ORIGINAL RESEARCH





Carbon Sequestration at a Forested Wetland Receiving Treated Municipal Effluent

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Abstract Secondarily treated municipal effluent has been discharged since 2006 into a 1439 ha cypress-tupelo forested wetland in coastal Louisiana. Changes in carbon stocks of trees and soils as well as emissions of methane and nitrous oxide were measured over a one-year period and compared to baseline conditions derived from the scientific literature. Methods and equations were applied from the American Carbon Registry (ACR) wetland carbon offset methodology 'Restoration of Degraded Deltaic Wetlands of the Mississippi Delta'. The cumulative carbon sequestered in the Project scenario was 4090 mt CO2e/y by trees and 13,752 mt CO2e/y by soils, while 32,982 mt CO2e/y of greenhouse gasses were emitted. The Baseline scenario sequestered 3790 mt CO2e/y by trees and 2435 mt CO2e/y by soils while emitting 70,870 mt CO2e/y in greenhouse gasses. The net difference between the Project and Baseline emissions was 11,617 mt CO2e/y if greenhouse gasses were omitted and 49,505 mt CO2e/y if greenhouse gasses were included. This study demonstrates the potential of using forested wetlands receiving treated municipal effluent for the net sequestration of carbon.

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Introduction

Recognition that recent global climate change and severe weather events have been exacerbated by human activities (Oreskes 2004; Emanuel 2005; IPCC 2013) has facilitated significant growth in emissions trading programs, collectively referred to as carbon markets, in order to stem emissions (Gillenwater et al. 2007). Projects that sequester carbon and reduce greenhouse gas emissions generate 'carbon offsets' that can be used to compensate for an emission made elsewhere (Murray et al. 2011). The carbon sequestered in vegetated coastal ecosystems, specifically mangrove forests, seagrass beds, and salt marshes, has been termed 'blue carbon' (Sifleet et al. 2011; Mcleod et al. 2011). In coastal Louisiana, blue carbon also refers to carbon sequestered in soils and trees of baldcypress (Taxodium distichum) and water tupelo (Nyssa aquatica) forested wetlands, as well as freshwater emergent, brackish and saltwater wetlands. Although their global area is one to two orders of magnitude smaller than that of terrestrial forests, the contribution of vegetated coastal habitats per unit area to long-term C sequestration is much greater, with an estimated 50% of the carbon in the atmosphere that becomes bound or 'sequestered' in natural systems being cycled into coastal areas and oceans (Nellemann et al. 2009; Mcleod et al. 2011).

Carbon finance has the potential to generate much needed revenue to support wetland restoration and conservation (Murray et al. 2011; Siikamäki et al. 2012; Mack et al. 2015). In 2012, the American Carbon Registry (ACR), a



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leading carbon market standard, certified the first wetland offset methodology, which provided potential for carbon market investment into wetland restoration projects (Mack et al. 2012). Deltaic wetlands are unique among coastal wetlands in that they provide relatively permanent geologic storage of carbon due to subsidence caused by the compaction of deltaic sediments, with burial rates as high as 17 mm y⁻¹ in the Mississippi River Delta (Shinkle and Dokka 2004; Tornqvist et al. 2008). Projects that increase vegetative productivity result in enhanced organic soil deposition, and geological subsidence of this organic soil results in carbon burial (Bridgham et al. 2006; Hansen and Nestlerode 2014). Critical research is needed to determine the real world viability of wetland carbon offset projects.

The majority of coastal forested wetlands in Louisiana are degrading (Chambers et al. 2005), mostly from the lack of seasonal inputs of freshwater, nutrients, and sediments from the Mississippi River (Shaffer et al. 2009b; Conner et al. 2014; Shaffer et al. 2016). Flood control levees built during the last two centuries have separated the Mississippi River from its floodplain, preventing seasonal flooding that would naturally occur (Kesel 1988, 1989; Mossa 1996; Day et al. 2007). This has caused saltwater intrusion, accretion deficits, and prolonged flooding of most of the remaining forested wetlands (Roberts 1997; Day et al. 2007; Shaffer et al. 2009a; Conner et al. 2014; Shaffer et al. 2016).

The use of natural forested wetlands to process and assimilate nutrients from treated municipal effluent has been used in Louisiana for over 50 years as a cost-effective means to improve overall regional water quality while providing freshwater and nutrients to hydrologically isolated and degrading wetlands (Day et al. 2004; Hunter et al. 2009a, 2009b; Shaffer et al. 2016). The nutrient component of municipal effluent increases wetland vegetative productivity (Rybczyk et al. 1996; Hesse et al. 1998; Lundberg 2008; Hunter et al. 2009b; Shaffer et al. 2015), and the freshwater component provides a buffer against saltwater intrusion events, especially during periods of drought, which are predicted to increase in frequency in the future due to global climate change (IPCC 2013).

The objective of this research was to demonstrate the potential of using forested wetlands receiving treated municipal effluent as wetland carbon offset projects. We did this using the methods and equations from an ACR certified carbon offset methodology (Mack et al. 2012).

Study Area

The Luling wastewater treatment facility is located in St. Charles Parish 30 km west of New Orleans (Fig. 1). The facility consists of a facultative oxidation pond with a chlorination and dechlorination disinfection system with an average

discharge of 6000 m³/d (1.6 MGD). Before 2006, the treatment plant discharged into Cousin Canal, which drains into Lake Cataouatche via the Louisiana Cypress Lumber Canal (Fig. 1).

The Luling Oxidation Pond needed to be upgraded for a variety of reasons, including population growth. St. Charles Parish initially considered re-routing the effluent from the oxidation pond through a large force-main to a larger conventional wastewater treatment plant located approximately 16 km away. Later, the Parish considered the more environmentally beneficial option was to discharge the treated effluent into an adjacent wetland property; however, additional funds to reimburse the landowner for the servitude were lacking. Therefore, to allow the facility to operate, the parties agreed that St. Charles Parish, on behalf of the landowner, would use its best efforts to pursue carbon offsets arising out of the discharge of treated effluent to compensate the landowner for the use of their land.

Starting in 2006, the treated municipal effluent was piped to an adjacent permanently flooded (20–50 cm) 1439 ha baldcypress-water tupelo dominated forested wetland (Fig. 2). Effluent is retained within the project boundaries by low-lying levees running along the northern, eastern, and western boundaries that prevent hydrological exchange with the surrounding landscape, except at the southern most extent of the project area where water freely flows out of the project area into the Louisiana Cypress Lumber Canal (Fig. 1). The primary project area encompasses 1439 ha of mostly forested wetlands with exception of 93 ha of emergent wetlands at the southernmost boundary (Fig. 2).

Methods

In general, the amount of carbon sequestered that can be counted towards carbon offsets depends on the difference between the carbon sequestration rate under business-as-usual practices, referred to as the 'baseline scenario', and the carbon sequestration rate that results from a restoration activity or the 'project scenario' (Murray et al. 2007; Murray et al. 2011; Mack et al. 2015). For this study, the project scenario was the discharge of treated municipal effluent into the receiving wetland and the baseline scenario was the present status of the wetlands without effluent as derived from peer-reviewed literature of the area.

There are five general carbon storage pools in wetlands: aboveground trees; aboveground herbaceous vegetation; surface litter; dead wood; and belowground organic soil that includes all organic matter from belowground productivity and also some organic matter produced aboveground that is buried as detritus. The carbon pools included for this project include aboveground biomass of trees and soil organic carbon, as well



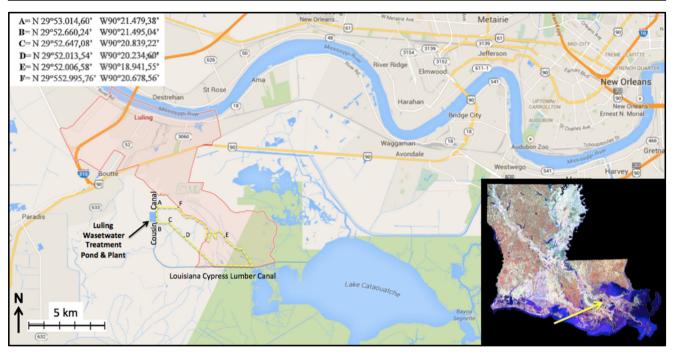


Fig. 1 The Luling wetland assimilation project (primary area delineated by the yellow dotted line; map source Google Maps). Letters indicate the locations where coordinates are given in the inserted box

as methane (CH_4) and nitrous oxide (N_2O) emissions. Nitrous oxide emissions have been included because N_2O is a potent GHG gas, 298 times as powerful as CO_2 , and measurement is required by all carbon accounting methods. Herbaceous vegetation, surface litter and dead wood were conservatively omitted since they were expected to either increase or not

change due to the project activity and also were expected to be incorporated into the soil organic carbon pool over the long-term and thus be counted.

This analysis used methods and equations described in the ACR wetland offset methodology 'Restoration of Degraded Deltaic Wetlands of the Mississippi Delta'

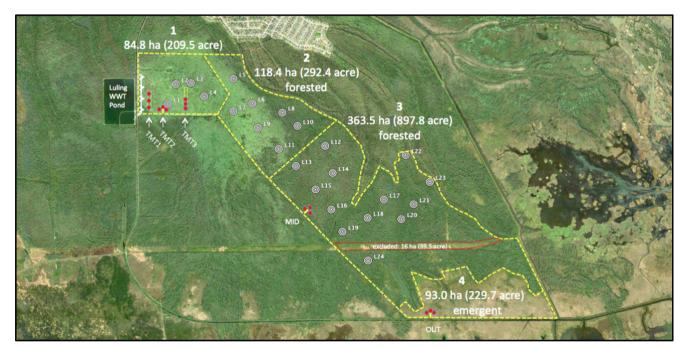


Fig. 2 Map of the wetlands receiving treated effluent (delineated by dashed yellow line; map source Google Earth). White circles and rectangular boxes indicate where tree biomass and soil accretion were

monitored. Red dots indicate where greenhouse gas sampling was carried out. The area has been delineated into four sections (1–4) based on hydrology and vegetation patterns



(Mack et al. 2012). This methodology applies to a wide range of restoration techniques including hydrologic management techniques that introduce freshwater, nutrients, and/or sediments to increase wetland productivity. All of the formulas given below were derived from the methodology modules BL-WR-HM, CP-S, CP-TB, and E-E. For all subsequent analyses, positive carbon fluxes indicate net fluxes into the wetland, whereas negative carbon fluxes indicate net fluxes to the atmosphere (Bridgham et al. 2006).

Project Scenario

A total of twenty-four 0.03 ha plots were delineated in a grid fashion across the project area where accretion and tree growth measurements were taken (Fig. 2). Six additional 0.04 ha plots were also used that had been delineated previously to meet state monitoring requirements. The number of plots was determined by application of the T-PLOTS tool, with a desired confidence interval of 90% and variance derived from past monitoring data of the assimilation wetland. Greenhouse gas sampling was carried out bi-monthly with three replicates at the TMT1, TMT2, TMT3, Mid, and Out (Fig. 2). Soil samples for bulk density and % carbon analysis were taken at the TMT1, TMT2, TMT3, and Mid sites. The project area was delineated into four sections based on hydrology and vegetation patterns to reduce within site variability and increase statistical power (Fig. 2).

Project Carbon Stock Change of Living Trees

The mean carbon stock of aboveground tree biomass was estimated based on field measurements of all trees in 30 fixed area plots using allometric equations (Fig. 2; Scott et al. 1985; Megonigal et al. 1997). The allometric equations express aboveground tree biomass as a function of diameter at breast height (dbh, ~1.3 m). The dbh of all trees in each plot were measured above and below (~5 cm) a metal identification tag in May 2013 and March 2014. The project tree carbon stock for the project area was calculated using eq. 1.

$$C_{\text{TREE}_{p}} = \sum_{i=1}^{n} \frac{44}{12} \left(F_{j}(DBH, H) * CF_{j} * (1 + R_{j}) \right)$$
 (1)

where:

 $C_{TREE,p}$ Carbon stock of living trees in sampling plot p;

mt CO2e

Ratio of molecular weight of CO2 to carbon;

dimensionless

 $F_i(DBH,H)$ Allometric equation for species j linking DBH

to aboveground biomass; ton d.m.

 CF_i Carbon fraction of biomass; dimensionless



 R_j Root-shoot ratio for tree species or group of species j; dimensionless - set to zero j 1, 2, 3, ... n tree species or group of species

These data were then applied to eq. 2 to calculate values of C_{TREEt1} and C_{TREEt2} for the start and end of the study. The carbon stock was calculated as the difference between the two years $(C_{TREEt2} - C_{TREEt1})$.

$$C_{\text{TREE}} = \frac{A_t}{A_p} \left(\sum_{p=1}^n C_{\text{TREE}_p} \right)$$
 (2)

where:

 C_{TREE} Carbon stock of living trees in the project area; mt

CO2e

 A_t Total project area; ha

 A_p Total area of sample plots; ha

p 1, 2, 3, ... n sample plots

Bulk Density

Bulk density samples were collected in triplicate at the TMT1, TMT2, TMT3 and Mid sites (Fig. 2). The top several cm of substrate was collected using a thin walled aluminum coring tube, brought to the laboratory, dried at 105 °C to a constant weight, and bulk density determined based on the weight and volume of the sample (Brady and Weil 2001; NRCS 2011).

Soil Carbon Fraction

The soil carbon fraction was determined using subsamples from the bulk density samples described above. The subsamples were weighed and analyzed for percent organic carbon by dry combustion (i.e., loss on ignition (LOI)) using a Neytech 85 M controlled-temperature furnace as detailed in NRCS (2011). Additional samples were analyzed for % carbon using a Vario EL Cube model elemental analyzer by Elementar (Chatterjee et al. 2009), as well as bulk density using the methods described above (Brady and Weil 2001; NRCS 2011).

Project Carbon Stock Change of Wetland Soils

Feldspar markers were put in place at 24 of the tree biomass plots when the trees were initially measured (DeLaune et al. 1983; Cahoon and Turner 1989; Conner and Day 1991). The thickness of material accumulated above the feldspar marker horizons was measured ten months later using a thin walled aluminum coring tube. The rate of vertical accretion was calculated by dividing the mean thickness of material above the

surface of the horizon by the amount of time the horizon had been in place (10 months) extrapolated to a yearly value. The carbon stock change of wetland soils was calculated using eq. 3.

$$\Delta C_{SOC} = \frac{44}{12} \sum_{i=1}^{n} \left(CF_{SOC_sample_{i,t}} *BD_{i,t} *Depth_{i,t} *Area_{i,t} *0.01 \right)$$
(3)

where:

 ΔC_{SOC} Cumulative soil carbon stock changes since start of project activities; mt CO2e 44/12 Ratio of molecular weight of CO₂ to carbon; dimensionless Carbon fraction of the sample; g C g⁻¹ d.m. CF_{SOC} sample,i Bulk density of soils; g cm⁻³ BD_i Depth to feldspar marker; cm Depth; Project area; m² Area; 0.01 Multiplier to convert units into ton C i 1, 2, 3, ... n strata in the project scenario – set

to 1 for this study

Project Emissions

Methane (CH₄) and nitrous oxide (N₂O) emissions were measured using the static chamber method (Smith et al. 1982; 1983a, b; Klinger et al. 1994; Livingston and Hutchinson 1995) at five locations in the study area; TMT1, TMT2, TMT3, Mid, and Out (Fig. 2). Gas chambers consisted of an inverted 5-gal bucket attached on top of a floating Styrofoam ring. A rubber septum inserted on the top of the chamber was used as a sampling port. Twenty-cm³ gas samples were taken as soon as chambers were placed on the water surface (T = 0), 30 min, and 60 min. These time intervals were selected based on preliminary results to optimize detection of GHGs. Gas samples were injected into pre-vacuumed 10-cm³ vacutainers and brought back to the laboratory for CH₄ and N₂O analysis using a gas chromatograph (e.g., Varian 3800) equipped with a dual Flame Ionization-Thermionic Specific (FID/TCD) system and an electron capture detector (ECD). Mean hourly rates were calculated as averages of the data collected, and total emissions were calculated by extrapolating the mean hourly rates to the respective time periods between sampling.

Greenhouse gas emission data were converted to CO₂ equivalents (CO2e) based on the 100-year Global Warming Potential (GWP) factors listed in the IPCC Fourth Assessment Report (IPCC 2007). Factors used were 25 for CH₄ and 298 for N₂O. These scaling factors represent the global warming

potential for CH_4 and N_2O over a 100-year time horizon. These greenhouse gas emission data were then applied to eq. 4.

$$f GHG_{E,t} = \sum_{i=1}^{n} f GHG_{CH_{4_i,t}} *GWP_{CH4} + \sum_{i=1}^{n} f GHG_{N_{2}O_i,t} *GWP_{N_{2}O}$$
(4)

where:

 $fGHG_{E,t}$ Rate of GHG emissions from the project area at monitoring event t; mt CO2e hr. Rate of CH_{4} emissions from stratum i at $GHG_{CH4\ i,t}$ monitoring event t; mt CO2e hr. Global warming potential for CH_4 (= 25 per GWP_{CH4} ACR Standard); mt CO2e (t CH_4)⁻¹ Rate of N_2O emissions from stratum i at $GHG_{N2O i,t}$ monitoring event t; mt CO2e hr. $^{-1}$ GWP_{N20} Global warming potential for N_2O (= 298 per ACR Standard); mt CO2e (t N_2O)⁻¹ 1, 2, 3, ... n strata in the project scenario $1, 2, 3, \dots n$ monitoring event

These results were then applied to eq. 5 to estimate the GHG emissions from the project area due to project activities.

$$\Delta GHG_E = \left(\frac{1}{n} \sum_{t=1}^{n} f GHG_{E,t}\right) *T_p *8766$$
(5)

where:

 ΔGHG_E Cumulative GHG emissions from the project area; mt CO2e T_p Time since start of project activities; yr 1,2,3, ...n monitoring event

Number of hours in a year

Baseline Scenario

A literature review was carried out to determine the baseline values for carbon sequestration from the forested wetlands in the Mississippi River delta plain (Table 1). These data were used as a reference of what would have occurred had the restoration activity not taken place. The review was assembled entirely from peer-reviewed literature. Summary statistics were carried out using JMP statistical software produced by SAS Institute, Inc. (Sall et al. 2012). The baseline parameters were applied to the amount of area of the various habitat types to determine baseline carbon sequestration.



Table 1 Baseline carbon sequestration derived from the scientific literature (metric tons CO2e/ha/y). s.e. = standard error

	Mean	Min	Max	s.e.	n	Source
Baseline Tree (Fresh - Forested):	6.7	1.5	11.9	0.99	10	Conner and Day (1976); Conner et al. (1981); Megonigal et al. (1997); Day et al. (2006); Hunter et al. (2009a, b); Shaffer et al. (2009b)
Baseline Soil (Fresh - Forested):	3.0	1.2	4.9	0.62	5	Craft and Casey 2000; Day et al. 2004; Noe & Hupp 2005; Lane et al. 2016
Baseline CH ₄ (Fresh - Forested):	-64.5	0.0	-228.0	36.80	6	Crozier and DeLaune 1996; Alford et al. (1997); Yu et al. (2008)
Baseline N ₂ O (Fresh - Forested):	-39.0	-1.5	-212.7	34.83	6	Lindau et al. 1994; Boustany et al. 1997; DeLaune et al. (1998); Lindau et al. (2008); Yu et al. (2008); Scaroni et al. (2011, 2014)
Baseline Soil (Fresh - Emergent):	7.9	2.2	11.4	0.74	8	Hatton et al. (1982, 1983); DeLaune and Smith (1984); Feijtel et al. (1985); Rybczyk et al. (2002); Nyman et al. (2006)
Baseline CH ₄ (Fresh - Emergent):	-131.2	-12.8	-251.0	32.36	6	DeLaune et al. (1983); DeLaune and Smith (1984); Feijtel et al. (1985); Crozier and DeLaune (1996)
Baseline N ₂ O (Fresh - Emergent):	-0.2	-1.0	-0.5	0.25	4	Smith et al. (1983a, b); DeLaune et al. (1989)

Results

Project Scenario

Project Carbon Stock Change of Living Trees

706 trees were measured to determine biomass. The results were fed into eq. 1 to provide an estimate of the carbon stock of living trees for each plot (Table 2).

The cumulative carbon stock changes of trees was 972.5 mt CO2e for section 1, 1135.8 mt CO2e for section 2, and 1981.9 mt CO2e for section 3 (Table 3; Fig. 2). The cumulative amount of carbon sequestered by trees in the project area was 4090.1 mt CO2e/y.

Bulk Density & Soil Carbon Fraction

Bulk density ranged from 0.12 to 0.19 g/cm³, with an average of 0.14 \pm 0.01 g/cm³ at site TMT1, 0.17 \pm 0.01 g/cm³ at site TMT2, 0.18 \pm 0.01 g/cm³ at site TMT3, and 0.13 \pm 0.003 g/cm³ at the Mid site. There was not a statistically significant difference in the bulk density of the soil between sites. The average for all sites combined was 0.16 \pm 0.01 g/cm³.

Percent loss on ignition (%LOI) ranged from 51.6 to 81.1%. Assuming and average carbon content of 50% for the %LOI fraction, percent carbon ranged from 25.8 to 40.5%, with an average of $36.3 \pm 0.02\%$ at TMT1, $33.3 \pm 0.04\%$ at TMT2, $30.0 \pm 0.03\%$ at TMT3, and $34.5 \pm 0.02\%$ at the Mid site. There was not a statistically significant difference in the %LOI of the soil between sites. The overall average was $33.6 \pm 0.01\%$.

The % carbon results from the autoanalyzer were $36.2 \pm 2.27\%$, which was very similar to the %LOI results of $33.6 \pm 0.01\%$, and the bulk density values from the two sampling efforts were also very similar, 0.16 ± 0.01 vs.

Table 2 Changes in carbon stock of living trees in sampling plots $(C_{TREE,p})$

Site	n	$C_{TREE,p1}$ mt CO2e	$C_{TREE,p2}$ mt CO2e	Plot Size ha	Section
TMT3	26	15.93	16.52	0.04	1
TMT3	14	9.84	10.14	0.04	1
TMT3	22	11.11	11.59	0.04	1
TMT1	20	16.91	17.40	0.04	1
TMT1	24	17.97	18.56	0.04	1
TMT1	17	17.31	17.64	0.04	1
L1	23	11.99	12.39	0.0333	1
L2	20	20.45	20.87	0.0333	1
L3	21	21.41	21.77	0.0333	1
L4	19	14.60	14.93	0.0333	1
L5	30	14.93	15.20	0.0333	2
L6	21	12.29	12.61	0.0333	2
L7	27	12.02	12.30	0.0333	2
L8	17	13.30	13.57	0.0333	2
L9	32	15.82	16.16	0.0333	2
L10	47	20.74	21.10	0.0333	2
L11	19	15.86	16.24	0.0333	2
L12	13	16.30	16.47	0.0333	3
L13	30	6.58	6.72	0.0333	3
L14	15	9.49	9.60	0.0333	3
L15	30	14.42	14.74	0.0333	3
L16	23	4.01	4.20	0.0333	3
L17	24	21.69	22.02	0.0333	3
L18	39	8.14	8.43	0.0333	3
L19	19	7.15	7.36	0.0333	3
L20	24	3.77	3.83	0.0333	3
L21	19	5.79	5.89	0.0333	3
L22	16	17.54	17.71	0.0333	3
L23	36	10.00	10.09	0.0333	3
L24	19	11.36	11.56	0.0333	3



Table 3 Accretion data used to estimate soil carbon stock (ΔC_{SOC})

Site	Depth cm	CF	BD	C_{SOC} kg CO2e/m ² /y	
L2	0.74	0.362	0.143	1.41	
L3	1.20	0.362	0.143	2.28	
L4	0.70	0.362	0.143	1.33	
L5	1.87	0.362	0.143	3.56	
L6	0.78	0.362	0.143	1.48	
L7	0.26	0.362	0.143	0.49	
L8	1.56	0.362	0.143	2.97	
L9	0.67	0.362	0.143	1.27	
L10	0.62	0.362	0.143	1.18	
L11	0.41	0.362	0.143	0.78	
L12	2.16	0.362	0.143	4.11	
L13	0.89	0.362	0.143	1.69	
L14	1.06	0.362	0.143	2.02	
L15	0.74	0.362	0.143	1.41	
L16	0.31	0.362	0.143	0.59	
L17	2.09	0.362	0.143	3.97	
L18	2.04	0.362	0.143	3.88	
L19	0.34	0.362	0.143	0.65	
L20	1.44	0.362	0.143	2.74	
L21	1.08	0.362	0.143	2.05	
L22	1.13	0.362	0.143	2.15	
L23	2.04	0.362	0.143	3.88	ΔC_{SOC}
L24	1.08	0.362	0.143	2.05	(mt CO2e/y)
				2.09 ± 0.24	13,752

 0.13 ± 0.01 g/cm³, respectively. For this analysis we used the % carbon results from the elemental analyzer, since it is presumed to be more accurate than the %LOI method, and we used an average of both sets of bulk density measurements (0.14 g/cm^3) .

Project Carbon Stock Change of Wetland Soils

Soil accretion ranged from 0.26 to 2.16 cm/yr. with a mean value of 1.09 ± 0.13 cm/yr. (Table 4). Carbon stock at the individual plots ranged from 0.49 to 4.11 kg CO2e/m²/y with a mean of 2.09 ± 0.24 kg CO2e/m²/y Extrapolated to the project area, this mean value provided a total soil carbon sequestration rate of 13,752 mt CO2e/y (Table 3).

Project Emissions

Methane emissions at TMT1 ranged from 2.2 to 272.3 mg/m 2 /h with a mean of 71.1 ± 41.4 mg/m 2 /h. Emissions decreased at TMT2 to 48.3 ± 27.3 mg/m 2 /h with a range of 1.74 to 177.2 mg/m 2 /h, but increased at TMT3 to 108.2 ± 42.4 mg/m 2 /h with a range of 7.4 to 286.3 mg/m 2 /h (Table 4). Methane emissions at the Mid site ranged from 1.49 to 28.38 mg/m 2 /h

with a mean of 9.0 ± 4.4 mg/m²/h, while at the Out site emissions ranged from below detection to 124.8 mg/m²/h with a mean of 54.6 ± 22.9 mg/m²/h. Extrapolation of these measurements to the entire study area indicates that total CH₄ emissions were 185.5 g CH₄/m²/yr.

Nitrous oxide (N₂O) emissions at TMT1 had a mean of $0.0987 \pm 0.0498 \text{ mg/m}^2/\text{h}$ and ranged from 0.0115 to $0.3416 \text{ mg/m}^2/\text{h}$ (Table 4). There was a decrease in N₂O emissions at TMT2, which ranged from below detection to $0.1921 \text{ mg/m}^2/\text{h}$ with a mean of $0.0542 \pm 0.0303 \text{ mg/m}^2/\text{h}$, followed by another decrease at TMT3, which had a mean of $0.0201 \pm 0.0122 \text{ mg/m}^2/\text{h}$ and ranged from below detection to $0.0790 \text{ mg/m}^2/\text{h}$. Nitrous oxide emissions at the Mid site were higher than at TMT1 with a mean of $0.0989 \pm 0.0961 \text{ mg/m}^2/\text{h}$ and a range of below detection to $0.5794 \text{ mg/m}^2/\text{h}$. Emissions at the Out site ranged from below detection to $0.0169 \text{ mg/m}^2/\text{h}$ with a mean of $0.0058 \pm 0.0029 \text{ mg/m}^2/\text{h}$. Extrapolation of these measurements to the entire study area indicates that total N₂O emissions were $0.76 \text{ g N}_2\text{O/m}^2/\text{yr}$.

The CH₄ and N₂O emissions described above were applied to eq. 4 to derive the rate of GHG emissions from the project area during each sampling event (f GHG_{E,t}; Table 4). These results were then applied to eq. 5 to determine the total emissions from the project area during the study period of -32,982 mt CO2e (Δ GHG_E; Table 4).

Project Carbon Stocks

The carbon stock changes and greenhouse gas emissions rates given above were used calculate net greenhouse gas emission reductions for the project of –15,140.13 mt CO2e/y if greenhouse gasses were included and 17,842 mt CO2e/y if they were omitted (Table 5).

Baseline Scenario

The baseline carbon stock change of the living trees was estimated to be 6.7 mt CO2e/ha/y (Table 1), which multiplied by the area of forested wetlands in the project area (566.7 ha) provided an estimate of the baseline carbon stock change for the project area of 3790 mt CO2e/y (Table 5). The baseline carbon stock change of wetland soils was estimated to 3.0 mt CO2e/ha/y at the freshwater forested wetlands and 7.9 mt CO2e/ha/y at the freshwater emergent wetlands (Table 1). These values were multiplied by the area of forested (566.7 ha) and emergent wetlands (93.0 ha), respectively, and summed to provide a baseline carbon stock change for the soils in the project area of 2435 mt CO2e/y (Table 5). The baseline CH₄ emissions were estimated to be -64.5 mt CO2e/ha/y at the freshwater forested wetlands and -131.2 mt CO2e/ha/y at the freshwater emergent wetlands (Table 1). Baseline N_2O emissions were estimated to be -39.0 mt CO2e/ha/y at the freshwater forested wetlands and -0.2 mt



Table 4 GHG emissions from the project area on dates sampled

Site	Date	CH ₄ Flux mg/m ² /h	N ₂ O Flux mg/m ² /h	StratArea m ²	<i>fGHG_{ch4}</i> mt CO2e/h	$fGHG_{n2o}$ mt CO2e/h	$fGHG_{E,t}$ mt CO2e/h	mt CO2e/y
TMT1	3/25/14	-2.22	-0.0713	185,000	-0.0103	-0.0039	-0.0142	
TMT1	5/20/14	-53.87	-0.0237	185,000	-0.2491	-0.0013	-0.2505	
TMT1	7/28/14	-272.27	-0.0830	185,000	-1.2593	-0.0046	-1.2638	
TMT1	9/17/14	-63.67	-0.0608	185,000	-0.2945	-0.0034	-0.2978	
TMT1	11/20/14	-24.98	-0.0115	185,000	-0.1155	-0.0006	-0.1162	
TMT1	1/27/15	-9.58	-0.3416	185,000	-0.0443	-0.0188	-0.0632	-2930.2
TMT2	3/25/14	-10.79	-0.0379	216,000	-0.0583	-0.0024	-0.0607	
TMT2	5/20/14	-60.63	-0.0824	216,000	-0.3274	-0.0053	-0.3327	
TMT2	7/28/14	-177.23	-0.0103	216,000	-0.9570	-0.0007	-0.9577	
TMT2	9/17/14	-34.04	bd	216,000	-0.1838	0.0000	-0.1838	
TMT2	11/20/14	-1.74	-0.0026	216,000	-0.0094	-0.0002	-0.0095	
TMT2	1/27/15	-5.1	-0.1922	216,000	-0.0275	-0.0124	-0.0399	-2314.8
TMT3	3/26/14	-7.41	-0.0218	227,000	-0.0420	-0.0015	-0.0435	
TMT3	5/20/14	-44.02	-0.0129	227,000	-0.2498	-0.0009	-0.2507	
TMT3	7/28/14	-286.25	bd	227,000	-1.6245	0.0000	-1.6245	
TMT3	9/17/14	-173.81	bd	227,000	-0.9864	0.0000	-0.9864	
TMT3	11/20/14	-87.14	-0.0071	227,000	-0.4945	-0.0005	-0.4950	
TMT3	1/27/15	-50.86	-0.079	227,000	-0.2886	-0.0053	-0.2940	-5397.0
MID	3/26/14	-1.58	-0.0028	5,039,000	-0.1995	-0.0042	-0.2037	
MID	5/23/14	-2.27	-0.0087	5,039,000	-0.2854	-0.0130	-0.2984	
MID	7/28/14	-28.38	bd	5,039,000	-3.5755	0.0000	-3.5755	
MID	9/18/14	-14.12	bd	5,039,000	-1.7782	0.0000	-1.7782	
MID	11/21/14	-1.49	-0.0028	5,039,000	-0.1873	-0.0042	-0.1915	
MID	1/27/15	-5.93	-0.5794	5,039,000	-0.7468	-0.8700	-1.6168	-11,197.2
OUT	3/27/14	-17.94	-0.0035	930,000	-0.4171	-0.0010	-0.4181	
OUT	5/30/14	-66.57	-0.0123	930,000	-1.5478	-0.0034	-1.5512	
OUT	7/29/14	-115.43	-0.0015	930,000	-2.6838	-0.0004	-2.6842	
OUT	9/23/14	-124.84	-0.0007	930,000	-2.9026	-0.0002	-2.9028	
OUT	11/19/14	-2.84	-0.0169	930,000	-0.0659	-0.0047	-0.0706	
OUT	1/27/15	bd	bd	930,000	0.0000	0.0000	0.0000	-11,142.8
							ΔGHG_E :	-32,982

CO2e/ha/y at the freshwater emergent wetlands (Table 1). These values multiplied by the area of forested (566.7 ha) and emergent wetlands (93.0 ha) wetlands were summed to

Table 5 Baseline, Project and Net emissions estimated using field data for the project activity and literature values for the baseline

	Baseline mt CO2e/y	Project mt CO2e/y	Net Cseq mt CO2e/y
ΔC_{TREE}	3790	4090	_
ΔC_{SOC}	2435	13,752	
Net w/out GHGs	6225	17,842	11,617
ΔGHG_E	-70,870	-32,982	
Net with GHG _s	-64,645	-15,140	49,505

provide baseline emissions in the project area of -70,870 mt CO2e/y (Table 5).

Net Project Carbon Sequestration

The total net greenhouse gas emissions reductions of the project activity were calculated with and without greenhouse gas emissions. The cumulative carbon sequestered by the trees and soils in the Project scenario was 4090 and 13,752 mt CO2e/y, respectively, while the greenhouse gasses were emitted at -32,982 mt CO2e/y. The Baseline scenario sequestered 3790 and 2435 mt CO2e/y by the trees and soils, respectively, while emitting -70,870 mt CO2e/y in greenhouse gasses. The net difference between the Project and Baseline emissions was 11,617 mt CO2e/y if greenhouse gasses are omitted and 49,505 mt CO2e/y if they are included (Table 5).



Discussion

This study demonstrates the potential of using forested wetlands receiving treated municipal effluent as wetland carbon offset projects for the net sequestration of carbon. Most of the carbon sequestered that we measured in this study was in the soils. Peat soils of wetland environments have the highest C content of all the soil orders (Bridgham et al. 2006) due to very high net primary production coupled with slow organic matter decomposition (Reddy and DeLaune 2008; Mitsch and Gosselink 2015). This makes wetland soils an important sink for atmospheric CO₂ (Bridgham et al. 2006; Hansen and Nestlerode 2014), especially in areas with high rates of subsidence. In this study, carbon sequestration by soils averaged 2.09 ± 0.24 kg CO2e/m²/y in contrast to the 0.84 ± 0.06 kg CO2e/m²/y sequestered by trees. This is similar to the findings reported by Day et al. (2004) for soils at the assimilation wetlands in Thibodaux, LA (2.24 kg CO2e/m²/y) and for the assimilation wetlands at Pointe aux Chene (2.56 kg CO2e/m²/y; Rybczyk et al. 2002). The carbon sequestered by the trees in this study was lower compared to the assimilation wetlands at Breaux Bridge (1.71–2.85 kg CO2e/m²/y; Day et al. 2004; Hunter et al. 2009a), or those at Amelia (2.73 kg CO2e/m²/y; Day et al. 2006), perhaps because these later projects benefit from long-term datasets making the methods used more accurate. Close agreement with these other studies indicates validity of the methods used by the ACR methodology (Mack et al. 2012).

Annual methane emission estimates from this study were 185.5 g CH₄/m²/yr., which is higher than, but comparable to other wetlands with nutrient inputs, such as 62.3 g CH₄/m²/yr. reported by Holm et al. (2016) for wetlands located 4 km eastward from our study area that receive river water from the Davis Pond Mississippi River Diversion, and 72.1 g $CH_4/m^2/vr$, reported by Kadlec & Wallace (2009) for ten free water surface treatment wetlands in Europe. However, natural wetlands without nutrient inputs have been shown to emit methane at comparably high rates; for example, Yu et al. (2008) reported mean emissions of 182.6 g $CH_4/m^2/yr$. at the Jean Lafitte National Historic Park and Preserve located south of New Orleans, Louisiana. Wang et al. (2008) reported emissions of >1000 g CH₄/m²/yr. at experimental treatment wetlands in Japan. Methane emission rates, however, may not be as important as previously thought; though CH₄ flux may have a warming effect on climate over decadal time scales, across centuries wetlands can be expected to act as net radiative sinks (Poffenbarger et al. 2011). The GWP of methane is dependent on the time interval over which the radiative forcing is integrated. Over a short-term integration period (ca. 20 years), the GWP of methane is estimated to be 21.8; however, the GWP of CH₄ falls to between 7.6 and 2.6 when considered over the time horizons of 100 to 500 years due to the decay of methane in the atmosphere over time (Whiting and Chanton 2001). Mitsch et al. (2013) demonstrated by dynamic modeling that methane emissions become unimportant within 300 years compared to carbon sequestration in temperate and tropical wetlands and that most wetlands become both net carbon and radiative sinks within that timeframe. The modeling done by Mitsch et al. (2013); however, may have underestimated the radiative forcing effect of methane (CH₄) emissions and overestimated soil C sequestration in freshwater wetlands (Bridgham et al. 2014). The implication of these studies is that the impact of methane on the warming of the planet may be exaggerated over the long-term by the use of the currently accepted GWP values.

There has been considerable discussion over the past decade about the effects of nutrient loading on coastal wetlands with regard to belowground productivity, soil strength, and soil organic matter decomposition (Darby and Turner 2008a, b, c; Swarzenski et al. 2008; Turner 2010; Deegan et al. 2012; Fox et al. 2012; Anisfeld and Hill 2012; VanZomeren et al. 2012; Day et al. 2013; Morris et al. 2013a; Graham and Mendelssohn 2014; Nyman 2014). In particular, there have been concerns that nutrient loading to coastal wetlands causes decreased belowground productivity and soil strength and increased soil organic matter decomposition, which decreases wetland resilience to disturbance and leads to increased subsidence and land loss (Darby and Turner 2008a, b, c; Deegan et al. 2012). However, there have been numerous studies showing either increased growth or no effect to baldcypress that are exposed to nutrient rich waters (Hesse et al. 1998; Lundberg et al. 2011; Keim et al. 2012). For example, Brantley et al. (2008) found significantly higher cypress growth downstream of effluent discharged from the Mandeville wastewater treatment plant. And Shaffer et al. (2009b) found increased growth rates in the Maurepas basin in areas receiving regular non-point source inputs, as did Effler et al. (2006) for trees given nutrient amendments. Hunter et al. (2009a) found slightly higher, but not significant, cypress growth at the Breaux Bridge assimilation wetlands.

Wetland projects have the potential for carbon sequestered to be released back to the atmosphere when a project has exposure to risk factors such as sea level rise and saltwater intrusion, hurricanes, fires, and damage from wildlife such as canopy insects (e.g., baldcypress leafroller (Archips goverana)) and nutria herbivory (Myocaster covpus; Evers et al. 1998; Lane et al. 2016). When vegetation death occurs, part of the soil organic carbon pool is decomposed (oxidized) and released as either CO2 or CH4 (Davidson and Janssens 2006; DeLaune and White 2011; Mcleod et al. 2011; Pendleton et al. 2012). These 'prevented emissions' may be claimed as carbon credits if project activities are successful in preventing the loss of the wetland soil horizon (Lane et al. 2016). Also, the direct result of the loss of wetlands is the loss of their sequestration capacity and GHG emissions, that is, as the wetland area becomes smaller so does the amount of



carbon it can sequester and GHGs it can release. This 'loss of sequestration capacity' can be calculated as the proportion of wetlands lost over a given period of time if restoration project activities were not to take place (Mack et al. 2012). Since the greatest soil carbon sink is subsidence, which permanently buries dead wood and other organic matter, much of these risk factors impact the ability for future carbon sequestration and not necessarily a reversal of carbon already sequestered and buried.

One of the greatest threats to restoration and sustainability of coastal wetlands worldwide is accelerating sea level rise (Blum and Roberts 2012; Day et al. 2016). Current eustatic sea-level rise (ESLR) is between 2 and 3 mm y^{-1} , and there is a strong scientific consensus that the rate of ESLR will accelerate in association with global warming (FitzGerald et al. 2008; Meehl et al. 2009; McCarthy 2009). Increasing eustatic sea-level rise is especially critical in the Mississippi Delta because it is augmented by high rates of geologic subsidence. Relative sea level rise (RSLR), which is the combination of ESLR and subsidence, ranges from 5 to 8 mm y⁻¹ in the region surrounding the project area (Shinkle and Dokka 2004). However, restoration projects that provide much needed freshwater and nutrients restore vital land building processes through increased vegetative productivity and soil accretion, which can offset or largely mitigate RSLR (Day et al. 2004; Izdepski et al. 2009). In addition, the discharge of freshwater into wetlands creates a buffer to saltwater intrusion events that can be lethal to freshwater wetlands.

The wise utilization of freshwater resources is necessary to provide reliable sources of water to freshwater forested and emergent wetlands to prevent ongoing saltwater intrusion and to increase vertical accretion through either direct sediment deposition or organic soil formation (Morris et al. 2013a, b; Nyman 2014). Without consistent freshwater input, most forested wetlands in coastal Louisiana will not survive. Even if saltwater impacts can be reduced, forested wetland soils need to accrete vertically if they are to survive in the long-term because regeneration cannot occur with permanent or semipermanent flooding (Conner et al. 2014). Currently, many sources of freshwater exist, such as secondarily treated municipal effluent, nonpoint source stormwater runoff, municipal stormwater, and river water. However, most of these sources are currently engineered to maximize drainage efficiency by bypassing wetlands using ditches and canals that discharge directly to lakes and rivers (Lane et al. 2015a, 2015b). Rerouting the water to maximize sheet flow over wetlands would reduce nutrient input to surface waters and thus improve regional water quality and increase wetland productivity, while decreasing impacts of saltwater intrusion, sea level rise, and subsidence.

Wetland restoration is a critical tool to combat wetland loss and is an effective climate change mitigation strategy. The results of this study demonstrate that the assimilation of treated municipal effluent by cypress-tupelo wetlands increases wetland productivity and enhances carbon sequestration. This project supports the inclusion of wetland restoration management approaches in the emerging carbon market and GHG policy regimes to supplement critical funding to facilitate rapid and effective climate change mitigation and adaptation.

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