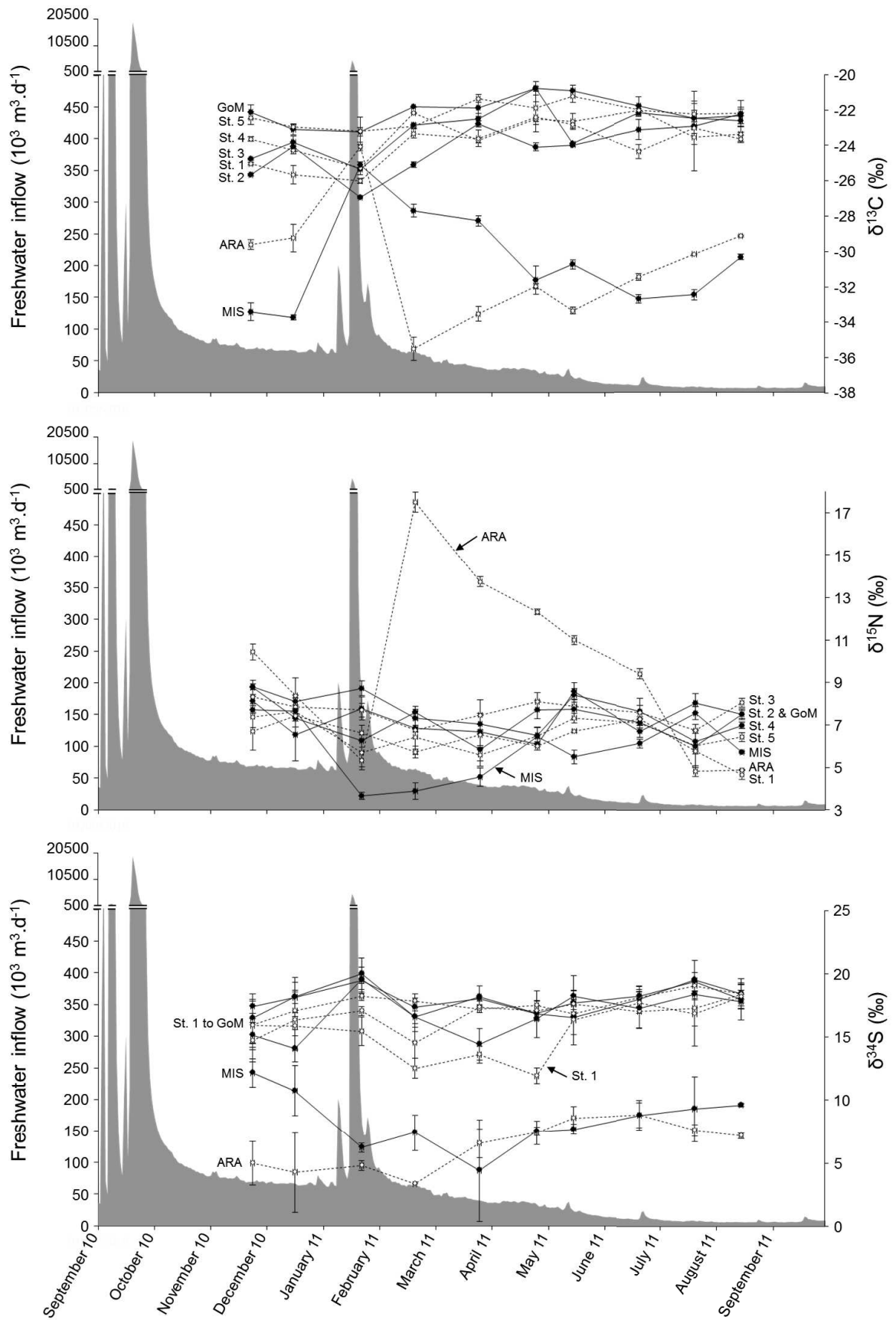




Figure 7

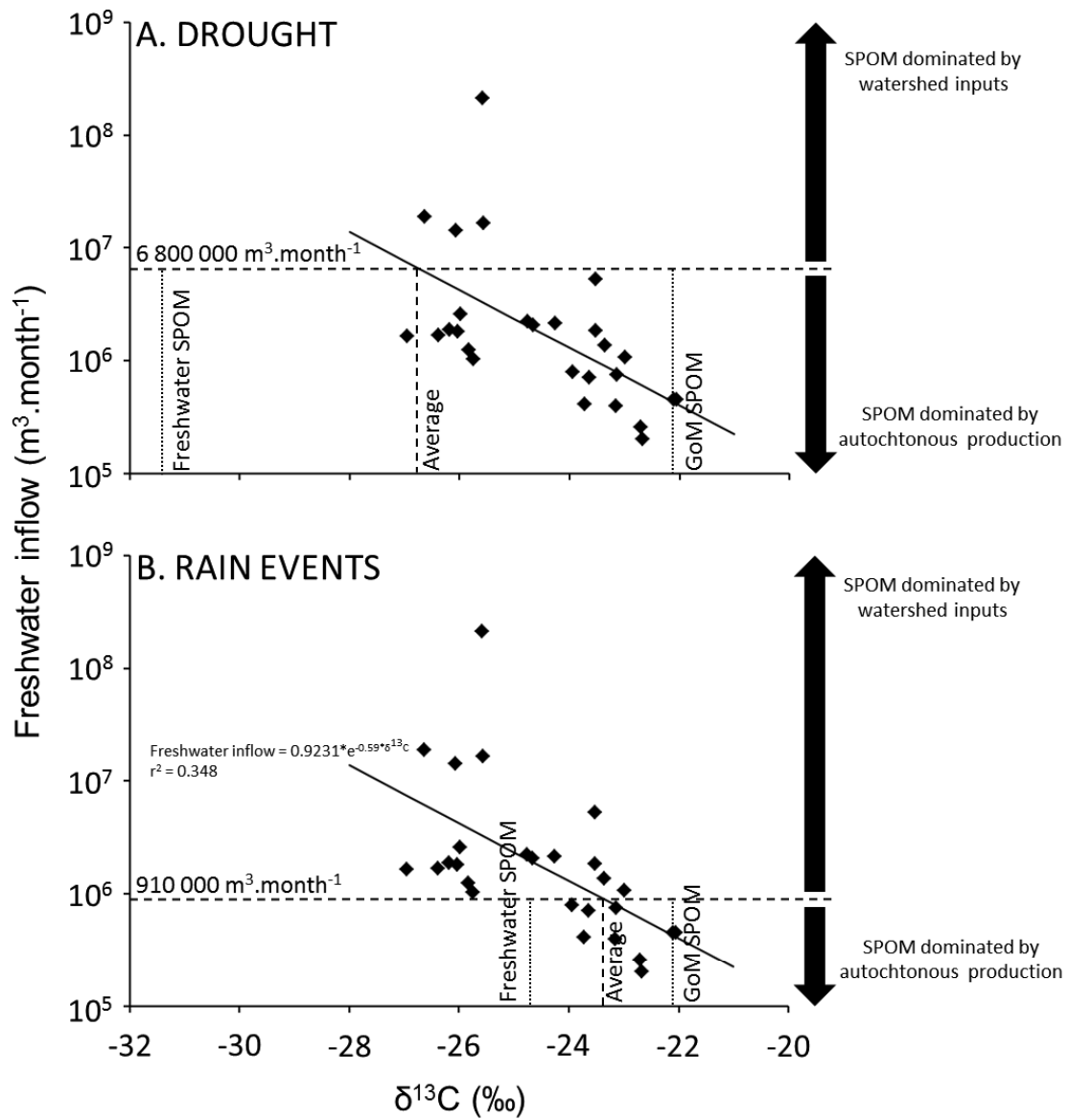
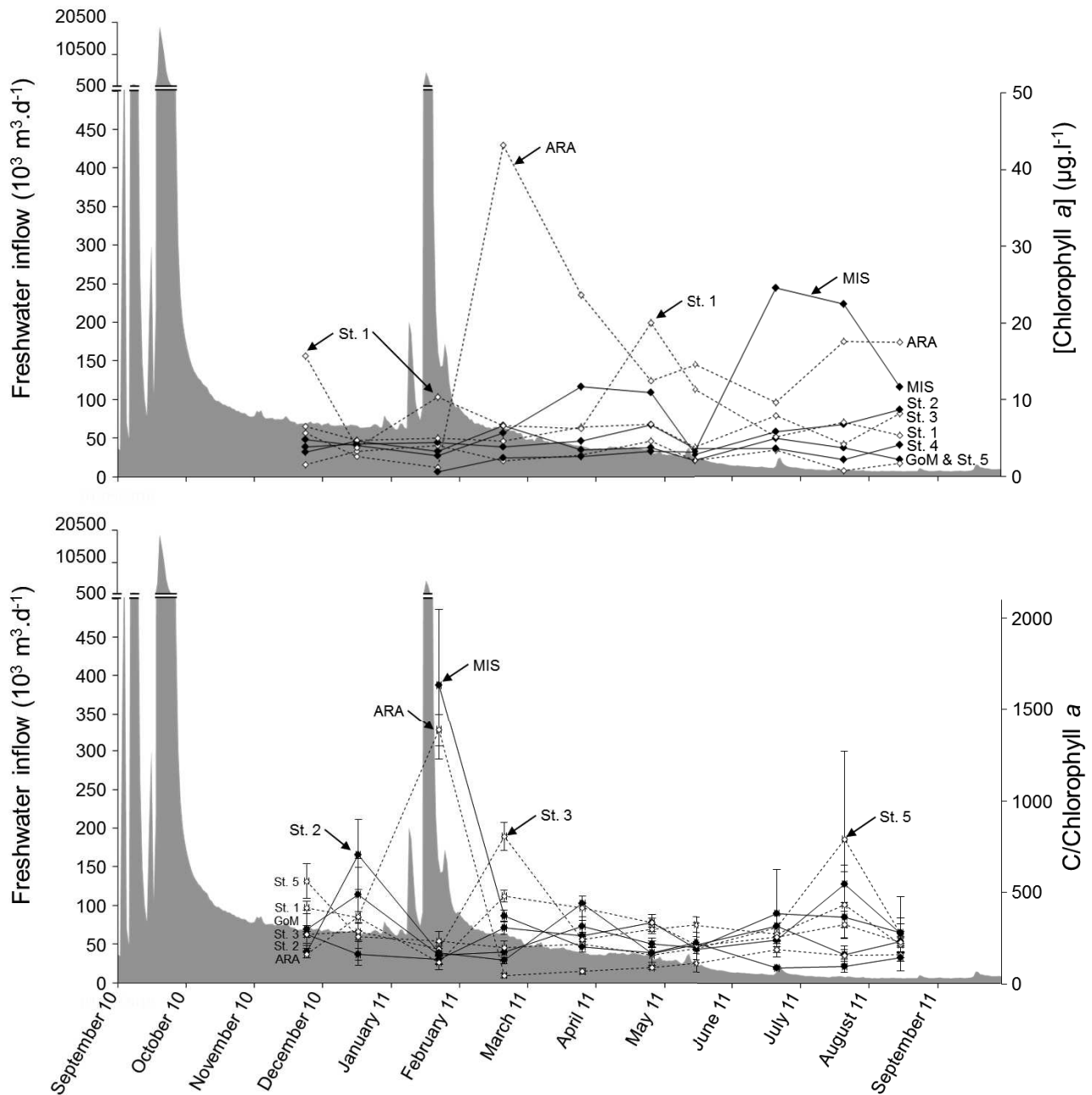


Figure 6



**Supplementary material for online publication only**

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