# CRABS ENGINEERING EFFECTS ON SOIL ORGANIC MATTER AND NUTRIENTS FLOW IN SUBTROPICAL MANGROVES FOREST

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#### **Abstract**

Burrowing activities of crabs has potential effect on biogeochemical cycles in sediments. A field study was conducted in the Sashiki Bay (Okinawa Island, southern Japan) to compare engineering impact of ocypodid *Uca vocans* and sesarma crabs *Perisesarma bidens* on the distribution of organic matter and nutrients processes in intertidal sediments. The organic carbon content tended to be higher in burrows than adjacent surface sediments of both crab species. Burrowing by *U. vocans* resulted in a massive release of NO<sub>3</sub><sup>-</sup> to the burrow sediments as compared to that of *P. bidens*. In contrast, an opposite effect on the NH<sub>4</sub><sup>+</sup> concentration was observed in both crab burrows and their adjacent surface sediments. These results suggest that physical structures and activities of ocypodid and sesarmid crabs may lead to a significantly contrasting influence on the organic matter and nutrient distribution in the subtropical mangrove forest.

Keywords: Burrow, Nutrients, Ocypodid crabs, Sediments, Sesarma crabs

#### INTRODUCTION

Burrowing ocypodid and grapsid crabs are among the most abundant macrofauna inhabiting the mangrove forests. Most of the sesarmid crabs (family: Grapsidae) and fiddler crabs (Ocypodidae) construct and maintain burrow structures in mangrove sediments with a significant engineering effect on their habitats and associated fauna and flora. Burrowing activities by these crabs potentially affect biogeochemical sediment cycles by modifying size distribution, affecting topography, improving aeration, reducing pore water salinity, providing microhabitats for other fauna and contributing to secondary production, thus controlling nutrient release and affecting mangrove productivity [1,2,3].

In mangrove forest, burrow construction by crabs creates unique habitats for bacteria that in turn, provide nutrients for primary production. In addition, they enhance the exportation of nutrients into the coastal zone. Burrows allow rapid water flow and the transportation of dissolved materials such as oxygen through the sediment. The availability of oxygen to the deeper sediments (anoxic-zone) creates a favorable environment for certain bacteria responsible for nutrient cycling. The benthic nitrogen cycle and the mineralization of organic matter are among the microbial processes that are stimulated by bioturbation [4]. Nitrification processes can be stimulated in burrow walls that are periodically aerated by ventilation and exposure to the NH<sub>4</sub><sup>+</sup> excreted by the inhabiting animal [5]. On the other hand, denitrification can be promoted by the facilitated NO<sub>3</sub><sup>-</sup> penetration into deep sediment layers that become anoxic periodically [6]. Crab burrows in particular, create ideal conditions for both nitrification and denitrification processes. Through these interactions crab burrows can effectively remove nitrogen loads from aquatic ecosystem [7].

Ecosystem engineers are organisms which directly or indirectly modulate the availability of resources (other than themselves) to other species, by changing the physical state in biotic or abiotic materials [8]. Crab bioturbation has been recognized as a typical example of ecosystem engineering as it biogeochemical gradients, redistributes food resources and bacteria [9]. Therefore this study examined the burrowing ecosystem engineering effect of ocypodid and grapsid crabs on sediment organic matter distribution and nutrient dynamics, under mangrove forest. I compared nutrients (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>,) and organic matter (TOC, TN and CN ratio) in: 1) surface sediments of the ocypodid (U. Vocans and U. dissumieri) and sesarmid crabs (H. formosensis and P. bidens); 2) their burrow wall sediments and 3) Upper and lower intertidal sediments.

## METHODOLOGY

Study site

The study was conducted in Sashiki Bay, in the southern part of Okinawa Island, Japan (26°N, 128°E), during autumn, 2006. The dominant

mangrove species is Kandelia obovata which occurs in the muddy upper tidal zone, and to a lesser extent, on the sandy middle and lower tidal flats. The mangrove forest grows in the small river/stream originating from the main bay. The watercourse depth is less than 80 cm at low tide and the salinity ranges from 24.5 to 30.1. This area is characterized by a subtropical climate with an average annual air temperature of 23.2°C. The highest temperatures occur from July (28.6°C) to August (28.5°C) and lowest from December (18.5°C) to February (16.8°C). The annual precipitation exceeds 100mm month <sup>1</sup> throughout the year and the average rainfall is 2127 mm year<sup>-1</sup>. In the Sashiki Bay, *U. vocans* is the dominant crab species which often constructs less permanent burrows in the open lower and mid-intertidal sandy flat. In contrast, P. bidens is abundant along the upper tidal muddy zone under the mangrove canopy where they occasionally construct burrows but appear to have preference for natural refuges in interstices of mangrove roots buttresses.

Sample collection

Surface sediments (~ 0.5 cm) of crab areas in the upper and lower intertidal zones during low tide were sampled in triplicate. Burrow wall sediments of ocypodid crabs (U. vocans) and the grapsid (P. bidens ) were collected from each habitat. Sediments was carefully scraped off of the opening shafts (to ~ 10 cm depth) during burrow wall sampling using sterile spatulas rinsed with methanol (99.7%). Since only a thin layer of the burrow was collected, three burrow samples were pooled in order to obtain enough samples for each replicate. All sediment samples were placed into polyethylene bags, and were temporarily stored in a cooler at -20°C in the laboratory until analyses for total organic matter (TOC), total nitrogen (TN), and inorganic nitrogen forms of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>.

Analytical methods

Sediment samples were first dried at 80°C to a constant weight and TOC and TN contents were analysed using a high-sensitivity C/N analyser (Shimadzu NC 80). Prior to analysis, 1 g of each sediment sample was treated with 20 ml of a 1 N HCl solution for 24 h to remove carbonates and bicarbonate.

For the measurement of sediment inorganic nitrogen ( $NH_4^+$  and  $NO_3^-$ ), extractions were performed in triplicate for each sediment type using the alkaline reagent, 2 M KCl. Freezedried sediment samples (2.5 g; < 1 mm) were shaken with 25 ml of Milli-Q water solution

(1:10 w/v soil to solution ratio) for 1 h in 50-ml bottles on a reciprocating shaker at 200 rev min-1. The soil extracts were then centrifuged at 2500 rpm for 15 min to obtain a clear supernatant liquid before filtering through a GF/C filter. The filtrate was stored in a small glass bottles in a  $-40^{\circ}$ C freezer prior to analysis. All extracts were analysed using a QuAAtro automatic water analyser (Bran+Luebbe GmbH, Norderstedt, Germany). Nutrient measurements are presented as  $\mu$  mol g<sup>-1</sup> dry weight sediments. Data analysis

Multi-dimensional scaling (MDS) was conducted based on a Bray-Curtis similarity coefficient using the PRIMER software (version 6). No transformation was performed on the data. The significance of differences in nutrients between crab habitats (upper and lower intertidal areas), burrow and surface sediments of each species were determined using a one-way analysis of similarity (ANOSIM).

Student's t-tests and one-way analysis of variance (ANOVA) were used to test for differences in TOC, TN, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> between burrow wall sediment, surface sediments at different habitats (upper and lower intertidal areas). Probabilities (p) of <0.05 were considered to be significant.

#### RESULTS

Crab burrow sediments of *U. vocans* and *P.* bidens were characterized by a significantly higher TOC than adjacent surface sediments (ANOVA, F = 404.0, df = 3, 8; p < 0.001). TOC in *U. vocans* burrows was nearly double those of surface sediments (38.1 and 22.1 mg g<sup>-1</sup> of dry wt sediments, respectively), however no difference was found between the surface sediments of *U. vocans* and *P. bidens* (Figure 1a). The TN contents significantly varied between crab species burrows and surface sediments (ANOVA, F = 449.6, df =3, 8; p <0.001; Figure 1b). While TN was higher in the burrows of P. bidens than surface sediments (1.9 and 1.8 mg g<sup>-1</sup> of dry wt sediments, respectively), U. vocans burrows revealed an opposite pattern. Surface sediments of *P. bidens* had a significantly higher TN content than that of *U. vocans*.

The levels of NH<sub>4</sub><sup>+</sup> were recognizably higher in all sediment categories as compared to NO<sub>3</sub><sup>-</sup> levels. There were significant differences in NH<sub>4</sub><sup>+</sup> concentrations between the surface sediments of both crabs' species. However, significantly lower concentration of NH<sub>4</sub><sup>+</sup> was

recorded in the burrows of U. vocans and P. bidens than surface sediments of their surroundings (ANOVA, F = 140, df =3, 13; p < 0.001; Figure 2a). The concentration of  $NO_3$  significantly varies in an opposite manner between burrow and surface sediments of both crabs (ANOVA, F = 59, df =3, 13; p < 0.001, Figure 2b). The highest concentration was recorded in the surface sediments of P. bidens (1.7  $\mu$  mol g<sup>-1</sup> of dry wt) and the lowest in the surface sediments of U. vocans (0.3  $\mu$  mol g<sup>-1</sup> of dry wt).

Results of MDS and ANOSIM from sediment nutrients revealed significant groupings of the crab habitat (ANOSIM, R = 0.95, p < 0.01). The stress value for the MDS analysis was 0.03, indicating a clear separation between the crab burrows and surface sediments of each crab species (Figure 3). The distribution of nutrients showed a significant difference between sediments of burrows, surface for both crab species and the upper and lower intertidal zone (ANOVA, F = 2650.1, df =2, 6; p < 0.0001). There was no significant difference in amount of total carbon between the upper and lower zone sediments (Figure 4a). In contrary, total nitrogen contents were significantly higher in the upper area of P. bidens than the lower area of U. vacans (1.8 and 1.0 mg g-1 of dry wt sediments respectively; Figure 4b) Similar trends were observed for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> with higher concentrations recorded in the upper intertidal areas (Figure 5a and b).

### **DISCUSSION**

Burrowing and feeding by benthic invertebrates influence known sediment to biogeochemistry [9,10]. Such bioengineering is reported to occur in crab species [11, 12], and is particularly associated with the release of nutrients and sediment nutrient regulations under mangrove forests [2, 3, 7, 13]. In the present study, burrows of both *U. vocans* and *P. bidens* are characterized by a significantly higher TOC than adjacent surface sediments. This result is in agreement with other reports on the contribution of crab burrows to sedimentary organic matter content (14, 15]. Ocypodid crabs are typical deposit-feeders consuming primarily detritus materials rich in nitrogen content by sorting diatoms, algae and bacteria [16]. As they scrape up the upper few centimeter of the surface sediments *U. vocans* probably removes some portions of organic materials, and in particular, nitrogen rich resources. This could explain the higher C/N ratios in the burrow and surface sediments of *U. vocans* than *P. bidens* as they mostly rely on mangrove leaves (detailed discussion see FA profile section 4.2).

Overall total nitrogen and NH<sub>4</sub><sup>+</sup> concentrations showed similar patterns of increase within the upper and lower intertidal zones, which showed that NH<sub>4</sub><sup>+</sup> is the dominant form of inorganic nitrogen in this area [7]. Both crab species showed a significant impact of burrowing by a lowered NH<sub>4</sub><sup>+</sup> concentration, as reported for the Helice formosensis [7] and other grapsid crabs [2]. Interestingly, *U. vocans* burrows were the most efficient in releasing NO<sub>3</sub> forms of nitrogen (3.5x more than surface sediments). Because of their selective habits to consume nitrogen rich sources, it is expected that they will deplete high quality carbon (algae), thus limiting the better substrates for competitive, heterotrophic bacteria, while providing a favourable environment for nitrifying bacteria which would increase nitrification [17]. On the other hand, ocypodids are active burrowing crabs which can improve oxygen content in a few centimeter of their burrow wall (11, 12, 18], thereby improving nitrification processes. In contrast, continuous increase in organic matter in the upper muddy area of the mangrove inhabited by P. bidens could stimulate a higher release of NH<sub>4</sub><sup>+</sup>. In addition, sediment oxygen consumption increases in autumn due to higher temperatures and the decomposition mangrove detritus, leading to a low redox condition and consequently, a reduction of NH<sub>4</sub><sup>+</sup> via the oxygen dependent process of nitrification becomes considerable lower [19].

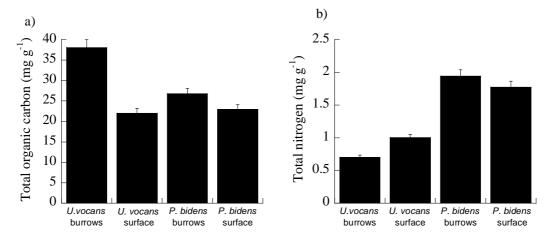
#### **CONCLUSION**

These results demonstrate a contrasting impact of burrowing ocypodid and sesarmid crabs. Our field observation revealed that burrowing activities these crabs and passive deposition of materials in their burrows have a significant impact on the organic matter and nutrient distributions in subtropical mangrove sediments. Both groups have accumulated higher organic matter in their burrows via the active and passive input of detritus materials as indicated by an increase in total carbon contents. They have the tendency to enhance the release of NO<sub>3</sub> or decrease NH<sub>4</sub><sup>+</sup> levels. However, *U. vocans* burrows exhibited a higher potential release of NO<sub>3</sub> and decrease NH<sub>4</sub> levels in their sediment than P. bidens.

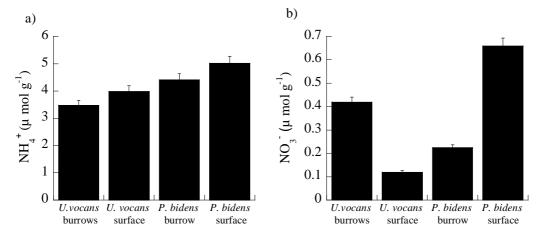
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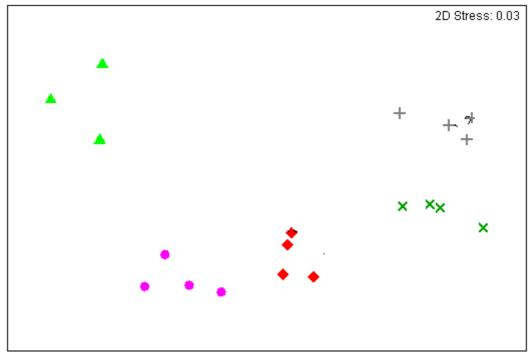
COE Program (University of the Ryukyus, Okinawa, Japan) and Japan Gasoline Company - Saneyoshi Scholarship Foundation to the first author.



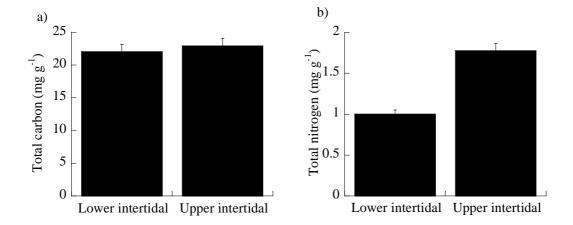
**Figure1:** Comparison of the total organic carbon (a) and total nitrogen (b) in crab burrow and surface sediments. Values are mean  $\pm$  SE (n = 3).



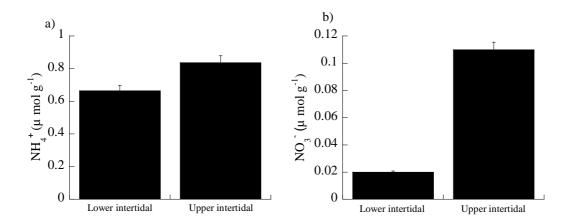
**Figure 2.** Comparison of the inorganic forms of nitrogen, a)  $NH_4^+$  and b)  $NO_3^-$  crab burrow and surface sediments. Values are mean  $\pm$  SE (n = 3).



**Figure 3:** MDS ordination of Bray-Curtis similarities of nutrients in sediments of the *U. vocans* burrow ( $\spadesuit$ ), *U. vocans* surface ( $\bullet$ ), *P. bidens* burrow ( $\spadesuit$ ), *P. bidens* surface (+) and surface without crabs (x).



**Figure4:** Comparison of the total organic carbon a) and b) total nitrogen contents in sediments of upper and lower intertidal areas. Values are mean  $\pm$  SE (n = 3).



**Figure 5:** Comparison of the inorganic forms of nitrogen, a)  $NH_4^+$  and b)  $NO_3^-$  concentrations in sediments of upper and lower intertidal areas. Values are mean  $\pm$  SE (n = 3).

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