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

MIS26-01 Room:104 Time:May 28 09:00-09:15

Ocean oxygen depletion due to decomposition of submarine methane hydrate

YAMAMOTO, Akitomo[1*] ; YAMANAKA, Yasuhiro[2] ; OKA, Akira[1] ; ABE-OUCHI, Ayako[1]

[1]Atmosphere and Ocean Research Institute, the University of Tokyo, [2]Faculty of Environmental Earth Science, Hokkaido University

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 tomethane 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_2 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 onmarine 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

Japan Geoscience Union Meeting 2015
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MIS26-02 Room:104 Time:May 28 09:15-09:30

Real reactions of seawater and mineral matter: coral reef ecology

ICHIKAWA, Kazuhiko[1*] ; HATTA, Masayuki[2]

[1]Grad. School Environmental Earth Sci., Hokkaido University, Sapporo,, [2]Grad. School Humanities and Sciences, Ochanomizu University,

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, $Ca^{2+} + HCO_3^- \leftrightarrow CaCO_3 + H^+$. Here solubility product $[Ca^{2+}][HCO_3^-]$ is much larger than $[Ca^{2+}][CO_3^{2-}]$ for reversible equation $Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3$. Our idea$*$) of proton dynamics demonstrated the increase of $[Ca^{2+}]$, and the decrease of major $[HCO_3^-]$ and minor $[CO_3^{2-}]$ with decreasing pH at a given P_{CO2} and $\sim$7.8 $<$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 organizum, Real reactions, Proton dynamics, Material balance

Japan Geoscience Union Meeting 2015
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MIS26-03 Room:104 Time:May 28 09:30-09:45

Phylogenetic composition of picophytoplankton in the Oyashio and Kuroshio transition regions

KATAOKA, Takafumi[1*] ; YAMAGUCHI, Haruyo[1] ; KUWATA, Akira[2] ; KAWACHI, Masanobu[1]

[1]National Institute for Environmental Studies, [2]Tohoku National Fisheries Research Institute

Eukaryotic picophytoplankton (less than 3 μm) 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

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

MIS26-04 Room:104 Time:May 28 09:45-10:00

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

SHIOZAKI, Takuhei[1*] ; KODAMA, Taketoshi[2] ; HASHIHAMA, Fuminori[3] ; HAMASAKI, Koji[1] ; FURUYA, Ken[2]

[1]The University of Tokyo, Atomosphare and Ocean Research Institute, [2]The University of Tokyo, Graduate School of Agricultural and Life Sciences, [3]Tokyo University of Marine Science and Technology, Department of Ocean Sciences

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 (Dekaezemacker 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

Japan Geoscience Union Meeting 2015
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MIS26-05 Room:104 Time:May 28 10:00-10:15

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

YOKOKAWA, Taichi[1*]

[1]Center for Marine Environmental Studies, Ehime University

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

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

MIS26-06 Room:104 Time:May 28 10:15-10:30

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

NOMAKI, Hidetaka[1*] ; NUNOURA, Takuro[1] ; HIRAI, Miho[1] ; JULIARNI, Juliarni[1] ; TAKAKI, Yoshihiro[1] ; INOUE, Kentaro[2] ; SUGA, Hisami[1]

[1]JAMSTEC, [2]The University of Tokyo

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 ^{13}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

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

MIS26-08 Room:104 Time:May 28 11:00-11:15

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

WONG, Shu-kuan[1*] ; SUZUKI, Shotaro[1] ; CUI, Yingshun[1] ; KANEKO, Ryo[1] ; KOGURE, Kazuhiro[1] ; HAMASAKI, Koji[1]

[1]Laboratory of Marine Microbiology, Atmosphere and Ocean Research Institute, The University of Tokyo

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 μm, 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

Japan Geoscience Union Meeting 2015
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MIS26-09 Room:104 Time:May 28 11:15-11:30

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

TAKASU, Hiroyuki[1*] ; MIYAJIMA, Toshihiro[1] ; NAGATA, Toshi[1]

[1]Atmosphere and Ocean Research Institute, The University of Tokyo

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

Japan Geoscience Union Meeting 2015
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MIS26-10 Room:104 Time:May 28 11:30-11:45

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

YAMAGUCHI, Yasuhiko T.[1*] ; BROEK, Taylor a. B.[1] ; MCCARTHY, Matthew D.[1]

[1]University of California, Santa Cruz

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

Japan Geoscience Union Meeting 2015
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MIS26-11 Room:104 Time:May 28 11:45-12:00

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

SATO, Naomi[1*] ; FUKUDA, Hideki[1] ; MIYAIRI, Yosuke[1] ; YOKOYAMA, Yusuke[1] ; NAGATA, Toshi[1]

[1]Atmosphere and Ocean Research Institute, the University of Tokyo

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. Pinattifida* 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 (Δ^{14}C) of each pinnate blade reflects Δ^{14}C of dissolved inorganic carbon (DIC) at the time of its formation, we hypothesized that Δ^{14}C values of the upper (older) blades formed under the influence of the Tsugaru warm water (characterized by high Δ^{14}C-DIC value) are high, whereas those of lower (younger) blades formed after the intrusion of Oyashio water (characterized by low Δ^{14}C-DIC value) are low.

To test this hypothesis, we cultured *U. Pinattifida* sporophyte in Otsuchi Bay between November 2013 and April 2014 and examined variability in Δ^{14}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 Δ^{14}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 Δ^{14}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 (δ^{13}C) varied widely (range, 4.7 ‰) among the blades. Because this range in δ^{13}C among blades largely exceeded the difference in δ^{13}C-DIC between Tsugaru warm water and Oyashio water (0.22 ‰), it was considered that the variability in the δ^{13}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 δ^{13}C and Δ^{14}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

Japan Geoscience Union Meeting 2015
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MIS26-12 Room:104 Time:May 28 12:00-12:15

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

HIRATA, Takafumi[1*] ; OHARA, Seiya[1] ; MUKOYAMA, Sho[1] ; HATTORI, Kentaro[1] ; SAKATA, Shuhei[1] ; SUZUKI, Toshihiro[2]

[1]Kyoto University, [2]Tokyo Institute of Technology

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 μm to 25 mm with the optimum spatial resolutions. With the 75 μm 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 μm 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 miving 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

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

MIS26-13 Room:104 Time:May 28 12:15-12:30

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

AGUSA, Tetsuro[1*] ; KUNITO, Takashi[2] ; INOUE, Suguru[1] ; MINH, Tu binh[3] ; TUE, Nguyen minh[1] ; HA, Nguyen ngoc[4] ; TU, Nguyen phuc cam[4] ; TRANG, Pham thi kim[3] ; TAKAHASHI, Shin[5] ; TUYEN, Bui cach[6] ; VIET, Pham hung[3] ; IWATA, Hisato[1] ; TANABE, Shinsuke[1]

[1]Center for Marine Environmental Studies (CMES), Ehime University, Japan, [2]Faculty of Science, Shinshu University, Japan, [3]Hanoi University of Science, Vietnam National University, Vietnam, [4]Faculty of Fisheries, Nong Lam University, Vietnam, [5]Faculty of Agriculture, Ehime University, Japan, [6]Research Institute for Biotechnology and Environment (RIBE), Nong Lam University, Vietnam

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 $<$0.1 - 502 μg/l, with about 39% of these water samples exceeding WHO drinking water guideline of 10 μg/l. Interestingly, 31% and 5% of groundwater samples had higher concentrations of manganese (400 μg/l) and barium (700 μg/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.

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MIS26-14 Room:104 Time:May 28 12:30-12:45

What factors decide the trace element levels in wildlife ?

WATANABE, Izumi[1*]

[1]Tokyo Univ. Agricul. Tech.

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 leaded 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

Japan Geoscience Union Meeting 2015
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MIS26-15 Room:104 Time:May 28 14:15-14:30

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

SUZUKI, Yoshinari[1*] ; WATANABE, Izumi[2]

[1]Faculty of Life and Environmental Science, Shimane University, [2]Graduate School of Agriculture, Tokyo University of Agriculture and Technology

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 root 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 μg/wet g on an average, and reported that Cu accumulation phenomenon in this animal wasn't due to environmental pollution.[1,2)] In this study, we focused on followed two points: The presence/absence of hepatotoxity 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 ℃ 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×g for 60 min at 4 ℃ . 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, 8.0×300 mm with a 8.0×35 mm guard column; Nomura Chemical, Tokyo) by eluted with 100 mM ammonium acetate, pH 6.5 (25 ℃) 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 μg/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 became 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 μg/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 μg/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, ceruroplasmin

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

MIS26-16 Room:104 Time:May 28 14:30-14:45

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

KUDO, Seiya[1*] ; WATANABE, Izumi[2] ; AZUMA, Nobuyuki[1]

[1]Faculty of Agriculture and Life Science, Hirosaki University, [2]Graduate School of Agriculture, Tokyo University of Agriculture and Technology

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 roman* *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

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(May 24th - 28th at Makuhari, Chiba, Japan)

MIS26-17 Room:104 Time:May 28 14:45-15:00

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

NAKAYAMA, Shouta[1*] ; YABE, John[2] ; IKENAKA, Yoshinori[1] ; BEYENE, Yared[1] ; BORTEY-SAM, Nesta[1] ; OROSZLANY, Balazs[1] ; MUZANDU, Kaampwe[2] ; CHOONGO, Kennedy[2] ; MWEENE, Aaron[2] ; KABALO, Abel[3] ; MIZUKAWA, Hazuki[1] ; ISHIZUKA, Mayumi[1]

[1]Graduate School of Veterinary Medicine, Hokkaido University, [2]School of Veterinary Medicine, University of Zambia, [3]Kabwe District Health Office, Ministry of Health

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

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

MIS26-18 Room:104 Time:May 28 15:00-15:15

Elimination of the heavy metals from coastal water by scallop cultivation

AZUMA, Nobuyuki[1*] ; IWASAKI, Chiko[1] ; KUDO, Seiya[1] ; INOUE, Hiromoto[2] ; NODA, Kaori[3] ; WATANABE, Izumi[4]

[1]Faculty of Agriculture and Life Science, Hirosaki University, [2]The United Graduate School of Agricultural Sciences, Iwate University, [3]Faculty of Science and Technology, Hirosaki University, [4]Graduate School of Agriculture, Tokyo University of Agriculture and Technology

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 (δ^{13}C) of Mutsu Bay specimens were higher than those of the Sea of Japan specimens. The nitrogen stable isotope ratios (δ^{15}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 δ^{15}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

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

MIS26-19 Room:104 Time:May 28 15:15-15:30

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

CID, Abigail[1*] ; SONG, Uhram[7] ; TAYASU, Ichiro[1] ; OKANO, Jun-ichi[2] ; TOGASHI, Hiroyuki[3] ; ISHIKAWA, Naoto F.[6] ; MURAKAMI, Aya[2] ; HAYASHI, Takuya[5] ; IWATA, Tomoya[5] ; OSAKA, Ken'ichi[4] ; NAKANO, Shin-ichi[2] ; OKUDA, Noboru[1]

[1]Research Institute for Humanity and Nature, [2]Ctr Ecol Res, Kyoto Univ, [3]Field Sci Educ Res Ctr, Kyoto Univ, [4]Univ Shiga Pref, [5]Faculty of Life and Envi Sci, Univ Yamanashi, [6]JAMSTEC, [7]Jeju Nat Univ

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 ($\delta^{18}O_p$) 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 $\delta^{18}O_p$ among sites within the river. Significant differences were also detected in the $\delta^{18}O_p$ 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

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

MIS26-20 Room:104 Time:May 28 15:30-15:45

Effects of environmental factors on production of dissolved N2-a product of denitrification. A case study in Tama River

NGUYEN CONG, Thuan[1*] ; KOBA, Keisuke[1] ; YANO, Midori[1] ; MAKABE, Akiko[1]

[1]Tokyo University of Agriculture and Technology, Japan

In aquatic ecosystems, denitrification, the nitrate (NO_3^-) reduction to dinitrogen gas (N_2), is considered as the important process to remove nitrogen to improve water quality. However, the denitrification also contributes to the emission of N_2O – a greenhouse gas. Many studies of N_2 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_2 production, we explore the relationships between dissolved N_2 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_4^+, NO_2^-, NO_3^-, DIN, DON, TDN, TOC were analyzed. Dissolved nitrogen gases through N_2/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_4^+: 0.26 – 1.78 μM/L, NO_2^-: 0.24 – 0.47 μM/L, NO_3^-: 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_4^+: 5.23 – 155.87 μM/L, NO_2^-: 5.53 – 22.08 μM/L, NO_3^-: 185.93 – 403.00 μM/L, DON: 14.56 – 64.09 μM/L). Nitrate dominated and accounted for 90.8 $\pm$ 11.7% of DIN, 82.4 $\pm$ 11.8% of TDN. Station 2 had the highest concentrations of nitrogen compounds expect NO_3^- 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_2/Ar ratio (37.36 $\pm$ 0.45) was lower than the theoretical average N_2/Ar ratio (38.05 $\pm$ 0.25). Unfortunately remarkable excess in N_2/Ar was not observed in our samples. We will present our preliminary isotopic results on NO_3^-, NO_2^-, NH_4^+ and TDN in the presentation to discuss the occurrence of denitrification in the presentation.

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MIS26-21 Room:104 Time:May 28 15:45-16:00

Behavior of organic phosphorus compounds in Lake Kasumigaura, Japan: A 31P nuclear magnetic resonance spectroscopy study

SHINOHARA, Ryuichiro[1*] ; IMAI, Akio[1] ; TOMIOKA, Noriko[1] ; KOHZU, Ayato[1] ; KOMATSU, Kazuhiro[1] ; SATOU, Takayuki[1] ; SANO, Tomoharu[1] ; MIURA, Shingo[1] ; SHIMOTORI, Koichi[1]

[1]National Institute for Environmental Studies

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 31P 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, 31P nuclear magnetic resonance (NMR)

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

MIS26-22 Room:104 Time:May 28 16:15-16:30

Quantifying nitrate dynamics in the changing lake Inawashiro

MATSUMOTO, Yoshiumi[1*] ; TSUNOGAI, Urumu[1] ; OHYAMA, Takuya[1] ; NAKAGAWA, Fumiko[1]

[1]Graduate School of Environmental Studies, Nagoya University

Lake Inawashiro in Fukushima (surface area:103.3 km^2, 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_3^- 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 $\Delta\ ^{17}O$ 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_3^- 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 $\delta^{15}N$ values of nitrate increased for around +1 ‰, implying surface NO_3^- was consumed through primary production. The observed large seasonal variation range in NO_3^- 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 $\Delta\ ^{17}O$ values were almost costant around +3.5 ‰ inspective to the depths and seasonals. The mixing ratio of atmospheric NO_3^- were about 14 %, implying the average residence time of NO_3^- 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 nitrare was fed into the water column though nitrification, while 14.8 Mmol of nitrate was simultaneousluy 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) caluculated 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

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MIS26-23 Room:104 Time:May 28 16:30-16:45

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

HOSONO, Takahiro[1*] ; ALVAREZ, Kelly[1] ; LIN, In-tian[2] ; SHIMADA, Jun[2]

[1]Priority Organization for Innovation and Excellence, Kumamoto University, [2]Graduate School of Science and Technology, Kumamoto University

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 $\delta^{13}C_{DIC}$ with increase of DIC was observed during denitrification (enrichment factor εN was -2.3 ‰), suggesting the contribution of C of trisodium citrate. No $SO_4{}^{2-}$ and $\delta^{34}S_{SO4}$ changes were observed. In the latter experiment, clear fractionation of $\delta^{13}C_{DIC}$ during DIC consumption and $\delta^{34}S_{SO4}$ during sulfur use of FeS_2-S (around 2 ‰) were confirmed through denitrification (εN = -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

Japan Geoscience Union Meeting 2015
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MIS26-24 Room:104 Time:May 28 16:45-17:00

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

ANDO, Kenta[1*] ; TSUNOGAI, Urumu[1] ; OHYAMA, Takuya[1] ; NAKAGAWA, Fumiko[1] ; UCHIYAMA, Shigeki[2] ; YAMASHITA, Naoyuki[2] ; SASE, Hiroyuki[2]

[1]Graduate School of Environmental Studies, Nagoya Univ., [2]Asia Center for Air Pollution Research

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 Polution 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 μm membrane filter, and were stored in the refrigerator until analysis. The triple oxygen an 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

Japan Geoscience Union Meeting 2015
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MIS26-25 Room:104 Time:May 28 17:00-17:15

Possibility of base cation depletion in nitrogen saturated forest

KOSHIKAWA, Masami K.[1*] ; WATANABE, Mirai[1] ; MURATA, Tomoyoshi[1] ; HAYASHI, Seiji[1]

[1]National Institute for Environmental Studies

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

Japan Geoscience Union Meeting 2015
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MIS26-26 Room:104 Time:May 28 17:15-17:30

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

KOBA, Ayami[1*] ; KOBA, Keisuke[2] ; INAGAKI, Yoshiyuki[2]

[1]Tokyo University of Agriculture and Technology, [2]Forestry and Forest Products Research Institute

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 CO2 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 (d15N). In soils collected from these two plots, nitrate pool sizes were quite small, while considerable amount of ammonium existed. Plants d15N varied among species; the dominant species (Hinoki) showed the low d15N, while other understory species had higher d15N. We compared d15N of plants with d15N of ammonium in the soil and found that Hinoki utilized the ammonium in organic soil with low d15N, while other understories utilized ammonium in deeper soil, suggesting the niche differentiation for N utilization in these plots. We will present the reults of water isotopes to investigate if similar niche-differentiation for water uptake can be determined or not in these plots in the poster.

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(May 24th - 28th at Makuhari, Chiba, Japan)

MIS26-27 Room:104 Time:May 28 17:30-17:45

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

INAGAKI, Yoshiyuki[1*] ; NOGUCHI, Kyotaro[1] ; MIYAMOTO, Kazuki[1] ; OKUDA, Shiro[1] ; NOGUCHI, Mahoko[1] ; ITOU, Takeharu[1]

[1]Forestry and Forest Products Research Institute

Thinning in hinoki cypress plantations may enhance soil water and nitrogen availabilities and affect water and nitrogen utilization strategy for remaining trees. Nitrogen concentration, δ^{15}N and δ^{13}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 $g^{-1}$and from 7.7 to 12.7 mg g^{-1}, in 2007 and 2009, respectively. Changes of nitrogen concentration between two periods ranged -1.2 to +2.1 mg g^{-1} 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. δ^{15}N in leaves ranged from -5.9 to -1.6 ‰ and from -6.0 to -2.0 ‰, in 2007 and 2009, respectively. Change of δ^{15}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. δ^{13}C ranged from-28.6 to -26.9 ‰ and from -28.5 to -26.2 ‰ in 2007 and 2009, respectively. Changes of δ^{13}C between two periods ranged from -0.9 to +1.5 ‰ and were correlated with thinning intensity positively, with δ^{13}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

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MIS26-28 Room:104 Time:May 28 17:45-18:00

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

NOGUCHI, Kyotaro[1*] ; MORISHITA, Tomoaki[1] ; KIM, Yongwon[2] ; MATSUURA, Yojiro[1]

[1]For. & For. Prod. Res. Inst., [2]IARC, Univ. Alaska Fairbanks

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_2.

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^{-2}). 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^{-2} y^{-1}, respectively; those of fine roots were 48.0, 47.0 and 64.5 g m^{-2} y^{-1}, respectively; those of forest floor mosses were 46.4, 33.3 and 37.7 g m^{-2} y^{-1}, respectively. Assuming that carbon concentrations in these components are 50% (0.5 g g^{-1}), below-ground carbon input was estimated to be 57.5, 51.0 and 66.5 g C m^{-2} y^{-1} 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

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MIS26-P01 Room:Convention Hall Time:May 27 18:15-19:30

In situ estimation of new and regenerated production in lakes using triple oxygen isotopes as tracers

NAKAGAWA, Fumiko[1*] ; TSUNOGAI, Urumu[1] ; KOMATSU, Daisuke[1] ; OHYAMA, Takuya[1] ; MIYAUCHI, Takanori[1] ; SAKUMA, Hiroki[1] ; MINAMI, Sho[1] ; TADENUMA, Yukie[2] ; UMEDA, Makoto[3] ; TANAKA, Atsushi[4]

[1]Graduate School of Environmental Studies, Nagoya University, [2]Faculty of Science, Hokkaido University, [3]Graduate School of Engineering, Tohoku University, [4]National Institute for Environmental Studies

The gross primary production rate is an essential parameter to study biogeochemical processes in hydrosphere, having strong relations with environmental changes in lakes and oceans, such as eutrophication and global warming. Supplying rates of fixed nitrogen, especially dissolved nitrate (NO_3^-) and ammonium (NH_4^+), to each hydrospheric environment often control each gross primary production rate. As a result, the primary production is often divided into the following two categories: “new production” that uses NO_3^- supplied either from atmosphere or from aphotic layer, and “regenerated production” that uses a recycled nitrogen in the form of NH_4^+ or dissolved organic nitrogen excreted or produced during biogeochemical processes within photic layer.

All the above-mentioned parameters had been traditionally estimated based on incubations of sampled water in bottles by adding isotope-labeled compounds such as $^{13}CO_2$ or $^{14}CO_2$ for the primary production rates and/or $^{15}NO_3^-$ or $^{15}NH_4^+$ for nitrogen uptake rates. In these approaches, however, sampled water in bottles is incubated under artificial conditions that must be somewhat different from actual in-situ conditions and the results could represent different rates from the original in aquatic environments. Moreover, the estimated values based on the incubation corresponds to instantaneous uptake rates when sampling was done so that large errors could be expected for the hydrospheric environments with significant temporal variations, otherwise we must increase a number of observations using time and costs.

In this study, we determined the parameters using natural isotopes in lake-dissolved materials instead of using incubations. Most of the oxygen-containing molecules on earth show mass-dependent relative variation between $^{17}O/^{16}O$ ratios and $^{18}O/^{16}O$ ratios. On the other hand, atmospheric O_3 photochemically produced from O_2 shows an anomalous enrichment in ^{17}O, so that residual atmospheric O_2 is slightly depleted in ^{17}O in comparison with the mass-dependent relative relation. Besides, at least one of the O atoms in atmospheric NO_3^- is derived from atmospheric O_3 owing to the contribution of O atoms from O_3 during the photochemical oxidation processes of NOx in atmosphere, so that the triple oxygen isotope ratios ($\Delta\ ^{17}O$ values) of NO_3^- also deviate from the mass-dependent relative relation. Since $\Delta\ ^{17}O$ value does not vary during the general mass-dependent reactions such as decompositions, we can estimate the mixing ratio between atmospheric O_2 and photosynthetic O_2 from $\Delta\ ^{17}O$ value of O_2 and that between atmospheric NO_3^- and remineralized NO_3^- from $\Delta\ ^{17}O$ value of NO_3^-. If we determine the $\Delta\ ^{17}O$ values of both dissolved O_2 and NO_3^- in a hydrospheric environment as well as supplying rates of atmospheric O_2 and NO_3^-, we can determine both the primary production rate and NO_3^- uptake rate simultaneously. One of the priorities of this $\Delta\ ^{17}O$ method is that the estimated rate corresponds to the average value of each rate, so that the values can be a more reliable and accurate than the values estimated from the incubation methods.

In this study, we determined both gross primary production rate and new primary production (NO_3^- uptake) rate simultaneously based on the $\Delta\ ^{17}O$ value of dissolved O_2 and NO_3^- in two oligotrophic lakes (Lake Shikotsu and Lake Kuttara) and one mesotrophic lake (Lake Biwa) in Japan. The regenerated production rate was then calculated by deducing the later from the former. Water samples were collected twice (spring and summer) in a year for each lake. Both primary production rates and NO_3^- uptake rates were determined from the vertical distribution of $\Delta\ ^{17}O$ values of O_2 and NO_3^- and their difference between the seasons. We found that the f-ratios (relative use of NO_3^- among the total use of nitrogen) were lower in oligotrophic lakes than in the mesotrophic lake.

Keywords: new production, regenerated production, gross primary production, lakes, triple oxygen isotopes, hypolimnion

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Nitrogen isotopic measurement of NOx gas with the filter-pack method

MURATA, Eriko[1*] ; KOBA, Keisuke[1] ; WATANABE, Mirai[2] ; NISHINA, Kazuya[2] ; KOHZU, Ayato[2] ; TAKENAKA, Chisato[3]

[1]Tokyo University of Agriculture and Technology, [2]NIES, [3]Nagoya University

Estimation of the nitrogen loss as N2 and NOx from ecosystem is quite important to close the nitrogen budget. However, due to the high spatio-temporal variations of the nitrogen dynamics in soils does not allow us to investigate the production/consumption processes of these gaseous forms of nitrogen. Although natural abundance of stable isotope is considered to be able to use for the investigation of the N dynamics with respect to gaseous nitrogen losses, nitrogen isotopic measurement of NOx is quite difficult due to its high reactivity. Here we present our preliminary work on the nitrogen isotopic measurement of NOx gas with the filter-pack method (Watanabe et al. 2006) together with the denitrification method (Sigman et al. 2001). NO gas produced from NaNO2 with known nitrogen isotopic ratio via several chemical treatments, then the trapped NO as NO2- and NO3- ions were converted to N2O with denitirifer, then nitrogen isotopic signature was measured by GC-IRMS. We found that the filter-pack method can be applied for the nitrogen isotopic measurement. We applied this method to measure nitrogen isotopic signature of atmospheric NOx and present these data in the poster.

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Seasonal and spatial variation of dissolved iron transformation kinetics in the Shizugawa bay and its adjacent rivers

LEE, Ying ping[1*] ; FUJII, Manabu[1] ; YOSHIMURA, Chihiro[1] ; KIKUCHI, Tetsuro[1]

[1]Tokyo Institute of Technology, Civil Engineering Department

Importance of Fe as an essential nutrient for microorganisms is well recognized such as cellular respiration, photosynthesis and nitrogen fixation. However, the extremely low solubility of thermodynamically stable Fe(III) in oxygenated and circumneutral pH natural waters resulted in low concentration of dissolved Fe in some coastal and oceanic seawaters. The bioavailability of Fe in natural waters can be affected by concentration of dissolved Fe and its redox kinetics, given that Fe(II) has much higher solubility and thus higher bioavailability than Fe(III) does. In the river-coastal dynamic system, changes in land cover, salinity gradients, types of riverine input or organic matter, and seasonal changes may affect to the Fe oxidation kinetics as the Fe(II) oxidation is affected by water qualities such as pH and organic matter. In this study, we mainly focus on the Fe(II) oxidation kinetics in the river-coastal system in order to grasp the seasonal and spatial scales of Fe bioavailability between two different aquatic environments.

The study area was located in Shizugawa bay, Miyagi prefecture (N38 ^{0} E141 ^{0}) and totally 6 stations from near to offshore (SB-1 to SB-6) were selected as sampling stations. Also, this study included upstream and downstream of three rivers: Hachiman upstream (HR Up) and downstream (HR Down), Oritate upstream (OR Up) and downstream (OR Down), and Mizushiri upstream (MR Up) and downstream (MR Down). All the surface water samples were collected manually with acid-wash 1 L Nalgene bottles for three consecutive seasons starting from summer year 2014. All water samples were filtered through 0.45 micrometer Milipore membrane and stored in the dark at 4 ℃ for further analysis.

Fe(II) oxidation rates were determined by measuring time course of Fe(II) concentration using a flow injection analysis (FIA) system with a luminal chemiluminescence detection. Water sample and luminol reagent were both simultaneously pumped into the system using a peristaltic pump with flow rate at 2.4 mL per min. Water sample and luminol mixing was conducted in the flow cell situated at the front of a photomultiplier tube (PMT, Hamamatsu) and the PMT signal was recorded by WA control v91 software. Calibration was conducted for each water sample from three standards addition of Fe(II) (varied between 0.5 and 40 nM depending on signal response of each water sample). The initial signal (i.e., signal when Fe(II) was added) was obtained by extrapolation of signal data collected after 50 seconds back to time zero using a regression line which was obtained from time versus log-transformed signal data. The linear response of initial signal to the nominal initial Fe(II) concentration was found. Thus, the signal value was converted to the Fe(II) concentration by using the linear regression equation. The Fe(II) oxidation rate constant (M^{-1} s^{-1}) for each standard Fe addition was derived by assuming a pseudo-first order reaction.

Fe(II) oxidation rates in Shizugawa for three consecutive seasons from summer to winter were shown in Figure 1. The Fe(II) oxidation rates shown here are the average of three standards addition of Fe(II). A distinct seasonal trend of Fe(II) oxidation was observed particularly in autumn season with higher Fe(II) oxidation rates. Generally, Fe(II) oxidation is a pH-dependent reaction. Supposedly, the oxidation rate at seawater pH ($>$7.9) should be higher than that for typical freshwater pH (e.g. pH ranges between 6.8 and 7.9 in the river investigated). In our study, however, the oxidation rates in coastal seawater tended to be slower compared to those in freshwater. The results of Fe(II) oxidation in freshwater indicated that water quality variables other than pH affect the oxidation process. These factors may include dissolved organic matter concentration and its chemical properties (binding strength) and/or interaction between Fe and other trace metals which will be investigated further.

Keywords: dissolved iron, oxidation, river, coastal

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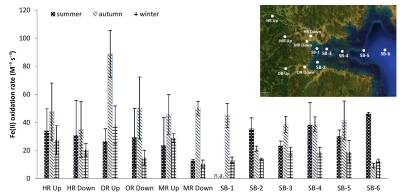

Japan Geoscience Union Meeting 2015
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MIS26-P04 Room:Convention Hall Time:May 27 18:15-19:30

Aerobic methane production by planktonic microbes under nitrogen and phosphorus starved conditions in a lake

ISHIDA, Dai[1] ; KHATUN, Santona[1*] ; IWATA, Tomoya[1]

[1]University of Yamanashi

Lake ecosystems are now recognized as an important source of atmospheric methane (CH_4), which account for about 6-16% of global methane emission from natural sources. In lake ecosystems, it has long been believed that CH_4 is produced only in anoxic environments (e.g., lake sediments and anoxic hypolimnion) by anaerobic methanogens. However, recent empirical and experimental works have revealed that planktonic microbes can produce methane in aerobic water columns of oligotrophic lakes through the use of methylphosphonic acid (MPn) by C-P lyase enzyme under P-limited conditions. But, there is no study examining the effects of cell nutritional conditions (N starved or P starved) on aerobic methane production by bacterioplankton.

We performed the batch-culture experiments to identify the effects of cell starvation on the rate of aerobic methane production under nitrogen and phosphorus limited conditions. Planktonic microbes collected from well-oxygenated water of Lake Saiko (Yamanashi Prefecture) were incubated with a growth medium (BG-11) for several months and used for the starvation experiment to make their cells N-starved or P-starved conditions by removing either element from the BG-11 medium. Then, we added MPn and/or inorganic nitrogen (N_i) and inorganic phosphorus (P_i) to confirm the response of N-starved or P-starved microbes to such experimental additions.

The results showed that although the cell nutritional conditions did not affect the production of CH_4, nutrient balance of lake water (N excess or P excess) greatly influenced the aerobic methane production. First, we confirmed aerobic CH_4 production in the MPn addition treatment, suggesting the active C-P lyase catalysis that converts MPn to methane and inorganic phosphate. Moreover, we found that MPn + N_i addition accelerated the aerobic CH_4 production. This is due probably to the fact that N_i addition promoted the biosynthesis of C-P lyase and/or made lake water more P-limited condition (increase of N/P ratio); both may contribute to increasing the MPn utilization by microbes. However, MPn + P_i addition did not increase the CH_4 production, indicating the opportunistic utilization of MPn alternative to P_i under phosphorus limited conditions. The present results suggest that the input of excess N into lake ecosystems promotes the metabolism of MPn by planktonic microorganisms, which leads to increase of aerobic methane production in phosphorus-limited oligotrophic lakes.

Keywords: Aerobic methane production, C-P lyase, methylphosphonic acid, phosphorus and nitrogen starvation, planktonic microbes

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MIS26-P05 Room:Convention Hall Time:May 27 18:15-19:30

Evaluation for load of bioavailable particulate phosphorus during rain events from Yasu river, at Lake Biwa catchment

CHISHIRO, Shinsho[1*] ; OSAKA, Ken'ichi[2] ; NAGAFUCHI, Osamu[2] ; OKUDA, Noboru[3]

[1]Enviromental Science Graduate, the University of Shiga Prefecture, [2]Department of ecosystem study, University of Shiga Prefecture, [3]Research Institute for Humanity and Nature

It is well known that primary production in Lake Biwa is limited by phosphorus, and that means phosphorus load into Lake Biwa influence on its environment. In general, it is considered that algae in lake use PO_4-P as a phosphorus nutrient, however, it has been revealed that a part of particulate phosphorus (PP) also might be used as nutrient in recent study. However, there are a few studies that quantify the load of bioavailable PP discharged through river in Japan. The purpose of this study is to quantify the load of bioavailable PP discharged through Yasu river into Lake Biwa during rainfall events. Water samples were collected at one to six hours interval in two rainfall event (May and July) in Yasu River using automatic river water collector. We measured several forms of PP by sequential extraction methods (ammonium chloride, bicarbonate dithionite, NaOH, HCl extraction) in river water sample. In rainfall event in May and July, about 70 - 90 % of PP was bioavailable and that part were larger than PO_4-P load from Yasu river, indicate that PP discharge from river have large impact on primary production in downward lake.

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Spatio-temporal variation of phosphate concentration at a high P concentration groundwater in the Hachirogata polder

HAYAKAWA, Atsushi[1*] ; ABE, Misato[1] ; ASANO, Ryoki[1] ; ISHIKAWA, Yuichi[1] ; HIDAKA, Shin[1]

[1]Akita Prefectural University

[Aim] The present study was conducted to elucidate spatio-temporal variation of phosphate (PO_4) concentration and the PO_4 release mechanism at a high PO_4 concentration groundwater in the Hachirogata polder, Akita, Japan.

[Materials and Methods] The study site was at a pristine wilderness area after the land reclamation in southwest part of the Hachirogata polder. Groundwater samples were collected once a month from December 2013 to December 2014 from seven groundwater wells at a depth of 3.1, 4.3, 5.6, 6.6, 12.4, 19.5, and 45.0 m, respectively. The 30 m (ϕ5 cm) boring core sample was collected from near the wells in Feb. 2013. We measured water temperature, pH, and electrical conductivity (EC) using a pH/EC meter (D-54,Horiba, Kyoto, Japan) and measured oxidation-reduction potential (ORP) using a ORP electrode (D-55, Horiba) on site. The water samples were also filtered through a 0.45 μm membrane filter on site. The concentrations of PO4 in the groundwater were determined using an autoanalyzer (QuAAtro2-HR, BLTEC, Osaka, Japan). The fresh boring sediment was extracted twice with distilled water (soil:water, 1:2.5 for pH and 1:5 for EC), and pH and EC of in the extract were determined using pH/EC meter (LAQUA F-74BW, Horiba). Water soluble P were determined using an autoanalyzer (QuAAtro2-HR, BLTEC, Osaka, Japan). Sediment samples were digested with a combination of HF-HNO_3-$HClO_4$ acids in Teflon beakers at 180 ℃ and element concentration in solutions were determined by ICP-OES (iCAP 6000, Thermo Fisher Scientific).

[Results and Discussion]

TP content in sediments increased in clay and silt layers while PO_4 content increased in sandy layers. This indicated that clay and silt layers were a P sink/source and released PO_4 was moving in sand layers. Sediment EC increased in clay layer and drastically increased from 21 m deeper layer likely influenced by sea water. Groundwater ORP showed a moderately reducing (-113 $\pm$ 42 mV) environment in all the wells. PO_4 concentration in groundwater was ranged from 5.7 to 18.2 mg L^{-1}, and the highest concentration was observed at the well of 6.6 m depth (18.2 $\pm$ 0.7 mg L^{-1}) at sandy layer, the second was at the well of 12.4 m depth (10.2 $\pm$ 0.9 mg L^{-1}). Positive correlation was observed between PO_4 and F^- concentrations in groundwater (r = 0.96, P <0.01, n = 7), indicated fluorapatite would be a PO_4 source. At the well of 6.6 m depth, Cl^- concentration was the lowest (29.8 $\pm$ 2.9 mg L^{-1}) value and was similar to the lake (Lake Hachiro) water (20.6 mg L^{-1}), suggesting that freshwater was preferentially flowing into the land through groundwater around the depth. At the well of 6.6 m depth that had little fluctuation of water qualities all year round, Na^+, bicarbonate and carbonate ion were higher but Ca^{2+} concentration was lower compare to other wells. At the well of 12.4 m depth that had a large fluctuation of water qualities, PO_4, pH, Na^+, bicarbonate and carbonate ion, and Fe increased while Ca^{2+} and ORP decreased when Cl^- concentration decreased from 72 to 33 mg L^{-1} during May to Oct. 2014. These phenomena indicated PO_4 release induced likely by Na-saturated sediment might be due to release of P associated with oxide surfaces or to dissolution Ca-P at clay layer by increasing pH and dissolution of $CaCO_3$ (from shell) by inflowing freshwater to the groundwater.

Keywords: phosphate, groundwater, polder, Na saturation, freshwater

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Spatial and temporal heterogeneity of the sources of streamwater sulfate in tropical dry forest catchment in Thailand

YAMASHITA, Naoyuki[1*] ; MOROHASHI, Masayuki[1] ; INOMATA, Yayoi[1] ; UCHIYAMA, Shigeki[2] ; KIEVUTTINON, Bopit[3] ; GARIVAIT, Hathairatana[4] ; SASE, Hiroyuki[1]

[1]Asia center for air pollution research, [2]Environmental Science Research Niigata, [3]Royal forest department in Thailand, [4]Environmental Research and Training Center in Thailand

In Southeast Asia an increase in emissions of sulfur (S) into the atmosphere may introduce new risks for the plant, soil and inland-water through acidification. However, the effect of the atmospheric S deposition on acidification by an increase in sulfate is poorly understood in tropical forests with possible S sources and processes in the internal cycle. S isotopic ratio (δ^{34}S) could be a good indicator to identify the source of sulfate in soil and inland-water because only dissimilatory S reduction results in a large fractionation of S isotope. Our objectives are to clarify the spatial and temporal variability of δ^{34}S in rainfall, throughfall, soil and stream water within the catchment and discuss the influence of the atmospheric S input on the stream in tropical forest.

Study catchment has been established at dry evergreen forest in Sakaerat silvicultural research station, northeastern Thailand. Anion-exchange-resin columns were installed for rainfall, throughfall, soil-water and stream-water through a year to collect and concentrate sulfate in the field. The sulfate retained in the resin was extracted by NaCl and precipitated as $BaSO_4$. We determined ^{34}S / ^{32}S of the $BaSO_4$ by mass spectrometer (IR-MS) and calculated δ^{34}S (‰) using the reference material (Canyon Diablo Troilite). Annual weighted-mean δ^{34}S was calculated from sulfate flux (kg ha^{-1} year^{-1}) and δ^{34}S in each period. We also determined δ^{34}S by the concentration method for the water samples of rainfall and streamwater in some cases.

Annual weighted-mean δ^{34}S and S deposition in rainfall were 4.1 ‰ and 6.4 kg ha^{-1} year^{-1}, respectively. δ^{34}S in streamwater was 4-5 ‰ higher than rainfall during late-wet and dry season, whereas δ^{34}S in rainfall and streamwater was mostly comparable during early and middle wet season. In late-wet and dry season, δ^{34}S in sub-soil water was particularly higher in the riparian zone near the outlet of the study catchment than in the area near the headwater and on the slope. Sulfate enriched ^{34}S might be increased due to bacterial dissimilatory S reduction in late wet season and retained in the sub-soil during dry season, which could be a main source for the streamwater sulfate during base-flow periods. Meanwhile, in early and middle wet season, streamwater sulfate could be directly affected by atmospheric S input. These heterogeneity of internal S dynamics should be considered to examine the effect of atmospheric deposition on soil and inland-water ecosystems in tropical dry forest. The project is supported by the grant from APN (ARCP2012-18NMY-Sase: ARCP 2013 -13 CMY -Sase).

Keywords: tropical dry forest, stream water, sulfur dynamics, stable sulfur isotope ratio, atmospheric deposition, soil water

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Long-term changes in nitrogen discharge from watershed of restored artificial forest

URAKAWA, Rieko[1*] ; TODA, Hiroto[2] ; ODA, Tomoki[1] ; OHTE, Nobuhito[3]

[1]Graduate School of Agricultural and Life Sciences, The University of Tokyo, [2]Institute of Agriculture, Tokyo University of Agriculture and Technology, [3]Graduate School of Informatics, Kyoto University

Forest restoration practice has a greatest impact on nitrogen (N) dynamics in forest ecosystems. While there are a number of studies surveying forest cutting and successive regrowth of vegetation, the extent of increase and its duration of N leaching differs among these studies (Oda et al. 2013). There are mainly three processes that affect N leaching from a forested watershed after cutting; (1) N input via precipitation, (2) N uptake by vegetation and (3) N mineralization and nitrification in soil. To understand the impact of forest cutting both quantitatively and periodically, it is necessary to investigate the long-term changes in these three processes before and after the practice.

In Japan, the area of old-aged forest is now increasing due to the decreases in forest activities and restoration practices (Forest Agency 2013). While old-aged sugi (*Cryptomeria japonica*) lowers nutrient uptake (Ohata 1996), soil N mineralization and nitrification retain considerably high rates (Oyanagi et al. 2004). Therefore, increase in N leaching from old-aged artificial forest is expected.

There are ”nitrogen saturated” forested watersheds in the suburban region (Ohrui and Mitchell 1997). Because the amount of cross-border pollutants from continental region is increasing, the nitrogen saturation might become widespread. Therefore, it is necessary to maintain the streamwater quality by enhancing nutrient uptake of forest stands by efficient restoration of artificial forest.

The objective of this study is to clarify the changes in N dynamics caused by cutting and restoration of the artificial forest. We investigated the changes in three processes before and after the partial cutting of old-aged forest which had been in a state of nitrogen saturation.

The study site locates in Field Museum Oyasan in Gunma prefecture, Japan. The watershed area is 1.8 ha, and sugi was planted on the lower to middle slopes, while hinoki (*Chamaecyparis obtusa*) was planted on the upper slope. The sugi plantation on the lowest slope (0.3 ha, 18% of the watershed area) was felled in 2000, and sugi was replanted the following year. The ages of replanted and un-cut old-aged stands are now 15 and 107, respectively.

The long-term hydro-chemical monitoring has been conducted in this site. The amount of N input via precipitation and N leaching from streamwater was estimated from Urakawa et al. (2012). N uptake by sugi before cutting was referred from Oyanagi et al. (2004) and that after cutting was estimated from the aboveground tree biomass surveyed in 2014. Soil net N mineralization and nitrification was measured by in situ incubation conducted intermittently before and after the cutting.

The amount of N leaching from streamwater, which was 10-15 kgN/ha/y before cutting, increased to 15-20 kgN/ha/y for 11 years after cutting, and declined to 10 kgN/ha/y in recent 3 years. By contrast, N input via precipitation maintained stable amount of 9-13 kgN/ha/y. Increases in annual amounts of N mineralization and nitrification were limited for 4 years after cutting, but from the fifth year of the cutting, these rates settled back to the level of the pre-cutting. Rapid growth of replanted sugi began from the 10th year after the cutting, suggesting that the significant decline in streamwater nitrate concentration in recent years attributed to increase in N uptake.

Forest restoration even in 20% of the area was suggested to recover the state of nitrogen saturation caused by aging stands and increase in N input.

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Keywords: long-term monitoring, forested watershed, forest restoration, nitrogen leaching, nutrient uptake by vegetation

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Measurement of inorganic nitrogen leaching and its origin in forest soil by resin core method and tension free method

FUJITA, Kento[1*] ; OSAKA, Ken'ichi[2] ; CHISHIRO, Shinsho[1] ; NAGAFUCHI, Osamu[2] ; NAKAMURA, Takashi[3] ; NISHIDA, Kei[3]

[1]Enviromental Science Graduate School, the University of Shiga Prefecture, [2]Department of ecosystem study, University of Shiga Prefecture, [3]International Research Center for River Basin Environment, University of YAMANASHI

Nitrogen loading from forest watershed can affect downstream ecosystem, therefore, to clarify the interaction between nitrogen cycle and nitrogen loading from forested ecosystem is important. In this study, we measured nitrogen leaching in forest soil by both resin core method and tension free lysimeter method and estimate nitrate origin by measuring oxygen isotope in nitrate in order to clarify the interaction between nitrogen cycle and nitrogen leaching process. Reisn core method are very useful method, however, there are a few studies that compared nitrogen leaching by method and other method. Moreover, there are a few studies that test of measurement of oxygen isotope in nitrate in resin core methods. This study was conducted at two forested watersheds (Aburahi-S and Surumi-A) in Shiga Prefecture. In Aburahi-S, we measured nitrogen leaching by resin core method and tension free lysimeter method, and nitrogen leaching were measured by only resin core method in Surumi-A.

The amount of nitrogen leaching were averaged 0.61±0.79kgN/ha in resin core method and 0.21±0.26kgN/ha in Aburahi-S from May 2014 to February. Little ammonium was not leaching in both methods. In tension free lysimeter method, there is the possibility that unsaturated flow was difficult to collected, and that may lead underestimated of nitrogen leaching at tension free lysimeter method. The results of nitrogen leaching at Surumi-A watershed and oxygen isotope of nitrated will be explain at presentation on this day.

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Temporal changes in the soil microbial biomass and N dynamics in eastern Hokkaido

WATANABE, Tsunehiro[1*] ; SHIBATA, Hideaki[1] ; TATENO, Ryunosuke[2] ; IMADA, Shogo[2] ; FUKUZAWA, Karibu[1] ; ODA, Tomoki[3] ; URAKAWA, Rieko[3] ; ISOBE, Kazuo[3] ; HOSOKAWA, Nanae[4] ; MAKOTO, Kobayashi[1] ; INAGAKI, Yoshiyuki[5]

[1]Field Science Center for Northern Biosphere, Hokkaido University, [2]Field Science Education and Research Center, Kyoto University, [3]Graduate School of Agricultural and Life Science, University of Tokyo, [4]Graduate school of Environmental Science, Hokkaido University, [5]Forestry and Forest Products Research Institute

1. Introduction

In arctic and alpine regions having seasonal snow cover, it has reported that the microbial activity in winter had impact on the annual nitrogen (N) cycling and the soil N availability in the growing season. However, the study focused on the soil microbial biomass and N dynamics from winter to spring in temperate region with seasonal snow cover is limited. In eastern Hokkaido in northern Japan, the soil often experiences soil freezing and freeze-thaw cycle due to the less snowpack in winter. Previous study in this region has reported that the ammonium production rate in soil increased in the late-winter compared to the much snowpack region. However, the pattern of soil microbial biomass and N dynamics from winter to spring is not clear. The objective of this study was to clear the pattern of soil microbial biomass and N dynamics and the relationship between their patter and the environmental factor.

2. Methods

This study was conducted on the Shibecha Experimental Forest, Kyoto University located in eastern Hokkaido, northern Japan. The main vegetation are Mongolian oak (*Quercus crispula*) and Sasa (*Sasa niponica*) that is understory vegetation. The study plots (5m×15m) were established in the slope of east and west. The plot number in each slope was six. The study period was from October 2013 to September 2014. In each plot, the soil moisture and temperature were measured using moisture sensor and temperature probe at 5 cm depth and 0 , 5 , 25 cm depth, respectively. The soil sampling from 0 to 10 cm was conducted in almost monthly. In same period, the exchange (collecting and setting) of resin was also conducted. The soil incubation from 0 to 10 cm and 10 to 20 cm was conducted by cylinder method. Collected interval of the incubated soil was from 1 to 2 month. We also measured snowpack and soil freezing depth in winter.

3.Results and discussion

The soil temperature at 0 and 5cm depth showed constantly 0 degree as snowpack increased. Microbial biomass C and N and inorganic N amount in the soil from 0 to 10 cm depth peaked in mid-winter. The inorganic N amount decreased from mid-winter to late-winter. On the other hand, both net N mineralization and nitrification rates showed higher trend in the growing-season than in the winter-season. These results indicated that the soil N utilization by living matter was significantly difference between winter- and growing-season. Although the microbial activity was inhibited by the low soil temperature in winter, the microbes would function as N sink because there is not competition for soil N resource between microbe and plant. The NH_4^+-N and NO_3^--N peaked in October and December, respectively. Then both inorganic N amounts, especially in NO_3^--N decreased rapidly, although the NO_3^--N leaching at 20 and 30 cm depth was not found in same period. Furthermore, the values of ratio of fungi to bacteria and net nitrification rate decreased from October to March. These results suggested that the change of microbial flora might be important for the N sink in winter. However, the microbial biomass C significantly decreased at end of the April that the critical disappearance of snowpack was measured. These results suggested that the microbe could not tolerate to the freeze-thaw cycle and dramatic rise of soil temperature in the winter-spring transition.

Keywords: nitrogen cycling, soil freezing, freeze-thaw cycle, nitrogen mineralization, nitrogen leaching

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Bamboo lodging associated with nitrogen saturation: its morphological and mechanical reasons

AIHARA, Yuki[1] ; YOH, Muneoki[1*]

[1]Tokyo University of Agriculture and Technology

[Introduction]
Nitrogen saturation, a situation of available nitrogen in excess of plant and microbial nutritional demand due to nitrogen deposition, has been suggested to affect plant growth and the root-shoot biomass allocation; an increase in foliar biomass and a decrease in fine root biomass under high nitrogen availability (Aber et al. 1989). In recent years, some reports have suggested that tree windthrow damages may be associated with increased nitrogen status (Braun et al., 2003; Meyer et al., 2008). Here, we report a phenomenon of lodging (falling down) of moso bamboo (Phyllostachys pubescens) in a forest site of nitrogen saturation. We suggest that the bamboo lodging is associated with the following morphological and mechanical anomalies; 1) an increase in branches-and-leaves biomass, 2) a decrease in root system, and 3) a decrease in bending strength.

[Materials and methods]
We studied the biometry of bamboo in a nitrogen-saturated site (Tama hill, Tokyo) and control sites (Fukushima and Izu), such as diameter at breast height (DBH), culm height, and mass of branches-and-leaves. Root density and soil nitrate concentration were measured for soil cores 25, 50, 75, 100cm away from culm. The total carbon and total nitrogen content of leaves and culms were measured with dry combustion method. As an index of mechanistic strength, Young's modulus (E) and flexural rigidity (EI) of culm were measured with a bending test of test piece.

[Results and discussion]
In a N-saturated site, leaf and culm nitrogen concentration were significantly higher than control sites. Any elongation growth, which was initially hypothesized, was not observed in a N-saturated site. However, some bamboos in a N-saturated site had significantly larger mass of branches-and-leaves. Very low root density associated with elevated nitrate concentration was also demonstrated, in contrast with a root mat in the soil surface observed in control sites. Culm density and culm thickness showed a negative correlation with bamboo nitrogen concentration. Consequently, culm flexural rigidity (EI) also declined with the increase in nitrogen concentration. A combination of these observed changes, a higher load of canopy, a lower culm strength and a lower uprooting resistance by root system, may be responsible for bamboo lodging observed in the N-saturated site. The results suggest that nitrogen saturation significantly affect morphologies and mechanical properties in bamboo to cause the lodging.

Keywords: nitrogen saturation, lodging, bamboo, fine root biomass, mechanistic strength, morphological change

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The impact of nitrogen saturation on tree roots, which lead to uprooting

OHNO, Satoshi[1*] ; YOH, Muneoki[1]

[1]Tokyo Univeresity of Agriculture and Technology

[Introduction]

Anthropogenic nitrogen deposition has been increasing for these decades (Galloway, 2004). Some adverse effects on plants have been reported, such as root / shoot ratio, severity of disease, plant-fungal relationships (Veresoglou et al., 2012; Gojon et al., 1994; van Diepen et al., 2010 etc.). However, most of these experiments have used herbs or seedlings; there have been few studies that targeted adult trees in forest ecosystems (Meyer et al., 2008 etc.). We investigated a possibility whether adult tree root biomass and physical properties are affected by elevated nitrogen concentration, which enhances uprooting risks.

[Materials and Methods]

Field research was conducted at a nitrogen-saturation site (Tama-hill, Tokyo), a middle N status site (Karibasaka, Saitama), and a nitrogen-limited control site (Ogawa, Ibaraki). Nitrate concentration in a stream for these watersheds was 280, 86, 16 μmol / L, respectively. The sites are deciduous broad-leaf forests with altitudes between 150 ? 650 m and slopes between 22 ? 35 degrees.

Roots and soils of 0 ? 40 cm depth, for konara oak (*Quercus serrata*) and cherry (*Cerasus jamasakura*), were taken 1 m apart from a stand with a core sampler (7.5 cm diameters). Live roots were sorted into two diameter class of $>$2 mm and $<$2 mm and measured for dry weight. Soil nitrate concentrations were measured for water extraction. Wood cores collected from root of these stands with borer auger (5 mm diameters and about 15 cm length) were measured for dry density and Young's modulus (mechanical strength). Angles of stem inclination were measured graphically as an indicator of uprooting risk.

[Results and Discusses]

Dry root weight (both $>$2 mm and $<$2 mm) decreased by 60% with the increase of soil nitrate concentration. Cherry's physical properties didn't show any significant changes with different soil nitrate concentrations. However, core's dry density from konara decreased significantly under higher soil nitrate concentrations ($p<0.01$). Young's modulus was also smaller under higher soil nitrate (more 50 μmol / kg soil) than lower soil nitrate concentration (less 50 μmol / kg soil) ($p<0.05$). Moreover, a part of Konara trees in Tama-hill (N-saturation site) showed distinct stem inclinations as contrast to Ogawa (N-limited site).

The results suggest that nitrogen saturation may have resulted in decreasing root biomass and physical properties to lead to higher uprooting risk.

Keywords: nitrogen saturation, tree root, uprooting

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Comparison of SRP (soluble reactive phosphorous) with orthophosphate in riverwater

ISHIMARU, Mana[1] ; MARUO, Masahiro[1*] ; OBATA, Hajime[2]

[1]School of Environmental Science, The University of Shiga Prefecture, [2]Atmosphere and Ocean Research Institute, The University of Tokyo

Introduction

Orthophosphate is essential nutrient for primary production in waters and it is thought to be the main chemical form of phosphorous directly available to organisms. In oligotrophic and mesotrophic waterbodies, phosphorous often plays as controlling factor of primary production. For the determination of phosphorous, spectrophotometric method based on the formation reduced form of phosphomolybdate (molybdenum blue) is usually applied Determined value is called as SRP: soluble reactive phosphorous, because various kinds of phosphorous compounds in water also reacts with molybdate by hydrolysis in acidic solution. Molybdemum blue method is very useful but this method does not show practical value of orthophosphate. The authors applied suppressed ion chromatography to determine solely orthophosphate. Obtained phosphate concentrations were compared with the values of SRP concentration for inflowing rivers of Lake Biwa, Japan (phosphorous limiting and mesotrophic freshwater lake).

Materials and Methods

River water samples were collected 4 times from April to November in 2014 at 7 inflow rivers (Yasu, Amano, Ta, Ane, Yogo, Nishino Creek of Yogo, Ado) of Lake Biwa, Shiga Prefecture, Japan. Water samples of Seta River, the only outflow river were also collected. Samples were filtered with a Nuclepore membrane filter (0.2?m pore size) and stored in a cool dark container below 10 degree in celcius. Orthophosphate concentration was measured by suppressed ion chromatography. Dionex AS-23A analytical column (250 was with electrochemical suppressor in electric suppression mode. Injection of high volume sample enhanced detection limit of orthophosphate to 10 nmol/L or less. SRP was measured according to the method JIS K0102 using ascorbic acid as reducing reagent. Micro glass cells of 50 mm path length (approximate volume: 3 mL), was used.

Results and discussion

Determined value of orthophosphate varied from 0.04 to 0.58 micro mol/L, while SRP showed values from 0.34 to 2.31 micro mol/L. There was so much difference between orthophosphate concentration and SRP in all river water samples collected. Ratios of orthophosphate to SRP in water differed between rivers sampled regardless of sampling season. In case of Yasu River, the ratio showed values from 0.06 to 0.14, while Ane River they were between 0.26 and 0.52. These differences might be caused by land use of watershed. In consideration of effects of river water quality to trophic status and primary production in Lake Biwa, these results might show the needs to consider direct impact of orthophosphate and indirect impact of other phosphorous compounds included in SRP separately.

Keywords: Lake Biwa, Inflow rivers, orthophosphate, SRP, Ion chromatography

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MORI, Yasushi[1*] ; SAKIKAWA, Kazuki[1] ; FUJI, Kazuya[1] ; ARAI, Miwa[3] ; KANEKO, Nobuhiro[2] ; FUJIE, Koichi[2]

[1]Okayama University, [2]Yokohama National University, [3]National Institute for Agro-Environmental Sciences

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Keywords: soil aggregate, macropore, soil moisture, infiltration

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Effects of group cutting on litterfall and organic horizon in the secondary forest dominated by hinoki cypress

NAKANISHI, Asami[1*] ; INAGAKI, Yoshiyuki[2] ; SHIBATA, Shozo[3] ; OSAWA, Naoya[4]

[1]Field Science Education and Research Center, Kyoto University, [2]Shikoku Research Center, Forestry and Forest Products Research Institute, [3]Kyoto University Graduate School of Global Environmental Studies, [4]Graduate School of Agriculture, Kyoto University

Nitrogen cycling in forest ecosystem is influenced by cutting and vegetation recovery after cutting. The growth of under vegetation after cutting of a coniferous forest may contribute to nutrient input by litterfall and the decomposition process of organic horizon (O-horizon). These changes may have a significant impact on nitrogen cycling in the forest ecosystem.

Litterfall and O-horizon were investigated to determine the cutting effects on nitrogen cycling at three different slope positions in the secondary forest dominated by hinoki cypress 10 years after group cutting to promote regeneration of broad-leaved tree species.

Carbon inputs by litterfall in the cutting plots were lower than those in the control plots. Nitrogen inputs by litterfall in the cutting plots were similar those in the control plots. In the cutting plots at the middle and lower positions where abundant tall-trees of broad-leaved species regenerated, carbon and nitrogen inputs by broad-leaved tree species leaf-litter were higher and litterfall C/N ratio was lower than those in the control plots, whereas those at the upper position where abundant woody shrubs and red pine regenerated did not differ between the control and cutting plots. Litterfall C/N ratio decreased with increasing nitrogen inputs by broad-leaved species leaf-litter.

Stocks of carbon and nitrogen and the mean residence time (MRT) of carbon and nitrogen in the O-horizon in the cutting plots were lower than those in the control plots at the same position. The MRT of carbon and nitrogen in the O-horizon was shorter with decreasing litterfall C/N ratio. The decrease of MRT of O-horizon in the cutting plots at the middle and lower positions was much more notable, whereas at the upper position that was smaller than in the other two positions.

The results suggest that the change in MRT of O-horizon between the cutting and control plots at each position 10 years after group cutting is strongly influenced by regenerated tree species in the cutting plots, but not by the difference of soil nutrition along a slope.

Keywords: group cutting, litterfall C/N ratio, organic horizon, regenerated tree species, nitrogen input, slope position

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Evaluating nutrient removal in a large river by in situ spiraling metric measurements

KOBAYASHI, Yuta[1*] ; IWATA, Tomoya[2]

[1]Department of Ecosocial System Engineering, University of Yamanashi, [2]Faculty of Life and Environmental Sciences, University of Yamanashi

Fluvial transports of excess nutrients can induce explosive growth of primary producers in aquatic ecosystems, thereby degrading the structure and function of downstream areas. River networks have traditionally been regarded as a conduit of such land-derived nutrient loads to coastal ecosystems. However, recent studies have increasingly identified the importance of in-stream processes that retain, transform and remove nitrogen and phosphorus from water column to benthic environments. Therefore, elucidating the pattern and mechanisms of nutrient removal processes in river networks has now become an important requirement to prevent the eutrophication of coastal waters.

Small headwater streams have been recognized as a vital element of riverine ecosystems as they are believed to be far more efficient at processing and transforming inorganic nutrients than large rivers. However, no study has hitherto directly quantified the nutrient removal rate in large rivers that exceed 18m^3/s in discharge. Therefore, the role of large rivers in controlling nutrient flux to downstream ecosystems has rarely been evaluated. In this study, we performed the in situ longitudinal measurements of dissolved inorganic nitrogen and phosphorus, as well as physico-chemical environmental gradients, to estimate the spiralling metrics (areal uptake rate, uptake velocity and uptake length) in the 6th-order mainstems of the Fuji River (Q >40m^3/s), central Japan.

The present result showed that the areal uptake rates of NH_4 and PO_4 in the Fuji River are relatively fast compared with those estimated in the 1st-to-5th order rivers by previous studies, although the net uptake rates of NO_3 were usually negative due probably to the stoichiometric imbalance of river waters. In contrast, the metrics of nutrient removal efficiency of NH_4 and PO_4 (uptake velocity and uptake length) did not differ from or even low relative to the previous findings, as a result of the high nutrient concentration and high water velocity in this steep terrain watershed. In the presentation, we will also introduce the analyses on the effects of spatial heterogeneity in river environments on the nutrient spiralling metrics in order to identify the hotspots of nutrient removal in this large river.

Keywords: spiraling metric, large river, nutrient, uptake rate, hot spot