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Title	Coupled nitrogen and oxygen isotope effects of anaerobic ammonium oxidation (anammox) [an abstract of dissertation and a summary of dissertation review]
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Degree Grantor	北海道大学
Degree Name	博士(工学)
Dissertation Number	甲第14449号
Issue Date	2021-03-25
Doc URL	https://hdl.handle.net/2115/81648
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	doctoral thesis
File Information	Kanae_Kobayashi_abstract.pdf, 論文内容の要旨

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学　位　論　文　内　容　の　要　旨

博士の専攻分野の名称　　博士（工学）　　氏名　小林　香苗

学　位　論　文　題　名

Coupled nitrogen and oxygen isotope effects of anaerobic ammonium oxidation (anammox)
(アナモックス細菌の窒素および酸素同位体分別の解析)

Natural abundance of stable nitrogen (N) and oxygen (O) isotopes (δ^{15}N and δ^{18}O) are invaluable biogeochemical tracers for assessing the N transformations in the environment. To fully exploit these tracers, the N and O isotope effects ($^{15}\varepsilon$ and $^{18}\varepsilon$) associated with the respective N transformation processes must be known. Anaerobic ammonium oxidation (anammox) and denitrification are the two major sinks of fixed N. In addition, anammox bacteria contribute to re-oxidation of nitrite to nitrate, because they fix CO_2 into biomass with reducing equivalents generated from oxidation of nitrite to nitrate. Nitrate production by anammox bacteria influences the nitrite and nitrate N and O isotope effects in freshwater and marine systems. Despite the significant importance of anammox bacteria in the global N cycle, $^{15}\varepsilon$ and $^{18}\varepsilon$ of anammox are not well known. Therefore, the never yet determined $^{15}\varepsilon$ and $^{18}\varepsilon$ associated with anammox were investigated in this study.

Firstly, the $^{15}\varepsilon$ were determined for ‘*Ca*. Scalindua sp.’, ‘*Ca*. Jettenia caeni’, and ‘*Ca*. Brocadia sinica’ growing in continuous enrichment cultures. All three anammox species yielded similar $^{15}\varepsilon$ values of NH_4^+ oxidation to N_2 ($^{15}\varepsilon_{NH_4^+ \rightarrow N_2}$ = 30.9 ~ 32.7 ‰) and inverse kinetic isotope effects of NO_2^- oxidation to NO_3^- ($^{15}\varepsilon_{NO_2^- \rightarrow NO_3^-}$ = - 45.3 ‰ ~ - 30.1 ‰). In contrast, the values of NO_2^- reduction to N_2 was significantly different among three species ($^{15}\varepsilon_{NO_2^- \rightarrow N_2}$ = 5.9 ~ 29.5 ‰), which is probably because individual anammox bacteria species might possess different types of nitrite reductase.

Secondly, the $^{18}\varepsilon$ were determined for ‘*Ca*. Scalindua sp.’, which is a putative marine species. Determination of $^{18}\varepsilon$ of anammox is more challenging because the $\delta^{18}O_{NO_2^-}$ value is affected by abiotic O isotope exchange between NO_2^- and H_2O (k_{eq}, $^{18}\varepsilon_{eq}$) and incorporation of a water-derived O atom into NO_3^- during NO_2^- oxidation to NO_3^- ($^{18}\varepsilon_{H_2O}$). In order to determine abiotic k_{eq}, $^{18}\varepsilon_{eq}$, and $^{18}\varepsilon_{H_2O}$, batch experiments with different $\delta^{18}O_{H_2O}$ values of medium were conducted. Oxygen isotope ratio measurements of NO_2^- and NO_3^- by the azide method and denitrifier method are sensitive to the δ^{18}O of sample water. However, the influence of $\delta^{18}O_{H_2O}$ on those measurements has not been quantitatively evaluated and documented so far. Therefore, the influence of $\delta^{18}O_{H_2O}$ of sample on δ^{18}O analysis of NO_2^- and NO_3^- were quantitatively evaluated. Then, the rate of abiotic O isotope exchange between NO_2^- and H_2O: k_{eq} = 1.13 × 10^{-2} (h^{-1}), as well as equilibrium isotope effects: $^{18}\varepsilon_{eq}$ = 11.9 ‰ were experimentally determined. To determine $^{18}\varepsilon$ of each reaction, batch culture experiments with different $\delta^{18}O_{H_2O}$ values of medium ($\delta^{18}O_{H_2O}$ = -12.6 ~ 110.1 ‰) were conducted for ‘*Ca*. Scalindua sp.’. A numerical model was developed for estimation of respective $^{18}\varepsilon$ of anammox reaction, resulting in $^{18}\varepsilon_{NO_2^- \rightarrow N_2}$ =8.4 ~10.0 ‰, $^{18}\varepsilon_{NO_2^- \rightarrow NO_3^-}$ = -3.0 ~ -1.2 ‰, and $^{18}\varepsilon_{H_2O}$= 25.8 ~ 27.8 ‰, respectively. These $^{18}\varepsilon$ values were determined for the first time in the world.

These obtained dual N and O isotopic effects could provide significant insights into the contribution

of anammox bacteria to the fixed N loss and nitrite reoxidation in (recycling N) in various natural environments.