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

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Abstract

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

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

Introduction

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

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

The processes responsible for C production and storage vary with time and are continuously changing 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).

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

content; in all samples, inorganic C was less than 5% of the total. Analytical errors (SD) were 0.25‰ for $\delta^{13}\text{C}$ 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 study only N:C ratios are reported and used in mixing diagrams (Perdue and Kroprivnjak 2007); for reference, C:N ratios are also given in some places alongside the N:C ratios

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

Temporal differences among C sources and soil C stability

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

Interstitial salinity

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

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

Data analyses

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

Results

Spatial differences among C sources

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

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

Mangroves soils fit well within the biogeochemical characteristics expected for N:C and $\delta^{13}\text{C}$ values for mangroves (C_3 plants; Fig. 4). Most of the soil C of mangroves seemed to derive from *in situ* production. Surface soil from swamp forests was also within the ranges that corresponded to values for C_3 plants, but deeper soil samples were located between values of C_3 plants, soil organic matter and C_4 plants, along with marsh soil (Fig. 4), suggesting multiple sources and processes involved in C storage for these soils.

Temporal differences among C sources and C stability

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

Peat swamps and marsh had large depth-related variations with $\delta^{13}\text{C}$ value differences of up to 6‰ (Fig. 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; $\delta^{15}\text{N}$ was variable. Finally, in all wetlands N:C increased since 1902; average increases in mangroves, peat swamps and marshes were 15.3%, 10.8% and 25%, respectively.

Discussion

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

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

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

The mangrove forest $\delta^{13}\text{C}$ values were constant throughout time, changing little in the past century. 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 that it was dominated by *A. germinans* and located in an elevated area far from the river edge. This site also had

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

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

The soil profile $\delta^{13}\text{C}$ values of peat swamp forest and marsh was notably different from those of mangroves. The soil profiles for the peat swamp forests had a large enrichment of $\delta^{13}\text{C}$ values (3‰) with time, a profile similar to oxic terrestrial soils (Nadelhoffer and Fry 1988). Thus, even though peat swamps generally have anoxic soils, our data shows that disturbances of this forest could have temporarily increased oxygen in the soil and thus, increased C decomposition. Alternatively, there may have been a change in source material. The soil profile of the marsh was highly variable, and showed an abrupt change from $\delta^{13}\text{C}$ values close to a peat swamp/mangroves (plants with C_3 metabolism) at the bottom of the core (-26.3 ‰), to those closer to a marsh grass (plants with C_4 metabolism) at the top of the core (-21.9 ‰). The large change in $\delta^{13}\text{C}$ suggests a change in plant community, from forest to grassland (Delegue et al. 2001). Fires in the Reserve are common in the dry season and usually affect peat swamps and marshes (L. Castro pers. comm). The data from the soil cores suggest the marsh could have been previously a forest that was degraded to secondary vegetation after a major disturbance and that the peat swamp forest could also have been disturbed by fires in the past. The multiple ash horizons found in the cores further support the idea that fire is a major disturbance to these ecosystems and their C sequestration potential.

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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 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

Figure Legends

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

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 upriver to downriver mangroves. An ash horizon (derived from the explosion of Santa Maria volcano at Guatemala in 1902) found at every site was used for dating the soil cores.

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

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

Figure 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. An ash horizon (derived from the explosion of Santa Maria volcano at Guatemala in 1902) found at every site was used for dating the mangrove soil (grey square).

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