

Dissolved organic matter and nutrient dynamics of a coastal freshwater forested wetland in Winyah Bay, South Carolina

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Abstract Seasonally flooded, freshwater cypress-tupelo wetlands, dominated by baldcypress (*Taxodium distictum*), water tupelo (*Nyssa aquatica*), and swamp tupelo (*Nyssa sylvatica* var. *biflora*) are commonly found in coastal regions of the southeastern United States. These wetlands are threatened due to climate change, sea level rise, and coastal urban development. Understanding the natural biogeochemical cycles of nutrients in these forested wetlands as ecosystems services such as carbon sequestration and nitrogen processing can provide important benchmarks to guide conservation plans and restoration goals. In this study, surface water and soil pore water samples were collected weekly from a cypress-tupelo wetland near Winyah Bay, South Carolina and analyzed for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), inorganic nitrogen, and phosphate during its flooding period between October 2010 and May 2011. DOC was further characterized by specific ultra-violet absorbance at 254 nm, spectral slope ratio (S_R) (ratio of two spectral slopes between 275–295 nm and 350–400 nm), E2/E3 ratio (ratio between A254 and A365), and fluorescence excitation-emission matrix. In addition, litterfall was collected on a monthly basis for a year while the biomass

of the detritus layer (i.e., decomposed duff lying on the wetland floor) was determined before and after the flooding period. Results of the field study showed that concentrations of DOC, DON, $\text{NH}_4^+\text{-N}$, and $(\text{NO}_2^- + \text{NO}_3^-)\text{-N}$ in the surface water were generally higher during the fall, or peak litterfall season (October to December), than in the spring season (March to May). Highest concentrations of 54.8, 1.48, 0.270, and 0.0205 mg L^{-1} , for DOC, DON, $\text{NH}_4^+\text{-N}$, and $(\text{NO}_2^- + \text{NO}_3^-)\text{-N}$ respectively, in surface waters were recorded during October. Lower SUVA, but higher S_R and E2/E3 ratios of DOC, were observed at the end of the flooding season comparing to the initial flooding, suggesting the wetland system converts high aromatic and large DOC molecules into smaller and hydrophilic fractions possibly through photochemical oxidation. A similar trend was observed in soil pore water, but the pore water generally had greater and relatively stable concentrations of dissolved nutrients than surface water. No obvious temporal trend in phosphate concentration and total nitrogen to total phosphorus ratio (N:P) were found. Results of the laboratory extraction and mass balance calculation suggested fresh litter was a major source of DOC whereas decomposed duff was the source of dissolved nitrogen in surface water. In summary, the biogeochemistry of this isolated cypress-tupelo wetland is not only driven by the vegetation within the wetland system but also by hydrology and weather conditions such as groundwater table position, precipitation, and temperature.

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Introduction

Seasonally flooded, freshwater cypress-tupelo wetlands, dominated by baldcypress (*Taxodium disticum*), water tupelo (*Nyssa aquatica*), and swamp tupelo (*Nyssa sylvatica* var. *biflora*) are commonly found in coastal regions of the southeastern United States. Although these wetlands represent <3 % of the southeastern US landscape (Tiner et al. 2002), seasonally flooded depressional wetlands are an important habitat for birds, mammals, reptiles, amphibians, invertebrates, and rare plant species (Semlitsch and Bodie 1998; Sutter and Kral 1994), especially supporting species not found in longer-hydroperiod wetlands (Snodgrass et al. 2000). Unfortunately, cypress-tupelo wetlands are threatened due to climate change, sea level rise, and coastal urban development (Titus and Richman 2001; Allen and Lu 2003; Doyle et al. 2007a; Dai et al. 2011). Sea level rise—an expected consequence of global warming—may likely increase salinity exposure in coastal freshwater-forested systems, resulting in tree mortality and forest dieback, eventually converting forested wetlands into salt marsh (Doyle et al. 2007b; Krauss et al. 2009). It has been estimated that ~58,000 km² of land along the Atlantic and Gulf coasts lie below the 1.5 m contour (Titus and Richman 2001). By the year 2100, global warming could raise sea level as high as 1.4 m, which could lead to loss of many coastal wetlands in low-lying states in the southeastern United States, including North and South Carolina (SC), Louisiana, Florida, and Texas (Titus and Narayanan 1996; Rahmstorf 2007).

In addition to serving an important habitat for many animals, cypress-tupelo wetlands play an important functional role in nutrient cycling and storage (Watt and Golladay 1999; Craft and Casey 2000; Ozalp et al. 2007). Most of the plant species within the wetland are deciduous species with high inputs of fresh litter between October and December. Depending on the vegetation density, tree composition, and hydroperiod of a wetland, annual leaf litterfall can range from 371 to 582 g m⁻² (Watt and Golladay 1999; Busbee et al. 2003). Approximately half of the annual leaf litter

would remain after 1 year of decomposition (Day 1982; Battle and Golladay 2001). Both fresh and decomposed litters are important sources of DOC and nutrients to wetland water (Goni and Thomas 2000; Davis et al. 2006).

The hydrology of depressional wetland systems is driven primarily by rainfall, shallow subsurface water flow, and evapotranspiration with no apparent surface water connection to perennial rivers or streams, estuaries, or oceans (Tiner et al. 2002). Typically, these cypress-tupelo wetlands are hydrologically recharged by rainfall in the fall after drying out during the summer due to evapotranspiration (Busbee et al. 2003; Battle and Golladay 2007). The seasonal wetting and drying cycles, or hydroperiod, of the wetland could affect leachability and microbial degradation on soil organic matter and litter materials (Watt and Golladay 1999; Battle and Golladay 2001, 2007; Chow et al. 2006). Studies have been conducted to evaluate the nitrogen and phosphorus cycling in litter materials within the cypress-tupelo forested wetland (Watt and Golladay 1999; Craft and Casey 2000; Anderson and Lockaby 2007, 2011; unpublished data), but few studies have examined dissolved nutrients in surface and subsurface waters. DOC and dissolved nutrients within forested wetlands are of particular interest because surface water is generally characterized by low turbidity but with a visible tea-colored organic composition, which is typical of these coastal isolated wetland systems. In fact, cypress-tupelo wetlands have been considered to be a main source of DOC and nutrients to adjacent estuaries (Wolaver et al. 1986; Williams et al. 1992; Goni et al. 2003).

Winyah Bay, a moderately-sized estuary along the southeastern coast located 97 km north of Charleston, SC, has experienced significant impacts by long-term sea level rise (Titus and Richman 2001; Doyle et al. 2007a). The boundary between the low-lying coastal forest and high marsh has moved up the topographical gradient over time. Sandy forested Spodosols are subjected to tidal inundation and salinization, and forest vegetation (e.g., *Pinus*, *Quercus*, and *Vaccinium*) is replaced by coastal salt marshes (e.g., *Juncus* and *Spartina*) (Gardner et al. 1992; Morris et al. 2002). One of the cypress-tupelo wetlands surrounding Winyah Bay has experienced a 30 % loss in forest area in the last 60 years corresponding to the rising sea level (Williams et al. submitted). The rate of wetland loss could be further increased with a rapid increase in sea level in the future.

Understanding the natural biogeochemical cycles of nutrients in this forested wetland will provide important functional information related to ecosystem services, such as carbon sequestration and nitrogen processing, and also serve as background for future conservation plans and restoration activities in the region. In this study, part of an ongoing long-term project examining sea level rise in coastal forests, DOC and nutrient dynamics of an isolated cypress-tupelo wetland in Winyah Bay were examined during its flooding period. The objectives of the study were (1) to examine temporal variations of dissolved nutrients and their relationships with environmental conditions such as temperature and rainfall, and (2) to determine relative dissolved nutrient contributions from fresh and decomposed litters.

Materials and methods

Study site

The study took place from July 2010 to June 2011 in a forested wetland—hereafter called Crabhaul wetland (33°21'N, 79°12'W)—on Hobcaw Barony which is a 7,082 ha wildlife refuge adjacent to Winyah Bay, SC (Fig. 1). This 24-ha ($2.4 \times 10^5 \text{ m}^2$) cypress-tupelo wetland, which represents a typical seasonally flooded wetland in coastal SC, is a natural depression dominated by baldcypress, water tupelo, and swamp tupelo. This wetland is considered to be highly productive with aboveground net primary productivity of $774 \text{ g m}^{-2} \text{ year}^{-1}$ and litterfall of $548 \text{ g m}^{-2} \text{ year}^{-1}$, recorded in a previous study (Busbee et al. 2003). The wetland is surrounded by longleaf pine (*Pinus palustris* Mill.) and American turkey oak (*Quercus laevis* Walt.) communities. Soil in Crabhaul wetland is of the Hobcaw series (fine-loamy, siliceous, thermic, Typic Umbraquults) (Stuckey 1982). Surrounding forests had 80 % soil organic matter at 0–1 cm but decreasing to 53 % at 8–9 cm (Goni and Thomas 2000). Detailed characterization of the site including tree composition, stem density, aboveground productivity, and hydrology of the Crabhaul wetland can be found in Busbee et al. (2003).

Weather and water level data

Surface water level of the Crabhaul wetland was continuously measured at 15-min intervals using a

Leupold and Stevens Type F water level chart recorder, which was installed between two experimental plots as described in the next section. Also, an in situ staff gage was installed about 50 m from the chart recording gage. Staff gage levels were recorded once a month during monthly litterfall collection. Rainfall (mm), ambient air temperature (°C), and solar radiation (W m^{-2}) were also recorded continuously with 15 min intervals using an Onset Hobo® U30/Wi-Fi weather station installed at the Clemson Baruch Institute of Coastal Ecology and Forest Science. The weather station was located in an open area and was less than 1.0 km away from the study wetland.

Vegetation sampling and water extractable nutrients

Two 20×25 -m plots were established in the central portion of the wetland in order to eliminate edge effects (Fig. 1). Five 0.5×0.5 -m litterfall traps with 1 mm mesh fiberglass screen bottoms were randomly placed in each of the plots. The traps were elevated 1 m to prevent inundation during flooding. Litterfall was collected monthly and a total of 10 samples were collected from the two plots each month. Detritus materials lying on the dry wetland floor (named duff thereafter) in July 2009 and June 2010 (before and after the flooding period) were also collected using 0.25×0.25 -m quadrats. Five quadrats were placed 3-m apart along a 15-m transect that was randomly placed within the 20×25 -m plots. A total of 10 samples were collected during each sample event. All collected litter and detritus materials were dried immediately upon returning to the laboratory at 70 °C for 2–3 days, and then weighed. Both litter and duff were recorded as g m^{-2} after suitable conversion.

Both dried litter and duff were ground and passed through a 0.5 mm sieve. Ten grams of dried materials were mixed with 100 mL Milli-Q water and shaken at room temperature for 8 h. The suspended liquids were centrifuged with relative centrifugal force at 3360 for 10 min and then filtered through a $0.45 \mu\text{m}$ polyethersulfone membrane filter (Supor-450). Extracts were stored at 4 °C before analyses. A prefix termed “water extractable” is added for dissolved nutrients obtained in this method in order to distinguish these parameters from those obtained in the field

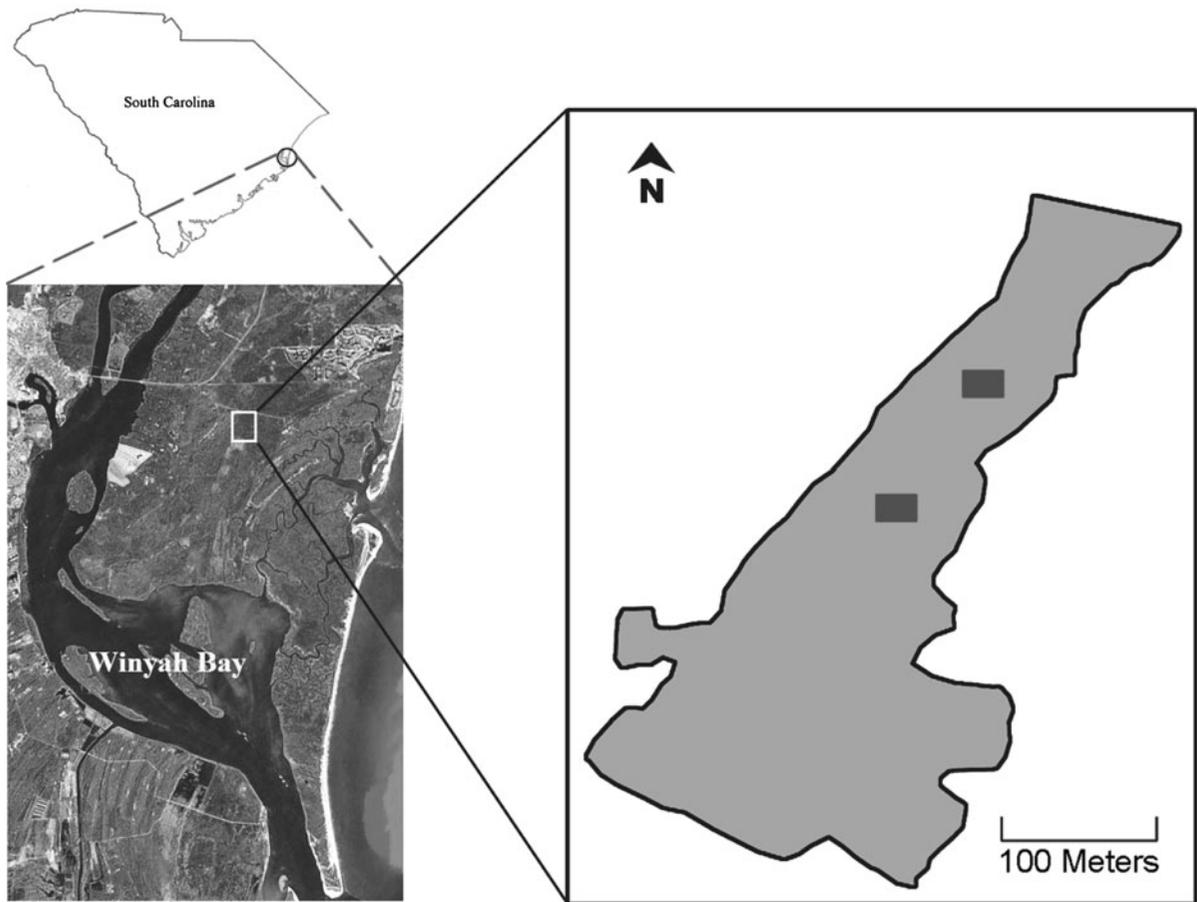


Fig. 1 The cypress-tupelo wetland located on Hobcaw Barony in the northern portion of Winyah Bay, South Carolina, USA. The boundary of the wetland is outlined on the right. The two

dark grey boxes within the wetland indicate the two 20 × 25-m plots. The map of Winyah Bay is adopted from Community Resource Inventory Online

measurements. For example, dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) from the water extraction are termed as water extractable organic carbon (WEOC) and water extractable organic nitrogen (WEON), respectively. Noticeably, a greater amount of WEOC and WEON could be obtained from our extraction method compared to other conventional extraction methods. Two to four mm sieved material with an extraction time between 30 min and 2 h has been typically used to examine readily leachable DOC from temporal forests and agricultural soils under non-flooded conditions (e.g. Hishi et al. 2004; Ghani et al. 2003). In contrast to those methods, smaller sieved materials and a longer extraction time were used here to complement the extensive soaking time of foliar litters in isolated wetlands, and to estimate total water soluble fractions from detritus materials.

Surface and soil pore water collection

Four surface and four soil pore water samples were collected within the two 20 × 25-m plots once a week (except in the month of December when only two sampling events were conducted) between October 1, 2010 and May 31, 2011 when the forested wetland was inundated. No surface water samples were collected between May and September as the area was not flooded. Surface water, about 5 cm below water surface, was collected in 125 mL pre-acid washed polyethylene bottles. Soil pore water was manually pumped from four pre-installed piezometers (two at each plot) that were inserted 50 cm below the soil surface when the wetland was dry. At least one liter of water was pumped and discarded from the piezometer before collecting into the 125 mL bottles. All samples

were immediately stored in an ice cooler and transported to the laboratory. Electrical conductivity (EC) and pH of non-filtered samples were measured upon arrival in the laboratory. The measured EC was corrected to EC at 25 °C (EC_{25}) using the temperature correction equation: $EC_{25} = EC_t/[1 + 0.021(t-25)]$ (Hayashi 2004). Waters were then filtered through a syringe filter (Millipore Millex-HV Hydrophilic PVDC, 0.45 μm). Filtrates were stored at 4 °C until further analysis.

Chemical analysis

Each filtered sample was analyzed for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) using a Shimadzu TOC/TN analyzer. Inorganic nitrogen, including $\text{NH}_4^+\text{-N}$, $(\text{NO}_2^- + \text{NO}_3^-)\text{-N}$, and $\text{PO}_4^{3+}\text{-P}$ were determined using a Syntex® Easychem™ discrete analyzer (EPA Methods 350.1, 325.2, and 365.1, respectively) (Eaton et al. 2005). DON was calculated by subtracting TDN from the sum of the inorganic nitrogen species. DOC was further characterized by Shimadzu UV-1800 visible and ultraviolet spectrophotometer scanning from 200 to 700 nm. Specific ultraviolet absorbance (SUVA) was calculated by normalizing ultraviolet absorbance at 254 nm to DOC concentration, recorded as $\text{L mg}^{-1} \text{m}^{-1}$. SUVA has been widely used as a surrogate of aromatic carbon content of DOC (Chow et al. 2008; Weishaar et al. 2003). Spectral slope ratio (S_R), which is correlated with molecular weight (MW) of DOC and to photochemically induced shifts in MW, was calculated as the ratio of two spectral slopes between 275–295 nm and 350–400 nm (Helms et al. 2008; Loiselle et al. 2009). In addition, the E2/E3 ratio, absorbance at 254 nm divided by absorbance at 365 nm, was calculated. The E2/E3 ratio, which is positively correlated with the quantum yields of singlet oxygen ($^1\text{O}_2$) but negatively correlated with hydrogen peroxide (H_2O_2) (Dalrymple et al. 2010), is an important index showing the photo-degradability and photo-reactivity of DOC. Also, selected samples were analyzed using fluorescence emission-excitation matrix. The components and redox index of DOC were quantified using the PARAFAC (fluorescence parallel factor analysis) model (Cory and McKnight 2005) with spectral correction (Cory et al. 2010). Samples were diluted with deionized water to the absorbance at 254 nm to 0.3 or lower, and were measured using a

Fluoromax-4 fluorometer or Shimadzu RF-5301 spectrofluorophotometer.

Statistical analyses

Differences in water quality parameters between surface and soil pore waters were determined using a paired-samples *t* test. For those parameters that did not conform to a normal distribution (Kolmogorov–Smirnov test) and homogeneity of variance (Levene test), a Wilcoxon signed rank test was used. To detect differences in water quality parameters in different times, ANOVA and a post hoc Tukey's test were used. A Kruskal–Wallis test and a non-parametric Tukey-type multiple comparisons test were used when parameters did not conform to a normal distribution and homogeneity of variance. Statistical methods were implemented using SPSS and the level of significance for all tests was 0.05.

Results

Weather conditions

South Carolina has a subtropical climate characterized by humid and hot summers and mild winters. In the study site at Hobcaw Barony, air and water temperatures dropped below 0 °C for only a few days in December and January with the lowest temperature being –2.5 °C. Temperature gradually increased after January, and average air temperature reached 20 °C in late April and 30 °C in July (Fig. 2a). The pattern of solar radiation was similar to temperature, with the lowest radiation in December and January and gradually increased through the end of the study period in June (Fig. 2b). Between July 1, 2010 and June 30, 2011, a total of 1,208 mm of rainfall was recorded at the weather station at the Baruch Institute of Coastal Ecology and Forest Science. The year of the study was considerably drier than the normal annual 1,312 mm of rainfall (SCDNR 2011).

The intensity of rainfall played an important role in controlling water level in Crabhaul wetland. Sharp increases in water level were observed with heavy rainfall (Fig. 2c, d). In particular, a storm with over 100 mm day^{-1} at the end of September increased the water level ~ 1 m, from –60 to 58 cm within 5 days. Also, intense storm events, such as the ones in

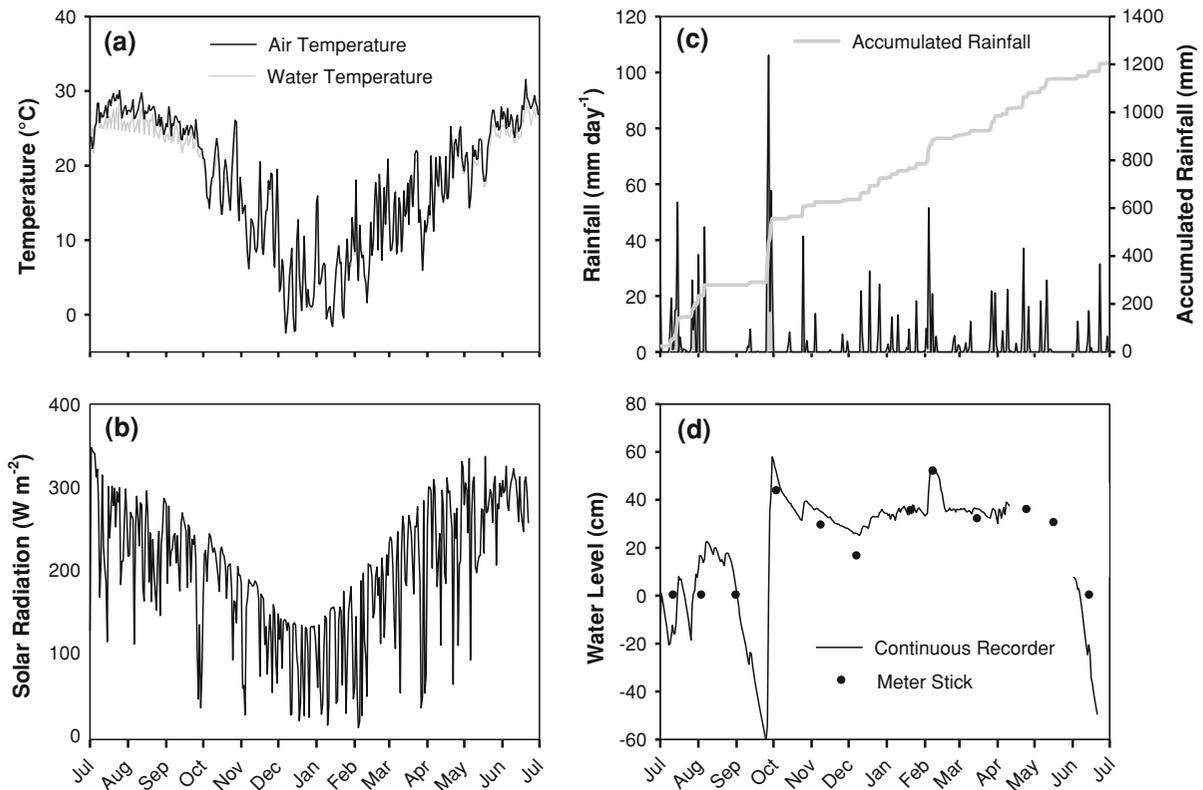


Fig. 2 Records of air and water temperatures (a), solar radiation (b), daily and accumulated rainfalls (c), and water level of Crabhaul forested wetland between July 2010 and June 2011

mid-July and mid-February, also caused sharp increases in water level. To correlate the water quality analyses to the weekly sampling event, accumulated rainfall of 30 mm or greater between two samplings were especially noted and summarized as follows: October 28, February 10, March 31, April 28, and May 14 with 46, 96, 62, 55 and 39 mm week⁻¹, respectively. Also, for the sampling event between December 8 and January 6, total accumulated rainfall was 106 mm with weekly rainfall amounts measuring ~30 mm in that period (i.e., the accumulated rainfall of December 8–15, December 16–22, and December 23–30, and December 31–January 6 were 28, 29, 31, and 18 mm, respectively). This seasonal wetland is usually dry in summer months from May to July due to high water uptake via evapotranspiration, as seen in rapid drops in water level in the months of September 2010 and June 2011 (Fig. 2d). However, occasional ponding and short-term storage after intense rain events were also observed in summer months. Water level in the system was relatively constant with a

surface water level of ~35 cm during the inundation period from October to May.

Litterfall and decomposed duff

The amount of decomposed duff collected prior to flooding was $1,168 \pm 138 \text{ g m}^{-2}$ (average \pm standard deviation, $n = 10$). The total litterfall in Crabhaul wetland between July 2010 and June 2011 was 833 g m^{-2} . Most of the leaf litterfall occurred in December in 2010 with an average of 356 g m^{-2} , representing 43 % of the total amount (Fig. 3). Litterfall in October and November had similar values and contributed 119 and 127 g m^{-2} of litter, which were 14 and 15 % of the total, respectively. A total of 72 % of litterfall was dropped in the first 3 months of the flooding period (i.e., October to December). During the flooding period between October and May, 85 % of litterfall occurred. At the end of the flooding period, decomposed duff was higher than collected prior to flooding with an average of

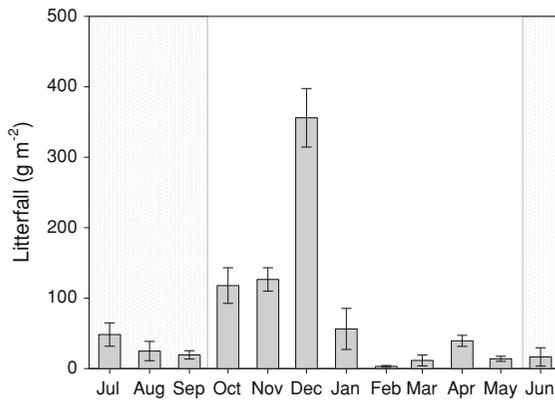


Fig. 3 Monthly litterfall records expressed as dry weight per unit area in Crabhaul wetland between July 2011 and July 2012. The values represent an average of 10 replicates with error bar as standard deviation. *Dotted areas* show the typical dry and non-flooding period of the wetland

$1,237 \pm 167 \text{ g m}^{-2}$, but the difference is statistically insignificant ($p > 0.05$).

WEOC, WEON, and other water extractable nutrients of fresh litter and decomposed duff are summarized in Table 1. A notable difference between litter and duff was found on the yield of WEOC. Litter produced $41.6 \pm 1.6 \text{ mg g}^{-1}$ WEOC and was four times greater than duff. However, no significant differences were found between litter and duff in WEON and water extractable inorganic nitrogen species. The carbon to nitrogen ratio of water extracts (WEOC : WEON) of fresh litter was 141 ± 12 ,

whereas WEOC: WEON of duff was only 32 ± 1 . In terms of optical properties of DOC, significantly lower SUVA but greater S_R and E2/E3 values ($p < 0.05$) were found in fresh litter than decomposed duff.

Electrical conductivity and pH

Surface and soil pore waters had similar and relatively low pH upon initial flooding but they gradually increased over time. There was a greater pH change in surface water than soil pore water in the 8-month flooding period. The pH in surface water increased from 6.40 ± 0.06 to 7.50 ± 0.03 while the pH in soil pore water increased from 6.44 ± 0.02 to 7.05 ± 0.08 . Except for the first sampling event, surface water always had a greater pH value than soil pore water. Differences between surface and soil pore waters were also observed in EC_{25} measurement. The EC_{25} of soil pore waters was generally greater than surface waters. The EC_{25} of surface water fluctuated between 0.213 ± 0.006 and $0.333 \pm 0.014 \text{ mS cm}^{-1}$ while the EC_{25} of soil pore water fluctuated between 0.237 ± 0.009 and $0.466 \pm 0.083 \text{ mS cm}^{-1}$. Intense rain events had a dilution effect on surface water but not the soil pore water (Fig. 4a). A statistically lower surface water EC_{25} ($p < 0.05$) occurred when accumulated rainfall was greater than 30 mm in the week (i.e., the sampling events listed in Sect. “[Weather conditions](#)”), ranging from 10 to 55 % decreases in EC_{25} . In addition, there was a greater standard

Table 1 Characteristics of water extractable nutrients and organic matter from fresh litter and duff (mean \pm standard deviation, $n = 5$)

	Fresh litter	Decomposed duff
Biomass (g-dry weight m^{-2}) ^a	833 ± 51	1168 ± 256
WEOC (mg g^{-1}) [*]	41.6 ± 1.6	9.4 ± 0.4
WEON ($\mu\text{g g}^{-1}$)	364 ± 30	368 ± 24
Water extractable $\text{NH}_4\text{-N}$ ($\mu\text{g g}^{-1}$)	30.2 ± 4.0	36.3 ± 2.9
Water extractable $\text{NO}_3\text{-N}$ ($\mu\text{g g}^{-1}$)	0.969 ± 0.117	3.38 ± 1.22
WEOC: WEON (mol mol^{-1}) [*]	141 ± 12	32 ± 1
SUVA ($\text{L mg-C}^{-1} \text{ m}^{-1}$) [*]	1.35 ± 0.05	2.46 ± 0.18
S_R [*]	1.00 ± 0.07	0.62 ± 0.02
E2/E3 [*]	7.04 ± 0.30	4.81 ± 0.10

Oven-dried litter or duff was mixed with deionized water in 1:10 mass to volume (10 g litter in 100 mL water) and shaken at room temperature for 8 h

Parameters marked with symbol (*) indicate the differences between fresh litter and decomposed duff are significant with $p < 0.05$

^a Value of fresh litter is the annual litterfall and it is the total value of monthly litterfall between July 2010 and June 2011, whereas value of decomposed duff is the total mass collected within the wetland prior to the flooding period

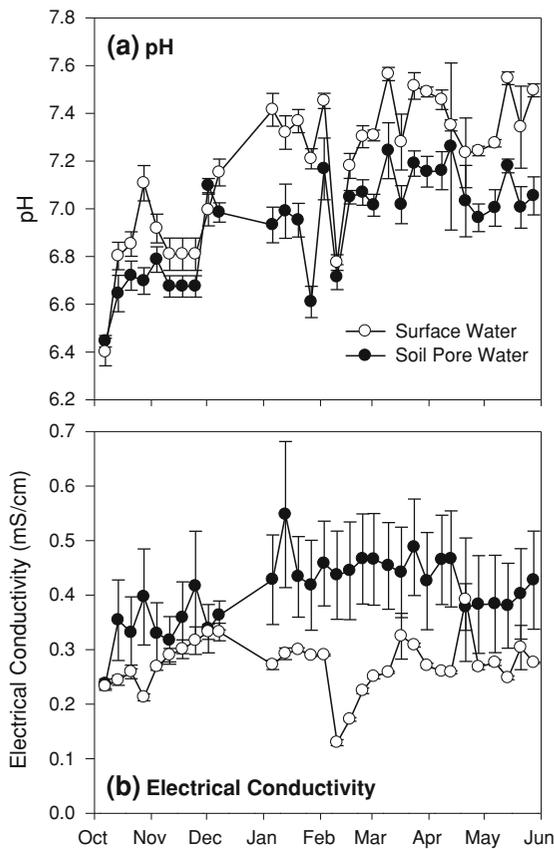


Fig. 4 pH and electrical conductivity measurements of weekly surface and soil pore water samples collected at Crabhaul forested wetland while flooded between October 2010 and May 2011. The values represent an average of 4 replicates with *error bar* as standard deviation

deviation in EC_{25} of soil pore waters, as shown by the error bars in Fig. 4b. Water samples from the piezometers installed on the south side of the wetland constantly had a greater EC_{25} than the piezometers on the north side, suggesting spatial heterogeneity of soil pore water.

Nitrogen dynamics

Temporal variations of inorganic species (NH_4^+-N and $(NO_2^- + NO_3^-)-N$) and DON in surface and soil pore waters are shown in Figs. 5a, b. The data can be roughly separated into two phases: an early flooding period with high inputs of litterfall (October to December) and a later flooding period with low inputs of litterfall (January to June). With the exception of NH_4^+ in surface water, higher concentrations occur early in the season but lower concentrations are found

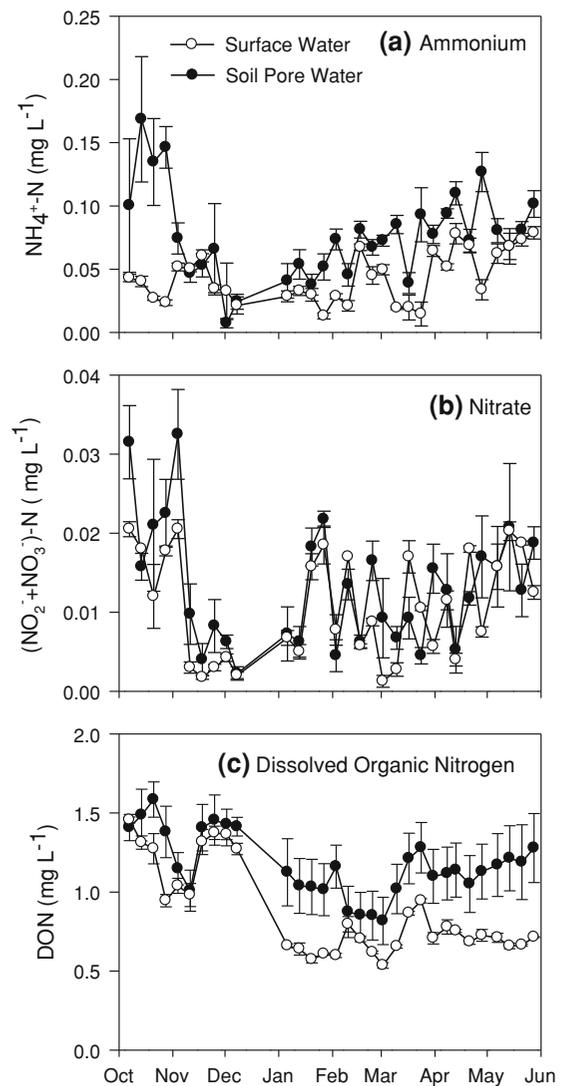


Fig. 5 Temporal variation of nitrogen species in surface and soil pore water samples at Crabhaul forested wetland while flooded between October 2010 and May 2011. The values represent an average of 4 replicates with *error bar* as standard deviation

later. Surface water DON was also greater in the early flooding period (Fig. 5c), fluctuating from 1.0 to 1.5 $mg L^{-1}$, whereas DON concentrations after January were generally lower than 1.0 $mg L^{-1}$.

In addition, surface water generally had a lower concentration of dissolved nitrogen than soil pore water. On average, surface water generally contained 0.043 ± 0.004 , 0.011 ± 0.001 , 0.86 ± 0.05 $mg L^{-1}$ of NH_4^+-N , $(NO_2^- + NO_3^-)-N$, and DON, respectively, whereas soil pore water contained 0.077 ± 0.006 , 0.013 ± 0.001 , and 1.16 ± 0.04 $mg L^{-1}$, respectively.

The differences in concentration were significant ($p < 0.05$) for $\text{NH}_4^+\text{-N}$ and DON but not for $(\text{NO}_2^- + \text{NO}_3^-)\text{-N}$. In terms of speciation, DON was the dominant species in both surface and soil pore waters, accounting for 94 and 93 % of TDN in molar unit, respectively. $\text{NH}_4^+\text{-N}$ was the dominant inorganic species and accounted for 5 and 6 % of TDN in surface and soil pore waters, respectively. $(\text{NO}_2^- + \text{NO}_3^-)\text{-N}$ only accounted for 1 % of TDN in both waters.

Carbon dynamics

The two phases become more obvious when inspecting DOC concentration in surface and soil pore waters. A higher DOC concentration, ranging from 42 to 55 mg L^{-1} , was observed in the months with the highest litterfall (October and December) (Fig. 6a). No difference was observed between surface and soil pore water. However, surface and soil pore water exhibited different dynamics in the later season. Concentrations of DOC in soil pore water dropped in January to $\sim 30 \text{ mg L}^{-1}$ but gradually increased towards the end of the season. In contrast, concentrations of DOC in surface water dropped rapidly and reached its lowest concentration at the end of January at 15 mg L^{-1} . Then DOC concentration rapidly increased to 40 mg L^{-1} after the storm event in mid-Feb and gradually decreased back to the range of 25–50 mg L^{-1} . In particular, a sudden increase in DOC concentration was observed in mid-Feb, and it was associated with the intensive 96 mm rain event during the week.

DOC/DON ratios in both surface and soil pore water were relatively stable in the range of 40 mol mol^{-1} during most of flooding season (Fig. 6b). The difference between waters was statistically insignificant ($p > 0.05$). A rapid increase of DOC/DON ratio was observed for both waters after the rain event in mid-Feb. DOC/DON ratio of surface and soil waters jumped to over 60 and 50, respectively, and remained at that level for ~ 1 month before decreasing back to the range of 40. Such changes did not occur in other rain events with similar or greater rainfall amounts.

Phosphate dynamics

Concentration of $\text{PO}_4^{3-}\text{-P}$ in both surface and soil pore waters fluctuated during the flooding period, ranging from less than 0.01 to 0.08 mg L^{-1} (Fig. 7a). Phosphate in soil pore water was generally greater than

surface water with few exceptions in the early season; however, the differences in most of the sampling event were statistically insignificant ($p > 0.05$). The ratio of nitrogen to phosphorus (N:P) also fluctuated with large error bars over the entire season. Neither any obvious temporal trend, nor differences between surface and soil pore waters were found (Fig. 7b). We conducted regression analyses on rain intensity at different time scales (i.e., 3, 5, 7, and 10 days before water sampling events) with phosphate and other nutrients but no significant correlation were found ($p > 0.05$).

Optical properties of dissolved organic carbon

As with other parameters, temporal trends in optical properties of DOC can be generally separated into early and later periods. In the early season, surface and

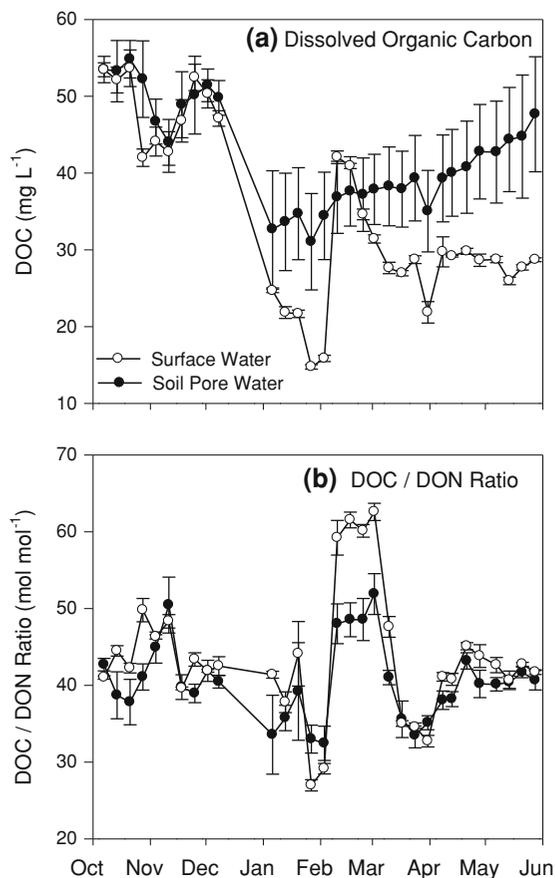


Fig. 6 DOC measurement and DOC/DON ratio of weekly surface and soil pore water samples collected at Crabhaul forested wetland while flooded between October 2010 and May 2011. The values represent an average of 4 replicates with error bar as standard deviation

soil pore waters had similar SUVA. Both had a high SUVA of $\sim 4.5 \text{ L mg-C m}^{-1}$ but gradually decreased by mid-January to about $3.0 \text{ L mg-C}^{-1} \text{ m}^{-1}$ (Fig. 8a). After Feb, the SUVA of both surface and soil pore waters increased but behaved differently. Soil pore water had a lower SUVA of about $3.7 \text{ L mg-C}^{-1} \text{ m}^{-1}$ but was relatively stable towards the end of the season, while surface water fluctuated during this period. S_R and E2/E3 ratios had similar two-period patterns and generally increased in the early season and then leveled off in January. Soil pore water was also relatively stable, but surface water exhibited sudden changes that matched the peaks in SUVA and S_R (Figs. 8b, c).

Similar to DOC concentrations, the optical properties of DOC in surface water were sensitive and exhibited rapid responses to changes in environmental conditions. Rapid changes in SUVA, S_R , and E2/E3 were observed in early February. The SUVA of surface water increased from 3.0 to 4.2 in a week and reached its highest value of 4.6 in early Feb.

In the same time period, S_R and E2/E3 dropped rapidly from 0.9 to 0.75 and 5.4 to 4.4 in a week, respectively. Such rapid changes were also found in mid-April associated with an intense rain event. Furthermore, lower SUVA but higher S_R and E2/E3 ratios of DOC were observed at the end of the flooding season comparing to the initial flooding.

Selected samples were analyzed using fluorescence emission-excitation matrix. Samples collected on April 28 are shown in Fig. 7. Peaks in humic acid-like ($E_m = 450 \text{ nm}$, $E_x = 300 \text{ nm}$) and fulvic acid-like ($E_m = 450 \text{ nm}$, $E_x = 250 \text{ nm}$) regions were observed in both surface and soil pore waters, whereas a small peak in microbial byproduct-like region ($E_m = 350 \text{ nm}$, $E_x = 270 \text{ nm}$) was only observed in surface water (Chen et al. 2003). The oxidized quinone-like, reduced quinone-like, and amino-acid like components of surface and soil pore waters are calculated as 31, 29, and 5 %, as well as 30, 32, and 2 % using Cory and McKnight (2005) model, respectively. The corresponding redox indexes were 0.518 and 0.479.

Discussion

Hydrologic influence on biogeochemistry

Hydroperiod has been considered an important factor influencing the breakdown of leaf litter in cypress-

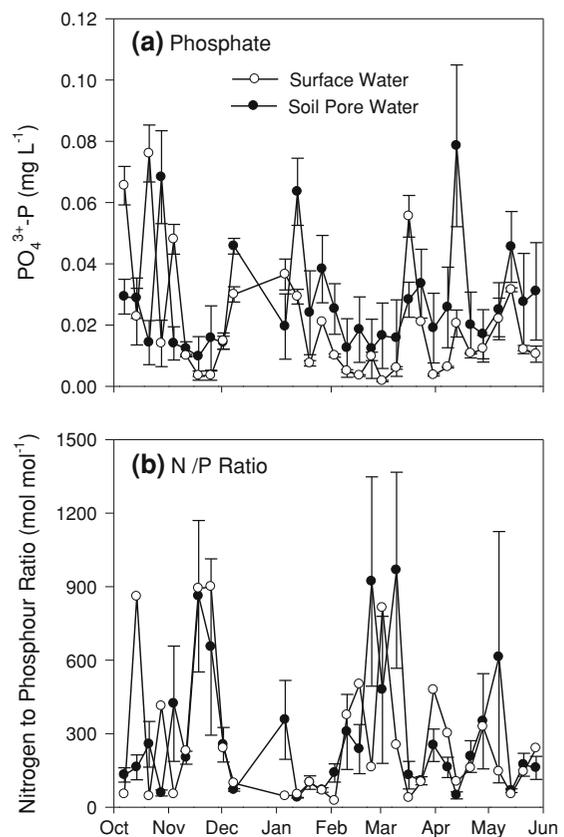


Fig. 7 Phosphate measurements and nitrogen to phosphorus ratio of weekly surface and soil pore water samples collected at Crabhaul forested wetland while flooded between October 2010 and May 2011. The values represent an average of 4 replicates with error bar as standard deviation

tupelo wetlands (Battle and Golladay 2001; Battle and Golladay 2007), and the hydrology of the cypress-tupelo wetland is driven primarily by rainfall and shallow subsurface water (Tiner et al. 2002; Busbee et al. 2003; Goni et al. 2003). Therefore, one would expect that rainfall and position of shallow subsurface water would be determining factors on the biogeochemistry in the wetland. Results from this study confirmed the hydrologic influence on varying concentrations and chemical characteristics of dissolved nutrients in the wetland. As shown in Fig. 8a, the highest DOC concentration was found early in the flooding season during October before greatest litter-fall occurred in December. High DOC concentration was probably attributed to the accumulation of nutrients during the relatively dry period from August to mid-September. High rain intensities in late September released DOC and nutrients of the detritus layers

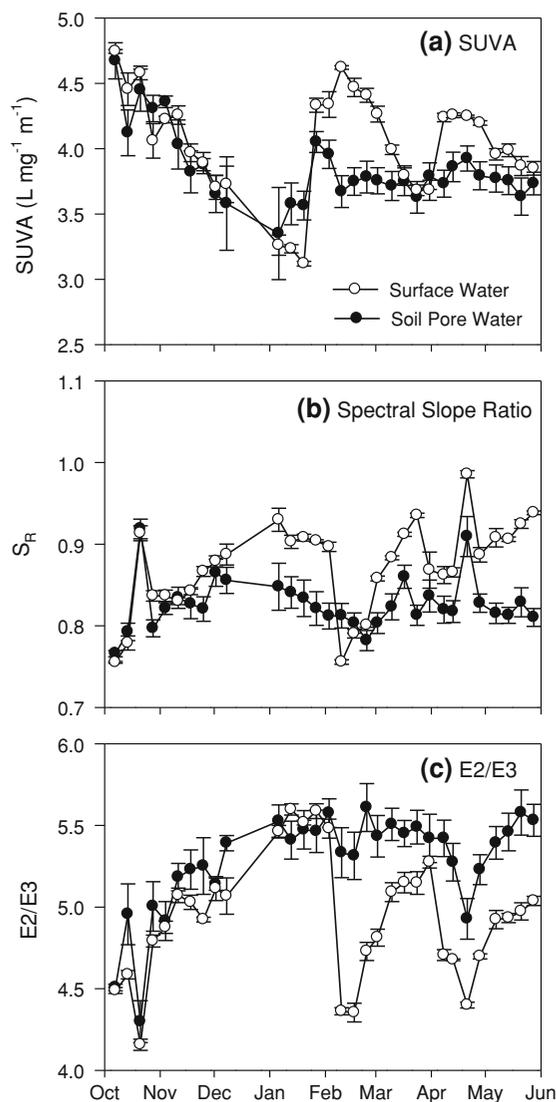


Fig. 8 Optical properties of surface and soil pore water samples at Crabhaul forested wetland while flooded between October 2010 and May 2011. The values represent an average of 4 replicates with *error bar* as standard deviation

into surface and soil pore waters. Such dry-wet effects on DOC are commonly observed in agricultural and forest ecosystems elsewhere (Chow et al. 2006, 2009).

Notably, water inputs from intensive rainfalls did not simply dilute the nutrients in the wetland. The storm event in early February was a good example in which rainfall was greater than 40 mm day^{-1} (or 96 mm week^{-1}) and temperature was below $10 \text{ }^{\circ}\text{C}$ (Fig. 2a, c). This event caused a relatively rapid increase in water level, from 33 to 53 cm in a few days

(Fig. 2d). The input of fresh water resulted in a dilution effect on the surface water, dropping EC_{25} from 0.28 to 0.12 mS cm^{-1} (Fig. 4b). However, a dilution effect on DOC or dissolved nutrients was not observed. Instead, DOC concentration increased from 15 to 42 mg L^{-1} after the rain event (Fig. 8a). Along with the increase in concentration, the DOC/DON ratio also increased twofold from below 30 to above 60. The DOC characteristics, in terms of SUVA, S_R , and E2/E3 ratios, expressed rapid changes in the same period of time.

In order to determine the source of this additional DOC, we compared chemical properties of surface water with litter extracts of the dominant vegetation within and outside the studied wetland. Baldcypress and water tupelo were the two dominant tree species (45 and 35 %, respectively) within the studied wetland with longleaf pine the major species of the surrounding forest (Busbee et al. 2003). Detailed procedures and chemical characterizations of litter extracts have been reported in our other publications (Dai 2012; Zhou et al. submitted). Briefly, DOC/DON ratio of baldcypress, water tupelo, and longleaf pine were 24, 121, and 125, respectively. SUVA and E2/E3 of the 3 samples were 0.79, 3.41, 1.19 and 7.81, 5.08, 13.87, respectively. Litter extract of water tupelo had strong peaks in humic acid-like ($E_m = 450 \text{ nm}$, $E_x = 320 \text{ nm}$) and fulvic acid-like ($E_m = 450 \text{ nm}$, $E_x = 250 \text{ nm}$) regions while strong peak in protein-like ($E_m = 320 \text{ nm}$, $E_x = 270 \text{ nm}$) region was observed in longleaf pine's extracts. High DOC/DON ratio, high SUVA, strong humic-acid like and fulvic-acid like peaks but low E2/E3 observed in the February storm (Figs. 6, 8, 9) suggests that the increase of DOC in surface water could be from water tupelo litter that accumulated in the wetland from the December litterfall. The freezing temperature in January restrained DOC and nutrient releases from fresh litters. The rapid drop in EC_{25} with warmer temperature in the February storm could increase the solubility and production of hydrophobic DOC from decomposing organic matter (Chow et al. 2003), resulting in the release of DOC into surface water. Although DOC could also be flushed from forest litter from the surrounding forest, DOC/DON ratio and optical properties of longleaf pine litter did not support this claim. In other rain events that occurred at the end of October and March, the EC_{25} as well as the concentrations of other nutrients and DOC decreased, indicating a dilution effect from rainfall.

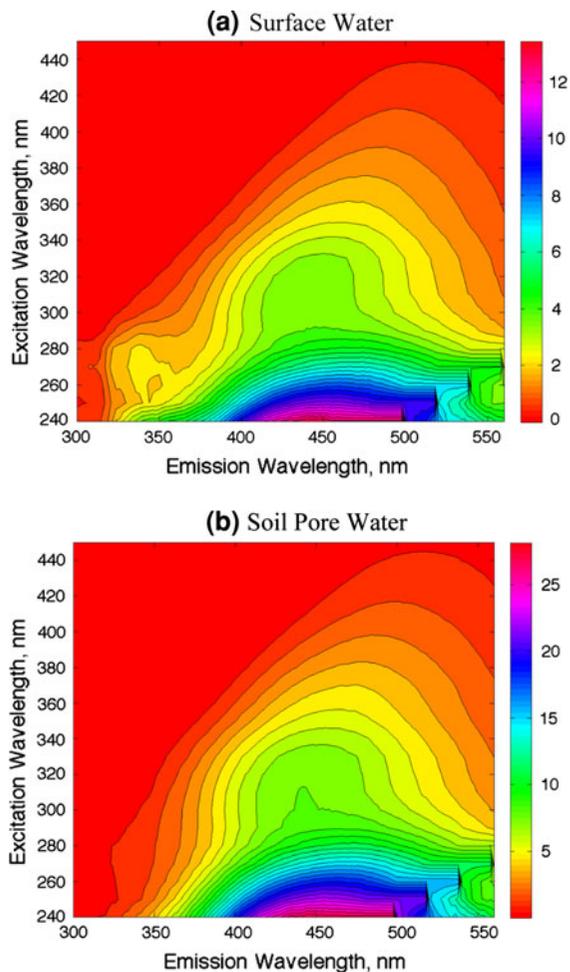


Fig. 9 Fluorescence excitation and emission matrix of surface and soil pore waters collected at Crabhaul forested wetland on April 28, 2011

Differences in nutrients dynamics between rainfall events suggest that litterfall is the major source of DOC and nutrients in the studied cypress-tupelo wetland but precipitation and temperature controlled the DOC and nutrient concentrations in the wetland.

Other factors on biogeochemistry

Lower SUVA but higher S_R and E2/E3 ratios of DOC observed at the end of the flooding season compared to the initial flooding could relate to the degradation of DOC. During the flooding period, the cypress-tupelo wetland was without canopy, and the surface water was shallow and static. Surface water was directly exposed to natural sunlight. In fact, solar radiation

increased from January towards the end of flooding season (Fig. 2b). The decreasing trend of SUVA and the increasing trend of S_R and E2/E3 ratio since January, with the exception of the intense rain event, suggested photobleaching of DOC in this shallow wetland (Waiser and Roberts 2004), converting high aromatic and large DOC molecules into smaller and hydrophilic fractions (Helms et al. 2008; Chow et al. 2008).

A large fluctuation observed in PO_4^{3+} -P concentration and N:P molar ratios in both surface and soil pore water was observed. We postulate that such an irregular pattern could relate to the solubility and adsorption of PO_4^{3+} -P. The N:P molar ratio of litter and sediments in cypress-tupelo wetlands was typically <200 (Craft and Casey 2000; Battle and Golladay 2001, 2007; Anderson and Lockaby 2011) but our results showed N:P molar ratios in water phases was frequently greater than 300 and even over 1,000 was occasionally recorded (Fig. 6b). A lower N:P ratio in solid versus aqueous phases suggests PO_4^{3+} -P was mainly bounded into suspended particles and sediments. These PO_4^{3+} -P bounded particles settled in the detritus layer, but any physical and chemical disturbances, such as rainfall and animal bioturbation activities, could mix and re-suspend solid particles and change water chemistry, and could temporally change the concentration of PO_4^{3+} -P in water phases (Froelich 1988; Chambers and Odum 1990; Abe 2010).

Biomass accumulation in forested wetlands

Biomass accumulation in wetlands is of great interest because wetlands can be an important carbon sink (Bridgman et al. 2006). Decomposed duff was collected and examined before and after the flooding period. Values shown in the results section indicate a greater amount of decomposed duff was found after 1 year, suggesting organic matter accumulation within the wetland. The biomass of decomposed duff lying in the wetland floor were comparable to another cypress-tupelo wetland study in the southeastern United States, which ranged from 351 to 1,842 $g\ m^{-2}$ (Watt and Golladay 1999). That study also suggested there was a biomass accumulation in the cypress-tupelo wetlands.

A simple mass balance model with a first-order kinetic decay was developed to examine biomass dynamics and accumulation of the study wetland.

Total biomass of detritus materials of the Crabhaul wetland (M_{Total}^t) in a particular month (t) is the sum of the monthly fresh litterfall (M_{Litter}^t) and decomposed duff (M_{Duff}^t)

$$M_{Total}^t = M_{Litter}^t + M_{Duff}^t \tag{1}$$

(M_{Litter}^t) is the monthly litterfall collected in situ as described in the methods section (Fig. 3). M_{Duff}^t is the sum of accumulated decomposed duff (m_{2009}^{Jul}) remaining on the wetland floor after the last flooding season and newly decomposed fresh litter (m_{2010}^t) prior to month t:

$$M_{Duff}^t = m_{2009}^{Jul} + m_{2010}^t \tag{2}$$

In other words, the decomposed duff was accumulated prior to July 2010 whereas newly decomposed fresh litter was a month or older duff deposited after July 2010. Using the first-order kinetic equation, their masses in a given month t can be expressed with the original masses, decay rate constant (k), and time exposed in the field (Δt):

$$[m_{2009}^t = m_{2009}^{Jul} \exp(-k(\Delta t))] \tag{3}$$

$$[m_{2010}^t = \sum M_{2010}^t \exp(-k(\Delta t))] \tag{4}$$

Combining Eqs. 1–4, the total biomass in the detritus layer can be calculated as:

$$M_{Total}^t = M_{Litter}^t + m_{2009}^{Jul} \exp[-k(\Delta t)] + \sum_i^{t-1} = July M_{Litter}^i \exp[-k(\Delta t_i)]$$

The decay rate constants of fresh cypress and tupelo litter found at the Crabhaul wetland were 1.10 and 0.697 year⁻¹, respectively (unpublished data). These values were comparable to other sites, which ranged from 0.327 to 1.61 and 0.57 to 2.04 year⁻¹ for cypress and tupelo, respectively (Day 1982; Battle and Golladay 2001, 2007; Ozalp et al. 2007). In the calculation, the decay rate of fresh litter is the sum of the fractions of the decay rate of cypress litter ($k_{cypress}$) and tupelo litter (k_{tupelo}) according to their populations in Crabhaul wetland ($k = f_{cypress}k_{cypress} + f_{tupelo}k_{tupelo}$). Cypress and tupelo were the two main tree species found in Crabhaul wetland with roughly $f_{cypress} = 0.38$ and $f_{tupelo} = 0.62$ in density, respectively (Busbee et al. 2003). Therefore, the decay rate constant for fresh litter used in the calculation is 0.849 year⁻¹ or 0.0708 month⁻¹. The decomposed duff generally has

a lower decomposition rate compared to fresh litter and a lower decay rate constant is expected for decomposed duff. In a review of the literature, no decay rate of decomposed duff was found. In this study, the lowest decay rate constant of mixed litter in a two-year cypress-tupelo wetland study (Day 1982) was used for the decay rate of decomposed duff, which was 0.327 year⁻¹, or 0.0423 month⁻¹.

Using October as an example, the total biomass of detritus materials in the wetland (M_{Total}^{Oct}) is the sum of fresh litterfall in October ($M_{Litter}^{Oct} = 118\text{gm}^{-2}$) and decomposed duff (M_{Duff}^{Oct}) accumulated before October. The (M_{Duff}^{Oct}) is the sum of decomposed duff prior to this flooding season (m_{2009}^{Oct}) and decomposed fresh litter in July, August, and September (m_{2009}^{Oct}). M_{2009}^{Jul} is the biomass of decomposed duff collected in July 2009 prior to the flooding period. Therefore, the total biomass of the detritus layer in October is:

$$\begin{aligned} M_{Total}^{Oct} &= M_{Litter}^{Oct} + M_{Duff}^{Oct} \\ &= M_{Litter}^{Oct} + m_{2009}^{Oct} + m_{2010}^{Oct} \\ &= M_{Litter}^{Oct} + m_{2009}^{Jul} \exp[-k(\Delta t)] \\ &\quad + \sum_{i=July}^{Sep} M_{Litter}^i \exp[-k(\Delta t_i)] \\ &= 118 + 1168 \exp[-0.042(3)] \\ &\quad + 48 \exp[-0.075(3)] + 25 \exp[-0.075(2)] \\ &\quad + 19 \exp[-0.075(1)] \\ &= 1,225 \text{ gm}^{-2} \end{aligned}$$

Based on this mathematical model and inputs of fresh litter biomass, we can estimate the total biomass of litter and duff in each month (Fig. 10a). At the end of the flooding period in June 2011, the M_{Duff}^{Jun} and M_{Total}^{Jun} , are estimated to be 1,253 and 1,269 g m⁻², respectively.

The calculated biomass closely matches the field measurement (1,237 g m⁻²) and both suggested biomass accumulation (69–101 g m⁻²) as duff in the study period (original duff biomass was 1,168 g m⁻²) (Fig. 10a). This value is in the range of organic C accumulation of an independent study on cypress-tupelo forested wetlands, which was between 20 and 79 g m⁻² year⁻¹ (Craft and Casey 2000). However, there could be a net loss or gain of biomass in detritus layer in the wetland and it depends on the primary productivity, bacterial and fungal activities, and hydrological period of a wetland, which could be

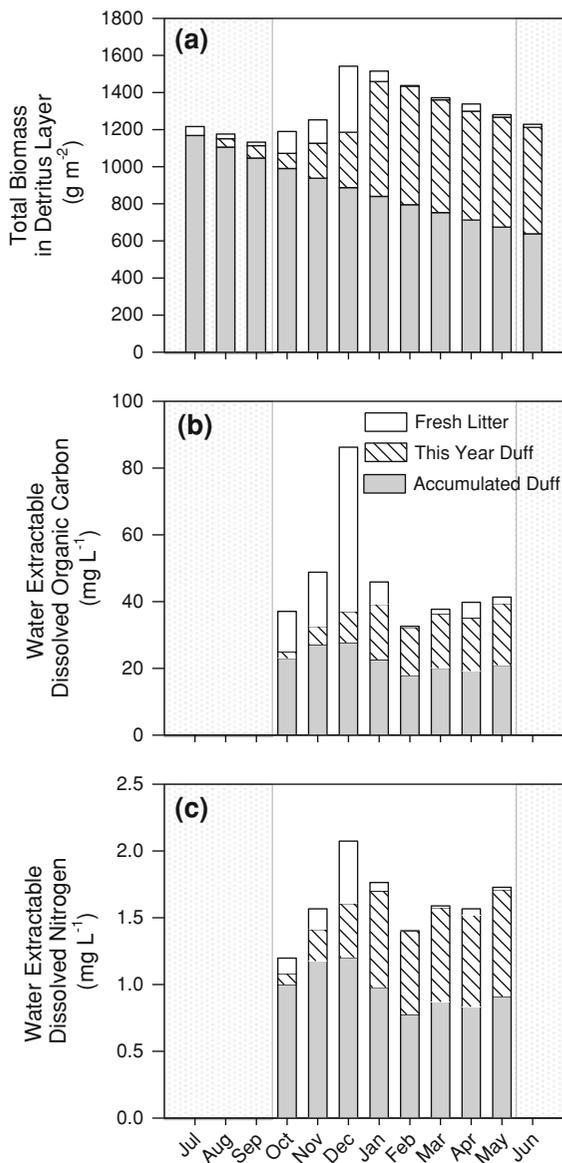


Fig. 10 Total biomass of the detritus layer during the flooding period was estimated using first-order decay (a). Contributions of DOC (b) and dissolved nitrogen (c) from fresh litter, the current year's duff, and accumulated duff was estimated using results from laboratory extraction. *Dotted areas* show the typical dry and non-flooding period of the wetland

climate dependent. For example, the litterfall in this year was significantly greater ($p < 0.05$) than a previous study that was conducted in year 2000, in which the total amount was 548 g m^{-2} and the largest input occurred in Nov (Busbee et al. 2003). With fresh litter inputs, our model suggests there could be a loss in detritus biomass in that year. Therefore, the duff

biomass accumulating in the wetland could be different for each year (Watt and Golladay 1999), and long-term monitoring is needed in determining carbon sequestration dynamics in this forested wetland, particularly under changing climate conditions.

Dissolved carbon and nitrogen contributions from detritus layer

Results from laboratory water extractions and field litter and decomposed duff measurements per unit area provided an estimation of relative DOC and dissolved nitrogen contributions from the detritus layer. Annual litterfall and accumulated decomposed duff were 833 and $1,168 \text{ g m}^{-2}$ in the study period, respectively. With a wetland area of $2.4 \times 10^5 \text{ m}^2$ and the results of extraction summarized in Table 1, a total of 832 and 264 kg of DOC could be produced from fresh leaf litter and accumulated decomposed duff annually. Similarly, a total of 79 and 114 kg TDN (mainly DON, as seen in Table 1) could be produced in a year. Calculations suggest that fresh litter is a major source of DOC, whereas decomposed duff is the source of dissolved nitrogen within the wetland.

However, the contributions of fresh litter and decomposed duff to dissolved nutrients were highly dynamic and changed over time (Chow et al. 2011). With the detritus biomass model as described above and water level records, the dissolved nutrient contribution by detritus layer for each month can be estimated. In the calculation, it was assumed that both current year newly decomposed duff (m_{2010}^t) and accumulated decomposed duff (m_{2009}^t) had the same yield on dissolved nutrients, as summarized in Table 1, and a monthly average of water level was used. Results are summarized as concentrations in order to compare to the field monitoring data, as shown in Fig. 10b, c. In October, the first month of flooding, the total biomass in the detritus layer was $1,225 \text{ g m}^{-2}$, with 118 , 78 , and $1,029 \text{ g m}^{-2}$ of fresh litter, newly decomposed duff, and accumulated decomposed duff, respectively (Fig. 10a). The calculation suggests that fresh litter, newly decomposed duff, and accumulated duff could contribute 12 , 2 , and 24 mg L^{-1} of DOC (Fig. 10b) and 0.12 , 0.08 , and 1.04 mg L^{-1} of TDN (Fig. 10c) in surface water, respectively. In this month, accumulated decomposed duff was the major source of DOC and TDN in the

wetland. In the month of December, the total biomass increased to $1,590 \text{ g m}^{-2}$, with 356, 288, and 945 of fresh litter, newly decomposed duff, and accumulated duff, respectively (Fig. 10a). They represent 49, 9, and 30 mg L^{-1} of DOC (Fig. 10b) and 0.47, 0.39, and 1.28 mg L^{-1} of TDN (Fig. 10c) in surface water, respectively. With the highest litterfall inputs, fresh litter was the major source of DOC in the wetland in December. Nevertheless, the accumulated duff was still the major source of TDN. Similar litterfall inputs were found in other cypress-tupelo forested wetlands (Watt and Golladay 1999; Craft and Casey 2000). With continued decay of accumulated duff and fresh litter inputs, newly decomposed duff was an important source of DOC and TDN at the end of the season.

Noticeably, although litterfall and water extraction experiments suggest highest DOC input occurred in December, actual but dynamics of dissolved nutrients were controlled by the hydrology and weather conditions, as discussed in Sects. “[Hydrologic influence on biogeochemistry](#)” and “[Other factors on biogeochemistry](#)”. Also, the calculation has not accounted for other biogeochemical processes such as DOC decomposition and photobleaching and other nutrient sources such as soils and throughfall. Also, an increase of artificial surface area by grinding the litter materials and mechanical agitation could enhance the release of dissolved nutrients from litter materials, resulting in an overestimation of availability of dissolved nutrient (Zsolnay 2003). Therefore, results in this section and in Fig. 10 only indicate the relative contributions of fresh and decomposed materials to DOC and TDN to the wetland system.

Conclusions

A 1-year monitoring study, accompanied by a laboratory extraction experiment and development of a mass balance model, was conducted in a healthy, isolated cypress-tupelo wetland near Winyah Bay, South Carolina. Results showed that the concentrations of dissolved nutrients, except phosphate, in wetland waters were generally higher upon initial flooding than later in the flooding season. Characteristics and concentrations of DOC in surface water were highly variable and sensitive in response to rain events. Sample analyses and subsequent calculations showed that fresh leaf litter was the major contributor of DOC, but decomposed duff

was the source of both organic and inorganic nitrogen. The highest litterfall were recorded in December, suggesting the greatest DOC inputs within the wetland system could occur in December, but actual dynamics of dissolved nutrients were controlled by the hydrology and weather conditions.

In summary, both the vegetation and the hydrology within the wetland system have determinant effects on the biogeochemistry of the isolated cypress-tupelo wetland. The results of this study are in agreement with other published research efforts but also warrant further investigation for a better understanding of the overall nutrient balances based on hydrologic inputs to, exports from, and internal reaction kinetics within these forested wetland systems. A better definition of the natural biogeochemical cycles of nutrients in this forested wetland will provide important information about the ecosystem services of these wetland systems in the coastal landscape.

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