

# Mud shrimp burrows as dynamic traps and processors of tidal-flat materials

K. Kinoshita<sup>1</sup>, M. Wada<sup>2,\*</sup>, K. Kogure<sup>2</sup>, T. Furota<sup>1</sup>

<sup>1</sup>Department of Biology, Faculty of Science, Toho University, Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan

<sup>2</sup>Ocean Research Institute, The University of Tokyo, Minamidai 1-15-1, Nakano, Tokyo 164-8639, Japan

**ABSTRACT:** Bacterial abundance, electron transport system activity (ETSA) and organic matter content (total organic carbon [TOC], total nitrogen [TN] and chl *a*) on the burrow wall of the mud shrimp *Upogebia major* were determined and compared with those in surrounding non-burrow sediments and on the tidal-flat surface. The values of each parameter in burrow sediment tended to decrease outwardly from the wall, while bacteria abundance was highest in the subsurface of the burrow wall. In summer, the abundance of bacteria on the burrow wall was double that in the non-burrow sediments. In winter, both bacterial abundance and ETSA were at the same level as those in non-burrow sediments. The levels of TOC and TN on the burrow wall were more than 3 times higher than those in non-burrow sediments, regardless of the season. However, there was no significant difference in chl *a* content between burrow wall and non-burrow sediments. These results suggest that fresh organic matter in the burrow wall is supplied from the tidal-flat surface, making the burrow environment a suitable niche for microbial populations in the sediment. The mud shrimp burrow functions as a trap for organic matter and thus, helps prevent the outflow of carbon and nitrogen from the tidal flat.

**KEY WORDS:** *Upogebia* · Burrow · Bacteria · ETSA · Organic matter · Tidal flat

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## INTRODUCTION

Burrows of thalassinidean mud shrimps are one of the most significant structures in marine intertidal and shallow subtidal sediments. The burrows of shrimps of the genus *Upogebia* have a distinct U or Y (upper U-shaped part plus a lower I-shaped part) shape (Ott et al. 1976, Dworschak 1983, 1987, Scott et al. 1988, Griffis & Suchanek 1991, Nickell & Atkinson 1995, Coelho et al. 2000, Kinoshita 2002). The burrow of *Upogebia major* (De Haan) is Y-shaped, similar to those of other *Upogebia* species, and its lower I-shaped part can extend deeper than 2 m below the sediment surface (Hamano 1990, Kinoshita 2002). The U-shaped part of the burrow is ideal for efficient unidirectional water flow (Dworschak 1981, Allanson et al. 1992), which keeps the burrow wall oxidized and provides suitable conditions for aerobic bacterial metabolic activities, such as nitrification (Aller et al. 1983, Koike & Mukai 1983).

To understand the ecological significance of mud shrimp burrows in the coastal environment, we focused on *Upogebia major*, which is one of the major constituents of the macrobenthic community on tidal flats in Japan. We report here on bacterial abundance, electron transport system activity (ETSA) and organic matter content (total organic carbon [TOC], total nitrogen [TN] and chlorophyll *a* [chl *a*]) of the burrow wall sediment. Comparison of the values of these parameters on burrow walls with those in the surrounding non-burrow sediments revealed the role of *U. major* burrows in relation to microbial activity and the cycling of materials on tidal flats.

## MATERIALS AND METHODS

**Study site.** A study site was chosen on a tidal flat in Shinhama Lagoon (35° 40' N, 139° 56' E) near the head of Tokyo Bay, Japan (Fig. 1). The tidal flat is at

\*Corresponding author. Email: mwada@ori.u-tokyo.ac.jp

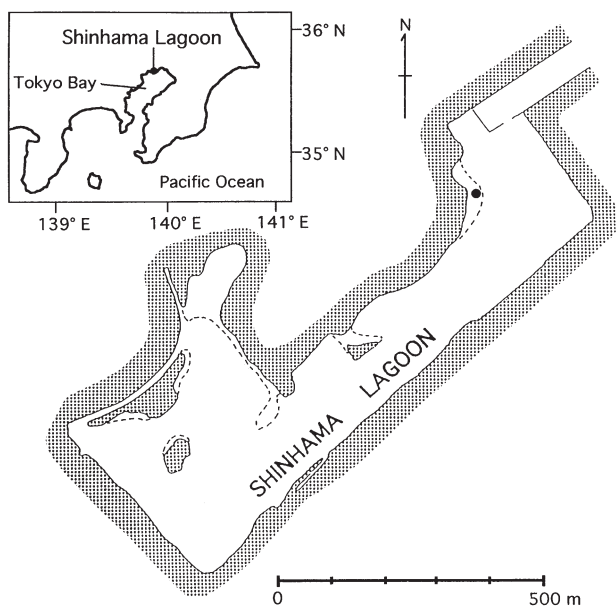


Fig. 1. Shinhama Lagoon and study site. Dotted lines show lowest tide line on the tidal flats

the northern end of the lagoon, where *Upogebia major* averages 36 shrimp  $m^{-2}$ , with a maximum of 142 shrimp  $m^{-2}$  (K. Kinoshita unpubl.). The sediment of the flat consists of coarse mud containing about 60% silty clay.

**Sampling procedure.** Samples were taken on 2 occasions during the spring ebb tide, in January and July 2000. The dates were chosen to encompass a large range of environmental conditions. Temperatures on the surface and at a depth of 10 cm in the sediment were both 6.5°C in January (winter), and 33.0 and 29.0°C, respectively, in July (summer).

Sediment cores including *Upogebia* burrows were taken by using a plastic pipe (inside diameter 10 cm). Collected sediments were carefully sectioned to find the burrow walls of *U. major* down to a depth of 10 cm. Subcores 1.5 cm long into the burrow wall were then taken using sterilized cut-off syringes (5 or 12 mm in diameter) (Fig. 2). The subcores were sliced into three 5 mm thick sections. Subcore samples were collected from 5 burrows. Two further sets of samples were taken from non-burrow sediments of the tidal flat (Fig. 2). These were collected at the surface and at depths of 1.5 and 10 cm. Subcores from 1.5 cm depth were sliced into sections as described above. The 10 cm-depth sediment samples were taken more than 5 cm from a burrow wall and in the present study, are referred to as standard sediment.

For counting bacteria, subcore samples were preserved in filtered (pore size, 0.22  $\mu m$ ) seawater with 2.5% glutaraldehyde immediately after sampling. All

samples were kept on ice and transported to the laboratory within 3 h of the time of collection. In the laboratory, samples for counting bacteria were stored at 4°C; the other samples were stored at -80°C.

**Laboratory experiments.** Subcores including intact burrow walls were kept in an aquarium (water volume = 50 l, water temperature = 20°C and salinity = 24) in the dark with constant aeration. The top section of each subcore (0 to 5 mm) was used to determine bacterial abundance, ETSA and organic matter content at 0, 3 and 6 d of incubation ( $n = 5$  for each sampling date).

**Analysis. Bacteria counting:** To liberate the bacteria cells from sediment particles, Polysorbate 80 (Bacto Tween 80, DIFCO) was added to the sample with a final concentration of 1  $mg\ l^{-1}$ . After mixing for 1 min, the sample was sonicated for 1 min and then mixed again for 1 min. The suspended sample was centrifuged at  $1600 \times g$  for 30 s, and the supernatant was diluted 50-fold with filtered seawater. Then, 500  $\mu l$  of the supernatant was mixed with 4' 6-diamidino-2-phenylindole (DAPI), with a final concentration of 5  $\mu g\ ml^{-1}$ , and kept for 30 min at room temperature (ca. 25°C). The filter was then placed on a glass slide and embedded in a non-fluorescent immersion oil. Bacteria cells were examined with an epifluorescent microscope (BH2-RFCA, OLYMPUS) with UV excitation. At least 200 single cells were counted on each slide at a magnification of 1250 $\times$ . Next, the sediment sample was centrifuged at  $3000 \times g$  for 35 min, and the precipitate dried at 110°C for 24 h to determine its dry

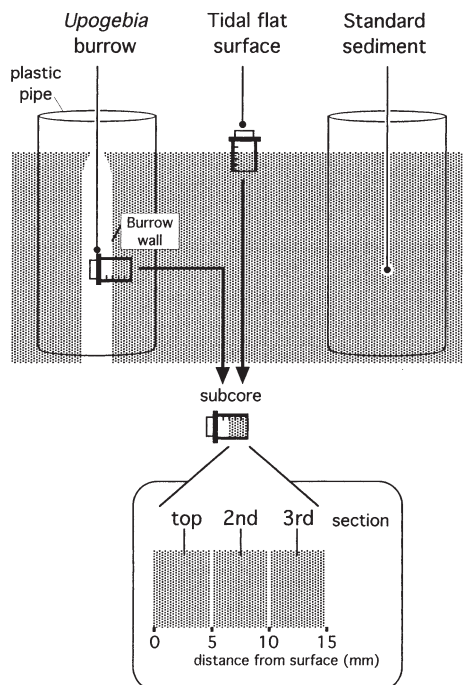


Fig. 2. Sediment sample locations

weight. Bacterial abundance was expressed as cells  $g^{-1}$  dry sediment.

**ETSA measurement:** ETSA was measured by using the method of Broberg (1985) with slight modifications. One gram of wet sediment was mixed with 10 ml of homogenization buffer (25  $\mu$ mol  $MgSO_4 \cdot 7H_2O$ , 0.5 mg PVP-40, 2 ml Triton X and 0.01 mol EDTA in 1 l of 0.1 M phosphate buffer [pH 8]), and sonicated (TOMY UD-200, 22 W) in an ice bath for 5 min. This core mixture was clarified by centrifuging at  $10\,000 \times g$  at  $4^\circ C$  for 10 min, after which the supernatant was further clarified at  $8000 \times g$  for 5 min. We mixed 1 ml of supernatant with 1 ml of substrate solution (7.6 g succinic acid sodium salt 2.1 mM NADH, 0.15 mM NADPH, 0.2% Triton X in 0.1 M phosphate buffer), 0.5 ml of 0.14% 2-(*p*-iodophenyl) 3 (*p*-nitrophenyl) 5 phenyl tetrazolium chloride (INT) solution and 1 ml of homogenization buffer, and then incubated at  $25^\circ C$  for 40 min. From a preliminary experiment, the concentrations of substrates described above were found to be sufficient to achieve  $V_{max}$  of the INT reduction with sediments used in the present study. Immediately after incubation, absorbance of the sample at 490 nm was read with a spectrophotometer (HITACHI U-3200). In calculating ETSA, the molar absorption coefficient of INT-formazan of  $15.9\text{ mM}^{-1}\text{ cm}^{-1}$  (Kenner & Ahmed 1975) was used. All ETSA values were determined within 3 d of field sampling.

**TOC and TN contents:** TOC and TN contents of the sediments were determined using a CHN analyzer (NA 1500, FISONs) with acetanilide (C = 71.09%, N = 10.36%) as a standard. Prior to analysis, sediment samples were treated with 1 N HCl for 24 h to remove carbonates, and subsequently dried at  $60^\circ C$  in a vacuum to eliminate HCl vapors.

**Chl a:** A 100 mg sediment sample was suspended in 5 ml *N,N'*-dimethylformamide (DMF) and stored at  $-20^\circ C$  for more than 24 h before analysis. After being brought to room temperature (ca.  $25^\circ C$ ), samples were vortexed for 30 s and centrifuged at  $3000 \times g$  for 35 min. The clear supernatant was used to determine absorbance at 750, 663.8 and 646.8 nm. Chl *a* concentrations were calculated from the equation given by Porra et al. (1989) and expressed as  $mg\ g^{-1}$  dry wt.

**Statistical analysis.** Comparisons of bacteria abundance, ETSA and organic matter content of the burrow wall and the surrounding non-burrow sediments were made by using the Mann-Whitney *U*-test. Similar comparisons were made for the top section of the burrow wall and the standard sediment.

## RESULTS

### Bacterial abundance, ETSA and organic matter contents in the burrow wall

Profiles of microorganisms (bacteria abundance and ETSA) and organic matter (TOC, TN and chl *a*) in burrow wall cores are shown in Fig. 3A. All parameters, except numbers of bacteria, tended to decrease out-

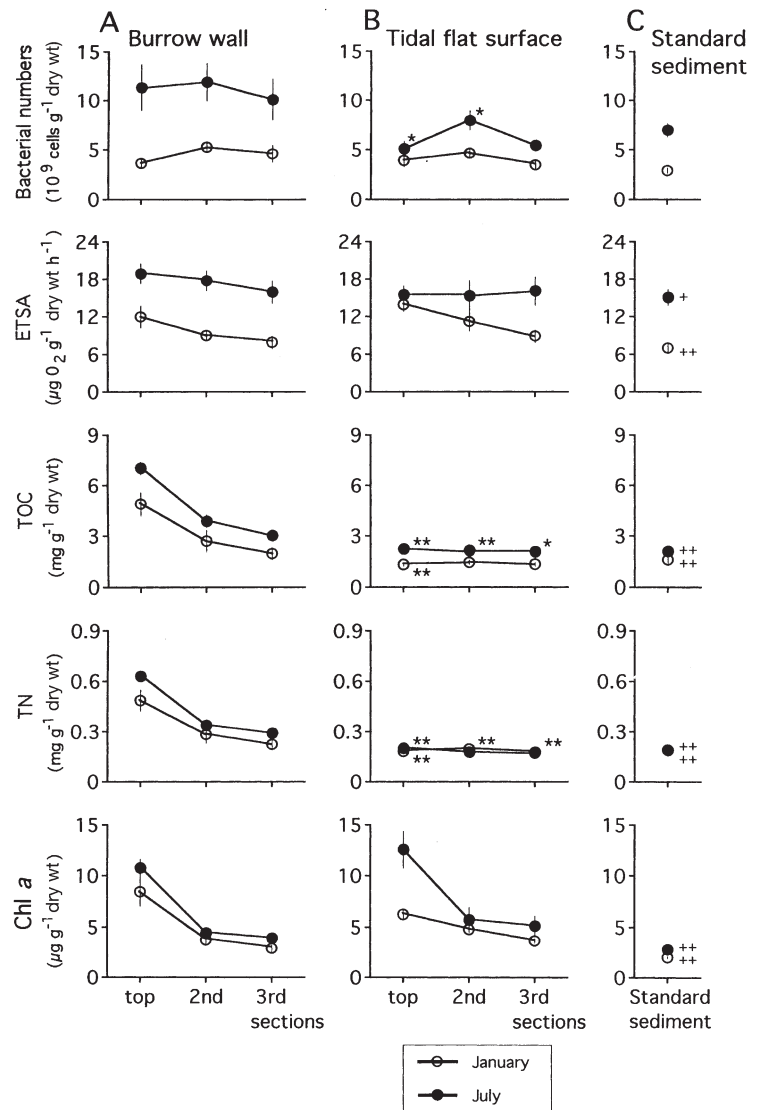


Fig. 3. Depth profiles of bacterial numbers, electron transport system activity (ETSA) and organic matter content (total organic carbon [TOC], total nitrogen [TN] and chl *a*) in subcores of the burrow wall (A), tidal flat surface (B) and standard sediment (C) in January and July 2000. Vertical bars indicate SE ( $n = 5$ ). Significant differences between the burrow-wall variables and tidal-flat surface are indicated by \*\* $p < 0.01$ , \* $p < 0.05$  (Mann-Whitney *U*-test). Significant differences between the top section of the burrow wall variables and standard sediment are indicated by \*\* $p < 0.01$ , \* $p < 0.05$  (Mann-Whitney *U*-test).

wardly from the burrow wall surface and showed higher values in July (summer) than in January (winter). The numbers of bacteria peaked in the second section (5 to 10 mm) rather than in the top one, although differences between the 2 sections were not statistically significant ( $p > 0.05$ ). More than twice as many bacterial cells were found in summer ( $1.13 \times 10^{10}$  cells  $g^{-1}$  dry wt sediment on average). ETSA ranged from  $18.9 \mu g O_2 g^{-1}$  dry wt sediment  $h^{-1}$  in the top section to  $7.9 \mu g O_2 g^{-1}$  dry wt sediment  $h^{-1}$  in the section farthest from the wall. ETSA values were higher in July by a factor of more than 1.5. TOC, TN and chl *a* levels declined most significantly between the top and second sections ( $p < 0.05$  in January and  $p < 0.01$  in July). The average C:N ratio was  $10.2 \pm 0.9$  SD, and remained approximately constant regardless of the season and distance from the wall.

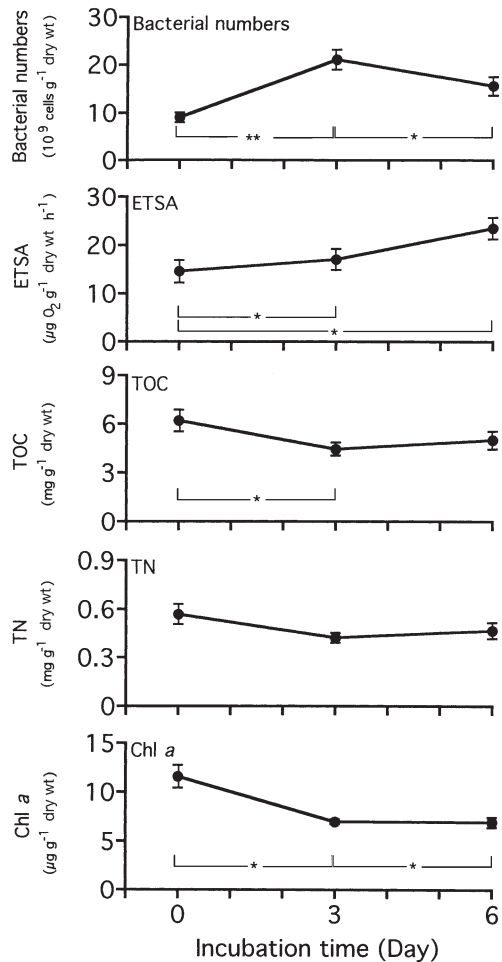


Fig. 4. Changes in bacterial numbers, electron transport system activity (ETSA) and organic matter levels in the top sections of the burrow subcores. Vertical bars indicate SE ( $n = 5$ ). Significant differences among subcores on Days 0, 3 and 6 are indicated by \*\* $p < 0.01$ , \* $p < 0.05$  (Mann-Whitney *U*-test)

### Comparisons between burrow wall and non-burrow sediments

Some differences between the burrow wall and tidal-flat surface were obvious (Fig. 3B,C). First, bacteria numbers in the burrow wall were nearly double those of the tidal-flat surface in summer ( $p < 0.05$ ), but were similar in winter. Second, TOC and TN accumulated in the inner 2 sections of the burrow wall, but were constant among sections of tidal-flat sediments ( $p < 0.01$ ). Chl *a* values were similar in the burrow wall and tidal-flat surface in both January and July.

Comparisons between the burrow wall and the standard sediment are shown in Fig. 3C. ETSA and organic matter in the top section of the burrow wall were clearly higher than those in the standard sediment. Levels of TOC, TN and chl *a* in the burrow wall were 3-times higher than those in the standard sediment. Although the numbers of bacteria cells in the top burrow-wall section were not significantly different from those in the standard sediment, statistically higher numbers of bacteria were found in the second section of the burrow wall ( $p < 0.01$ ).

### Changes in microbiological parameters and organic matter content under laboratory conditions

Results of the laboratory experiments are shown in Fig. 4. Microbiological variables at 3 and 6 d incubation were higher than those at the beginning of incubation. Bacteria numbers increased by a factor of 2.3 in the first 3 d, followed by a slight decrease to Day 6. ETSA gradually increased to 1.6 times the initial value by Day 6. In contrast, TOC, TN and chl *a* decreased by 40% in the first 3 d.

## DISCUSSION

### Burrow wall

Upogebiids have been shown to draw large volumes of water through the U-part of their burrows (Dworschak 1981, Allanson et al. 1992). Thus, the burrow wall should be well oxygenated. The surface of the burrow walls was brown with a thickness of 1 mm and no iron sulfide was found in the sediment cores, indicating that the burrow and surrounding sediments were under oxidizing conditions. Thus, our data on bacterial abundance and ETSA in the burrow wall should reflect the aerobic metabolism of sediment microorganisms. Generally, ETSA represents the respiratory potential in all living microorganisms (Packard 1985), including autotrophic phyto

(mostly benthic diatoms), heterotrophic meiobenthos and microbenthos (protozoans and bacteria). Although the relative contribution of each biological component was not determined in the present study, autotrophic organisms do not seem to have been responsible for the ETSA. On the burrow wall, chl *a* levels in summer were similar to those in winter, while ETSA and bacteria numbers differed greatly between the 2 seasons (Fig. 3A). Furthermore, the laboratory experiments showed that, within a short period of incubation, chl *a* decreased, while both ETSA and bacterial abundance increased. These results strongly suggest that ETSA of the burrow wall was mainly derived from the activity of heterotrophic rather than autotrophic components. Autotrophic activities in the burrow environment would be limited by insufficient light.

The decrease in the chl *a* level to less than half of the initial value within 3 d of incubation indicates that chl *a* in the burrow wall is a labile fraction of the organic matter. Chl *a* abundance in the burrow wall was comparable to that of the surface sediment, implying that the burrow of *Upogebia major* receives a continuous supply of fresh phytodebris. Microscopic observation revealed the presence of intact benthic diatoms (*Navi-cula* sp.) on both the burrow wall and the tidal-flat surface (Kinoshita pers. obs.). In addition, preliminary chromatographic analysis revealed that the pigment composition of the burrow wall was nearly the same as that of the tidal-flat surface and had several unknown peaks. It is most likely that the majority of chl *a* measured in the present study was derived from diatoms found in the tidal flat.

The increase in bacteria numbers, in contrast to the decrease in chl *a*, in the laboratory experiments strongly suggests that the phytodebris introduced into the burrow wall was used by bacteria. Population dynamics of the bacteria cells *in situ* may, however, be influenced not only by substrate, but also by other factors, including physical disturbance caused by the irrigation activity of *Upogebia major* and grazing pressure from eukaryotic microorganisms. Such organisms, nematodes and foraminifera, were found in the top section of the burrow wall. The lower bacterial abundance in the top section of the burrow wall, in contrast to the higher ETSA, may be attributed to predation on bacteria by coexisting microorganisms.

#### Comparison between the burrow wall and tidal-flat surface sediment

Bacteria numbers and ETSA in the burrow wall were comparable to those of the surface sediment in winter, whereas in summer, their levels in the burrow wall were up to 4-times higher than those in the surface

sediment. This seasonal difference suggests that temperature has a substantial impact on microbial biomass and metabolism on the burrow wall. On the tidal-flat surface, however, there were fewer significant differences in the microbiological parameters between the 2 seasons, suggesting that factors other than temperature have a greater effect on microbiological processes.

The constant distribution of TOC and TN in the tidal-flat sediment implies that the surface sediment is well mixed by physical and/or biological disturbance. Physical disturbance, particularly water movement, may erode the tidal-flat surface, resulting in less accumulation of organic matter in the sediment. This assumption is supported by our preliminary experiment that showed approximately 0.9 g of sediment particles were trapped at the present study site in a 9 mm diameter, artificial burrow tube within 1 h (data not shown). Thus, it is likely that sediment of the tidal-flat surface is in a substrate- (organic carbon and nitrogen) limited condition because physical disturbance has restricted the abundance and activities of microorganisms. Taking all these factors into account, *Upogebia* burrows can be viewed as a trap for benthic primary production and organic matter that would otherwise be washed out of the tidal flat by erosion. The present results are in good agreement with other reports on the accumulation of phytopigments and organic matter in biogenic structures in the sediment (Suchanek 1983, Takeda & Kurihara 1987, Dobbs & Guckert 1988, Dworschak & Ott 1993, Felder & Griffis 1994).

#### Turnover time of TOC in the burrow wall

The results of our laboratory experiments were used to estimate the turnover time of organic matter in burrow sediment. Assuming that the decrease in TOC in the burrow wall after 6 d was balanced by the increase in ETSA (Fig. 4), the reduction of TOC (RT) could be expressed by the formula  $RT = 0.39 \times ETSA$ . The time required for TOC in the wall to reach the level in the standard sediment in winter and summer (Fig. 3C) was calculated to be 29 and 28 d, respectively. These values might be underestimates as the decomposition rate may decrease as the labile fraction of organic matter becomes smaller.

#### CONCLUSION

The burrow of *Upogebia major* has higher bacterial abundance and ETSA than the surrounding non-burrow sediments. The burrow accumulates considerable organic carbon and nitrogen, probably derived from

the tidal-flat surface. Fresh phytodetritus forms the labile fraction of the organic matter and is likely to be efficiently utilized by bacterial populations in the burrow wall sediment. As a consequence, the burrow wall of the mud shrimp provides a suitable niche for heterotrophic microorganisms. Thus, the burrow functions as a trap for organic matter, reducing outflow of carbon and nitrogen from the tidal flat.

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