

**Changes in relative nitrogen:phosphorus requirements for  
phytoplankton growth with absolute nutrient levels and their  
macromolecular basis**

Mengqi Jiang

Abstract:

**Introduction**

Nitrogen (N) and phosphorus (P) are two predominant elements utilized in the biochemical functions of phytoplankton, and the main nutrients limiting phytoplankton growth in aquatic ecosystems. Clarifying the pattern of relative N-to-P requirements for phytoplankton growth is of great significance for eutrophication mitigation and management of aquatic systems. Relative N-to-P requirement for phytoplankton growth is considered as an essential trait determining species dominance within ecosystems and explaining phytoplankton response to nutrient availability. These requirements vary with environmental trophic statuses, though this variation remains unclear. Moreover, the underlying macromolecules basis of the relative N-to-P requirement for phytoplankton growth was also poorly understood. The aim of the present study is to clarify the pattern

of relative N-to-P requirement for phytoplankton growth under different trophic statuses, and to discuss about the underlying macromolecules basis.

## **Materials and methods**

In order to clarify the pattern of relative N-to-P requirement for phytoplankton growth under different trophic statuses, we evaluated the relative N-to-P requirements under different absolute nutrient levels using previous and current experimental data on eight phytoplankton species (three studied by us [*Chlorella vulgaris*, *Anabaena variabilis*, and *Microcystis aeruginosa*] and five extrapolated from the previous studies).

To study the underlying macromolecules basis of the relative N-to-P requirements for phytoplankton growth, *C. vulgaris* was used as a phytoplankton model to verify the growth-rate hypothesis (GRH). Growth parameters, including OD<sub>680</sub>, chlorophyll, and biomass carbon (C), were used to assess the responses of *C. vulgaris* to various trophic statuses. Furthermore, cellular macromolecules (protein, RNA, and major pigments) were measured to determine whether the GRH can be applicable to phytoplankton growth under low-nutrient conditions and the macromolecular basis underlying phytoplankton N:P requirements.

## **Results**

Relative N-to-P requirements for phytoplankton growth decreased as absolute nutrient levels increased. Thus, N may be crucial for enhancing phytoplankton growth under low trophic statuses, whereas P may be the primary limiting factor of phytoplankton growth under sufficient nutrient conditions. This result applies to single species as well as species assemblages, which are independent of species shifts occurring along water N:P gradients.

Results also indicated that *C. vulgaris* biomass correlated positively with biomass N and protein content under P starvation, though *C. vulgaris* biomass had insignificant relationship with biomass P and RNA concentrations under N starvation. Reduced RNA content under P starvation sustained protein synthesis and biomass production during sufficient N supply. Structural equation modeling (SEM) revealed that protein acted as the essential mediator when chlorophyll was used as a proxy for *C. vulgaris* biomass; it was not essential with biomass C as the proxy.

## **Discussions**

Results of the present study demonstrated that the shift from N requirements to P requirements is not clear, but it varies depending on trophic status, with lower nutrient supply leading to higher N requirements than P requirements. This conclusion may help identify which element, N or P, would be firstly reduced for mitigating eutrophication in aquatic ecosystems.

Further, we additionally demonstrated the inapplicability of GRH for *C. vulgaris* under N starvation and the higher requirement for N than P under low nutrient levels, addressing the potential causality of N, protein, and biomass under P starvation. Our results indicated that N, rather than P, would be the primary element limiting phytoplankton growth under low-nutrient conditions. If this phenomenon is applicable for all phytoplankton species, it could be valid for controlling phytoplankton biomass in aquatic ecosystems, and could improve biomass management of blooming phytoplankton by emphasizing a specific nutrient.