

# **Absence of nitrate assimilation ability in *Synechococcus* revealed by metagenomics and NanoSIMS analysis in the HNLC subarctic Pacific Ocean**

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*Synechococcus* is a cosmopolitan picocyanobacterium inhabiting waters from the tropics to the poles and contributes substantially to marine primary production. To adapt to diverse environments, *Synechococcus* lineages are known to gain and lose functional genes, resulting in metabolic differentiation within the genus. However, most studies have focused on subtropical regions, and their ecological adaptations in high-latitude High Nutrient Low Chlorophyll (HNLC) regions remain poorly explored. Importantly, metabolic potential inferred from metagenomic data is rarely validated in situ.

Here, we combined comparative metagenomics with single-cell measurements of nitrogen assimilation to investigate the survival strategy of *Synechococcus* in the subarctic HNLC Pacific Ocean. Metagenomic mapping and pathway enrichment analyses revealed that the major genomic differences between the subarctic HNLC and subtropical nitrogen-depleted regions are associated with nitrogen metabolism and molybdoprotein synthesis. Specifically, *Synechococcus* populations in the subarctic HNLC region largely lack genes required for nitrate assimilation, including those for nitrate/nitrite transport, reduction, and associated molybdenum cofactor, while possessing non-canonical ammonium transporter genes. Consistently, NanoSIMS analysis demonstrated that picophytoplankton, including *Synechococcus*, actively assimilated ammonium but showed minimal nitrate uptake in the subarctic HNLC region, likely minimizing cellular iron demand. This contrasts with subtropical populations, where *Synechococcus* preferentially utilizes nitrate under nitrogen-depleted conditions. The differential nitrogen preference under contrasting nitrogen and iron availability may also reflect the absence of competition for ammonium with *Prochlorococcus* in subarctic waters. Such metabolic flexibility, including the loss of nitrate assimilation capacity, likely underpins the ecological success and broad biogeographic distribution of *Synechococcus*.