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Comparative Analysis of Chemical and Microbial Profiles in Estuarine Sediments Sampled from Kanto and Tohoku Regions in Japan

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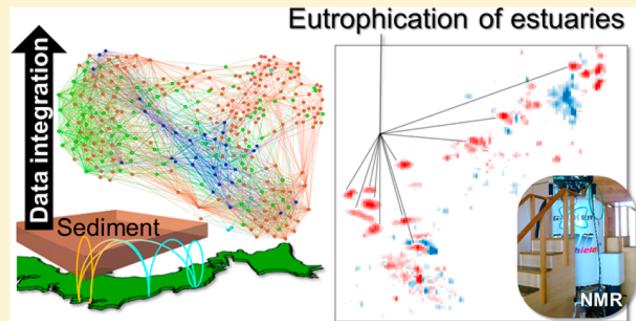
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Supporting Information

ABSTRACT: Estuarine environments accumulate large quantities of organic matter from land masses adjoining the sea, and this is consumed as part of the detritus cycle. These environments are rich in biodiversity, and their ecosystem services greatly benefit humans. However, the estuarine environments have complicated aqueous ecosystems, thus the comprehensive evaluation of biotic interactions and stability is difficult using conventional hypothesis-driven approaches. In this study, we describe the advancement of an evaluation strategy for characterizing and visualizing the interactions and relationships among the microorganisms and chemicals in sediment ecosystems of estuarine environments by a combination of organic matter and elemental profiling as well as microbial profiling. We also report our findings from a comparative analysis of estuarine and coastal environmental samples collected from the Kanto and Tsunami-affected Tohoku regions in Japan. The microbial-gated correlation deployed from the coefficient of microbiota from the correlation matrix and network analysis was able to visualize and summarize the different relationships among the microbial communities, sediment organic matter, and element profiles based on geographical differences in Kanto and Tohoku regions. We demonstrated remarkable estuarine eutrophication in the Kanto region based on abundant sediment polypeptide signals and water nitrogen ions catabolized by microbiota. Therefore, we propose that this data-driven approach is a powerful method for analyzing, visualizing, and evaluating complex metabolic dynamics and networks in sediment microbial ecosystems and can be applied to other environmental ecosystems, such as deep sea sediments and agronomic and forest soils.



Estuarine environments accumulate large quantities of organic matter from land masses, which is consumed as a part of the detritus cycle. Eutrophic estuarine environments are rich in biodiversity, and humanity greatly benefits from the ecosystem services they provide.¹ Such environments are formed in part by particulates suspended in river water entering the area and deposited on underlying sediments; these are distributed by tidal movements. Particulates include humic substances, which are formed by chemical and biological transformation of plant and animal matter and constitute 60%–75% of total soil organic matter.² Assessment of the biotic interactions and stability of aqueous ecosystems is difficult using conventional hypothesis-driven approaches because of the complicated nature of the systems involved.³ Novel data-driven approaches are, therefore, required to improve our ability to evaluate the conservation, regeneration, and exploitation of ecosystem services provided by estuarine environments.⁴

Organic matter present in sediments plays a major role in geochemical carbon cycles. It also affects atmospheric CO₂ and

O₂ levels in the oceans on a long timescale.⁵ In addition, the response of benthic communities to organic enrichment affects the aquaculture industry.⁶ The relationship between benthic biodiversity and deep-sea ecosystem efficiency, which reflects the ability of an ecosystem to exploit the available energy (food sources), has previously been investigated, with researchers reporting that sediment organic matter influences ecosystem functioning.⁷ Technological advances in the analytical methods used for evaluating the sediment organic matter include solid- and solution-state nuclear magnetic resonance (NMR) and Fourier transform cyclotron resonance mass spectrometry.⁷ These methods have been widely used to characterize humic substances in various environments, including soils and fresh and salt water sediments.⁸

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In addition, sediment organic matter acts as both a major bioavailable source and a sink in global biogeochemical cycles. The humic sediments contribute to the binding of metals by adsorption, complication, and chelation. At the same time, dissolved organic ligands, such as low-to-medium molecular weight carboxylic acids, amino acids, and fulvic acids, can form soluble metal complexes. Estuarine sediments are generally considered a sink for anthropogenic metals introduced into the environment. Several studies have also suggested that metals in sediments are available for detritus feeders and can, therefore, enter estuarine food webs. Microbial biofilms in intertidal systems also play an important role in binding metals and the transfer of these to the food web.⁹ Therefore, considering the interactions between organic chemicals, heavy metals, and microbial communities in estuarine environments is of great importance.

As would be expected, there is a strong relationship between the presence of organic matter and microbial flora of estuarine sediments. Microbial flora are present in a variety of environments, including soils, the oceans, and symbiotic ecosystems, and these microorganisms are responsible for driving the biogeochemical cycling of elements on Earth.¹⁰ For example, the biodegradation of aromatic compounds, which are the most prevalent and persistent environmental pollutants, by microorganisms is a major mechanism by which organic pollutants are removed from contaminated sites.¹¹ One of the challenges that microbial ecologists faced is the identification of microorganisms that perform specific metabolic processes in the natural environment.¹² The characteristics of organic matter present in estuarine sediments are determined by its original configuration and the geochemical environment of the sediment¹³ and by microbial inputs; therefore, methods for evaluating how the composition and structure of sediment organic and inorganic matter is related to the community structure and function of microorganisms are of great interest.

In this study, we describe a data-driven approach to evaluate estuarine environments that involves integrating sediment organic compositional and structural analyses data with mineral composition and microbial community analysis findings. In addition, we describe a comparative analysis of estuarine and coastal environments performed using this approach. To this end, NMR-based organic component analyses were combined with inductively coupled plasma-optical emission spectrometry (ICP-OES)-based ionomic and pyrosequencing-based microbial community analyses of estuarine and coastal environments in the Tohoku and Kanto regions of Japan.

The Kanto region is the most populated area in Japan, and its rivers, thus, contain greater amounts of polluted water. In contrast, the Tohoku region has a relatively cold, wet climate and a smaller population than the Kanto region. In 2011, the Great East Japan Earthquake triggered a massive tsunami that was accompanied by rolling of sediments and destruction of several ecosystems. A method that is able to evaluate estuarine and coastal environments, which are relatively stable, as well as those that have been recovered from such massive disasters would be of great benefit. This study describes a method for evaluating correlations among the structural and mineral components of sediment organic matter and microbial communities present in the same samples.

MATERIALS AND METHODS

Sampling Sites and Methodology. The sediment samples used in this study were obtained from 12 estuarine

and port sites: two sites on the Tama River in Tokyo (Tt1 and Tt2), four sites on the Tsurumi River in Kanagawa (Kt1–Kt4), one site on the Sagami River in Kanagawa (Ks1), one site at the Hisanohama port in Fukushima (Fh1), one site on the Abukuma River in Miyagi (Ma1), two sites on the Natori River in Miyagi (Mn1 and Mn2), and one site at the Torinoumi ports in Miyagi (Mt1) (Figure S2 of the Supporting Information). In addition, time-course sampling was performed at Kt1. The selected rivers were all identified in the River Act as being important for conservation or the economy by the Ministry of Land, Infrastructure, Transport and Tourism (<http://www.mlit.go.jp/>) and were all relatively wide and calm. The sediment samples were collected using a small dredge sampler (developed in-house) from the surface layer of sediment at the bottom of the estuaries and ports; 10 samples were collected from each sampling site.

Organic and Inorganic Chemicals and Microbial Community Analyses. The sediment organic matter samples were isolated from 10 g of surface sediments by extraction using 0.1 M NaOH, as described previously.¹⁴ The extracted samples (60 mg) were dissolved in DMSO-*d*₆ solvent (600 μL).¹⁵ The two-dimensional (2D) ¹H-¹³C heteronuclear single-quantum coherence (HSQC)¹⁶ spectra were collected on a Bruker 700 MHz NMR spectrometer. Inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis was performed to evaluate the distribution of metals at each site, as described previously.¹⁷ Denaturing gradient gel electrophoresis (DGGE) was performed to evaluate the microbial community composition at each of the sites, as described previously.¹⁸ An amplicon analysis of 16S rRNA genes was performed using a next-generation pyrosequencing technique based on the FLX system (this analysis was outsourced to Operon Biotechnology Company Ltd., Tokyo, Japan). The total elemental analysis of the sediments was performed using a CHNS/O Elemental Analyzer (vario MICRO cube, Elementar Analysensysteme GmbH).¹⁹ Nitrate, nitrite, and ammonia levels were evaluated by a DR-2800 Portable Spectrophotometer (HACH Company), according to the manufacturer's instructions. The detailed analytical procedures were described in the Supporting Information.

Data Preprocessing and PCA of Individual Analytical Data. The HSQC spectra were binned using FT2DB on the ECOMICS web-based toolkit (<https://database.riken.jp/ecomics/>)²⁰ as follows. The HSQC data were analyzed by subdividing the spectra into sequential 0.04 ppm designated regions for ¹H chemical shifts of 0.0–8.0 ppm and sequential 0.4 ppm designated regions for ¹³C chemical shifts of 8.0–138.0 ppm.²¹ These HSQC data (normalized to a constant sum, or the total intensity of the signals was constant between each datum) as well as ICP and DGGE data were statistically evaluated with PCA using "R" software (<http://www.R-project.org/>), as previously reported.²² Parameters of the models are described in Table S3 of the Supporting Information.

Whether the data were normally distributed was tested using the Kolmogorov-Smirnov normality test. The presence of significant differences between groups was tested using a Student's *t* test for parametric data. For nonparametric data, the test for statistical significance was performed using a Mann-Whitney U test. Because of the possibility of obtaining false-positives with tests such as Student's *t* test and Mann-Whitney U test for multiple comparisons, a posthoc Benjamini-Hochberg correction was performed.

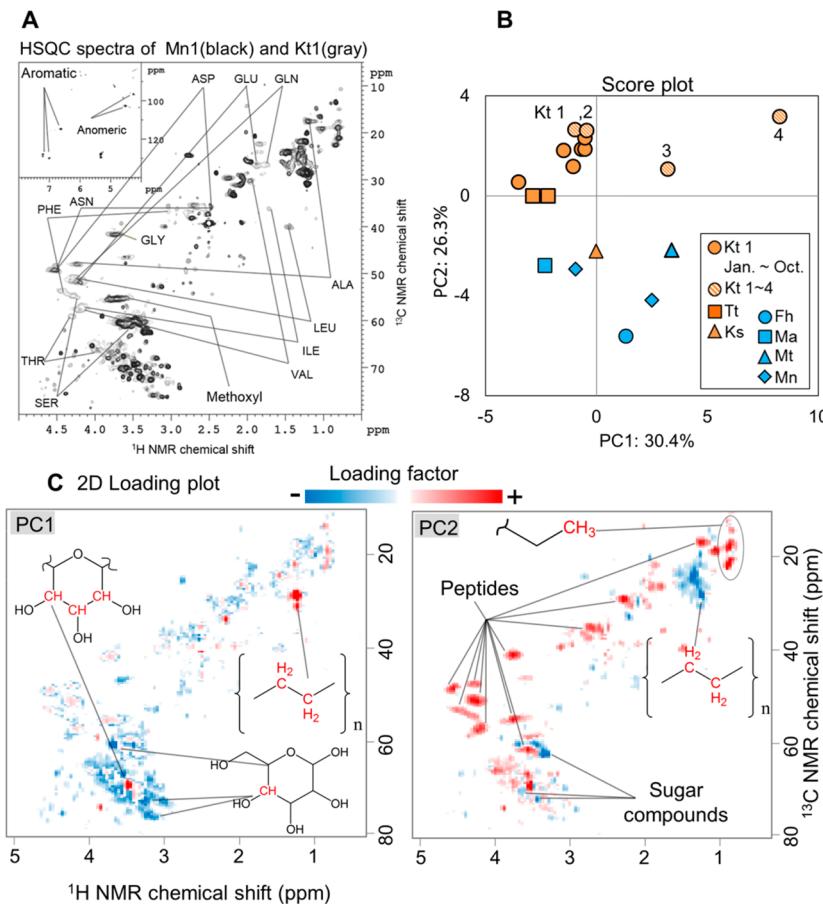


Figure 1. Organic matter profiles of geographically and seasonally varied sediments computed from HSQC spectra. (A) Overlay of HSQC spectra from Kt1 and Mn1. (B) The PCA score plots of geometrically and seasonally varied sediments computed from HSQC spectra. (C) Loading plots of (B) on 2D-HSQC profiles. Left: PC1; right: PC2.

Integration of Various Chemical and Microbial Data by Correlation and Network Analysis. To link the organic information, elemental variability, and microbial diversity variations, we performed a correlation analysis based on the NMR/HSQC peak intensity, ICP/elemental analysis, spectrophotometer/ammonium, nitrite, and nitrate concentrations, and pyrosequencing/microbial analysis data.²³ The statistical analyses involved the calculation of correlations, according to the Pearson correlation coefficients method and were performed using “R” software. The correlation network was constructed using Gephi (0.8) (<https://gephi.org/>) to enable the integration and visualization of all data. A hierarchical cluster analysis (HCA) was performed using “R” software for statistical computing based on Euclidean distances with group averages.

RESULTS AND DISCUSSION

Characterization of Persistent Organic Matter Deposited in the Sediments. Solution-state 2D NMR approaches were applied to identify the signatures of persistent organic matter (not only humic substances but also carbohydrates, peptides, and lipids) in the sediments. Figure 1A shows the solution-state HSQC NMR spectra of the DMSO-dissolved organic extracts from estuarine sediments sampled from the Kanto and Tohoku regions. The HSQC spectra showed that carbohydrates, peptides, and lipids were abundant in the sediment organic matter; these were possibly derived from plant, animal, and microbial sources. In addition, an abundance

of complexes from human sewage residues may have contributed to the spectral findings. The spectra also showed an extremely small number of simple carbohydrates, and an encircled region in a lignin methoxyl group was observed. Comparison of the HSQC spectra between the Kanto and Tohoku regions revealed some differences, including multiple peptide peaks (possibly derived from raw garbage and dead organisms) in the Kanto region and a characteristic lipid peak in the Tohoku spectra.

This study focused on the variations in “relative” abundance based on HSQC NMR spectra, capturing the relative variation in signal intensity among sampling sites. To this end, all spectral data were processed by PCA to determine the individual characteristics of the samples because PCA can facilitate simultaneous comparison of a large amount of NMR spectral data and can statistically identify both similarities and dissimilarities over time as well as geographical variations. The PCA score plots showed that the organic matter profiles in the Kanto and Tohoku regions were clustered in positive and negative directions, respectively, for PC2 (Figure 1B). From the loading plot analysis, large peptide signals were attributed to the Kanto region, whereas the characteristic peaks of carbohydrates and lipid compounds were attributed to the Tohoku region (Figure 1C). These clusters may have been due to a difference in the temperature and activity of the microbial layer or differences in substance inflows. In addition, the PC1 axis in the loading plot analysis showed that the amount of carbohydrates and peptides increased from positive to negative. Sites Kt1–Kt4

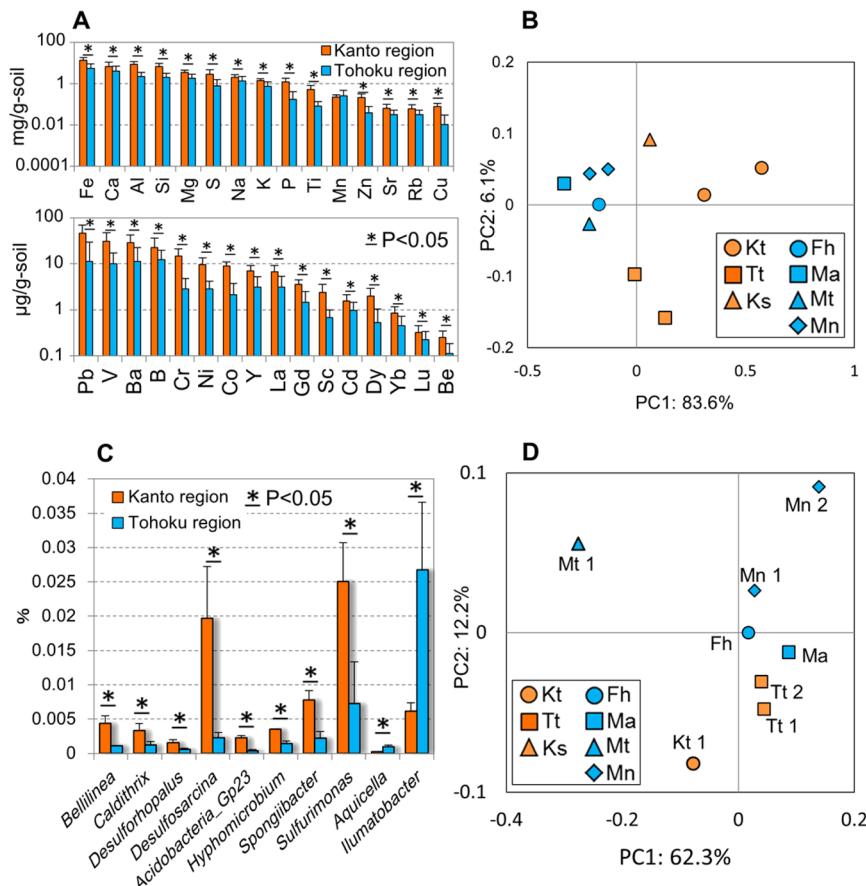


Figure 2. Mineral and microbial profiling of geographically varied sediments. (A) Bar plots comparing average concentrations from Kanto (orange) and Tohoku (blue) sampling sites measured by ICP-OES. Significant differences were calculated using the Mann–Whitney U test. (B) The PCA score plot for the mineral profiles was computed from seven geographically varied sediment samples. (C) Bar plots comparing the average percentages from Kanto (orange) and Tohoku (blue) sampling points based on pyrosequencing targeting for microbial 16S rRNA genes. Significant differences were calculated using the Student's *t* test. (D) The PCA score plot for microbial profiles was computed from seven geographically varied sediment samples.

(representing the sediments collected 0, 2, 4, and 8 km from the mouth of the Tsurumi river) on the PCA score plot aligned from positive to negative in the upper portion of the plot. In particular, Kt3 and Kt4 aligned with the PC1 and PC2 positive axis in the PCA score plot. These samples contained a relatively small amount of carbohydrates and peptides compared with the other sampling sites. This indicated that organic matter was readily deposited in the estuary. Furthermore, less variation was observed in the time-series samples taken from January to October at site Kt1 compared with those observed not only between individual estuarine sediments but also between the sediment sites along the Tsurumi river. These findings indicate that long-term data related to differences between regional basins is reflected in sediment organic matter.

Characterization of Elemental and Inorganic Compound Profiles. Although the variations of elemental profiles were already characterized by PCA on the basis of the ICP-OES-based ionomic approach in Figure S3A (see supplementary results and discussion), the elements included in the sediment samples from the individual sampling sites were further analyzed to obtain more detailed information about the characteristics at each site (Figure 2A and Table S1 of the Supporting Information). Several minerals, such as Fe, Ca, Si, and Mg, were commonly detected in sediments from both the Kanto and Tohoku regions. Almost all elements, except Mn,

tended to be more abundant in the Kanto region. The differences of these elements in the sediments were significant between the Kanto and Tohoku regions. Moreover, organic elements (i.e., C, H, N, S, and O) were more abundant in the Kanto region (Figure S4, panels A and B, and Table S1 of the Supporting Information). It was, therefore, determined that differences were based on topographical variations and geological formations in each region and/or because of differences in adsorption, binding, and accumulation of estuarine sediments because accumulation may be related to the composition and structure of humic organic matter.²⁴

In addition, variations in the elemental profiles of the surface water samples taken from the individual sampling sites were evaluated by an ICP-OES-based ionomic approach in combination with multivariate statistical analysis (Figure S5 and Table S1 of the Supporting Information). The elemental profiles in surface water were likely to be clustered according to differences between the Kanto and Tohoku regions. Some elements in particular, such as Fe, Ca, K, and Mg, were more abundant in the Tohoku region compared with the Kanto region. In contrast, the amount of phosphorus was significantly greater in the Kanto region, which may be linked to the degree of eutrophication associated with artificial effluent inputs given the difference in population and industrial activity in each area.²⁵ This is also supported by ammonium, nitrite, and nitrate

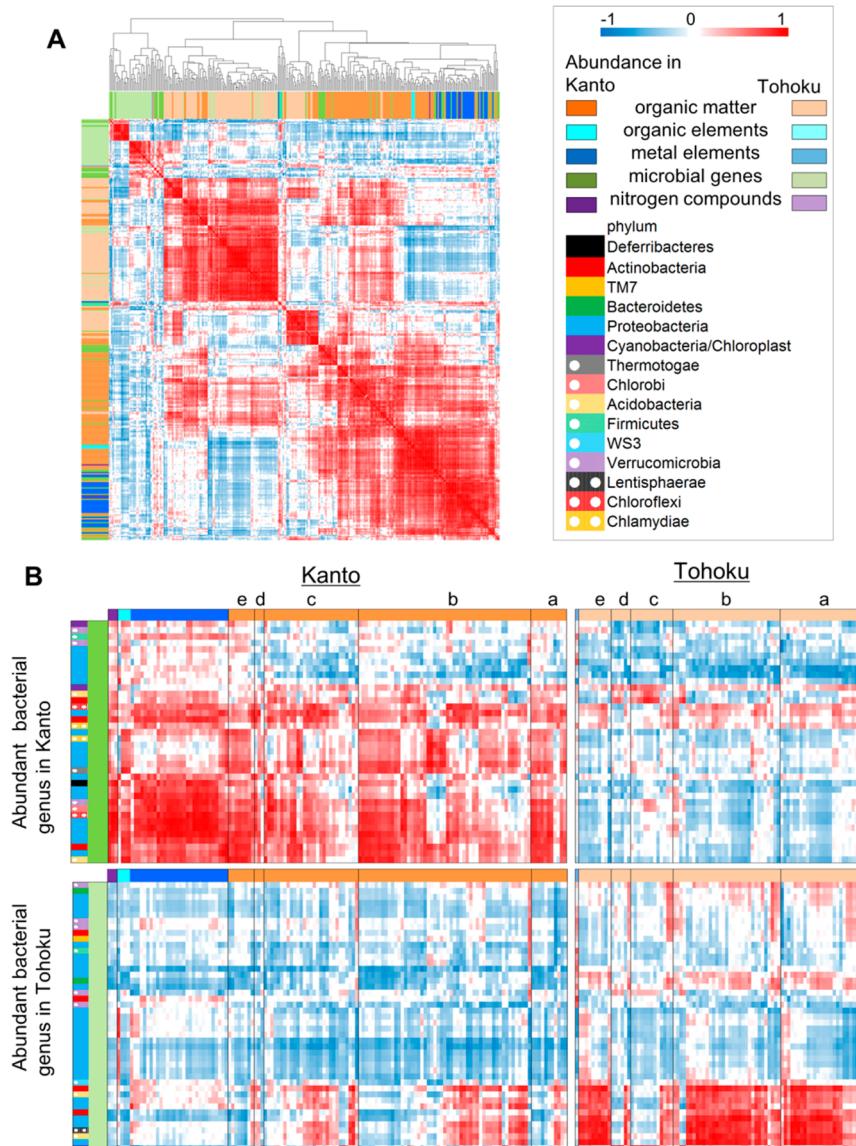


Figure 3. 2D Correlation heat maps computed for all measured numerical values from seven geographically varied sediments. (A) 2D Correlation maps depicting the relationships among organic matter numerical values from HSQC spectra (orange), total organic element numerical values from CHNS/O total element analyzer (cyan), mineral numerical values from ICP-OES (blue), microbial community numerical values from pyrosequencing (green), and nitrogen compounds by spectrophotometric analysis (purple). (B) Comparison of 2D correlation maps for Kanto and Tohoku sediments depicted by the relationships between microbial community parameters (upper, Kanto; lower, Tohoku) against organic and inorganic parameters (left, Kanto; right, Tohoku). (a) Aliphatic linker or side-chain region, (b) amino acid or peptide region, (c) carbohydrate region, (d) anomeric region, (e) aromatic region by HSQC spectra, (f) mineral by ICP-OES, and (g) organic matter by CHNS/O analysis. Other colors are the same as those for (A).

concentrations of the surface water samples from the individual sampling sites because those from the Kanto region had significantly higher amounts of ammonium, nitrite, and nitrate (Figure S4, panels C and D, and Table S1 of the Supporting Information). It has been considered that nitrogen ions are often produced by deamination reactions of high molecular peptide by microorganisms. Furthermore, several metal elements were more abundant in sediments from the Kanto region than in surface water samples from the Tohoku region. These observations may be because of the composition and structure of the sediment organic matter as well as microbial community and other environmental differences.

Characterization of Microbial Community Profiles. Although variations in the microbial community profiles were already characterized by PCA based on the DGGE-based

community analysis shown in Figure S3B (see supplementary results and discussion), the microbial communities included in the sediment samples from each individual sampling site were also analyzed to obtain more detailed information regarding the taxonomic characteristics at each sampling site. Proteobacteria and Cyanobacteria were the most abundant bacterial phyla and were commonly detected in both the Kanto and Tohoku regions (Figure S6 of the Supporting Information). However, some differences between the Kanto and Tohoku regions were noted (Figure 2C and D). For example, sulfate-reducing bacteria *Desulfosarcina* sp.²⁶ and *Desulfuromonas* sp.,²⁷ sulfate-oxidizing bacterium *Sulfurimonas*,²⁸ and other bacteria such as *Spongibacter* sp.²⁹ were relatively more abundant in the Kanto region, whereas aerobic bacteria such as *Ilumatobacter* sp.³⁰ were relatively more abundant in the Tohoku region (Figure

2C). Because the flow velocity between the rivers does not differ, these differences may be due to physical and biochemical differences in the sediment (such as particle and mineral size). The configuration of the sediment organic matter is also believed to be involved in the formation of sediment microbial communities, which in turn affects other animals and water quality.

Correlation and Network Analysis of Integrated Data.

In the sediments of estuarine environments, organic matter is intricately linked to minerals, metal ions, and microbial communities. We, therefore, evaluated the correlations and relationships among organic matter, elements, and microbial communities in sediment ecosystems. To this end, a correlation and network analysis was performed to identify the elemental, microbial community, nitrogen ions, and organic component profiles, and these profiles were compared between the Kanto and Tohoku regions (Figures 3, 4, and Figure S7 of the Supporting Information). The homogeneous correlation analysis identified relationships between organic, elemental, and microbial community profiles (Figure 3A). For example,

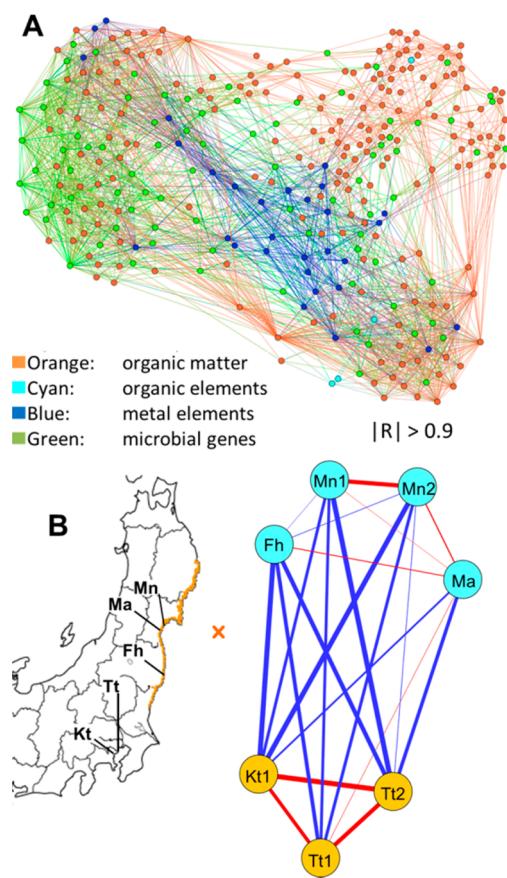


Figure 4. Correlation network analysis computed for all measured numerical values from seven geographically varied sediments. (A) Correlation network analysis of 325 measured numerical variables was computed against seven geographically varied sampling points. The colors are the same as those for Figure 3. (B) Correlation network analysis of seven geographically varied sampling points computed against 325 measured numerical variables. Red and blue lines show a positive and negative correlation, respectively. The line width denotes the relative strength of the correlations. The orange cross on the map is the epicenter of the Great East Japan Earthquake (March 11, 2011), and the orange line shows the area affected by the tsunami (more than 4 m in height).

organic components had a strong positive correlation with nitrogen and *Filomicrobium* sp. *Filomicrobium* sp. is an aerobic bacterium (e.g., lives in the brackish water of the Baltic Sea³¹), and it was correlated with the greatest number of organic matter signals. In particular, it was strongly correlated with lipid linker and aromatic region signals. *Lentisphaera* sp., which affects oxygen during the biodegradation of fuels in a corroding environment,³² showed a strong correlation with lipid linker and peptide signals. In addition, high correlations with lipid, peptide, carbohydrate, and aromatic region signals were observed for the aerobic bacteria *Conexibacter* sp.,³³ *Acidobacteria* Gp10,³⁴ *Arenicella* sp.,³⁵ *Spongibacter* sp.,²⁹ *Shewanella* sp.,³⁶ and *Mycobacterium* sp. It is believed that these microorganisms play an important role in estuarine bottom ecosystems because organic lipids are an energy source.³⁷

From the homogeneous correlation analysis (Figure 3A), we further analyzed the data using a microbial-gated correlation (Figure 3B) and network analyses (Figure 4). A “microbial-gated” correlation analysis tests for correlations among multiple data set across heterogeneous measurements made by different types of instruments (i.e., ICP, NMR, CHNS/O, and pyrosequencing in this study). The correlation coefficients are calculated following usual methods such as a Pearson’s product-moment correlation coefficient and a Spearman’s rank-correlation coefficient and are visualized in Figure 3A. Subsequently, the coefficients of microbiota are obtained from the correlation matrix (i.e., all the correlation information related to microorganisms). At this point, clustering analyses, such as HCA and PCA, and statistical analyses, such as significance tests, are performed based on the parameters of interest. In this study, for example, the parameters of interest are the two groups (i.e., Kanto and Tohoku groups), and HCA was performed for this grouping (Figure 3A). On the basis of this grouping, the correlation coefficient matrix “gated” by the microorganisms was arranged by sorting each chemical component (the first sorting was performed on the basis of regions of each chemical shift observed in the HSQC spectra, Figure S7 of the Supporting Information), subsequently by arranging and combining the organic and inorganic elemental data, and finally clustering the data based on the relative abundance of each microbe in the Kanto and Tohoku regions (Figure 3B). This approach enables us to understand in a visual way the relationship between microbiota and the other components in the Kanto and Tohoku regions.

Characteristic bacteria that strongly correlated with sediment organic matter signals in the Kanto or Tohoku regions were visualized by the microbial-gated correlation and network analysis. Furthermore, these bacteria were observed to show different patterns of correlation with organic structure, depending on the species. For example, sulfate-reducing bacteria such as *Desulfosarcina* sp. (Figure 2C) and *Desulfobulbus* sp.³⁸ were abundant in Kanto and had a high positive correlation with sulfur and iron elements and some lipids, peptides, carbohydrates, and aromatic region signals. Because the abundance and functional activity of sulfate-reducing bacteria is associated with the accumulation of iron sulfide,³⁹ the mineral correlation patterns of these sulfate-reducing bacteria were reasonable. Moreover, the suggestions that these sulfate-reducing bacteria utilize some organic components in the sediments was supported by the correlation analysis. In addition, anaerobic bacteria such as *Caldithrix* sp.⁴⁰ and *Bellilinea* sp.⁴¹ were also correlated with organic matter and in a manner similar to that of the sulfate-reducing bacteria,

suggesting that the function of the microbial community is revealed by the correlation pattern. Increase or decrease of these bacteria has a strong correlation with nitrogen ions (ammonium, nitrite, and nitrate). Further, these nitrogen ions have a strong correlation with the NMR signals of random coil shifts of polypeptides (Table S2 of the Supporting Information). Polypeptide signals correlated with nitrogen ions had a stronger correlation than that among other organic signals. Thus, it was suggested that these polypeptides, deposited in estuarine sediments, were degraded and converted by above-mentioned bacteria into inorganic nitrogen, representing a major eutrophication factor. Namely, aliphatic and acidic amino acid chemical groups in polypeptides had high correlation rather than aromatic, sulfur-containing, and basic amino acid chemical groups. Furthermore, bacteria such as *Caldilinea* sp., *Saccharophagus* sp.,⁴² and *Arthrobacter* sp.,⁴³ are strongly correlated with carbohydrates and may be involved in the degradation of polysaccharides. These bacteria showed a different correlation pattern than others (i.e., they showed a stronger correlation with the different carbohydrate signals than other bacteria). In contrast, photosynthetic bacteria such as *Bacillariophyta* sp.⁴⁴ and *Chlorophyta* sp. showed exceedingly small correlations with lipid, carbohydrate, and peptide structures. This is considered reasonable because photosynthetic bacteria do not utilize sediment organic matter.

In the network analysis, which was performed using the open-source software Gephi, microbial and chemical relationships and networks were visualized according to a homogeneous correlation (Figure 4A). These networks can be interpreted as significant detail in Figure 3A in this study with advantages for mining large amounts of data. Figure 4A shows individual data based on regional differences, and Figure 4B shows correlations at each site based on data variation. Figure 4B shows that estuaries located in the same region had a positive correlation, and Kanto and Tohoku were separated by a negative correlation. Site Fh showed a relatively weak positive correlation with site Ma, but negatively correlated with the Kanto region. The network analysis captured the trends and characteristics of the individual sampling sites, although the individual sampling sites showed relatively weak correlations among each other. The analytical strategy, including the microbial-gated correlation and network analysis, described in this study is a powerful approach for analyzing, visualizing, and evaluating the complex metabolic dynamics and networks in sediment microbial ecosystems. In particular, the microbial-gated correlation analysis is useful for comparing the relationships among (two or more) groups and categories. In this study, we adopted a “microbial”-gated correlation analysis; however, “elemental”- and “sediment organic matter”-gated correlation analyses are also possible, by modifying the “gating” (deploying) elements to ICP data or organic matter in sediment from NMR data, respectively, and can be used to characterize, visualize, and compare the relationships among the components of sediment ecosystems. This analytical strategy will enable us to evaluate and characterize not only estuarine sediment ecosystems but also other environmental ecosystems, such as deep and shallow sea sediments and agronomic and forest soils. The analytical strategy promises to be useful for deciphering complicated metabolic dynamics, networks, and interactions in a variety of microbial ecosystems.

CONCLUSIONS

Our findings indicate that a microbial-gated correlation and network analysis can visualize and summarize the different relationships among organic and inorganic elements and microbial communities in estuarine sediments based on geographical differences. Namely, we demonstrated remarkable estuarine eutrophication in the Kanto region by stronger HSQC signals of sediment polypeptides and water nitrogen ions catabolized by microbiota. The development of new methodologies incorporating this approach will provide a promising new field in environmental science and technology.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

The manuscript was written with contributions from all authors. All authors have approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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