Physical Protection in Aggregates and Organo-Mineral Associations Contribute to Carbon Stabilization at the Transition Zone of Seasonally Saturated Wetlands

Anna I. Kottkamp · C. Nathan Jones · Margaret A. Palmer · Katherine L. Tully

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Abstract
Wetlands store significant soil organic carbon (SOC) globally due to anoxic conditions that suppress SOC loss. However, stored SOC may become vulnerable to decomposition where climate and land use change alter wetland hydrology. Seasonally saturated wetlands experience fluctuating hydrologic conditions that could promote physicochemical mechanisms known to stabilize terrestrial SOC. These wetlands are therefore likely to be important for SOC storage at the landscape-scale. This study examined physicochemical stabilization of SOC within five seasonally saturated wetlands across a hydrologic gradient from the frequently saturated basin edge to the rarely saturated upland. At each wetland, we monitored water level and collected soil samples from the top two mineral horizons across five transect points to quantify physical protection of SOC in aggregates and organo-mineral associations between SOC and iron (Fe). As expected, both SOC concentrations and SOC stocks from 10–50 cm decreased across the transect from frequently saturated soils to rarely saturated soils. However, SOC stocks from 0–10 cm increased along this gradient, indicating diverging SOC dynamics throughout the soil profile. The majority of SOC was associated with macroaggregates across the transect, suggesting that macroaggregates are likely to physically protect wetland SOC during seasonal drying. By contrast, Fe-associated SOC was low across the transect, though modest accumulations of Fe (5 mg Fe g−1 soil) were observed in the transition zone where saturation was most dynamic throughout the year. Our results suggest that SOC stabilization occurs via physical protection within macroaggregates and, to a lesser extent, organo-mineral associations during dry periods in and around seasonally saturated wetlands. As climate scenarios predict intensified wet and dry cycles in many wetlands, understanding SOC stabilization is critical to predicting vulnerability to future change.

Keywords Soil organic carbon (SOC) · Seasonally saturated wetlands · SOC stabilization · Physical protection of SOC in aggregates · Organo-mineral associations · SOC stocks

Introduction
Wetlands are a major component of the terrestrial carbon (C) cycle and sequester disproportionately large C stocks for their area (Mitsch et al. 2013). Given their C sequestration potential, wetland soils are the focus of current conservation and restoration efforts to help offset rising C emissions (Griscom et al. 2017). Research to date has generally focused on soil organic C (SOC) of permanently saturated wetlands, where anoxia and subsequently slow decomposition are strong environmental drivers of SOC storage. However, a considerable yet frequently overlooked subset of wetlands are seasonally saturated and therefore experience dry, oxic conditions annually (e.g., vernal pools, Delmarva bays, prairie potholes) (Zedler 2003; Brooks 2005;...
Calhoun et al. 2017). Under the existing understanding of wetland SOC storage, periodic drying of seasonally saturated wetlands might be expected to stimulate C emissions and SOC loss (Miao et al. 2017). Contrary to this expectation, seasonally saturated wetland soils sequester large SOC stocks (e.g., Pearse et al. 2018; Tangen and Bansal 2020), though little is known about the mechanisms that support long-term SOC storage during dry periods in seasonally saturated wetlands. As more extreme wetland wetting and drying cycles are expected with changes in land use and climate (e.g., Fennessy et al. 2018; Lee et al. 2020), understanding mechanisms of SOC storage in seasonally saturated wetlands will be critical to predicting the vulnerability of SOC to future change.

Given the unique condition of annual drying in seasonally saturated wetlands, mechanisms known to control SOC dynamics in upland (terrestrial) soils may also contribute to SOC stabilization during dry periods in these systems. Clay content has been correlated with SOC stabilization on mineral surfaces in upland soils (Oades 1988) and is assumed to also play a major role in wetland SOC sequestration (Daugherty et al. 2019). However, clay content alone does not distinguish mechanisms of SOC stabilization, especially in seasonally saturated soils where C cycles are influenced by numerous hydrologic and biogeochemical factors (e.g., LaCroix et al. 2019). Beyond clay content, two physicochemical mechanisms of SOC stabilization control long-term storage of SOC in upland soils: (1) physical protection of SOC within aggregates and (2) organo-mineral associations (Kaiser and Guggenberger 2000; Six et al. 2000; Moni et al. 2010; Schmidt et al. 2011). Because environmental conditions (i.e., anoxia) are dominant controls on SOC storage in saturated soils, the contribution of physicochemical mechanisms of SOC stabilization is understudied in wetlands. Even less is known about the potential for physical protection of SOC in aggregates and organo-mineral associations under the dynamic biogeochemical conditions of seasonally saturated wetlands.

Physical protection of SOC in aggregates is a dominant control on SOC storage in upland soils. Aggregates are associations between soil particles such as clay, silt, and organic matter which may protect SOC for years to decades by limiting microbial access to C, creating occluded anoxic microsites, and reducing decomposition (Puget et al. 2000; Lehmann and Kleber 2015; Ebrahimi and Or 2016). Aggregates may be physically separated into size classes associated with SOC of distinct ages and historical composition: macroaggregates are a dynamic, younger fraction related to plant detritus, while microaggregates represent a more biologically processed, older fraction (Six et al. 2004; von Lützow et al. 2006, 2007). Research from terrestrial soils suggests that aggregate formation and stability are influenced by soil moisture and wet-dry cycles (Denef et al. 2001, 2002), organic inputs (Park et al. 2007), biological activity (Blankinship et al. 2016), and interactions with clay and mineral oxides (Wagner et al. 2007). Several studies indicate the potential for physical protection of SOC within aggregates in permanently saturated wetlands (e.g., Hosslrer and Bouchard 2010; Maietta et al. 2019), but the extent of aggregate-associated SOC in and around seasonally saturated wetlands is unknown.

Organo-mineral associations are a second type of physicochemical mechanism of SOC stabilization that has almost exclusively been studied in terrestrial soils (Kaiser and Guggenberger 2000). Strong interactions with iron (Fe) can stabilize SOC for up to hundreds of years (Torn et al. 1997; Kleber et al. 2005; Mikutta et al. 2006; Wagai and Mayer 2007; Kögel-Knabner et al. 2008). The strength of association between SOC and Fe depends on the type and amount of soil Fe, ranging from adsorption or coprecipitation of SOC with Fe (hydr)oxides to chelation (Kaiser and Guggenberger 2000; Mikutta et al. 2014; Sodano et al. 2017). Mineral–mineral and organo-mineral associations also contribute to stable aggregate formation, as organic matter may act as a binding agent between clay minerals (Denef et al. 2002). Permanently saturated wetland soils tend to be depleted in mineral oxides due to reduction and subsequent leaching losses (Mitsch and Gosselink 2015). The reduction of Fe3+ in wetlands may destabilize SOC by facilitating anaerobic metabolism as an alternative terminal electron acceptor. Reduced Fe2+ may further promote the oxidative activity of certain enzymes (Wang et al. 2017) and inhibit compounds which would otherwise constrain microbial decomposition (Wang et al. 2019). However, Fe (hydr)oxides may be retained in soils that are only intermittently saturated, such as the transition zone between seasonally saturated wetland and upland. Subsequently, Fe and aluminum (Al) (hydr)oxides are better predictors of SOC levels than clay content in such high moisture, acidic soils (Rasmussen et al. 2018). If Fe and Al (hydr)oxides are present in seasonally saturated wetlands, these associations could contribute to the stability of wetland SOC during dry periods.

To characterize the contribution of physicochemical mechanisms to SOC storage during annual dry periods in seasonally saturated wetlands, our objectives were to (1) quantify total SOC and hydrologic characteristics, (2) examine the role of physical protection of SOC in aggregates, and (3) determine the extent of organo-mineral associations from the frequently saturated edge of the wetland basin to the rarely saturated upland. We investigated associations between SOC, aggregates, and Fe across a gradient of mean water levels using aggregate size fractionation and selective Fe extractions at five seasonally saturated wetlands. Across the wetland-upland gradient, we focused our study on the intermittently saturated transition zone. Transition zone soils tend to contain intermediate SOC stocks compared to...
the basin and upland and are likely important sites for SOC storage at the landscape scale (Webster et al. 2011; Fenstermacher 2012; LaCroix et al. 2019; Chanalburt et al. 2020); however, the role of SOC stabilization mechanisms in transition zone soils has not been quantified. We hypothesized that both aggregate- and Fe-associated C would be greatest in the hydrologically dynamic transition zone. Given the annual drying of seasonally saturated wetland systems, we expected the physicochemical mechanisms of SOC stabilization studied here to be most relevant when under dry, oxic soil conditions, which may become increasingly prevalent under future climate change conditions.

Methods

Study Sites

This study was located on the Delmarva Peninsula in the eastern U.S.A., which is bordered by the Chesapeake Bay on the west and the Atlantic Ocean on the east. Mean monthly temperature ranges from 1.3 °C (January) to 25.1 °C (July). Mean annual precipitation is 1105 mm and is distributed evenly through the year (Supplementary Fig. 1). Study wetlands were < 5 km apart; the region contains numerous seasonally saturated freshwater wetlands. Water table fluctuations in this system are primarily driven by seasonal evapotranspiration (Lee et al. 2020), with maximum surface water expression in late spring and wetland drawdown over the growing season from May to September (Brooks 2005).

We selected five seasonally saturated wetlands with similar mineral soils (Order: Ultisols, Suborders: Udults and Aquults), depressional shape (Fig. 1b), and vegetation (Maple-Oak forest). Given the acidity of the soils (Supplementary Fig. 2), calcium carbonates were assumed to be negligible.

Field Methods

Establishing Sample Points Along Transect at each Wetland

At each wetland, we established one 20–25 m transect with five evenly spaced sample points (4–5 m apart) extending from the frequently saturated “basin edge” (sample point A on the transect) to the rarely saturated “upland” (sample point E on the transect; Fig. 1a). The basin edge sample point was located at the minimum extent of ponded water observed during the 2017 water year, where A horizons were > 15 cm thick. The upland was designated as the elevated area with sandy soils around each wetland (Stolt and Rabenhorst 1987). Specifically, the upland sample point was located where (a) upland trees and understory vegetation were present and (b) no hydromorphic features were found in the upper 0.5 m of the soil (Supplementary Fig. 3).

Characterizing Hydrologic Conditions at each Sample Point

To characterize hydrologic conditions at sample points along the wetland transects, we used a combination of one year of continuous water level measurements, surveyed sample point elevations, and a simple interpolation procedure. Across all wetlands, we measured wetland and upland water levels at 15-min intervals throughout water year 2018 (Oct 1, 2017–Sept 31, 2018) using HOBO U20 water level loggers (HOBO, Onset Computer Corporation, Bourne, MA). We measured wetland water levels in surface water stilling wells located near the center of each wetland, and we measured upland water level in wells located near the upland sample point (Fig. 1a). We also measured relative elevations of sample points along each transect using a TOPCON RL-H5A Laser Level (Topcon Positioning Systems, Livermore, CA). Finally, we estimated mean daily water level at each sample point by interpolating between the edge of surface water inundation and the upland water level in the well. Notably, this procedure accounted for the dynamic expansion and contraction of surface inundation along each transect. Further, while seasonally saturated wetlands may experience temporary groundwater mounding (Phillips and Shedlock 1993; Rosenberry and Winter 1997), this approach assumed linearity given the relatively small spatial scale and low-relief landscape. Interpolated water levels were comparable to observed water levels as measured biweekly from May to August 2018 (R² = 0.87, Supplementary Fig. 4). For each transect point at each wetland, we calculated the mean water level for water year 2018 from daily water levels (n = 365 at each sample point). Higher, more positive values for mean water level indicated frequently saturated conditions at the basin edge, while lower, negative water levels indicated rarely saturated conditions at sample points towards the upland. A link to water level data, surveyed transect elevations, and interpolation procedure can be found in the Availability of Data and Material section.

We examined continuous hydrologic data (e.g., mean water level) instead of categorical transect points (e.g., A, B, etc.), as mean water level varied not only across the transect within wetlands but also at a given transect point among wetlands. This allowed us to characterize the relationship between hydrologic conditions and physicochemical mechanisms of SOC stabilization (e.g., physical protection in aggregates and organo-mineral associations). In addition to mean water level, we quantified hydrologic variability as “saturation events” in the upper 0.5 m of soil by calculating the number of times that water level rose above a threshold (mean water level > -0.5 m) in water year 2018. To characterize reducing conditions, Fe
oxide coated Indicator of Reduction In Soils (IRIS) films were deployed along each transect in April 2019 and analyzed after 30 d (Appendix A) (Castenson and Rabenhorst 2006; Rabenhorst 2018). In the upper 0.5 m of the soil profile, all metrics of hydrology and reducing conditions indicated frequently saturated conditions at sample points A and B, rarely saturated conditions at sample points D and E, and intermittently saturated conditions consistent with a transition zone near sample point C (Fig. 2; Supplementary Table 1).

Soil Sampling

Soils were collected with a gouge auger in the top 0.5 m of soil to represent the top two mineral horizons at sample points A–E at each of the five wetlands in June 2018 (Fig. 1a). We sampled by pedogenic horizon instead of arbitrary depth limits given the differences in soil type between transect points. In general, the first mineral horizon was approximately 5–20 cm in depth and the second mineral horizon was approximately 20–45 cm in depth.

Fig. 1 Cross-section schematic of theoretical sampling design (Panel A). Elevation gradient shown is exaggerated. Panel B shows actual location of wells and transects in wetlands that vary in topographic relief. Basin outline is the minimum extent of ponded water for each wetland.
horizons were excluded from analysis as they were high in fresh organic matter inputs and low in the mineral matrices necessary for stabilization. Three replicate cores from each of the A–E points were separated by horizon; replicates were combined and gently homogenized to obtain a representative sample for each horizon (Supplementary Fig. 3; Supplementary Table 2). Subsamples of 200 g were set aside for aggregate analysis.

Additional samples for bulk density were collected using a McCauley peat sampler at sample points A and B and using a 7.62 cm diameter sharpened aluminum core at sample points C–E. Bulk density samples were separated into three depth increments (0–10 cm, 10–30 cm, and 30–50 cm) and corrected for compaction using a simple linear correction (Walter et al. 2016). Bulk density was calculated by dividing dry mass by core volume for each increment, subtracting roots and rocks (Poeplau et al. 2017).

Soil Chemical Analysis

Soil moisture levels can fluctuate daily in seasonally saturated wetlands, and, as it was not within the scope of the study to collect multiple soil samples through time, we decided to air-dry all soils prior to analysis. Drying soils before aggregate analysis standardized soils and allowed soils to be stored while avoiding the effects of freezing on SOC. Standardization was necessary because aggregate stability in field-moist soils may be a function of antecedent water content (Gollany et al. 1991). However, we recognize that drying samples may have promoted the formation of stable macroaggregates (Beare and Bruce 1993) and novel Fe–C complexes following Fe$^{2+}$ oxidation (Kaiser et al. 2015). Therefore, by analyzing air-dried soils, we present the upper limit for both aggregate-associated C and Fe–C associations across the transect, to characterize the maximum potential contribution of physical protection and organo-mineral association to SOC stabilization in wetlands that dry seasonally.

Air dried soils were passed through a 2-mm sieve (excluding large roots and rocks) for analysis of pH, texture, C, nitrogen (N), and Fe. We measured pH using an Orion 9165BNWP Sure-Flow Combination pH probe on field moist soils with a 1:2 soil to solution (0.01 M CaCl$_2$) ratio. Texture was determined by the hydrometer method (Gee and Bauder 1986). Bulk C ($C_{\text{bulk}}$) and N were measured using dry combustion (LECO CHN-2000 analyzer; LECO Corp, St. Joseph, MI) with helium as the carrier gas and with a detection limit of 0.02% for total N and 0.01% for total C.
Physical Protection of SOC Determined by Aggregate Size Class Separation

We examined water-stable aggregates on mineral horizons in the upper 0.5 m using the wet sieving protocol established by Six et al. (2000) and modified for wetland soils by Maietta et al. (2019). Across the transect, some soils were drier while others were saturated at the time of collection. Dry soils were passed through a 4.75 mm sieve within 24 h of sampling and dried for 7 d. Saturated soils were dried for 3 d at room temperature, passed through a 4.75 mm sieve, then dried at room temperature.

Soils were wet-sieved within two months of collection into four size classes: (1) large macroaggregates (≥ 2000 μm), (2) small macroaggregates (250–2000 μm), (3) microaggregates (53–250 μm), and (4) silt/clay particles (<53 μm; includes only silt and clay sized particles not part of larger associations). Sieved samples were dried at room temperature with fans to evaporate excess water, then dried in a 65 °C oven for 7 d before weighing. Aggregate samples were then gently crushed, and roots were separated using a 1 mm sieve. Remaining mass for each size class was weighed and analyzed for C and N using dry-combustion (LECO CHN-2000 analyzer; LECO Corp, St. Joseph, MI). We measured sand content for all size classes > 53 μm using the pipette method on a 5 g subsample and then subtracted the mass of the sand to avoid overestimating aggregate mass (Appendix B) (Elliott et al. 1991; Six et al. 2002).

Organo-Mineral Associations Between Fe–C Determined by Extractions

We measured soil Fe concentrations using a dithionite-citrate-bicarbonate (DCB) extraction to estimate “total extractable” Fe oxides (FeDCB) following the method by Darke and Walbridge (1994). A separate extraction with acid ammonium oxalate (AAO) was used to estimate “poorly crystalline” Fe species (FeAAO; Darke and Walbridge 1994; Coward et al. 2018; Hall et al. 2018). For both extractions, the supernatant was filtered through a 0.45 μm nylon filter (Tisch Scientific, North Bend, OH) and stored at 4 °C until analysis of organic C concentration with a TOC/TN Analyzer (Shimadzu Corporation, Kyoto, Japan; Sugimura and Suzuki 1988). Fe concentrations were measured with AA spectrometry as described above.

Calculations and Statistical Approach

We calculated the proportion of total C in bulk soil (Cbulk) that was associated with each aggregate size class (Caggregate/Cbulk) (Table 1, Eq. 1). We also calculated the proportion of Cbulk that was associated with extracted Fe and used this normalized value for analyses (Cextract/Cbulk; Table 1, Eq. 2). We tested the main effect of mean water level, where a higher mean water level indicated more frequently saturated soils towards the basin edge and a lower, more negative water level indicated more rarely saturated soils towards the upland. We also tested the main effect of horizon by categorizing horizons in order of depth: first mineral horizon (1), second mineral horizon (2), and a higher mean water level indicated more frequently saturated soils (mean water level ≤ 0.5 m) and “rarely saturated” soils (mean water level > 0.5 m). Thus, for concentrations of Fe and associated C (e.g., FeAAO, FeDCB, FeDCB, Cextract/Cbulk), we performed separate LME models on frequently saturated and rarely saturated soils to describe the effects of hydrology on organo-mineral associations.

We also used simple linear regression to test the relationship between SOC stocks and mean water level. Preliminary data exploration showed differences between 0–10 cm and 10–50 cm depth increments; therefore, we conducted separate analyses for SOC stocks from 0–10 cm and 10–50 cm.
| Property                  | Variable                        | Abbreviation | Unit                      | Source or Equation | Description                                                                 |
|--------------------------|---------------------------------|--------------|---------------------------|--------------------|-----------------------------------------------------------------------------|
| Bulk Soil C              | Bulk soil C concentration       | $C_{\text{bulk}}$ | %; g C \cdot g^{-1} soil | CHN Analyzer       | Concentration of C in the bulk soil sample                                   |
|                          | Bulk soil C:N                   | $C:N_{\text{bulk}}$ | ratio; g C \cdot g^{-1} N | CHN Analyzer       | Ratio of C:N in bulk soil                                                  |
|                          | Soil C stock                    | $C_{\text{stock}}$ | kg C \cdot m^{-2}         |                    | Carbon stock in each length increment, calculated by multiplying the C concentration in each depth increment by the bulk density and sample length |
| Aggregate Size Class     | Aggregate fraction mass         | $g_{\text{aggregate}}$ | g aggregate              | mass$_{\text{aggregate}}$ | Mass of aggregate size class after air drying and removal of rocks (> 1 mm) |
|                          | Aggregate C:N                   | $C:N_{\text{aggregate}}$ | ratio; g C \cdot g^{-1} N | CHN Analyzer       | Ratio of C:N within an aggregate size class                                |
|                          | Aggregate C concentration       | $C_{\text{aggregate}}$ | mg C \cdot g^{-1} soil   | CHN Analyzer; $(C_{\text{mass}}_{\text{aggregate}})/\text{mass}_{\text{total}}$ | Concentration of C in each aggregate size class per gram of bulk soil, excluding rocks |
|                          | Normalized aggregate-associated C | $C_{\text{aggregate}}/C_{\text{bulk}}$ | mg C \cdot g^{-1} C | $(C_{\text{mass}}_{\text{aggregate}})/\sum(C_{\text{mass}}_{\text{aggregate}})$ (Eq. 1) | Proportion of C associated with an aggregate fraction, normalized to C of the entire sample. Indicates the relative proportion of C in each aggregate fraction, normalized for differences in bulk soil C across the transect |
| Bulk Soil Fe             | Poorly crystalline Fe           | $Fe_{\text{AAO}}$ | mg Fe \cdot g^{-1} soil   | Atomic absorption spectrometer | Concentration of poorly crystalline Fe species, extracted by AAO |
|                          | Total extractable Fe            | $Fe_{\text{DCB}}$ | mg Fe \cdot g^{-1} soil   | Atomic absorption spectrometer | Concentration of total extractable Fe species (crystalline and poorly crystalline), extracted by DCB |
|                          | Percent poorly crystalline Fe   | $Fe_{\text{AAO}}/Fe_{\text{DCB}}$ | % | $Fe_{\text{AAO}}/Fe_{\text{DCB}} \times 100$ | Percent of total extractable Fe that is poorly crystalline |
| Fe–C Association         | Fe-associated C                 | $C_{\text{Dit-HCl}}$ | mg C \cdot g^{-1} soil   | TOC Analyzer       | Concentration of C extracted with Dit-HCl, an inorganic extraction for total extractable Fe |
|                          | Extracted Fe                    | $Fe_{\text{Dit-HCl}}$ | mg Fe \cdot g^{-1} soil   | Atomic absorption spectrometer | Concentration of Fe extracted with Dit-HCl |
|                          | Normalized Fe-associated C      | $C_{\text{Dit-HCl}}/C_{\text{bulk}}$ | mg C \cdot g^{-1} C | $C_{\text{Dit-HCl}}/C_{\text{bulk}}$ (Eq. 2) | Proportion of C associated with $Fe_{\text{Dit-HCl}}$, normalized to bulk soil C |
|                          | Extractant C:Fe                 | $C_{\text{Dit-HCl}}/Fe_{\text{Dit-HCl}}$ | ratio; g C \cdot g^{-1} Fe | $C_{\text{Dit-HCl}}/Fe_{\text{Dit-HCl}}$ | Ratio of extracted C to extracted Fe; indicates the kind of association between C and Fe |
Initially, this LME model included wetland as a random effect to account for differing wetland intercepts. However, the random term did not significantly improve the model (log-likelihood ratio test, \( P > 0.05 \)) (Zuur et al. 2009). Therefore, we removed the random term and conducted a simple linear regression.

Statistical analysis was conducted using R statistical software v3.5.3 (R Development Core Team 2019). Plots were created with the tidyverse and cowplot packages (Wickham 2017; Wilke 2019).

**Results**

**Hydrologic Variability was Greatest at Sample Points with a Mean Water Level Near -0.5 m**

Temperature in water year 2018 was within the 30-year mean (mean monthly range: -1 °C in January to 26 °C in July 2018; Supplementary Fig. 1). Precipitation over the year was 1366 mm, which was higher than average due to high precipitation in July and September 2018. Water levels varied ~1 m over the year at each sample point, indicating dynamic hydrology typical for seasonally saturated wetlands of the region (Fig. 2a). Water levels rose from November to February and remained high until May. Water levels fell from June to September 2018 but rose in response to storm events in July and September.

Saturation within the upper 0.5 m of the soil profile was most variable at sample points with a mean water level of -0.5 m (Fig. 2b) compared to both the more rarely saturated sample points (e.g., yellow and green lines, Fig. 2a) and the more frequently saturated sample points (e.g., dark blue lines, Fig. 2a). Accordingly, low Fe oxide reduction on IRIS films at sample point C (Supplementary Table 1) indicated heterogeneity of reducing conditions at the transition zone between frequently and rarely saturated soils.

**SOC Concentrations were Highest in Frequently Saturated Soils and Declined Towards the Rarely Saturated Upland**

Soil \( C_{\text{bulk}} \) concentration was greatest in frequently saturated soils and decreased with decreasing mean water level toward rarely saturated soils \( (P = 0.001, \text{Supplementary Table 3}; \text{Fig. 3a}) \). With increasing horizon depth, \( C_{\text{bulk}} \) concentrations decreased, as is typical for soils not affected by fluvial processes \( (P < 0.0001, \text{Supplementary Table 3}) \). However, we observed contrasting relationships by soil depth between SOC stocks and mean water level. In the top 0–10 cm, SOC stocks were lowest in the frequently saturated soils and increased as mean water levels declined \( (P = 0.001, \text{Supplementary Table 4}; \text{Fig. 3b}) \). Conversely, SOC stocks in the 10–50 cm depth increment were greatest in frequently saturated soils and decreased with decreasing mean water level \( (P < 0.0001, \text{Supplementary Table 4}; \text{Fig. 3c}) \).

Bulk soil clay (%) was greatest in frequently saturated soils and decreased with decreasing mean water level \( (P < 0.0001, \text{Supplementary Table 5}; \text{Supplementary Fig. 2}) \), while percent sand tended to increase. For soil pH, there was a significant interaction between horizon and mean water level. As mean water level decreased, soil pH decreased in
the first mineral horizon but increased in the second mineral horizon \((P = 0.0017,\) Supplementary Table 6; Supplementary Fig. 2).

**Physical Protection of SOC in Macroaggregates was Greatest in Frequently Saturated Soils**

Among size classes, small and large macroaggregates contained the highest \(C_{\text{aggregate}}\) concentrations, while microaggregates and silt/clay contained lower \(C_{\text{aggregate}}\) concentrations \((P < 0.0001,\) Supplementary Table 7). In the large macroaggregate size class only, \(C_{\text{aggregate}}\) concentrations were greatest in frequently saturated soils and decreased as water levels declined \((P = 0.00098,\) Supplementary Table 8; Fig. 4a–d).

Normalized \(C_{\text{aggregate}}/C_{\text{bulk}}\) proportions \((\text{mg C g}^{-1}\text{ bulk C}; \text{Table 1, Eq. 1})\) decreased with decreasing water level toward rarely saturated soils in the large macroaggregates but increased in all smaller size classes \((P < 0.05\) for all tests, Supplementary Table 9; Fig. 4e–h). Among size classes, normalized \(C_{\text{aggregate}}/C_{\text{bulk}}\) proportions were highest in small macroaggregates and lowest in the smallest size classes \((P < 0.0001;\) Supplementary Table 7). Normalized \(C_{\text{aggregate}}/C_{\text{bulk}}\) proportions declined in the deeper horizon in large macroaggregates but increased in microaggregates \((P < 0.05,\) Supplementary Table 9; Fig. 4e).

**Fe-Associated C was Highest in Rarely Saturated Soils and Declined Sharply Toward the Frequently Saturated Soils**

We conducted separate statistical analyses for frequently saturated soils \((\text{mean water level} > -0.5\text{ m})\) and rarely saturated soils to understand the effect of mean water level on Fe species. In frequently saturated soils, Fe concentrations of all extracted species were low \(<0.5\text{ mg Fe g}^{-1}\text{ soil};\) Fig. 5a; Supplementary Fig. 5). In rarely saturated soils, \(Fe_{\text{Dit-HCl}}\) concentrations were highest where mean water level was approximately 0.5 m below the soil surface \((\text{transition zone}),\) but concentrations decreased as mean water level declined \((P < 0.0001,\) Supplementary Table 10; Fig. 5a). We observed similar patterns in poorly crystalline \(Fe_{\text{AAO}}\) and total extractable \(Fe_{\text{DCB}}\) concentrations \((\text{Supplementary Table 11; Supplementary Table 12; Supplementary Fig. 5})\). Across the entire transect, the proportion of poorly crystalline extractable Fe oxides \((Fe_{\text{AAO}}/Fe_{\text{DCB}})\) was highest in frequently saturated soils and decreased with decreasing mean water level \((P < 0.0001,\) Supplementary Table 13; Supplementary Fig. 5).

Fe-associated C followed similar patterns to the extractable Fe species and was also analyzed separately in frequently and rarely saturated soils \((\text{Fig. 5b})\). In frequently saturated soils, Fe-associated \(C_{\text{Dit-HCl}}\) concentrations showed no significant changes with decreasing water level. In rarely saturated soils, Fe-associated \(C_{\text{Dit-HCl}}\) concentrations showed no significant changes with decreasing water level. In rarely saturated soils, Fe-associated \(C_{\text{Dit-HCl}}\) concentrations showed no significant changes with decreasing water level.

![Fig. 4](image-url) Aggregate C \((C_{\text{aggregate}})\) and normalized Aggregate-Associated C \((C_{\text{aggregate}}/C_{\text{bulk}})\) vs. mean water level \((\text{from frequently saturated soils to rarely saturated soils})\), by aggregate size class. Solid lines represent significant differences across mean water level in the LME models. Statistical results in Supplementary Information.
soils, Fe-associated \( C_{\text{Diit-HCl}} \) concentrations decreased as mean water levels declined (\( P = 0.00035 \), Supplementary Table 14). To understand the relative amount of total C associated with Fe across the entire transect, we calculated the normalized \( C_{\text{Diit-HCl}} / C_{\text{bulk}} \) proportion (mg C g\(^{-1}\) bulk C; Table 1, Eq. 2). There was no significant association between mean water level and the proportion of \( C_{\text{Diit-HCl}} / C_{\text{bulk}} \) in either the frequently or rarely saturated soils (\( P > 0.05 \), Supplementary Table 15; Fig. 5c). However, when we compared \( C_{\text{Diit-HCl}} / C_{\text{bulk}} \) proportions across frequently versus rarely saturated soils as separate groups, a greater proportion of bulk soil C was associated with Fe in rarely saturated soils than frequently saturated soils (Welch’s two sample t-test, \( P < 0.05 \)).

Discussion

Seasonally saturated wetlands play an important role in SOC storage and stabilization (Pearse et al. 2018; Chanlabut et al. 2020), yet the mechanisms underpinning these processes during seasonally dry, oxic conditions are understudied. We observed patterns of correlation across the wetland-upland gradient which suggest that SOC in seasonally saturated wetlands may be stabilized during dry periods by physical protection within macroaggregates and, to a lesser extent, by organo-mineral associations with Fe. Specifically, the proportion of total C within large macroaggregates was highest in frequently saturated soils, suggesting that macroaggregates are likely to physically protect wetland SOC during seasonal drying. In contrast, Fe-associated C was low across the transect, peaking in the transition zone where saturation is most dynamic throughout the year. Overall, this research highlights the potential role of physicochemical mechanisms of SOC stabilization in wetlands that dry annually, indicating the need for research on how these mechanisms influence the resilience of wetland SOC to future change.

SOC Changes Across the Gradient from Frequently Saturated to Rarely Saturated Soils

Our results demonstrate that mean water level can be used as a continuous indicator of hydrologic conditions to examine linear and nonlinear trends in soil characteristics across wetlands, building on prior studies studying SOC across discrete topographic categories (e.g., Webster et al. 2008, 2011; Pearse et al. 2018; LaCroix et al. 2019; Chanlabut et al. 2020). Low relief, wet forests are ubiquitous throughout the eastern U.S., but given their complexity they have typically been neglected in forest C models (e.g., Hurtt et al. 2019) and may be poorly represented by upland SOC models (Trettin et al. 2001). As remote sensing and geospatial methods improve wetland inundation detection (e.g., Lang and McCarty 2009; Lang et al. 2013; Evenson et al. 2018), relationships between SOC and hydrology could lead to more accurate models of SOC stocks in seasonally saturated wetlands.

We found contrasting trends by soil depth across the hydrologic gradient, wherein SOC stocks of surface soils (0–10 cm) were highest in the rarely saturated upland, while SOC stocks from 10–50 cm were greatest in frequently saturated soils (Fig. 3b–c). As expected, wetland SOC stocks from 10–50 cm decreased from frequently saturated to rarely saturated soils. In the lower depth
increment, we hypothesize that these strong trends in SOC storage are predominately due to trends in saturation and anoxia across the hydrologic gradient, as all metrics studied here indicate saturation and reducing conditions increase toward the basin edge (Supplementary Table 1). However, SOC stocks from 0–10 cm increased from frequently saturated to rarely saturated soils, which we speculate is from higher root growth in the shallow organic horizons of upland soils than in the basin edge (LaCroix et al. 2019). Our results were consistent with research from similar seasonally flooded wetlands which found selective preservation of chemically reduced organic matter with high molecular weight in wetland surface horizons, indicating that anoxic conditions are a dominant control on SOC accumulations in wetland soils (LaCroix et al. 2019).

The trends in SOC stocks observed here suggest that the overall relationship between hydrology and SOC is continuous rather than binary (e.g., wetland vs. upland). We observed greater SOC stocks from 10–50 cm in frequently saturated and transition zone soils than rarely saturated soils (Fig. 3). Our findings reinforce the SOC storage potential of intermittently flooded mineral soil wetlands found by Chanlabut et al. (2020), in which intermittently flooded soils contained higher SOC than permanently flooded soils. While the transition zone makes up a small portion of the area of an individual wetland, the transition zones of many seasonally saturated wetlands may contribute to important biogeochemical functions at the landscape scale (e.g., Tangen and Bansal 2020) and therefore must be included to improve wetland SOC models and expand targets of wetland conservation for SOC storage (e.g., Bridgam et al. 2006).

We observed strong relationships between mean water level and SOC characteristics, though soil texture also changed across the transect. Clay content is related to SOC stabilization (Oades 1988), as SOC may be protected from microbial decomposition by being bound to clay surfaces or sequestered in aggregates under anaerobic conditions (Weil and Brady 2017). However, bulk soil C was not correlated with percent clay along the transects studied here (Supplementary Fig. 6). Instead, we found relationships between macroaggregate content and SOC (Fig. 4). Thus, our method of examining aggregate size classes distinguished between the relative importance of SOC stabilization via free clay mineral surfaces (silt/clay fraction) and physical protection within aggregates (micro- and macroaggregate fractions). Our results suggest that percent clay alone as a proxy for C stabilization may not capture spatial variability of SOC in seasonally saturated wetlands. Rather, mean water level may be a better proxy for SOC in seasonally saturated wetlands due to its effects on anoxic conditions and SOC stabilization mechanisms in these systems.

**Large Macroaggregates Contained the most SOC in Frequently Saturated Soils**

Our observations suggest physical protection of SOC in large macroaggregates contributes to SOC stabilization in frequently saturated soils. Specifically, we observed that large macroaggregates (> 2 mm diameter) contained the most SOC across the transect and were correlated with hydrologic gradients. Both C concentration (Caggregate) and the proportion of total C (Caggregate/Cbulk) in large macroaggregates were highest in frequently saturated soils and were intermediate in the transition zone (Fig. 4). Large macroaggregates were greatest in surface soils, consistent with the expectation that large macroaggregates incorporate fresher organic inputs and are the most dynamic aggregate size class (Six et al. 2004). Because soils were dried prior to analysis for standardization, these results represent an upper limit for macroaggregate-associated C in sampled wetlands. While few studies have quantified macroaggregates in mineral wetland soils, Cui et al. (2019), Maietta et al. (2019), and Hossler and Bouchard (2010) found that large macroaggregates store significantly more SOC than other aggregate size classes in organic wetland soils. Additionally, Maietta et al. (2019) found that macroaggregate C was highest in the lowest elevation marsh sample point, consistent with the increase in large macroaggregates toward the frequently saturated soils found in the present study. We note, however, that several of the wettest soils studied here did not contain large macroaggregates; therefore, we expect that hydrology, in addition to other factors such as root activity and microbial biomass (e.g., Blankinship et al. 2016), contributes to aggregate stability in wetland soils.

Physical protection of wetland SOC within macroaggregates may be promoted by the influx of organic-rich water and frequently saturated conditions at the basin edge. The influx of C-rich water provides organic constituents that may increase macroaggregate stability by increasing sorption between organic matter and minerals at aggregate surfaces and by strengthening intra-aggregate structures through the diffusion of dissolved organic C (Park et al. 2007). Previous research in the study wetlands found high concentrations of dissolved organic C (Hosen et al. 2018), which we expect would contribute to macroaggregate stability. Soil saturation also influences aggregate formation and stability. Research from agricultural soils indicates that aggregates tend to increase under moderately high soil moisture compared to dry conditions (Blankinship et al. 2016). Further, multiple cycles of saturation and drying also tend to increase aggregates (Denef et al. 2001, 2002), which may be due to stronger interactions between organic matter and clay particles (Wagner et al. 2007) and greater macroaggregate cohesion with increased microbially-derived biomass (Cosentino et al. 2006). However, research on aggregates in
dry agricultural soils may not translate to wetland soils, as the wet-dry cycles in these studies are typically around 14 d (consistent with irrigation and subsequent drying). The influence of longer (annual) hydrologic cycles, such as those of seasonally saturated wetlands, is a critical area for new research on aggregates, as intact aggregates may mitigate the effects of annual wet-dry cycles and reduce C emissions during seasonally dry conditions (Navarro-García et al. 2012).

We found that the three smaller aggregate size classes were associated with the majority of SOC in rarely saturated, upland soils but were less responsive to the hydrologic gradient than large macroaggregates. The proportion of bulk soil C associated with smaller aggregate size classes (C_{aggregate}/C_{bulk}) increased from frequently saturated to rarely saturated soils, but C concentration in smaller size classes did not change across the gradient (Fig. 4). As expected, SOC associated with smaller aggregate size classes contained lower C concentrations and C:N than macroaggregates, indicating that microaggregates and silt/clay fractions consist of older, microbiologically derived C (Supplementary Table 12; Jastrow 1996; Six et al. 2000). Notably, microaggregates likely contribute to long-term SOC stabilization through strong adsorption reactions on mineral surfaces, underscoring both the importance of smaller aggregate size classes to long-term physical protection of SOC as well as the connection between aggregation and organo-mineral associations (Arachchige et al. 2018; Totsche et al. 2018).

**Organo-Mineral Associations were Relatively Low Across the Transect, with Minor Accumulations in Intermittently Saturated Transition Zone Soils**

Overall, total extractable Fe concentrations in our study wetlands were on the low end (0–5 mg Fe g\(^{-1}\) soil) of the typical range for wetlands in the Northeast and mid-Atlantic (0–12 mg Fe g\(^{-1}\) soil) (National Cooperative Soil Characterization Database 2017; LaCroix et al. 2019). Thus, our results suggest a relatively minor potential for organo-mineral associations in transition zone and upland soils that is constrained by low Fe concentrations. As we dried soils prior to analysis, these values represent the maximum levels of Fe that we would expect to find in the sampled soils, regardless of saturation level. Unsurprisingly, we observed the lowest total extractable Fe concentrations in frequently saturated soils (<1 mg Fe g\(^{-1}\) soil), where saturated conditions promote reduction and translocation of Fe (Fiedler and Sommer 2004; Chen et al. 2017). In contrast, modest accumulations of total extractable Fe (up to 5 mg Fe g\(^{-1}\) soil) were observed in the transition zone. Approximately 50% of the Fe oxides in the transition zone were poorly crystalline (Fe_{AAC}/Fe_{DCl}), indicating increased surface area available for transient association with C (Fig. 3; Supplementary Fig. 5) (Coward et al. 2017; Hall et al. 2018). We expect that minor accumulations of Fe in transition zone soils are the result of periodic groundwater mounding that causes Fe to flow from uplands towards ponds (Phillips and Shedlock 1993) as well as seasonally dry conditions in upper transition zone soils that promote Fe (hydr)oxide retention. Additionally, research in permafrost soils suggests that vertical translocation may promote Fe accumulation at redox interfaces such as the upper soils of the transition zone (Herndon et al. 2017).

Constrained by low Fe (hydr)oxide levels, Fe-associated C concentrations were low overall (Supplementary Fig. 5) but were highest in the transition zone and decreased linearly toward the upland (C_{DCl-HCl}; Fig. 5b). Our results add to existing evidence that Fe may associate with SOC in places where Fe is present, such as redox interfaces like the transition zone (e.g., Riedel et al. 2013). While Fe-associated C concentrations were highest in the transition zone, the proportion of total SOC that was Fe-associated (C_{DCl-HCl}/C_{bulk}) was highest in the rarely saturated soils, indicating that a greater fraction of SOC is in stabilizing organo-mineral associations in upland soils (up to 30%; Fig. 5c). Of note, high proportions of poorly crystalline Fe and high ratios of C:Fe in all samples (>1, Supplementary Table 16) indicate that most associations between C and Fe are transient and therefore susceptible to dissolution under seasonally saturated conditions when anoxic conditions are instead expected to drive SOC storage and stability (Kaiser and Guggenberger 2007; Wagai and Mayer 2007).

Organo-mineral associations in frequently saturated soils were even lower (<1 mg Fe g\(^{-1}\) soil) than in transition zone and rarely saturated soils. Only 10% of bulk soil C was associated with Fe_{DCl-HCl} in frequently saturated soils (Fig. 5c). These results corroborate other studies in mineral wetlands finding a lack of Fe (hydr)oxides in the wetland basin (e.g., LaCroix et al. 2019), suggesting that SOC in these frequently saturated soils are largely unprotected by organo-mineral associations. In fact, reactive and reduced Fe may stimulate C loss under saturated conditions, as has been suggested in some drained and restored wetlands (Anthony and Silver 2020). Subsequent oxidation of Fe\(^{2+}\) may increase C mineralization by generating reactive oxygen species and, under strongly acidic conditions, promoting the release of soil DOC (Hall and Silver 2013). Thus, we expect the SOC-stabilizing influence of organo-mineral associations to promote SOC stabilization primarily during seasonally dry soil conditions, which may increase in frequency and magnitude in the future.

While extractable Fe concentrations in the present study were relatively low, the role of organo-mineral associations in SOC stabilization may be underestimated in wet soils with greater concentrations of Fe (e.g., Kramer and Chadwick 2018). For example, one study found total extractable Fe concentrations of up to 25–30 mg Fe g\(^{-1}\) soil in vernal pools.
(Hobson and Dahlgren 1998), though Fe is rarely reported in studies of wetland SOC and thus the extent of wetlands with Fe concentrations of this magnitude is unknown. Further, while our results represent the upper limit of Fe oxides in sampled wetlands, future research including in-situ measurements (e.g., Anthony and Silver 2020) could characterize temporal dynamics of Fe–C associations in seasonally saturated wetlands.

**Physicochemical Stabilization Mechanisms in Wetlands that Dry Seasonally may Impact Landscape-Scale SOC Storage**

As small, depressional wetlands constitute a disproportionately high percentage of the total wetland perimeter length in many landscapes (Cohen et al. 2016), our results suggest that areas with fluctuating saturation play an important role in landscape-scale SOC stabilization. Given the relatively strong correlation between mean water level and SOC content, we posit that hydrology is a major driver of SOC storage and stabilization in these seasonally dynamic systems. Additionally, we identified correlations between mean water level and SOC associated with physicochemical stabilization mechanisms. Of the two mechanisms studied here, a greater proportion of total SOC was physically protected within macroaggregates (Caggregate/Cbulk) than was associated with Fe (CFe-HCl/Cbulk), indicating that macroaggregates contribute more to SOC storage in wetlands that dry seasonally. These results are a “snapshot” of the potential for physicochemical stabilization within a system that has been shaped by many annual cycles of soil formation, leading to a dynamic stability of SOC (Dynarski et al. 2020). Given how little is known about the stability of SOC in seasonally saturated wetlands, this study provides an initial assessment of physicochemical stabilization in a hydrologically dynamic wetland system. Future research on the molecular composition and age of wetland soil organic matter would clarify where organic matter has persisted in the soil for longer than would be expected given its molecular composition or anoxic conditions alone, indicating the potential for physicochemical controls on SOC stability.

The future of stabilized SOC in wetlands is uncertain because the hydrologic regime of seasonally saturated wetlands is vulnerable to changes in land use and climate (Kolka et al. 2018). Under current hydrologic conditions, we expect SOC of seasonally saturated wetlands to be stabilized by physical protection in aggregates and by organo-mineral associations where Fe or Al oxides are abundant. However, as more extreme weather events are predicted with climate change (USGCRP 2017), increased incidence of severe precipitation, droughts, and warmer temperatures may lead to longer dry periods and more rapid transitions between wet and dry conditions. In transition zones and rarely saturated soils, we expect the effects of increased drying conditions to be moderated by associations between Fe and SOC in the short term. In the long term, increased storm events and subsequent leaching losses of Fe from upland soils may render SOC more susceptible to loss as DOC (Kramer and Chadwick 2018) or via respiration. In frequently saturated soils, the low abundance of Fe–C associations may lead to enhanced SOC loss with increased drying (e.g., Fissore et al. 2009), though macroaggregates may mitigate some of these effects. Overall, our work highlights the complex implications of future hydrologic change on SOC emissions and stabilization in wetlands (Bridgham et al. 2006; Kolka et al. 2018).

**Conclusions**

Hydrologic conditions influence physicochemical mechanisms of SOC stabilization, which our research suggests may contribute to SOC stability during dry, oxic conditions in seasonally saturated wetlands. Overall, our results highlight distinct patterns in aggregate-associated C and, to a lesser extent, Fe-associated C from frequently saturated soils to the hydrologically dynamic transition zone, suggesting that these mechanisms merit further study in soils that experience both saturated and dry conditions throughout the year. These results are not only relevant for the seasonally saturated wetlands in this study, but likely extend to floodplain wetlands as well (Scott et al. 2019).

The role of global wetlands as a future C sink or source is uncertain, as climate and land use change alter wetland area and biogeochemical processing (e.g., Dahl 2011; Kolka et al. 2018; Moomaw et al. 2018). While the wetlands in this study are relatively small in area, up to one-fifth of wetland area in this region experiences seasonally dynamic hydrology (MD iMAP 2016), and seasonally saturated wetlands are globally ubiquitous (Calhoun et al. 2017). Therefore, the seasonally saturated areas of many small wetlands are likely to have a large cumulative impact on SOC stocks at the landscape scale. Additionally, small wetlands are disproportionately subject to loss from human activity (e.g., Van Meter and Basu 2015), causing a decline in wetland perimeter-to-area ratios and altering SOC storage. While wetland restoration tends to focus on large wetlands, our results indicate that small, seasonally saturated wetlands have a capacity for SOC storage and stability that must not be overlooked in conservation efforts. Our research emphasizes hydrologically dynamic soils of seasonally saturated wetlands as a critical but understudied component of landscape-scale wetland SOC storage and highlights the potential for physicochemical stabilization of SOC across the wetland–upland continuum.
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Author’s Contributions Kotkamp: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing-Original Draft, Visualization. Palmer: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing-Review & Editing, Supervision, Project administration, Funding acquisition. Jones: Formal analysis, Data Curation, Writing- Review & Editing, Visualization. Tully: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing- Review & Editing, Supervision, Project administration.

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Data Availability Water level data can be found at www.data.jpeg.org/10.5281/zenodo.3879223. All soil sample data is available in the Supplementary Information (SI).

Code Availability Code used for hydrologic analysis can be found at www.data.jpeg.org/10.5281/zenodo.3879223.

Declarations

Ethics Approval NA

Consent to Participate NA

Consent to Publication NA

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