

Origin of nitrous oxide dissolved in the ocean deduced from concentration and isotope ratios

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Nitrous oxide (N₂O) is a trace gas that contributes to both global warming and stratospheric ozone depletion. Its major sources are nitrification and denitrification by microorganisms in soils and waters. Emission of N₂O from the ocean to the atmosphere is estimated to take account for 20% of global N₂O sources. Based on the distribution of N₂O dissolved in the surface seawater, most of the emission is considered to occur near the eutrophic, suboxic water mass like eastern tropical Pacific, where N₂O is produced by denitrification. However, N₂O concentration shows its maximum in middle layer (600-1000 m) in many sea areas, and the origin of the concentration maximum is not clear. In addition, N₂O is also supersaturated in deep water and its concentration has been found to be different among sea areas. In this study, we focus attention on isotope ratios of N₂O (oxygen isotope ratio, delta-¹⁸O, difference in abundance of isotopomers ¹⁴N¹⁵N¹⁶O and ¹⁵N¹⁴N¹⁶O, Site Preference (SP), and average nitrogen isotope ratio, delta-¹⁵N) as informative parameters reflecting microbiological metabolisms, and made an analysis of origin of N₂O in the middle and deep ocean by the use of distribution of isotope ratios as well as concentration.

In addition to published data obtained in the western North Pacific, subtropical North Pacific, eastern tropical North Pacific, eastern subtropical North Pacific, and eastern subtropical South Pacific, we obtained depth profiles of N₂O and its isotope ratios in the Southern Ocean, equatorial Pacific, and North Pacific by analyzing the samples collected in cruises KH01-3, MR02-K06, and MR07-04, respectively. Comparison of the profiles indicated that (1) the dissolved N₂O concentration reaches its maximum in the layer of sigma-theta = 26.5-27.5, which corresponds to the depth of 100-300 m in the Southern Ocean and equatorial Pacific and 700-1500 m in other regions, and (2) the maximum concentration is about twice and 4-7 times as large as saturation concentration for atmosphere-ocean equilibrium in the southern (Southern Ocean and South Pacific) and other stations, respectively. Isotope ratios at the N₂O concentration maxima also shows a variation among the ocean areas, but there is no significant relationship between the concentration and isotope ratios. This means that the origin of N₂O maxima observed in the Pacific is not identical, and in situ production plays more important role than lateral advection or diffusion. Assuming that the N₂O excess is caused by in situ production, we estimated isotope ratios of the produced N₂O as 7-11 permil, 50-65 permil, and 18-35 permil for delta-¹⁵N, delta-¹⁸O, and SP, respectively. The SP value was higher in the northern stations than in the southern stations. On the other hand, average concentration and isotope ratios of N₂O in the deep layer (below 2000 m) show a significant correlation with the age of the water mass estimated from ¹⁴C concentration, suggesting that N₂O is produced during the deep water circulation. Estimated isotope ratios for the N₂O produced were 10 permil, 62 permil, 29 permil for delta-¹⁵N, delta-¹⁸O, and SP, respectively.

The estimated delta-¹⁵N and SP of N₂O produced in the middle and the deep layer almost agree with the values of N₂O produced during nitrification (NH₂OH oxidation) by ammonia-oxidizing archaea (AOA), which were recently obtained by laboratory incubation experiments. However, the estimated delta-¹⁸O value in the middle/deep water is about 20 permil higher than that of AOA-produced N₂O, and SP of N₂O in the middle layer shows lower value in some areas. We therefore cannot exclude the contribution from bacterial nitrification and denitrification (nitrite reduction by nitrifier or denitrifier). Further researches on isotopic fractionation during the N₂O production by AOA is needed with respect to its variation among species and dependence of environmental factors.

Keywords: Nitrous oxide, ocean, stable isotope ratio, nitrification, denitrification, ammonia-oxidizing archaea

Potential use of N_2^* as a constraint of sedimentary denitrification in the deep ocean

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Measurements of the concentration of dissolved N_2 in the ocean have the potential to provide an important constraint on the magnitude of marine nitrogen fixation and denitrification, i.e., the main source and sink processes of fixed nitrogen in the ocean. The reason is because N_2 is consumed by nitrogen fixation and produced by denitrification. However, the use is impeded by the observation that, to first order, the oceanic distribution of N_2 is governed by air-sea exchange (diffusive and bubble-mediated), which is driven primarily by temperature-induced changes in the solubility. The effect of gas exchange on N_2 can be largely captured by considering simultaneous measurements of inert noble gases, such as argon (Ar) or neon (Ne), as this is the primary process governing their distribution. This offers the opportunity to use the difference in saturation anomaly between N_2 and one of the noble gases to determine the amount of fixed nitrogen removed or added.

We here define a new tracer N_2^* ($=N_2^{obs} - (N_2^{sat}/Ar^{sat}) * Ar^{obs}$) to assess the marine nitrogen cycle, and aim to investigate if the new tracer can be used for the purpose by using a global 3-dimensional ocean circulation model (OGCM) and the observations of N_2 and Ar. We explicitly incorporate the air-sea exchange processes of N_2 and Ar into OGCM, and prepare the previously simulated nitrogen fixation (Deutsch et al., 2007), and water-column and benthic denitrification fields which are calculated based on satellite-based estimates of organic-matter production, observed dissolved oxygen and nitrate concentrations combined with simple models of water-column and benthic remineralization.

Available observations of N_2^* in the Atlantic and Pacific except for oxygen minimum zones (OMZs) where water-column denitrification occurs showed the following features: 1) N_2^* gradually increases with depth from surface to deep waters, 2) N_2^* in the deep Atlantic is higher than that in the deep South Pacific, and 3) there is a south to north increase in N_2^* in the deep Pacific.

In order to evaluate the role of each source and sink in controlling the features of observed N_2^* , we carried out a suite of simulations. These simulations demonstrate that the features are determined mostly by the deep water sedimentary denitrification with minor contributions of shallow to intermediate-depth sedimentary denitrification and water-column denitrification, and nitrogen fixation has little impact on those. Thus, it seems like N_2^* can be the tracer of deep sedimentary denitrification in addition to water-column denitrification in OMZs.

Keywords: marine nitrogen cycle, denitrification, ocean general circulation model

Island mass effect and nitrogen fixation

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Most of the tropical and subtropical ocean is well stratified almost throughout the year, restricting upward nutrient supply from deeper water and making the surface environment oligotrophic. In the oligotrophic ocean, biological production has been recognized to be enhanced around islands where unique nutrient supply occurs, and that is referred to as the island mass effect (Doty and Oguri, 1956). Sufficiently strong and steady current results upwelling in the lee of island (Hasegawa et al., 2004, 2009). Hasegawa et al. (2009) demonstrated that Karman vortex streets in the lee develop phytoplankton blooms on a scale of several hundred kilometers. Other mechanisms for the enhanced productivity are nutrient supply from land drainage (Dandonneau and Charpy, 1985) and volcanic dust deposition (Lin et al., 2011).

Among phytoplankton assemblage, diatoms dominate in the local upwelling by the island mass effect (Furuya et al., 1986) because diatoms grow more rapidly than other algae under light-saturated and nutrient-replete conditions. Hence, the diatoms bloom is considered to link high fish production around oceanic islands. On the other hand, recent studies showed another aspect of the island mass effect (Shiozaki et al., 2010, 2013). Shiozaki et al. (2010) demonstrated that high abundance of *Trichodesmium* occurred mainly around oceanic islands and the nitrogen fixation activities enhanced comparing with in the open ocean. Diazotrophs utilize dinitrogen gas as a nitrogen source, and thus their growth are not limited by nitrogen as with other algae. However, diazotrophs require much more iron than nondiazotrophs because nitrogenase, a key enzyme of nitrogen fixation contains iron in its reactive center. Iron concentration in the water around islands is higher than in the open ocean, and hence diazotrophs would be abundant. Besides, Shiozaki et al. (2013) recently found that high abundance of diazotrophs not only occur around islands, but also in areas far remote from oceanic islands owing to advective transport of diazotrophs. The diazotrophs distribution is different from diatoms which are locally abundant around islands. In this presentation, we discuss influences of the island mass effect to diazotrophs activity and its importance in biogeochemical cycling in the oligotrophic ocean.

Keywords: island mass effect, nitrogen fixation, oligotrophic ocean, new production

Trophic fractionation of carbon and nitrogen isotope ratios along food chains in marine, lake and grassland ecosystems

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Evidence suggests that analysis of Stable Isotopes (SI) have the potential to reveal complex interactions, including trophic interactions and energy or mass flow through ecological communities. However, the magnitude of trophic fractionation of carbon isotopes in natural ecosystems remains unclear and requires further study with emphasis on kinetic isotope fractionation during feeding processes on food chains. To examine the relationship between carbon and nitrogen stable isotope ratios ($d^{13}C$ and $d^{15}N$) along food chains, we analyzed data collected from both from marine and terrestrial ecosystems.

For marine ecosystems, we analyzed data from four oceanic regions: the Oyashio waters at the western North Pacific (samples collected from March to October 2009), the warm-core ring 86-B derived from the Kuroshio extension region (preserved samples), and previously published data from the Gulf of Alaska, Antarctic Ocean. The statistical analysis suggested a strong similarity in slopes of $d^{15}N$ versus $d^{13}C$ ($d^{15}N/d^{13}C$) among regions: $d^{15}N = 1.53[+/-0.25] d^{13}C + [\text{ecosystem specific constant}]$ ($p < 0.001$). For terrestrial ecosystems, we conducted statistical analysis for previously published data from Lake Biwa, Lake Baikal and Mongolian grassland, which showed similar slope value of $1.61[+/-0.41]$ as the marine ecosystems. We attribute this similarity to common physiological aspects of feeding processes (*e.g.*, 'kinetic isotope effects' inherent in the processes of amino acid synthesis). We also compared seasonal differences seasonal in $d^{15}N/d^{13}C$ for the euphotic layers of the Oyashio waters. The $d^{15}N/d^{13}C$ slope of the food chain during the spring bloom differs from its common value in other seasons. We suspect that the $d^{15}N/d^{13}C$ slope of food chain may reflect 'ecological factors' due to strong seasonal factors such as spring bloom, and appearance of various zooplankton with different life history.

If we could better understand both carbon and nitrogen trophic fractionation within ecosystems, the stable isotope ratios may help to elucidate migratory behavior of higher trophic levels such as fishes in marine ecosystems as well as frame work of biogeochemical cycles in question.

Keywords: $d^{15}N$ - $d^{13}C$ relationship, food chain, isotopic fractionation

Earth's biogeochemical processes revealed by radiotracer-based activity measurement

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The biological fluxes on Earth are driven largely by microbial redox reactions. Microbial activity can sometimes demonstrate a recognizable level (e.g., fermentation), whereas most of the microbial processes show only a small effect on natural environment in the short term but have an impact on a longer time scale (i.e., over geological time scale). However the mechanisms regulating their operation and maintenance of elemental cycling on Earth remain poorly understood. In this regard, radioisotope tracer-based measurement is useful as a sensitive tool for the detection of microbial activity and related elemental cycling in the environment. This method can discriminate between assimilation and dissimilation processes based on the incorporation of radio-labelled substrate and excretion of the waste product from cells, respectively. Radiotracer-based analysis has been utilized since the early 1970's, and gone out of use with the development of molecular microbial ecology. Especially, microbial community genome sequencing (i.e., metagenomics) can provide less biased information of microbial community and function, which prompt further investigation of microbial ecosystem in extreme environment including deep subsurface and the outer space. These latest techniques will emphasize the importance of microbial activity measurement in extreme environment again. We have already started to evaluate the potential microbial activity of methane and acetate production, sulfate reduction, CO₂ uptake and organic matter mineralization in a deep-sea hydrothermal field, cold-seep and deep subsurface. The detection limit for each analytical method is above the level of nmol/L/day. Understanding these biogeochemical processes is crucially important to us as microbial life would globally catalyze and potentially provide environmental transformations.

Keywords: radiotracer, activity measurement, biogeochemical cycle

Determination of elemental composition of diatom opal and its implication on the geochemical cycle in the Oceans

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Settling particles play an important role in the oceanic material cycle. They contact with seawater and exchange elements during their settlement through a seawater column. Although diatoms account for over half of primary productivity in the oceans, it is almost unknown how much diatoms and diatomous opal are involved with the cycle in the oceans. According to the study using REEs, chemical alternation of siliceous matter depends on degree of aggregation (Akagi et al., 2011). Therefore, it is expected that settling particles at extremely high diatomaceous productivity keep unaltered opal as a result of increase of settling speed and decrease of surface area. This study aims to understand the chemistry of the unaltered opal and involvement in the elemental cycle in the ocean by analyzing sediment trap samples collected in the Bering Sea and North Pacific Ocean.

Concentration of 55 elements in the unaltered diatom opal could be determined (e.g. Al, Zn, REEs). This study for the first time disclosed that diatom opal contains these elements at a much higher concentration than considered. Comparing with concentration in hard tissue of coral (1/10000-1000 times concentration in the upper crust), that in diatom opal is much closer to that of the crust (1/100-1 times concentration). It might be able to understand vertical profiles by considering alternation/dissolution of diatom opal.

The behavior of elements in a water column was analyzed by a box model, which consists of four boxes of alumino-silicate, opal, dissolved matter and oxide. Assuming a steady state condition of the water column, concentration of elements in fresh diatom opal (M/Si) was expressed as a function of these in the crust and the proportion of amounts of elements incorporated to those dissolved. The formula could explain the change in the observed value in terms of chemical character of each element.

Keywords: diatom opal, vertical distribution, settling particles

Speciation of iron(II) in oxic inland water

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Introduction

In oxic freshwater, iron(II) is thought to be a minor component in total iron concentration because it is immediately oxidized to iron(III) species in hydrated ionic form. But iron(II) has higher potential bioavailability for primary production than iron(III), because of its high solubility and relatively organic weak complexation compared with that of iron(III) which sometimes forms very stable organic complex unavailable for phytoplankton. In practice, iron(II) was detected in some oxic freshwater. In Lake Biwa (Shiga, Japan), 5 to 20 nmol/L of iron(II) was determined by solid phase extraction as PDTS (Ferrozine) complex and spectrophotometry (Maruo M., Obata H. et al., *Verh. Internat. Verein. Limnol.*, 30, 1379-1383, 2010). Also in Lake Kinneret (Israel), some ten nmol / L of Fe(II) was detected with the same reagent. On the other hand, Iron (II) was detected in the range 0.1 to 0.9 nmol/L by flow injection with chemiluminescence detection (Emmenegger L. et al., *Limnol. Oceanogr.*, 46, 49-61, 2001) in daytime in surface water of Lake Greifen (Switzerland). Iron speciation in freshwater is largely regulated by pH, dissolved oxygen and characteristics of dissolved organic compounds in the water, so the difference in the concentration of Fe(II) between these two methods might be caused by chemical species of iron(II) detected. In this study, iron(II) in freshwater was determined by these two methods simultaneously.

Experimental

In Lake Biwa (av. depth 41.2 m, max. 103.6 m), waters were sampled at station T1 (35°22'N; 136°6'E; maximum depth 90 m) in the north basin of the lake on Feb., 2009 and Sep. 2010 by the research ship Hassaka (Univ. of Shiga Pref.). All water samples were collected in a metal-free Niskin sampling bottle. Collected samples were filtered immediately shipboard by a capsule filter (pore size 0.2 micrometer). Iron (II) measurement by chemiluminescence (King D.W. et al., *Environ. Sci. Technol.*, 29, 818-824, 1995) was performed onboard. For spectrophotometric method (Maruo M. et al., *Bunseki Kagaku*, 59, 1143-1147, 2010), buffer (pH 6.8) and PDTS solution were added to filtered water. Treated samples were ice-cooled to avoid oxidation of iron(II). At the laboratory, samples were passed through the Sep-pak PS-1 column to concentrate iron(II)-PDTS complex. The complex was eluted from the column and eluate was introduced to a long pass flow cell (1000 mm pathlength; LPC-1, Ocean Optics Co.), and absorption at 562 nm was read by a spectrophotometer (USB4000-VIS-NIR, Ocean Optics).

Results and Discussion

Iron(II) concentrations obtained from two methods were clearly different from each other. Chemiluminescence detection gave iron(II) concentrations below 1 nmol/L, while spectrophotometry based on Iron(II)-PDTS complexation showed 10 - 100 times higher values than those from chemiluminescence detection. This difference might be caused by complexation of iron(II) with some ligands in natural water or existence of reactive colloidal iron hydroxides. Without addition of the reagents, iron(II) concentration in Lake Biwa waters showed no significant change within 6 hrs after sampling in a dark condition after filtration (Maruo et al., 2011). This finding also suggests a stability of iron(II) by complexation with natural ligands in the water. Concerning the strong ligands for iron(II), the effect of natural strong ligand like EDTA may retard the ligand exchange of iron(II) into PDTS (Oji B., Yoh M., *Abstracts of 70th Meeting of the Japanese Society of Limnology*, 202, 2005). We also made sure that in iron(II) concentration at 25-100 nmol/L, it took 48hrs to form iron(II)-PDTS complex completely. Even by PDTS method, some portion of iron(II) strongly complexed with organic ligands might be missed.

Keywords: oxic environment, inland water, iron(II), complexation, Lake Biwa, chemical analysis

Decomposition process of labile DOC derived from phytoplankton

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Dissolved organic carbon (DOC) is one of the largest carbon pools in lakes. The elucidation of its source is very important for our understanding of the lacustrine carbon cycle. One of the sources of DOC is phytoplankton. Phytoplankton supplies DOC to water column directly through extracellular release and cell lysis, and indirectly via bacteria. In order to estimate the contribution of phytoplankton to lake DOC, the production and decomposition processes of DOC derived from phytoplankton need to be well understood. These processes, however, are difficult to investigate because DOC accumulation during phytoplankton decomposition is generally too small. In order to detect and examine this small amount of DOC, we have conducted decomposition experiments using ¹³C tracer in which products by natural phytoplankton communities were decomposed. Previous studies revealed the production process of refractory (R-) DOC. These studies showed that 1.3% of newly fixed carbon by phytoplankton became R-DOC and remained in water column for long time. In the present study, the production and decomposition processes of labile (L-) DOC derived from phytoplankton were investigated.

Phytoplankton communities were collected monthly for a year from Lake Kasumigaura, one of the most eutrophic lakes in Japan. The collected samples were incubated *in-situ* for 24h to label the newly fixed carbon with ¹³C. The samples were subsequently incubated in the dark for 100 days (20°C). The subsamples were collected from the cultures at intervals of 1 to 30 days. The concentration and ¹³C atom% of DOC in each subsample was measured and used for the calculation of the residual amount of newly fixed carbon.

An example of the change in newly fixed carbon is shown in Fig. 1 as an example. Most of newly fixed carbon was particulate organic carbon (POC) at the end of *in-situ* incubation (i. e. the start of dark incubation). The POC concentration, however, decreased drastically as soon as the sample has transferred into the dark condition, while the concentration of DOC showed increase until day 12. The DOC concentration showed gradual decrease after that, but a part of it remained until day 100. We tried to simulate the change in DOC concentration by the consecutive reaction shown in Fig. 1, where k_a is the decay constant of L-POC (d^{-1}), k_b is the decay constant of L-DOC (d^{-1}), a is the conversion efficiency from L-POC to L-DOC and b is the conversion efficiency from L-DOC to R-DOC. The values of a and k_b are especially important to understand the dynamics of L-DOC derived from phytoplankton. Using the least-squares method, a and k_b were estimated to be 0.069 and 0.037 d^{-1} , respectively, in the examination shown in Fig. 1. We also estimated these parameters in the other examinations and revealed that a ranged from 0.030 to 0.13 and k_b ranged from 0.016 to 0.058 d^{-1} . The relationships between these two parameters and phytoplankton community composition were not clear.

The amount of L-DOC derived from phytoplankton in a system can be estimated from primary productivity, a , k_b and water residence time. The values of a and k_b obtained from the present study indicate that the most of L-DOC in water column is derived from phytoplankton in L. Kasumigaura.

Keywords: phytoplankton, labile DOC, decomposition

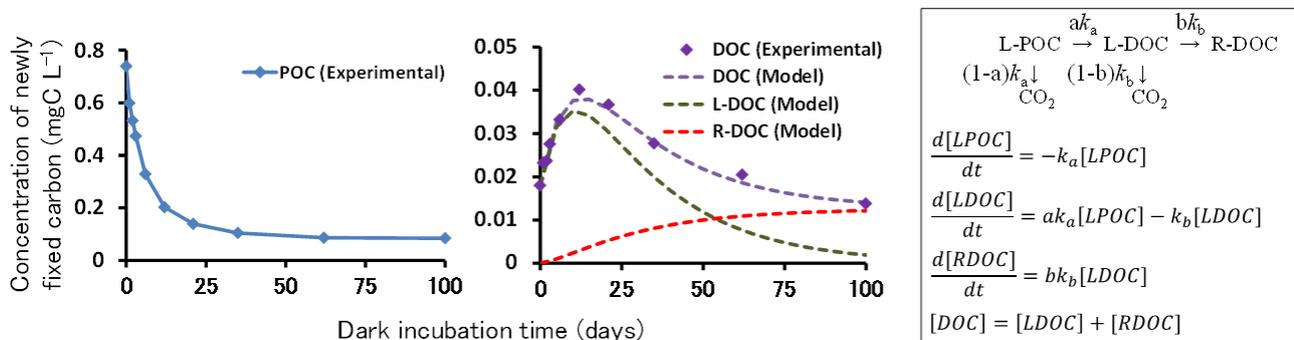


Fig. 1. Change in newly fixed carbon during experiment (Result from Oct. 2010 exam.). Equations used to simulate the DOC change are shown in the box.

Decomposition process of macroalgal dissolved organic matter

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[Introduction]

Productivity of macroalgae per community area is comparable with that of tropical rain forest which is one of the most productivity areas in biosphere on the earth's surface. Since the habitat of macroalgae is mainly limited to coastal shallow regions, macroalgae would have great contribution to coastal biogeochemical cycle. Our research group has previously focused on the fate of photosynthetic products of macroalgae to understand their role in coastal ecosystems, and we showed macroalgae release about 40% of their products as dissolved organic matter (DOM).

Since DOM constitutes a major component of marine organic matter, its dynamics is related with the reservation and transport processes of organic carbon. In addition, a part of marine DOM (e.g., humic substances) affects the activity of marine organisms by absorbing ultraviolet radiation. Macroalgae would contribute to above processes through the release of DOM, but the fates of macroalgal DOM in marine environments such as bacterial and photochemical decomposition have been unknown. Here we summarize our past research on decomposition of macroalgal DOM together with recent results.

[Materials and methods]

Collection of macroalgal DOM

We targeted a brown alga, *Ecklonia cava* Kjellman, which is commonly found in north Pacific including our research area (Oura bay, Shimoda, Japan). We covered transparent bag on *E. cava*, and recovered seawater after 2-4 days. In addition, we set the bags without *E. cava* as control samples. The seawater sample was filtered through glassfiber filter.

Bacterial decomposition experiment

Filtrates were stored under dark at 20°C, and subsamples were collected several times during 30 days. Concentration of dissolved organic carbon (DOC) and spectra of fluorescent dissolved organic matter (FDOM) were measured by a total carbon analyzer (Shimadzu TOC 5000A) and a fluorometer (Hitachi F-4500), respectively.

Photochemical decomposition experiment

Filtrates were additionally filtered through a filter (pore size: 0.2 micrometer) to eliminate bacteria, and artificial sunlight was irradiated with Atlas XLS+. During 24 hours, subsamples were collected in several times, and we measured DOC concentration and fluorescent spectra.

[Results and discussion]

Collection of macroalgal DOM

DOC concentration and fluorescent intensity of humic-like peak of seawater in the bag covering on *E. cava* increased to higher concentration than those of control sample.

Decomposition of macroalgal DOM

In the decomposition experiment under dark, the DOC concentrations gradually decreased, and remaining fractions on day 30 were 39-80%. On the other hand, intensity of humic-like peak increased with time. Photochemical decomposition experiment was carried out for 24 h, and largest decrease in DOC concentration was found in the initial 4 hours. Decrease rate of DOC became slow in the latter period of the experiment, and most part of DOC remained at the end of the experiment (72% of initial concentration). Fluorescent intensity of humic-like peak also decreased in the period from 0 to 4 h, but another peak at relatively shorter wavelength appeared after 4 h.

Conclusion

These two types of decomposition experiment showed that most part of macroalgal DOM resists for decomposition within a few day timescale. Since macroalgal DOM is rapidly transported from coastal to offshore area (in case of Oura bay: a half day), resistance of macroalgal DOM suggests that most part of macroalgal DOM is exported to broad area. In addition, the wavelengths and fluorescent intensity of humic-like material altered along decomposition process, suggesting change in the impact of macroalgal DOM on UV penetration by bacteria and photochemical decomposition.

Keywords: macroalgae, dissolved organic matter, bacterial decomposition, photochemical decomposition, dissolved organic carbon, fluorescent dissolved organic matter

Relationship between fluorescence characteristics and molecular weight of FDOM produced by bacteria

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Three bacterial strains were isolated from coastal surface seawater. These strains were inoculated into the liquid media amended with organic substrates and incubated in the media for a week. After the incubation, the production of fluorescent dissolved organic matter (FDOM) in the samples was confirmed by using excitation-emission matrix (EEM) spectroscopy. Simultaneously, alterations in molecular weight of the samples were analyzed by high-pressure size-exclusion chromatography (HPSEC) with fluorescence and absorbance detectors. The parallel factor analysis of the EEM spectra revealed that two strains produced the component associated with visible humic-like fluorescence and the rest of one strain made the two components which were related to visible and UV humic-like fluorescence. The fluorescence chromatograms derived from former two strains exhibited a single peak, whereas the chromatogram for the latter strain showed multiple peaks. The peak that attributes to the component associated with UV humic-like fluorescence consisted of lower molecular weight than the peaks derived from visible humic-like fluorescence. This finding suggests that the fluorescence characteristics of the bacterially-derived FDOM were related to its molecular weight. The HPSEC results with absorbance detection at 260 and 280 nm showed that the bacterial strains transformed organic substrates into low molecular weight compounds that included aromatic carbon content. The variation of the ratio of the fluorescence intensity to the absorbance among the peaks was found in their chromatograms, indicating that a content of aromatic carbon affects fluorescence intensity of FDOM. Thus, it is important for the qualitative analysis of FDOM to explore the relationship among aromatic carbon contents, molecular weights and fluorescence characteristics of FDOM

Keywords: Bacteria, Fluorescent dissolved organic matter, Excitation-emission matrix spectroscopy, Molecular weight

Study on migration of particulate organic matter in the Kushiro River using radiocarbon and stable carbon isotopes

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Knowledge of organic carbon dynamics in river basins is necessary to study the role of rivers in global biogeochemical cycles and ecosystem in coastal marine environment. The recent studies have pointed out an importance of snowmelt and rainfall on the transport of particulate organic matter (POM) in river systems. Therefore, it is important to understand the sources of POM, its transport behavior and controlling factors. Carbon isotopic compositions of POM are useful tools as tracers of the sources and behavior of POM in river systems. The objective of this study is to elucidate the transport behavior of POM from wetland to coastal ocean, because wetland is an extremely important carbon pool in terrestrial environment.

River water samples were collected at a fix station near Iwaboki observatory during spring to summer in 2004-2009. The station is located at the southern end of the Kushiro Wetland in eastern Hokkaido, Japan. Suspended solids were concentrated by single-bowl continuous-flow centrifugation and then freeze-dried. Aliquots of powdered samples after 1M HCl treatment were measured for C and N using an elemental analyzer. Stable carbon isotopic ratios were analyzed sub-samples of CO₂ gas generated during graphite production using a mass spectrometer. Radiocarbon of organic matter was determined using accelerator mass spectrometer at the NIES and JAEA. Radiocarbon values were reported as Delta¹⁴C corrected for sample delta¹³C.

The Delta¹⁴C and delta¹³C of particulate organic matter in suspended solids from the Kushiro River ranged from -76 to +6 permil and -29.0 to -27.8 permil, respectively. In order to understand the transport processes of particulate organic matter, we discussed relationship between the water level and carbon isotopes. The Delta¹⁴C value in April 2004, 2005 and August 2009 is higher than the other samples. The above sampling date corresponds to higher water level with spring snowmelt and long rainy period. On the other hand, delta¹³C value decreased with increasing water level. These results indicate that when the water level increases, the contribution of organic matter in the surface soil increases at the Kushiro Wetland area.

Keywords: river water, POM, suspended solids, coastal marine sediments, terrestrial organic matter, land-ocean interaction

Humic substances may control dissolved iron distributions in the global ocean: Implications from numerical simulations

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This study used an ocean general circulation model to simulate the marine iron cycle in an investigation of how simulated distributions of weak iron-binding ligands would be expected to control dissolved iron concentrations in the ocean, with a particular focus on deep ocean waters. The distribution of apparent oxygen utilization was used as a proxy for humic substances that have recently been hypothesized to account for the bulk of weak iron-binding ligands in seawater. Compared to simulations using a conventional approach with homogeneous ligand distributions, the simulations that incorporated spatially variable ligand concentrations exhibited substantial improvement in the simulation of global dissolved iron distributions as revealed by comparisons with available field data. The improved skill of the simulations resulted largely because the spatially variable ligand distributions led to a more reasonable basin-scale variation of the residence time of iron when present at high concentrations. The model results, in conjunction with evidence from recent field studies, suggest that humic substances play an important role in the iron cycle in the ocean.

The sources of amino acids in marine sediments estimated from nitrogen isotopic composition of amino acids and chlorin

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Amino acids are the building blocks of proteins and peptides and key compounds in microbial metabolisms. Amino acids represent one of major fractions of sedimentary organic matter and are important in undergoing organic matter mineralization in marine sediments. However, our understanding is still limited about the biogeochemical dynamics of amino acids in marine sediments, in part because currently available methods are not sufficient to constrain sources and transformation processes of amino acids in sediments.

Here, as a new method to estimate sources of amino acids in marine sediments, this study reports down-core profiles of compound-specific nitrogen isotopic composition ($\delta^{15}\text{N}$) of total hydrolysable amino acids (THAA) in marine sediments of the Japan Sea (a surface sediment and a 7-m-long piston core; ca. 46,500 years). The $\delta^{15}\text{N}$ profiles of THAA were compared with a down-core $\delta^{15}\text{N}$ profile of chlorin pigment (pyropheophytin a), which reflects the $\delta^{15}\text{N}$ values of organic matter produced by photosynthetic organisms in the past ocean.

Significant correlations were observed between $\delta^{15}\text{N}$ of amino acids and chlorin pigment in the piston core samples ($r^2 = 0.87$ for phenylalanine, 0.78 for glutamic acid, 0.77 for alanine, and 0.62 for glycine; $n = 13$). This result suggests that the major source of THAA is organic matter produced by the organisms in the past ocean (i.e., necromass) and that contribution of in situ sedimentary microbial production to THAA is less than 15% below 1 m depth in the core. The offset values of $\delta^{15}\text{N}$ between amino acids and chlorin pigment in the sediments of 1-7 mbsf (e.g., $\delta^{15}\text{N}$ -phenylalanine - $\delta^{15}\text{N}$ -chlorin = +7.3 permil in average) suggest that the source organisms of THAA contain not only photosynthetic algae and animals but also heterotrophic or chemoautotrophic microbes in the past ocean (water column and surface sediments).

Keywords: Organic matter, Nitrogen isotope, Amino acids, Photosynthetic pigments, Marine sediments, Paleoceanography

QUANTITATIVE ESTIMATION OF METHANOGENIC POTENTIAL USING FACTOR F430

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Methanogenesis is a final step of decomposition of organic matter by organisms, which is mediated by methanogenic archaea. It is suggested that a large proportion of methane in marine gas hydrate originates from the microbially mediated methanogenesis, based on isotopic compositions of methane. Therefore, investigations of distribution and activities of methanogenic archaea are important for better understanding of carbon cycles and mechanisms of gas hydrate formation. So far presence and activities of methanogenic archaea have been inferred using molecular biologic techniques, membrane lipids and tracer experiments with isotopically-labelled substrates however, it was difficult to clarify their in situ distribution and activities quantitatively.

Coenzyme F430 that is a Ni porphinoid with five carboxyl groups catalyses a final step of methanogenic reactions (Thauer, 1998). Because of its unique structure, functionality and lability, F430 can be a biomarker for a quantitative estimation of in situ methanogenic potential and activities of living methanogens (Takano et al., in press). In this study, we developed quantitative analysis of F430 and applied the method into methanogenic industrial sludge and environmental samples.

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Keywords: Methanogenic archaea, F430, Methanogenic potential

Physiological and genetic properties of key methanogenic Archaea for global methane emission from rice paddy fields.

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Rice paddy fields (RPFs) are one of the major sources of the greenhouse gas methane: it contributes 10-25% of the global methane emissions to atmosphere. Methane emission from RPFs is caused by the microbial production of methane, as the end products of anaerobic degradation of organic compounds. Cultivation-independent molecular studies suggested that one of the orders of methanogens, the order *Methanocellales*, which previously recognized as uncultured group Rice Cluster I (RC-I), is identified as the predominant methanogen in RPFs. However, despite their numerical significance and cosmopolitan dispersal, RC-I methanogens had so far escaped isolation.

Previously, we successfully isolated a novel methanogen, belonging to the RC-I lineage from a Japanese rice paddy field. To enrich the RC-I methanogens from rice paddy samples, we attempted to mimic the in situ conditions of RC-I on the basis of the idea that methanogens in such ecosystems should thrive by receiving low concentrations of substrate (H₂) continuously provided by heterotrophic H₂-producing bacteria. For this purpose, we developed a coculture method using an indirect substrate (propionate) in defined medium and a propionate-oxidizing, H₂-producing syntroph, *Syntrophobacter fumaroxidans*, as the H₂ supplier. By doing so, we significantly enriched the RC-I methanogens and eventually obtained a methanogen within the RC-I group in pure culture. That was the first report on the isolation of a methanogen within the RC-I group. We determined further morphological and physical properties of the isolate, and proposed the isolate as a novel mesophilic hydrogenotrophic methanogen *Methanocella paludicola*.

In addition, we reported complete genome sequence of *M. paludicola*. The genome sequence of *M. paludicola* consists of a single circular chromosome of 2,96 Mbp containing 3004 protein-coding sequences (CDS). Genes for most of the functions known in the methanogenic archaea were identified, e.g. a full complement of hydrogenases and methanogenesis enzymes. Comparative genome analysis among the previously determined methanogen genomes pointed to the genome-wide relatedness of *M. paludicola* to the orders *Methanosarcinales* and *Methanomicrobiales* methanogens in terms of the genetic repertoire. Meanwhile, the unique evolutionary history of *M. paludicola* was also traced in an aspect by the comparative genome analysis among the methanogens. The physiological and genetic properties of *M. paludicola* would provide better understanding how RC-I methanogens contribute global methane emission from RPF environments.

Keywords: methane, methanogenic archaea, rice paddy fields, cultivation

Variation in delta N-15 among the soil organo-mineral particles of various sizes in two volcanic-ash soils

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Stabilization and destabilization of soil organic matter (SOM), representing the largest carbon pool of terrestrial ecosystem, exert strong control on the biogeochemical cycling of biogenic elements. Growing evidence suggest that SOM stabilization is largely controlled by the interaction of organic matter (OM) and soil minerals. Volcanic-ash soils are characterized by a high contents of OM, short-range-order (SRO) minerals (e.g., allophane/imogolite), and organo-metal complexes. Previously, we showed that SOM is mainly stabilized in the soil particles of <2 micro-m size class where SRO minerals and organo-metal complexes were enriched relative to larger-size particles. Furthermore, we found that the decline of C:N ratio and 5 permil enrichment of N-15 towards finer particle size fraction. These results suggest that the OM in finer-sized particles appears to be more strongly altered by microbial metabolism. How the microbial transformation of SOM is linked to soil mineralogical property remains poorly understood, however. Here we hypothesized that N-rich OM is stabilized by interacting with metal ions (Al and Fe) and the organo-metal interaction, in turn, controls the variation in delta N-15 among the soil particles of various sizes. To test this, we chose two types of volcanic-ash soils - one is rich in SRO minerals and the other rich in layer-silicate minerals and organo-metal complex. We used particle-size fractionation to separate organo-mineral particles and characterized each by selective-dissolution, solid-state C-13 NMR, isotopic (N-15, C-13, C-14), and microscopic techniques. Our results showed that: (i) OM was mainly stabilized in <2 micro-m size fractions in both mineral type soils, (ii) OM content showed strong positive correlation with the content of organically-complexed metals, (iii) C:N ratio, N-15, and C-14 values were correlated with one another among the size fractions of both soils. Based on these results, we will discuss the possible factors and processes controlling the variation in N-15 among the organo-mineral particles and its relation to SOM stabilization in the studied soils.

Keywords: soil organic matter, organo-mineral associate, particle-size fractionation, short-range-order minerals, organo-metal complexes, carbon stabilization

Organic matter accumulation in volcanic ash soil revealed by C-13 tracer experiments and density fractionation analysis

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Organic matter (OM) enters into soil system mainly as plant detritus, root exudates, and microbial metabolites and detritus. The OM experiences both microbial degradation and the interaction with soil mineral particles, leading to the formation of organo-mineral particles that have a wide range of size, density, and chemical reactivity. Physical fractionation studies have shown that the OM present in organo-mineral particles (e.g., microaggregate in high-density fraction) are more stable against microbial degradation compared to mineral-free OM (e.g., plant detritus in low-density fraction). Little is known, however, the rate at which the OM is stabilized in organo-mineral particles and the transfer of C among various types of particles present within a soil. We thus conducted a laboratory soil incubation experiment to quantify the rate at which easily-metabolizable C transfer among the organo-mineral particles of three different density classes.

We added ¹³C-labelled glucose (99 ¹³C atom%, 0.1915 mmol ¹³C g⁻¹soil as solution) to a typical volcanic-ash soil (2-mm sieved, 10 g moist) and incubated for 30 d at 35 oC and 50% water holding capacity. After the incubation, soil was separated according to particle density using sodium polytungstate into low (<1.8 g mL⁻¹), middle (1.8-2.5 g mL⁻¹), high (>2.5 g mL⁻¹) density fraction. We measured the mass, isotopic ratio (¹³C/¹²C) and total C content of the density fractions as well as the amount of CO₂ respired during the incubation by alkali trap method. This experiment was conducted in triplicate.

The density separation showed that 85 percent of total soil C was recovered in the mid-density fraction, whereas 10 and 5% was recovered in the low- and high-density fractions, respectively. The highest concentration of short-range-order minerals (e.g., allophane/imogolite) was found in the mid-density fraction, which imply that high specific surface areas and sorption capacity of the short-range-order minerals contributed to the large OM accumulation in this fraction. Of the ¹³C added to the soil, half was mineralized to CO₂ during the 30-day incubation. The other half of added ¹³C was recovered from soil density fractions with greater partitioning into lower density (37, 13, 0.8% of the ¹³C in the low-, mid- and high-density fractions, respectively). Highest ¹³C recovery in the low-density fraction suggests rapid assimilation of added glucose by the active microbial communities in and subsequent turnover of the labeled C within the low-density fraction. Our results support the hypothesis that microbial degradation of newly-added OM is accompanied by the progressively intimate interaction with mineral particles.

Keywords: soil organic matter, C-13 glucose, density fractionation, soil microorganism, organo-mineral particles, short-range-order minerals

Mineralogical variations in tropical soils on African Shield

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Mineralogical variations and their influence on soil fertility status are not fully understood in tropical soils on African Shield. A total of 124 horizon samples from 32 pedons were collected from the South-Cameroon (SC) and Adamawa plateaus (ADM) in Cameroon, where highly-weathered clayey soils (Ferralsols, on the basis of WRB soil classification) are known to be dominant. We found that three parameters to indicate the presence of mica in soil clays, i.e., mica: kaolinite ratio based on XRD analysis, total K content, and Radiocesium Interception Potential, all showed a significant positive correlations with cation exchange capacity (CEC) of soil clay, indicating that nutrient retention ability of soil is largely dependent on the residual amount of mica. Furthermore, soil clays of ADM contained fewer amount of mica, while contained larger amount of Al-hydroxides (gibbsite) and Fe-oxides (goethite) with higher degree of Al-substitution than that of SC. These relationships strongly suggest that dissolution of 2:1 phyllosilicates including mica (i.e. desilication) and accumulation of Al-dominant clays (i.e. aluminization) proceeds to a greater extent in soils on ADM than those on SC. The difference may be attributed to the geological ages when plateau was formed. Our findings revealed that tropical soils on African Plateau showed large variations in their mineralogical properties, although they have never been paid many attentions. Reconsideration of mineralogical control on the soil fertility status would be required to evaluate precisely the nutrient dynamics in tropical soils on African Shields.

Keywords: Cameroon, Mica, Desilication/Aluminization, X-ray diffraction analysis, Differential thermal analysis, Radiocesium Interception Potential

Modification of the DNDC-Rice model to evaluate the nitrogen balance at a paddy field

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The DNDC (DeNitrification-DeComposition)-Rice model simulates the processes of carbon and nitrogen turnover in ecosystems for estimating greenhouse gas emissions from paddy fields, and can be used to simulate the N balance of a paddy field. In this study, we validated DNDC-Rice using field observation data, including N balance data, to reveal problems when using the model to evaluate a paddy field's N balance. To validate the N balance components of DNDC-Rice, we used data collected at the Mase paddy flux site (36.03N, 140.01E), in the middle of the Kanto Plain of Japan's Ibaraki Prefecture, in 2009. Before the validation, a process for adsorption of ammonium (NH_4^+) ions by clay was modified based on the results reported by Katayanagi et al. (2012) *Soil Sci. Plant Nutr.* 58:360-372. The modified DNDC-Rice simulated the dry weight of roots, stems, and grains well, but overestimated leaf dry weight. The normalized root-mean-square errors (nRMSEs) for the root, stem, grain, and leaf dry weights were 13, 16, 7, and 60%, respectively. DNDC-Rice also overestimated the leaf area index (LAI) and leaf N content, with nRMSEs of 125 and 37%, respectively. The overestimation of leaf dry weight and LAI resulted from overestimation of N uptake by rice and of N allocation to leaves. The high N uptake could be due to either a high available soil N content, crop N recovery from the soil or both. At harvesting, the simulated N balance (= N input - N output) was -38.8 kg ha^{-1} , which was much lower than the N balance determined by observations and from relevant literature (12.8 kg ha^{-1}). The underestimation of the N balance resulted mostly from the model's inability to calculate dry N deposition and N fixation as inputs and from overestimation of grain N uptake and underestimation of N_2 emissions through denitrification as outputs. Based on the result, the model has been being modified using the data collected at Tsukuba FACE (Free-Air CO_2 Enrichment) experimental site. Tsukuba FACE (35.97N, 140.00E) was established in farmers' rice fields in Tsukubamirai City located near the Mase site. The elevated $[\text{CO}_2]$ treatments were imposed on experimental plots in Tsukuba FACE to evaluate responses of rice and carbon and nitrogen cycles to high CO_2 and soil temperature. The modification based on the data collected at Tsukuba FACE will make it possible to predict future carbon and nitrogen dynamics at paddy fields more accurately.

Keywords: nitrogen balance, continuous flooding paddy field, DNDC-Rice

Rapid decomposition of organic matter in N-rich forest and cropland soils as revealed by cellulose filter incubation

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The microbial decomposition of organic matter plays important roles in soil C cycles. The decomposition activity of soil microorganisms may increase in response to the increased N deposition or fertilization. By using filter paper of cellulose (major constituent of plant litter) as a standard substrate for soil microorganisms, effects of N availability on microbial decomposition activity can be compared between different forest and cropland types. The mass loss of filter papers buried in the surface soils was measured in the N-rich cropland and three forest sites varying in N deposition in Japan. The rates of cellulose decomposition were higher in the cropland soil than in three forest soils. Despite a small biomass of the cropland soil microorganisms, their decomposition ability is considered to be enhanced by higher soil temperature and N availability. Among three forest soils, decomposition rate on cumulative degree-day basis was greatest in the suburban forest receiving high N deposition (20 kg N ha⁻¹ yr⁻¹). The mineralization experiment of ¹⁴C-glucose showed that the mineralization rates of glucose increased with soil microbial biomass, which contrasts with results of cellulose decomposition. This suggests that the rate-regulating factors of cellulose decomposition are different from those of glucose mineralization. Cellulose can be rapidly decomposed in the forest soils receiving high N deposition, as well as N-rich cropland soils. The N input from the external sources may be taken into account as one of rate-regulating factors of organic matter decomposition in the suburban forests.

Keywords: soil respiration, soil organic matter, organic matter decomposition, N saturation, cellulose

Morphological changes in bamboo with nitrogen saturation

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[Introduction]

In recent years, nitrogen saturation, a state where the amount of available nitrogen exceeds plant and microbial nutritional demand due to nitrogen deposition, has been reported. In a nitrogen-saturated forest, Tama hill, bamboo (*Phyllostachys pubescens*) lodging is observed. In the case of rice, it is known that leaf mass is increased and internodal growth is promoted under high nitrogen nutrition, resulting in rice lodging. Thus, we hypothesized that similar morphological changes by nitrogen saturation would have caused a lodging phenomenon for bamboo, a family of rice. Based on this assumption, we studied the cause of bamboo lodging from the following morphological changes; 1) promotion of extension growth, 2) the increase in mass of branches-and-leaves, 3) decrease of roots.

[Method]

We studied the biometry of bamboo in a nitrogen-saturated site (Tama, Tokyo) and control sites (Fukushima and Izu). Diameter at breast height (DBH), each internode length, culm height, and mass of branches-and-leaves, and root mass in the soil surface (0-5 cm) were measured. The total carbon and total nitrogen content of leaves were measured with the dry combustion method.

[Result and discussion]

No significant difference was found in the leaf nitrogen concentration, culm height, and the mass of branches-and-leaves for bamboos standing straight among the N-saturated and control sites. Lodging bamboo in the Tama hill, however, had significantly larger mass of branches-and-leaves. Very low root mass in the soil surface was also measured and a root mat as observed in the soil surface in a control site was hardly seen in the N-saturated site. It is considered that increase in the load of the aboveground by the increase in mass of branches-and-leaves and the fall of the bearing power of the underground by the decrease of root mass is responsible for bamboo lodging as observed in the N-saturated site. Further research is necessary to study the strength of bamboo culm, mass of branches-and-leaves of lodging individual, and mass of whole root.

Keywords: nitrogen saturation, bamboo, morphological change, lodging, overgrowth, biometry

Effect of nitrogen fixation activity on green needle decomposition of Japanese cedar

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Decomposition of green needles in Japanese cedar was investigated. Experimental sites were established at Shirosato, Ibaraki and Odate, Akita. The former site is no snow and the later site is much snow in winter. Litter bag experiment was conducted using green needles of Japanese cedar, and set on the ground surface and in the air.

A decomposition rate was not differing among treatments in first year but higher in the ground installed than those of air. A decomposition rate in Katsura was higher than in Odate. It was reflected difference of mean annual temperature among sites. Decomposition was proceeding even in during winter at Odate, but not at Katusra. It was considered that decomposition was progressed by water condition and physical destruction in the snowpack. A nitrogen fixation activity in remained needles was appeared three months after installation and was maximum at 6-10 month after. These values among sites were not differing. A nitrogen contents in remained needles was increased following installing time, was higher in the ground installed samples than those of air, but no relationship was observed with nitrogen fixing activity. Nitrogen content was constant in each treatment at sampling time. It was considered that increments of nitrogen contents between initial and air installed needles were derived from nitrogen fixing from air, and increments between air and ground installed needles were derives immobilization of soil microbes.

Keywords: Immobilization, litter, snow, thinning

Proton budgets for a Japanese cedar stand and a Japanese red pine stand receiving high nitrogen deposition

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To evaluate the effects of atmospheric nitrogen (N) inputs on soil acidification, the proton budgets for a Japanese cedar (*Cryptomeria japonica*) stand (Cs site) and Japanese red pine (*Pinus densiflora*) stand (Ps site) in Gunma prefecture, central Japan, were studied by measuring biogeochemical fluxes (atmospheric deposition, canopy leaching, vegetation uptake and leaching from soil). Proton budgets were estimated for two individual compartments of the forest ecosystems: vegetation canopy and soil horizon with root zone.

Nitrogen inputs below the canopies at Cs site and Ps site were 2.0 kmol ha⁻¹ yr⁻¹ and 3.4 kmol ha⁻¹ yr⁻¹, respectively, which have almost been equal to those in nitrogen-saturated forests in northwestern Europe. Its outputs from the soil horizon at Cs site and Ps site were 3.9 kmol ha⁻¹ yr⁻¹ and 2.5 kmol ha⁻¹ yr⁻¹, respectively. These results indicate that the ecosystems were nitrogen-saturated, and that a net loss of N occurred at Cs site.

The dominant proton sources in vegetation canopies were atmospheric H⁺ deposition at Cs site, and leaching of anions at Ps site. In soil layers, the dominant proton sources were base-cation uptake by vegetation and nitrification of NH₄⁺ derived from organic N at Cs site, and nitrification of atmospheric NH₄⁺ at Ps site, respectively. The sum of internal proton sources within the soil-vegetation system at Cs site and Ps site was 15.5 kmol_c ha⁻¹ yr⁻¹ and 6.9 kmol_c ha⁻¹ yr⁻¹, respectively. These internal proton sources were 4.9 times at Cs site and 1.6 times at Ps site as large as the sum of external sources (atmospheric H⁺ deposition, uptake of atmospheric NH₄⁺ by the canopy and nitrification of atmospheric NH₄⁺).

These proton sources were neutralized almost completely within the system mainly by base-cations release from the canopy or the soil, and uptake of NO₃⁻ by vegetation at the both sites. These results suggest that the forest ecosystems studied have the higher capacity to neutralize acid than nitrogen-saturated forests in northwestern Europe. It is concluded that soil acidification due to N deposition is unlikely to occur at present at the sites.

Keywords: atmospheric deposition, nitrification, nitrogen saturation, proton budget, soil acidification

Factors influencing spatial distribution of soil nitrogen dynamics in a natural mixed forest stand in northern Japan

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Nitrogen (N) dynamics in forest soils is often spatially heterogeneous. Such heterogeneity is important as a component of forest biodiversity and succession. Several factors, including plants, influence spatial heterogeneity of N in soils. Many studies have reported that tree species affect soil N dynamics, and that net N mineralization and nitrification are negatively correlated with litter Carbon (C) :N ratio, lignin:N ratio, and soil C:N ratio. As organic materials in soils are largely derived from forest floor, species-specific litter traits would affect soil N dynamics. Therefore, the spatial pattern of canopy tree composition in a natural mixed forest stand would be related to the spatial heterogeneity of soil N dynamics. In addition, understory vegetation also play a role in N cycling, and litterfall of both overstory and understory vegetation mixes in mixed forests. Hence, spatial pattern of both plant and litterfall would influence spatial heterogeneity of N dynamics of soils in a mixed forest stand. However, these relationships are not well understood.

We established two experimental sites (site A & B) in cool-temperate natural mixed stands of Uryu experimental forest of Hokkaido University. Site A is dominated by coniferous trees with dense understory of *Sasa senanensis*, while site B has more broadleaved trees with dense understory of both *S. senanensis* and *Viburnum furcatum*. Within each site, we conducted two types of surveys (survey 1 & 2). In survey 1, we collected data and samples on vegetation, litterfall, forest floor, and mineral soils in 15 plots at each site to understand the mechanisms of plants influencing soil N dynamics. Survey 2 focuses on understanding the spatial relationships between vegetation and soil N, and sampling were conducted in each of 50 grids covering entire site. The samples were analyzed for their physical and chemical properties such as pH, C, N, base cations and aluminum (Al) concentration (survey 1). Also, soil microbial respiration rates, net N mineralization and nitrification rates were measured with laboratory soil incubation.

In site A, the biomass of *S. senanensis* was high near broadleaved trees and in areas with less coniferous trees. In site B, the *V. furcatum* densities were low under conifer-dominated area, and the biomass of *S. senanensis* were low in the *V. furcatum* dense area. The spatial distribution of both overstory and understory vegetation influenced that of litterfall. The C:N ratio of coniferous litter was significantly higher than other litter types. The Calcium (Ca) concentration was the highest in *V. furcatum* litter, while it was the lowest in *S. senanensis* litter. Both *V. furcatum* and coniferous tree litter contained higher level of Al than others. Forest floor under coniferous trees were thick, while it was thin under *S. senanensis* and *V. furcatum* dominate area with nearby broadleaved trees. The C:N ratios of total litterfall were positively correlated with the thickness of FH layer, indicating that litterfall with higher C:N ratio decomposes more slowly. The FH layer thickness was negatively correlated with soil nitrate pool and net nitrification rates in both sites, suggesting that where litter decomposition is faster, nitrate production is higher. The spatial relationships between vegetation and soil N dynamics in survey 2 indicated that soil nitrate pool is higher under *S. senanensis* and *V. furcatum* dominate area with nearby broadleaved trees, and is lower in conifer-dominated area. These relationships may be due to the difference of litter C:N ratio and decomposition rates. As *V. furcatum* litter contained the highest level of Ca, Ca might promote nitrification in soils. Our results indicated that spatial pattern of both plant and litterfall influence spatial distribution of nitrate pool in soils through the difference in litter decomposition rate in cool-temperate natural mixed forest of northern Hokkaido.

Keywords: soil nitrogen dynamics, spatial heterogeneity, understory vegetation, litterfall, mixed forest