

博士論文の内容の要旨

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論文題目	Carbon cycle and interactions of surface hydrosphere and deep-subsurface: key molecule and isotopic ratio approaches (バイオマーカーおよび同位体比分析を用いた表層水圏および深部地下圏における炭素循環と相互作用の解明)

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Microorganisms play a role in the hydrosphere's carbon cycle by producing, decomposing, and recycling organic matter. Therefore, it is important to understand microorganisms' functions for solving environmental problems such as eutrophication and global warming. In this thesis, we firstly assessed microorganism's functions on the carbon cycle in the surface and deep subsurface hydrosphere using microbial-specific compounds (biomarkers) and their isotopic ratios; i) origin and fate of dissolved organic matter in lake water, ii) foraging strategy of zooplankton in oligotrophic pond, iii) deep methanogenesis in an organic- and iodine-rich aquifer, iv) planktonic microbial methane production in a eutrophic lake. Although the carbon cycle in these hydrospheres has been studied independently, it is thought that there is an interaction between the surface and the deep subsurface hydrosphere. We elucidated the interaction of the carbon cycle between the surface and deep subsurface hydrosphere.

Dissolved organic matter (DOM), accounting for more than 95 % of the total organic carbon in the hydrosphere, plays an essential role in the surface hydrosphere's carbon cycle. However, the source and formation of high-molecular-weight dissolved organic matter (HMW-DOM) and its interaction with other organic components such as particulate organic matter (POM) and low-molecular-weight DOM (LMW-DOM) have been poorly understood so far. To elucidate DOM's origin and interactions, we performed the sterol compositions and their isotopic ratio analyses for phytoplankton, POM, HMW-DOM, and total-DOM isolated from three lakes in different aquatic environments. 24-ethylcholesta-5,22-dien-3 β -ol (stigmaterol) was dominant in HMW-DOM, which was the different trend in other components, suggesting that HMW-DOM has a different origin and interaction from other components. Moreover, from the carbon and hydrogen isotope analysis, we reveal autochthonous algae as a significant source of the abundant stigmaterol in the lakes. These results suggest that HMW-DOM in summer is derived from autochthonous phytoplankton rather than allochthonous terrestrial plants and that HMW-DOM likely has few interactions with POM and LMW-DOM in the lake water.

Phytoplankton and zooplankton, the primary source of DOM, are the most important primary producers and primary consumers in lake water ecosystems. The biomass of the primary consumers is limited to that of the primary producers, while it has been reported that many zooplankton are present in lakes where the biomass of phytoplankton is extremely low. In this study, trophic position (TP) of the daphnia *Daphnia longispina* and the copepod *Acanthodiaptomus pacificus* in an oligotrophic lake, Shirakoma-ike, was investigated via stable nitrogen isotope composition analysis of amino acids, to understand the foraging strategy of these two dominant freshwater zooplankton species in an oligotrophic lake. The surface water of this lake is frozen in winter, and the life cycle and trophic behavior are different between these two species: the daphnia is found only in spring-autumn, whereas the copepod is found in a whole season. We found that the TP is 2.1 ± 0.0 for the daphnia in spring-autumn and 2.3 ± 0.3 for the copepod in a whole season. These results reveal strong herbivory for the daphnia compared to dietary plasticity and facultative omnivory for the

copepod. The latter is suggested to feed on phytoplankton for spring and autumn (TP=2.1±0.0) and on both phytoplankton and heterotrophic microbes for summer and winter (TP=2.6±0.0). The foraging strategy is thus different between daphnia and copepods in this pond.

The surface hydrosphere is a eukaryotic-dominated ecosystem, while the deep subsurface hydrosphere is a prokaryotic-dominated ecosystem. Methanogenic archaea produce methane in an anaerobic environment, which is the final step in the decomposition of organic matter. Although insights into methanogenesis and the methane cycle in the deep subsurface are important for understanding early life development on Earth, the ecology of deep subsurface environments is still unclear because of a limited number of surveys utilizing culture-based approaches. We performed coenzyme factor 430 (F430) analysis and radiocarbon measurements to identify deep microorganisms, including methanogenic archaea, and assessed the origin of methane in a deep organic- and iodine-rich aquifer in a forearc basin at the Southern Kanto gas field, Boso Peninsula, Japan. Both lipid analysis and small-subunit rRNA gene sequencing indicated that the biomass of domain archaea was less than 10% of the total prokaryotes, while methanogenic archaea (e.g., *Methanomicrobia*) was detected in the gene sequencing. High concentrations of native F430 (1.67×10^4 femto mol/L) in the absence of the F430 epimer strongly suggested high active methanogenesis potential mediated by the subsurface microbes. Radiocarbon measurements of methane and dissolved inorganic carbon (DIC) revealed ^{14}C -depleted (both $\Delta^{14}\text{C}_{\text{methane}}$ and $\Delta^{14}\text{C}_{\text{DIC}}$, <-997.4 ‰), suggesting that the entire deep habitat and methanogenesis represents an isolated subterranean microbial ecosystem.

Deep methane is potentially transported to the surface hydrosphere and affects the surface carbon cycle; however, it is poorly understood how deep methane affects the carbon cycle in the surface hydrosphere. We quantitatively evaluated the impacts of deep methane on the carbon cycle in a eutrophic lake's surface hydrosphere using the radiocarbon measurements. The $\Delta^{14}\text{C}$ of deep methane and carbon dioxide seeping from the bottom of the lake were depleted ($\Delta^{14}\text{C}_{\text{methane}}$, <-972.1 ‰), and DIC and cyanobacterial bloom (Aoko) collected from surface lake water contained ^{14}C -depleted carbon ($\Delta^{14}\text{C}_{\text{DIC}}$, -630.6 to -103.1 ‰ and $\Delta^{14}\text{C}_{\text{aoko}}$, -110.3 ‰), suggesting that 10–60% of DIC is affected by deep carbon, which is also propagated through phytoplankton into lake ecosystems. Also, we detected F430 in cyanobacterial bloom ($6.8\text{--}35 \times 10^2$ femto mol/g-wet), suggesting the coexistence of methanogenic archaea with cyanobacterial bloom in the lake water under aerobic conditions. This result may provide insight into the methane paradox, in which the accumulation of methane under aerobic conditions has been reported in oceans and lakes.