

## Geochemical and molecular biological characterization of nitrogen dynamics in (had)opelagic sediments

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Great progress has been made in understanding the nitrogen cycle in oceanic waters by the recent identification of ammonia-oxidizing archaea and anaerobic ammonia oxidizer (anammox), and by the following comprehensive approaches to clarify the abundance and activity of each component in the nitrogen cycle. However, nitrogen dynamics in marine sedimentary habitats is still uncertain. To further characterize nitrogen dynamics in the deep-sea sediments, we have quantified i) gene abundance of putative nitrifiers, denitrifiers and anammox, and ii) potential rate of denitrification in the hadopelagic sediment cores taken from the Ogasawara Trench (water depth of 9760m). We have also determined nitrogen and oxygen stable isotopic compositions of nitrate in the interstitial water in the hadopelagic sediments. Abundance of potential proteobacterial denitrifiers correlated with that of nitrifiers through the depth, and anammox also likely co-occurred with nitrifiers. Further, nitrate isotope compositions suggest the enrichment of <sup>18</sup>O by nitrification process and co-occurrence of nitrification and denitrification in nitrate reduction zone. The data suggest that aerobic and anaerobic processes of the nitrogen cycle coupled in the nitrate reduction zone in the hadopelagic sediments.

## Niche separation of nitrifiers and anammox in deep-sea sediments.

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We revealed the distribution patterns of nitrifiers and anammox along with geochemical gradients in a hadopelagic sediment core from the Ogasawara Trench (Nunoura et al. 2013). The results presented novel insights into the inorganic nitrogen cycle in deep-sea sediments as shown below. 1) Thaumarchaeotes and *Nitrospina* predominates in the ammonia and nitrite-oxidizing communities, respectively. 2) The pore-water nitrate recorded isotopic signatures of nitrification. 3) Abundance of anammox was likely regulated by not only by redox potential but also by nitrite supply from ammonia oxidation. 4) Maximum abundance of denitrifier occurred at sediment surface.

The purpose of this study is to know the roles of benthic microbial inorganic nitrogen cycle in diverse deep-sea environments. In this study, we compared pore water chemistry, and abundance and composition of nitrifier and anammox populations in 6 distinct regions, and will discuss about the roles of dynamic nitrogen cycle in deep-sea benthic environments.

Keywords: nitrification, anammox

## Ecology of viruses in deep-sea hydrothermal vents

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Since the discovery of ubiquitous and highly abundant viruses in aquatic ecosystems, many studies have been conducted to discern the role of viruses within aquatic microbial communities. As a result, viruses are now recognized to be significant components of all aquatic ecosystems. It has been suggested that they affect global nutrient and biogeochemical cycles in the world's oceans, and play a role in regulating abundance and composition of microbial communities. Viruses can also mediate lateral gene transfers and drive the diversification of microbial communities and the co-evolution between viruses and hosts.

Deep-sea hydrothermal vents are sites having great microbial biomass, high productivity, and physiologically and genetically high diversity, contrasting sharply with the surrounding sparsely populated deep-sea environments. The primary production in the deep-sea vent ecosystem is sustained by chemolithoautotrophic microorganisms that utilize reduced chemical compounds from the earth interior as energy sources. To date, the biogeochemical processes, ecophysiological functions, and evolutionary significance of deep-sea vent microbial communities have been extensively studied, but the ecological and evolutionary impacts of viruses on the deep-sea vent microbial communities remain to be fully elucidated.

Here, I provide an overview of current hot research topics related to viruses in aquatic ecosystems, and then introduce our studies on the viral functions and ecology in deep-sea hydrothermal vents in addition to several previous studies on virus-host interactions.

Keywords: virus, hydrothermal vent, chemolithoautotrophs

## Iron redox cycling and subsurface offshore transport in the eastern tropical South Pacific oxygen minimum zone

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Iron (Fe) is well known as an essential element involved in a number of biochemical processes in the ocean such as nitrogen metabolism. The distribution of dissolved Fe in seawater depends on the nature and magnitude of the sources and sinks, and the transport mechanisms. The thermodynamically favored oxidation state of Fe, Fe(III), is strongly hydrolyzed and its removal is mainly constrained by the formation of strong complexes with natural organic ligands such as humic substances and siderophores. These organic ligands control not only the solubility of dissolved Fe in seawater, but also the bioavailability of Fe(III) for phytoplankton. Fe(III) in seawater can be reduced to Fe(II), which is more soluble and kinetically labile, although is rapidly oxidized in the oxygenated seawater. Recent studies have suggested that dissolved Fe(II) substantially exists in surface seawater (e.g., Hansard et al., 2009), suboxic layers in oxygen minimum zones (OMZs) (e.g., Kondo and Moffett, 2013), hypoxic shelf waters and sediments (Lohan and Bruland, 2007), hydrothermal vents and shallow submarine eruption (Santana-Casiano et al., 2013). Since Fe(II) is more bioavailable than Fe(III), the existence of Fe(II) could provide a big advantage for the organisms in these environments even though it is ephemeral. These results suggest the importance to investigate chemical and redox speciations of Fe to elucidate carbon and nitrogen cycles in the ocean.

The distribution of dissolved Fe, Fe(II) and Fe(III)-binding organic ligands were investigated in the upper 1000 meters of the eastern tropical South Pacific from January to March 2010, during El Nino event. Dissolved Fe concentrations were exceedingly low in surface waters, showed minima near chlorophyll maximum, and increased below that depth. While high rates of nitrogen fixation have been inferred for this region from models, our data suggest that surface Fe is much too low to support diazotrophs. Dissolved Fe and organic Fe(III) ligands concentrations at mid-depth were elevated in the nearshore stations, where virtually all dissolved Fe(III) was bound to these ligands. Maxima in the concentration of Fe(II) were seen in the oxygen-deficient and high-nitrite layers of the OMZ. Fully 8 to 68% of dissolved Fe existed as Fe(II) in the samples collected at these depths. Dissolved Fe concentration was higher in the OMZ where Fe(II) and nitrite were present. We propose that this region, the most reducing part of the OMZ, plays an important role in subsurface, offshore Fe transport.

Keywords: iron, Fe(II), oxygen minimum zone, eastern tropical South Pacific, organic ligand

## Biogeochemical cycles on the deep-sea floor revealed by isotope labeling experiments

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Deep-sea benthic food webs are mainly sustained by sinking aggregates of phytodetritus derived from the water column. Although the majority of organic matter is consumed before reaching the deep-sea floor, phytodetritus still transports a significant amount of fresh material from the surface ocean to the seafloor. A portion of the phytodetritus is converted to benthic biomass, and the remaining refractory organic matter not utilized by the benthic community is preserved in the sedimentary record. The activity of the benthic community is thus expected to be an important factor in controlling the quality of organic matter, and biogeochemical cycles on the deep-sea floor. We carried out some different types of *in situ* isotope labeling experiments to reveal these benthic processes. Results quantitatively demonstrated the fate of phytoplankton, bacteria, dissolved organic carbon, and dissolved inorganic carbon on the deep-sea floor.

Keywords: Sediment-water interface, Benthos, isotope tracer, Biogeochemical cycle

## Nitrogen isotopic record of chlorophylls as a tool for understanding of nitrogen dynamics in the oceanic photic zone

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Nitrogen isotope record of chlorophylls has a large potential as a tool for reconstructing the nitrogen cycle and its dynamics in the photic zone. In this study, we determined the nitrogen isotopic compositions of chlorophyll *a* ( $\delta^{15}\text{N}_{chl}$ ) and pheophytin *a* ( $\delta^{15}\text{N}_{Phe}$ ) as well as nitrate ( $\delta^{15}\text{N}_{NO_3}$ ) collected from two sites (S1 and K2) in the northwest Pacific as a case study. Both chlorophyll *a* and pheophytin *a* were extracted from the particulate organic matter (POM) and purified by the fraction collector of high-performance liquid chromatography. The nitrogen isotopic composition of the isolated chlorophylls was determined by our ultra-sensitive elemental analyzer / isotope ratio mass spectrometry. The estimated isotopic fractionation associated with the chlorophyll synthesis is -7.9 ‰ to -13.1 ‰, confirming the previous studies. However, the  $\delta^{15}\text{N}$  of POM is not consistent with those of chlorophylls, suggesting that the POM from both sites is a mixture of phytoplankton and other materials like detritus of zooplankton. The  $\delta^{15}\text{N}_{chl}$  value provides pure  $\delta^{15}\text{N}$  signature of phytoplankton, which is crucial for better understanding of nitrogen dynamics in the surface ocean. Chlorophylls are also buried and preserved in the sediments for long, and thus useful for the reconstruction of nitrogen cycle in the surface ocean in the geological past. In this presentation, we will summarize the evidence and discuss advantages and pitfalls of this tool for the future use in the oceanography and paleoceanography.

Keywords: nitrogen isotope, oceanic photic zone, nitrogen dynamics, photosynthetic pigments, nitrate

## Heterotrophic bacterial production and extracellular enzymatic activity in sinking particulate matter

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Heterotrophic activities on sinking particulate matter (SPM) have important role for flux of SPM. To demonstrate regional differences in heterotrophic activities on SPM, we measured heterotrophic bacterial production (HBP) in seawater and SPM as well as potential extracellular enzyme activity (EEA) in SPM on a transect along 155E in the western North Pacific Ocean in the subarctic (44N), the Kuroshio Extension area (35N), and the subtropical gyre (20N).

Samples were collected from the western North Pacific Ocean during cruise KH08-2 (Leg 2) on R/V Hakuho-maru from 23 August to 16 September 2008.

Hydrographic data were provided by a shipboard CTD profiler equipped with a carousel multi-sampling system. We obtained water-column depth profiles of dissolved nutrients including nitrate, phosphate, and silicate, Chl a, bacterial cell abundance (BA), and HBP.

We deployed standard cylindrical multi-traps, with eight acrylic trap tubes mounted at each depth. The traps were set vertically on the array line at three targeted depths of 50 m, 200 m, and 500 m at 44N, and 100 m, 200 m, and 500 m at 35 and 20N. The upper deployment depths were chosen to be just under or near the bottom of the euphotic zone. The euphotic zone was defined as the depth at which photosynthetically active radiation was 1% of the value just below the surface.

Before deployment, all trap tubes except tube for HBP and EEA in SPM on each array were filled with seawater that had been collected from 4 m below the surface at each station using the ship's pump, pre-filtered through a 0.2- $\mu$ m capsule cartridge filter to minimize biological contamination, and mixed with sodium chloride to a final concentration of 4% (w/v) to create a density gradient. Trap tube at each depth was used for collecting samples for measuring HBP and EEM in SPM, and was filled with seawater filtered as described above that was collected just before deployment from the depth corresponding to the target layer of trap deployment with a 12-L Niskin bottle. The arrays were attached to a buoy and allowed to drift freely for 24 h at 44N, and 48 h at 35 and 20N.

Upon recovery, the traps were stored upright in the dark and left to settle for 1 h. After the contents had settled, the upper portion of the trap volume above the collection cup was gently drained by siphoning. During the siphoning, only about trap tube for HBP and EEA, an aliquot of the supernatant was subsampled approximately 30 cm from the top of the tube. After siphoning was complete, the upper cylinder of the trap tube was separated from the collection cup. The particle-rich water in each collection cup was pre-screened through a 500- $\mu$ m-mesh sieve to remove swimmers and then mixed to disrupt large amorphous particles. The pre-screened filtrates were used for measurements of total mass flux of SPM, particulate organic carbon (POC) and nitrogen (PON) content, and HBP and EEA (leucine aminopeptidase (LAPase),  $\alpha$ -glucosidase (BGase), lipase, and alkaline phosphatase (APase)).

Depth-integrated HBP in seawater from the surface to 500 m was comparable between the locations, whereas HBP in SPM at 44N was substantially lower than at the other sites. We found the highest POC export flux and export efficiency to bathypelagic depths, and the lowest water temperatures, at 44N. We found significant correlations between LAPase activity, BGase activity, POC flux and particulate organic nitrogen flux. LAPase activity was two orders of magnitude higher than BGase activity, with a BGase:LAPase activity ratio of 0.027. There were no significant correlations between HBP and EEA in SPM except for lipase, and lipase activity was significantly correlated with temperature. We propose that hydrographic conditions are an important factor controlling heterotrophic bacterial activity and export efficiency of organic carbon to the deep ocean, as are the sources and abundance of SPM produced in the euphotic zone via primary production.

**Keywords:** Sinking particulate matter, Sediment trap, Heterotrophic bacterial activity, Extracellular enzyme activity, western North Pacific

## Enigmas concerning sterols and their surrogates in eukaryotic cell membranes

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A large fraction of eukaryotes and bacteria respectively possess sterols and hopanoids, which function as potent stabilizers of cell membranes. Sterols are also associated with fluidity and permeability of eukaryotic cell membranes, and are key to fundamental eukaryotic-specific cellular processes such as phagocytosis. Several steps of *de novo* sterol biosynthesis require molecular oxygen. For example, the epoxidation of squalene is the first oxygen-dependent step in the sterol pathway; the epoxidized squalene is then cyclized to either lanosterol or cycloartenol by the enzyme oxidosqualene cyclase. In contrast, prokaryotic hopanoid biosynthesis does not require molecular oxygen as a substrate, and the squalene is directly cyclized by the enzyme squalene-hopene cyclase.

Until now, it was unclear how bacterivorous unicellular eukaryotes that are abundant in anoxic or low oxygen environments could carry out phagocytosis. These eukaryotes cannot obtain sterols from food bacteria as the latter generally lack them and sterols cannot be synthesized *de novo* in the absence of molecular oxygen. We have previously provided evidence that the molecule tetrahymanol is synthesized by some anaerobic/microaerophilic eukaryotes and possibly functions as an analogue of sterols in these organisms. Nevertheless, neither sterol, nor tetrahymanol, nor their related molecule has been found in the other anaerobic/microaerophilic eukaryotes, and so it is still enigmatic how these organisms maintain their fluid and permeable membrane system specific to eukaryotes.

One more area of confusion is regarding sterols in bivalves with chemosynthetic bacteria inhabiting areas of deep-sea hydrothermal vents and methane seeps, such as *Calyptogena* spp. and *Bathymodiolus* spp. In general, bivalves cannot synthesize sterols *de novo* and it is necessary for them to obtain these molecules from small eukaryotic prey. On the other hand, *Calyptogena* spp. and *Bathymodiolus* spp. mainly or exclusively acquire nutrients produced by their bacterial symbionts, rather than from eukaryotes rich in sterols. Nevertheless, these "chemosynthetic bivalves" contain sterols. More curiously, *Calyptogena* spp. have intermediate metabolites of phytosterols (24-methylenecycloartanol, cycloeucalenol, and obtusifoliol), while *Bathymodiolus* spp. have high amounts of cholesterol typical of animals. Little attention has been given to how chemosynthetic bivalves produce or acquire these kinds of sterols.

In my talk, I will discuss potentially controversial topics regarding sterols and their surrogates in eukaryotic cell membranes, which do not appear in biochemical and geochemical textbooks.

Keywords: eukaryotes, sterols, tetrahymanol, cell membrane

## Structural differences of humic acid isolated from estuarine sediments at several fields around Ariake Sea

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Material transfer and circulation of coastal areas, and the form and state of the organic matter in the estuary tidal flat area, is attracting attention at various angles from the biological importance of the river estuaries. Analysis and evaluation of the sediment material and coastal water has been carried out around the river basin. So far, we have analyzed the chemical structure of humic substance in the Chikugo River basin near or vary by region. The Ariake Sea, from the fact that environmental issues such as hypoxic water and red tide has occurred, environmental analysis have been made from various points of view in recent years. However, research of organic matter deposition simultaneous in a wide range of areas of the Ariake Sea coast is a few instances.

In this study, humic acid fraction were extracted from the surface sediment of the tidal flat areas, including rivers and estuaries tidal flats, tidal flats as well as less affected by other rivers a broad area of the northern half of the Ariake Sea. Then, the extracted humic acids were analyzed such as stable isotopic analysis and elemental composition, and regional differences were compared. And the use of humic acid as environmental indicator was evaluated from the point of some differences to the several conventional analyses of the environmental indicators at the coastal area.

Sediment samples were collected at a total of seven locations of tidal flat (estuaries at Hayatsue-gawa, Rokkaku-gawa, Hama-gawa, Kikuchi-gawa, Shira-kawa, and tidal flat at Arao and Tara) and two places of the downstream of Chikugo River from May 2011 to August 2013. Humic acid fraction were prepared according to the IHSS soil humic acid extraction method. Multiple analysis, such as the elemental analysis, stable isotope ratios, ultraviolet-visible absorption analysis (application of (A<sub>2</sub>/A<sub>4</sub>) ratio of 270nm/407nm that has been proposed by Fookan and Liebezeit (2000)) were applied to the humic acid of coastal areas.

Correlation derived from the source materials was observed between stable isotope ratio, and the atomic ratio calculated from elemental analysis, ultraviolet-visible absorption ratio and the regional differences of humic acid. Contribution of terrigenous organic matter is poor at Hama-gawa estuary Tara and Arao tidal flat. Further, trend in nitrogen isotope ratio is different from the other regions and the 2 points (Hama-gawa mouth and Tara tidal flat). Conditions such as denitrification and nitrogen sources is somewhat different in the Ariake Sea northwest side was suggested.

Keywords: Ariake Sea, estuarine, stable isotope ratio, UV, humic substance

## Origin of fluorescent dissolved organic matter in forested headwater stream during base-flow period

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In this study, we focus on fluorescent dissolved organic matter (FDOM) such as humic substances (HSs) and aromatic amino acids, which constitutes the main portion of streamwater DOM. Our objective is to estimate the origin of streamwater FDOM during baseflow period, by comparing its composition with soil infiltration water, saturated groundwater and bedrock spring water.

Our study site is Inokawa watershed (watershed area 503 ha) in The University of Tokyo Chiba Forest. We collected stream water samples and bedrock spring water at 142 points in the watershed in 2009, 2010 and 2012, and also soil waters and groundwater in Fukuroyamasawa Experimental Watershed (2ha) which is one of most headwater hollows. Rainwater was collected at the weather station in the watershed. The water samples were filtered with 0.45mm membrane filters and analyzed for DOC concentration by wet-oxidation method, and Excitation-Emission Matrix (EEM) using 3D-spectrofluorometry. EEMs were compiled and further analyzed by Parallel Factor Analysis based on Murphy et al., (2013), and decomposed into five components with distinctive fluorescence spectra. Chemical characteristics of components were identified by comparing their spectral shapes with previous studies as follows: C1 as humic acid type HS-like, C2 as fulvic acid type HS-like, C3 as microbial-derived HS-like, C4 as tryptophane-like and C5 as tyrosine-like.

Groundwaters and bedrock spring waters were classified into three groups based on the ratio of three HS-like components, as C1-dominant group, C2-dominant group and C3-dominant group. Although groundwater in Fukuroyamasawa watershed belonged to C1-dominant group, and showed seasonal change in DOC concentration, the composition of HS-like components of groundwaters and bedrock spring waters in three groups were temporally relatively stable. This suggests that these groups can be used as end-members in identification of the origin of streamwater FDOM.

Ratios of three HS-like components in streamwaters fell in between groundwater groups and soil waters in about half of the samples. In other samples, however, ratios could not be explained by mixing of such hillslope end-members. FDOM of those streamwaters had higher abundance of C1 and C2, and also relatively higher DOC concentrations, suggesting that it was originated not only from soil and/or groundwater in the hillslope, but also from organic materials in the stream such as deposited litters, woody debris and/or other organic-containing sediments. As to aromatic amino acid-like components, streamwater FDOM tended to have lower C5/C4 ratio relative to hillslope waters, and often had C5 undetectable, suggesting that C5 was more labile than C4 in stream environment.

This study showed that HS in streamwater is produced not only in hillslope but also in stream itself, and in-stream produced HS can show different fluorescence spectral characteristics from hillslope-produced HS.

**Keywords:** fluorescent dissolved organic matter (FDOM), forested watershed, streamwater chemistry, excitation-emission matrix (EEM), parallel factor analysis (PARAFAC)

## Pseudopolarographic estimation of copper complexing ligands in freshwater of Lake Biwa, Japan

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Pseudopolarography (Croot P. L. et al., *Mar. Chem.*, 67, 219-232 (1999), Wiramanaden C. I. E., et al., *Mar Chem.*, 110, 28-41 (2008)) is useful method to detect metal (copper) complexation that is very stable compared with that detected by other methods: AdCSV: adsorptive cathodic stripping voltammetry, ion selective electrode etc. in water. It was applied in seawater analysis especially for coastal area where large amount of organic material with high complexing capacity was detected. Also in freshwater lake, there is high potential of existence of very stable copper complexes in water, as it sometimes includes high concentration of sulfur containing compounds and concentration of competing metals such as calcium and magnesium are very low compared with those in seawater. Existence of very stable ligands was investigated using freshwater sampled in Lake Biwa, Japan.

As reference ligands, EDTA, DPTA and CDTA were used at pH 8.8 using borate buffer solution. Copper was deposited on HDME (hanging mercury drop electrode) by varying potential from -0.2 to -1.5 V, and deposition time was 420 s. After deposition, deposited copper was stripped by scanning from the deposition potential to 0 V. Peak height was plotted against deposition potential, and half wave potential was determined. By comparing the half wave potential with that of reference ligands, stability of copper complexing ligands in the sample was estimated.

Half wave potentials measured by references were -0.4 V for EDTA, -0.58V for CDTA, and -0.65 V for DTPA, respectively. By measuring water sampled at north basin of Lake Biwa, half wave potentials at -0.5 V and -1.1 V was obtained for surface water. Only single half wave potential at -0.5 V was obtained for waters at 2m and 10m depth. Existence of strong ligands that has stability close to EDTA was detected all samples tested. These ligands were also detected by AdCSV using salicylaldoxime as competing ligands. But ligand detected at half wave potential at -1.1 V is not detected or undetectable. It might suggest significance of very stable complexes in water of Lake Biwa.

Keywords: freshwater, Lake Biwa, copper, ligand, electroanalysis

## Isotopic composition of chlorophylls as a new indicator of energy flow in stream ecosystems

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In most freshwater (e.g., stream) ecosystems, periphytic algae attached to a substrate (periphyton) play an important role as benthic primary producer. However, the energy flow, which is transferred from periphyton to animal consumers through trophic pathways, has not yet been adequately assessed because few studies have traced algal signatures from periphyton matrix to food webs. Here we present a new application of the isotopic composition of chlorophylls in periphyton to the tracer of *in situ* primary production. Chlorophylls can be used as a biomarker of photosynthetic autotrophs, including periphytic algae. We purified chlorophylls from periphyton matrix using a high performance liquid chromatograph (HPLC), and measured carbon and nitrogen stable isotope ratios of chlorophylls, pheophytins, the bulk of periphyton, and algal grazing specialists (e.g., *Epeorus latifolium*: mayfly larva) using an elemental analyzer coupled with an isotope ratio mass spectrometer (EA/IRMS). We will compare the results with traditional isotope maps, and discuss the potential of the isotopic composition of chlorophylls in aquatic food web studies.

Keywords: periphyton, photosynthetic pigments, biomarker, HPLC, stable isotopes

## Biodiversity indicators of trophic structure measured by stable isotope ratios

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The term "biodiversity" is considered as multi-level diversity, ranging from genetic, species, to ecosystem level. However, it is difficult to measure arbitrary level of biodiversity, therefore, biodiversity assessment at species level is often applied to an ecosystem. Biodiversity assessment at species cannot directly be related to ecosystem function, thus, a grouping method, such as functional feeding group (FFG), is often used in stream ecology.

Our project, funded by the Environment Research and Technology Development Fund (4D-1102), aimed at developing a method to evaluate functions of biodiversity in watershed ecology, especially streams. Stable isotope tools have been used to study watershed ecology, which covers researches on nutrient cycling and food web structure among forest, river, lake and coastal ecosystems. Recently, nitrogen isotope ratios of individual amino acids have been measured to estimate trophic positions of animals. However, this technique has not been applied to complex food web analysis, such as freshwater systems, which are based on both autochthonous and allochthonous productions. We have proved that this method is applicable to various freshwater food webs, including the system to which the bulk-isotope method could not be applied. Application of the method to archived biological specimen allows us to study long-term trophic changes in the ecosystem. Natural abundance of radiocarbon is another signature that separates carbon sources in freshwater ecosystems.

We suggest that a trophic structure estimated by various isotope signatures, together with estimated biomass of each taxonomic group, is an alternative index of describing biodiversity in watershed ecosystems.

Keywords: Stable isotope ratios, Food web, Trophic position

## Vertical distribution of the triple oxygen isotopic compositions of DO in oligotrophic/mesotrophic environments

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In order to quantify the gross production rate of dissolved oxygen molecules (DO) in hydrosphere beneath thermocline, vertical distributions of the triple oxygen isotopic compositions of DO in oligotrophic/mesotrophic lakes were determined, together with their temporal variations.

Keywords: oligotrophic lake, mesotrophic lake, dissolved oxygen, triple oxygen isotopes, vertical profile, seasonal variation

## Biogeochemistry on glaciers and icesheets ? Microbial process of glacier darkening and material cycles

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Glaciers and icesheets have been reported to shrink worldwide, probably caused by recent global warming. They are inhabited by diverse organisms, which adapted to the cold environment. Snow and ice algae grow photosynthetically on their surface and sustain heterotrophic microbes. Organic matter including their bodies and products can reduce surface albedo and accelerate melting of glaciers. Thus, shrinkage of glaciers and icesheets is not only due to climate change, but also possibly due to change of glacier ecosystems. Therefore, it is important to assess quantitatively biogeochemical process of carbon and nitrogen cycles on glaciers. In this talk, I would like to review our present knowledge on glacial ecosystems including Asian and polar glaciers and discuss possible reasons of recent darkening of the Greenland icesheet.

Keywords: glacier, Greenland, albedo, algae, microbe, carbon cycle

## How does anthropogenic nitrogen input affect the nutrient dynamics and food web structures?

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In last five decades, impacts of anthropogenic nutrient inputs on river ecosystems have continuously been a major concern for the governments and residents of the catchments in Japan. Major sources of anthropogenic nitrogen (N) include leachate from forest ecosystem, surplus fertilizers and sewage. Impacts of anthropogenic N inputs on nutrient dynamics and food web structures were investigated using stable N isotope techniques in the Arida river catchment, Japan. Riverine survey utilizing 5 regular sampling points showed that  $\delta^{15}\text{N}$  of nitrate ( $\text{NO}_3^-$ ) increased from forested upstream ( $\sim 2\text{‰}$ ) to the downstream ( $\sim 7\text{‰}$ ) due to the sewage loads and fertilizer effluents from agricultural area. Correspondingly the  $\delta^{15}\text{N}$  of benthic algae and aquatic insects increased toward the downstream. This indicates that primary producers of each reach strongly relied on the local N sources and it was utilized effectively in their food web. Simulation using a GIS based mixing model considering the spatial distributions of human population density and fertilizer effluents revealed that strongest impacts of N inputs was originated from organic fertilizers applied to orchards in the middle to lower parts of catchment. Differences in  $\delta^{15}\text{N}$  between primary producers and predators were  $\sim 6\text{-}7\text{‰}$  similarly at all sampling points. Food web structural analysis using food network unfolding technique based on observed  $\delta^{15}\text{N}$  suggested that the structure of nutrient pyramid did not differ significantly along the riverine positions, while the members of species in each trophic level changed and the impact of anthropogenic N input was visible along the river.

Keywords: river ecosystem, nitrogen input, stable isotope, food web

## Aerobic methane production in oxygenated water column of a lake ecosystem

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Methane is a potent GHG with about twenty times the global warming potential of carbon dioxide. Globally, half of CH<sub>4</sub> emissions are linked to industry and the extraction of fossil fuels, while the remainder of emissions is related to natural sources such as wetlands, freshwaters, oceans, forests, and termites. Among such various natural sources, lake ecosystems are now recognized as the important source of atmospheric CH<sub>4</sub>, evading the 8-48 Tg CH<sub>4</sub> yr<sup>-1</sup> (6-16% of total natural CH<sub>4</sub> emissions and greater than oceanic emission)(Bastviken et al. 2004). Therefore, identifying the pathways and mechanisms of CH<sub>4</sub> production in lake ecosystems is prerequisite to predict the GHG concentrations in the atmosphere and the resultant global warming in the future of the earth.

In lake ecosystems, the majority of methane production has long been believed to occur in anoxic sediments via methanogenesis. However, we have recently found the novel pathway of methane production in aerobic environments with well-oxygenated water in oligotrophic lakes. In particular, in lakes with phosphorus-deficient conditions, dissolved CH<sub>4</sub> concentrations often exhibit a large subsurface maximum during the stratified period. Moreover, seasonal occurrence of the CH<sub>4</sub> maximum was closely related to the abundance of planktonic microbes (such as *Synechococcus*) in the oxygenated water, suggesting active methane production by microbes even in the presence of O<sub>2</sub>. Furthermore, the microcosm experiments confirmed the aerobic methane production when methylphosphonic acid (MPn) was added to the P-deficient lake water, suggesting the expression of *phn* genes encoding a carbon-phosphorus (C-P) lyase pathways for P utilization and producing methane from MPn. These findings are contradict to the conventional theory of methane production (methanogenesis in the absence of oxygen) but correspond to the recent findings on the aerobic CH<sub>4</sub> production in the North Pacific gyre (Karl et al. 2008); this study showed that marine microorganisms use MPn as a source of phosphorus when inorganic phosphate is scarce and generate CH<sub>4</sub> as a byproduct of MPn metabolism.

In this session, we will present such novel methane production pathway observed in an oligotrophic lake, central Japan. Spatial and temporal dynamics of dissolved methane and planktonic microbes, as well as the laboratory microcosm experiments show the causal relationships between aerobic microorganisms, their phosphonate metabolism, and aerobic methane production in lake ecosystems.

Keywords: Aerobic methane production, cyanobacteria, *Synechococcus*, methylphosphonic acid, P-deficient lake

## Effect of fertilizer use and N deposition on global terrestrial nitrogen cycling in 1960-2010

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Human activities have considerably disturbed terrestrial nitrogen cycling especially after the industrial revolution. Because Harbor-Bosch techniques and fossil fuel combustions have been large sources of reactive nitrogen to the terrestrial ecosystems. The recent N loading derived from these sources on terrestrial ecosystems was estimated 2 times higher than biogenic N fixation in terrestrial ecosystems (Gruber et al., 2009). In this study, we evaluated N fertilizer and N deposition on global terrestrial N cycling using ecosystem model 'VISIT' and global datasets. For the cropland, we made spatial temporal explicit N fertilizer input data (as NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> respectively) made by FAO statistics, historical land-use dataset and global crop calendar in SAGE dataset. For N deposition, we used global grid data from Galloway et al. (2004) with simple interpolation in time-series. From the simulation results, we evaluated historical N cycling changes by land-use changes and N depositions in N cycling (e.g., N leaching, N<sub>2</sub>O, NO) at global scale.

Keywords: N fertilizer, N deposition, N<sub>2</sub>O, Land use change, N leaching

## The diversity-stability relationship in soil microbial community investigated by a diversity-manipulation experiment

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How biodiversity influences the stability of ecosystem processes is the central question in environmental science, but empirical investigations on the biodiversity-stability relationship in soil microbial community is still limited. To investigate the diversity-stability relationship in soil microbial community, microbial community composition was manipulated using taxon-specific biocides, and changes of community functions (i.e., soil decomposition activities) against changes in external environmental factors (i.e., plant materials to be decomposed) imposed to the soil microbial communities were investigated in a microcosm experiment.

Distilled water, bactericide (oxytetracycline) and fungicide (cycloheximide) were added to forest soils to create communities that are intact (i.e. fungi and bacteria are coexisting), fungi dominated and bacteria dominated, respectively. For decomposition substrates, fresh leaves of eastern hemlock and sugar maple were collected from the same location as the soil collection. The leaves, whose chemical qualities differ from each other, were dried, powdered, then mixed to fixed proportions to produce the substrate quality variations. The substrates were then added to each microbial community, and soil decomposition activity (soil respiration rate and activities of acid phosphatase, *N*-acetyl-glucosamidase,  $\beta$ -*D*-glucosidase and cellbiohydase) was measured after the substrate addition.

Soil respiration rates of the bacterial and fungal communities showed highly significant change along the substrate quality variation, but those of the coexisting community changed less significantly. Dependence of the enzyme activity on the substrate quality in the coexisting community was the weakest in general. These results indicated that the decomposition activity of the coexisting community was generally more stable than those of the less-diverse communities. In addition, microbial community compositions, which were estimated by soil lipid profile, changed more flexibly along the substrate quality variation for the coexisting community. These results can be interpreted as that, for the coexisting community, substrate quality influenced the microbial composition, and in turn, the shift in the microbial composition buffered the influences of the changes of substrate quality. The results could indicate that belowground microbial diversity as well as aboveground plant biodiversity is essential for the stability of terrestrial ecosystem processes, which are driven by the interaction of production and decomposition.

Great cautions should be taken because the specificity of the taxon-specific biocides used in this study was not perfect. For example, there must be many bacteria species that could not be inactivated by the addition of the bactericide. In order to understand the diversity-stability, or diversity-function, relationship in microbial community, more sophisticated methodology to manipulate microbial community composition is required. Limitations of current methodologies as well as possible techniques for the better manipulation of microbial community composition will be discussed in this presentation.

Keywords: biocides, enzyme activity, diversity, soil microbial community, soil respiration rate, stability

## Determination of phosphorus species and bioavailability in allophanic and non-allophanic Andisols

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Andisols have high phosphorus (P) retention capacity due to abundant active aluminums (Al) and irons (Fe). Such characteristics result in a significant inhibition of plant growth in Andisols, if not properly managed. Andisols are categorized into two groups on the basis of the difference in the clay mineral compositions. One group is called as allophanic Andisols, including allophane and imogolite in the clay fraction. The other is called as non-allophanic Andisols, including Al- and Fe- humus complexes and 2:1 phyllosilicates. These soil colloids are considered a major cause of high P retention capacity of allophanic and non-allophanic Andisols. Soil P forms have been investigated using chemical extraction methods. Chemically extracted P fractions of H<sub>2</sub>O-P and NaHCO<sub>3</sub>-P are considered readily soluble P, while NaOH-P is modelately labile P associated with Al and Fe, and HCl-P is apatite-like P. However, there are no studies determining chemical species and hosting phases of P in allophanic and non-allophanic Andisols at the molecular levels. This study was conducted to characterize the species and sorption hosts of P in allophanic and non-allophanic Andisols using Hedley's sequential P extraction method, solution <sup>31</sup>P-NMR and X-ray absorption near-edge structure (XANES) spectroscopy. For revealing the behavior of P in soils precisely, it is required to separate the different soil colloids along with their density and then identify P speciation and hosting mineral phases. This study used a density separation method that can classify soil colloids including humus and Al/Fe (oxy)hydroxides by their density.

The total concentration of P in the allophanic and non-allophanic Andisols was 6.2 g P kg<sup>-1</sup>. The sequential fractionation of bulk soil showed that the largest P pool of both allophanic and non-allophanic Andisols was NaOH-P. The density fractions of 2.0-2.25, 2.25-2.5, and >2.5 g cm<sup>-3</sup> accounted for 88% of allophanic Andisols, and among five fractions, the 2.0-2.25 g cm<sup>-3</sup> fraction was largest (44%). On the other hand, the density fractions of 1.8-2.0, 2.0-2.25, 2.25-2.5, and >2.5 g cm<sup>-3</sup> accounted for 88% of non-allophanic Andisols. The sequential fractionation of allophanic Andisols showed that the NaOH fraction had a large proportion of inorganic P (Pi, 76-92%) and organic P (Po, 72-99%). The sequential fractionation of non-allophanic Andisols also showed NaOH-Pi (46-83%) and Po (54-97%) were consisted largely of phosphorus pool, with exceptions in >2.25 g cm<sup>-3</sup> fractions. The results combined with the density separations and sequential extraction indicated that i) P in allophanic and non-allophanic soils is primarily associated with Fe and Al minerals, ii) Pi and Po in the 2.0-2.25 g cm<sup>-3</sup> fraction accounted largely for the total P of allophanic Andisols (Pi: 61%, Po: 68%), iii) Pi and Po in 1.8-2.0 g cm<sup>-3</sup> fraction accounted largely for allophanic Andisols (Pi: 48%, Po: 64%). According to the solution <sup>31</sup>P-NMR results, orthophosphate monoester accounted largely for Po in allophanic and non-allophanic Andisols. Further investigations on XANES and NMR spectroscopy will be presented for more detailed P speciation in the soils.

Keywords: phosphorus, ecosystem, Andisols

## Development of a carbonized wood passive sampler for atmospheric mercury

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### [Intro]

UNEP and WHO require reduce the amount used and mercury emission because it is a toxic metal. As a result, reduce and discharge in the world but Southeast Asia and China increase coal production and used by gold mining, And so increase emission to atmosphere. Emitted mercury to atmosphere is Hg<sup>0</sup> (elemental mercury) with over 95%. It can be transported and deposited to remote place from the sources because calculated atmospheric residence time of Hg<sup>0</sup> was estimated about 1 to 2 year. Furthermore deposited Hg can be converted to organic mercury and accumulated in the food chain, posing a potential threat to human's health. As a result, it is important to monitoring atmospheric mercury pollution. The present, atmospheric mercury sampler is active sampler with gold amalgam collection glass tube, but it is difficult to sampling cover wide area for high costs and need electrical power. So we made simple passive sampler for mercury monitoring with carbonized wood and experimented.

### [Method]

Sticked a wood (*c.japonica*) to acrylic laboratory dish with double-stick tape after it had been cut to 2.5cm×4.5cm×1.5cm and carbonized at 300 °C 2h in a electric muffle furnace. We conducted Uryu Exoerimental Forest of Hokkaido University, Sapporo campus of Hokkaido University, Kumagaya campus of Rissho University, Kuniiriyama in Gunma , Kanazawa University, Tottori University, Hiroshima University and Chiang Mai University (Thailand). Moreover, we compared the active sampler at Center for Environmental Science in Saitama.

### [Result]

Mercury concentration in propose passive sampler increased as day passed at All conducted sites. Mercury concentration in part of carbonized woods were 0.39 (for 33 days), 0.44 (for 66 days), 0.63 (for 95days), 0.86 (for 127 days), 0.91 ng Hg cm<sup>-2</sup> (for 158 days), and correlation coefficient was 0.95 at Center for Environmental Science in Saitama.

Atmospheric mercury concentration range were 2.0 to 2.6 ng Hg m<sup>-3</sup> during experiment. Absorption mercury speed into carbonized wood was uniform in steady atmospheric mercury. Propose passive sampler and active sampler were correlated, slope was  $y=14.7x$ , correlation coefficient was 0.95. Propose passive sampler was agreement with the data obtained by an active sampler by these results.

Keywords: mercury, carbonized wood, passivesamplerq, monitoring of air pollution

## Mechanisms and regulating factors of dissolved organic matter production in beech forest soils in northern Kyoto

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In forest ecosystems, most of the organic matter supplied to the organic layer mineralizes to CO<sub>2</sub>, but a proportion (~30%) is leached as dissolved organic matter (DOM), as soil water percolates. DOM plays important roles in carbon and nutrient cycling in forest soils, however, the controlling factors and mechanisms of DOM production remain to be clarified. Since DOM contains high concentrations of aromatic compounds derived mainly from lignin, the roles of microorganisms in lignin solubilization and DOM production were investigated under field condition.

The concentrations and fluxes of dissolved organic carbon (DOC) in soil solution were quantified under beech forest in northern Kyoto. The activities of lignin-degrading enzymes, lignin peroxidase (LiP) and manganese peroxidase (MnP), and fungal community composition were analyzed.

The DOC fluxes increased in the organic layer (344 kg C ha<sup>-1</sup> yr<sup>-1</sup>), followed by a decrease with depth in the mineral soil layers (20 kg C ha<sup>-1</sup> yr<sup>-1</sup>). The seasonal fluctuation of DOC concentrations showed that DOC production increased in summer with increasing temperature, highlighting the importance of microbial activity to DOM production. The activities of both lignin-degrading enzymes, MnP and LiP, were detected in the organic layers, and several potential producers of enzymes, namely basidiomycete fungi, were also identified. These findings could support the central roles of fungi in lignin solubilization and DOC production in organic layers under beech forest in northern Kyoto, where the large fluxes of DOM leaching was observed.

## Soil nitrite transformation along a forest slope and controlling factors

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We conducted a tracer study to clarify the spatial heterogeneity of nitrite ( $\text{NO}_2^-$ ) dynamics in forest soils. Because of its reactive nature,  $\text{NO}_2^-$  does not usually accumulate in forest soils. This low concentration and experimental difficulties of accurate quantification have hampered quantitative detailed analyses of gross  $\text{NO}_2^-$  production and consumption in terrestrial environments. However,  $\text{NO}_2^-$  is an intermediate in many N transformation processes including nitrification and denitrification. Furthermore  $\text{NO}_2^-$  can also be reduced to gaseous N and react with organic matter not only biologically but also chemically. Thus  $\text{NO}_2^-$  dynamics may control whole N retention/emission characteristics in forest soils.

We added  $^{15}\text{NO}_2^-$  to mineral top soils derived from a slope of a Japanese cedar forest. Primary properties of soils such as concentration of inorganic N, pH and water content differed geographically; N concentration, pH and water content are lower in the upper soils.  $\text{NO}_2^-$  production and consumption rates gradually increased from upper slope to lower slope. Quite short mean residence time of  $\text{NO}_2^-$  implies that  $\text{NO}_2^-$  consumed very rapidly anywhere in slope. The dominant pathway of  $\text{NO}_2^-$  consumption change geographically. It is suggested that the conversion to DON and gaseous N is more important in upper soils. On the other hand, conversion to  $\text{NO}_3^-$  (nitrification) is dominant in lower soils.

At this presentation, we focus on geographical difference of  $\text{NO}_2^-$  dynamics and their regulation by environmental factors.

Keywords: Forest soil, Nitrite,  $^{15}\text{N}$  tracer, Dissolved organic nitrogen, Nitrification

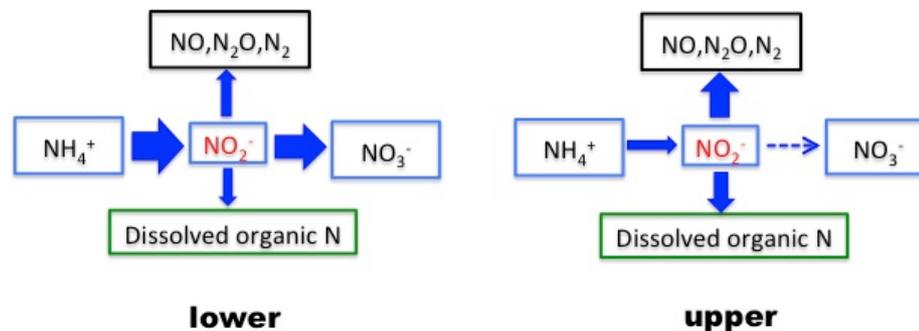


Fig.1 Schematic picture of nitrite dynamics along a forest slope.  
 Difference between upper and lower soils.

## The availability of atmospheric nitrate in a forested ecosystem

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Nitrogen is an important element for forest ecosystems; shortage of nitrogen limits plant growth [Vitousek and Howarth, 1991], however, nitrogen discharged from forested ecosystems link to various environmental problems, such as eutrophication of aquatic ecosystems and deterioration of drinking water quality. Recently, atmospheric nitrogen deposition into terrestrial ecosystems is increasing [Galloway et al., 2008]. However, influence of the increase of atmospheric nitrogen deposition on forested ecosystem is not clear because the interaction between nitrogen input/output and inner nitrogen cycle is not sufficiently understood. In this study, to clarify the interaction between nitrogen input/output and inner nitrogen cycle, we investigate the atmospheric nitrate discharge rate from forested watershed and discuss the availability of atmospheric nitrate deposited into forested watersheds.

Keywords: forested watershed, stable isotope, nitrate, atmospheric deposition

## Estimation of leaf biomass and nitrogen uptake in a hinoki cypress forest

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Nitrogen uptake by aboveground vegetation in forest ecosystems is determined from nitrogen loss by litterfall plus nitrogen allocated to aboveground biomass increment. Nitrogen in litterfall is accurately estimated by littertrap while that in stem increment is by measurement of tree growth. In contrast, estimation of leaf biomass increment is difficult. Although there is close correlation between leaf biomass and cross-sectional area at lowest live branch in the crown, it is difficult to measure diameter at the lowest live branch. Recently, the simple method to estimate leaf biomass is developed by using tree height, height at lowest live branch and diameter at breast height. The applicability of this method depends on tree species and information about hinoki cypress is not known. In this study, we determined the equation for estimating leaf biomass by this method to hinoki cypress trees in Kochi prefecture, southern Japan. In addition the obtained equation is applied for a hinoki cypress forest where tree biomass and litterfall is measured for 20 years.

In two areas at different altitude in Kochi prefecture, 14 hinoki trees were felled and leaf biomass was measured. The relationship between leaf biomass ( $W_{\text{leaf}}$ ), and tree height ( $H$ ), height at lowest live branch ( $H_b$ ), and cross-sectional area at breast height ( $A_{1.3}$ ) was determined by following equation.

$$W_{\text{leaf}} = 1.02[0.0364AB^{1.10}] \quad (r^2=0.926, p<0.0001)$$

$$AB = A_{1.3} [(H - H_b) / (H - 1.3)].$$

This equation is applied for a hinoki cypress forest in Kochi prefecture. Leaf biomass is determined at seven periods between 21 to 41 years old. Leaf production is calculated as leaf-litterfall plus increase of leaf biomass. Nitrogen uptake was calculated as sum of nitrogen in litterfall, increase of nitrogen in leaf and stem biomass. Nitrogen use efficiency of aboveground biomass was calculated as sum of leaf and stem production divided by nitrogen uptake. There was no significant correlation between forest age and leaf biomass, leaf production, stem production, nitrogen uptake and nitrogen use efficiency. However, nitrogen uptake was positively correlated with leaf biomass and leaf production. Nitrogen uptake was not correlated with stem production. These results suggest that nitrogen use of hinoki cypress does not show clear trend in relation to stand age but hinoki cypress utilize nitrogen efficiently to sustain stem production when nitrogen uptake is lower.

Keywords: hinoki cypress, leaf biomass, Stem, nitrogen, forest age