Field and lab procedures for DOC and optical properties

Surface water was collected by bucket or bottle from approximately 0.2 m depth and immediately syringe-filtered through a pre-combusted and pre-rinsed Whatman GF/F filter (~0.7 μm nominal pore size) and stored in pre-cleaned and rinsed 60 mL opaque brown plastic bottles. Replicates were not collected. Samples were kept on ice in the field and then refrigerated at ~ 4 degrees C in the laboratory until analysis. Samples were analyzed for fluorescence within two weeks of collection. DOC measurements were made within three weeks at the Southeast Environmental Research Center's water quality lab at Florida International University with a Shimadzu TOC-V CSH TOC analyzer using a high temperature combustion method or on a TOC-5000 TOC analyzer in the Jaffe lab also using a high temperature combustion method. UV-Vis absorbance scans were collected on a Varian Cary 50 Bio spectrophotometer and collected over a range of l=200 nm to l=800 nm in a 1-cm quartz cuvette. A blank scan (Milli-Q water) was subtracted from each sample spectrum and spectra were baseline normalized using the average absorbance between 700-800 nm.

Fluorescence EEMs were collected on a Horiba Jobin Yvon SPEX Fluoromax-3 spectrofluorometer using the methods of Maie et al. (2006) and Yamashita et al. (2010). Briefly, EEMs were collected over an excitation wavelength (l_{ex}) range of 240–455 nm with an increment of 5 nm and an emission range of λ_{ex} + 10 nm to λ_{ex} + 250 nm with an increment of 2 nm in a 1 cm quartz cuvette. The excitation and emission slit widths were set to 5.7 nm and 2 nm, respectively. Fluorescence scans were collected in signal/reference ratio mode with an integration time of 0.25 s and reported in quinine sulfate units (QSU). EEMs were corrected for instruments optics and inner-filter effects according to Ohno (2002) and Raman normalized and blank subtracted using Matlab v2009a software. EEMs were modeled using Matlab v2009a and fit to an eight component PARAFAC model described in Chen et al. (2010) and Yamashita et al. (2010) that is comprised of Florida coastal Everglades samples. The Fluorescence Index (FI), which indicates DOM source (McKnight et al. 2001), was calculated as the ratio of the emission intensities at 470 nm and 520 nm at an excitation wavelength of 370 nm (Jaffe et al. 2008). A higher FI value (e.g., 1.8) indicates a microbial DOM source while a lower value (e.g., 1.2) indicates a terrestrial source; intermediate values indicate a mixed DOM source.

Floc Sampling and Analytical Procedures

Sampling:

Quarterly floc samples will be collected from at SRS2, SRS6, TS2 and TS6 using pre-established methods (FCE2). Floc samples were collected using a transparent plastic corer (inner diameter of 2.5 cm) which was pushed about 10 cm below the soil/sediment surface. The corer was capped at the top end to create suction and retrieved. The floc and soil/sediment layers were visible in the transparent corer. A plunger with a slightly smaller diameter to that of the core tube was inserted and pushed through the floc until it was resting on top of the consolidated soil/sediment layer. With the plunger still in place, excess water was removed and the corer was tilted to decant the floc into clean, 1 L Teflon jars. This procedure was repeated at several randomly selected locations at each site enough times to obtain about 1 L of floc composite for each sampling event. For this sampling protocol, the definition of floc as consisting mostly of decaying macrophyte tissue, soil particles, algae and bacteria, was adopted. All easily suspended materials, potentially including benthic periphyton, resting on top of the consolidated soil/sediment layer in the core were considered part of the floc. All samples were transported to the laboratory on ice where they were frozen and freeze-dried. The samples were ground and stored in a freezer until prepared for the procedures described below.

Bulk Measurements:

During each sampling event, floc depth was obtained by measuring the sediment and floc height in the core. After the removal of the floc layer, the sediment height was measured again. Floc depth was obtained by subtracting the initial and final height measurements of the material in the corer. Floc bulk density was also measured for all quarterly samples. Floc density was obtained by using the following equations:

$$\text{Floc bulk density} = \text{floc dry weight (g)}/\text{floc volume (cm}^3\text{)}$$

The floc dry weight was the weight of the floc obtained after freeze-drying. The floc volume was obtained by using the formula for the volume of a cylinder:

$$\text{Floc volume} = \pi r^2 h$$

where π is 3.14, r is the radius (1/2 of i.d.) of the core (1.25 cm) and h is the floc height measured in the field (cm).

A subsample was used to obtain the ash free dry weight (AFDW) and the % organic matter (%OM) content. Subsamples were dried at 70 °C to determine the sample dry mass and ashed at 500 °C for 5 hours to determine the AFDW. The percent OM (%OM) was obtained by using the following relationships:

$$\%\text{AFDW} = [\text{AFDW (g)}/\text{total sample dry mass (g)}] \times 100$$

$$\%\text{OM} = 100\% - \%\text{AFDW}$$

Standard Operating Procedures for Sedimentary and Particulate Organic Matter Characterization

Field collection

Sampling sites are typically reached by small boat or airboat, depending on season (water levels) and location.

a. *Sediments (Collected for Biomarkers and/or Stable Isotope Analysis)*
   Surface sediments (0-2 cm) are collected with an Eckman Dredge (Wildco) or subsampled from a core. Sediment cores are collected manually by inserting a plastic core tube at least 10 cm into bottom sediments. The top of the core is capped, and manually extracted from the sediments. The bottom of the core is capped. Flocculent material (floc) is allowed to settle, then floc and overlying water are removed either by decanting or pipetting. Cores are extruded and sliced in 2 cm intervals. Sediments are immediately transferred to clean, solvent rinsed glass jars with Teflon-lined lids and stored on ice in the field.

b. *Water Column Suspended Particles (Collected for Biomarkers, Total Suspended Particles (TSP), Particulate Organic Matter, and/or Stable Isotope Analysis)*

Large volumes (25-50 L) of water samples are collected in acid and base-washed polyethylene carboys. Water is strongly agitated, then filtered through a pre-combusted 142 mm GF/F glass fiber filter to collected suspended particulate organic matter. Simultaneously, triplicate 1 L samples of water from the same location is collected in acid and base-washed polyethylene bottles. These triplicate water samples are filtered through pre-weighed, pre-combusted 47 mm GF/F glass fiber filters. All filters are stored on ice in the field. When stable carbon isotope analysis of suspended POM is desired, 10 L of water is collected from each site in a polyethylene bottle and filtered through a 90 mm, pre-combusted GF/F glass fiber filter. Filters are stored in clean glass jars with Teflon lined caps or folded and stored in pre-combusted aluminum foil and placed on ice in the field.

Upon return to the laboratory, sediment and particle samples on filters are immediately frozen and stored until processing. Prior to processing, sediments and filters are freeze-dried, and sediments are homogenized with a mortar and pestle.

Laboratory Methods

a. *Bulk Parameters (C, N, $\delta^{13}C$, $\delta^{15}N$, TP)*

Freeze-dried, homogenized sediment samples are split into separate samples for carbon and nitrogen analysis. Samples for carbon analysis are acidified with 10% HCl until effervescence ceases, then rinsed with ultrapure (Milli-Q) water until reaching neutral pH. Samples are re-dried in an oven at 60 °C. Samples for nitrogen analysis remain unacidified. Samples are weighed into small tin cups (mg size samples) for bulk parameter analysis. Total organic carbon (TOC), total nitrogen (TN), and isotopic composition were measured concurrently with a Carlo Erba NA 1500 Elemental Analyzer coupled to a Finnigan MAT Delta isotope ratio mass spectrometer at Florida International University's Stable Isotope Laboratory. Unacidified samples were analyzed for TN and $\delta^{15}N$ and acidified samples are analyzed for TOC and $\delta^{13}C$. All isotopic values are presented using the standard δ notation with Pee Dee Belemnite (PDB) and atmospheric air as standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. Total phosphorus (TP) content is measured for with colorimetric

analysis following dry oxidation and an acid hydrolysis extraction (Fourqurean et al. 1992). For isotopic measurements of suspended particulate organic matter collected on filters, each filter was put into a 600 mL beaker and a clean razor blade along with DI water was used to scrape the material away. As much color was removed from the filter as possible. The sample was dried in an oven at 60 ˚C, and ground into a fine powder with an agate mortar and pestle. The sample was split for C and N analysis and treated as described above.

b. *Lipid Biomarker Analysis*

Lipid biomarkers are extracted from the freeze-dried filters or sediments for 24 hours in Optima Grade Methylene Chloride (350 mL) using a Soxhlet extraction. Activated copper is added to the round bottom flask during the extraction to eliminate sulfur. Total extracts are then saponified with 0.5N KOH and separated into a fatty acid fraction through liquid-liquid extraction with methylene chloride. After acidification with HCl to pH <2, neutral lipids are partitioned into methylene chloride and isolated. The neutral lipid fractions are further separated into aliphatic hydrocarbon, aromatic hydrocarbon, wax and methyl ester, ketone/aldehyde, and alcohol and sterol fractions by adsorption chromatography over silica gel. The fatty acid fraction is concentrated by rotary evaporation and methylated with freshly distilled diazomethane. The alcohol and sterol fractions were derivatized with BSTFA (Aldrich) and 5 uL of pyridine (Fisher) prior to analysis by gas chromatography-mass spectrometry (GC/MS).

c. *Instrumental and Analysis*

Lipid biomarkers in the fatty acid and neutral lipid fractions are identified and quantified using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer operating in electron ionization (EI) mode at 70 eV. The gas chromatograph was equipped with either a Restek Rtx®-5MS or Restek Rtx-1 capillary column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness). The GC oven is ramped from an initial temperature of 60 °C to 300 °C at a rate of 6 °C/min, followed by a hold at 300 for 20 minutes. Identification of lipids is

performed by comparison of chromatographic retention times with authentic standards and mass spectra of standard and previously reported compounds. Quantification is performed by comparison of peak areas between reported compounds and internal standards.

Dissolved Black Carbon Analyses:

Sampling:

Surface water samples for dissolved black carbon (DBC) are collected in 2.5L glass bottles or pre-cleaned 2L plastic bottles. The bottles were pre-acid cleaned and rinsed with a small portion of water sample three times immediately before sampling. After sampling, bottles were properly labeled, stored on ice and transported to the laboratory.

Sample processing:

Samples were filtered immediately in the lab through pre-combusted glass fiber filters (GF/F, 0.7 μm) into pre-acid cleaned glass bottles. 60 ml of sample were transferred into amber polycarbonate Nalgene brown bottles (pre-acid and base cleaned) and saved in refrigerator for dissolved organic carbon (DOC) analysis. The remaining water samples were acidified to pH = 2 using concentrated HCl (32%, CAS plus grade). Dissolved organic matter from samples was extracted by solid phase extraction (SPE) using Varian Bond Elut PPL (1g) cartridges. The cartridges were conditioned by MeOH (20ml, optima grade), and 0.01N HCl (40 ml) was used to wash out MeOH right before applying the water samples. Filtered and acidified samples were gravity fed through the cartridges at a flow rate < 15 ml/min. Immediately after absorption, salts were rinsed off the cartridges with 0.01N HCl (40 ml). The cartridges were dried under ultra-high purity nitrogen gas stream, and eluted by 20 to 30 ml MeOH ensuring that the eluent became colorless, and stored at -18 ºC for further analysis.

DOC analysis:

Dissolved organic carbon (DOC) concentration of all water samples were analyzed by a non-purgeable organic carbon method on a TOC-5000A analyzer (Shimadzu, Japan). 4 ml of water samples were acidified to pH<2 with 3N HCl to convert inorganic carbon into CO_2. After being

sparged by compressed air (ultra-zero grade, Airgas), 100ul pre-treated samples were injected and oxidized in the TOC-5000A furnace, filled with pre-conditioned platinum-catalyst, at 680 ºC. The combustion products, including CO_2, was carried by the ultra-zero grade compressed air and detected by a non-dispersive infrared CO_2 detector

Benzenepolycarboxylic acids method:

An equivalent of 20-80 umol C of MeOH extracts after SPE were filled into 1 ml glass ampoules, dried under an ultra-high purity N_2 stream, and sealed after an addition of 0.5 ml nitric acid (65%, CAS plus grade). The samples were digested in a programmable GC oven (Hewlett Packard HP 5890 Series II GC) that was set to heat the ampoules to 160°C in 30 min and remain for 6 hours. Benzenepolycarboxylic acids (BPCAs), the oxidation products, were separated and quantitatively detected by a high-performance liquid chromatography system coupled with a three dimensional PDA detector (Surveyor, Thermo Scientific). A C_{18} column (3.5µm, 2.1×150mm, Waters Sunfire) and a binary gradient consisting of mobile phase A (4mM Tetrabutylammonium bromide, 50mM Sodium acetate and 10% MeOH) and mobile phase B (100% MeOH) were employed to achieve chromatographic separation.