The effect of nutrient supply dynamics on the competition between *Synechococcus* sp. and *Micromonas pusilla*

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ABSTRACT

Picophytoplankton constitute the most abundant functional group in the ocean, often dominate planktonic biomass and primary production in tropical and subtropical areas, and they represent a substantial contribution to the global export of carbon. We have a limited understanding about the factors that control picophytoplankton community structure. Previous studies have identified that light and temperature are the main factors explaining *Prochlorococcus* and *Synechococcus* distributions, whereas nutrient concentration would only play a minor role. However, nutrient concentrations are not necessarily representative of actual nutrient supply rates in the oligotrophic ocean. To test the role of nutrient supply dynamics in controlling picoplankton community structure, we maintained mixed cultures of the cyanobacterium *Synechococcus* and the picoeukaryote *Micromonas pusilla* growing under continuous nutrient limitation (chemostats) but experiencing different regimes of intermittent nutrient pulses. Here we describe the resulting competitive dynamics, as well as the nutrient uptake kinetics of the two species. Our results suggest that the dynamics of nutrient supply play a role in controlling the abundance of different groups of picophytoplankton in the ocean.

INTRODUCTION

Prochlorococcus, *Synechococcus* and the picoeukaryotes constitute the picophytoplankton (cells with diameter < 2 µm), the most abundant and smallest component of the phytoplankton community. As a functional group, picophytoplankton are relevant for global carbon exportation [1] and dominate phytoplankton biomass and primary production in the vast oligotrophic ocean. Therefore, picophytoplankton are a key component to understand nutrient cycling in marine ecosystems.

The factors that control the distribution and abundance of different picophytoplankton groups remain to be understood. Previous correlation studies revealed the importance of temperature and light in global picoplankton distribution [2]. However, the role of nutrients has not been adequately assessed, due to the difficulty to measure nutrient supply in the field and the fact that nutrient concentrations and supply rates are often disconnected in oligotrophic waters [3].

Both physiological studies [4] and nutrient competitive experiments in other groups of plankton [5] indicate that nutrient injection is a strong ecological determinant of the taxonomic composition of eukaryotic phytoplankton. A conceptual framework relating turbulence, nutrient supply and taxonomic selection was proposed by Ramon Margalef in the 70's [6], although the applicability of this fundamental principle to picophytoplankton has been little explored. To contrast the hypothesis that nutrient supply dynamics affects picophytoplankton community structure, we followed 2 approaches. First, we conducted experiments in chemostats to investigate the competitive dynamics between *Synechococcus* and *Micromonas pusilla* under different regimes of nutrient-limited growth. Second, we characterized the kinetics of nutrient uptake under steady–state, nutrient-limited growth.

MATERIAL & METHODS

Two ultraoligotrophic picoplankton strains were selected from North Atlantic Gyre: A picoeukaryote (*Micromonas pusilla*, RCC450) and a cyanobacteria (*Synechococcus sp.*, RCC2366). The strains were obtained from the Station Biologique De Roscoff. Both cultures grew in a modified PCR-S11 medium, in which ammonium was replaced by nitrate and the f/2 vitamin stock was added. To ensure nitrogen limitation, the N/P ratio was fixed to 5. Dissolved inorganic nitrogen final concentration in the medium was 50 µM. Cultures were acclimated in the culture chamber for 3 weeks to this medium in experiment conditions (21ºC and a PAR of 115 µmol photons/m^2/s).

Strains were introduced in independent chemostats (*Sartorius Biostat Plus)* and when the cultures reached steady state condition we conducted the uptake experiments. The dilution rate was 0.225 d^{-1}. Cells were enumerated using a FACSCalibur flow cytometer (Becton Dickinson Instruments) [7].

Nutrient uptake curves were estimated using short-term independent incubations. Bulk nitrate was measured in 7 incubations (0.5, 1, 1.5, 2.5, 5, 10, 25 µM) for 40 min [8]. Bulk nitrate samples were obtained by a gentle filtration of medium onto Nylon filters (0.45 µm nominal diameter). Samples were frozen until analysis in SFA (Segmented Flow Analyzer).

The uptake kinetics parameters (V_{max} and K_s) were calculated assuming a Michaelis-Menten model [9].

After the steady state was achieved again, cultures were gently mixed, using a peristaltic pump and autoclaved tubing.

Competition for nitrate was tested under steady state conditions and nitrate pulse conditions. Nitrate spike magnitude was constant in the experiment (5 µM) but different spike frequency was used (0.5 and 2 day^{-1}) to represent different degrees of perturbation.

RESULTS & DISCUSSION

- Chemostat experiments

Under steady state conditions, the abundance of *Synechococcus sp.* increases steadily, whereas that of *M. pusilla* decreases. Thereafter, population dynamic was altered by providing pulses of nitrate. Deviation from steady-state nitrate supply rapidly switched the outcome of competitive interactions, allowing *M. pusilla* to increase its abundance (Fig 1). These results were consistent in two independent experiments.

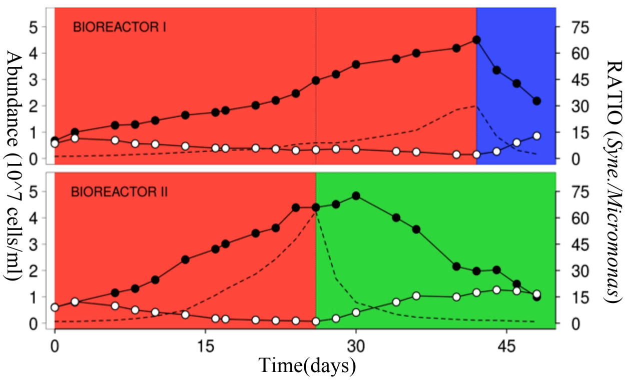

Fig. 1. Population abundance of *Micromonas pusilla* (○) and *Synechococcus sp.* (●) competing under nitrate-limiting (red area) and two different nitrate-pulsing conditions: 0.5 spikes day^{-1} (green) and 2 spikes day^{-1} (blue). The dotted line represents the *Synechococcus*:*Micromonas* ratio.

- Nitrate uptake experiments

Maximum uptakes rates (V_{max}) were 1.4 femtograms N/cell/h for *Synechococcus* and 12.5 femtograms N/cell/h for *Micromonas pusilla*. These values are slightly higher than previous studies obtained in batch cultures [4]. Semi-saturation constants (Ks) were 1.35 µM for *Micromonas pusilla* and 0.45 µM for *Synechococcus* sp. Uptake kinetic parameters vary with physiological state, previous nutrient status, and phytoplankton species, thus complicating the interpretation of field results or even senescence experiments, where the physiological state of collected organisms is generally unknown [10,11]. Conducting these experiments in steady state, nutrient-limited condition is crucial to determine nutrient uptake kinetics that are representative of field conditions in ultraoligotrophic environments.

Our results suggest that the variability of nutrient supply dynamics (our nutrient spikes) play a role in controlling the relative dominance of different picophytoplankton functional groups in the oligotrophic ocean.

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