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Evaluating permanganate oxidizable carbon (POXC)'s potential for differentiating carbon pools in wetland soils

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ABSTRACT

Soil carbon (C) storage is a globally important ecosystem service with the potential to contribute to climate change mitigation. Wetlands are heavily researched hot spots for soil C storage. Despite the growing number of wetland soil C inventories, most studies focus only on total C quantification; there is limited application of methods that evaluate differences in C stability and vulnerability to mineralization within the C pool. Permanganate oxidizable C (POXC) is a well-established soil health indicator in agriculture shown to be sensitive to changing conditions or management regimes and may prove equally informative in wetland studies. This research quantified POXC in six diverse wetland soils that differed greatly in organic matter content and spanned both freshwater and saltwater habitats, then evaluated the relationship between POXC and basic soil C properties, microbial indicators, and physical and chemical fractionation metrics. Results showed POXC averaged ~ 37 times greater in wetlands than upland agricultural soils, but was less robust in differentiating between individual wetlands than total C or organic matter content. Rather, the ratio of POXC to soil organic C may be a more informative metric for evaluating the proportion of slightly processed C in wetland soils. Significant correlations were found between POXC and almost all other soil properties measured, suggesting POXC could be a rapid, reliable, and economical proxy for other analyses. Overall, POXC shows potential for providing novel information about wetland soil C stability, but requires additional research to improve interpretability. Applying POXC analysis in time series data collection and before-after-control impact experiments may be particularly informative for wetland management.

1. Introduction

Improving the understanding of soil carbon (C) cycling and storage is a common goal among soil researchers and managers alike, whether it is to improve plant productivity, qualify for economic energy incentives, or understand ecosystem functions and services. Among ecosystems, wetlands receive substantial attention because the mass of global C they contain a disproportionately large (e.g., 20–30 % of the total soil carbon pool; Lal 2008), given their limited areal coverage (5–8 % of land surface; Mitsch and Gosselink, 2005). Interest in wetlands has resulted in a rapid expansion of knowledge about how soil C storage and cycling may vary with wetland habitat type, latitude, plant composition, age, and other factors (Bennett and Chambers, 2023; Breithaupt et al., 2023, 2020; Chmura et al., 2003; Hinson et al., 2017; Nahlik and Fennessy, 2016; Page et al., 2011; Steinmuller et al., 2022, 2020). However, as the understanding about conditions influencing wetland soil C dynamics expands, new questions arise concerning the form and persistence of stored soil C.

Soil inorganic C (SIC), mainly in the form of $CaCO_3$, cycles in the soil on geologic timescales due to the weathering of Ca silicates or pedogenic carbonate formation in association with plants (Monger, 2014). Meanwhile, soil organic C (SOC) is part of a highly heterogenous and dynamic pool of soil organic matter (SOM) that can include both living biomass and necromass (e.g., plant roots, litter, exudates, microbial biomass and

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byproducts, soil macrofauna, etc.). This creates a diversity of molecular and biochemical properties within the SOM, and equally variable turn-over rates, all of which exists in a state of continuous processing and decomposition (Lehmann and Kleber, 2015). To better represent the wide spectrum of SOM properties and stabilities, researchers developed several muti-pool conceptual models that vary in complexity. The earliest and simplest conceptualizations often differentiated "labile" compounds (rapidly mineralized; carbohydrates, amino acids, peptides, amino sugars, and lipids) from "recalcitrant" compounds (resistant to decomposition; lignin, suberin, resins, fats, and waxes) (Rovira and Vallejo, 2002; Wander, 2004). More recent models focus on functional turn-over rates, incorporating physical and chemical mechanisms for SOC protection, rather than molecular structure alone (Dungait et al., 2012; Kleber, 2010; von Lützow et al., 2007). These functional classification systems often use terms such as: biologically active, rapid, metabolic, or particulate organic matter (POM), to describe SOC pools that turn-over quickly (weeks to years), and terms like passive, very slow, stable, persistent, or mineral-associated organic matter (MAOM) are used to describe SOC pools that reside in the soil long-term (centuries to geologic time scales (Cotrufo and Lavallee, 2022; Lavallee et al., 2020; Parton et al., 1988; Wander, 2004). Being able to characterize the degree of soil decomposability (or vulnerability to C mineralization) with a consistent and simple method in wetlands could greatly advance the accuracy of C storage estimates, models, and the general understanding of the role wetlands play in global C budgets.

The breadth of existing research addressing the concentration and composition of SOM and the processes governing it is extensive. However, the compartmentalization of soil science into subdisciplines hinders the rate of universal advancement and integration of knowledge across soil systems (Baveye and Wander, 2019). There is evidence of the limited knowledge transfer between soil subdisciplines in wetland science, which has only recently begun to investigate the relevance of SOM-mineral interactions (e.g., MAOM) as a mechanism for SOC persistence (Kida and Fujitake, 2020; Lacroix et al., 2019; Mirabito and Chambers, 2023), despite substantial evidence of its critical importance in non-wetland soils (Castellano et al., 2015; Cotrufo et al., 2013; Lehmann and Kleber, 2015; Schmidt et al., 2011). The role of physical protection of SOC via aggregation is another example of a concept that is well establishing in terrestrial soil literature (Cambardella and Elliott, 1993; Six et al., 1999; von Lützow et al., 2007), but less commonly addressed in wetland soil literature (except see Hossler and Bouchard 2010; Wright and Hanlon 2013; Maietta et al. 2019; Kottkamp et al. 2022).

Permanganate oxidizable C (POXC) is a common metric used for agricultural soil assessment to evaluate changes in SOM due to management activities and was initially considered an indicator of the biologically active (i.e., labile, rapid turn-over) C pool in soils (Conteh et al., 1999; Culman et al., 2021; Fine et al., 2017). Although other methods exist for quantifying the 'labile' or 'active' C pool in soils (e.g., CO_2-C rate, microbial biomass C (MBC), enzyme assays, carbohydrates, POM), the rapidity and reliably of POXC has led to its inclusion as a soil health indicator (SHI) in agricultural soils (Culman et al. 2021). Considered a 'Teir 2′ SHI, POXC is an accepted biological metric for assessing the capacity of a soil to function as a living, sustainable ecosystem for plants, animals, and humans (Shafer et al., 2021). The method for quantifying POXC is well-established, after having been repeatedly tested and modified by numerous researchers in both laboratory (Gruver, 2015; Tirol-Padre and Ladha, 2004; Weil et al., 2003) and field (Mandal et al. 2011) settings.

Recent literature refutes some of the earlier claims that the reactant, potassium permanganate ($KMnO_4$), is targeting "labile" C compounds with a faster turnover rate, but instead indicates it is a strong oxidizing agent targeting specific functional groups common in SOM, such as diverse aromatic compounds, some primary and secondary alcohols, glycol, aldehyde, and keto groups, as well as aliphatic-rich compounds (Christy et al., 2023; Hurisso et al., 2016; Tirol-Padre and Ladha, 2004). As evidence is lacking to support a strong relationship between molecular size and POXC, it is no longer considered a direct indicator of active or bioavailable C (Christy et al., 2023). For example, lignin and tannins are highly reactive to permanganate oxidation, while cellulose, carbohydrates, and proteins are unreactive or minimally reactive (Christy et al., 2023; Tirol-Padre and Ladha, 2004). Despite this recent shift in understanding of exactly what compounds $KMnO_4$ is oxidizing, POXC still shows a consistent and strong correlation with many common soil physicochemical and biological properties in agricultural soils, including those indicative of biologically active (labile C), and thus continues to be endorsed as a key indicator of soil health and sensitive tracker of changes in SOM storage (Conteh et al. 1999; Weil et al. 2003; Mandal et al. 2011; Culman et al. 2012).

In response to the need for greater knowledge and methodological technology transfer across soil subdisciplines, this study sought to: 1) quantify POXC in diverse wetland soils (three freshwater and three saltwater wetlands with variable SOM content), and 2) determine the relationship between POXC and several other soil physicochemical properties commonly reported in wetland research. Specifically, the relationship between POXC and standard soil C properties (SOM, SOC, total C, total C:N ratio, and extractable dissolved organic C (DOC)) were evaluated. In the three freshwater wetlands, biological metrics of soil microbial communities and processes were quantified and tested for relationships to POXC, including MBC, potential soil respiration (CO_2-C) and methane flux (CH_4-C). In the three saltwater wetland soils, physical and chemical fractionation metrics of SOC (i.e., POM, MAOM, cellulose, hemicellulose, lignin, and ash content) were quantified and tested for relationships to POXC. We hypothesized: 1) POXC would differ among these diverse wetland soils, and 2) show strong positive correlations with other common soil physicochemical properties. We anticipated POXC would be most strongly correlated with MBC and CO_2-C, as direct measurements of C-driven biological activity in wetland soil. We also predicted a strong positive relationship between POXC and organic matter content (OMC) and SOC, as seen by other investigators studying agricultural soils.

2. Methods

2.1. Site description and field sampling

The studied wetlands represent a wide range of SOC contents in both freshwater (inland) and saltwater (coastal) regions in Florida, USA. Site selection leveraged ecosystems with research access and permission granted, where prior data could inform SOC to maximize the represented range of soil properties. Although the three wetlands represented in each region were geographically near, each was unique in soil physicochemical properties, and sometimes dominant plant community, condition, history, and hydrology (Table 1). Due to logistical constraints, not all analyses could be completed on all soils, but a subset (freshwater wetlands) was chosen for a more intensive evaluation of biological metrics, and another (saltwater wetlands) was chosen to evaluate fractionation metrics. This approach has the advantage of evaluating salinity regime effects while incorporating diverse SOM ranges reflective of environmental conditions.

All soils were collected in 10-cm diameter x 1 m long polycarbonate tubes using the push-core method, extruded in the field, and sectioned so only the 0–10 cm depth segment was retained. Samples were stored in polyethylene bags on ice and transported to the laboratory, then stored at 4 °C until analysis. The freshwater wetlands were each sampled in July 2022 and consisted of five cores collected from haphazardly selected locations within a 20 m area. The coastal wetlands were each sampled in June 2022 and consisted of 11 stratified random samples within a 1200 m^2 area.

Table 1
General properties of the wetland soils studied.

Region	Wetland Name	Location	Dominant Vegetation	Visual Soil Character	USDA Soil Classification	Notes
Fresh-water	Bayhead Swamp	28°36′25.00″N, 81°12′6.84″W	*Persea palustris; Myrica cerifera*	Sapric organic	sandy, siliceous, hyperthermic Histic Humaquepts	Continuous flooded; natural and pristine
	Cypress Dome	28°36′13.84″, 81°11′40.97″W	*Taxodium distichum* var. *imbricarium*	Well mixed coarse sand and hemic organic	sandy, siliceous, hyperthermic Histic Humaquepts	Intermittent flooded; impacted by reduced water inputs
	Basin Marsh	28°36′33.47″N, 81°11′57.20″W	*Ecleocharis* spp.	Thin organic epipedon over fine sand	siliceous, hyperthermic Spodic Psammaquents	Young basin marsh; natural and pristine
Salt-water	Dredge-Created Marsh	29°42′36.19″N, 85° 0′56.06″W	*Juncus roemerianus; Bolboschoenus robustus*	High silt/clay content	Sandy or sandy-skeletal, siliceous, euic, thermic Terric Sulfisaprists	Created (~40 year ago) dredge coastal wetland
	Estuarine Marsh	29°46′5.67″N, 84°52′46.92″W	*Juncus roemerianus*	Fibric organic soil	Fine, mixed, superactive, nonacid, thermic Typic Sulfaquents	Natural estuarine wetland
	Coastal Marsh	29°43′22.83″N, 84°53′26.91″W	*Juncus roemerianus; Bolboschoenus robustus*	High silt/clay content	Sandy or sandy-skeletal, siliceous, euic, thermic Terric Sulfisaprists	Natural intertidal wetland; stagnation and stress

2.2. Permanganate Oxidizable C (POXC)

The POXC is quantified as the change in color absorbance of $KMnO_4$ (a strong oxidizing agent that is a deep purple color). As it reacts with SOC, the C is oxidized from C^0 to C^{+4}, while the Mn is reduced from Mn^{7+} to Mn^{4+} (Eq. 1); this reaction changes the color of the solution toward a light pink. The reduction in color is proportional to the amount of SOC in the sample that was oxidized (Mandal et al. 2011).

$$3C + 4KMnO_4 + 2H_2O = 4KOH + 4MnO_2 + 3CO_2$$

Significant prior research has tested, modified, and optimized the specific details of the POXC method, resulting in slight variations in $KMnO_4$ concentration, absorbance wavelength, soil preparation, shaking time, etc. (Weil et al. 2003; Tirol-Padre and Ladha 2004; Gruver 2015; Culman et al. 2021). For example, the early POXC methods used higher concentrations (e.g., 0.333 M $KMnO_4$; Blair et al., 1995), while subsequent work found greater sensitivity with more dilute concentrations (0.02 M $KMnO_4$; Weil et al. 2003). Most of this methodological testing was performed in agricultural soils with lower SOC content. We found through previous trials that 0.02 M $KMnO_4$ was appropriate for sandy/terrestrial soils, but the high amount of POXC in organic-rich soils required a higher concentration to maintain measurable color in the solution (Bhadha, personal communication). Therefore, we used 0.2 M $KMnO_4$ for all our wetland soils, as has been done previously for cultivated muck soils (Bhadha et al. 2018).

All soils (0–10 cm) were analyzed for POXC following oven drying at 70 °C until constant weight, then grinding to particle size using a mortar and pestle and/or SPEX SamplePrep 8000 M Mixer/Mill (SPEX SamplePrep LLC, Metuchen, NJ). Note that air drying soils is typically recommended, but the high moisture content of wetland soils makes this challenging; oven drying allows for a consistent moisture status across all soils tested, which is the most critical consideration for data interpretation (Weil et al. 2003). Approximately, 1 g of the dried and ground soil was added to a 40 mL Nalgene centrifuge tube, then 10 mL of deionized (DI) water and 10 mL of 0.2 M $KMnO_4$ was added. Tubes were vortexed for 5 s, placed on an orbital shaker for 2 min at a rate of 150 RPM, then centrifuged at 5,000 RPM for 10 min at 20 °C. Following centrifugation, a 0.5 mL subsample of the supernatant was removed via pipette into a 20 mL scintillation vial, using care to ensure there were no soil particles in the solution. The supernatant was diluted with 20 mL of DI water to be in range of our standard solutions (0–2 mM $KMnO_4$). Oxidation of $KMnO_4$ was quantified by pipetting 200 μL of sample into a clear, flat-bottom, 96-well plate in replicates of 3 using a multi-channel micropipette. A 6-point standard curve (also in replicates of 3) was included on each plate and standards were made fresh daily. Absorbance was measured on a BioTek Synergy HTX spectrophotometer (BioTek Instruments, Inc., Winooski, VT) at a wavelength of 550 nm; samples were run within 24 h of being in contact with $KMnO_4$.

Following analysis, POXC was calculated as follows:

$$\text{POXC}\,(\text{mg g}^{-1}\text{soil}) = \frac{((\text{mM stock} - (\text{mM sample}*\text{dilution factor}))*20\text{mL}*9\text{mg}}{(\mathit{Soilweight}(g)*1000)}$$

Where, mM stock = concentration of $KMnO_4$ added to the centrifuge tube; mM sample = concentration of $KMnO_4$ left in the sample after analysis (calculated using the slope and y-intercept of the standard curve); dilution factor = total number of dilution (in centrifuge tube + scintillation vial); 20 mL=volume of solution in the centrifuge tube; 9 mg = mass of carbon oxidized by 1 mM of KMnO4 changing from $Mn^{7+} \rightarrow Mn^{4+}$; Soil weight = mass (g) of soil used for analysis.

2.3. Standard Soil Properties

Standard soil properties were those commonly quantified physicochemical soil parameters in relation to soil C that were determined for all soils in this study: soil OMC, SOC, total C, total N, total C:N ratio, and extractable DOC. Briefly, OMC was quantified via loss-on-ignition (LOI) using a subsample of the 0–10 cm core that was first oven dried at 70° C to constant weight, then ground on a SPEX SamplePrep 8000 M Mixer/Mill (SPEX SamplePrep LLC, Metuchen, NJ). Approximately 0.5 g of the dried ground sample was placed in a muffle furnace at 550° C for 3 h and percent OMC was calculated as the difference in mass before and after burning. The dried ground soil was also used to determine total C, total N, C:N, and SOC through analysis on a Vario Micro Cube CN Analyzer (Elementar Americas Inc., Mount Laurel, NJ). Total C and N analysis was performed on unburned soils. To determine SOC, 5 mg subsamples of soil were weighed into silver tins and underwent an acid fumigation using 12 M HCl for 6 h within a desiccator. After fumigation, samples were left out to dry for 24 h prior to being analyzed. The C:N ratio was calculated as the mass ratio of total C and N. Extractable DOC was quantified on field-wet soils within 48 h of collection. A 2.5 g sample was placed in a 40 mL centrifuge tube with 25 mL of 2 M KCl. Samples were placed on an orbital shaker at 100 RPM for 1 h, then centrifuged at 5000 RPM for 10 min at 10° C. The supernatant was vacuum filtered through a 0.45 μm membrane filter, acidified with H_2SO_4 and analyzed on a Shimadzu TOC-L (Shimadzu Instruments, Kyoto, Japan).

2.4. Soil biological process metrics

All freshwater wetland soils were analyzed for MBC and potential CO_2-C and CH_4-C production; all analyses were initiated within 48 h of soil collection. The fumigation-extraction method developed by (Vance et al., 1987) was applied (with modification) to determine MBC. Duplicate 3 g wet weight samples were placed in 40 mL centrifuge tube; one was fumigated with 0.5 mL chloroform for 24 h, while the other was non-fumigated. Both samples were extracted with 25 mL of 2 M KCl and analyzed on a Shimadzu TOC-L (Shimadzu Instruments, Kyoto, Japan). Both potential CO_2-C and CH_4-C production were quantified in microcosms created in 120 mL glass serum bottles with approximately 10 g of field moist soil and 20 mL of DI water (Hurst et al., 2022; Steinmuller et al., 2020). Briefly, the DI water and headspace were purged with O_2-free N_2 gas to create an anaerobic environment and bottles were incubated with a slight overpressure at 25° C while on an orbital incubator shaker rotating at 150 RPM. Headspace was extracted at 4 time points during the 8-d incubation and analyzed on a Shimadzu 2014 GHG Analyzer equipped with a Flame Ionized Detector (FID) and Methanizer (Shimadzu Scientific Instruments, Kyoto, Japan). Rates were calculated as the slope of the regression line between CO_2-C and CH_4-C concentrations over time using the Ideal Gas Law and Henry's Law (for CO_2-C).

2.5. Soil physical and chemical fractionation metrics

All coastal wetland soils underwent physical and chemical fractionation to determine the amount of soil C as POM and MAOM, and acid detergent fiber (ADF) analysis to determine the percent of soil mass that was cellulose, hemicellulose, lignin, and ash. The POM and MAOM were analyzed following the method developed by (Mirabito and Chambers, 2023). The MAOM was defined as the heavy fraction (density > 1.85 g cm^{-3}) of the < 53 μm soil fraction after dispersion with 0.5 % sodium hexametaphosphate and vortexing with glass beads. Density fractionation was performed using 25 mL of 1.85 g cm^{-3} sodium polytungstate. All soil not included in the MAOM fraction was considered POM. Soil C content in each fraction was analyzed on a Vario Micro Cube CN Analyzer (Elementar Americas Inc., Mount Laurel, NJ) and calculated based on the soil mass in each fraction. Cellulose, hemicellulose, lignin, and ash content were quantified using ADF (Cotrufo et al., 2015; Van Soest et al., 1991). Briefly, 0.4 g of oven dried ground soil was digested in 35 mL of an acid-detergent solution for 1 h at 100° C, then filtered using Gooch crucibles while rinsing with DI water and 10 mL of acetone. Samples were oven dried at 70° C until constant mass and mass loss was considered hemicellulose. Next, the samples were soaked in 72 % H_2SO_4 solution for 3 h, then filtered with Gooch crucibles while rinsing with DI water. Following drying at 70 °C until constant mass, mass loss was considered cellulose. The remaining mass of the sample was placed in the muffle furnace and combusted at 550 °C for 3 h. The mass of the sample remaining was recorded and was determined as ash content and the mass loss due to combustion was determined to be lignin.

2.6. Statistical analyses

All statistical analyses utilized R version 4.03 (R Foundation for Statistical Computing, Vienna, Austria) within RStudio (RStudio Team, 2020). Data visualization was done using R package "ggplot2" (Wickham, 2016) and the R package "tidyverse" (Wickham et al., 2019) for used for data wrangling. For comparisons within each laboratory analysis, generalized linear models (glm) with a gaussian distribution and an identity link were used to compare across different sites. Models were made using the "stats" package (R Core Team, 2023) and selected based on model performance using R packages "AICcmodavg" package (Mazerolle, 2023) to compare AIC scores and "performance" package (Lüdecke et al., 2021) to determine model fit based on normality, homogeneity, and collinearity. Pairwise comparisons across sites were made using the Tukey method within the "emmeans" package (Lenth, 2020). Principle component analysis (PCA) was done using the princomp function within the "stats" package (R Core Team, 2020) to compare correlation between variables. Matrix of correlations were created using the "Hmisc" package and the rcorr function to retrieve Pearson correlation coefficient values (r) and P-values (Harrell and Dupont, 2024). Results are reported using mean values and standard errors.

3. Results

The following communicates study results in terms of 1) POXC content, 2) wetland soil properties, 3) PCA relationships, and 4) Pearson product correlations. Mean (±standard error) POXC ranged from 21.7 ± 2.4 mg g^{-1} in the freshwater Basin Marsh, to 40.7 ± 1.1 mg g^{-1} in the saltwater Estuarine Marsh (Fig. 1a). As a result, Estuarine Marsh POXC was statistically greater than all other wetlands except the Coastal Marsh ($p = 0.178$), but POXC concentrations did not differentiate between any of the other wetlands.

The observed POXC concentrations represented an average of 6.2 to 22.6 % of the SOC pool, and the ratio of POXC:SOC was inversely related to the total SOC (Table 2). For example, the most organic-rich soil (the Bayhead Swamp) had POXC representing only 6.2 % of the SOC, while the least organic soils (the Basin Marsh and Dredge-Created Marsh) had ~ 22 % of the SOC in the POXC pool.

Beyond POXC, the 6 wetlands studied exemplified a wide range of naturally occurring wetland soil properties (Table 2). For example, mean OMC ranged from 15.2 ± 0.6 % in the Basin Marsh, to 80.3 ± 0.5 % in the Bayhead Swamp. The soil OMC was composed of 43–73 % TC (mean = 56.3 ± 4.4 %), and the majority of TC (≥90 %) was SOC. The positive correlations between TC, SOC, and OMC were strong (all with $r \geq 0.93$; $p < 0.001$; data not shown), as seen visually by the similarity in the data distributions for OMC, TC, and SOC (Fig. 1b, 1c, and 1d, respectively). Extractable DOC ranged from 0.11 ± 0.01 (Dredge-Created Marsh) to 0.84 ± 0.04 mg g^{-1} (Cypress Dome). The strongest correlate of DOC was TN ($r = 0.85$, $p < 0.001$), while OMC, TC, and SOC also had $r > 0.6$ ($p < 0.001$). Total N ranged from 4.8 ± 0.7 mg g^{-1} (Dredge- Created Marsh) to 25.6 ± 0.8 mg g^{-1} (Cypress Dome; similar to DOC) while TC:TN ratios were lowest in the Cypress Dome (15.5 ± 0.1) and highest in the Bayhead Swamp (38.4 ± 0.6).

A PCA revealed that when all 6 wetlands and their basic physicochemical properties were plotted in two dimensions, 87.2 % of the variation in the data could be explained along two components (Fig. 2a). Component 1 explained 65.9 % of the variability and had a strong positive correlation with all the variables measured, except C:N. Variability in POXC was most closely aligned with variability in DOC and TN, but was a slightly weaker predictor than DOC and TN (as indicated by the shorter arrow). When only the three freshwater wetlands were plotted, along with the biological process metrics measured in each of them, all three freshwater wetlands plotted as distinct clusters in space (Fig. 2b). A strong positive relationship with Component 1 (explaining 67.5 % of the variability in the data) was again visible for most of the parameters, with POXC plotting close to MBC, SOC, TC, OMC, and CO_2. Component 2 (explaining 26.9 % of the variability) was a stronger predictor of CN and CH_4, and to a lesser degree TN and DOC. The saltwater wetland data, along with the physical and chemical fractionation metrics, showed weaker clustering by wetland (Fig. 2c). Again, most of the measured properties plotted as positively correlated with Component 1 (explaining 63.5 % of the variability), with POXC closely aligned with many other soil properties, including OMC, SOC, TC, TN, cellulose, and POM-C and −N, DOC, and hemicellulose. Ash had an inverse correlation with these variables along Component 1, and MAOM-C and −N showed a strong correlation with Component 2, which explained 11.7 % of the variability.

Pearson's product correlations with POXC showed similar trends to the PCAs (Table 3). All parameters were positively related to POXC except for ash, which showed a strong negative correlation, and C:N and

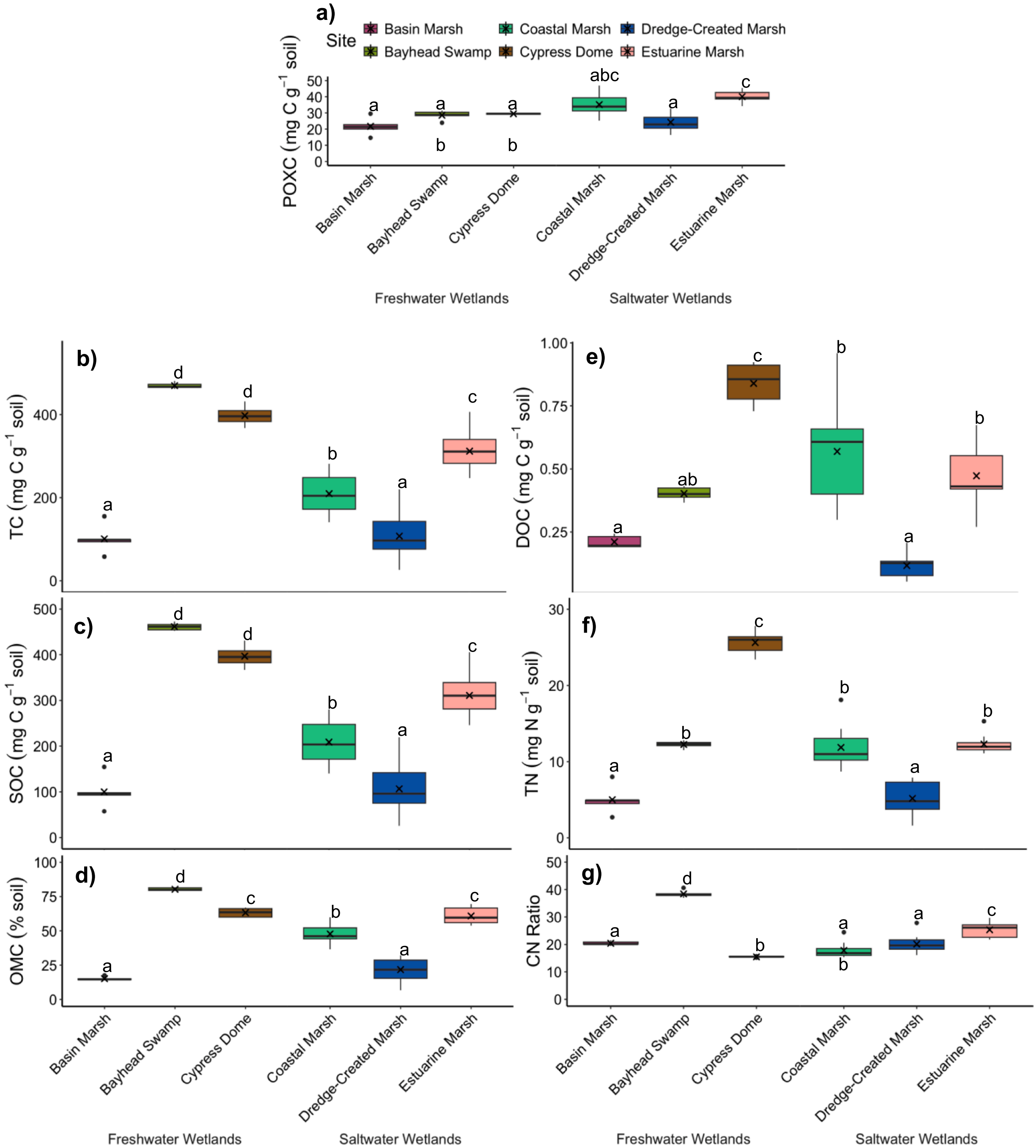

Fig. 1. Box plots of basic soil properties quantified on all wetland soils by wetland type. Boxes represent 1st, 2nd, and 3rd quartiles of the data distribution, x represents the mean, and tails and dots represent the full variance of the data distribution, x represents the mean, and tails and dots represent the full variance of the data. Different letters represent significantly different means ($p \leq 0.05$) based on general linear models (glm) and Tukey pairwise comparisons. N=5 for all freshwater wetlands; n = 11 for all saltwater wetlands.

CH_4, which showed no correlation. The strongest and most consistent correlate of POXC was OMC ($p \leq 0.001$, regardless of how the data was combined for analysis), followed closely by TC and SOC (all $p < 0.01$). The physical/chemical fractionation analyses conducted on the saltwater wetland soils showed slightly greater correlations with POXC (e.g., POM-C, hemicellulose, cellulose, lignin and ash, all $p < 0.001$) than the biological metrics measured in the freshwater wetland soils (e.g., MBC and CO_2, $p < 0.01$), but both are considered strong correlates (r values > 0.6). These trends are also evident when viewed graphically (Figs. 3 and 4).

The patterns in data distribution between the three saltwater wetlands were generally consistent across all parameters (e.g., the Coastal Marsh and Estuarine Marsh had similar averages, which were greater than that of the Dredge-Created Marsh). As the only exception, ash showed the inverse pattern (Fig. 4i). Meanwhile, the biological metrics of MBC and CO_2 produced larger differentiation among the freshwater wetlands (e.g., the Basin Marsh was substantially lower than the other two, and the Bayhead Swamp and Cypress Dome were statistically different; Fig. 3b and 3c) than POXC did (Fig. 3a).

Table 2
Mean (standard error) of soil properties quantified in all six wetlands. All data is the 0–10 cm depth segment.

Soil Prop-erty	Units	Freshwater (n = 5) Bayhead Swamp	Cypress Dome	Basin Marsh	Saltwater (n = 11) Dredge-Created Marsh	Estuarine Marsh	Coastal Marsh
Bulk Density	$g\ cm^{-3}$	0.10 (0.004)	0.14 (0.006)	0.32 (0.007)	0.42 (0.044)	0.11 (0.007)	0.09 (0.010)
texture	N/A	N/A	sand	sand	silt	silt	silt
OMC	%	80.3 (0.5)	63.3 (1.5)	15.2 (0.6)	20.3 (2.8)	59.7 (2.1)	46.6 (2.2)
TC	$mg\ g^{-1}$	469.6 (3.3)	397.6 (11.0)	100.9 (15.6)	100.2 (16.7)	304.8 (15.6)	201.5 (15.0)
SOC	$mg\ g^{-1}$	461.3 (3.7)	396.8 (11.0)	99.8 (15.5)	99.2 (16.7)	303.8 (15.6)	200.5 (15.0)
POXC	$mg\ g^{-1}$	28.6 (1.3)	29.5 (0.2)	21.7 (2.4)	22.6 (2.2)	40.7 (1.1)	34.7 (1.7)
POXC: SOC	N/A	6.2	7.4	21.7	22.6	13.4	17.3
DOC	$mg\ g^{-1}$	0.40 (0.01)	0.84 (0.04)	0.21 (0.01)	0.11 (0.01)	0.48 (0.04)	0.55 (0.06)
TN	$mg\ g^{-1}$	12.2 (0.2)	25.6 (0.8)	5.0 (0.9)	4.8 (0.7)	12.2 (0.4)	11.5 (0.8)
TC:TN	N/A	38.4 (0.6)	15.5 (0.1)	20.4 (0.4)	20.4 (1.0)	24.9 (0.9)	17.5 (0.8)

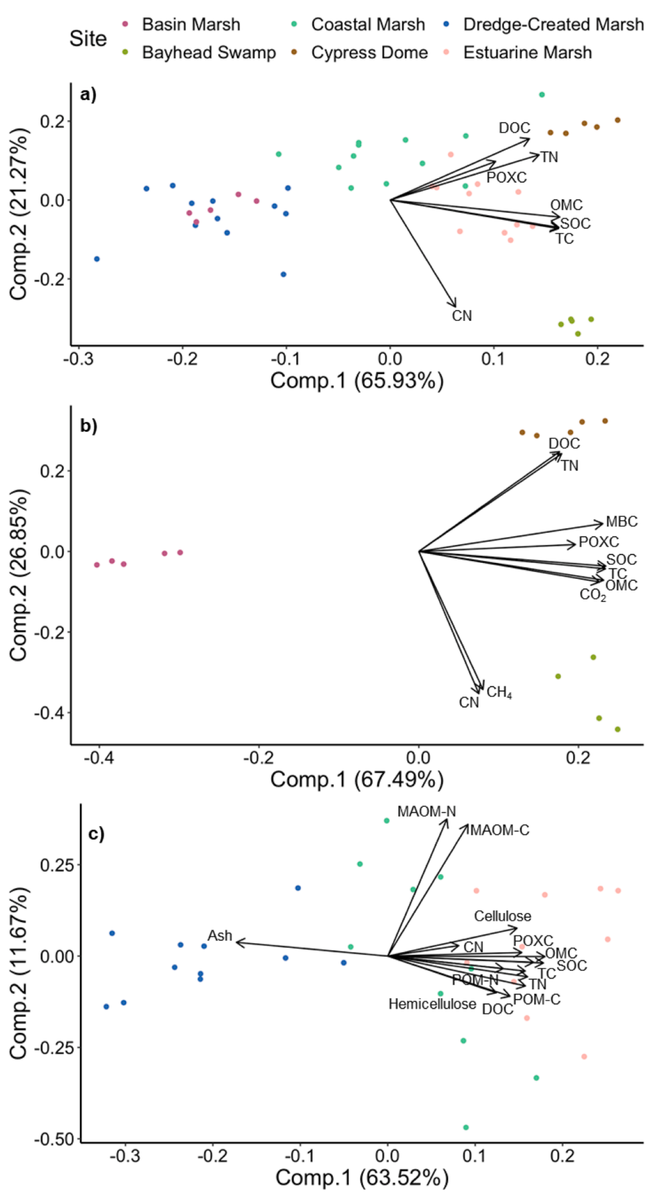

Fig. 2. Principle component analysis (PCA) to compare correlation between variables in 2 dimensions. Analysis including basic soil properties quantified for all six wetlands (a), biological metrics quantified for freshwater wetlands only (b), and soil physical and chemical fractionation metrics for saltwater wetlands only (c).

Table 3
Pearson correlation coefficient values (r) and p-values according to wetland site data included. Underlined indicates significance and $\alpha \le 0.05$; bold and underline indicates significance at $\alpha \le 0.01$.

Parameter	All Wetlands r	p	Freshwater Only r	p	Saltwater Only r	p
OMC	0.56	<0.001	0.78	0.001	0.83	<0.001
TC	0.34	0.02	0.77	0.001	0.68	<0.002
SOC	0.35	0.02	0.77	0.001	0.68	<0.003
DOC	−0.19	0.19	0.6	0.02	0.58	<0.004
TN	0.33	0.02	0.02	0.12	0.72	<0.005
TC:TN	0.01	0.94	0.23	0.43	0.07	0.68
MBC	–	–	0.77	0.001	–	–
CO_2 Flux	–	–	0.72	0.004	–	–
CH_4 Flux	–	–	0.20	0.5	–	–
MAOM-C	–	–	–	–	0.52	0.001
POM-C	–	–	–	–	0.67	<0.001
MAOM-N	–	–	–	–	0.44	0.008
POM-N	–	–	–	–	0.52	0.001
hemicellulose	–	–	–	–	0.67	<0.001
cellulose	–	–	–	–	0.73	<0.002
lignin	–	–	–	–	0.74	<0.003
ash	–	–	–	–	−0.85	<0.004

4. Discussion

Numerous prior studies in agricultural soils have elucidated the utility of POXC as an informative indicator of soil management techniques, often deemed as the best and most rapid response variable to changes in crop rotation management, tillage practices, compost amendments, and overall crop productivity (Culman et al., 2012; Fine et al., 2017; Hurisso et al., 2016; Jensen et al., 2019). Although POXC is consistently shown here, and in the literature, to be a strong correlate of common soil C pool indicators (e.g., OMC, TC, SOC), it has also been touted as providing additional and unique data regarding SOM quality (Fine et al. 2017). Specifically, POXC is considered by others to be a better indicator than other biological metrics (e.g., mineralizable C, MBC) of the abundance of the slightly processed OM pool and the C occluded with fine minerals, with the implication being that these pools are indicative of long-term C storage and stabilization (Culman et al. 2012; Hurisso et al. 2016). This study sought to evaluate if POXC may be an equally useful and reliable method for comparing wetland soils with unique soil properties, potentially providing complementary or additional information about soil C pool quality and stability.

4.1. Wetland soils all contain large POXC fractions

Direct comparisons of POXC concentrations across published studies are imperfect due to slight variations in methodology. However, the POXC concentrations observed in the wetlands evaluated in this study, compared to prior (mainly agricultural) studies, suggests POXC abundance is significantly greater (on average, ~ 37 times higher) in wetland

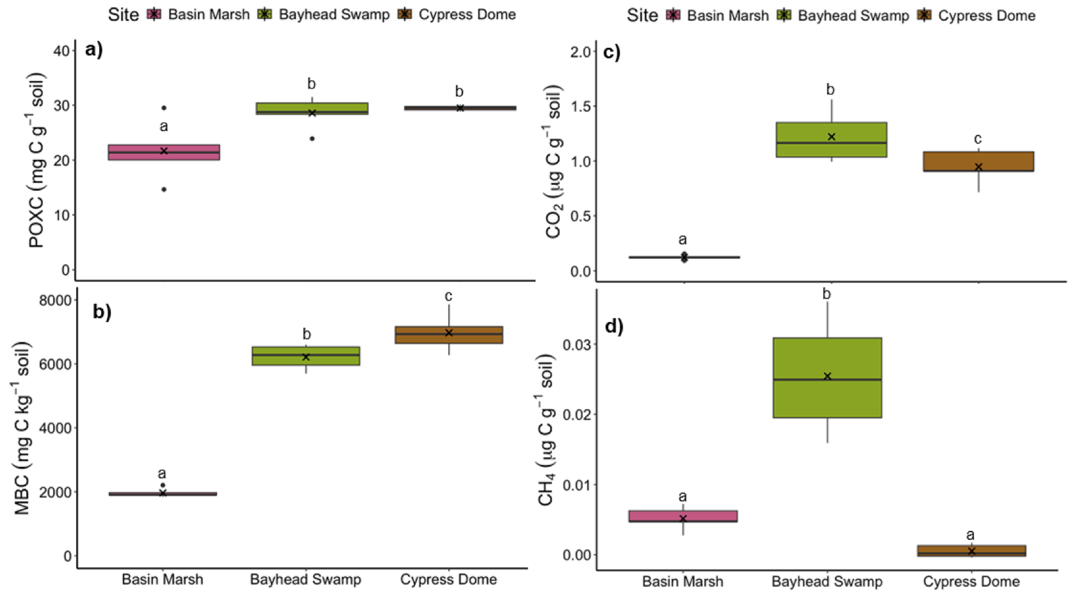

Fig. 3. Box plots of biological metrics quantified on freshwater wetlands only, by wetland site. Boxes represent 1st, 2nd, and 3rd quartiles of the data distribution, x represents the mean, and tails and dots represent the full variance of the data distribution. Different letters represent significantly different means ($p \leq 0.05$) based on general linear models (glm) and Tukey pairwise comparisons ($n = 5$).

soils than most terrestrial soils (Table 4). The only prior study with comparable POXC concentrations to the current study was by Bhadha et al. (2018), which was conducted in 'muck' (organic rich) agriculture soils formed by the drainage of the Everglades wetlands. That study was used as a model for the current research, including increasing the concentration of $KMnO_4$ used from 0.02 M (commonly seen in traditional upland agricultural soils (Weil et al. 2003)) to the 0.2 M. This higher concentration helped to compensate for the larger size of the POXC pool, thus ensuring enough color remained after the oxidation reaction to accurately quantify the change via spectrophotometry (Bhadha et al. 2018).

The significantly greater OMC of wetland soils relative to the upland agricultural soils is the most obvious explanation for the significantly greater POXC concentrations, given the strong positive correlation between these properties. However, waterlogging may also contribute to more POXC because it directly influences soil redox conditions, and thus the rates and pathways for C mineralization, the solubility of metals that often associate with the SOM, and the release of C as DOC (De-Campos et al., 2012; Kögel-Knabner et al., 2010; Reddy and DeLaune, 2008). A study by Wilson et al. (2011), investigated experimental flooding of an Australian floodplain soil and found flooding caused a rapid (within 1 d) increase in POXC, relative to non-flooded conditions, and the flooded treatment POXC remained ~ 29 % higher throughout the 24-d study. It was concluded that POXC is a sensitive indicator to flooding (and associated changes in soil C availability (Wilson et al., 2011)). Others have noted that POXC values increase when soil structure is disturbed (Gruver, 2015), so the general lack of structural development through the occurrence of natural aggregates in most wetlands may also contribute to a higher proportion of POXC in wetland soils, relative to uplands (Mirabito and Chambers, 2023). Finally, recent laboratory experiments indicate POXC increases over the course of the decomposition process (Christy et al., 2023) so the particularly high concentrations of POXC in wetlands may be a result of the anaerobic conditions preserving decomposition products over long periods of time.

4.2. POXC is strongly related to other common soil C metrics

Using multiple methods, including PCAs (Fig. 2), correlations (Table 3), and visual interpretations (Figs. 1, 3, and 4), it is evident that POXC concentrations in wetland soils are positively related to many other commonly measured soil C parameters, including OMC, TC, SOC, DOC, TN, MBC, CO_2, soil physical fractions (MAOM-C/N, POM-C/N), and soil chemical fractions (hemicellulose, cellulose, lignin). These finding are consistent with numerous previous studies that found strong correlations between POXC and TC, SOC, lignin, POM-C, C mineralization/CO_2, and TN, among other parameters not measured in the current study (e.g., soluble carbohydrates, infiltration, water stable aggregates, dehydrogenase activity) (Culman et al., 2012; Mandal et al., 2011; Morrow et al., 2016; Tirol-Padre and Ladha, 2004; Weil et al., 2003). Despite the consistency of these correlatives, there are other soil properties with mixed findings. For example, some found strong correlations between POXC and MBC (Weil et al. 2003; Mandal et al. 2011), while other studies found no relationship (Tirol-Padre and Ladha 2004). The strength of the relationship with DOC also varies somewhat depending on extraction method and specific study (Culman et al., 2012; Morrow et al., 2016). Mixed results for the relationship with DOC are not surprising given the heterogenous nature of DOC, which can comprise both labile and stable compounds (Kalbitz et al., 2003). Based on the work of Tirol-Padre and Ladha (2004) that tested what types of substrates $KMnO_4$ can oxidize, the strong correlation between POXC and cellulose found in the current study was unexpected, because cellulose does not contain the glycol groups $KMnO_4$ oxidizes.

4.3. Possible benefit of POXC analysis in wetland science

With so many statistically significant relationships, no individual parameter(s) stand-out as having the best relationship with POXC. This highlights the question: what is the potential benefit of POXC in wetland science? Here, we propose three possibilities for the utility of POXC quantification in wetlands that warrant additional research: 1) POXC could be useful in monitoring soil C changes over time; 2) POXC, and particularly the ratio of POXC:SOC, may provide unique information about SOC processing and stability; and 3) POXC measurement is simpler, faster, and more cost effective than many of the soil parameters it correlates with, making it a potential proxy for other analyses. Collectively, these benefits yield significant opportunities to further inform wetland soil C resource management in a variety of contexts.

In agriculture, POXC is most often used to identify changes/differences in soil C pools resulting from different soil management practices (e.g., tillage, cover crops, amendments); it has been shown in numerous

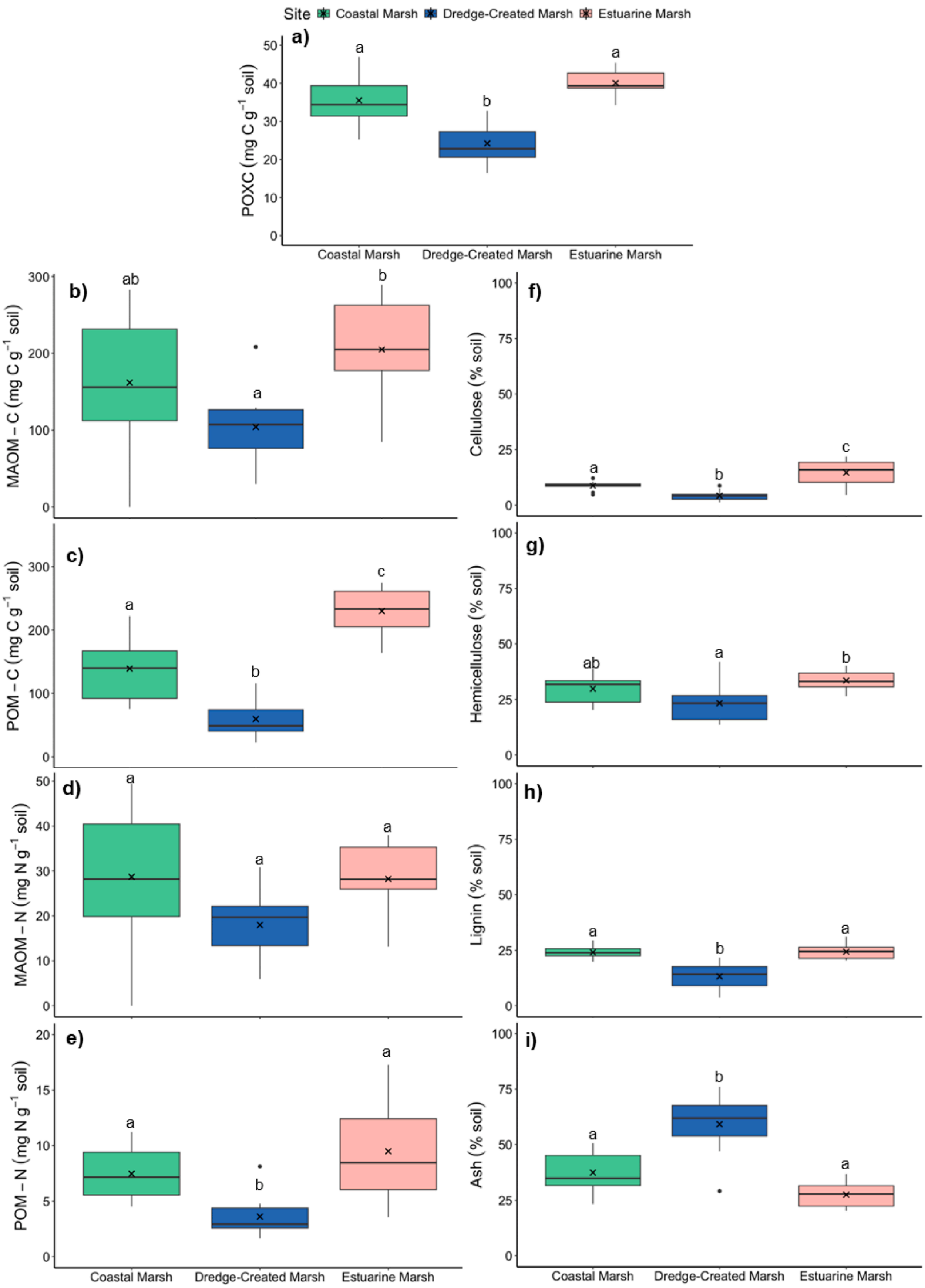

Fig. 4. Box plots of biological metrics quantified on saltwater wetlands only, by wetland habitat. Boxes represent 1st, 2nd, and 3rd quartiles of the data distribution, x represents the mean, and tails and dots represent the full variance of the data distribution. Different letters represent significantly different means ($p < 0.05$) based on general linear models (glm) and Tukey pairwise comparisons ($n = 5$).

studies to be more sensitive to changes in management practices than many, or all, other metrics studied (Culman et al. 2012; Morrow et al. 2016; Fine et al. 2017). In this study, we compared different wetland soils based on one discrete sampling (i.e., an observational study design) rather than tracking a single wetland soil over time (such as a before-after-control-impact) or a space-for-time study design. The later study designs, which focuses on temporal or management-driven changes in POXC, is most often employed in agricultural studies (Culman et al. 2012; Plaza-Bonilla et al. 2014; Hurisso et al. 2016; Bhadha et al. 2018) and may prove equally useful in wetlands. Most natural wetlands are not actively managed, but POXC could be helpful in monitoring soil C dynamics in created or restored wetlands that are subjected to hydrologic management (e.g., for wildlife habitat) or wetlands used in water treatment or bioremediation.

Future wetland research should test POXC over time or depth in response to independent variables (such as water level, plantings,

Table 4
Comparison of literature and observed concentrations of POXC. NA=not available.

Study	Soil description	$KMnO_4$ Conc.	POX-C	SOC content	POXC: SOC
		M	*mg g⁻¹*	*mg g⁻¹*	*%:%*
Bhadha et al., 2018	muck agricultural soils (FL, USA)	0.2	11–37	NA	NA
Chambers et al., 2023 **(this study)**	6 wetlands soils (USA)	0.2	21–41	99–461	6–23
Culman et al., 2012	53 agricultural soils (USA)	0.02	0.4–0.8	10–24	~4
Hurisso et al., 2016	76 agricultural soils (USA)	0.02	0.3–0.8	6–56	1–4
Mandal et al., 2011	Diverse upland soils (Spain)	0.01	0.2–0.4	6–12.5	2.7–3.4
Morrow et al., 2016	5 agroecosystems (NW USA)	0.02	0.1–0.5	8–28	~1–2
Weil et al., 2003	209 agricultural soils (W Hemisphere)	0.02	0.6–1.5	~15–40	~3–4
Wilson et al., 2011	Floodplain soils (Australia)	0.033	2.3–2.9	~39	~6

chemical loading rate, etc.), rather than an across wetlands comparison, such as performed herein. This recommendation comes from the fact our first hypothesis (POXC would differ among the diverse wetland soils studies) was generally not supported by the data. The observed variation in POXC concentrations between the 6 wetlands sampled in this study was low, and significantly lower than the variation among other C pools measured. For example, OMC content had a relative standard deviation (RSD) among the 6 wetlands of 53.6, which was more than twice that of deviation in POXC (RSD=24.4), resulting in fewer statically significant differences between wetlands for POXC, compared to OMC.

Since POXC concentrations showed little discrimination between wetlands, considering the ratio of POXC:SOC may be more informative in high OMC soils. The POXC pools in the studied wetlands represent ~ 6 to 23 % of the entire SOC pool, compared to ~ 1 to 6 % of the SOC pool in previously studied upland soils (Table 4). This wide disparity in POXC:SOC is primarily a product of variation in the denominator (i.e., POXC concentrations remained fairly consistent among wetlands, despite large differences in SOC content). As a result, the wetlands with the lowest OMC (highest mineral content; the Dredge-Created Marsh, Basin Marsh, and Coastal Marsh) had the highest percentage of the SOC pool represented by POXC. In wetlands, high mineral content soils are often suggestive of either younger wetlands, those subject to mineral soil deposition (e.g., floodplains, storm surge areas), or wetlands with more intermittent hydroperiods (less reduced conditions) that prevent excessive accumulation of SOM. A higher ratio could also indicate more of the SOC has undergone at least some degree of microbial processing (Culman et al., 2021). Enhanced microbial processing in wetland soils often reflects greater electron acceptor availability. For example, a varied hydroperiod that allows for aerobic respiration during periods of lowered water table could both enhance microbial activity and decrease the total SOC pool (Boudreau et al., 2024; Davila and Bohlen, 2021). A positive relationship between POXC:SOC and SOC persistence is therefore hypothesized. This ratio may function similarly to the ratio of MAOM:POM, which has also been shown to decrease as the total soil OMC increases (Cotrufo and Lavallee, 2022). Furthermore, others have found a link between POXC and smaller particle size fractions and occluded soils (Culman et al. 2012), such as those responsible for MAOM formation. Additional research is warranted to elucidate the ecological significance of the POXC:SOC in wetland soils and how informative it may be in quantifying C stability.

Finally, because POXC is strongly correlated with many other soil physicochemical properties, it could be an easier and more economical alternative to analyses that require more time (e.g., CO_2 flux rate, MBC, physical, chemical, or density fractionation) or require specialized instrumentation (e.g., SOC, OMC, DOC). Some additional benefits of the POXC method include, 1) it can be done in the field with no instrumentation, only a color chart (Mandal et al. 2011), 2) it does not involve any hazardous chemicals, 3) it can be done with dried soil samples, extending the potential shelf time between collection and processing (Weil et al. 2003), and 4) it is as analytically, spatially, and temporally reliable as other common soil nutrient tests (Hurisso et al. 2018). In order to use POXC as quantitative proxy for other soil physicochemical properties, more in-depth studies would be need to be performed to establish a robust numeric relationships between metrics across a wide range of concentrations and locations, similar to other efforts where loss-on-ignition has been established as a proxy for total C in wetlands (Breithaupt et al. 2023).

5. Conclusions

Numerous studies have investigated and validated POXC as a reliable soil health metric in agricultural soils. This study tested the utility of the method to differentiate wetland soil properties related to C storage and cycling. Analyzing soils from six unique wetlands that varied substantially in OMC and type (freshwater and saltwater) revealed consistently high POXC concentrations (ranging from 22 to 41 mg g^{-1}), which was up to an order of magnitude greater than most agricultural soils studied. As a strong positive correlate of OMC, TC, SOC, DOC, and others, POXC concentrations are assumed to be elevated in wetlands because these soils naturally accumulate organic matter as a result of persistent anaerobic conditions slowing the rate of decomposition. Unexpectedly, despite high variability in OMC among the wetlands studied, POXC concentrations between wetlands resulted in minimal statistical differentiation between sites. This may indicate POXC is better suited for quantifying changes in a single system over time, or the impacts of disturbance or management activities, than it is suited for observing differences across many soils. Additionally, the ratio of POXC:SOC may be more informative than the POXC concentrations alone, indicating how much of the existing soil C is in a more processed (and potentially stable) form. Finally, the simplicity, reliability, and economic benefits to measuring POXC over other soil properties suggest it could be a useful proxy measurement for other properties, as well as informative of actionable wetland soil management alternatives. Additional research is needed to evaluate the ecological significance of POXC in wetland soils, including experimental manipulations to see if changes in POXC may function as an ‘early indicator’ of changes in wetland C storage or stabilization.

CRediT authorship contribution statement

Lisa G. Chambers: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Anthony J. Mirabito:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shannon Brew:** Writing – review & editing, Methodology, Investigation. **Chelsea K. Nitsch:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Jehangir H. Bhadha:** Writing – review & editing, Methodology, Conceptualization. **Nia R. Hurst:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Jacob F. Berkowitz:** Writing – review & editing, Resources, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

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