

Minireview

Impacts of Macrobenthic Bioturbation in Marine Sediment on Bacterial Metabolic Activity

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Marine sediment in coastal zones is a habitat for various seaweeds, microalgae, invertebrates, and microorganisms. It is characterized by high biomass and diversity, and high rates of turnover of elements such as carbon, nitrogen and sulfur. One of the factors leading to this extensive biological activity is bioturbation, the process whereby benthic animals form local heterogeneous physical structures and topographic features. In this review, the basis of bioturbation and element cycles in marine sediment, and the relationship of bioturbation with microbial activity will be described.

Key words: bioturbation, marine, sediment, benthos, element cycle

What is bioturbation?

The surface of the earth is shaped by geographical, meteorological and biological factors. The positions of mountains, continents and oceans primarily depend on the movements of plates on the planet, whereas on a smaller scale, the earth's surface is more influenced by meteorological events such as rain, snow, and wind. Living organisms also play major roles, but on still much smaller scales. Virtually all plants on land have roots that inevitably form a specific construction in the soil. We can easily find holes made by ants, earthworms, moles and so on everywhere. A similar situation is found on shore lines or in marine sediment. The movement of water makes ripples on a soft bottom. The bottom's surface is constantly being covered with sinking materials. This process is primarily influenced by the movement of water, physicochemical factors and also biological factors in the upper water column. Some animals like crabs, shells, and polychaetes make tubes in the sediment.

Bioturbation is the process whereby the local physical

structures or topographic features of soil or sediment are modified by biological activities. In the strict sense, a biological process that stabilizes a structure is not regarded as bioturbation. For instance, some benthic organisms make firm tube structures that make sediment stable. However, in this review, such processes are considered as bioturbation, because these constructions alter biological processes and material cycles, which are our major interest in this review. Here, we would like to focus on the bioturbation of marine benthic organisms, with special emphasis on their impact on bacterial activities.

The word, "Bioturbation" was first used by Richter⁶⁶. However, the first conceptual work might be the one by C. Darwin in the 19th century⁸). Darwin^{14,15}) observed the behavior of earthworms and found that they make tubes in the soil, ingest soil material and discard fecal material around the holes they produce. In this way, worms cultivate the soil. His work not only clarified the significance of earthworms in terrestrial environments but also stimulated subsequent works on invertebrates in the sea (e.g., ref. 16).

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Bioturbation in aquatic sediment

In coastal aquatic environments, sediment is being constantly disturbed by the activities of animals. For instance, deposit-feeding clams move into sediment⁷⁶. The depth to which they dig varies with the species and environment. Sea cucumbers ingest sand and leave the feces as surface deposit⁵³. Hermit crabs move on sand and leave traces. Another example is birds. When they feed on small animals on or in sediment, they leave holes.

Once the surface sediment is altered, environmental changes and subsequent biological processes occur. For instance, rays, dugongs and whales exploit sediment and leave meter-scale holes^{64,81}. Marine snow or large particulate matter tends to accumulate in such holes, where meiobenthos may then gather. Therefore, the activity of organisms results in the formation of structurally and biologically different environments which may distribute unevenly⁶⁸. In general, such alterations of the environment cause changes to the biological processes of smaller organisms. Thus a change made by one benthic organism may elicit continuous changes by other types of organisms¹¹.

Table 1 shows the characterization of habitats, some fauna and typical feeding modes of benthic organisms in shelf environments. Except for benthic algae, all animals are dependent on the food present in the upper water column or sedimented materials on the bottom. Suspension feeders and deposit feeders ingest these materials, respectively. Benthic organisms often make tubes, burrows, or holes in the sediment. Such structures vary depending on the feeding habitats of benthic organisms. For instance, deposit feeders tend to make deep holes that result in a vertical mixture of sediments. Polychaetes and ghost shrimps discard sediment materials to the surface around the hole, resulting in the formation of mounds. Irregular erosion or sedimentation processes may proceed in such an area. Therefore, the change in the surface structure or size of sediment particles

may alter the community structure of benthic organisms⁷³.

Suspension feeders such as spoon worms and mud shrimps draw water into their burrows and ingest the particulate matter present in it²¹. Their burrows are generally rigid because cementing materials excreted by the animal harden the wall inside. These structures offer a stable habitat for some smaller organisms, thereby leading to an increase in diversity in environments^{24,32}.

The morphology of burrows is of interest^{55,69}. In general, larger benthic organisms burrow deeper into the sediment and contribute more to the mixing of materials. However, smaller organisms can also contribute considerably when their density is high. For instance, the density of a small polychaete, *Capitella* sp. I can reach up to one million/m²⁷⁷. Smaller benthic organisms also have more adaptability to environmental variability. Therefore, they can be pioneers in open habitats, where larger animals may settle later. These pioneers may serve as prey for the larger organisms or may make the environment moreoxic⁶⁵.

Geochemical cycles in marine sediments

Figure 1 represents general chemical characteristics of marine sediments in coastal areas. Without disturbances, three zones, i.e., an oxidized zone, a redox potential discontinuity (RPD) zone and an anoxic zone, tend to develop. Bioturbation may break this zonation and/or enhance the exchange of water and materials through these zones. Accompanying this exchange, the chemical conditions in the sediment are altered. Reduced compounds or ions that accumulate in the anoxic zone are transported upward with the interstitial water. Ammonium ion (NH₄⁺), phosphate ion (PO₄³⁻), ferrous ion (Fe²⁺), manganese ion, and sulfide ion (SH⁻) are good examples^{2,4,22,29,35,36,43}. Conversely, chemical elements such as oxygen and oxidized compounds including nitrate ion (NO₃⁻), ferric ion (Fe³⁺) and sulfate ion (SO₄²⁻) are transported downward into the

Table 1. Typical fauna, sediment type and feeding mode of benthic organisms in shelf environments

	Typical fauna	Sediment type	Feeding mode
Group I	Barnacles—juvenile amphipod Clams	Muddy gravel	Surface and subsurface deposit feeding
Group II	Tube-dwelling polychaete	Mud with high water and organic content	Surface and subsurface deposit feeding
Group III	Barnacles—Juvenile and adult Tube-dwelling amphipods	Sand and gravel	Suspension feeding
Group IV	Sand dollar	Sand	Suspension feeding

(Modification of ref 51, Table 13.1)

sediment^{3,4,22,34,42,43,48}.

These exchanges enhance or suppress the functions of certain bacterial groups. Among various chemicals and elements, oxygen plays by far the most important role in controlling microbial processes and also element cycles, mainly because it serves as the electron acceptor in the aerobic respiratory chain. The concentration of oxygen affects the relative contribution of aerobic and anaerobic bacteria. Among anaerobes, methanogens and sulfate reducing bacteria are widely distributed in anoxic zones and contribute to the carbon and sulfur cycles. Because methanogens are quite sensitive to oxygen, the introduction of oxygen excludes those groups. Sulfate reducers that are involved in reducing sulfate to sulfide, are less sensitive to oxygen. Therefore it is common for methanogens to be distributed just beneath the sulfate reducing bacteria in sediment. The transport of oxic seawater from the upper water column into the sediment suppresses production of sulfide, which is toxic to most living organisms. In the presence of oxygen, reduced sulfur compounds or sulfur itself can be used as an electron donor by sulfur-oxidizing bacteria. Close coupling of the reduction and oxidation of sulfur occurs on a minute scale. Thus oxygen concentrations affect the sulfur cycle in the sediment. Matsui *et al.*⁴⁹ have investigated how the tubes constructed by a polychaete, *Diopatra cuprea*, affect microbial community structures, especially sulfate-reducing bacteria. By applying culture-independent molecular techniques to both sandy and muddy sediments, they clarified that the community structures were strongly influenced by the characteristics of the sediment and by the tubes constructed by the worm.

The nitrogen cycle is also dependent on the oxygen con-

centration. Under oxic conditions, ammonium ion or nitrite is utilized by nitrifying bacteria as electron donor or energy source, and eventually oxidized to nitrate. Nitrifying bacteria are distributed throughout the water column and also in the upper layer of sediment. Under anoxic conditions, nitrate, in turn, is utilized as an electron acceptor by denitrifying bacteria. The final product of this process is nitrogen gas, which is released into the atmosphere. As in the sulfur cycle, the oxidation and reduction of nitrogen compounds may be closely coupled in RPD zone in sediment^{1,41} (Fig. 1). By applying computer axial tomography (CAT-scan) to intertidal benthic communities, Mermillord-Blondin *et al.*⁵¹ determined on a scale of millimeter the heterogeneous distribution of biogenic structures and sediment characteristics. One small grain or tiny particle of sediment can be the site for such a heterogeneous distribution of biogeochemical processes, because such a gradient can be formed in one particle. Dollhopf *et al.*¹⁹ recently reported that in salt marsh sediment, nitrification and denitrification potential rates were strongly correlated with one another, and coupled nitrification-denitrification was stimulated by macrofaunal burrowing activity. They further proposed that the burrowing and high concentrations of ferric ion (Fe^{3+}) stimulate nitrification, and thus increase nitrogen removal through coupled nitrification-denitrification in salt marshes.

In addition to these “classical” cycles, several new pathways have been discovered for methane and ammonium oxidation in the last decade. Those discoveries will be described briefly here because both have ecological implications in marine sediment.

Methane had been regarded to be oxidized by methanotrophs, which are obligate aerobes. This group possesses

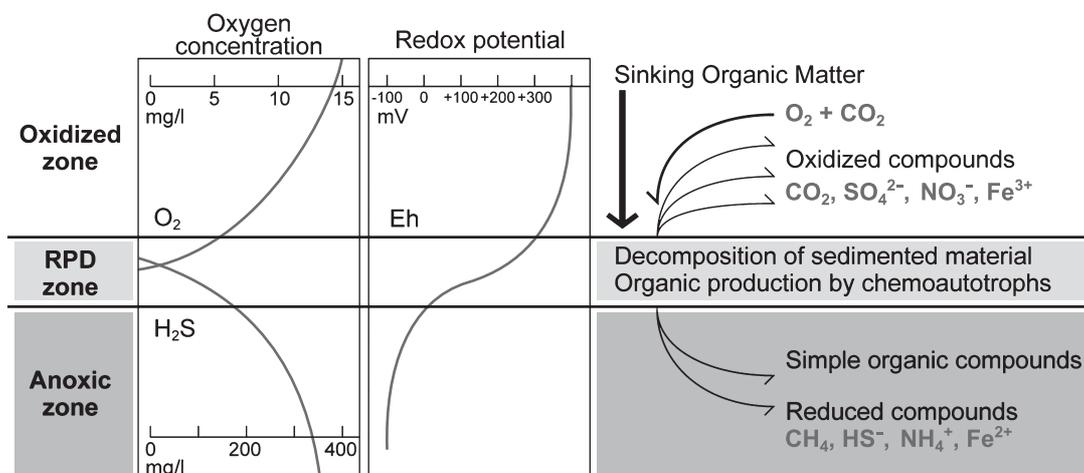


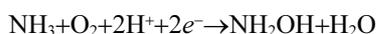
Fig. 1. Physicochemical characteristics and element cycle in and above marine sediment. (redrawn from ref. 56)

methane monooxygenase which converts methane to methanol by introducing oxygen. However, a pathway discovered recently is the anaerobic oxidation of methane (AOM)⁶. During AOM, methane is oxidized with the concurrent reduction of sulfate as follows:

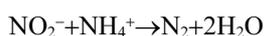


AOM proceeds in a consortium composed of sulfate-reducing bacteria and archaea called anaerobic methanotrophs (ANME), which are closely related to methanogenic archaea of the order Methanosarcinales. The mechanism of AOM is still not clarified, but it is hypothesized that it proceeds via a reversal of methane formation using the same or similar enzymes⁵⁴. Since sulfate is the electron acceptor, AOM is limited to zones where both sulfate and methane are available. Although the contribution of AOM to global methane cycles still needs to be elucidated, a recent study at Hydrate Ridges, Pacific Ocean indicates that 50–100% of methane transported to the surface as dissolved gas may be consumed by this process.

Another example of a pathway discovered recently is the anaerobic oxidation of ammonium. Ammonium oxidation or nitrification has been regarded as follows:



This step is accomplished in the presence of oxygen by the enzyme, ammonia monooxygenase, in nitrifying chemolithotrophic bacteria. Also new is the anaerobic ammonium oxidation (anammox) to nitrogen gas as follows:



A stoichiometric analysis using isotopically labeled nitrogen compounds indicated that NO_2^- , not NO_3^{2-} is used as a direct oxidant of ammonium ion¹². This was found first in waste water treatment facilities⁷¹, and then in the Black Sea⁴⁴ and various marine environments including the water column^{13,44}. The bacteria responsible for this reaction have yet to be isolated, but are affiliated with the order Planctomycetales. As nitrite is usually produced by the reduction of nitrate, these bacterial groups may be present in the RPD zone. It has been estimated that anammox makes a substantial contribution to nitrogen loss in marine environments.

It is also worth noting that the “common” notion of electron donor and acceptor couples has been drastically altered by recent studies on bacteria-metal interactions^{46,78}. For instance, manganese, uranium, chromium, technetium, cobalt, selenium and arsenic can serve as electron acceptors for microbes. Although these relatively newly discovered processes are present in both coastal and deep-sea sediment, there

has been so far little attempt to directly address the influence of marine benthic organisms on either process.

Influence of bioturbation on the sediment

As was discussed above, benthic organisms may modify environmental conditions at the bottom or in sediment in the sea. Then how do such modifications affect physical and chemical conditions and microbial processes?

The consequences of deposit feeding and suspension feeding are primarily reflected in particle size and sediment structures. The deposit feeders, such as clams and sea cucumbers ingest sedimented materials and release finer particles as fecal materials. These materials create a very loose unstable layer in the top few centimeters of the sediment and are easily resuspended with the movement of water. In coastal environments, the resuspension and redeposition of sedimented materials are quite often observed⁵. Therefore, environments dominated by deposit feeders tend to be unstable. For most suspension feeders, this environment is difficult to invade or settle because resuspended fine particles clog their filtering structures. For bacteria, finer particles offer large surfaces for attachment and subsequent decomposition⁷⁵. In general, as particle size decreases, the organic content of the sediment and bacterial numbers increase¹⁷.

Suspension feeders, on the other hand, are generally known as “sediment stabilizers”. This group of organisms including some polychaetes and anemones, often make rigid tubes which stabilize the sediment, especially when tube builders attain a high density. The inner surface of the tubes can be stable substrata suitable for meiobenthos and bacterial cells⁶⁵.

The bioturbation enhances the exchange of water between the upper water column and sediment. This generally leads to an expansion of the oxic zone in the sediment^{22,25,31,70,74}. However, if a highly reduced anoxic zone has developed, the surface oxic layer may become thinner³⁵. When such sediment is ingested by benthic organisms, the inside of their feces may remain in a reduced state even after egestion into oxic sediment. This heterogeneous condition leads to a diversification of microorganisms in the environment³³.

The number of microorganisms in detrital material generally decreases when such matter is ingested by benthic organisms²⁸. However, the number increases in some cases because of a high organic concentration in the gut^{60,61}. These changes in number are accompanied by changes in community structure^{18,20}. Plante and Mayer⁶² measured the

bacteriolytic rates of digestive fluids of a polychaete, *Arenicola marina*, by turbidimetric assay and found seasonal variations. It is assumed that the relative sensitivity to digestive enzymes in the gut may be an important factor controlling microbial community structures in sediment^{50,62}.

Structure of burrows and microbial activity

Some benthic organisms, such as polychaete or crustacean, build tubes or burrows which are stable due to cementing materials excreted by the organisms^{37,63}. These tubes stabilize the upper part of the sediment structure. For the quantitative estimation of the microbial activities in or on the surface of these tubes, those formed by relatively large organisms are suitable because it is easier to treat samples without destroying the structure. Here, we summarize some of the recent progress in microbial research in such an environment.

A) Accumulation of bacterial cells in the burrow and enhancement of aerobic metabolism

The actions of benthic animals draw the water from the upper water column into their burrows. Suspended materials such as particulate matter or plant debris in the water are transported simultaneously. Some of them became embedded into the inner surface of the burrow^{4,7,72}. At sites where the concentration of accumulated organic matter is high, bacterial activity and growth are stimulated. The bacterial cells thus produced are ingested by benthic organisms. This phenomenon is called "gardening", meaning that the animal "cultivates" bacteria as a source of food³⁰. This is analogous to the "cultivation" of fungi by some groups of ants⁹. Although this intriguing hypothesis is quite interesting, its experimental confirmation is not easy, as quantitative proof that the ingested bacteria contribute to the additional growth of the benthic organisms is required^{28,60}.

The organic waste or mucus egested by the animal may be entrapped in the inner surface of the burrow. Such materials also serve as a source of organic nutrients for bacterial cells and stimulate their growth⁶⁵. As long as oxic conditions are maintained at the inner surface of the burrow, aerobic metabolic processes may continue. Under these conditions, sharp gradients of oxygen and redox potential as shown in Fig. 1, are usually formed just below the surface. In these gradients, bacterial groups conducting oxidation (nitrification) and reduction (denitrification) of nitrogen are both present and each reaction may proceed simultaneously. Similarly, both the reduction of sulfate and oxidation of sulfur occur in this gradient. The analysis of this environment

is important for understanding geochemical cycle of elements^{39,63}.

B) Respiratory activity in the burrow system

Several methods of measuring the metabolic activity of whole microorganisms in sediment are available. The oxygen consumption rate is most widely used. However, the two most popular methods, i.e., Winkler titration⁸² and oxygen electrode methods pose problems. Winkler titration is suitable for water samples, but not for samples of sediment or mud²⁶. Although the materials can be suspended in ambient water and analyzed, environmental conditions may be affected by this treatment. The electrode makes it possible to show in fine detail the distribution of oxygen in space, however, the continuous monitoring of oxygen does not always provide reliable data because of instrumental drift with time (e.g., ref. 23).

Another approach is to measure the electron transport system activity (ETSA) of living organisms⁵⁸. A chemical whose optical or chemical characteristics change after reduction is added to the sample. After incubation, the chemical is extracted and quantified by a suitable method. For instance, tetrazolium compounds, such as 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT)⁵⁸ and 5-cyano-2,3-ditoly) tetrazolium chloride (CTC)⁶⁷, are converted to insoluble chromogenic or fluorogenic compounds (formazan) after reduction by the respiratory chain in bacteria or mitochondria. These chemical reactions are generally quite sensitive and need only a small amount of water, sediment or biological sample^{10,27,47,57,59,80}. Assuming that ETSA limits the rate of aerobic respiration, the quantity of formazan can be converted to oxygen reduction or oxygen consumption rates⁵⁸. If oxygen consumption is stoichiometrically balanced with CO₂ production, this also provides a rough estimation of the degradation of organic compounds in the environment.

Recently, Kinoshita *et al.*³⁸ have applied the ETSA technique to microbial communities in the burrow formed by a mud shrimp, *Upogebia major* in estuarine ecosystems. The density of *Upogebia major* can reach as high as 100/m² and burrows can extend 2 meters below the surface. The concentration of organic compounds was significantly higher in the burrows than in the sediment around the burrow (Fig. 2). A high concentration of chlorophyll a (Chl a) on the inner surface of the burrow indicates that detrital materials are continuously transferred into the burrow and cemented there. It seems reasonable to assume that the movement of the animal and water lead to the establishment of such a characteristic environment. When a horizontal column of

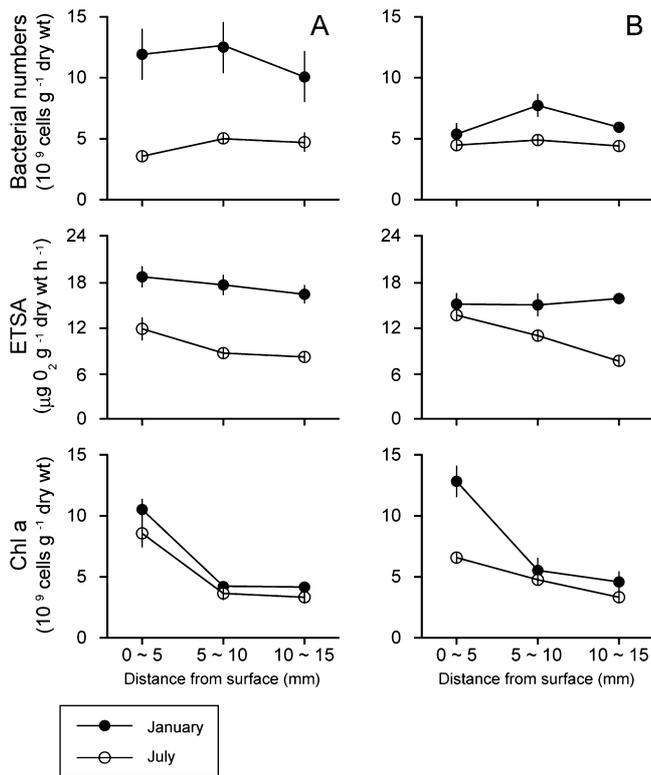


Fig. 2. Depth profile of bacterial number, electron transport system activity (ETSA) and chlorophyll a concentration in a subcore of burrow wall (A) and tidal flat surface (B). (redrawn from ref. 37)

sediment in the burrow was obtained and analyzed, the ETSA was highest on the inner surface of the burrow and decreased with distance from the surface. The activity, however, was not proportional to bacterial biomass. The maximum number was found in the subsurface sediment (5–10 mm in depth), not in the top surface layer (0–5 mm)³⁸⁾ (Fig. 2). There may be several possible explanations for this difference. First, anaerobic bacteria and anaerobic processes were more dominant in the subsurface sediment, than at the surface. Second, the bacteria at the surface maintain much higher levels of metabolic activities than the bacteria in the subsurface sediment. Third, not only bacteria but also other microorganisms such as meiobenthos or protozoa contributed to the respiration or ETSA at the surface. Fourth, the bacterial cells at the surface were constantly ingested by predators, whereas such an effect is much smaller in the subsurface sediment. These explanations, however, still need confirmation. More elaborate studies in combination with the careful separation of each microbial component may help to clarify the biological processes inside of the burrow.

Another approach to quantifying and visualizing the ac-

tivity of benthic organisms and microbes was developed recently. Since formazan has a distinctive color, a specific site of strong ETSA can be visualized if an appropriate device is constructed. Wu *et al.*⁸³⁾ prepared a microcosm system with sediment and *Capitella* sp. I, which is a small polychaete widely distributed in sediment with a high organic load, such as coastal polluted areas or aquaculture sites⁷⁷⁾. These worms are deposit feeders and make U-shape burrows of approximately 1–2 mm in diameter. Their fecal materials are accumulated around the mouth of the burrow. Because the burrow structures are rather fragile and small, it is difficult to take the same approach used for *Upogebia major* by Kinoshita *et al.*³⁸⁾. Wu *et al.*⁸³⁾ directly added a tetrazolium compound into the microcosm and incubated the system. Formazan was quantified after 7 days of incubation, and the sites that accumulate the most formazan were observed visually. They found that the accumulation was quite prominent in the burrow and fecal materials of *Capitella* sp. I. Microscopic observation clarified that formazan was deposited heavily around bacterial cells, indicating that bacteria were mostly responsible for the ETSA and oxygen consumption. Although the inside of the burrow generally retained high levels of activity, there seemed to be some local spots of weak activity as well. This strongly indicates that macro- and micro-organisms and their activities are not homogeneously distributed in terms of space and time. This approach makes it possible to observe the behavior of individual benthic organisms and simultaneously measure their impact on the respiratory activity of microorganisms in the system.

Future direction of research on bioturbation

The first extensive and quantitative scientific work on marine organisms was conducted on sublittoral benthic fauna at the beginning of the 12th century by a Danish biologist, C.G.J. Petersen. Since then, various studies on the taxonomy, physiology, reproductive mechanisms, ecology and behavior of benthic organisms have been conducted. Recent progress in molecular technology has also revealed phylogenetic relationships among macro benthic organisms sharing a similar body structure or similar taxonomical characters⁴⁰⁾. Even among organisms of the same species, the presence of geographic and phylogenetic variations has been reported⁴⁰⁾. Thus new molecular techniques should expand our knowledge of their evolution and also functional diversities. In the near future, we may be able to show how marine organisms with slightly different functions distribute closely and heterogeneously in marine environments.

We still have very limited information on the interaction

of individual organisms with their environment and with other organisms. For instance, most biologists are mainly interested in the behavior of organisms from an ethological point of view. Research into interactions with other organisms has been left mainly because of methodological difficulties and also a lack of correlative studies among scientists of different scientific backgrounds. For this reason, the biogeochemical implications of burrows or tubes made by benthic organisms have drawn little attention among marine chemists and marine microbiologists. Virtually any abiotic or biological structure formed as a result of biological activity should offer suitable conditions for particular living organisms. Interdisciplinary works among scientists of different fields with modern techniques and new approaches will lead to the discovery of new aspects of interactions among organisms and their significance in geochemical cycles.

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References

- 1) Aller, R.C. 1988. Benthic fauna and biogeochemical processes in marine sediment: the role of burrow structures. pp. 301–338. In T.H. Blackburn, and J. Sorensen (ed.), Nitrogen cycling in coastal marine environments. John Wiley and Sons Ltd, New York.
- 2) Aller, R.C. and J.Y. Yingst. 1985. Effects of the marine deposit-feeders *Heteromastus filiformis* (Polychaeta), *Macoma baltica* (Bivalvia) and *Tellina texana* (Bivalvia) on aerated sedimentary solute transport, reaction rates, and microbial distributions. *J. Mar. Res.* **43**: 615–645.
- 3) Anderson, J.M. 1976. Importance of the denitrification process for the rate of degradation of organic matter in lake sediments. pp. 357–362. In, H.L. Golterman and Junk Puddoc, Hague, (ed.) Interactions between sediments and fresh water.
- 4) Bird, F.L., P.I. Boon and P.D. Nichols. 2000. Physicochemical and microbial properties of burrows of the deposit-feeding Thalassinidean ghost shrimp *Biffarius arenosus* (Decapoda: Callinassidae). *Estuar. Coast. Shelf Sci.* **51**: 279–291.
- 5) Bloesch, J. 1982. Inshore-offshore sedimentation differences resulting from resuspension in the eastern basin of Lake Erie. *Can. J. Fish. Aquat. Sci.* **39**: 748–759.
- 6) Boetius, A., K. Ravensschlag, C.J. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann, B.B. Jørgensen, U. Witte and O. Pfannkuche. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* **407**: 623–626.
- 7) Branch, G.M. and A. Pringle. 1987. The impact of the sand prawn *Callinassa krussi* STEBBING on sediment turnover and on bacteria, meiofauna, and benthic microflora. *J. Exp. Mar. Biol. Ecol.* **107**: 219–235.
- 8) Cadde, G.C. 2001. Sediment dynamics by bioturbating organisms. pp. 127–148. In K. Reise (ed.), Ecological comparisons of sedimentary shores, Ecological studies, Vol. 151, Springer-Verlag, Berlin Heidelberg.
- 9) Chapela, I.H., S.A. Rehner, T.R. Schultz and U.G. Mueller. 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* (Wash. D.C.) **266**: 1691–1695.
- 10) Christensen, J.P. 1983. Electron transport system activity and oxygen consumption in marine sediments. *Deep Sea Res.* **30(2A)**: 183–194.
- 11) Cullen, D.J. 1973. Bioturbation of superficial marine sediments by interstitial meiobenthos. *Nature* **242**: 323–324.
- 12) Dalsgaard, T. and B. Thamdrup. 2002. Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. *Appl. Environ. Microbiol.* **68**: 3802–3808.
- 13) Dalsgaard, T., B. Thamdrup and D.E. Canfield. 2005. Anaerobic ammonium oxidation (anammox) in the marine environment. *Res. Microbiol.* **156**: 457–464.
- 14) Darwin, C. 1837. On the formation of mound. *Trans. Geol. Soc. Lond.* **5**: 505–509.
- 15) Darwin, C. 1881. The formation of vegetable mound through the action of worms with observations on their habits. *IndyPublish.com. USA.* 140 p.p.
- 16) Davison, C. 1891. On the amount of sand brought up by lobworms to the surface. *Geol. Mag.* **8**: 489–493.
- 17) DeFlaun, M.F. and L.M. Mayer. 1983. Relationship between bacterial and grain surfaces in intertidal sediments. *Limnol. Oceanogr.* **28**: 873–881.
- 18) Dobbs, F.C. and J.B. Guckert. 1988. Microbial food resources of the macrofaunal-deposit feeder *Ptychodera bahamensis* (Hemichordata: Enteropeustea). *Mar. Ecol. Prog. Ser.* **45**: 127–136.
- 19) Dollhopf, S.L., J.-H. Hyun, A.C. Smith, H.J. Adams, S. O'Brien and J.E. Kostka. 2005. Quantification of ammonia-oxidizing bacteria and factors controlling nitrification in salt marsh sediments. *Appl. Environ. Microbiol.* **71**: 240–246.
- 20) Duchene, J.C., P. Imboud and D. Delille. 1988. Associated bacterial microflora of a subantarctic polychaete worm *Thelepus setosus*. *Arch Hydrobiol.* **112**: 221–231.
- 21) Dwarschak, P.C. 1983. The biology of *Upogebia pusilla* (Peta-gna) (Decapoda, Thalassinidea) I. The burrows. *Pubbl. Stn. Zool. Napoli Mar. Ecol.* **4**: 19–43.
- 22) Edwards, R.W. 1958. The effect of larvae of *Chironomus riparius* MEIGEN on the redox potentials of settled activated sludge. *Ann. Appl. Biol.* **46**: 457–464.
- 23) Emerson, S., C. Stump, B. Johnson and D.M. Karl. 2002. In situ determination of oxygen and nitrogen dynamics in the upper ocean. *Deep-Sea Res.* **49**: 941–952.
- 24) Fisher, W.K. and G.E. MacGinitie. 1928. The natural history of an echiuroid worm. *Annals Mag. Nat. Hist.* **(10) 1**: 204–213.
- 25) Flint, R.W. and R.D. Kalke. 1986. Biological enhancement of estuarine benthic community structure. *Mar. Ecol. Prog. Ser.* **31**: 23–33.
- 26) Furuya, K. and K. Harada. 1995. An automated precise Winkler titration for determining dissolved oxygen on board ship. *J. Oceanogr.* **51**: 375–383.
- 27) Graf, G. and W.B. Kiel. 1984. Heat production, activity of the electron-transport-system (ETS), the ratio heat production/ETS-activity, and ATP-turnover as useful tools in benthic ecological field studies. *Arch. Hydrobiol. Beih.* **19**: 249–256.
- 28) Grossmann, S. and W. Reichardt. 1991. Impact of *Arenicola marina* on bacteria in intertidal sediments. *Mar. Ecol. Prog. Ser.* **77**: 85–93.

- 29) Henriksen, K., J.I. Hansen and T.H. Blackburn. 1980. The influence of benthic infauna on exchange rates of inorganic nitrogen between sediment and water. *Ophelia Suppl.* **1**: 249–256.
- 30) Hylleberg, J. 1975. Selective feeding by *Abarenicola pacifica* with notes on *Abarenicola vagabunda* and a concept of gardening in lugworms. *Ophelia* **14**: 113–137.
- 31) Hylleberg, J. and K. Henriksen. 1980. The central role of bioturbation in sediment mineralization and element re-cycling. *Ophelia, Suppl.* **1**: 1–16.
- 32) Itani, G. 2003. Symbiotic relationship in burrows. In pp. 233–253, A. Crustacea Asakura, (ed.), Tokai Univ. Press, Tokyo. (in Japanese)
- 33) Jørgensen, B.B. 1977. Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. *Mar. Biol.* **41**: 7–17.
- 34) Kikuchi, E. 1987. Effects of the brackish deposit-feeding polychaetes *Notomastus* sp. (Capitellidae) and *Neanthes japonica* (Izuka) (Nereidae) on sedimentary O₂ consumption and CO₂ production rates. *J. Exp. Mar. Biol. Ecol.* **114**: 15–25.
- 35) Kikuchi, E. and Y. Kurihara. 1977. In vitro studies on the effects of tubificids on the biological, chemical and physical characteristics of submerged ricefield soil and overlying water. *Oikos* **29**: 348–356.
- 36) Kikuchi, E. and Y. Kurihara. 1982. The effects of the oligochaete *Branchiura sowerbyi* BEDDARD (Tubificidae) on the biological and chemical characteristics of overlying water and soil in a submerged ricefield soil system. *Hydrobiologia* **97**: 203–208.
- 37) King, G.M. 1986. Inhibition of microbial activity in marine sediments by a bromophenol from a hemichordate. *Nature* **323**: 257–259.
- 38) Kinoshita, K., M. Wada, K. Kogure and T. Furota. 2003. Mud shrimp burrow as dynamic traps and processors of tidal-flat materials. *Mar. Ecol. Prog. Ser.* **247**: 159–164.
- 39) Koike, I. and H. Mukai. 1983. Oxygen and inorganic nitrogen contents and fluxes in burrows of the shrimps *Callinassa japonica* and *Upogebia major*. *Mar. Ecol. Prog. Ser.* **12**: 185–190.
- 40) Kojima, S., I. Hayashi, D. Kim, A. Iijima and T. Furota. 2004. Phylogeography of an intertidal direct-developing gastropod, *Battillaria cumingi*, around the Japanese Islands. *Mar. Ecol. Prog. Ser.* **276**: 161–172.
- 41) Kristensen, E. 1988. Benthic fauna and biogeochemical processes in marine sediments: microbial activities and fluxes. pp. 275–299. In Nitrogen cycling in coastal marine environments. T.H. Blackburn and J. Sorensen (ed.), John Wiley and Sons Ltd, New York.
- 42) Kristensen, E. 1985. Oxygen and inorganic nitrogen exchange in a *Nereis virens* (Polychaeta) bioturbated sediment-water system. *J. Coastal Res.* **1**: 109–116.
- 43) Kristensen, E. and T.H. Blackburn. 1987. The fate of organic carbon and nitrogen in experimental marine sediment systems: Influence of bioturbation and anoxia. *J. Mar. Res.* **45**: 231–257.
- 44) Kuypers, M.M., G. Lavik, D. Woebken, M. Schmid, B.M. Fuchs, R. Amann, B.B. Jørgensen and M.S. Jetten. 2005. Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. *Proc. Natl. Acad. Sci. USA* **102**: 6478–6483.
- 45) Kuypers, M.M., A.O. Sliemers, G. Lavik, M. Schmid, B.B. Jørgensen, J.G. Kuenen, J.S.S. Damsté, M. Strous and M.S. Jetten. 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. *Nature* **422**: 608–611.
- 46) Lovley, D.R. 2002. Dissimilatory metal reduction: from early life to bioremediation. *ASM News* **68**: 231–237.
- 47) LaFerla, R., M. Azzaro and G. Chiodo. 1996. Microbial activity in the euphotic zone of the Mediterranean Sea. *Microbiologia* **19**: 243–249.
- 48) Martens, C.S. 1976. Control of methane sediment-water bubble transport by macrofaunal irrigation in Cape Lookout Bight, North Carolina. *Science* **192**: 998–999.
- 49) Matsui, G.Y., D.B. Ringelberg and C.R. Lovell. 2004. Sulfate-reducing bacteria in tubes constructed by the marine infaunal polychaete *Diopatra cuprea*. *Appl. Environ. Microbiol.* **70**: 240–246.
- 50) Mchenery, J.G. and T.H. Birkbeck. 1982. Characterization of the lysozyme of *Mytilus edulis*. *Comp. Biochem. Physiol.* **71B**: 583–589.
- 51) Mermillod-Blondin, F., S. Marie, G. Desrosiers, B. Long, L. de Montety, E. Michaud and G. Stora. 2003. Assessment of the spatial variability of intertidal benthic communities by axial tomodensitometry: importance of fine-scale heterogeneity. *J. Exp. Mar. Biol. Ecol.* **287**: 193–208.
- 52) Miller, C.B. 2004. *Biological Oceanography*. Blackwell, Carlton, Australia. 402 p.p.
- 53) Myers, A.C. 1977. Sediment processing in a marine subtidal sandy bottom community: II, Biological consequences. *J. Mar. Res.* **35**: 633–647.
- 54) Nauhaus K., T. Treudel, A. Boetius and M. Kruger. 2005. Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I and ANME-II communities. *Environ. Microbiol.* **7**: 98–106.
- 55) Nickell, L.A. and R.J.A. Atkinson. 1995. Functional morphology of burrows and trophic modes of three thalassinidean shrimp species, and a new approach to the classification of thalassinidean burrow morphology. *Mar. Ecol. Prog. Ser.* **128**: 181–197.
- 56) Nybakken, J.W. 2001. *Marine Biology* 5th ed. Benjamin Cummings. San Francisco, USA. 516 pp.
- 57) Olanczuk-Neyman, K.M. and J.H. Bosjan. 1977. Measuring respiratory electron-transport-system activity in marine sediment. *Neth. J. Sea Res.* **11**: 1–13.
- 58) Packard, T.T. 1985. Measurement of electron transport activity of microplankton. pp. 207–261. In H.W. Jannasch and P.J.L. Williams (ed.), *Advances. aquatic microbiology*, Vol 3. Academic Press, London.
- 59) Pamatmat, M.M. and H.R. Skjoldal. 1974. Dehydrogenase activity and adenosine triphosphate concentration of marine sediments in Lindaspollene, Norway. *Sarsia* **56**: 1–11.
- 60) Plante, C.J., P.A. Jumars and J.A. Baross. 1990. Digestive associations between marine detritivores and bacteria. *Annu. Rev. Ecol. Syst.* **21**: 93–127.
- 61) Plante, C.J. and L.M. Mayer. 1994. Distribution and efficiency of bacteriolysis in the gut of *Arenicola marina* and three additional deposit feeders. *Mar. Ecol. Prog. Ser.* **109**: 183–194.
- 62) Plante, C.J. and L.M. Mayer. 1996. Seasonally variability in the bacteriolytic capacity of the deposit feeder *Arenicola marina*: environmental correlates. *Aquat. Microb. Ecol.* **11**: 1–109.
- 63) Probert, P.K. 1984. Disturbance sediment stability and trophic structure of soft-bottom communities. *J. Mar. Res.* **42**: 893–921.
- 64) Reidenauer, J.A. and D. Thistle. 1981. Response of a soft-bottom harpacticoid community to stingray (*Dasyatis sabina*) disturbance. *Mar. Biol.* **65**: 261–267.
- 65) Rhoads, D.C. and L.F. Boyer. 1982. The effects of marine benthos on physical properties of sediments: A successional perspective. pp. 3–52. In P.L. McCall and M.J.S. Trevesz (ed.), *Animal-sediment relations*, Plenum.

- 66) Richter, R. 1952. Fluidal-textur in sediment-gesteinen und ber sedifluktion überhaupt. Notizbl Hess L-Amt Bodenforsch **6**: 67–81.
- 67) Rodriguez, G.G., D. Phipps, K. Ishiguro and H.F. Ridgway. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. Appl Environ Microbiol. **58**: 1801–1808.
- 68) Savidge, W.B. and G.L. Taghon. 1988. Passive and active components of colonization following two types of disturbance on an intertidal sandflat. J. Exp. Mar. Biol. Ecol. **115**: 137–155.
- 69) Shimoda, K. and A. Tamaki. 2004. Burrow morphology of the ghost shrimp *Nihonotrypaea petalura* (Decapoda: Thalassinidea: Callianassidae) from western Kyushu, Japan. Mar. Biol. **144**: 723–734.
- 70) Sorensen, J. 1978. Occurrence of nitric and nitrous oxides in a coastal marine sediment. Appl. Environ. Microbiol. **36**: 809–813.
- 71) Strous M., J.A. Fuerst, E.H. Kramer, S. Logemann, G. Muyzer, K.T. van de Pas-Schoonen, R. Webb, J.G. Kuenen and M.S. Jetten. 1999. Missing lithotroph identified as new planctomycete. Nature **400**: 446–449.
- 72) Suchanek, T.H. 1983. Control of seagrass communities and sediment distribution by *Callianassa* (Crustacea, Thalassinidea) bioturbation. J. Mar. Res. **41**: 281–298.
- 73) Tamaki, A. 1995. The alteration of habitat by the ghost shrimps and its influence on benthic communities. pp. 129–171. In Y. Takekado, I. Tanida, A. Tamaki, H. Mukai and Z. Kawabata (ed.), Ecology of habitat. Ecology of Symbiosis-Ser7. Heibonsha, Tokyo. (in Japanese)
- 74) Tamaki, A. and K. Suzukawa. 1991. Co-occurrence of the cirrulanid isopod *Eurydice nipponica* BRUCE & JONES and the ghost shrimp *Callianassa japonica* ORTMANN on an intertidal sand flat. Ecol. Res. **6**: 87–100.
- 75) Tjetjen, J.H. 1979. Microbial-meiofaunal inter-relationships: A review. pp. 130–140. In R.R. Colwel and J. Foster (ed.), Aquatic Microbial Ecology, Univ. Maryland, College Park.
- 76) Trueman, E.R. 1968. Burrowing habit and the early evolution of body cavities. Nature **218**: 96–98.
- 77) Tsutsumi, H., S. Wainright, S. Montani, M. Saga, S. Ichihara and K. Kogure. 2001. Exploitation of a chemosynthetic food resource by a polychaete, *Capitella* sp. I. Mar. Ecol. Prog. Ser. **216**: 119–127.
- 78) Wackett, L.P., A.G. Dodge and L.B. Ellis. 2004. Microbial genomics and the periodic table. Appl. Environ. Microbiol. **70**: 647–655.
- 79) Wares, J.P. and C.W. Cunningham. 2001. Impacts of macrobenthic bioturbation in marine sediment on bacterial metabolic activity. Evolution **55**: 2455–2469.
- 80) Weiser, W. and M. Zech. 1976. Dehydrogenases as tools in the study of marine sediments. Mar. Biol. **36**: 113–122.
- 81) Weitkamp, L.A., R.C. Wissmar, C.A. Simenstad, K.L. Fresh and J.G. Odell. 1992. Gray whale foraging on ghost shrimp (*Callinassa californiensis*) in littoral sand flats of Puget Sound, USA. Can. J. Zool. **70**: 2275–2280.
- 82) Winkler, L.W. 1888. Die Bestimmung des im Wasser gelosten Sauerstoffes. Ber. Dtsche. Chem. Ges. **21**: 2843–2855.
- 83) Wu, S.S., H. Tsutsumi, K. Kita-Tsukamoto, K. Kogure, K. Ohawada and M. Wada. 2003. Visualization of the respiring bacteria in sediments inhabited by *Capitella* sp. I. Fish. Sci. **69**: 170–175.
- 84) Young, D.K. and D.C. Rhoads. 1971. Animal-sediment relations in Cape Cod Bay, Massachusetts. I. Transect study. Mar. Biol. **11**: 242–254.