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沖縄トラフ熱水プルーム中の微生物群集
Unique microbiome in the hydrothermal plumes in Okinawa Trough sediment hosted back arc hydrothermal systems

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海底ー海洋境界の物質循環において重要な海底熱水が噴出後に海水と混合してできる熱水プルーム中では、熱水中に含まれるイオウ、メタン、水素などの還元物質の酸化によりエネルギーを得る微生物が増殖する。特にイオウをエネルギー源にする SUP05 はさまざまな熱水プルームで報告され、伊豆小笠原弧の熱水プルームでは増加する微生物細胞の８割以上がこの微生物で占められている。一方、沖縄トラフの熱水系では、SUP05 が占める割合は低く、メタンをエネルギー源にする*Methylococcus*が出現することが明らかになっているが、未だに増殖する微生物の最大で約半分はわかっていない。そこで、本研究では今後の海底熱水鉱床開発に向けた熱水域の深海生態系のベースライン調査も含め、これまでに取得した沖縄トラフ熱水系における７サイトの熱水プルーム試料について NGS による高解像度微生物群集解析を行った。プルーム中では、*Thaumarchaeota*種の入れ替わり、*Thermogemmatispora*の増加傾向が認められ、一部のプルームでは、*Surfurimonas*の増加も認められた。熱水由来の無機化学成分利用可能性のあるこれらの微生物に加え、*Caulobacter*、*Sphingomonas*、*Marinobacter*などの従属栄養微生物の増殖も認められた。

キーワード: 熱水プルーム, 次世代シーケンサー, 微生物群集構造解析, 沖縄トラフ
Keywords: hydrothermal plume, MiSeq, microbial community structure, Okinawa Trough

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微生物による OCS 分解時の同位体分別係数の決定
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. Therefore, OCS should be validated for prediction of climate change, but the global OCS budget is imbalance. 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 composition and isotopic enrichment factor 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 enrichment factor of OCS during degradation via microorganisms, we performed laboratory incubation experiments using OCS-degradingmicroorganism.

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 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 the gas chromatography-isotope ratios mass spectrometry system using ^{32}S, ^{33}S, and ^{34}S fragment ions. Isotopic enrichment factor is determined by correspond to the Rayleigh isotope fractionation model.

In this experiment, The isotopic compositions (^{33}S and ^{34}S) of OCS were increased during degradation of OCS, indicated 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, Rayleigh isotope fractionation model were applied to determine isotopic enrichment factors ($^{33,34}\varepsilon = (^{33,34}S\text{-}_{33,34}S_{initial})/\ln f$). It is worth noting that$^{33}\varepsilon$ and$^{34}\varepsilon$ values determined by the experiments which showed 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 comparison of $^{33}\varepsilon$ and $^{34}\varepsilon$ values using some strains and the atmospheric implications for the OCS degradation in the present atmosphere are discussed.

キーワード: 硫化カルボニル, 同位体分別係数, 微生物
Keywords: Carbonyl sulfide, Isotopic fractionation factor, Microorganism

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Aspergillus oryzae の窒素安定同位体比における窒素可給性の影響
Nitrogen availability influences natural abundance 15N 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 15N (d15N) 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 D15N which means the difference between d15N of SMB (Soil Microbial Biomass) and d15N of microbial substrate (K2SO4 extractable nitrogen from soil) and microbial substrate C/N, and this result suggested D15N 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 15N because microbes release NH4+ which is depleted in 15N. However, previous study about the relationship between d15N-biomass and d15N-NH4+ in C/N controlled pure culture is conducted only by Collins et al. (2008) who used E. coli, and they could not detect d15N-NH4+ in a low concentration. Thus, the relationship between nitrogen availability and d15N-biomass is unclear. The purpose of our study is to reevaluate if biomass becomes enriched in 15N when microbes release NH4+ which is depleted in 15N. 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 d15N-biomass, NH4+ concentration and d15N-NH4+. In C/N5 and 10 where NH4+ concentration increased over time, we found that biomass was strongly enriched in 15N and NH4+ is depleted in 15N. Conversely, in C/N 30, 50 and 100 where microbes hardly released NH4+, we found that d15N-biomass got the almost same value of initial d15N-glycine. In the presentation, we will discuss more detail about the carbon and nitrogen mass balance during our experiment.

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埋没腐植層における土壌微生物の無機化ポテンシャルの定量的解析
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 ^{14}C-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 ^{14}C-glucose solution (100 μL; 10 - 300000 μM) was added to 1 g of field-moist soil and incubated for 24 h at 20 ℃. The $^{14}CO_2$ 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 (μM) in soil solution, V_{MAX} is the maximum mineralization rate (nmol g^{-1} h^{-1}), and K_M is the Michaelis constant (μM) 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, suggestion 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 μM. 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 μM 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