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ScienceDirect
Estuarine, Coastal and Shelf Science 73 (2007) 316–324
ESTUARINE COASTAL AND SHELF SCIENCE
www.elsevier.com/locate/ecss

Bioturbation activity by the grapsid crab *Helice formosensis* and its effects on mangrove sedimentary organic matter

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Received 3 October 2006; accepted 22 January 2007
Available online 21 March 2007

Abstract

Grapsid crabs are one of the most abundant and potentially the most important group of macrofauna inhabiting mangrove forests. A field study was conducted in the Manko wetland (Okinawa Island, southern Japan) to investigate how the burrowing crab *Helice formosensis* affects the sedimentary fatty acid (FA) and physicochemical characteristics of subtropical mangrove sediments. Multi-dimensional scaling (MDS) results from FA profiles revealed clear differences between burrow compartments and sediments with and without crabs. The impacts of burrowing were demonstrated by higher percentages of bacterial, vascular plant, and macroalgal FA markers in the burrow compartments and crab sediment areas. Conductivity and redox potential were significantly higher in sediments of the burrow opening shaft than in the burrow chamber. We found a similar pattern in surface sediments with crabs, but not in surface sediments without crab habitats. These results suggest that *H. formosensis* significantly influences the physicochemical properties, FA composition, and organic matter profiles of its surrounding environment.
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Keywords: bioturbation; fatty acid markers; *Helice formosensis*; organic matter; sediments

1. Introduction

In coastal aquatic environments, sediment is regularly disturbed by animal activities through the bioturbation process. This process has a profound influence on the profiles of organic matter (OM), nutrients, and oxidized and reduced chemical compounds in the sediment (Kristensen and Blackburn, 1987; Bird et al., 2000; Kinoshita et al., 2003). Most chemical processes within sediments are mediated by microorganisms. Their functioning conditions are in turn significantly modified by burrowing macroinvertebrates (Steward et al., 1996; Marinelli et al., 2002; Kinoshita et al., 2003). Microbial populations in burrow environments are larger than in surface sediments (Phillips and Lovell, 1999; Dworschak, 2001; Kinoshita et al., 2003). In addition, substrate penetrability, OM, and water content are also higher in crab burrow beds when compared to nearby areas without crabs (Bortolus and Iribarne, 1999; Botto and Iribarne, 2000).

Grapsid crabs are among the most important macrofauna inhabiting mangrove forests (Jones, 1984; Smith et al., 1991; Tan and Ng, 1994). Due to their relatively high abundance, species diversity, and intense activity, intertidal grapsoids are potential sediment disturbance agents that cause significant topographical changes (Warren and Underwood, 1986; Lee, 1998), thereby influencing nutrient cycling and altering the surrounding soil properties (Robertson, 1986; Smith et al., 1991). These crabs also form an important link in energy flow within the mangrove ecosystem by processing leaf litter (Lee, 1998).

To assess the ecological effect of bioturbation activities by grapsid crabs on sedimentary OM content and physicochemical properties of mangrove sediments, we focused on *Helice formosensis*, one of the most abundant organisms in the Manko tidal flat on Okinawa, Japan. We used the fatty acid (FA)

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doi:10.1016/j.ecss.2007.01.016

biomarker approach to examine various sources of OM and their potential contribution to the sediment OM pool (Parkers, 1987; Volkman et al., 2000; Meziane and Tsuchiya, 2002). We also assessed the physicochemical characteristics of sediment by comparing: (i) sediments between burrow components; and (ii) sediments that were or were not inhabited by the crabs.

2. Materials and methods

2.1. Study site

The study was conducted in the Manko wetland in the southern part of Okinawa Island, Japan (26°11′ N, 127°40′ E) during autumn 2004. This region has a subtropical climate, with an average annual temperature of 23.2 °C. The lowest and highest monthly mean temperatures are recorded in February (16.8 °C) and July (28.8 °C), respectively. Precipitation exceeds 100 mm month^{-1} throughout the year, and the average rainfall is 2086 mm year^{-1}. The tidal flat is located at the confluence of the Kokuba and Noha Rivers. The main watercourse depth at low tide is 80 cm with a salinity range from 10 to 31. The sediment characteristics are black, soft clay or sandy mud (Islam et al., 2004). The mangrove forest found on both banks in the lower course is dominated by *Kandelia candel* with a few patches of *Rhizophora stylosa*, *Bruguiera gymnorhiza*, and *Excoecaria agallocha*. Among other invertebrates, grapsid (*Helice formosensis*, *Perisesarma bidens*, *Chasmagnathus convexus*, *Neosarmatium sonithi*, and *Sesarmops infermedium*) and ocypopid (*Uca lactea perplexa*, *Uca dussumieri dussumieri*, and *Uca chlorophthalma crassipes*) crabs are commonly found in this tidal flat. In particular, *H. formosensis* construct deep burrows in more compact, sandy mud in the landward extreme of the tidal mangrove forest. At this site, their burrow density ranges between 16 and 36 holes^{-1} m^2 (Mchenga, 2005). They form and maintain "*U*" or "*Y*"-shaped semi-permanent burrows, which are exposed to air during low tide. The morphology and dimensions of *H. formosensis* burrows were examined in a previous study (Mchenga, 2005) and are summarized in Table 1.

2.2. Sample collection and measurements

Sampling and measurements were carried out during low tide. Three samples were randomly collected from surface sediments (~1 cm in depth) inhabited by crabs and from those without crabs. For burrow sampling, three burrow replicates were used. A thin layer of the burrow wall opening (BO) was carefully scraped off of the opening shafts (to approximately 10 cm depth) using sterile spatulas. Sediment samples were pooled from two openings of the same burrow (Fig. 1). For the burrow chamber sample, similar burrows were filled with a polyester resin and carefully excavated after 24 h (casting method of Dworschak, 1983). After the cast was removed, a 1-cm slice of sediment was collected from the bottom of the burrow chamber.

Redox potential (Eh) was measured using an electrode Oxidation-reduction potential (ORP) meter (model RM 20P; TOA, Tokyo, Japan). Measurements were made by gently inserting the electrode into a burrow opening until it came in contact with the burrow wall. Burrow chamber measurements were made by pushing the electrodes into the sediment once the cast was removed. The Eh of surface sediment with and without crabs was measured by inserting the electrode into the surface (~0.5 cm depth). The electrode was allowed to stabilize for 5–10 min before each reading. Prior to measurements, the Ag/AgCl Pt electrode potential was calibrated using an ORP check solution (quinhydron solution). Similarly, temperature, pH, conductivity, and resistance were measured *in situ* using a pH meter (model PH 81; Yokogawa, Tokyo, Japan) and a conductivity meter (model ES-51; Horiba, Kyoto, Japan). Salinity was measured using the Practical Salinity Scale. Sediment samples were placed in a 20-ml syringe (with filter paper), pushed with a plunger until a few drops of water were extracted, and measured using a refractometer (model IS/Mill-E IUCHI, Japan).

2.3. Determination of OM content

Total organic carbon (TOC) and total nitrogen (TN) contents in sediments were analyzed using a Shimadzu high-sensitivity C:N analyzer (NC 80). Sediments were first dried at 80 °C to a constant weight and then used to determine water

Table 1
General description of *H. formosensis* burrows. *HD: Distance between borrow openings (a single burrow is formed by two openings)

Burrow component	Mean ± SE	Range
Burrow diameter (BD) in cm	3.0 ± 3.6	1.8–4.5
Total length (TL) in cm	29.7 ± 8.2	18.4–41.3
Burrow shaft length in cm	17.0 ± 5.0	10.2–27.3
Burrow handle length in cm	12.7 ± 5.3	7.1–22.5.3
Hole distance (HD) in cm*	12.4 ± 1.0	5.5–18.4
Burrow volume (BV) in g l^{-1}	3.0 ± 1.3	0.8–4.5
Burrow density (hole/m^2)	26.2 ± 2.1	16–36
Burrow surface area (cm^2)	162.5 ± 36.9	116–210.9

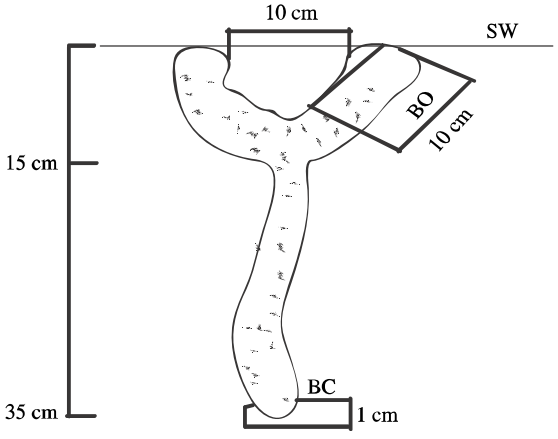


Fig. 1. Diagrammatic representation of *Helice formosensis* burrow and sampling design. BO: burrow opening shaft; BC: burrow chamber, and SW: surface sediments inhabited by crabs.

content. Samples were later ground to a fine powder and passed through a 0.25-mm sieve. Prior to analysis, sediment samples were treated with 2 N HCl for 24 h to remove carbonates and bicarbonate. A subsample was ignited in an electric muffle furnace (FUL 220FA) at 550 °C for 3 h to obtain the percentage loss on ignition (% LOI) as a measure of total organic matter (TOM).

2.4. Lipid extraction

Triplicate samples of approximately 10 g of wet weight sediment were used for lipid extraction following a modified procedure of Bligh and Dyer (1959). Extraction was conducted ultrasonically for 20 min with a mixture of chloroform:methanol:distilled water (1:2:1; 20 cm^3; v:v:v). With the addition of a distilled water:chloroform mixture (1:1; 10 cm^3; v:v), an aqueous-organic two-layer system was formed. Lipids were transferred to the lower chloroform layer, and the separation was improved by centrifugation (2000 rpm for 5 min). After evaporation of the solvent, the extracts were dried under nitrogen and weighed for total lipid content using an electronic balance (ER-182A; A&D Company, Broomfield, CO, USA). The lipids were then saponified under reflux (2 h, 100 °C) with a 2 mol dm^{-3} NaOH solution in methanol and distilled water (2:1, v:v). After acidification with an ultrapure HCl solution (37.5%), 2 × 2 cm^3 of chloroform were successively added to recover the lipid. The solvent was then evaporated under a nitrogen stream, and FAs were converted to methyl esters under reflux using 1 ml of 14% BF3-methanol for 10 min. Saponification and methylation were performed to obtain total FAs (Meziane et al., 2002; Mfilinge et al., 2005).

FA methyl esters (FAMEs) were purified using the high performance thin-layer chromatography technique (HPTLC) using Merck (Darmstadt, Germany) plates coated with silica gel. The solvents used for developing were a mixture of hexane:diethyl ether:acetic acid (70:30:1). Bands containing FAMEs were scraped and collected in a mixture of chloroform:methanol (2:1, v:v) at 40 °C for 60 min. FAMEs were then isolated in the same solution until analysis by gas chromatography. For all samples, a second plate was prepared to estimate the proportion of FAMEs in the total lipids (Yamashiro et al., 1999). After drying, the developed plate was scanned using a flatbed scanner (GT-9000; Epson, Tokyo, Japan) and Adobe Photoshop software (version 6.0; San Jose, CA, USA). The resulting image file was imported into NIH image (version 6) to estimate the relative contribution of FAs, as a proportion of total lipids, by integrating the chromatogram (Meziane et al., 2002).

The FAMEs were separated and quantified by a gas chromatograph (GC 14.B; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID). Separation was performed with a free fatty acids phase (FFAP) polar capillary column (30 m × 0.32 mm internal diameter, 0.25-μm film thickness) with helium as a carrier gas. After injection at 60 °C, the oven temperature was raised to 150 °C at a rate of 40 °C min^{-1}, then to 230 °C at 3 °C min^{-1}, and finally held constant for 30 min. The flame ionization detector was held at 240 °C. The peaks of the FAMEs were then identified by comparing their retention times with those of authentic standards (Supelco Inc., Bellefonte, PA, USA).

FA nomenclature is designated by X:$Yn-z$, where X is the number of carbon atoms followed by the number of double bonds (Y) separated by a colon. The position of double bonds nearest the methyl terminus is given by a suffix of the form $n-z$, where z is the distance from the end of the chain. The FA results are given as the mole percentage of total FAMEs. Total FAME content was expressed as an absolute dry weight (μg g^{-1}) following the conversion of initial wet weight to dry weight sediments and estimation of the contribution of individual FAs in the total lipids.

2.5. Data analysis

Differences in OM, total lipid content, and the concentration of individual or groups of FAs between surface sediments with and without crabs and the burrow components (opening shafts and chambers) were tested using a one-way analysis of variance (ANOVA). Post-hoc Tukey (HSD) and Fisher's (LSD) tests were used to detect differences between treatments when significant differences were found. Results were considered significant if $p < 0.05$.

Multivariate analyses of FA profiles were performed using the PRIMER software (version 6). Multi-dimensional scaling (MDS) was conducted based on a Bray-Curtis similarity coefficient. No transformation was performed on the data. Differences between habitats (surface sediments with and without crabs) and burrow compartments (opening shafts and chambers) were determined using one-way analysis of similarity (ANOSIM).

3. Results

3.1. Physicochemical properties

The ANOVA resulted in no significant differences in temperature or pH between burrow compartments and sediment types (Table 2). However, salinity was significantly lower in the burrow chamber than in the burrow opening shaft or the surface sediments with and without crabs (ANOVA, $F = 4.9$, $df = 3$, $p < 0.05$). The Eh of the burrow opening shaft (upper 10 cm) was significantly higher (200.3 mV) than surface sediments with and without crabs (ANOVA, $F = 59.2$, $df = 3$, $p < 0.001$). Redox potential values significantly decreased with depth in the burrow chamber (−42 mV), but the Eh of surface sediments inhabited by crabs was not significantly different from those without crab burrows (Table 2). Ionic conductivity was higher in sediments of the burrow shaft (5.4 s^{-1} cm) than in the burrow chamber (0.4 s^{-1} cm) and was significantly different between surface sediment with (3.8 s^{-1} cm) and without (3.0 s^{-1} cm) crabs (ANOVA, $F = 107.01$, $df = 3$, $p < 0.001$). In contrast, resistance measurements exhibited the opposite pattern (Table 2). Sediment water content was significantly higher in sediments of the burrow opening shaft and surface areas with crabs (21.5 and

Table 2
Physicochemical properties of the grapsid crab burrow and surface sediments. BO: burrow opening shaft, BC: burrow chamber, SW: surface sediments inhabited by crabs, SO: surface sediments without crabs. Values are means ±1 SE, $n = 6$. Same letter at the top indicates no significant difference at $p < 0.05$; ANOVA followed by post hoc Turkey (HSD) and Fisher's (LSD) test

	H. formosensi BO	*H. formosensi* BC	SW	SO
Temperature (°C)	19.3 ± 0.2^{a}	19.3 ± 0.3^{a}	19.3 ± 0.0^{a}	20.0 ± 0.0^{a}
Salinity	23.3 ± 0.1^{a}	17.1 ± 0.1^{b}	20.0 ± 0.2^{a}	25.4 ± 1.1^{a}
pH	7.5 ± 0.2^{a}	7.3 ± 0.2^{a}	7.5 ± 0.0^{a}	7.7 ± 0.1^{a}
Redox potential (mV)	200.3 ± 3.3^{a}	-42 ± 2.4^{b}	104.4 ± 23.9^{c}	119.2 ± 28^{c}
Conductivity (s/cm)	5.4 ± 1.3^{a}	0.4 ± 0.0^{b}	3.8 ± 0.4^{c}	3.0 ± 0.3^{d}
Resistance (Ohms/cm)	0.2 ± 0.1^{a}	2.9 ± 0.1^{b}	0.2 ± 0.0^{c}	0.2 ± 0.0^{d}
C:N ratio (mol:mol)	9.1 ± 0.0^{a}	11.0 ± 1.3^{a}	9.6 ± 0.3^{a}	9.6 ± 0.4^{a}

22.7%, respectively) than in the burrow chamber and surface areas without crabs (ANOVA, $F = 8.1$, df = 3, $p < 0.01$; Fig. 2a). TOM concentration ranged between 19.3 and 25.3% and was not significantly different between the burrow opening shaft and the surface areas with and without crabs. However, TOM was significantly lower in sediment of the burrow chamber (ANOVA, $F = 4.4$, df = 3, $p < 0.05$, Fig. 2b) and was associated with a higher C:N ratio.

3.2. FA composition

The mean percentage compositions of identified individual FAs (≥14:0) that accounted for >0.2% of the total FAMEs in the profiles for all sediment samples are presented in Table 3. Results of MDS and ANOSIM revealed significant grouping of the sediment types (ANOSIM, $R = 0.815$, $p < 0.01$). The stress value for the MDS analysis was 0.05, indicating differences between burrow compartments and surface sediment habitats (Fig. 3). The total amount of FAMEs in the sediment of the burrow opening shafts was significantly higher than in the burrow chamber or surface areas with and without crabs (ANOVA, $F = 58.3$, df = 3, $p < 0.001$). However, the total amount of FAMEs in sediment with and without crabs did not significantly differ (Fig. 4). Palmitic acid (16:0) ranged between 19.5 and 27.9% and was present in significantly higher proportions than other FAs. The amount of palmitic acid was highest in sediment with crabs, especially in comparison to values in the burrow compartments (ANOVA, $F = 10.4$, df = 3, $p < 0.01$). Another abundant FA was monounsaturated 18:1n−9 (12.9%) and 18:1n−7 (10.6%), which were detected in the sediments of the burrow chamber and opening shaft, respectively. Values of these FAs in the burrow sediments were significantly different from those in the surface sediments with and without crabs (ANOVA, $F = 40.2$, df = 3, $p < 0.001$).

In the total FAME composition, saturated FAs (SAFAs) were the most dominant, comprising between 37.4 and 46.6% in all sediment samples. The SAFAs were significantly higher in the crab burrow chamber than in the burrow opening shaft or in sediments with and without crabs (ANOVA, $F = 5.4$, df = 3, $p < 0.02$). Polyunsaturated FAs (PUFAs) accounted for the lowest average concentration, ranging between 6.0 and 12.6%. In addition, monounsaturated FAs (MUFAs) had a significantly higher contribution, particularly in sediment of the burrow opening shaft and chamber (25.3 and 24.4%, respectively). Similarly, sediment from areas with crabs contained a significantly higher contribution of MUFAs than sediment without crabs (ANOVA, $F = 45.9$, df = 3, $p < 0.001$). Sediment from the burrow opening shaft consisted of relatively higher concentrations of bacterial markers branched with an odd number of carbons (∑15:0 iso, anteiso and 17:0 iso, anteiso) and 18:1n−7 than sediment from the burrow chamber (ANOVA, $F = 20.3$, df = 3, $p < 0.001$). However, there was no significant difference between sediments with and without crab habitats (Fig. 5a). FAs 18:2n−6, 18:3n−6, and 18:3n−3 (indicators of green

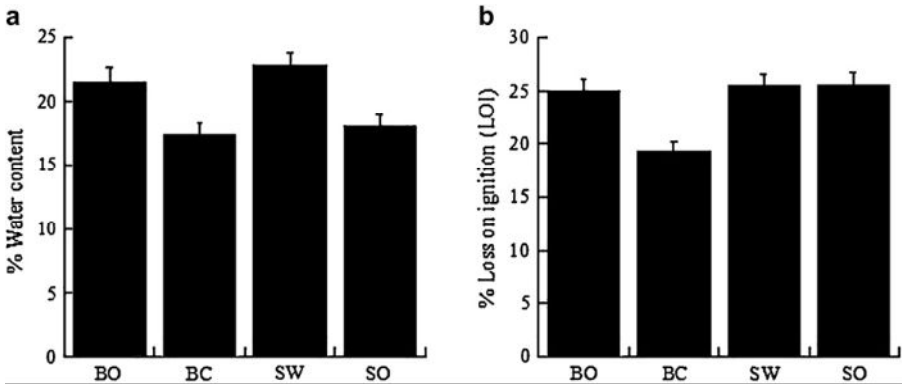


Fig. 2. Percentage water content (a); and percentage loss on ignition (LOI) (b) in sediment of the burrow opening shaft (BO), burrow chamber (BC), surface with crabs (SW), and surface area without crab habitat (SO). Values are means ±1 SE.

Table 3
Fatty acid (FA) composition (mol% of total FAMEs) in sediments of the burrow opening shaft, burrow chamber, and surface areas with and without crab habitat. Values are means ±1 SE, $n = 3$. – Not detected

Fatty acids	*H. formosensi* Burrow opening	*H. formosensi* Burrow chamber	*H. formosensi* Surface sediments	Surface sediments without crabs
Saturated				
14:0	2.7 ± 0.2	1.3 ± 0.7	1.1 ± 0.7	1.7 ± 0.1
15:0	1.6 ± 0.2	1.0 ± 0.4	1.4 ± 0.5	0.4 ± 0.0
16:0	19.5 ± 1.1	24.8 ± 2.9	27.9 ± 1.3	27.5 ± 0.4
17:0	1.2 ± 0.4	0.7 ± 0.2	1.3 ± 0.8	1.8 ± 0.7
18:0	6.1 ± 1.8	10.1 ± 0.8	4.7 ± 0.2	3.3 ± 0.1
20:0	0.4 ± 0.3	0.4 ± 0.3	–	0.2 ± 0.0
22:0	0.6 ± 0.4	0.4 ± 0.4	0.5 ± 0.4	0.4 ± 0.0
24:0	1.0 ± 0.7	2.0 ± 0.1	2.1 ± 0.9	2.0 ± 0.0
26:0	1.0 ± 0.4	2.8 ± 1.5	1.0 ± 0.3	0.4 ± 0.0
28:0	2.0 ± 0.4	1.5 ± 0.6	1.0 ± 0.8	0.2 ± 0.1
30:0	1.2 ± 0.9	1.5 ± 1.2	1.2 ± 1.7	–
Sum	37.4 ± 6.9	46.6 ± 9.2	42.3 ± 7.6	37.9 ± 1.5
Branched				
15:0 i	4.5 ± 1.3	3.9 ± 1.5	3.4 ± 1.0	1.5 ± 0.1
15:0 a	2.2 ± 1.0	1.9 ± 0.8	3.9 ± 2.2	1.0 ± 0.0
17:0 i	2.6 ± 1.1	1.8 ± 0.3	1.0 ± 0.5	1.5 ± 0.4
17:0 a	1.5 ± 0.3	2.1 ± 1.0	1.1 ± 0.1	1.5 ± 0.2
Sum	10.8 ± 3.6	9.7 ± 3.6	9.4 ± 3.9	5.5 ± 0.6
Monounsaturated				
16:1*n*−7	4.3 ± 1.7	5.7 ± 1.4	4.1 ± 0.3	2.5 ± 0.4
17:1	1.8 ± 1.3	–	–	0.4 ± 0.0
18:1*n*−9	8.6 ± 0.4	12.9 ± 5.0	7.3 ± 1.2	5.1 ± 0.4
18:1*n*−7	10.6 ± 0.4	5.8 ± 0.7	7.6 ± 0.4	7.3 ± 0.2
Sum	25.3 ± 3.9	24.4 ± 7.1	19.0 ± 1.9	15.4 ± 0.9
Polyunsaturated				
16:2*n*−4	1.1 ± 0.4	1.1 ± 0.5	0.8 ± 0.1	1.0 ± 0.3
18:2*n*−6	2.4 ± 0.8	0.9 ± 0.4	1.8 ± 0.1	1.6 ± 0.0
18:3*n*−6	1.7 ± 0.1	–	–	0.3 ± 0.0
18:3*n*−3	0.2 ± 0.3	3.2 ± 0.6	0.7 ± 0.2	0.6 ± 0.1
18:4*n*−3	–	0.5 ± 0.2	0.6 ± 0.5	0.5 ± 0.0
20:4*n*−6	0.7 ± 0.5	–	0.8 ± 0.6	1.9 ± 0.1
20:5*n*−3	1.1 ± 0.1	–	6.3 ± 2.4	6.6 ± 0.3
22;2	1.6 ± 2.3	–	–	–
Sum	8.8 ± 4.5	6.0 ± 2.0	11.1 ± 3.9	12.6 ± 0.9
16:1*n*−7/16:0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
18:2*n*−6 + 18:3*n*−3	2.6 ± 1.1	4.1 ± 0.9	2.5 ± 0.3	2.2 ± 0.1

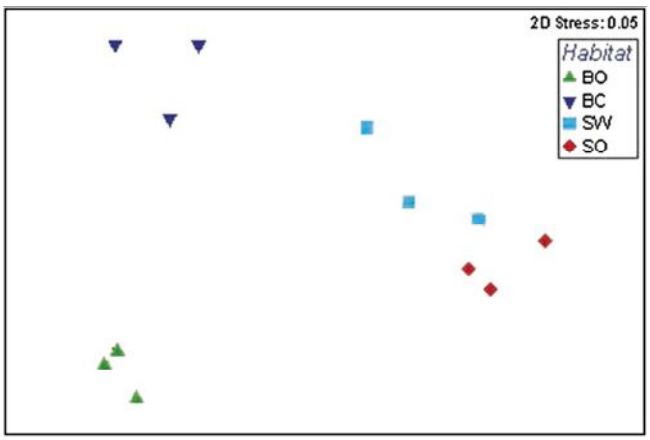


Fig. 3. MDS ordination of Bray-Curtis similarities of fatty acid profiles in sediments of the burrow opening shaft (BO), burrow chamber (BC), surface with crabs (SW), and surface without crab habitat (SO). Values are means ±1 SE.

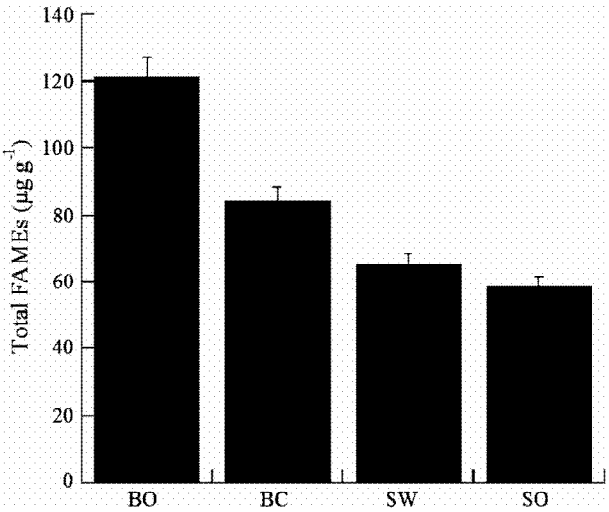


Fig. 4. Total fatty acid methyl esters (FAMEs) in sediment of the burrow opening shaft (BO), burrow chamber (BC), surface with crabs (SW), and surface without crab habitat (SO). Values are means ±1 SE.

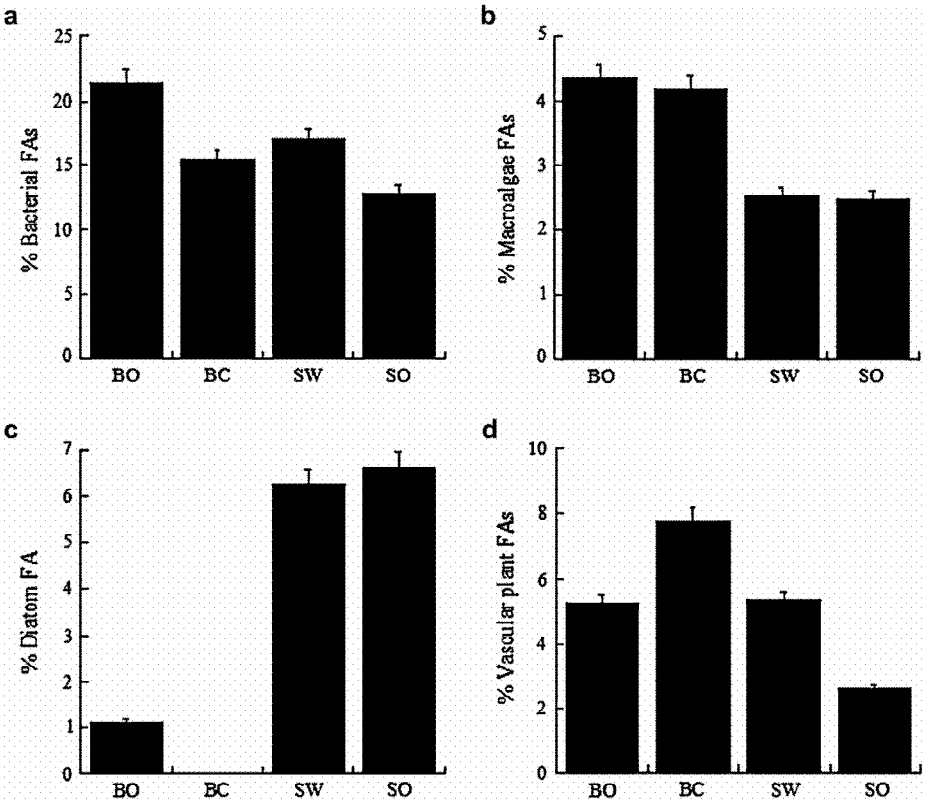


Fig. 5. Contribution of selected fatty acid (FA) biomarkers of organic matter sources in sediments: (a) bacterial FA; (b) macroalgal FA; (c) diatom FA; and (d) vascular plant markers. Values are means ±1 SE.

macroalgae) were higher in sediments of burrow compartments than in surface sediments with and without crabs (ANOVA, $F = 9.3$, df $= 3$, $p < 0.01$). In contrast, there was no significant difference between sediments with and without crab habitats (Fig. 5b). The contribution of eicosapentanoic acid 20:5$n-3$ (diatom marker) was relatively low (ranging between 1.1 and 6.6%), with the highest amount recorded in sediments without crabs, which was significantly different from sediment from the burrow compartments (ANOVA, $F = 15.6$, df $= 3$, $p < 0.001$, Fig. 5c). Long chain FAs (LCFAs) $\geq C_{24}$ in length (vascular plant markers) were relatively higher in sediments of the burrow chamber than in the burrow opening shaft or surface sediments with and without crabs (Fig. 5d).

4. Discussion

4.1. Effect of burrowing crabs on sediment properties

Bioturbation by burrowing macrofauna has a major impact on the sediment. Their activity affects the environmental conditions within their burrows and the surrounding surface sediment. Similar to other burrowing crustaceans, such as *Callianasa* (Dworschak, 2001) and *Upogebia* (Kinoshita et al., 2003), crabs have a significant effect on sediment composition, habitat structure, and benthic organisms. For instance, the grapsid crab *Chasmagnathus granulatus* has an important effect on meiofaunal distribution, OM content, and nitrogen loading in sediments (Botto and Iribarne, 1999; Botto et al., 2005, 2006). The results of this study suggest that *Helice formosensis* activities have a significant impact on Eh by improving the oxygen conditions of the burrow opening shaft. According to Myers (1977), burrowing fauna increases the Eh zone both by deepening the surface oxidizing layers and creating oxic microenvironments. Montague (1982) found that fiddler crab (*Uca* spp.) burrow walls were aerobic and concluded that burrowing activity provided more oxygen, thereby enhancing salt marsh growth. Sediment in plots of fiddler crabs had elevated redox potential in the upper 7 cm (~225 mV) and gradually decreased (−70 mV) at 16 cm depth (Nielsen et al., 2003). Many studies have reported an enhanced Eh associated with bioturbation activities (Kristensen, 1988; Bird et al., 2000; Dworschak, 2001). Similarly, *in situ* redox measurements conducted in this study demonstrated that *H. formosensis* burrow shaft sediments were more oxidized than the burrow chamber and surface sediments. However, no difference was found between surfaces with and without crab activity.

The higher conductivity levels in the sediment of the burrow opening shaft and surface areas inhabited by crabs versus surface sediment without crabs emphasize the effect of *Helice formosensis* burrowing. Previous studies have suggested that burrowing by crabs can potentially enhance sediment drainage and oxidation levels (Montague, 1982; Bertness, 1985; Iribarne et al., 1997; Bortolus and Iribarne, 1999). Bioturbation activity by invertebrates maintains a higher hydraulic conductivity in sediments and promotes water-sediment exchanges

(Nogaro et al., 2006). Our measurements revealed a higher water content in the burrow opening shaft and surface sediment inhabited by crabs than without crabs, which may be related to the burrowing activities of *H. formosensis*. This finding is consistent with other studies, such as those examining thalassinidean shrimps (Tamaki et al., 1992; Atkinson and Taylor, 2004), fiddler crabs (*Uca uruguaynsis*), and grapsid crabs (*Chasmagnathus granulatus*; Botto and Iribarne, 2000).

A comparison of sediment-water salinity contents revealed lower salinities in the burrow and in areas with crabs than in areas without crabs. Salinity is one of the most important factors controlling the vertical and horizontal distribution of grapsid crabs (Jones and Simons, 1982; Omori et al., 1998; Mia et al., 2001). While habitat characteristics clearly affect the distribution of crabs, the reverse is also true. Grapsid crabs may affect the salinity of their habitat, particularly in densely inhabited areas. Bridger and Allen (2006) suggested that diffusion/conductivity may be an important mechanism controlling the distribution of salinity in sediments. Montague (1982) reported that the salinity of fiddler crab burrow water was two-fold lower than the interstitial water, suggesting that crab activity tended to lower soil salinity. Although sediment biogeochemical processes were not quantified in this study, other findings indicate that colonization by bioturbating macrofauna can enhance nutrient exchange in sediment (Hansen and Kristensen, 1997). For example, fiddler crab burrow wall sediments appeared to be a zone of intense redoxidation of reduced compounds such as Fe (III) and sulfide compounds (Gribsholt et al., 2003; Nielsen et al., 2003).

TOM content did not differ between surface sediment with and without crab habitats. In contrast, previous studies reported increased OM content in the burrow environments of crabs (Bortolus and Iribarne, 1999; Botto and Iribarne, 2000; Botto et al., 2005) and thalassinidean shrimps (Dworschak, 2001; Kinoshita et al., 2003). However, the moderate amounts of TOM detected in the burrow chamber of *Helice formosensis* may suggest passive input of detritus material (Botto et al., 2006), which may decrease the amount of OM in surface sediment inhabited by crabs. The occurrence of a higher C:N ratio in sediment of the burrow chamber strongly supports the possibility of input of fresh OM of a vascular/mangrove plant origin (Wetzel, 1983; Mfilinge et al., 2005).

4.2. Effect of burrowing crabs on sedimentary FA and OM sources

Generally, sediment of the burrow opening shaft had higher amounts of total FAMEs than the burrow chamber and surface areas with or without crabs. Previous studies have shown that bioturbation activity can have a significant impact on the burial and degradation of sedimentary lipid components (Sun et al., 1999; Grossi et al., 2003). In addition, the degradation of OM components, including lipids, is generally faster under aerobic rather than anaerobic conditions (Harvey and Macko, 1997; Sun et al., 1997). Therefore, our FAME profile results can be attributed to the combined effects of burrowing activity. The contribution of odd-branched FAs ($\sum$15:0 iso, anteiso and 17:0 iso, anteiso) and MUFAs (18:1$n-7$) were highest in the burrow compartment sediment, intermediate in the surface environments with crabs, and lower in surface areas not inhabited by crabs. The occurrence of these markers is related to an increase in bacterial input (Carrie et al., 1998; Mfilinge et al., 2005). Invertebrate burrow sediments are sites of increased bacterial activity and abundance (Phillips and Lovell, 1999; Dworschak, 2001; Marinelli et al., 2002; Kinoshita et al., 2003; Papaspyrou et al., 2005), most likely due to burrow properties and faunal activity. Here, high Eh and water content were detected in the burrow opening shaft and the surface sediment inhabited by crabs, which may provide a suitable environment for bacterial growth (Watling, 1991). Moreover, detritus in *Helice formosensis* burrows may serve as a dietary source for bacteria and thus stimulate their growth and activity (Kogure and Wada, 2005). Although microbial community structure was not categorized in this study, some researchers have suggested that FA 18:1$n-7$ and odd-branched FAs (15:0 iso, anteiso and 17:0 iso, anteiso) are useful markers of aerobic, anaerobic, and sulfate-reducing bacteria (Canuel, 2001; Pinturier-Geiss et al., 2002). Thus, burrow sediments may provide both an aerobic and an anaerobic zone, where contribution from these bacterial groups is expected.

In this study, PUFAs were generally less abundant in all sediment types. This is likely due to their labile nature (Smith et al., 1983), which subjects them to rapid losses through zooplankton grazing and/or bacterial degradation. PUFAs are therefore not expected to be preserved in their original abundances, but their presence could be related to a fresh input of algae, diatoms, and dinoflagellates to sediments (Carrie et al., 1998; Meziane and Tsuchiya, 2000, 2002). All sediment types contained a low input of a diatom biomarker (PUFA 20:5$n-3$) and a low 16:1$n-7$/16:0 ratio (<0.2). However, the presence of 20:5$n-3$ together with a high amount of SAFAs (16:0 and 18:0) may suggest a potential metazoan input, particularly meiofauna in the sediment (Goulden et al., 1999; Pinturier-Geiss et al., 2002).

Because grapsid crabs are omnivorous (Dahdouh-Guebas et al., 1999), a possible input from diatoms, meiofauna, and macroalgal detritus cannot be excluded from the burrow sediments. Likewise, burrow structure may have an influence on meiofaunal distribution, similar to *Chasmagnathus granulatus* burrows (Botto and Iribarne, 1999). Macroalgal FA indicators (18:2$n-6$, 18:3$n-6$, and 18:3$n-3$) were considerably more abundant in the burrow compartments than in the surface sediments with and without crabs. This result is most likely related to *Helice formosensis* feeding behavior. Although an analysis of the gut contents of this crab accounted for 64 to 85% animal materials and only 2.1 to 2.5% algal materials (Mia et al., 2001), recent FA analyses revealed that 18:2$n-6$ and 18:3$n-3$ are also dominant FAs in mangrove leaves (Hall et al., 2006; Meziane et al., 2007). Mangrove leaves constituted an important nutritional component in the diet of some Grapsidae, such as *Parasesarma erythrodactyla* (Hall et al., 2006). Therefore, a higher contribution of these markers could possibly be associated with both feeding activity by crabs and

passive accumulation of mangrove leaf detritus in the burrow. Alternatively, the total amount of these two FAs may suggest the input of terrestrial material in burrow sediments (Budge and Parrish, 1998). FA 18:2*n*−6 may originate from agricultural fields (Meziane and Tsuchiya, 2002) located near the study site (Tashiro et al., 2003).

LCFAs ($>C_{24}$) are generally used as biomarkers for materials of vascular plant origin. They are associated with the waxy leaf coatings of higher plants and are therefore considered indicative of vascular plant input (Meziane and Tsuchiya, 2002). Relatively high amounts of C_{26}, C_{28}, and C_{30} (which are associated with degraded OM input) were detected in sediment of the burrow chamber, indicating the presence of mangrove detritus inside the burrow. This finding may be partly attributed to the trapping of fine particles and detrital materials due to crab burrowing activities (Botto and Iribarne, 2000; Botto et al., 2006). Moreover, a higher amount of LCFAs in sediment inhabited by crabs than in sediment without crabs reinforces the impact of crabs on the degradation of mangrove detrital material.

It should be noted that changes in the sediment FAME profile related to changes in specific taxonomic groups are complicated, because individual FA markers may occur in more than one species (Bossio et al., 1998). Therefore, the application of the FA technique together with PCR-based approaches (with primers for particular groups using 18S or 16S RNA genes and microscopic examination of washed and fixed sediments) would provide the maximum information on the contributions of different sediment-based biota (Papaspyrou et al., 2006).

Our examination of various FA sources and physicochemical characteristics of sediment clearly indicate that grapsid crabs (*Helice formosensis*) are important agents of bioturbation in their surrounding environment. Their burrowing activity significantly influenced physicochemical properties, FAs, and OM content of sediments, thereby affecting the sediment properties of subtropical mangrove estuaries. Our study elucidates the ecological effect of grapsid crabs on sediments in subtropical mangrove estuaries and broadens our understanding of the role of bioturbation activities by macroinvertebrates on sediment biogeochemical processes.

Acknowledgments

This study was supported by a Ministry of Education, Culture, Sports, Science and Technology (MEXT) Scholarship Grant and the 21st Century COE Program (University of the Ryukyus) to the first author. We also thank Dr. T. Meziane for help with the sampling design and FA analysis protocols, Mr. A. Sakdullah for technical assistance, and Ms. Kimberly K. Takagi and Ms. J. Bouchard for a critical reading of the manuscript.

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