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Interactive comment on "Inhibition of nitrogenase by oxygen in marine cyanobacteria controls the global nitrogen and oxygen cycles" *by* I. Berman-Frank et al.

Anonymous Referee #1

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The presented data did not prove that suboptimal functioning of the enzyme nitrogenase will have any effect on the overall atmospheric levels of nitrogen and oxygen. To my opinion, the authors did not show that the inability of Trichodesmium to perform nitrogen fixation at maximum rates at 21% O2, overrules other regulatory mechanisms, acting on ecosystem level. Furthermore, the title claims that cyanobacteria are controlling the nitrogen en oxygen cycles, while this ms only deals with Trichodesmium. Atmospheric gas concentrations are not the result of nitrogen-limited primary production rates alone, but result from the balance between production and loss rates. A higher standing stock of algal or cyanobacterial biomass does not necessarily yield a higher net oxygen production of the total population. In addition, to increase the atmospheric oxygen level with one percent, it may well be that the available inorganic

carbon in the atmosphere and ocean will not be sufficient. Moreover, following the authors line of reasoning, the non optimal functioning of photosynthesis should be discussed as well, since photosynthesis in Trichodesmium most probably will not reach its potential maximum at the irradiance used within the experiments presented in this ms. This would lead to the same conclusion as for non-optimal functioning of nitrogenase. The authors also mention in this ms that other enzymes, like rubisco and D1, have the same high turn over and therefore inefficiency as nitrogenase and claim that the three are the cause of the upper bound of gas concentrations. However, the tendency within article is that “the crippled enzyme” nitrogenase is responsible. I am irritated by the use of words as “crippling effect”, “chronically crippled” and “enzymatic inefficiency”, by which the authors try to create a negative atmosphere in the ms, while they did not try to measure the cause for the observed changes in enzyme activities at the different oxygen concentrations. To my knowledge there are three possibilities for the observed differences: 1) Irreversible inactivation 2) A shift in the ratio of the two forms of the enzyme due to an active post translational modification (if present in Trichodesmium) 3) Substrate limitation of the enzyme nitrogenase. Only irreversible inactivation may be assigned as crippling to my opinion. Looking at fig 1C, it is very likely that substrate limitation was the cause of the decreasing activity at increasing O2 concentrations in that experiment. A competition for reducing equivalents between nitrogenase and terminal electron donors, like cytochrome oxidase, can be the reason for differences in activity at the given oxygen levels. A limitation of carbohydrates (as source of reducing equivalents) can be the result of incubation conditions rather than the physiological inability to protect nitrogenase against inactivation. Moreover, it is not shown or nitrogenase indeed was inactivated in the experiments presented. I also do not understand what iron has to do with the story of the “crippled enzyme”. After inactivation, nitrogenase is rapidly degraded by proteases. This rapid breakdown will make the iron molecules available, to be incorporated in new proteins within the same cell. The protein may be nitrogenase, if necessary, but may also be another Fe based protein. Thus, a high turn-over rate of nitrogenase may lower the cellular iron

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demand, since the iron atom can be used by different type of proteins during the complete 24 hour period, also at times when no nitrogen fixation is found. So it may be a highly optimized regulatory system to balance C and N fixation on the protein level. One problem with post translational modification may be that the enzyme is not turned over within a short time period, while the enzyme is not active. This can lower the efficiency of iron atoms, and thus argues against the modification strategy as most efficient strategy under iron limiting conditions. In addition, under iron limiting conditions the energetic costs of protein synthesis becomes irrelevant, since iron is limiting and not energy (carbohydrates + ATP). The defense mechanisms against inactivation of nitrogenase may be energetically expensive, but seems effective. Not much is known about the nitrogenase concentration in cyanobacteria. The part that nitrogenase can be 10-40% of the total protein in diazotrophs is not relevant in the discussion, since that value is based on non-cyanobacterial organisms. The ratio nitrogenase/total protein may be completely different in cyanobacteria. If indeed nitrogenase accounts for 10-40% of the protein in filaments of Trichodesmium (the budget should be made for whole filaments or populations, since it is the population which is studied for growth and primary production, not individual cells) this would imply that nitrogenase must account for 100% of the protein in nitrogen fixing cells, since only a fraction of the cells fix N2 (according to Berman Frank et al 2001b). To my knowledge it is not known how much cells within the population fix nitrogen but, if only nitrogen fixing cells contain 10-40% nitrogenase per total protein, and 10% of the cells within a population fix nitrogen it would mean that nitrogenase only account for 1-4% of the total protein content within a Trichodesmium population. I wonder whether the symbiotic heterotrophic diazotrophic organisms, together with their hosts, will yield the 10-40% nitrogenase contents if the total symbiotic system is considered and not only the N2 fixing bacteria. The authors also mention that, under anaerobic and micro-aerobic conditions the respiratory requirements are not met and substrates essential for nitrogen fixation are not produced, causing a decline in the enzymes performance. Which substrates do the authors have in mind? To my knowledge, there are three substrates for nitrogenase: N2, reducing

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equivalents and ATP. From these three, only the production of ATP in the dark requires oxygenic respiratory activity. However, since N2 fixation takes place in the light in Trichodesmium, and photosystem 1 can produce ATP by cyclic photophosphorilation, I can not see why ATP limitation will become a problem under anaerobic conditions. Most probably, growth under anaerobic conditions is not possible, independent of the N-source. If the authors can show that they are able to grow Trichodesmium under non-nitrogen fixing conditions they may blame the “crippled” nitrogen fixation for the inability of Trichodesmium to grow anaerobically. However, that is not possible with the dataset presented in this ms. The authors did not give a proper description of the experimental set-up, what makes it hard to judge the value of the presented results. The material and methods refers to Berman frank et al. 2001b to define growth conditions, but in that article two treatments are described (when I exclude the nitrate treatment). In one treatment, Trichodesmium IMS101 is grown at 14L/10D at 40μmol photons m-2 s-1, while in the other treatment Trichodesmium is grown at 12D/12L at 80 μmol photons m-2 s-1. So which condition is used for the Trichodesmium incubations? Furthermore, ARA is measured according to Berman-Frank et al 2001a. This article states that ARA incubations took place at growth irradiances and lasted 2 hours, and that the samples are taken at the middle of the light period. How can the authors, with such a long incubation time’s claim that the potential activity (is that defined by max activity at a determined oxygen optimum?) reduces within minutes at 30% O2 (pg 267, sentence 6)? Did the authors take samples at shorter time intervals? That should be indicated in the methods. It is also not clear how the ARA incubations under different O2 concentrations were carried out, since Berman Frank et al. 2001a did not present O2 experiments. In the legend of fig 1C the authors mention a short time effect (1-2h) on nitrogen fixation activity, but in what sense does that experiment differ from the measurements described in fig 1D, except that the cultures have been bubbled with different O2 concentrations for 1 hour before the onset of the acetylene reduction measurement. Can that change really cause the difference in results? Or were the data of 1C the result of samples taken in the morning, far before noon, when Trichodesmium

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still performs gene transcription (but has little build up of carbohydrates), while in 1D the incubation started at noon (according to Berman-Frank 2001a)? Concerning the data in fig 1D, I can not see any dramatic “crippling effect” measured by the authors at O2 concentrations below 30% O2. It is hard to find these data, since the authors, to my opinion without any good reason, filled the graph with field data measured by others (done under the same light conditions?) and laboratory data of complete different organisms (with different protection strategies and most probably complete different nitrogen fixation rates per biomass). Looking at the dark triangles, the rates fluctuate between 80% and 100% of the maximum value. For the range 0-30% O2, my guess would be that there is no significant correlation between O2 concentration and nitrogenase activity after doing the appropriate statistical tests. I also wonder where the line originates from in graph 1D. Is the line a result of a fit? If so, from which data? In fig 2, growth is represented as negative doubling time for 0, 5 and 50% O2, to indicate that the biomass decreased during the incubation. From that graph + legend I conclude that a doubling time of -2 means that the biomass was halved in two days, while a doubling time of -13 indicates that the biomass was halved in thirteen days. This would mean that the biomass almost did not decrease at 0% O2, while the decrease of biomass at 5% and 50% O2 was much faster. Can the authors explain why the biomass decreased more at 5% O2, relative to 0%, while it can be expected that the respiratory metabolism can be active during the night at 5% O2, and therefore allow cell maintenance and DNA transcription/replication during that period, while that may not be possible at 0% O2?

Interactive comment on Biogeosciences Discussions, 2, 261, 2005.