

## 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  $^{18}\text{O}$  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 obtusifolol), 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

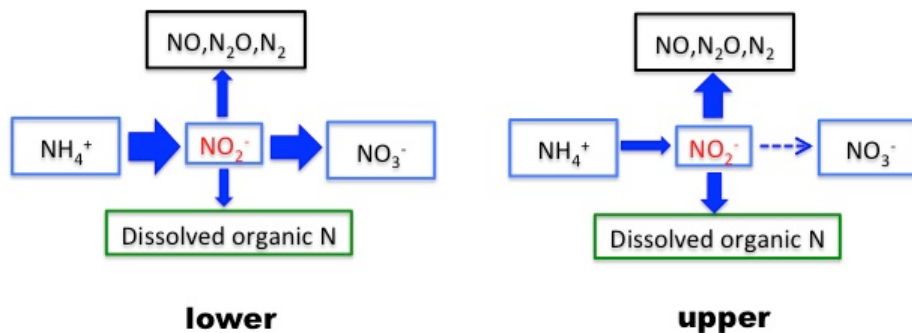


Fig.1 Schematic picture of nitrite dynamics along a forest slope.  
Difference between upper and lower soils.

## 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

## A metabolic model of stable isotope dynamics

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Carbon and nitrogen stable isotope analysis have been a powerful tool used for identifying food-web structures. Our recent study suggested that the ratios of trophic fractionation of carbon and nitrogen isotopes ( $\Delta\delta^{15}\text{N}/\Delta\delta^{13}\text{C}$ ) throughout food chain are similar in various ecosystems (Wada et al. 2013), although the general mechanisms determining isotopic incorporation rates and discrimination factors are poorly understood.

Here, we developed a mechanistic model of the isotopic fractionation in metabolic processes that are common to animals composing most grazing food chains. Particularly, we calculate fluxes of carbon and nitrogen stable isotopes within an organism by following fluxes of molecules involved in some of physiological reactions: the synthesis of amino acids and their carbon skeletons, the rates of which are governed by energy-producing systems such as glycolysis, the TCA cycle, and oxidative phosphorylation, that is, the ratio of the rate of amino-acid syntheses to that of energy-yielding processes. The active metabolic pathways above are assumed to be changed by the conditions of supply (diet quantity and quality) and demand (growth rate).

The model result suggests that the instant isotopic composition of animals are sensitive to the change of their diet composition and growth rate, but on the other hand, the isotopic composition converges as the integrating period becomes long. With further temporal scaling-up, in turn, the isotopic compositions of animal body reflect the spatio-temporal variability due to their life history, migration and foraging patterns. This gives mechanistic insight to what information we can acquire from the observation.

## Tracing environmental history of macroalgae by the use of radiocarbon and stable isotope ratio analyses

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In the bays located along the Sanriku coastal area (northeast Japan), where the Kuroshio and Oyashio mix in a complex manner, environmental conditions (e.g. water temperature, irradiation and nutrients) may largely change depending on which current predominantly enters into the bays. Changes in environmental conditions, in turn, may exert a large influence on growth of and interactions between organisms residing there. However, knowledge is limited regarding relationships between shifts in hydrographic conditions and physiological responses of organisms to environmental variability. The purpose of this presentation is to report our attempt to trace environmental history of individual macroalgae (wakame, *Undaria pinnatifida*), a widespread benthic primary producer and an important aquaculture product in the Sanriku area, by a combined use of radiocarbon and stable isotope ratio analyses. The key concept was to use distinct <sup>14</sup>C abundance between the two water currents, i.e., <sup>14</sup>C abundance of dissolved inorganic carbon in the Oyashio water is lower than that in Kuroshio water due to upwelling of old deep water. We assumed that <sup>14</sup>C abundance profile of pinnate blades of macroalgae (blades near the top are older than those near the bottom) reflects a temporal change in dissolved inorganic carbon <sup>14</sup>C (hence, shift in water current) via photosynthetic fixation. We also analyzed carbon and nitrogen stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of the blades to gain insights into changes in physiological state of the macroalgae during their growth.

We collected sporophytes of *U. pinnatifida* cultured between October 2012 and March 2013 at two stations (one located near the bay center and the other located near the river mouth) in Otsuchi Bay. One individual (length, ca. 190 cm) was collected at each station. For each individual, a tip of each pinnate blade was cut, treated with HCl, reduced to graphite, and served for determination of radiocarbon composition ( $\Delta^{14}\text{C}$ ) profile using an accelerator mass spectrometer.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the corresponding samples were also measured using a stable isotope ratio mass spectrometer.

$\Delta^{14}\text{C}$  of pinnate blades of the saprophyte collected near the bay center varied between 0 and 40 permil.  $\Delta^{14}\text{C}$  values were high for the blades located at the upper and lower parts of the macroalgae, whereas they were low for the blades situated at the middle part. These results indicate that the sporophyte experienced the intrusion of the Oyashio water during the period of the development of the middle blade. Similar to the pattern in  $\Delta^{14}\text{C}$ ,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were also lowest at the middle part, although the position of the minimum was skewed toward bottom relative to the position of the  $\Delta^{14}\text{C}$  minimum. There was a significant positive correlation between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , suggesting that the variation in stable isotope ratios reflected macroalgal physiological responses and associated shifts in isotope fractionation. Collectively, these results were interpreted as an indication that the physiological state of the saprophyte was altered with a time lag after the intrusion of the Oyashio water into the bay. In contrast,  $\Delta^{14}\text{C}$  profile of pinnate blade was complex for a saprophyte collected near the river mouth. For this individual, there was no clear pattern in distribution of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among blades. Complex variations in isotopic compositions for this individual might be ascribed to the influence of inflow river water.

Keywords: macroalgae, Sanriku coast, water current, radiocarbon, stable isotope

## Accumulation of humic-like fluorescent dissolved organic matter in the Japan Sea interior

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Marine dissolved organic matter (DOM) is the largest reduced carbon reservoir in ocean. Most marine DOM is produced by marine biota and is resistant to rapid microbial degradation. Thus, it is crucial to know the dynamics of recalcitrant DOM for determining whether the marine DOM reservoir is stable or not. Even though there have been several hypotheses regarding with the recalcitrant mechanism of marine DOM, the microbial production of recalcitrant DOM (defined as microbial carbon pump) has been considered as the main process. Humic-like fluorescent DOM (FDOM<sub>H</sub>) has found to produce during microbial incubation. Even though FDOM<sub>H</sub> has known to easily degrade by sunlight, linear relationships between fluorescence intensity of FDOM<sub>H</sub> and indicators of microbial remineralization, e.g., apparent oxygen utilization (AOU), have been observed throughout the ocean. These experimental and observational results imply that FDOM<sub>H</sub> is a product of microbial carbon pump. Another important source of FDOM<sub>H</sub>, especially in coastal environments and marginal seas, is riverine supply. Even though the major fractions of FDOM<sub>H</sub> have been considered to be photo-degraded in coastal environments, substantial contribution of terrestrial FDOM<sub>H</sub> into ocean interior has been suggested. Thus, in addition to accumulation of in situ produced FDOM<sub>H</sub>, recalcitrant terrestrial FDOM<sub>H</sub> might occur in deep ocean, especially in marginal seas. However, it is not clear whether recalcitrant autochthonous and/or terrestrial FDOM<sub>H</sub> is accumulated in deep ocean of marginal seas or not.

We determined vertical profiles of FDOM<sub>H</sub> at 5 stations in the Japan Sea and 5 stations in the western North Pacific using excitation emission matrix fluorescence with parallel factor analysis (EEM-PARAFAC). Seawater samples from surface to bottom waters of the Japan Sea and the western North Pacific were collected during T/S Oshoro-maru (C184) and R/V Taisei-Maru (KT-11-17) cruises, respectively. Two FDOM<sub>H</sub> were obtained after EEM-PARAFAC and assigned as traditional terrestrial and marine (microbial) FDOM<sub>H</sub>, respectively. In the Japan Sea, levels of both FDOM<sub>H</sub> were lowest in surface waters, gradually increased with depth below surface waters, and were highest in waters distributed depths greater than 2000 m that were corresponding to the lower part of Japan Sea Proper Water (JSPW), i.e., lower part of the Japan Sea Deep Water (JSDW) and the Japan Sea Bottom Water (JSBW). Levels of both FDOM<sub>H</sub> were linearly correlated with AOU in the JSPW, suggesting that both FDOM<sub>H</sub> were produced in situ in the JSPW. Interestingly, levels of both FDOM<sub>H</sub> in the JSPW were similar or slightly higher compared with those in deep waters of the western North Pacific, even though AOU in the JSPW were significantly lower than those in deep waters of the western North Pacific. Such distributional characteristics of FDOM<sub>H</sub> in the JSPW imply that FDOM<sub>H</sub> is accumulated in the interior of the Japan Sea. We will discuss possible origin and accumulation mechanism of FDOM<sub>H</sub> in the Japan Sea interior.

Keywords: Japan Sea, Dissolved Organic Matter, Humic-like fluorescence

## Sources of hydroxyl radical photochemically produced in headwater streams from nitrogen-saturated forest

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Hydroxyl radical ( $\cdot\text{OH}$ ) is the most oxidative reactant among the active oxygen species and oxidation reactions with  $\cdot\text{OH}$  are involved in important biogeochemical processes. In this study  $\cdot\text{OH}$  photoformation rate ( $R_{\text{OH}}$ ) was determined in headwater stream samples from nitrogen (N)-saturated forests, 1) to quantify the sources of  $\cdot\text{OH}$  in headwater streams and 2) to evaluate the nitrate ( $\text{NO}_3^-$ )-induced enhancement of  $\cdot\text{OH}$  formation in stream water caused by N saturation in forested watersheds. Stream water fulvic acid extracted from the forested watersheds was used to quantify the contribution of dissolved organic matter (DOM) to  $R_{\text{OH}}$ . The results showed that almost all (97%; 81-109%)  $R_{\text{OH}}$  sources in our headwater stream samples were quantitatively elucidated; the photolysis of  $\text{NO}_3^-$  (55%; 34-75%), nitrite [N(III)] (2%; 0.5-5.2%), and DOM-derived  $\cdot\text{OH}$  formation, from which photo-Fenton reactions (18%; 12-26%) and the direct photolysis of fluorescent dissolved organic matter (FDOM) (22%; 10-40%), was successfully separated. FDOM, which accounted for 53% (24-96%) of DOM in total organic carbon bases, was responsible for  $\cdot\text{OH}$  formation in our headwater streams. High  $\text{NO}_3^-$  leaching caused by N saturation in forested watersheds increased  $R_{\text{OH}}$  in the headwaters, indicating that N-saturated forest could significantly change photoinduced and biogeochemical processes via enhanced  $\cdot\text{OH}$  formation in downstream water.

Keywords: hydroxyl radical, dissolved organic matter, nitrate, photo-Fenton reaction, stream, photoinduced processes

## Effects of clear-cutting on the loss of ion and DOC from cool-temperate forested watershed in northern Japan

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Nitrate and dissolved organic carbon (DOC) concentrations in stream water before and after clear-cutting of trees and subsequent strip-cutting of understory vegetation, dwarf bamboo (*Sasa* spp.) were investigated to understand the effect of these disturbances on biogeochemical processes in forested watershed in Teshio Experimental Forest in northern Japan. Trees of 8 ha watershed except riparian zone were clear-cut in January-March of 2003. *Sasa* was strip-cut in October of 2003 and larch seedlings were planted on the cut line immediately after the *Sasa* cutting. Stream water was sampled every two or three weeks from 2002 to 2013. Tree-cutting did not cause a significant increase of nitrate concentration in stream water during the growing season after the cutting. Subsequent *Sasa*-cutting caused significant increase of stream nitrate concentration to ca. 15 micro mol L<sup>-1</sup>. At the cut site, it has been reported that *Sasa* compensated the decrease in tree fine root biomass. Thus, we suggest that nitrogen uptake by *Sasa* was very important in mitigating nitrogen leaching after tree-cutting, and the decline of this nitrogen uptake after *Sasa*-cutting lead to marked nitrate leaching to the stream. However, after that stream nitrate concentration fluctuated in the range of <0.1 to >20 micro mol L<sup>-1</sup> depending on date and year, and was especially high in 2007 throughout the year. It did not get back to pre-cutting level. Cation (K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) concentration and pH fluctuated much depending on the flow rate and changes by both cutting were not observed. On the other hand, ammonium was detected in 2007 and synchronized with increase in nitrate concentration. DOC concentration in stream water was not changed after both cuttings of tree and *Sasa* and had clear seasonal pattern that peaked in late summer. Stream DOC concentration increased in growing period with low runoff from late May to August and then decreased after runoff increased in fall, indicating that dilution by the runoff reduce stream DOC concentration after late summer. However, DOC concentration remained low during winter when runoff was stably low, suggesting that high temperature also promote DOC production in soil during the early summer. DOC loss from ecosystem was not influenced by the cutting of trees and *Sasa* in this watershed owing to the adsorption to the soil at the cut area. These results indicate the response to cutting is different between NO<sub>3</sub><sup>-</sup> and DOC due to the different source area of these solutes in the watershed with cool climate and the gentle basin topography.

Keywords: nitrate, DOC, cation, *Sasa*, stream discharge

## Microbial contributions to biochemical commonalities of decaying organic matter

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Natural organic matters including dead organisms are decayed (decomposed) under various environmental conditions, but some parts of them, residues, remain for long time. Organic matter residues are major components of organic matter in soil and marine ecosystems, and play important functions and roles. The residues are originated from various organisms, organs, and cellular components, and in various stages of organic matter decay. Recently, it has been suggested that organic matter produced by microorganisms increase its percentage in residues during decay process. However, researches are limited especially on the quality of the produced organic matter and its producing processes. In this research, we investigate biochemical differences between original organic matters and organic residues and biochemical changes of organic matter during decaying process to clarify microbial contributions to the organic matter residues. Biochemical molecular compositions of original organic matters was more variable than those of organic residues in soil and water: for example, glycine:lysine (Gly/Lys) ratio and glucosamine:galactosamine (GlcN/GalN) ratio of original organic matters varies widely with origins, while those of decayed residues indicates considerably narrower ranges for both ratios. An incubation test, litterbag experiment, in a terrestrial ecosystem showed a clear trend that Gly/Lys ratio increased and GlcN/GalN ratio decreased during decay for all three species litter. These changes were also observed for litterbag experiments conducted in water, suggesting that it might be uniformly-observed biochemical directivity for decaying organic matter. In addition, amino acids and amino sugars are biomoleculars, which increase during decaying processes, suggesting that this directivity results from microbial products. Another biochemical directivity was observed for molecular weight distribution of decaying organic matters, suggesting that organic residues derived from microbial products contribute to biochemical directivities and commonalities of decaying organic matter in various environmental conditions.

Keywords: Organic matter decay, Soil, Ocean, Amino acids, Amino sugars, Molecular weight distribution

## Spatial modelling of water, nitrogen and sediment for systematic conservation of multiple ecosystem services

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Spatial modelling and analysis of multiple ecosystem service (ES) under land use and climate changes provides useful support for decision making in sustainable planning, management and policies of large landscapes. This study aimed to integrate the GIS modelling approach of spatial explicit ESs (water yield, and retention of nitrogen (N) and sediment) into system conservation model under various land use and climate changes in Teshio river watershed located in northern Hokkaido, Japan. In this study, we applied hydrology and material flow model (Soil and Water Assessment Tools, SWAT model), land use change model (CLUET) and system conservation model (Marxan). The multiple scenario includes three different land use maps in past (1976), current (2006) and future (2036), and three climate change scenarios (short-term (2010-2039), mid-term (2040-2069), and long-term (2070-2099)).

Our results indicated that various land use and climate change scenarios showed different impact on ES and system conservation in the watershed. The forest land use change significantly affected on magnitudes and spatial patterns in water yield, sediment and N retention. It was suggested that south western and northern part of the studied watershed should be conserved to match the given conservation targets of multiple ESs (0.3 and 0.5 of maximum ES values). The protection area to satisfy each ES conservation target increased with increase of differences between each ES and maximum ES values under land use and climate changes. Our results indicated that the land distribution and area of optimal ES protection for multiple ESs were totally different from those for single ES. The conservation area for multiple ESs was more compact than those for single ES. The proposed approach in this study provided useful information to assess the responses of ESs and system conservation under the land use and climate changes. The system conservation area of ES protection for multiple ESs provided an effective trade-off tool between environmental protection and agriculture expansion.

Keywords: Ecosystem services, SWAT, Marxan, Land use and climate change



## Spatial variability of mineralization and nitrification in soil nitrogen along the hillslope in Japanese cedar forest

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**Introduction** Precise understandings of mechanism of nitrogen (N) cycle is one of the most important subjects for ecosystem conservation in forests and rivers. Especially, the responses of N mineralization and nitrification to environmental changes are especially important due to their role in entire N cycling. Previous studies suggest that nitrification and mineralization have spatial variation in forest, which are controlled by the geophysical condition such as topography and water condition. The aim of this study is to clarify the mechanisms behind the spatial variety of nitrification and mineralization rate in soil along the hillslope.

**Methods** The field observations and soil samplings were conducted at Fukuroyamasawa Experimental Watershed (Catchment Area 0.8ha) which belongs to the University of Tokyo, Chiba forest. Dominant vegetation on the slope consisted of *Cryptomeria japonica* plantation. Along the hillslope (entire length: 110m), soil samples were collected at organic layer (O-layer) and mineral layer (0-10cm) at 10m intervals. After measuring water content and pH, the  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentration of soil extract (by 2M KCl solution) were measured. The net mineralization and nitrification rate were measured by laboratory incubations (28 days). Then, the gross mineralization and nitrification rate were determined using the  $^{15}\text{N}$  pool-dilution method.

**Results** Soil moisture content was higher at the down slope part. Similarly, the pH value was higher at down slope part. The pool size of  $\text{NO}_3^-$  was significantly low at the up slope part, and gradually increased along the slope toward the lower portion. On the other hand, the pool size of  $\text{NH}_4^+$  did not have visible pattern along the slope. Moreover, there was not significant spatial variations in net and gross mineralization rate over the hillslope while net and gross nitrification had significant spatial pattern with higher rate at the down slope part.

**Discussion** Difference in the spatial patterns of mineralization and nitrification suggested that nitrification is more sensitive to the geophysical conditions such as the soil moisture content. We are attempting to explain the mechanisms of these spatial patterns from the spatial distributions of related microbial communities in the next step of this study

## Nitrogen mineralization rates in forest soils in the Japanese archipelago measured by field incubation

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### 1. Introduction

To predict the effect of climate change on nitrogen dynamics in the forest ecosystem, it is necessary to investigate nitrogen mineralization and nitrification at various locations allowing for modeling of nitrogen dynamics in soils. In this study, we selected 20 sites from the Japanese archipelago and conducted field incubation for measuring net nitrogen mineralization and nitrification. We also considered whether parameters obtained from laboratory incubation were applicable in the field by comparing the ratios between field and laboratory incubations (Urakawa et al., 2013).

### 2. Method

We conducted buried bag method to investigate field net nitrogen mineralization and nitrification rate. In autumn 2012, we established an experimental plot (20 \*20 m) at each site, and at each plot, five soil sampling locations were established. At each sampling location, mineral soil samples were collected from 0-10, 10-30, 30-50 cm depths. Soil samples were sieved (4 mm mesh) to remove roots and gravel, and composited soils from five plots into one sample at each depth. After collecting soils for the initial extraction, buried bags were made and buried in the plots again. We collected them in spring, summer, and autumn 2013, and measured net nitrogen mineralization and nitrification for three seasons (autumn 2012 - spring 2013, spring - summer, summer - autumn). Soil samples were extracted with 2M-KCl solution (1:10) and concentrations of ammonium nitrogen and nitrate nitrogen were measured by colorimetric method. Net nitrogen mineralization and nitrification amount of each season were summed to evaluate the annual amount.

Simultaneously with the field incubation, inorganic nitrogen leaching was investigated by resin column method. Resin columns containing ion exchange resin was installed at depths of 0 and 50 cm. Inorganic nitrogen absorbed by ion exchange resin was extracted with 1M-KCl and concentration of inorganic nitrogen was analyzed by colorimetric method.

### 3. Results and Discussion

Annual net nitrification in 0-50 cm layer ranged widely from 40 to 140 kgN ha<sup>-1</sup> y<sup>-1</sup>. Nitrification amount in 10-50 cm layer was comparable to that in surface soil layer (0-10 cm) due to large bulk density and thickness, while nitrification in 0-10 cm layer accounted for about a half of that of all soil layers.

There was a significant positive correlation between nitrification measured by field and laboratory incubation. This suggests that estimation of field nitrification using parameters obtained from laboratory incubation is possible.

### 4. Reference

Urakawa et al. (2013) Characteristics of nitrogen mineralization rates and controlling factors in forest soils in Japanese archipelago, 2013 AGU Fall Meeting, San Francisco, 9-13 December 2013

Keywords: forest soil, nitrogen mineralization, nitrification, field incubation, nitrogen leaching, ion exchange resin

## Analysis of transportation and consumption processes of atmospheric nitrate in forested watershed by using oxygen isotop

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Some studies reported that atmospheric nitrogen deposition into terrestrial ecosystem has recently increased due to increase of anthropogenic emission of nitrogen compound into atmosphere. However, dynamics of atmospheric nitrogen deposition in forested watersheds is not clearly understood. Moreover, that leads our poor understanding of influence of increasing atmospheric nitrogen deposition on nitrogen cycle in forested ecosystem and nitrogen discharge from forested ecosystem. The purpose of this study is to clarify the mechanisms of transportation and consumption of atmospheric nitrate deposition in forested ecosystems. We collected rainfall, throughfall, surface water, soilwater (10cm, 30cm), groundwater, spring water and streamwater at a forested watershed planted with Japanese cypress in central Japan at biweekly. Samples were analyzed for total nitrogen, dissolved nitrogen, nitrate, ammonium, nitrite, and oxygen isotope of nitrate. Isotope analysis was conducted at ICRE in University of Yamanashi. We also collected soil at several month intervals, and measured nitrate, ammonium in soil and net mineralization rate, and net nitrification rate. We are planning to present transportation rate of nitrogen compounds and atmospheric nitrate through the forested watersheds and discuss the interaction between nitrogen cycle and atmospheric nitrate deposition in forested ecosystem.

Keywords: oxygen isotope of nitrate, transportation and consumption processes of nitrogen, forested watershed

## The effect of soil freeze-thaw on nitrogen transformation through the root litter changes

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Soil freeze-thaw cycles are considered to alter soil nitrogen (N) cycle through physical disturbances of soil, changes in root litter quality, inhibition of microbial N immobilization and others. However, these mechanisms have not been well elucidated yet. Plant litter is important substrate for N mineralization by soil microbes. It has been reported that root litter mass is quantitatively comparable to those of leaf litter in various forest ecosystems. Previous studies suggested that physical disruption of root litter by freeze-thaw cycle in winter affect soil N dynamics through the change in substrate availability. In this study we aimed to clarify that effect of root litter on the rates of soil N mineralization and nitrification under various conditions of soil freeze-thaw.

This study was conducted at Shibeche experimental forest, Kyoto University located in Eastern Hokkaido, Japan. The study site is dominated by Natural oak (*Quercus crispula*) with dense dwarf bamboo (*Sasa niponica*) as understory vegetation. Soil is Humic Andosol. In July 2013, we collected 0-10cm mineral soil and fine root (<2mm) of oak in the 2500 cm<sup>2</sup> square plot. The collected soil was sieved to 2mm to remove coarse gravel and coarse organic matter. Fine roots for incubations were separated manually from the organic matter. The fine roots were added to 25g soil as 0, 5 and 15 mg g soil<sup>-1</sup>, respectively. The soil were exposed to three different freeze-thaw treatments: +5 °C ~-5 °C, -5 °C ~0 °C, -5 °C constant and +5 °C constant for 7days in low temperature incubator. After these freeze-thaw treatments, the soil were incubated at +5 °C for 2 days. For the samples exposed at +5 °C ~-5 °C and -5 °C constant were also incubated at +5 °C for 7 days and at +10 °C for 2 and 7 days. Each treatment had four replications. Soils were extracted using potassium chloride (KCl) before and after incubations, and were measured for ammonium (NH<sub>4</sub>) and nitrate (NO<sub>3</sub>) concentrations in the extracts. Net production rates of NH<sub>4</sub> and NO<sub>3</sub> were calculated as differences of NH<sub>4</sub> and NO<sub>3</sub> contents in soil between before and after the incubations. After the freeze-thaw treatment, roots were extracted using distilled water, and were measured for dissolved organic nitrogen (DON) in the extracts.

Root litter addition significantly increased the net NH<sub>4</sub> production incubated at 5 °C for 2 days after all freeze-thaw treatments (15 mg added >0mg added) with maximum at -5 °C ~0 °C treatment followed by -5 °C constant treatment. However, these effect were not observed in higher incubation temperature (10 °C) and longer incubation period (7days), rather dominated by N immobilization in those treatments. Similarly, the root litter additions significantly increased the net NO<sub>3</sub> production rate (nitrification rate) incubated at 5 °C for 2 days after all freeze-thaw treatments (15 mg added >0mg added) with maximum at +5 °C ~-5 °C treatment. However, these effect were not observed in higher incubation temperature (10 °C) and longer incubation period (7days) as same as the effects to the NH<sub>4</sub> production. The DON supply by water extraction from root litter tended to be large at -5 °C ~0 °C treatment.

These results indicated that increases of soil freeze-thaw cycles with root litter addition increased the net NH<sub>4</sub> production and nitrification. It was suggested that DON supply from root litter by soil freeze-thaw cycle related to these impacts. These effect seems to be remarkable in shorter period (2 days) and lower temperature (5 °C incubation). Furthermore, net NH<sub>4</sub> production, the sum of net NH<sub>4</sub>+NO<sub>3</sub> productions and DON supply from root rate were higher at -5 °C ~0 °C treatment than those at +5 °C ~-5 °C treatments, implying that magnitude (temperature ranges) of freeze-thaw cycle was not simple explain variables to impact of freeze-thaw on the microbial NH<sub>4</sub> production and nitrification activities.

## Estimation of trace gas fluxes in the forest of Mount Fuji using the multi layer model

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We measured vertical profiles of nitrogen oxide, NO, nitrogen dioxides, NO<sub>2</sub>, ozone, O<sub>3</sub>, and VOC in the atmosphere in Fujiyoshida and Hokuroku forest observation sites at the foot of Mt. Fuji in summer 2012. The concentration of ozone increased gradually with the height, but the concentrations of NO and NO<sub>2</sub> did not changed obviously. VOCs showed characteristic vertical profiles. We calculated O<sub>3</sub> fluxes at parts of in and under the canopy, and around the surface layer as  $-2.6 \pm 3.2 \text{ nmol m}^{-2} \text{ s}^{-1}$ ,  $0.2 \pm 2.9 \text{ nmol m}^{-2} \text{ s}^{-1}$ ,  $-8.7 \pm 5.2 \text{ nmol m}^{-2} \text{ s}^{-1}$ , respectively.

Keywords: forest, atmosphere, nitrogen oxides, ozone, VOC, vertical profile

## Localization of delta-34S value distribution in tree ring of Japanese cedar and evaluation on the S deposition history

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Anthropogenic sulfur emissions have been changed with human activities and affected sulfur dynamics in terrestrial ecosystems. Therefore, the information on sulfur deposition change should be important for understanding of the effects of anthropogenic sulfur on its dynamics. The stable sulfur isotope ratios ( $\delta^{34}\text{S}$ ) in tree rings are a useful archive for the history of sulfur deposition (Kawamura et al. 2006), since the  $\delta^{34}\text{S}$  of various origins have specific values and there is few isotopic fractionation through absorption of sulfur by plant. However, only few studies have been conducted about the  $\delta^{34}\text{S}$  in tree ring, and factors affecting the  $\delta^{34}\text{S}$  in tree ring have not been understood.

The aim of this study is to clarify the localization of  $\delta^{34}\text{S}$  distribution in tree ring. We also perform the evaluation of sulfur deposition history at locations received heavy anthropogenic sulfur deposition using tree ring.

The investigation was carried out at two study sites, Yokkaichi (YOK) and Inabu (INA) in central Japan. Both study sites have different histories of sulfur deposition. YOK had been affected by quite high anthropogenic sulfur deposition during 1960s. INA is located about 60 km NE of main urban area (Nagoya City). Three disk samples were obtained from Japanese cedar (*Cryptomeria japonica*) stump in 2013 at YOK and in 2012 at INA. The stumps at YOK were 63-year-old cut down in 2012 and those at INA were 170-year-old cut down in 2007. In addition, at INA, three 40-year-old living stems were cut down in 2013 at INA and the disk samples were obtained. After washing and dried, the tree ring samples were divided into 5 year increments from bark toward the pith, and ground using power mill. The ground samples were digested with  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  on a hot plate and after filtration  $\text{BaCl}_2$  was added to obtain the  $\text{BaSO}_4$ . The  $\delta^{34}\text{S}$  values (VCDT) were measured using EA-IRMS.

To evaluation the localization of  $\delta^{34}\text{S}$  in sapwood, heartwood and pith, the data from the stump and the living wood samples at INA were compared. These samples showed the different localization of  $\delta^{34}\text{S}$  against the age. There were no difference of  $\delta^{34}\text{S}$  between the sapwood (living wood) and the heartwood (stump) at the same age. However, the  $\delta^{34}\text{S}$  values of the pith (living wood) were higher than those of heartwood (stump). This result indicated that the specific composition of sulfur compound might be consisted in pith and the  $\delta^{34}\text{S}$  of the pith should be unsuitable for evaluation of sulfur deposition history.

The  $\delta^{34}\text{S}$  values in ring at YOK declined from the late 1950s to early 1970s and then increased again. This trend was almost homologized in ring at INA and air  $\text{SO}_2$  concentration at near the YOK. In contrast, the minimum value of at YOK (-7.3 ‰) was lower than that at INA (-1.6 ‰). These results should be reflected by the deposition history of anthropogenic sulfur with low  $\delta^{34}\text{S}$  value at each site.

Keywords: Tree ring, Sulfur isotope, Morphology, Sulfur deposition

## Distribution of radiocesium in a small forest at Namie town in Fukushima Prefecture

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Fresh leaf/needle, litter, surface soil, stream water and bottom sand were monthly collected in a deciduous broadleaf forest and an evergreen needleleaf forest in Fukushima Prefecture during non-snowfall period. The concentration of radiocesium (<sup>134</sup>Cs and <sup>137</sup>Cs) was measured by commercially available NaI(Tl) scintillation detector.

The air dose rate at a broadleaf forest (5.64  $\mu$ Sv/h) was higher than that at a needleleaf forest (4.11  $\mu$ Sv/h) in November 2012. The average concentration of radiocesium in each sample was also higher at broadleaf forests than at needleleaf forests. The order of the concentration of radiocesium was litter > surface soil > fresh leaf/needle > bottom sand at both sites, indicating that radiocesium was accumulated in litter. Radiocesium was not detected in precipitation, throughfall, and stream water.

Surface soil samples at each sampling point were taken using a scraper plate in April and December 2013. Samples were taken with 0.5 cm increments for the depth of 0-5 cm and 1.0 cm increments for the depth of 5-10 cm. The maximum concentration was found at the surface at the broadleaf forest in April and December 2013. The maximum concentration was also found at the surface at the needleleaf forest in April 2013 but at 1-1.5 cm in December 2013, indicating that the radiocesium in surface soil penetrated deeply at the needleleaf forest. These differences were likely caused by the soil type and the composition of tree species at the sampling points.

A photostimulable phosphor (PSP) image plate was used to record a two-dimensional image of radioactivity distribution on the leaf/needle and root of the broadleaf tree samples. We used the CR<sup>x</sup>25P (General Electric Company). The image of the needleleaf sample of Japanese cedar showed some high intensity spots on the needles, indicating the presence of radioactive dusts attached onto the plant's surface. On the other hand, the image of the broadleaf showed uniform distribution, suggesting that contamination with radiocesium occurred internally.

In the presentation, we will also report about the runoff processes of the radiocesium with the stream bottom sand.

## Monitoring of atmospheric mercury pollution using a leaf camphor tree ( *Cinnamomum camphora* (L.) Sieb. )

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Source of mercury is divided into two anthropogenic sources such as incineration and sludge of fossil fuel and natural sources, such as by volcanic activity. Mercury discharged from these sources is present in the gaseous atmosphere mainly. On the other hand, trees are accumulated by adsorption or absorption in the leaves and bark of contaminants in the atmosphere. I am thought to absorb atmospheric pollution from the pores in the case of accumulation by the leaves. I was aimed at performing mercury pollution monitoring of air by measuring the mercury content in the leaves in this study. The absorption in the two years up fallen leaves from the deployment of new buds and evergreen broad-leaved tree, using the camphor tree( *Cinnamomum camphora* (L.) Sieb. ) accumulation is expected to indicators of mercury pollution in the atmosphere. I was monitoring for the full year, including the winter an increase in use of fossil fuels is expected by this. It was possible sampling points you ' ve covered as being human influenced due to its proximity to urban areas, in Rissho University campus is located in Kumagaya, Saitama Prefecture. In addition, a point of performing region comparison was a sampling Kirryu City in Gunma, Ogose town in Saitama, Ueno Park in Tokyo, Sarue Park in Tokyo and Katsuura City in Chiba.

### 1. Changes in mercury concentration due to dry and weight change due to drying temperature of leaf

After drying for five hours, respectively 70 °C leaves, at 130 °C, it was found that 60 minute in 70 °C , it is 10 minute at 130 °C to constant weight. Mercury concentration at each temperature was 33.4ngg<sup>-1</sup> at 70 °C, 33.0ngg<sup>-1</sup> at 130 °C.

### 2. Mercury concentration in the leaves within the site-specific mercury concentration in leaves

I was measuring the mercury concentration of each site by dividing the top, middle, at the bottom toward the petiole from the tip of the leaf camphor tree. As a result, mercury concentration was 62.0ngg<sup>-1</sup> at the top, 67.0ngg<sup>-1</sup> at middle, 66.5ngg<sup>-1</sup> at the bottom. In addition, I compared the mercury concentration in leaves in removing the mercury deposited in the leaf and total mercury concentration in the leaves. It is a leaf inside was revealed that much of the mercury contained in the leaves.

### 3. Changes in mercury concentration in the leaves by the time series variation

I investigated the time series changes in mercury concentration accumulated in the leaves by the use of leaves of different leaf age.

Keywords: mercury, camphor tree, environment



MIS21-P16

Room:Poster

Time:April 28 18:15-19:30

## Restoration of Soil Physical Properties by No-tilled Management in Tropical Sugarcane.

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Keywords: Non-till, Sugarcane, Infiltration

## A study of soil organic matter stabilization using physical fractionation, isotopic, and spectroscopic approaches

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Volcanic-ash soil (Andisol) is unique among the world soil types due to the strong physical stability of organo-mineral aggregate structure at micro and submicron scales (Asano and Wagai, 2013, Geoderma) and its high capacity to store organic matter (OM) even in upland surface horizons under warm, moist climate regime where microbial heterotrophic activity is high. Several hypotheses have been proposed to account for these features of Andisol including (i) strong interaction of OM with dissolved metals (Al, Fe) and/or short-range-order (SRO) minerals that are quite abundant in this soil type, and (ii) preservation of recalcitrant compounds such as char.

Here we present some highlights from the 3-year project (GR091, NEXT Program, JSPS) examining the mechanisms of soil OM stabilization with a focus on organo-mineral interactions at various spatial and temporal scales using multiple analytical methods and experimental approaches. After careful consideration of the degree of soil aggregate disruption levels, we physically fractionated Andisol surface horizon sample based on particle size and density. Chemical composition of each physical fraction was assessed by elemental analysis, selective dissolution of inorganic phases, and solid-state <sup>13</sup>C-NMR. The origin and degree of microbial alteration of OM was estimated from C and N stable isotope ratios while the turnover time of C was assessed by radiocarbon measurements. Physical features of soil mineral and organo-mineral aggregate surfaces were characterized by specific surface area (N<sub>2</sub>-BET), XPS, and microscopic methods. We also conducted tracer experiments to further assess the residence time of the OM in each density fractions. Based on these results, we will discuss the progression of organo-mineral associations from fresh plant detritus to the aggregates of varying structure and stability for the studied Andisol.