

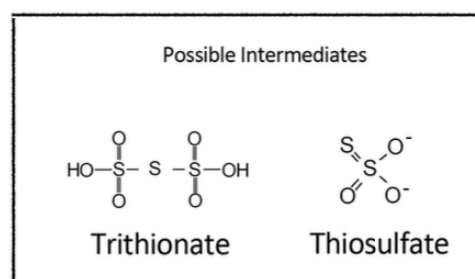
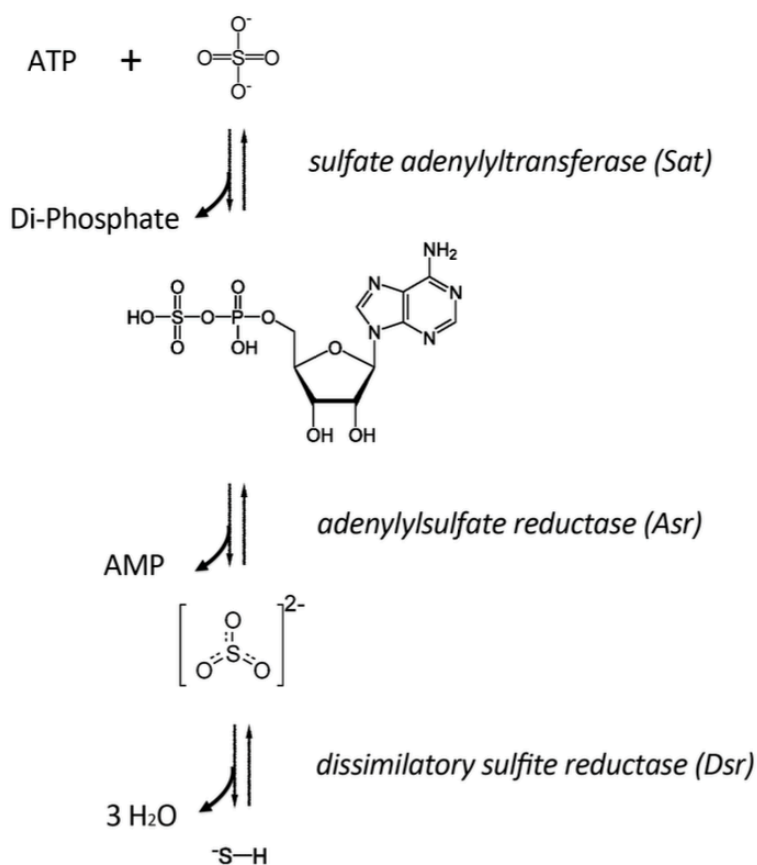
Biochemistry meets geochemistry: Sulfur isotope fractionation factor of the APS reductase and implications for interpreting biological sulfur isotopes signals.

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Sulfur isotopes have a long history of use to gauge the occurrence and extent of biogeochemical processes on Earth, and the advent of modern techniques including multiple isotope measurements and secondary isotope mass spectrometry indicate that much knowledge remains to be obtained through their analysis. A significant portion of microbial sulfur isotope fractionation occurs through the process of dissimilatory sulfate reduction (DSR); however very little data are available which describe the extent and variability of sulfur isotope fractionation at the level of the individual enzymes which carry out intracellular sulfur conversions. Here, I will present new data on the fractionation of sulfur by the enzyme adenylylsulfate reductase, which catalyzes a two electron reduction of adenylylsulfate to form sulfite (Asr in the figure below). The results will be interpreted in the context of enzyme evolution, cellular physiology, and also associated geochemical implications.

Keywords: Enzymes, Isotopes, Sulfate Reduction, Biogeochemistry



Microbial community function and response associated with metalloid redox transformations in the contaminated environment

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Arsenic (As) and antimony (Sb) are both naturally occurring toxic elements and are considered to be priority pollutants of interest by the USEPA. Although the concentrations of these toxic metalloids in the environments are generally low ($\sim 15 \mu\text{g g}^{-1}$ As and $< 1 \mu\text{g g}^{-1}$ Sb in soils), the elevated levels of As and Sb have been released via natural processes and anthropogenic activities. Both As and Sb can exist in four oxidation states (-III, 0, III and V), while they are mainly found in two oxidation states, trivalent (III) and pentavalent (V) in natural systems. The trivalent forms, As(III) and Sb(III) are highly reactive with thiol-containing proteins and are considered more toxic to biota than As(V) and Sb(V).

Despite their toxicity, microorganisms have developed mechanisms to tolerate and catalyze redox transformation of As and Sb. In this study, we characterized various microbial metalloid redox transformation pathways associated with As and Sb-impacted environments. The presence of indigenous microbial populations capable of metalloids transformation was examined by using both molecular biological and cultivation approaches. The genes coding for arsenite oxidase (*aioA*), which mainly catalyzes As(III) oxidation coupled to O_2 reduction, as well as anaerobic arsenite oxidase (*arxA*), known to catalyze As(III) oxidation coupled to nitrate reduction or photosynthesis, have been recovered from mine tailing soils. Successful cultivation of various As(III)-oxidizing bacteria confirmed the microbial attribute in As oxidation. The indigenous microbial populations catalyzing Sb redox transformation were also identified by successful cultivation of aerobic and anaerobic Sb(III)-oxidizing isolates and anaerobic enrichment cultures capable of reducing Sb(V). Furthermore, the soil microbial community response to co-contamination with As and Sb was examined by combined geochemical, cultivation and genomic approaches. The results showed varying inhibitory effects of co-contamination depending on the Sb chemical forms on As(III) oxidation rates, which were associated with selection of distinct As(III)-oxidizing population. Our study revealed the diversity of microbial metalloid redox pathways associated with polluted environments, indicating the potential importance of biological processes in geochemical cycling of As and Sb.

Keywords: Arsenic, Antimony, arsenite oxidase, arsenate reductase

Diversity and metabolic and genomic characteristics of prokaryotes in deep granitic rock independent of photosynthesis

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Granite is a main constituent of the upper terrestrial crust, which potentially harbor one of the largest microbial habitats throughout the Earth's history. Microbial investigations of deep granitic aquifers require drilling to avoid contamination from surface microorganisms. Although drilling from underground facilities is commonly practiced to avoid the contamination, the construction of underground facilities tends to cause mixing of shallow and deep groundwater. In the granitic basement where the Mizunami underground research laboratory (URL) was constructed, a highly fractured domain (HFD) and a sparsely fractured domain (SFD) were horizontally drilled from underground tunnels at 300 meter below ground level (mbgl). Based on previous biogeochemical site-characterizations, it was revealed that groundwater mixing is not evident in the SFD borehole (SFDB), and that sulfate reduction was biologically mediated in the HFD borehole (HFDB) before the construction. The purpose of this study is to reveal the diversity and metabolic and genomic features of microbial communities inhabiting the deep granitic environment at the Mizunami URL.

First of all, microbial communities inhabiting the SFD borehole (SFDB) were characterized. As the underground drilling introduced O₂ in the aquifer, the influence of drilling was clarified by monitoring temporal shifts over 4 years. Immediately after drilling, aerobic β -proteobacterial species were dominant, while the phylum Nitrospirae became dominant after 3 years, the close relatives of which were detected exclusively from deep subsurface environments. One-week incubation of the Nitrospirae-dominated community with ¹³C-labeled bicarbonate and 1% H₂ and subsequent single-cell imaging with nanometer-scale secondary ion mass spectrometry (NanoSIMS) demonstrated that the assimilation of ¹³C-labeled bicarbonate. From these results, it is implied that the granitic aquifer hosts microbial communities isolated from the photosynthetic ecosystem.

Secondly, microbial communities found in the HFDB were microbiologically and hydrogeochemically investigated. The HFDB groundwater was dominantly colonized by archaea suspected to mediate anaerobic methane oxidation (AOM), because of their phylogenetic relationship with anaerobic methanotrophic archaea subtype-2d (ANME-2d). To demonstrate whether AOM is mediated by the subsurface archaea, statistical analyses of microbial distributions and environmental factors, metabolic activity measurements of AOM and metagenomics-enabled genomic reconstruction were performed. The correlation between archaeal abundance and sulfate concentration was statistically validated. Two-week incubation of microbial cells with ¹³C-labeled methane demonstrated anaerobic oxidation of methane (AOM) linked to sulfate reduction. A draft genome of the subsurface archaea contained functional genes required for AOM. In addition, the subsurface archaea were dominantly found in SFDB groundwater outflowing during drilling, which excludes the possibility that facility construction and underground drilling artificially stimulated the growth of the subsurface archaea. It is therefore concluded that a microbial ecosystem energetically dependent on methane does exist in the deep granitic environment. The deep granitic biosphere revealed in this study is strongly suggested to be nearly independent of photosynthesis-derived organic matter. However, this inference is inconsistent with the dominance of Parcubacteria, many members of which are reported to thrive near-surface environments supplied with photosynthetic organic matter. Phylogenetic analysis demonstrated that Parcubacteria lineages detected from the Mizunami groundwater were novel and distantly related to those from the near-surface biosphere. As Parcubacteria is positioned at the root of a universal tree of life recently resolved by

genomic advancement, it is likely that the early life might be hosted in the granitic biosphere before the emergence of phototrophic prokaryotes. It is further suggested that the deep granitic environment has been a stable microbial habitat, even when meteorite bombardment was frequent on Earth before 3.5 billion years ago.

Keywords: Deep biosphere, Anaerobic oxidation of methane, Candidate phyla radiation

Cultivation of microbial dark matters from marine sediments: novel bacteria affiliated with uncultured groups of the phylum *Chloroflexi*

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Subseafloor sediments are known to be a critical component of marine biogeochemical cycles across Earth. A number of previous biochemical and molecular biological studies have demonstrated that subseafloor sedimentary environments possess an enormous microbial biomass, comprised of microorganisms with unique function and phylogenetic diversity. However, the detailed physiological and metabolic properties of individual species are largely unknown because most subseafloor sedimentary microbes have evaded conventional cultivation methods. As a novel approach to cultivation of uncharacterized subseafloor microorganisms, we have implemented a down-flow hanging sponge (DHS) continuous-flow bioreactor system to initiate *in vitro* enrichment and successfully obtained cultures of target organisms [1, 2, 3]. In this presentation, we report cultivation, partial physiological and genomic characterization, of two new bacterial strains belonging to uncultivated lineages of the phylum *Chloroflexi* with high prevalence across subseafloor environments and yet undefined ecological function.

One of the *Chloroflexi* bacteria is affiliated with an uncultured group of the class *Anaerolineae*, and it has been in pure culture (strain MO-CFX2). The strain was isolated from a methanogenic community enriched in a DHS reactor, which was originally obtained from subseafloor sediments off Shimokita Peninsula, Japan. Strain MO-CFX2 grew in an anaerobic medium containing yeast extract and peptone. The genome reveals that strain MO-CFX2 may degrade glucose and lactate and also respire halogenated compounds. Currently, we are going to characterize these and other physiological traits in detail.

The other *Chloroflexi* bacterium belongs to an uncultured lineage of the class *Dehalococcoidia*, and it is being purified (strain MK-GIF9). This strain was obtained from an anaerobic methane-oxidizing DHS reactor inoculated with biomass collected from Nankai Trough methane seep sediments. The genomic analysis of strain MK-GIF9 indicates that the bacterium can use only several amino acids as energy sources. We also found a glycine fermentation pathway that produces formate as an end product. Based on the genomic analysis, we suspected that the growth of strain MK-GIF9 can be promoted if the metabolic byproduct (formate) is continuously removed by a formate-scavenging partner (*e.g.*, methanogens). Indeed, co-cultivation of MK-GIF9 with a formate-utilizing methanogen successfully stimulated methanogenic glycine degradation and promoted growth of strain MK-GIF9. The specialized ability to cooperatively degrade amino acids sheds light on the poorly understood fate of amino acids in subseafloor sediments. Taken together with the ubiquity and predominance of *Chloroflexi* bacteria in subseafloor environments, our results implicate that amino acids including glycine may be one of the important precursors for methane production in subseafloor sediments.

References; [1] Imachi et al., (2011) *ISME.J.* 5., 1923 –1925. [2] Aoki et al., (2014) *PLOS ONE.* 9, (8), e105356 [3] Inagaki et al., (2015) *Science.* 349, (6246), 420 –424.

Keywords: Amino acids, Chloroflexi bacteria, Methanogenesis

Expanding the Frontiers of the Virosphere: Viruses in the Subsurface

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Viruses are the most abundant biological entities on Earth and have been identified everywhere that life has been found. In surface environments, viruses are known to be mediators of global biogeochemical cycling by infecting and killing host cells, thereby releasing biomass-bound carbon, nitrogen, and other elements. However, the role that viruses play in subsurface systems remains poorly understood. Viral infection of subsurface microbial communities may be widespread as shown by the presence of viral sequences, integrated prophage, as well as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) sequences. Full genomes of 56 Bacteria and Archaea isolates from various subsurface environments were analyzed for viral genes and CRISPR sequences. Integrated viral genes were detected in 50% of subsurface genomes, and CRISPR sequences were detected in 79%, suggesting that viral infection may be prevalent and may play an important role in the subsurface. The activity of subsurface viruses is also likely correlated with the local geochemical conditions which influence host metabolism. Viruses and cells were enumerated in groundwater from a shallow aquifer at Rifle, Colorado (USA). Viruses and cells were positively correlated (Spearman's $r_s=0.46$, $p<0.05$ and $r_s=0.54$, $p<0.05$; respectively) to dissolved organic carbon (DOC) concentrations, with both being more abundant in organic-rich regions of the aquifer. Experimental stimulation of microbial activity within a section of the aquifer through the injection of oxygenated groundwater resulted in an increase in virus-to-cell ratio by 1.8-3.4 times, indicating that subsurface viruses are responsive to changes in geochemical conditions. A possible consequence of subsurface viral infection may be cell lysis, which would result in a flux of organic matter from biomass to environmental pools of dissolved organic matter/particulate organic matter (DOM/POM). As a result, viruses have the potential to influence carbon biogeochemistry in subsurface systems. This process may be especially important in deep subsurface environments where energy and nutrients are limiting. In order to expand the frontiers of the virosphere, it will be necessary to determine the nature of viruses under conditions of extreme energy limitation in the deep subsurface and how viruses may be playing a role in the functioning of the deep subsurface ecosystem.

Keywords: subsurface, virus, biogeochemistry, organic carbon

Ecology of willow in the Arctic and its feedback to Indigirka river condition and its tributaries using stable isotopic methods

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In northeast Siberian Arctic, rapid warming have been observed (Serreze and Barry, 2011; ACIA, 2004; IPCC, 2013) and Yana-Indigirka-Kolyma lowland located there has a large area. River changes is one of key parameters to control material cycling through water level changes. From our former work (Morozumi, in preparation), we found willow growing widely along river, so that willow there can be good records of river condition. Foliar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are known as integrated indicators of environment, meanwhile plant $\delta^{18}\text{O}$ is expected to record source water isotope ratio.

This study was conducted in taiga-tundra ecosystem along Indigirka river (70.63°N, 147.91°E). Three transect sites were set up along mainstream and also tributary. In each site, sampling was made at three points along a transect from river to land. Willow current year shoot were collected, other samples including river water, soil water, willow stem were collected weekly in summer, both 2015 and 2016. In the end of July in both year, there are also widely random sampling of willow current year shoot. In 2015, N content ranged from 1.5% to 3.9% and $\delta^{15}\text{N}$ from -5.6‰ to 5.3‰ showed clear spatial variation with high N content and $\delta^{15}\text{N}$ near river and low N content and $\delta^{15}\text{N}$ on land in 2015. No clear differences was observed for willow foliar $\delta^{13}\text{C}$ which ranged from -31.1‰ to -25.3‰. In 2016, same trend as last year was found in foliar $\delta^{15}\text{N}$ (from -6.84‰ to 4.91‰) in large area and showed clear spatial variation from water logging points ($0.6 \pm 2.6\%$), near water points ($-2.3 \pm 1.05\%$) and land points ($-3.8 \pm 0.9\%$). This maybe caused by denitrification and enhanced leaching in waterlogging points. Willow foliar $\delta^{13}\text{C}$ (-31.6‰ to -25.7‰) and N content (1.4% to 4.3%) didn't show difference. In summer of 2015, the $\delta^{18}\text{O}$ value of mainstream was little different from tributaries, because the relative high water level in summer of 2015. Soil water $\delta^{18}\text{O}$ at the nearest location of river was affected by river water, and in addition, stem water $\delta^{18}\text{O}$ varied with that of 20cm-soil water ($R^2=0.55$, $p<0.001$). At the points by river, cellulose $\delta^{18}\text{O}$ of current year shoot along tributary was little higher than along mainstream. The points by river also has lower $\delta^{18}\text{O}$ compared to the points on land. These together proved $\delta^{18}\text{O}$ of willow could record $\delta^{18}\text{O}$ of river. In future work, tree-ring studies to reconstruct water level and the water level differences between mainstream and its tributaries will be challenged.

This study showed the physiology and phenology of willow growing in Indigirka lowland and tried to reveal the relationship with river and willow growing from stable C and N isotopic aspect. And also tried to figure out source water changes which related to river water level changes by $\delta^{18}\text{O}$ ratios of willow.

Keywords: Eastern Siberia, Taiga-Tundra ecosystem, Willow, Stable isotope, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, $\delta^{18}\text{O}$

Experimental study of hypervelocity impact of meteoritic material into liquid water in an open system for better understanding of the fate of extraterrestrial organics in the Hadean ocean

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Unravelling the origin(s) of prebiotic organic materials that constituted protocells on the early earth is important to constrain the conditions for the emergence of life and the chemical diversity of life in the universe. One proposes that biologically relevant organic materials were mostly produced through atmospheric chemistry and mineral-water interactions from simple compounds (N_2 , CO_2 , CO , CH_4) available in the early earth, whereas the other proposes that a significant amount of extraterrestrial organic materials, that were produced in the early solar system, protoplanetary disk, and molecular clouds, were delivered to the early earth and served as key components of protocells. If the latter hypothesis is correct, most of the extraterrestrial organic materials in meteorite should have been survived from thermal decomposition during hypervelocity impact into the Hadean ocean (>3 km/s), because geological evidences suggest that the ocean was already present but the continent was almost absent in the Hadean earth. However, the fate of extraterrestrial organic materials during oceanic impact is poorly understood due to the lack of experimental knowledge about the physicochemical processes associated with the hypervelocity impact of meteorite into liquid water. For better understanding of the physicochemical processes associated with the hypervelocity impact of meteorite into liquid water and the fate of impactor, we have newly establish a methodology of experimental impact in an open system that can simulate the Pressure-Temperature path of oceanic impact most faithfully. In this meeting, we report experimental results that polycarbonate and stainless steel projectiles impacted into liquid water at a velocity of 4-6 km/sec.

Keywords: hypervelocity impact, hedean ocean, meteorite

Evolution of amino acids biosynthetic systems—Diversity of serine biosynthetic enzymes

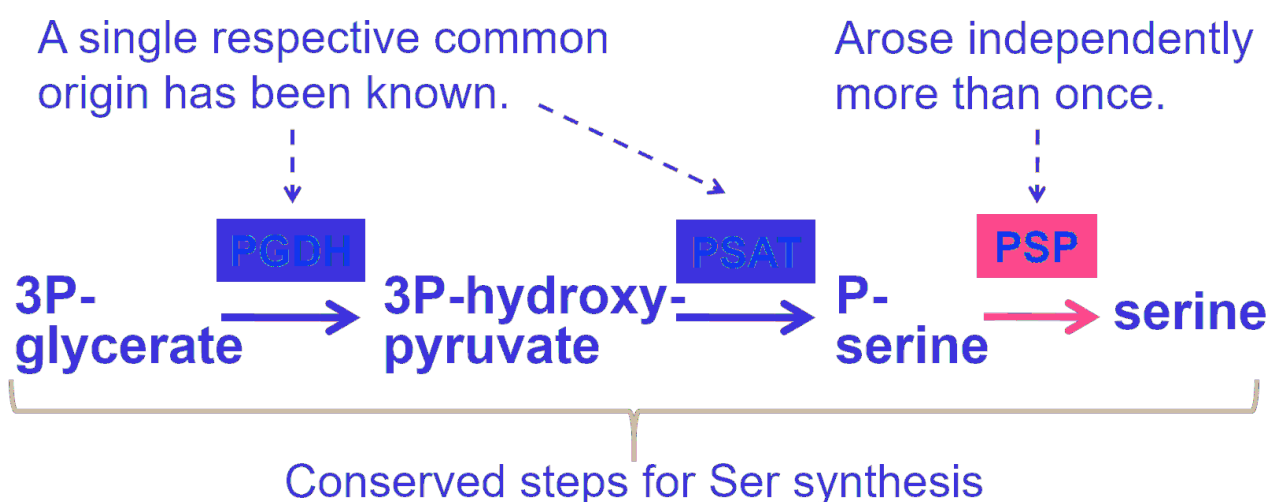
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Amino acids are the building blocks of proteins and fundamental for life on Earth. Therefore, amino acid biosynthetic pathways are essential systems. Exploring ancestral amino acid biosynthetic pathways is important for understanding the evolution of early life on Earth. Ancestral serine biosynthetic systems were investigated by analyzing their conservation and diversity in modern organisms, resulting in the discovery of more diversity among serine producing enzymes than previously known. Here, I present on the newly discovered diversity of serine biosynthetic enzymes and consider what this means for the evolution of life.

A variety of organisms are known to synthesize serine from 3-phosphoglycerate, an intermediate of glycolysis/gluconeogenesis, by a three-step enzymatic reaction (Fig). Each respective enzyme had been thought to arise from a common origin. However, detailed genomic analysis using the MGD database revealed that a variety of organisms lack a gene for phosphoserine phosphate (PSP), an enzyme catalyzing the last step of the reaction, while possessing the first two enzymatic genes. One representative is a thermophilic and hydrogen-oxidizing autotrophic bacterium belonging to the phylum Aquificae. Detailed biochemical analyses revealed that Aquificae possess a novel protein that shares the same function which has no homology to the classical PSP. Furthermore, genomic analyses revealed that the novel PSP exists not only in Aquificae but is also distributed among diverse bacterial phyla which lack the classical PSP. The distribution of classical and novel PSPs suggests that both PSPs arose independently before the division of existing bacterial phyla.

Keywords: amino acid, evolution of life, metabolism



The microbial nitrogen pump in the ocean: Role of heterotrophic bacteria in the ocean DON pool indicated by compound-specific $\delta^{15}\text{N}$ analysis of amino acids

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Dissolved organic nitrogen (DON) is one of the most important - but perhaps least understood - components of the modern ocean nitrogen cycle. We explored the use of compound-specific nitrogen isotope analysis of amino acids ($\delta^{15}\text{N}_{\text{AA}}$) of DON as a new approach to examine relative sources and transformation processes of the main detrital organic nitrogen form in the ocean water column. We measured $\delta^{15}\text{N}_{\text{AA}}$ distributions in high-molecular-weight DON (HMW DON) and suspended particulate organic nitrogen (PON) samples from various oceanic regions including the oligotrophic open ocean (the North Pacific Subtropical Gyre) and a coastal upwelling system (the California Current System). A new analytical approach using HPLC purification of amino acids achieved far greater $\delta^{15}\text{N}_{\text{AA}}$ measurement precision for DON than earlier work, allowing us to resolve previously obscured differences in $\delta^{15}\text{N}_{\text{AA}}$ signatures, both with depth and between ON pools. The $\delta^{15}\text{N}_{\text{AA}}$ values of DON in the various oceanic regions and depths (surface to mesopelagic) suggest mainly heterotrophic sources, with the mesopelagic and upwelled DON bearing signatures of far more degraded material. These results contrast with a previous proposal that DON are essentially "pre-formed" in the surface ocean, undergoing little further change with depth. Together with the results of amino acid enantiomers (D/L), these results suggest that transformation by heterotrophic bacteria is the main source for DON in the ocean.

Keywords: Dissolved Organic Matter, Marine Nitrogen Cycle, Amino Acids, Heterotrophic Bacteria, Compound-Specific Isotope Analysis, Deep Ocean

Iron Isotope Signatures of Gastropods at the Hydrothermal Vent

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Variation in iron isotope ratio ($\delta^{56}\text{Fe}/^{54}\text{Fe}$) of biological samples can reflect principally the differences in the Fe absorption efficiency from food sources. In the marine environment, several pioneering studies have revealed that $\delta^{56}\text{Fe}$ of marine organisms did not vary significantly among different organs of an organism, or among different trophic levels, due to the availability of Fe being very limited in seawater. In contrast, Fe abundance in hydrothermal vent fields (~10%) is many fold higher than most of seawater (~10⁻⁷%). This evoked us to consider that the Fe bio-cycle at hydrothermal vents is likely to be different from most other marine environment. However, the detailed understanding concerning the Fe absorption and metabolism in organisms inhabiting hydrothermal vents remained obscure.

To investigate the Fe bio-cycle at the Fe-enriched hydrothermal vent environment, the Fe isotope ratio of two vent endemic snails with thioautotrophic endosymbionts were measured. This included the ‘scaly-foot gastropod’ (*Chrysomallon squamiferum*) (n=5) and *Gigantopelta aegis* (n=5), which are members of the same family Peltospiridae and live side-by-side in the Longqi vent field, Southwest Indian Ridge. The $\delta^{56}\text{Fe}$ of various tissues (muscle, gill, blood, heart, endosymbiont-containing oesophageal gland), shell, and scales on the foot were measured using a multiple collector ICP-mass spectrometer (MC-ICP-MS), after the decomposition of organic component and the purification of Fe through an anion-exchange chromatography.

The resulting of $\delta^{56}\text{Fe}$ for muscle, gill, blood, and heart from the ‘scaly-foot’ was approximately the same as the $\delta^{56}\text{Fe}$ value of the environment, indicating high absorption efficiencies of Fe. On the other hand, the $\delta^{56}\text{Fe}$ of such tissues in *Gigantopelta aegis* was about 1‰ lower than the ‘scaly-foot’ and the environment, indicative of lower Fe absorption efficiencies. No differences in $\delta^{56}\text{Fe}$ was detected in the endosymbiotic bacteria hosting oesophageal gland between the ‘scaly-foot’ and *Gigantopelta aegis*; but only in the ‘scaly-foot’ the isotopic ratio was different from other tissues. These results suggest that the ‘scaly-foot’ has high Fe absorption efficiency from the environment but low Fe supply efficiency to its endosymbionts, whereas *Gigantopelta aegis* has low Fe absorption efficiency but high Fe supply efficiency to its endosymbionts. The Fe isotope signatures obtained here revealed clear differences in the absorption efficiency of dietary Fe, between two closely related snails in the same environment with similar lifestyle. These signatures will also be discussed in relation to two key adaptations of these snails, namely hosting endosymbiotic bacteria in an internalized organ and having an iron-compound layer on the shell and scales.

Keywords: Fe stable isotope, Fe bio-cycle, hydrothermal vent, scaly-foot gastropod, *Gigantopelta aegis*, multiple collector ICP-mass spectrometer (MC-ICP-MS)

Multi-element, compound-specific isotope analysis of chloropigments: Insights into the biogeochemical cycle of the microbial mat in the shallow hypersaline environment

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In the last several decades, compound-specific isotope analysis of source-specific organic compounds (biomarkers) enabled scientists to access essential information of modern and past environments. Among numerous biomarkers, chloropigments are particularly powerful because they are synthesized only by photoautotrophs, and their tetrapyrrole nucleus is composed of carbon and nitrogen, thus providing constraints on the physiology of the photoautotrophs and the biogeochemical cycles of the photic zone from both carbon and nitrogen aspects. Another unique characteristic of the chloropigments is that they are metallo-organic complexes with Mg^{2+} as a central metal ion, potentially providing the opportunity to investigate into the biogeochemical processes that involve Mg. In this study, we investigated carbon, nitrogen, and magnesium isotopic compositions ($\delta^{13}C$, $\delta^{15}N$, $\delta^{26}Mg$) of the chloropigments isolated from the hypersaline benthic microbial mat and gypsum crusts formed in the solar salterns of Trapani, Italy. The mats and the gypsums exhibit stratification of yellow, green, and pink layers, which are inhabited mainly by photosynthetic bacteria such as cyanobacteria and purple sulfur bacteria, together with diverse chemotrophic and heterotrophic microorganisms. We measured isotopic compositions of chlorophyll *a* (Chl *a*) originating from cyanobacteria living in the upper layers, and bacteriochlorophyll *a* (BChl *a*) from purple sulfur bacteria living directly below. The relationship between Chl *a* and BChl *a* were similar for both $\delta^{13}C$ and $\delta^{15}N$ in all examined samples. Lower $\delta^{13}C$ of BChl *a* (ave. -22.9%) compared to Chl *a* (ave. -17.2%) suggests that purple sulfur bacteria are assimilating ^{13}C -depleted carbon source supplied by mineralization of the organic matter in the deeper parts of the mats and gypsums. Striking difference of $\delta^{15}N$ between Chl *a* (ave. 13.6%) and BChl *a* (ave. -2.5%) indicate that different substrate is utilized by cyanobacteria and purple sulfur bacteria. We infer that cyanobacteria are assimilating ^{15}N -enriched ammonium because $\delta^{15}N$ of nitrate ranged between -0.3 to 3.8% , indicating that nitrate is not the main nitrogen source. Unlike $\delta^{13}C$ and $\delta^{15}N$, $\delta^{26}Mg$ of Chl *a* and BChl *a* did not show any clear trend; $\delta^{26}Mg$ of Chl *a* varied between -1.77% and -0.39% and that of BChl *a* -2.13% and -0.11% , on DSM3 scale. Moreover, $\delta^{26}Mg$ of Mg^{2+} in the brine ranged between -0.88 to -1.12% , indicating that there are apparently both positive and negative isotopic fractionation between the source Mg and the chloropigments. Although more fundamental studies are necessary to understand the mechanisms determining Mg isotopic signature of the chloropigments, our results imply that $\delta^{26}Mg$ of chloropigments may vary substantially in response to the changing physiological states and environmental conditions of the photoautotrophs.

Keywords: chloropigments, carbon isotope, nitrogen isotope, magnesium isotope, microbial mat, hypersaline environment



Perspective: The macrofaunal activity effects on the ecosystem at extreme environments

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Drastic chemical reactions occurred at extreme environments such as vents, seeps and large organic-falls (e.g. whale-falls and sunken woods). The most of reactions thought to be performed by microbial activities at least at the close proximity of sediment-water interface. It would be true, however, the process will be enhanced by macrofaunal activities. I will demonstrate those macrofaunal activities and its behavior at the extreme environments.

The macrofauna adapted to those environments often have chemosymbiotic bacteria, which usually utilize sulfides and oxygen, for their food (or energy) source. Those macro-organisms have tolerant with high concentrations of hydrogen sulfide and low oxygen level, and thus, the organisms can take the symbiotic microbes to their favorable position (generally the position is equal to the redox boundary). In the case of vent shrimp *Rimicaris*, which have epibiotic bacteria in their gill chambers, swims just around chimneys with active venting to keep their position to cultivate harboring bacteria.

In addition, the host macro-organisms would maintain the place of reactions, e.g. in their body or burrow, and pumping up/down fluids containing reductants/oxidants to control appropriate amount of chemical species for symbiotic microbes. Infaunal solemyid bivalves make Y-shaped burrow and they have ability to suck oxygenated sea-water into the sediment and pumping up pore water which contains hydrogen sulfide through their burrow.

In other cases, bone-eating worms, e.g. *Osedax*, and boring bivalves mechanically and/or chemically decompose hard substrates, e.g. bones of whales and sunken wood, and accelerate decay of the large organic carcasses, e.g. whale carcasses, and sunken wood trunk, and create new environments for other organisms.

Some of those macrofaunal activities at the extreme environments have also been found in the fossil record and can be traced back more than 100 million years. However, long-term relationships between macro-organisms and microbes at extreme environments with chemical and physical aspects have been paid a little attention so far.

Keywords: Extreme Environment, Vent, Seep, Invertebrates

Eukaryotic diversity in late Pleistocene marine sediments around a shallow methane hydrate deposit in Japan Sea

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Eukaryotic DNA has been detected from detrital organisms such as planktonic remains, spores, cysts, and pollen from marine and lacustrine sediments, associated with the intense upwelling and the high primary productivity. However, a large proportion of deposited eukaryotic DNA is aerobically biodegraded in shallow marine sediments. Cold seep sediments are often anaerobic near the sediment-water interface, so eukaryotic DNA in such sediments is expected to be preserved. As the abundance and biodiversity of eukaryotic DNA in deeply buried marine sediments are largely unknown, we investigated marine sediments at depths up to 31.0 meters below the seafloor (mbsf; 97 ka) around a shallow methane hydrate deposit in the eastern Japan Sea. Quantitative PCR analysis revealed the reproducible recovery of eukaryotic DNA in deep marine sediments in the vicinity of the methane hydrate deposit. Pyrosequencing of an 18S rRNA gene variable region generated total 9,366 reads from 5 samples, which was sufficient to cover the biodiversity based on rarefaction curves. Phylogenetic analysis revealed that most of the eukaryotic DNA originated from radiolarian genera of the class Chaunacanthida, which have SrSO₄ skeletons, the seagrass genus *Zostera*, and the seaweed genus *Sargassum*. Eukaryotic DNA originating from planktonic fauna and land plants were also detected. Diatom sequences closely related to *Thalassiosira* spp., known as indicator species for cold climates, were obtained from sediments deposited during the last glacial period (MIS-2). Land plant sequences of the genera *Alnus* and *Ulmus* were found in sediments deposited during the warm interstadial period (MIS-3). These results suggest the long-term persistence of eukaryotic DNA from terrestrial and aquatic sources in marine sediments associated with cold seeps, and that the genetic information from eukaryotic DNA from deeply buried marine sediments associated with cold seeps can be used to reconstruct environments and ecosystems from the past.

Keywords: Ancient DNA

Wind erosion alters ecosystem carbon balance and carbon sequestration potential in a temperate grassland

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Wind erosion and deposition of soil could greatly alter soil C pool and ecosystem C balance in arid and semi-arid ecosystems. Land use change is one of the most important driving forces influencing the intensity of wind erosion in those regions. However, how wind erosion under different land use scenarios will affect ecosystem C balance and its capacity for future C sequestration are poorly quantified. Here, we established an experiment in Xinlinhot, Inner Mongolia, and simulated different intensity of land use in grassland: control, 50% of aboveground vegetation removal (50R), 100% vegetation removal (100R) and tillage (TI). We monitored lateral and vertical carbon flux components from 2013 to 2016. We found that wind deposition resulted in net C gain during growing seasons, whereas wind erosion induced net C loss. Adding up the lateral C flux across the whole year, ecosystems under control behaved as a C sink of $44.85 \text{ g C m}^{-2} \text{ yr}^{-1}$. However, under relative degradation ecosystems, the disturbance managements resulted in a net C loss, and the loss strength increased from $3.35 \text{ g C m}^{-2} \text{ yr}^{-1}$ under 50R to $135.86 \text{ g C m}^{-2} \text{ yr}^{-1}$ under TI and the erosion intensity increased rapidly with experimental duration. Land use also significantly altered the biological vertical carbon flux. The net ecosystem exchange (NEE) shifted from a net uptake of $86.85 \text{ g C m}^{-2} \text{ yr}^{-1}$ under control to a net emission of $35.12 \text{ g C m}^{-2} \text{ yr}^{-1}$ under TI treatment. With the increase in land use intensity, the contribution of lateral C flux to ecosystem C balance increased from 34% under control to 79% under TI. Wind erosion caused by land use changes not only result in dramatically surface soil C loss, but also significantly decrease soil C sequestration potential by altering soil texture. Permanent losses of organic carbon sequestration potential were 0.10 kg C m^{-2} , 0.12 kg C m^{-2} and 0.31 kg C m^{-2} in 50R, 100R, and TI, respectively. Overall, our study demonstrated that wind erosion could result in irreversible soil degradation and shape the landscapes of arid and semi-arid regions in the long term. Appropriate land use is critical to protect grassland ecosystems from being crashed.

Keywords: temperate grassland, land use changes, wind erosion/deposition, C balance, C sequestration potential

Evaluation the physiological effects of extremely high temperature on temperate paddy rice using temperature gradient chamber

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Changes in growths and physiological responses of food crops impacted by climate change are critical for the yield ability. In particular, understanding the effects of high temperature on temperate paddy rice will be important for the food security in East Asia. Under the warmed condition, the lower quantity and quality of gain yield have been reported. The heat wave of 2016 in South Korea was the worst in over 22 years. To understand the effects of extremely high temperature on temperate paddy rice, we examine the measurement data for 2016 from TGC (Temperature Gradient Chamber) where the air temperature was raised gradually 0°C to 3°C. NDVI (Normalized Difference Vegetation Index), PRI (Photochemical Reflectance Index) and chlorophyll fluorescence observations were analyzed how the high temperature affected the physiological activities of rice. The result was shown that the stress in photosynthesis efficiency was increased according to warm up conditions. Further, the infertile ears were mostly occurred in the 3°C higher temperature condition than 2016. Moreover, the remained photosynthate by those infertility caused the more tillers and the second ears emerged apart from the infertility ears. However, the second ears had generally low yield ability. The chlorophyll content and vegetation index did not decline even in the late grain filling period. Our result represented that the warmed condition in 2016 would be the critical limit for the stable yield of temperate paddy rice. Continuous TGC experiments will be expected to enhance understandings in the growth and physiological response of crops in the future climate change, and become a foundation of the adaptive technology development.

Keywords: Global warming, Heat stress, temperature gradient chamber(TGC)