# Oxygen isotope effect and exchange of a marine anammox species; "*Ca.* Scalindua sp."

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Natural abundance of stable nitrogen and oxygen isotopes 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 nitrogen transformation processes must be known. Anaerobic ammonium oxidation (anammox) and denitrification are the two major sinks of fixed nitrogen (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, the N and O isotope effects of anammox are not well known. Especially, the oxygen isotope effect of anammox metabolism has never been determined yet. This is probably because the O isotope effect of nitrite is affected by three simultaneously occurring reactions; (1) nitrite reduction to $N_2$ gas, (2) nitrite oxidation to nitrate, and (3) incorporation of a water-derived O atom into nitrate during nitrite oxidation to nitrate, and thus is difficult to determine. Furthermore, the $\delta^{18}O_{NO2}$ value is affected by abiotic O isotope exchange between nitrite and water. Here we analyzed the O isotope effects and oxygen atom exchange associated with anammox metabolism by a marine anammox species "*Ca.* Scalindua sp.".

The rate of abiotic oxygen atom exchange rate was measured using the medium with different $\delta^{18}O$ values ($\delta^{18}O_{H2O}$= -12.3, 27.1, 60.0, and 114.3‰) at a temperature of 30°C and pH=7.5 which was same as the experimental condition of anammox batch incubation. The approach of $\delta^{18}O_{NO2}$ to isotope equilibrium ($\delta^{18}O_{NO2,\ eq}$) is modeled with the following formula: $\delta^{18}O_{NO2} = \delta^{18}O_{NO2,eq} + (\delta^{18}O_{NO2,initial} - \delta^{18}O_{NO2,eq})*\exp(-k_{eq}*\mathrm{Time})$. $k_{eq}$ (in units of $h^{-1}$) represents the rate constant for abiotic equilibration of oxygen atoms **(Fig. 1)**. The model fitting approach of $\delta^{18}O_{NO2}$ at different medium $\delta^{18}O$ values yielded a rate constant ($k_{eq}$) of (1.13 ±0.007) ×$10^{-2}$ ($h^{-1}$), and the equilibrium oxygen isotope effect between nitrite and water ($^{18}\varepsilon_{eq}$) of 12.95 ±0.16‰ (tentative result).

To determine oxygen isotope effects of (1) nitrite reduction to $N_2$ gas ($^{18}\varepsilon_{NO2\rightarrow N2}$) and (2) nitrite oxidation to nitrate ($^{18}\varepsilon_{NO2\rightarrow NO3}$), anammox batch experiments were conducted using the medium with different $\delta^{18}O$ values ($\delta^{18}O_{H2O}$= -12.3, 27.1, 60.0, and 114.3‰) in triplicate. In these batch experiments, we used highly enriched (Percoll-separated) cultures (>99.9%) of "*Ca.* Scalindua sp.". The stoichiometric ratios of consumed nitrite and consumed ammonium ($\Delta NO_2^-/\Delta NH_4^+$, average 1.35) and produced nitrate and consumed ammonium ($\Delta NO_3^-/\Delta NH_4^+$, average 0.29) agreed with previously observed stoichiometry of anammox process **(Fig. 2)**. During the anammox reaction, $\delta^{18}O$ of produced nitrate appeared to depend on the $\delta^{18}O_{H2O}$ of medium. This observation suggested that a water-derived O atom was incorporated into nitrate during nitrite oxidation to nitrate. Rapid increase in $\delta^{18}O$ of nitrite overtime was observed in high $\delta^{18}O_{H2O}$ media as compared to abiotic exchange. A numerical model is currently being developed to estimate the oxygen isotope effect of each reaction ($^{18}\varepsilon_{NO2\rightarrow N2}$ and $^{18}\varepsilon_{NO2\rightarrow NO3}$). The obtained O isotopic effects of a marine anammox species "*Ca.* Scalindua sp." could provide significant insights into the contribution of anammox bacteria to the fixed N loss and $NO_2^-$ reoxidation (N recycling) in the ocean.

Keywords: Anammox, Oxygen isotope effect, Nitrogen cycle

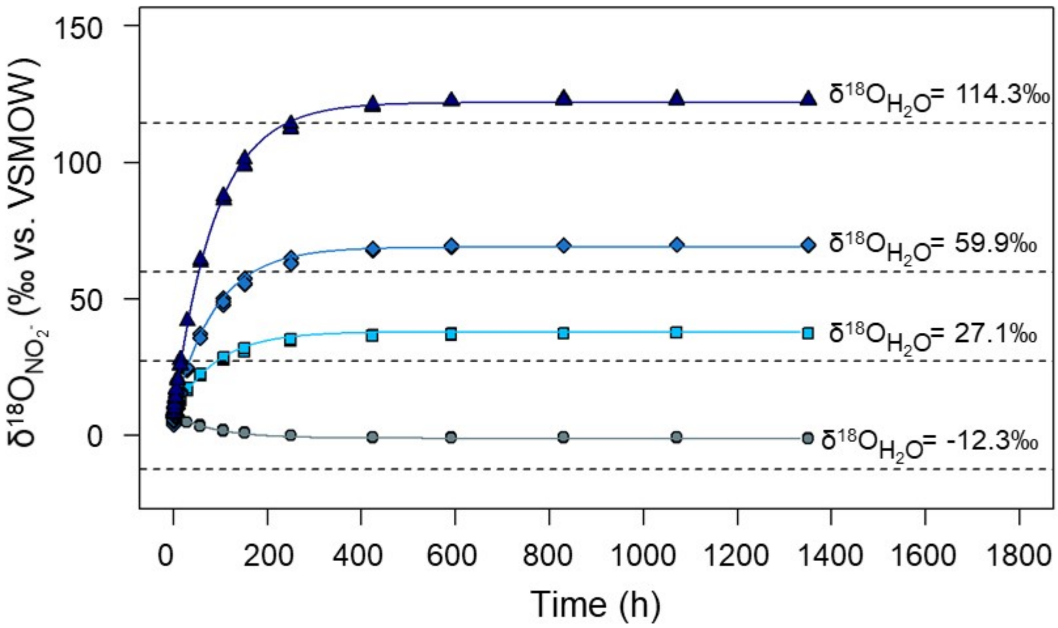


Fig. 1 Time course behavior of the $\delta^{18}O_{NO2-}$ values in the abiotic oxygen isotope exchange experiments

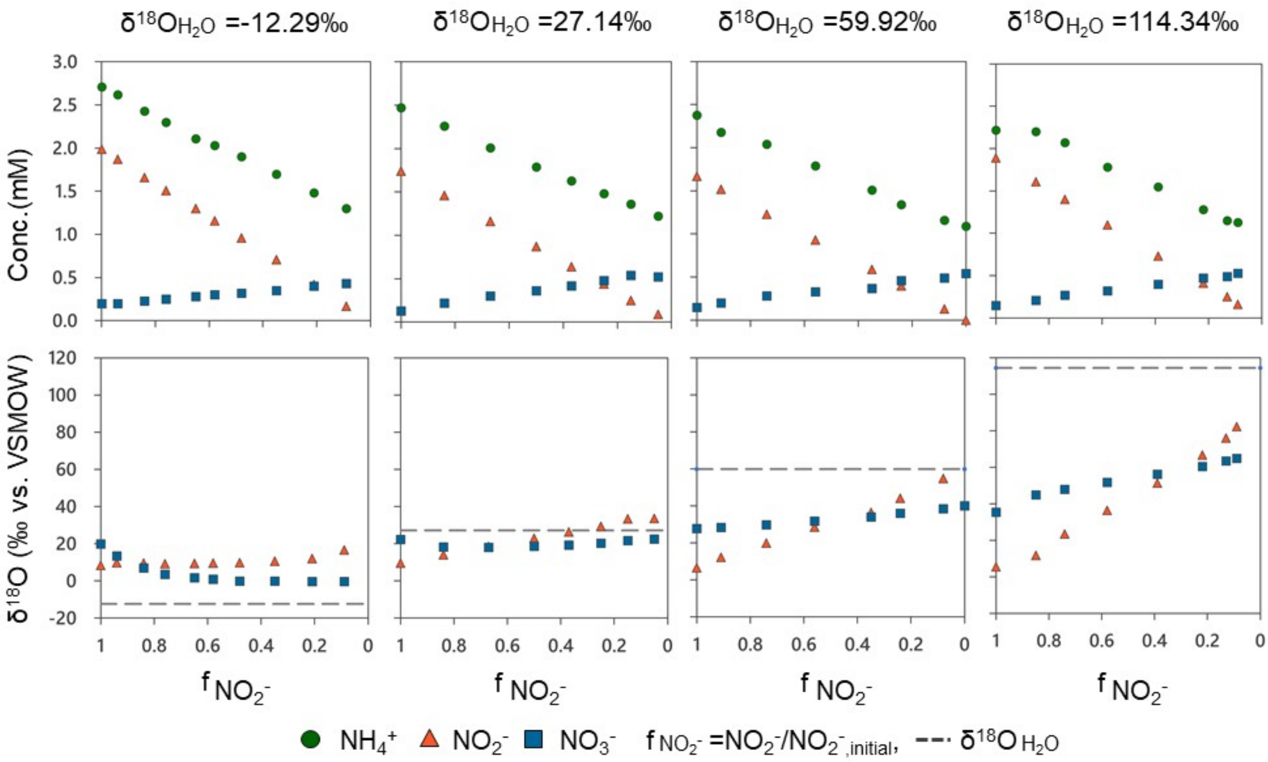


Fig. 2 Anammox stoichiometry, and time course behavior of the $\delta^{18}O_{NO2-}$ and $\delta^{18}O_{NO3-}$ value during batch experiment.