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Soil carbon characterization in a subtropical drained peatland

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ABSTRACT

Peatlands store a significant portion of the global carbon (C) budget. Peatland drainage triggers soil subsidence and is a threat to soil conservation. Differences in C composition as soils get shallower in drained peatlands are not clearly understood. Soil C quality has been considered as one of the possible causes for changes in soil subsidence rates in drained peatlands. The purpose of this research was to characterize soil C in drained peats experiencing soils subsidence by using a fractionation procedure and ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) spectroscopy. The study was done in the Everglades Agricultural Area (EAA), located south of Lake Okeechobee in south Florida, US. The EAA is a subtropical peatland that has been drained for more than 100 years. Subsidence rates at the EAA have shown a reduction in recent decades possibly due to higher water tables and increases in inorganic and recalcitrant C (Shih et al., 1998). Analyses were performed for the top and bottom of sampled peat soils collected from shallow and deep soil areas within the EAA. Hot water (80 °C) and acid extractions were used to determine operational C fractions with residues characterized by magic angle spinning (MAS) ^{13}C NMR spectroscopy before and after extraction to quantify C compound classes. Recalcitrant C is the dominant C pool in EAA soils with 85% of total carbon (TC) on average, while acid extracted C, hot water extracted C, and inorganic C comprise 19%, 1%, and 0.7% of TC, respectively. Shallow soils had significantly less organic matter (OM), hot water extracted C, and recalcitrant C, and showed a reduction of OM and C fractions deeper in the soil profile. The ^{13}C NMR spectra analyses indicate that average C compounds in EAA peats are *O*-alkyl C (36.9%), followed by alkyl C (28.2%), aromatic C (24%), and carboxyl C (10.8%), with shallow and deep soils having a similar distribution of these C compounds. Extracted recalcitrant C fractions of both shallow and deep soils showed an increase in aromatic C (34.3%) and a reduction in *O*-alkyl C (26.6%). Subsidence reduced OM in shallow soils and can reduce soil C, including the recalcitrant C pool, which might affect N cycling and nutrient availability. Addition of fresh biomass into the soil (e.g. sugarcane crop residue) might increase labile C reducing subsidence and improving soil conservation in the EAA and other drained peatlands.

1. Introduction

Soils store between 1500 and 2400 Pg C, which is a significant portion of the global carbon budget (Ciais et al., 2014). Estimates indicate that 529 Pg C are stored in wetlands worldwide, this significant amount of C can potentially be released to the atmosphere due to human activities such as land use change (Bridgham et al., 2006). Many of these organic soil wetlands developed during the deglaciation period (8000–12,000 years ago), although most peatlands are in boreal high latitudes, coastal areas that experienced sea level rise, and flooding, such as Florida, also contain histosols (organic rich soils) (Armentano and Menges, 1986). The slow rates of decomposition in wetlands are conducive to the formation of peat soils. Peat soils have high organic matter content; due to slow decomposition, dark color, low bulk density, large porosity that decreases with increasing degree of decomposition, high nutrient storage capacity seen by high cation exchange capacity,

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Abbreviations: EAA, Everglades Agricultural Area; NMR, Nuclear Magnetic Resonance; MAS, Magic Angle Spinning; OM, organic matter; IC, inorganic carbon; HW ex, hot water extracted carbon; HCl ex, HCl extracted carbon; Rec C, recalcitrant carbon.

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and generally low pH (Lucas, 1982; Stephens et al., 1984; Kasimir-Klemedtsson et al., 1997). Peat accumulation in peatlands occurs when organic soils increase in mass by lateral spread or vertical accretion (Armentano, 1980). Peat accumulation rates of peatlands vary depending on climate conditions, being generally higher in tropical regions; for example, rates between 0.54 and 1.89 mm yr^{-1} have been reported for Southeast Asia (Dommain et al., 2011), compared to estimates of 0.2 to 1 mm yr^{-1} for temperate peatlands and 0.2 to 0.8 mm yr^{-1} for boreal peatlands (Page et al., 2006). Under flooded conditions, slower oxygen diffusion rates into the soil limit the availability of electron acceptors and aerobic bacterial activity, which slows down organic matter decomposition and is conducive to peat accumulation (Tate, 1980; Reddy and DeLaune, 2008). Therefore, peatland drainage reverses these conditions and triggers soil subsidence, which is the reduction in elevation in the soil resulting from a combination of leaching (due to water erosion), burning, compaction (due to the use of machinery and tillage), shrinkage (occurring to peats above the water table), consolidation (which occurs below the water table due to the loss of buoyancy), and oxidation (decomposition under aerobic conditions) processes in the soil (Stephens et al., 1984; Pronger et al., 2014).

Soil subsidence might change soil C composition in drained peatlands by degrading labile C compounds and enriching soils with recalcitrant C, more resistant to oxidation, therefore lowering subsidence rates (Shih et al., 1998). The Everglades Agricultural Area (EAA) located in south Florida, U.S. is a subtropical drained peatland that has experienced a reduction in subsidence rates from 2.5 cm yr^{-1} in the 1950s to 1.45 cm yr^{-1} (Shih et al., 1998; Snyder, 2005). The EAA comprises approximately 36% of the original Everglades ecosystem (Daroub et al., 2011). Peat accumulation in the EAA began 5000 years ago (Gleason and Stone, 1994), and occurred at an average rate of 0.08 cm yr^{-1} before human intervention (McDowell et al., 1969). Peat deposits in the EAA are deeper in the north and shallower at its southern edge (Aich et al., 2013). Drainage of the EAA began in 1917 with the construction of four major canals (Light and Dineen, 1994). Because of drainage, EAA peats have experienced soil subsidence for more than 100 years. The initial drainage of the EAA produced accelerated subsidence controlled by shrinkage and compaction of the drained peats (Stephens et al., 1984). A more constant subsidence rate of 2.5 cm yr^{-1} controlled by oxidation took place afterwards (Stephens, 1956; Stephens and Speir, 1970). From 1978 subsidence rates have been reduced possibly due to an increase in inorganic and recalcitrant C content, and higher water tables (water tables kept closer to the soil surface) due to the implementation of best management practices for phosphorus reduction, such as flooding fallow fields, and a longer retention of rainwater in the fields before being drained (Shih et al., 1998; Snyder, 2005; Daroub et al., 2011).

The diverse compounds that comprise soil organic matter can be separated using different fractionation techniques (Swift, 1996). Some of these techniques include density fractionation (Golchin et al., 1994; Helfrich et al., 2006), aggregate size fractionation (Wright and Inglett, 2009), and acid hydrolysis. By separating organic matter into labile compounds, that experience fast biodegradability (e.g., carbohydrates, and proteins), and recalcitrant compounds, that experience slower biodegradability (e.g., lignin, and humic substances), it is possible to determine how easily organic matter can be degraded (Rovira and Vallejo, 2000). The acid hydrolysis method has been widely used to determine labile and recalcitrant organic C pools (Oades et al., 1970; Silveira et al., 2008; Belay-Tedla et al., 2009; Knox et al., 2015), most commonly 6 M Hydrochloric acid (HCl) is used to separate labile (hydrolyzed compounds) from recalcitrant (unhydrolyzed compounds). The hot water extraction obtains labile C, which includes soluble carbohydrates and amines (Ghani et al., 2003), and has been found to be highly correlated with microbial biomass carbon and total carbon (Sparling et al, 1998; Gregorich et al., 2003).

The chemical composition of C compounds in organic soils has been studied with techniques such as Fourier Transformed Infrared spectroscopy (Normand et al., 2017), Pyrolysis-gas chromatography-mass spectroscopy (Zhang et al., 2019), and with ^{13}C NMR spectroscopy (Golchin et al., 1994; Kögel-Knabner, 1997; Knicker and Skjemstad, 2000). Solid-state ^{13}C NMR spectroscopy allows determination of the chemical moieties present in soils and organic matter based on the intensities observed over the measured ^{13}C chemical shift range. In soil studies, five chemical regions have been defined: alkyl region (0–45 ppm), which represents aliphatic C in fatty acids, amino acids or paraffinic structures; methoxyl region (45–60 ppm); carbohydrate region (60–110 ppm); aryl (aromatic) region (110–160 ppm), which includes aromatic and phenolic compounds, and carboxyl region (160–220 ppm), which represents aliphatic esters and carbonyl-C of lignin, hemicelluloses, lipids, and proteins (Kögel-Knabner, 1997). The use of ^{13}C NMR spectroscopy allows determination of the degree of decomposition in soils by comparing changes in spectral regions (Golchin et al., 1994; Helfrich et al., 2006; Normand et al., 2017). The relative abundance of the ^{13}C NMR spectral regions has also been used to establish the origin of the C inputs going into the soil (Helfrich et al., 2006), and to identify the effect of fires in soil organic matter (Knicker and Skjemstad, 2000; Rovira et al., 2012). The application of ^{13}C NMR spectroscopy along with C fractionation procedures allows the characterization of C pools. Studies that have combined these two techniques have shown changes in spectral regions such as increases in the alkyl-C region (Rovira and Vallejo, 2002; Silveira et al., 2008), and the aromatic C region (Silveira et al., 2008; Dodla et al., 2012) after acid extraction. On the other hand, the carbohydrate (*O*-alkyl C) region has been found to decrease after acid extraction (Silveira et al., 2008).

Differences in C composition as soils get shallower in drained peatlands are not clearly understood. The determination of the labile and recalcitrant C pools in drained peats can be useful to assess the soils' vulnerability, and to better understand the role of C composition in oxidation changes that can contribute to subsidence. The detailed identification of C compounds in drained peats using ^{13}C NMR spectroscopy can provide information on the impact of drainage to these soils, and changes in soil depth to C composition. The understanding of C fractions and C composition in subsiding EAA peats is important to assist in the development of soil conservation strategies to minimize oxidation and nutrient transport out of the EAA. Additionally, the composition of the labile and recalcitrant soil C pools and how they change with soil depth in the EAA is unknown. The objectives of this study were to: (1) compare C pools on shallow and deep drained peats using a C fractionation procedure, and (2) characterize organic C pools by using ^{13}C NMR spectroscopy in drained EAA peats. We hypothesize that shallow soils will have higher recalcitrant and inorganic C compared to deep soils due to the complete drainage under cultivation and proximity to the bedrock of shallow soils compared to deep soils. After more than 100 years of drainage, C composition in EAA peats is expected to be enriched in alkyl C while the more labile C components such as the carbohydrates are expected to be less represented in the ^{13}C NMR spectra.

2. Materials and methods

2.1. Study area

The EAA encompasses an area of 280,000 ha located in south Florida between Lake Okeechobee in the north, and the water conservation areas in the south and the east. The EAA coordinates are: 26° 25′ 16″–26° 57′ 01″ N, 80° 22′ 46″–80° 56′ 57″ W (Fig. 1). The EAA peats originated from sawgrass (*Cladium mariscus* (L.) Pohl ssp. *jamaicense* (Crantz) Kük) deposits and are known as Everglades peats (Gleason and Stone, 1994; Aich et al., 2013). The majority of the EAA (56% of its area) is cultivated with sugarcane (Glaz and Lingle, 2012) with the remaining being used for vegetables, pasture, and rice production (Bottcher and Izuno, 1994; Daroub et al., 2011; Bhadha et al., 2016). Peat soils in the EAA are classified according to the Soil taxonomy key by the Soil Survey Staff of the United States Department of Agriculture as *euic hypothermic*

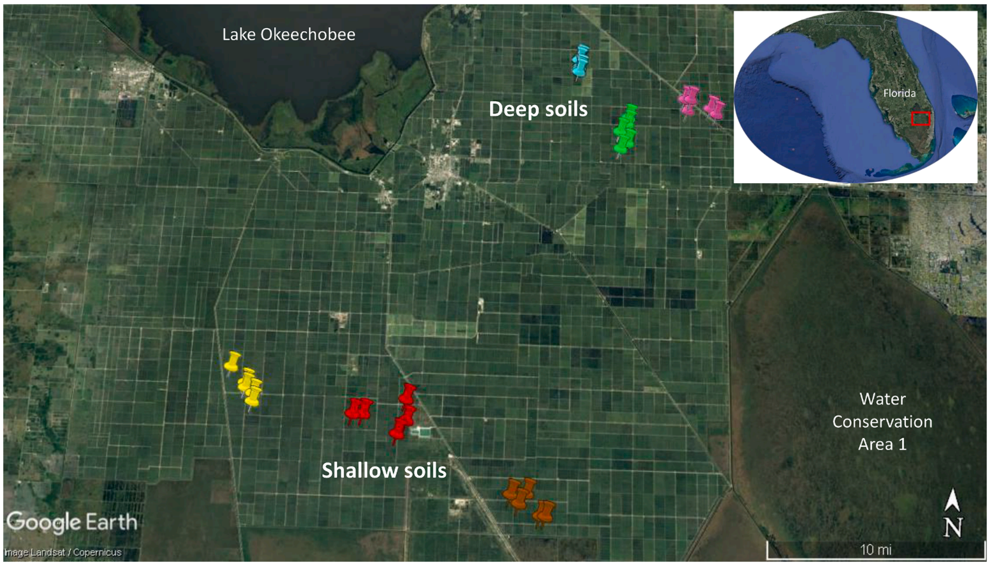

Fig. 1. Map of Florida (top right) and the Everglades Agricultural Area showing soil sampling locations. Colors indicate different farms. Each location is a 40-acre field.

haplosaprists, with a high degree of decomposition, a lack of horizon development, relatively high pH (5.5 to 8) (Snyder, 1994) compared to other peat soils (3.7 to 7.8) (Lucas, 1982), and mean annual soil temperatures higher than 22 °C with a fluctuation of more than 6 °C between summer and winter (Rice et al., 2005). This study was done in soils from the Dania, Lauderhill, and Pahokee series, which are all underlain by limestone rock, have a mineral content less than 35%, and differ based on O horizon depth: Pahokee (91–130 cm), Lauderhill (51–91 cm), and Dania (<51 cm) (Rice et al., 2005).

2.2. Sample collection

Soils from six farms were sampled within the EAA. Farms were selected based on average soil depth data collected between 2013 and 2016 to represent the dominant depths, and soil series found in EAA peats. Shallow soils (Dania soil series, average soil depth: 25 cm) were

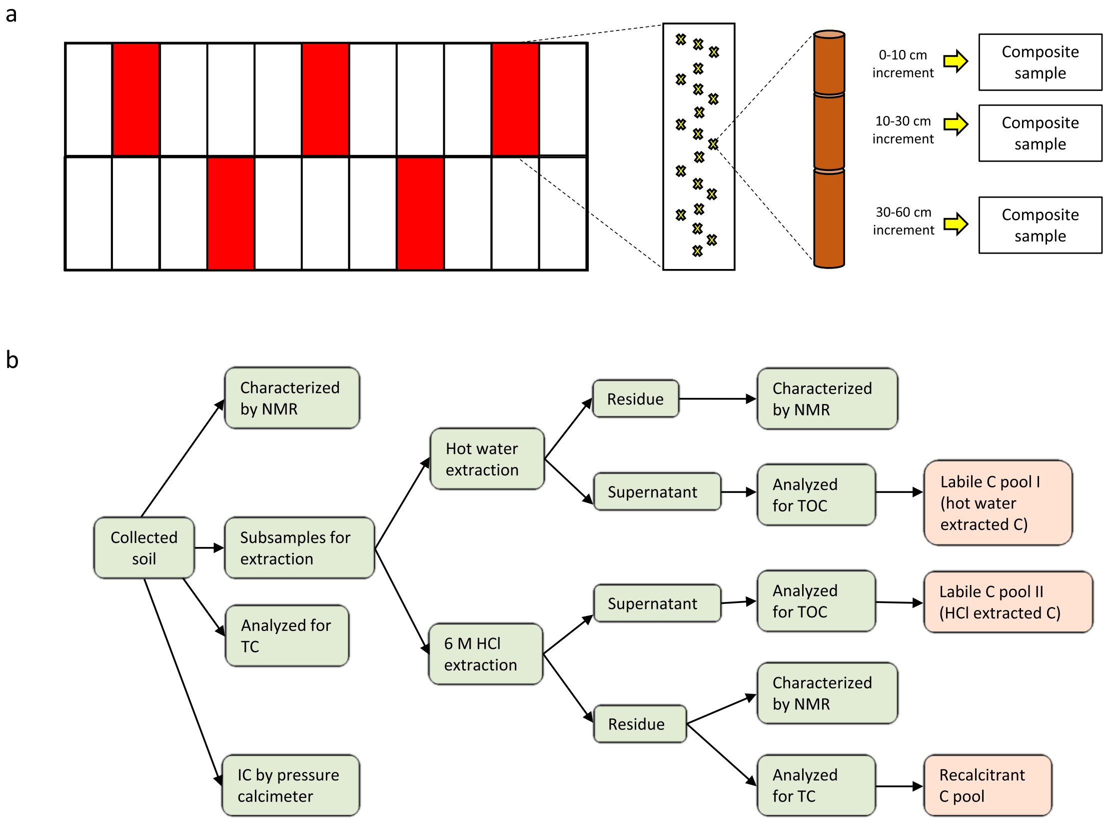

Fig. 2. Diagram of (a) experimental design of a deep soils farm. Red rectangles are selected 40-acre fields, yellow X mark 20 soil cores collected at each 40-acre field, and (b) Carbon fractionation. Hot water extraction based on Ahn et al. (2009), HCl extraction based on Silveira et al. (2008). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

collected from three farms located south of the EAA, while deep soils (Lauderhill and Pahokee soil series, average soil depth: 85 cm) were collected from three farms located north of the EAA (Fig. 1). At the moment of sampling, all soils were drained and under sugarcane cultivation. In each farm, five 40-acre fields were sampled in a zigzag pattern across the entire field, obtaining 20 soil cores, these 20 cores were separated in increments and combined into composite samples (one per increment for each 40-acre field) (Fig. 2a). Cores were collected keeping a buffer zone of 30 m from canals, roads, and ditches. Soil cores were separated in two increments (0–10 cm, and 10–30 cm) for shallow soils, and three increments (0–10 cm, 10–30 cm, and 30–60 cm) for deep soils, if soils were less than 30 cm (for shallow soils) or 60 cm (for deep soils), coring stopped upon hitting the bedrock. A total of 75 samples were collected, 30 samples from shallow soils: (3 farms X 5 40-acre fields X 2 increments), and 45 samples from deep soils: (3 farms X 5 40-acre fields X 3 increments). Soil samples were immediately stored on ice upon collection. At the laboratory, soil samples were air dried (72 hrs. at 21 °C), homogenized, sieved (2 mm), and visible plant materials were removed following the procedure described by Silveira et al. (2008). Soil samples were then refrigerated in airtight polyethylene containers at 4 °C until extractions were performed.

2.3. Soil analyses

Organic matter content (OM) was determined with the loss on ignition method using a muffle furnace at 550 °C (Nelson and Sommers, 1996). Soil pH was determined using a standard electrode (Orion 8157BNUMD, Thermo Fisher Scientific) in 1:1 soil/deionized water (DI) slurries (USEPA, 2004). Total carbon (TC) and total nitrogen (TN) contents in soil samples were determined using a CHNS-O elemental analyzer Costech ECS 4010 (Costech Analytical Technologies, Valencia, CA, USA). Inorganic carbon (IC) was determined using the pressure calcimeter method (Loeppert and Suarez, 1996). Solubilized solutions from the C fractionation procedure described below were analyzed for total organic C (TOC) with a TOC-VCPH/CPN (Shimadzu Corp., Kyoto, Japan).

2.4. Carbon fractionation

An extraction procedure using hot water and Hydrochloric acid (HCl) was used to determine two labile C pools (hot water extracted C and HCl extracted C), and a recalcitrant C pool (Fig. 2b). The hot water extraction was performed using a modified procedure described by Ahn et al. (2009). Three grams of air-dried soil were mixed with 30 ml of DI in centrifuge tubes and mixed in a shaker for 30 min. at 30 rpm. Tubes were then placed in a hot water bath at 80 °C for 16 hrs. Thereafter, the tubes were shaken for 10 min. and centrifuged for 10 min. at 8,000 rpm. After centrifugation, the solubilized extract was filtered using 0.7 µm pore size filters. The residue of the extraction was dried in an oven at 60 °C for 24 hrs and preserved at room temperature, while the solution was acidified and stored at 4 °C until analyzed for TOC.

The HCl extraction was performed based on the procedure described by Silveira et al. (2008). One gram of air-dried soil was mixed with 25 ml of 6 M HCl and set in a block digester for 4 hrs at 105 °C. After digestion, the content was centrifuged for 10 min. at 6,037 rpm. The centrifugation residue was washed four times with DI water and centrifuged. The combined solution containing the solubilized solution and the washings was then filtered and diluted to 100 ml with DI water. The residue after extraction (designated as the Recalcitrant C) was oven dried at 60 °C for 24 hrs and stored at room temperature until analyzed for TC and C structure and composition using ^{13}C NMR. The solution was stored at 4 °C and analyzed for TOC (HCl extracted C).

A recalcitrant C index was calculated to correct for the mass of mineral material lost in the HCl extraction based on Paul et al. (2006) using the following calculation:

$$\text{Rec. C index} = \left(\frac{\text{Recalcitrant C conc.}(\text{g } kg^{-1}) * \text{Mass of soil after HCl extraction}(\text{g})}{\text{C conc. prior to HCl ext. } (\text{g } kg^{-1}) * \text{Mass of soil prior to HCl ext. (g)}}\right) * 100 \tag{1}$$

2.5. Solid state ^{13}C NMR

Soil samples from two farms (one representing shallow soils and one representing deep soils) were selected, based on their consistent soil depth (23.3 ± 11.5 cm for shallow soils farm, and 85 ± 20 cm for deep soils farm; mean ± SD), for ^{13}C NMR analyses. Composite samples obtained by combining the five 40-acre plots sampled in each farm were used for the analyses. For shallow soils two soil depths (0–10, and 10–30 cm) were analyzed, while for deep soils three soil depths (0–10, 10–30, and 30–60 cm) were analyzed. The ^{13}C NMR analyses were performed on soils prior to extraction, after hot water extraction, and after HCl extraction (Fig. 2). A total of 15 samples were analyzed by magic angle spinning (MAS) solid state ^{13}C NMR spectroscopy. Analyses were performed using a 600 MHz Bruker Avance III spectrometer with a 3.2 mm E-free MAS H/C/N probe. Between 25 and 30 mg of dried and finely ground soil samples were packed into zirconia rotors for the analyses. Spectra were collected using Cross Polarization with Total Sideband Suppression (CPTOSS) (Dixon et al., 1982) and referenced using an external adamantine standard. The MAS rate was regulated to 8 kHz and 4.8 µs π-pulses were used for sideband suppression with delays set to appropriately rotor synchronize the TOSS pulses. Ramped ^{1}H cross polarization (Metz et al., 1994) from 43.7 to 62.5 kHz, a static 62.5 kHz ^{13}C field, and a 1.5 ms contact time was utilized prior to the TOSS pulses for optimal signal enhancement. Proton decoupling (100 kHz) was applied during the 20 ms of signal acquisition time. Spectra were collected at −20 °C to enhance the Boltzmann distribution, averaging 8,192 scans with a 2 s recycle delay. Spectra was normalized dividing it by the mass of soil and adding 100 to scale them. To determine the percentage contribution of the major C functional groups the spectral regions were integrated as follows: alkyl C (0–45 ppm), *O*-alkyl C (45–110 ppm), aromatic C (110–160 ppm), and carboxyl C (160–220 ppm), based on Kögel-Knabner (1997) and Knicker (2011).

2.6. Statistical analyses

Statistical analyses were performed in the SAS 9.4 software (SAS Institute, 2015). A nested design (soil depth nested within location) linear mixed model was used to perform comparisons between C fractions and soil physicochemical parameters. The model considered farms and the 40-acre plots at each farm as random factors. The Tukey multiple comparison procedure was used to determine significant

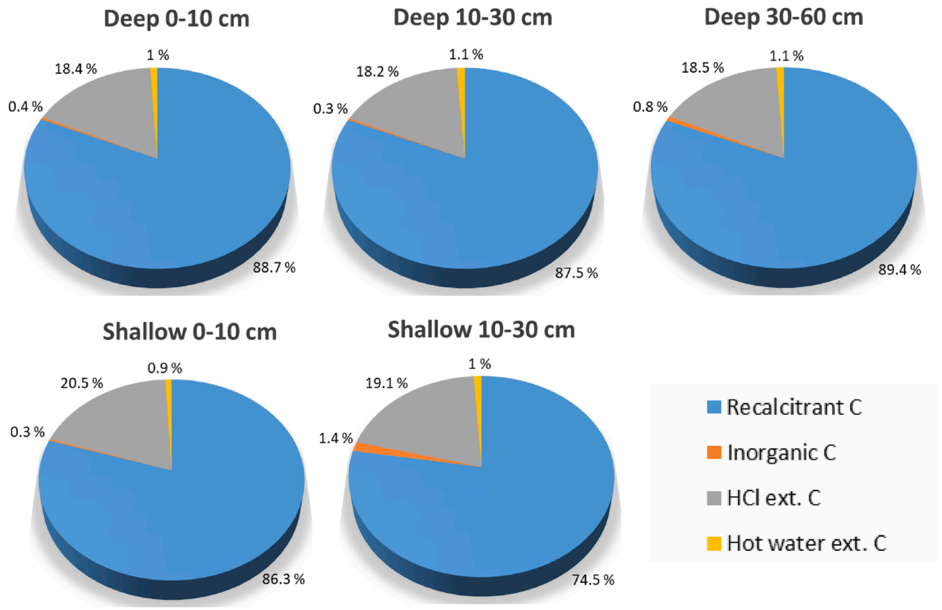

Fig. 3. Average percentage of total carbon for carbon fractions for deep and shallow soils at all soil increments.

differences between means. Data for comparisons of pH and recalcitrant C index was squared root transformed and log transformed, respectively, to meet model assumptions. Pearson correlation and multiple regression analyses were performed among C fractions and soil parameters. The multiple regression model used the same random factors (farms and plots) as the nested design described above. Differences were considered significant if $p < 0.05$.

3. Results

The dominant C fraction in both deep and shallow soils at all soil depths was recalcitrant C (Fig. 3), accounting for 85 ± 10% (mean ± SD) of the TC for all soils combined. The second most dominant C fraction for both soils and all increments was HCl extracted C which represented an average of 19 ± 2% of the TC. The hot water extracted C accounted for an average of 1 ± 0.2% of the TC, and was the second lowest fraction in all soils and increments, except for the shallow soils in the 10–30 cm increment, where inorganic C was higher than hot water extracted C. Inorganic C was the smallest of all fractions in deep soils at all soil increments, and in shallow soils at the 0–10 cm increment accounting for an average of 0.7 ± 0.8% of the TC.

Deep soils had higher OM content compared to shallow soils (Table 1). Similarly, hot water extracted C and recalcitrant C concentrations were higher in deep soils compared to shallow soils. TC, HCl extracted C and IC concentrations were not statistically different between deep and shallow soils. The recalcitrant C index was not statistically different between deep and shallow soils despite the observed differences in recalcitrant C concentration between the shallow and deep soils. There were no statistical differences between shallow and deep soils with respect to pH: 7.35 ± 0.15 (shallow soils) compared to 7.07 ± 0.16 (deep soils), and C:N ratio: 14.3 ± 1 (shallow soils) compared to 13.5 ± 1 (deep soils) (Table 1).

Table 1
Comparison of soil parameters and carbon fractions between deep and shallow soils.

Parameter (units)	Deep soils	Shallow soils	p value
OM (%)	79 ± 2	68 ± 2	0.01 *
Total C (g C kg^{-1})	404 ± 18	361 ± 20	0.17
HW ex. C (g C kg^{-1})	4.4 ± 0.2	3.3 ± 0.2	0.01 *
HCl ex. C (g C kg^{-1})	74 ± 2	71 ± 2	0.35
Rec. C (g C kg^{-1})	357 ± 6	289 ± 9	< 0.01 *
Inorganic C (g C kg^{-1})	3 ± 0.9	2.1 ± 0.8	0.5
pH	7.07 ± 0.16	7.35 ± 0.15	0.27
Rec. C index	88.5 ± 2.7	80.4 ± 3.3	0.09
C:N ratio	13.5 ± 1	14.3 ± 1	0.57

All soil depths combined. Values are estimated means ± standard error. Significant differences are marked with a star. Abbreviations: Organic matter (OM), hot water extracted carbon (HW ex. C), HCl extracted carbon (HCl ex. C), recalcitrant carbon (Rec. C).

Comparisons between soil increments in shallow soils showed that TC, OM, HCl extracted C, and recalcitrant C concentrations are significantly higher in the 0–10 cm (386 ± 20 g kg^{-1}, 75 ± 3%, 78 ± 3 g kg^{-1}, and 330 ± 10 g kg^{-1}, respectively ; means ± SE) compared to the 10–30 cm increment (335 ± 20 g kg^{-1}, 61 ± 3%, 64 ± 3 g kg^{-1}, and 249 ± 10 g kg^{-1}; means ± SE), while IC concentration was higher in the 10–30 cm (4.9 ± 1 g kg^{-1}) compared to the 0–10 cm increment (1 ± 1 g kg^{-1}) (Fig. 4). The hot water extracted C concentration did not show a significant difference between soil increments in shallow soils. For deep soils there are differences in soil increments for TC in which the 30–60 cm increment (398 ± 18 g kg^{-1}) had a lower concentration compared to the 0–10 and 10–30 cm (414 ± 18, and 406 ± 18 g kg^{-1}, respectively), and in IC in which the 30–60 cm increment (3.3 ± 0.9 g kg^{-1}) had a higher concentration compared to the 0–10 and 10–30 cm (1.6 ± 0.9, and 1.4 ± 0.9 g kg^{-1}, respectively). There were no significant differences between soil increments for HCl extracted C, recalcitrant C, hot water extracted C, and OM concentrations (Fig. 5).

Results from Pearson correlation analyses show that OM content has a strong positive correlation with TC and all C fractions' concentrations and a negative correlation with IC concentration (Table 2). Similarly, total C concentration, hot water extracted C, HCl extracted C, and recalcitrant C concentrations have positive correlations with each other. In contrast, IC concentration was negatively correlated with both HCl extracted C and recalcitrant C concentrations. Soil pH exhibited a positive correlation with IC concentration, and negative correlations with OM, TC, and the other C fractions' concentrations, except for HCl extracted C concentration with which there was no statistically significant correlation. The C:N ratio has a positive correlation with OM, TC, and all C fractions' concentrations except for HCl extracted C, and a negative correlation with pH. The recalcitrant C index has a positive correlation with OM, hot water extracted C, HCl extracted C, and the recalcitrant C concentrations, and a negative correlation with pH.

Results from the multiple regression analyses (Eqs. (2) to (6)), which

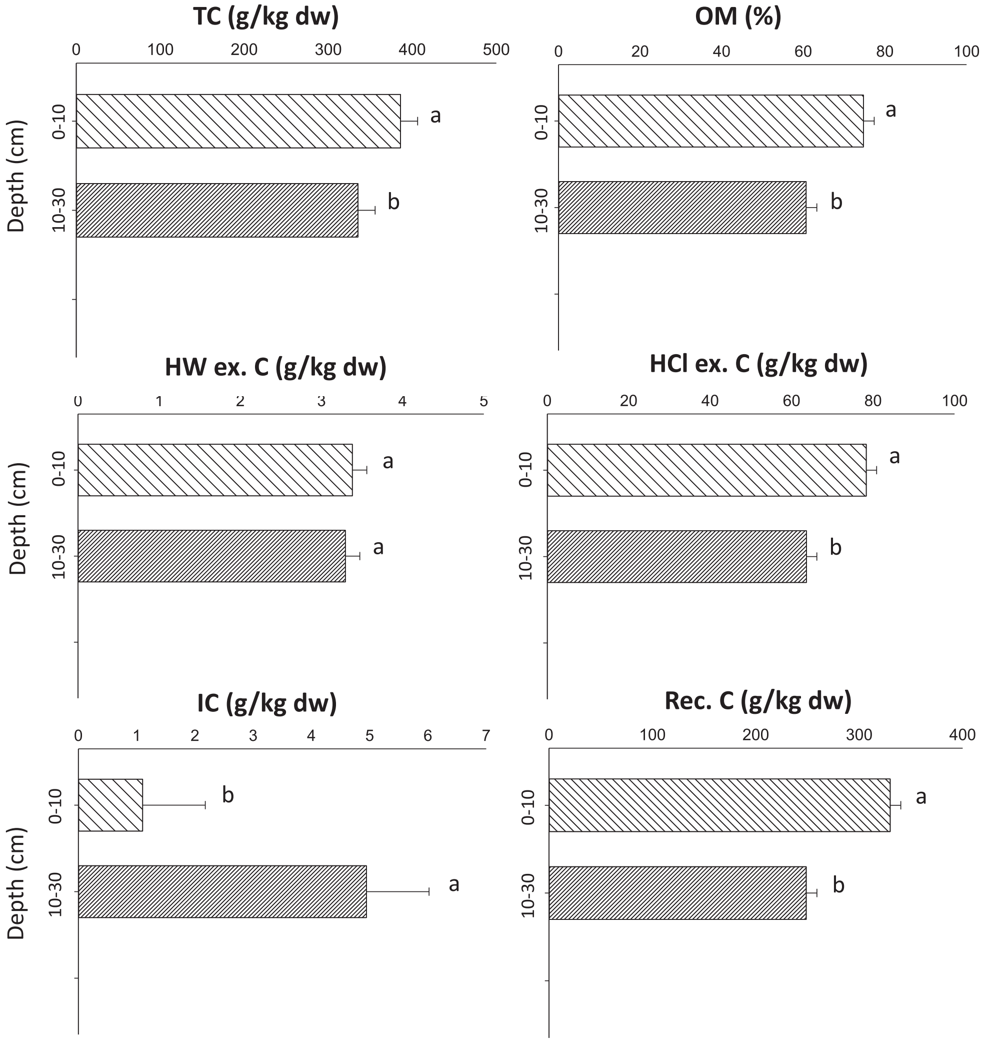

Fig. 4. Total carbon, organic matter and C fractions for shallow soils at different soil depths. Total C (A), organic matter (B), hot water extracted C (C), HCl extracted C (D), inorganic C (E), recalcitrant C (F). Letters indicate significant differences between soil depths at p value < 0.05.

were performed based on correlations, indicate that OM content is the most useful predictor of TC and the C fractions. The TC concentration has a small negative relation with hot water extracted C concentration and pH and a strong positive relation with OM content. For HCl extracted C concentration, besides OM content, the recalcitrant C concentration is also important to explain about 65% of changes in HCl extracted C concentration. Similarly, recalcitrant C concentration is also explained by HCl extracted C concentration. The IC concentration has a strong negative relation with OM content, a moderate positive relation with HCl extracted C concentration, and a positive relation with pH.

$$\text{TC} = -0.169(\text{HW ex.}) + 0.723(\text{OM}) - 0.187(\text{sqrt pH}) \tag{2}$$

$$\text{HW ex.} = 0.722(\text{OM}) \tag{3}$$

$$\text{HCl ex.} = 0.653(\text{Rec C}) + 0.679(\text{OM}) \tag{4}$$

$$\text{Rec C} = 0.363(\text{HCl ex.}) + 0.488(\text{OM}) \tag{5}$$

$$\text{IC} = 0.27(\text{HCl ex.}) - 0.977(\text{OM}) + 0.402(\text{sqrt pH}) \tag{6}$$

The ^{13}C NMR spectra depicted in Fig. 6 shows that soils with lower TC content have lower intensities in the spectra. In this study, shallow soils, and particularly the 10–30 cm increment, had lower intensities compared to deep soils. Shallow and deep soils at all soil increments did not differ in the peak distribution seen in the spectra. However, the lower peak height in shallow soils compared to deep soils is more evident in the aromatic region (110–160 ppm) (Fig. 6a). The ^{13}C NMR spectra of the soil prior to extraction and the residue after hot water extraction do not differ in their distribution (Fig. 6a and b). These spectra have peaks in the total alkyl region (0–45 ppm) with a small contribution from the terminal methyl region (0–25 ppm). There are also peaks in the methoxyl region (45–60 ppm), two peaks in the carbohydrate region (60–90 and 90–110 ppm), two peaks in the aromatic region (110–140 and 140–160 ppm), with the 140–160 ppm representing phenolic compounds, and a peak in the carboxyl region (160–220 ppm). The ^{13}C NMR spectra of the recalcitrant C (Fig. 6c) showed differences with respect to the spectra of the soils prior to extractions and the residue after hot water extraction; the two carbohydrate peaks were considerably reduced as was the terminal methyl region, while there were gains in the aromatic peaks.

The *O*-alkyl C region was the dominant spectral region both in the soil prior to extractions, at 36.9 ± 0.4% of the total spectrum (mean ± SD), and in the residue after hot water extraction, 36.4 ± 1.5% of the total spectrum (Table 3). The second dominant region in the ^{13}C NMR spectra for these two soils was the alkyl-C region, comprising 28.2 ± 1.1%, and 28 ± 1.7% of the total spectrum for the soil prior to extraction and the hot water extracted residue, respectively. For recalcitrant C, the dominant region was the total aromatics (110–160 ppm) with 34.3 ±

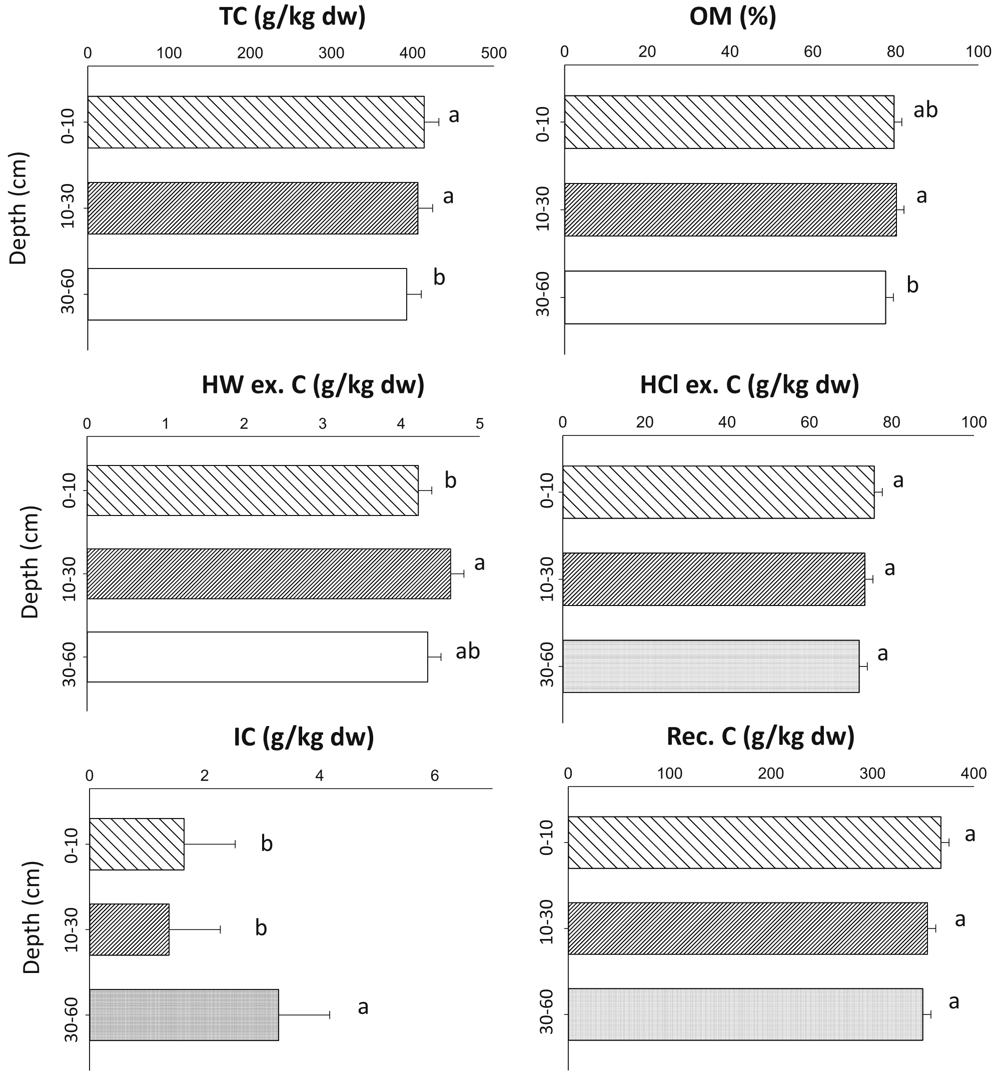

Fig. 5. Total carbon, organic matter and C fractions for deep soils at different soil depths. Total C (A), organic matter (B), hot water extracted C (C), HCl extracted C (D), inorganic C (E), recalcitrant C (F). Letters indicate significant differences between soil depths at p value < 0.05.

Table 2
Pearson correlation coefficients for soil parameters and carbon fractions.

Parameter †	TC	HW	HCl	Rec C	IC	C:N	OM	Sqrt pH
HW	0.59 ***							
HCl	0.46***	0.37*						
Rec C	0.73***	0.63***	0.69***					
IC	−0.12	−0.14	−0.34**	−0.38***				
C:N	0.5***	0.27*	0.07	0.25*	0.43***			
OM	0.86***	0.71***	0.66***	0.89***	−0.35**	0.36**		
Sqrt pH	−0.64***	−0.46***	−0.1	−0.48***	0.23*	−0.28*	−0.52***	
Log rci	0.08	0.33**	0.6***	0.73***	−0.43***	−0.13	0.48***	−0.03

†: total carbon (TC), hot water extracted carbon (HW), HCl extracted carbon (HCl), recalcitrant carbon (Rec C), inorganic carbon (IC), organic matter (OM), pH square root transformed (Sqrt pH), recalcitrant carbon index log transformed (Log rci). * significant at $p = 0.05$, ** significant at $p = 0.01$, *** significant at $p < 0.001$.

0.9%, while the *O*-alkyl C was 26.6 ± 0.4%. The alkyl C showed a slight reduction in the recalcitrant C spectra with respect to the soil prior to extraction and the hot water extracted residue, while the carboxyl region in the recalcitrant C spectra showed a slight increase with respect to the other two soils (Table 3).

4. Discussion

Most of the C in the studied peats at all soil depths was found to be in the recalcitrant C pool. The dominance of this pool over labile pools in C fractionation procedures using acid hydrolysis has been reported for several soils. A comprehensive study by Paul et al., (2006) which included data from different types of soils found that recalcitrant C ranged between 30 and 80% of the total organic C. In a group of Florida soils including histosols, Knox et al. (2015) found recalcitrant C concentrations between 50 and 96% of the TC. Additionally, dominance of recalcitrant C concentrations over labile C pools have also been found in wetland soils (Dodla et al., 2012). Isotopic studies in the EAA found that

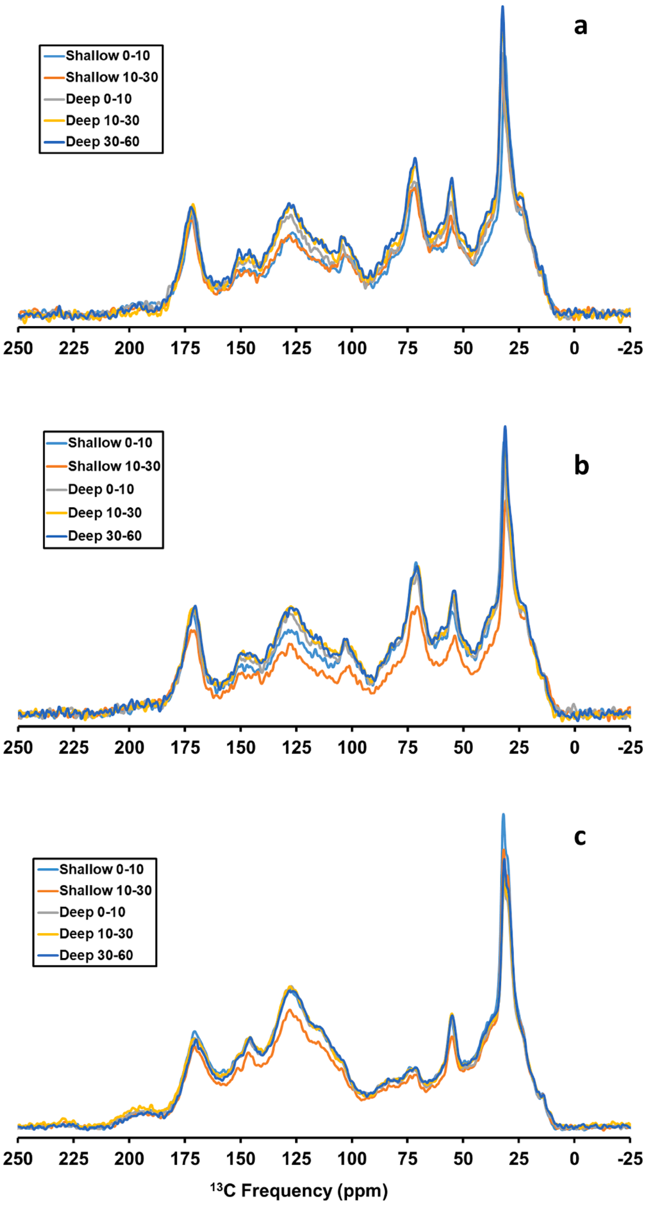

Fig. 6. Nuclear Magnetic Resonance spectra of a) soils prior to extraction b) soils after hot water extraction, and c) soils after HCl extraction (recalcitrant C). Different colors indicate different soils (shallow or deep) at different soil depths (0–10 cm, 10–30 cm, and 30–60 cm). Overlaid spectra were normalized to the total weight of each soil sample.

Table 3
Abundance of major carbon functional groups in Nuclear Magnetic Resonance spectra.

Carbon pool	Soil	Depth (cm)	Carbon functional group			
			Alkyl C	*O*-alkyl C	Aromatic C	Carboxyl C
Prior to extractions	Shallow	0–10	28.9	36.5	22.5	12.1
	Shallow	10–30	29.9	37.2	22.3	10.6
	Deep	0–10	26.9	36.3	25.1	11.7
	Deep	10–30	27.9	37.4	25.3	9.4
	Deep	30–60	27.3	37.3	25	10.3
Residue after HW. Extraction	Shallow	0–10	28.7	37.9	22.5	10.9
	Shallow	10–30	31	33.6	22	13.4
	Deep	0–10	27.1	37	24.9	11
	Deep	10–30	26.2	37.1	25.5	11.2
	Deep	30–60	27.1	36.4	25.3	11.2
Recalcitrant C	Shallow	0–10	27.2	26.2	34	12.6
	Shallow	10–30	29.2	26.2	32.6	12
	Deep	0–10	25.5	26.7	35.4	12.4
	Deep	10–30	25.5	26.5	34.8	13.2
	Deep	30–60	26.7	27.4	34.7	11.3

Values represent abundance as percent of total spectra. Alkyl C: 0–45 ppm, *O*-alkyl C: 45–110 ppm, Aromatic C: 110–160 ppm, and Carboxyl C: 160–220 ppm of spectra, respectively.

current peat originated mostly from Sawgrass (Wright and Inglett, 2009), dominance of recalcitrant C shows that current peat is mostly the humified product of the original peat with little contribution of crop residues. The proportion of recalcitrant C has been found to increase at the expense of the labile C pools in cultivated soils (Guimarães et al. 2013), and to vary depending on agricultural practices with tilled soils having less recalcitrant C compared to no till soils (Paul et al., 2006). The high abundance of recalcitrant C in the EAA soils is possibly a consequence of the prolonged drainage and cultivation of these soils. Peatland drainage is conducive to the loss of younger topsoil and the exposure of older and recalcitrant OM (Leifeld et al., 2012). Management practices, such as tillage, that affect recalcitrant C should be minimized to preserve this C and soil.

Reported values for hot water extracted C as percentage of TC vary depending on soil type and land use. Estimates of hot water extracted C for wetland soils range between 1 and 4% for Georgia, US (Silveira et al. 2008), 1.2 to 4.3% for Louisiana, US (Dodla et al., 2012), and 0.5 to 0.9% for the Everglades' marshland (Sihi et al., 2016). Results from this study (1 ± 0.2%) are close to the Everglades' values reported by Sihi et al. (2016). For HCl extracted C, studies in wetlands have found values between 16 and 49% of the TC (Dodla et al., 2012), and between 24 and 32% of the TC (Silveira et al., 2008). Results from this study (19 ± 2%) are closer to the lower range of these reported values. The low percentage of IC found is due to the high OM content of these peats which can be as high as 79% in deep soils. In general, the two labile C pools studied in the EAA soils are lower compared to natural wetlands, an observation that may be due to the intense cultivation of these soils, which can affect soil aggregation exposing previously protected OM to microbial oxidation, reducing this way labile C (Guimarães et al., 2013) or to the low input of fresh biomass into the soils. For example, Zhang et al. (2019) found that the transition from marsh to sugarcane crops in the EAA (which decreases biomass input to the soils) reduced water extracted organic C (a component of the labile C pools). It is a common practice to burn the sugarcane crop prior to harvest in the EAA minimizing residual biomass. Incorporating sugarcane residue may increase labile C pools in these soils, although this practice can also make sugarcane crops more vulnerable to freezing (Gilbert et al., 2010) and delay growth (Sandhu et al., 2017).

The TC concentrations found in this study (189 to 456 g C kg^{-1}) are similar to concentrations previously reported for EAA soils (316 to 431 g C kg^{-1}) (Hu et al., 2017), for the Everglades (404 to 438 g C kg^{-1}) (Sihi et al., 2016), and for other peatlands: (308 to 517 g C kg^{-1}) (Kalisz et al. 2015) and (418 to 476 g C kg^{-1}) (Sparling et al., 1998). The shallow and deep EAA soils did not differ in TC concentrations. Both shallow and deep soils showed a reduction of TC concentration with depth, possibly due to the incorporation of bedrock fragments closer to the bedrock. The reduction of TC with depth has also been reported in EAA peats (Hu et al., 2017) and in cultivated soils (Paul et al., 2001). The progressive subsidence will make these soils shallower, thereby reducing TC concentrations, this will also reduce the labile and recalcitrant C fractions' concentrations which are strongly correlated with TC. Lower availability of labile material can have implications in the cycling of other nutrients, such as N; drained peatlands that experience subsidence and C oxidation have been found to have lower C:N ratios, which can result in higher N leaching (Tiemeyer and Kahle, 2014; Brown et al., 2017). This could also be the case in the EAA, particularly in shallow soils.

Hot water extracted C concentrations (1.5 to 5.9 g C kg^{-1}) are similar to those reported by Kalisz et al. (2015) for a drained peatland, which were between 3.9 and 6.1 g C kg^{-1}, and lower than values reported for natural peatlands by Sparling et al. (1998), which were between 6.2 and 12.2 g C kg^{-1}. Lower concentrations of hot water extracted C compared to natural peatlands can be explained by the mineralization of readily available C under aerobic conditions. The difference between deep soils, which have significantly higher hot water extracted C concentration, compared to shallow soils, indicates that as soils get shallower the most labile C pool is reduced. There is no clear trend in soils at different depths in hot water extracted C, however the highest concentrations were found in the 10–30 cm depth in the deep soils. This can be due to the influence of plant roots (Kalisz et al., 2015), or to the redistribution of harvest residue, which can be responsible for higher C concentrations in the subsurface of EAA soils (Wright and Inglett, 2009). Differences in management in cultivated lands produce differences in hot water extracted C (Ghani et al., 2003; Gregorich et al., 2003). An improved management of these peats, allowing the incorporation of fresh biomass inputs may potentially increase the concentration of this readily available C.

Concentrations of acid extracted C found (30 to 87 g C kg^{-1}) are higher than concentrations reported for wetlands (6.4 g C kg^{-1}) (Ahn et al., 2009), and (1.4 to 26.6 g of C kg^{-1}) (Silveira et al., 2008). The higher HCl extracted C concentration found in these soils is due to their higher TC content compared to the wetlands studied by Ahn et al. (2009) and Silveira et al. (2008), and the strong positive correlations between TC, OM, and HCl extracted C concentrations. The presence of labile materials such as fatty acids, proteins, and polysaccharides, represented in the HCl extracted C pool (Paul et al., 2006), can partly be attributed to belowground biomass from crops (especially sugarcane) left after harvest and incorporated into the soil. The pattern found in shallow soils with lower HCl extracted C concentration in the 10–30 cm depth compared to the 0–10 cm depth is explained by a similar trend found in TC and OM.

Concentrations of recalcitrant C found (84 to 270 g C kg^{-1}) are within the range of values reported by Knox et al. (2015) for a variety of Florida soils (0.67 to 502 g of C kg^{-1}). Recalcitrant C concentrations have a similar trend to HCl extracted C, with lower concentrations in the 10–30 cm depth of shallow soils compared to topsoils. Recalcitrant C concentration has been found to strongly correlate with TC content (Paul et al., 2006; Knox et al., 2015). In this study, correlation analyses also show that recalcitrant C concentration is strongly correlated with TC and OM content (All of these showing a decline in the 10–30 cm increment of shallow soils) (Fig. 4), which explains the pattern found in recalcitrant C. Deep soils were found to have higher recalcitrant C concentrations than shallow soils. The recalcitrant C pool in these soils, which comprises most of the C, diminishes as soils get shallower. This can potentially affect C availability and biogeochemical processes.

The reduction in the recalcitrant C pool in shallow soils results in lower recalcitrant C indexes for shallow soils, which was contrary to this study's original hypothesis of higher recalcitrant C concentration in shallow soils. The recalcitrant C index has been found to decrease with depth in the soil profile (Paul et al., 2006; Rovira and Vallejo, 2007) due to higher mobility down the profile of labile C, and higher root input in deeper horizons (Rovira and Vallejo, 2007). The reduction of recalcitrant C concentrations, particularly in the 10–30 cm increment of shallow soils, drives the difference in recalcitrant C index between shallow and deep soils. The negative correlation between inorganic C and recalcitrant C index shows how the reduction of organic C (here an increase in IC concentration) produces a decrease in the recalcitrant C index.

The C:N ratio found in this study is within the range of C:N ratios reported by Kalisz et al. (2015), and similar to the ratio reported for EAA peats under sugarcane cultivation (15 ± 0.3) (Santos et al., 2020). Even though there were no significant differences between shallow and deep soils for C:N ratio, lower values generally were found in shallow soils which might be related to the lower C content in these soils. Additionally, lower N crop uptake found in shallow EAA peats (Jennewein et al., 2020), can result in lower C:N ratios (Wright and Inglett, 2009). The reduction of C concentration in shallow soils could potentially produce differences in C:N ratios as it has been reported in other drained peatlands (Tiemeyer and Kahle, 2014; Wang et al., 2016; Brown et al., 2017). The results obtained in both the recalcitrant C index and the C:N ratio are possibly driven by C dynamics.

Results from multiple regression analyses show that OM content is the most useful predictor of TC and all the studied C fractions. Organic matter content has a positive relationship with TC and all C fractions except IC concentration. A reduction of OM such as the one seen in shallow soils can therefore reduce TC, labile C pools, and recalcitrant C, and increase IC concentrations. Recalcitrant C and HCl extracted C concentrations have a reciprocal positive relation showing the importance that recalcitrant C can have on the availability of labile C pools, particularly HCl extracted C. As expected, pH had a positive relation with inorganic C concentration. Closer to the limestone bedrock there are more rock particles interacting with the soil, more IC, and a higher pH. The Increase in pH in EAA soils as they get progressively shallower (7.35 ± 0.15 for shallow soils, and 7.07 ± 0.16 for deep soils; mean ± SD) is a concern because higher pH could affect nutrient availability, especially phosphorus and micronutrients.

The pattern found in the ^{13}C NMR spectra of the soils prior to extraction dominated by the *O*-alkyl C region has also been found in peat soils in Sweden (Normand et al., 2017). An increase in recalcitrant C has been related to increases in alkyl and aromatic C (Bera et al., 2019), therefore, the result of aromatic C being dominant in the recalcitrant C pool was expected. Alkyl C has been found to increase with depth in peatland soils with a simultaneous reduction in *O*-alkyl C (Leifeld et al., 2012; Normand et al., 2017). Leifeld et al. (2012) found that a peatland drained 140 years ago, did not show a pattern of reduction in abundance of *O*-alkyl C with depth. These findings are similar to the results of no change in spectra regions with depth in the EAA and indicate that the remaining peat is older OM enriched in recalcitrant C with no stratification. An isotopic study of EAA peats with ^{15}N by Wright and Inglett (2009) found that surface soils are more decomposed than subsurface soils, The ^{13}C NMR results did not show differences in decomposition with depth, a solid state ^{15}N NMR analysis would inform on changes in nitrogen (N) compounds in EAA peats and their relation with decomposition as it has been suggested by studies with pyrolysis–gas chromatography-mass spectroscopy in EAA peats (Zhang et al., 2019).

O-alkyl C, which have been associated with labile C increases in soils (Bera et al., 2019) is highly susceptible to changes due to land management. Well preserved peatlands exhibit a decline in *O*-alkyl C with depth (Leifeld et al., 2012), while rewetted drained peatlands show increases in *O*-alkyl C in surface soils coming from the newly added plant material (Negassa et al., 2019). *O*-alkyl C does not show a pattern of decline at depth in EAA peats possibly due to the lack of fresh biomass returned to the soil during farming practices. The peak found in the carboxyl region of the spectra is similar to results from Wang et al. (2011) for Louisiana marshes. Heller et al. (2015) found a higher ratio of carboxyl to alkyl C in drained peatlands having a high degree of decomposition, which is also the case in the studied soils. The lower peak in the aromatic region of shallow soils compared to deep soils found in soils prior to extractions in this study, is in accordance with results from the C fractionation which show that recalcitrant C is significantly lower in shallow soils. Considering that aromatic C is a constituent of the recalcitrant C pool (Leifeld et al., 2012; Bera et al., 2019) this result emphasizes that as soils get progressively shallower, the recalcitrant C pool is also diminished.

The ^{13}C NMR analysis of recalcitrant C (soils after HCl extraction) showed an enrichment in aromatic C, and a decline in *O*-alkyl C. This pattern was also found by Silveira et al. (2008) in wetland soils, additionally, the observed reduction in terminal methyl C found in recalcitrant C might be due to the degradation of algal origin compounds (Silveira et al., 2008) or to the input of crop residues low in these compounds (Courtier-Murias et al., 2013). On the other hand, the selective enrichment of aromatic C can be due to the resistance to hydrolyzation of lignin and lignin-derived compounds (Rovira and Vallejo, 2002). Sugarcane residue has been found to contribute to subsurface soil C in the EAA (Wright and Inglett, 2009). The transition from marsh vegetation to sugarcane crops in the EAA has increased the contribution of aromatic and alkyl C compounds to the soil organic C, while reducing the contribution of carbohydrates (Zhang et al., 2019); Therefore, enrichment of aromatic C in the recalcitrant C pool can partly be attributed to sugarcane residue inputs into the soil.

5. Conclusions

This study's hypothesis that shallow peats have higher recalcitrant C and inorganic C compared to deep peats was rejected because subsidence affects all C pools including the recalcitrant C pool; as a result, recalcitrant C was actually lower in shallow peats. Our hypothesis of alkyl C being dominant in EAA peats was rejected, the dominant C functional group in EAA peats is *O*-alkyl C. The most dominant C pool in these drained peats is recalcitrant C, while the labile C pool is non-dominant. Recalcitrant C accounts for 85% of the TC on average, and is enriched in aromatic C compounds, and depleted in *O*-alkyl C. The dominance of this C pool is a consequence of the prolonged drainage and cultivation experienced by these peats during the last century. The recalcitrant C pool is however susceptible to be reduced due to soil subsidence as shown by lower recalcitrant C found in shallow soils compared to deep soils and in the 10–30 cm compared to the 0–10 cm increment in shallow soils. The labile C pools represent on average 1% (hot water extracted C), and 19% (HCl extracted C) of TC and were in the lower range of the reported values for other soils. Proximity to the bedrock increased inorganic C in both shallow and deep soils but no significant differences were found between shallow and deep peats. Deep peats are more homogeneous with less changes in C pools and OM with depth. For shallow peats, significant differences were found in OM, TC, recalcitrant C, HCl extracted C, and IC between the top (0–10 cm) and the bottom (10–30 cm) increments. Organic matter is the most important factor controlling C fractions in these peats. A reduction in OM can be considered an indicator of organic C reduction. The reduction of OM and C fractions, particularly recalcitrant C, as soils get shallower, shows that peatland soil C is vulnerable to subsidence even when the remaining peat is in proximity to the bedrock. The decrease in C pools can also affect C:N ratios, and pH which can also affect nutrient availability. Further research on the contribution of crop residues to C pools, and on changes in N compounds as soils get shallower, can contribute to understand C dynamics and composition on this and other drained peatlands. Management strategies e.g. adding crop residues into the soil, should be implemented to protect these peats from subsidence.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114758.

References

Ahn, M.-Y., Zimmerman, A.R., Comerford, N.B., Sickman, J.O., Grunwald, S., 2009. Carbon mineralization and labile organic carbon pools in the sandy soils of a North Florida watershed. Ecosystems 12 (4), 672–685.

Aich, S., McVoy, C.W., Dreschel, T.W., Santamaria, F., 2013. Estimating soil subsidence and carbon loss in the Everglades Agricultural Area, Florida using geospatial techniques. Agric. Ecosyst. Environ. 171, 124–133. https://doi.org/10.1016/j.agee.2013.03.017.

Armentano, T.V., 1980. Drainage of organic soils as a factor in the world carbon cycle. Bioscience 30 (12), 825–830. https://doi.org/10.2307/1308375.

Armentano, A.T.V., Menges, E.S., 1986. Patterns of change in the carbon balance of organic soil-wetlands of the temperate zone. Ecology 74 (3), 755–774. https://doi.org/10.2307/2260396.

Belay-Tedla, A., Zhou, X., Su, B., Wan, S., Luo, Y., 2009. Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the US Great Plains subjected to experimental warming and clipping. Soil Biol. Biochem. 41 (1), 110–116.

Bera, T., Vardanyan, L., Inglett, K.S., Reddy, K.R., O'Connor, G.A., Erickson, J.E., Wilkie, A.C., 2019. Influence of select bioenergy by-products on soil carbon and microbial activity: a laboratory study. Sci. Total Environ. 653, 1354–1363.

Bhadha, J., Trotta, L., Vanweelden, M., 2016. Trends in rice production and varieties in the Everglades Agricultural Area. EDIS doc. SL-439. Florida Extension Serv., Inst. of Food and Agric. Sci., Univ. of Florida, Gainesville.

Bottcher, A.B., Izuno, F.T., 1994. Everglades Agricultural Area (EAA): Water, Soil, Crop, and Environmental Management. University Press of Florida, Gainesville, Florida.

Bridgham, S.D., Megonigal, J.P., Keller, J.K., Bliss, N.B., Trettin, C., 2006. The carbon balance of North American wetlands. Wetlands 26 (4), 889–916. https://doi.org/10.1672/0277-5212(2006)26[889:TCBONA]2.0.CO;2.

Brown, R., Zhang, Z., Comeau, L.P., Bedard-Haughn, A., 2017. Effects of drainage duration on mineral wetland soils in a Prairie Pothole agroecosystem. Soil Tillage Res. 168, 187–197. https://doi.org/10.1016/j.still.2016.12.015.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Heimann, M., 2014. Carbon and other biogeochemical cycles. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, pp. 465–570.

Courtier-Murias, D., Simpson, A.J., Marzadori, C., Baldoni, G., Ciavatta, C., Fernández, J. M., Plaza, C., 2013. Unraveling the long-term stabilization mechanisms of organic materials in soils by physical fractionation and NMR spectroscopy. Agric. Ecosyst. Environ. 171, 9–18.

Daroub, S.H., Van Horn, S., Lang, T.A., Diaz, O.A., 2011. Best management practices and long-term water quality trends in the Everglades Agricultural Area. Crit. Rev. Environ. Sci. Technol. 41 (S1), 608–632.

Dixon, W. T., Schaefer, J., Sefcik, M. D., Stejskal, E. O., McKay, R., 1982. Total suppression of sidebands in CPMAS C-13 NMR. J. Magnetic Resonance (1969), 49 (2), 341–345.

Dodla, S.K., Wang, J.J., DeLaune, R.D., 2012. Characterization of labile organic carbon in coastal wetland soils of the Mississippi River deltaic plain: relationships to carbon functionalities. Sci. Total Environ. 435, 151–158.

Dommain, R., Couwenberg, J., Joosten, H., 2011. Development and carbon sequestration of tropical peat domes in south-east Asia: links to post-glacial sea-level changes and Holocene climate variability. Quat. Sci. Rev. 30 (7–8), 999–1010.

Ghani, A., Dexter, M., Perrott, K.W., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol. Biochem. 35 (9), 1231–1243.

Gilbert, R. A., Kingston, G., Morgan, K., Rice, R. W., Baucum, L., Shine, J. M., Subiros, J. F., 2010. Effect of harvest method on microclimate and sugarcane yield in Florida and Costa Rica. In Proc. Int. Soc. Sugar Cane Technol, Vol. 27, pp. 1–10.

Glaz, B., Lingle, S.E., 2012. Flood duration and time of flood onset effects on recently planted sugarcane. Agron. J. 104 (3), 575–583. https://doi.org/10.2134/agronj2011.0351.

Gleason, P.J., Stone, P., 1994. Age, origin, and landscape evolution of the Everglades peatland. In: Davis, S.M., Ogden, J.C. (Eds.), Everglades: The Ecosystem and Its Restoration. St. Lucie Press, Boca Raton, Florida, pp. 149–197.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994. Study of free and occluded particulate organic matter in soils by solid state 13C CP/MAS NMR spectroscopy and scanning electron microscopy. Soil Res. 32 (2), 285–309.

Gregorich, E.G., Beare, M.H., Stoklas, U., St-Georges, P., 2003. Biodegradability of soluble organic matter in maize-cropped soils. Geoderma 113 (3–4), 237–252.

Guimarães, D.V., Gonzaga, M.I.S., da Silva, T.O., da Silva, T.L., da Silva Dias, N., Matias, M.I.S., 2013. Soil organic matter pools and carbon fractions in soil under different land uses. Soil Tillage Res. 126, 177–182.

Helfrich, M., Ludwig, B., Buurman, P., Flessa, H., 2006. Effect of land use on the composition of soil organic matter in density and aggregate fractions as revealed by solid-state 13C NMR spectroscopy. Geoderma 136 (1–2), 331–341.

Heller, C., Ellerbrock, R.H., Roßkopf, N., Klingenfuß, C., Zeitz, J., 2015. Soil organic matter characterization of temperate peatland soil with FTIR-spectroscopy: effects of mire type and drainage intensity. Eur. J. Soil Sci. 66 (5), 847–858.

Hu, J., VanZomeren, C.M., Inglett, K.S., Wright, A.L., Clark, M.W., Reddy, K.R., 2017. Greenhouse gas emissions under different drainage and flooding regimes of cultivated peatlands. J. Geophys. Res. Biogeosci. 122 (11), 3047–3062. https://doi.org/10.1002/2017JG004010.

Jennewein, S.P., Bhadha, J.H., Lang, T.A., McCray, J.M., Singh, M.P., Cooper, J., Daroub, S.H., 2020. Impacts of flooding, nitrogen-fertilization, and soil-depth on sugarcane nutrients grown on Histosols. J. Plant Nutr. 43 (3), 429–443.

Kasimir-Klemedtsson, Å., Klemedtsson, L., Berglund, K., Martikainen, P., Silvola, J., Oenema, O., 1997. Greenhouse gas emissions from farmed organic soils: a review. Soil Use Manag. 13, 245–250.

Kalisz, B., Lachacz, A., Glazewski, R., 2015. Effects of peat drainage on labile organic carbon and water repellency in NE Poland. Turk. J. Agric. For. 39 (1), 20–27.

Knicker, H., Skjemstad, J.O., 2000. Nature of organic carbon and nitrogen in physically protected organic matter of some Australian soils as revealed by solid-state 13C and 15N NMR spectroscopy. Soil Res. 38 (1), 113–128.

Knicker, H., 2011. Solid state CPMAS 13C and 15N NMR spectroscopy in organic geochemistry and how spin dynamics can either aggravate or improve spectra interpretation. Org Geochem. 42 (8), 867–890.

Knox, N.M., Grunwald, S., McDowell, M.L., Bruland, G.L., Myers, D.B., Harris, W.G., 2015. Modelling soil carbon fractions with visible near-infrared (VNIR) and mid-infrared (MIR) spectroscopy. Geoderma 239, 229–239.

Kögel-Knabner, I., 1997. 13C and 15N NMR spectroscopy as a tool in soil organic matter studies. Geoderma 80 (3–4), 243–270.

Leifeld, J., Steffens, M., Galego-Sala, A., 2012. Sensitivity of peatland carbon loss to organic matter quality. Geophys. Res. Lett. 39 (14).

Light, S.S., Dineen, J.W., 1994. Water control in the Everglades: a historical perspective. In: Davis, S.M., Ogden, J.C. (Eds.), Everglades: The Ecosystem and Its Restoration. St. Lucie Press, Boca Raton, Florida, pp. 47–84.

Loeppert, R. H., Suarez, D. L., 1996. Carbonate and gypsum. In: DL Sparks (ed.), Methods of soil analysis. Part 3. Chemical methods. SSSA Book Ser. 5. SSSA, Madison, Wisconsin, pp. 437–474.

Lucas, R. E., 1982. Organic soils (Histosols) formation, distribution, physical and chemical properties and management for crop production. Res. Report 435. Mich. State Univ. East Lansing, Michigan.

McDowell, L.L., Stephens, J.C., Stewart, E.H., 1969. Radiocarbon chronology of the Florida Everglades peat 1. Soil Sci. Soc. Am. J. 33 (5), 743–745.

Metz, G., Wu, X.L., Smith, S.O., 1994. Ramped-amplitude cross polarization in magic-angle-spinning NMR. J. Magn. Reson., Ser A 110 (2), 219–227.

Negassa, W., Acksel, A., Eckhardt, K.U., Regier, T., Leinweber, P., 2019. Soil organic matter characteristics in drained and rewetted peatlands of northern Germany: chemical and spectroscopic analyses. Geoderma 353, 468–481.

Nelson, D. W., Sommers, L. E., 1996. Total carbon, organic carbon, and organic matter. In: DL Sparks (ed.), Methods of Soil Analysis Part 3. Chemical Methods SSSA Book Ser. 5. SSSA, Madison, Wisconsin, pp. 961–1010.

Normand, A.E., Smith, A.N., Clark, M.W., Long, J.R., Reddy, K.R., 2017. Chemical composition of soil organic matter in a subarctic peatland: influence of shifting vegetation communities. Soil Sci. Soc. Am. J. 81 (1), 41–49.

Oades, J.M., Kirkman, M.A., Wagner, G.H., 1970. The use of gas-liquid chromatography for the determination of sugars extracted from soils by sulfuric acid. Soil Sci. Soc. Am. J. 34 (2), 230–235.

Page, S.E., Rieley, J.O., Wüst, R., 2006. Lowland tropical peatlands of Southeast Asia. Develop. Earth Surface Process. 9, 145–172.

Paul, E.A., Collins, H.P., Leavitt, S.W., 2001. Dynamics of resistant soil carbon of Midwestern agricultural soils measured by naturally occurring 14C abundance. Geoderma 104 (3–4), 239–256.

Paul, E.A., Morris, S.J., Conant, R.T., Plante, A.F., 2006. Does the acid hydrolysis–incubation method measure meaningful soil organic carbon pools? Soil Sci. Soc. Am. J. 70 (3), 1023–1035.

Pronger, J., Schipper, L.A., Hill, R.B., Campbell, D.I., McLeod, M., 2014. Subsidence rates of drained agricultural peatlands in New Zealand and the relationship with time since drainage. J. Environ. Qual. 43 (4), 1442–1449. https://doi.org/10.2134/jeq2013.12.0505.

Reddy, K.R., DeLaune, R.D., 2008. Biogeochemistry of Wetlands: Science and Applications. CRC Press, Boca Raton, Florida.

Rice, R. W., Gilbert, R. A., Daroub, S. H., 2005. Application of the Soil Taxonomy Key to the Organic Soils of the Everglades Agricultural Area. EDIS doc. SS-AGR-246. Florida Extension Serv., Inst. of Food and Agric. Sci., Univ. of Florida, Gainesville.

Rovira, P., Vallejo, V.R., 2000. Examination of thermal and acid hydrolysis procedures in characterization of soil organic matter. Commun. Soil Sci. Plant Anal. 31 (1–2), 81–100.

Rovira, P., Vallejo, V.R., 2002. Labile and recalcitrant pools of carbon and nitrogen in organic matter decomposing at different depths in soil: an acid hydrolysis approach. Geoderma 107 (1–2), 109–141.

Rovira, P., Vallejo, V.R., 2007. Labile, recalcitrant, and inert organic matter in Mediterranean forest soils. Soil Biol. Biochem. 39 (1), 202–215.

Rovira, P., Romanyà, J., Duguy, B., 2012. Long-term effects of wildfires on the biochemical quality of soil organic matter: a study on Mediterranean shrublands. Geoderma 179, 9–19.

Sandhu, H.S., Singh, M.P., Gilbert, R.A., Subiros-Ruiz, F., Rice, R.W., Shine Jr, J.M., 2017. Harvest management effects on sugarcane growth, yield and nutrient cycling in Florida and Costa Rica. Field Crops Res. 214, 253–260.

Santos, A.D.C., McCray, J.M., Daroub, S.H., Rowland, D.L., Ji, S., Sandhu, H., 2020. Nitrogen Assessment of Shallow Florida Histosols. Commun. Soil Sci. Plant Anal. 51 (14), 1916–1929.

SAS Institute, 2015. Version 9.4. SAS Inst., Cary, NC.

Shih, S.F., Glaz, B., Barnes Jr, R.E., 1998. Subsidence of organic soils in the Everglades Agricultural Area during the past 19 years. Soil Crop Sci. Soc. Florida Proc. 57, 20–29.

Sihi, D., Inglett, P.W., Inglett, K.S., 2016. Carbon quality and nutrient status drive the temperature sensitivity of organic matter decomposition in subtropical peat soils. Biogeochemistry 131 (1–2), 103–119.

Silveira, M.L., Comerford, N.B., Reddy, K.R., Cooper, W.T., El-Rifai, H., 2008. Characterization of soil organic carbon pools by acid hydrolysis. Geoderma 144 (1–2), 405–414.

Snyder, G.H., 1994. Soils of the EAA. In: Bottcher, A.B., Izuno, F.T. (Eds.), Everglades Agricultural Area (EAA): Water, Soil, Crop, and Environmental Management. University Press of Florida, Gainesville, Florida, pp. 27–41.

Snyder, G.H., 2005. Everglades agricultural area soil subsidence and land use projections. Soil and Crop Sci. Soc. Florida Proc. 64, 44–51.

Sparling, G., Vojvodić-Vuković, M., Schipper, L.A., 1998. Hot-water-soluble C as a simple measure of labile soil organic matter: the relationship with microbial biomass C. Soil Biol. Biochem. 30 (10–11), 1469–1472.

Stephens, J.C., 1956. Subsidence of organic soils in the Florida everglades. Soil Sci. Soc. Am. J. 20 (1), 77–80. https://doi.org/10.2136/sssaj1956.03615995002000010019x.

Stephens, J.C., Speir, W.H., 1970. Subsidence of organic soils in the USA. Land Subsidence Affaissement du sol 89, 523–534.

Stephens, J.C., Allen, L.H., Chen, E., 1984. Organic soil subsidence. Rev. Eng. Geol., VI 107–122. https://doi.org/10.1130/REG6-p107.

Swift, R. S., 1996. Organic matter characterization. In: DL Sparks (ed.), Methods of Soil Analysis Part 3. Chemical Methods SSSA Book Ser. 5. SSSA, Madison, Wisconsin, pp. 1011–1069.

Tate, R.L., 1980. Microbial oxidation of organic matter of Histosols. Adv. Microb. Ecol. 4, 169–201.

Tiemeyer, B., Kahle, P., 2014. Nitrogen and dissolved organic carbon (DOC) losses from an artificially drained grassland on organic soils. Biogeosciences 11 (15), 4123–4137. https://doi.org/10.5194/bg-11-4123-2014.

United States Environmental Protection Agency, 2004. Method 9045D, revision 4. Soil and Waste pH.

Wang, J.J., Dodla, S.K., DeLaune, R.D., Hudnall, W.H., Cook, R.L., 2011. Soil carbon characteristics in two Mississippi river deltaic Marshland profiles. Wetlands 31 (1), 157–166.

Wang, H., Richardson, C.J., Ho, M., Flanagan, N., 2016. Drained coastal peatlands: a potential nitrogen source to marine ecosystems under prolonged drought and heavy storm events—a microcosm experiment. Sci. Total Environ. 566, 621–626.

Wright, A.L., Inglett, P.W., 2009. Soil organic carbon and nitrogen and distribution of carbon-13 and nitrogen-15 in aggregates of Everglades Histosols. Soil Sci. Soc. Am. J. 73 (2), 427–433.

Zhang, Z., Wang, J.J., Lyu, X., Jiang, M., Bhadha, J., Wright, A., 2019. Impacts of land use change on soil organic matter chemistry in the Everglades, Florida-a characterization with pyrolysis-gas chromatography–mass spectrometry. Geoderma 338, 393–400.