Salt Marsh Soil Organic Carbon is Regulated by Drivers of Microbial Activity

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Running Head: SOC is Regulated by Drivers of Microbial Activity

ABSTRACT

Soil organic carbon is the foundation for soil health and a livable climate. Organic carbon is concentrated in coastal wetland soils, but dynamics that govern carbon persistence in coastal ecosystems remain incompletely understood. Whether microbial activity results in a gain or loss of carbon depends on environmental conditions that regulate microbial community attributes. We sought to identify which drivers of microbial activity have the greatest impact on organic carbon content in salt marsh soils. To address this question, we used the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analyses) Statement to compile data on soil and ecosystem characteristics from 50 studies of over 60 salt marshes located around the world. We conducted a meta-analysis with structural equation modeling, including mediation and moderation analyses, to identify environmental drivers of salt marsh soil organic carbon content. High salinity, pH, nitrogen, and phosphorus were associated with increased microbial biomass carbon and soil organic carbon. Correlations between microbial biomass and organic carbon were strengthened by soil salinity and nitrogen, and weakened by soil water content. These results suggest that environmental conditions that control microbial growth and activity have potential to preserve or degrade organic carbon in salt marsh soils.

Keywords: blue carbon, wetlands, soil ecology, meta-analysis, structural equation modeling

SUSTAINABILITY STATEMENT

This study can inform the development of plans which ensure healthy coastal soils that mitigate climate change and support ecological and human communities, satisfying multiple UN Sustainability Goals, including climate action and life on land. Carbon loss is occurring broadly in salt marsh ecosystems. Over 50% of salt marsh habitats in the United States have already been lost to anthropogenic development (Kennish, 2001). The balance between soil carbon sequestration and loss may be shifted by environmental conditions that govern plant productivity and microbial carbon processing (Macreadie et al., 2017; Spivak et al., 2019). Human perturbation can cause salt marshes to switch from carbon sinks to carbon sources and reduce the carbon burial capacity of salt marsh soils (Hopkinson et al., 2012; Mcleod et al., 2011). Conservation of soil carbon, on the other hand, preserves ecosystem function (Gurmu, 2020; Lal, 2016). Coastal ecosystem conservation may be bolstered by sustainable land management practices geared at retaining salt marsh soil carbon, for example, through programs like REDD+ which value carbon stored in a particular ecosystem (Duchelle et al., 2018). Soil carbon conservation can serve as an economically persuasive proxy for ecosystem and biodiversity conservation.
INTRODUCTION

Soils are living reservoirs of organic carbon that provide the vital foundation for a habitable planet. Healthy soils are productive ecosystems that support plant growth, animal health, and environmental quality (Doran, 2002). At the root of healthy soils is soil organic carbon (SOC): the primary component of organic matter and a common indicator of soil quality. SOC supports functional and productive ecosystems (Lal, 2004). A review of global SOC stocks reports the median estimate as 1500 petagrams of carbon, which is more than half of the ~2500 Pg carbon stored in terrestrial ecosystems as a whole (Scharlemann et al., 2014; Watson et al., 2000). The large size of the soil carbon pool means that small changes in the balance between SOC accumulation and loss may substantially influence the atmospheric concentration of carbon dioxide and other greenhouse gases, either mitigating or worsening the effects of anthropogenic climate change (Davidson & Janssens, 2006). Since soil carbon can be gained or lost, SOC is an incomplete measure of soil health when measured alone. SOC dynamics are regulated by microorganisms, and the effect of microbes on SOC is tuned by the character of the soil environment. Measurements of microbial biomass and activity, as well as environmental conditions that regulate these factors, provide deeper insights into the health status of a given soil.

Soil microorganisms depend on SOC to sustain their growth, and soil formation relies on the activities of soil organisms. Soil microorganisms can be envisioned as a collective pump through which carbon is transformed, and these microbes act as “double agents,” as the metabolic activities of a microbial community can cause both carbon accrual and loss (Liang et al., 2017; Liang & Zhu, 2021). Decomposition has historically been framed as a cause of carbon loss, but soil microbial activity can promote long-term carbon storage by consolidating molecular compounds that can be stabilized in the SOC pool (Kallenbach et al., 2016; Liang et al., 2017). A large portion of SOC in terrestrial soils consists of fungal and bacterial necromass (Kindler et al., 2006; Schweigert et al., 2015). Microbial biomass carbon (MBC) is thus an apt metric for estimating the size of a soil microbial community and the amount of carbon contained therein. While a large body of literature exists on environmental controls on carbon cycling in upland soils and offshore marine ecosystems, less is known about the impact of these same controls over microbial processing of carbon in salt marsh sediments, where SOC is concentrated.

Coastal and marine ecosystems have such high concentrations of soil carbon that they have invoked attention and a new name for this subset of the global carbon pool: blue carbon (Nellemann & Corcoran, 2009). Though coastal wetlands occupy relatively little land area, their total rate of carbon sequestration is similar to that of terrestrial forest ecosystems which are 30-100 times larger in land area. Salt marsh soils sequester approximately 218 g carbon m$^{-2}$ per year with a range of 18-1713 across 96 sites: nearly fifty times the rate of carbon burial in terrestrial forest soils (Chmura et al., 2003; Duarte et al., 2005; Mcleod et al., 2011). High rates of carbon burial in salt marsh soils are typically attributed to accumulation of organic materials from tidal and plant deposits and saline, anoxic conditions that suppress microbial decomposition of organic matter and respiration of CO$_2$ (Spivak et al., 2019). A more complex story arises when microbial generation of SOC is considered. A more comprehensive understanding of feedback between microbes and coastal soil conditions will advance efforts to conserve or build blue carbon in a changing world (Roner et al., 2016).

Retention of coastal blue carbon is a priority in a world marked by persistent coastal development and environmental changes which accelerate loss of coastal soil carbon. Anthropogenic perturbations like coastal development, sea level rise, and eutrophication modify the structure and function of coastal wetlands and effect constituent microbial communities (Cabral et al., 2018; Martone & Wasson, 2008; Paerl et al., 2002; Ridge et al., 2017). Such perturbations may switch marsh soils from carbon sinks to carbon sources: a positive feedback which worsens climate change (Mcleod et al., 2011). Soil carbon loss is facilitated by enhanced microbial respiration and tidal leaching of dissolved organic matter when coastal ecosystems are disturbed or converted to other land uses (Pendleton et al., 2012). Widespread human deterioration of coastal wetlands has already resulted in the loss of over 50% of salt marsh habitats that once grew in the United States (Kennish, 2001). Remaining salt marshes are estimated to sequester
between 4.8 and 87.2 Tg carbon annually (Chmura et al., 2003; Duarte et al., 2005; Mcleod et al., 2011). Continued degradation underlies the pressing need to preserve vital ecosystem services provided by surviving coastal wetlands.

To maintain or build vital blue carbon stocks, we need an improved understanding of the interplay among SOC, environmental factors, and microbial indices. We know that salinity regulates microbial community structure and function in coastal estuarine soils (Xue et al., 2023; Zhang et al., 2021). Salt marsh plant community composition drives changes in soil quality relative to unvegetated soil and regulates microbial community composition (Gu et al., 2022). Moisture regulates greenhouse gas emissions from salt marsh soils (Xue et al., 2023). Furthermore, nitrogen, phosphorus, and carbon cycling are intrinsically linked, while addition of nitrogen and phosphorus affects microbial processes in salt marsh soils (Caffrey et al., 2007; Finzi et al., 2011). However, the interacting effects of these environmental drivers on microbial carbon processing in salt marsh soils is not fully understood.

To test the hypothesis that blue carbon is affected by environmental drivers through microbial biomass and activity, we evaluated (1) how the relationship between environmental drivers and SOC was mediated by microbial biomass and and (2) how the relationship between SOC and microbial biomass responded to environmental drivers. To evaluate these research questions, we performed quantitative meta-analysis and collected data on a wide range of environmental characteristics known to affect soil microbial activity. The analysis included ecological variables which are known to affect the dynamics of soil microbial carbon processing, such as moisture, salinity, and pH. Linear and structural equation modeling were applied to the compiled data set to test predictions about ecological controls on carbon dynamics in salt marsh ecosystems. Using model output, we determined the degree to which microbial ecology affects long-term salt marsh soil carbon storage, and constructed a model for microbial soil health in coastal wetlands (Figure 1).

MATERIALS AND METHODS

We employed the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) Statement to select papers for a quantitative meta-analysis of salt marsh soil ecology (Moher et al., 2009). The PRISMA Statement is a useful methodology for conducting systematic reviews and analyses which comprises a checklist and flowchart for selecting studies for inclusion in the analysis. A search was performed on the Web of Science on 22 November, 2019 using the following constraints: TS = (salt marsh OR intertidal marsh) AND TS = (microbial activity OR metabolism OR flux OR organic matter OR microbial biomass OR carbon dioxide OR methane OR nitrous oxide). 2,858 papers were screened for duplicates, and duplicates were removed, resulting in 2,847 papers. These articles were screened by title and abstract for relevance to the review, resulting in 426 full-text articles which were then assessed for eligibility using the same qualifiers. Relevance to the review was determined on the basis of whether papers reported microbial indicators of soil health (microbial biomass or greenhouse gas emissions). 120 papers remained for the qualitative review, and this review was used to set the inclusion criteria for the quantitative analysis and to identify the variables to be extracted for review.

We first identified variables relevant to the microbial ecology of coastal wetland soils that were frequently reported in the literature using the 120 papers selected from full-text articles for inclusion in the qualitative synthesis. Commonly reported variables relevant to microbial ecology included plant biomass, soil temperature, salinity, sampling depth, bulk density, pH, moisture, nitrogen, phosphorus, carbon, microbial biomass, and field and laboratory fluxes of carbon dioxide (CO$_2$), methane (CH$_4$) and nitrous oxide (N$_2$O). Environmental parameters such as geographic coordinates, study type (in situ experimental, ex situ experimental, or observational), salt marsh type (natural, constructed, restored, drained, etc.), marsh zone (high, middle, low), and dominant plant species were also recorded.

Articles were included in the quantitative analysis as they met the following criteria: (1) soil samples were derived from a salt marsh, (2) study results were organized in a manner permissible to
numeric data extraction, (3) authors explicitly reported overlaying dominant plant species, and (4) authors
reported at least two of the three following indicators of soil health: soil carbon content, microbial
biomass, and/or greenhouse gas emissions (CO\(_2\), CH\(_4\), or N\(_2\)O). Using these criteria, 50 articles were
included in the quantitative analysis.

We compiled the mean value and unit of measurement for characteristics reported for associated
sets of soil samples. The standard error of the mean and sample size were also recorded when available.
Data were extracted from graphs using WebPlotDigitizer where numeric data were not explicitly reported
(Rohatgi, 2021). Unpublished data generously provided by the authors of several studies accompany
published data points. Since each of the data points collected for this study are sourced from primary
research articles which generally report mean values for associated sets of soil samples, sample sizes
differ between data points, sample sizes often differ for different variables in the same data point, and the
nature of association between averaged samples is contingent on study design (i.e. study authors may
report mean values for soil samples that are grouped by tidal zone, season, depth, etc.). Differences in
sample size and sampling scheme were accounted for by including the mean results from each study as a
single data point.

All linear regression analyses and structural equation models were performed in R (R Core Team,
2021) with packages here, MASS, tidyverse, corrr, corrrplot, knitr, tidySEM, lavaan, ggplot and
processR (Lissa, 2022; Moon & Hong, 2021; Müller & Bryan, 2020; Murdoch et al., 2021; Ripley et al.,
2022; Rosseel et al., 2022; Wei et al., 2021; Wickham et al., 2021; Wickham & RStudio, 2021; Wright,
2021; Xie, 2021). The regressions were run by excluding NA values. The fact that not all authors report
the same variables constrains our ability to build large models that holistically consider covariance
between soil characteristics. We instead performed pairwise regressions between each set of soil
characteristics to manually parse out covariances. Although over 30 variables were collected, only a
subset of variables were used in the analysis due to data sparsity and limitations on the sample size of
certain variables, a problem inherent to the collection of data related to dozens of environmental
variables. Ultimately, underrepresented variables (n<15) were excluded from the analysis. Excluded
variables included available phosphorus, in situ CH\(_4\), in situ N\(_2\)O, and several measures of microbial
biomass (phospholipid fatty acids, colony forming units, bacterial density count, and moles ATP). The
remaining data were converted to common units when possible. Carbon to nitrogen ratio (C:N) was
reported directly in some studies and calculated manually for other studies where both SOC and TN were
reported. Microbial biomass carbon (MBC) was log-transformed to meet the assumptions of linear
regression. Soil respiration relative to soil carbon content was found as the ratio of the CO\(_2\) production
rate (incubation only; CO\(_2\) μg g\(^{-1}\) h\(^{-1}\)) to soil organic carbon (SOC; mg g\(^{-1}\)). Linear models were fit to each
pairwise combination of variables.

Mediation analysis was performed with lavaan in R to determine whether microbial biomass
carbon explains the observed associations between environmental conditions (ENV) and soil organic
carbon (Figure 2a). Microbial carbon pump theory (Liang et al., 2017; Liang & Zhu, 2021) and empirical
evidence on microbial responses to environmental drivers were central to model construction (Spivak et
al., 2019). Path models were constructed which included an environmental condition (ENV) as an
exogenous explanatory variable, SOC as the primary response variable, and MBC as the mediating
variable. ENV includes pH, nitrogen, phosphorus, moisture, temperature, and other markers of soil health,
but only one of these characteristics was applied in each model. Both MBC (path A) and SOC (paths C
and A*B) were dependent on ENV across models. Mediation therefore tests the assumptions that (1)
MBC depends on ENV and (2) SOC depends on MBC. Thus, three regression pathways exist in each
mediation model: path “A” regresses MBC on ENV, path “B” regresses SOC on MBC, and path “C”
regresses SOC on ENV. Path C corresponds to the direct effect of the environmental condition on SOC,
and the indirect “mediating” effect is explained by paths A*B. A significant indirect effect implies that
MBC mediates the relationship between the explanatory environmental variable and the response variable
SOC.

Moderation analysis was conducted using the processR macro in R to evaluate how soil
conditions affect the association between microbial biomass carbon and soil organic carbon (Figure 2b)
Moderation tests the effect of a moderating variable on the correlation between a response variable and its predictor variable by assessing how the association between these two variables changes under differing values of the moderating condition. Moderating variables may affect the strength or direction of a correlation between a predictor and response variable (Baron & Kenny, 1986). Using this framework, a simple structural equation model was designed that included microbial biomass carbon as the focal predictor variable and soil organic carbon as the outcome variable. Several permutations were made to this baseline model wherein the moderation effect of various environmental variables was tested on the relationship between MBC and SOC. Environmental conditions tested as moderators include salinity, moisture, pH, total nitrogen, total phosphorus, belowground biomass, and temperature. Mediation and moderation models had different sample sizes because each regression was separately modeled in mediation analysis, whereas all three variables were required for the regression in moderation analysis.

RESULTS

Out of 2,847 candidate studies, 120 studies screened by title and abstract were included in a qualitative synthesis which was used to determine inclusion criteria for quantitative review. 50 studies met the inclusion criteria for quantitative synthesis, and data from these 50 studies were extracted for statistical analysis (Figure 3). Assembled data encompassed over 271 data points and thirty environmental variables, including indicators of soil health such as soil organic carbon, microbial biomass, and greenhouse gas flux (Table 1). Over sixty salt marshes were represented in the resulting dataset. Sites from which soils were collected were distributed widely across Asia, Europe, and North America (Appendix B). Salt marshes occupy coastlines around the globe, but geographic sampling bias persists due to sociopolitical factors beyond the scope of this review. Since the data were compiled in November 2019, the quantitative portion of this review does not comprise the findings of more recent literature.

Table 1. Soil characteristics compiled from fifty published studies of salt marsh ecosystems.

<table>
<thead>
<tr>
<th>Soil characteristic</th>
<th>Unit of measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marsh condition</td>
<td>Natural, regenerated, reclaimed, restored, constructed, drained, impounded</td>
</tr>
<tr>
<td>Latitude</td>
<td>Latitude</td>
</tr>
<tr>
<td>Soil sampling depth</td>
<td>Sample depth min, max, range, average (cm)</td>
</tr>
<tr>
<td>Location in marsh</td>
<td>High/middle/low marsh, mudflat, channel</td>
</tr>
<tr>
<td>Plant biomass</td>
<td>Belowground plant biomass (BGPB; mg cm⁻²)</td>
</tr>
<tr>
<td>Dominant vegetation</td>
<td>Dominant plant species</td>
</tr>
<tr>
<td>Plant growth form</td>
<td>Shrub, forb, graminoid, tree, mix, none</td>
</tr>
<tr>
<td>Plant invasive status</td>
<td>Native, invasive, mix, none</td>
</tr>
<tr>
<td>Microbial biomass</td>
<td>Microbial biomass carbon (MBC; mg kg⁻¹)</td>
</tr>
<tr>
<td></td>
<td>Phospholipid fatty acids (PLFA; ng g⁻¹ or nmol g⁻¹)</td>
</tr>
<tr>
<td></td>
<td>Colony-forming units (CFU; g⁻¹)</td>
</tr>
<tr>
<td></td>
<td>Bacterial density count (BDC; 10⁹ g⁻¹)</td>
</tr>
<tr>
<td></td>
<td>16s rRNA copy number (16s; 10⁷ g⁻¹)</td>
</tr>
<tr>
<td></td>
<td>Moles adenosine triphosphate (ATP; 10⁻¹¹ g⁻¹)</td>
</tr>
<tr>
<td>Carbon dioxide (CO₂)</td>
<td>In situ CO₂ emission (mg m⁻² h⁻¹)</td>
</tr>
<tr>
<td></td>
<td>In vitro CO₂ emission (ug g⁻¹ h⁻¹)</td>
</tr>
<tr>
<td>Methane (CH₄)</td>
<td>In situ CH₄ emission (ug m⁻² h⁻¹)</td>
</tr>
<tr>
<td></td>
<td>In vitro CH₄ emission (ug kg⁻¹ d⁻¹)</td>
</tr>
<tr>
<td>Nitrous oxide (N₂O)</td>
<td>In situ N₂O emission (ug m⁻² h⁻¹)</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>In vitro $N_2O$ emission (ug kg$^{-1}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature</strong></td>
</tr>
<tr>
<td>Degrees °C</td>
</tr>
<tr>
<td><strong>Salinity</strong></td>
</tr>
<tr>
<td>Electrical conductivity (EC)</td>
</tr>
<tr>
<td>Parts-per-thousand (ppt)</td>
</tr>
<tr>
<td><strong>Redox status</strong></td>
</tr>
<tr>
<td>Millivolts (mV)</td>
</tr>
<tr>
<td><strong>Bulk density</strong></td>
</tr>
<tr>
<td>Bulk density (g cm$^{-3}$)</td>
</tr>
<tr>
<td><strong>pH</strong></td>
</tr>
<tr>
<td><strong>Moisture</strong></td>
</tr>
<tr>
<td>Mass %</td>
</tr>
<tr>
<td><strong>Total carbon</strong></td>
</tr>
<tr>
<td>Total carbon (TC; mg g$^{-1}$)</td>
</tr>
<tr>
<td>Soil organic matter (SOM; mg g$^{-1}$)</td>
</tr>
<tr>
<td><strong>Total nitrogen</strong></td>
</tr>
<tr>
<td>Total nitrogen (TN; mg g$^{-1}$)</td>
</tr>
<tr>
<td>Total organic nitrogen (TON; mg g$^{-1}$)</td>
</tr>
<tr>
<td>Available nitrogen (NH$_4$+NO$_3$; mg kg$^{-1}$)</td>
</tr>
<tr>
<td><strong>Total phosphorus</strong></td>
</tr>
<tr>
<td>Total phosphorus (TP; mg kg$^{-1}$)</td>
</tr>
<tr>
<td>Available phosphorus (AP; mg kg$^{-1}$)</td>
</tr>
<tr>
<td><strong>Carbon : nitrogen</strong></td>
</tr>
<tr>
<td>Weight / weight</td>
</tr>
<tr>
<td><strong>CO$_2$ : SOC</strong></td>
</tr>
<tr>
<td>In vitro CO$_2$ emission / total organic carbon</td>
</tr>
</tbody>
</table>

Aggregate data were structured to accommodate the diverse methods used to collect and report soil characteristics. The sample size of individual soil assays, the number of samples in each associated group of soils, and the condition which defined each associated group of soils differed between studies. For example, some authors grouped soils by their location in the tidal zone, while others categorized groups by study site, sampling season, or soil depth. These variables were recorded but not used in the final analysis. Authors generally reported mean values (often with SE, SD, CV) for associated groups of soil samples. For these reasons, each group of soil samples was regarded as a single data point, irrespective of sample size or classification scheme. Variables like soil moisture and redox status vary with tidal conditions, so relationships to these variables must be interpreted carefully as the values reported were frequently aggregated across multiple timepoints or locations.

Meta-analysis was designed (1) to test the hypothesis that long-term soil carbon storage is subject to environmental drivers of microbial activity and (2) to identify environmental conditions that regulate the effect of soil microbes on SOC. Summary statistics (mean, median, range, and standard deviation) for each measured soil health indicator are reported in Table S1, and distributions for measured indicators are displayed as histograms in Figure S1. Like many environmental attributes, few variables were normally-distributed. Most measured indices were heavily right-skewed: the frequency of low values for a given indicator is high, and few data points yield a high value for that given indicator. Most variables were unimodally distributed, but variables including total and available phosphorus (TP and AP) displayed multiple peaks.

Soil organic carbon content was associated with many environmental attributes.

Soil variables were correlated between salt marshes, and correlations were evident in data collected from marshes around the world (Figure 4). High soil salinity, nitrogen, and phosphorus, for example, were associated with increased microbial biomass and SOC. The relationship between SOC and soil salinity, however, depended on the technique employed to measure salinity: salinity measured as parts-per-thousand was positively but weakly correlated with SOC. In contrast, salinity measured as electrical conductivity (EC) displayed a negative correlation with SOC at a similar magnitude.

SOC was correlated with many indicators relevant to the activities of soil microbes (Figure S2). SOC was most strongly and positively associated with total carbon, total nitrogen, and total phosphorus. Bulk density was strongly and inversely related to SOC, and a positive linear relationship was found between SOC and log-transformed microbial biomass carbon. Other measures of microbial biomass (e.g.
PLFA, CFU, BDC) were not sampled frequently enough to support regression (pairwise n < 15). Carbon to nitrogen ratio (C:N) was positively associated with SOC. pH was negatively associated with SOC over a range of 5.6 to 9.38. Soil temperature positively correlated with SOC, in contrast to patterns observed in terrestrial soils. SOC correlated positively with in situ $N_2O$ and $CO_2$ flux, but it was not related to in situ $CH_4$ or in vitro greenhouse gas emissions. SOC was not significantly correlated with soil moisture, latitude, or plant biomass.

While summary statistics for linear regressions between all pairwise variables are reported in Table S2, the residuals of many of linear models were not normally-distributed, indicating that relationships between SOC and other soil attributes may be better explained by non-parametric or non-linear fits (Table S2; Figure S3).

**Microbial biomass explains some associations between environmental characteristics and SOC.**

Mediation analysis was applied to test the prediction that microbial biomass explains soil organic carbon content. Path models were constructed to assess the response of SOC to MBC while controlling for environmental covariates (Figure 2). Best performing models suggest that microbial biomass mediates the effects of plant biomass, soil nitrogen, salinity, temperature, pH, and bulk density on soil organic carbon. Table 2 lists summary and fit statistics for each model applied. The mediation model in which salinity explained SOC via MBC performed best, and both direct and indirect effects of salinity were significant. Additional environmental variables with significant direct and indirect effects on SOC include belowground plant biomass, bulk density, and pH. In other models, SOC is explained by either a direct or indirect effect, but not both. Moisture, for example, is directly associated with SOC, but the effect of moisture on MBC does not explain substantial variance in SOC. In contrast, variables like aboveground plant biomass and soil nitrogen explain SOC primarily through indirect effects via changes in MBC.

**Table 2.** Summary table of mediation path model fits. Models were designed to test the mediation effect of microbial biomass carbon (M) on the relationship between explanatory environmental variables (X) and the response variable, soil organic carbon (Y). AIC describes overall model fit (lower is better). Path coefficients are labeled A, B, and C, and p-values of direct (C) and indirect effects (A*B) are reported.

<table>
<thead>
<tr>
<th>X</th>
<th>M</th>
<th>Y</th>
<th>sample size</th>
<th>AIC</th>
<th>A (M ~ X)</th>
<th>B (Y ~ M)</th>
<th>C (Y ~ X)</th>
<th>p-value (C)</th>
<th>p-value (A*B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>MBC</td>
<td>SOC</td>
<td>203</td>
<td>1618</td>
<td>0.26</td>
<td>0.61</td>
<td>0.07</td>
<td>0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>pH</td>
<td>MBC</td>
<td>SOC</td>
<td>208</td>
<td>1506</td>
<td>-0.18</td>
<td>0.56</td>
<td>-0.40</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Moisture</td>
<td>MBC</td>
<td>SOC</td>
<td>196</td>
<td>1575</td>
<td>-0.02</td>
<td>0.63</td>
<td>0.09</td>
<td>0.09</td>
<td>0.81</td>
</tr>
<tr>
<td>Temperature</td>
<td>MBC</td>
<td>SOC</td>
<td>182</td>
<td>1447</td>
<td>-0.11</td>
<td>0.66</td>
<td>0.23</td>
<td>0.00</td>
<td>0.13</td>
</tr>
<tr>
<td>AGPB</td>
<td>MBC</td>
<td>SOC</td>
<td>177</td>
<td>1419</td>
<td>0.17</td>
<td>0.65</td>
<td>-0.08</td>
<td>0.20</td>
<td>0.02</td>
</tr>
<tr>
<td>Bulk density</td>
<td>MBC</td>
<td>SOC</td>
<td>185</td>
<td>1400</td>
<td>-0.49</td>
<td>0.44</td>
<td>-0.39</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>BGPB</td>
<td>MBC</td>
<td>SOC</td>
<td>175</td>
<td>1384</td>
<td>0.26</td>
<td>0.69</td>
<td>-0.22</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Salinity</td>
<td>MBC</td>
<td>SOC</td>
<td>185</td>
<td>1380</td>
<td>0.54</td>
<td>0.87</td>
<td>-0.44</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Environmental drivers of microbial activity moderate correlations between microbial biomass and soil organic carbon.

Moderation analysis was performed across conditions to test the hypothesis that environmental drivers of microbial activity moderate the effect of microbial biomass on the size of the soil organic carbon pool. Figure 2b depicts a simplified version of the moderation test: an environmental variable influences the relationship between microbial biomass and soil organic carbon. Test results are highlighted in Table 3. Moderation analysis showed a significant interaction effect between salinity (ppt) and the effect of MBC on SOC. The relationship between MBC and SOC was stronger under high salinity. Moisture weakened the relationship between MBC and SOC: a significant negative moderation effect was observed between moisture and SOC ~ MBC. Nitrogen and pH positively moderated the relationship between microbial biomass carbon and SOC. There was a small moderation effect of phosphorus on the effect of MBC on SOC. There was no significant moderation by soil temperature or belowground plant biomass on SOC ~ MBC.

Table 3. Summary table of moderation results. Blue entries represent significant positive moderation results, while red entries represent significant negative moderation results.

<table>
<thead>
<tr>
<th>Moderator</th>
<th>N</th>
<th>Model R^2</th>
<th>Model p-value</th>
<th>Moderation coefficient</th>
<th>Moderation p-value</th>
<th>Condition effect at 16th percentile moderator</th>
<th>P-value effect at 16th percentile moderator</th>
<th>Condition effect at 50th percentile moderator</th>
<th>P-value effect at 50th percentile moderator</th>
<th>Condition effect at 84th percentile moderator</th>
<th>P-value effect at 84th percentile moderator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>34</td>
<td>0.66</td>
<td>&lt;0.01</td>
<td>-0.25</td>
<td>&lt;0.01</td>
<td>5.03</td>
<td>&lt;0.01</td>
<td>1.29</td>
<td>0.06</td>
<td>-0.64</td>
<td>0.48</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>40</td>
<td>0.74</td>
<td>&lt;0.01</td>
<td>2.32</td>
<td>&lt;0.01</td>
<td>0.29</td>
<td>0.19</td>
<td>-1.02</td>
<td>0.19</td>
<td>-0.20</td>
<td>0.76</td>
</tr>
<tr>
<td>pH</td>
<td>28</td>
<td>0.71</td>
<td>&lt;0.01</td>
<td>5.56</td>
<td>&lt;0.01</td>
<td>0.24</td>
<td>0.48</td>
<td>0.54</td>
<td>0.48</td>
<td>7.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Salinity</td>
<td>22</td>
<td>0.36</td>
<td>0.04</td>
<td>0.66</td>
<td>0.03</td>
<td>0.19</td>
<td>-1.93</td>
<td>0.02</td>
<td>-1.03</td>
<td>0.21</td>
<td>2.87</td>
</tr>
<tr>
<td>Temperature</td>
<td>11</td>
<td>0.63</td>
<td>0.06</td>
<td>-0.25</td>
<td>0.22</td>
<td>0.10</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Belowground plant biomass</td>
<td>10</td>
<td>0.87</td>
<td>&lt;0.01</td>
<td>0.65</td>
<td>0.09</td>
<td>0.48</td>
<td>0.65</td>
<td>1.16</td>
<td>0.16</td>
<td>5.41</td>
<td>0.02</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>13</td>
<td>0.96</td>
<td>&lt;0.01</td>
<td>-0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>1.06</td>
<td>0.20</td>
<td>0.93</td>
<td>0.23</td>
<td>-0.86</td>
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</table>

DISCUSSION

Microbial relationships to soil carbon within salt marshes are sensitive to environmental conditions. In support of our hypothesis, MBC mediated the relationships between environmental conditions and SOC, and the relationship between MBC and SOC varied among environmental conditions. Overall, our results suggest that the effect of microorganisms on salt marsh soil carbon content is subject to key soil characteristics which are known to regulate microbial activity.

Mediation and moderation analyses enabled assessment of the impact of microbial biomass and its drivers on SOC. Covariation between environmental variables obscures these relationships. Structural equation modeling (SEM) circumvents the covariance problem by allowing a user to test proposed causal pathways while using Bayesian inference techniques to account for confounding factors. Using mediation...
analysis, a form of SEM, we identified the degree to which microbial biomass carbon (MBC) explained observed relationships between SOC and soil characteristics (Table 2). However, feedbacks exist between SOC and MBC over longer time periods. SOC is produced by microbial transformation of carbon stocks, and the production of MBC depends on the availability of substrate. Therefore, interpretation of mediation and moderation results must be taken in the context of the co-dependence of SOC and MBC.

Microbial biomass C mediated the relationships between SOC and salinity, pH, nitrogen, and belowground plant biomass, known drivers of microbial activity that may affect SOC via changes in microbial biomass and community function. Though SOC and salinity were not correlated, salinity had a positive effect on SOC via mediation by MBC, and the relationship between MBC and SOC was stronger under high salinity. Prior work has demonstrated reduced SOC content, suppressed SOC decomposition, and mixed effects on MBC with increasing salinity (Morrissey et al., 2014; Tripathi et al., 2006; Zhao et al., 2017). Soil characteristics with correlations to SOC not explained by MBC include moisture, phosphorus, and temperature. Associations between moisture and temperature with SOC may not have been affected by MBC because these characteristics were variable over time, and recorded values were often mean values that were aggregated across timepoints or locations. The finding that the relationship between SOC with nitrogen but not phosphorus was mediated by MBC suggests that nitrogen may be more limiting in salt marsh soils.

Moderation analysis tested how relationships between MBC and SOC varied under different values of moderating soil characteristics (Table 3). Positive moderators included nitrogen, pH, salinity, and belowground plant biomass, and these tightened the strength of the correlation between SOC and MBC under high levels of the moderator. Nitrogen as a positive moderator may suggest that nitrogen availability enables microbial growth that is associated with carbon content, or it may suggest increased dependence of microbial growth on a diminishing source of bioavailable carbon under nitrogen saturation (Kopáček et al., 2013). Negative moderators, like moisture, weakened the association between MBC and SOC. While this meta-analysis could not account for geographic location, the effect of moisture on the relationship between MBC and SOC may reflect the effects of location in the tidal platform. Since SOC and MBC are co-dependent, a positive interaction could suggest that under high levels of the moderator there is a net positive contribution of microbes to long-term carbon storage, or it could imply increased dependence of microbial growth on substrate availability. Whether positive moderators are conducive to microbial activities that promote long-term carbon storage in salt marsh soils is yet to be determined.

Linear correlations also revealed some unexpected relationships between soil health indicators, likely due to the characteristic nature of saline wetland systems. Greenhouse gas emissions, for example, varied under different environmental regimes. Relationships between gas flux and soil health indicators depended on whether emissions were measured in situ or in vitro: for example, SOC was positively correlated to in situ N₂O and CO₂ flux, but it was not related to in situ CH₄ or in vitro gas emissions. In vitro measures may not be related to SOC due to variability between incubation conditions. While the response of SOC and greenhouse gas emissions to various perturbations is well-documented, the dependence of greenhouse gas flux on SOC is not. Increased flux due to increased SOC may be explained by increased microbial biomass, but gas emissions were not well-represented enough in the papers used in this study to support this analysis. In situ flux of CO₂ and CH₄ were positively linked, and they were negatively correlated to N₂O. All measured gasses had emission levels which increased with soil moisture and temperature. Moisture and temperature are major determinants of soil gas emissions, with temperature generally increasing flux rates, but soil moisture has variable effects on flux rates which depend on the gas measured (Oertel et al., 2016; Schaufler et al., 2010). Some metrics of greenhouse gas flux were enhanced by plant biomass, SOC, and nutrient content. This is not surprising as microbial activity depends on substrate availability (Soong et al., 2020). Additionally, SOC was positively correlated to soil temperature, in contrast to observations made in inland soils, where SOC and temperature tend to be negatively or not correlated (Burke et al., 1989; Fissore et al., 2008; Kirschbaum, 1995; Nichols, 1984), a relationship that is confounded by other climate and plant community factors (Hartley et al., 2021; Jobbágy & Jackson, 2000). The positive correlation between temperature and SOC may be explained by compensatory mechanisms: despite increased levels of decomposition due to high
temperatures, higher plant biomass at lower latitudes may result in high SOC. The relationship may also be a result of limiting oxygen availability in wetland soils.

Several important variables were left out of this study due to lack of representation in the literature. Sulfur cycling, for example, is a major biogeochemical process that occurs in marine sediments and in the saturated sediments of coastal wetlands (Jørgensen et al., 2019; Wasmund et al., 2017). Microbial sulfate reduction is positively associated with soil salinity, and it inhibits methanogenesis via microbial competition for energy sources (Poffenbarger et al., 2011; Sivakumar et al., 2019). Interactions between sulfate-reducers and methanogens under different regimes of salinity could be explored in future studies. The effects of geographic and temporal variability (beyond latitude) were not considered, yet location and seasonality are factors known to drive soil composition and microbial activity (Fierer & Jackson, 2006; Zhang et al., 2023). Furthermore, plant species composition and marsh status (natural, degraded, restored) were not included in the final analysis and may be useful considerations for future study. Studies exhibited wide variability in methodologies and site attributes, and partitioning the data on these variables may add specificity to the findings of future studies.

The paradigm that attributes coastal blue carbon accrual to environmental restrictions on microbial activity does not paint a full picture of the roles played by microbes to regulate the permanence of the soil carbon pool. Since microbes are generally thought of as agents of soil carbon loss, “most research has historically focused on minimizing microbial outputs from the soil carbon pool” (Conant et al., 2011). The results of this study indicate that microbial biomass can positively contribute to the size of the soil carbon pool and suggest that this effect can be modulated by engineering the soil microenvironment to harbor conditions that are conducive to microbially-mediated long-term carbon storage. Wetland soil conditions regulate microbial processing of coastal blue carbon (Moffett et al., 2010; Seyfferth et al., 2020), and microbially-conscious land management practices commonly applied in terrestrial systems are often successful at enhancing key indices of soil health, but the extent to which microbial manipulation can affect soil carbon storage in coastal wetland soils is not yet clear (Bhattacharyya et al., 2022; Gougoulias et al., 2014; King, 2011). Adjusting environmental conditions that moderate the effect of MBC on SOC, such as nitrogen, pH, salinity, moisture, or belowground plant biomass, may be key to this practice.

Managing coastal wetlands to support microbial processes that enhance the grasp of soil on accumulated carbon may be critical to retaining coastal blue carbon in the face of drastic environmental change. Salt marsh soil characteristics exist in a network of ecological interrelationality, evidenced by strong associations found between many measured indicators that were consistent across study sites (Figure 4). The relationship between MBC and SOC was furthermore moderated by marsh conditions (Table 3). Since (1) microbial activity affects SOC, (2) relationships between MBC and SOC are moderated by environmental conditions, and (3) environmental moderators can be effectively measured and modeled, a microbial framework may be key to retaining blue carbon permanence in coastal ecosystems.

Research on soil health and land management strategies aimed at developing or maintaining this aspect of ecosystem health could include microbial indicators as well as environmental controls on microbial activity. SOC is not sufficient as a sole indicator of soil health, as it has limited value unless monitored over time or measured in combination with other metrics of health. Microbial biomass and community features such as microbial diversity can be measured alongside indicators of microbial activity such as carbon dioxide, methane, and nitrous oxide flux to build a more comprehensive view of soil health. While microbial indicators are important, we propose that environmental drivers of microbial function identified in this study should also be considered key indicators of coastal soil health, as these variables may provide insight into the longevity of coastal blue carbon. Nitrogen, salinity, and moisture were identified through moderation analysis as environmental drivers of microbial carbon processing. These factors, as well as phosphorus, pH, temperature, and other factors that correlate with MBC and SOC, are candidate indicators of coastal soil health. With a comprehensive soil health index that includes microbial indicators and environmental drivers of microbial carbon processing, we can more effectively...
evaluate the efficacy of restoration projects and determine strategies for improving desired ecosystem functions.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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DATA AVAILABILITY

The data underlying this article are available in Open Science Framework at https://osf.io/ and can be accessed with DOI 10.17605/OSF.IO/S25ZC

AUTHOR CONTRIBUTION STATEMENT

Hailey Erb: conceptualization (lead), data curation (lead), formal analysis (lead), methodology (lead), visualization (lead), writing – original draft preparation (lead). Kristen DeAngelis: conceptualization (supporting), funding acquisition (lead), project administration (lead), supervision (lead), writing – review & editing (equal). Ashley Keiser: supervision (supporting), writing – review & editing (equal).

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DATA CITATION


Figure 1. Model of microbially-mediated carbon dynamics in salt marsh soils. Microbes facilitate the transfer of plant-derived organic material into soil carbon pools. The nature of microbial processing of organic material can be estimated using greenhouse gas emissions as a proxy for activity. Green arrows denote transfer of carbon between functional pools, and black arrows indicate greenhouse gas fluxes. Dotted arrows indicate partial relationships: (1) some carbon is abiotically partitioned into the stable soil carbon pool, one that turns over relatively slowly, and (2) only a portion of a given soil microbial community partakes in the active growth and metabolism that lends to the emission of greenhouse gasses from living soils. Created with BioRender.com.
Figure 2. (a) Representative path model to test mediation hypothesis. In mediation, a response variable (Y) is regressed on an explanatory variable (X). A mediator (M) regressed on X is also predictive of Y. Path C represents the direct effect of X on Y, and paths A*B explain the indirect effect, or the mediation effect, of M on Y. (b) Graphic depicting a representative structural equation model that tests the moderation effect of environmental conditions on the relationship between microbial biomass carbon and total soil organic carbon. ENV denotes an environmental indicator such as salinity or moisture, MBC denotes microbial biomass carbon (log-mg kg⁻¹), and SOC denotes soil organic carbon (mg g⁻¹).
Figure 3. PRISMA flow diagram of the process used to gather data. 2,835 papers identified through an initial search on the Web of Science and 23 additional papers were filtered through systematic screening according to the PRISMA statement (Moher et al., 2009). Data used for subsequent SEM were extracted from the 50 papers which satisfied prerequisites for inclusion in quantitative synthesis.
Figure 4. Correlogram that displays correlation coefficients (Pearson’s r) between salt marsh soil health indicators. Circle size corresponds to the magnitude of correlation. Color corresponds to the directionality of each relationship (negative = red; positive = blue). Uncertain correlations (n < 10) are denoted with a gray question mark.