

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

**E. Breitbarth et al.**

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We gratefully acknowledge the comments of the anonymous reviewer, which greatly improved the revised version of our manuscript. We believe that we were able to incorporate the reviewers' comments to a large extent. We would like to respond to the following reviewers' comments in detail:

The reviewer pointed out that it is crucial to identify, which 4 hours of the light cycle were used to measure nitrogen fixation in *Trichodesmium* since nitrogen fixation rates vary greatly over the course of the light cycle. Agreeably, this is a very important point. The incubations for the results presented here were always performed during the same four hours of the day and the peak time of nitrogen fixation was previously

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determined in our lab. We can not rule out that the timing of the peak rates of nitrogen fixation may have shifted as a function of temperature. However, the growth rates measurements also presented in our manuscript integrate (net)nitrogen fixation rates over the daily cycle and on timescales of several weeks. This is in our view more important in demonstrating sustained growth at a given temperature than short-term nitrogen fixation measurements. Of additional importance is that all nitrogen fixation measurements were made on exponentially growing cultures and that growth rates and nitrogen fixation rates were determined in separate experiments. We have clarified these points in the revised method section, and think that this information is now clearly accessible to the reader.

Further, all growth rate measurements were calculated from temperature acclimated cultures. Stock cultures were grown at 25°C, though. Transfers to higher or lower temperatures were either made directly from those, or also later during the experiments from cultures that had been incubated at the upper or lower temperature tolerance range. By comparing the growth rates for cultures at a specific temperature that had their last transfer from either 25°C or from the respective higher or lower temperatures we verified that all cultures used in the analysis were fully temperature acclimated. We now also clarified in the methods section that the media had no dissolved nitrogen sources added. Growth rates were calculated from the increase in biomass during a time interval. In the results section 3.1 we state that we found no significant differences in the growth rates at each specific temperature (this information is now added to the text) between the different biomass parameters used. Clearly, growth rates differ significantly as a function of temperature.

The reviewer argued that we have no measurements for nitrogen fixation between 29 and 34°C and that 30°C therefore could not be set as the upper limit to optimum growth. Our conclusions are largely based on growth rate data, with the nitrogen fixation data supporting this trend. Since we present growth rates at 17, 20, 21, 24, 27, 30, 31, 32, 34, and 36°C (Figure 1), we are confident about our interpretation. Although more

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data points between 27 and 30°C would be desirable, only one of the three nitrogen fixation measurement series shows a maximum at 30°C, while the other two peak at 27°C. However, Further, the reviewer suggested to extend the optimum temperature range for growth to 32°C. Our results show that the chlorophyll-*a* specific growth rates are significantly lower at 31 and 32°C than at 24°C. Compared to those, the rates at 27 and 30°C are significantly higher and the temperature function of growth rate decreases sharply above 30°C. Based on this we derived the optimum range of 24–30°C used in the calculations. These results are now clearer in the new Figure 1, which we revised based on the reviewer's suggestions.

We made several changes regarding the varying number of sampling points for elemental stoichiometry between temperature treatments to the results section. Fig. 2 shows all available data from the growth experiments we performed. We found it valuable to present and to use all data, regardless of growth phase, as the data still show a clear trend of elemental stoichiometry versus temperature. Further, in some of the experiments the exponential growth phase was very short, yielding a very limited amount of data points, which would make regression analysis problematic. The contribution of the exponential growth phase data to the stoichiometric ratios derived from all data points of an experiment was insignificant for most experiments. Generally, the number of data points at the different temperatures is different, because we grew more cultures at certain temperatures for various associated experiments over the course of a year, and also because cultures grew much slower/longer at certain temperatures, yielding more data points than at others. Selecting specific data points for the analysis would have resulted in a bias since the selection could never be absolutely objective and therefore we found it important to use all data and provide them to the reader (Fig. 2). The slopes of regression (originating from Fig. 2) are plotted in Fig. 3 and serve the purpose to clearly illustrate the trend of different stoichiometries versus temperature, which would not be visible using Fig. 2 alone.

Additionally, we want to point out that the reason for the high POC:PON ratio at 17°C

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is unknown and that this ratio needs to be treated as an exception from the general trend, because *Trichodesmium* does not grow at such low temperatures, but rather experienced a slow decay in biomass. Our statement that at lower temperatures POC:PON ratios are reduced is based on data from the temperature range at which *Trichodesmium* can achieve biomass increase and excludes the data point at 17°C. However, we believe it is worth reporting this value, since *Trichodesmium* has been encountered in waters with temperatures below 20°C, which has always been attributed to drift with currents and not to local net growth.

Finally, we agree that it would be very interesting to discuss multiple scenarios and possibly also seasonality in the discussion of global temperature changes. Unfortunately this was not possible for us at this stage and is beyond the scope of this manuscript.

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