

Interactive comment on “Physiological constraints on the global distribution of *Trichodesmium* – effect of temperature on diazotrophy” by E. Breitbarth et al.

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We gratefully acknowledge the comments by Dr. M. Voss, which were very valuable to improve our manuscript. We followed most suggestions for minor changes and further made numerous small alterations in the text based on Dr. Voss' comments. In the following, we reply to Dr. Voss' major comments:

We presented three different specific growth rates (chlorophyll-*a*, PON, and POC based) for several reasons: Ocean Biogeochemical Circulation Models are not standardized with regard to the biomass parameter used. Chl-*a* is a common biomass measure, but so is POC. Chlorophyll-*a* is often used a measure of biomass in field assays, however, the chlorophyll concentration per cell can vary as a function of light

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or temperature. As we discussed in the manuscript, phytoplankton acclimate to different temperatures by adjusting their chl-*a*:POC stoichiometry. Likewise, the POC:PON stoichiometry changed with incubation temperature (see also Figs 2 and 3). Therefore using i.e. chl-*a* as a biomass parameter in a model could potentially create difficulties, especially when addressing questions related to carbon cycling. The agreement observed between the growth rates measurements derived from the different biomass measurements indicated that the cells were well acclimated to the growth temperature and could achieve balanced growth in the exponential growth phase.

Dr. Voss asked why we used the acetylene reduction assay (ARA), which determines gross nitrogen fixation, instead of measuring the net fixed nitrogen uptake that is retained in the cells by applying the $^{15}\text{N}_2$ method. We found it important to determine both, direct cellular increase of PON (next to POC, and chl-*a*) in media that is free of added dissolved nitrogen, and the gross nitrogen input by *Trichodesmium* via nitrogen fixation determined by ARA. We briefly address in the manuscript that cells may retain more or less of the newly fixed nitrogen due to i.e. changing membrane permeability as a function of temperature. Applying the ARA, we found that the nitrogen fixation temperature relationship in *Trichodesmium* reflects similar patterns as shown in the growth rate temperature relationship. Applying the $^{15}\text{N}_2$ method could have masked a potential difference between the gross nitrogen fixation/input relationship and growth rate temperature relationship by *Trichodesmium*. Overall, ARA provided a fast and convenient way to assess nitrogen fixation in our work. It should be noted though that our main interpretations are largely based on the growth rate data. Moreover, while it is common to estimate nitrogen fixation per unit volume in order to calculate oceanic nitrogen input in OBCMs, we saw the need to standardize N_2 fixation per unit biomass in our experiments. Not all cultures reach the same biomass due to different growth rates at different temperatures and thus reporting N_2 fixation per unit volume would not have revealed a clear temperature nitrogen fixation relationship. In our opinion, nitrogen fixation measurements should always be standardized to POC to allow for a most detailed data interpretation and application thereof to modeling approaches. There rates can

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easily converted to a volumetric parameter later on, if biomass (POC) concentrations are computed.

The goal of this study was to establish the direct physiological effect of temperature on *Trichodesmium* growth rate and to obtain a relationship between temperature and growth rate in this species. Prior to our study, this was lacking in the published literature, because most of the information on the effect of temperature on *Trichodesmium* is from field studies where the direct physiological effects of temperature on the growth rate are confounded with the indirect effects of temperature on other physical parameters such as the light field and water column stratification. Our laboratory study should not however be interpreted in isolation from the indirect factors, therefore we briefly discuss other possible effects of temperature in the field in addition to the direct effect on growth rate. Over all, we hope that we were able to sufficiently state that it was necessary to perform controlled laboratory experiments where only temperature is a variable factor and indirect effects of temperature in the ocean on light and nutrient availability due to stratification and mixed layer depth can be ruled out. However, we saw the need to discuss these indirect effects as light and nutrient availability are interconnected with water temperature in the ocean via water column stability and upwelling processes. Our stronger focus on discussing light is based on its direct relevance to temperature via chromatic acclimation of the cells. We did not dwell deeper in the subject of nutrient limitation of *Trichodesmium*, since we did not access any effects of temperature nutrient interrelations and numerous publications in this area exist.

Further, we now slightly modified and simplified the genetics section. We can not completely rule out the possibility that other *Trichodesmium* strains may have different temperature adaptations than *Trichodesmium* IMS-101, or that other diazotrophs may gain significance due to surface ocean temperature changes in the future.

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