Carbon sequestration in a surface flow constructed wetland after 12 years of swine wastewater treatment

Gudigopuram B. Reddy, Charles W. Raczkowski, Johnsely S. Cyrus and Ariel Szogi

ABSTRACT

Constructed wetlands used for the treatment of swine wastewater may potentially sequester significant amounts of carbon. In past studies, we evaluated the treatment efficiency of wastewater in a marsh–pond–marsh design wetland system. The functionality of this system was highly dependent on soil carbon content and organic matter turnover rate. To better understand system performance and carbon dynamics, we measured plant dry matter, decomposition rates and soil carbon fractions. Plant litter decomposition rate was 0.0052 g day^{-1} ($\pm$0.00119 g day^{-1}) with an estimated half-life of 133 days. The detritus layer accumulated over the soil surface had much more humin than other C fractions. In marsh areas, soil C extracted with NaOH had four to six times higher amounts of humic acid, fulvic acid and humin than soil C extracted by cold and hot water, HCl/HF, and Na pyruvate. In the pond area, humic acid, fulvic acid and humin content were two to four times lower than in the marsh area. More soil C and N was found in the marsh area than in the pond area. These wetlands proved to be large sinks for stable C forms.

Key words | carbon sequestration, constructed wetlands, swine wastewater

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INTRODUCTION

North Carolina ranks second in swine production after Iowa and the industry generates a high amount of waste. Swine producers currently flush the waste from swine houses into an anaerobic lagoon and spray the lagoon wastewater onto agricultural fields. Field applications have resulted in excessive soil nutrient accumulations, especially phosphorus (P), which can potentially run off, or leach below the root zone, and pollute surface waters or groundwater. In most situations, producers lack the acreage to apply manure nutrients to crops at recommended nutrient rates to avoid high nutrient accumulations. To find a solution to this problem, we evaluated the efficiency of an artificial wetland that treated wastewater from an anaerobic lagoon that received effluent from an indoor production facility (Reddy et al. 2001). Results showed 75% N, 45% P, and 50% chemical oxygen demand removal (Reddy et al. 2001; Poach et al. 2004). We concluded that crop recommended application rates could be achieved without soil nutrient accumulations when small to marginal production operations use these wetlands.

During the last five decades, atmospheric levels of CO_2 and CH_4 have increased and are believed to be contributing to climatic changes. This notion led to an interest in research that searches for ways to increase C sequestration in terrestrial systems to help reduce atmospheric levels. It is not known how much carbon can be sequestered in constructed wetland systems on a long-term basis. The cycling process of C in wetlands is complex. A few studies have focused on components of the wetland carbon cycle and key relationships have been developed. For example, biomass input versus resulting carbon sequestration rates have been reported (Whiting & Chanton 2001; Euliss et al. 2006; Kayranli et al. 2010). These studies have discovered that the amount of C sequestered depends on the rate and concentration of both exogenous C deposition (wastewaters) and endogenous C inputs (plant detritus and microphyte biomass). It also depends on the rate of biomass decomposition and of carbon emission. Also, the efficiency of wetlands is largely dependent on the amount of organic matter present. In addition, components within the wetland carbon cycle may serve as a C sequestration offset (Rosso & Stenstrom 2008); e.g., the slow organic matter decomposition in

wetlands is offset by the release of CO_2 from the living vegetation (Whiting & Chanton 2001; Kayranli et al. 2010).

The dynamics of C in wetlands varies greatly, being time dependent and affected by hydrology (Whiting & Chanton 2001; Kayranli et al. 2010), biomass input, and amount and concentration of wastewater (Euliss et al. 2006; Villamar et al. 2015). Carbon sequestration is evident by the amount of carbon compounds that wetlands accumulate and the type of C fraction. The complex soil organic carbon (SOC) pool is divided into labile and recalcitrant pools. Microbes readily use the labile pool while microbial consumption from the recalcitrant pool is very slow. The labile pool resident time could be months to decades, whereas the resident time for the recalcitrant pool could be hundreds of years (Cheng et al. 2007). Resident time depends on plant biomass productivity, rate of organic matter decomposition, climatic conditions, and input of sources other than plant material. Studies have found the amount of recalcitrant SOC to be less than the amount of labile SOC (Melillo et al. 2002). Fang et al. (2005) found about equal amounts of each, while Leifeld & Fuhrer (2005) found greater amounts of recalcitrant SOC.

Wetlands have aerobic and anaerobic conditions that may facilitate C accumulation. Therefore, understanding how wetlands operate as a carbon sink and the types of carbon fractions that dominate the wetland ecosystem will help improve wetland construction design and management for optimal carbon sequestration and wetland efficiency. The objectives of this study were to: (1) determine plant biomass decomposition rates, (2) estimate C sequestration potential, and (3) determine the distribution of C among the labile, intermediate, and recalcitrant fractions in the detritus layer and in the soil, of a constructed wetland that treated swine wastewater for 12 years.

MATERIALS AND METHODS

Study site

This experiment was conducted at the North Carolina A&T State University swine unit, Greensboro, NC, USA. The number of sows during the study period at the swine unit ranged between 200 and 250. The manure handling system consisted of a receiving pit followed by primary and secondary anaerobic lagoons. Six surface flow wetland cells, 40 m in length by 11 m in width, with a marsh–pond–marsh (MPM) arrangement (Figure 1), were constructed in 1995. The inlet and outlet marsh areas were 10 m long with a wastewater depth of 15 cm, and the pond area was 20 m long with a wastewater depth of 75 cm.
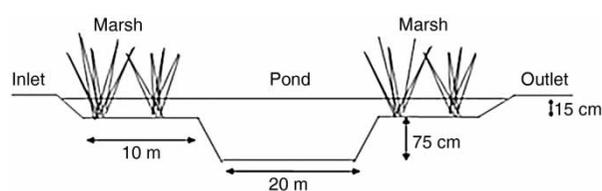

Figure 1 | Schematic showing the MPM design of constructed wetland cells used in the study.

In March 1996, the marsh sections were planted with a mix of cattails (Typha latifolia L.) and American bulrushes (Schoenoplectus americanus). To establish equilibrium in the wetland ecosystem, the cells were continuously loaded with wastewater having a nitrogen equivalent rate of 5 to 6 kg N ha^{-1} day^{-1} from March 1996 through April 1998 (Reddy et al. 2001). Loading consisted of pumping wastewater from either the primary or the secondary lagoon into an 80,000-l storage tank located uphill from the cells, and transported into cells by gravitational flow through an underground piping system. Over the years, the cattails predominated over the bulrushes and eventually the marsh areas had only cattails.

A study that began in May 1998 treated cells with swine wastewater at different daily N loading rates. Experimental methods and results are described in Reddy et al. (2001) and Poach et al. (2004). The varied loading rates were applied until April 2010, constituting a 12-year period of wastewater treatment. Loading each year was discontinued during mid-December through mid-April because of frequent freezing conditions and cattails being dormant during that period. In April 2010, the soil and detritus samples were taken for the C sequestration study.

Soil sampling

An auger having a diameter of 4.4 cm was used to collect soil samples from within each of the two marsh areas and from the pond area. Four samples collected at random within each of the three sampling areas were composited into one sample in each area (marsh inlet, pond, and marsh outlet). Sampling depths were the upper 4 cm layer of detritus, and soil depths of 0 to 6 cm and 6 to 12 cm. The wet detritus slush and soil samples were placed in polythene bags, transported in an ice chest to the laboratory, and stored in the refrigerator at 4 °C until being processed for chemical analysis. Plant debris was removed from all soil samples and then air-dried and crushed to pass through a 2 mm sieve. For bulk density, an Ehland sampler was used to collect undisturbed soil cores having a diameter of 7.5 cm. Three cores were collected from the marsh and the pond areas.

Soil analyses

Soil pH was determined using an Orion 3 Star pH meter and a 2:1 water:soil mix. Total carbon (TC) and total nitrogen (TN) were determined using a CHN analyzer (Perkin Elmer 2400 Series II model). Ammonium (NH_4) and nitrate (NO_3), extracted using a 2.0 M KCl solution, and orthophosphate (PO_4), were measured with a flow injection analyzer (Lachat Instruments QuikChem 8500 Series 2). Microbial biomass carbon (MBC) was determined using the procedure described by Vance et al. (1987). In general, field-moist soil that was chloroform ($CHCl_3$)-fumigated or un-fumigated was shaken for 30 minutes as a mix of 1:5 soil to 0.5 M K_2SO_4 extract solution. Fumigated soil was placed in a separate canning jar for microbial CO_2 production. Soil MBC was calculated as the quantity of CO_2 evolved following fumigation divided by an efficiency factor of 0.41 (Franzluebbers et al. 1999).

Soil organic matter fractionation

The sequential fractionation scheme illustrated in Figure 2 was used to extract SOC fractions from air-dried soil. Soil C was fractionated as (1) cold water (CW) and hot water (HW) extracted labile C, which is readily decomposable organic matter (Ghani et al. 2003) and rapidly responds to changes in C supply, (2) intermediate C (HCl/HF extracted), which is bound to soil minerals (Goh & Reid 1975), and (3) passive C, i.e., fulvic and humic acids extracted using Na pyruvate and NaOH. The residual C, the fourth fraction, was considered as humin C. These fractions were sequentially extracted in a five step process: (1) shaking 4 grams of air-dried soil in 30 ml of cold distilled water for 16 hours on an end-over-end shaker, and centrifugation to obtain the cold C fraction, (2) heating the residual soil in 30 ml of water at 80 °C for 16 h to remove the fraction extractable with hot water, (3) extracting the intermediate or slow carbon fractions containing hemicellulose and cellulose with a 1:1 hydrochloric/hydrofluoric acid solution, (4) extracting the resistant carbon fractions containing polyphenols and lignin with sodium pyrophosphate and sodium hydroxide, and (5) measuring the leftover C presumed to be humin C. The resistant fractions, specifically the humic and fulvic acids and the humin, were considered recalcitrant soil organic matter (SOM) fractions.

Plant decay

Cattail shoots were removed from a 0.5 m^2 area chosen at random within inlet and outlet marsh areas and weighed. Moisture content from subsamples was determined by
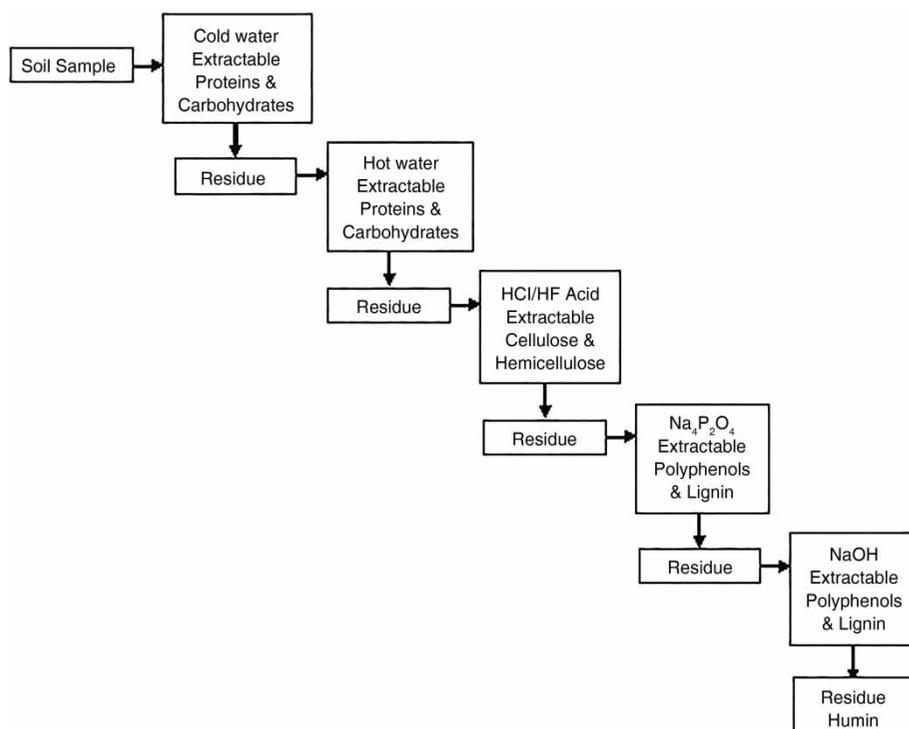

Figure 2 | Schematic of the SOM fractionation procedure used in the study (Murata et al. 1995; Murata & Goh 1997).

weighing, drying in a forced-air oven at 65 °C for 48 hours, and reweighing. The remaining fresh plant material was cut into lengths of 5 cm, and 100 g of this material (25 g dry weight equivalent) was placed inside nylon 1 mm mesh bags of size 30×30 cm. In the inlet and outlet marsh areas, three mesh bags were buried under the 4 cm detritus layer that had formed over the years. Mesh bag placement was on December 15, 2012. One bag was removed from each marsh area on April 4, July 17, and September 11, 2013. Immediately after mesh bag removal, contents were oven dried at 65 °C for 48 h and weighed. A subsample of the dried material was ashed at 500 °C in a muffle furnace to determine the soil content and adjust each sample to an organic matter content by weight (Aber et al. 1990).

Biomass decomposition rates were assumed to follow the first-order kinetic equation, which is given by:

$$\frac{dm}{dt} = -k \cdot m$$

The mass of the material at any given time is thus calculated by:

$$\ln(m_t) - \ln(m_0) = -k \cdot t \text{ or } \ln\left(\frac{m_t}{m_0}\right) - k \cdot t$$

where m_t is the mass (g) at time t (day), m_0 is the initial mass (g), k is degradation reaction constant (g day^{-1}), and t is time (day).

The time required for 50% plant litter mass to decompose is referred to as half-life (days) and is determined by:

$$\ln\left(\frac{m_t}{m_0}\right) = \ln\left(\frac{1}{2}\right) = -k \cdot \tau$$

$$\tau = \frac{0.693}{k} \text{ (days)}$$

RESULTS AND DISCUSSION

Soil properties

Soil pH values ranged from 6.7 to 7.7 and no significant differences were found between soil depths in marsh or pond areas (Table 1). The soil bulk density was higher in the pond area than in the marsh and it was due to the lack of vegetation in the pond area. The amount of NH_4^+, NO_3^-, and PO_4^- in the marsh areas was three to four times higher in the upper soil depth than in the lower depths, whereas, in the pond area, the nutrient concentrations did not differ much among the soil depths. The pond area was 75 cm deep and therefore anaerobic conditions prevailed in this area. Hence, nitrification did not occur as much as in the marsh area.

Plant decay

Cattails are known to contain approximately 28.7% cellulose, 23.4% hemicellulose, and 10.1% lignin (Suda et al. 2009). One of our previous studies showed that the plant decomposition function is supported by the abundance of heterotrophic bacteria (Dong & Reddy 2010); however, fungi that aid in the decomposition process may also prevail in this wetland.

Cattail decay in this study followed a linear trend with a daily decay of 0.27% and a 73% total decay during the 270-day period (Figure 3). Unexpectedly, the decomposition rate was constant at 0.27% daily despite differences in wetland temperature during the 270-day period. The coolest temperatures occurred during the first 110 days of late winter and early spring months. The temperature range during the first 110 days was 1.2 to 11.2 °C, and 5.6 to 17.2 °C during the 110- to 270-day period (Figure 4). The decay was higher after 120 days due to higher temperatures. The calculated average decay coefficient over the 270-day period was 0.0052 g d^{-1} with a standard deviation of 0.00119 g d^{-1}, and

Table 1 | Physical and chemical properties measured in two soil depths within marsh and pond areas after 12 years of swine wastewater treatment in a constructed wetland cell

Soil property	Marsh area				Pond area			
	0–6 cm		6–12 cm		0–6 cm		6–12 cm	
pH	6.69	(0.1)*	6.91	(0.1)	7.74	(0.1)	7.57	(0.1)
NH_4^+, mg kg^{-1}	18.6	(18.1)	3.6	(1.7)	42.8	(8.3)	56.4	(21.3)
NO_3^-, mg kg^{-1}	5.0	(4.6)	2.8	(2.3)	0.2	(0.2)	0.1	(0.1)
PO_4^{-2}, mg kg^{-1}	322.5	(76.4)	81.0	(46.1)	137.5	(53.4)	102.0	(17.8)
Bulk density, g cm^{-3}	1.32	(0.1)	1.33	(0.1)	1.73		1.74	

*Numbers in parentheses are standard errors.
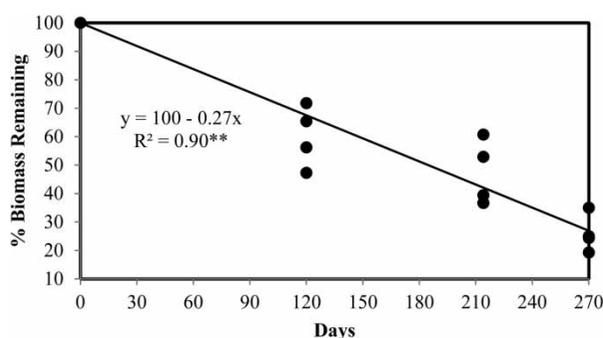
Figure 3 | Percent cattail shoot biomass remaining over time (days).

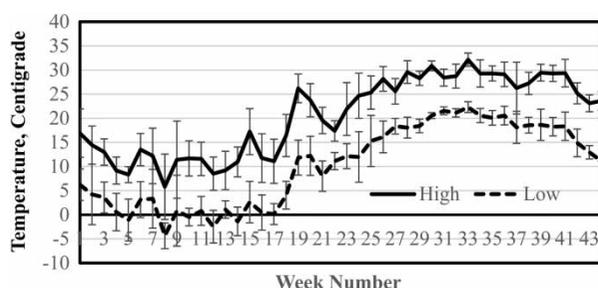$$y = 100 - 0.27x$$
$$R^2 = 0.90**$$

Figure 4 | Average weekly temperature during the December 2012 to September 2013 plant decay study period. Vertical bars are standard deviations.

the calculated half-life was 133 days. This decay rate is higher than a reported aerial decay rate for *T. latifolia* but similar to the submerged decay rate (Chimney & Pietro 2006). Alvarez & Becares (2006) found a similar decay co-efficient of 0.0043 g d^{-1} for *T. latifolia*. The cattail decay rate that we obtained was much lower than those of studies cited by Ruppel *et al.* (2004).

Soil and plant C and N

Wastewater was the major carbon source in the pond while in the marsh area C originated from the wastewater and the decomposing cattail biomass. Also, we reiterate that each year wetland loading was done during a 7-month period

due to freezing conditions and absence of cattail activity during the December through April months.

Soil TC, TN, and MBC results are shown in Table 2. Differences in TC occurred between soil depths and it was higher in the marsh area than in the pond area. Contents of TN and MBC were also higher in the marsh area but differences were not as large as with TC. In general, averaged across depths, TC content accumulated over 12 years was 21 Mg ha^{-1} (i.e., 1.75 Mg ha^{-1} yr^{-1}) in the marsh area and 12.1 Mg ha^{-1} (i.e., 1 Mg ha^{-1} yr^{-1}) in the pond area. TN contents in the marsh and pond areas were 2.0 Mg ha^{-1} and 1.7 Mg ha^{-1}, respectively, while the concentrations of MBC were 579.8 mg kg^{-1} and 445.7 mg kg^{-1}. The MBC was less than 2% of the TC in both wetland areas. Bernal & Mitsch (2008) reported a C accumulation of 2.42 Mg ha^{-1} yr^{-1} in created riverine wetlands. Also, Mitsch *et al.* (2012) reported a stable C accumulation of 2.19 and 2.69 Mg C ha^{-1} yr^{-1} for planted and non-planted wetlands, respectively. These values are consistent with ours, which were 1.75 Mg ha^{-1} yr^{-1} in marsh area and 1 Mg ha^{-1} yr^{-1} in the pond area, respectively, based on accumulations per year.

These C and N contents constitute end result estimates following a highly dynamic sequence of events during the 12-year period, involving major N cycle processes including immobilization and mineralization (Aber *et al.* 1990; Hunt *et al.* 2003), nitrification (Dong & Reddy 2010), denitrification (Hunt *et al.* 2006) and ammonia volatilization (Poach *et al.* 2004). The larger contents of these soil parameters: TC, TN, and MBC, in the marsh area were likely due to the cattail biomass added to the wetlands after they senesced in November each year.

Plant dry matter measurements (Table 3) showed that C content was slightly higher in the cattails from the wetland outlet than the inlet area. The wetlands are facilitated with gravity flow and the differences may be due to the bottom slope that could have caused a greater accumulation of water and nutrients in the outlet, resulting in a higher total nutrient accumulation in the harvested biomass. Maucieri *et al.* (2014) have also reported similar observations. Further,

Table 2 | TC, TN, and MBC in two soil depths within marsh and pond areas after 12 years of swine wastewater treatment in a constructed wetland cell

Soil property	Marsh area				Pond area			
	0–6 cm		6–12 cm		0–6 cm		6–12 cm	
TC, Mg ha^{-1}	25.1	(3.1)	16.8	(0.4)	14.5	(6.2)	9.7	(2.7)
TN, Mg ha^{-1}	2.5	(0.2)	1.6	(0.1)	1.8	(0.4)	1.6	(0.1)
MBC, mg kg^{-1}	606.9	(35.9)	552.8	(32.9)	476.2	(13.1)	415.2	(3.6)

*Numbers in parentheses are standard errors.

Table 3 | TC and TN accumulation in cattail plants collected in 2013 in swine wastewater inlet and outlet locations of a constructed wetland cell

| Location | $Mg\ ha^{-1}\ yr^{-1}$ | | | |
	Total N		Total C	
Inlet	5.1	(0.2)	0.2	(0.1)
Outlet	7.1	(1.5)	0.3	(0.1)
Averaged across depth	6.1	(0.85)	0.25	(0.1)

*Numbers in parentheses are standard errors.

high levels of nutrients entering the wetland system denote higher plant biomass resulting in higher nutrient accumulations in the vegetation (Lee et al. 2014). Averaging the data from inlet and outlet, cattails were observed to accumulate 6.1 Mg of TN and 0.25 Mg of TC $ha^{-1}\ yr^{-1}$ (Table 3). Maucieri et al. (2014) reported a dry matter organic carbon content of 3.9 Mg ha^{-1} in the aerial parts of common reed (Phragmites australis (Cav.)).

SOC fractionation

Swine wastewater contains many organic compounds that contribute to the formation of stable carbon structures. These include carbohydrates, aliphatic and aromatic hydrocarbons, proteins, N-acetyl amino sugars, lipids, and fulvic acid (Leenheer & Rostad 2004). The higher C content of the upper depth, and the lower C content in the pond area than in the marsh area, indicate that both swine wastewater and plant decomposing biomass and roots are significant C contributing sources. It is likely that C accumulation in lower depths is more related to plant root turnover. It is a known fact that high plant biomass, reducing soil conditions, and slow decomposition rates make wetlands effective C sinks. Clearly, a constructed wetland with a 7-month yearly loading period like the one used in this study will have a lower C accumulation than a continuous plant-based operated wetland. Hossler & Bouchard (2010) stated that poorly developed soil properties could be limiting soil C accumulation in constructed wetlands with variable hydrology. However, our study shows that when vegetated marsh areas are included, plant decomposition occurs at a fairly rapid rate, significantly adding to the stable carbon pool. The carbon-rich detritus layer, which is a combination of plant litter and the organic matter received from the wastewater settling on the wetland surface, contributes to this enhanced carbon accumulation in the marsh areas.

SOC dynamics assessments require temporal chemical fractionation of SOM constituents. Our fractionation assesses the SOM status after 12 years of wetland function. This fractionation provides knowledge of the potential of this system to sequester carbon in stable forms. The organic matter fractionation of the 4 cm detritus layer is illustrated in Table 4.

The highest amount of C was found as humin C. Needless to say, this is the most stable and recalcitrant form of C. Other C fractions were substantially lower in content ($<5\ mg\ g^{-1}$). Basically, all the labile organic matters of the detritus layer of these wetlands decompose rapidly and a significant amount of humin accumulates. In both soil depths of the marsh area, the highest content of carbon was found as soluble fulvic and insoluble humic acids (Figure 5).

The soil humin C content was two-fold lower than the NaOH extracted C. The SOC extracted with CW, HW, HCl-HF, and sodium pyruvate ($Na_4P_2O_4$) was less than 500 mg kg^{-1} and represents less than 10% of total C. Humic and fulvic acids extracted with NaOH were 16 to 20 times higher than humic and fulvic acids extracted with sodium pyruvate. Evidently, most SOM was at a high level of decomposition and C was mostly present as humin C. Marsh area Eh values ranged from -143 to $+250$ mV during the wastewater loading period, and during the non-loading winter period Eh values were <300 mV; i.e., redox potential was within the range where organic matter decomposition occurs.

The organic matter fractionation for the pond area differed from that in the marsh area (Figure 6). The Na pyruvate extracted C was five times lower in the pond area than marsh. Humin content was also much lower in the pond area.

In general, the lower content of soil C compared to the marsh area was in large part due to the absence of vegetation in the pond area. Therefore, most of the C that accumulated in the pond area soil was from the swine wastewater, a source of organic matter that lacks compounds containing significant lignin content. The pond C extracted with NaOH likely originated from fulvic acid, aliphatic hydrocarbons, aromatic hydrocarbons, proteins, and lipids,

Table 4 | Carbon fractionation in detritus layer of wetland marsh areas

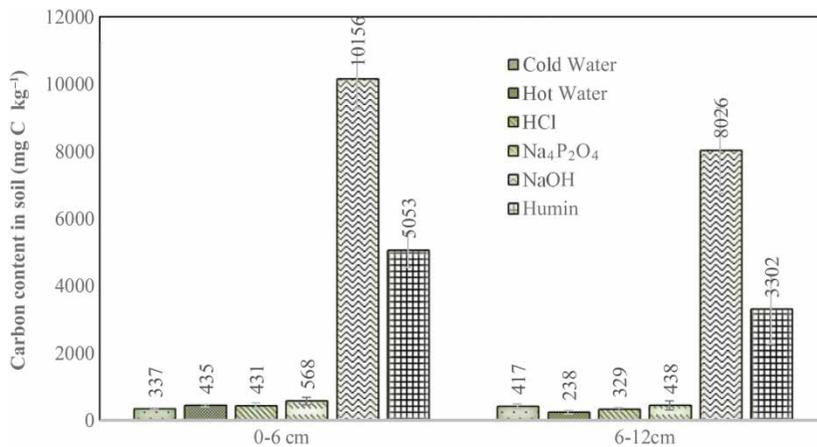| Fractions	Carbon content ($mg\ kg^{-1}$)	
CW	414.74	(40.47)
HW	2,554.01	(770.46)
HCl	1,143.85	(290.04)
$Na_4P_2O_4$	5,364.43	(487.80)
NaOH	37.22	(33.66)
Humin	65,101.03	(17,622.55)

*Numbers in parentheses are standard errors.

Figure 5 │ Carbon fractionations in two soil depths of wetland marsh areas. Mean values of carbon content (mg C/kg) are shown above each bar and vertical bars are standard errors.
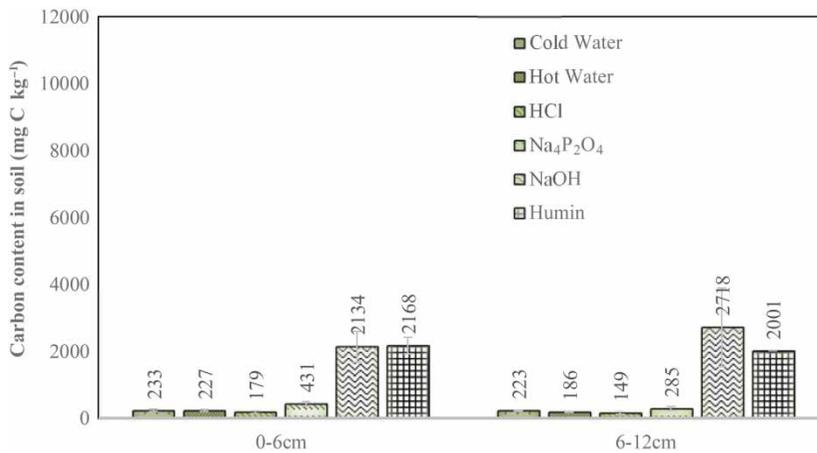

Figure 6 │ Carbon fractionations in two soil depths of pond area. Mean values of carbon content (mg C/kg) are shown above each bar and vertical bars are standard errors.

which are common in swine wastewater (Leenheer & Rostad 2004).

layer in continuous marsh wetlands can be used as a soil amendment for improving soil quality.

CONCLUSIONS

In conclusion, the labile C decomposes readily and humic substances accumulate in these wetlands. Our previous studies have shown that these MPM wetlands are efficient in removing nutrients, but less efficient than continuous marsh wetlands. We can also conclude based on this study, that continuous marsh wetlands would accumulate more stable forms of carbon than MPM wetlands. Dissolved organic C and MBC are important indicators of an active C pool and, although they constitute a small part of total C, the turnover rates are important to the cycling of nutrients in wetland soil. Being high in humin content, the detritus

ACKNOWLEDGEMENTS

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