

Interactive comment on “Adding nitrate and phosphate separately or together in the Central Indian Ocean: a nutrient enrichment experiment” by S. Tang et al.

S. Tang et al.

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We are truly grateful to the reviewer's comments and thoughtful suggestions. Our responses to the comments and questions are given below. Specific comments 1. The experiments were carried out in non-trace element clean conditions. The influence of this approach on the results should be addressed in the discussion. It has been confirmed that the concentration of trace element is very low in the surface seawater. With the addition of specific nutrient, the influence of original nutrients in the seawater on the results is very little and could be ignored, including the trace elements as micronutrients. And it is accepted by the researchers conducting enrichment exper-

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iment. Furthermore, as described in the manuscript, the experimental barrels were made of acrylic, and rubber tubes are used for sub sampling. They are all washed three times with surface seawater beforehand. Thus, the influence of trace element on the results could be ignored. 2. The authors have used regressions of chlorophyll or growth rate values measured in one barrel against values in temperature, N, P, or N:P in the same tank over time. This is incorrect, because measured values over the time series are not independent. Discussion and conclusions based on this analysis should be revised. The chlorophyll a concentration and phytoplankton growth rate is influenced by temperature, nutrient concentration, N: P, and other parameters. As pointed out by the reviewer, these values are interrelated. It is similar in the actual surface seawater. So results and conclusions from comparing two values during the enrichment experiment directly are more useful than that by limiting other values to understand the process in the actual surface seawater. 3. (1) The work would be more interesting if information were presented on what phytoplankton taxonomic groups responded to nutrient additions. (2) The experiment was continued for a time period that appears too long for investigations of simple phytoplankton nutrient limitations and it is likely community composition changed over this time period. It is unclear why such long time period was chosen when community dynamics, trophic transfer etc. are not discussed. (3) Calculating growth rate at day 17 by difference of chlorophyll a measured at days 17 and 0 is a reflection of net accumulation of growth after 17 days of growth, death and sedimentation, grazing, and new growth (of probably different phytoplankton groups) after recycling of nutrients. Discussing such values as “growth rates” is misleading. For the goals of the study as presented, the time period until the end of the exponential growth phase is relevant. (1) Taxonomic data of phytoplankton is not obtained during the experiment. So authors discuss only the responses of total phytoplankton to nutrients addition. (2) With such a long time experiment, data after phytoplankton bloom could be obtained, which was seldom reported before. Although phytoplankton classification and trophic transfer is absent, the results could also provide more information about biogeochemical process after phytoplankton bloom such

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as the variation of phytoplankton growth and nutrient concentration. (3) Phytoplankton growth rate (R) is calculated as follows: $R = \ln(\text{Chl}t/\text{Chl}i)/t$ where t is the incubation time and Chlt is the concentration of Chlorophyll a at time t. During the whole course of experiment, whether before phytoplankton bloom or after that, phytoplankton growth, death and sedimentation, as well as grazing all co-occur. So phytoplankton rate is always the result with the effect of these factors during the experiment. Maybe the influence of these factors to R after bloom is more obvious than that before bloom, which needs more data to prove. Thus, R after bloom is thought as important as that before bloom, which could provide significant information about phytoplankton growth after bloom. 4. (1) Abstract should state what the results and conclusions mean in the broader context of phytoplankton nutrient limitation in oceans/Indian Ocean. (2) It would be relevant and interesting to hear how the results relate to previous information on phytoplankton and nutrient limitations in Indian Ocean. (3) Are there reasons why we might expect the area to be N vs. P limited? (4) What might the results tell us about the biogeochemistry of the basin? (1) The results and conclusions are significant to understand the biogeochemical process when phytoplankton growing in the local sea area. And this point has been supplemented in the Abstract in the revised manuscript. (2) Authors have discussed the relevance between our results and that from previous work in the manuscript. See the first paragraph in the section of Phytoplankton growth, section of The influence of N: P to phytoplankton growth, etc. (3) The reason why N vs. P limited is discussed has been illustrated or referred in the manuscript and authors' comments¹. Firstly, nitrogen and phosphorus play a particularly important role in limiting biological productivity, and nutrients limitation of phytoplankton varies in different sea areas. Secondly, as reviewer said, "discussion on phytoplankton nutrient limitations in the Indian Ocean is more limited than many other ocean basins". (4) As concluded in the manuscript, results from enrichment experiment prove that phytoplankton growth is nitrogen limited rather than phosphorus limited around the sampling location. In addition, N: P could not control the growth of phytoplankton community completely. Furthermore, discussions in the manuscript

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are based mainly on the data from the nutrient enrichment experiment. However, the data about actual surface seawater is absent, including nutrient, chlorophyll a, temperature, and other parameters. Thus, to well understand the biogeochemical process during phytoplankton growing, data from field measuring and monitoring are urgently needed. 5. Introduction should mention Fe limitation of phytoplankton growth occurs in many ocean areas (citing appropriate references) and state whether this work does or does not address potential Fe limitations. Fe is one of the most important limiting nutrients for phytoplankton growth and Fe limiting has been proved in the many HNLC zones. Our work is designed to study the influence of nitrogen and phosphorus to the growth of phytoplankton in the experimental sea area. And our discussion focuses on this topic in the manuscript. So authors mainly review researches on N and P limiting rather than Fe limiting in the section of Introduction. Materials and methods (pages and rows refer to the authors' revised paper) 1. P2 R11-12 Please clarify: "After nitrate was consumed, substantial amount of phytoplankton survived with the supplied phosphorus" and corresponding section in the main text. Live phytoplankton requires both nitrogen and phosphorus. P7 R12 Here phytoplankton continued to increase although NO₃ was low. It would be appropriate to discuss nitrogen recycling and potential influence of NH₄ and DON supporting chlorophyll a. Authors agree that live phytoplankton requires both nitrogen and phosphorus. Without data about nitrogen concentration in other forms, authors don't meet to further discussion on the nutrient supply for the live phytoplankton after bloom in B4. The idea "... substantial amount of phytoplankton survived with the supplied phosphorus..." was corrected. Accordingly, corresponding sentences are revised. I. Abstract: "After nitrate was consumed, substantial amount of phytoplankton survived with the supplied phosphorus" is deleted in the abstract. II. First paragraph in the section 3.1: "managed to survive" is changed to "existed". III. Second paragraph in the section 3.3: "Our inference is that phytoplankton continued to live on phosphate" is change to "Maybe nitrogen in other forms, such as ammonia and dissolved organic nitrogen, supported the growth of these phytoplankton with the phosphate". 2. P4 R15 Here it is stated

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FeSO₄ was added to the barrels. Is this an error in the text? Iron additions/limitations are not discussed in the introduction, results and discussion. This is an error, and "FeSO₄·7H₂O" is deleted. 3. P4 R17 More detail should be provided on nutrients and chlorophyll a methods. How were samples collected and stored? Where analyses done onboard or later? Were replicates run? What instruments were used? What were the detection limits? NH₄⁺ concentration seems high. How was potential background contamination in NH₄⁺ measurements addressed? In addition to stating these points in the text, please include a citation for all nutrient and chlorophyll methods. Details are supplemented in the third paragraph in the section of Materials and Methods. The NH₄⁺ concentration in the surface seawater is 0.67 μM, which seems not to be much higher than that in other sea area. 4. P4 Were the barrels covered? Were they mixed during the 17 d they were incubated? Sedimentation may have caused a major bias if mixing was not done. The barrels were not covered, which is supplemented in the revised manuscript. The experimental water was mixed just after nutrient addition. Because northeast monsoon prevail there with strong waves and swell during the experiments, the sensible shake of the ship often happens. So authors don't think that sedimentation could cause a major bias for the results of the experiment. 5. P4 Information should be provided on any statistical methods used, including tests of data assumptions. Relative information is supplemented in the last paragraph in the section of Materials and Methods. 6. Table 2. It is shown that 5 mM concentration of nitrogen was added. However, nutrient measurements show concentrations were <14 μM in the barrels with nitrogen addition, starting from day 1. Similarly, P addition is shown as 0.7 mM, but concentrations in P addition barrels were <3 μM. Perhaps the numbers are nutrients added per barrel? Please clarify the nutrient additions as calculated final concentrations in barrels at the beginning of the experiment. Initial concentrations of nitrate and phosphate with nutrient addition in different barrels are shown in table 2 to clarify the nutrient additions. 7. Table 3: (1) Average rate of phytoplankton growth is shown. Each growth rate is calculated from the difference in chlorophyll a at the initial time point and at time t. Average growth rate is, however, not informative for

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treatments in which phytoplankton experiences an exponential growth phase, then crashes, followed by low chlorophyll levels. These trends show instantaneous growth rates must vary widely. Why not use maximum growth rate for the exponential part of the growth curve only and compare maximum growth rates among treatments? (2) At what time point is N: P from in Table 3? (1) Maximum growth rate represent only the highest extent nutrient addition could stimulate phytoplankton growth in the experiment. And average rate of phytoplankton growth could reflect the general effect during the whole course of the experiment. It is thought that both of them are useful to discussion the influence of nutrient addition to phytoplankton growth. (2) N: P in table 3 refers to the average during the experiment, which is illustrated in the revised manuscript. 8. Table 4. This test does not appear valid because data in each test are from the same barrel, thus autocorrelation is an issue. Authors think these tests are useful to estimate the influence of temperature or N: P to phytoplankton growth in the experiments. And it is taken for granted that data for correlative analysis are from the same barrel. See also the answer to question 2 in Specific comments. 9. Technical comments Proofreading English would improve readability. Authors read the manuscript carefully and revised some sentences. Please see the revised paper in the supplement.

Please also note the supplement to this comment:

<http://www.ocean-sci-discuss.net/6/C1013/2010/osd-6-C1013-2010-supplement.pdf>

Interactive comment on Ocean Sci. Discuss., 6, 2649, 2009.

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