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#### **Short Communication**

## High salinity leads to accumulation of soil organic carbon in mangrove soil



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#### HIGHLIGHTS

- Soil organic carbon (SOC) dominates carbon pool in mangrove forests.
- A new SOC accumulation mechanism in mangrove soils is proposed.
- High salinity flocculates and accumulates humic substances (HS) in mangrove soils.
- Selective accumulation of biologically-refractory HS is important for carbon cycle.
- More organic geochemical researches are needed for mangrove SOC studies.

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#### ABSTRACT

Although mangrove forests are one of the most well-known soil organic carbon (SOC) sinks, the mechanism underlying SOC accumulation is relatively unknown. High net primary production (NPP) along with the typical bottom-heavy biomass allocation and low soil respiration (SR) have been considered to be responsible for SOC accumulation. However, an emerging paradigm postulates that SR is severely underestimated because of the leakage of dissolved inorganic carbon (DIC) in groundwater. Here we propose a simple yet unique mechanism for SOC accumulation in mangrove soils. We conducted sequential extraction of water extractable organic matter (WEOM) from mangrove soils using ultrapure water and artificial seawater, respectively. A sharp increase in humic substances (HS) concentration was observed only in the case of ultrapure water, along with a decline in salinity. Extracted WEOM was colloidal, and  $\leq$ 70% of it re-precipitated by the addition of artificial seawater. These results strongly suggest that HS is selectively flocculated and maintained in the mangrove soils because of high salinity. Because sea salts are a characteristic of any mangrove forest, high salinity may be one of mechanisms underlying SOC accumulation in mangrove soils.

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#### 1. Introduction

Researchers during the past 25 years have confirmed that mangrove forests rank among the forests with most carbon-rich soils in the tropics (Donato et al., 2011). Comprising only 0.7% of the global tropical forests (Giri et al., 2011), mangrove forests account for approximately 11% of the global organic carbon burial in

\* Corresponding author. E-mail address: fujitake@kobe-u.ac.jp (N. Fujitake). the coastal ocean (Duarte et al., 2005) and the total export of terrestrial carbon into the ocean (Jennerjahn and Ittekkot, 2002). Mangrove forests store more carbon than other ecosystems per unit area, particularly in soils; among a mean whole-ecosystem carbon stock of 956 t C ha<sup>-1</sup>, soil organic carbon (SOC) constitutes 75% of the carbon pool (Alongi, 2014).

The mechanism underlying SOC accumulation remains to be elucidated. The possible mechanisms from an ecological view point can be as follows: (1) high net primary production (NPP) and typical bottom-heavy biomass allocation (Ong et al., 2004) and (2) low soil respiration (SR) rate because of anoxic conditions (Alongi

et al., 2000; Chen et al., 2014). However, the latter possibility is being challenged with the claim that SR is severely underestimated because of the leakage of dissolved inorganic carbon (DIC) in groundwater (Bouillon et al., 2008; Maher et al., 2013).

Mangrove forests are distinguished from terrestrial forests by the fact that they occur in seawater-logged, saline soils (indeed, they are the only known woody halophytes). This fact leads to the proposition of an organic geochemical explanation for the mechanism underlying SOC accumulation: SOC flocculation is caused in soils by high salinity. It is well known in organic geochemistry that dissolved organic matter (DOM) in a river precipitates (flocculates) and gets removed during estuarine mixing by interacting with metal cations in seawater (Sholkovitz, 1976). Assuming that the same mechanism is responsible for SOC accumulation, we hypothesized that SOC would be released in water if the factor that flocculates SOC is eliminated, i.e., if salinity of the mangrove soil is lowered.

#### 2. Methods

#### 2.1. Sampling of soil

The present study was conducted in a mangrove forest occurring along the Fukido River, Ishigaki Island, Okinawa, Japan (24°29′N, 124°14′E). Ishigaki Island is characterized by a subtropical climate with an annual mean temperature of 23.8 °C and annual precipitation of 2188 mm. The catchment area has mostly remained undeveloped, and few anthropogenic materials flow into the study area, which is affected by the semidiurnal tide. At high tide, the area is submerged in water, whereas at low tide, every soil surface is exposed to the air, except the soil within holes. Top soils (0-20 cm) were collected in triplicate at low tide on August 25, 2015, with moisture maintained to prevent oxidation by sealing in storage bags to be carried to the laboratory. The general characteristics of the soils were as follows: total organic carbon (TOC), 3.0%–3.9%; total nitrogen, 0.09%-0.14%; carbon to nitrogen ratio, 22-31; and soil texture, sandy loam to sandy clay loam (clay content, 10%–17%). Details about the site can be found elsewhere (Kinjo et al., 2005; Nakamura et al., 2008).

#### 2.2. Sequential extraction

Water extractable organic matter (WEOM) was sequentially extracted from the triplicate samples with ultrapure water and artificial seawater (ASW). ASW was prepared according to the method by Deslouis et al. (2006), with the composition of NaCl (0.5 M), CaCl<sub>2</sub>  $(1.05 \times 10^{-2} \text{ M})$ , and MgCl<sub>2</sub>  $(5.5 \times 10^{-2} \text{ M})$  (all Special Grade; Wako, Japan). Subsample was hand-picked of visible roots and leaves, and an aliquot (30-g dry weight) was placed into a polypropylene copolymer centrifuge tube (Nalgene, USA) with N<sub>2</sub> gas. Centrifugation was performed at 12,000 rpm for 10 min. Approximately 50% of the total water contained in the subsample was collected as "pore water." Ultrapure water or ASW (approximately 120 mL) was then added to the remaining solid to obtain a suspension with a solid-liquid ratio of 1:5, following which the suspension was sparged with N<sub>2</sub> gas for 5 min to remove dissolved oxygen. After 10 min of shaking, centrifugation was performed at 9000 rpm for 10 min, and the supernatant was collected and weighed. Ultrapure water or ASW of weight same as that of the supernatant was re-added to the centrifuge tube, and six sequential extractions of WEOM were conducted for each soil. In a preliminary experiment, it was observed that salinity of supernatants decreased exponentially and reached lowest after six extraction by ultrapure water. Each sample (pore water and supernatants) was immediately vacuum filtered through a glass fiber filter (GFF; 0.3-μm pore size; ADVANTEC, Japan). The WEOM solutions were analyzed for electric conductivity (EC), the specific ultraviolet absorbance at 254 nm (SUVA<sub>254</sub>; Weishaar et al., 2003), and humic substances (HS), which are reported to be the most susceptible to estuarine flocculation in DOM (Fox, 1983), using the published method (Tsuda et al., 2012). Briefly, 0.4 mL of purified DAX-8 resin was weighed in a glass vial with a Teflon screw cap, and 20 mL of the WEOM solution was added and acidified with 1 mL 1 M H<sub>2</sub>SO<sub>4</sub> (Wako, Japan) to adjust the pH to <2. After shaking for 24 h, the dissolved organic carbon (DOC) concentration of the supernatant (non-HS [NHS] fraction) was analyzed after filtration through GFF. HS concentration was calculated as the difference between the bulk DOC concentration and NHS concentration. Organic carbon contamination from the system was also determined with ultrapure water as a blank and used for correction. The experiment was conducted in triplicate. The validity of the method for samples with different salinity has been confirmed (Kida et al., 2016). The pore waters were analyzed for EC, SUVA<sub>254</sub>, and DOC because of their limited volume.

#### 2.3. Precipitation experiment

An additional WEOM solution was prepared with 240 g (dry weight) of the soil by sequential extraction using ultrapure water (solid-liquid ratio of 1:5). The first two solutions (high salinity, pale colored) were discarded, and the third and fourth solutions (low salinity, brown colored) were mixed to obtain low salinity sample to be used further for precipitation experiment by raising salinity. This procedure generated approximately 2 L of the GFF-filtered solution, with an EC value of 16.7 mS m<sup>-1</sup> and DOC concentration of 16.9 mgC  $L^{-1}$ . Subsequently, 20-mL aliquots of the solution and different volumes of ASW were pipetted into glass vials to obtain the desired EC values (54–715 mS  $m^{-1}$ ). After 12 h, the solutions were vacuum filtered through GFF. The precipitation time to reach equilibrium was optimized by a preliminary experiment. The precipitation ratio was calculated by mass balance between the initial DOC concentration and that of each filtrate after correction by the volumes of ASW added. The experiment was conducted in triplicate.

#### 2.4. Analytical measurements

DOC was analyzed by the combustion Pt-catalytic oxidation method in a TOC-V<sub>CPH</sub> total organic carbon analyzer (Shimadzu, Japan). First, each sample was sparged with a carrier gas for 90 s in the built-in syringe of the analyzer to remove any DIC prior to combustion. Calibration was performed by running four standards of a potassium hydrogen phthalate solution (Special Grade; Wako, Japan) over an appropriate range and one laboratory blank (ultrapure water). The analytical precision of the replicate measurements (n = 3–5) was less than 2.0% for the coefficient of variation. The measurements of UV absorption at 254 nm were performed using a JASCO V-630 UV—visible spectrophotometer (JASCO, Japan) with a 1-cm path length, acid-cleaned, quartz cuvette at ambient temperature. Measurements were baseline-corrected using ultrapure water before sample measurement.

#### 3. Results and discussion

Fig. 1 demonstrates the changes in chemical parameters of the WEOM solutions obtained by sequential extraction. The chemical parameters of the pore waters were as follows (average  $\pm$  standard deviation): EC, 3.7  $\pm$  0.3 S/m; DOC, 13.6  $\pm$  8.2 mg C L<sup>-1</sup>; and SUVA<sub>254</sub>, 0.75  $\pm$  0.17 L mgC<sup>-1</sup> m<sup>-1</sup>. Through the six sequential extractions, chemical parameters of the WEOM solutions experienced

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