

1 Source and stability of soil carbon in mangrove and freshwater wetlands of the Mexican

2 Pacific coast

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12

13 **Abstract**

14 Wetlands can store large quantities of carbon (C) and are considered key sites for C sequestration. However, the  
15 C sequestration potential of wetlands is spatially and temporally variable, and depends on processes associated  
16 with C production, preservation and export. In this study, we assess the soil C sources and processes responsible  
17 for C sequestration of riverine wetlands (mangroves, peat swamp forest and marsh) of La Encrucijada Biosphere  
18 Reserve (LEBR, Mexican south Pacific coast). We analysed soil C and nitrogen (N) concentrations and isotopes  
19 ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) from cores dated from the last century. We compared a range of mangrove forests in different  
20 geomorphological settings (upriver and downriver) and across a gradient from fringe to interior forests. Sources  
21 and processes related to C storage differ greatly among riverine wetlands of the Reserve. In the peat swamp forest  
22 and marsh, the soil C experienced large changes in the past century, probably due to soil decomposition, changes  
23 in plant community composition, and/or changes in C sources. In the mangroves, the dominant process for C  
24 accumulation was the burial of *in situ* production. The C buried in mangroves has changed little in the past  
25 hundred years, suggesting that production has been fairly constant and/or that decomposition rates in the soil are  
26 slow. Mangrove forests of LEBR, regardless of geomorphological setting, can preserve very uniform soil N and  
27 C for a century or more, consistent with efficient C storage.

28

29 Keywords: carbon sequestration, marsh, peat swamp, isotopes, blue carbon, decomposition

30

## 31 **Introduction**

32 Wetlands are one of the most carbon (C) rich ecosystems on the planet (Donato et al. 2011; Mcleod et  
33 al. 2011; Page et al. 2011). Wetlands can be highly productive and store large quantities of C (e.g. McKee et al.  
34 2007) and are generally considered key sites for C sequestration, increasing their C content over time (IPCC,  
35 2007). However, C accumulation in wetlands is by no means a linear process as C is not only accumulated, but  
36 also transformed and exported through respiration and tidal export (Bouillon et al. 2008; Alongi 2011). The  
37 spatial and temporal variability in carbon production and export is large, and it has been associated to multiple  
38 factors including temperature, rainfall, and nutrient inputs (Saenger and Snedaker 1993; Kayranli et al 2010;  
39 Adame and Lovelock 2011). Thus, the potential of wetlands as sites for C sequestration is also spatially and  
40 temporally variable, dependent on processes associated with C production, preservation and export (Alongi 2011;  
41 Breithaupt et al. 2012).

42 The processes associated with C export and storage in wetlands are reflected in soil characteristics. For  
43 instance, in mangrove soils, if local production is high and terrestrial inputs are low, most of soil carbon will be  
44 composed of mangrove detritus (McKee et al. 2007). But if sedimentation is considerable and marine  
45 connectivity is strong, phytoplankton and seagrass detritus will have a significant contribution to the C soil  
46 (Kristensen et al. 2008). Microbial biomass can also be an important contributor to soil surface C (Wooller et al.  
47 2003). Riverine wetlands are characterized by productive forests with large amounts of organic material and  
48 suspended sediment inputs (Woodroffe 1992; Eyre 1993). Thus, it is likely that soil C of riverine wetlands  
49 originates from both local production and terrestrially derived material (Ewel et al. 1998), but few studies have  
50 tested this idea quantitatively. In this study, we assessed the characteristics of soil C within riverine wetlands  
51 (mangroves, swamp forest and marsh) using C and N (nitrogen) concentrations and isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ).  
52 Additionally, we compared a range of mangrove forests: from upriver mangroves with a dominant riverine  
53 influence to downriver mangroves with a stronger marine influence. We also compared mangroves within a  
54 gradient between fringe and interior forests. We tested whether highest autochthonous contributions (mangrove  
55 detritus) to the soil C occurred in fringe mangroves located upriver, where production is highest (Tovilla et al.  
56 2007).

57

58 The processes responsible for C production and storage vary with time and are continuously changing  
59 due to the dynamic nature of tropical coastlines (Woodroffe 1992). Tropical wetlands are often impacted by

60 storms and experience rapid changes in erosion and sediment deposition, which are likely to change the  
61 composition of soil C in time (Gonneea et al. 2004; McKee et al. 2007). Additionally, large anthropogenic  
62 impacts in the past century have significantly changed the function of tropical wetlands (Duke et al. 2007;  
63 Kurnianto et al. 2015), with soil changes expected to occur as a result of climate change (Alongi 2008). In this  
64 study, we assess the changes of soil C in the last century using sediment cores from wetlands in La Encrucijada  
65 Biosphere Reserve (LEBR) in the south Pacific coast of Mexico. The questions addressed in this study are: Do  
66 soil C sources of riverine wetlands vary across geomorphological settings and between fringe and interior  
67 forests? And, have the soil C sources and processes associated with C storage changed during the past century?  
68

69 **Methodology**

70 *Study site*

71 LEBR is located in Chiapas, along the south Pacific coast of Mexico at 14° 43' N, 92° 26' W. The  
72 Reserve has an area of 144,868 ha with five coastal lagoons connected to seven river systems. The climate of  
73 LEBR is warm and humid with most precipitation occurring in the summer months (June - October). The tidal  
74 regime is mixed, semidiurnal with a maximum tidal range of 1.8 m. The mean annual temperature of the region is  
75 28.2°C, with a mean annual minimum of 19.2°C and a mean annual maximum of 36.5°C; mean annual  
76 precipitation is 1567 mm (Sistema Meteorológico Nacional - Comisión Nacional del Agua, station No. 7320,  
77 1951-2010).

78

79 *Spatial differences among C sources*

80 LEBR is characterised by large areas of freshwater and estuarine wetlands including mangroves, marsh  
81 and peat swamp forests. The mangrove forest is dominated by tall (20-40 m) *Rhizophora mangle* trees (Tovilla et  
82 al. 2007). To assess differences in C sources among wetland types, we sampled mangrove forests dominated by  
83 *R. mangle* (six sites) and *Avicennia germinans* (one site), a swamp forest dominated by *Pachira aquatica* and a  
84 marsh dominated by the grass *Typha dominguensis* (Fig. 1, see site details in Adame et al. 2015b). To assess  
85 differences in soil C sources among geomorphological settings, we sampled a gradient of mangrove forests from  
86 upriver to downriver mangroves. The sites were classified based on their location and interstitial salinity from the  
87 most riverine to the most marine forest: Panzacola, Teculapa, Paistalon, Esterillo, Santa Chila, Las Palmas and  
88 Zacopulco (Fig. 1). To assess differences in soil C sources among a gradient from fringe to interior forests, at  
89 each site we collected six soil cores every 25 m along a 125 m-transect perpendicular to the water edge. In total,  
90 54 cores were collected. Sampling was conducted during December 2012.

91

92 The soil cores were collected using a peat auger consisting of a semi-cylindrical chamber of 6.4 cm-  
93 radius attached to a cross handle. Cores were systematically divided into depth intervals of 0 - 15 cm, 15 - 30 cm,  
94 30 - 50 cm, and > 50 cm. A soil sample (~ 5 g) within each interval was collected. Samples were air dried in the  
95 sun and then homogenized by grinding. Samples were analysed for C%, N%,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in an elemental-  
96 analyser isotope ratio mass spectrometer (Costech Elemental Combustion System 4010, Continuous Flow-Stable  
97 Isotope Ratio Mass Spectrometer, Michigan Technological University, Forest Ecology Stable Isotope  
98 Laboratory). Samples were analysed before and after being treated with hydrochloric acid to estimate inorganic C

99 content; in all samples, inorganic C was less than 5% of the total. Analytical errors (SD) were 0.25‰ for  $\delta^{13}\text{C}$   
100 and 0.5‰ for  $\delta^{15}\text{N}$ . Results for soil C and N concentrations have been published in Adame et al. (2015b). In this  
101 study only N:C ratios are reported and used in mixing diagrams (Perdue and Kroprivnjak 2007); for reference,  
102 C:N ratios are also given in some places alongside the N:C ratios

103

104 Carbon sources within the soil were assessed with biogeochemical source plots and mixing models  
105 (Monacci et al. 2011), using published values for mangrove detritus, seagrass, soil organic matter and  
106 phytoplankton as the possible end members (Fry 2006). Sources can have high spatial and temporal variability  
107 among sites and can lead to errors when used in different locations (Gonneea et al. 2004). However, the aim of  
108 this study was to make a relative comparison of sites across geomorphological settings, thus the published values  
109 were useful to observe relative changes in C sources among locations.

110

#### 111 *Temporal differences among C sources and soil C stability*

112 Temporal differences were analysed within each site using the soils obtained at different depths. The soil  
113 cores were dated with the use of a natural marker that consisted of a volcanic ash horizon of about 1-2 cm that  
114 was clearly identified in all the cores. The ash horizon was deposited during the eruption of Santa Maria Volcano,  
115 Guatemala in 1902, which was one of the four largest volcano eruptions of the 20th Century (Volcanic  
116 Explosivity Index of 6 out of 7; Williams and Self, 1983). As a result of the eruption, a recognizable ash deposit  
117 is found along the Mexican Pacific coast, northwest of the volcano. The ash occurred between 30-50 cm from the  
118 soil surface, so most of our samples were deposited within the past hundred years. The exception was the Las  
119 Palmas site, where sediment accumulation was slower and the ash horizon was found at 15-20 cm. The  
120 estimation of dates for marsh and peat swamp forests was less clear, because these vegetation types frequently  
121 suffer from fires and thus have confounding ash horizons. So it can only be assumed that the data from these  
122 ecosystems falls within the past century if sedimentation rates are of the same magnitude as those of mangroves.  
123 The natural volcanic ash marker allowed us to compare changes among sites and locations that occurred within  
124 the last century.

125

#### 126 *Interstitial salinity*

127 Salinity was measured to classify the mangrove sites within a geomorphological gradient (upriver to  
128 downriver mangroves). Salinity was measured in two periods, first at the beginning of December 2013 and then

129 in February 2014, both within the dry season. Salinity was measured within each plot from interstitial water, by  
130 extracting it from 10-30 cm depth with a syringe and an acrylic tube. Salinity was measured using an YSI-30  
131 multiprobe sensor (YSI, Xylem Inc. Ohio, USA).

132

### 133 *Data analyses*

134 Normality was assessed using Normality Probability Plots and Shapiro-Wilk tests. Differences among  
135 sites were analysed with a one-way ANOVA and Bonferroni post-hoc tests, with site as the fixed effect and depth  
136 as the random effect of the model. Conversely, differences among depths were analysed with site as the random  
137 effect and depth as the fixed effect. When data was not normally distributed despite transformations, non-  
138 parametric Kruskal-Wallis tests were used. The relationship between parameters (salinity and surface soil  $\delta^{13}\text{C}$ )  
139 was analysed with linear regression. Statistical tests were performed with SPSS Statistics (version 21, IBM, New  
140 York, USA). Data are reported as mean  $\pm$  standard error.

141

142 **Results**

143 ***Spatial differences among C sources***

144 Surface soil mean  $\delta^{13}\text{C}$  values were  $-28.4 \pm 0.2$  ‰ for mangroves (range:  $-29.5$  to  $-26.9$  ‰),  $-28.0 \pm 0.3$   
145 ‰ for the peat swamp (range:  $-28.7$  to  $-27.4$  ‰), and  $-21.9 \pm 0.9$  ‰ for the marsh (range:  $-22.7$  to  $-19.4$  ‰).  $\delta^{15}\text{N}$   
146 values were  $-0.8 \pm 0.1$  ‰ (range:  $-2.2$  to  $0.2$  ‰),  $-0.5 \pm 0.5$  ‰ (range:  $-1.9$  to  $0.9$  ‰) and  $-1.2 \pm 0.1$  ‰ (range:  
147  $-1.4$  to  $-0.9$  ‰), respectively. Surface N:C ratios were  $0.037 \pm 0.006$  for mangroves,  $0.057 \pm 0.005$  for peat  
148 swamp and  $0.062 \pm 0.002$  for marsh. The corresponding mean C:N ratios were 27.0, 17.5 and 16.0.

149

150 There were no consistent differences between  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values from upriver and downriver  
151 mangroves (Fig. 2). However, there was a small 1-2 ‰  $\delta^{13}\text{C}$  variability among sites ( $F_{6, 18} = 5.47$ ,  $p < 0.002$ ).  
152 Mangrove surface soil  $\delta^{13}\text{C}$  was significantly correlated with interstitial salinity ( $R^2 = 0.75$ ;  $p < 0.011$ ; Fig. 3),  
153 such that lower  $\delta^{13}\text{C}$  values were measured in soils with low interstitial salinity. The  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values were  
154 similar across fringe and basin mangroves, with variations  $< 1\%$ .  $\delta^{15}\text{N}$  values were similar among sites ( $Z_{6, 162} =$   
155  $7.24$ ;  $p = 0.299$ ). Mangrove soil N:C ratios also showed only a small difference ( $< 0.02$ ) among sites and depths.  
156 Overall, all mangrove sites were fairly similar and showed only small variations in soil parameters.

157

158 Mangroves soils fit well within the biogeochemical characteristics expected for N:C and  $\delta^{13}\text{C}$  values for  
159 mangroves ( $\text{C}_3$  plants; Fig. 4). Most of the soil C of mangroves seemed to derive from *in situ* production. Surface  
160 soil from swamp forests was also within the ranges that corresponded to values for  $\text{C}_3$  plants, but deeper soil  
161 samples were located between values of  $\text{C}_3$  plants, soil organic matter and  $\text{C}_4$  plants, along with marsh soil (Fig.  
162 4), suggesting multiple sources and processes involved in C storage for these soils.

163

164 ***Temporal differences among C sources and C stability***

165 There was a distinct difference among the  $\delta^{13}\text{C}$  values of the wetland communities with depth. Over the  
166 past century, mangrove sites consistently had  $\delta^{13}\text{C}$  values close to  $-28$  ‰ (range:  $-25.6$  to  $-31.4$  ‰) with no  
167 significant downcore changes ( $F_{4, 19,5} = 2.39$ ,  $p = 0.086$ ; Fig. 5). Only one of the sites, Las Palmas, showed a  
168 trend of  $\delta^{13}\text{C}$  increase with time (Fig. 2).  $\delta^{15}\text{N}$  values changed with depth ( $Z_{4, 162} = 10.66$ ;  $p = 0.031$ ), with lowest  
169 values in the layer below 50 cm, although the difference was small ( $< 2$  ‰).

170



171 Peat swamps and marsh had large depth-related variations with  $\delta^{13}\text{C}$  value differences of up to 6‰ (Fig.  
172 5). In the peat swamp soil,  $\delta^{13}\text{C}$  values increased with time, while in the marsh soil the  $\delta^{13}\text{C}$  values decreased;  
173  $\delta^{15}\text{N}$  was variable. Finally, in all wetlands N:C increased since 1902; average increases in mangroves, peat  
174 swamps and marshes were 15.3%, 10.8% and 25%, respectively.

175

176

177 **Discussion**

178           The soil analyses indicate that the sources for soil C and the processes related to C storage and stability  
179 differed between riverine wetlands of the Reserve. In mangroves, the dominant process for C accumulation is the  
180 burial of *in situ* production. The C buried in mangroves has changed little in the past hundred years, suggesting  
181 that in the long-term decomposition rates are fairly slow. In the peat swamp forest and the marsh, the soil C has  
182 experienced large changes in the past century, probably due to differing decomposition rates, changes in  
183 community composition or C sources.

184

185           Geomorphological setting did not have a noticeable impact in the soil C of mangroves. The soil  $\delta^{13}\text{C}$   
186 values throughout the sites lie within the reported values for mangrove leaves (-28.8 to -26.7‰; Fry and Cormier  
187 2011; Lovelock et al. 2011; Adame et al. 2015) suggesting that most of the soil is derived from autochthonous  
188 production. Similarly, in Twin Cays Belize, where mangrove peat is mostly comprised of autochthonous  
189 production (McKee et al. 2007), the isotopic composition is fairly constant within the sediment column and  
190 shows that mangrove peat has been the main C source for a long time (Wooller et al. 2003b; Monacci et al.  
191 2011). This result contrasts with other locations such as Yucatan, Mexico, where mangrove peat is a  
192 combination of mangrove detritus and soil particulate matter with contributions varying within the past century  
193 (Gonneea et al. 2004). Thus, although it is likely that fringe upriver mangroves have high terrestrial inputs  
194 (Adame et al. 2010), our data shows that *in situ* production and burial exceeds external C inputs into the soil of  
195 mangroves of LEBRE, irrespective of geomorphological setting.

196

197           The small difference in  $\delta^{13}\text{C}$  values in the surface soil was correlated with salinity and is likely to  
198 indicate differences in stomatal conductance, carboxylation processes (McKee et al. 2002) and water availability  
199 (Fry and Cormier 2011). Surface samples (0-15 cm) have a higher percentage of live roots (up to 63% in the first  
200 40 cm; Tamooch et al. 2008), thus, the  $\delta^{13}\text{C}$  variations are likely to be a result of the processes of the live tree,  
201 while deeper samples are mostly comprised of dead roots and organic matter whose  $\delta^{13}\text{C}$  values are likely to  
202 reflect decomposition and storage processes.

203

204           The mangrove forest  $\delta^{13}\text{C}$  values were constant throughout time, changing little in the past century.  
205 Only one of the sites, Las Palmas, showed a  $\delta^{13}\text{C}$  increase with time (Fig. 2). Las Palmas was a distinct site in  
206 that it was dominated by *A. germinans* and located in an elevated area far from the river edge. This site also had

207 the ash horizon (marker of the year 1902) at a much shallower depth than any other site (Fig. 2), suggesting low  
208 C production and/or accumulation in these *A. germinans* mangroves with low inundation frequency. Apart from  
209 Las Palmas, there was consistent and unchanging  $\delta^{13}\text{C}$  values among forests and throughout time, contrasting  
210 with the typical profile of terrestrial oxic soils which have a characteristic 1-3 ‰  $\delta^{13}\text{C}$  increase with depth as a  
211 result of biogenic decomposition (Nadelhoffer and Fry 1988). Soils also lacked the strong (3-5 ‰) increases in  
212  $\delta^{15}\text{N}$  with depth, which are typical of oxic terrestrial soils (Nadelhoffer and Fry 1988), and instead were fairly  
213 constant. This result, along with the decrease of N:C ratios with depth suggests limited decomposition (Mariotti  
214 et al. 1980).

215

216 The decrease of N:C with depth, or increase with time, in all the wetlands could also suggest increased  
217 nitrogen inputs, which is in accordance of increased agricultural activity in the river catchment within the past  
218 century (UNESCO, 2013). Increased agricultural activity within the river catchment has likely resulted in  
219 increased N and phosphorus inputs within the river and into the wetlands. The  $\delta^{15}\text{N}$  values close to 0‰ of the soil  
220 profiles are consistent with nitrogen fixation as the main long-term source of N for these wetlands (Fry 2006),  
221 with more N fixation perhaps promoted by stronger anthropogenic phosphorus inputs.

222

223 The soil profile  $\delta^{13}\text{C}$  values of peat swamp forest and marsh was notably different from those of  
224 mangroves. The soil profiles for the peat swamp forests had a large enrichment of  $\delta^{13}\text{C}$  values (3‰) with time, a  
225 profile similar to oxic terrestrial soils (Nadelhoffer and Fry 1988). Thus, even though peat swamps generally  
226 have anoxic soils, our data shows that disturbances of this forest could have temporarily increased oxygen in the  
227 soil and thus, increased C decomposition. Alternatively, there may have been a change in source material. The  
228 soil profile of the marsh was highly variable, and showed an abrupt change from  $\delta^{13}\text{C}$  values close to a peat  
229 swamp/mangroves (plants with  $\text{C}_3$  metabolism) at the bottom of the core (-26.3 ‰), to those closer to a marsh  
230 grass (plants with  $\text{C}_4$  metabolism) at the top of the core (-21.9 ‰). The large change in  $\delta^{13}\text{C}$  suggests a change in  
231 plant community, from forest to grassland (Delegue et al. 2001). Fires in the Reserve are common in the dry  
232 season and usually affect peat swamps and marshes (L. Castro pers. comm). The data from the soil cores suggest  
233 the marsh could have been previously a forest that was degraded to secondary vegetation after a major  
234 disturbance and that the peat swamp forest could also have been disturbed by fires in the past. The multiple ash  
235 horizons found in the cores further support the idea that fire is a major disturbance to these ecosystems and their  
236 C sequestration potential.

237

238 Overall, during the last hundred years, C:N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variations were smallest in mangroves and  
239 largest in marshes. Mangrove C sources, accumulation and/or decomposition appear to be fairly stable during in  
240 the past century. But the peat swamp forest and the marsh showed large variation in time in their isotopic values  
241 and N:C, which suggests changes in C sources/processes associated with C storage.

242

243 In conclusion, soil of riverine wetlands within LEBR had diverse sources and processes associated with  
244 C burial and sequestration. The peat swamp and marsh have undergone multiple changes in the past century,  
245 presumably various rates of decomposition due to exposure to oxic conditions during the dry season, changes in  
246 plant community due to major disturbances such as fires, and possible nutrient enrichment from upstream  
247 agricultural activities. In mangroves, most of the soil C is mangrove detritus and has remained fairly unchanged,  
248 suggesting that decomposition is slow and that most C comes from autochthonous production. Mangrove soils  
249 within the reserve sequester every year 1.3 ton of C per  $\text{ha}^{-1}$  (Adame et al. 2015b), with average accumulation  
250 rates near 0.34 cm per year, which appears to remains stable for at least 100 years. This study shows that  
251 mangrove soils can preserve very uniform N and C characteristics for a century or more, consistent with efficient  
252 C storage.

253

254 **Figure Legends**

255

256 Figure 1. Sampling locations within La Encrucijada Biosphere Reserve, Mexico. Seven mangrove forests, one  
257 peat swamp and one marsh were sampled. The mangrove forests were located in a gradient from upriver to  
258 downriver.

259

260 Figure 2. Profile of mean values for soil N:C,  $\delta^{13}\text{C}$  (‰),  $\delta^{15}\text{N}$  (‰) for seven mangrove forests that ranged from  
261 upriver to downriver mangroves. An ash horizon (derived from the explosion of Santa Maria volcano at  
262 Guatemala in 1902) found at every site was used for dating the soil cores.

263

264 Fig 3. Correlation between surface soil  $\delta^{13}\text{C}$  (‰) values and interstitial salinity (mean of two sampling periods  
265 during the dry season) of seven mangrove forests within La Encrucijada Biosphere Reserve, Mexico.

266

267 Fig 4. Possible soil C source of mangrove forests (circles), peat swamps (squares) and marsh (triangles) within La  
268 Encrucijada Biosphere Reserve, Mexico (based on Monacci et al. 2011). Mangrove values were obtained from  
269 Wooller et al. 2003, Fry and Cormier 2011, Lovelock et al. 2011 and Adame et al. 2015.

270

271 Fig 5. Profile of mean values for soil N:C,  $\delta^{13}\text{C}$  (‰),  $\delta^{15}\text{N}$  (‰) for mangroves, a peat swamp forest and a marsh.  
272 An ash horizon (derived from the explosion of Santa Maria volcano at Guatemala in 1902) found at every site  
273 was used for dating the mangrove soil (grey square).

274

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276

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284

285

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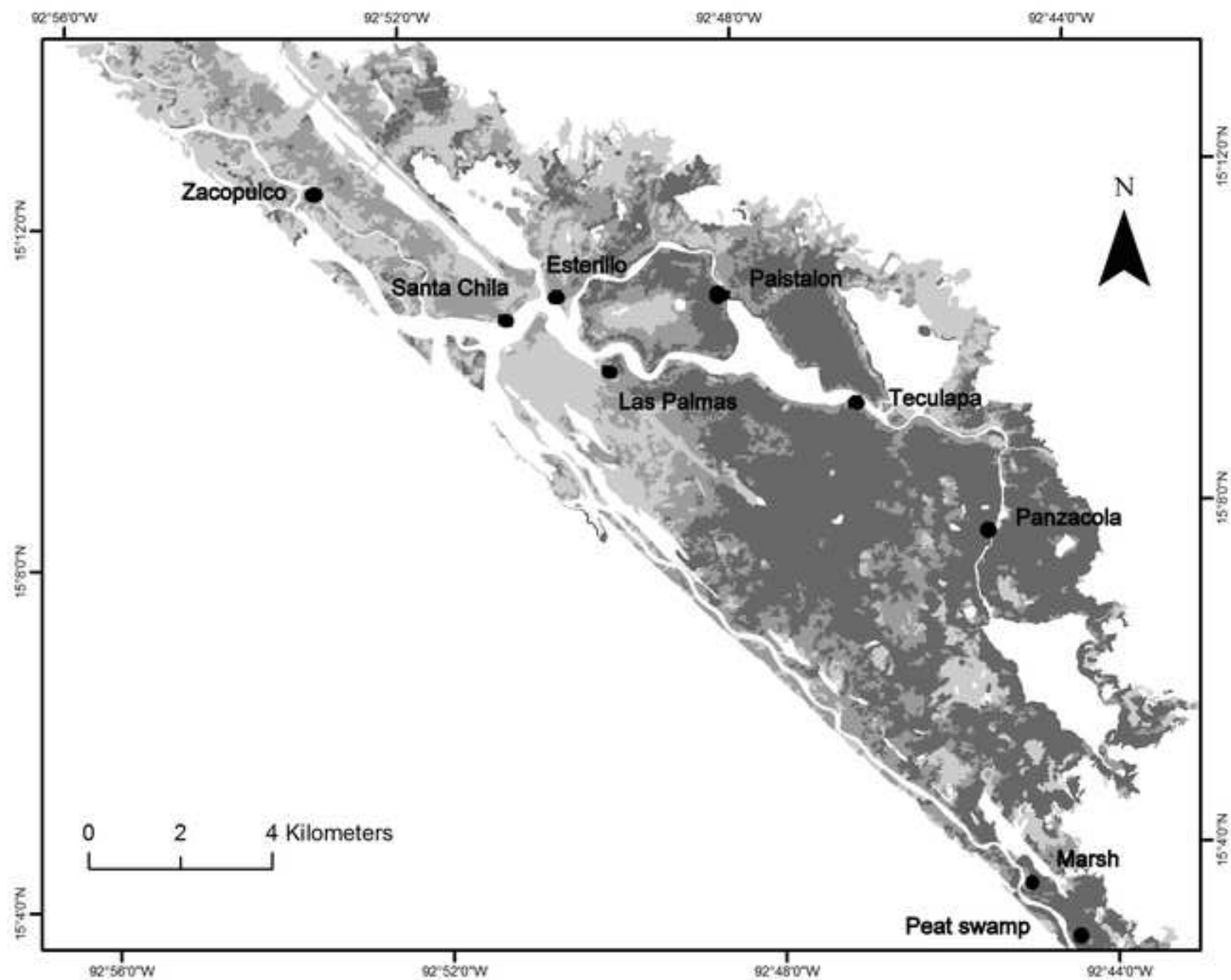
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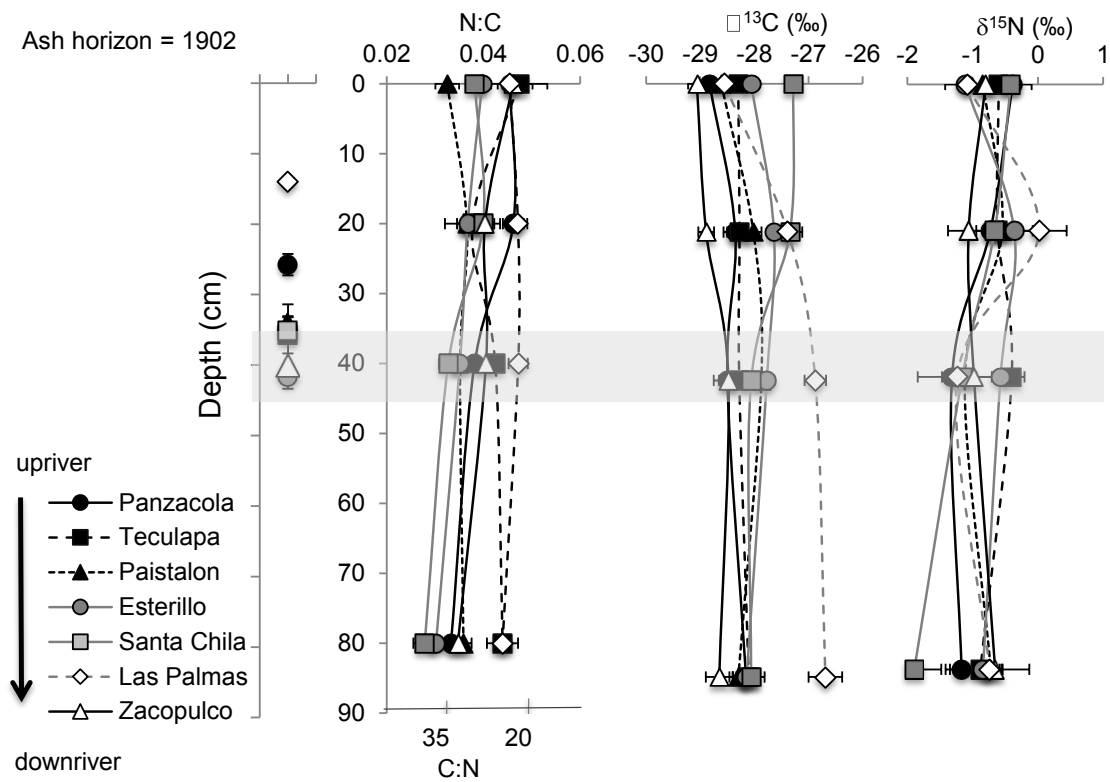
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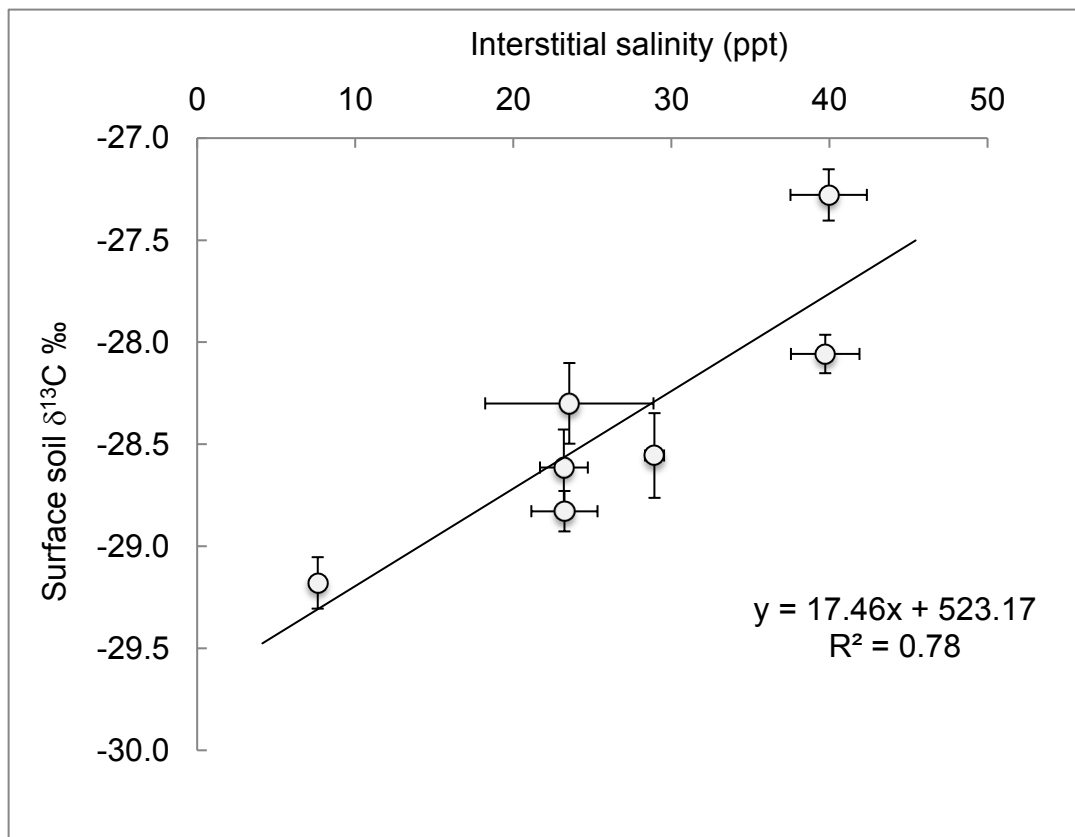
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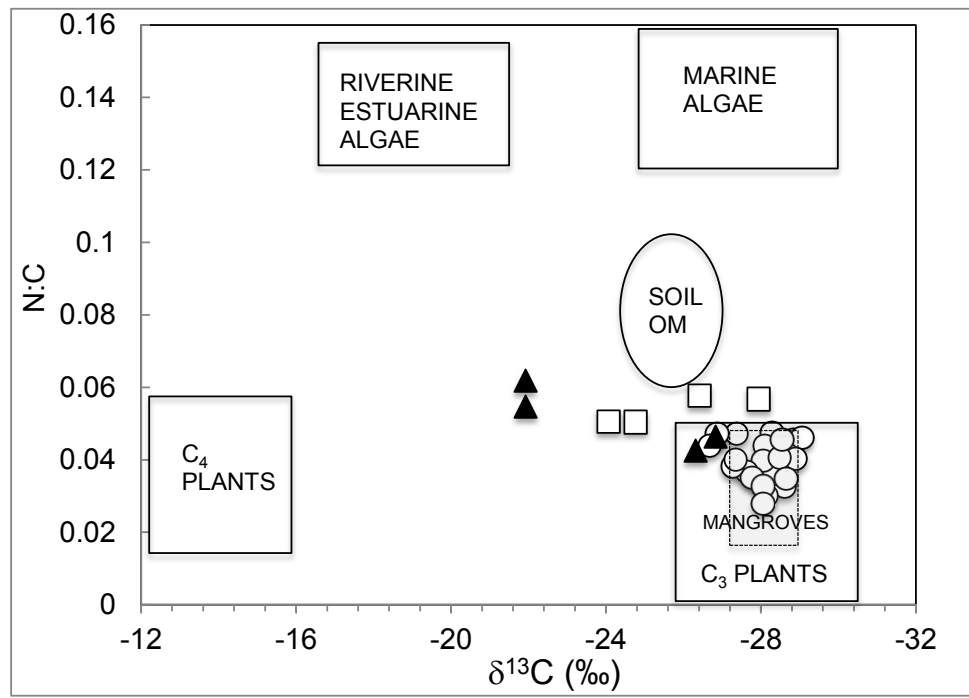
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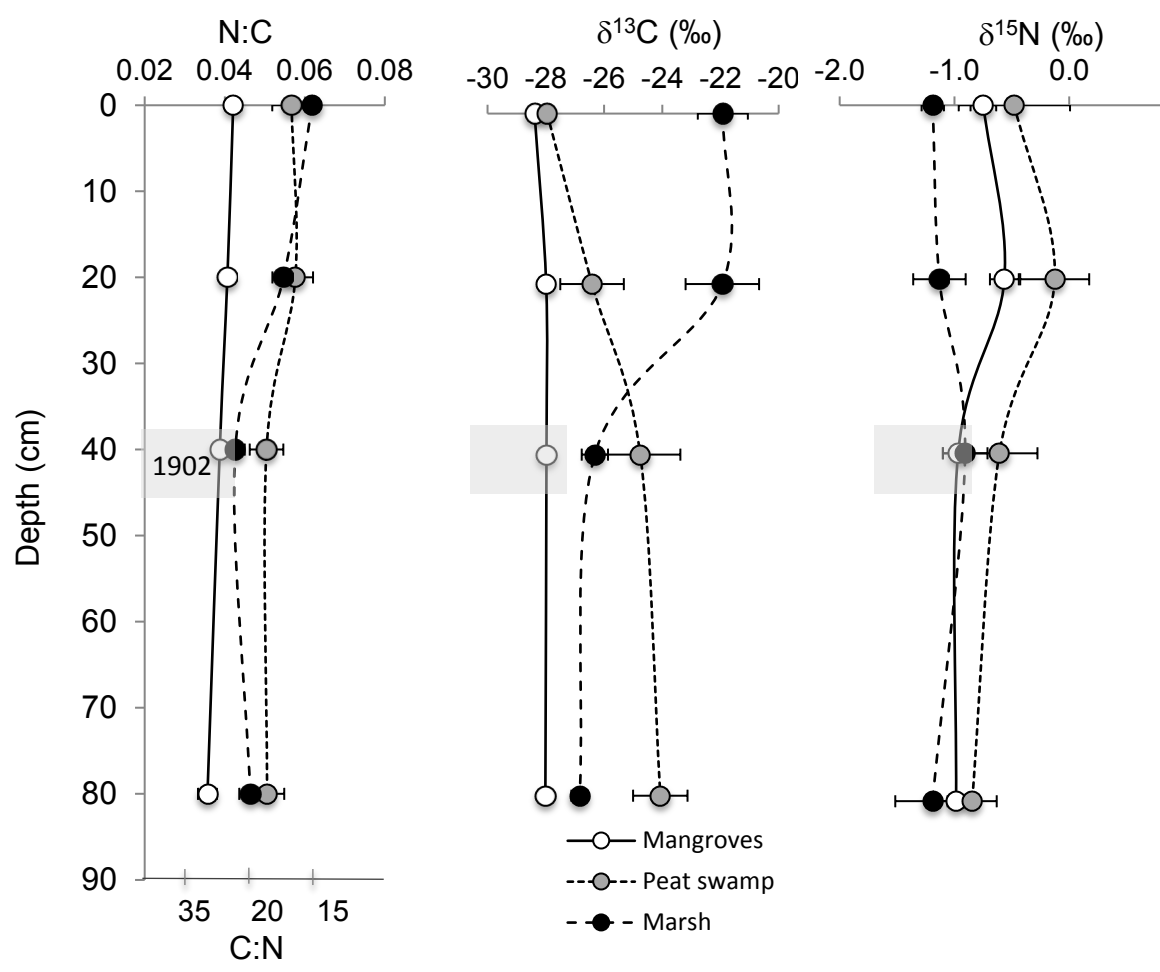
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