

## Ocean oxygen depletion due to decomposition of submarine methane hydrate

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Global warming could decompose submarine methane hydrate and cause methane release into the ocean. The released methane causes oxygen depletion via oxidation; however, its global impact is yet to be quantitatively investigated. We have projected the potential impact of oxygen depletion due to methane hydrate decomposition via numerical modeling. We find that the global methane hydrate inventory decreases by approximately 70% (35%) under four times (twice) the atmospheric CO<sub>2</sub> concentration and is accompanied by significant global oxygen depletion on a timescale of thousands of years. In particular, we demonstrate the great expansion of suboxic and hypoxic regions, having adverse impact on marine organisms and ocean biogeochemical cycles. This is because hydrate decomposition primarily occurs in the Pacific Ocean, where present-day seawater has low oxygen concentration. Besides the decrease in oxygen solubility and reduced ventilation associated with global warming, the process described in this study is also important in oxygen depletion.

Keywords: methane hydrate, global warming, ocean oxygen depletion

## Real reactions of seawater and mineral matter: coral reef ecology

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We need to elucidate which marine calcifying organisms can carry out the actual fixation of atmospheric carbon dioxide or not. The carbon dioxide species dissolved into seawaters are starting material of reversible reaction between calcification and decalcification. In our bottom-up research the real enhanced skeleton formation was actually observed from individual primary corals to each tubular colony. The proton transfer in coral reef-building seawaters controls all reversible acid/base dissociation reactions (Chem. Eur. J. 2014, 20, 13656-13661\*). After the true real reactions among different chemical species in seawaters were identified on the basis of material energetics and biology of marine calcifying organisms, a reasonable overall reaction should be estimated as material balance. From our data of base/acid titration (Chem. Eur. J. 2007, 13, 10176-10181\*\*), light microscope observation and culture experiment\*) it was become clear that the enhanced skeleton production of each coral polyp skeleton and each colony is controlled by reversible reaction between calcification and decalcification,  $\text{Ca}^{2+} + \text{HCO}_3^- \leftrightarrow \text{CaCO}_3 + \text{H}^+$ . Here solubility product  $[\text{Ca}^{2+}][\text{HCO}_3^-]$  is much larger than  $[\text{Ca}^{2+}][\text{CO}_3^{2-}]$  for reversible equation  $\text{Ca}^{2+} + \text{CO}_3^{2-} \leftrightarrow \text{CaCO}_3$ . Our idea\*) of proton dynamics demonstrated the increase of  $[\text{Ca}^{2+}]$ , and the decrease of major  $[\text{HCO}_3^-]$  and minor  $[\text{CO}_3^{2-}]$  with decreasing pH at a given  $P_{\text{CO}_2}$  and  $\sim 7.8 < \text{pH} < \sim 8.4$ . Thus stable variation of seawater pH over geological and laboratory timescales is actually real in reef-building seawaters under no anthropogenic influence on atmospheric carbon dioxide. \*) Suwa, Hatta and Ichikawa. \*\*) Ichikawa.

Keywords: Calcification, Marine organism, Real reactions, Proton dynamics, Material balance

## Phylogenetic composition of picophytoplankton in the Oyashio and Kuroshio transition regions

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Eukaryotic picophytoplankton (less than 3  $\mu\text{m}$ ) is ecologically and biogeochemically significant component in the marine microbial food web. Recently, studies about marine microbial diversity have been accelerated using molecular techniques, but basic information of picophytoplankton about diversity is still limited because of (i) lacking the 18S rDNA data in public database and (ii) fragile trait of the cell preventing sample collection. In this study, we investigated the phylogenetic diversity of surface community in one of the productive region of Japan, Oyashio and Kuroshio transition region. For the spatiotemporal comparison, seawater samples were collected from four geographically different sites with seasonal replicates (five seasons): Oyashio, Oyashio — Kuroshio transition regions, and mouth and head of the Sendai Bay. In order to better analyze the picophytoplankton community, we applied an efficient approach based on pyrosequencing of the 18S rDNA amplicon using flowcytometry sorting of cryopreserved cells. From the cleaned 10,000 reads came from the sorted 2,500 cells, 90 — 120 operational taxonomic units (OTUs: 95% cut off) were observed in each site and dominated by three higher level taxonomic groups: Stramenopiles (31 — 43%), Alveolata (16 — 35%) and Rhizaria (7 — 12%). Of the total of 217 OTUs, 40 OTUs were common among sites, and those included 21 OTUs common among five seasons, indicating spatially and temporally widespread distributing OTUs in this area. On the other hand, 21 — 38 OTUs were detected only in a site, indicating the local population. Multivariate analyses of OTUs compositions showed seasonal change of the community in each site (nMDS) and showed that the compositions were grouped by seasons ( $p < 0.01$ ) rather than by geographical difference ( $p = 0.5$ ). Thus, the phylogenetic composition of picophytoplankton in the Oyashio — Kuroshio transition region were composed of widespread and local phylotypes, and dynamically changed among seasons.

Keywords: Picophytoplankton, Flowcytometry, Pyrosequencing, 18S rDNA, Spatiotemporal distribution, Oyashio-Kuroshio transition region

## Cyanobacterial and non-cyanobacterial nitrogen fixation play a different role on marine primary production

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Marine primary production is limited by nitrogen availability, and it generally increases with new nitrogen input. The new nitrogen sources in the open ocean are nitrogen fixation and nitrate supplied from deep water. Due to the well-stratified condition in the tropical and subtropical oligotrophic region, nitrate input from deep water is little, and nitrogen fixation becomes important as new nitrogen source. Therefore, in theory, primary production would increase when nitrogen fixation becomes active in the oligotrophic region. In the South Pacific subtropical ocean, active nitrogen fixation occurs in both eastern and western region (Dekazemacker et al. 2013; Shiozaki et al., 2014). Meanwhile, satellite observations demonstrate that primary production is higher in the western region than in the eastern, suggesting that the contribution of nitrogen fixation to primary production would be different between the two regions.

In the present study, we examined primary production, nitrate-based production, and nitrogen fixation with accompanying measurements of nutrients and the diazotroph community in the eastern and western South Pacific subtropical ocean. In both regions, surface nitrate was depleted and nitrate-based production was similar. On the other hand, nitrogen fixation tended to be higher in the eastern region than in the western. Although primary production was elevated in the middle of western subtropical region where active nitrogen fixation occurred, it was not in the eastern region. These results indicated that nitrogen fixation did not enhance primary production in the eastern region. We quantified the *nifH* gene of three representative cyanobacterial diazotrophs, UCYN-A, UCYN-B, and *Trichodesmium* using a qPCR technique in both regions. In the western region, the three diazotrophs were widely distributed and abundant, that is, these three cyanobacterial diazotrophs played a key role in the nitrogen fixation. However, those abundances were nearly the detection limit of the analysis in the eastern region except some stations where abundance of UCYN-A was high, suggesting that non-cyanobacteria dominated the diazotrophs community in the eastern region. Therefore, the different contribution of nitrogen fixation to primary production was attributable to the diazotrophs community structure.

Keywords: nitrogen fixation, primary production, *nifH* gene, oligotrophic ocean

## Microbial control of carbon flux in the meso- and bathypelagic zone

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Prokaryotes (bacteria and archaea) play important roles in major carbon flux of the meso- and bathypelagic zone. Previous studies have revealed that patterns on prokaryotic production and biomass in the meso- and bathypelagic zone displayed strong regional variation consistent with sinking particulate organic matter flux variations. In general, the prokaryotic organic carbon consumption accounted for 50 — 100 % of the sinking POC fluxes (Yokokawa et al. 2013 *Limnol Oceanogr*). However this prokaryotic mediated flux of carbon have yet to be incorporated explicitly in carbon flux models. Incorporation of prokaryote processes to carbon flux models has been partly hampered due to the paucity of large-scale, high-resolution geographical variation data regarding prokaryotic abundance and production distributions in the oceans. Here I present some highlights from my previous studies examining the variation in prokaryotic production and biomass across oceanic regions. I also discuss novel approaches for determining activities of a specific functional group of prokaryote.

Keywords: prokaryotic community, carbon cycle, microbial oceanography

## Benthic prokaryote community and their roles on biogeochemical cycles under the oxygen minimum zone

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We investigated the impacts of the oxygen minimum zone (OMZ) on the benthic prokaryotic communities and biogeochemical cycles off India. Surface sediments were collected from three sites; core of the OMZ (water depth of 530 m), lower part of the OMZ (water depth of 800 m), and lower boundary of the OMZ (water depth of 1150 m). Porewater nutrient concentrations, organic matter contents, and diversity and abundances of microbial SSU rRNA and their functional genes were examined using the sediment cores down to 10 cm depth. In situ experiments using <sup>13</sup>C-labeled bicarbonate were also carried out at the same stations to evaluate carbon fixation rates at each site. The results demonstrated variability of benthic microbial communities with different carbon fixation rate across oxygen gradient of the bottom water.

Keywords: Oxygen minimum zone, sedimentary microbes, nitrogen cycle

## Characterizing the biological and microbial community dynamics in the coastal sea surface microlayer

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The sea surface microlayer is a thin surface film located at the interfacial point between the sea surface and the atmosphere. Compared to the underlying water (UW) below it, the SML is a unique but harsh environment; with elevated meteorological stresses and biologically and chemically enriched. Thus, it is widely recognized that the physical, chemical and biological processes in the SML are very different compared to UW even with just a few centimeters difference in depth. The proximity of this thin layer to the atmosphere also makes this layer highly dynamic and one of the most important layer to control the air-sea biogeochemical exchanges and climate-related processes. This biofilm-like thin layer with a depth of less than 1000  $\mu\text{m}$ , this layer have found to exist in most aquatic habitat and oceanic environments. This layer was found to be composed of hydrated gelatinous layer entangled in a matrix of dissolved organic matter composed mainly of transparent exopolymer particles (TEP). While few research have shown that the bacterial community in the SML possessed different functional genes compared to the underlying water others, in mesocosm experiments, have shown that bacterioneuston responded differently when introduced to experimentally-induced carbon dioxide loading scenarios in mesocosm experiments. However, little is still known about the microbial structure in this layer and their contribution towards the global biogeochemical cycles. In our research, bacteria community structure in the SML (bacterioneuston) at Aburatsubo Inlet, Misaki during summer and winter were examined using high throughput sequencing. In contrast to conditions in UW that remained constant throughout the sampling period, SML was highly dynamic with fluctuations in biological matter concentrations and bacterial communities. At times when the SML was enriched with biological matter and distinct bacterioneuston communities were formed. When the SML was enriched, rare bacterial groups including those that could play a role in biogeochemical cycles were more abundantly found in the SML and the diversity of these groups increased in proportion to the magnitude of biological matter enrichment in the SML.

Keywords: Surface microlayer, Microbial community structure, Biological enrichment

## Amino acid composition of natural bacterial assemblages and particulate organic matter in the western North Pacific

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Amino acid compositions were determined for natural marine bacterial assemblages (bacterium-size particles separated from other organisms and particles) and suspended particulate organic matter (POM) collected at subarctic and subtropical stations in the western North Pacific. We found that L-proline (L-Pro) content was remarkably high [38 - 57% of total hydrolysable amino acids (THAA)] in natural marine bacterial assemblages. These values were much higher than the corresponding values reported in the literature or those determined by ourselves for isolated bacterial strains (typical range, 4.3 - 8.8%). In POM, L-Pro content was low (<5% of THAA) in the upper layer (0 - 200 m), whereas it was high (24 - 26% of THAA) at the depth of 1000 m. Determination of enantiomeric amino acids in POM revealed that the ratio of D-/L- amino acids at the depth of 1000 m (0.054 - 0.061) was higher than that in the upper layer (0.012 - 0.039). These results confirm and add to the previous proposition that amino acid composition is systematically altered during bacterial reworking of marine organic matter, indicating that, in addition to the conventional indicator using enantiomeric amino acid ratio, L-Pro content can be a new indicator of the enrichment of POM by the organic matter derived from bacteria. Our results also underscore the importance of identifying bacterial constituents rich in L-Pro, which might play an important role in biochemical processes mediated by uncultured natural marine bacteria.

Keywords: marine bacteria, amino acids, proline, enantiomers, particulate organic matter



## Sources of dissolved organic nitrogen in the ocean indicated by nitrogen isotopic analysis of amino acids

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Estimating sources of marine dissolved organic matter (DOM) is one of crucial steps for mechanistic understanding of marine biogeochemical cycles. Bacteria have been suggested as important sources of marine DOM, but nature of the source Bacteria (e.g., heterotrophic v.s. autotrophic) currently remains uncertain. While compound-specific isotope analysis of amino acids (CSI-AA) can be a powerful tool for elucidation of the source of marine DOM, it has been difficult due to the large analytical errors of CSI-AA associated with the complexity of marine DOM. Here we developed a new method for precise d15N-AA analysis of marine DOM by coupling HPLC purification and GC-IRMS, and then applied the method to high-molecular-weight (HMW) DOM samples collected at the Gulf of Mexico and the North Pacific Subtropical Gyre. d15N-AA values and patterns of the HMW-DOMs were significantly different between the surface and the mesopelagic depths, indicating that their sources are different. Especially, the d15N-AA signatures of the mesopelagic HMW-DOMs suggest that they are product of resynthesis by heterotrophic Bacteria, rather than remnant of DOM produced by autotrophic Bacteria.

Keywords: Dissolved Organic Matter, Nitrogen Cycle, Amino Acids, Isotopes, North Pacific Subtropical Gyre, Gulf of Mexico

## Reconstructing the environmental history of macroalgae by the use of dual carbon isotope tracers

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The Sanriku coastal region, one of the world's greatest fishing grounds, is largely influenced by Oyashio and Kuroshio. These waters with distinct properties seasonally enter into the bays along the coast, and exert large influences on ecosystem dynamics in this region. Prominent examples include the intrusion event of Oyashio water into the bays (replacement of Tsugaru warm water with cold Oyashio water) during winter. This event has been suggested to cause fundamental alterations in environmental conditions (e.g., water temperature and nutrient concentrations) in the bays, which in turn may elicit complex physiological responses of coastal biota. However, the link between physiological responses of organisms to this oceanographic event has yet to be clarified fully. In order to gain insights into the relationship between the Oyashio intrusion event and coastal ecosystem dynamics, the present study used carbon isotopic signatures of *Undaria Pinnatifida* (wakame), a widespread and commercially important macroalgae in the Sanriku region. The growth of *U. Pinnatifida* occurs at the basal point of sporophyte where the meristem is located. Near this basal growing point, a pair of pinnate blades are formed to spread toward opposite directions from the central axis. Following the formation of new blades at the growing point, older blades are forced to move toward the apical end. Therefore, the blades near the apical end (upper blades) are older than those near the bottom (lower blades). Assuming that the radiocarbon isotopic signature ( $\Delta^{14}\text{C}$ ) of each pinnate blade reflects  $\Delta^{14}\text{C}$  of dissolved inorganic carbon (DIC) at the time of its formation, we hypothesized that  $\Delta^{14}\text{C}$  values of the upper (older) blades formed under the influence of the Tsugaru warm water (characterized by high  $\Delta^{14}\text{C}$ -DIC value) are high, whereas those of lower (younger) blades formed after the intrusion of Oyashio water (characterized by low  $\Delta^{14}\text{C}$ -DIC value) are low.

To test this hypothesis, we cultured *U. Pinnatifida* sporophyte in Otsuchi Bay between November 2013 and April 2014 and examined variability in  $\Delta^{14}\text{C}$  among different pinnate blades formed during different periods. Our results indicated that the lower blades formed after the Oyashio water intrusion, which appeared to occur in early March as indicated by a marked shift in salinity and temperature, had significantly lower  $\Delta^{14}\text{C}$  values compared to the upper blades formed before the event. These results are consistent with our hypothesis and suggest a possibility that the blade-order-dependent  $\Delta^{14}\text{C}$  variability in a sporophyte can be used as a new tool to reconstruct the timing of the Oyashio intrusion event in Sanriku bays. Our results also showed that the carbon stable isotopic signature ( $\delta^{13}\text{C}$ ) varied widely (range, 4.7 ‰) among the blades. Because this range in  $\delta^{13}\text{C}$  among blades largely exceeded the difference in  $\delta^{13}\text{C}$ -DIC between Tsugaru warm water and Oyashio water (0.22 ‰), it was considered that the variability in the  $\delta^{13}\text{C}$  of blades primarily reflected the variability in the extent of isotope fractionation, which was presumably related to changes in physiological state (growth rate) of the sporophyte. Furthermore, our data showed that there was a significant negative correlation between  $\delta^{13}\text{C}$  and  $\Delta^{14}\text{C}$  of the blades, suggesting that the growth rate of the sporophyte increased after the intrusion of Oyashio water. Dual carbon isotopic signatures of the blades of sporophyte could be potentially useful to reconstruct the timing of the Oyashio intrusion event and to examine physiological responses of macroalgae to this oceanographic event.

Keywords: microalgae, radiocarbon, carbon stable isotope, Sanriku region, Oyashio, Tsugaru warm current

## Multi-scale elemental mapping analysis for biochemical tissue samples using laser ablation-ICP-mass spectrometry

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Laser ablation sampling technique combined with ICP-mass spectrometry (LA-ICPMS) has become one of the most sensitive and versatile analytical tool for elemental imaging for minerals, fossils or various biological tissue samples. Laser sampling under the atmospheric pressure conditions can provide high analytical capability to accept large-sized samples ranging from 10  $\mu\text{m}$  to 25 mm with the optimum spatial resolutions. With the 75  $\mu\text{m}$  laser beam, from major elements (e.g., C, Na or Ca) to trace-elements (e.g., Ni, Se or Mo) can be monitored. With newly developed square-shaped laser beam can provide flat sample surface even after the laser ablation. After the survey scan using the square-shaped laser pit, elemental imaging with high-spatial resolution can be achieved by the laser ablation using the 5 ? 10  $\mu\text{m}$  pit sizes without any additional sample preparation procedures. With the present analytical protocol, multiple elemental images with different spatial resolution can be obtained. Only the problem is that the determination of elemental concentrations from the sample. Element concentrations would be very important to estimate the absolute amount or rate of elemental metabolism within and among the organs. The quantitative elemental imaging, however, had been retarded by the heterogeneous sampling (variation in the sampling depth or volume), mainly due to the difference in the hardness or color of the samples. To overcome this, we have developed the soft-ablation sampling technique.

With the soft ablation technique, biochemical tissue samples, placed onto the glass substances, were preferentially ablated by the laser ablation under the highly controlled energy fluence (soft ablation). Hence, no laser ablation was made on the glass substrate, because the energy fluence employed for the laser ablation of the biochemical samples was significantly lower than the energy threshold for the glass materials. With the preferential and total ablation of only biochemical samples, we can manage to obtain the homogeneous depth and volume of the sampling.

To take a full advantage of the quantitative imagings, we have developed new software to obtain the imaging data from the repeated line profiling analysis. With the present software, possible correlation among the analytes can be easily evaluated from only the specific area, or lines. Moreover, possible contamination or secondary mixing of the elements can also be tested. Another advantage of the present software is to accept almost all the time-profiling information achieved by various analytical techniques. Analytical features achieved by the combination of the LA-ICPMS technique and the present software will be demonstrated.

Keywords: laser ablation, ICP-mass spectrometry, Elemental Mapping, Multiple Scale, New Software

## Contamination by arsenic, manganese and barium in groundwater and human health risk in Vietnam

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In this study, we investigated contamination by arsenic and other trace elements in groundwater and in the Red River and the Mekong River Deltas, Vietnam. In addition, we evaluated human health risk from consumption of the contaminated groundwater. Concentrations of arsenic in groundwater were in the range of <math><0.1 - 502 \mu\text{g/l}</math>, with about 39% of these water samples exceeding WHO drinking water guideline of  $10 \mu\text{g/l}$ . Interestingly, 31% and 5% of groundwater samples had higher concentrations of manganese ( $400 \mu\text{g/l}$ ) and barium ( $700 \mu\text{g/l}$ ) than WHO guidelines for drinking water, respectively. Concentrations of arsenic, manganese and barium in hair of local residents were positively correlated with those in groundwater. Estimation using hazard quotient showed that about 43 % of groundwater samples have potential human health risks associated with intakes of these elements. These results suggest that people in these regions are exposed to arsenic, manganese and barium through the consumption of groundwater and hence potential health risks of these elements are of great concern for these local people.

## What factors decide the trace element levels in wildlife ?

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To understand the background levels of trace elements (heavy metals) in the organs and tissues of wildlife is a one of the important parameter for evaluation of ecological risk assessment. It is well known that there is a species-specific accumulation and sensitivity of chemicals including artificial pollutants and unique species-specific hyper-accumulation in wildlife. When ecological risk assessments are done without understanding above species-specific aspects, it may be leded inaccurate results. This concern applies to the case of trace elements including heavy metals.

This presentation focus on the actual trace element concentrations in organs and tissues in wild animals, which parameters are affective to determine these levels using some cases. These were suggested that not only trace element levels in diet reflecting surrounding environment, but characteristics of animal grouping such as genus, family and order, and other factors of environment affecting to behavior and physiologic aspects including inter element relationships also.

The approaches using above perspectives for example, accurate understanding of trace element accumulation in wildlife pose the important clues to not only field of ecological risk assessment but field of chemical evolution of animals also. Therefore these attempts might provide to understand interaction between environment and organisms and advance on hints of chemical evolution of organisms too.

Keywords: trace elements, heavy metals, wildlife, species specific accumulation, element specific accumulation

## Specific copper accumulation in liver of Formosan squirrel (*Callosciurus erythraeus*)

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### Introduction

Copper is one of the essential elements and liver is the central organ of Cu homeostasis, regulating both storage and excretion. Wilson disease (WD) is well known as disorder of Cu homeostasis. WD patient and its animal models, LEC rat, accumulate Cu in their livers because of the decrease of Cu excretion to bile and defective supply of Cu to ceruloplasmin (Cp; Cu excretion route to bloodstream) due to hereditary mutation of ATP7B gene. Bedlington terrier accumulates excess hepatic copper because of mutation of COMMD1 gene, which is involved in Cu excretion to bile with cooperating with ATP7B.

We have discovered that Formosan squirrel (*Callosciurus erythraeus*), living in Japan and Taiwan, accumulated Cu in their liver at 420  $\mu\text{g}/\text{wet g}$  on an average, and reported that Cu accumulation phenomenon in this animal wasn't due to environmental pollution.<sup>1,2)</sup> In this study, we focused on followed two points: The presence/absence of hepatotoxicity caused by Cu accumulation and the distribution and the chemical form of Cu in the liver of Formosan squirrel with using HPLC-ICP MS.

### Experimental

Thirty seven wild-living Formosan squirrels were trapped alive in Kamakura, Kanagawa, Japan under permission from the Kamakura City Hall. After blood and bile were collected, liver tissues were removed, and then samples were stored at  $-80\text{ }^{\circ}\text{C}$  until chemical analysis. About 0.1 g of liver tissues was preserved in 10 % natural buffered formalin before washed for pathological test. The activities of ALT, AST, and Cp in the serum were determined with UV absorbance methods. About 2.0 g of liver samples were homogenized, then supernatant samples were prepared by ultracentrifuging at  $105,000\times\text{g}$  for 60 min at  $4\text{ }^{\circ}\text{C}$ . A portion of each fraction, serum and bile were wet-digested, then digested solution was diluted with Milli-Q water to 10mL. Concentrations of Cu, Zn and Cd were determined by the ICP-MS (HP-7500, Agilent, Japan). The distributions of Cu and other metals in the liver supernatants were determined on gel filtration HPLC column (Develosil 100Diol-5,  $8.0\times 300\text{ mm}$  with a  $8.0\times 35\text{ mm}$  guard column; Nomura Chemical, Tokyo) by eluted with 100 mM ammonium acetate, pH 6.5 ( $25\text{ }^{\circ}\text{C}$ ) at the flow rate of 1.0 mL/min, with in-line detection with an ICP MS.

### Results and discussion

Hepatic concentrations of Cu ranged from 6.3 to 1740  $\mu\text{g}/\text{wet g}$ . From the result of HE stain, cellular infiltrations were shown in 14/27 liver of specimens in all. However, these were reversible degeneration and any gross anatomical changes, such as jaundice, hypertrophy and so on, were not shown. Moreover, cellular infiltrations didn't become severe according to hepatic Cu accumulation. Again, serum ALT and AST activity did not correlate with hepatic Cu concentration. These findings suggested that this species had any Cu detoxication mechanism.

Normally, excess Cu was detoxicated by metallothionein (MT), which is a family of low molecular weight, mainly cytoplasm-located, heavy metal-binding proteins. In the case of LEC rat and BT, most of accumulated Cu was bound to MT. Therefore, we focused on Cu bound to MT in this species. In the specimens, whose hepatic Cu concentration is higher than 100  $\mu\text{g}/\text{wet g}$ , 60 % of Cu distributed in the insoluble fraction. From HPLC-ICP MS analysis, the Cu bound to MT increased according to hepatic Cu accumulation until about 500  $\mu\text{g}/\text{wet g}$  of Cu were accumulated in the liver. Then amount of Cu bound to other protein, which is soluble and heavier than MT, increased.

From analysis of serum Cu distribution and Cp activities, serum Cu were bound to Cp despite low activity of Cp. Moreover, it was examined that Cu was excretion to bile in this species. These results suggested that Cu accumulation mechanism of Formosan squirrel was different from those of LEC rat and BT. Excess Cu in the liver of Formosan squirrel mainly bound to other than cytosolic MT.

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Keywords: Formosan squirrel, copper accumulation, copper homeostasis, species-specific, metallothionein, ceruloplasmin

## Estimating the Natal Sites of Clearwing Moths by using Trace Elements and the Invasive Pattern of Currant Clearwing Moth

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Some species of clearwing moths (Lepidoptera: Sesiidae) are known as destructive pests. For example, *Glossosphecia romanovi* is a pest of a grape tree. *Sesia yezoensis* is also observed in the same area as the previous species, but it is not a pest because its host plants are not fruit trees but willows. The currant clearwing moth *Synanthedon tipuliformis* has known as a pest of red and black currants. It was originally confined to Europe, but was introduced to Australia, New Zealand, USA and Japan along with the spread of the currant cultivation. In Japan, this species was firstly recorded from Hokkaido Island in 2008 and also found in the northern and central parts of Honshu Island within a few years. We estimated their natal sites using the elements in their bodies as tracers and evaluated their adult dispersal patterns. These are important information for the pest control and preventing to spread the invasive species.

By using ICP-MS (Agilent, 7500cx), levels of various trace elements were determined in bodies of clearwing moths (*Glossosphecia romanovi*, *Sesia yezoensis* and *Synanthedon tipuliformis*) collected from Aomori and Akita Prefectures in northern Japan.

The 4 element (Ni, Zn, Sn, and Pb) levels of *G. romanovi* in the vineyards were markedly higher than those in the non-vineyard areas, and the two groups could be clearly discriminated by these element levels. These elements might be introduced by the past and/or present agricultural managements, the exhaust gas of vehicles, and so on. Moreover, we could estimate their natal sites locally by multiple statistical analysis, and an individual which had apparently migrated from the non-vineyard area to the vineyard were detected. However, in the case of *S. yezoensis*, the differences between their natal sites were indistinct. This was probably because the host plants of this species were various willows (family Salicaceae). The differences between their natal sites might be masked with the differences between plants on which they had fed. These results suggested that the discrimination method using the trace elements were used effectively for stenophagous species such as *G. romanovi* rather than euryphagous species.

We could also discriminate between the currant clearwing moths in the each sampling sites by using the trace elements. Then, there might be no individual which had immigrated from another sites in spite of the short distances between the sampling sites (about 1.4 - 2.7 km). Therefore, it was considered that this species did not have high dispersal potential and the rapid invasion was caused by artificial import of its larvae with currant trees.

Keywords: migration, invasive species, clearwing moth, heavy metal, ICP-MS

## Lead (Pb) poisoning in children from townships around an extensive lead-zinc mine in Kabwe, the Republic of Zambia

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Childhood lead (Pb) poisoning is a serious public health concern worldwide. Young children under the age of 7 years are particularly vulnerable to Pb poisoning because of behavioral factors, such as frequent hand-to-mouth activities and biological factors including greater gastrointestinal absorption compared to adults and developing neurological systems. Lead exposure among children is associated with developmental abnormalities including impaired cognitive function, reduced intelligence, impaired hearing and reduced stature.

In Kabwe, Zambia, the capital of Central Province, extensive contamination of Pb in soils, wild rats as well as offal of cattle and chicken in townships in the vicinity of a lead-zinc mine has been reported and poses a serious health risk to children in these townships. We have previously reported that the concentrations of Pb (9-51188 mg/kg) in Kabwe soil (n=101) were much higher than benchmark values. Pb levels in tissues of Kabwe cattle were higher than those in other Zambian towns. Moreover, mean concentrations of Pb exceeded maximum levels for human consumption in some organs including muscle in free-range chickens, in contrast to low levels in broiler chickens, suggesting Pb exposure. Therefore, this study investigated blood lead levels (BLLs) in children in townships around the Pb-Zn mine in Kabwe and to identify children with BLLs that require medical intervention so as to mitigate the toxic effects of Pb.

The study was approved by the University of Zambia Research Ethics Committee and the Ministry of Health, Zambia. After informed and written consent was obtained from the parents or guardians, blood samples up to 3 mL (17 samples at Chowa, 100 samples at Kasanda and 129 samples at Makululu) were collected by qualified laboratory technicians from the children at clinics in the study areas. For each child, data on the age, sex and residential area were recorded. The blood samples were promptly transferred and stored at the laboratory of the Kabwe District Health Offices. The samples were transported to Japan and analyzed for Pb concentrations by ICP-MS.

Almost all of the sampled children in the current study had indications of Pb poisoning, with BLLs exceeding 5 microgram/dL. Children in these areas could be at serious risk of Pb toxicity as 18% of the sampled children in Chowa, 57% (Kasanda) and 25% (Makululu) had BLLs exceeding 65 microgram/dL. Eight children had BLLs exceeding 150 microgram/dL with the maximum being 427.8 microgram/dL. When children were grouped according to age, younger children between the ages of 0-3 years accumulated higher BLLs than their older counterparts (4-7 years). Significant negative correlation between age and BLLs supported this finding. This study demonstrated that childhood Pb poisoning in Kabwe is among the highest in the world. Although clinical cases and deaths due to Pb poisoning among children in Kabwe are rare, these findings indicate that more studies are needed to establish the health effects of Pb poisoning in children exposed to Pb pollution in townships around the Pb-Zn mine in Kabwe.

Given that Pb poisoning among children in Kabwe was extensive, it is recommended that chelation therapy be commenced in the children with BLL exceeding 45 microgram/dL prior to the onset of symptoms to reduce morbidity and prevent mortality in the affected children. This can be achieved for each child by devising and implementing an individualized plan of follow-up, especially for those children with extremely high BLLs.

Keywords: Zambia, Kabwe, Lead, Children, Mining



## Elimination of the heavy metals from coastal water by scallop cultivation

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Mutsu Bay in northern Japan is a semi-enclosed shallow water basin connected to Tsugaru channel between the Sea of Japan and Pacific Ocean. The problem of seawater eutrophication has not been appeared in Mutsu Bay because the human population density around the bay is relatively low. However, Mutsu Bay is one of Japan's most famous areas for the scallop cultivation, and about 100,000 tons of the scallops which took up inorganic substances in seawater are landed every year. For example, it is known that the mid-gut glands of scallops accumulate high levels of cadmium. We determined the nitrogen and carbon stable isotope ratios and the trace element concentrations of fish and measured the material cycles in Mutsu Bay and the Sea of Japan.

The Japanese whiting *Sillago japonica* were collected from the coastal areas (Mutsu Bay: 7 sites, the Sea of Japan: 10 sites) of Aomori Prefecture, northern Japan, in 2012 and 2013. We determined the nitrogen and carbon stable isotope ratios of their muscles by DELTA-plus Isotope Ratio Mass Spectrometer coupled with NC2500 Elemental Analyzer (Thermo Fisher Scientific), the levels of 25 elements (Li, Mg, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Mo, Cd, In, Sn, Sb, Cs, Ba, Tl, Pb, Bi) in their livers by ICP-MS (Agilent, 7500cx), and the mercury levels in their livers by the cold vapor technique with an automatic mercury analyzer (Nippon Instruments Corporation, RA-3220A).

The carbon stable isotope ratios ( $\delta^{13}\text{C}$ ) of Mutsu Bay specimens were higher than those of the Sea of Japan specimens. The nitrogen stable isotope ratios ( $\delta^{15}\text{N}$ ) were not different in most of the sampling sites, but the ratios were obviously higher in the particular site of Mutsu Bay. It was considered that this phenomenon was locally caused by the unnatural nitrogen supply.

Moreover, the levels of 15 elements (Li, Mg, Ca, Mn, Fe, Co, Cu, Zn, Rb, Sr, Mo, Cd, Cs, Hg, Pb) in the livers of Mutsu Bay specimens were significantly lower ( $p < 0.01$ ,  $U$  test) than those in the Sea of Japan specimens. Especially, the levels of cadmium and mercury for  $\delta^{15}\text{N}$  values were obviously low in the Mutsu Bay specimens. This result suggested that some elements such as cadmium were brought out from Mutsu Bay by the landing of cultured scallops.

In Iwasaki fishing port (one of the sampling sites in the Sea of Japan), the levels of 8 elements (V, Fe, Co, Cu, Ga, Cd, Hg, Pb) of the specimens captured in 2012 were significantly higher ( $p < 0.01$ ,  $U$  test) than those in 2013. In March 2012, a cargo ship was stranded nearby Iwasaki fishing port, and the oil spilled into the ocean.

Keywords: essential trace element, stable isotope, Japanese whiting, Japanese scallop, element elimination

## Spatial distributions of REE, heavy metals and oxygen isotope of phosphate in the Yasu river, Shiga, Japan

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### Introduction

Yasu river is the largest river that flows through the Lake Biwa. The Lake Biwa is the largest freshwater lake in Japan. The land use pattern within the Yasu river system has been gradually changing since 1960s. This study reports the spatial distribution of rare earth elements (REE), heavy metals and oxygen isotope of phosphate ( $\delta^{18}\text{O}_p$ ) in the Yasu river to give insights on the surface geological processes in the river.

### Methodology

Surface river water samples were collected from 66 sites in the Yasu river on October 2012 with acid-cleaned polyethylene bottles. Nitric acid was added to the filtered sample to make 2% solution and elements were directly analysed using Agilent 7500cx inductive couple plasma mass spectrometer. Dissolved inorganic phosphate from selected sites were extracted and converted to silver phosphate. Oxygen isotope analysis of these silver phosphate samples were performed using a thermal conversion elemental analyzer coupled to a continuous flow isotope ratio mass spectrometer via a helium stream.

### Results and Discussion

There were no direct correlations among land use pattern, nutrients and elemental concentration. The concentrations of REE and heavy metals were generally constant throughout the Yasu river system. However, the concentrations of some elements, such as Na, Ca, Y and Sn, were high in urban areas but not always on the same site. The elemental concentrations of water from the mouth of Lake Biwa were similar to the average concentrations all over the Yasu river system. On the other hand, river waters showed a marked variation in their  $\delta^{18}\text{O}_p$  among sites within the river. Significant differences were also detected in the  $\delta^{18}\text{O}_p$  among a variety of potential P sources, showing this technique is applicable to trace P sources in the river ecosystems.

Keywords: rare earth elements, Yasu river, Land use, oxygen isotopes of phosphate, metals, geological cycling

## Effects of environmental factors on production of dissolved N<sub>2</sub>-a product of denitrification. A case study in Tama River

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In aquatic ecosystems, denitrification, the nitrate (NO<sub>3</sub><sup>-</sup>) reduction to dinitrogen gas (N<sub>2</sub>), is considered as the important process to remove nitrogen to improve water quality. However, the denitrification also contributes to the emission of N<sub>2</sub>O – a greenhouse gas. Many studies of N<sub>2</sub> production in river were conducted to estimate the removal of nitrogen under natural condition via the denitrification. To gain more information of key factors for the N<sub>2</sub> production, we explore the relationships between dissolved N<sub>2</sub> and environmental factors in Tama River.

Water samples in Tama River from eight stations (from stn 1 near the mouth of the Tama River (in Kawasaki City) to stn 8 – Mid-Tama River (in Ohme City)) were collected on 13th November, 2014. Environmental parameters as temperature, pH, EC, concentrations of DO, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, DIN, DON, TDN, TOC were analyzed. Dissolved nitrogen gases through N<sub>2</sub>/Ar ratios were analyzed by Membrane Inlet Mass Spectrometer (MIMS) system.

Types of the river water were divided two groups. Upstream stations (stn 6 to 8) located in Mid-Tama River showed low concentrations (TDN: 51.36 – 78.09 μM/L, NH<sub>4</sub><sup>+</sup>: 0.26 – 1.78 μM/L, NO<sub>2</sub><sup>-</sup>: 0.24 – 0.47 μM/L, NO<sub>3</sub><sup>-</sup>: 45.33 – 67.84 μM/L, DON: 3.94 – 11.35 μM/L). Downstream stations (stn 1 to 5) showed high concentrations (TDN: 261.63 – 590.75 μM/L, NH<sub>4</sub><sup>+</sup>: 5.23 – 155.87 μM/L, NO<sub>2</sub><sup>-</sup>: 5.53 – 22.08 μM/L, NO<sub>3</sub><sup>-</sup>: 185.93 – 403.00 μM/L, DON: 14.56 – 64.09 μM/L). Nitrate dominated and accounted for 90.8 ± 11.7% of DIN, 82.4 ± 11.8% of TDN. Station 2 had the highest concentrations of nitrogen compounds except NO<sub>3</sub><sup>-</sup> concentrations. Water quality of this site was affected by sewage of plants which were in upper near this site. TOC values were also divided two groups as groups of nitrogen compounds (0.41 – 0.50 mg/L for upstream stations, while 1.71 – 2.21 mg/L for downstream stations).

Measured average N<sub>2</sub>/Ar ratio (37.36 ± 0.45) was lower than the theoretical average N<sub>2</sub>/Ar ratio (38.05 ± 0.25). Unfortunately remarkable excess in N<sub>2</sub>/Ar was not observed in our samples. We will present our preliminary isotopic results on NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and TDN in the presentation to discuss the occurrence of denitrification in the presentation.

## Behavior of organic phosphorus compounds in Lake Kasumigaura, Japan: A $^{31}\text{P}$ nuclear magnetic resonance spectroscopy study

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Phosphorus (P) is an essential nutrient for all living organisms in lakes. In the surface water, particulate P is the major P fraction usually accounting for more than 80% in total P in eutrophic lakes.

The goal of this study is to clarify how nucleic acid-P compounds in suspended particles change with the productions of microorganisms in a shallow eutrophic lake. In particular, primary productions by phytoplankton are the greatest biological productions in surface water in lakes, yet information on P compounds composition through productions of phytoplankton is limited. The current study therefore concurrently analyzes P compounds with  $^{31}\text{P}$  NMR spectroscopy, particulate organic C (POC), biomass of *M. aeruginosa* by the quantitative polymerase chain reaction (qPCR) technique as a possible contributor of nucleic acid-P in Lake Kasumigaura. We hypothesized that (1) concentrations of nucleic acid-P compounds change with production of microorganisms in a shallow, eutrophic lake; and (2) phytoplankton species composition, including *M. aeruginosa*, could also alter P composition in suspended particles.

Keywords: Phosphorus,  $^{31}\text{P}$  nuclear magnetic resonance (NMR)

## Quantifying nitrate dynamics in the changing lake Inawashiro

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Lake Inawashiro in Fukushima (surface area:103.3 km<sup>2</sup>, maximum depth:94.5 m) had been characterized by low pH around 5.0. The pH, however, has been increasing for these 20 years. Present pH is around 6.8. In addition, with the neutralization, annual variation range of NO<sub>3</sub><sup>-</sup> concentration in surface water increased from 3.6 μmol/L in 2007-2008 to 5.2 μmol/L in 2011-2012 (Fukushima Prefectural Institute of Environmental Reserch, 2008 and 2012), implying primary production is increasing in the lake water column. The purpose of study is to quantify both the gross assimilation rate and gross nitrification rate in the lake using Δ <sup>17</sup>O of nitrate.

Water sampling was carried out in both June and September, 2014. Water samples were filtered through GF/F filters and stored in cold storage until analysis. The nitrate concentration were determined with Shimadzu Prominence HIC-SP. Each isotopic composition of nitrate was determined with CF-IRMS system using the Chemical Conversion method (Tsunogai et al., 2010).

While NO<sub>3</sub><sup>-</sup> concentrations in the lake water column were almost constant at 14.0 μmol/L from surface to the bottom in June, those in 0-30 m decreased to 8.0 μmol/L in September. The δ<sup>15</sup>N values of nitrate increased for around +1 ‰, implying surface NO<sub>3</sub><sup>-</sup> was consumed through primary production. The observed large seasonal variation range in NO<sub>3</sub><sup>-</sup> at the surface (6 μmol/L) supported the past observation of the increasing trend. The total amount of nitrate in the lake water column also decreased from 79.9 to 72.7 Mmol during the period between the observations. On the other hand, the Δ <sup>17</sup>O values were almost constant around +3.5 ‰ inspective to the depths and seasonals. The mixing ratio of atmospheric NO<sub>3</sub><sup>-</sup> were about 14 ‰, implying the average residence time of NO<sub>3</sub><sup>-</sup> in lake was long and nitrogen nutrient is not a limiting nutrient for the primary production in the lake. The observed mixing ratio indicated that 6.2 Mmol of remineralized nitrate was fed into the water column though nitrification, while 14.8 Mmol of nitrate was simultaneously removed from the water column by assimilation, during the period between the observations. The assimilation amount between the observation interval (14.8 Mmol) correspond to only 30 % of the annual amount of assimilation (48.5 Mmol) calculated assuming steady state in the lake. As a result, assimilation in the lake proceed at an almost constant rate throughout the year, otherwise the nitrogen cycling in the lake water column is not under the steady state condition.

Keywords: Inawashiro lake in Fukushima, nitrate stable isotopes, nitrogen cycling, triple oxygen isotopes, assimilation, nitrification

## Nitrogen, carbon, and sulfur isotope fractionation during heterotrophic and autotrophic denitrification reactions

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In batch culture experiments, we studied the isotope fractionation of nitrogen in nitrate, carbon in dissolved inorganic carbon, and sulfur in sulfate during heterotrophic and autotrophic denitrification of two bacterial strains (*P. aerofaciens* and *T. denitrificans*). Heterotrophic denitrification experiments were conducted with trisodium citrate as electron donor, autotrophic denitrification experiments were carried out with iron disulphide as electron donor. For heterotrophic denitrification experiments a complete nitrate reduction was accomplished, however bacterial denitrification with *T. denitrificans* is a slow process in which the degree of denitrification achieved in seventy days was 60 %. In the former experiment, systematic change of  $\delta^{13}\text{C}_{DIC}$  with increase of DIC was observed during denitrification (enrichment factor  $\epsilon_N$  was -2.3 ‰), suggesting the contribution of C of trisodium citrate. No  $\text{SO}_4^{2-}$  and  $\delta^{34}\text{S}_{\text{SO}_4}$  changes were observed. In the latter experiment, clear fractionation of  $\delta^{13}\text{C}_{DIC}$  during DIC consumption and  $\delta^{34}\text{S}_{\text{SO}_4}$  during sulfur use of  $\text{FeS}_2$ -S (around 2 ‰) were confirmed through denitrification ( $\epsilon_N = -12.5$  ‰). The results of this batch experiment study are useful to understand the anaerobic bacterial denitrification processes in contaminated groundwater flow systems where a carbon source and/or pyrite are present. However, in natural aquifers, other anaerobic microbial activities such as sulfate reduction and methanogenesis would take place after or in the middle of the progress of the denitrification reaction, which play a decisive role changing isotope ratios of carbon and sulfur. Nevertheless, obtained results can be applicable in environments where complex simultaneous anaerobic reactions would not occur after, in the middle of the denitrification reaction, or at organic poor land that prevent further heterotrophic bacterial reactions to proceed.

Keywords: N-C-S stable isotope ratios, batch culture experiments, groundwater, denitrification

## Tracing the source of nitrate eluted from the forest ecosystem under high deposition rate of atmospheric nitrogen

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Forest ecosystems are deficient in fixed-nitrogen in general (Vitousek and Howarth, 1991). Excess input of fixed-nitrogen, however, often produced "nitrogen saturation" (Aber et al., 1989) in forest ecosystems. Nitrate concentrations dissolved in streams and rivers eluted from nitrogen saturated forest increased due to either increased leaching rate of nitrogen preserved in forest soils and/or increased direct drainage rate of atmospheric nitrated deposited onto forest. In order to evaluate the direct drainage of atmospheric nitrate from forest under high deposition rate of atmospheric nitrogen, we determined both concentrations and triple oxygen isotopic compositions of nitrate in the stream water eluted from the forest around Lake Ijira, Gifu Prefecture. Within Long-Term Monitoring sites of Transboundary Air Pollution and Acid Deposition by the Ministry of the Environment in Japan, Lake Ijira have been characterized by the highest deposition rate of atmospheric nitrogen.

Samples were collected once in two weeks from March, 2013, to February, 2014, at two rivers, RW1 (Kamagadani river) and RW3 (Kobora river) eluted from the forest around Lake Ijira. Water samples were filtered throughout 0.45  $\mu\text{m}$  membrane filter, and were stored in the refrigerator until analysis. The triple oxygen and nitrogen isotopic composition of dissolved nitrate was determined using Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS) system (Komatsu et al., 2008). Dissolved nitrate in the samples were chemically converted to nitrous oxide introduced into the system (Tsunogai et al., 2010).

The triple oxygen isotopic compositions of nitrate in both rivers (RW1 and RW3) were about +1~2 ‰, confirming the direct drainage of atmospheric nitrate from the forest. Seasonal variation in the triple oxygen isotopic composition was not significant during the observation. Calculated mixing ratios of atmospheric nitrate within total nitrate dissolved in the river water was around 5.8% at RW1 and 4.0% at RW3, respectively. RW3 catchment can be characterized by lower elution rate of atmospheric nitrate, as well as by lower elution rate of total nitrate, to the streams and rivers. In accordance with the decrease in the nitrate concentrations in 2013 from those in 2012 at both sites, the triple oxygen isotopic compositions also decreased in 2013. Therefore, both direct drainage rate of atmospheric nitrate and elution rate of remineralized nitrate in forest soils have been reduced simultaneously in the forest probably due to some kind of "recovery" in the forest ecosystems.

Keywords: forest ecosystem, nitrogen saturation, atmospheric nitrate, triple oxygen isotopic composition, Lake Ijira

## Possibility of base cation depletion in nitrogen saturated forest

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In our previous study, we found that acidic deposition in Japan has already resulted in elevated concentrations of acid anions (nitrate ion and sulfate ion) in stream waters, and a high level of Ca (instead of Al) is serving as a major counterion for acid anions (Koshikawa et al., *Appl Geochem*, 22, 1209-1216, (2007)). However, an additional loading of acidic deposition may result in shortage of Ca (essential element for plants) and mobilization of Al (toxic element for plants and fishes). Mt. Tsukuba is known as a system under "nitrogen saturation", where high concentration of nitrate ion in stream water has been observed since more than 25 years ago (Muraoka and Hirata, *J Hydrol*, 102, 235-253, (1988)). Bedrock of some catchments in Mt. Tsukuba is granite. Capacity of granite to supply Ca and other base cations is relatively low. Therefore, we have launched studies on Ca budget and source analysis of Ca, concerning possibility of Ca depletion in some granitic catchments in Mt. Tsukuba.

Keywords: Ca, nitrate ion, Sr isotope, stream water, soil solution



## 15N natural abundances and N use by plants in forested ecosystems

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Supply of nitrogen to plants often limits the primary productivity for plants in terrestrial ecosystems (Vitousek and Howarth 1991). Thus, the better understanding on how plants can utilize this limiting resource is quite important to project the changes in ecosystem functions with environmental changes such as the increase in nitrogen deposition and in CO<sub>2</sub> concentrations. We applied the isotopic approach to get insights into the niche differentiation for nitrogen uptake in the forest where nitrogen is considered to strongly limit the plants' productivity. In two plots (control and 50% cut), we sampled soils and plants for the measurements of nitrogen isotopic signatures (d<sup>15</sup>N). In soils collected from these two plots, nitrate pool sizes were quite small, while considerable amount of ammonium existed. Plants d<sup>15</sup>N varied among species; the dominant species (Hinoki) showed the low d<sup>15</sup>N, while other understory species had higher d<sup>15</sup>N. We compared d<sup>15</sup>N of plants with d<sup>15</sup>N of ammonium in the soil and found that Hinoki utilized the ammonium in organic soil with low d<sup>15</sup>N, while other understories utilized ammonium in deeper soil, suggesting the niche differentiation for N utilization in these plots. We will present the results of water isotopes to investigate if similar niche-differentiation for water uptake can be determined or not in these plots in the poster.

## Effects of thinning on stable N and C isotope ratios and nitrogen concentration in leaves of hinoki cypress plantation

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Thinning in hinoki cypress plantations may enhance soil water and nitrogen availabilities and affect water and nitrogen utilization strategy for remaining trees. Nitrogen concentration,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  are used as indices of nitrogen uptake, sources of soil nitrogen and water use efficiency, respectively and changes of water and nitrogen utilization of remaining trees can be evaluated from these leaf properties. We investigated leaf properties before and after thinning in hinoki cypress plantations in Kochi prefecture. Six treatments with tree replicates were established (no thinning and 50% thinning at a lower-elevation area and no thinning, 35% thinning, 50% thinning and 50% row thinning at a higher elevation area). Thinning was conducted before growing season in 2008 and leaf samples were collected by slingshot in 2007 and 2009. Leaf nitrogen concentration ranged 7.9 to 13.4 mg g<sup>-1</sup> and from 7.7 to 12.7 mg g<sup>-1</sup>, in 2007 and 2009, respectively. Changes of nitrogen concentration between two periods ranged -1.2 to +2.1 mg g<sup>-1</sup> was correlated with nitrogen concentration in 2007 negatively and with percentage of thinning positively. The result suggests that nitrogen uptake of remaining trees should enhance where nitrogen availability is limited before thinning practice.  $\delta^{15}\text{N}$  in leaves ranged from -5.9 to -1.6 ‰ and from -6.0 to -2.0 ‰, in 2007 and 2009, respectively. Change of  $\delta^{15}\text{N}$  between two periods ranged from -0.6 to 0.8 ‰ but was not related with thinning intensity nor initial nitrogen concentration. The result suggests that soil nitrogen sources are not significantly affected by thinning practice.  $\delta^{13}\text{C}$  ranged from -28.6 to -26.9 ‰ and from -28.5 to -26.2 ‰ in 2007 and 2009, respectively. Changes of  $\delta^{13}\text{C}$  between two periods ranged from -0.9 to +1.5 ‰ and were correlated with thinning intensity positively, with  $\delta^{13}\text{C}$  in 2007 negatively and nitrogen concentration in 2007, negatively. The results indicate that water use efficiency of remaining trees should not decrease in response to increase in soil water availability after thinning. The results suggest that water use efficiency should increase after thinning where water limitation is not severe and leaf photosynthetic ability as indicated by higher nitrogen concentration should increase where nitrogen limitation is severe. From these findings we concluded that thinning in hinoki cypress plantations with low soil nitrogen availability is a suitable management to improve nitrogen nutrition of remaining trees.

Keywords: hinoki cypress, thinning, nitrogen concentration, carbon isotope ratio, nitrogen isotope ratio

## Below-ground carbon input in black spruce stands with different fire history in interior Alaska

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Permafrost forests account for more than 20% of forested area in boreal biome. Those permafrost forests have a key role in carbon dynamics of terrestrial ecosystems due to their huge carbon stock in permafrost (frozen soil). However, recent reports suggested that permafrost ecosystems would be vulnerable to climate change (e.g. rising temperature) and disturbances such as forest fire, which may result in increased carbon release including greenhouse gasses such as CO<sub>2</sub>.

In interior Alaska, permafrost is present at poorly drained north-facing slope or bottom lands, where black spruce stands have been established. Forest fire is a process needed for regeneration of the black spruce stands, which is considered to occur every 100-200 years. However, recent reports suggested that fire frequency in the permafrost regions was likely to increase in the past decades and effects of the fire would vary with varied fire intensity. Thus, it would be needed to clarify effects of different fire history or different fire intensity on black spruce stands for better understanding of carbon dynamics in permafrost forest ecosystems.

In this study, we examined below-ground carbon input in three black spruce stands, which experienced fire in 2004, 1999 and around 1920 (intact 90-year black spruce stand). The fire in 2004 and 1920 was stand replacing fire, whereas fire intensity in 1999 was low and the fire burned the stand only partially. As a result, above-ground biomass in 2004- and 1999-fire stands were 8% and 38% of that in 1920-fire stand (ca. 2.6 kg m<sup>-2</sup>). We established study plots in those three stands in the summer of 2009. In each plot, production rates of litterfall, fine roots and forest floor mosses were estimated, which are major components of below-ground carbon inputs in the black spruce stands on permafrost.

In the 2004-, 1999- and 1920-fire plots, estimated production rates of litterfall were 20.5, 21.8 and 30.3 g m<sup>-2</sup> y<sup>-1</sup>, respectively; those of fine roots were 48.0, 47.0 and 64.5 g m<sup>-2</sup> y<sup>-1</sup>, respectively; those of forest floor mosses were 46.4, 33.3 and 37.7 g m<sup>-2</sup> y<sup>-1</sup>, respectively. Assuming that carbon concentrations in these components are 50% (0.5 g g<sup>-1</sup>), below-ground carbon input was estimated to be 57.5, 51.0 and 66.5 g C m<sup>-2</sup> y<sup>-1</sup> in the 2004-, 1999- and 1920-fire plots, respectively. These results suggested that the amounts of below-ground carbon input could recover to the level before fire during the 5-10 years after the forest fires, although decreases in above-ground biomass was still evident even after the low-intensity fire (1999-fire plot). The quick recovery of below-ground carbon input is likely to be attributed to increased contribution of understory vascular plants on production of litterfall and fine roots, and to changes in species composition for production of forest floor mosses after the forest fires.

Keywords: permafrost, forest fire, litterfall, fine roots, forest floor mosses