Disturbance of deep-sea environments induced by the M9.0 Tohoku Earthquake.

Authors

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Supplementary information for *Kawagucci et al.*

Contents of Supplementary Information

1. Supplementary Methods

To know reference microbial cell densities in the deep-sea water of the Japan Trench regions, the deep-sea water (10 m above the seafloor) was sampled by the same method as in this study from 4 different stations from Stns. R, N1-N3 and JKEO (Stn. F: 36˚59.95'N-143˚11.83'E at a water depth of 6800 m; Stn. 39N: 38˚59.87'N-144˚3.86'E at a depth of 6680 m; Stn. E: 38˚15.27'N-144˚2.24'E at a depth of 7415 m; Stn. SE: 38˚15.97'N-144˚15.23'E at a depth of 6495 m) at a time of about 70 days after the 3.11 Tohoku Earthquake during the JAMSTEC YK11-E03 R/V Yokosuka cruise.

For extraction of microbial DNA, quantitative PCR of 16S rRNA gene, and 16S rRNA gene clone analysis, a portion (2 L) of deep-sea water was filtered with a 0.22-μm-pore-size, 47-mm-diameter cellulose acetate filter (Advantec, Tokyo, Japan), was preserved onboard at -80 ˚C prior to DNA extraction. From the frozen filters with microbial communities, DNA was extracted by using the Ultra Clean Mega Soil DNA Isolation kit (MO Bio Laboratory, Solana Beach, CA, USA), following the manufacturer's instructions with minor modification. In the purification step, extracted DNA was concentrated by using spin filter unit provided in Ultra Clean Soil DNA Isolation kit (MO Bio Laboratory), and was eluted in 50μl. Quantitative PCR of archaeal and entire prokaryotic 16S rRNA genes was performed using 7500 Real Time PCR System following a method constructed by Takai & Horikoshi (2000) with minor modifications described previously (Nunoura et al., 2008). For amplification standards of archaeal and prokaryotic 16S rRNA genes, 16S rRNA gene mixtures described previously were used (Takai and Horikoshi, 2000).

Prokaryotic 16S rRNA gene clone analysis was conducted with another PCR experiment. Both bacterial and archaeal genes were simultaneously amplified from DNA extracts by PCR using LA Taq polymerase with GC buffer (TaKaRa Bio, Otsu, Japan). The oligonucleotide primers used were the mixtures of various derivatives of

previously designed 530F and 907R primers (Lane, 1985); the 530F primer mixture, (GTGCCAGCAGCCGCGG, GTGBCAGCCGCCGCGG, YTGCCAGCCGCCGCGG, GTGCCAGCAGCWGCGG, GTGCCAGCAGTCGCGG, GTGCCAGAAGMMTCGG and GTGGCAGTCGCCACGG), and 907R primer mixture (CCGYCAATTCMTTTRAGTTT, CCGYCTATTCCTTTGAGTTT, CCGYCAATTTCTTTRAGTTT, CCGYCAATTCCCTTRAGTTT, CCGYCAATTCCTTMAAGTTT and CCGCCAATTCCTTTGAATTT). Thermal cycling was performed under the following conditions: after initial preheating for 5 min at 96˚C, denaturation at 96 ˚C for 25 sec, annealing at 50 ˚C for 45 sec, and extension at 72 ˚C for 30 sec for a total of 35 cycles, and final extension at 72˚C for 7 min. The PCR cycle numbers represent almost the minimum cycle numbers providing enough amplified products for the cloning based on the preliminary PCR amplification experiments using the same templates. The amplified rRNA gene products from several separate reactions at the least number of thermal cycles were pooled and purified as previously described (Takai et al., 2001). Cloning and sequencing were also followed by the procedure described by Takai et al. (2001). Approximately 450 nucleotides of cloned rRNA gene fragments were determined for single strand using M13M4 oligonucleotide primer and the sequence similarity analysis was conducted among the clone sequences by using a similarity program in the GENETYX MAC software ver. 12 (Genetyx Corporation, Tokyo, Japan). The sequences having >97% similarity were identified as the same phylotype. The sequences of the representative phylotypes were imported into the ARB software program (Ludwig et al., 2004) and were phylogenetically classified into certain taxonomic unit using Hugenholtz's small subunit rRNA sequence database and phylogenetic classification.

To assess difference in phylogenetic context of the post-earthquake deep-sea microbial communities in the bottommost deep-sea water, an online tool, UniFrac, was used for the principal coordinates analysis (PCoA). The UniFrac analysis was based on the phylogenetic tree that was reconstructed from the representative phylotypes in the clone libraries using the neighbor-joining method by ARB.

3. Supplementary Results

In the approx. 70-days-after deep-sea bottom water environments at 4 different stations from Stns. R, N1-N3 and JKEO, the increase of microbial cell densities in the bottom water samples was not obviously found. The average microbial cell density in the deep-sea water of the Japan Trench at 10 m above the seafloor at about 70 days after the largest earthquake (4 other stations) was determined to be $1.1 \pm 0.12 \times 10^4$ cells ml^{-1}

(Fig. 2). This average microbial cell density in the deep-sea water was used as a reference in an attempt to know the temporal and spatial variation of deep-sea microbial communities in biomass after the gigantic earthquake (Table S1).

The archaeal and prokaryotic 16S rRNA gene numbers in the whole microbial DNA assemblages were also determined (Table S1). The results showed quite similar patterns in spatial and temporal variation of microbial populations in the deep-sea bottom water environments as observed in the microbial cell densities (Fig. 2 and Table S1). In addition, the quantitative PCR showed that the archaeal rRNA gene proportions to the whole prokaryotic rRNA gene communities at the bottommost deep-sea water were 53.8, 33.7, 63.4, 63.5 and 89.7% at Stns. JKEO, R, N1, N2 and N3. These results suggested that all the deep-sea water microbial communities except for in the bottommost water at Stn. R could be dominated by the archaeal population.

3. Supplementary Discussion

The deep-sea temperature-salinity structures of our stations showed no spikes indicating inputs of hot fluid and/or freshwater (Fig. S2). Although local decrease of dissolved molecular oxygen (DO) in the depth profiles was found near the seafloor at Stns. N1 and N2, the parallel decrease in seawater density (Fig. S2) suggested that little in-situ DO consumption occurred through aerobic microbial activity, as observed in the Deepwater Horizon oil spil[20]. The depth of the slight pycnocline at Stn. N1 seems to be consistent with that of the methane and manganese peaks (Figs. 2 and S2). The disturbed vertical structure of seawater density would result from the usual internal wave induced by the interaction between tidal flow and topography (Vlasenko et al., 0225), although a tsunami-associated disturbance of the seawater structure cannot completely be ruled out as a possible alternative cause.

The 16S rRNA gene clone analysis of the bottommost deep-sea water revealed diverse but generally similar prokaryotic phylotype compositions among the stations (Fig. 3). The abundance of archaeal phylotypes in the whole microbial rRNA gene clone libraries was a little lower than that determined by the quantitative PCR analysis but supported the potential abundance of archaeal population in each of the deep-sea water microbial communities (Fig. 3). Commonly in all the bottommost deep-sea water samples, the phylotypes of *Gammaproteobacteria*, *Alphaproteobacteria*, *Bacteroidetes*, *Planctomycetes* and several subgroups of Marine Group I (MGI) archaea (or thaumarachaea) were identified as the predominant prokaryotic phylogenetic groups. There have been reported a few investigations of deep-sea planktonic prokaryotic phylotype compositions at depths of >1000 m (e.g., Lopez-Garcia et al., 2001) and no

previous example on the deep-sea planktonic microbial communities in the trench regions. Thus, it is difficult that the microbial phylotype compositions in the deep-sea water of the Japan Trench are compared to those in other geologically and geographically different deep-sea environments. Nevertheless, the similar predominant prokaryotic phylogenetic groups were also found in the deep-sea water samples (>2000 m) in the North and mid Atlantic (Gallangher et al., 2004, Agogué et al. 2011) and in the Antarctic (Lopez-Garcia et al., 2001). Thus, the planktonic microbial phylotype composition in the deep-sea water of the Japan Trench regions may represent the cosmopolitan nature.

In comparison of the bottommost water phylotype compositions among all the stations, some phylogenetic groups were detected in the deep-sea water samples at the trench landward slope stations. We tentatively defined signature phylogenetic groups in the post-earthquake deep-sea water as the phylogenetic groups that were identified in at least two stations of four trench landward slope stations and of which habitat endemism was obviously differentiated from the planktonic microbial communities in the ordinal, oxic deep-sea water habitats. For instances, SUP05 phylogroup within *Gammaproteobacteria* are the predominant planktonic microbial components with the possible sulfur-oxidizing chemolithoautotrophic potentials in the deep-sea hydrothermal fluid plumes (Sunamura et al., 2004) and in the oxygen minimum zones of ocean (Walsh et al., 2009), and may represent a fresh planktonic sulfur-oxidizing population that dominates the microbial communities in responding to the earthquake-induced sulfide input in the deep-sea water or to the sulfate reduction in colloidal sediment particle diffused by earthquake impact described below. Similarly, the zeta-proteobacterial phylotype was closely related with a deep-sea iron-oxidizing chemolithoautotroph, *Mariprofundus ferrooxydans* (Emerson et al., 2007), and with a number of rRNA gene phylotypes that dominated microbial communities in deep-sea iron-oxide mats of the hydrothermally active seafloors (Emerson et al., 2010). It is a possible inference that the zeta-proteobacterial phylotype in the bottommost deep-sea water is brought by the diffusing sediments with the existing seafloor microbial communities sustained by the ferrous iron-rich fluid seepages or is a newly dominating planktonic population sustained by chemical influx of ferrous iron to the deep-sea water via the earthquake-driven subseafloor fluid migration. Other potential signature phylogenetic phylogroups were *Desulfobacterales* and *Desulfuromonales* phylogroups within *Deltaproteobacteria* and *Arcobacter* phylogroup within *Epsiloproteobacteria*. The similar proteobacterial rRNA gene sequences have been found in the shallow sediments at and around the cold-seep chemosynthetic animal (*Calyptogena* sp.)

colonies (Li et al., 1999; Inagaki et al., 2002). In addition, the phylogenetically related *Desulfobacterales*, *Desulfuromonales* and *Arcobacter* species are frequently identified as significant populations in the deep-sea benthic microbial communities of the anoxic and oxic-anoxic interface zones (Kuever et al., 2005a; 2005b; Vandamme et al., 2005; Wirsen et al., 2002). These proteobacterial phylotypes could be the existing benthic microbial components in the shallow sediments before the earthquakes and could be spread into the deep-sea water with the earthquake-induced sediment diffusion. In addition, the previously uncultivated euryarchaeota, DHVE5 and DHVE6, were initially found in the deep-sea hydrothermal vent chimneys and sediments hosting low temperatures of diffusing fluids (Takai and Horikoshi, 1999). However, several investigations clarified the occurrence of these previously uncultivated archaeal phylotypes in non-hydrothermal, but relatively reduced, marine sedimentary environments (Nakayama et al., 2011; Teske and Sørensen, 2008) although, intriguingly, typical subseafloor archaeal phylogroups such as the DSAG (MBGB), SAGMEG and MCG (Teske and Sørensen, 2008) are absent in these deep waters. Thus, it seemed likely that these uncultivated archaeal phylogroups also represented the benthic microbial components spread into the deep-sea water by the earthquake-induced sediment diffusion, and grow in the colloidal microhabitats.

Probably the growths and functions of these signature microbial phylotypes and other phyloype components would be activated by the direct and indirect chemical inputs through the earthquake-induced sediment diffusion or subseafloor fluid discharge. The increased microbial populations (microbial cell densities and prokaryotic 16S rRNA gene numbers) in the bottom water of the Japan Trench landward slope would represent such an activated response of the microbial community to the earthquake-induced environmental disturbances. In particular, the bottommost deep-sea water at Stn. R at the times of 36 and 98 days after the M9 event exclusively showed the increased abundance of bacterial 16S rRNA gene number in the whole prokaryotic 16S rRNA gene number (66.3 and 59.8 %, respectively) (Table S1). Since the 36-days-after bottommost deep-sea water at Stn. R had the highest LTA value (the greatest sediment diffusion), the potential enrichment of bacterial populations in the planktonic microbial communities is likely associated with the greatest influence of sediment diffusion. The certain chemical inputs in the deep-sea water such as the preserved subseafloor reduced chemical substances (e.g., CH_4, H_2, sulfide, ferrous iron and organics) may trigger the selective activation that would more effectively work for the growths and functions of the bacterial components than the archaeal componentsin the planktonic microbial community.

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208 **4. Supplementary Table**

209 Table S1 Microbial cell density and archaeal and prokaryotic 16S rRNA gene number in

210 whole microbial DNA assemblage in deep-sea bottom water environments

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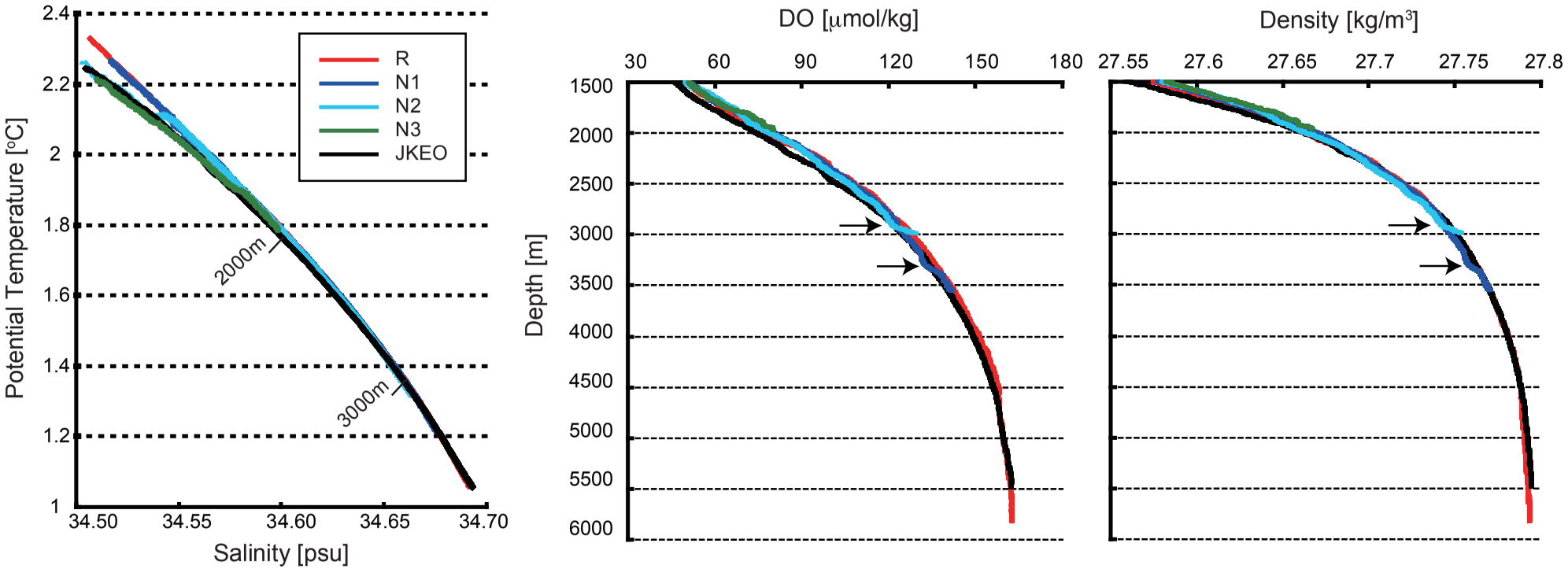
Station	Days after the M9 event	Altitude	Microbial cell density	Prokaryotic 16S rRNA gene number	Archaeal 16S rRNA gene number	Proportion of archaeal/prokaryotic 16S rRNA gene numbers
	(day)	(m)	(cells/ml)	(copy/ml)	(copy/ml)	(%)
JKEO	36	10	1.46×10^4	1.92×10^4	1.03×10^4	53.6
JKEO	36	381	1.56×10^4	1.32×10^4	0.94×10^4	71.2
JKEO	36	1381	2.01×10^4	1.77×10^4	0.83×10^4	46.9
R	36	10	3.01×10^4	8.58×10^4	2.89×10^4	33.7
R	98	10	0.83×10^4	0.87×10^4	0.35×10^4	40.2
R	36	110	3.32×10^4	n.d.*	n.d.	n.d.
R	36	310	1.62×10^4	n.d.	n.d.	n.d.
R	36	510	1.73×10^4	n.d.	n.d.	n.d.
R	36	760	1.96×10^4	3.47×10^4	1.69×10^4	48.2
R	98	760	0.99×10^4	0.78×10^4	0.37×10^4	48.1
R	36	1260	1.47×10^4	n.d.	n.d.	n.d.
N1	36	10	2.68×10^4	6.56×10^4	4.16×10^4	63.4
N1	36	160	1.71×10^4	n.d.	n.d.	n.d.
N1	36	280	1.60×10^4	n.d.	n.d.	n.d.
N1	36	400	1.77×10^4	n.d.	n.d.	n.d.
N1	36	550	2.12×10^4	3.60×10^4	2.37×10^4	65.8
N1	36	980	1.68×10^4	n.d.	n.d.	n.d.
N2	36	10	3.30×10^4	7.99×10^4	5.08×10^4	63.6
N2	98	10	1.90×10^4	1.72×10^4	0.92×10^4	53.5
N2	36	110	2.12×10^4	n.d.	n.d.	n.d.
N2	36	200	2.56×10^4	n.d.	n.d.	n.d.
N2	36	320	2.49×10^4	n.d.	n.d.	n.d.
N2	36	480	2.41×10^4	3.40×10^4	3.03×10^4	89.1
N3	36	10	2.04×10^4	5.08×10^4	4.56×10^4	89.8

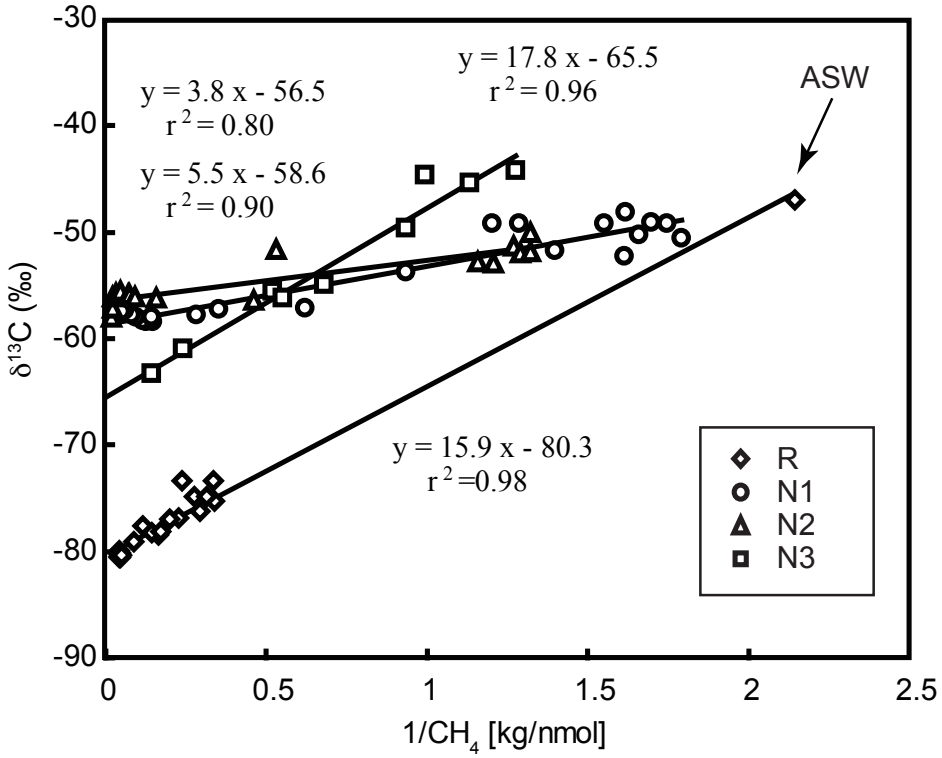
N3	36	110	2.93×10^4	n.d.	n.d.	n.d.
N3	36	950	6.38×10^4	10.2×10^4	7.99×10^4	78.3
F	70	10	1.22×10^4	n.d.	n.d.	n.d.
E	70	10	1.11×10^4	n.d.	n.d.	n.d.
39N	70	10	1.08×10^4	n.d.	n.d.	n.d.
SE	70	10	0.93×10^4	n.d.	n.d.	n.d.

*n.d.; not determined.

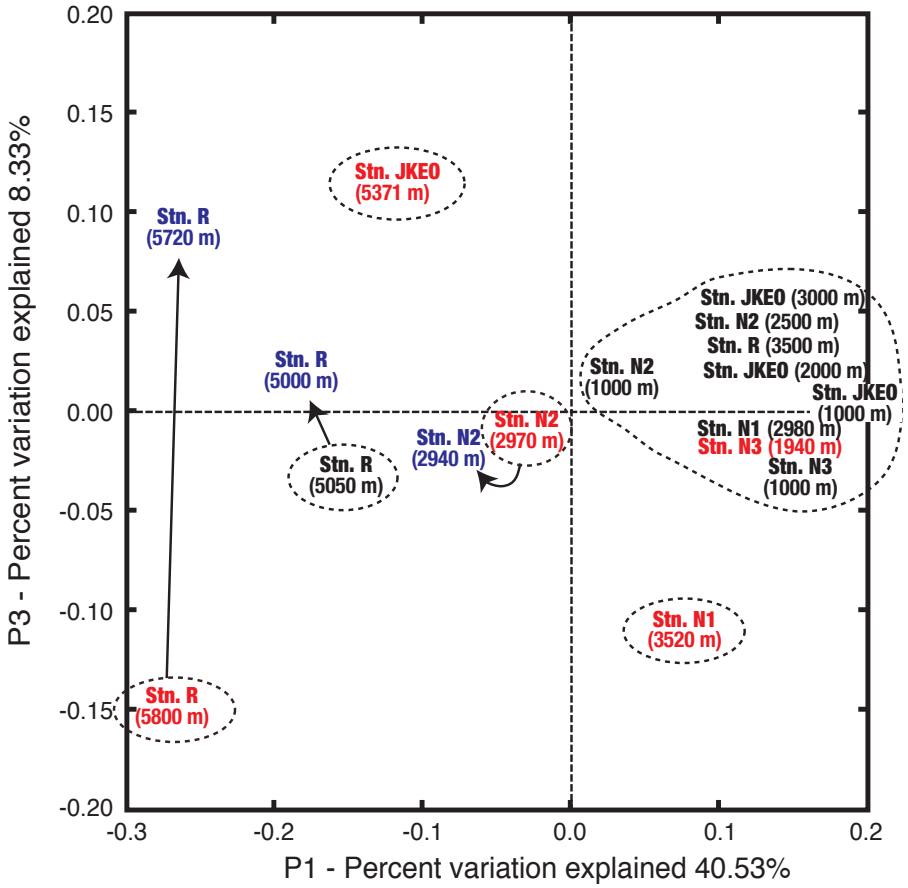
5. Supplementary Figures



Supplementary Figure S1: Potential temperature, salinity, dissolved molecular oxygen and density in deep-sea water at the stations. The depths where decreases of dissolved molecular oxygen (DO) and density simultaneously occur at Stns. N1 and N2 are indicated by arrows.



Supplementary Figure S2: A Keeling plot of concentration and stable carbon isotopic composition of methane in deep-sea bottom water. A least-square line fitted to the data from each station is presented with the resulting equation and correlation coefficient.



Supplementary Figure S3: Comparison of post-earthquake prokaryotic phylotype compositions in the deep-sea bottom water environments of the Japan Trench region. The distribution pattern of the phylotype compositions at different stations was determined by the principal coordinates analysis using an online tool, UniFrac (Lozupone C et al., 2005). Red colors indicate the compositions in the 36-days-after deep-sea bottommost water at Stns. R, N1-3 and JKEO. Blue colors indicate the compositions in the 98-days-after deep-sea water at Stns. R and N2. Dot line areas show 6 types of brief classification of deep-sea water planktonic microbial phylotype compositions.

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