Effects of Anthropogenic Nutrient Loading on Nitrogen and Phosphate Limitation of phytoplankton from the West Falmouth Harbor and Quashnet River Estuaries

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Abstract

High levels of nitrate loading to the Mashapaquit Creek and West Falmouth Harbor are causing changes in the composition of primary producers and the amount of annual net primary production (Howes, 2005). While the Quashnet River system experiences some level of anthropogenic nutrient loading from its watershed, nitrate concentrations in the incoming groundwater are not anywhere near as high (Table 1). Microalgal community response to nutrient loading in these two systems was examined with microcosm nutrient enrichment experiments (fertilization with nitrogen, phosphorous, or both) with water sampled along a salinity gradient in the two sites; biomass and dissolved inorganic nutrient concentrations were quantified at the end of a 21 day incubation period in a growth chamber. Phosphorous enriched microcosms failed to produce meaningful outcomes for this experiment but results from the nitrogen enrichments suggest that phytoplankton from the Mashapaquit Creek may have internal stores of nitrate from before they were removed from their natural environment.

Introduction

Anthropogenic nitrogen loading to coastal estuaries is a subject of particular interest because nutrient inputs can cause complex changes in nitrogen limited brackish systems (Valiela, 1995). Fertilization can increase net primary production of both macro and microalgae and eutrophication of coastal systems can lead to shifts in species composition and diversity, hypoxia in stratified bottom waters (leading to fish kills), and release of the greenhouse gas N_2O (Lee and Valiela et al., 1997) so understanding the processes affecting primary producers in systems with high nutrient loading can be important in assessing the health of a system. The major source of nitrate in the watershed of the West Falmouth Harbor (WFH) comes from effluent irrigation at the Falmouth Wastewater Treatment Plant (FWTP) though there are also non-point sources coming from septic systems and lawn fertilizers (Howes et al, 2005). These non-point sources also play a role in loading the Quashnet River with nutrients, however, the nitrate rich effluent plume from the FWTP do not influence this system.

To examine nutrient limitation on phytoplankton growth in the Mashapaquit Creek (MC) and the Quashnet River (QR) I sampled water along a salinity gradient of 0-27ppt and ran microcosm incubations with water from each site separated into control, nitrogen enriched, phosphorus enriched, and nitrogen and phosphorus enriched groups. It is generally true that phosphorus is most limiting in freshwater environments and nitrogen is most limiting in ocean or estuarine systems, as is exemplified in research done by Nina Caraco and her colleagues and advisors at the Boston University Marine Program at the Woods Hole Marine Biological Laboratory on phytoplankton nutrient limitation in Falmouth area brackish ponds along a salinity gradient (Caraco et al, 1987).

Ivan Valiela and collaborators from the Boston University Marine Program have done studies in recent years on macroalgal enzyme response to long term high nutrient exposure. Valiela and colleagues collected several species of macroalgae from sub-estuaries of the Waquoit Bay and tested their response to nutrient enriched exposure *in situ* and found that macroalgae from areas of high anthropogenic loading uptake nutrients more rapidly in enrichment experiments because of increased activity of nitrate reductase and glutamine synthetase (Valiela and Thompson, 1999; Teichberg et al, submitted).

Phytoplankton have high turnover rates so unlike macroalgae one individual organism does not generally show measurable adaptation to its environmental conditions within its lifetime but a mélange of genera of phytoplankton growing together as a community can show ‘evolutionary’ change on short time scales as those that are well adapted to the conditions can quickly reproduce, outnumbering and out-competing species less well adapted in a matter of days. Nuisance macroalgal blooms are not the only potential dangers in eutrophic systems, excess phytoplankton growth can also be toxic, cause hypoxia, or cause high turbidity that blocks out light for benthic producers, so understanding how phytoplankton respond to long term exposure to high nutrient loads is important to understanding the potential effects of eutrophy.

My collaborator Greg Henkes and I sampled groundwater from shore sampling wells along the Mashapaquit Creek and adjacent to Snug Harbor and I combined that with data on the groundwater inputs to the Quashnet River (Eng, personal communication) to show the relation of the nutrient concentrations found in the control incubations to the loading from groundwater inputs (Table 1).

Methods

We collected water from 4 sites along a salinity gradient in the Mashapaquit Creek at low tide as well as one fully saline site in the Snug Harbor portion of the West Falmouth Harbor (Fig 2), and 4 sites along a salinity gradient in the Quashnet River (Fig 1). A refractometer and hydrolab were used to measure salinity and other ambient conditions at each site just before water was collected in 20L carboys and transported back to the lab where it was kept refrigerated overnight.

Exact salinity of the samples was tested again the next day with a hydrolab immediately before the carboys were filtered through a 250μm screen (to remove zooplankton) into clean, clear, 2-liter soda bottles. For each site 2 bottles were left as control, 2 were fertilized with 5mL of 20,000μM NH_4NO_3 (for a final concentration of 100μM N), 2 were fertilized with 5mL of 40,000μM KH_2PO_4 (for a final concentration of 100μM P), and 2 were fertilized with both N and P (adapted from Caraco et al., 1987). Once all of the bottles were ready they were placed in on a shaker table, to keep the organic and inorganic matter well mixed, in a growth chamber where they received 14 hours of light a day on a regular schedule with a constant temperature of 15°C for 3 weeks.

At the end of the incubation the bottles were filtered through Whatman GFF filters to test for chlorophyll a which were immediately put into centrifuge tubes on ice in a dark cooler. After all filters had been on ice for at least 24 hours they were extracted in 35mL of 90% acetone and placed back in the dark cooler for 16-20 hours before they were read on a spectrophotometer (Lorenzen, 1966). Sub-samples were taken of the filtrate as well and preserved to later test for ammonium and phosphate colorometrically

(Solarzano, 1969 and Murphy and Riley, 1962 respectively) and nitrate using a Lachat flow injection analyzer (Wood et al. 1967).

Groundwater was hand pumped from permanent groundwater wells that range from ~1-9m deep and each has several pieces of tubing coming from different depths down the groundwater table. Samples were filtered through a swinnex with a 25mm diameter 47μm pore size GFF filter before being preserved for later analysis of nitrate and ammonium.

Results

DIN concentrations in groundwater seeps to the WFH were at least an order of magnitude higher than in the seeps to the QR (Table 1) and higher in the control incubations in the MC/WFH than in the QR (Figs 5, 6). Control incubation ammonium levels were highest in the MC1 site, closest to the ground well site that had by far the highest ammonium concentration in the seep. Control incubation nitrate levels were highest in the WFH site and the MC4 site, which were also closest to the seeps with the highest nitrate levels. Phosphate load from groundwater was higher in the QR, averaging around 17.2 uM (Eng, personal communication), than in the MC/WFH where it averaged about 0.66 uM (Kayfetz, unpublished data). Phosphate concentrations in the control incubations did not differ significantly between sites (Fig 7) and were much lower than concentrations input from groundwater in both systems.

Most sites showed a negative response to phosphorous alone enrichment with respect to the control while others showed a very small positive response compared to the control (Fig 4). With nitrogen enrichment (N) alone chlorophyll a concentrations were at least double the control concentrations and in the two most saline sites chlorophyll a concentrations were 5-10 times higher (Fig 4). Incubations fertilized with both nitrogen and phosphorous (N+P) had the highest levels of chlorophyll in all but the two most saline sites (Fig 3).

Ammonium concentrations in nitrogen fertilized incubations from sites in the MC/WFH were close to or lower than the levels present in the controls while in the QR they were always much higher in the nitrogen enriched incubations. Phosphate enriched incubations consistently had lower ammonium than the controls except in the two most saline sites (WFH ~27ppt and QR4 ~20ppt) while nitrogen and phosphorous enriched bottles also had lower ammonium than the controls with the exception of the freshwater sites (Fig 5). Except for the very high nitrate loading sites (MC4 and WFH), control and phosphorous enriched incubations had negligible concentrations of nitrate at the end of the incubation period; bottles enriched with nitrogen and phosphate almost all had less nitrate than the nitrogen alone enrichments (Fig 6).

Phosphate concentrations in phosphate enriched bottles were only lower than the initial concentration added (~100uM) in the mid-salinity MC sites (Fig 7) which were also the sites with the highest absolute growth of all phosphate treatments (Fig 3). Concentrations were also generally lower in N+P compared to just P enriched bottles and lower in N enriched bottles compared to the control (Fig 7).

Discussion

The correlation between DIN concentrations in seeps and differences in control incubation levels of ammonium and nitrate in the MC suggest that despite the tidal flushing that is characteristic of that system the groundwater inputs can have a localized effect; so it appears that a multi-site approach is essential for studying sites where the groundwater nutrient inputs vary so widely on a spatial scale. Spread between groundwater DIN concentrations in QR seeps was very small compared to the MC seeps and levels of nutrients in the QR control incubations varied more widely between replicates than between sites, suggesting that where groundwater inputs are similar along the seeps the spatial component within the estuary is less significant for seeing different responses.

Phosphorous enrichment alone seemed to inhibit growth more than promote it (Fig 4), probably because K_2HPO_4 was used for these fertilizations and K^+ has a very similar structure and charge to NH_4^+ so it is possible that the high levels of K^+ relative to NH_4^+ inhibited proper ammonium uptake. A survey of the literature found evidence that potassium free compounds, such as Na_2HPO_4, used to fertilize algal growth instead of K_2HPO_4 support growth even when many times the amount of phosphate used in this experiment was added while phytoplankton populations fertilized with K_2HPO_4 crashed when concentrations of the chemical were comparable to this experiment (Lehman, 1976). Interestingly, the same levels of monobasic potassium phosphate used in the P treatments did not inhibit growth in the treatments fertilized with both nitrogen and phosphorous; this is probably because relative NH_4^+ concentrations were high enough that ammonium uptake was not inhibited by the presence of K^+. Phosphate treatments must have had some growth response since phosphate was taken up to some degree in the MC sites and in most sites more ammonium and nitrate was immobilized in the microcosms treated with phosphate compared to the controls. Since the P treatments overall were not successful it is difficult not to confound the results of the N+P treatment with the potential results of a phosphorous alone treatment. However, growth response to the N+P treatment did steadily decrease with increasing salinity, which is consistent with the results that Nina Caraco and her colleagues saw with N+P fertilization along a salinity gradient and not with the P only treatment (Caraco et al., 1987).

Nitrogen only treatments in the two most saline sites were most successful compared to N treatments in the other sites (Fig 4) since nitrogen is generally the most limiting nutrient in seawater. Some of the fresher-water sites responded positively to N enrichment, though never as strongly as to N+P, and the responses were less consistent (there was a larger spread between replicates). In fact from the pattern of response to N+P enrichment, it is clear that phosphorous remained essential for maximum growth until salinities were above 10.4 at least, whether inherently or because of the demand initiated by a nitrogen fertilized bloom.

In the MC/WFH ammonium concentrations in the nitrogen and nitrogen and phosphorous fertilized treatments were close to or less than levels present in the control (Fig 5), even though the treatment was to increase the concentration by 50µM NH_4^+. While it seems from this data that the MC/WFH phytoplankton communities could have been more efficient in taking up ammonium than those in the QR it is impossible not to confound this result with the difference in biomass and therefore biological demand in the MC microcosms. It is possible that in microcosms from the freshwater sites less ammonium was taken up in the N+P bottles compared to the N bottles because N was a less limiting agent.

Nitrate was added to N and N+P incubations at 50µM and except for the MC4 and WFH microcosms, where control incubation nitrate concentrations were around 6 and 22 µM respectively, the available nitrate without fertilization was very low, especially compared to the amount added in the experiment. Interestingly, the final concentrations of nitrate in N treatments generally fell between about 5-15µM and the average nitrate concentration in the QR N treatments was lower than in MC N treatments. This alone may be the most telling piece of data as it shows that even though phytoplankton from the MC have been historically exposed to higher nitrate loading conditions (Table 1) and the fact that the MC microcosms had greater biomass (Fig 3) and should therefore show a higher biological demand for nutrients, microalgae from the QR took up more nitrate proportionally than microalgae from the MC. This may, in fact, be evidence that phytoplankton communities exposed to high nitrate concentrations in their native waters may have already begun to saturate their nitrogen storage capacity while nitrate starved phytoplankton have a greater capacity to withdraw and store nitrate when it is made available.

Conclusion

Examination and comparison of physiological characteristics of microalgae in nutrient loaded and pristine systems may be the next step to answering the questions that inspired this research as well as possibly some of the questions raised by the results. Enzyme response studies, like those being done on macroalgae in the Waquoit Bay sub-estuaries (Teichberg, submitted), could shed light on nutrient uptake dynamics of phytoplankton communities without the confounding factors of removing the algae from their environment or running microcosm experiments with highly variable initial populations. Even more simply, quantification of the C:N ratios of phytoplankton (while being careful to exclude zooplankton and microbes) in high nutrient waters and low nutrient waters could shed light on the capacity for phytoplankton to store nutrients and how that affects their growth response to changing conditions.

Acknowledgements

Thanks to Ken Foreman for advising this project, helping with some of my fieldwork, and constructing an awesome shaker-table platform for my growth chamber. In addition, Laura Whitman and Gregory Henkes for their indispensable help with other aspects of my field work and to Clara Funk, Jen Culbertson, Rich McHorney, and Beth Bernheardt for all of their guidance and assistance in the lab. Special thanks to the guys at the Falmouth Waste Disposal Plant for letting me dumpster dive in their recycling bin, Carolyn Solchenberger for her donation of a couple dozen Pepsi bottles, and to the Boston University Marine Program for donating the rest of the bottles necessary for my work. Finally a big thanks to all of the Ecosystems Center PIs who taught me in the SES course; I can not imagine being more satisfied with the knowledge and experience gained in a semester.

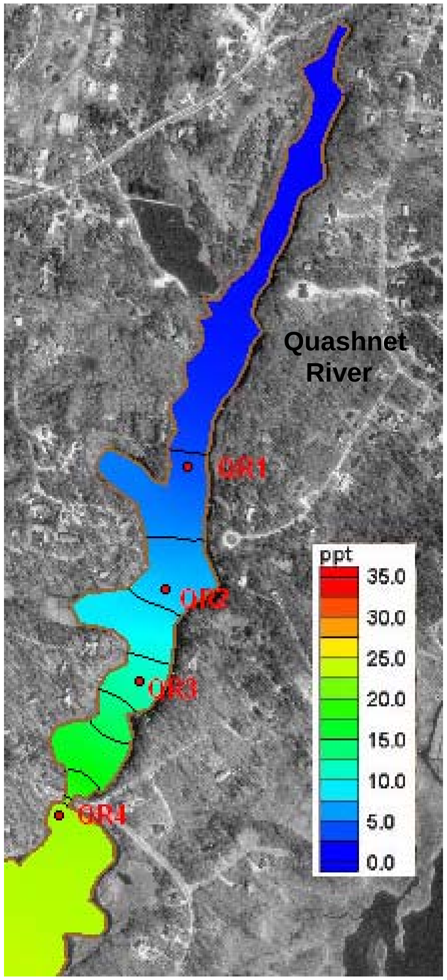

Figure 1. Salinity gradient in the Quashnet River with approximate sampling sites marked in red. (modified from Howes et al., 2004)

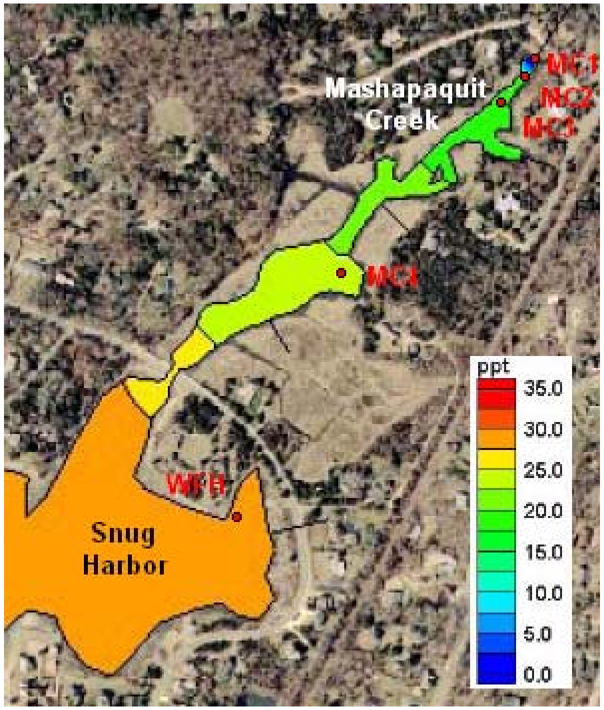

Figure 2. Salinity gradient in the West Falmouth Harbor Watershed with approximate sampling sites marked in red. (modified from Howes et al., 2005)

West Falmouth Harbor

Site	NH_4 (uM)	NO_3 (uM)
1.1	1.34	290
1.2	3.87	167
1.3	39.8	96.0
1.4	1.13	12.6
2.1	2.49	44.4
2.2	2.23	325
2.3	0.89	117
2.5	6.77	229
3.1	10.3	190
3.2	5.79	602
3.3	4.19	359
3.4	0.76	463
4.4	0.81	146
5.1	1.90	155
5.2	4.04	40.2
5.3	3.48	83.4
5.4	8.87	39.6

Quashnet River

Site	NH_4 (uM)	NO_3 (uM)
1 E	9.30	6.05
2 E	5.13	-
3 E	4.73	-
4 E	5.20	-
5 E	6.34	6.82
1 W	1.45	6.19
2 W	2.16	5.47
3 W	0.19	3.58
4 W	1.14	22.6
5 W	6.26	15.4

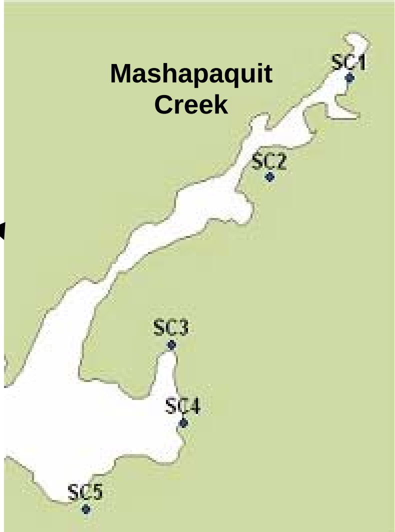

Table 1. Dissolved inorganic nitrogen (DIN) concentrations in the groundwater seeps to the Mashapaquit Creek/Snug Harbor and to the Quashnet River (Eng, personal communication). Well sites around the West Falmouth Harbor, shown on the map on the bottom right, each contain 4-5 sub-wells ranging from 1-9m deep and numbered in order of increasing depth. Quashnet River was sampled at 5 sites, approximately equidistant from one another, on both the east bank (E) and west bank (W); site 1 was closest to the headwaters and site 5 closest to the outlet into the Waquoit Bay.

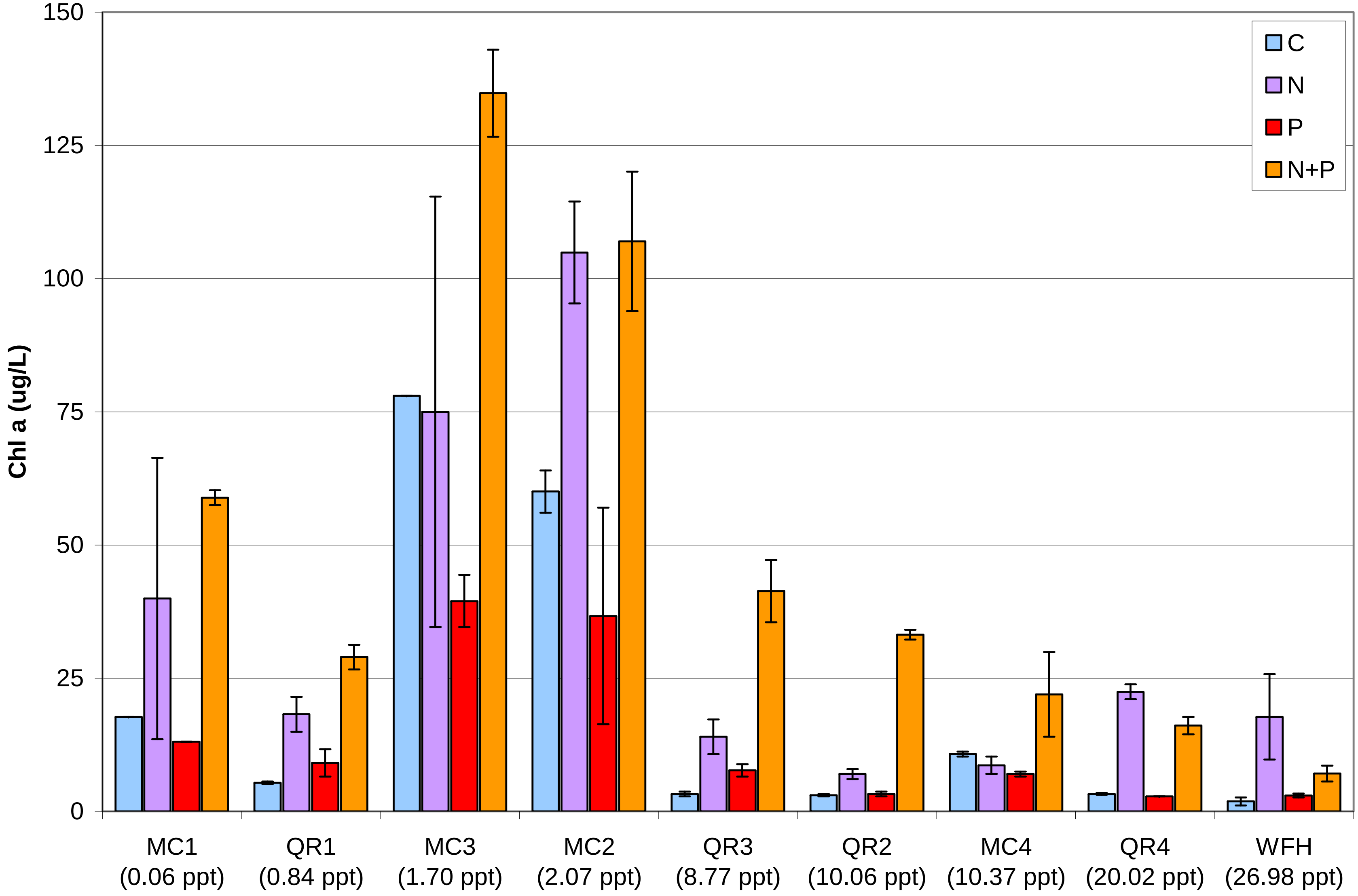

Figure 3. Average chlorophyll a in each treatment group at the end of the 21 day incubation period. Error bars show the spread of the two replicate treatments. Sites are arranged from lowest to highest salinity.

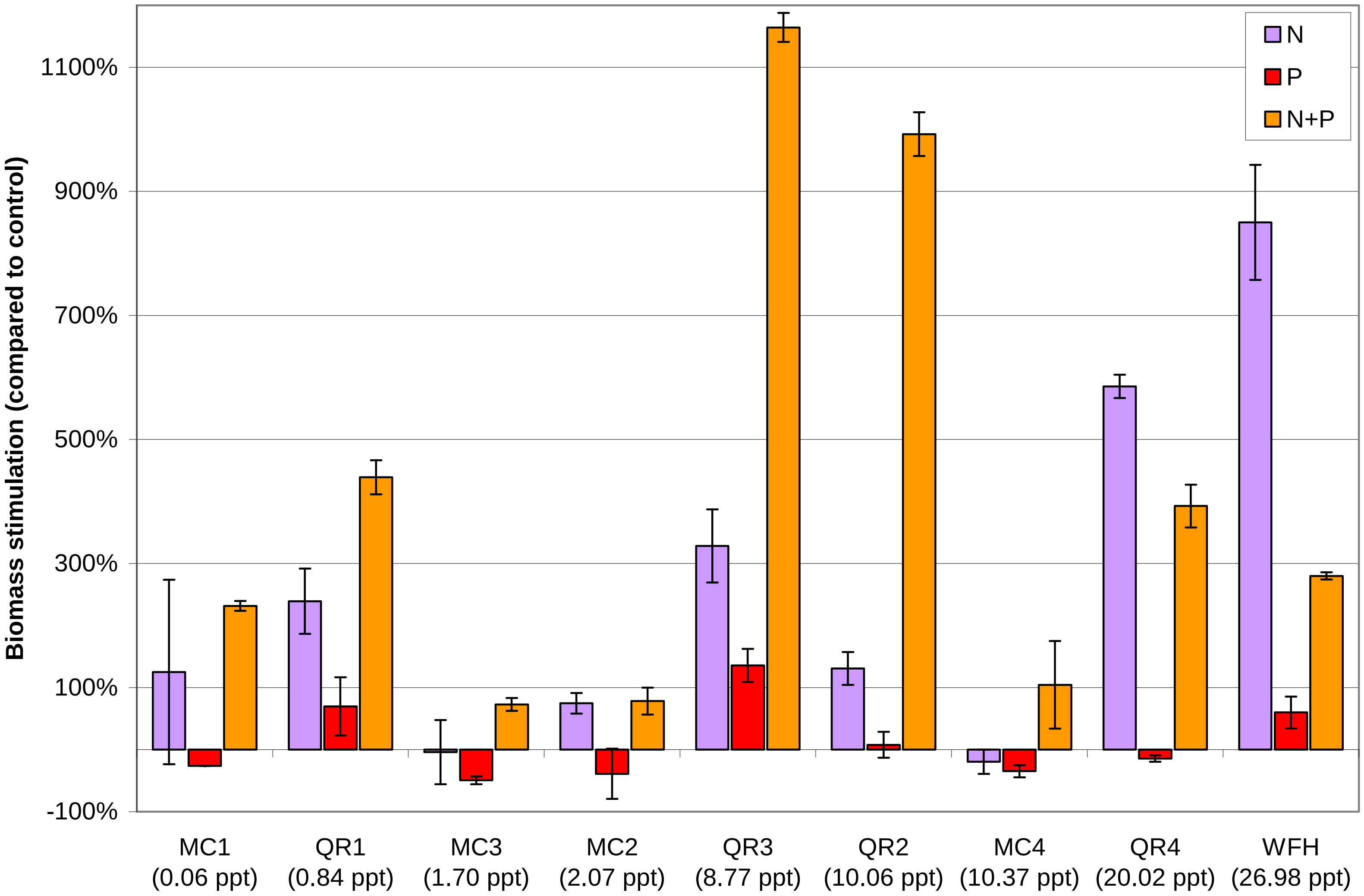

Figure 4. Average biomass stimulation of each treatment group compared to the average control, based on chlorophyll a levels at the end of the 21 day incubation period. Error bars show the spread of biomass stimulation the replicate treatment with highest Chl a with respect to the control replicate with highest Chl a and biomass stimulation of the replicate treatment with the lowest Chl a to the control replicate with the lowest Chl a.

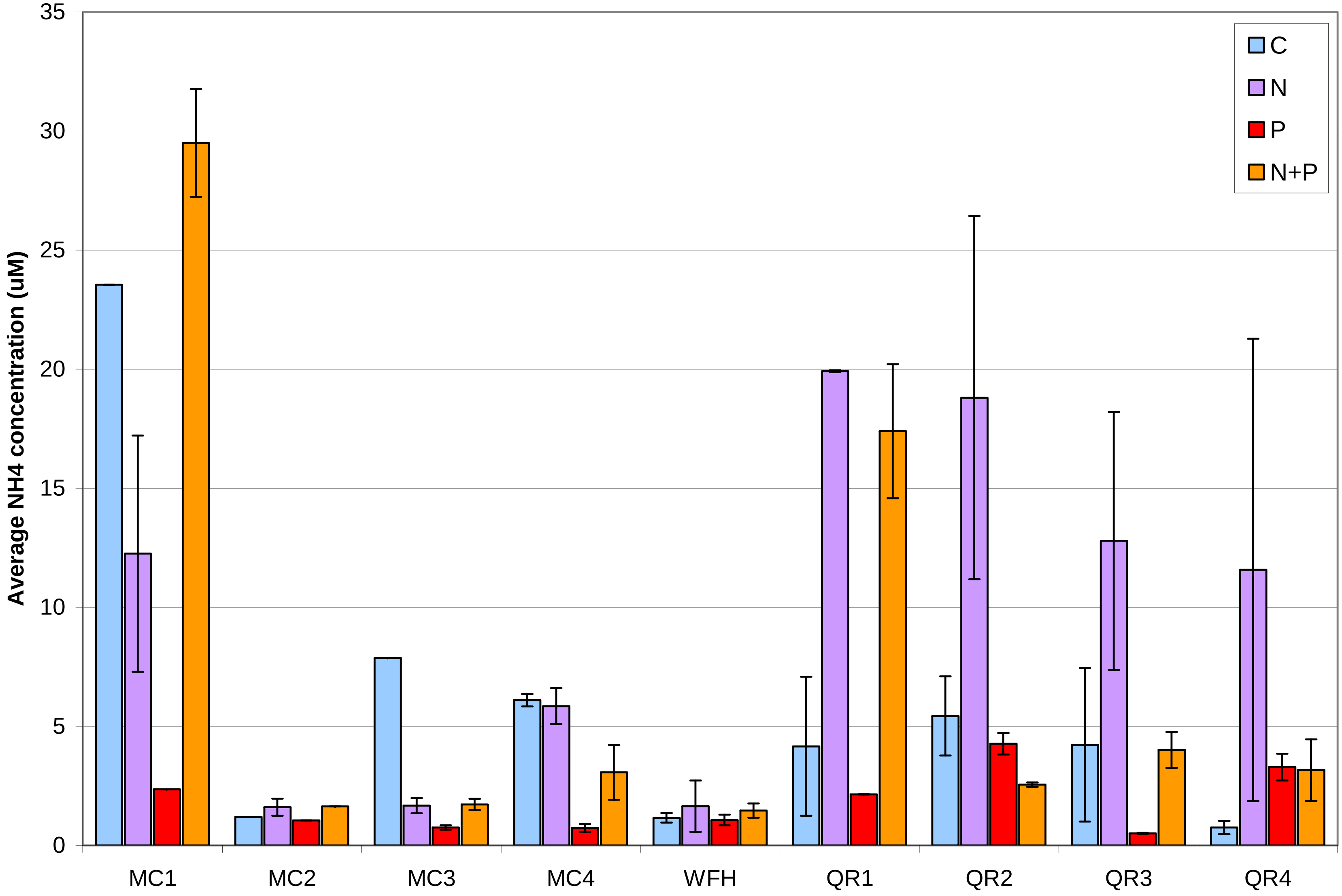

Figure 5. Average ammonium concentration in each treatment group at the end of the 21 day incubation period. Error bars show the spread of the two replicate treatments.

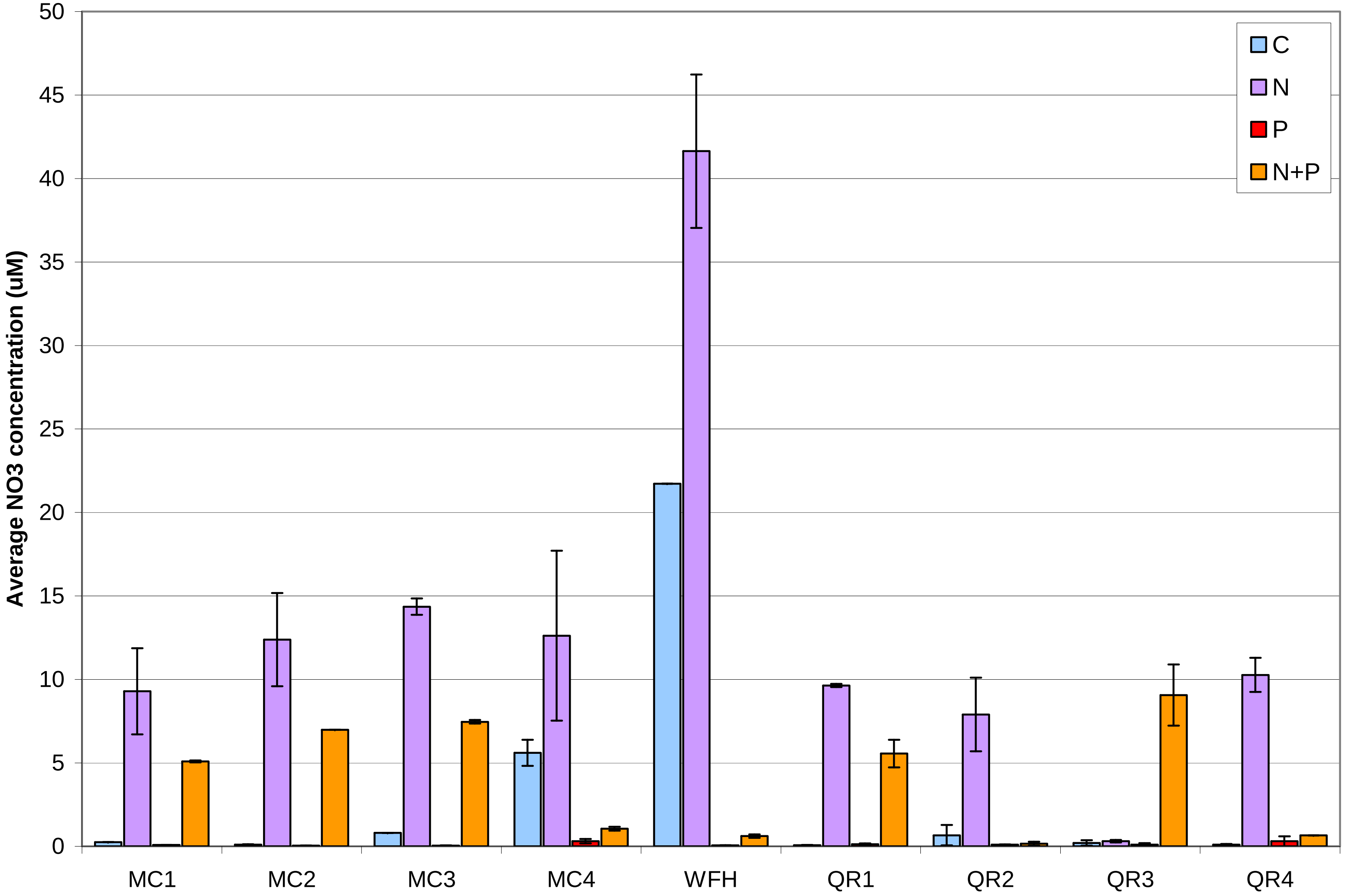

Figure 6. Average ammonium concentration in each treatment group at the end of the 21 day incubation period. Error bars show the spread of the two replicate treatments.

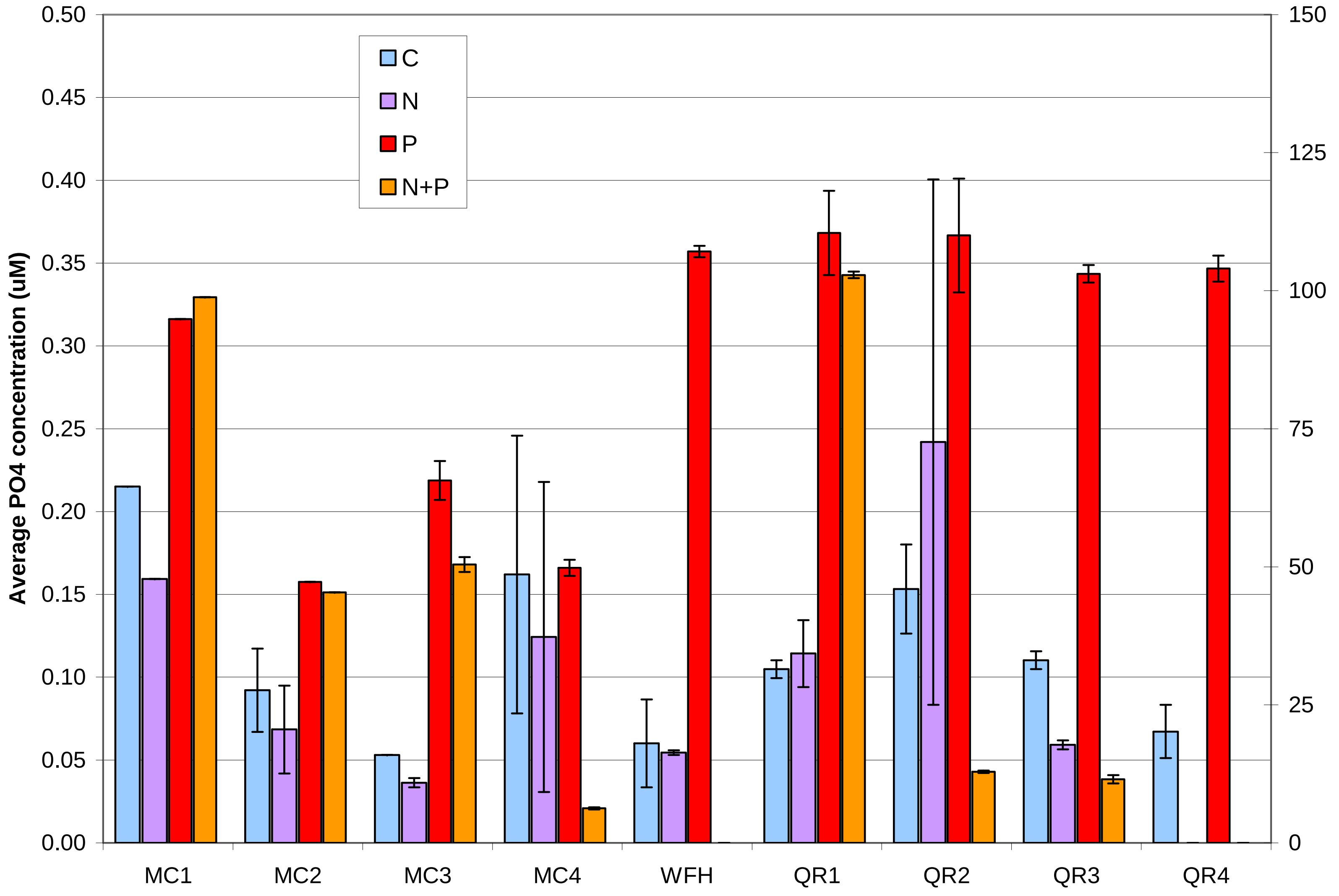

Figure 7. Average phosphate concentration in each treatment group at the end of the 21 day incubation period. Error bars show the spread of the two replicate treatments. Control and nitrogen enriched groups (blue and purple bars) are plotted on the left hand y-axis and phosphate and nitrogen and phosphate enriched groups (red and orange bars) are plotted on the right had x-axis.

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