MIXED INDICATIONS OF MINERAL NUTRIENT LIMITATION IN A MICROCOSM EXPERIMENT USING EASTERN MEDITERRANEAN SURFACE WATER.

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Abstract

Microbial food web responses to additions of phosphate, nitrate, and glucose were investigated in a microcosm experiment using eastern Mediterranean surface water. Glucose alone gave no stimulation of bacteria, and there was free, bioavailable silicate. The food web thus appeared not to be constrained by carbon limitation of bacteria or by silicate limitation of diatoms. Adding phosphate alone led to an increase in both chlorophyll and primary production, suggesting the presence of a bioavailable N-reserve of ≈ 800 μmol-N m^{-3}. Turnover-time for orthophosphate, however, increased when phosphate doses > 10 μmol-P m^{-3} were added, and decreased when nitrate was added alone, resembling the pattern expected for N-limitation. Mixed indications as to whether the osmotroph organisms were phosphate or nitrogen-limited were thus found.

Keywords: Eastern Mediterranean, phosphorus, phytoplankton, bacteria

Deep water nitrate:phosphate ratios in the eastern Mediterranean basin have been shown to be well above Redfield (1) Combined with the observation that surface heterotrophic bacterial communities in the eastern basin (2), and phytoplankton as well as bacterial communities in the north-western Mediterranean (3) appear phosphate-limited, this has led to the suggestion that the Mediterranean surface ecosystem may be relatively simple in the sense that osmotrophs are limited by phosphate, rather than nitrogen, organic carbon, silicate, or combinations of these (4) (Fig. 1). As part of a more critical investigation of these suggested relationships, we here report results from a microcosm study designed primarily to test the response to phosphate addition of the microbial ecosystem in eastern Mediterranean surface water.

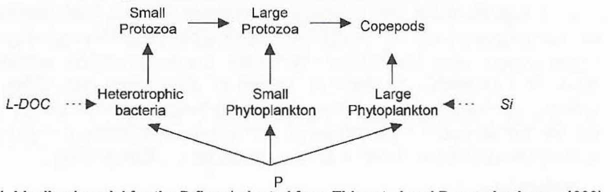

Fig. 1. Idealised model for the P-flow (adapted from Thingstad and Rassoulzadegan ,1999) proposing that, in the Mediterranean, the system is particularly simple with all osmotrophs (heterotrophic bacteria, small and large phytoplankton) are P-limited. If so, P-flow should not be constrained by labile DOC (L-DOC) limiting bacterial growth rate, silicate (Si) limiting diatom growth rate, or nitrogen limiting any of the three osmotroph groups.

Experimental design and rationale

Seawater pumped from 10m-depth 30 km west of Haifa during May 14 2000, was filtered on 125 μm mesh plankton nets to remove large grazers, and dispensed into 25L high-density polyethylene carboys that had been pre-treated with seawater, acid washed, and rinsed with seawater from the sampling station. The carboys were incubated in a circulating seawater (21°C) tank under subdued natural light conditions. Nutrient additions were made as single doses on Day 0 (May 15), according to the scheme in Table 1. Chl-a was determined by HPLC, primary production from ^{14}C-incorporation in light-dark bottles incubated at mid-day, and orthophosphate turnover-time from incorporation of ^{33}P after short-term incubation with $H_3{}^{33}PO_4$. Based on the hypothesis of a P-limited state of the food web, the four treatments with increasing phosphate dose (#2, #3 and #4) were expected to reveal an increasing response relative to the untreated control (#1). For a sufficiently large P-dose, the system was expected to be shifted into N-deficiency. In this case, continued growth was expected in the corresponding Treatments #6, #7, or #8, supplied with excess nitrate-N. Treatment #9 was given glucose as a negative control to confirm the expectation of no bacterial response to the addition of a degradable carbon source.

Results and discussion

No response could be found in bacterial biomass or activity to the addition of glucose alone (#9, data not shown. As evidenced by the diatom bloom induced, the free silicate in the collected water (1.1 mmol-Si m^{-3}) was bioavailable. Together, these observations support the hypothesis that the system was not constrained, neither by organic-carbon limitation of heterotrophic bacteria on the left, nor by silicate limitation of diatoms on the right side of Fig. 1. An increasing response to increasing phosphate-alone addition was found in both chlorophyll and in primary production (Fig.2). Saturation of the response occurred for additions around 50 μmol-P m^{-3}. Assuming a Redfield ratio of 16:1, this corresponds to the mobilisation of an N-reserve around 800 μmol-N m^{-3}. However, adding nitrate alone (#5) also had a stimulatory effect on both primary production and chl-a (Fig.2). Also, turnover-time of orthophosphate increased in carboys with phosphate added alone in doses >10 μmol-PO_4 m^{-3} (Fig. 3A), while adding nitrate alone (Fig. 3B) led to a decrease in turnover-time. Except for the absence of significant effects from adding 10 μmol-PO_4 m^{-3} alone (#2), the response-pattern seen in orthophosphate turnover-time (Fig.3) corresponds to what would be expected in a system with nitrogen-limited osmotrophs stimulated in their P-uptake by the added nitrate.

Table 1. Nutrient additions to experimental carboys in µmol P, N or C m-3. Additions made as single additions of orthophosphate, nitrate or glucose on Day 0 to triplicate carboys.

P alone	#1:P:0	#2:P:10	#3:P:50	#4:P:500
Excess N	#5:P:0,N:1000	#6:P:10,N:1200	#7:P:50,N:2000	#8:P:500,N:1100
Glucose	#9:P:0,N:0,C:10000			

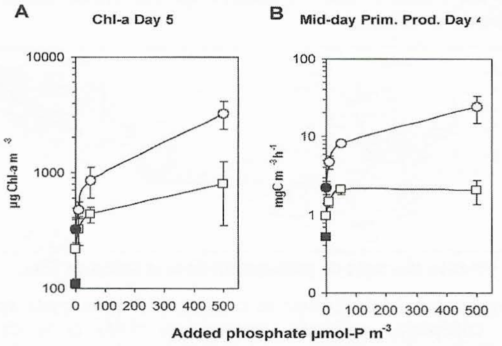

Fig. 2. (A) Chl-a on Day 5, and (B) mid-day primary production on Day 4 as functions of phosphate added (O)alone, or (O)in combination with excess nitrate. Nitrate alone (#5) indicated by filled circle and value on Day 0 by filled square. Error bars represent SE of mean of triplicate carboys. Note logarithmic scale on y-axes.

Conclusions

Our experiment supported the suggestion that neither organic-carbon limitation of bacteria nor silicate limitation of diatoms constrained the functioning of the photic zone microbial food web. As hypothesised, we also found a positive phytoplankton response from adding phosphate alone. Seen in isolation, these results suggest a P-limited system with a bioavailable N-reserve in the order of 800 μmol-N m^{-3}. This simple picture was however complicated by a response both in chl-a and in primary production to adding NO_3 alone (#5), an additional effect of nitrate in all treatments with NO_3 and PO_4 together (#6, #7 and #8), and a response pattern in orthophosphate turnover-time resembling that expected under N-limitation. The possible role of N as a co-limiting factor can thus not be ruled out. Interpretation was complicated by changes in the control treatment (#1, no additions). Sampling, prefiltration, and confinement, alone or in combination, may thus have disturbed the state of the food web present in the collected water. Two Lagrangian experiments are planned (May 2001, May 2002) that will be unaffected by these problems.

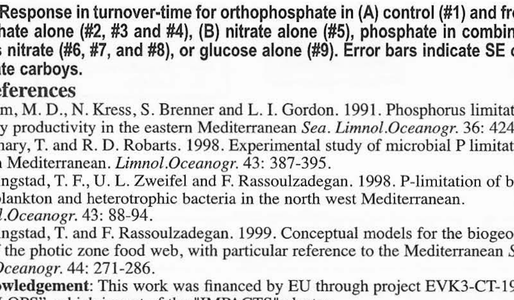

Fig. 3. Response in turnover-time for orthophosphate in (A) control (#1) and from adding phosphate alone (#2, #3 and #4), (B) nitrate alone (#5), phosphate in combination with excess nitrate (#6, #7, and #8), or glucose alone (#9). Error bars indicate SE of mean of triplicate carboys.

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