

—Original Paper—

Microbial metabolism inferred from chemical and isotopic compositions of pore water around bananas discovered on the deep-sea floor in the Tenryu Submarine Canyon

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We describe in detail the pore water chemistry in the sediments below a white mat around a bunch of bananas at a water depth of 2,200 m in the Tenryu Submarine Canyon. We infer the metabolism of microbes in the sediments around the bananas based on the chemical and isotopic compositions of the pore water. On the basis of the relation between ammonia and total carbonate (ΣCO_2) concentrations in the pore water, we identified that an excess ΣCO_2 was distributed around the bananas that cannot be explained by the decomposition of organic matter derived from marine organisms, indicating that the bananas decomposed to generate the excess ΣCO_2 . We conclude that the bananas built a local organic-rich environment, stimulating the activity of organotrophic bacteria.

Keywords : deep-sea floor, banana, pore water, chemical composition, microbial metabolism

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1. Introduction

Organic matter produced at the surface of the ocean is primarily decomposed prior to arriving on the deep-sea floors, and biomasses at this deep level are extremely low relative to that of the surface ocean (Gage and Tyler, 1991); however, in hydrothermal systems and cold seep areas, tremendous high-density biomasses have been discovered (Lonsdale, 1977; Suess et al., 1985). These biological communities comprise ecosystems furnished by chemosynthetic microbes utilizing hydrogen sulfide or hydrocarbons in hydrothermal or cold seeping fluid, respectively, as their energy sources (Sibuet and Olu, 1998; Tunnicliffe et al., 1998; Van Dover, 2000). Moreover, the whale fall or cargo of shipwrecked vessels have been reported as energy sources of benthic communities on the deep-sea floor (Smith and Baco, 2003; Dando et al., 1992). Metabolisms of these benthic communities have been studied very well (e.g., Smith et al., 2015), but interstitial water around these communities have rarely been studied.

Vertical profiles of chemical components in interstitial water provide us various information; fluid migration including methane-hydrate chronicle from conservative components (e.g., Toki et al., 2017), metabolism including microbially-unknown processes

from biochemical components (e.g., Martens and Berner, 1974), or formation process of authigenic and foraminiferal carbonates from various isotopic compositions (Gieskes et al., 2005; Gieskes et al., 2011). In this present study, we sampled the pore water around a bunch of bananas discovered during a dive survey in the deep ocean, clarifying a peculiar microbial metabolism around the bananas *via* geochemical interpretation.

2. Geological settings

The Tenryu Submarine Canyon is part of the great erosional valley on the seafloor located in the eastern Nankai Trough (Fig. 1b). The Nankai Trough is a plate boundary between the Philippine Sea Plate and the Eurasia Plate (Fig. 1a), and an accretionary prism has been forming on the landward plate by accreted sediments scraped off from the subducting Philippine Sea Plate (Ranken et al., 1984; Seno, 1977). The Tenryu Submarine Canyon cuts deeply across the Nankai accretionary prism, and the ongoing accretionary processes of the accretionary prism can be described by direct observations during submersible surveys along the walls of the Tenryu canyon in exact detail (Kawamura et al., 2009). Three major faults, the Kodaiba Fault, Tokai

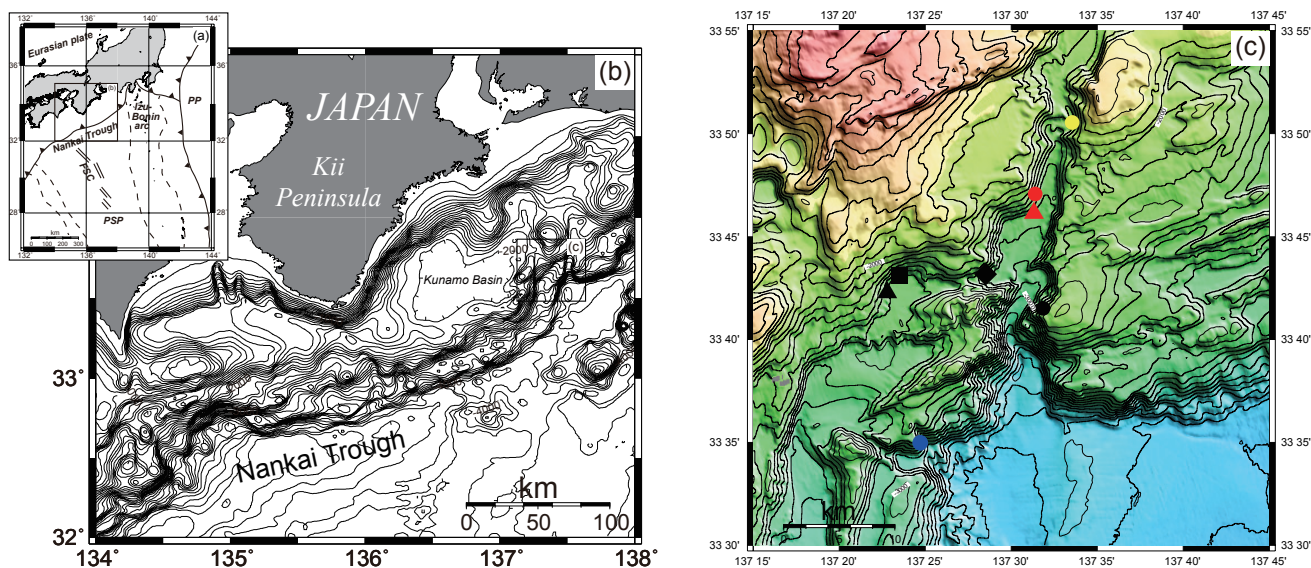


Fig. 1. (a) Schematic map showing the plate motion around Japan Islands. A square indicates the range of Fig. 1b. (b) Bathymetric map showing the eastern Nankai Trough. A square indicates the range of Fig. 1c. (c) Close up of the Tenryu Submarine Canyon showing sampling sites during the YK05-08 cruise. Red plots indicate the locations of sites where bacterial mat were found on the seafloor. A blue one indicates the location of a site where a *Calyptogena* colony was found. Black ones indicate those where nothing special was found on the seafloor. A yellow one indicates where a bunch of bananas was found on the seafloor.

Thrust, and Tenryu Frontal Thrust, are all active, and several chemosynthesis-based benthic communities have been discovered along the faults (Kawamura et al., 1998; Le Pichon et al., 1987; Sibuet et al., 1988).

3. Samples

For our samples, we collected MBARI-type push cores (<http://www.mbari.org/push-cores/>; length = 35 cm) from the surface of the seafloor in the Tenryu Submarine Canyon during the YK05-08 cruise of the *R/V Yokosuka* (JAMSTEC) between June 20, 2005 and July 08, 2005. Sampling sites are shown in Table 1 and Fig. 1c. Detailed tracks and photos have been reported in Kawamura et al. (2009). We conducted 6 dives, and sampled 8 cores during the cruise (Table 1); 2 cores from bacterial mats, 1 core from *Calyptogena* colony, and 4 cores from the normal seafloor for a reference. During dive 892, we found a white patch on the seafloor in the Tenryu Canyon (Fig. 2a), and sampled the surface sediment by a push core from the patch (Fig. 2b). Subsequently, we retrieved a bunch of bananas from the patch (Figs. 2c and 2d). The sediment sample with a length of 14.5 cm was obtained from around the patch, of which 5 cm of the surface above comprised black mud including a fragment of a banana, which had a strong odor like hydrogen sulfide (H₂S). As shown in Fig. 3, below 5 cm, we found light gray clay without any odor.

4. Analytical methods

We squeezed the sediment in a 60-cm³ syringe using a C-clamp to extract the pore water into a 10-cm³ syringe as quickly after recovery as possible (Manheim, 1968). Subsequently, the pore water was distributed into two bottles for water and gas analyses. Using aliquot for water analysis, we measured both the ammonia (= NH₃ + NH₄⁺) concentration and pH/alkalinity using standard shipboard techniques and procedures (Gamo and Gieskes, 1992; Gieskes et al., 1991). When these samples were brought back to our onshore laboratory at Hokkaido University, we measured the concentrations of chloride (Cl⁻) and sulfate (SO₄²⁻) ions using ion chromatography (Tsunogai and Wakita, 1995). Further, the concentration and carbon isotope ratio (¹³C/¹²C) of CH₄ were determined using an

irm-GC/MS system (HP6890 and Finnigan MAT252) with an open-split interface (Finnigan Combustion III with some modifications) (Tsunogai et al., 2002). Next, we calculated the concentration of ΣCO₂ (= H₂CO₃ + HCO₃⁻ + CO₃²⁻) using these pH and alkalinity values. Finally, we determined the ¹³C/¹²C value of ΣCO₂ using the irm-GC/MS system (Ijiri et al., 2003). The ¹³C/¹²C value was calibrated to the Vienna Pee Dee Belemnite (VPDB) standard and converted into conventional delta notation (δ¹³C) using the standard correction procedure given below (Coplen, 2011):

$$\delta^{13}\text{C} = ((^{13}\text{C}/^{12}\text{C})_{\text{sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{VPDB}}) - 1 \quad (1)$$

The analytical precisions are shown in Table 1.

5. Results

From our analyses, we found that Cl⁻ concentrations showed a value that is equivalent to that of overlying seawater (Fig. 4a). Further, SO₄²⁻ remarkably declined to zero at 15 cm below the seafloor (cmbsf) at the bacterial mat site, and sulfate was a little lower than that of overlying seawater at 1.5 cmbsf at the banana site; however, at the other sites, SO₄²⁻ showed values equivalent to that of overlying seawater (Fig. 4b). Further, as shown in Fig. 4d, the concentrations of ΣCO₂ were high at the surface of the banana site and the deep layer of the bacterial mat site. Similarly, CH₄ concentrations were high at the bacterial mat site and the *Calyptogena* site (Fig. 4e). The δ¹³C values of ΣCO₂ (δ¹³C_{CO2}) decreased to -50‰ at 15 cmbsf at the bacterial mat site and again increased below 15 cmbsf (Fig. 4f). In addition, the δ¹³C_{CO2} values decreased to -20‰ at the *Calyptogena* site and decreased to approximately -15‰ at the surface of the banana site. Finally, the δ¹³C_{CH4} values declined to -80‰ at the bacterial mat; however, they nearly stayed constant between -60‰ and -40‰ at the other sites (Fig. 4g). Ammonia increased gradually with depth in all cores (Fig. 4c).

6. Discussion

In Fig. 5, we plotted the relation between ammonia and ΣCO₂ concentrations in the pore water for each site in this study. When organic matter derived from marine

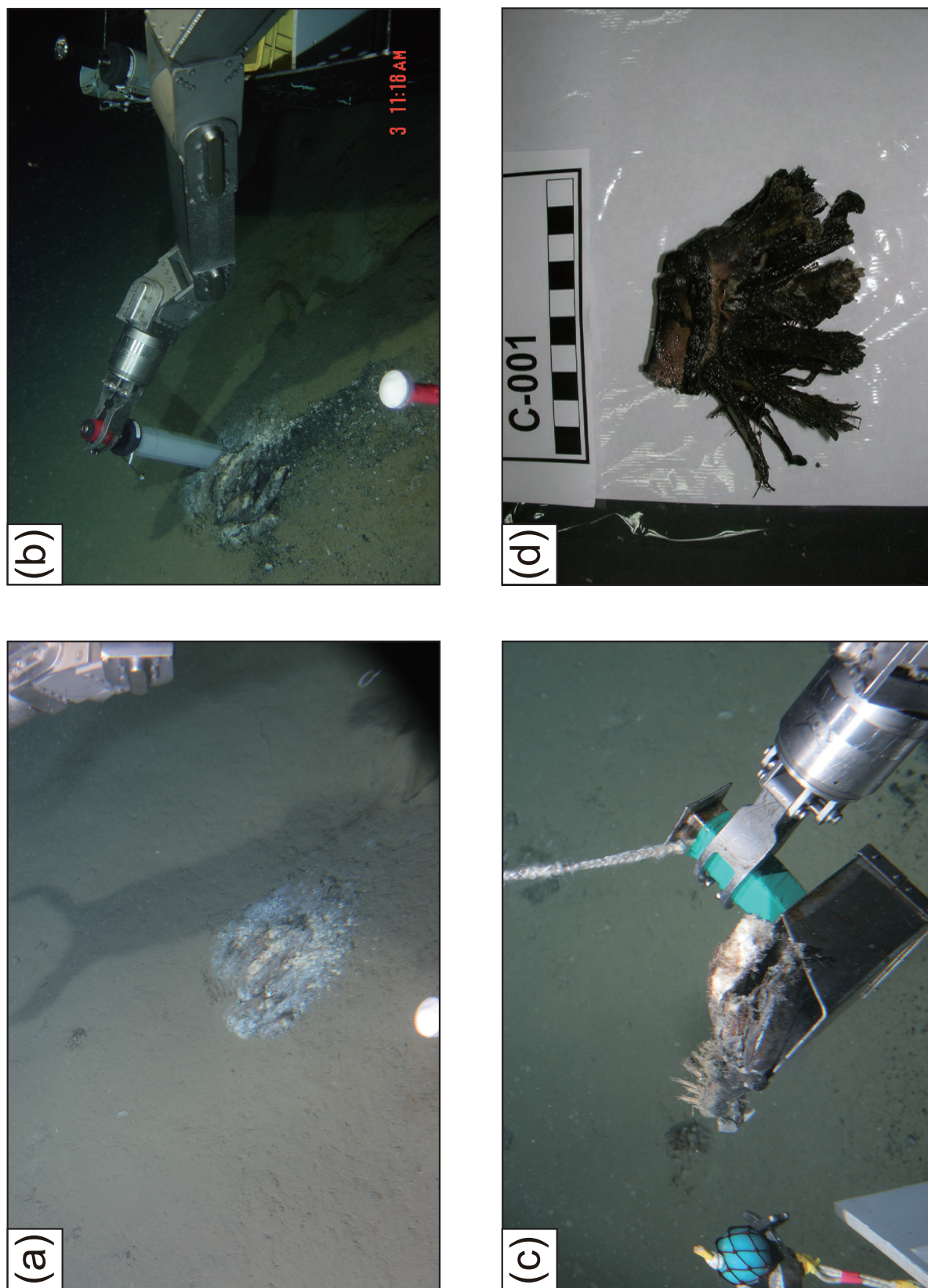
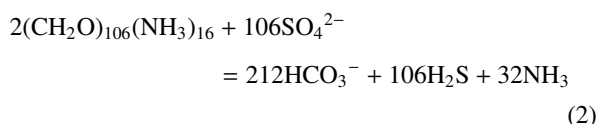


Fig. 2. (a) A picture taken from the submersible for white sediment around the bananas found in the Tenryu Canyon. (b) A picture for sampling the white sediment using an MBARI-type push corer. (c) A picture for sampling the bananas using a scoop as a payload of a manned submersible *Shinkai6500*. (d) A photo for a spoiled peduncle of the bananas recovered from the deep-sea floor in the Tenryu Canyon. A tick of the scale located in the upper part of the peduncle indicates 1 cm.

organisms is decomposed in the sediment, it reacted according to the equation given below, generating ΣCO_2 and ammonia at a ratio of 106:16, i.e., the Redfield ratio (Redfield, 1934):



The Redfield ratio was based on organic matter in seawater (Redfield, 1934), but in interstitial water, ammonia adsorbs to a surface of sediment particles (Rosenfeld, 1979), or a ratio of carbon to nitrogen (C/N ratio) of sediment shows regionally variation (e.g., Sampei and Matsumoto, 2001). Considering these circumstances, we showed the normal data as a reference by small crosses in Fig. 5. These data are unpublished data obtained during YK02-02, YK03-03,

YK05-08, YK06-03, and YK10-09 cruises. The analytical methods and the sampling procedures are the same as those of this study, and the sampling points have already been reported (Toki et al., 2014; Toki et al., 2004). The “normal data” mean chemical compositions of interstitial water from sediments of the seafloor without bacterial mats, tube worms, *Calyptogen* colony, or carbonate chimney.

Considering that the ΣCO_2 concentration in overlying seawater was approximately 2.4 mmol/L and ammonia was less than 100 $\mu\text{mol/L}$ (Table 1), we draw a regression line through those of seawater as a dashed line in Fig. 5. The slope is 6.3, which is slightly smaller than the Redfield ratio ($106/16 = 6.625$), but the deviation of 4.5% is corresponding to anything more than the analytical errors (Table 1). Hereafter, we recognize the regression line as the standard, and discuss the ratio of ΣCO_2 concentration to ammonia concentration in the other sites. The ratios in

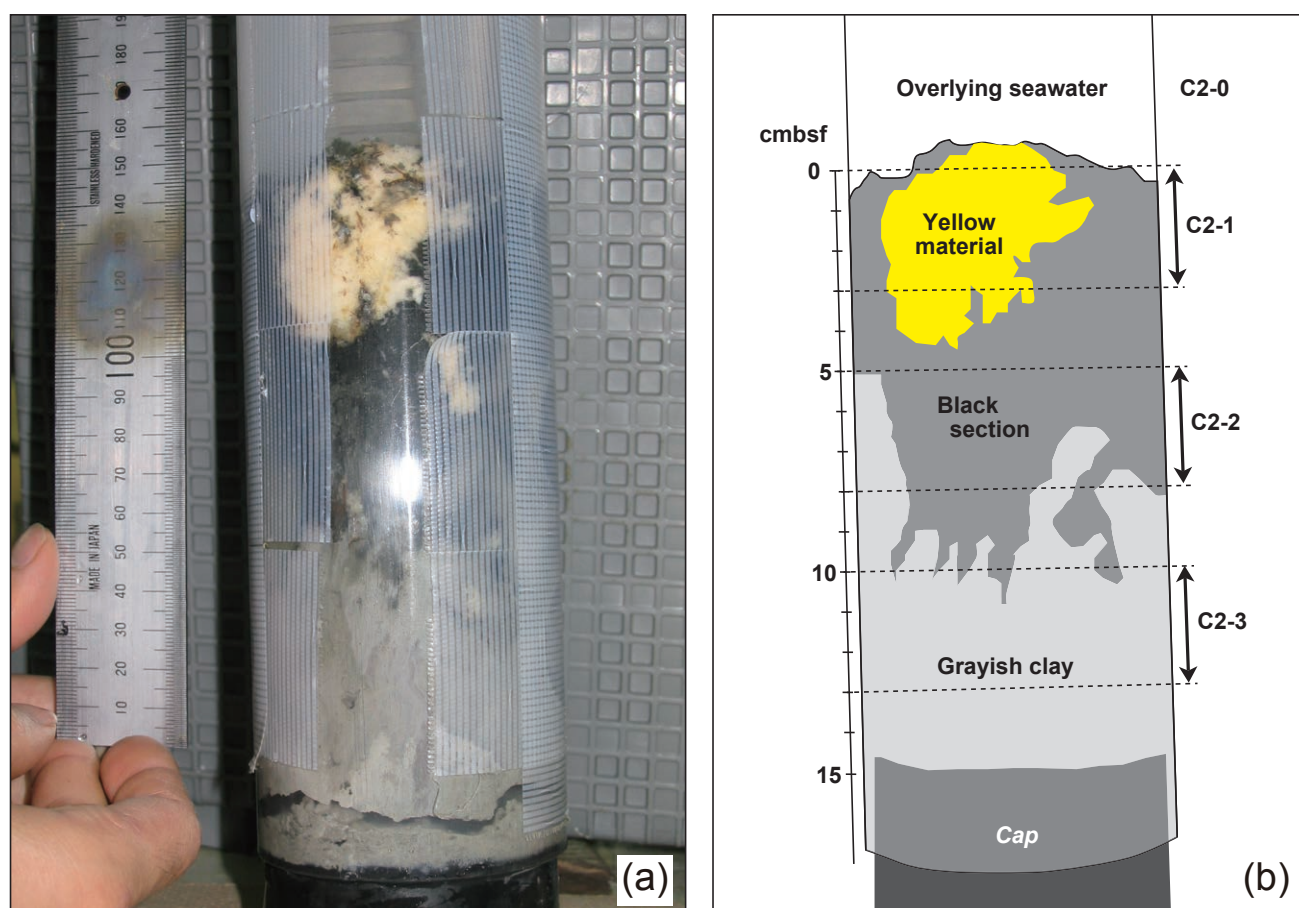


Fig. 3. (a) A photo from a lateral view of sediment sample cored from the deep-sea floor around the bananas in the Tenryu Canyon. (b) A description about the cored sediment shown in Fig. 3a; there is yellow material above ~4.5 cm from the top, followed by a black section above ~10.5 cm which is probably containing iron sulfides (Gieskes et al., 2005; Gieskes et al., 2011), below which the sediment becomes grayish in color (normal sediment). Total length is ~14.5 cm. Dashed lines indicate the sample intervals; 0–3 cm for C2-1 sample, 5–8 cm for C2-2 sample, and 10–13 cm for C2-3 sample, as listed in Table 1. The depths of the samples in Table 1 correspond to averages of the intervals of the samples.

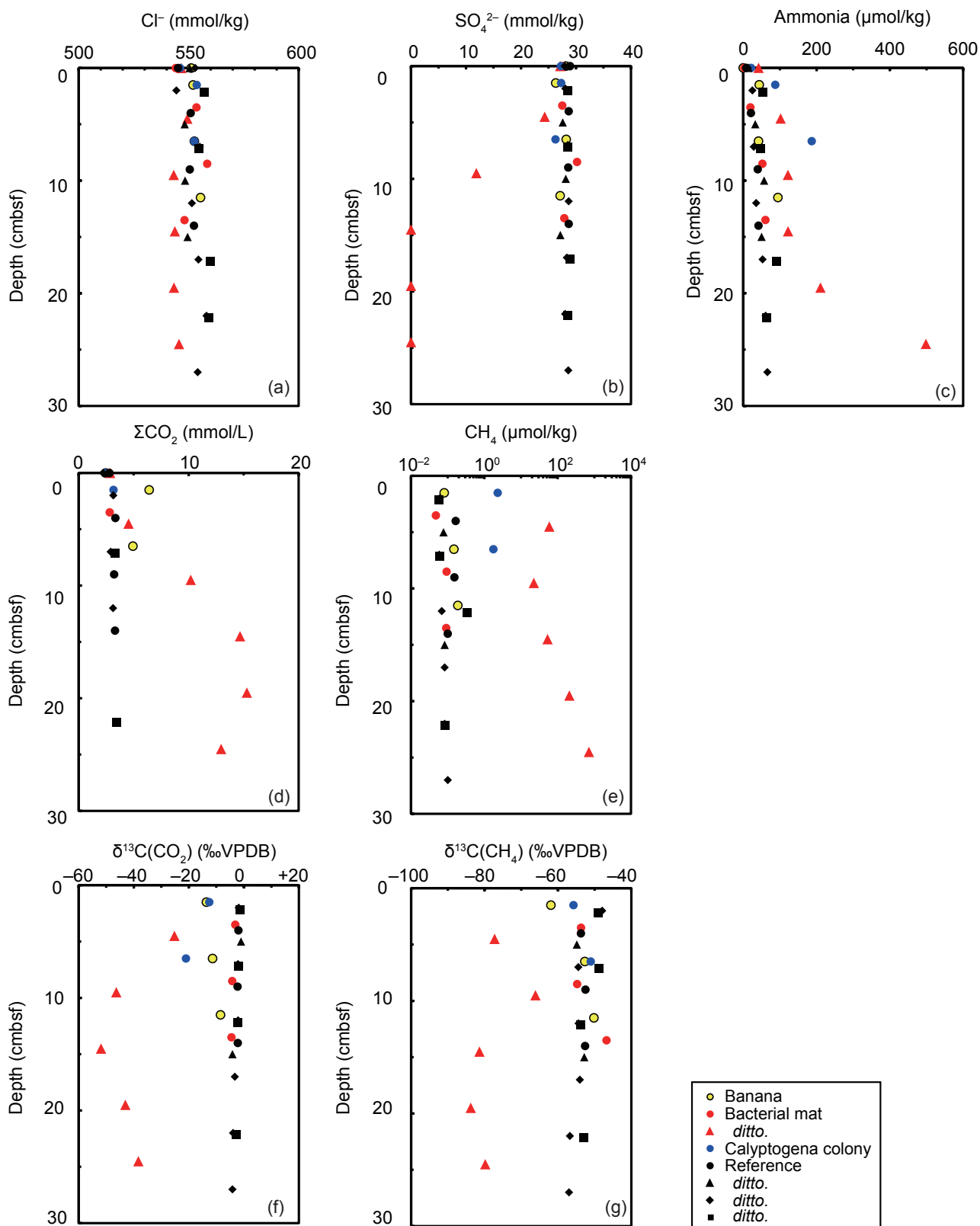


Fig. 4. Vertical profiles of chemical and isotopic compositions of the pore water. A yellow plot represents the data of chemical compositions in the interstitial water from the banana site; red ones from the bacterial mat sites, a blue one from the *Calyptogena* site, and black ones from the normal seafloor.

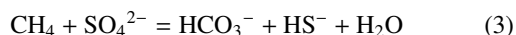
Table 1. List of water samples with analytical results, together with the precision for each analysis.

Date	Dive	Observer	Site	Latitude	Longitude	Depth m	Type	Sample No.	Depth cmbsf	pH	Alkalinity mmol/L	Ammonia $\mu\text{mol/L}$	Cl ⁻ mmol/kg	SO ₄ ²⁻ mmol/kg	CH ₄ mmol/kg	ΣCO ₂ mmol/L	δ ¹³ C(CH ₄) ‰VPDB	δ ¹³ C(CO ₂) ‰VPDB
										0.02	2%	8%	4%	7%	3%	0.3‰	0.3‰	
2005.6.23	885	K. Kawamura	Normal seafloor	33 41.7443	137 31.8594	2525	MBARI (Black)	C1	0	8.4	2.8	8.2	545	28.9	-	2.8	-	-
									1	4.0	3.1	20.9	551	28.7	0.16	3.3	-53.7	-2.0
									2	9.0	3.1	39.4	550	28.6	0.15	3.2	-52.5	-2.3
2005.6.24	886	S. Kawamura	Normal seafloor	33 42.5249	137 22.6863	2531	MBARI (Black)	C1	0	8.2	2.6	14.4	550	28.5	-	2.6	-	-
									1	5.0	-	32.8	548	27.6	0.08	-	-54.8	-1.1
									2	10.0	-	57.0	548	28.2	-	-	-	-
2005.6.25	887	Y. Dilek	Normal seafloor	33 43.4168	137 23.2218	2379	MBARI (Yellow)	C3	0	8.1	2.2	12.3	553	27.9	-	2.2	-	-
									1	2.0	3.1	25.0	544	28.1	0.06	3.1	-47.9	-1.8
									2	7.0	2.8	28.7	555	28.5	0.06	2.9	-54.4	-2.1
									3	12.0	3.0	35.3	551	28.7	0.07	3.1	-54.4	-2.1
									4	17.0	-	52.9	554	28.4	0.08	-	-54.0	-3.3
									5	22.0	-	61.1	558	28.0	0.08	-	-56.7	-3.9
2005.7.3	892	K. Kawamura	Banana	33 50.8677	137 33.6037	2194	MBARI (Red)	C2	0	7.7	2.4	N.D.	551	28.1	-	2.5	-	-
									1	1.5	6.0	43.5	552	26.3	0.08	6.4	-61.9	-13.6
									2	6.5	4.7	41.5	552	28.2	0.15	4.9	-52.7	-11.4
									3	11.5	-	94.4	555	27.1	0.19	-	-50.2	-8.5
									5	22.2	3.3	64.4	559	28.5	0.08	3.4	-53.0	-2.7
2005.7.5	893	R. Anma	Bacterial mat	33 46.5791	137 31.4786	2538	MBARI (black)	C2	0	8.1	2.7	3.1	544	27.6	-	2.8	-	-
									1	3.5	2.7	19.4	553	27.5	0.05	2.8	-53.7	-3.1
									2	8.5	-	52.0	558	30.2	0.09	-	-54.8	-4.3
2005.7.7	894	Y. Ogawa	Calyptigena	33 35.0135	137 24.6949	3058	MBARI (Red)	C3	0	8.2	2.8	41.8	547	27.1	-	2.9	-	-
									1	4.5	4.4	102	549	24.3	59.1	4.5	-77.3	-25.2
									2	9.5	9.9	122	543	22.3	22.3	10.2	-66.1	-46.3
									3	14.5	14.3	122	544	N.D.	53.0	14.7	-81.4	-51.9
									4	19.5	14.9	211	543	N.D.	209	15.3	-83.7	-43.0
5	24.5	12.6	498	546	N.D.	710	12.9	-79.8	-38.3									
2005.7.7	894	Y. Ogawa	Calyptigena	33 35.0135	137 24.6949	3058	MBARI (Red)	C3	0	8.2	2.5	21.3	546	27.3	-	2.5	-	-
									1	1.5	3.1	87.4	554	27.3	2.31	3.2	-55.8	-12.6
								2	6.5	7.9	186.6	552	26.3	1.76	-	-	-51.1	-21.0

-: No data

N.D.: Not detected

the pore water from the *Calyptogenia* and reference sites in this study are comparable to those of the standard ratio. However, at the banana and bacterial mat sites, a surplus ΣCO_2 is distributed to an extent beyond the degradation of organic materials by marine organisms. This surplus was also detected at cold seep areas (Kulm et al., 1986) due to anaerobic methane oxidation (AOM) from ΣCO_2 without ammonia generation, which is governed by the following equation:



At the bacterial mat site, the CH_4 concentration was considerably high and the SO_4^{2-} concentration significantly diminished (Figs. 4d and 4b), suggesting that high ΣCO_2 concentration detected at the bacterial mat site resulted from AOM. In addition, when AOM occurred, $\delta^{13}\text{C}_{\text{CO}_2}$ values as low as -30‰ were detected in the pore water (Martin et al., 1997; Suess and Whiticar, 1989). At the bacterial mat site, the $\delta^{13}\text{C}_{\text{CO}_2}$ values showed considerably low values (less than -50‰), as shown in Fig. 4f; however, at the banana site, such features were not observed. Instead, the ΣCO_2 concentration was higher (Fig. 4d) and the $\delta^{13}\text{C}_{\text{CO}_2}$ value was lower (Fig. 4f) than those of the reference sites, respectively, without a high-level CH_4 concentration. This suggests that high ΣCO_2 concentration with a relatively low

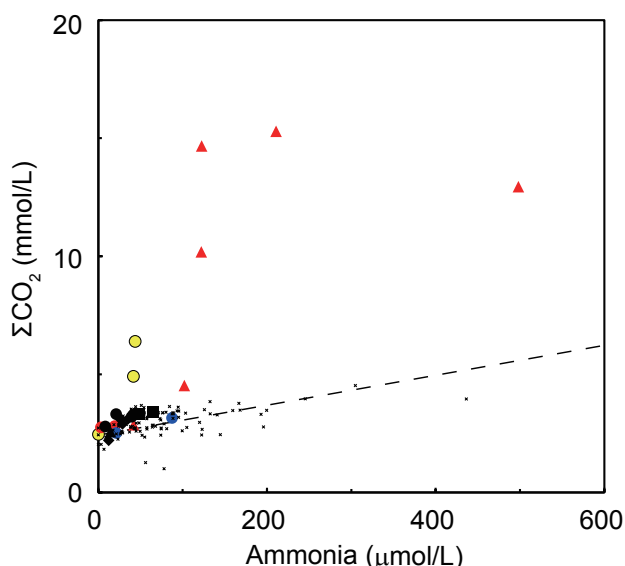
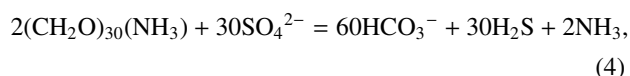


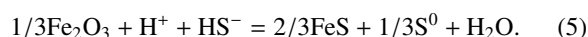
Fig. 5. Relation between ammonia and ΣCO_2 concentrations in the pore water. Symbols as in Fig. 4, together with the data from the normal seafloor in the Nankai Trough as small crosses (see the main text for more information).

$\delta^{13}\text{C}_{\text{CO}_2}$ value was not due to the presence of CH_4 , which needs a carbon source other than CH_4 at the banana site.

Given the above, we first argue where the carbon source is in the sediment column at the banana site. When we investigate the vertical profiles shown in Fig. 4, we can find a peak of ΣCO_2 concentration at the top of the sediment column with a relatively low $\delta^{13}\text{C}$ value, as shown in Figs. 4d and 4f, indicating that the carbon source at the banana site is located at the top of the column. In general, terrestrial plants have high C/N ratios and low $\delta^{13}\text{C}$ values relative to those of marine organisms (Fry and Sherr, 1984; Hedges et al., 1986; Parsons et al., 1961). Further, banana is one of the terrestrial plants with a C3 cycle and has a C/N ratio of approximately 30 and a $\delta^{13}\text{C}$ value of approximately -30‰ (Ilori et al., 2007; Tixier et al., 2013). Based on these facts, the degradation of the banana on the seafloor would cause surplus ΣCO_2 generation at the surface sediment of the banana site. In particular, the surface sediment after its recovery smelled like H_2S , suggesting that sulfate reduction occurred for generating H_2S at the surface sediment, which is expressed in the following equation:



where a banana represents $(\text{CH}_2\text{O})_{30}(\text{NH}_3)$, because it is one of the terrestrial plants having a C/N ratio of ~ 30 (Ilori et al., 2007). In addition, the generated H_2S could react with Fe ions to form a precipitate by the following stoichiometric formula (Gieskes et al., 2005; Gieskes et al., 2011):



The produced iron sulfide could be distributed as a black mud at 0–10.5 cmbsf (Fig. 3). Native sulfur is theoretically produced as a by-product, but we could not find it in the sampled sediments (Fig. 3). Indeed sulfate concentration slightly decreased at 1.5 cm (± 1.5 cm) below the seafloor (Fig. 4b), but the black mud stretched to deeper as shown in Fig. 3, which could be due to artificial “bioturbation”. The black mud was very fine and buoyant, while the underlying clay was relatively hard. As a coring had been conducted, the black mud possibly was dragged along the inner of the corer, so the black mud could seem to stretch to deeper. Thus, sulfate reduction would occur near the deeper part of the bananas (eq. 4), however, considering the bananas were placed on the seafloor (Fig. 2), the upper part of the bananas

would undergo decomposition by dissolved oxygen, aerobic oxidation. This is expressed in the following equation:



The ratio of ΣCO_2 to ammonia generated by the aerobic oxidation according to Eq.(6) is also stoichiometrically 30, explaining the excess ΣCO_2 relative to the marine organism degradation observed at the banana site.

Based on the above considerations, we inferred the redox reaction around the bananas (Fig. 6), where aerobic and anaerobic oxidation occurs in a locally organic-rich environment. This environment could be caused by the bananas sinking down to the deep-sea floor. Since bananas are a tropical plant and they don't grow wild around this area in Japan, possible route of the bananas could be (1) dropping down from a tree and carried on the Kuroshio Current, or (2) dropping from a cargo ship during shipping. The Kuroshio Current is one of the strongest current in the world flowing through the west side of the North Pacific Ocean, and it flows from the east coast of Philippines to northeast off Japan. In the former case, bananas dropped down from a tree could have been flowed on the current, and have sunk down at the site. The Kuroshio Current snakes the way day by day, and the pathway is reported every day on a website of Japan Meteorological Agency. Data of an axis of the Kuroshio can be seen on another website (<http://www.mirc.jha.or.jp/>), and the Kuroshio axis draft away from the Japan coast, and it flowed through far south from the banana site during the sampling campaign in

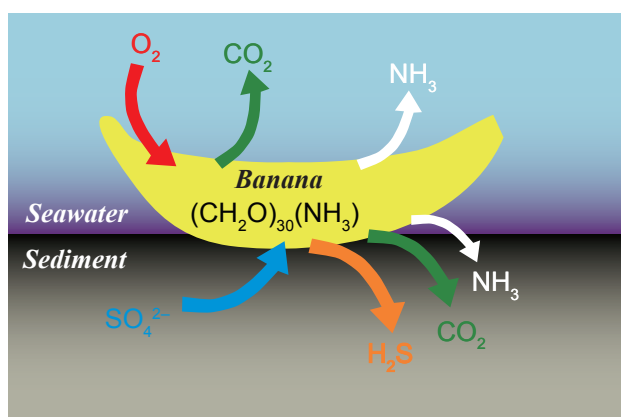


Fig. 6. Schematic showing microbial metabolism around the bananas in the Tenryu Canyon.

July 2005. The Kuroshio snake has initiated since August 2004. Therefore, if the bananas had been carried on the Kuroshio axis, they could have been dropped earlier than July 2004. But if the bananas were attributable to such a natural event, much more bananas should be widespread along the Kuroshio Current. We have never heard of dropped bananas on the deep-sea floor, and the phenomenon could be caused from an accident, that is, the second possible route. We can monitor the positions of sailing ships on a website (<http://www.marinetraffic.com/>), and some ships are sailing over the banana site based on the data. Probably, the bananas could be dropped down from one of these ships. To this point, this phenomenon was responsible for having an anthropogenic impact on the deep-sea floor ecosystem. For deep-sea floor bacteria intrinsically earning a sparse living by utilizing considerably little organic matter, the bananas could have been a “treat from heaven” and the bacteria could gather together around the bananas from the ambient seawater or pore water. The bacterial mat, however, could not flourish on a sustainable basis because the bananas had finite mass. The bacteria clustering around the bananas could be back to the seawater or pore water and live on little organic matter. Such an incidental phenomenon could not have a significant impact on the deep-sea floor ecosystem because the phenomenon has a low frequency of occurrence. Conversely, if this phenomenon had a high frequency of occurrence, it would create substantial contact between anthropogenic materials and deep-sea floor ecosystems; thus, anthropogenic materials having a substantial impact on the deep-sea floor ecosystem cannot be denied. We must continue to observe this impact without missing uncommon phenomenon to understand the influence of this phenomenon on deep-sea environments during seafloor exploration.

7. Conclusion

During the YK05-08 cruise, we collected a surface sediment around a bunch of bananas discovered in the Tenryu Canyon. The sediment was squeezed to extract the pore water just after recovery. Then, we measured ammonia, pH/alkalinity, Cl^- , SO_4^{2-} , CH_4 concentrations, and $\delta^{13}\text{C}$ of CH_4 and ΣCO_2 in the pore water. Based on the vertical profiles of chemical components in the pore water, CH_4 and ΣCO_2 increased, whereas SO_4^{2-} decreased in the deeper part of the sediment column at the bacterial mat site. In contrast,

considerably high ΣCO_2 concentration was detected at the surface of the column at the banana site. Based on the ratios of ΣCO_2 to ammonia, an excess of ΣCO_2 was detected that was unable to be explained by organic matter degradation from marine organisms at the banana and bacterial mat sites.

Further, as reported in cold seep areas, AOM could result in excess ΣCO_2 at the bacterial mat, where CH_4 was enriched, SO_4^{2-} diminished, and $\delta^{13}\text{C}_{\text{CO}_2}$ was lower than -50‰ in the deeper side of the column; these results support the AOM occurrence at the bacterial mat site. On the other hand, at the banana site, CH_4 was at a normal level for deep-sea environments, implying the input of a carbon source rather than methane oxidation. This carbon source would be located at the top of the sediment column based on the vertical profiles of chemical constituents in the pore water at the banana site. Bananas, one of the terrestrial plants, have higher C/N ratios than those of marine organic matter; thus bananas could be a possible carbon source during degradation. At the banana site, the bananas broke down to form ΣCO_2 , thereby leading to a high-concentration anomaly of ΣCO_2 at the surface of the banana site. Despite the strong H_2S odor and black sulfide precipitation at the surface sediment of the banana site, sulfate depletion was a little observed in the pore water, suggesting that the bananas broke down using sulfate and oxygen in the deep-sea water and the interstitial water in the sediment. Therefore, the bananas would extraordinarily enrich the organic matter in the deep-sea environment, building a highly condensed microbial community on the deep-sea floor.

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