Goldschmidt 2023 Abstract
https://doi.org/10.7185/gold2023.18961

Rare methane isotopologue signals produced by mixed methanotrophic populations from a subtropical freshwater reservoir

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Methanotrophy plays an effective role in reducing the export of methane from subsurface environments to the atmosphere, attenuating greenhouse effect over geological and contemporary time scales. In terrestrial, freshwater environments, sulfate is often scarce, thereby facilitating methane oxidation potentially coupled with the reduction of oxygen or other electron acceptors. Clarifying the pathways and regulatory factors of methanotrophy using stable isotope approaches remains challenging as multiple community members and electron acceptors could have generated comparable isotopic patterns. In this study, we measured the abundances of doubly substituted methane isotopologues and companion community compositions derived from the incubations of mixed methanotrophic populations previously enriched with methane under anoxic conditions from the Feicui reservoir in Taiwan. The incubation experiments were carried out with ~100% methane under anoxic conditions and 40% methane and 60% oxygen under oxic conditions. The bulk isotope patterns for anoxic and oxic conditions were similar in that both $\delta^{13}C$ and $\delta^{2}H$ values of methane increased through time. Accordingly, the apparent fractionation factors (α) were calculated to be 0.991–0.996 for carbon, and 0.908–0.982 for hydrogen. In contrast, $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$ variation patterns for anoxic and oxic conditions were distinct. The values for anoxic conditions varied along the equilibrium line at temperature sectors higher than the life limit, whereas the values for oxic conditions were distributed above the equilibrium line. The isotopologue fractionation factors (γ) ascribed to the deviation from the rule of geometric mean were close to the unity for both isotopologues under anoxic conditions, and were 1.000 for $^{13}CH_3D$ and 0.977 for $^{12}CH_2D_2$ under oxic conditions. Methanotrophic populations under anoxic conditions shifted from the initial coexistence of types I and II methanotrophs to the predominance of type II methanotrophs at the end. For comparison, type I methanotrophs dominated over type II ones under oxic conditions. Our results combined with previous results for anaerobic methane oxidation coupled with sulfate reduction demonstrate that the coupled rare methane isotopologues could be an effectively diagnostic tool to distinguish the pathways and even populations of methane oxidations under different environmental conditions.