Japan Geoscience Union Meeting 2015
(May 24th - 28th at Makuhari, Chiba, Japan)

BGM22-01 Room:105 Time:May 26 09:00-09:15

Multiple analyses to chase the signature of direct impact of rainfall into groundwater in Mt. Fuji

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A huge amount of groundwater is stored in subsurface environment of Mt. Fuji, the largest volcanic (basalt) mountain in Japan. There distribute many springs flushing out between lava (high permeability) and underlying older lava (low permeability) at various points of the foot. Based on the concept of piston flow transport an apparent residence time was estimated to ca. 30 years by ^{36}Cl/Cl ratio (Tosaki et al., 2011). However, this number represents an averaged value of the residence time of groundwater mixed before flushing out. On the other hand, we found that pH of spring water in the lower part of the foot of Mt. Fuji decreased shortly after the typhoon in August 2011 which suggested the newly supplied rainwater was mixed into groundwater. Thus, we try to chase signature of direct impact of rainfall into groundwater from multiple analyses to elucidate the routes of groundwater under the torrential rainfall. Though analyses of groundwater chemistry show just an averaged value, microbial DNA analysis could suggest the routes of transport; if thermophilic microbial DNA is detected this suggests at least a part of groundwater must originated from the environment $>$ca. 40^{o}C (=600 m deep in Mt. Fuji). Thus, we employed three different tracers; stable isotopic analysis (δ^{18}O and δD), chemical analysis (concentration of silica) and microbial DNA analysis.

Stable oxygen isotopic ratio of shallow groundwater became higher than usual value reflecting torrential rainfall and the concentration of silica decreased after the torrential rainfall amounting more than 300 mm. In addition, the density of Prokaryotes in shallow groundwater apparently increased. These findings indicate a direct impact of rainfall into groundwater was observed after torrential rainfall with more than 300 mm in the studied geological setting. This did not appear when rainfall did not exceed 100 mm/day. Increase in the density of Archaea at deep groundwater after the torrential rainfall suggests a possible mixing of deep groundwater which was pushed by piston flow transport as an indirect impact of rainfall into deep groundwater, if it is true to this geological setting that Archaea is predominant in deep subsurface environment as was suggested. Microbial DNA can give possible information about the route of groundwater.

Keywords: groundwater, strong rainfall, abnormal flash out

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BGM22-02 Room:105 Time:May 26 09:15-09:30

Higher diversity and abundance of denitrifying microorganisms in environments than considered previously

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Denitrification is an important process in the global nitrogen cycle. The genes encoding NirK and NirS (*nirK* and nirS), which catalyze the reduction of nitrite to nitric oxide, have been used as marker genes to study the ecological behavior of denitrifiers in environments. However, conventional polymerase chain reaction (PCR) primers can only detect a limited range of the phylogenetically diverse *nirK* and *nirS*. Thus, we developed new PCR primers covering the diverse nirK and nirS. Clone library and qPCR analysis using the primers showed that nirK and nirS in terrestrial environments are more phylogenetically diverse and 2-6 times more abundant than those revealed with the conventional primers. RNA- and culture-based analyses using a cropland soil also suggested that microorganisms with previously unconsidered *nirK* or *nirS* are responsible for denitrification in the soil. PCR techniques still have a greater capacity for the deep analysis of target genes than PCR-independent methods including metagenome analysis, although efforts are needed to minimize the PCR biases. The methodology and the insights obtained here should allow us to achieve a more precise understanding of the ecological behavior of denitrifiers and facilitate more precise estimate of denitrification in environments.

Keywords: denitrification, nitrite reductase gene, nirS, nirK

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BGM22-03 Room:105 Time:May 26 09:30-09:45

Soil microbes shape nitrogen isotopic signatures of soils: a linkage between the ecological stoichiometry and d15N.

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Nitrogen (N) is an essential, although ecologically limiting, nutrient in many terrestrial ecosystems. It is thus critically important to understand N cycles in terrestrial ecosystems to project their responses to expected changes in environments such as the increase in anthropogenic N input and CO2 concentration. Natural abundance of N isotopes (d15N) has been used to get insights into N cycles in the ecosystems because the d15N signature can provide unique information on the naturally-occuring processes in the intact ecosystem. Interpretations of global dataset of plant d15N (e.g. Craine et al. 2009) and soil d15N (e.g. Houlton and Bai 2009, Craine et al. 2015) have been proposed to explore the important flux/parameter in N cycles which are difficult to measure (such as N availability and denitrification loss). In most of these cases, the rule of the thumb in d15N interpretation is that soil loses 15N-depleted N during decomposition (more strictly, mineralization and leaching/denitrification loss), which is also the fundamental concept for marine sediment d15N (e.g. Robinson et al. 2012, Tesdal et al. 2013). Even this ”15N-depleted N loss” concept is easy to follow, the direct (experimental) evidence for the isotopic fractionation during N mineralization or decomposition is suprisingly scarse. Although long-term lab incubation of soil samples revealed the expected d15N increase with the decrease in N concentration (Nadelhoffer and Fry 1988), litter-bag experiments (Melillo et al. 1989; Connin et al. 2001) did not show this expected d15N change during litter decomposition. Thus the gap between field observations and lab experiments in the d15N trend calls the review of the fundamental concept for the interpretation of soil d15N.

In the presentation, I will summarize the d15N data we obtained in the last five years on soil bulk N, several extractable organic N (EON), extractable inorganic N (EIN) in soils and soil microbial biomass (SMB), which are now relatively easy to measure with denitrifier method (Sigman et al. 2001, Houlton et al. 2006). The d15N of SMB is generally higher than d15N of other N compounds, which should be interpreted as a consequence of carbon and N stoichiometry (or N mineralization; Dijkstra et al. 2008). This high d15N of SMB can complement the interpretationo of soil d15N variations – the large d15N differences between organic layers and mineral soils often observed in soil profiles, the low d15N in wet/cold ecosystems and the high d15N in dry/hot ecosystems in the global soil d15N trend, and the high d15N of the microbially-processed soil fractions.

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BGM22-04 Room:105 Time:May 26 09:45-10:00

Isotopic fractionations during nitrogen removal in the activated sludge

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Anammox is considered to be an important nitrogen removal pathway in the ecosystem. However, it is still unknown how much the anammox can contribute to the total nitrogen loss in the ecosystem. Natural abundance of stable isotopes can be a promising tool to investigate the relative contribution of anammox and denitrification in the intact ecosystem, although the isotopic fractionation factors during anammox which are necessary to interpret isotopic signatures are not fully known. Here we reported nitrogen and oxygen isotopic fractionation factors during anammox occuring in the activated sludge. We incubated the sludge anaerobically to trace the changes in concentrations and isotopic signatures of ammonium, nitrite and nitrate during the anammox process. We found the large isotopic fractionations for ammonium oxidation and nitrite reduction by anammox. In addition, the inverse isotopic fractionation during nitrite oxidation to nitrate was observed. We will discuss these factors with comparison of the latest study on anammox isotopic systematics (Brunner et al. 2013) in the presentation.

BGM22-05 Room:105 Time:May 26 10:00-10:15

Single cell genomic analysis for the marine ammonia-oxidizing archaea

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Ammonia-oxidizing Archaea (AOA) is one of the most dominant lineages in aquatic microbial ecosystem in the dark oceans. Potential niche separation of AOA has been observed along with the concentration/flux of electron donors such as ammonia and urea. However, isolates and enrichments of AOA belong to the lineages that adapt relatively high concentration/flux of electron donors. In this study, single cell genomic analysis was applied to the AOA population that inhabit sea surface to hadal water in the Izu Ogasawara Trench in order to know the machinery of niche separation of the AOA lineages in the dark ocean.

Keywords: genome, ammonia oxidation, archaea

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BGM22-06 Room:105 Time:May 26 10:15-10:30

Rare earth elements and bacterial C1 metabolic system

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The rare earth elements (REEs) include lanthanum (La) and cerium (Ce) as light REEs. La and Ce are very low abundant in the universe, but relatively higher abundant in the earth crust including soils. Recently, it has been reported that the product of xoxF gene in Methylobacterium. extorquens AM1 is La- and Ce-dependent methanol dehydrogenase (MDH) (Nakagawa et al. 2012), which is different from classical Ca-dependent MDH encoded by mxaF gene. Although bradyrhizobia are ubiquitous bacterial in the environments, they are often associated with legume and non-legume plants. In the present work, we examine the effects of La and Ce on C1 metabolism (methanol oxidation) of these bradyrhiozbia. We used six strains of Bradyrhizobium oligotrophicum S58, Bradyrhizobium sp. BTAi1, Bradyrhizobium sp. ORS278, Bradyrhizobium sp. RP5, Bradyrhizobium sp. RP7, and Bradyrhizobium sp. WD16. The former three strains formed root nodules of an aquatic legume plant (Aeschynomene indica), while the latter three strains are endophytes in paddy rice roots. They are also able to survive in oligotrophic environments such as soils (Okubo et al. 2013). BLASTN search were conducted on the genomes of six strains by the DNA sequences of xoxF and mxaF gene in M. extorquens AM1. As a result, the former three strains of the aquatic legume plant (A. indica symbionts) have xoxF gene that presumably encodes La- and Ce-dependent methanol dehydrogenase (MDH), while the latter three strains of rice endophytes have both xoxF and mxaF gene. Culture experiments supported these results: The cell growth of B. oligotrophicum S58, Bradyrhizobium sp. BTAi1 and Bradyrhizobium sp. ORS278 (A. indica symbionts) was enhanced by La or Ce in HM medium containing methanol as a sole carbon source. They utilized methanol in the medium. On the other hand, the growth enhancement of bradyrhizobial rice endophytes (Bradyrhizobium sp. RP5, Bradyrhizobium sp. RP7, and Bradyrhizobium sp. WD16) by La or Ce additions were not observed in the same culture condition, probably because the existence of classical Ca-dependent MDH encoded by mxaF gene. We constructed two types of xoxF mutants, xoxF::omega and delta xoxF of B. oligotrophicum S58 by using omega cassette and sac markerless system, respectively. In the presence of La or Ce in HM medium supplemented with methanol, the growth of xoxF::omega mutant decreased as compared with that of wild-type strain of B. oligotrophicum S58. On the other hand, the growth of delta xoxF mutant increased as compared with that of wild-type strain S58. This apparent discrepancy indicates two suggestions in methanol catabolism in B. oligotrophicum S58. Firstly, the polar effect of omega cassette probably induced the repression of gene for formaldehyde catabolism, which located on downstream of the xoxF gene in xoxF::omega mutant. Secondly, there are other xoxF genes for La- or Ce- dependent MDHs. Indeed, we found redundant xoxF gene candidates on the genome of B. oligotrophicum S58 by extensive survey. Finally, we want to discuss the geobiological significance of light REEs in environmental bacteria.

Keywords: Rare earth elements, Bacteria, C1 compound metabolism, Bradyrhizobium, Methanol, Methanol dehydrogenase

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BGM22-07 Room:105 Time:May 26 11:00-11:15

Phototoxicity of chlorophylls: a major photobiochemical constraint on the energy flux from photosyntheis

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The “entrance of energy flux” driving biological systems in Earth’s biosphere had shifted largely to solar radiation since the invention of the mechanism of photosynthesis. Chlorophylls as photosensitizers serve as central and indispensable factors in photosynthesis, which enables conversion of photon energy to chemical potential that is conserved in organic matters. Particularly, the emergence of oxygenic photosynthesis, recruiting water molecules ubiquitous in Earth’s environments as the terminal electron donor, is regarded as a major innovation of Earth’s biosphere by accelerating photosynthetic primary production, that is, drastically increasing the flux from solar energy. Yet, generated molecular oxygen in compensation for this innovation is rather incompatible with chlorophylls, for chlorophylls photosensitizing normal molecular oxygen (triplet oxygen) to generate highly toxic reactive species called “singlet oxygen” (*i.e.*, phototoxicity of chlorophylls). Modern plants (*i.e.*, all oxygenic phototrophs including cyanobacteria and eukaryotic phototrophs) have developed elaborated mechanisms that protect against phototoxicity of chlorophylls[1]. Paradoxically, the first oxygenic phototrophs must have already invented any mechanism against the phototoxicity in prior to oxygenation of Earth’s atmosphere. Moreover, in order to draw energy flux from photosynthesis to the subordinated ecosystem, which is presumably conducted by heterotrophs, it requires intake of organic matters deriving in phototrophs into the cells. Heterotrophic, particularly phycophagic protists (*i.e.*, unicellular eukaryotes), plays important roles in the modern aquatic ecosystem through phagocytosis of algal cells, which perhaps was a much more important process in early ages before emergence of metazoan planktons. Although the process taking chlorophyll-containing matters into the cell inevitably accompanies the risk of the phototoxicity, yet any such a problem is generally observed in the environment in practice. We recently discovered a metabolic process converting phototoxic chlorophylls to non-phototoxic derivatives, 13^2,17^3-cyclopheophorbide enols (CPEs), associated with phagocytosis of algae by protists[2,3]. This metabolism is found to be shared by a very wide range of heterotrophic protists that virtually distribute among almost all major supergroups of Eukarya. In fact, CPEs are turned to be highly abundant pigments in any aquatic environment, suggesting importance of the phycophagic process by protists in the energy flux. Furthermore, production of CPEs is also reported from phototropic protists[4,5]; we observed that the “CPE metabolism” functions in some secondary algae such as Euglenophyceae during self-degradation processes of own plastids. We infer the CPE metabolism of the algae must be inherited from the ancestral phycophagic protists. In summary, although plesiomorphy of the CPE metabolism in Eukarya must carefully be examined after accumulations of studies through various approaches, we argue possible importance of metabolism(s) for detoxification of chlorophylls among eukaryotes both in early radiation enabling ingestion of oxygenic phototrophs and in evolution of eukaryotic phototrophs enabling retention of chlorophyll-containing organelle by controlling its phototoxicity, hence being a major factor allowing expansion and sophistication of the flux originating from solar energy.

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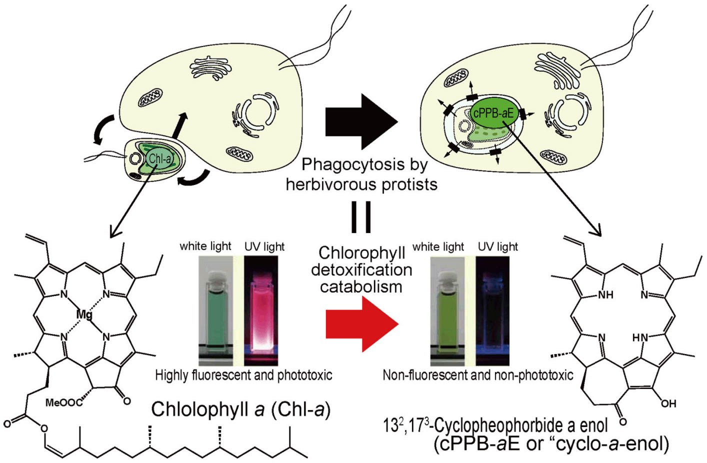
Keywords: chlorophyll, oxygen, phototoxicity, protist, microalgae, cyclopheophorbide enol


Chl-a
cPPB-aE
Phagocytosis by herbivorous protists
=
Chlorophyll detoxification catabolism
white light
UV light
Highly fluorescent and phototoxic
white light
UV light
Non-fluorescent and non-phototoxic
Mg
N
NH
HN
MeOOC
OH
Chlolophyll *a* (Chl-*a*)
$13^2,17^3$-Cyclopheophorbide a enol
(cPPB-*a*E or “cyclo-*a*-enol)

BGM22-08 Room:105 Time:May 26 11:15-11:30

Microbiological transformation of antimony and its geochemical implications

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Antimony (Sb) is a naturally occurring toxic element and is considered to be a priority pollutant of interest by the USEPA. Although the concentrations of Sb in soils are generally low ($<$1 mg kg^{-1}), elevated levels of Sb have been released via mining activities and other anthropogenic activities due to its increasing industrial use. Antimony is commonly associated with arsenic (As) in the environment and both elements have similar chemistry and toxicity. Increasing numbers of studies have focused on microbial roles in As transformations, while microbial-Sb interactions are still not well understood. To gain insight into microbial roles in the geochemical cycling of Sb, soils from an old stibnite (Sb_2S_3) mine tailing area (Ichinokawa mine, Ehime, Japan) were characterized geochemically and examined for the presence of Sb-transforming microbial populations. Total concentrations of Sb and As were higher in the surface soil (0-3 cm: 2280 and 1240 mg kg^{-1}, respectively) and decreased with depth (9-12 cm: 330 and 133 mg kg^{-1}). Bacterial community profiles, examined by cultivation-independent analysis using 16S rRNA gene-based denaturing gradient gel electrophoresis, did not show substantial differences through depth (0-12 cm). After the aerobic enrichment culturing with Sb(III) (100 μM), pure cultures of *Pseudomonas*- and *Stenotrophomonas*-related isolates with Sb(III) oxidation activities were obtained. Anaerobic enrichment cultures capable of reducing Sb(V) (2 mM) were also obtained, in which the precipitation of antimonite [Sb(III)] as antimony trioxide was observed. These results demonstrate that indigenous microorganisms associated with stibnite mine soils are capable of Sb redox transformations and contribute to the speciation and mobility of Sb *in situ*.

Keywords: Antimony, Arsenic, Microbial antimony oxidation, Microbial antimony reduction, Soil bacterial community

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BGM22-09 Room:105 Time:May 26 11:30-11:45

Ecological and mineralogical characteristics of Fe-oxidizing microbial communities in a shallow hydrothermal mound

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Microbial Fe-oxidation has been mainly studied at deep-sea hydrothermal vents because the low concentration of oxygen and high concentration of ferrous ion was preferable for Fe-oxidizing bacteria that have to compete with abiotic Fe-Oxidation. However, microbial Fe-oxidization in shallow, fully oxygenated environments has been still largely unknown. In this study, we aim to reveal the ecology and role for mound formation of Fe-oxidizing bacteria at iron oxyhydroxide-rich hydrothermal mounds developing in Nagahama Bay, Satsuma-Iwojima where we observed dense assembly of twisted stalks, typical signature of microbial Fe-oxidation. Core samples were taken from the iron oxyhydroxide-rish mound and used for sequencing and microscopic analysis. Microscopic observation indicated the highest occurrence of stalk structure was observed at around 20 cm from the surface. Sequencing of 16S rRNA gene of prokaryotic communities ($>$100,000 reads/sample) revealed that Anaerolineae known as obligately anaerobic heterotroph was highly dominated at ˜40% throughout all depths down to 40 cm from the surface of the mound, inferring anaerobic circumstances in the sediment. We also found Fe-oxidizing Zetaproteobacteria in all depths and its population was determined to be up to 4%. Network analysis of microbial communities revealed that appearance of the Zetaproteobacteria coincided with some anaerobic sulfur reducing bacteria, indicating that the Zetaproteobacteira lived in ecological niche of oxic-anoxic interface in the mounds. Seismic data indicated that those mounds grow ˜1cm/yr which is much faster than the abiotic deposition occurring at the surrounding diffuse hydrothermal venting seafloor. Overall, our results indicated that Zetaproteobacteria may accelerate deposition of Fe species in hydrothermal fluid and formation of iron oxyhydroxide-rich mounds in the Nagahama-bay, Satsuma-Iwojima.

Keywords: Hydrothermal mound, Fe-oxidizing bacteria, microbial ecology