

## Linking stoichiometric organic carbon–nitrogen relationships to planktonic cyanobacteria and subsurface methane maximum in deep freshwater lakes

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Our understanding about the source of methane (CH<sub>4</sub>) in freshwater ecosystems is being revised because CH<sub>4</sub> production in oxic water columns, a hitherto inconceivable process of methanogenesis, has been discovered for lake ecosystems. The present study surveyed nine Japanese deep freshwater lakes to show the pattern and mechanisms of such aerobic CH<sub>4</sub> production and subsurface methane maximum (SMM) formation. The field survey observed the development of the SMM around the metalimnion in all of the study lakes. Generalized linear model (GLM) analyses showed a strong negative nonlinear relationship between dissolved organic carbon (DOC) and dissolved inorganic nitrogen (DIN), as well as a similar curvilinear relationship between DIN and dissolved CH<sub>4</sub>, suggesting that the availability of organic carbon controls N accumulation in lake waters thereby influences the CH<sub>4</sub> production process. The microbial community analyses revealed that the distribution of picocyanobacteria (i.e., *Synechococcus*), which produce CH<sub>4</sub> in oxic conditions, was closely related to the vertical distribution of dissolved CH<sub>4</sub> and SMM formation. Moreover, a cross-lake comparison showed that lakes with a more abundant *Synechococcus* population exhibited a greater development of the SMM, suggesting that these microorganisms are the most likely cause for methane production. Thus, we conclude that the stoichiometric balance between DOC and DIN might cause the cascading responses of biogeochemical processes, from N depletion to picocyanobacterial domination, and subsequently influence SMM formation in lake ecosystems.

Keywords: dissolved organic carbon, Dissolved inorganic nitrogen, phosphonate, subsurface methane maximum, stoichiometry, *Synechococcus*