

Unique microbiome in the hydrothermal plumes in Okinawa Trough sediment hosted back arc hydrothermal systems

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Deep-sea hydrothermal plume harbors chemolithoautotrophic microbes depending on high concentration of sulfur, methane and hydrogen supplied from hydrothermal fluid. The most major constituent of the plume microbe is SUP05, which utilize sulfur as energy source. It occupy more than 80% of the elevated microbial population in Izu-Mariana hydrothermal plume. In the Okinawa trough hydrothermal plume, their contribution to the elevated plume microbes is lower than those of Izu-Mariana plume and methane utilizing *Methylococcus* play more important roles. However these two groups are responsible for 50-70% of the elevated microbial population in the Okinawa plume. In this work, I examine the microbial community structure analysis based on 16S rRNA gene using NGS with higher resolutions. The microbial community structures are varied among 7 hydrothermal and 1 methane seep sites. Potential chemolithoautotrophs (*Thaumarchaeota*, *Thermogemmatispora*, *Surfurimonas*) and heterotrophs (*Marinobacter*, *Caulobacter*, *Sphingomonas*) are detected. These data will be useful for baseline microbial community structures in hydrothermal plume against the deep sea mining in the future.

Keywords: hydrothermal plume, MiSeq, microbial community structure, Okinawa Trough

Sulfur isotopic fractionation in carbonyl sulfide during microbial degradation

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Carbonyl sulfide (OCS) is the most abundant gas containing sulfur in the atmosphere, with an average mixing ratio of 500 p.p.t.v. in the troposphere. OCS is suggested as a sulfur source of the stratospheric sulfate aerosols (SSA) which plays an important role for Earth of radiation budget and for ozone depletion. OCS, therefore, should be validated for prediction of climate change, but the global OCS budget is imbalanced. It is known that some microorganisms in soil can degrade OCS, but the mechanism and the contribution to the OCS in the air are still uncertain. Isotopic compositions and isotopic fractionation factors are used to trace the sources and transformations of atmospheric trace gases. Recently, we developed new method measuring sulfur isotopic composition of OCS using fragmentation ions S^+ , and this method can be used to investigate its sources and sinks in the troposphere. In order to determine sulfur isotopic fractionation factors for OCS during degradation via microorganisms, we performed laboratory incubation experiments using OCS-degrading microorganisms.

Bacterium strains, which have OCS degradation activity, were cultured on the slant in a glass tube. After forming the colonies, headspace were replaced with N_2/O_2 (80:20 mixture) and 0.03% of CO_2 , and then approximately 4000 p.p.m.v. of OCS were added to the batch. The concentrations of OCS were measured using gas chromatograph equipped with a flame photometric detector, and headspace gases were collected in the helium purged vials for isotope analysis at the same time of concentration measurements. For isotope analysis, we injected the OCS samples to on-line purification system connected to the gas chromatography-isotope ratios mass spectrometry. The sulfur isotope ratios in OCS were determined by measuring the fragment ions $^{32}S^+$, $^{33}S^+$, and $^{34}S^+$ using triple faraday collector cups. Isotopic fractionation factors were determined by the Rayleigh equation.

The isotopic compositions ($\delta^{33}S$ and $\delta^{34}S$) of OCS were increased during degradation of OCS, indicating that reaction for $OC^{32}S$ was faster than that for $OC^{33}S$ and $OC^{34}S$. On the basis of the concentration of OCS and its isotopic compositions, the Rayleigh isotope fractionation model were applied to determine isotopic fractionation constants ($^x\varepsilon = (\delta^xS - \delta^xS_{initial}) / \ln f$, where x indicates 33 or 34). It is worthy noting that $^{33}\varepsilon$ and $^{34}\varepsilon$ values determined by the experiments shows no significant deviations from mass-dependent relationship, indicating that OCS degradation via microorganisms is not mass-independent fractionation (MIF) process. This result suggests this reaction is not contributed to the MIF signatures observed in sulfur for sulfate aerosol samples and/or Archaean rock records.

At the presentation, the comparisons of variability of $^{33}\varepsilon$ and $^{34}\varepsilon$ values for different strains are presented, and the atmospheric implications for the OCS degradation in the present atmosphere are discussed.

Keywords: Carbonyl sulfide, Isotopic fractionation factor, Microorganism

Nitrogen availability influences natural abundance ^{15}N of *Aspergillus oryzae*

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Nitrogen availability controls nitrogen mineralization and nitrification which are important reaction for nitrogen cycle in the soil (Schimel and Bennet. 2004). To evaluate nitrogen availability, soil C/N ratio and net nitrogen mineralization are usually used. However, difficulty in extracting the nitrogen source pools for soil microbes and getting the field circumstance information by using laboratory culture experiment create the difficulty in evaluating the nitrogen availability accurately. Then, the natural abundance of ^{15}N ($\delta^{15}\text{N}$) has been used for evaluating the nitrogen availability as a tool of getting the field circumstance information. Dijkstra et al. (2008) showed negative correlation between $\delta^{15}\text{N}$ which means the difference between $\delta^{15}\text{N}$ of SMB (Soil Microbial Biomass) and $\delta^{15}\text{N}$ of microbial substrate (K_2SO_4 extractable nitrogen from soil) and microbial substrate C/N, and this result suggested $\delta^{15}\text{N}$ could be a good indicator for nitrogen availability. They explained this phenomenon that mineralization is the dominant process for soil microbes at the high nitrogen availability sites, and SMB becomes enriched in ^{15}N because microbes release NH_4^+ which is depleted in ^{15}N . However, previous study about the relationship between $\delta^{15}\text{N}$ -biomass and $\delta^{15}\text{N}$ - NH_4^+ in C/N controlled pure culture is conducted only by Collins et al. (2008) who used *E. coli*, and they could not detect $\delta^{15}\text{N}$ - NH_4^+ in a low concentration. Thus, the relationship between nitrogen availability and $\delta^{15}\text{N}$ -biomass is unclear. The purpose of our study is to reevaluate if biomass becomes enriched in ^{15}N when microbes release NH_4^+ which is depleted in ^{15}N . In this study, we cultured Fungi (*Aspergillus oryzae*) who has large biomass in the forest soil in C/N controlled pure culture (C/N5, 10, 30, 50, 100) for 4 days. We used glycine and glucose as a nitrogen and carbon source. And we measured mainly changes in $\delta^{15}\text{N}$ -biomass, NH_4^+ concentration and $\delta^{15}\text{N}$ - NH_4^+ . In C/N5 and 10 where NH_4^+ concentration increased over time, we found that biomass was strongly enriched in ^{15}N and NH_4^+ is depleted in ^{15}N . Conversely, in C/N 30, 50 and 100 where microbes hardly released NH_4^+ , we found that $\delta^{15}\text{N}$ -biomass got the almost same value of initial $\delta^{15}\text{N}$ -glycine. In the presentation, we will discuss more detail about the carbon and nitrogen mass balance during our experiment.

Biodegradation activity of organic matter in the buried humic horizons in volcanic ash soils

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Over ten thousand years, Hokkaido soils have been formed through deposition of volcanic ash. The surface soil layer in the past has been buried in deeper part of soil profile. The buried humic horizons contain the high amounts of organic matter (OM) and they serve as a large C reservoir. The microbial degradation activity can generally decrease with soil depth, and OM in the deeper soil horizons is stable due to limitations of organic matter input. In contrast, microbial activity in the buried humic horizons may not be low, because the high amounts of OM can fuel soil microbial activity. We aim to examine the microbial mineralization kinetics in the buried humic horizon using ¹⁴C-tracer incubation.

Soil samples (volcanic ash soil) were collected from the soil profiles in three forest sites and one pasture site in Hokkaido, Japan. These "fresh" field moist, un-sieved soils were used for mineralization kinetic studies. A ¹⁴C-glucose solution (100 μ L; 10 - 300000 μ M) was added to 1 g of field-moist soil and incubated for 24 h at 20 °C. The ¹⁴CO₂ production was trapped in the scintillation vial containing NaOH and determined by liquid scintillation counting. The experiments were performed in triplicate. The data of mineralization kinetics were fitted to a single Michaelis-Menten equation: $V = V_{MAX}C/(K_M + C)$, where V is the mineralization rate (nmol g⁻¹ h⁻¹), C is the substrate concentration (μ M) in soil solution, V_{MAX} is the maximum mineralization rate (nmol g⁻¹ h⁻¹), and K_M is the Michaelis constant (μ M) representing the concentration at which 1/2 V_{MAX} is achieved. We also measured soil microbial biomass, fungal/bacterial (F/B) ratio, and fine root biomass.

Fine root biomass in the soil profiles decreased with depth, suggesting the low rates of organic substrate supply in the buried humic horizon. Their Michaelis-Menten kinetic parameters (V_{MAX} and K_M) varied widely from 303 to 18598 nmol g⁻¹ h⁻¹ and 198 to 1294 μ M. The parameters decreased with soil depth. The kinetic parameters of the surface soil horizon exhibited high mineralization capacity, while parameters of the buried humic horizons were similar to those of the other soil types (11 to 2406 nmol g⁻¹ h⁻¹ for V_{MAX} and 198 to 30786 μ M for K_M). The both of V_{MAX} and K_M parameters were correlated positively with microbial biomass-C and -N, respectively. This indicates microbial biomass is a primary factor regulating the potential degradation activity in the buried humic horizons. Microbial biomass decreased with soil depth, consistent with the low input of fresh organic substrates in the deeper soil horizons. The higher F/B ratios were observed in the buried humic horizon, compared to the surface horizon. Since F/B ratios has influence on the mineralization kinetic parameters due to differences of substrate use efficiency and growth speed between fungi and bacteria, the high potential degradation activity relative to the other soil types may be due to high F/B ratios in the buried humic horizons. The high potential degradation activities of soil microorganisms in the buried humic horizons suggest that OM decomposition can be accelerated by addition of easily-biodegradable OM which stimulates soil microbial activity.

Keywords: the buried humic horizon, volcanic ash soil, ¹⁴C-glucose, microbial mineralization kinetics, soil organic matter