

## Iron isotopic signature of red blood cell samples from shark and seal : new tracer for biological cycle of Fe in marine

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Among the essential metal elements, Fe is one of the most important essential elements for all organisms because its flexible redox activity contributes to a cell respiration, photosynthesis, nitrogen fixation, and hemoglobin enhances the efficiency of oxygen transport in blood. Recent Fe isotope studies have revealed that the  $^{56}\text{Fe}/^{54}\text{Fe}$  and  $^{57}\text{Fe}/^{54}\text{Fe}$  isotope ratios for the terrestrial plants or animals were systematically decreased with increasing the nominal trophic level (about 1‰/amu per trophic level). The systematic decrease in the  $^{56}\text{Fe}/^{54}\text{Fe}$  isotope ratio can be attributed to the preferential absorption of lighter Fe isotopes from nutrients or dietary foods [1, 2]. However, the same is not true on the marine organisms. Despite the quite limited number of Fe isotope data, there were no significant difference in the reported  $^{56}\text{Fe}/^{54}\text{Fe}$  ratio data between the marine organisms (phytoplankton, shrimp and tuna samples [3]) and the seawater samples [4], suggesting very small change in the  $^{56}\text{Fe}/^{54}\text{Fe}$  ratio against the trophic level. This is contrasting to the  $^{56}\text{Fe}/^{54}\text{Fe}$  ratio for the terrestrial plants or animals. To investigate the possible correlation in the  $^{56}\text{Fe}/^{54}\text{Fe}$  isotope ratio and the trophic level for the marine organisms, the  $^{56}\text{Fe}/^{54}\text{Fe}$  ratio for marine organism with high-trophic level is highly desired. In this study, we have measured the  $^{56}\text{Fe}/^{54}\text{Fe}$  and  $^{57}\text{Fe}/^{54}\text{Fe}$  ratios for red blood cell (RBC) samples from the high-trophic level animals of various ages (15 shark and 13 seal samples of various ages). After the series of chemical procedures, including sample decomposition, chemical purification, and the adjustments of the Fe valence, the  $^{56}\text{Fe}/^{54}\text{Fe}$  and  $^{57}\text{Fe}/^{54}\text{Fe}$  isotopic ratios were obtained by a multiple-collector ICP-mass spectrometry (MC-ICPMS). The resulting  $^{56}\text{Fe}/^{54}\text{Fe}$  ratio for shark and seal samples were ranging from -1.11‰ to -2.56‰ and -0.70‰ to -1.26‰, respectively. For shark samples, there were no significant differences in the measured  $^{56}\text{Fe}/^{54}\text{Fe}$  ratio between the male and female samples. This is contrasting with the  $^{56}\text{Fe}/^{54}\text{Fe}$  ratio for the terrestrial animals, including human RBC samples. For the seal samples, no correlation in the resulting  $^{56}\text{Fe}/^{54}\text{Fe}$  ratio and both the age and body length was found. More importantly, the resulting  $^{56}\text{Fe}/^{54}\text{Fe}$  ratios for the shark and seal samples were significantly higher than those for the high-trophic level terrestrial animals. We will discuss the difference in the correlation of the  $^{56}\text{Fe}/^{54}\text{Fe}$  ratio with trophic level between the terrestrial and marine organisms.

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Keywords: stable isotope geochemistry, iron isotopes, red blood cell, marine environment, biocycle of Fe, essential element

## Processes of stromatolite formation examined from the modern analogs

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Stromatolites, macroscopically laminated domal, columnar and lentic shaped sediments, are an abundant sedimentary facies in Precambrian carbonates. Despite of 100-yr-long study history of taxonomy, paleontology, and sedimentology for understanding early biosphere and ocean chemistry, the detailed formation processes have not been well defined because the initial structures and chemical features were obscured due to recrystallization, deformation, and scarcity of microfossils. After the discovery of the modern stromatolite at restricted marine settings such as Bahamas and Western Australia (e.g. Logan, 1961), the detailed processes for the stromatolite formation have revealed, for example, growth by trapping and binding of detrital particles in the surface cyanobacterial mat, and lamina formation by cyanobacterial phototactic behavior and intermittent sediment supply. These processes were often adopted for interpreting the genesis of ancient stromatolites, however, its applicability is doubtful because the fabrics consisting of in situ precipitated carbonate minerals is different from the modern ones. Therefore, it is necessary to seek and investigate other modern analogs having similar fabrics to ancient stromatolites.

Travertines, carbonate precipitates from hot spring, have common fabrics to the ancient stromatolites in terms of sub-mm order lamination and scarcity of detrital particles. Travertines are inorganically precipitated by mechanical degassing of carbon dioxide from the water enriched with DIC and calcium ion. Previous studies mainly focusing on calcite travertines have indicated that cyanobacterial daily biofilm formation overcoming the inorganic mineral precipitation formed daily lamination (Takashima and Kano, 2008). However, aragonite is another component of travertine and was the primary mineral of some ancient stromatolites (Grotzinger, 1989). Here, I study aragonite travertines to understand the geomicrobiological processes forming laminated textures.

Aragonite travertines consist of radially expanded needle crystals, which is different from calcite fabrics called as dendrite consisting of tree-like aggregation of rhombic crystals. Sequences of sampling through day and night showed the lamination in the aragonite travertines was also formed daily cycle. There are two types of processes forming lamina formation in aragonite travertines; one is cyanobacterial direct role for daily lamination by their daily migration, another is cyanobacterial indirect role by daily production of organic materials (Okumura et al., 2011). It was also observed rapid decomposition of microbes in the travertine, which prevent preservation of microfossils in the sediment. The textural features of the aragonite travertines such as radially expanded needle crystals and scarcity of microfossils are common in some Precambrian stromatolites.

Despite of differences in water chemistry, hydrological setting, and mineralogy, geomicrobiological processes in the daily-laminated travertines show one detailed microbial behavior for interpreting the Precambrian stromatolites. If the relationship between stromatolite texture and microbial processes is clearly confirmed by geomicrobiological studies for modern analogs, it leads new possible interpretation for the stromatolite microbiology and the early biosphere.

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Keywords: stromatolite, travertine, modern analogs, cyanobacteria, lamination

## Millennial-scale effect of microbes on soil organic matter buried by volcanic ash

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Soil is considered to be the major carbon (C) reservoir accounting for about 70% of terrestrial C. Turnover time of C accumulated in soil often exceeds thousands years, thus much concerns have recently been directed to the mechanism of long-term sequestration and preservation of organic matter in soil. Microbes are the major contributor for changing the characteristics of preserved organic matter and influencing its stability. However, because soil typically comprise a mixture of old and young organic residues, it is difficult to analyze long-term effect of microbes on soil organic matter. Buried soil allows us to study the long-term changes in soil organic matter, because supply of young organic matter is limited. Thus, in this study, we analyzed hydrolyzable amino acids (AA) and amino sugars (AS) in soils buried for hundreds to thousands years in order to clarify the long-term effect of microorganisms on soil organic matter. Buried soils were sampled in Tomakomai, Memuro, and Abashiri in Hokkaido Island. Carbon content in buried soil ranged from 1.12 to 8.06%, unrelated to the soil age or the type of volcanic ash, suggesting strong influences other than time and parent material. The positive correlation between yield of AA and AS suggests a strong influence of microorganisms on chemical changes in organic matter in buried soils. Ratio of GlcN/GalN was lower in older soils, suggesting grater progress of diagenesis in soils buried for a longer period. This was supported by the lower yield of AA and AS in older soils. These observations suggest that soil organic matter sequestered for hundreds to thousands years is influenced partly by microorganisms, and is diagenetically altered to more uncharacterizable compounds.

Keywords: Soil organic matter, Carbon sequestration, Buried soil, Microbial contribution, Diagenetic alteration

## Nature of organo-mineral interaction in volcanic-ash soil. Par II. Particle size and aggregate hierarchy

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Soil organic matter (SOM) is the largest carbon pool of terrestrial ecosystem. Stabilization of SOM is largely controlled by interaction of organic matter (OM) and soil minerals. Volcanic-ash soils are characterized by a high SOM content and consist mainly of short-range-order minerals such as allophane, imogolite and ferrihydrite. Allophane/imogolite are unique nanoclays of hollow spherule/tube structures with the diameter of <5 nm and have extensive, variable-charge surfaces. Due to the dominance of these nanoclays, SOM stabilization process in volcanic soils may differ drastically from that in non-volcanic soils consisting of well-crystalline minerals. Particle-size fractionation is an effective approach to distinguish different types of organo-mineral associate. These nanoclays form stable micro-aggregates and are difficult to disperse, which makes the effectiveness of this approach obscure. Here we hypothesized that, with an appropriate dispersion technique, major portions of SOM is stabilized in finer size fractions (<2 micro-m) as sub-micron aggregates of short-range-order minerals with microbially-processed organic matter in volcanic-ash soils. To test this, we chose a typical allophanic Andisol in Japan and characterize each particle-size fraction by selective-dissolution, isotopic (N-15, C-13, C-14), and spectroscopic techniques. Results showed that: (i) total organic carbon and nitrogen were mainly stabilized in finer size fractions (<2 micro-m), (ii) <0.2 micro-m and 0.2-2micro-m sized fractions largely consist of the association between OM and short-range-order minerals, (iii) the decline of C:N ration and enrichment of N-15 towards finer fraction indicate that the OM in finer-sized aggregates appeared to be more strongly altered by soil microbial activity. Based on these results, we attempt to provide a speculative synthesis on the progression in organo-mineral associations and the development of aggregate hierarchy in the volcanic-ash soil.

Keywords: soil organic matter, organo-mineral associate, particle size fraction, allophanic soil, carbon stabilization

## Isotopic study on atmospheric nitrate discharge from forested watersheds

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Clarifying the source of discharged nitrate is important to discuss the influence of increasing nitrogen deposition on forest ecosystem, because in the forested ecosystem where discharged nitrate derived from atmospheric nitrate, increasing atmospheric nitrate deposition is difficult to accumulate into the forest ecosystem and influence on nitrogen saturation might be small. However, increasing atmospheric nitrate may directly increase the amounts of discharged nitrate from such forest ecosystems. On the other hand, in the forested ecosystem where discharged nitrate derives from nitrified nitrate, increase of nitrate deposition should not immediately increase nitrate discharge from watershed because atmospheric nitrate is taken up by nitrogen cycle in such forest ecosystems at least one time. However, atmospheric nitrate should easily accumulate in such forest ecosystem.

In this study, we conducted the observation of several storm events and quantitatively clarify the source of nitrate discharged from forest ecosystems at storm events.

Keywords: isotope, nitrogen, forest, biogeochemistry

## Effects of clear-cutting on nitrate dynamics in a forested watershed using the triple oxygen isotopes as tracers

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Temporal variations in the triple oxygen isotopic compositions of nitrate in stream water were investigated to understand the effect of clear-cutting on trees (January to March, 2003) and subsequent stripcutting of understory dwarf bamboo (*Sasa senanensis*) (October 2003) on biogeochemical processes of forested watershed in northern Japan. In accordance with the significant increase in stream nitrate concentration up to 15 micromols L<sup>-1</sup> in spring 2004, probably in response to the *Sasa*-cutting (Fukuzawa et al., 2006), we found significant increase in the <sup>17</sup>O anomalies. The maximum anomaly (+14 permil) suggest that the direct drainage of atmospheric nitrate occupied more than 50% of total nitrate being exported from the forested watershed during spring 2004. Similar increases in both concentrations and the <sup>17</sup>O anomalies were found in spring 2005 as well. On the other hand, we found little anomalies in the other seasons, irrespective to increases in stream nitrate concentration. That is to say, most portion of the increased nitrate, being exported from the forested watershed during the seasons other than spring, was remineralized nitrate: those preserved in the forested ecosystem as either organic-N or ammonium and then been converted to nitrate. The annual export flux of atmospheric and remineralized nitrate from the forest ecosystem increased to more than 20-times and 4-times, respectively, from those prior to the *Sasa*-cutting. These results suggest that *Sasa* is important not only for preventing nitrogen leaching from soil, but also for enhancing biological consumption of atmospheric nitrate before being exported from forest ecosystem, especially when significant quantities of atmospheric nitrate were added to forest floor through the spring thaw.

Keywords: forest ecosystem, nitrogen leaching, triple oxygen isotopic compositions, nitrate, clear-cutting of trees, *Sasa*

## The response of litter dynamics of Sasa and trees to the long-term atmospheric nitrogen deposition in forest ecosystem

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Nitrogen is fundamental nutrient for plants and microbes in forest ecosystem. However, it has been concerned that increased nitrogen deposition cause a change of the internal cycling between soil and plant system such as enhancement of litter decomposition by increase nitrogen content in the litter and nitrogen leaching from soil to stream water. It has been known that responses of the forest ecosystem to nitrogen deposition vary with an amount of the nitrogen entering into the forest ecosystem, the period and comprised main plant species. In forest ecosystem of northern Hokkaido, Sasa dwarf bamboo on the forest floor is an important component of vegetation, in addition to the overstory tree. It has been reported that Sasa has a role reducing a change of short-term soil nitrogen increment after the forest management due to the nutrient absorption by Sasa. However, the influence the change of long-term soil nitrogen environment on Sasa litterfall and litter decomposition is not understood. This aim of this study is to clarify the influence the long-term nitrogen addition to soil on the litter dynamics of Sasa and tree in the forest ecosystem.

Nitrogen additional experiment was conducted on Nakagawa experimental forest of Hokkaido University in northern Hokkaido. The study area is a cool-temperate climate. Predominant tree species are birch (*Betula ermanii*), acer (*Acer mono*), oak (*Quercus crispula*) and fir (*Abies sachalinensis*). The forest floor is covered with understory vegetation, Sasa (*Sasa senanensis*) having high density and tall structure. Study sites were established in flat ridge of the experimental watershed (1.43 ha) and the adjacent control watershed (1.06 ha), respectively. Nitrogen of 5 g N m<sup>-2</sup> year<sup>-1</sup> (particulate form of NH<sub>4</sub>NO<sub>3</sub>) has been added to the whole watershed at the snowmelt season from 2002. We investigated above-ground standing stock of Sasa and litterfall of Sasa and trees in each watershed. The litter decomposition experiment of Sasa and trees using litter samples collected from both watersheds conducted on the control watershed to avoid the directly effect of the nitrogen addition. Stream water was collected in the outlet of each watershed to analyze the nitrogen export from each watershed.

The nitrogen amount of above-ground of Sasa and litterfall of trees were significantly higher in treatment site than in control site. The nitrate concentration in stream water was not significantly different between the watersheds before and 1-year after the first nitrogen addition, but tended to increase in the treatment watershed from three years after the initial addition. These results suggested that the nitrogen absorption of Sasa and trees and the nitrogen leaching from soil to stream increased by long-term nitrogen addition. The litter decomposition rate of tree leaf was faster in treatment watershed than that in the control watershed. The nitrogen content in initial litter increased in tree leaf, suggesting that the increased nitrogen content in initial litter influenced on enhancement of litter decomposition of tree leaf. On the other hand, those of Sasa leaf and culm litter were not significantly different between the watersheds. These results suggested that Sasa litter have some degree of the resilience capacity to maintain the nitrogen dynamics of the soil-vegetation system through the stable nitrogen concentration of litter against the atmospheric nitrogen deposition.

Keywords: Biogeochemistry, Understory vegetation, Nitrogen cycling, Litterfall, Litter decomposition, Litter-bag method

## Atmospheric mercury accumulation by carbonized trunk

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In March 1945, Tokyo was bombed by B29, and most of all plants of Tokyo was fired. At that time, *Ginkgo biloba* was also fired. The fired trunk *G.biloba* was carbonized.

Mercury that in the bark evaporates to the atmosphere when it is fired, and we expect mercury(Hg<sup>0</sup>)contains in the carbonized *G.biloba* trunk because biochar has ability of get the environmental pollutants.

In this study, We collected the carbonized tree trunk and contain of mercury in the carbonized tree trunk was analyzed by thermal decomposition amalgamation.

Keywords: Mercury, Carbonized trunk, Mercury accumulation



## Effect of winter climate on nitrogen mineralization in forest soil, evaluation from nitrogen mineralization ratio

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A nitrogen mineralization ratio which is nitrogen mineralization rate to total nitrogen content were 0.4 - 11.9% in Japanese forest soil under field condition, and there were 0.9 - 5.8 in cool temperate forest. These values show 3.0-6.5% in the result of newly investigation in this forest. Values of nitrogen mineralization rate estimated from multiple regression model were higher than those of measurement values in the field condition. We consider winter climate affect nitrogen mineralization in the soil, and discussed nitrogen mineralization rate in winter season and seasonal changes of nitrification and ammonification rate. Nitrogen mineralization rate in surface soil was measured with resin core method in Japanese cedar and deciduous broadleaf stand at Katsura experimental site (KT), Ibaraki and in Japanese cypress forest at Terasawa-yama forest, Shinshu University (TR), Nagano. Soil type was dry to slightly wetted brown forest volcanic ash mixture in KT and moderately moist brown forest soil derived granite in TR.

Annual nitrogen mineralization rate were 182-367mgN kg<sup>-1</sup>at KT, and 167-264 mgN kg<sup>-1</sup> at TR. It was high in summer and low in winter with temperature fluctuation. Seasonal changes was not clear at TR. Nitrification was dominated and decreased in spring in the soil located lower to mid slope position distributed Japanese cedar and cypress at both of site. On the contrary, Ammonification was dominated in upper slope position of deciduous forest at KT. Higher nitrogen mineralization ratio was indicated at KT. These values were 3.0-5.0% at KT and 5.7-6.5% at TR.

As mentioned before, nitrogen mineralization ratio was higher in this study site than those of other site in cool temperate forest. Nitrogen mineralization rate in winter was not differing from another season. Snow depth was small but daily average temperature was below 0°C in winter at both site in this research. It was considered that repeated soil freezing and melting introduce the changes of organic matter quality in soil and easily mineralizable properties was highly increased compared heavy snow site. We considered these processes will affect nitrogen mineralization in these sites.

Keywords: Field incubation, nitrogen mineralization ratio, winter

## Geochemical studies on nitrate in a basin with tropical glaciers in Bolivia, using $^{15}\text{N}$ , $^{17}\text{O}$ and $^{18}\text{O}$

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Water resources in La Paz, the capital of Bolivia, are highly dependent on runoff from its surrounding glaciers, since the city locates in a semi-arid area in the Altiplano with an annual precipitation of about 500 mm/y. This study aims at determination of nitrate cycles using its stable isotopic compositions in Tuni Lake and its basin, which is one of the important water resources of La Paz.

Samples were collected from downstream rivers of the glaciers four times (September, 2010 and March, September, November 2011). The sampling points were located from an edge of the glacier to the inflow point to the lake. Samples were filtered through a 0.2 micro meter pore-size membrane filter and stored in a refrigerator until analysis. To determine the stable isotopic compositions of nitrate, the sample nitrate was chemically converted to nitrous oxide using a method originally developed for  $^{15}\text{N}/^{14}\text{N}$  and  $^{18}\text{O}/^{16}\text{O}$  isotope ratios of seawater and freshwater nitrate (McIlvin and Altabet, 2005) with slight modification (Tsunogai et al. 2008). The stable isotopic compositions of nitrous oxide were determined using our Continuous-Flow Isotope Ratio Mass Spectrometry system (Komatsu et al. 2008).

Nitrate in glacial ice had smaller triple oxygen anomalies (+6 permil at Huayna Potosi Glacier and +11 permil at Condoriri Glacier) relative to those in deposited nitrate (+25 permil), which corresponds to a mixing ratio of atmospheric nitrate to total nitrate of 45%, indicating the significance of non-atmospheric origin of nitrate (e.c. microbial oxidation of ammonium) within the glacier. The triple oxygen anomalies of nitrate in downstream river showed systematic variation between two basins ranging from +1.3 to +7.3 permil in Huayna Potosi basin and from +0.4 to +5.5 permil in Condoriri basin, respectively. The variation may be attributable to the difference in biological activity between the basins.

Keywords: nitrogen cycle, triple oxygen isotopic compositions, nitrogen isotopic composition, nitrate, glacial melt water, Bolivia

## EM Sounding Characterization of Soil Environment toward Estimation of Potential Non-point Pollution Sources

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Land management would affect water environment through infiltration of rainwater/irrigation water or surface water runoff at heavy rainfall event. Soil survey is, however, laborious and take long time for wide area research. In this research, we employed electro-magnetic (EM) sounding to conduct soil survey effectively and estimate potential environmental load, which would be discharged to water environment. A multi-frequency EM sounding was performed to measure electrical conductivity (EC) distribution at the soil surface. Soil sampling at 10,30 and 50cm depth, and stream water sampling was also conducted.

As results, EM sounding successfully measured land surface EC distribution, showing most of their properties was represented by land surface EC. EC showed the amount of soluble chemicals at the land, which was the result of fertilizer application, poor infiltration, and so on. Thus high EC was estimated as potential environmental load for water environment. EC decreased when the interval became longer at the combination use of paddy and upland field. EC was higher at non-thinning operation forest than that of well-managed forest. The forest floor had lower infiltration than well-managed one, moreover, floor weed was poor at those site. Thus forest with non-thinning operation would affect water environment through surface runoff at the storm event. When ion concentrations at stream water were compared with land EC, Na and Cl were inversely correlated with land EC. Because those two ions were affected by rainwater at Japan sea coast area, this means well-infiltration for low EC site and poor- infiltration for high EC site. In general through EM sounding survey, surface land EC distribution, land conductivity and ion concentration at stream water successfully connected each other. It would be beneficial when this technique is used for watershed management.

Keywords: Electromagnetic sounding, Soil Environment, Non-point sources

## Monitoring of atmospheric mercury pollution using carbonized tree and Chinese ink

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We report the results on the Accumulation of mercury by carbonized tree trunk and old paper written by Chinese ink.

The mercury accumulated on the trunk of tree disappears during forest fire because of high temperature more than

300C. After forest fire, the carbonized tree trunk free from mercury starts to accumulate atmospheric mercury.

The content of mercury accumulated on the carbonized tree trunk seems to be reflect atmospheric mercury pollution

at the site after the forest fire. The old paper written by Chinese ink also accumulates atmospheric mercury. Especially in the case of DAIHUKUCHO which has records of the used year, the mercury content of the cover page seems to be reflect the atmospheric mercury pollution of the recorded year at the site.

Keywords: carbonization, Chinese ink, mercury, monitoring, air pollution, forest fire

## Topography controls stream NO<sub>3</sub><sup>-</sup> concentration and sediment denitrification at headwater streams

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[Aim] The effects of topography and distribution of electron donors (DOC, and reduced sulfur) for denitrification on stream NO<sub>3</sub><sup>-</sup> concentration and sediment denitrification were examined at 34 headwater streams in the Lake Hachiro watershed. [Materials and Methods] Study catchments are 34 head streams (0.07-16.9 km<sup>2</sup>) in the lake Hachiro watershed at Akita, Japan. Stream water at each catchment was sampled four times a year from 2010 to 2011. Water discharge (L/s) was estimated from width, depth, and velocity measured at 8-10 points along a cross-sectional transect of the stream. At each catchment, sediment samples for denitrification assays were collected once in July, 2011 from the top 5 cm of the streambed across the width of the stream. Dissolved N<sub>2</sub>O in stream water was measured in December, 2011. Water and sediment samples were placed on ice, transported back to the laboratory, and refrigerated overnight until denitrification assays and water analysis were begun. The denitrification assays of the sediments were determined using the acetylene inhibition technique, which inhibits the final step in the conversion of N<sub>2</sub>O gas into N<sub>2</sub> gas. To determine the difference among catchments in the amount of organic C available to the denitrifying organisms, we defined denitrification potential (DP) as the denitrification rate that occurred under anaerobic conditions with abundant NO<sub>3</sub><sup>-</sup> at 25 deg C. Samples of fresh, homogenized soil (15 g) were placed into 150-mL glass bottles. A 50-mL aliquot of solution containing nitrate (5 mg-N L<sup>-1</sup> as KNO<sub>3</sub>) with chloramphenicol was added to the bottles. The bottles were purged with O<sub>2</sub>-free ultrapure N<sub>2</sub> for 3 min to ensure anaerobic conditions, and acetylene (C<sub>2</sub>H<sub>2</sub>) gas was added to a final concentration of 10% v/v (10 kPa) in the headspace. We extracted the headspace gas with a gas-tight syringe, and calculated denitrification rates from the linear portion of the curve for N<sub>2</sub>O production as a function of time. The stream water sample was filtered through 0.45-µm membrane filters and concentrations of dissolved components were determined. The fresh sediment samples were extracted in distilled water (soil:water, 1:5 w/v), and the concentrations of water-extractable soil organic carbon (WSOC) was determined. Easily oxidizable-S (EOS) content in the sediments, which can be an electron donor for denitrification as reduced sulfur, was measured by the difference between H<sub>2</sub>O<sub>2</sub>-S and H<sub>2</sub>O-S content. Topographic index (wetness index) in each catchment was calculated using 10 m grid digital elevation model (DEM) by GIS. [Result and Discussion] Stream NO<sub>3</sub><sup>-</sup> concentrations among catchments had a large spatial variability ranging from 0.06 to 0.52 mg N L<sup>-1</sup>. Stream NO<sub>3</sub><sup>-</sup> concentrations were negatively correlated with topographic index significantly ( $r=0.56$ ,  $p<0.01$ ), indicated NO<sub>3</sub><sup>-</sup> was reduced in a catchment which had a gentle slope area. Dissolved N<sub>2</sub>O in stream water was positively correlated with topographic index, which supported denitrification in such a catchment. A significant positive correlation was also observed between sediment DPs and topographic index. The WSOC content in the sediment, the primary predictor of denitrification rates, increased with topographic index and affected sediment DPs. NO<sub>3</sub><sup>-</sup> concentrations also had a negative correlation with EOS content in the sediments indicating sulfur denitrification in catchments, however, the distribution of EOS did not related topography. Multiple regression analysis showed topography and EOS content in the sediments affected concentration of stream NO<sub>3</sub><sup>-</sup> significantly.

Keywords: nitrate, headwater stream, denitrification, topography

## Comparison of the water quality of rivers between the river influenced by the polluted air from urban area and the river

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The source of Arakawa river in Saitama prefecture and the source of Fujigawa river in Yamanashi is common. It is high mountain district located in Western part of Kanto district. However, there is a big difference of water quality between the both rivers. Especially nitrate concentration in Fujigawa river is lower than that of Arakawa river.

This difference suggested the shielding effect of high mountain district on the transportation of polluted air containing nitrate and the other pollutants from the Tokyo area.

## Relationships between biomass of *Sasa* dwarf bamboo (*Sasa senanensis*) and multiple ecosystem functions in forest soil

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There is a growing recognition that understanding the relationships among multiple ecosystem services is crucial in order to avoid unintended tradeoffs in ecosystem management. Understanding the functions and processes behind interactions among multiple services is therefore important. Understory vegetation plays an important role in ecosystem processes in forest soil. However, it has not been paid much attention in previous ecological works. *Sasa* dwarf bamboo is a common understory plants in Japanese forests. They usually form dense undergrowth and dominate forest floor. In Hokkaido, Northern Japan, *Sasa* is a major component of forest ecosystem which occupies some 90% of forest area. Understanding the contribution of *Sasa* to ecosystem functions in forest soil is essential to help establish a future ecological management. Our objective is to understand the multiple functions in forest surface soil contributed by *Sasa* and the mechanism behind the interactions of among these functions. We hypothesized that the ecosystem functions in surface soil are correlated with the aboveground biomass of *Sasa*. The ecosystem functions examined in this study were carbon sequestration, soil fertility and buffering of soil acidity in surface soil of forest.

We established experimental sites in Uryu Experimental Forest of Hokkaido University, northern Hokkaido, Japan. The vegetation of the site is a cool-temperate mixed forest with dense *Sasa* understory. According to the biomass of *Sasa*, three plots with five sampling spots (1m<sup>2</sup>) were established as Large (L), medium (M) and small biomass (S) plots. The L plot was located in a gap area, whereas the S plot was located under a dense canopy of coniferous stand. The distance between the three plots is within 10m. The mean *Sasa* biomass of L, M and S plot were 1.61, 0.46 and 0 kgDW m<sup>-2</sup>, respectively. Parameters describing each ecosystem functions were measured from samples of litter layer and mineral soil at the depth of 0-10cm (Upper soil) and of 10-20cm (Lower soil) from the plots.

With carbon sequestration parameters, the sum of litter dry mass and the dry root mass was higher in S plot than other plots. Soil microbial respiration rate was higher in L plot than in S plot. In regards to soil fertility, the concentration of inorganic nitrogen in upper soil was higher in L and M plots than S plot. Nitrogen mineralization rate was highest in M plot in Upper soil. With buffering of soil acidity, although there was no significant difference in the soil pH, the ratio of exchangeable (K+Ca+Mg)/exchangeable-Al increased as the *Sasa* biomass increased. The soil and litter in L plot contained higher Ca than M and S plots, while the soil and litter in S plot was high in Al than other two plots. This means that the soil acidity decreased as *Sasa* biomass increased. High Ca concentration in soil in L plot probably contributed reducing soil acidity in the plot. Ca in soil might be supplied from *Sasa* litter as litter in L plot had high Ca concentration. The reason why soil acidity was higher in S plot could be explained by supply of organic acid from the coniferous leaves. One possible relationship among multiple ecosystem functions was found between soil fertility and acid buffering functions. The increase of soil acidity due to the small biomass of *Sasa* (with higher density of coniferous trees) reduced soil microbial activity which led less fertility. Further analysis will be carried out and discussion will be included in the presentation.

Keywords: biogeochemistry, forest ecosystem, understory vegetation

## Microbial processes in the biogeochemical dynamics of amino acids in marine sediments

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Sedimentary organic matter (OM) is the major reservoir of carbon and nitrogen in the global biogeochemical cycle. For example, burial of organic matter in sediments plays a role in controlling atmospheric CO<sub>2</sub> and O<sub>2</sub> levels on long time scales. There remains, however, much debate about the controlling factors of OM preservation and the processes of OM degradation in marine sediments. Amino acids are major nitrogenous components in OM and also the important intermediate during the OM degradation by microbes. Composition, D/L ratio, and compound-specific isotopic composition have been used as the indicators of amino-acids biogeochemistry in marine sediments.

In this presentation, we report our results of composition, D/L ratio, and nitrogen isotopic composition (d<sup>15</sup>N) of amino acids in the surface sediments collected at off-Akita, Japan Sea (KT10-06) and the deep-subsurface sediments collected at off-Boso, Northwestern Pacific (CK09-03). We observed difference of d<sup>15</sup>N and/or D/L ratio between total hydrolysable amino acids (THAA) and dissolved/extractable amino acids. Based on the results of culture experiments of microbes for amino-acids d<sup>15</sup>N and simple mass-balance calculation, we estimated the contribution to the dissolved/extractable amino acids from the microbial biomass in the sediments and that from the THAA. For example, in the surface sediments in Japan Sea, we observed 3-5 permill enrichment in the d<sup>15</sup>N of free glutamic acids (Glu) from the THAA-Glu d<sup>15</sup>N. Based on the <sup>15</sup>N-enrichment factor (8 permill) of Glu in the cultured microbes, we estimated that the release from microbial biomass would contribute to 30-40% in the free Glu. These results suggests that recycle of amino acids by microbes would be significant during the degradation of amino acids.

Keywords: Organic matter, Nitrogen cycle, Nitrogen isotope, Marine sediments, Amino acids, Deep biosphere



## Analysis of production and emission processes of nitrous oxide at the beginning of flood irrigation by isotopomer ratios

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### 1. Introduction

Agricultural soil is the largest anthropogenic source of nitrous oxide (N<sub>2</sub>O) which contributes to global warming and ozone depletion. N<sub>2</sub>O is produced by microbial processes of nitrification as a byproduct of hydroxylamine oxidation, and denitrification as an intermediate product of nitrite reduction and is further reduced to N<sub>2</sub>. In rice paddy soils, N<sub>2</sub>O emissions were observed in association with water supplies and drainage practices (for midseason aeration or for rice harvest). N<sub>2</sub>O emitted at the beginning of flood irrigation is considered to be derived from denitrification, but production and consumption processes of N<sub>2</sub>O in the soil or transport process to soil surfaces are not fully understood.

The purpose of this study is to clarify both contribution of denitrification for N<sub>2</sub>O emissions and transport process of N<sub>2</sub>O in the soil at the beginning of flood irrigation by analyzing isotopomer ratios. It is suggested that isotopomer ratios of N<sub>2</sub>O (bulk nitrogen and oxygen isotope ratios,  $d^{15}N^{bulk}$  and  $d^{18}O$ , and intramolecular <sup>15</sup>N site preference, SP) are useful parameters that provide information about microbial metabolisms described above. We tried to figure out temporal changes of production and consumption processes of N<sub>2</sub>O or its transport process affected by a water supply using spatial variability of soil moisture content.

### 2. Materials and methods

N<sub>2</sub>O fluxes were measured by closed chamber method 7 days after the beginning of a water supply on Apr. 20, 2011 at 8 plots of paddy soils in Tsukubamirai, Ibaraki Prefecture, Japan. The soil type was a Gray lowland soil. Isotopomer ratios of soil-emitted N<sub>2</sub>O were calculated from those of N<sub>2</sub>O in chamber air and ambient air assuming mixing of soil-emitted gases and ambient air in chambers. To measure concentrations and analyze isotopomer ratios of N<sub>2</sub>O in the soil, soil gases at 10 cm depth were collected by soil gas samplers with silicon tubes at 6 plots. N<sub>2</sub>O flux was monitored every 2 h with an automated chamber system at a plot. Soil samples from 0-10 cm depth at each plot were collected and used for extraction with 10% KCl solution for analysis of the soil nutrient content. The water filled pore space (WFPS) was calculated from the volumetric water content and porosity.

### 3. Results and discussion

High N<sub>2</sub>O emissions (80-217 mcgN/m<sup>2</sup>/h) were observed at plots where groundwater level was 1 cm depth. On the other hand, low N<sub>2</sub>O emissions (6-60 mcgN/m<sup>2</sup>/h) and high N<sub>2</sub>O concentrations of soil gases (134-160ppm) were observed at plots where water depth were 1-5 cm. Isotopomer ratios of N<sub>2</sub>O emitted from soil surfaces were close to the values of N<sub>2</sub>O in the soil, although they indicated relatively higher values than those of N<sub>2</sub>O in the soil at a plot where water depth was 5 cm. Isotopomer ratios of N<sub>2</sub>O at plots where groundwater level was 1 cm depth showed values near the range of the reported values of N<sub>2</sub>O which produced by denitrification in pure culture. On the other hand, isotopomer ratios of N<sub>2</sub>O at water depth were 1-5 cm were relatively higher than those of N<sub>2</sub>O at plots where groundwater level was 1 cm depth. It was thought to be due to consumption of a part of N<sub>2</sub>O by denitrification which is associated with elevations of isotopomer ratios of residual N<sub>2</sub>O by isotopic fractionation. N<sub>2</sub>O concentrations and isotopomer ratios in the soil were near the values of N<sub>2</sub>O in ambient air at the plots where WFPS were low.

Therefore, it was considered that N<sub>2</sub>O was produced by denitrification at shallow depth with the increase of WFPS, and emitted to air by the effects of both upward advection by increasing ground water levels and molecular diffusion. N<sub>2</sub>O emissions became low and N<sub>2</sub>O accumulated in the soil when soil surfaces were covered with water. It was assumed to be due to low diffusion coefficient of dissolved N<sub>2</sub>O. In addition, it was thought that a part of N<sub>2</sub>O was consumed by reduction to N<sub>2</sub> under anaerobic conditions and residual N<sub>2</sub>O accumulated in the soil.

Keywords: Nitrous oxide, Denitrification, Isotopomer

## Stable isotopes of nitrate in natural mosses

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Natural mosses have been employed as reactive and accumulative indicators of atmospheric pollutants. Using the denitrifier method, the concentration, nitrogen and oxygen isotopes of moss nitrate were measured to elucidate the sources of nitrate trapped in natural mosses. Oven drying, not lyophilization, was recommended to dry mosses for moss nitrate analysis. Preliminary investigation suggested that moss nitrate can respond to nitrate availability in different habitats. Nitrate in terricolous mosses showed isotopic ratios as close to those of soil nitrate, reflecting the utilization of soil nitrate. Isotopic signatures of nitrate in corticolous and epilithic mosses could elucidate atmospheric nitrate sources and strength of anthropogenic NO<sub>x</sub> emission at urban-rural scales. However, mechanisms and isotopic effects of moss nitrate utilization must be further verified to enable the application of moss nitrate isotopes for source identification and partitioning.

Keywords: Nitrogen deposition, moss, nitrate, biomonitoring, stable isotopes, nitrate reductase

## Characterizing the dynamics of dissolved organic matter in fjord systems, New Zealand

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Dissolved organic matter (DOM) comprises the largest pool of organic matter in a wide range of aquatic environments. In oceanic environments, major fractions of DOM have been considered to be marine origin even though rivers convey substantial amounts of terrestrial organic matter to the coastal ocean (0.4 PgC yr<sup>-1</sup>). Thus, there might be removal mechanisms of terrestrial DOM in coastal environments. However, dynamics, i.e., sources and sinks, of DOM in coastal environments have not been well understood. The characterization of the DOM dynamics in coastal environments is challenging due to intricate structure/composition of terrestrial and marine DOM. Therefore, we need a better technique for evaluating the terrestrial and marine DOM separately. Fluorescence technique can successfully separate the terrestrial DOM from marine one, and thus, has been widely used in coastal environments. Here, we applied excitation emission matrix (EEM) fluorescence combined with parallel factor analysis (PARAFAC) for characterizing the dynamics of DOM in fjord systems, New Zealand. In addition, we compared obtained fluorescence results with stable carbon isotope values of DOC.

In June 2007 (Austral winter), water samples were collected from surface to bottom layer at fjord systems in Fjordland National Park, New Zealand. Water samples were immediately filtered through pre-combusted GF/F filters and were kept on ice, returned to the laboratory, and stored at 4 °C until analysis. EEM spectra were obtained using a Horiba Jovin Yvon SPEX Fluoromax-3 fluorometer. PARAFAC, which can statistically identify fluorescent components in EEMs, were applied for 53 water samples obtained from fjord systems. As a result, two terrestrial humic-like, one marine humic-like, and one protein-like components were obtained. Isotopic analysis ( $\delta^{13}\text{C}$ ) of dissolved organic carbon (DOC) were performed on a modified OI Analytical model 1010 wet oxidation TOC analyzer interfaced with a Finnegan MAT Delta Plus IRMS with a CONFLO III continuous flow interface.

Relationships between salinity and fluorescence intensity differed among the four components. Levels of humic-like components were higher in low salinity waters and vice versa and showed negative relationships with salinity, indicating that major sources of humic-like components are riverine water in this system. Levels of protein-like component did not show clear relationship with salinity, suggesting that substantial contribution of autochthonous protein-like component in addition to riverine input.

On the vertical axis, fluorescence intensity of all components showed highest levels in the surface water, sharply decreasing with increasing depth. However, in the deep layer (depths greater 50 m), increases in fluorescence intensity with depth were observed for humic-like components, but not for protein-like component. The increase in fluorescence intensity with depth in deep layer was highest at the stations with the lowest water exchange rates, while it was almost absent at the sites most strongly influenced by tides and flushing with oceanic water. Interestingly,  $\delta^{13}\text{C}$  of DOC in deep layers were lower than those in subsurface layers. These results strongly suggest that DOM is physically desorbed and/or generated through microbial oxidation from terrestrial POM during its sinking processes.

Keywords: dissolved organic matter, fluorescence, stable carbon isotope, fjord

## Real time, continuous measurements of CO<sub>2</sub> and H<sub>2</sub>O isotopes in a forest using laser absorption spectrometers

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Measurements of CO<sub>2</sub> and H<sub>2</sub>O isotope compositions are very powerful methods for investigating the carbon and water cycles. We had been deployed two infrared absorption laser spectrometers (Aerodyne Inc. for CO<sub>2</sub> and Los Gatos Research Inc. for H<sub>2</sub>O) in the red-pine forest at the foot of Mt. Fuji for 10 days from the end of July, 2010 and had successfully measured CO<sub>2</sub> and H<sub>2</sub>O isotopologues (<sup>16</sup>O<sup>12</sup>C<sup>16</sup>O, <sup>16</sup>O<sup>13</sup>C<sup>16</sup>O and <sup>18</sup>O<sup>12</sup>C<sup>16</sup>O for CO<sub>2</sub>, D<sub>2</sub>O and H<sub>2</sub><sup>18</sup>O for H<sub>2</sub>O). The CO<sub>2</sub> isotope laser spectrometer can measure the isotope ratios (δ<sub>13</sub>C, δ<sub>18</sub>O) of ambient air CO<sub>2</sub> in 10-second integration time with a precision of 0.1 permil in real-time. The height of the observation tower is 30 m. Air was sampled every 260 seconds from six vertical height locations from above the forest canopy to 2 m above the ground. The total interval time was 30 minutes including measurements of standard gases for the calibration. The 30-minutes interval measurements of the CO<sub>2</sub> and H<sub>2</sub>O isotope ratios were repeated continuously during the 10 days. We did Keeling plot analysis regarding with δ<sub>13</sub>C every a few hours and found that the keeling plot intercepts showed clearly a diurnal pattern. We will discuss the details of the results at the meeting.

Keywords: CO<sub>2</sub> isotopes, H<sub>2</sub>O isotopes, forest, laser spectroscopy, ecosystem, atmospheric CO<sub>2</sub>

## The distribution and circulation of Ca in ecosystem of the acidified drainage basin in Guangdong province, south China

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This study was undertaken to determine the elemental and strontium isotopic compositions ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) in rainwater, stream water, plant, soil, and bedrock of the acidified drainage basin (Dinghushan: DHS) and the slightly acidified drainage basins (Heishiding: HSH and Conghua: CH) in Guangdong province, south China and to clarify the contribution of atmospheric deposition to Ca and Sr circulation in the acidified ecosystem. In order to assess the distribution of the plant-available elements in soils, a sequential extraction procedure that extract the water-soluble, exchangeable, bound to carbonates, bound to Mn oxide, organic, bound to amorphous Fe oxide, bound to crystalline Fe oxide and residual silicates components from soil was performed.

Strontium and Ca in the plant-soil ecosystem are mostly derived from atmospheric deposition in DHS, HSD and CH basin. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of dominant tree leaves were identical to those of the exchangeable fraction in soils. Their ratios were lower than those of bedrock and bulk soil and were close to those of rainwater. The contribution of the atmospheric deposition to plant and the exchangeable fraction of soil in DHS are up to 90%, because sandstone, shale and granite that have low Ca and Sr concentrations are composed of the bedrock in DHS, HDS and CH, respectively.

The lack of plant-available Ca, Mg, K, and P and the leaching of Cu, Al, Fe and Zn from Fe-oxide to mobile fraction occur in the DHS acidified soil. The pH values of soils showed that DHS soil is more acidified than HSD soil. In acidified soil from DHS, the percentages of the water-soluble and exchangeable Ca, Mg, and K decreased. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of bulk soil were same to that of the weathered sandstone in DHS, indicating that almost of Sr and Ca in the DHS acidified soil were distributed into silicate. On the other hand, the percentages of Cu, Al, Fe, Zn and P bound to oxide decreased and those of the water-soluble and exchangeable Cu, Al, Fe and Zn and P bound to organic matter increased in DHS.

The chemical compositions of stream water in DHS and HSD are affected by bedrock such as sandstone, shale and granite involving low Ca contents. Especially, the acidified drainage basin has an influence on the chemical compositions of stream water in DHS. The pH values of stream water were 3.86-4.58 and 6.5 in DHS and HSD, respectively. Calcium concentrations of stream water were less than 3 ppm in both sites. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of stream water were lower than those of exchangeable fraction in soils, and were close to those of rainwater. These results indicate that most Ca and Sr in stream water are originated from atmospheric depositions. In DHS, Al and Zn concentration were high in stream water. These elements are derived from high contents of exchangeable fractions in acidified soil.

## Effects of thinning on delta-<sup>15</sup>N in leaves of hinoki cypress and understory vegetation

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Light, water and nutrient availability for remaining trees increases after thinning in hinoki cypress plantation. Soil nitrification often increases after thinning. Abundance of understory vegetation also increases. These changes may affect nitrogen use strategy of hinoki cypress and understory vegetation but there is no information about hinoki cypress plantation in Japan. If nitrification increases after thinning, the nitrate should have lower delta<sup>15</sup>N while remaining ammonium should have higher delta<sup>15</sup>N. The preferential uptake of nitrate and ammonium should affect delta<sup>15</sup>N of plants.

In this study, we investigated changes of delta<sup>15</sup>N in hinoki cypress plantations at two different altitude (Tengu 1150m, Furumiya 710m). Two adjacent plots (20m x 20m) were located. One plot was thinned in 2002 and the other was remained as a control. Organic layer and surface soil at 5cm depth were samples in 2002. Leaves of hinoki cypress and understory vegetation (*Lindera triloba* in Tengu and *Lindera sericea* in Furumiya) were collected in July from 2002-2007. Nitrogen concentration of these samples were measured by NC analyze while delta<sup>15</sup>N of them were measured by IRMS.

The results suggest that leaf delta<sup>15</sup>N in understory vegetation is more affected by thinning than hinoki cypress. Higher delta<sup>15</sup>N in leaves suggests preferential uptake of nitrate by understory vegetation in thinned forest in Tengu or reliance of nitrogen sources in the upper layer of soil, i.e. organic horizon by hinoki cypress in Furumiya. These findings indicate that measurement of delta<sup>15</sup>N in leaves provide valuable insights about the nitrogen sources for plants.

Keywords: hinoki cypress, understory vegetation, thinning, <sup>15</sup>N

## Nature of organo-mineral interaction in volcanic-ash soil. Par I. density and surface property of particles

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Organic carbon (OC) in soil is mainly present as aggregates of organic matter with soil mineral particles. Volcanic-ash soil is characterized by allophane/imonogolite, the smallest soil minerals on earth, and by unusually high organic carbon (OC) concentration compared to non-volcanic soils. Based largely on correlation studies, the high OC of volcanic-ash soil is generally explained by protective effect of the inorganic constituents unique to this soil (e.g., short-range-ordered minerals and dissolved aluminum) via sorption of OC on mineral surfaces and organo-metal complexation. Yet little direct evidence is available on the mechanism of OC stabilization or the nature of organo-mineral interactions in volcanic-ash soil. Fractionation of soil according to particle density is an effective approach to distinguish the OM of different degrees of mineral associations and to elucidate SOM stabilization processes. Here we examined a surface (Ap) horizon of an allophanic Andisol in central Japan by isolating six density fractions (from  $<1.6$  g/cc to  $>2.5$  g/cc). We previously reported the progressive increase in delta C-13, N-15, and radiocarbon age from low to higher density. At this presentation, we focus on physical and mineralogical characteristics of the organo-mineral aggregates. We use N<sub>2</sub> gas sorption approach to assess the extent of organic coverage on mineral surfaces in addition to the measurements of the specific surface area. Using selective dissolution techniques (acid oxalate and pyrophosphate), we quantify the abundance of short-range-order minerals and to estimate the amount of organic matter sorptively stabilized by these minerals in each fraction. Together with SEM/EDS characterization of each fraction, we attempt to summarize the progression of organo-mineral interaction from fresh plant detritus to stable organo-mineral aggregates.

Keywords: soil organic matter, carbon sequestration, nano particle, volcanic ash, specific surface area

## Testing the use of $^{15}\text{N}$ natural abundance acorn as an indicator of nitrogen saturation of forests

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Testing the use of  $^{15}\text{N}$  natural abundance acorn as an indicator of nitrogen saturation of forests

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The natural abundance of stable N isotopes ( $\delta^{15}\text{N}$ ) has been used for interpreting N cycles in forest ecosystems because isotopic fractionations during microbial transformations such as mineralization, nitrification, and denitrification can leave different  $\delta^{15}\text{N}$  for each N pool (Nadelhoffer and Fry 1994). It has been conceived that the  $\delta^{15}\text{N}$  of plants can reflect the isotopic signatures of soil (Hogberg et al. 1997), thus can be used as an indicator of N dynamics, especially N availability and progress of N saturation (Pardo et al. 2006, Craine et al. 2009). Foliar  $\delta^{15}\text{N}$  values have been used as the representative for plant body. However, ideal foliar sampling is sometimes difficult due to the large canopy and possible heterogeneity of leaf chemistry in a canopy. Compared with leaves,  $\delta^{15}\text{N}$  of acorns would have some advantages as the  $\delta^{15}\text{N}$  of plant body; the strategy of the acorn production would be different from the one for leaves, which can allow us to get more insights into N economy of the plants including retranslocation of N, and less decomposability of acorns that can allow us to use the dropped acorns as the samples, making the sampling efforts much easier. However, the information on  $\delta^{15}\text{N}$  of acorns together with other  $\delta^{15}\text{N}$  of leaves and soil N pools is totally limited. Thus, we selected several watersheds with different N status and collected acorns together with soils and plant leaves to see if acorn  $\delta^{15}\text{N}$  can be similar with that of leaves to be used as an indicator of N status.

Sampling was carried out in Kamigamo Experimental forest (Kyoto Univ) in Kyoto, several forests in Fukushima prefecture, FM Tamakuryo (TUAT) in Hachioji, Tokyo. We collected soils, leaves and acorns. We measured  $\delta^{15}\text{N}$  of ammonium ( $\text{NH}_4^+\text{-N}$ ), nitrate ( $\text{NO}_3^-\text{-N}$ ), acorn, leaves, and the bulk soil. The  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  was measured using the denitrifier method. The  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  was measured using ammonia diffusion, followed by persulfate oxidation of recovered  $\text{NH}_4^+$  onto a glass fiber filter and the denitrifier method.

We tentatively consider the concentration of dissolved inorganic nitrogen (DIN:  $\text{NH}_4^+$  +  $\text{NO}_3^-$ ) in the soil as an indicator of the nitrogen saturation. We thought progress status of nitrogen saturation as below, Kyoto, Fukushima, and Hachioji. Between Hachioji and Fukushima, we found the decreasing in  $\delta^{15}\text{N}$  of acorns, which is the same trend observed for leaves (Takebayashi et al. 2010). This results suggest that  $\delta^{15}\text{N}$  of acorns can have a possibility to be used as an indicator of nitrogen saturation. However, the variation in  $\delta^{15}\text{N}$  of acorns in a forest was quite large compared with that of leaves. This large variation is difficult to interpret but we suspect that this large variation possibly due to the strong N retranslocation in acorn production and will discuss the potential of the use of this variation in  $\delta^{15}\text{N}$  in the presentation.

Keywords: Nitrogen, Forest, Soil, Plant, Acorn