Water Table Drawdown Alters Soil and Microbial Carbon Pool Size and Isotope Composition in Coastal Freshwater Forested Wetlands

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Loss of coastal wetlands is occurring at an increasingly rapid rate due to drainage of these wetlands for alternative land-uses, which also threatens carbon (C) storage in these C-rich ecosystems. Wetland drainage results in water table drawdown and increased peat aeration, which enhances decomposition of previously stabilized peat and changes stable C isotope profiles. The effect of water table drawdown on the pool size and δ^{13}C signature of plant C, soil organic C (SOC), and microbial biomass C (MBC) across a range of organic and mineral soils has not previously been reported in coastal freshwater forested wetlands. To this end, litter, roots, and soils were collected from organic and mineral soil horizons in two coastal freshwater forested wetlands in North Carolina with different hydrological regimes: (1) a natural bottomland hardwood forest (natural); and (2) a ditched and drained, intensively-managed wetland for loblolly pine silviculture (managed). We found that hydrology and soil horizon, and to a lesser degree micro-topography, was important in shaping observed differences in size and ^{13}C signature of soil and microbial pools between the natural and managed wetland. The natural wetland had higher SOC and MBC concentrations in the litter, surface organic, and mineral horizons compared to the managed wetland. In the managed wetland, ^{13}C of SOC was enriched across most of the soil profile (Oa and mineral soil horizons) compared to the natural wetland, suggesting enhanced decomposition and incorporation of microbially-derived inputs into stable SOC pools. Root C concentration decreased with soil depth, while root ^{13}C signature became enriched with soil depth. In the litter and Oe horizon of the natural wetland, MBC was higher and ^{13}C of MBC and SOC was enriched in hummocks compared to hollows. The ^{13}C of MBC and SOC tended to be enriched in upper soil horizons and depleted in lower soil horizons, particularly in the managed wetland. We conclude that drainage of these coastal wetlands has enhanced the breakdown of previously stabilized C and has the potential to alter regional C storage, feedback to climate warming, and ecosystem response to changing environmental conditions.

Keywords: wetland drainage, microbial biomass carbon, loblolly pine silviculture, microtopography, δ^{13}C, soil organic carbon, land-use change

INTRODUCTION

Globally, wetlands store large quantities of carbon (C) in belowground pools over a small land area (Johnson and Kern, 2003; Lal, 2005; Yu et al., 2011), providing a critical role in mitigating atmospheric greenhouse gas concentrations through long-term sequestration in soils. The ability of forested wetlands to store large amounts of atmospheric C stems largely from high organic matter inputs and reduced soil organic C (SOC) degradation in saturated soils (Trettin et al., 1996; Yu et al., 2006; Miao, 2013). Coastal freshwater forested wetlands along the southeastern US are particularly important to regional C storage (Johnson and Kern, 2003), and provide myriad ecosystem and economic services (Tiner, 1984; Richardson, 1994; Jansson et al., 1998; Mitsch and Gosselink, 2000; Snodgrass et al., 2000; Hinton et al., 2013; Tanentzap et al., 2014). As such, the value of wetlands to regional and global ecosystem health is undeniable. Unfortunately, these wetlands are currently under accelerated threat from sea level rise, increased frequency/intensity of extreme storm events and an array of anthropogenic disturbances, most notably drainage for alternative land-uses (Dahl, 2011), threatening the C storage capacity of these ecosystems.

It has been estimated that 87–89 million hectares (ha) of wetlands existed in the conterminous United States prior to European settlement, approximately half of which were lost by the 1950's (Tiner, 1984; Dahl, 1990). Drainage has further reduced the extent of freshwater forested wetlands in the conterminous US (~9.7% managed since the mid-1970s; Dahl et al., 1991; Dahl, 2011) and around the world (Gorham, 1991; Minkkinen and Laine, 1998; Holden et al., 2004). Wetland drainage occurs for various reasons, including agricultural and silvicultral purposes, urbanization, and expansion of coastal communities. Between 2004 and 2009, drainage for loblolly pine (*Pinus taeda* L.) silviculture accounted for a majority (38%) of total freshwater forested wetlands converted in the southeastern US compared to development (26%), agriculture (13%), sea level rise (4%), and other upland land uses (19%) (Dahl, 2011). Pine silviculture in the southeastern US is instrumental in providing wood, paper, and energy supplies for the US and internationally, thus earning the label "wood basket of the US" (Fox, 2000; Fox et al., 2004). Thus, advanced loblolly pine silviculture is of great importance to local and regional economies, but the drainage of natural forested wetlands has poorly quantified environmental costs in terms of loss of C (Armentano and Menges, 1986) and climate change impacts that could alter long-term stability of coastal regions worldwide.

Hydrology is a key regulating factor of wetland primary productivity and decomposition processes (Day and Megonigal, 1993; Kasimir et al., 2018), greenhouse gas emissions (Fenner and Freeman, 2011; Dai et al., 2012; Miao, 2013; Kasimir et al., 2018), and C storage potential (Yu et al., 2006). Microorganisms are also a key component of the SOC cycle, controlling organic matter decomposition, nutrient mineralization and immobilization, and SOC stabilization. Rates of aerobic microbial activity, decomposition, and soil respiration are typically lower in saturated soils (Freeman et al., 2004; Fenner and Freeman, 2011; Miao, 2013; Minick et al., 2016). Microbial biomass, which correlates with the potential for SOC decomposition (Neely et al., 1991), also tends to be lower in saturated soils compared to unsaturated soils (Poret-Peterson et al., 2007). Wetland drainage results in drawdown of the water table, enhancing soil aeration and stimulating breakdown of previously stable SOC (Gorham, 1991; Trettin et al., 1996; Fenner and Freeman, 2011; Kasimir et al., 2018). This results in loss of SOC as increased (aerobic) heterotrophic respiration releases C to the atmosphere as CO_2. Therefore, it is generally considered that wetland drainage leads to losses of SOC (Gorham, 1991; Trettin et al., 1996; Trettin and Jurgensen, 2003), but this has not always been observed (Minkkinen and Laine, 1998; Toberman et al., 2008, 2010) and depends on time since drainage and post-drainage land use (Minkkinen and Laine, 1998; Noormets et al., 2010) or other edaphic factors such as pH and concentration of phenolic compounds (Toberman et al., 2008, 2010). For instance, more intense disturbance after drainage of forested wetlands in the southeastern US has resulted in large losses of SOC (Trettin et al., 1996) compared to less intense disturbances (Noormets et al., 2010). Given that drainage changes the vertical distribution of water in the soil profile, with clear potential to influence wetland SOC storage, analysis of SOC cycling as a function of land-use change is necessary to understand long-term ecosystem C storage potential.

Stable C isotope profiles of SOC pools provides a robust indicator of the degree of microbial processing of SOC and is a widely used tool in soil ecological research, integrating across several biogeochemical process (Ehleringer et al., 2000). For instance, the length of previous exposure to altered environmental conditions (such as changes in hydrology after wetland drainage; Minkkinen and Laine, 1998; Noormets et al., 2010) can greatly affect organic matter decomposition rates and hence its isotopic signature (Melillo et al., 1989). Aerated soils typically display ^{13}C enrichment of SOC with increasing soil depth (O'brien and Stout, 1978; Natelhoffer and Fry, 1988; Melillo et al., 1989; Wang et al., 2015). Enrichment of SOC with soil depth may result from numerous independent or interacting processes including: (1) breakdown of ^{13}C-depleted C compounds by microbes during decomposition; (2) isotopic fractionation (discrimination) by soil microbes, in which the light C isotope (^{12}C) is preferentially utilized during metabolism and respiration of SOC while the heavy carbon isotope (^{13}C) remains in the residual SOC pool; (3) incorporation of microbially-derived, ^{13}C-enriched compounds into SOC pools; and (4) via inputs of ^{13}C-depleted CO_2 to the atmosphere as fossil fuels are burned (the Suess effect; Werth and Kuzyakov, 2010; Lerch et al., 2011; Keeling et al., 2017; Minick et al., 2017a). Soil microbial biomass C also tends to be enriched in ^{13}C compared to bulk soil (Werth and Kuzyakov, 2008) and mounting evidence suggests a much greater contribution of microbial biomass C to stable C pools than previously considered (Schmidt et al., 2011). This indicates that ^{13}C enrichment of SOC with depth results from a greater degree of decomposition and reflects contributions of ^{13}C-enriched microbial products and necromass to SOC, partially explaining the observed enrichment of SOC

pools with soil depth (Natelhoffer and Fry, 1988; Werth and Kuzyakov, 2008; Schmidt et al., 2011). By contrast, under saturated or anaerobic soil conditions, breakdown of organic matter is suppressed and therefore should result in different patterns of SOC ^{13}C natural abundance with soil depth as organic material is preserved in a less decomposed state (von Fischer and Tieszen, 1995; Esmeijer-Liu et al., 2012; Hobbie et al., 2017). For instance, Krüger et al. (2014) found that depth profiles of ^{13}C were uniform in peatland soils, although some enrichment from surface peat soils to deeper peat soils is not completely unexpected as fresh plant material is slowly decomposed over long periods of time (Hobbie et al., 2017). Suppressed breakdown of SOC in wetlands should lead to ^{13}C of SOC being more similar to that of plant-derived organic materials, particularly of non-soluble, ^{13}C-depleted, recalcitrant C compounds such as lignin (Benner et al., 1987; von Fischer and Tieszen, 1995; Bowling et al., 2008; Segnini et al., 2013). Microbial stable C isotope composition should also provide an indicator of the type of C compounds soil microorganisms are metabolizing (Bowling et al., 2008; Coyle et al., 2009; Werth and Kuzyakov, 2010), as well as reflect the balance between the ^{13}C of immobilized C substrates and respired CO_2. Unfortunately, ^{13}C analysis of microbial biomass C and SOC in wetlands have rarely been studied simultaneously or under different land-use regimes. Thus, it is unclear how hydrology affects ^{13}C profiles of soil and microbial C pools in managed vs. natural wetland systems, leaving a major gap in our knowledge of how land-use change alters wetland SOC stabilization.

Finally, hummock and hollow micro-topography is a common feature of wetlands, and has been shown to influence SOC cycling due to effects on soil aeration through interactions with fluctuations in the water table (Miao et al., 2017), which has important implications for wetland C biogeochemistry and C cycling processes (Keiluweit et al., 2017). Similarly, in intensively managed pine plantations of the lower coastal plain the soil is bedded into rows to increase soil aeration and reduce competition, increasing tree seeding survival and growth (Fox et al., 2007). Bedding of pine plantations creates more spatially uniform micro-topography, compared to a natural forested wetland, with alternating high (bed) and low (interbed) zones, which can also influence soil C and nutrient cycling (Minick et al., 2015, 2017b).

The objective of this study was to characterize the effects of intensive management on SOC cycling and storage in coastal forested wetlands of the US Southeast. We hypothesized that: (1) the lower water table and improved aeration in the managed wetland would promote decomposition of existing SOC and greater microbial biomass; (2) the enhanced breakdown of SOC in the managed wetland would result in the enrichment of SOC ^{13}C as ^{13}C enriched microbial products are incorporated into the SOC pool; and (3) the δ^{13}C of microbial biomass will reflect the signature of the substrate, i.e., be more enriched in the managed wetland. To this end, plant litter, roots and soils were sampled from two micro-topographic positions and numerous organic and mineral soil horizons (e.g., depth) in a natural and managed forested wetland along the lower coastal plain of North Carolina to quantify the C concentration and ^{13}C isotopic signature of vegetation, SOC and microbial pools.

METHODS

Site Description

The natural forested wetland was located at Alligator River National Wildlife Refuge (ARNWR) in Dare County, North Carolina (35°47′N, 75°54′W), with the Alligator River to the west, Albemarle Sound to the north, and the Croatan Sound to the east (**Figure 1**). The refuge was established in 1984 and is characterized by a diverse assemblage of pocosin (wetland fed by rainwater or groundwater, acidic peat and mineral soils, oligotrophic, woody vegetation) wetland types and swamp forests (Allen et al., 2011). ARNWR has a network of roads and canals, but in general contains vast expanses of minimally disturbed forested- and shrub-wetlands. Thirteen plots were established in a 4 km^2 area in the middle of a bottomland hardwood forest surrounding a 35 m eddy covariance flux tower (US-NC4 in the AmeriFlux database; Miao, 2013). Of the 13 plots (7 m radius), the five central plots were utilized for this study (**Figure 1**). Over-story plant species composition was predominantly composed of black gum (*Nyssa sylvatica*), swamp tupelo (*Nyssa biflora*), bald cypress (*Taxodium distichum*), with occasional red maple (*Acer rubrum*), sweet gum (*Liquidambar styraciflua*), white cedar (*Chamaecyparis thyoides*), and loblolly pine (*Pinus taeda*). The understory was predominantly fetterbush (*Lyonia lucida*), bitter gallberry (*Ilex albra*), red bay (*Persea borbonia*), and sweet bay (*Magnolia virginiana*) (**Table 1**). The mean annual temperature and precipitation from an adjacent meteorological station (Manteo AP, NC, 35°55′N, 75°42′W, National Climatic Data Center) for the period 1981–2010 were 16.9°C and 1,270 mm, respectively. These wetlands are characterized by a hydroperiod that operates over short time scales and is driven primarily by variation in precipitation. Soils are classified as a Pungo series histosols (very poorly drained dystic thermic typic Haplosaprist) with a deep, highly decomposed muck layer overlain by a shallow, less decomposed peat layer and underlain by highly reduced mineral sediments of Pleistocene origin (Riggs, 1996). Ground elevation is <1 m above sea level. Periodic *in situ* measurements of redox potential indicate that standing water is relatively aerated (Eh = 175–260 mV), while surface soils of hummocks are more aerated (Eh = 320 mV) than hollows (Eh = 120–150 mV) compared to deeper organic soils (Eh = 50–90 mV). Micrometeorological variables (precipitation, water table depth) were measured at a micro-met station established at the center of the study site from January 1, 2016 to December 31, 2016. Both precipitation and water table data were collected at 30-min intervals. Water table depth was measured with a pressure water level data logger (Infinities, Port Orange, FL, USA). Precipitation was measured with a tipping bucket rain gauge (TE-525, Texas Electronics, TX, USA).

The managed wetland was an intensively managed loblolly pine plantation located in Washington County, NC (35°48′N, 76°40′W) within the lower coastal plain mixed forest province (Noormets et al., 2010, 2012; Sun et al., 2010; **Figure 1**). In the late 1800's/early 1900's, the area was clearcut and ditched for agriculture. Since the mid-1960's, this site has been owned and operated by Weyerhaeuser NR Company for commercial timber production and is currently in its fourth rotation of loblolly pine.
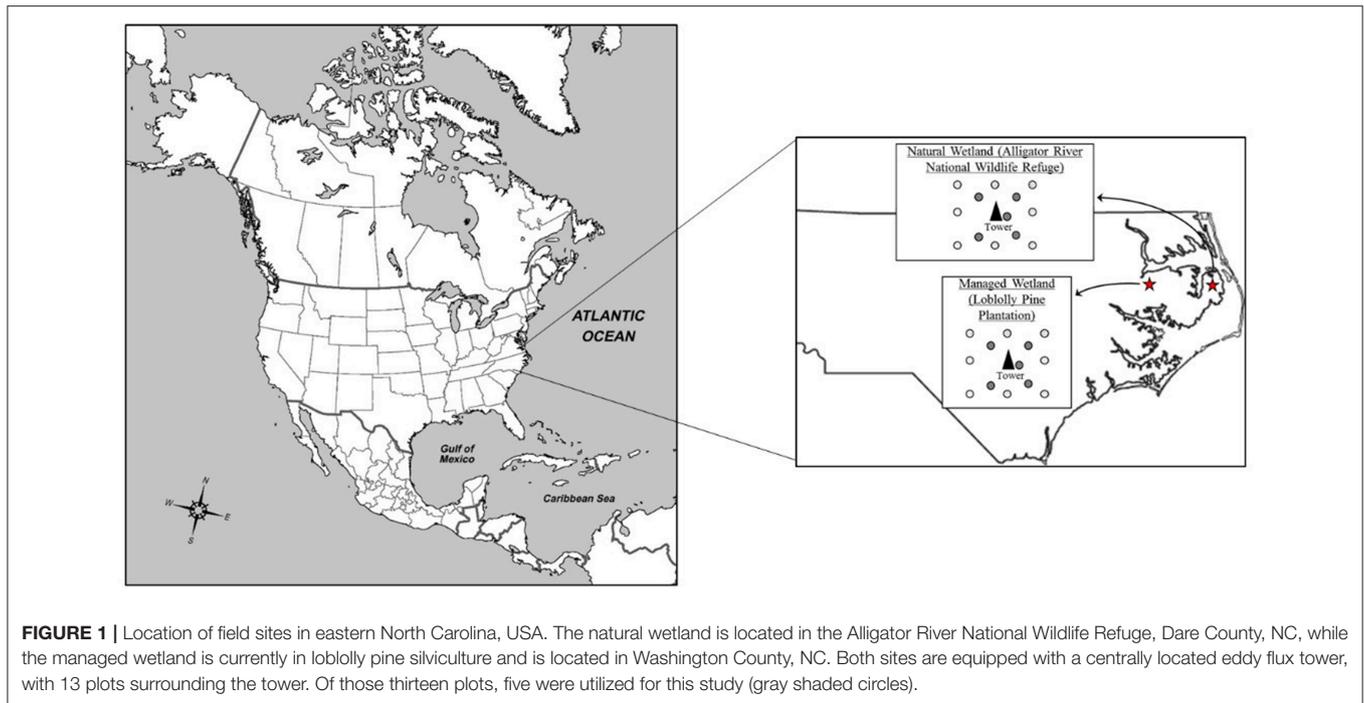
FIGURE 1 | Location of field sites in eastern North Carolina, USA. The natural wetland is located in the Alligator River National Wildlife Refuge, Dare County, NC, while the managed wetland is currently in loblolly pine silviculture and is located in Washington County, NC. Both sites are equipped with a centrally located eddy flux tower, with 13 plots surrounding the tower. Of those thirteen plots, five were utilized for this study (gray shaded circles).

The pine plantation watersheds are managed with a network of parallel ditches (0.9–1.3 m deep; 90 m spacing) and more widely spaced roadside canals. Drainage lowers the height of the water table, and improves site access and tree productivity by reducing stresses caused by excessive soil water, especially during winter months. The current loblolly pine stand is also part of the Ameriflux core sites (US-NC2 in the Ameriflux database) and was planted in 1992, on bedded rows, with 2-year-old half sibling seedlings after clear-cutting the previous mature pine plantation. Planting spacing was 1.5 by 4.5 m. Bedding is common best management practice along the southeastern lower coastal plain (Fox et al., 2007) in order to increase growth and survival of seedlings. Bedding creates unique micro-topography in managed pine forests, resulting in high (bed) and low (interbed; space between beds) micro-topographic locations, which influence SOC stabilization (Minick et al., 2017b) and nitrogen (N) cycling (Minick et al., 2014). Thirteen plots were established in the mature loblolly pine stand surrounding a 28 m eddy flux tower. Of these 13 plots (7 m radius), five central plots were utilized for this study (**Figure 1**). The understory was primarily composed of young red maple (*A. rubrum*) and other non-woody plants such as devil's walking stick (*Aralia spinosa*), pokeweed (*Phytolacca americana*), beautyberry (*Callicarpa americana*), giant cane (*Arundinaria macrosperma*), and meadow grass (Poa spp.) (Domec et al., 2015). The histic-mineral soil at this site is classified as a Belhaven series histosol (loamy, mixed, dystic, thermic Terric Haplosaprists) with a highly decomposed organic matter layer underlain by loamy marine sediments. Belhaven and Pungo soil series are typically found within the same soil map of this region, with Belhaven series soils having somewhat higher permeability due to the loamy soil texture (USDA Soil Survey Staff, 2008). The soils are very poorly drained

with a ground elevation of 4–6 m above sea level. The long-term (1945–2010) average annual precipitation was 1290 ± 199 mm, evenly distributed throughout the year. Long term mean annual temperature averaged 15.5°C, with a monthly high temperature occurring in July (26.6°C), and a monthly low occurring in January (6.4°C). Micrometeorological variables (precipitation, water table depth) were measured at a micro-met station established at the center of the study site from January 1, 2016 to December 31, 2016. Water table depth was measured hourly with a pressure water level data logger (Infinities, Port Orange, FL, USA). Precipitation was measured continuously by two tipping bucket rain gages (TE-525, CSI; Onset1 Data Logging Rain Gauge, Onset Computer Corporation, USA), and one backup manual rain gage (Forestry Suppliers Inc., USA).

Litterfall and Fresh Litter

For both sites, average annual litterfall (**Table 1**) was quantified over numerous collection periods in 2015 and 2016 from three litter baskets (2,122 cm^2) located within each of the five plots. After collection, litter was dried at 50°C until constant weight and the dry weight of litter was recorded.

In 2016, fresh litter was collected for C concentration and ^{13}C isotopic signature. This litter was collected from young trees or from newly fallen branches with litter still attached. Fresh litter was dried at 50°C until constant weight and then ground to a fine powder in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). Ground fresh litter samples were analyzed in duplicate for C concentration and δ^{13}C isotopic signature on a Picarro G2201-*i* Cavity Ring-Down Spectroscopy (CRDS) isotopic CO_2/CH_4 analyzer (Picarro Inc., Sunnyvale, CA, USA) interfaced with a Picarro Combustion Module (Picarro Inc., Sunnyvale, CA,

TABLE 1 | Site characteristics, including forest age, dominant overstory vegetation, tree height (m), diameter at breast height (>5 cm), tree density (trees per hectare >2.5 cm; TPH), average annual litter fall (2015–2016), and depth of soil horizons (cm).

	Natural wetland	Managed wetland
Location	35°47'N, 75°54'W	35°48'N, 76°40'W
Forest type/land-use	Pocosin swamp	Loblolly pine plantation
Elevation	<1 m	4–6 m
Forest age (year)	~80	26
Overstory vegetation	*Nyssa sylvatica, Nyssa biflora, Taxodium dstichum, Acer rubrum, Liquidambar styraciflua, Chamaecyparis thyoides*	*Pinus taeda*
Height (m)	21 (0.5)	21 (0.3)
Diameter (cm)	17 (0.5)	27 (1.1)
Density (TPH)	2,320 (800)	2,673 (412)
Litter fall (kg ha^{-1} yr^{-1})	4,595 (1,139)	9,503 (1,199)
SOIL HORIZON DEPTH (cm)		
Oi	2 (0.2)	2 (0.2)
Oe	8 (0.4)	4 (0.5)
Oa1	20 (3.5)	4 (0.4)
Oa2	21 (3.5)	12 (1.7)
Oa3	3 (0.0)	3 (0.0)
A	10 (1.7)	10 (1.2)
AB	11 (2.1)	7 (1.3)
B	9 (0.6)	10 (0.1)

Site level values are presented with standard error in parenthesis.

USA). For δ^{13}C analysis, certified standards were used to develop a standard curve from the expected and measured δ^{13}C values ($R^2 > 0.999$). These standards included USGS 40 (L-glutamic acid) (δ^{13}C = −26.39‰; USGS Reston Stable Isotope Laboratory, Reston, VA, USA), protein (δ^{13}C = −26.98‰; Elemental Microanalysis Ltd, Okehampton, UK), urea (δ^{13}C = −48.63‰; Elemental Microanalysis Ltd, Okehampton, UK), atropine (δ^{13}C = −18.96‰; Costech Analytical Technologies, Inc, Valencia, CA, USA), and acetanilide (δ^{13}C = −28.10‰; Costech Analytical Technologies, Inc, Valencia, CA, USA). For C concentration, atropine standards were weighed out over a range of C concentrations that encompassed the expected C concentrations of the unknown samples. A standard curve for C concentration was also developed from the expected and measured C concentration of the atropine standards ($R^2 > 0.99$). All unknown sample C concentration and δ^{13}C values were adjusted using the linear equation derived from the appropriate standard curve. Soil, root, and microbial biomass C samples were analyzed using the same procedure (see below).

Soil Sample Collection and Processing

Soils at both the natural and managed forested wetlands were sampled in early 2016. A single time point measurement such as this is not uncommon in studies where the objective is to capturte forest management (or vegetation) effects on soil C pools (see Krüger et al., 2014; Hobbie et al., 2017, for examples of other studies which measured SOC pools at wetland sites) and

not necessarily seasonal dynamics. The SOC pool is typically quite stable while microbial biomass can vary seasonally as microbial activity is sensitive to changes in temperature and moisture (see Miao, 2013; Miao et al., 2017). Our results highlight clear differences though between the two wetland sites in MBC and δ^{13}C of MBC and therefore provide a reasonable index for comparing the effects of forest management on microbial biomass.

In the natural wetland, two soil samples were collected from high (hummock) and low (hollow) micro-topographic positions in early 2016 within each of the five plots. First, surface litter (Oi horizon) was collected within a 0.25 × 0.25 m^2 quadrant. In the natural wetland, rooting density is very high at the surface (Domec et al., 2015) rendering traditional coring methods ineffective. Therefore, after surface litter was collected a saw was used to cut a 0.25 × 0.25 m^2 monolith (Oe horizon) down to ~8 cm depth. Below the Oe horizon was an approximate 20 cm deep space containing very large coarse roots and water and/or air. This was particularly evident in hummocks. Coarse roots in this region (root mat) were also cut within the 0.25 × 0.25 m^2 quadrant and collected for analysis. Finally, the organic (Oa horizon) and mineral (A, AB, and B) soil horizons below the root mat were sampled with a Macaulay auger. The Oa horizon samples were subdivided into three sub-horizons: Oa1, which was directly below the coarse root mat but still contained a large amount of coarse and fine roots; Oa2, which comprised the bulk of the soil profile below Oa1, but was more homogenous with little root biomass; and Oa3, which was the 3 cm portion above the mineral A horizon. In some cases, the Oa3 horizon may be better characterized as an OaA as some vertical mixing of the upper organics and lower mineral soil had occurred. Mineral soil samples were collected from the A, AB, and the top 10 cm of B mineral horizons.

In the managed wetland, two soil samples were collected from high (bed) and low (interbed) micro-topographic positions in early 2016 from each of five plots. A dense surface rooting zone was not present at the managed wetland as was at the natural wetland. Therefore, a 5 cm diameter by 1.5 m long PVC corer was used to sample organic and mineral soils. Surface litter samples were first collected from a 0.25 × 0.25 m^2 quadrant. Organic (Oe and Oa) and mineral (A, AB, and B) soil horizons were then sampled beneath the collected litter using the PVC corer and subdivided into the same horizons as the natural wetland: Oe, Oa1, Oa2, Oa3, A, AB, and top 10 cm of B mineral horizons.

Root and Soil Characteristics

Live and dead roots were removed from the samples and soil was removed from roots by rinsing in distilled water (dH$_2$O). Roots were then dried to constant mass and ground to a fine powder in a Wiley mill. Ground root samples were analyzed in duplicate for C concentration and δ^{13}C isotopic signature on a CRDS isotope analyzer. After roots were removed, the remaining fresh soil from each of the two samples per plot and horizon were composited and homogenized. Soil pH was measured on fresh soil samples with a glass electrode in a 1:2 mixture (by mass) of soil and dH$_2$O. Gravimetric soil water content was determined by drying a fresh subsample for 24 h at 105°C.

After microbial biomass analysis (see below), the remaining soil was dried at 70°C. Air dried soils were homogenized by passing through a 2 mm sieve. Soil organic matter content was measured by loss on ignition at 600°C for 4 h. A subsample of the dried, homogenized soil was ground to a fine powder before C concentration and δ^{13}C isotopic signature on a CRDS isotope analyzer.

Soil Microbial Biomass C

The chloroform fumigation extraction (CFE) method was adapted from Vance et al. (1987) in order to estimate MBC and δ^{13}C. Briefly, one subsample of fresh soil (~2 g dry weight each) was placed in a 50 mL beaker in a vacuum desiccator to be fumigated. Another subsample was placed into an extraction bottle for immediate extraction in 0.5 M K_2SO_4 by shaking for 1 h and subsequently filtering through Whatman #2 filter paper to remove soil particles. The samples in the desiccator were fumigated with ethanol-free chloroform ($CHCl_3$) and allowed to incubate under vacuum for 4 d. After the 4 d incubation, fumigated samples were extracted similar to that of unfumigated samples. Filtered 0.5 M K_2SO_4 extracts were frozen at -20°C until further analysis. Upon thawing, extracts were dried at 60°C in a ventilated drying oven and then ground to a fine powder with mortar and pestle. Ground samples were analyzed for C concentration and δ^{13}C isotopic signature on a CRDS isotope analyzer. Microbial biomass C was determined using the following equation:

$$MBC = EC/k_{EC}$$

where the chloroform-labile pool (EC) is the difference between C in the fumigated and non-fumigated extracts, and k_{EC} (extractable portion of MBC after fumigation) was estimated as 0.45 (Joergensen, 1996).

δ^{13}C of MBC was estimated as the δ^{13}C of the C extracted from the fumigated soil sample in excess of that extracted from the non-fumigated soil sample using the following equation:

$$\delta^{13}C_{MBC}(\%) = (\delta^{13}C_f{\times}C_f - \delta^{13}C_{nf}{\times}C_{nf})/(C_f - C_{nf})$$

where C_f and C_{nf} is the concentration (mg kg^{-1} soil) of C extracted from the fumigated and non-fumigated soil samples, respectively, and $\delta^{13}C_f$ and $\delta^{13}C_{nf}$ is the ^{13}C natural abundance (%) of the fumigated and non-fumigated soil samples, respectively.

Statistical Analysis

All data were analyzed using repeated-measures ANOVA (PROC MIXED package). Each of the two sites and two micro-topographical sampling locations per site were treated separately as treatments (Trt) and analyzed as fixed effects, with soil horizon (Hrz) as the repeated measure (Littell et al., 2000; Schabenberger and Pierce, 2001; Sainju and Singh, 2008). Soil horizons (or soil depths) in a soil profile are spatially correlated due to lateral and horizontal movement of organic and mineral soil materials. Therefore, we chose to treat soil horizon as a repeated measure for modeling this correlation in space (Littell et al., 2000;

Schabenger and Pierce, 2001). Data were log transformed where necessary to meet assumptions of ANOVA. All data were plotted as means of raw data. If significant main effects or interactions were identified in the repeated-measures ANOVA ($P < 0.05$), then differences were tested using least square means *post-hoc* analysis with a Tukey's adjustment. Regression analysis (PROC REG) was used to relate soil C pools, isotopic signature, and soil water content. All statistical analysis were performed using SAS 9.4 software (SAS Institute, Cary, NC, USA).

RESULTS

Tree biomass and annual litterfall was ~40 and 50% higher, respectively, in the managed compared to natural wetland (**Table 1**). On average, organic horizons were deeper in the natural wetland (54 cm to mineral soil) compared to the managed wetland (25 cm to mineral soil; **Table 1**). Water table depth was different between the two sites, with the natural wetland having continuous water table depths above zero cm and completely flooded at numerous time periods throughout 2016 (**Figure 2A**). Alternatively, water table depth in the managed wetland was never above zero cm and was punctuated by seasonally deep water table depths (**Figure 2B**). Water table depth was clearly influenced by precipitation for both sites, with large precipitation events leading to quick decreases in water table depth followed by a gradual increase (**Figures 2A,B**). Annual cumulative precipitation for 2016 was 1,126 and 1,444 mm in the natural and managed wetlands, respectively. Similar temporal patterns of precipitation and fluctuations in water table depth were found for each site, indicating that hydrologic regimes across the region were similar during this study (**Figures 2A,B**).

The natural wetland was highly acidic, ranging from 4.2 to 4.9, with minimal differences between soil horizons (**Tables 2**, **3**). The managed wetland was also acidic, ranging from 4.0 to 5.6, with differences between soil horizons primarily driven by higher pH in litter and B horizons (**Table 3**). Litter and B horizon pH were higher in the managed wetland compared to the natural wetland. Soil organic matter content decreased moving down the soil profile for both the natural and managed wetland (**Tables 2**, **3**). Soil organic matter content was similar between sites and sampling locations in the litter and organic horizons, but was approximately two times higher in the mineral horizons (A, AB, and B) of the natural wetland compared to the managed wetland. Soil water content ranged from 20 to 90% and generally decreased with depth (**Table 3**). In the natural wetland, soil water content was higher in all soil horizons compared to the managed wetland (**Table 3**). Not surprisingly, hollows in the natural wetland had higher water content in the litter and Oe horizon compared to that of the hummocks (**Table 3**).

Soil organic C concentration ranged from 7.6 to 516.0 g C kg soil^{-1} (**Figure 3A**) and was influenced by micro-topography and soil horizon (**Table 2**). A clear decline in SOC concentration occurred with depth at both sites and micro-topographic positions (**Figure 3A**). In the litter, Oe, A, AB, and B horizons, both hummocks and hollows in the natural wetland had

higher SOC concentration compared to the managed wetland (**Figure 3A**). Microbial biomass C also declined with depth (**Figure 3B**) and was influenced by drainage, microtopography, and soil horizon (**Table 2** and **Figure 3B**). In the litter horizon, MBC was similar between the hummock of the natural wetland and the bed and interbeds of the managed wetland, but lower in the hollow of the natural wetland (**Figure 3B**). Interestingly, MBC in the Oe horizon in the hummock of the natural wetland was two to three times higher than other micro-topographic positions in the natural or managed wetland (**Figure 3B**). The managed wetland tended to have higher MBC in the Oa2 horizon compared to the natural wetland, while the natural wetland had

higher MBC in the AB mineral horizon (**Figure 3B**). Finally, no difference in site or micro-topography was found for root C concentrations, although a significant effect of horizon was found (**Table 2**), evident as a decrease in root C concentration with depth (**Figure 3C**).

Surface litter (Oi horizon) $\delta^{13}C$ ranged from -30.7 to $-30.3‰$ (**Figure 4A**). Soil $\delta^{13}C$ ranged from -30.0 to $-26.2‰$ across all sites and micro-topographical locations (**Figure 4A**). Differences in soil $\delta^{13}C$ between site, micro-topography, and soil horizon were observed below the litter layer and continued down to the B mineral horizon (**Table 2** and **Figure 4A**). In the Oe horizon, ^{13}C of hummocks was enriched ($-29.1 \pm$

FIGURE 2 | Seasonal dynamics of precipitation (daily cumulative) and water table depth (daily average) in 2016 from the **(A)** natural wetland and **(B)** managed wetland located in coastal North Carolina. In the natural wetland, the average height of hummocks was 12.8 cm, hollows was −3.8 cm, and the site average was 8.7 cm, relative to the elevation of the water level data logger location at the ground micrometeorological station (Miao et al., 2017). Similar information for the managed wetland micro-topography is not available.

TABLE 2 | Results (*F*-values and significance) of repeated-measures ANOVA testing effects of all site (e.g., natural and managed wetland) and sampling location (e.g., hummock, hollow, bed, or interbed) combinations (Trt), soil horizons (Hrz), and interactions for soil pH, soil organic matter (SOM), soil water content (SWC), soil organic C (SOC) and microbial biomass C (MBC) pools, ^{13}C isotopic signature of each pool, and difference between ^{13}C signature of each pool.

Source	pH	SOM	SWC	SOC	MBC	Root C	δ^{13}C SOC	δ^{13}C MBC	δ^{13}C Root C	MBC–soil δ^{13}C	Root–soil δ^{13}C	MBC–root δ^{13}C
Trt	9.0***	14.0***	107.6***	13.5***	3.65*	0.95	66.1***	12.7***	4.0**	3.1*	30.5***	16.2***
Hrz	15.0***	250.3***	391.6***	264.0***	166.8***	20.8***	78.9***	5.6**	5.4***	19.1***	1.4	6.8***
Trt*Hrz	2.6**	2.7**	3.95***	4.0***	3.1**	1.53	8.5***	3.3**	0.8	4.72***	1.4	2.4**

*P < 0.05, **P < 0.01, ***P < 0.001.

0.29‰) compared to hollows of the natural wetland (−29.7 ± 0.14‰) and interbeds of the managed wetland (−29.9 ± 0.07‰; **Figure 4A**). In the Oa1 horizon of the managed wetland, the beds were more enriched in ^{13}C than the hollows of the natural wetland. Beyond the Oa1 horizon, both the beds and interbeds of the managed wetland were more enriched in ^{13}C down to the deepest mineral horizon sampled. This pattern became more apparent with depth, as the δ^{13}C signature of soils continued to become enriched in the managed wetland, while no change in δ^{13}C of soils was found with depth past the Oa1 horizon in the natural wetland (**Figure 4A**). The greatest differences between sites were in the A and AB horizons, with the δ^{13}C of the natural wetland ∼-28.9‰ and that of the managed wetland ∼-26.5‰.

For δ^{13}C of MBC, a high degree of variability was found but differences due to site, micro-topography, and soil horizon were observed (**Table 2**). In the Oe horizon, ^{13}C of MBC was depleted in the hollows of the natural wetland compared to all other sites and micro-topographic combinations. In both the Oa1 and the Oa2, ^{13}C of MBC in the hummocks and hollows of the natural wetland were highly depleted compared to that of the bed and interbed of the managed wetland (**Figure 4B**). After the Oa2 horizon, the δ^{13}C of MBC in the managed wetland became more depleted while little difference between soil horizons was found for the natural wetland, except that the MBC in litter and Oe horizons of hummocks tended to be enriched in ^{13}C compared to all other depths.

The δ^{13}C isotopic signature was also determined for fresh litter from four major tree species in the natural wetland, loblolly pines in the managed wetland, and live plus dead roots from both sites. For the natural wetland, δ^{13}C of fresh litter ranged from −31.6‰ in *Nyssa* to −28.3‰ in *Taxodium* (**Table 4**). The δ^{13}C value of loblolly pine litter was −30.0 ± 0.01‰ and −31.4 ± 0.04‰ from the natural and managed wetland, respectively (**Table 4**). The δ^{13}C of roots ranged from −30.7‰ in Oa1 to −27.1‰ in the B horizon (**Figure 4C**). Root δ^{13}C exhibited an overall difference between site and micro-topography (**Table 2**). Across all horizons, δ^{13}C of roots was different between hummocks (−28.4 ± 0.18‰) and hollows (−28.2 ± 0.17‰) of the natural wetland compared to beds (−29.0 ± 0.17‰) of the managed wetland. Across horizons, a similar trend was observed in ^{13}C of roots between sites and micro-topographic positions, with the most depleted ^{13}C values in the Oe horizon (−29.4 ± 0.23‰) compared to Oa2 horizon (−28.6 ± 0.23‰) and the most

enriched values in the A (−28.3 ± 0.16‰), AB (−28.1 ± 0.24‰), and B (−27.9 ± 0.17‰) mineral horizons (**Figure 4C**).

The differences in δ^{13}C between SOC, MBC, and root C pools were calculated. A positive value indicates enrichment of ^{13}C in the first pool compared to the second pool, while a negative value indicates depletion. For the difference between SOC and MBC, an interaction between site, micro-topography, and horizon was observed (**Table 2**). In the litter and Oe horizons, MBC tended to be enriched in the heavy isotope compared to SOC (**Figure 5A**). In both the Oa1 and Oa2 horizons, MBC was depleted in the heavy isotope compared to SOC in the natural wetland, but enriched in the managed wetland (**Figure 5A**). At both sites, MBC was depleted in the heavy isotope compared to SOC in the mineral horizons (**Figure 5A**).

Root-C in the natural wetland was enriched in ^{13}C compared to SOC while the opposite was true for the managed wetland (**Figure 5B**). Furthermore, an overall difference was found for each site by micro-topography combination (**Table 2**), with the difference in δ^{13}C between SOC and root-C being more positive in hummocks (0.6 ± 0.20‰) and hollows (0.9 ± 0.18‰) of the natural wetland compared to beds (−0.9 ± 0.18‰) and interbeds (−1.1 ± 0.18‰) of the managed wetland (**Figure 5B**). The difference between the δ^{13}C signature of root-C and MBC was also influenced by horizon (**Table 2**), but most strongly in the managed wetland (**Figure 5C**). In the managed wetland, MBC was enriched in the heavy isotope compared to root-C from the Oe horizon down to the Oa3 horizon, but was only enriched in the hummock of the Oe horizon in the natural wetland (**Figure 5C**). Otherwise, MBC was depleted in the heavy isotope compared to root-C in the natural wetland and in the mineral horizons of the managed wetland.

Strong relationships were found between SOC concentration, isotopic signature, and soil water content (**Figures 6, 7**), particularly in the managed wetland. Overall, correlations were higher in the managed wetland site and lower in the natural wetland, especially that of the hummocks in the natural wetland. SOC was positively correlated with MBC, with a much stronger relationship in the managed wetland (**Figure 6A**). SOC content exhibited a negative relationship with SOC δ^{13}C (**Figure 6B**), with ^{13}C becoming more depleted as SOC concentration increased. In the managed wetland, SOC concentration was positively related with MBC δ^{13}C (**Figure 6C**), although no relationship was found in the natural wetland. Finally, the difference between SOC and MBC δ^{13}C was positively correlated

TABLE 3 | Soil pH, organic matter content (%), and water content (%) for samples collected from organic and mineral horizons from a natural coastal freshwater forested wetland (Natural Wetland) and an intensively managed loblolly pine plantation (Managed Wetland) in coastal North Carolina, USA.

Horizon	pH				SOM Content (%)				Soil water content (%)			
	Natural wetland		Drained wetland		Natural wetland		Drained wetland		Natural wetland		Drained wetland	
	Hummock	Hollow	Bed	Interbed	Hummock	Hollow	Bed	Interbed	Hummock	Hollow	Bed	Interbed
Litter	4.8 ± 0.1^{Ab}	4.3 ± 0.1^{Bb}	5.6 ± 0.3^{Aa}	5.6 ± 0.2^{Aa}	—	—	—	—	67 ± 3^{Bb}	90 ± 1^{Aa}	48 ± 5^{BCc}	55 ± 5^{BCc}
Oe	4.2 ± 0.1^{Ba}	4.3 ± 0.1^{Ba}	4.2 ± 0.2^{DCEa}	4.5 ± 0.2^{CDEa}	95 ± 0.4^{Aa}	89 ± 7^{Aa}	84 ± 1^{Aa}	87 ± 2^{Aa}	85 ± 1^{Ab}	90 ± 1^{Aa}	66 ± 2^{Ad}	78 ± 2^{Ac}
Oa1	4.4 ± 0.1^{ABa}	4.4 ± 0.1^{Ba}	4.0 ± 0.1^{Eb}	4.3 ± 0.1^{Ea}	63 ± 12^{Ba}	67 ± 5^{Aa}	69 ± 4^{ABa}	64 ± 6^{Ba}	84 ± 4^{Aa}	86 ± 2^{Aa}	61 ± 1^{Ab}	64 ± 3^{Bb}
Oa2	4.3 ± 0.1^{ABa}	4.4 ± 0.2^{ABa}	4.2 ± 0.1^{DEa}	4.4 ± 0.1^{DEa}	56 ± 10^{BCa}	45 ± 8^{Ba}	60 ± 3^{Ba}	53 ± 7^{Ba}	78 ± 6^{Aa}	74 ± 5^{Ba}	60 ± 3^{Ab}	61 ± 1^{BCb}
Oa3	4.4 ± 0.1^{ABb}	4.4 ± 0.2^{Bb}	4.6 ± 0.2^{Cab}	4.7 ± 0.0^{BCa}	40 ± 2^{Ca}	32 ± 4^{Ba}	28 ± 4^{Ca}	29 ± 2^{Ca}	74 ± 5^{ABa}	71 ± 6^{BCa}	45 ± 5^{Cb}	50 ± 5^{Cb}
A	4.4 ± 0.2^{ABa}	4.4 ± 0.2^{ABa}	4.5 ± 0.2^{DCa}	4.7 ± 0.1^{BCDa}	21 ± 3^{Da}	20 ± 5^{Cab}	10 ± 2^{Dc}	11 ± 2^{Dbc}	60 ± 4^{BCa}	59 ± 5^{Ca}	31 ± 3^{Db}	29 ± 3^{Db}
AB	4.5 ± 0.3^{ABa}	4.5 ± 0.3^{ABa}	5.0 ± 0.1^{Ba}	5.0 ± 0.1^{ABa}	19 ± 4^{Da}	15 ± 2^{Ca}	7 ± 2^{Db}	8 ± 3^{EDb}	52 ± 6^{Ca}	45 ± 4^{Da}	20 ± 2^{DEb}	24 ± 3^{DEb}
B	4.7 ± 0.2^{ABb}	4.9 ± 0.3^{Aab}	5.4 ± 0.2^{Aa}	5.4 ± 0.2^{Aa}	9 ± 1^{Ea}	8 ± 2^{Da}	3 ± 1^{Eb}	4 ± 1^{Eb}	36 ± 1^{Da}	32 ± 1^{Ea}	21 ± 1^{Eb}	20 ± 1^{Eb}

Data represent mean with standard error (n = 5). Significant differences between soil horizons at each site and micro-topographic location (e.g., hummock, hollow, bed, or interbed) at each soil horizon are indicated by superscript lower case letters (P < 0.05). Significant differences between soil horizons at each site and sampling location are indicated by superscript upper case letters (P < 0.05). Significant differences between each site and micro-topographic location are indicated by superscript lower case letters (P < 0.05).

to the SOC concentration (**Figure 6D**). Interestingly, in the managed wetland site MBC tended to be depleted in ^{13}C compared to SOC at concentrations ranging from 0 to ~200 g kg^{-1}, but became increasingly enriched compared to SOC at concentrations >200 g kg^{-1}, up to ~500 g kg^{-1}. No relationship was found between SOC δ^{13}C and MBC δ^{13}C in the natural wetland, and only a weak negative relationship existed for beds [Adj. $r^2 = 0.15$; $F_{(1,339)} = 5.6$; $P = 0.02$] and interbeds [Adj. $r^2 = 0.24$; $F_{(1,35)} = 10.7$; $P = 0.002$] of the managed wetland.

Soil water content, expressed as a percentage of total soil, exhibited a positive, exponential relationship with SOC concentration at both sites and micro-topographical positions (**Figure 7A**). Likewise, soil water content and MBC also had a positive relationship (**Figure 7B**), but to a lesser extent than that with SOC. A negative correlation was found between soil water content and SOC δ^{13}C, except for that of hummocks in the natural wetland, where no relationship was identified (**Figure 7C**). Soil water content and MBC δ^{13}C were positively correlated in the managed wetland, while no relationship was found at either micro-topographical position in the natural wetland (**Figure 7D**). Soil water content also was highly positively correlated with soil organic matter content, across all sites and micro-topographic positions [Adj. $r^2 = 0.81$; $F_{(1,128)} = 534.9$; $P < 0.0001$]. The relationship was even stronger when sites and micro-topographic positions were separated, as indicated by analysis for hummocks [Adj. $r^2 = 0.88$; $F_{(1,27)} = 211.2$; $P < 0.0001$] and hollows [Adj. $r^2 = 0.92$; $F_{(1,29)} = 330.6$; $P < 0.0001$] of the natural wetland, and beds [Adj. $r^2 = 0.83$; $F_{(1,33)} = 170.0$; $P < 0.0001$] and interbeds [Adj. $r^2 = 0.88$; $F_{(1,33)} = 258.0$; $P < 0.0001$] of the managed wetland.

DISCUSSION

Land-use change in coastal wetlands disrupts processes that enhance C storage in these C-rich ecosystems, potentially resulting in release of greenhouse gases from the soil to the atmosphere. In order to predict changes in the SOC balance of drained wetlands, an understanding of how SOC and microbial pools respond is necessary. We hypothesized that drainage would enhance decomposition of existing SOC as increased soil aeration due to water table drawdown would be more favorable to microbial activity, conferring changes in the SOC and MBC pool size and δ^{13}C isotopic signature. As a result of the hypothesized enhancement of microbial activity under drained conditions, enrichment of SOC ^{13}C was expected as new microbial products and biomass (formed under the more favorable decomposition conditions) were incorporated into the SOC pool (Schmidt et al., 2011). This was evident by the ^{13}C of SOC in the managed wetland being more enriched in Oa and mineral horizons, but not in the litter or Oe horizon. By contrast, ^{13}C of SOC in the Oe horizon was enriched in the hummocks of the natural wetland compared to the hollows and the interbed of the managed wetland, although no other micro-topographical effects on SOC pool size or isotopic signature were detected. Furthermore, ^{13}C of MBC in the Oe horizon of hummocks in the natural wetland was the same as that of the managed wetland, indicating the
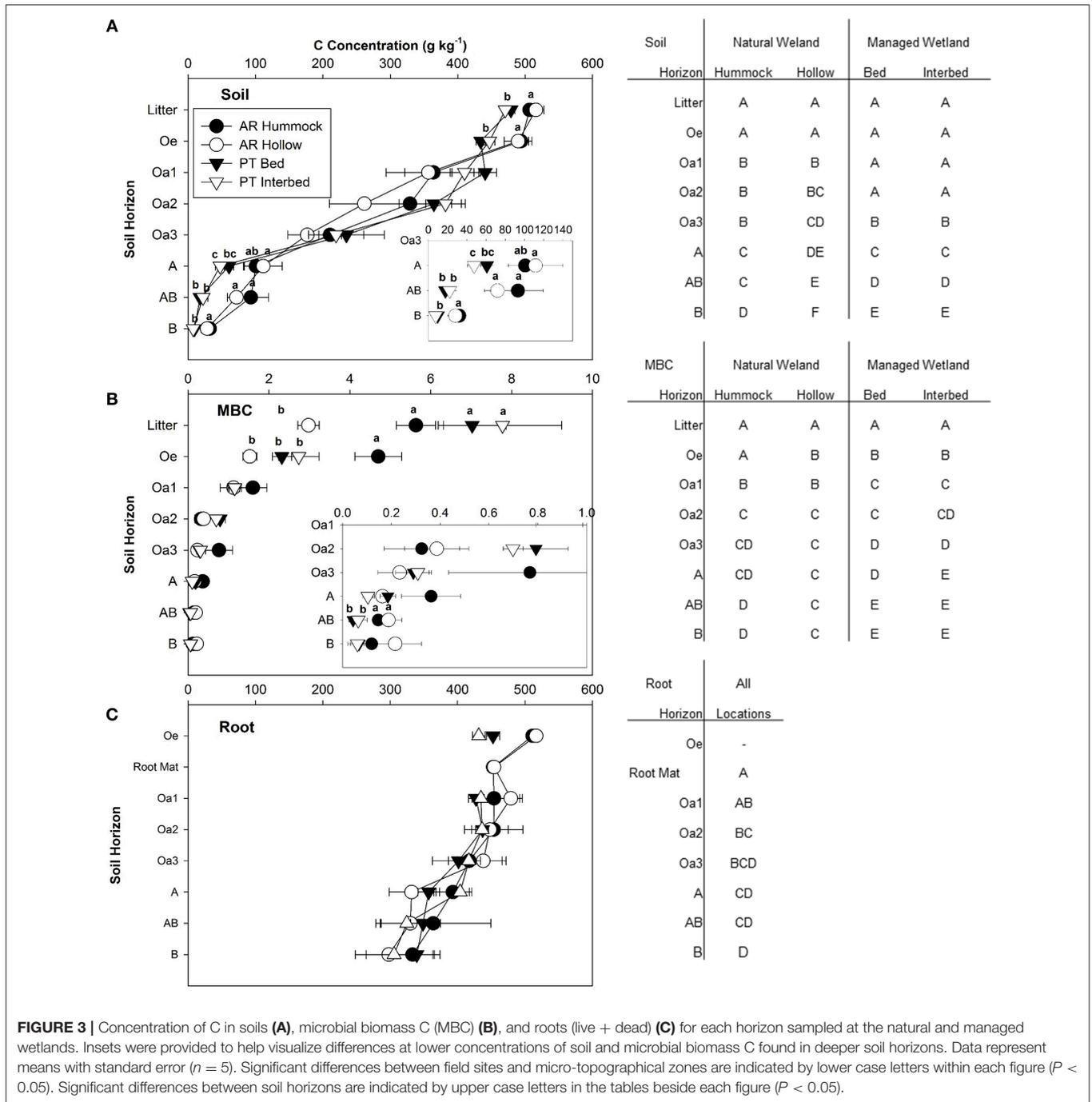
FIGURE 3 | Concentration of C in soils **(A)**, microbial biomass C (MBC) **(B)**, and roots (live + dead) **(C)** for each horizon sampled at the natural and managed wetlands. Insets were provided to help visualize differences at lower concentrations of soil and microbial biomass C found in deeper soil horizons. Data represent means with standard error ($n = 5$). Significant differences between field sites and micro-topographical zones are indicated by lower case letters within each figure ($P <$ 0.05). Significant differences between soil horizons are indicated by upper case letters in the tables beside each figure ($P < 0.05$).

potential contribution of microbial biomass to the SOC pool and its influence on the ^{13}C signature of SOC. Litter and root ^{13}C were more similar to that of SOC with depth in the natural wetland, indicating slowed decomposition and preservation of plant-derived organic matter under more saturated conditions found in the natural wetland. It is important to note that in this study live and dead roots were not separated, but the decrease in root C concentration and enrichment of root ^{13}C with depth at both sites suggest an increasing contribution of dead, partially

decomposed roots to the total live + dead root pool with soil depth. Interestingly, MBC in the natural wetland was similar to or greater than that of the managed wetland in the litter and Oe horizon, respectively, with micro-topography as a clear source of spatial heterogeneity. These results provide evidence of the important role hydrology and micro-topography play in controlling SOC patterns in coastal freshwater forested wetlands, and shed light on how drainage of freshwater wetlands in coastal regions alter soil and microbial carbon pools and storage.
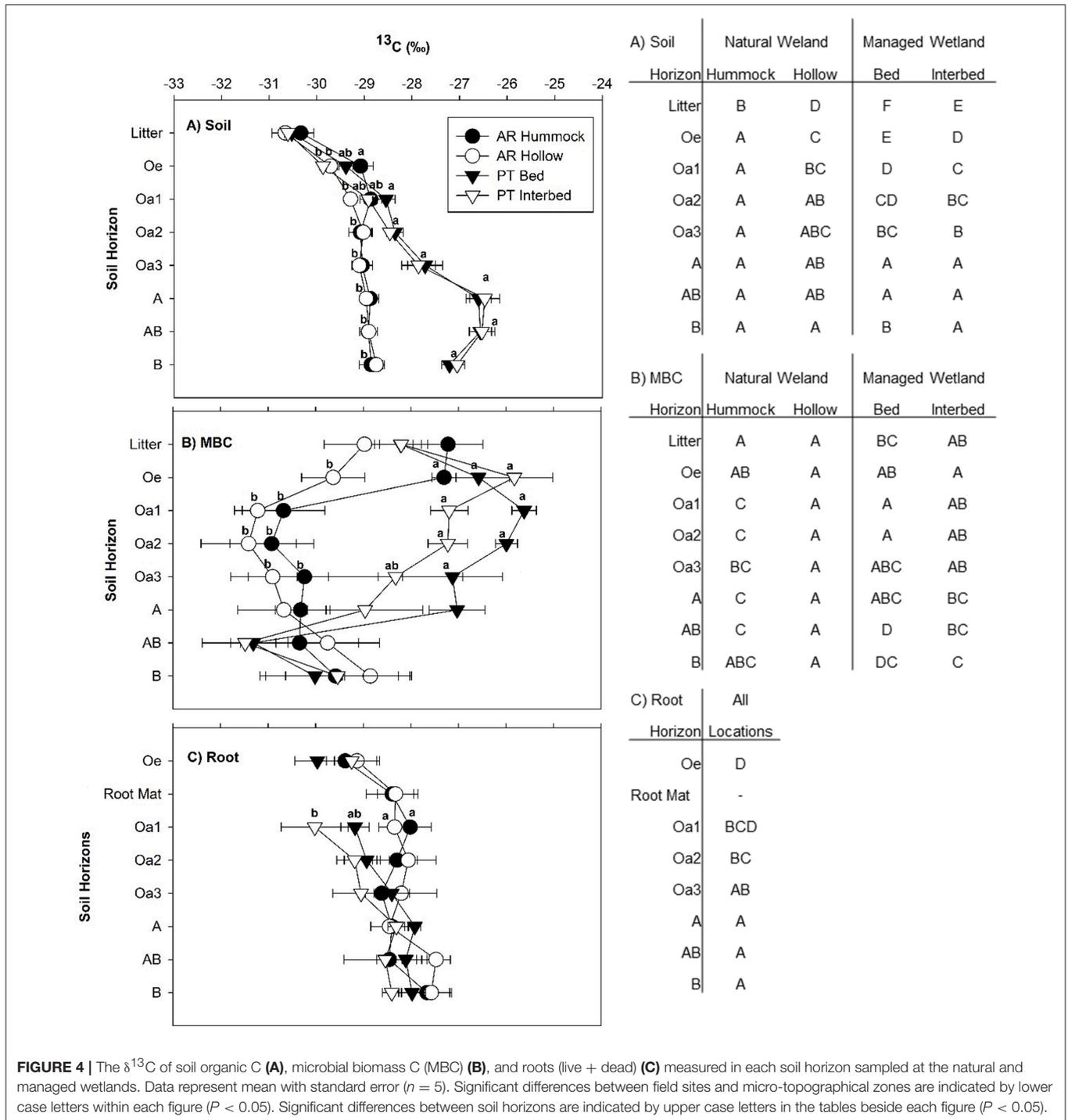
FIGURE 4 | The δ^{13}C of soil organic C **(A)**, microbial biomass C (MBC) **(B)**, and roots (live + dead) **(C)** measured in each soil horizon sampled at the natural and managed wetlands. Data represent mean with standard error (n = 5). Significant differences between field sites and micro-topographical zones are indicated by lower case letters within each figure (P < 0.05). Significant differences between soil horizons are indicated by upper case letters in the tables beside each figure (P < 0.05).

Concentration of SOC and MBC decreased rapidly with depth at both wetland sites regardless of micro-topographical position, similar to what has been found in tropical and temperate wetlands (Bernal and Mitsch, 2008; Krüger et al., 2014), as well as in loblolly pine plantations of the lower coastal plains of North Carolina (Minick et al., 2017b). We found that MBC was positively correlated with SOC, as more C can support a

larger microbial population (Poret-Peterson et al., 2007), with the greatest degree of variability in MBC in the litter layer. For MBC, concentrations ranged from 0.04 g kg^{-1} to 7.23 g kg^{-1} in the natural wetland and from 0.01 to 12.13 g kg^{-1} in the managed wetland. Our wide ranging values for MBC span litter to organic to mineral soils and are in agreement of soils from other wetland sites (0.9 and 6.0 g kg^{-1}; Groffman et al.,

The C concentration and δ^{13}C of fresh litter for major tree species in the natural and managed wetland.

	Species	C (g kg^{-1})	δ^{13}C (‰)
Natural wetland	*Acer rubrum*	469 ± 6.1[bc]	−30.3 ± 0.02[c]
	Nyssa biflora	460 ± 4.2[c]	−31.5 ± 0.02[e]
	Taxodium distichum	488 ± 11.1[b]	−28.3 ± 0.02[a]
	Pinus taeda	512 ± 5.1[a]	−30.0 ± 0.01[b]
Managed wetland	*Pinus taeda*	513 ± 2.4[a]	−31.4 ± 0.04[d]

Data represent average with standard error (n = 5). Significant differences between tree species are indicated by superscript lower case letters (P < 0.05).

1996; Rokosch et al., 2009), organic soils from upland hardwood forest spodosols (7.7–10.8 g kg^{-1}; Fisk et al., 2015), mineral soils from coastal North Carolina (0.04–0.56 g kg^{-1}; Li et al., 2004; Zhang et al., 2018), forest floor from loblolly pine plantations (8.1–13.7 g kg^{-1}; Allen and Schlesinger, 2004), and mineral soils from loblolly pine plantations across the southeastern US (0.05–0.73 g kg^{-1}; Lee and Jose, 2003; Rifai et al., 2010). In the natural wetland, higher MBC was found in the litter (Oi) and surface organic horizon (Oe) of hummocks compared to hollows. Furthermore, MBC in litter and Oe horizons from hummocks of the natural wetland were similar or higher, respectively, compared to the managed wetland. This suggests that hummocks are biogeochemical hotspots in natural wetlands, acting as localized zones of intense plant and microbial activity with large and biologically active C pools (Kim and Verma, 1992; Belyea, 1996; Belyea and Clymo, 2001; Miao et al., 2017; Minick et al., 2018). These results indicate a robust separation of organic and mineral soil horizons with distinct chemical and biological properties, reflecting the type of C within the soil profile and its chemical properties (Trettin and Jurgensen, 2003).

In support of our second hypothesis, the ^{13}C signature of SOC changed minimally with depth in the natural wetland, but became increasingly enriched with depth at the managed wetland, similar to that observed in upland ecosystems (Wang et al., 2015). This is not necessarily surprising, as hydrologic conditions in wetland soils tend to maintain SOC in a form more closely resembling that of original plant-derived organic matter, whereas less saturated soils have faster decomposition rates which process soil organic matter into amorphous organic materials. von Fischer and Tieszen (1995) and Krüger et al. (2014) found minimal or inconsistent effects of soil depth (down to 60 cm) on the ^{13}C isotopic enrichment of SOC with soil depth in anaerobic soils of forested wetlands in Puerto Rico, while Hobbie et al. (2017) found consistent enrichment of ^{13}C of peat up to ~100 cm depth in a northern forested bog. The slight increase in δ^{13}C below the Oe horizon in the natural wetland suggests some degree of decomposition (Alewell et al., 2011), which was also evident by the lack of fibrous material upon visual inspection of the Oa compared to Oe soil horizons. Further, no differences in δ^{13}C between hummocks and hollows were found below the Oe horizon of the natural wetland, suggesting that these deeper soil horizons were more frequently submerged than the hummock Oe horizon which promotes leaching of water soluble, ^{13}C-enriched

organic compounds (e.g., carbohydrates) and retention of lower quality, non-extractable ^{13}C-depleted organic compounds (e.g., lignin, phenol; Lerch et al., 2011). In the managed wetland, ^{13}C enrichment of SOC with depth suggests enhanced decomposition (Natelhoffer and Fry, 1988; von Fischer and Tieszen, 1995) and incorporation of ^{13}C-enriched microbial biomass into SOC (von Fischer and Tieszen, 1995; Schmidt et al., 2011). As SOC concentration increased, ^{13}C of SOC became more depleted, reflecting the presence of ^{13}C-depleted plant-derived chemical constituents of higher C concentration in a less decomposed state (Hobbie et al., 2017). Alternatively, SOC of more decomposed, lower C substrates were ^{13}C-enriched indicating that this C was of a different nature than that of less decomposed, higher C substrates (Hobbie et al., 2017). Enrichment of ^{13}C SOC with depth may also reflect the assimilation of ^{13}C-depleted CO_2 into new plant biomass and deposition of this organic matter to surface soils as atmospheric CO_2 has become more depleted in ^{13}C over time due to continued burning of fossil fuels (Keeling et al., 2017). In the natural wetland, no difference was found between the δ^{13}C of the Oe surface horizon (~2–10 cm depth, **Table 1**) and any other surficial soil horizon in either hummocks (above the water table) or hollows (below the water table), suggesting that the decline in δ^{13}C of atmospheric CO_2 has not influenced observed patterns of δ^{13}C of SOC with depth at this wetland site. In the managed wetland, the change in δ^{13}C of surface litter to the mineral soil was ~-4‰, while the average change in δ^{13}C of atmospheric CO_2 since pre-industrial times (1880's) was −2‰ (Keeling et al., 2017). Therefore, this only accounts for ~2‰ of this difference, leaving the remaining 2‰ difference as a result of biological processes during decomposition.

Dominant vegetation type, the associated water-use efficiency (WUE), and water availability have also been shown to impact stable C isotope composition of plant biomass with potential impacts to SOC (Farquhar and Richards, 1984; Ni and Pallardy, 1991). For instance, Peri et al. (2012) suggested that a negative relationship between SOC ^{13}C and water availability resulted from a decline in water-use efficiency at wetter sites promoting depletion of ^{13}C in plant biomass and soil. Alternatively, under drought stress or at drier sites, trees reduce stomatal aperture in order to lessen water loss during photosynthesis, with the effect of reducing photosynthetic ^{13}C isotopic discrimination and enriching ^{13}C of plant biomass and potentially that of SOC (Farquhar and Richards, 1984; Gebrekirstos et al., 2011; Werner et al., 2012). The δ^{13}C values of deciduous and pine tree litter from a temperate forest ranged from −27‰ (*Pinus* taeda and *Pinus echinata*) to −30‰ (*Nyssa sylvatica*) (Garten and Taylor, 1992), although their analyses were of tree species growing along water stressed ridge tops which exhibited higher water-use efficiency and ^{13}C-enriched biomass compared to lower elevation, non-water stressed environments. In the current study, loblolly pine litter from the managed wetland was depleted in ^{13}C (−31.4 ± 0.04‰) compared to that of the natural wetland (−30.0 ± 0.01‰; **Table 3**), indicating that these pines have lower water-use efficiency as a result of high water availability in comparison to those measured in Garten and Taylor (1992). Furthermore, differences in bulk root ^{13}C between sites were

A) MBC ^{13}C - SOC ^{13}C	Natural Weland		Managed Wetland	
Horizon	Hummock	Hollow	Bed	Interbed
Litter	A	A	AB	AB
Oe	AB	AB	A	A
Oa1	C	C	A	B
Oa2	C	C	A	B
Oa3	BC	BC	AB	BC
A	C	BC	BC	DC
AB	C	BC	D	D
B	BC	ABC	DC	DC

A) MBC ^{13}C - Root ^{13}C	Natural Weland		Managed Wetland	
Horizon	Hummock	Hollow	Bed	Interbed
Litter	-	-	-	-
Oe	A	A	A	A
Oa1	B	AB	AB	A
Oa2	B	B	AB	A
Oa3	B	AB	AB	AB
A	B	AB	BC	BC
AB	B	AB	CD	BC
B	B	AB	D	C
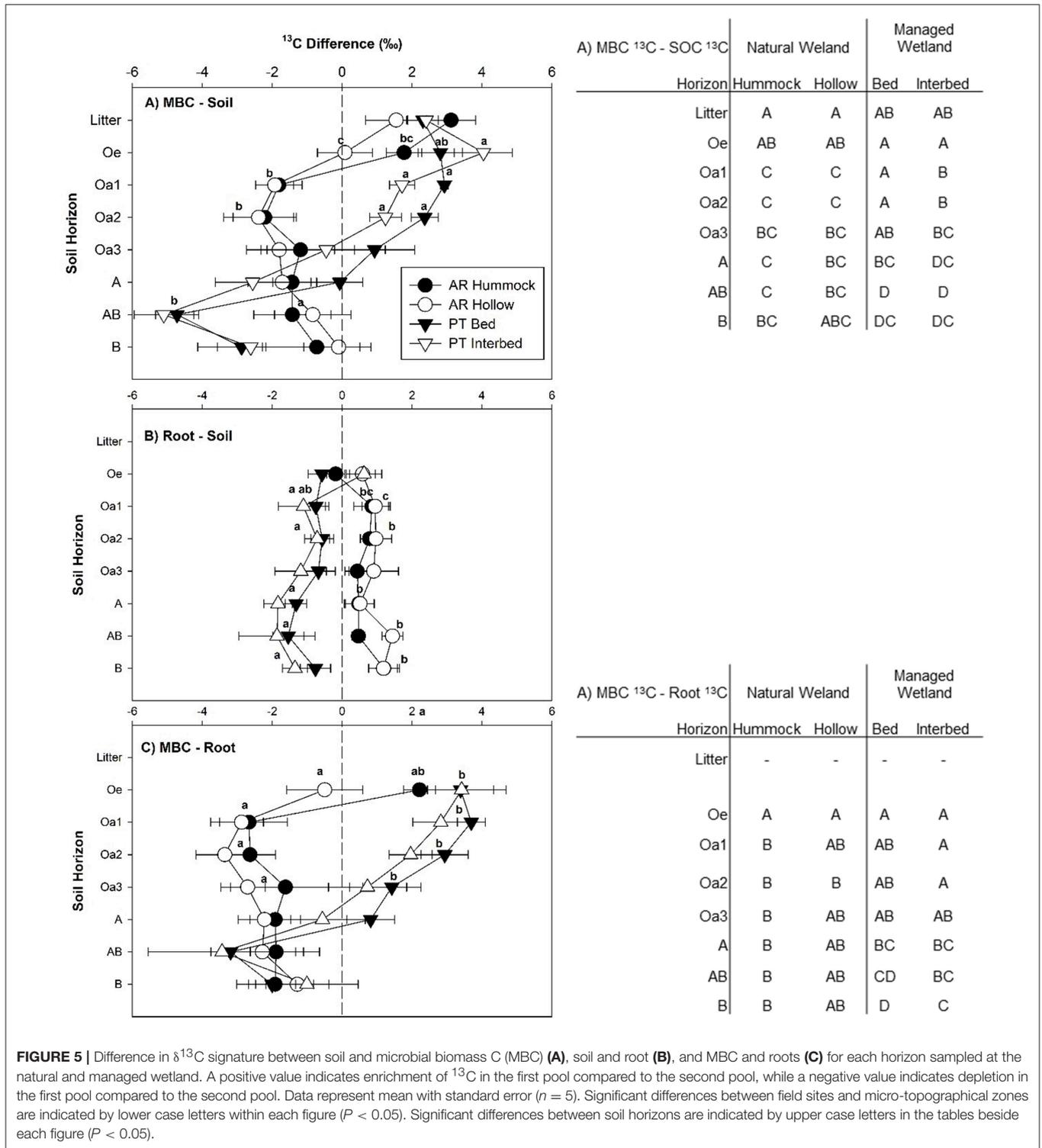
FIGURE 5 | Difference in δ^{13}C signature between soil and microbial biomass C (MBC) **(A)**, soil and root **(B)**, and MBC and roots **(C)** for each horizon sampled at the natural and managed wetland. A positive value indicates enrichment of ^{13}C in the first pool compared to the second pool, while a negative value indicates depletion in the first pool compared to the second pool. Data represent mean with standard error ($n = 5$). Significant differences between field sites and micro-topographical zones are indicated by lower case letters within each figure ($P < 0.05$). Significant differences between soil horizons are indicated by upper case letters in the tables beside each figure ($P < 0.05$).

minimal and, as such, ^{13}C of plant tissue does not explain the patterns of ^{13}C enrichment of SOC at the managed wetland. Therefore, the major driver of the observed patterns of SOC ^{13}C with soil depth between the managed and natural wetland is likely the effect of drainage on decomposition dynamics and

not nuanced differences between plant species or WUE effects on plant biomass ^{13}C.

The δ^{13}C signature of MBC varied minimally between soil horizons at both wetland sites, but was influenced by drainage and micro-topography. In the organic horizons, ^{13}C of MBC

FIGURE 6 | Regression analysis comparing relationships across all soil horizons between soil organic carbon (SOC) and microbial biomass C (MBC) pool size **(A)**, δ^{13}C of SOC **(B)**, δ^{13}C of MBC **(C)**, and **(D)** the difference in δ^{13}C between MBC and SOC (positive value indicates MBC is enriched in ^{13}C compared to SOC and negative value indicates MBC is depleted in ^{13}C compared to SOC). Adjusted R^2- values are presented in parenthesis for any significant correlation found for the four site by micro-topographic location combinations.

was enriched in the managed wetland compared to the natural wetland, with one major exception. In the natural wetland, ^{13}C of MBC in the Oe horizon from the hummock was similar to that of the managed wetland, but enriched compared to hollows of the natural wetland. The ^{13}C of MBC was also depleted in the mineral horizons compared to the organic horizons of the managed wetland and similar to that of the natural wetland. This may reflect the saturation of mineral horizons of the managed wetland due to a relatively high water table even under drained conditions, given the depths of mineral soils sampled (**Table 1**) are below the water table numerous times throughout year (**Figure 2**). We also found that ^{13}C of MBC is generally enriched compared to SOC at high SOC concentrations (primarily in the managed wetland) when SOC more closely resembles litter (or is litter). But as SOC concentration decreased, MBC tended to be depleted in ^{13}C compared to SOC. For example, SOC with concentrations below 200 g kg^{-1} in the managed wetland (which were associated with mineral soil horizons) were enriched in ^{13}C compared to MBC, while SOC with concentrations above 200 g kg^{-1} (which were associated with organic soil horizons) were depleted in ^{13}C compared to MBC. By comparison, in

the natural wetland, MBC tended to be ^{13}C-depleted compared to SOC for all soil horizons except the Oe. Previous studies have found that ^{13}C of microbial biomass is typically enriched compared to the bulk SOC pool (Dijkstra et al., 2006; Bernal and Mitsch, 2008; Werth and Kuzyakov, 2010; Lerch et al., 2011), but results from our study only partially agree with these observations. The ^{13}C-enrichment of MBC compared to SOC indicates microbes are degrading ^{13}C-enriched biochemical constituents under non-saturated or aerobic conditions (Bowling et al., 2008). Furthermore, the ^{13}C of MBC is expected to parallel that of SOC (Dijkstra et al., 2006), but little to no relationship was found between MBC ^{13}C and SOC ^{13}C in our study. Therefore, these results do not support our third hypothesis, in that ^{13}C of MBC did not necessarily reflect the ^{13}C of SOC. As noted, Dijkstra et al. (2006) found a strong positive correlation between ^{13}C of MBC and SOC in A horizon mineral soils which were characterized by a much smaller range of SOC concentrations (1.4–4.4%) and wider range of ^{13}C (−3.0 to −24.5‰) compared to our study. We are not aware of any studies which compared MBC isotope composition across soil horizons in wetland soils or soils spanning such

FIGURE 7 | Regression analysis comparing relationships across all soil horizons between soil water content and soil organic carbon (SOC) content **(A)**, microbial biomass C (MBC) **(B)**, $\delta^{13}C$ of SOC **(C)**, and $\delta^{13}C$ of MBC **(D)**. Adjusted R^2-values are presented in parenthesis for any significant correlation found for the four site by micro-topographic location combinations.
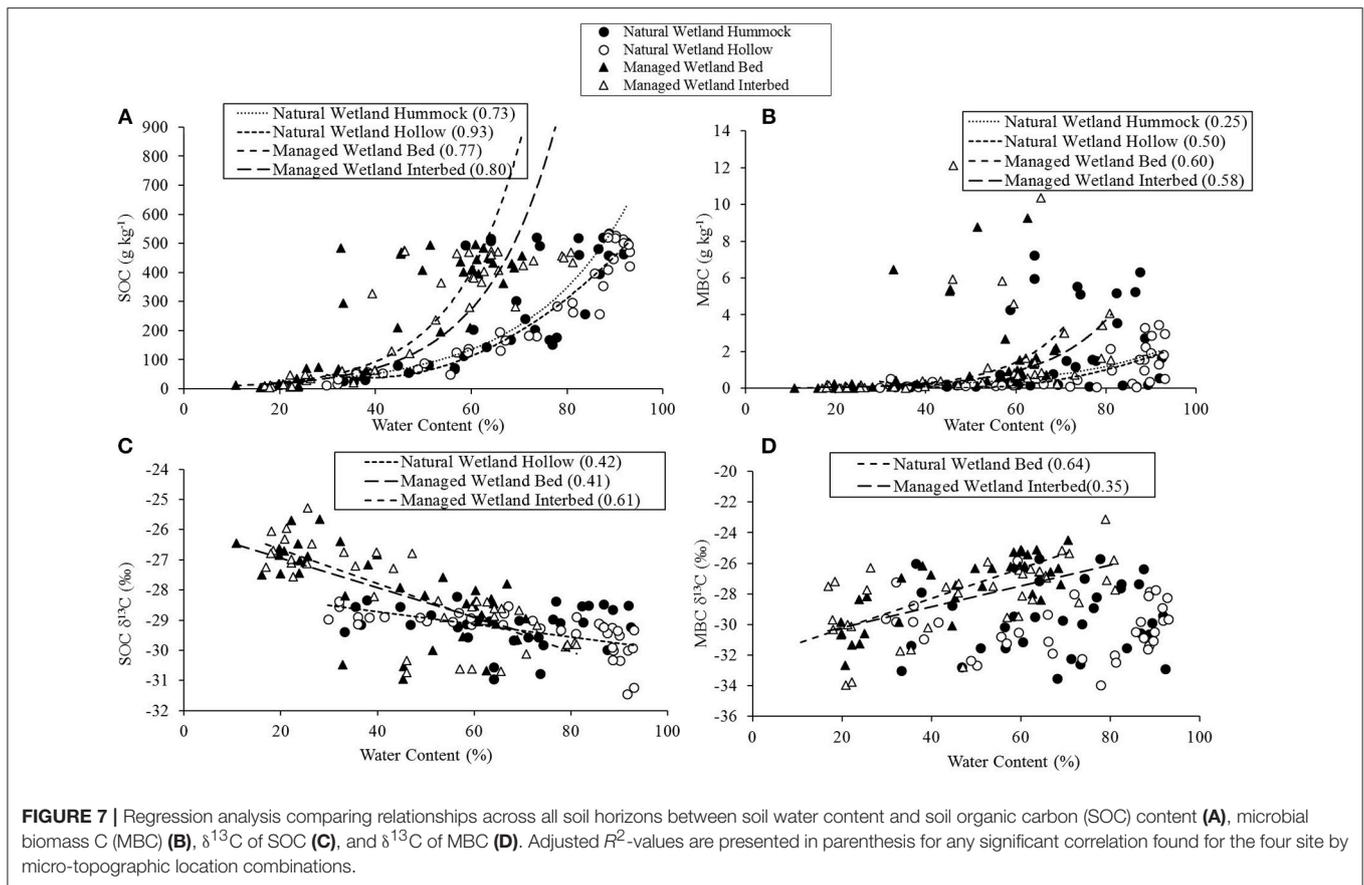
a large range of SOC concentrations. Therefore, our results shed new light into the pattern of MBC and SOC with soil depth in natural and managed wetland ecosystems and over a wide range of SOC and MBC concentrations, but narrow range of ^{13}C.

Only a few studies have reported depleted ^{13}C signatures of microbial biomass in comparison to SOC (Blair et al., 1985; Wick et al., 2003; Werth and Kuzyakov, 2010). Werth and Kuzyakov (2010) reported that the chloroform-fumigation extraction procedure resulted in an enriched microbial ^{13}C signature compared to bulk soil while the chloroform-fumigation incubation procedure resulted in a depleted microbial ^{13}C signature compared to bulk SOC. The current study utilized the chloroform-fumigation extraction procedure and measured a shift from an enriched to depleted MBC ^{13}C signature with depth at both sites, indicating that these results were not likely due to experimental artifacts. The ^{13}C-depletion of MBC compared to SOC with soil depth likely indicates: (1) microbes have shifted substrate use to more ^{13}C-depleted biochemical constituents under saturated or anaerobic conditions (von Fischer and Tieszen, 1995; Bowling et al., 2008; Werth and Kuzyakov, 2008), and as previously mentioned, leaching of ^{13}C-enriched compounds (e.g., sucrose, starch; Bowling et al., 2008; Lerch et al., 2011) under saturated conditions could be one explanation for the observed patterns of SOC ^{13}C and MBC ^{13}C in the natural

wetland; and/or (2) potential shifts in microbial community structure (Boschker et al., 1999).

A change in the microbial community structure could also confer changes in microbial extracellular enzyme production and substrate use (Lerch et al., 2011; Singh et al., 2018), and therefore drive observed patterns of MBC ^{13}C under different environmental conditions found in coastal forests (Boschker et al., 1999; Wang et al., 2015). Soil organic C is also a complex mixture of organic compound classes, which exhibit a range of ^{13}C (Bowling et al., 2008), and therefore changes in the microbial community structure and/or enzyme capacity of the soil microbial community could alter microbial C substrate use and $\delta^{13}C$ of microbial biomass. Unpublished data from the natural wetland indicate that fruiting bodies from various fungal groups (e.g., basidiomycetes, saprotrophic fungi) are enriched in ^{13}C compared to SOC, which has been found in numerous other studies (Gleixner et al., 1993; Hobbie et al., 1999), but a shift to a bacterial dominated microbial community is likely under saturated conditions like those found in deeper soil horizons. Dijkstra et al. (2006) and others (Gleixner et al., 1993; Hobbie et al., 1999; Kohzu et al., 1999) have all suggested that various fungal groups (e.g., mycorrhizal, basidiomycetes, saprophytic fungi) are enriched compared to the SOC pool, while some bacterial groups (as measured by PLFA) can be either enriched or depleted compared to the decomposing substrate (Blair et al.,

1985; Boschker et al., 1999; Wick et al., 2003; Lerch et al., 2011). Therefore, a shift to a more bacteria-dominated microbial community may also explain the observed shift in MBC to depletion in ^{13}C compared to SOC under saturated conditions. This shift also likely occurs higher up in the soil profile in the natural wetland due to the higher water table, as indicated by the more depleted MBC in the upper soil horizons of the natural wetland compared to the managed wetland.

In conclusion, we found that hydrology, and to a lesser degree micro-topography, was important in shaping the size and stable C isotopic signature of soil and microbial C pools in these natural and managed coastal freshwater forested wetlands. We have highlighted that within the natural wetland, characterized by hummock-hollow micro-topography, surface soils of hummocks are a highly active zone of microbial soil C processing, comparable to or greater than that of drained wetlands. Our data suggest that management of wetlands for loblolly pine production leads to more advanced breakdown of SOC, although the effects of this on the total ecosystem C balance remains unknown. The decreased saturation of soils in drained wetlands likely leads to more processing of organic matter compared to the natural wetland (Kasimir et al., 2018), and therefore a greater contribution of microbes to the soil organic matter pool over time, resulting in the observed enrichment of ^{13}C in SOC with depth. It is possible that enhanced breakdown of SOC in managed wetlands may be counterbalanced by the growth of highly productive loblolly pine trees and enhanced above- (e.g., litterfall) and below-ground (e.g., root productivity and turnover) inputs (Noormets et al., 2015), but this will require future studies to characterize all C pools in the critical zone of these ecosystems (Minkkinen and Laine, 1998). Taken together, our results suggest that saturated soils in natural forested wetland ecosystems along the lower coastal plains of the southeastern US influence C isotopic composition across the soil profile by slowing decomposition of plant-derived C compounds, and alter the relationships between soil chemical and biological properties that are typically observed in non-wetland ecosystems (Dijkstra et al., 2006; Bernal and Mitsch, 2008; Werth and Kuzyakov, 2010). As coastal wetlands are rapidly being altered by humans, and simultaneously threatened by climate change and sea level rise, understanding the relationships between organic C inputs, microbial community composition and function, as influenced by hydrology and management, is fundamental to our capacity to project impacts on regional-to-global SOC cycling and storage. Essential to this challenge will be to expand such studies to other similar sites across the region to increase confidence in the conclusions and practical application of these results.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study. KM wrote the first draft of the manuscript. KM and XL collected the samples from the field. KM performed laboratory analysis. BM performed the water table analysis. All authors contributed to manuscript revision and approved the submitted version.

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