ABSTRACT
学 位 論 文 要 旨

Nitrification and denitrification in river ecosystems elucidated by natural abundance of stable isotopes
安定同位体を用いた河川生態系における硝化および脱窒の検討

Department of Symbiotic Science of Environment and Natural Resources,
Major Chair of Environmental Conservation
NGUYEN CONG THUAN

Rivers function to transport nitrogen (N) from land to ocean accompanied with the diverse biogeochemical processes occurring such as mixing, assimilatory uptake, nitrification, heterotrophic denitrification, and N sedimentation. The question is what can be useful tools to get insights into the occurrence of N biogeochemical processes in rivers. Concentrations of N compounds can provide the fundamental information on their enrichment levels, but they cannot always provide information on the N process occurrence. The rates of the N processes can be estimated in the laboratorial experiments, but the obtained results might not reflect truly the actual occurrences in natural conditions in rivers. The N processes taking place result in isotope fractionations between substrates and products (expressed as isotope fractionation factors), and isotope fractionation factors can be unique and different for the different N processes. Therefore, the observation of the changes and differences in isotopic compositions ($\delta^{15}N$ and $\delta^{18}O$) among N compounds is expected to be a useful tool to understand the occurrence of the N processes in rivers. In this study I measured natural abundance of stable isotopes of N compounds to elucidate N dynamics in rivers in Japan.

To identify microbial processes of N_2O production along the urban Tama River, concentrations and isotopic ratios of N_2O and its substrates (dissolved inorganic N; DIN, namely NH_4^+, NO_2^-, and NO_3^-), abundances of functional genes of nitrifiers (*amoA*-bacteria) and denitrifiers (*nirK*, *nirS*, and *nosZ*), dissolved organic carbon (DOC) and abundances of protein and humic components of dissolved organic matter (DOM) were measured in the surface water samples collected in 2014. The results showed that both $\delta^{15}N$ and $\delta^{18}O$ of N_2O fell within the expected $\delta^{15}N$ and $\delta^{18}O$ ranges for N_2O production by denitrification, thus I concluded that denitrification was the dominant process of N_2O production along this River. A positive correlation between *nirK* gene abundance and N_2O concentration supported the importance of denitrification for the N_2O production. The DOC and protein and humic components of DOM did not control dissolved N_2O, but at least protein-DOM (with high lability and bioavailability) supported the

occurrence of denitrification.

To identify the biological processes causing changes in concentrations of DIN with distances along the Fuji River, concentrations and isotope ratios of DIN species (NH_4^+, NO_2^-, and NO_3^-) were measured in the water samples collected in the reach of 5.5 km in June, October, November, and December, 2015 using the longitudinal Lagrangian approach. The N isotope fractionations for NH_4^+ consumption ($^{15}\varepsilon_{AC}$) were also estimated to give additional evidence for biological processes of NH_4^+ consumption. I also estimated the spiraling metrics such as uptake length, velocity and rate for NH_4^+ to explore the relations between spiraling metrics and the $^{15}\varepsilon_{AC}$. The results showed the decrease in NH_4^+ concentrations accompanied with the increase in $\delta^{15}N$ of NH_4^+ in all sampling times, which indicated that NH_4^+ was consumed by biological processes. The significant correlation between $\delta^{15}N$ of NH_4^+ and $\delta^{15}N$ of NO_3^-, and the lower $\delta^{15}N$ of NO_2^- than those of NH_4^+ and NO_3^-, illustrated that nitrification was the main process of NH_4^+ consumption and NO_3^- accumulation. I reported the $^{15}\varepsilon_{AC}$ of 4.0 to 8.9‰ in river environments, although the variation of the $^{15}\varepsilon_{AC}$ was not explained clearly in this study. Interestingly, I found the $^{15}\varepsilon_{AC}$ had positive relations with uptake velocity and rate for NH_4^+, but need more evidences to confirm the possibility that $^{15}\varepsilon_{AC}$ can be a good parameter for uptake velocity.

To investigate the variation of the $^{15}\varepsilon_{AC}$ across rivers with different NH_4^+ concentrations, the investigation in four other large rivers (the Saigawa River, the Arakawa River, the Chikuma River, and the Tama River) was done in 2016. This investigation found that in rivers with low NH_4^+ concentrations (ca. 1.5 μM), NH_4^+ consumption was considered to be negligible which is confirmed by the constant NH_4^+, NO_2^- concentrations with distances. The any change in isotope ratios and concentrations of DIN species might be due to abiotic processes such as mixing, dilution, and release N from sediment. In rivers with moderate-to-high NH_4^+ concentrations (above 5 μM), nitrification was the dominant process for NH_4^+ consumption and NO_3^- production. No clear evidence from concentration and isotope data of DIN species for occurrence of assimilation and denitrification were found in the large rivers. The long area uptake length for NH_4^+ (2.4 km to 29.2 km in rivers with moderate-to-high NH_4^+ concentrations) together with insignificant NO_3^- consumption implied nutrient N removal efficiency was low in the large rivers. It was confirmed that the spiraling metrics for NH_4^+ controlled significantly the $^{15}\varepsilon_{AC}$. It is further suggested that the estimated $^{15}\varepsilon_{AC}$ can predict NH_4^+ uptake levels in rivers.

This study demonstrates that the isotopic measurements of N compounds are the powerful tools to get insights into the occurrence of the N biogeochemical processes in rivers.