Sediment acidification on Majuro Atoll

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Soil acidification often occurs due to various factors such as NO_x and SO_x from anthropogenic combustion substances. In the process of rock weathering or nitrification, protons are also released which causes soil acidification. It is difficult to evaluate each factor independently in industrial countries, since the many factors are affected one another, which makes it difficult to evaluate each factor. On the other hand, it is relatively easy to discuss anthropogenic impacts and natural effects independently in the case of atoll, since atoll sediments are composed of bio-clastic sand such as foraminifera and coral that are made from calcium carbonate ($CaCO_3$), which exhibits simpler composition compared with other areas with more heterogeneous geology. Here, we investigated the degrees of acidity in Majuro Atoll, one of the atolls of Marshall Islands. In Majuro Atoll, human residence has lasted for about 2000 years, which is the longest history among atolls around the world (Yamaguchi et al., 2005, 2009). However, the traditional life style in the atoll has changed and more industrial products such as motor vehicles have been used after 20th century.

In this study, we focused on factors which have a possibility to change surface environment of reef islands in atoll in terms of both artificial and natural factors. We evaluated the correlation between the elements contained in the sediment and sediment pH at different depths. We also examined the mechanism to acidify the pH of the sediment.

Samples were taken at different depth of the sediments in both inland area and ocean side of each reef island which are named as Laura, Calalen, and Jelto. All the surface layers are black-colored and well-vegetated. The grain size is medium to fine sand.

We examined concentrations of major elements such as calcium (Ca) and magnesium (Mg) by XRF. Concentration of inorganic ions (NO_3^-, SO_4^{2-}, and NH_4^+) were measured by ion chromatography and absorption spectrophotometry, respectively. Concentration of organic acid was calculated by pH titration. Mineral composition of foraminifera, which is the main component of the sediment was examined by XRD. Porosity of foraminifera were calculated using X-ray micro-Computed Tomography (μ-CT) and Image J. Stable isotope of nitrogen (^{14}N and ^{15}N) and triple oxygen isotopes (^{16}O, ^{17}O, and ^{18}O) in nitrate contained in the sediment were measured by the denitrifier method (Hattori et al., 2016) to identify the origin of the nitrate. ^{13}C solid state Nuclear Magnetic Resonance (NMR) spectroscopy were used to identify the organic matter which was concentrated in the upper layer.

As a result, sediment pH decreased near the surface layer and the concentration of Mg decreased. Mg-calcite fraction to total $CaCO_3$ (calcite + magnesian calcite (Mg-calcite)) of foraminifera also decreased near the surface. In contrast, the porosity of foraminifera, concentrations of NO_3^-, NH_4^+, TOC, and organic acid increased in the upper layer, whereas concentration of SO_4^{2-} fluctuated and especially became higher in the lower layer which is close to the water-table. Therefore, SO_4^{2-} may be originated from seawater, which did not induce acidification of the sediment. According to NMR analysis, organic matter enriched in the upper layer was identified as fulvic acid. Both the concentrations of nitrate and organic acid as fulvic acid had high correlation with sediment pH in the range from pH 7.5 to 8.0. Therefore, it is considered that both factors contribute to the acidification and dissolution of foraminifera test in the upper layer. From the analysis of the stable isotopes, the average of $\Delta^{17}O$ was close to 0‰ over all the areas, which suggested that nitrate was produced by bacterial activities. Therefore, we can conclude that highly concentrated nitrate produced during the excess nitrification and subsequent release of proton during the process should be the main factor to acidify the sediments on Majuro Atoll.

Keywords: sediment acidification, reef island, nitrification

Comparison of recalcitrant dissolved organic matter produced by three model marine bacterial strains

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Marine dissolved organic matter (DOM) constitutes one of the largest reduced organic carbon pools on the earth’s surface. The recalcitrant DOM (RDOM) has been considered to be major fraction of marine DOM and play an important role as a slow cycling carbon reservoir. However, source and production mechanism of marine RDOM have not been fully understood. Bacterial production of RDOM has recently been proposed as a carbon sequestration process. The concept of this process called microbial carbon pump (MCP) was derived from the previous studies of in vitro culture experiments in particular using natural microbial community. Since specific species who contribute MCP cannot identify from incubation experiments with microbial community, it is still unclear whether difference in bacterial species can affect RDOM production. In this study, to clarify similarity/dissimilarity of production efficiencies and composition of DOM derived from different bacterial species, we conducted batch culture experiments using three model marine bacterial strains, namely *Alteromonas macleodii* (*A. macleodii*) and *Vibrio splendidus* (*V. splendidus*) affiliated with ubiquitous Gammaproteobacteria, and *Phaeobacter gallaeciensis* (*P. gallaeciensis*) belonging to Alphaproteobacteia. Incubations were conducted in the dark at 25°C for 1–2 weeks with glucose (1 mmol C L^{-1}) as the sole carbon source in addition to inorganic nitrogen and phosphate. Bacterial numbers, dissolved organic carbon (DOC) concentrations, and excitation-emission matrices (EEMs) of DOM were monitored during incubations. Subsamples were taken from triplicate bottles at 8 points of time. DOC concentrations were drastically decreased during the exponential growth phases and relatively stable during the stationary phases, irrespective of differences in bacterial strains. While, bacterially derived DOM from glucose, namely residual DOC concentration in experiment was considerably high for *V. splendidus* (205 ±11 μmol C L^{-1}) compared with other two strains (51 ±4 μmol C L^{-1} for *A. macleodii*, 75 ±3 μmol C L^{-1} for *P. gallaeciensis*), suggesting that efficiencies of RDOM production were possibly different among bacterial species. EEMs during/after incubations showed the bacterial production of fluorescent DOM. Interestingly, fluorescent peak positions and number of peaks, including refractory humic-like fluorophores, were different among three strains. For example, two strains belonging to Gammaproteobacteria produced humic-like fluorophore emitted at >500 nm wavelength, while these fluorophores were not produced by the strain of Alphaproteobacteria. These in vitro culture results with different model bacterial strains imply that production efficiency and composition of RDOM are different among bacterial species or class levels.

Keywords: Marine carbon cycle, Dissolved organic matter, Microbial carbon pump, Marine bacterial isolate

Quantifying nitrogen cycling rate in streams water by using triple oxygen isotopes as tracers

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Nitrate (NO_3^-) can be an important source of information for understanding the biogeochemical cycles within the catchment area of the stream. In addition, the nitrate concentration in the stream water is important to primary production, and an excess of nitrate can lead to eutrophication in downstream areas, including receiving lakes, estuaries and oceans. However, nitrate concentration in stream water is determined through a complicated interplay of several processes within the catchment area including the deposition of atmospheric nitrate ($NO_3^-{}_{atm}$), the production of remineralized nitrate ($NO_3^-{}_{re}$) through microbial nitrification, the removal of nitrate through assimilation by plants and microbes, and the removal of nitrate through denitrification by microbes. Therefore, interpretation of the processes regulating nitrate concentration in stream water is not always straightforward.

Recent progresses in the stable isotope analysis enable us to use the stable isotopic compositions of nitrate ($\delta^{15}N$, $\delta^{18}O$, $\Delta^{17}O$) as tracers to understanding the origin of nitrate in stream water. Especially, the excess ^{17}O ($\Delta^{17}O$) tracer, in which isotopic fractionations during assimilation and denitrification have been canceled, can be a robust tracer to quantify mixing ratios of $NO_3^-{}_{atm}$ in stream water nitrate accurately. That is to say, we can quantify the absolute concentration of $NO_3^-{}_{atm}$ in the stream water by using both $\Delta^{17}O$ value and nitrate concentration ($NO_3^-{}_{total}$) in stream water.

In this study, we applied the absolute concentration of $NO_3^-{}_{atm}$ in streams as tracers to quantify nitrogen cycling rates in streams. If the supplying rate of nitrate through nitrification and the uptake rate of nitrate through either assimilation or denitrification are balanced, the nitrate concentration in the stream water is stable. The absolute concentration of $NO_3^-{}_{atm}$, however, should decrease because what produced by nitrification is $NO_3^-{}_{re}$. Therefore, we can estimate the nitrogen cycling rate (i.e., supplying rate of nitrate through nitrification and uptake rate of nitrate through assimilation) by quantifying the changes in the concentrations of $NO_3^-{}_{atm}$ and nitrate along with the stream flow. So as to verify this hypothesis, we quantified both nitrate concentrations and the stable isotopic compositions of nitrate ($\delta^{15}N$, $\delta^{18}O$, $\Delta^{17}O$) along with the stream flow at Yasu river, one of the representative inflow in Lake Biwa, to quantify nitrogen cycling rates of nitrification, denitrification, and assimilation in the stream water. We also determined the nitrogen cycling rates by using the traditional ^{15}N tracer method to verify the accuracy of the estimated values.

Keywords: nitrate, triple oxygen isotopes, stream water, nitrification, assimilation, denitrification

Exploration of the relationship between isotopic signatures of inorganic nitrogen and the spiraling metrics in the river

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Spiraling metrics are important for the better understanding of the biogeochemical processes in rivers, although it is quite demanding to measure these parameters. We explore the possibility to use the natural abundance of 15N and d18O of inorganic nitrogen (ammonium, nitrate and occasionally nitrite) as a proxy of the spiraling metrics. We measured concentrations and isotopes ratios of ammonium, nitrate and nitrite in a reach of Fuji River to figure out how concentrations and isotope ratios of these inorganic nitrogen can change according to their spiraling. We continuously collected water samples in the reach of ca. 6.5 km in the Fuji River by directly tracking a specific parcel of water following the longitudinal Lagrangian approach in June, October, November and December of 2015. We found significant inverse correlations between ammonium concentrations and d15N of ammonium in all sampling periods, implying that ammonium was removed by nitrification and assimilation. The d15N of nitrite measured in December were lower than both d15N of ammonium and nitrate, suggesting the inverse isotopic fractionation during nitrite oxidation in the nitrification. Both concentrations and d15N of nitrate significantly increased with the distance in all sampling periods, indicating the consumption of nitrate in parallel with nitrification. We will present more data collected from different rivers to explore the usefulness of isotope ratios as proxies of the spiraling metrics in the presentation.

Keywords: nutrient spiral, inorganic nitrogen, stable isotope

High concentrations of phosphate in streams of hilly areas, which stimulate high primary production by benthic algae on pond soils

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Phosphate is an essential mineral nutrient for all life forms. However, little phosphate is present in river waters, because it is strongly adsorbed on particulates such as oxides and hydroxides of iron and aluminum. Wakamatsu et al. (2006) reported low concentrations of phosphate in many headwater streams in Japan, which was presumed to vary depending on geological features. Here, we report high concentrations of phosphate in streams of hilly areas, which are created through a topographical feature of hilly areas. The reduction of Fe under humid soils resulted in the release of phosphate, which could act as a prime source of phosphate in a watershed. Moreover, we report an occurrence of significant primary production by benthic algae on sediments of a shallow pond, which may be stimulated by phosphate released from sediments.

Keywords: phosphate, hilly area, primary production, river, reduction

A nobel methane production pathway in freshwater ecosystems

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Climate change caused by the increasing of greenhouse gases (GHGs) in the atmosphere is the major problem of the 21st century. Methane (CH_4) is one of the powerful GHGs. Freshwater ecosystems (*i.e.*, lakes and ponds) are recently identified as one of the most important natural sources of atmospheric CH_4 , accounting for 18% of total annual CH_4 emission to the atmosphere. It has long been believed that CH_4 is mainly produced by CH_4-producing archaea (i.e., methanogens) in anaerobic lake sediments. However, our laboratory recently revealed the novel CH_4 production by photosynthetic microorganisms (i.e., cyanobacteria) in aerobic lake waters. Similar findings also confirmed that the planktonic microbes in the North Pacific subtropical Gyre have C-P lyase that cleaves the C-P bond of methylphosphonic acid (MPn) and produce methane aerobically as a byproduct of MPn decomposition. However, the pathways and organisms responsible for the aerobic methane production in freshwater ecosystems are still unknown. The ability of CH_4 production by freshwater organisms was examined for ten axenic planktonic microbes by three batch-culture experiments (Experiment 1, 2 & 3). In Experiment 1, to confirm the ability of aerobic methane production ability by planktonic microbes, we compared the CH_4 production of P-starved microbes between inorganic phosphorus (P_i) and MPn addition treatments. In Experiment 2, to identify the enzymatic reaction of aerobic methane production, we measured the CH_4 production of P-starved microbes for various phosphonate addtition treatments (MPn, EPn, 2-AEPn and DMMPn). Finally, in Experiment 3, inorganic nitrogen (N) and P_i was added with MPn to identify the effects of nutrient stoichiometry on aerobic methane production by planktonic microbes. In Experiment 1, the aerobic methane production was observed only in the MPn-add treatment for all microbes, while there was no CH_4 production in control and Pi-add treatments. Therefore, it is confirmed that the most planktonic microbes are able to decompose MPn to produce CH_4 under Pi-starved condition. Experiment 2 also revealed that the test organisms are able to cleave the C-P bond of MPn as a substitute for P_i, thereby producing CH_4 or C_2H_6 gases. However, the Protein BLAST search revealed that the test organisms have no C-P lyase (*phn*) genes, implying that different enzymes may function for the degradation of phosphonates. Therefore, further biochemical and proteomic analyses are necessary to identify the metabolic pathway. Finally, the effect of nutrient stoichiometry on CH_4 production was confirmed in Experiment 3. In particular, CH_4 production rate was accelerated in the MPn+N-addition treatment, indicating that N availability controls the MPn decomposition.

The present study revealed that the planktonic microbes have the ability to produce CH_4 aerobically by cleaving the C-P bond of phosphonates, whereas N availability increases CH_4 production. Therefore, the hitherto unknown CH_4 production by planktonic microbes in aerobic freshwater ecosystems represents a contemporary fact to amend the global CH_4 budget.

Keywords: Aerobic methane production, Planktonic microbes, Freshwater ecosystems, Methylphosphonic acid

Structure and functions of a methane-driven microbial food chain in rice field soil

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Methane oxidation is a key process controlling methane emission from wetlands into the atmosphere. Methanotrophs, responsible for aerobic methane oxidation, do not only oxidize but also assimilate methane. Once assimilated, methane carbon may be utilized by other organisms. Here we present the evidence of a methane-driven microbial food chain in a rice field soil and its potential impact on methanotrophs. Stable-isotope probing of nucleic acids using ^{13}C-labelled methane demonstrated that methane carbon is incorporated not only into methanotrophs but also into non-methanotrophic bacteria, phagotrophic protists, bacteriovorus nematodes in a rice field soil. Methane carbon could be also incorporated into the capsid gene of T4 type bacteriophages. These results suggest that methane carbon is linked to the soil microbial food chain that involves cross feeding, grazing, and viral lysis—once assimilated. The rice rhizosphere—the active site of methane oxidation—can have a distinct community of phagotrophic protists involved in a methanotrophic food chain that is influenced by elevated atmospheric CO_2; this implies a variation of prey-predator interaction in the methanotrophic food chain. Protistan grazing shapes the community structure of methanotrophs most likely by selective grazing. Protistan grazing also has a potential to affect methane oxidation in the surface of water-saturated rice field soil. Altogether, a series of our study suggests the potential importance of microbe-microbe interactions in methane dynamics in a rice field soil.

Keywords: Paddy soil, Protists, Protozoa, Methane oxidaiton, Foodweb

Biogeochemical arsenic cycling unraveled by microbial genome analysis

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Arsenic is released from anaerobic sediments into groundwater as As(III) (arsenite), which threatens the health of millions of people in southern Asia. It is widely accepted that certain anaerobic bacteria, such as dissimilatory iron-reducing bacteria and dissimilatory As(V) (arsenate)-reducing bacteria, play important roles in arsenic release in nature. Although respiratory arsenate reductase genes (*arrA*) closely related with *Geobacter* species have been detected frequently in arsenic-rich sediments, it is still unclear whether they directly participate in arsenic release, mainly due to lack of pure cultures capable of arsenate reduction. Previously, we isolated *Geobacter* sp. OR-1 from Japanese paddy soil. Strain OR-1 also utilized soluble Fe(III) and ferrihydrite as electron acceptors, and catalyzed dissolution of arsenic from arsenate-adsorbed ferrihydrite. Furthermore, inoculation of strain OR-1 into sterilized paddy soil successfully restored arsenic release. In this study, we analyzed draft genome sequence of strain OR-1, and found two distinct “arsenic islands”, the genomic regions highly enriched with arsenic-metabolizing genes. One consisted of *arrAB*, and they are also flanked with genes for arsenic resistance (*arsADR* and *acr3*) Another island consisted mainly of genes for arsenic resistance including that for a detoxifying arsenate reductase ArsC. Transcriptional analysis revealed that most of these genes were expressed specifically in the presence of arsenic, and that the expression of *arrA* was more than 30 times higher in the presence of arsenic. Comprehensive proteomic analysis by means of 1D SDS-PAGE and LC-MS/MS showed that not only arsenic-metabolizing proteins but also those involved in oxidative stress response, protein folding, molecular chaperones, phosphate uptake, and sulfur metabolism were expressed cooperatively in the presence of arsenic. Our results shed light on how microbes cope with this toxic metalloid, and play an important role in the biogeochemical cycling of arsenic.

Keywords: arsenic, microbes, genome analysis, arsenate reduction, transcriptional analysis, proteomic analysis

Biological nitrogen fixation coupled to chemolithotrophic sulfur metabolisms in a thermophilic microbial community in a hot spring

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Thermophilic microbial communities are potent model systems for ecological and evolutionary analysis in ecosystems. One important physical component related to evolutionary and ecological dynamics in these systems is the availability of fixed nitrogen, which can only be acquired from the atmosphere (from N_2) by the action of nitrogenase, an enzyme which might have emerged as early as 3.5 billion years ago. Molecular based studies have suggested a distribution of nitrogen-fixing bacteria and archaea in hydrothermal vents and geothermal springs, however, the activities and ecological consequences of these metabolisms are poorly understood. Here, we detected and characterized nitrogen-fixing activity of chemosynthetic microbial communities developed at 74°C at sulfidic and slightly alkaline hot spring water.

N_2-fixation (nitrogenase activity) was determined using the acetylene reduction assay. Collected microbial communities were incubated in hot spring water in a sealed vial in situ or at 70°C in the laboratory. Nitrogenase activities were detected in conditions where moderate amounts of methane were produced, but not detected under conditions where methane production was at the observed maximum or minimum. These results suggest that nitrogen fixation occurs within a limited range of redox levels in the communities.

Addition of molybdate, an inhibitor of anaerobic sulfur metabolisms which have sulfite as an intermediate (e.g. sulfate reduction, and sulfur disproportionation), inhibited the nitrogenase activity of the communities. Dispersion of cell aggregates of the communities also decreased the nitrogenase activity, but the activity was partially recovered by amendment with H_2 and CO_2. These suggest that the nitrogenase activity in the communities is coupled with hydrogen-autotrophic and anaerobic sulfur metabolisms. In addition, cell aggregation may contribute to efficient interactions which support the currently unknown N_2-fixing microbe(s).
Taken together, our findings provide new insight into the ecological contributions of anoxic sulfur metabolisms in chemosynthetic thermophilic microbial communities.

Keywords: nitrogen fixation, sulfur metabolism, thermophiles, geothermal spring

Climate and geochemical controls on soil phosphorus in Bornean tropical rainforest soils

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Phosphorus (P) is a major limiting nutrient controlling primary productivity in terrestrial ecosystems especially on highly-weathered soils. It was recently suggested that P depletion during soil weathering may also control long-term ecosystem evolution and plant species diversity. However, the connection between soil P forms and availability, forest productivity, and other ecosystem properties remains uncertain. The stock and chemical forms of soil P in ecosystem development is a fundamental issue because it affects plant and microbial strategies for acquiring P.

Here we examined how climate and parent materials control soil P in tropical rainforest ecosystems on Mt. Kinabalu, northeastern Borneo. We selected the sites developed on two chemically-contrasting rock types (ultramafic igneous and acidic sedimentary rocks) along an elevation gradients. We will present the results of soil P concentration (relative to C and N) as well as P forms assessed by liquid-state 31P NMR spectroscopy using NaOH/EDTA extracts.

Keywords: Phosphorus, 31P liquid-state NMR spectroscopy, Tropical forest ecosystems, Biogeochemical cycling, Ultramafic rock

Elemental and mineralogical diversity of serpentine soils in East Asia

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Serpentinite is an ultrabasic rock composed of ferromagnesian minerals with extremely high concentration of Mg and transition metals. Mainly due to the anomalies, special vegetation communities called serpentine flora can be developed on serpentinite geological substrate. Because serpentine minerals are relatively unstable in ordinary temperature and pressure, they may transform or neoform into secondary silicates or oxides during soil formation process with much higher rate than other felsic minerals such as quartz, feldspars and micas. In East Asia, where it has high temperature and much precipitation, mierals in serpentine soils are weathered easier than those in soils from other regions. Thus, mineral diversity of serpentine soils is expected to be very high in East Asia depending on the climate conditions. However, there has been no comprehensive study to elucidate diversity of these soils in this region. The objective of this work is to investigate the variation of elemental and mineral composition of serpentine soils in East Asia to understand their diversity in the region.

Samples were collected from Japan (Hokkaido, Kyoto, Kochi), Malaysia (Mt. Kinabalu' s 4 sites with different elevation) and Indonesia (Kuaro). Soil samples collected from each horizon and rock samples were collected from near respective soil sampling sites. The elemental compositions of the samples were determined by AAS or ICP-AES after wet-digestion or by XRF directly. A ratio of ferrous (Fe^{2+}) to total Fe was determined by a photochemical method using 1, 10-phenanthroline. The Fe in free oxides (Fe_d) was extracted by dithionite-citrate-bicarbonate (DCB) method. The Fe_d content was determined by ICP-AES. Mineral composition of rocks and clays fractionated from each soil sample was identified by XRD. Principal component analysis (PCA) was used to obtain basic information on difference of elemental concentrations in the samples.

The rock samples showed similar elemental composition regardless of sampling sites. Namely, sum of SiO_2 , MgO and Fe_2O_3 was 96±0.8% of total weight, and their ratio was about 8: 5: 1. The ratios of ferrous to total Fe were over 0.3 in the rock samples and the Fe_d contents were 12±1.5 g kg^{-1}. In the soil samples, the SiO_2 content varied largely from 45% to 5%, which was associated with the decrease in MgO content from 40% to almost zero, while the increase in Fe_2O_3 content from 15% to 80%. Furthermore, the increase in Fe_2O_3 content was proportional to the increase in the Fe_d content from 40 to 200 g kg^{-1} and the decrease in the proportion of the ratio of ferrous to total Fe from 0.2 to 0.01. Thus, elemental composition of serpentine soil was considerably different from parent rocks. The difference in the elemental condition was more evident in soils at lower latitude with similar elevations or at lower elevation with the same latitude (i.e. Kinabalu soils). PCA revealed that the first two PCs accounted for 77% of the total variance. The contents of Si and Mg had high positive loadings and those of Fe, Ni, Cr, and Mn had high negative loadings to PC1, indicating that PC1 is associates with degree of mineral weathering. The contents of Na, K, Al, and Ti had negative loadings to PC2. Because these elements were poor in all the rock samples and were relatively abundant in surface soil in Japan, PC2 may be associated with an incorporation of exotic minerals, probably as aeolian materials. Indeed, XRD revealed that quartz and mica were present in the Japanese clay samples, although major clay components were serpentine and talc. Thus, serpentine soils in East Asia were found to have highly variable elemental and mineralogical composition, largely different from those of serpentine in most cases. Such information of elemental and mineralogical diversity would be helpful to establish land management strategies in serpentine areas in East Asia.

Keywords: Soil, Serpentinite

Development of global dataset of soil clay minerals for biogeochemical studies

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Clay minerals play important roles in terrestrial biogeochemistry and atmospheric physics, but their data have been only partially compiled at global scale. We present a comprehensive global dataset of clay minerals in the topsoil and subsoil at different spatial resolutions. The data of soil clay mineral composition were gathered through a literature survey and aggregated by soil orders of the Soil Taxonomy for each of the nine clay mineral groups: chlorite, gibbsite, kaolinite, mica/illite/mica, quartz, smectite, vermiculite, non-crystalline, iron oxide, and others. Using a global soil map, a global dataset of soil clay minerals distribution was developed at resolutions of 2' to 2° grid cells. The data uncertainty associated with data variability and assumption was evaluated using a Monte Carlo method, and validity of the clay mineral distribution obtained in this study was examined by comparing with other datasets. The global soil clay data offer spatially explicit studies on terrestrial biogeochemical cycles, dust emission to the atmosphere, and other interdisciplinary earth sciences.

Keywords: Clay mineral, Soil

Probing the Future: Response of Soil Organic Carbon and Nitrogen dynamics to elevated CO_2 in a paddy field

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Rice is the staple food for approximatively half of people on Earth and, because of the projected increase in the world population, the sustainability of paddy fields ecosystems is of great importance. Continuous increase in atmospheric CO_2 concentration is likely to alter rice ecosystems productivity directly via photosynthesis or indirectly via global climate change. Less is known for how elevated CO_2 (eCO_2) affect soil C pool which is linked to soil N availability and long-term soil fertility. Free Air CO_2 Experiments (FACE) give an opportunity to simulates future atmospheric CO_2 concentrations in open-field environment by maintaining an elevated CO_2 concentration throughout the field. Here we report results from a rice paddy FACE at Tsukuba, Ibaraki started in 2010. We examined how the eCO_2 altered soil C and N stocks as well as the fate of rice-derived C over a four year period.. We analyzed surface (Ap) horizon samples from 2010, 2012 and 2014 and determined their C and N contents. The soils were fractionated into three density fractions (i.e. free light fraction, occluded light fraction and heavy fraction) that correspond to soil organic matter pools with different degrees of decomposition state and mineral association. The free light fraction corresponds to relatively recent plant residues which were not extensively decomposed by soil microorganisms. The occluded light fraction is composed of organic matter that was entrapped in soil aggregates. This organic matter is physically entrapped into soil aggregates. The heavy fraction is enriched in mineral particles and the organic matter present in this fraction is associated with mineral particles and underwent more pronounced microbial transformation. Because the CO_2 added in this experiment derived from fossil C, its carbon isotopic signature differed from natural atmospheric CO_2. This isotopic signature allowed us to track the flow of photosynthesized C into different soil organic matter pools and to estimate mean residence time of C in each fraction.

Both soil C and N contents progressively decreased over the four year period due presumably to a shift in management (i.e. removal of rice straw form the field since 2010). It is worth mentioning that this decrease in soil organic matter was 25% more pronounced in the control plot compared to the plot exposed to eCO_2, suggesting that eCO_2 significantly enhanced organic matter input to the soil. The decrease in N contents was not as pronounced relative to C, leading to an increase in C:N ratios. The higher C:N ratios of soil and plant observed are likely to promote N immobilization by soil microbial community, which might lowered N availability for rice.

Further soil analysis using stable isotopes measurements revealed that, after four years, significant proportions of original soil C were replaced with recent C at different degrees among the three soil density fractions. The mean residence time of C was on average 6.5, 120 and 56 years in free light fraction, occluded light fraction and heavy fraction, respectively. About 60% of soil organic matter was distributed in the heavy fraction and the relatively rapid turnover of C in this fraction was not expected. The heavy fraction contains the organic matter bound with mineral particles, which is more stable into the soil. We found that, after four years, about 7% of the C in the heavy fraction was originating from C assimilated by rice plants since 2010.

The relatively rapid incorporation of newly added carbon into the heavy fraction and the higher soil C

contents measured under eCO_2 suggest that the soil may be acting as a C sink under the open-field eCO_2 conditions. As it corresponds to a negative feedback for the rise in atmospheric CO_2 concentration, further research investigating this process are necessary.

Keywords: Soil, C and N cycling, Free Air CO2 Experiment (FACE), Density fractionation, 13C, Climate change

Heterogeneity of organo-mineral particles within submicron aggregate among contrasting soil types

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Soil organic matter (SOM) accounts for a major portion of terrestrial C and is considered to be stabilized against microbial degradation due partly to its interaction with soil minerals. These organo-mineral interactions contribute to the formation of heterogeneous organo-mineral aggregate at various space scales down to submicron level. Many early studies showed hierarchical structure of organo-mineral aggregates that are bound together by various binding agents. Yet how organic compounds of microbial and plant origins interact with mineral phases within soil aggregate structure at the spatial scale relevant to microbial extracellular enzymes (tens of nanometers) remain unclear.

Here we focused on the sonication-resistant organo-mineral aggregates that are enriched in organic matter (OM) collected by particle size fractionation from four soil types of contrasting minerology. We hypothesize that spatial variation of C, Fe and Al and it’ s chemical composition differ among the soil types due to the differences in the mode of organo-mineral associations.

We compare top soils (A horizon) from four soil types: allophanic Andisol, non-allophanic Andisol, Mollisol, Ultisol. The recovered particle size fractions are observed by scanning transmission X-ray microscopy (STXM) and near-edge X-ray absorption fine structure (NEXAFS). We will discuss common patterns and differences based on the mapping of these elements as well as that of carbon chemical composition among the four soils.

Keywords: soil aggregate, soil organic matter, organo-mineral associate, STXM, carbon stabilization

Paleodiet study based on isotopic ratio analysis of bone collagen from Malagasy extinct species

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Madagascar is well known for the unique ecological system and approximately 80% of plants and animals in Madagascar are the endemic species. During the past 2000 years, at least 17 genuses of vertebrates became extinct, which led to demise of animals weighting over 12 kg excepting crocodile *Crocodylus niloticus* (Burney and MacPhee, 1988). The main factors of the megafaunal extinction in the island of Madagascar would be human activities (e.g. over-kill, fire) or environmental changes (e.g. aridification, vegetational transition) (Crowley, 2010; Burney et al., 2004). Previous research reveals that the vegetational shift coincided with the extermination of megafauna (Geoffrey Clark, personal communication). However, the relationship between them has remained less understood.

Here we reconstructed the changes in the feeding habits of extinct species at the level of C3 or C4 plants to evaluate the correlation between feeding habitats and vegetational shift as well as human arrival. Fossil bones of extinct pygmy hippo *Choeropsis liberiensis* were collected from Taolambiby, Ambolisatra and Itampolo in southwestern Madagascar and used for isotopic ratio analysis. Collagen was extracted from the bones, and carbon and nitrogen stable isotopic ratios of collagen were measured using EA/IRMS at Japan Agency form Marine-earth Science and Technology. Radiocarbon dating of bone collagen was also employed using Single-Stage AMS at Atmosphere and Ocean Research Institute, The University of Tokyo.

Radiocarbon dates of bone collagen show the range from 2750-1130 cal BP. The δ^{15}N values were 9.6-10.1‰ in Taolambiby (*n*=2), 11.1-12-3‰ in Itampolo (*n*=6) and 12.48-12.57‰ in Ambolisatra (*n*=3) without the date of 2750 cal BP, suggesting that the trophic level of hippos would not change in the same region. The δ^{13}C values were -18.9--19.7‰ in Taolambiby, -17.2--17.9‰in Ambolisatra and -12.7--14.8‰ in Itampolo. The range of δ^{13}C values in fossil bones of pure C3 feeders would be approximately -31--17‰ through the δ^{13}C values in modern C3 plants from Beza Mahafaly, located about 20 km off eastern Taolambiby (Crowley et al., 2011). To take into account of the isotopic fractionation, the values in C4 feeders should be higher than that of C3 feeders. In comparison with the range of C3 feeders and our results, hippos in Taolambiby and Ambolisatra would be C3 feeders, while hippos in Itampolo would feed on both C3 and C4 plants, indicating that the diet of hippos in southwestern Madagascar did not change at the level of C3 or C4 plants in the same region. Previous studies revealed that human migrated to Taolambiby ca. 2300 cal BP (Burney et al., 2004). The extinction window of hippos in southwestern Madagascar is estimated 1250-950 cal BP (Geoffrey Clark, personal communication). Furthermore, pollen records in Ambolisatra show that the vegetational change occurred around ca. 1250 cal BP (Geoffrey Clark, personal communication). Therefore, our results suggest that hippos would not shift their diet at the level of isotope composition in their tissues from 2600 cal BP to 1100 cal BP, corresponding the timing after human arrival until hippos' extinction. Moreover, the δ^{13}C values different among locations suggest that hippos would feed on local plants without distinction of C3 or C4 plants. Hence, vegetational shift would not have an impact on the isotope composition of their tissues, and food shortage may not directly cause hippos’ extinction.

Keywords: Madagascar, Holocene, extinction, bone collagen, stable isotope ratio, feeding habit

Leaf biomass of hinoki cypress forests along a slope gradient in Kochi Prefecture.

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Leaf biomass in forest ecosystems is estimated by several techniques. Leaf biomass is known to be proportional to the basal area at the crown base. The basal area at the crown base can be indirectly predicted from tree height, height at the base crown and diameter at the breast height of trees. From these principles a simple method for leaf biomass estimation is developed for hinoki cypress trees. The method is applied for 18 hinoki cypress forests in two areas at different elevations in Kochi prefecture, southern Japan. Leaf biomass raged from 14.1 to 19.2 Mg/ha at the high elevation area and from 12.6 to 18.2 Mg/ha at the low elevation area. Leaf biomass was not different between the two areas or among different slope positions. The linear regression was applied for log-transformed leaf biomass and stem biomass in each forest. The slope of the regression lines ranged from 0.75 to 1.03 at the high elevation area and from 0.92 to 1.05 at the low elevation area. The slope of the regression was positively correlated with relative yield index which is correlated with height growth of trees. The results indicate that smaller trees have greater amount of leaves per unit of stem biomass at the low productivity sites and smaller trees have fewer leaves at the high productivity sites. This mechanism explains relatively constant leaf biomass along a slope gradient.

Keywords: Leaf biomass, hinoki cypress forest, slope

The stable isotopic compositions of nitrate extracted from natural plants : Quantifying the contribution of atmospheric nitrogen oxides to nitrogen assimilated by plants

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Nitrogen is one of the essential elements for organisms in general. Nitrate is the representive nitrogen nutrient assimilated by plants. While most of nitrate assimilated by plants must be supplied from soils, plants could uptake atmospheric nitrogen oxides from leaves and use them for the source of nitrogen. Traditionally, such assimilation pathways in plants of have been studied using artificial isotope tracers such as ^{15}N. The ^{15}N tracer methods, however, need tedious and time consuming incubation expariments. Besides, such experiments could often alter the growing environment from natural. In order to clarify both the source and the assimilation pathway of nitrate in plants, we used the natural stable isotopes of nitrate extracted from plants as tracers, instead of artificialy concentrated isotopes, with special emphasis on quantifying the contribution of atmospheric nitrogen oxides to plants by measuring triple oxygen isotopic compositions of nitrate.

Leaf samples were collected from conifer needles (*Metasequoia* and *Cypress*), broad leaves (*Quercus glauca* and *Prunus yedoensis*) and herbage (*Sasa*) once every month from April, 2016, to April 2017 from the same plant individual at Nagoya University.The leaf samples were washed immediately after each sampling using Milli-Q water and dried, and then nitrate was extracted using Milli-Q water, while insoluble materials were removed through filiteration.The stable isotopic compositions including the triple oxygen isotopic compositions of the extracted nitrate were determined using Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS) system in Nagoya University (Tsunogai et al., 2010).

The nitrate concentrations per dry weight of plants were ranged from 0.1 to 0.5 μmol g^{-1}. We could not find clear seasonal variations in the concentrations. The isotopic compositions of nitrate extracted from the plants were highly deviated from those in soil water. The δ^{15}N values showed large variation from -25‰ to +10‰ (vs. air) among the plants. *Quercus glauca* and *Metasequoia* showed definitely lower δ^{15}N values in nitrate compared with those dissolved in soil water (-5‰ to +5‰). Besides, the δ^{18}O values were significantly higher than those dissolved in soil water (-10‰ to 0‰ vs. VSMOW) , ranging from +60‰ to +80‰ in the whole plants. Furthermore, the Δ^{17}O values always showed large anomalies (+13‰ to +20‰). We concluded that most of the nitrate extracted by the method contain nitrate derived from atmospheric nitrogen oxides.

Keywords: Plant, Nitrogen oxides, Triple oxygen isotopic composition

Effects of flooding on iron solubilization in soils

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Solubilization of iron (Fe) oxides in soils has strong influences on phosphorus (P) sorption and plant productivity (Fe^{2+} toxicity). Seasonal flooding is hypothesized to change the red-ox status of Fe^{3+} (Fe^{2+}) and Fe oxide stability to varying extents under different ecological conditions. The stability of Fe oxides were thermodynamic analyzed using Fe^{2+} concentrations, Eh, and pH in soil solution for a variety of seasonally-flooded soils in arctic, temperate, and tropical regions (forest and paddy system). In continuous permafrost zone (Canada), permafrost-affected soils of black spruce forest are seasonally flooded due to summer thawing impermeable permafrost table. The surface soil solutions were under-saturated with short-range order Fe oxides (ferrihydrite). The soil solutions in deeper horizons are supersaturated with short-range order Fe oxides, consistent with the high concentrations of oxalate-extractable Fe oxides. In tropical forest soils (Indonesia), soil solutions are under-saturated with short-range order Fe oxides, but they are supersaturated with crystalline Fe oxides (esp., lepidocrocite). This suggests solubilization and re-precipitation of short-range order Fe oxides. This process is promoted in the soils of riparian zone. The highly reducing condition of tropical paddy soils (Indonesia) promoted solubilization of goethite and hematite, which caused Fe^{2+} toxicity. The red-ox cycles in temperate paddy soils (Japan) caused solubilization of lepidocrocite and supersaturation with short-range order Fe oxides after drainage. This process caused P solubilization and translocation of Fe oxides in deeper horizons.

Keywords: paddy soil, iron, phosphorus

Seasonal evolution of the N_2/Ar ratio in the upper ocean of the western subarctic Pacific: a modeling study

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The N_2/Ar saturation ratio (ΔN_2/Ar) in seawater provides a powerful constraint on water column and benthic denitrification. To use ΔN_2/Ar as a tracer of denitrification, accurate knowledge of the influence of abiotic processes, such as air-sea heat flux related to diffusive gas exchange, turbulent mixing, sea-level pressure variation, and bubble injection, on the distribution of these two gases in the upper ocean is required. To this end, we investigate the contribution of each of these abiotic processes to the seasonal evolution of N_2 and Ar saturation anomalies and ΔN_2/Ar in the western subarctic Pacific using a one-dimensional model. Variations in surface heat flux and sea-level pressure tend to create an undersaturation of N_2 and Ar in the mixed layer from winter to early spring, when the mixed layer depth reaches its maximum; this undersaturation is carried to depths below the mixed layer. Mixing induces a small supersaturation of both gases in and below the mixed layer. Because these processes affect both gases in a very similar manner, they lead to only very small ΔN_2/Ar anomalies in and below the mixed layer. In contrast, bubble-mediated gas exchange leads to higher supersaturation of N_2 than Ar, and it accounts for almost all the ΔN_2/Ar anomalies in and below the mixed layer. The contribution of bubble-mediated gas exchange thus needs to be well understood when using ΔN_2/Ar as a tracer for oceanic denitrification.

Keywords: N2/Ar ratio in the upper ocean, abiotic process, seasonal evolution

Nitrification and denitrification processes in sediment and its influence on nitrogen dynamics in Lake Biwa

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It has been well known that excess nitrogen loading on aquatic ecosystems causes severe environmental problems such as harmful algae outbreak and deterioration of water quality. Denitrification is a microbial process that reduce nitrate to di-nitrogen. Nitrification is important microbial process for denitrification because that produces nitrate from ammonium. These mean that co-occurrence of nitrification and denitrification reduces nitrate and ammonium concentration in aquatic ecosystem. In this study, we conducted sediment incubation experiments of sediment to determine nitrification rate and denitrification rate at sediment in Lake Biwa. We also corrected lake water at 14 depths from May 2015 to Dec. 2016 in a month interval at first sedimentary basin of Lake Biwa, and analyze total nitrogen, dissolved nitrogen, ammonium, nitrate and nitrogen and oxygen isotopes of nitrate.

Nitrate concentration was higher in the deeper layer (50-80 m) than the surface layer (0-10 m) at all observation period, and the difference increased in latter stratification period. Moreover, nitrogen isotopes ratio of nitrate increased and oxygen isotope ratio of nitrate decrease in deeper layer at latter stratification period. These results mean new nitrate was generated at deeper layer at stratification period. The results of sediment incubation experiments and nitrate mass balance in water column show that increase of nitrate in deeper layer at stratification period was result of nitrification in water column. Moreover it is considered that the influence of sedimentary denitrification on nitrate consumption in water column was minor.

Keywords: nitrification, denitrification, sediment, Lake Biwa

Nitrogen and phosphorus dynamics in the mainstem of the Fuji River estimated by *in situ* spiralling metric measurements

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Excess land-derived nitrogen and phosphorus often stimulate the primary production of downstream ecosystems such as lakes and coastal waters, thereby causing the eutrophication problems therein. Therefore, elucidating the role of river networks in processing the fluvial nutrients supplied from terrestrial ecosystems has become an important prerequisite for the ecosystem management of downstream ecosystems. Nitrogen and phosphorus dynamics in lotic ecosystems have long been described uniquely by three major variables of spiralling metrics (areal uptake rate, U; uptake velocity, v_f; uptake length, S_w). Areal uptake rate (U) represents the net uptake rates of nutrient atoms by benthic compartments, while uptake velocity (v_f) and uptake length (S_w), respectively, refer to nutrient uptake efficiency relative to concentration and the longitudinal travel distance of a dissolved nutrient atom before removal from the water column. However, until recently, there are only a few studies that have undertaken to measure the spiralling metrics in large rivers at the downstream end of the river networks, especially in large rivers of mountainous watersheds with high relief, as are watersheds in Japan. The objective of the present study is to evaluate the roles of large rivers in fluvial nutrient transports by estimating the nutrient spiraling metrics (U, v_f, S_w) in the mainstem of a sixth-order river (discharge = 37−53 $m^3 s^{-1}$) in a Japanese high-relief watershed.

The field study was conducted during May and December 2015 in the mainstem and tributaries of the Fuji River system in central Japan. We monitored the longitudinal changes of inorganic nitrogen (ammonium, nitrite, and nitrate) and phosphorus (phosphate) concentrations by directly tracking a specific parcel of water and by continuously collecting the samples from the parcel along the river course. Assuming that nutrient removal reactions in river channels obey first-order kinetics, we estimated nutrient spiralling metrics from the longitudinal change of the natural logarithm of nutrient concentrations with distance downstream. In this presentation, we will show the pattern and dynamics of nitrogen and phosphorus in the Fuji River by using the estimated nutrient spiralling metrics. The results emphasize the seasonal dynamics of nutrient uptake and the importance of substrate limitation and nitrification in phosphorus and nitrogen uptake in the river system. Based on these findings, we discuss the functional roles of large river ecosystems in longitudinal nutrient transports from terrestrial to coastal ecosystems.

Keywords: nitrogen, phosphorus, river, spiral metrics

Clarifying sources of methane enriched in oxic water columns by using stable carbon and hydrogen isotopes as tracers

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Although methane is one of the representative greenhouse gases in atmosphere, there are still many uncertainties on the emission sources on earth surface. The hydrosphere is the major source of atmospheric methane, and thus slight changes on the hydrospheric environment will cause a drastic change on the atmospheric methane concentration and thus on global warming. Consequently, we must increase our knowledge on the sources of methane enriched in hydrosphere, together with the behaviors of methane in hydrosphere.

Generally, methane is produced in anoxic environments, while being decomposed in oxic environments. Nevertheless, supersaturation of methane in water relative to that in equilibrium with atmospheric concentration have been frequently found in oxic oceans and lakes. Various studies had been done in past to clarify the reasons of this contradiction, being called as ‘methane paradox’.

So as to clarify the sources and behaviors of methane in such oxic hydrosphere, the δ^{13}C values of methane have been frequently used as a tracer in past studies, while the studies using δD values of methane enriched in oxic hydrosphere were limited in past. By using both δ^{13}C and δD values of methane simultaneously as tracers, the changes in the values of δ^{13}C and δD during oxidation (partial removal of methane in oxic water column) can be corrected. In this study, we used stable isotopes (δ^{13}C and δD) of dissolved methane in the water columns of Lake Biwa, Ise Bay, and Mikawa Bay as the tracers to specify the sources of methane supersaturated in the oxic water columns and to clarify the behavior of methane in the oxic water columns. Besides, I defined a new parameter $\Delta(2,13)$(= $\delta D - 11 \times \delta^{13}C$) in which variations in both δ^{13}C and δD values during methane oxidation had been corrected and used this parameter as the tracer to differentiate the sources for each methane dissolved in the water columns. To clarify the sources in the studied fields, I assumed following three methane sources as the possible sources of methane supersaturated in the oxic water columns, (1) river input, (2) lake-floor (or seafloor) sediments, and (3) sinking particles in the water columns, and determined the stable isotope ratios of methane supplied from each. Then, I compared them with those in the water columns, and concluded that methane supersaturated in Lake Biwa and Ise Bay surface water columns is likely to be supplied via river. On the other hand, it was impossible to assume river input as the major source of methane in Mikawa Bay water showing the δD values (-327~-287‰) substantially lower than those dissolved in river input (-175‰). I concluded that the seafloor sediments should be highly responsible for the source of methane enriched in the Mikawa Bay water columns.

Keywords: methane , hydrogen isotopes, carbon isotopes

Elucidation of nitrate dynamics in a temperate region watershed with heavy snowfall using triple oxygen isotopes as tracers

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Atmospherically deposited nitrogen to the terrestrial environment due to human activity has been increased over the last decades. It is important to elucidate the response of ecosystems towards nitrogen deposition. In this study, a triple oxygen isotope approach was used as a tracer for environmental fate of atmospheric NO_3^- in a temperate forest with heavy snow for the years 2015 and 2016. The $\Delta^{17}O$ values of NO_3^- for precipitation and throughfall ranged from 22 to 32‰ and reflect the seasonal variation between summer (minimum) and winter (maximum), this is attributed to the changes in atmospheric formation pathways of NO_3^- over seasons. Based on $\Delta^{17}O$ values of NO_3^- in litter layer and mineral soil at 25, 55, and 90 cm depths respectively, calculated fraction of $NO_3^-{}_{atm}$ (f_{atm}) shows that nitrification mainly occurs in the litter layer in the summer. In the winter, on the other hand, relatively high $\Delta^{17}O$ values of NO_3^- in litter layer were observed, indicating that nitrification does not occur in the litter layer due to the existence of snowmelt water. Although different f_{atm} for litter layer were observed over the seasons, f_{atm} of stream water were constant (approximately 10%) in both winter and summer. In addition, gross nitrification rates (GNR) based on f_{atm} for stream water and total NO_3^- input for this study site in summer were lower than those in winter, suggesting higher nitrification activity in winter due to higher loads of NO_3^- and NH_4^+ inputs in the latter season. So far, nitrogen and oxygen isotopic values for biologically produced NO_3^- showed no significant correlation, indicating no detectable trend of assimilation by plants and/or denitrification.

Keywords: stable isotopes, fraction of atmospheric nitrate, gross nitrification rate, biologically produced nitrate

Using triple nitrate isotopes to determine nitrate sources in the streamwater of tropical dry forest

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In southeast Asia a nitrogen emission and deposition increased since 1980s. This could lead to the nutrient imbalances and the degradation of stream-water quality in tropical rain/dry forest which is largely distributed in the area. Quantifying the contribution of atmospheric nitrogen deposition to nitrogen discharge has not been well examined in tropical forest area. Oxygen isotope anomaly ($\Delta^{17}O$) would be a good technique for distinguishing the atmospheric nitrate from the microbe-oriented nitrate. Using this technique, existing studies has determined the fraction of atmospheric nitrate in the stream water mainly in temperate forest located in mid-latitude. However, an applicability of this technique is largely unknown in tropical area. Our objectives of the study are 1) to examine the applicability of $\Delta^{17}O$ techniques in tropical forest and 2) to clarify the contribution of the atmospheric nitrate to the stream water nitrate in the forest established in tropical savanna climate.

The study catchment was established in dry evergreen forest of the Sakaerat silvicultural research center, Nakohn Ratchasima Province in northeastern Thailand (35 ha). The altitude of the study catchment ranges 600 to 680 m. Annual mean precipitation was 1370 mm and annual mean temperature was 25.5 ° C. Climate type was tropical savanna. The period between November and March is extremely dry, with monthly precipitation less than 50 mm. Using bulk sampler, we collected a precipitation in the morning of the day just after the rain event was observed. We mixed the daily precipitation into the monthly composite samples and stored it at freezer. Meanwhile, nitrate in soil water and stream water was collected by anion exchange resin method. The resins for soil water and stream water were put during ca. 6 month and 2 weeks, respectively. We extracted the nitrate from the recovered resins from the field after the period. We measured nitrate concentration and isotopic compositions of the nitrate for each water and extracts samples. $\delta^{15}N$ and $\delta^{18}O$ of nitrate were determined by the denitrifier method. And $\Delta^{17}O$ was measured in Washington University after we transformed nitrate of the sampler to nitrous oxide by the denitrifier method.

As a result, in precipitation $\Delta^{17}O$ -NO_3^- in precipitation was about 21‰ and did not largely fluctuate during wet season. In soil water $\Delta^{17}O$-NO_3^- was 1.3 and 1.4‰ at surface and sub soil, respectively, on the slope. These values decreased on riparian area. This tendency simply suggested that the contribution of atmospheric nitrate steeply declined in soil water in this study catchment. As for the seasonality, $\Delta^{17}O$-NO_3^- of soil water was higher in dry season compared to wet season. Finally, $\Delta^{17}O$-NO_3^- in the stream water ranged 6 to 12‰ during wet season. We also found the significantly relationship between $\delta^{15}N$ and $\delta^{18}O$ in the stream water, which suggested the strong contribution of the denitrifying processes within the study catchment.

We assumed that this technique was effective for distinguishing the atmospheric nitrate from microbe-oriented nitrate even in the tropical forest ecosystems. Meanwhile, in this study $\Delta^{17}O$-NO_3^- of precipitation (21‰) was relatively lower than the value in existing report at mid-latitude. In this

presentation we are going to discuss the fraction of atmospheric nitrate to surface water nitrate by a quantitative approach.

Keywords: Oxygen isotope anomaly, Tropical dry forest, Nitrogen deposition

The influence of dry deposition and wet deposition on streamwater nitrate concentration in forested watersheds

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Nitrogen exported from forest is considered to affect the eutrophication in downstream ecosystems, therefore, the understanding of nitrogen export processes from forest is important. Recently, nitrogen deposition to terrestrial ecosystem is increasing and that enhance nitrate concentration exported from some forested watersheds. Nitrogen deposition to forest generally consists of dry and wet deposition of nitrogen, however, the influence dry deposition on nitrogen export from forested watershed has not clear compared with the influence of wet deposition. In this study, we measured nitrogen compounds concentrations in stream water, througfall and air in forested watersheds near highway to clarify the influence of dry deposition and wet deposition on streamwater nitrate concentration. We collected streamater in 23 sites, throughfall in 13 sites and nitrogen oxide aerosol in 8 sites at north part of Shiga prefecture. Stremawater was collected from March 2016 to November 2016 in a month interval, and throughfall and aerosol were collected from August 2016 in two weeks interval. Nitrate concentrations were higher in streamwater from forested watershed near highway. We will discuss the relationship between streamwater nitrate concentrations and wet and dry depostion in presentation.

Keywords: nitrate, streamwater, dry deposition, wet deposition, forested watershed

Development of stable N isotope model for forest ecosystem

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Since the Industrial revolution, reactive nitrogen in the environment has increased due to the combustion of fossil fuels and increased use of chemically synthesized fertilizers, the amount of which has been doubled in the whole land before the industrial revolution. Excessive reactive nitrogen is a cause of various environmental problems such as generation of nitrous oxide which is a greenhouse gas, nitrate nitrogen contamination of groundwater, eutrophication of closed water area, and decline of biodiversity. Even in forest ecosystems, nitrogen saturation has been confirmed in many suburban areas, but it is still difficult to accurately measure the nitrogen balance in forest ecosystems. This is partilly because it is difficult to quantify the denitrification in forest ecosystem. For this reason, we have also great uncertainties in the nitrogen cycle of the ecosystem (biogeocemical) models. However, the ratio of natural N isotopes is considered to reflect information on N loss rates (i.e., denitrification, leaching) in some extent. So, Houlton et al. (2015 in Nature Climate Change) proposed that implementing the ratio of natural N isotopes in ecosystem model and validate these values could improve their representation of N cycling.

In this study, to implement d15N calculation scheme to ecosystem model "VISIT", we measured the delta15N of leaves, litter and soils, as a validation dataset, in five forest experimental sites across Japanese archipergo (Teshio Experimental Forest of Hokkaido Universiy Ryukyu , Fujiyoshida, Mt. Tsukuba, Tenryu Field of Shizuoka University, Yona Field of Universiy of Ryukyu). First, to estimate soil d15N using VISIT, we implemented Houlton & Bai (2010 in Global Biogeochemical Cycle) scheme to VISIT model. However, the simulation results of soil d15N in VISIT showed smaller values, compared to observation in all site. We will report further progress in the representation 15N in the modified VISIT model.

Keywords: Nitrogen isotope ratio, Forest ecosystem

Seasonal variation of O_3 flux in red pine forest

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The emission and absorption of trace gases at the biosphere affects to atmospheric chemistry, and thus it makes influence with potential indirect effects on carbon cycle and climate (Ollinger et al., 2002). We observed O_3 flux with the gradient method at a meteorological tower in red pine forest (Site Code: FJY) through 2016. We also measured CO_2 flux at the same meteorological tower for validation of the system by comparison with CO_2 flux determined by the eddy covariance method.

The heights of the forest canopy and the meteorological tower were about 25 m and 32 m. Concentrations of O_3 and CO_2 were measured at two heights (26 m and 34 m) above the canopy by an ultraviolet absorption O_3 analyzer (Thermo: 49C) and an infrared absorption CO_2 analyzer (Licor:LI-820). The O_3 instrument was calibrated before the observation, and the CO_2 instruments were calibrated every three weeks at the observation site. The air was sampled every 300 seconds from each two vertical heights and supplied to the analytical instruments through PFA tube. Concentration of CO_2 was also measured by an infrared absorption CO_2 analyzer (Licor: LI-6262) at 26.5 m to determine CO_2 fluxes by the eddy covariance method. Wind speed and wind direction were measured at 26.5 m and they were used to obtain fluxes by the gradient and eddy covariance methods.

The CO_2 fluxes in the day time (9:00-16:00) in 2016 were observed with the gradient and the eddy covariance method as -0.10 ± 0.08 mol m^{-2} d^{-1} and -0.25 ± 0.16 mol m^{-2} d^{-1}, respectively. The CO_2 flux obtained by the gradient method was slightly lower and more scattered than CO_2 flux obtained by the eddy covariance method; however these values reasonably agreed. We made sure the flux observation system with gradient method worked properly.

O_3 concentration showed a seasonal variation and was in a maximum in May. However the primary result showed that O_3 deposition in the red pine forest in the day time (9:00-16:00) had a maximum in July. The peak of O_3 deposition delayed to the peak of O_3 concentration, which was also obserbed in mixed forests of conifers and broad leaved trees inTeshio, Japan (K. Takagi, 2016).

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Keywords: ozone, flux, forest

Carbon stock of coarse woody debris in Japanese forests

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The national forest soil inventory project of Japan was launched from 2006 in order to support reporting to the United Nations Framework Convention on Climate Change (UNFCCC). The second phase of the project had been started from FY2011 to FY2015. The project is collecting data from Japanese forests with respect to three carbon compartments in pedosphere; soil, litter, coarse woody debris (CWD). Here, we report our preliminary results of the analyses of CWD compartments based on 2636 plots in the second phase of the project.

The CWD data used in this study include dead wood, stump, and blighted tree. We obtained the average CWD carbon stock of 0.75±0.98 kg m^{-2}, that is 8.3% of total soil carbon stock.

In secondary forests, CWD was produced by mortality dues to environmental stress, snow damage, wind damage, disease, insect pests, competition and aging. In contrast, CWD in plantation was generated by thinning or large-scale felling for forest management. The CWD in secondary forests, cedar plantation, and Japanese cypress plantation has average carbon stocks of 0.60±0.83 kg m^{-2}, 1.16±1.24kg m^{-2}, and 1.14±0.94kg m^{-2}, respectively. These results suggest that the CDW carbon stock differs largely between secondary forest and plantation. Considering other conditions such as management practice and above ground biomass, we plan to report characteristics of CWD carbon stock in Japanese forest through different types and ages.

Keywords: soil carbon stock, forest ecosystem, inventory

表 1　枯死木の調査方法とサイズ計測箇所

枯死木の種類	**調査方法**	**サイズの測定方法**
倒木	ラインインターセクト法	ライン上の直径
根株	ベルトトランセクト法	直径、地際直径、斜面上部高、斜面下部高
立枯木	ベルトトランセクト法	胸高直径、高さ

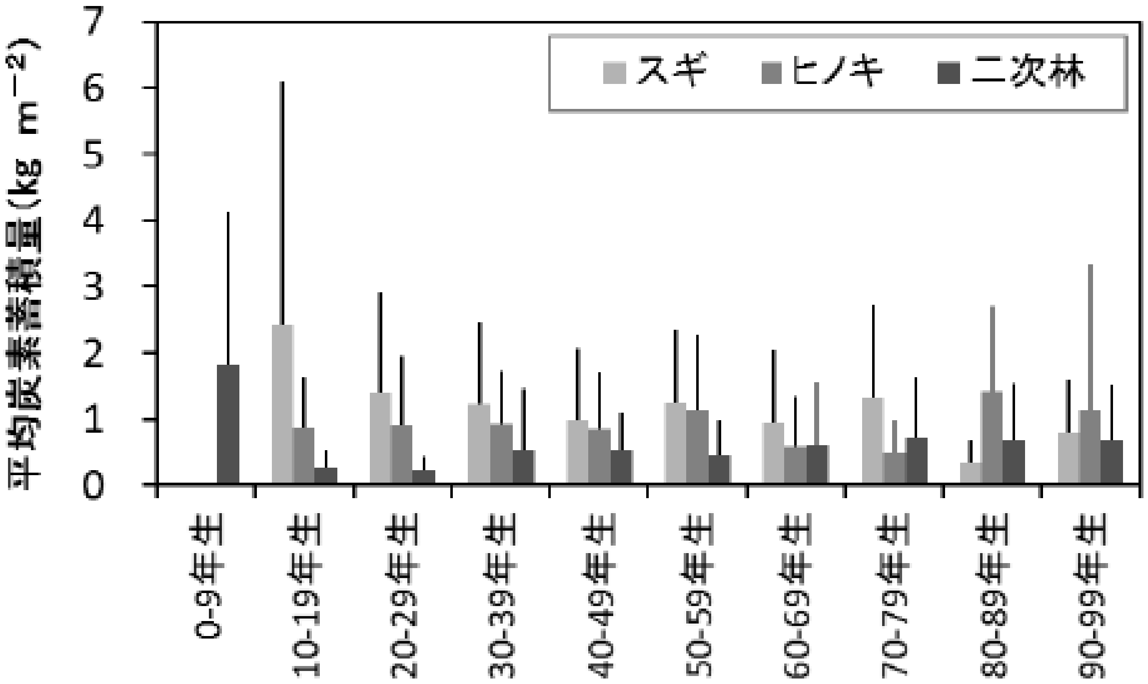

図 1 林齢による枯死木の平均炭素蓄積量

エラーバーは標準偏差を示す。

The sensitive soil chemical properties reflect the vegetation changes caused by overgrazing in Mongolia.

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Human activities and climate changes have altered grassland ecosystems in Mongolia. Especially, overgrazing causes deterioration of vegetation and soil degradation. The process of soil degradation with vegetation deterioration in Mongolian grassland is not clearly understood. We need to find out which soil properties can be a sensitive index of soil degradation to promote sustainable use of grassland in Mongolia. Therefore, to obtain preliminary data for establishing a soil degradation index, we observed soil profile morphology and measured soil physicochemical properties under different grazing pressure in the steppe grassland Mongolia.

Keywords: soil physicochemical properties , soil degradation, overgrazing, Mongolia

Comparison of Different Degraded Grassland Soils in the Qinghai-Tibet Plateau

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The Qinghai-Tibetan Plateau, the largest geomorphological unit on the Eurasian continent, is an important part of the global terrestrial ecosystem. In recent years, the degraded grassland area has reached about 4.251×10^7 hm^2, accounting for 33% of the available area.

Objective of this study are (1) To identify morphological characteristics and physicochemical properties of soils in alpine degraded grassland (2) To investigate the change of soil micromorphology under different degradation grassland.

Therefore, we chosen 3 site from the Hequ horse farm in the eastern Qinghai-Tibet plateau, there are lightly degraded grassland(HQ1-L), moderately degraded grassland(HQ2-M) and heavily degraded grassland(HQ3-H). HQ1-L in the winter pasture, HQ2-M close to the nest, surrounded by more serious desertification, HQ3-H plots selected in the cow enclosure, the surface vegetation was destroyed. Soil samples from each horizon were systematically collected for physicochemical analysis and 100 cm^3 core samples were taken from 0-5cm surface soils for the micromorphology analysis.

Vegetation coverage decreased with grassland degradation. The characteristics of the OA layer are root mat, because the dominant species of *Kobresia* belongs to the *Cyperaceae* genus, this is easy to form the root mat. In the HQ3-H degraded grassland, a large number of vegetation degradation, secondary vegetation instead of dominant species, the OA horizon was disappeared.

In conclusion, the exchangeable cations, CEC, total nitrogen (TN) and organic carbon (OC) were decreased with grassland degradation degrees; however, the pH value is exactly the opposite. While, from the micromorphology of soil thin section, with the soil degradation, the porosity obviously decreases while the degree of soil microstructure is also reduced, resulted in mineral ions and free water supply to plant growth are also restricted. Also the activity of soil animals was decreased with grassland degradation.

Keywords: Qinghai-Tibet Plateau, Soil, Grassland, Degradation

Effect of epigeic earthworm casting on soil properties of subsoil from an Andosol

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The burrowing and feeding activity of earthworms have the beneficial effect on the soil properties. It is reported that earthworm cast forms a stable aggregate with large size and it increases the water holding capacity, water permeability. Earthworm casts have higher available nutrients and microbial activity than surrounding soils and have a higher rate of decomposition of organic matter. In addition, it is reported that bacterial composition is different between the intestine of large earthworm and surrounding soils. However, the influence of earthworms on the interactions among organic matter - minerals - microorganisms in soil is still unclear. In this study, we conducted the breeding experiment of earthworm to show the effects of earthworm on; (1) the soil aggregation and soil organic matter content, (2) soil enzyme activity and microbial community.

Metaphire hilgendorfi was collected and kept in a rearing container with soil and litter for 2 weeks. After breeding, control soil and earthworm cast were sampled for microscopic observation, measurement of pH, EC, total nitrogen, organic carbon, β-glucosidase and protease activity, and evaluation of microbial diversity using biology eco-plate.

Keywords: earthworm, soil aggregates, microbial activity

Soil carbon loss induced from artificial macropore installation

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Soil carbon loss induced from artificial macropore installation

Keywords: macropore, infiltration, carbon storage

Which fraction of soil organic matter is more vulnerable to rhizosphere priming effect?

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Rhizosphere priming effect (RPE) is defined as the stimulation or suppression of soil organic matter (SOM) decomposition by living roots. It remains unclear which fraction of SOM is more vulnerable to rhizosphere priming. We conducted two experiments in continuous 13CO2 labeling growth chamber to compare the intensity of RPE for the active (or labile) vs. slow (or recalcitrant) SOM. A sandy loam (Alfisol) was incubated at 20oC and 80% water holding capacity for different periods, which created a gradient in the relative proportion of active vs. slow SOM in the remaining soils. We then grew sunflower (Helianthus annuus) and soybean (Glycine max) in these remaining soils for 50 days under the same environmental conditions to compare the RPE of these two plant species on the decomposition of soils that varied in the lability of SOM. In both experiments, as the incubation proceeded from 1 to 8 to 14 months (in experiment 1) and the soil changed from freshly-sampled soil to two-year-incubated soil (in experiment 2), the intensity of RPE increased significantly even after accounting for the changes in root biomass or root-derived CO2. This result suggests that the slow (or recalcitrant) fraction of SOM is likely more vulnerable to rhizosphere priming compared to the active (or labile) fraction of SOM. Although the underlying mechanisms of this finding await further investigation, our study clearly shows that the main component of SOM (slow or recalcitrant SOM, decadal turnover) is vulnerable to rhizosphere priming. Therefore, the RPE has the potential to substantially regulate both short-term and long-term soil carbon dynamics.

Keywords: soil organic matter decomposition, rhizosphere priming effect, labile soil carbon, recalcitrant soil carbon