



# Characterization of labile organic carbon in coastal wetland soils of the Mississippi River deltaic plain: Relationships to carbon functionalities

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## HIGHLIGHTS

- ▶ Labile organic C fractions and slowly released C increased with increasing total soil organic C in wetlands.
- ▶ Extraction-oriented labile organic C methods related more closely to each other than with aerobically mineralizable C.
- ▶ Various molecular organic C moieties contributed to labile and acid hydrolysable C fractions differently.
- ▶ Recalcitrant organic C accounted for 51–84% of total organic C in Mississippi River deltaic plain wetland soils.

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## ABSTRACT

Adequate characterization of labile organic carbon (LOC) is essential to the understanding of C cycling in soil. There has been very little evaluation about the nature of LOC characterizations in coastal wetlands, where soils are constantly influenced by different redox fluctuations and salt water intrusions. In this study, we characterized and compared LOC fractions in coastal wetland soils of the Mississippi River deltaic plain using four different methods including 1) aerobically mineralizable C (AMC), 2) cold water extractable C (CWEC), 3) hot water extractable C (HWEC), and 4) salt extractable C (SEC), as well as acid hydrolysable C (AHC) which includes both labile and slowly degradable organic C. Molecular organic C functional groups of these wetland soils were characterized by <sup>13</sup>C solid-state nuclear magnetic resonance (NMR). The LOC and AHC increased with soil organic C (SOC) regardless of wetland soil type. The LOC estimates by four different methods were positively and significantly linearly related to each other ( $R^2 = 0.62\text{--}0.84$ ) and with AHC ( $R^2 = 0.47\text{--}0.71$ ). The various LOC fractions accounted for  $\leq 4.3\%$  of SOC whereas AHC fraction represented 16–49% of SOC. AMC was influenced positively by O/N-alkyl and carboxyl C but negatively by alkyl C, whereas CWEC and SEC fractions were influenced only positively by carboxyl C but negatively by alkyl C in SOC. On the other hand, HWEC fraction was found to be only influenced positively by carbonyl C, and AHC positively by O/N-alkyl and alkyl C but negatively by aromatic C groups in SOC. Overall these relations suggested different contributions of various molecular organic C moieties to LOC in these wetlands from those often found for upland soils. The presence of more than 50% non-acid hydrolysable C suggested the dominance of relatively stable SOC pool that would be sequestered in these Mississippi River deltaic plain coastal wetland soils. The results have important implications to the understanding of the lability and refractory character of SOC in these wetlands as recent studies suggest marsh SOC to be an important C source in fueling hypoxia in the northern Gulf of Mexico.

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## 1. Introduction

Wetland soils, consisting of 20 to 25% of the terrestrial soil carbon, play a major role in global carbon cycle despite comprising a relative small portion of the total land area (Amthor and Huston, 1998). High

rates of organic matter input coupled with slow mineralization rate have made wetland soils an important C sink for atmospheric CO<sub>2</sub> (Reddy and DeLaune, 2008). Low oxygen, acidic peat and lack of nutrients are among the major factors contributing to the slow mineralization of soil organic matter (SOM) in wetlands (Bridgman and Richardson, 2003; Yavitt et al., 2004). On the other hand, the amount of C sequestered in these soils could be significantly impacted by relative distribution of soil organic carbon (SOC) fractions with different lability for microbial mineralization (Rovira and Vallejo, 2007; Silveria et al., 2008; Wang et al., 2011). Therefore, adequate characterization of wetland soil labile organic C (LOC) is essential to the

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understanding of the C transformation and overall biogeochemistry in these soils.

SOM is generally considered as a continuum of heterogeneous substances with variable molecular structure and elemental composition in various stages of decomposition and reformation (Oades, 1988; McLauchlan and Hobbie, 2004; Fang et al., 2005). Based on resistance to mineralization, SOM has been divided into labile, intermediate and recalcitrant organic carbon pools. Soil labile organic C pool is the most active fraction of SOC with rapid turnover rates, and governs production and flux of CO<sub>2</sub> from soils to atmosphere (Zou et al., 2005; Silveria et al., 2008). The lability of SOM has been found to be dependent on both chemical recalcitrance and physical protection from microbial degradation (McLauchlan and Hobbie, 2004; Rovira and Vallejo, 2007; Silveria et al., 2008). Under oxic conditions, organic compounds with simple structure such as polysaccharides followed by proteins and lipids are rapidly utilized by microbes (Harvey et al., 1995) whereas other compounds with high molecular weight, irregular structure, and aromatic structure are recalcitrant (Krull et al., 2003; Dodla et al., 2008). Since LOC can respond rapidly to changes in C supply, it has been considered to be important indicators of soil quality (Hanes, 2005; Zhang et al., 2006; Dodla et al., 2009). Soil LOC has a turnover time of less than few years as compared to recalcitrant carbon with a turnover time of several hundreds to thousands of years (Parton et al., 1987).

Different chemical and biological approaches have been used to estimate the amount of LOC in a given soil (McLauchlan and Hobbie, 2004). Among those, aerobically mineralizable carbon (AMC) by biological incubation has been extensively used (Alvarez and Alvarez, 2000; Robertson et al., 1999). This procedure has been designated as a reference method for LOC although different incubation times have been used (McLauchlan and Hobbie, 2004). Other methods, especially simple chemical procedures, such as water (cold/hot) extraction (Gregorich et al., 2003; Wang and Wang, 2007; Silveria et al., 2008), salt extraction (Fang et al., 2005; Jinbo et al., 2006) as well as permanganate digestion (Ettema et al., 1999; Weil et al., 2003; Zou et al., 2005) of SOM have also been proposed for determining the LOC fraction. In addition, acid hydrolysable C has been used to approximate both labile and slowly-degradable C consisting primarily of young SOM (Sollins et al., 1999; Paul et al., 2006; Ran et al., 2007). Since organic C in soil is stabilized by various physical and chemical mechanisms, the C extracted by various fractionation methods can differ in the amount and type of C extracted from a given soil. Several scientists have questioned whether chemical extractions of SOM represent fractions that clearly define turnover rates and contributions to nutrient cycling (Ladd et al., 1977; Cadisch et al., 1996; von Lützow et al., 2007). Others have also stated that organic matter in soil does not degrade as a single pool but rather as a composite of multiple rates among the various classes of organic matter (Harvey et al., 1995). Nonetheless, to date, the majority of previous characterizations on LOC were conducted on upland soils and very little work has been done on LOC characteristics in wetland soils, especially at coastal zones, where soils are constantly influenced by different environmental factors such as redox fluctuations and salt water intrusions. How these different LOC characterization methodologies are related for coastal wetland soils is not currently known. In addition, there has been little work conducted to identify the relationship between the organic C determined by the various techniques and the distribution of C functional groups in soil, which has important implications to transformation dynamics of SOC (Wang et al., 2011; Dodla and Wang, in press). Therefore, the nature of organic C determined by these methods has not been fully clarified (von Lützow et al., 2007).

The Mississippi River deltaic plain coastal wetlands sequester large amounts of organic C through vertical accretion (Nyman et al., 1990). However a significant portion of C previously sequestered in these soil profiles is lost through marsh deterioration (DeLaune and White, 2011). The wetland loss is primarily as a result of subsidence in the Mississippi River deltaic plain plus a similar rate of eustatic sea level rise. The dissolved organic matter (DOM) and sediment-associated particular

organic matter (POM) lost from marshes could be a significant source of organic C in fueling hypoxia in the northern Gulf of Mexico (Wilson and Allison, 2008; Bianchi et al., 2009, 2010). In order to survive the subsidence, these wetlands have to accrete soil organic matter at a similar rate to the rise in water level. Understanding how different LOC methodologies compare in these wetlands and how bulk organic matter functionality contributes to specific LOC pools would help to characterize the overall dynamics of SOC in these coastal wetlands. Therefore, the objectives of this study were to evaluate the relations among the LOC fractions in Mississippi River deltaic plain coastal wetland soils estimated by different methods and to compare amount of LOC to the slowly mineralizable C and the C pool resistant to degradation. In addition, the relationship between LOC by the different methods and C functional groups characterized by <sup>13</sup>C solid-state nuclear magnetic resonance (NMR) was also investigated.

## 2. Materials and methods

Soil samples across different wetland types including forested swamp (FS), freshwater marshes (FM), brackish marsh (BM) and salt marsh (SM) were collected along a salinity gradient from the Louisiana gulf coast Mississippi River deltaic plain (Fig. 1). These wetland sites were selected so that the profiles cover a range of organic C and C:N ratio that represented ecosystems with different vegetative types. The vegetation was dominated by a mixture of *Panicum hemitomon* and *Sagittaria lancifolia* in FM, *Taxodium distichum* in the FS, and *Spartina patens*, and *Spartina alterniflora* in BM and SM, respectively. The soils in these sites were dominated by Barbary muck (very-fine, smectitic, nonacid, hyperthermic Typic Hydraquents), Lafitte muck (Euic, Thermic Typic Medisaprists), Allemands muck (clayey, smectitic, Euic, hyperthermic, Terric Haplosaprists), and Timbalier muck (Euic, hyperthermic, Typic Haplosaprists), in FS, FM, BM and SM respectively. All these wetlands are seasonally flooded (≥ 6 months in a year) and mostly stay saturated throughout the remaining months. At each site, composite soils were collected from three to four depths and the sampling depth was based on horizon development in the profile. All the soils were thoroughly mixed in the laboratory, and distinguishable plant roots, stems and leaves were removed. Soil pH, electric conductivity (EC), bulk density, and LOC were determined using field-moist soil samples whereas other properties were determined using freeze-dried samples. Bulk density was measured by gravimetric method based on soil volume. Total C and N were determined by dry combustion at 900 °C using a TruSpec CN analyzer (LECO, St. Joseph, MI). All the soils contained negligible amount of CaCO<sub>3</sub> as determined by treating with 1 N HCl. Thus the total C determined in all the soils was treated as total soil organic carbon (SOC). Selected physical and chemical properties of these soils are presented in Table 1.

### 2.1. Estimation of labile organic carbon

Soil LOC was determined by four different methods namely 1) cold water extraction, 2) hot water extraction, 3) salt extraction, and 4) aerobic mineralization under incubation. Field moist soils were employed in these methods but the results were reported based on dry soil basis after correction for soil moisture. Cold water extractable carbon (CWEC) was determined using a procedure described by Bijay-Singh et al. (1988). Specifically, field moist soil samples of 10 g were first mixed with 30-mL deionized water in Erlenmeyer flasks and shaken for 30 min. After shaking, the mixtures were centrifuged, filtered through 0.45 µm filter, and the filtrates were collected for analyzing total organic C (TOC). For hot water extractable C (HWEC), the modified procedure outlined by Wang and Wang (2007) was used. Briefly, 10 g of field moist soil samples was mixed with 30 mL of deionized water in a 100-mL centrifuge tubes at 70 °C for 18 h. The mixtures were centrifuged and the supernatants were filtered through 0.45 µm filter for analysis of TOC. Salt-extractable carbon (SEC) was extracted using 0.5 M K<sub>2</sub>SO<sub>4</sub> in

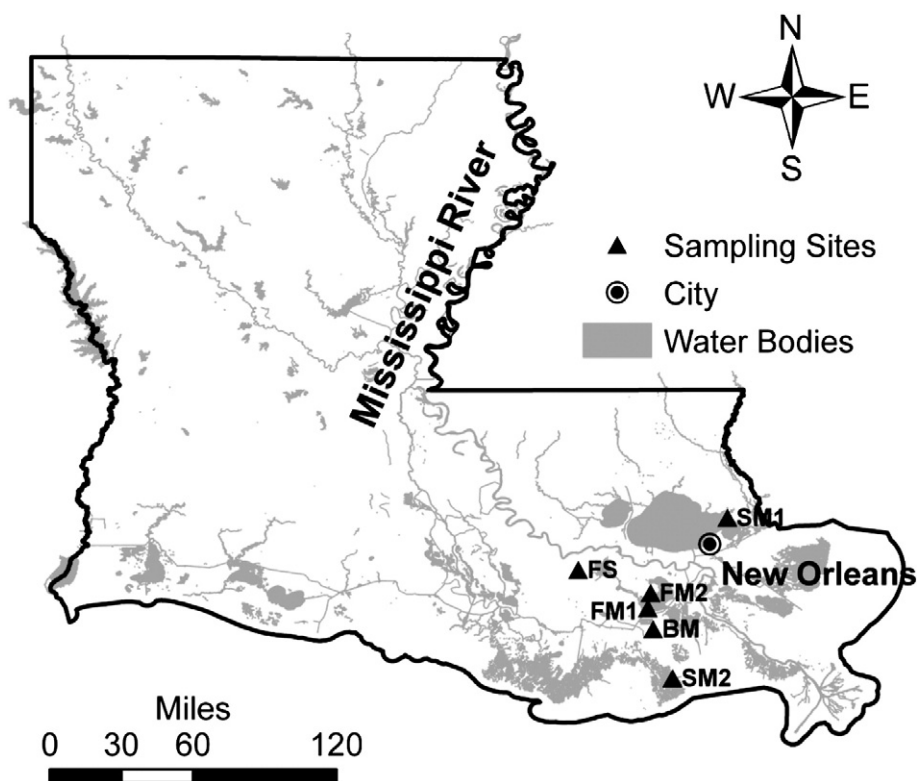


Fig. 1. Sample locations for this study.

Erlenmeyer flasks based on soil (moist): solution ratio of 1:3 (Fang et al., 2005) and shaken for 30 min. The mixtures were subsequently centrifuged, filtered through 0.45  $\mu\text{m}$  filters and analyzed for TOC. Total C in all the filtrates for the three extract methods were quantified using a Shimadzu TOC analyzer ( $V_{\text{CSH}}$  C analyzer, Shimadzu, Kyoto, Japan).

Aerobically mineralizable carbon (AMC) was determined by aerobically incubating 10 g of field moist soil sample in a 250-mL air tight glass jar that had a special septum for collecting gas sample using syringe. The headspace of each jar was flushed with compressed air and

the sample mixture was then incubated in dark for 12 days at room temperature ( $22.5 \pm 0.5$  °C). At the end of incubation, gas samples were collected from the headspace and analyzed for  $\text{CH}_4$  and  $\text{CO}_2$  using a CP3800 gas chromatography (GC) equipped with an FID detector (Varian Inc., Palo Alto, CA). The GC injector, column, methanizer and detector temperatures were maintained at 60 °C, 60 °C, 400 °C and 250 °C respectively. The amount of C mineralized (dry soil weight basis) was based on the total C released over 12 days (McLauchlan and Hobbie, 2004).

In addition to LOC estimate, acid hydrolysable carbon (AHC) was determined by mixing soil samples in digestion tubes with 6 N HCl based on 1:10 soil to solution ratio followed by refluxing for 16 h (Sollins et al., 1999; Cheng et al., 2007). The mixtures were then cooled to room temperature ( $22.5 \pm 0.5$  °C), and the residual soil from each tube was separated by vacuum-filtering using Whatman # 50 filter papers. The residual samples were then washed with de-ionized water and oven-dried at 80 °C. After drying, the residual samples were weighed and analyzed for total C by dry combustion using a TruSpec CN analyzer (LECO, St. Joseph, MI). Labile organic C was calculated by difference in total C between soil samples with and without acid hydrolysis on a dry soil basis.

## 2.2. Solid state nuclear magnetic resonance spectroscopy

All  $^{13}\text{C}$  solid-state nuclear magnetic resonance (NMR) spectra were collected using a Bruker Avance wide bore 400 MHz spectrometer based on a procedure described by Wang et al. (2011). Specifically, the resonance frequencies of  $^1\text{H}$  and  $^{13}\text{C}$  were 400 and 100 MHz, respectively. Cross polarization magic angle spinning (CPMAS) was used to acquire the  $^{13}\text{C}$  NMR spectra. Spinning side bands were eliminated using the total suppression of sidebands (TOSS). Prior to analysis soils were freeze dried, grounded and passed through a 250  $\mu\text{m}$  sieve. No prior HF treatment was conducted since the soils were low in paramagnetic elements. Samples were loaded into 4 mm zirconium rotors and analyzed using a double resonance CP-MAS probe. The spectra were

**Table 1**  
Physico-chemical properties of the selected wetland soils.

Site	Soil depth cm	pH	EC <sup>a</sup> dS m <sup>-1</sup>	BD <sup>b</sup> g cm <sup>-3</sup>	C g kg <sup>-1</sup>	N	C/N
FS	0–25	6.2	1.1	0.24	75.4	5.5	13.7
	25–50	6.1	1.3	0.44	56.3	3.7	15.2
	50–75	6.1	1.5	0.49	41.7	2.5	16.7
FM1	0–50	5.8	1.9	0.09	413	14.0	15.6
	50–100	6.2	1.4	0.11	311	13.2	14.3
	100–150	6.3	2.2	0.10	348	12.5	14.8
FM2	0–25	6.1	2.7	0.07	371	24.5	15.1
	25–50	6.4	3.5	0.19	212	11.8	17.9
	50–100	6.6	4.5	0.16	312	18.6	16.8
BM	100–150	6.6	4.4	0.12	317	21.2	14.9
	0–75	6.8	2.6	0.08	386	23.0	16.8
	75–125	7.2	4.8	0.07	437	28.0	15.6
SM1	125–150	7.4	6.1	0.08	387	24.0	16.1
	150–175	7.3	7.0	0.11	278	21.0	13.2
	0–50	7.4	8.3	0.25	143	8.0	17.9
SM 2	50–100	7.3	13.1	0.22	133	8.9	14.9
	100–150	7.4	13.0	0.49	72.3	5.8	12.5
	0–50	7.3	38.9	0.16	159	8.7	18.3
	50–75	7.4	42.5	0.20	119	6.7	17.7
	75–100	7.3	34.6	0.11	255	14.3	17.8
	100–150	7.9	42.3	0.76	12.1	0.80	15.1

<sup>a</sup> EC, electric conductivity.

<sup>b</sup> BD, bulk density.

collected at a spinning speed of 5 kHz, with 1 ms contact times, and 2 s recycling times. A total of 72,000 scans were collected for each sample. The  $^{13}\text{C}$  chemical shifts were referenced to tetra methyl silane and calibrated with the glycine carbonyl signal, set at 176.03 ppm. Within the 0 to 230 ppm chemical shift range, C atoms were assigned to various molecular groups in to aliphatic C (0–50 ppm), O/N-alkyl C including alcohols, amines, carbohydrates, ethers, methoxyl and acetal carbon (50–108 ppm), total aromatic C (108–165 ppm), carboxyl C (165–190 ppm) and carbonyl (190–230 ppm) (Kögel-Knabner, 1997; Silveria et al., 2008 Pedersen et al., 2011). All other assignments were assigned based on reference literature or calculated via ChemDraw Ultra 9.0 by CambridgeSoft. Carbon distribution was summarized based on spectral peak area for all C groups. Amount of each functional group C per unit soil was calculated based on their relative distribution and the total C in soil.

### 2.3. Statistical analysis

Data Analysis was conducted using SAS software (SAS 9.1, SAS Institute, Cary, NC) using *Proc Reg* procedure. Stepwise regression analysis was used in identifying the most significant factors. The significance level for all statistical analysis was at  $\alpha = 0.05$ .

## 3. Results and discussion

The physico-chemical characteristics of the wetland soils used in this study varied among sites and within the soil profile (Table 1). The amount of SOC was generally higher in FM and BM soils than SM and FS soils with the range from 12.1 g kg<sup>-1</sup> soil in the 100–150 cm layer of SM2 to 437 g kg<sup>-1</sup> in the 75–125 cm layer of BM soil. The C:N ratios ranged from 12.5 to 18.3 among the soils. The pH of the FS and FM soils was slightly acidic ranging from 5.8 to 6.6, whereas that of BM and SM soils was slightly alkaline ranging from 7.2 to 7.9 except for the top layer (0–75 cm) of the BM where the pH was 6.8. Overall, soil pH tended to increase toward the coast along with the increasing salinity gradient. The EC of soils ranged

from 1.1 dS m<sup>-1</sup> in FS soil profile to 42.5 dS m<sup>-1</sup> in SM2 soil profile. Bulk density of the soils was higher in FS and SM soils but lower in FM and BM soils and ranged from as low as 0.07 g cm<sup>-3</sup> in the FM soil to 0.76 g cm<sup>-3</sup> in the SM soil. These characteristics are similar to those commonly found for coastal wetland soils (Reddy and DeLaune, 2008).

### 3.1. Labile C estimates by different methods and inter-relationships

All LOC estimates determined by different methods for the studied wetland soils were significantly ( $p < 0.05$ ) related to SOC with  $R^2 \geq 0.72$  except for AMC, which had a slightly lower  $R^2$  value of 0.56 (Fig. 2). These positive correlations suggest that LOC fractions in these soils increased with increasing amounts of SOC, a relationship that was also reported for upland and forest soils (McLauchlan and Hobbie, 2004; Hanes, 2005; Wang and Wang, 2007). Because of the correlations of SOC with LOCs and large ranges of SOC distributions in these soils (Table 1), LOC fraction estimates as determined by different methods are presented as percentage of total SOC (Table 2).

Labile OC fractions differed among the sites and soil depths. Aerobically mineralizable C (after 12 d incubation), CWEC and SEC were generally <0.50% of SOC except for the bottom layer of SM2 where AMC, CWEC and SEC were 0.54%, 2.12% and 1.40% of SOC, respectively. On the other hand, SEC was less than CWEC (about 40–70% of CWEC). The lower SEC was likely due to the coagulation effect of smaller organic compounds under high salt concentration (Dou et al., 2008). In addition, there appeared to be a general trend in that the AMC fraction in the surface soil layers of these wetland sites was higher than subsurface layers except for SM2. Since the AMC fraction measures the directly available C to microbes, it was possible that the LOC in deeper layers might have been consumed due to the longer residence time of the substrate. On the other hand, presence of more active microorganisms in the surface soil layers than in the deeper layers of soil could have also led to higher AMC. As for higher AMC value observed for the 100 to 150 cm depth of SM2, it was likely that some LOC leached from the layers above accumulated at this depth as this layer was dominated with sediment containing low SOC (Table 1).

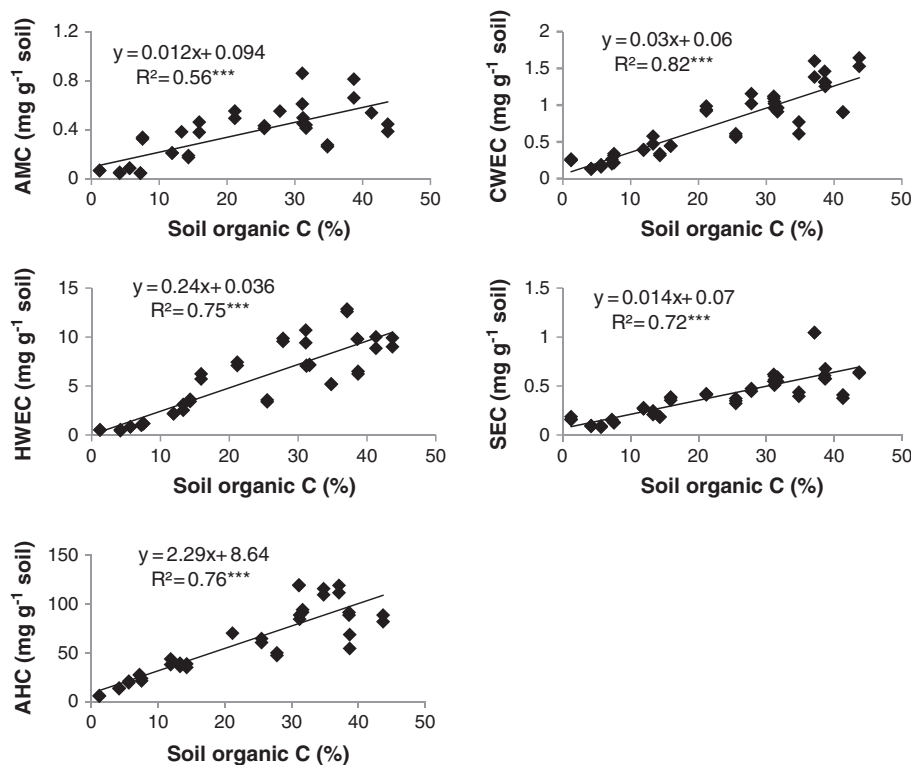


Fig. 2. Linear regressions between labile organic C pool by different methods and soil organic carbon. Three asterisks indicate statistical significance at 0.001 level.



**Table 2**

Labile organic carbon fractions estimated by different methods and acid hydrolysable carbon fraction. The values in the parenthesis indicate the standard deviation.

Site	Soil depth cm	AMC % C	CWEC	HWEC	SEC	AHC
FS	0–25	0.44(0.013)	0.38(0.093)	1.56(0.037)	0.17(0.002)	29.8(2.32)
	25–50	0.15(0.001)	0.31(0.036)	1.49(0.012)	0.15(0.010)	35.8(2.02)
	50–75	0.11(0.012)	0.32(0.003)	1.20(0.187)	0.22(0.013)	32.7(1.05)
FM1	0–50	0.13(0.025)	0.22(0.005)	2.29(0.198)	0.09(0.003)	50.0(2.60)
	50–100	0.18(0.044)	0.31(0.006)	3.24(0.293)	0.17(0.014)	29.4(0.10)
	100–150	0.07(0.003)	0.19(0.03)	1.49(0.010)	0.12(0.008)	32.2(1.14)
FM2	0–25	0.66(0.022)	0.4(0.042)	3.43(0.049)	0.28(0.005)	31.0(1.43)
	25–50	0.25(0.020)	0.45(0.022)	3.44(0.113)	0.20(0.003)	33.1(0.45)
	50–100	0.15(0.011)	0.32(0.020)	2.27(0.014)	0.18(0.021)	27.7(0.96)
BM	100–150	0.13(0.006)	0.3(0.012)	2.26(0.015)	0.18(0.010)	29.3(0.57)
	0–75	0.49(0.029)	0.34(0.026)	2.54(0.004)	0.15(0.005)	23.3(0.50)
	75–125	0.09(0.009)	0.35(0.018)	2.17(0.146)	0.14(0.002)	19.4(1.08)
SM1	125–150	0.18(0.027)	0.32(0.009)	2.51(0.049)	0.16(0.018)	15.9(2.58)
	150–175	0.19(0.076)	0.37(0.033)	2.29(0.078)	0.16(0.006)	17.4(0.67)
	0–50	0.13(0.008)	0.20(0.010)	2.18(0.106)	0.11(0.002)	25.6(1.87)
SM2	50–100	0.20(0.12)	0.34(0.046)	1.82(0.309)	0.16(0.016)	28.2(1.81)
	100–150	0.06(0.002)	0.30(0.056)	1.31(0.038)	0.20(0.008)	37.6(0.86)
	0–50	0.26(0.037)	0.28(0.004)	3.77(0.236)	0.24(0.003)	–
	50–75	0.18(0.002)	0.33(0.001)	1.84(0.035)	0.23(0.007)	34.2(3.40)
	75–100	0.17(0.073)	0.23(0.013)	1.37(0.053)	0.14(0.014)	24.5(1.10)
	100–150	0.54(0.022)	2.12(0.030)	4.34(0.133)	1.4(0.035)	49.0(3.66)

Between CWEC and HWEC, the latter was 4 to 13 times greater than the former and ranged from 1.2 to 4.3% of SOC (Table 2). These results were similar to approximately 0.5% CWEC fractions of SOC found for forest soils (Wang and Wang, 2007). The HWEC fractions were also close to those agricultural, pasture (Curtin et al., 2006) and forest soils (Wang and Wang, 2007). Our results suggest that these characterization methodologies quantify similar fractions of LOC as they do for upland soils.

In addition, these LOC estimates (on per unit of dry soil basis) by different methods were generally related (Table 3). The three extraction-oriented measurements of LOC (CWEC, HWEC and SEC) were strongly correlated among each other ( $R^2 = 0.79–0.84$ ) whereas the incubation-oriented AMC was generally related, to a less extent ( $R^2 = 0.62–0.69$ ), with the three extraction-oriented measurements of LOC (Table 3). The later results likely suggest that the AMC measures slightly different fractions of LOC in nature compared to CWEC, HWEC and SEC although all these methods yielded small quantities of LOC especially CWEC, SEC and AMC. On the other hand, the CWEC and SEC were highly correlated with each other ( $R^2 = 0.84$ ), indicating that both SEC and CWEC approximated similar type of wetland LOC. Also, the strong linear relationship between HWEC and CWEC ( $R^2 = 0.83$ ) for these wetland soils of different profiles, indicates a very close linkage between LOC values extracted by the two methods even though the HWEC was much greater than the CWEC (Table 2). Regression analyses showed that there was no significant relationship between pH, EC, bulk density, C:N ratio, and AMC, CWEC, HWEC, and SEC (data not shown).

The AHC fraction, which comprises both LOC and slowly mineralizable C pool, accounted for 16 to 49% of SOC in these wetland soils (Table 2). There was no specific trend or significant difference in AHC

in the soil profiles among the different wetland sites except for SM1 where AHC increased with soil depth. Previous studies had showed increase in AHC along depth in soil profiles of different land uses (Tan et al., 2004; Rovira and Vallejo, 2007). In this study, the lack of consistency and large variability in AHC in the majority of these wetland soil profiles could be due to the disturbance caused by tidal movement in past history. We did, however, observe a positive curve linear relationship between soil bulk density and AHC ( $y = 10.7x^2 + 24.3x + 22.9$ ,  $R^2 = 0.73$ ). The latter could reflect the physicochemical protection provided by soil mineral matter to the acid hydrolysable C compounds. The AHC fractions observed for these wetland soils were similar to those reported for upland soils (Xu et al., 1997; Paul et al., 2001). In addition, following acid hydrolysis, it was revealed that C/N ratios of these wetland soils almost doubled as compared to the initial values (13.7–17.9 before HCl hydrolysis vs. 20.3–35.9 after HCl hydrolysis). The increase in C/N ratio signified that the majority of N containing compounds were hydrolyzed by 6 N HCl (McLauchlan and Hobbie, 2004). Despite the relatively large difference in extraction quantity, the slowly releasing AHC (on per unit of dry soil basis) showed positive correlations ( $R^2 = 0.61–0.71$ ) with the LOC estimates determined by the three extraction methods, as well as with the AMC ( $R^2 = 0.47$ ) at a less degree (Table 3), suggesting the difference of AHC from the LOC fractions.

### 3.2. Relationship between LOC and carbon functional groups

The distribution of C functional groups relative to total SOC in these soils is presented in Table 4. In all cases, these soil profiles were dominated with O/N-alkyl C followed by approximately similar percentages of

**Table 3**Linear relationships between different LOC methods<sup>a</sup>.

X\Y	CWEC	HWEC	SEC	AHC
AMC	$y = 1.54x + 0.123$ $R^2 = 0.62^{***}$	$y = 12.36x + 0.41$ $R^2 = 0.69^{***}$	$y = 0.72x + 0.101$ $R^2 = 0.67^{***}$	$y = 107.5x + 20.1$ $R^2 = 0.47^{***}$
CWEC		$y = 7.41x - 0.021$ $R^2 = 0.83^{***}$	$y = 0.47x + 0.05$ $R^2 = 0.84^{***}$	$y = 58.2x + 16.3$ $R^2 = 0.61^{***}$
HWEC			$y = 14.1x - 0.063$ $R^2 = 0.79^{***}$	$y = 7.80x + 19.08$ $R^2 = 0.70^{***}$
SEC				$y = 120.3x + 12.4$ $R^2 = 0.71^{***}$

<sup>a</sup> Three asterisks indicate statistical significance at  $P < 0.001$ .

**Table 4**  
Relative distribution of C functional groups in different wetland soils.

Site	Soil depth cm	Alkyl %	O/N-alkyl	Aromatic	Carboxyl	Carbonyl
FS	0–25	25.56	38.16	22.78	10.08	3.43
	25–50	29.42	29.85	24.10	12.05	4.58
	50–75	25.38	32.31	28.20	9.59	4.53
	0–50	22.72	50.58	17.27	8.13	1.30
FM1	50–100	20.13	50.46	20.89	7.36	1.15
	100–150	21.42	47.04	22.07	7.64	1.83
	0–25	20.91	48.75	18.96	9.96	1.42
	25–50	27.84	31.77	23.83	12.22	4.34
FM2	50–100	26.10	36.90	24.94	10.80	1.26
	100–150	27.68	35.34	24.66	11.21	1.12
	0–75	19.37	47.75	21.86	9.66	1.36
	75–125	23.50	39.13	24.41	11.27	1.69
BM	125–150	24.82	36.39	25.96	11.13	1.70
	150–175	27.16	32.58	25.78	12.57	1.91
	0–50	26.87	43.51	21.09	7.51	1.01
	50–100	26.46	41.96	21.95	8.17	1.46
SM1	100–150	29.95	37.79	22.51	8.66	1.09
	0–50	20.53	40.49	23.09	11.05	4.83
	50–75	20.50	39.85	26.33	10.94	2.39
	75–100	31.63	32.22	23.85	9.42	2.88
SM2	100–150	—	—	—	—	—

alkyl C and aromatic C followed by carboxyl C and carbonyl C. Significant multi-variate relationships between these different relative C functional groups and relative LOC fractions determined by the various methods are given in Table 5. Aerobically mineralizable C, which represents the actual portion of labile C pool available to the microorganisms, showed positive correlations with O/N-alkyl C and carboxyl C but a negative correlation with alkyl C (Table 5). This result was consistent with the observation that polysaccharides are more labile than any other C moieties (Silveria et al., 2008). It also showed that carboxylic C, likely small molecular organic acids, contributed significantly to the AMC. The hydrophilic nature of carboxylic functional group makes carboxyl compounds more susceptible to microbial degradation (von Lützow et al., 2007). On the other hand, the negative effect of alkyl C on AMC suggests the less soluble but more recalcitrant nature of aliphatic C in these wetland soils. Aliphatic C has also been shown to be recalcitrant to biodegradation and often concentrated as a result of decomposition and humification of plant residues in upland soils (Baldock et al., 1997; Kögel-Knabner, 1997).

The three extractable LOC methods showed different relationships with molecular C moieties from the AMC (Table 5). Like the AMC, both CWEC and SEC fractions were positively affected by carboxyl C and negatively by alkyl C. Clearly the positive effect of carboxyl C on CWEC and SEC signifies a strong contribution of soluble carboxyl C compounds such as organic acids to the cold water and salt extractable C in these wetland soils. Previous studies have shown that the cold-water extractable organic C from upland forest and agricultural soils was composed mainly of hydrophilic fraction of C which includes carbohydrates, amino sugars, and low molecular weight organic acids (Herbert and Bertsch, 1995; Huang et al., 1998; Fröberg et al., 2003; Hanes, 2005). The CWEC from compost materials contained high molecular substance fulvic acids in addition to polysaccharides (Said-Pullicino et al., 2007). However in this study, the lack of a significant effect of O/N-alkyl C fractions on CWEC and SEC indicates that the contribution from O/N-alkyl C to the cold water and salt extractable LOC was less important in the SOM of these wetland soil profiles even though they are generally hydrophilic and present as the dominant molecular fraction (Table 4). This observation was different from those often observed for upland soils (von Lützow et al., 2007).

In addition, unlike the CWEC and SEC, the HWEC fraction showed a completely different relationship from the AMC by positively relating to % carbonyl C in the SOC, which was mainly ketonic and aldehyde compounds. This result was likely due to the ability of hot water to hydrolyze and cleave esters of various organic materials (Siskin and Katritzky, 1991; Stange et al., 2001). Previously the HWEC fraction from several upland soils including those from pasture and forest was found to be primarily composed of polysaccharides and N-containing compounds in soil solution (Ghani et al., 2003; Balaría et al., 2008). It also contained an additional C fraction that was weakly adsorbed to mineral surfaces and humic acids (Ghani et al., 2003; von Lützow et al., 2007). The decomposition data of HWEC from soil growing maize also showed that the amount of biodegradable SOC in the HWEC was greater than that in the CWEC (Gregorich et al., 2003). Because of generally much greater yield of LOC in the hot water extraction than the cold and salt extractions, the HWEC was suggested as a valuable and rapid indicator of LOC (von Lützow et al., 2007). We did not measure directly the degradability and molecular components of HWEC in this study. However, the LOC fraction in these wetland soils as extracted by hot water was clearly influenced by different molecular moieties as opposed to the AMC as well as the CWEC and SEC (Table 5) even though the HWEC (on per unit of dry soil basis) was reasonably correlated with the latter three (Table 3). This could imply that various molecular C moieties of SOC contribute to the HWEC fraction differently in these wetland soils as compared to those in upland soils.

The AHC, which measures both labile and slowly releasable C, was positively correlated with the O/N-alkyl C and alkyl C but negatively

**Table 5**

Statistically significant relationships between labile carbon and carbon functionalities for studied wetland soils.

Method		R <sup>2</sup>	p
AMC	= 0.017 (O/N-alkyl C) + 0.148 (carboxyl C) – 0.094 (alkyl C) + 2.29	0.38	0.048
CWEC	= 0.105 (carboxyl C) – 0.046 (alkyl C) + 3.34	0.45	0.006
HWEC	= 0.165 (carbonyl C) + 1.46	0.28	0.017
SEC	= 0.056 (carboxyl C) – 0.029 (alkyl C) + 2.10	0.40	0.017
AHC	= 2.719 (alkyl C) + 1.287 (O/N-alkyl C) – 6.1 (aromatic C) + 342.8	0.65	0.001

correlated with the aromatic C groups in SOC of these wetland soils (Table 5). This relationship clearly shows the difference of the AHC from the LOC fractions as measured by aerobic mineralization and the three extraction methods (CWEC, HWEC and SEC). Besides O/N-alkyl C, the positive contribution of alkyl C to the AHC in these wetland soils indicates different impact of HCl extraction from those reported for other soils. In a recent study of comparing residues of an upland soil and a wetland soil after 6 N HCl extractions, relative distribution of O/N-alkyl C was found to be significantly reduced in both soils but that of aromatic C was enriched in the wetland soil whereas that of aliphatic C distribution was enriched in the upland soil (Silveria et al., 2008). Other studies have also shown that the HCL extraction hydrolyzed nitrogenous compounds besides polysaccharides (Otto and Simpson, 2007) and non hydrolysable C fractions of various soils and plants following HCl extraction were mainly the materials consisting of aromatic and alkyl C groups such as lignin and wax (Ran et al., 2007; von Lützow et al., 2007). Our study, however, suggests that alkyl C is likely extracted by HCl hydrolysis from these gulf coast Mississippi River deltaic plain wetland soils.

It should be indicated that due to easy measurement of TOC and AHC, the difference between TOC and AHC, or non-hydrolysable C, has been suggested as a quick assessment of soil recalcitrant C even though the non-hydrolysable SOC could be changed by certain management techniques such as afforestation and cultivation and the acid extraction could not separate functional SOM pools since HCl hydrolysis releases organic C stabilized by different mechanisms (Paul et al., 2006; von Lützow et al., 2007). The negative contribution of aromatic C to the AHC as shown in this study appears to support the recalcitrant nature of non-hydrolysable C in these wetland soils. Based on this assessment, the non-hydrolysable C fraction ranged from 51 to 84% of total SOC for these wetland soil profiles, which was greater than 24 to 32% previously reported for the hardwood forest wetlands in Georgia (Silveria et al., 2008). This result suggests that the majority of SOC in these Louisiana Mississippi River deltaic plain wetlands would be sequestered into long term storage as a result of accretion process. However, the latter conclusion is purely based on the chemical evaluation of SOC of these wetlands as described without considering other environmental factors. Recent studies have indicated that significant transport of marsh OC, including both DOC and POC, to the coast especially at these marsh edges due to erosion from tidal/riverine flushing (Wilson and Allison, 2008; Bianchi et al., 2009). The input of the marsh OC to the Louisiana shelf from these sites could contribute as an important source of OC to fuel hypoxia in the northern Gulf of Mexico (Bianchi et al., 2010). Nonetheless the results from this study has important implications as to the understanding of the liability and refractory character of SOC in these wetlands since such properties closely affect actual contribution of marsh SOC to the oxygen consumption variability as it relates to hypoxia.

The relatively close correlation of CWEC (or SEC) with AMC and their similar relation to SOC functionality suggest that CWEC (or SEC) could be used for assessing the liability of wetland SOC and size of potential DOC. On the other hand, the use of AHC along with non-acid hydrolysable C (TOC minus AHC) may be used for assessing the

recalcitrant carbon of these wetland soils as well as potential liability of POC subjected to transport from marshes due to erosions.

#### 4. Conclusions

This study demonstrated that among four commonly used methods for estimating upland soil LOC, the three extraction-oriented measurements (CWEC, HWEC and SEC) were more closely related to each other ( $R^2 = 0.79–0.84$ ) than to the incubation-oriented AMC ( $R^2 = 0.62–0.69$ ) when employed for characterizing the Gulf coastal wetlands; a result was likely attributed to the associated difference in the contribution of different soil molecular organic C moieties to each method in these wetlands. Aerobically mineralizable C, the reference LOC method, showed positive correlations with O/N-alkyl C and carboxyl C but a negative correlation with alkyl C, whereas CWEC and SEC fractions only had positive correlation with carboxyl C and a negative correlation with alkyl C in SOC. On other hand, the HWEC fraction was 4 to 13 times of CWEC, and had a primarily positive contribution of carbonyl C in the SOC of these wetlands. Despite the relatively large difference in extraction quantity, the slowly releasing AHC was correlated with the LOC estimates by all four methods ( $R^2 = 0.47–0.71$ ). The AHC was positively influenced by the O/N-alkyl and alkyl C but negatively influenced by the aromatic C groups in SOC of these wetlands. Overall, various molecular C moieties of SOC contribute differently to the CWEC, SEC, HWEC as well as AHC fractions in relevance to AMC in these wetland soils as compared to those often observed in upland soils. Cold water extractable C and SEC did show the most similar influence of SOC functionalities as AMC as opposed to HWEC and AHC in these wetland soils, and could be used for the closest approximation of AMC for LOC. The LOC range represented 0.1–4.3% of total SOC whereas AHC accounted for 16–49% in these Mississippi River deltaic plain coastal wetlands. LOC fractions and AHC increased with increasing SOC regardless of wetland soil type or profile depth. The non-acid hydrolysable C (TOC minus AHC) may be used for assessing the potentially recalcitrant C in these wetland soils. The dominance of non-acid hydrolysable C suggests that the majority of SOC in these wetlands is relatively recalcitrant. While recent studies have suggested that these wetlands especially marshes, due to erosion from tidal/riverine flushing, could be a significant source of OC contributing to hypoxia in the northern Gulf of Mexico, our study implies that actual contribution of marsh SOC to the oxygen consumption may be variable depending on the liability and recalcitrance of the lost SOC.

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