Response to RC1

The authors investigated the combined effects of light and temperature on the growth, N2 fixation and photosynthesis in the marine diazotroph, Trichodesmium. Light and temperature are two of the most environmental drivers for this species as for other marine primary producers. However, the combined effects of these two factors have surprisingly little been documented on Trichodesmium. This work fills such gap. The new finding from this work is that the thermal responses in Trichodesmium are strongly dependent on light exposures when grown under different light and temp levels. The parameters derived from the measurement are of significance in predicting the re- sponses of Trichodesmium to ocean physical environmental changes associated with global changes. Generally, this work has been well performed and delivers a clear message, but some revisions are needed before being considered acceptable for publication at BG:

1. Line 65, "... where light intensity could be as low as 2 μmol quanta m-2 s-1". What's the source of this number?

Response: We used the following equation to get this number:

E(d) = E0 * exp(-k * d)

E(d) is the light intensity (µmol quanta m^{-2} s^{-1}) at depth d(m); k is the light extinction coefficient; E0 is the surface solar irradiance. We assumed that the water column was homogenous, extinction coefficient was 0.05 m^{-1} (common value reported for subtropical and tropical pelagic oceans (Olson et al., 2015)) and surface solar irradiance was 2000 µmol quanta m^{-2} s^{-1}.

In the revised manuscript, we added references at lines 56-57

2. Line 69, "... Trichodesmium's N2 fixation and growth,". It's better to delete " 's N2 fixation and growth".

Response: We revised this paragraph. See lines 71-78

3. Line 115 – 118. In the treatment "light-limiting, 31 oC", the N2 fixation rate under growth condition was obtained through an indirect and unusual way. I recommend that the authors should also take the N2 fixation rate measured at >31oC into consideration (maybe use the average of this and that measured at 30 oC), although such modification may alter the Figure 1b, and require revision of related text.

Response: We re-analyze the short-term thermal response for N_2 using nonlinear mixed effects model from which we can directly obtain the N_2 fixation rate under growth conditions for all treatments.

4. Line 122. "...Aliquots of 1.5 m ..." should be "1.5 ml".

Response: corrected.

5. The authors should describe the statistical analysis techniques they used in the Material and methods. Although I can roughly deduce the used statistical techniques from the text in Results, the authors should explicitly present them, which will help readers evaluate their results and conclusions.

Response: It was a serious mistake that we omitted the crucial paragraphs describing how we analyzed the data. In the revised manuscript, we added paragraphs at lines 147-164 describing how we analyzed our data.

6. Figure 3. It seems that the selections of temperature gradients are different among different

treatments, which is uncommon. Why? Will this affect the interpretation of the data?

Response: We found that the temperature was not homogeneous in the multi-zone chambers that were used to measure the response of N_2 fixation to short-term temperature changes (Figure 3), so we used the actually measured temperatures rather than the pre-set temperatures.

No, this should not be a problem.

7. Line 202-205. How did the authors get the numbers ">28% and 7%-20%"? The cited literatures do not provide such numbers.

Response: We got these numbers from the figures in the cited references (Figure 3 in Davis & McGillicuddy, 2006; Fig 8 and 10 in Olson et al., 2015), although they do not show up in the text.

8. Table 1. In the text, the light treatments were referred as "light limiting" and "light saturating", but in this Table they were denoted as "LL" and "HL". It will be better to keep them consistent.

Response: Revised accordingly. See Table 2 (Table1 in original manuscript) and Table 3 in the revised manuscript.

9. Fig 3b. The temperature norm of N2 fixation in the treatment "light-limiting, 31 oC" is quite different from those in other treatments, which deserves more discussion. However, authors didn't put much attention on this phenomenon.

Response: We guess that the unusual performance in treatment "light-limiting, 31 ^{o}C" might be related to the nitrogenase damage which was induced by the high growth temperature and exacerbated by the light limitation. The quantity of the functional nitrogenase might be not enough to form the expected N_2 fixation peak.

All in all, this work focused on a valuable but previously overlooked scientific topic and obtained some interesting results. If the authors can properly deal with the concerns listed above, I think it will be qualified to be published in BG.

Response to RC2

General comments: This manuscript by Yi et al. examines how light availability (tested at two levels of light intensity) interacts with the effects of warming (along a gradi- ent of three temperatures) in a marine N2 fixer (Trichodesmium erythraeum IMS101) across a time scale of about ten generations. The experiment is in its essence a two- driver question, where either driver might intrinsically decrease or increase metabolic performance, but the

cumulative effect is unknown. The findings and the results are straightforward, with a clearly identifiable general trend. While theoretically relevant (e.g. changes in temperature may coincide with changes in light intensity), it is not quite clear why the authors chose these two drivers over other sets of drivers until much later on in the manuscript. It would also have been nice to see a more explicit evaluation over whether the changes in temperature/light level constitutes an environmental deterioration or amelioration and how that impacts on how they interact. Still, the results are quite interesting, especially since they cover a range of phenotypic traits (growth rates, N2 fixation rates, photosynthetic machinery). However, I have major concerns about how the results are presented: the methods do not indicate how the data were analysed, and the results appear largely as post-hoc output. The latter would indicate that the authors used an ANOVA or similar test, which is indeed indicated more clearly once in line 185, but details are nowhere to be found. For example, a statement about the data is followed up simply by (p<0.05,tukey HSD method). It is impossible to glean from this what kind of data were compared and what the original model looked like. As the main question is about interactive effects, and the data are hierarchical in nature (e.g. differently acclimated samples used in a short-term assay), the authors would have needed some kind of mixed model approach. The closest the text ever gets to describing how the data were handled is in line 129 'parameters can be ob- tained through non-linear least squares regression in R language'. Which packages did the authors use to do so? How did they fit their data to the Eiler curve? Similarly, the authors mention the Sharpe-Schoolfield model, but that would be no easy feat with only 5 temperatures (it is a 4 parameter equation). More information would have been crucial here! It clearly worked well, as the fits in Figure 3 don't look too bad. However, we then need to also know how different these curves are from each other. For this, one needs to either extract the parameters and compare them (and describe how!) or run a non-linear mixed effects model (and describe how). As it stands, the handling and analysis of data is not at all traceable. I will provide suggestions on how to deal with this issue in the detailed comments below.

Response:

We are grateful for the referee's constructive comments and suggestions on our manuscript. We have studied them carefully.

As the referee points out, it would be better if we had explained why we chose light and temperature over other drivers at the beginning of the manuscript. We have revised the Introduction to handle this issue.

It was a serious mistake that we omitted the crucial paragraphs describing how we analyzed the data. We performed the two-way ANOVA with normality (Shapiro-Wilk test) and variance homogeneity (Levene's test) tests to determine whether light, temperature and the interaction of light and temperature affected the phenotypic traits (Figure 1), including growth rate, effective photochemical efficiency and N_2 fixation rate. Then, post hoc (Tukey) test was used to do the pairwise comparisons. As with the data in Table 1 and Figure 2, 3, 4, we first extracted the parameters from the non-linear fitting to individual measurement. Then, the two-way ANOVA and Turkey test were used to determine the effects of light, temperature and their interaction on these parameters. The data analysis was done using the R language (version 3.5.3) with the built-in functions, including 'aov', 'shapiro.test' and 'TukeyHSD', function 'nlsLM' from package 'minpack.lm (version 1.2-1)' (line 113) and function 'leveneTest' from package 'car (version 3.0-2)'. We argue that our data analysis processes were appropriate for most of the tested physiological traits.

Also, these statistical methods are widely used in other similar work, such as (Hong et al., 2017; Hoppe et al., 2018; Trimborn et al., 2019). Hoverer, we agree with the referee that the part involving the Sharpe-Schoolfield model might be problematic. Using 5 data points to fit a 4-parameter equation was overparameterized. We are grateful that the referee suggests an alternative statistical method to handle this problem, that is, non-linear mixed effects model. We have used this method to re-analyze our data, which did not change our main results and conclusions.

In the revised manuscript, we added paragraphs at lines 147-164 describing how we analyzed our data and presented the results in a more traceable way.

Technical comments and corrections, further suggestions:

Throughout: please double-check use of singular/plural and use of present tense and past tense. Please be careful with the vocabulary used. What is ‘acclimation’, what is ‘short term’? How are either of these different from ‘acute’? Be consistent throughout in how you use these words. You could, for example, define them in the introduction and then stick to that definition.

Response: In our manuscript, “acclimation” means that the cells had been maintained under the growth condition for more than 10 generations with their growth rates being stable. “acute” and “short-term” referred to processes that occur within several hours. We defined these terms at lines 73-76.

Abstract Line 13: Consider telling the reader which phenotypes from the get go. Line 16: ‘range of 23-31’ could be misleading, just state the three temperatures Line 16/17: ‘when the acclimation . . . [. . .]. . . to growth temperature was evaluated by short-term

Response: We have revised the manuscript accordingly at lines 14-17.

Line 22: “cells growing under low light levels while distributed deep in the euphotic zone or under cloudy weather conditions might be more susceptible to ocean warm- ing”: I would be careful about that, the study refers to response of acclimated cells at different conditions, not to acute or immediate responses (at least for the growth re- sponse), especially when we consider that these cells can actively migrate along the water column.

Line 23: Point out explicitly that this is true for ocean warming occurring on the timescales of a few generations, or, as in your assays, short term responses within the same generation in mere hours. Mention scenarios when this is applicable upfront (mixing, heat waves..)

Response: We measured such phenotypical traits as growth, N_2 fixation, effective quantum yield of *Trichodesmium* cells that had acclimated (over 10 generations) to different light intensity and temperature levels. Additionally, we also measured the response of N_2 fixation to short-term (hours) temperature changes. The former is related to the long-term environmental changes, such as global warming, and the latter is more related to strongly disturbed weather conditions, such as cyclones, and marine heat waves. Studies showed that strong cyclones would be more frequent and stronger in the warmer oceans (Elsner et al., 2008; Knutson et al., 2010; Wehner et al., 2018).

We have revised these sentences to clarify the ambiguity at lines 21-25.

Introduction Line 29: might not be all that ‘obvious’ to all readers. Consider elaborating.

Response: We have revised this at lines 30-35.

Line 39: The 1960s are not a century ago yet, plus the literature cited after this statement is pretty recent? Specifically: Is there a reference for the 1960 discovery of diazotrophy in Trichodesmium?

Response: Modern interest in *Trichodesmium* dates back to the 1960s with the recognition that *Trichodesmium* is diazotrophic.

Yes, (Dugdale et al., 1964; Dugdale et al., 1961). We have revised this part and cited these original papers at lines 36-37.

Line 41/42: ‘In the IPCC…[…]’ consider rephrasing to ‘The IPCC scenario […] predicts..[..]’

Response: We have followed this comment at lines 39-40.

Line 43: I am not sure Collins et al 2013 is the correct reference here, as it is focused on the long-term implications of global climate change, not so much the ocean physics

Response: The acclimated phenotypic traits, such as growth rate, N_2 fixation rate etc., were related to this reference with respect to long term implication. Superimposed on this, we also measured the response of N_2 fixation to short-term (hours) temperature change, which was more related to strongly disturbed weather conditions.

Line 44: ‘consequences’ on what? Consider elaborating.

Response: We have revised this at lines 39-44.

Line 50-52: different responses to warming more due to relation between traits and environment, than only “because of the spatial heterogeneity of present temperatures and projected warming”. Clarify it is also a matter of local adaptation.

Response: Yes. Local adaptation is another factor affecting organism’s response to climate change. We have revised and clarified this at lines 45-51 and 76-78.

Line 68: clearly state that Trichodesmium is ACTIVELY able to migrate vertically.

Response: revised accordingly at lines 57-58

Methods:

Line 75: Are three replicate populations enough to assess within species variability? Was this decision based on pilot studies? Were the cultures clonal? Mixed?

Response: We only used one strain of *Trichodesmium* (IMS101), which was clonal

when isolated decades ago, but likely resembles a mixed population now. In our work, the population referred to independent replicate cultures. In the revised manuscript, we used term "cultures" to avoid the confusion. "Three replicate cultures" is widely used in similar studies.

Line 77: Would be crucial to know where these three temperatures lie on the thermal tolerance/performance curve. The 2007 and 2014 studies just state that these are temperatures that this specific Trichodesmium can live in?

Response: According to these two papers, we can locate these three temperatures on the thermal tolerance curve. This was described at lines 45-47.

Line 77: Might have been better to have used a third light intensity toward the Iopt, just for the sake of comparison and to underpin the basic response to temperature of Trichodesmium.

Response: If Iopt means "optimal light intensity", the high light level in our study is within the range of "optimal light intensity" for this *Trichodesmium* strain. We have clarified this in the revised manuscript at lines 85-86

Line 77: 160 μmol quanta m-2 s-1 seem like quite a low light intensity to be saturating, although they report in the supplementary a pilot study that seems confirm the state- ment. Nevertheless, the cultures for the pilot study were not aerated while it seems to be a constant for Trichodesmium culturing in all other papers (formation of cells^t aggregates and consequently maybe self-shading effects?).

Response: The value, 160 μmol quanta m^{-2} s^{-1}, is consistent with the values reported and used by other researchers (Garcia et al., 2011; Kranz et al., 2010; Cai et al., 2015; Breitbarth et al. 2008). Additionally, given the self-shading effects after the formation of cells aggregate, if 160 μmol quanta m^{-2} s^{-1} is saturating for cultures without aeration, it should also be saturating for cultures with aeration.

Line 84: 'cyanobacteria were floating singly' consider rephrasing to 'cyanobacteria floated as single filaments'

Response: revised accordingly at line 91

Line 85: Was there a round of pre-acclimation prior to the acclimation phase? Pre-acclimation is a crucial step to avoid carry-over from the previous culture conditions. See for example Trimborn et al 2019, Front. Mar. Sci https://doi.org/10.3389/fmars.2019.00167, Schaum and Collins 2014, Proc Biol Sci.281(1793): 20141486, Scheinin et al 2015 https://doi.org/10.1098/rsif.2015.0056, Lenski 2017 The ISME Journal volume 11, pages2181–2194(2017)

Response: Yes. All independent cultures were built up from a stock culture which had been kept in 100 μmol quanta m^{-2} s^{-1} and 25 °C. Subsequently, growth rate of each independent culture was continuously monitored. After the culture was established in the new conditions for 10 generations and its growth rate was stable for more than three consecutive dilutions, we believed that the culture adequately acclimated to the new conditions and started to take samples (see lines 91-93 in the revised manuscript).

Therefore, carry-over effect should not be a problem here.

Line 86: How were the growth rate curves fitted? Missing info

Response: In the original manuscript, this was described in lines 91-94. We provided more details about how we obtained the growth rate in the revised manuscript at lines 100-105 and 148-149.

Line 94: should be ‘before applying the natural logarithm’ instead of ‘ before natural logarithm’. Generally, how does using Chla as a proxy for growth deal with cells having more Chla per cell?

Response: Indeed, Chla:cell ratio was different between cultures grown under different conditions. However, when using Chla as a proxy for growth, what matters is Chla:cell ratio within the culture. For a specific culture, once it acclimates to its growth condition, its Chla:cell ratio is relatively stable. The main variation is the cell cycle-related variation, which can be eliminated by fixing the sampling time and taking samplings during consecutive dilutions. Practically, using Chla as a proxy for growth has also been proven to be a proper method (Breitbarth et al., 2007).

Line 99: ‘acute’ as stated above, be mindful of vocabulary used. Define once, then stick to it.

Response: we made the corresponding revisions to the manuscript as mentioned above.

Line 102: is 0.5 to read 50 minutes or 30 minutes? This seems really short for a 25mL vial to equilibrate to the correct temperatures!

Response: 25 ml was further dispensed into 5 vials, so it was 5ml-culture that equilibrated to the target temperature in 30 minutes (line 110-111 in the revised manuscript). We had tested this, and it turned out that 30 minutes was enough.

Line 107: The Padfield paper is pivotal, but it is not about the Schoolfield equation per se (it is about adaptation to warming and uses the Schoolfield as a tool).The second correct reference is Sharpe, P. J. & DeMichele, D. W. Reaction kinetics of poikilotherm development. J. Theor. Biol. 64, 649–670 (1977).

Response: The paper mentioned by the referee (Sharpe, P. J. & DeMichele, D. W. Reaction kinetics of poikilotherm development. J. Theor. Biol. 64, 649–670, 1977) is the origin of the Schoolfield equation, but modifications have been made by Schoolfield et al. (1981) and Padfield et al. 2015. In our study, the modified Schoolfield equation was used. We add the original paper at lines 117-118.

Line 113: Which package was used for the “optimize” function? Which version?

Line 114: If used correctly, the Sharpe-Schoolfield output should not require the ‘optimize’ function, but simply, rates at Topt can be obtained by re-arranging the equation. It is really not clear at all here how the data were fitted to the Sharpe- Schoolfield (it clearly went well as the figure looks correct). To me, it would make sense to either extract the parameters (Ea, Eh, Topt. Tc) and then compare them via a mixed model (e.g. parameter growth

temp*light with replicate within treatment as the random effect) or fit a non-linear mixed effects model where lnNrate schoolfield.high(ln.c,Ea,Eh,Th,temp=K,Tc=your Tc value) and, to begin with fixed = list(ln.c + Ea + Eh + Th growthtemp*light). You can then compare AICcs of your mod- els (e.g. test also additive effect, each on their own, and just the intercept) and chose the best one. If you compare extracted parameter values, then the MuMin dredge function will come in handy!

Response: "optimize" is a function in package "stats" in R language.

The analytical solution to T_{opt} given by Padfield et al. 2015 assumes that E_a is less than E_h (because of the existence of $\log(1-E_a/E_h)$ in the solution), which was not always satisfied in our original data analysis. However, we just found that this analytical solution was incorrect, and gave the correct one in the revised manuscript at lines 127. In the revised manuscript, with the correct analytical solution to T_{opt}, we have used the nonlinear mixed effects model to re-analyze the short-term thermal response for N_2 fixation (lines 155-160). We appreciate the referee's constructive suggestion.

Line 116: Why was it not possible to measure N2for samples at 31° C? At what time were the samples taken? I know N2 fixation-related genes show a strong circadian cycle, maybe a similar mechanism is involved?

Response: We found that the temperature was not homogenous in the multi-zone plant chambers that were used to determine the responses of N_2 fixation rate to acute temperature changes, so we used the accurately measured temperatures (which did not cover 31 °C) to do the model fitting. Base on the model, we were able to get the predicted N_2 fixation rates corresponding to the growth temperature.

We took all the samples in the middle of the light phase for all the treatments, and the circadian rhythm did not play a role here.

As the authors stated into the nice small "meta-analysis", there is a huge within strains variation, why don't you used more strains? Alternatively, more isolates instead of three if you wanted to assess for within strains variations?

Response: Almost all laboratory studies exploring the effects of temperature on *Trichodesmium* use the strain IMS 101, so we are not able to use more strains. We interpret the "huge within strains variation" as inter-laboratory variations, which probably comes from the differences in methodological details, such as aeration vs. no-aeration, LED vs. fluorescent lamp etc. However, for a certain study, variations within strains are small. Even with such huge inter-laboratory variations, there is still a trend that light limitation leads to less sensitivity of growth rate to temperature changes in *Trichodesmium* IMS 101.

Line 129: See comments above – how were the data dealt with? Again, you can either extract parameters and compare via a mixed model, or run a non-linear mixed model starting with the most complex model and then working your way down to the most simple model. For all other phenotypic traits (the ones where you are not fitting a slope), a mixed model

seams the way to go!

Response: We extracted parameters and compared via two-way ANOVA and Turkey test. We added the paragraphs describing how we analyzed these data at lines 161-164.

Results: Throughout: When giving a value, also give the standard deviation or standard error. When referring to the result of statistical test, just giving the post-hoc value is not enough, as that only refers to ONE specific pair-wise comparison. If reporting one specific pair-wise comparison, we need to know which one!

Response: In the revised manuscript, "Results" have been revised accordingly, making it traceable.

Line 140: Might be worth starting out with whether the combined effect of light limita- tion was indeed interactive, or additive, or if one out of the two described the data best. Without the appropriate reporting of the stats involved, this is impossible to tell.

Line 141: see above. Strictly speaking, this is not a temperature range, but three tempera- tures, 23,27,and 31oC.

Response: We followed this suggestion and revised the text at lines 167-180

Line 145: How much is 'slightly'? Line 152: How much higher is higher? Line 168: What was the variation around this 1.4 oC increase? Line 183: Add SD or standard error to these values

Response: We have reported the values in the form of mean plus SD or SE, and used more precise vocabularies and specific values to describe our findings in the revised manuscript at lines178-180, 209-211 and 228-230.

Line 159: Is acute the same as short-term here? Pick a word, then stick to it.

Response: Yes. We followed this suggestion and revised throughout our text.

Line 178: be mindful of the tense. Should be 'were able to sustain'

Response: Revised.

Line 185: again, not clear what the p value refers to, or what was actually tested in the two way ANOVA

Response: Revised accordingly throughout our text.

Discussion Line 191: "negative growth effects" seems a strong statement, maybe better use "reduced"

Response: Revised accordingly at lines 233-234.

Line 196: level should be levels

Response: Corrected.

Line 202: "temperature is lower" than surface?

Response: Yes. We have clarified this in the revised manuscript at line 247.

Line 206: maybe I didn^{t}t get it, but “respectively” to what?

Response: These two values were obtained from two papers cited in this sentence. We have corrected this in the revised manuscript at lines 250-252.

Line 210: This is a very nice and clear summary (the additive vs interactive bit), how- ever, without the correct statistical approach it is impossible to tell whether the data ac- tually support this conclusion!

Response: We provided this critical information in the revised manuscript at lines 147-164.

Line 232: May need a reference here

Response: A reference was added at lines 276.

Line 235: Should be equivalents, not equivalent

Response: Corrected.

Line 250: what is the difference here between ‘acclimated” and “short-term”? Youmentioned both “short-term temperature norms” and “acclimation” throughout the pa- per (e.g. Table S1). Please clarify.

Response: We apologize for having used the confusing wordings. Now, we have clarified this, as indicated in the above response, at lines 73-76

Line 257: ‘a bit different’ is too vague Line 258: not sure if ‘and/or’ is the correct choice of words here. Plus, it should be ‘on the time scales of acclimation processes’ . Consider adding that here, this is approximately 10 generations. Line 259: What about within-strain variation?

Response: We revised these at lines 295-305.

Line 266: ‘to some extent’ is a bit vague, may need a bit more information here.

Response: We have revised this part at lines 310-315

Tables Spell out HL and LL as high light and low light

Response: Revised accordingly.

You clearly have the data from the light curves in the table, so explaining how you actually got them should not cause too much agony (we hope).

Response: We explained how we got them in detail in the revised manuscript at lines 147-164.

Figures Might be worth mentioning the software the figures were produced in.

Response: Software is R (3.5.3) and the packages are ggplot2 (3.2.1) and plot3D(1.1.1).

Figure 1 The lettering of the subpanels as a, b,c, is highly confusing with the signifi- cance levels using the same lettering. Might be easier to present the significance levels as a table? What are the slopes in this graph? How were they fitted?

Response: Revised accordingly.

We just linked the near points with lines, so these lines mean nothing special. We removed the slopes/lines to rule out the confusions.

Figure 2 Spell out what a.u. stands for. Consider adding confidence intervals to model fits

Response: a.u. refers to artificial unit. We added the confidence intervals in Figure 2.

Figure 4 Not clear where the interactions are. Again, the significance levels are a bit distracting and probably better displayed in a table.

Response: The information provided in original Figure 4 was presented in Table 3 in revised manuscript

Figure 5: a) Probably good idea to highlight the symbol for this study in bold b) –d) why are there no SDs or confidence intervals?

Response: We redrew the original "Figure 5 panel a" accordingly as Figure 4 in the revised manuscript. Original "Fig 5 panel b-d" were removed for reasons (see lines 295-304).

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Light availability modulates the effects of warming in a marine N_2 fixer

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Abstract. ~~As a group of photosynthetic N_2 fixers (diazotrophs),~~ *Trichodesmium* species, as a group of photosynthetic N_2 fixers (diazotrophs), play an ~~especially~~ important role in the marine biogeochemical cycles of nitrogen and carbon, especially in oligotrophic waters. How ongoing ocean warming may interact with light availability to affect *Trichodesmium* is not yet clear. We grew *Trichodesmium erythraeum* IMS 101 at three temperature levels of 23, 27 and 31 °C under ~~two~~ growth limiting and saturating light levels of 50 and 160 µmol quanta m^{-2} s^{-1}, respectively, for at least 10 generations, and then measured physiological performances, including specific growth rate, N_2 fixation rate and photosynthesis. Light availability significantly modulated the growth response of *Trichodesmium* to temperature, with the specific growth rate peaking at ~27 °C under the light–saturating conditions, while growth of light–limited cultures was non–responsive across the tested temperatures ~~range of 23–31 °C~~(23, 27 and 31 °C). Short-term thermal responses for N_2 fixation indicated that both high growth temperature and light intensity increased the optimum temperature (T_{opt}) for N_2 fixation and decreased its susceptibility to supra–optimal temperatures (deactivation energy, E_h). Simultaneously, all light–limited cultures with low T_{opt} and high E_h were ~~When the acclimation of N_2 fixation to growth temperatures was evaluated by short–term temperature norms, the optimum temperature (T_{opt}) for N_2 fixation increased by 0.6–1.4 °C in the cells grown under high levels of temperature and light, and the susceptibility to supra–optimal temperatures (deactivation energy, E_h) was decreased by 56%–61%. However, light limitation decreased the T_{opt} by 0.5–1.8 °C and increased the supra–optimal temperature susceptibility by 33%–71%. This made all light–limited cultures~~ unable to sustain N_2 fixation during short–term exposure to high~~er~~ temperatures (33–34 °C) that are not lethal for ~~cultures~~ the cells grown under light–saturating conditions. Our results imply that *Trichodesmium* spp. growing under low light levels while distributed deep in the euphotic zone or under cloudy weather

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conditions might be less sensitive to long-term temperature changes that occur on the time scale of multi-generation but more susceptible to abrupt (less than one generation time span) temperature changes, such as those induced by cyclone and heat waves.~~more susceptible to ocean warming.~~

1. Introduction

In vast areas of the oceans, primary production is usually limited by availability of nitrogen (Moore et al., 2013). In addition to recycling within the euphotic zone, biologically available nitrogen sources can be supplied to phytoplankton from upwelling, aerosol deposition and N_2 fixation by diazotrophic prokaryotes, supporting new primary production (Dugdale and Goering, 1967). *Trichodesmium* is one of the major diazotrophic organisms occurring in the pelagic oceans (Zehr, 2011).~~Obviously, biological N_2 fixation is an important component of the marine biological CO_2 pump (Sohm et al., 2011).~~ ~~—~~*Trichodesmium* is a genus of filamentous cyanobacteria that exists as both single filaments and colonies consisting of tens to hundreds of trichomes, and that is broadly distributed in oligotrophic tropical and subtropical oceans (Capone et al., 1997). ~~Among the diazotrophs occurring in the pelagic oceans, *Trichodesmium* is the most well studied group (Bergman et al., 2013; Capone et al., 1997; Zehr, 2011), and has long been recognized as one of the major diazotrophic organisms in the open oceans (Martínez-Pérez et al., 2016; Zehr, 2011).~~ Its contribution to local new production can even be more important than that of nitrate diffusion in some regions (Capone et al., 2005; LaRoche and Breitbarth, 2005; Mahaffey et al., 2005), and it thus plays a significant role in ~~global~~ marine ecosystems and biogeochemical cycles of nitrogen and carbon (Sohm et al., 2011; Zehr, 2011).

Trichodesmium has attracted tremendous research interest ~~for about a century, especially~~ since its discovery as diazotroph in the early 1960s (Dugdale et al., 1961; Dugdale et al., 1964)~~(Bergman et al., 2013; Capone et al., 1997)~~. Recently, considerable research attention has been focused on evaluating effects of the ongoing ocean climate changes, including sea surface warming associated with global warming, on this keystone organism (Fu et al., 2014; Hutchins and Fu, 2017; Jiang et al., 2018). ~~In t~~The IPCC RCP 8.5 scenario predicts that~~,~~ upper ocean temperature will increase by about 3 °C on average by the end of the 21st century, and the strongest ocean warming will happen in tropical and subtropical regions (Collins et

al., 2013). Because of its important role in marine biogeochemical cycles and marine ecosystems, ~~Understanding~~ understanding the responses of *Trichodesmium* to ocean warming and their underlying mechanisms will be critical to evaluating the potential ~~consequences~~ implications of climate changes on marine primary productivity, food web dynamics and biogeochemical cycles.~~.~~

Previous studies demonstrate that without resource limitation the growth versus temperature curve is unimodal in *Trichodesmium* with lower and upper tolerance limits separately at 18-20 °C and 32-34 °C and optimum temperature at 26-28 °C (Breitbarth et al., 2007; Chappell and Webb, 2010; Fu et al., 2014).~~Field observations demonstrate that *Trichodesmium* occurrence is generally restricted to waters with sea surface temperatures (SST) between 20 °C and 30 °C (Breitbarth et al., 2007; Capone et al., 1997). *Trichodesmium's* lower limit is set by the physiological constraint of thermal tolerance, whereas the upper limit is set by the present SST maximum (Breitbarth et al., 2007). Laboratory studies show that the upper limit of *Trichodesmium* thermal tolerance is approximately 32–34 °C (Breitbarth et al., 2007; Fu et al., 2014). The growth rate versus temperature curve of *Trichodesmium* is unimodal, with an optimum temperature at ~27 °C (Breitbarth et al., 2007; Fu et al., 2014; Jiang et al., 2018). Because of the~~Based on these findings and the spatial heterogeneity of present temperatures and projected warming of *Trichodesmium's* habitat (Capone et al., 1997; Collins et al., 2013), the effects of ocean warming on *Trichodesmium* can be spatially diverse, generally benefiting those occurring in relatively high latitude but being harmful to those occurring near to the equator (Breitbarth et al., 2007; Fu et al., 2014 Thomas et al., 2012). However, this pattern can be distorted and complicated by resource limitations. For example, it is shown that iron limitation, which is commonly experienced by *Trichodesmium* in nature (Hutchins and Boyd, 2016; Sohm et al., 2011), substantially increases the optimum temperature in *Trichodesmium* (Jiang et al., 2018).~~the responses of *Trichodesmium* to warming have been suggested to be spatially diverse (Breitbarth et al., 2007; Fu et al., 2014; Jiang et al., 2018; Thomas et al., 2012).~~

Similar to iron availability, light is also among the key environmental drivers for *Trichodesmium* (Cai and Gao, 2015; Cai et al., 2015; Breitbarth et al., 2008). *Trichodesmium* can be distributed from the sea surface down to 150 m depth where light intensity at noon ranges from > 2000 µmol quanta m^{-2} s^{-1} to < 10 µmol quanta m^{-2} s^{-1} (Davis and McGillicuddy, 2006; Olson et al., 2015). Moreover, *Trichodesmium* is known to be able to partially regulate its vertical position in water column by buoyancy adjustment (Villareal et al. 2003). Currently, ocean warming effects on *Trichodesmium* have been widely

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examined under single, saturating light conditions, providing important knowledge on this diazotroph's physiological responses to temperature changes (Breitbarth et al., 2007; Fu et al., 2014; Jiang et al., 2018; Levitan et al., 2010). However, how it responds to warming under both light–limiting and saturating conditions is also of general significance, but has been rarely studied (Boatman et al., 2017).

The ~~response of~~ growth ~~to environmental change~~of phytoplankton is a holistic result of many biochemical and physiological activities, ~~which can differ from short acclimation times to long adaptation periods.~~so its responses to environmental changes are dependent on species-specific physiology. Normally, warming increases enzyme activities, accelerating biochemical reactions (Gillooly et al., 2001). For phytoplankton, reduced growth at high temperature might be the result of less carbon availability due to the higher thermal sensitivity of respiration compared to that of photosynthesis (Padfield et al., 2015). This negative effect of high temperature might be exacerbated by directly reduction of photosynthesis under light limitation. ~~This reflects an organism's acclimation and adaptive strategies to deal with environmental change (Somero, 2010). Thermal acclimation potentials of photosynthesis and respiration can be key growth responses to temperature changes in phytoplankton (Padfield et al., 2015).~~ In the diazotroph *Trichodesmium*, besides photosynthesis and respiration, N_2 fixation process might also play a critical role in its growth response to environmental changes~~, such as warming~~.~~. Nevertheless, l~~Little has been documented on this aspect (Jiang et al., 2018).

~~Light is well known to modulate the responses of photosynthetic organisms to environmental change, and light levels and photoperiodicity are particularly important in regulating metabolic activities related to N_2 fixation capacity in *Trichodesmium* (Breitbarth et al., 2008; Cai and Gao, 2015). *Trichodesmium* spp. can be distributed from the sea surface down to 120 m depth (Davis and McGillicuddy, 2006; Olson et al., 2015), where light intensity could be as low as 2 μmol quanta m^{-2} s^{-1}. Ocean warming effects on *Trichodesmium* have been widely examined under single, constant light conditions, providing important knowledge on this diazotroph's physiological responses to temperature changes (Breitbarth et al., 2007; Fu et al., 2014; Jiang et al., 2018; Levitan et al., 2010). However, how it responds to warming under both light–limiting and saturating conditions is also of general significance, considering its dynamic vertical distribution.~~ In ~~the present work~~this study, we explored the combined effects of temperature and light in *Trichodesmium erythraeum* IMS 101. We measured the specific growth rate, photosystem functions and N_2 fixation rate in *Trichodesmium* cultures acclimated to two

light levels and three temperatures. Moreover, we measured their short-term thermal responses for N_2 fixation. In this paper, "acclimation" and "acclimated" indicate that cultures were given enough time (several weeks) to respond to the environmental changes so that balanced growth was achieved, and "short-term" refers to acute processes and changes occurring within hours. Although adaptation (over several hundreds of generations) has been demonstrated to be critical in evaluating the responses in phytoplankton to environmental changes (Hutchins et al., 2015; Li et al., 2017; Padfield et al., 2015; Schaum et al., 2018; Tong et al., 2018), it is beyond the scope of this study.we investigated how temperature and light interactively affect *Trichodesmium*'s N_2 fixation and growth, and found that the cells acclimated to different temperatures and light levels exhibited differential physiological performances in terms of growth, photosynthesis and N_2 fixation, and differential thermal acclimation potential of N_2 fixation.

2. Material and methods

2.1 Culture conditions

Three replicate populationsTriplicate cultures of *Trichodesmium erythraeum* (strain IMS101, originally isolated from the North Atlantic Ocean by (Prufert–Bebout et al. 1993)) were established under six different culture conditions. These included factorial combinations of three temperatures (23±1, 27±1 and 31±1 °C) and two light intensities (saturating light, 160 ± 20 µmol quanta m^{-2} s^{-1} and limiting light, 50 ± 6 µmol quanta m^{-2} s^{-1}). These three growth temperatures are representatives of present and future temperatures of *Trichodesmium* habitats (Breitbarth et al., 2007; Fu et al., 2014). The limiting and saturating light levels were established based on a pilot experiment (Supplementary Fig. S1(a)) and previous studies (Cai et al., 2015; Breitbarth et al., 2008; Garcia et al., 2011). All the cultures were run semi–continuously by continual dilutions (every 2–4 days) in the artificial seawater medium YBCII without combined nitrogen (Chen et al., 1996) in 1–L glass flasks maintained in plant growth chambers (HP300G–C, Ruihua, China). Light was provided by LED tubes (FSL, China) with a 12:12 Light:Dark cycle. Different levels of light intensity were achieved using neutral density filters. Cultures were continuously bubbled with air (outdoor) so that the cyanobacterial filaments were floating singlyfloated as

125 single filaments. The cells were allowed to acclimate to each condition for at least 10 generations. Acclimation was confirmed by balanced growth with stable specific growth rate. before Then, we started the sampling and data collection.

2.2 Chlorophyll–a (Chl–a) concentration and specific growth rate

Chl–a concentration was spectrophotometrically quantified by gently filtering the cells onto glass–fiber filters (GF/F, Whatman), followed by extraction in pure methanol at 4 ℃ for 24 h and centrifugation at 6000g for 10 minutes. The 130 absorbance spectrum of the supernatant was determined from 400 nm to 700 nm using a spectrophotometer (DU800, Beckman, USA). Chl–a concentration was calculated as: [Chl–a] (μg mL^{-1}) =12.9447*(A_{665}–A_{750}), where A_{665} and A_{750} were respectively the absorbances at 665 and 750 nm (Ritchie, 2006).

Because Chl-a concentration is a good proxy for biomass in *Trichodesmium* (Breitbarth et al. 2007), Chl-a concentrations measured at different days were analysed using Eq. (1) to obtain the specific growth rate:

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$$\ln(\text{Chl-a}(t)) = \mu * t + b \quad (1)$$

where Chl-a(t) is the Chl-a concentration at time t (d), μ is the specific growth rate (d^{-1}), and b is interpreted as the natural logarithm of Chl-a concentration at day 0. Because the cultures were semi–continuously maintained, Chl–a concentrations at each time point was corrected by the dilution ratios with the assumption of no dilutions. Specific growth rate was calculated as the slope of the linear regression of the natural log of Chl–a versus time during consecutive dilutions (Hong et al., 2017). 140 Because the cultures were semi–continuously maintained, Chl–a concentrations at each time point was obtained by taking dilution ratios into account before natural logarithm.

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2.3 Short–term thermal response for N_2 fixation temperature norm of N_2 fixation

N_2 fixation rates were determined using the acetylene reduction assay assuming a ratio of 4:1 to convert ethylene production 145 to N_2 fixation (Capone, 1993). To examine the responses of N_2 fixation in the cells grown at different temperatures and light levels to acute short-term temperature changes, we simultaneously measured N_2 fixation at five temperatures ranging from 19 to 35 ℃. For each populationreplicate, a 25ml aliquot of the culture was taken and dispensed into five vials. Five vials each of which contained 5ml culture were separately placed in five different zones of two multi–zone culture chambers

(HP100–2 and HP100–3, Ruihua, China) and allowed to equilibrate to different target temperatures for ~~0.5~~30 ~~h~~minutes. Pilot experiments had showed that ~~0.5 h~~30 minutes was enough for temperature equilibrium. After temperature equilibration, each vial was spiked with 1 ml (12.5% of headspace volume) pure acetylene and incubated for another ~~0.5 h~~30 minutes under the growth light level. The quantity of ethylene produced was determined using a gas chromatograph with flame ionization detector (Clarus 580, PerkinElmer, USA).

Typically, the ~~acute~~ short-term thermal response curves for N_2 fixation were unimodal and negatively skewed, which could be accommodated to a modified version (Padfield et al., 2015; Schaume et al. 2018) of the Sharpe–Schoolfield model (~~Padfield et al., 2015;~~ Schoolfield et al., 1981; Sharpe and DeMichele, 1977):

$$N(T) = N(T_c) * \exp\left(E_a * \left(\frac{1}{kT_c} - \frac{1}{kT}\right)\right) / \left(1 + \exp\left(E_h\left(\frac{1}{kT_h} - \frac{1}{kT}\right)\right)\right) \quad (~~1~~2)$$

where N(T) is the N_2 fixation rate (μmol N_2 mg Chl–a^{-1} h^{-1}) at temperature T (Kelvin, K), E_a is the activation energy (electron volt, eV) for N_2 fixation, being indicative of the steepness of the slope leading to a thermal optimum, E_h is the deactivation energy (electron volt, eV) characterizing high temperature induced inactivation above deactivation temperature T_h (K), and $N(T_c)$ is the N_2 fixation rate at ~~the~~ an arbitrary reference temperature T_c (here, T_c = 25 °C) used for normalization. According to Eq. (2), the optimum temperature (T_{opt}, K) corresponding to the maximal N_2 fixation rate (N_{max}, μmol N_2 mg Chl–a^{-1} h^{-1}) is:

$$T_{opt} = E_h * T_h / (E_h + k * T_h * \ln(E_h/E_a)) \quad (3)$$

Additionally, we also obtained the N_2 fixation rate at growth temperature (N_{growth}) by bring corresponding growth temperatures to Eq. (2). ~~E_a, E_h, T_h and $N(T_c)$ are the parameters obtained through non–linear least squares regression using the 'nlsLM' function in the 'minpack.lm' package, and optimum temperature (T_{opt}) for N_2 fixation was calculated using 'optimize' function in R language. Bringing the T_{opt} into Eq. (1) gives the maximal N_2 fixation rate (N_{max}). Similarly, we can also get the N_2 fixation rate corresponding to the respective growth temperature (N_g). Unexpectedly, the thermal response curves of N_2 fixation in light–limited populations grown at 31 °C cannot be described by the Eq. (1) (more details in Sect. 3.3). Therefore, for this treatment, we use N_2 fixation rate measured at 30 °C (the assay temperature closest to 31°C) as its N_g.~~

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2.4 Chl–a fluorometry

Photosystem II (PSII) effective quantum yield (Φ_{PSII}) and photosynthetic relative electron transport rate (rETR) were

175 measured by using the Multiple Excitation Wavelength Chlorophyll Fluorescence Analyzer (MULTI–COLOR–PAM, Walz, German) equipped with the US–T temperature control unit (Walz, Germany). Aliquots of 1.5 ml of the culture were taken to determine effective quantum yield (Φ_{PSII}) under actinic light levels that were the same as those of the growth conditions. Then, Φ_{PSII}(E) values were successively measured at seven levels of light intensity (E) ranging from 0 to 1064 μmol quanta m^{-2} s^{-1}. Samples were allowed to acclimate to each light level for 3 minminutes before Φ_{PSII}(E) measurements (Suggett et a.,

180 2007). Relative electron transport rate (rETR) at each light level was calculated as: rETR = E * Φ_{PSII}(E) (Ralph and Gademann, 2005). The light response curve of rETR was analysed according to the model of (Eilers and Peeters, 1988):

$$rETR = \frac{E}{a*E^2+b*E+c} \quad (24)$$

~~where a, b and c are parameters that can be obtained through non- linear least squares regression in R language.~~

Photosynthetic parameters including photosynthetic light harvesting efficiency (α), rETR maximum ($rETR_{max}$) and light

185 saturation point (E_k) can be calculated as:

$$\alpha = \frac{1}{c} \quad (35)$$

$$rETR_{max} = \frac{1}{b+2*\sqrt{a*c}} \quad (46)$$

$$E_k = \frac{c}{b+2*\sqrt{a*c}} \quad (57)$$

During the measurements, sample temperature was maintained at the corresponding growth temperatures using the US–T

190 temperature control unit (Walz, Germany).

2.5 Statistical analyses

Statistical analyses were performed with the R language (version 3.5.3). [Chl-a] versus time in each of the triplicate cultures for each treatment was fitted to Eq. (1) using function “lm” in package “stats” to get the specific growth rate (μ). The significance of differences in specific growth rate (μ) between treatments was tested using two-way analyses of variance

195 (ANOVA) (function “aov” in package “stats”), followed by Tukey’s test for pairwise comparison (function “TukeyHSD” in package ‘stats’). The homogeneity of variance assumption and the residuals normality assumption were separately checked by

Levene's test (function "leveneTest" in package "stats") and Shapiro-Wilk test (function "shapiro.test" in package "stats"). The significance level was set to 0.05.

To test whether parameters $N(T_c)$, E_a, E_h, T_h and T_{opt} differ between different treatments, we fitted short-term thermal

200 responses for N_2 fixation to Eq.(2) using nonlinear mixed effects models ("nlme" package). Models included random effects on each of the parameters of Eq.(2) by replicate. The structure of the fixed effects of the nonlinear mixed effects model was determined by trying all possible models (625 models) and selecting the one with the lowest small sample-size corrected Akaike information criterion (AICc) (see Supplementary Table S3 for all tested models' AICc). AICc was calculated using function "AICc" in package "MuMIn".

205 Light curve of rETR in each of the triplicate cultures for each treatment was fitted to Eq. (4) using function "nls" in package "stats". The significance of differences in photosynthetic light harvesting efficiency (α), rETR maximum ($rETR_{max}$), light saturation point (E_k) and effective quantum yield (Φ_{PSII}) between treatments was tested using the same statistical methods as those for μ. The significance level was set to 0.05.

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3. Results

210 **3.1 Specific growth rate and N_2 fixation rate**

Specific growth rates of *Trichodesmium* IMS 101 were significantly affected by growth light intensity (two-way ANOVA, $F_{1,12} = 662.7$, $P < 0.001$), growth temperature (two-way ANOVA, $F_{2,12} = 22.0$, $P < 0.001$) and the interaction between these two drivers (two-way ANOVA, $F_{2,12} = 18.0$, $P < 0.001$) (Table 1). High growth light intensity increased specific growth rates of *Trichodesmium* IMS 101 by 63% at 23 °C (Tukey's test comparing light-saturated and light-limited growth rates at 23ºC,

215 $P < 0.001$), 111% at 27 °C (Tukey's test comparing light-saturated and light-limited growth rates at 27 °C, $P < 0.001$) and 88% at 31 °C (Tukey's test comparing light-saturated and light-limited growth rates 31 °C, $P < 0.001$), respectively (Fig. 1(a)). The interaction between growth light intensity and temperature on specific growth rate was indicated by the totally different temperature effects between light-saturated and light-limited cultures. Light–saturated growth rates of *Trichodesmium* IMS 101 were maximal at 27 °C with a value of 0.52 ± 0.02 d^{-1} (±SD), being higher by 29.5% (Tukey's test

220 comparing growth rates between light-saturated cultures grown at 27 °C and 23 °C, $P < 0.001$) and 21.3% (Tukey's test

comparing growth rates between light-saturated cultures grown at 27 °C and 31 °C, $P < 0.001$) than those at 23 °C and 31 °C, respectively. However, light–limited growth rates ranged from 0.23 ± 0.02 d^{-1} (±SD) to 0.25 ± 0.01 d^{-1} (±SD), with no significant differences between the tested temperatures (Tukey's test comparing growth rates among light-limited cultures grown at three temperatures, $P > 0.05$ for all three comparisons).

225 Overall, N_2 fixation rates at growth temperature (N_{growth}) (Fig. 1(b)) were significantly higher in cultures grown under high light intensity compared to those grown under low light intensity (two-way ANOVA, $F_{1,12} = 149.9$, $P < 0.001$). Also, growth temperature significantly affected N_{growth} (two-way ANOVA, $F_{1,12} = 3912.3$, $P < 0.001$). Different thermal effects between light-saturated and light-limited cultures indicated a significant interaction between light and temperature on N_{growth} (two-way ANOVA, $F_{2,12} = 112.7$, $P < 0.001$). For light-saturated cultures, the N_{growth} peaked at 27 °C with a value of 17.1 ± 0.5

230 µmol N_2 mg Chl–a^{-1} h^{-1} (±SD), which was higher by 39% and 17% than those at 23 °C (Tukey's test comparing N_{growth} between light-saturated cultures grown at 27 °C and 23 °C, $P < 0.001$) and 31 °C (Tukey's test comparing N_{growth} between light-saturated cultures grown at 27 °C and 23 °C, $P < 0.001$), respectively. However, for light-limited cultures, the value of N_{growth} at 27 °C (6.8±0.2 µmol N_2 mg Chl–a^{-1} h^{-1}(±SD)) was similar to that at 23 °C (6.3±0.1 µmol N_2 mg Chl–a^{-1} h^{-1}(±SD)) (Tukey's test comparing N_{growth} between light-limited cultures grown at 27 °C and 23 °C, $P = 0.54$) but significantly higher

235 than that at 31 °C (3.8±0.4 µmol N_2 mg Chl–a^{-1} h^{-1}(±SD)) (Tukey's test comparing N_{growth} between light-limited cultures grown at 27 °C and 31 °C, $P < 0.001$).Specific growth rates of *Trichodesmium* IMS 101 in light–saturated cultures were higher than those in light–limited cultures by 63% at 23 °C, 111% at 27 °C and 88% at 31 °C, respectively ($P<0.05$, Tukey's HSD method; Fig. 1(a)). Within the temperature range of 23–31 °C, light–saturated growth rates of *Trichodesmium* IMS 101 were maximal at 27 °C with a mean value of 0.52 d^{-1}, 29.5% and 21.3% higher than those at 23 °C and 31 °C, respectively

240 ($P<0.05$, Tukey's HSD method). However, light–limited growth rates ranged from 0.23 – 0.25 d^{-1}, without showing any maximal value at any temperature levels tested and with no significant differences across the different temperatures ($P>0.05$, Tukey's HSD method), although the mean growth rate at 31°C was slightly lower than that at 23 °C or 27 °C.

N_2 fixation rates at growth temperature (N_g) were higher under the growth–saturating light than under the limiting level by 94% at 23 °C, 149% at 27 °C and 128% at 31 °C ($P<0.05$, Tukey's HSD method; Fig. 1(b)). Light availability also

245 modulated the effects of growth temperature on N_g (Fig. 1(b)). As with specific growth rate, the N_g peaked at 27 °C under

light–saturating conditions, but N_g was insensitive to growth temperature change under the light–limiting conditions (P>0.05, Tukey's HSD method).

3.2 PSII effective quantum yield (Φ_{PSII}) and rETR light response curves

Compared to light–saturated cells, light–limited cells had higher values of Φ_{PSII} (Fig. 1(c); two-way ANOVA, $F_{1,12} = 233.2$,

250 $P < 0.001$)(P<0.05, Tukey's HSD method; Fig. 1(c)). Meanwhile, under both light regimes, Φ_{PSII} in *Trichodesmium* populations cultures grown at 31 °C was significantly higher than that in cultures grown at 23 °C and 27 °C (two-way ANOVA, $F_{2,12} = 22.1$, $P < 0.001$). No interaction between growth light intensity and temperature on Φ_{PSII} was found (two-way ANOVA, $F_{2,12} = 1.8$, $P = 0.211$).

(P<0.05, Tukey's HSD method; Fig. 1(c)). The rETR light response curve of *Trichodesmium* IMS 101 was influenced by

255 growth temperature in both light–saturated (Fig. 2(a)) and light–limited (Fig. 2(b)) treatments. This thermal impact was mainly reflected in the $rETR_{max}$(two-way ANOVA, $F_{2,12} = 31.2$, $P < 0.001$), which tended to be higher in cultures acclimated to 31 °C than those in cultures acclimated to 23 °C or 27 °C (Table 2; Fig. 2). Additionally, high growth light intensity tended to decreased the $rETR_{max}$ (two-way ANOVA, $F_{1,12} = 31.2$, $P < 0.001$), especially for cultures grown at 27 °C (Tukey's test comparing $rETR_{max}$ between light-saturated and light-limited cultures grown at 27 °C, $P < 0.001$). Both high light

260 intensity (two-way ANOVA, $F_{1,12} = 6.0$, $P < 0.05$) and high temperature (two-way ANOVA, $F_{2,12} = 5.0$, $P < 0.05$) significantly increased the E_k (Table 2).populations acclimated to 31 °C (P<0.05, Tukey's HSD method; Table 1).

3.3 Short–term temperature normthermal response of for N_2 fixation

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Optimum temperature (T_{opt}) for N_2 fixation in *Trichodesmium* IMS 101 was affected by both growth temperature and light intensity (Table 3). Generally, T_{opt} for N_2 fixation in light-saturated cultures were higher than that in light-limited cultures,

265 whereas warming effects on N_2 fixation T_{opt} differed between light-saturated and light-limited cultures. For light-saturated cultures, elevations of growth temperature raised the T_{opt} for N_2 fixation. A 4 °C warming was accompanied by 0.5-0.8 °C increase of T_{opt}, which was 28.7 ± 0.2 °C (±S.E.M), 29.5 ± 0.2 °C (±S.E.M) and 30.0 ± 0.3 °C (±S.E.M)) for the cells grown at at 23 °C, 27 °C and 31 °C, respectively. Under limiting light level, T_{opt} in cultures grown at 27 °C (T_{opt} = 28.6 ± 0.2 °C (±S.E.M)) was higher than that in cultures grown at 23 °C (T_{opt} = 28.2± 0.2 °C (±S.E.M)), but T_{opt} in cultures grown at 31 °C

270 (T_{opt} = 27.8± 0.2 °C (±S.E.M)) was the lowest among all treatments. As expected, the maximal N_2 fixation rate (N_{max}) in light-saturated cultures was higher than that in light-limited culture (Table 3). The temperature effect on N_{max} was also dependent on the light availability. Light-saturated N_{max} was highest in cultures grown at 27 °C (N_{max} = 19.3 ± 0.4 μmol N_2 mg Chl–a $^{-1}$ h^{-1} (±S.E.M)), being higher by 21% and 32% than those grown at 23 °C (N_{max} = 16.0 ± 0.3 μmol N_2 mg Chl–a $^{-1}$ h^{-1},(±S.E.M)) and 31 °C (N_{max} = 14.6 ± 0.4 μmol N_2 mg Chl–a $^{-1}$ h^{-1},(±S.E.M)), respectively. However, N_{max} for light-

275 limited cultures was similar among different temperature treatments (Table 3).

The value of deactivation energy (E_h) for N_2 fixation, reflecting the thermal susceptibility to supra–optimal temperatures, was affected by both light availability and growth temperature, but not by their interaction (Table 3). E_h tended to be lower in *Trichodesmium* cultures grown under high temperature and high light intensity. With the highest T_{opt} (30.0 ± 0.3 °C (±S.E.M)) and the lowest E_h (1.47 ± 0.14 eV(±S.E.M)) among all treatments, light–saturated cultures acclimated to 31°C

280 were the only cultures that were able to maintained considerable N_2 fixation rates at assay temperatures as high as 34 °C (Fig. 3). In addition, both light availability and growth temperature affected the deactivation temperature (T_h) for N_2 fixation in *Trichodesmium* and no interaction between these two drivers was found on T_h (Table 3). T_h in light-saturated cultures tended to be higher than that in light-limited cultures regardless of the growth temperature. T_h was lower in cultures grown at 31 °C compared to that in cultures grown at 23 or 27 °C under both light levels. The activation energy (E_a) for N_2 fixation

285 was affected by growth temperature but not by growth light intensity (Table 3). The values of E_a for N_2 fixation increased from 0.49 ± 0.04 eV (±S.E.M) to 0.91± 0.05 (±S.E.M) and 1.07± 0.04 eV(±S.E.M) as growth temperatures increased from 23 °C to 27 °C and 31 °C.

Generally, the N_2 fixation rates exposed to acute temperature changes were well- described by the modified Sharpe–Schoolfield model (Eq. (1)) in *Trichodesmium* IMS 101 grown under the saturating (Fig. 3(a)) and limiting (Fig. 3(b)) light

290 levels. The only exception is the light-limited cultures grown at 31 °C whose N_2 fixation rates showed nearly insensitivity to temperature changes within an assay temperature range of 23–30 °C, which made the model fitting unsuccessful (Fig. 3(b), Fig. 4).

Under saturating light level, the maximal N_2 fixation rate (N_{max}) corresponding to the optimum temperature (T_{opt}) was highest in cultures grown at 27 °C, with a mean value of 19.3 μmol N_2 mg Chl–a $^{-1}$ h^{-1}, 21% and 30% higher than those

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295 grown at 23 °C and 31 °C, respectively (Fig. 4(a); P<0.05, Tukey's HSD method). T_{opt} for N_2 fixation in light-saturated cultures was slightly but significantly increased by growth temperature increase (Fig. 4(b)). An 8 °C warming (from 23 °C to 31 °C) was accompanied by 1.4 °C increase of T_{opt} (from 28.7 °C to 30.1 °C) (p<0.05, Tukey's HSD method). Simultaneously, the values of deactivation energy (E_h), reflecting the thermal susceptibility to supra-optimal temperatures, in cultures acclimated to 31°C were 61% and 56% lower than those in cultures acclimated to 23 °C and 27 °C, respectively

300 (Fig. 4(c)). Higher T_{opt} and lower E_h in light-saturated cultures acclimated to 31°C made them the only treatment that could maintain considerable N_2 fixation rates at assay temperatures as high as 34 °C (Fig. 3(a)). Light limitation decreased the N_{max} by 7.9 and 12.1 μmol N_2 mg Chl-a^{-1} h^{-1}, decreased T_{opt} by 0.5 and 1.1 °C, and increased E_h by 3.3 and 1.4 eV at 23 °C and 27 °C, respectively (Fig. 4(a-c); Table S1). There is no thermally driven difference in N_2 fixation T_{opt}, N_{max} and E_h in light-limited cultures (p>0.05, Tukey's HSD method). Although we cannot derive the N_2 fixation T_{opt}, N_{max} and E_h in the light-

305 limited cultures grown at 31 °C, it is evident that acclimation to 31 °C did not help light-limited cultures maintain N_2 fixation rates during the short-term exposure to supraoptimal temperatures (Fig. 3(b)). None of the light-limited cultures can sustain N_2 fixation rate at an assay temperature of 34 °C (Fig. 3(b)). Unlike N_{max}, T_{opt} and E_h, the activation energy (E_a), representing the thermal dependence of metabolic activity within the range of temperature below the deactivation temperature (T_h) for N_2 fixation, was not affected by light availability, but was increased by higher growth temperature

310 regardless of the light levels (Fig. 4(d)). The mean values of E_a for N_2 fixation increased from 0.50 eV to 1.07 eV as growth temperatures increased from 23 °C to 31 °C (p<0.05, Tukey's HSD method) in light-saturated cultures. These values in light-limited cultures increased from 0.43 eV at 23 °C to 1.01 eV at 27 °C. Surprisingly, the T_h, above which temperature increase induces negative effects on N_2 fixation, was affected by neither growth temperature nor light availability (p>0.05, Two-way ANOVA; Supplementary TableS1).

315 **4 Discussion**

In this study, light availability not only affected growth rate and N_2 fixation directly, but also modulated their responses to temperature changes in *Trichodesmium* IMS 101. Reduced energy supply due to light limitation leads to lowered nitrogen fixation and thus reduced growth in the diazotroph. The specific growth rate and N_2 fixation rate were maximal at 27 °C for

saturating light–grown cells, but were virtually insensitive to temperature changes across the tested temperature (23, 27 and 31 °C) ~~within the thermal range of 23–31 °C~~ for light–limited ~~growth~~ cultures. ~~It appears that reduced energy supply due to light limitation leads to lowered nitrogen fixation and thus negative growth effects on the diazotroph.~~

The interactions between temperature and light on *Trichodesmium* demonstrated in this work are relevant to natural light and temperature variations and to *Trichodesmium* global change physiology and biogeography. Light supplies energy for photosynthesis, growth and other key activities, such as N_2 fixation in cyanobacterial diazotrophs. The observed phenomenon that the growth rate becomes less sensitive to temperature changes (Fig 1(a) and Fig. 4) ~~(Fig. 5(a))~~ in *Trichodesmium* IMS 101 under limiting light levels can be attributed to insufficient energy supply ~~being insufficient~~ for the cells to respond to temperature changes. While thermal biological responses are mainly based on enzymatic performance, light limitation suppresses syntheses of enzymes (Raven and Geider, 1988), and thus subsequently limits thermal responses. Although light–limited phytoplankton cells typically allocate more resources to light–harvesting systems to compensate for light shortages, at very low irradiances this compensation cannot prevent light harvesting capacity from being a limiting factor for enzyme synthesis and growth (Raven and Geider, 1988). Field investigations show that vertical distributions of *Trichodesmium* can reach to depths greater than 100 m, where light is absolutely limiting and temperature is lower compared to surface temperature (Olson et al., 2015; Rouco et al., 2016). According to the typical values of surface ~~light dose~~solar irradiances and vertical extinction coefficient in tropical and subtropical oceans (Olson et al., 2015), the daily light dose received by the light–limited cultures in our study corresponds to that received by *Trichodesmium* at a depth of 50–60 m. The contribution of biomass and N_2 fixation by *Trichodesmium* at depths greater than 50 m might ~~be >28% and~~range from 7~~%–~~% to ~~20~~28%~~, respectively~~ (Davis and McGillicuddy, 2006; Olson et al., 2015). Therefore, the evaluation of potential warming effects on *Trichodesmium* should not be constrained to the populations inhabiting light–saturated environments (upper tens of meters) (Breitbarth et al., 2007; Jiang et al., 2018), making 3–Dimensional models indispensable. In existing 3–Dimensional model studies involving *Trichodesmium* (Boyd and Doney, 2002; J. K. Moore et al., 2001), the combined effects of temperature and light on *Trichodesmium* biological activities are simply assumed to be additive, which is proven to be inappropriate in this work. ~~Although~~ While the absolute values of N_2 fixation rate under light limiting and saturating levels cannot be directly compared on the basis of Chl–a content, since lower light level resulted in more cellular Chl–a

content (Supplementary Fig. S1(b)), comparison of the thermal response patterns can generate~~s~~ ~~some~~ useful information ~~that can be used to~~for improv~~e~~inge model predictions of diazotrophic responses to ocean climate changes.

~~Temperature norms or thermal windows~~Thermal responses for organisms are known to be useful in evaluating thermal acclimation potential and probing low and high temperature tolerances (Gunderson et al, 2010; Somero, 2010; Way and Yamori, 2014). In this work, the shape of the short–term ~~temperature norm~~thermal response curves for ~~of~~ N_2 fixation is normalization–independent because cells were exposed to different assay temperatures for only one hour, hardly changing the elemental stoichiometry or cellular pigments component. When exposed to abrupt temperature gradients, the light-saturated cells acclimated to higher temperature ~~and light levels~~ exhibited higher T_{opt} values (~~Fig. 4(b)~~Table 3) and lower thermal susceptibility to supra–optimal temperatures (E_h; Table 3~~Fig. 4(c)~~). This indicates an increased capability for the diazotroph to tolerate short-term warming impacts. However, ~~this is only true under light–saturating conditions, and~~ light limitation made~~would make~~ the cells more susceptible to warming due to decreased T_{opt} and increased E_h for N_2 fixation (Table 3~~Fig. 3(b)~~). Moreover, with light limitation, acclimation to high temperature did not help *Trichodesmium* cells tolerate short-term supral-optimal temperature. ~~.~~On the other hand, Chl–a fluorescence data shown that the PSII ~~of~~ in light–limited cultures ~~were~~ was as healthy as ~~those of~~that in cells grown under saturating light (Fig. 1(c), 2), and it has been shown that damage to PSII usually occurs at temperatures above 45 °C (Yamori et al., 2014). Therefore, the collapse of N_2 fixation at high temperature was not likely caused by the dysfunction of the photosystems, but might be caused by the uncoupling of adenosine triphosphate (ATP) synthesis to electron transport, since~~. This is because~~ proton leakiness of the thylakoid membrane has been frequently proposed as a problem at high temperature (Yamori et al., 2014). This is consistent with the observation that supra–optimal temperature inhibition of N_2 fixation was aggravated by light limitation (Fig. 3). In addition, damage to nitrogenase at high temperatures might also be one of the reasons responsible for the faster drop of N_2 fixation at high temperature in light–limited cultures (Gallon et a., 1993). This is because the extra investment of resources in repair of damaged nitrogenase could not be supported under light–limiting conditions (Fig. 3(b)). Therefore, light availability exerts critical control on the acclimation potential of N_2 fixation in *Trichodesmium* to warming.

Acclimation to different temperatures also affected the activation energy (E_a) for N_2 fixation in *Trichodesmium* IMS 101 (~~Fig. 4(d)~~Table 3). For *Trichodesmium* species, N_2 fixation can be controlled by supply of ATP/reducing equivalents,

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mainly coming from photosynthesis, and the inherent catalytic capacity of the nitrogenase. ~~Both of these may have~~These two processes may exhibit different temperature dependence, i.e. different E_a. The E_a of the controlling process determines the N_2 fixation E_a (Hikosaka, et al,, 2006; Staal et al., 2003). Therefore, the differences in N_2 fixation E_a between cultures grown at different temperatures may reflect that N_2 fixation was primarily controlled by different processes in cultures acclimated to different temperatures. Preliminary evidence supporting this hypothesis came from the various effects of assay light intensity on the values of E_a for N_2 fixation between light–limited cultures grown at 23 °C and 27 °C (Supplementary Table S1~~2~~, Fig. S2). For *Trichodesmium* grown under limiting light level, the lower E_a values in ~~populations~~ cultures acclimated to 23 °C was significantly elevated by the increased assay light intensity~~,~~ which can provide more ATP/reducing equivalents (Supplementary Table S1~~2~~; Fig. S2(a)). This suggests the constraint should~~is~~ be the ~~the~~ supply of ATP/reducing equivalents. The higher E_a values in ~~populations~~ cultures acclimated to 27 °C were insensitive to the assay light intensity changes, suggesting N_2 fixation ~~is~~ should not be controlled by the supply of ATP/reducing equivalents at this optimal temperature, but may possibly be controlled by inherent catalytic capacity of the nitrogenase (Supplementary Table S1~~2~~; Fig. S2(b)).

The short–term ~~temperature norms of~~thermal responses for N_2 fixation mirror thermal shock responses. If cells are ~~allowed to~~ exposed to the thermal changes for longer time, acclimation will definitely change the thermal responses for ~~the temperature norms of~~ N_2 fixation in *Trichodesmium* (Breitbarth et al., 2007; Fu et al., 2014; Staal et al., 2003). To compare the short–term and acclimated ~~temperature~~ thermal responses for N_2 fixation~~norms~~, we calculated the corresponding values of E_a ~~(Fig. 5(b))~~, E_h ~~(Fig. 5(c))~~ and T_{opt} ~~(Fig. 5(d))~~, being respectively 0.93 ± 0.64 eV(±S.E.M), 1.86 ± 1.19 eV(±S.E.M) and 27.1 ± 1.0 °C(±S.E.M),~~0.93eV, 1.86 eV and 27.1 °C,~~ for fully–acclimated N_2 fixation within the range of 20–34 °C growth temperatures in *Trichodesmium* IMS 101~~, as reported by~~ (Breitbarth et al., ~~(~~2007~~)~~). These values of E_a and E_h are comparable to those derived from short–term ~~temperature norms of~~thermal response for N_2 fixation ~~rate~~ in the same strain grown under light–saturating condition and 31 °C in our study (~~Fig. 5(b–d)~~Table 3), but the T_{opt} value~~s~~ ~~are~~ is lower than ~~those~~ that from short–term ~~temperature norms~~thermal response. On the other hand, we have tried to derive values of E_a, E_h and T_{opt} for acclimated N_2 fixation rates in another three *Trichodesmium erythraeum* strains (strains RLI, KO4-20 and 21-75) (Fu et al., 2014), but the model fitting failed to converge. Instead of been negatively skewed, the thermal response curves of acclimated

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N_2 fixation in these three *Trichodesmium* strains are nearly symmetrical.~~Additionally, the values of E_a, E_h and T_{opt} for acclimated N_2 fixation rates in another three *Trichodesmium* strains were respectively estimated to be 2.76–4.06 eV, 0.54–0.94 eV and 26.1 °C (Fu et al., 2014), being a bit different from the values mentioned above.~~ These comparisons show that ~~temperature norms of~~thermal response for N_2 fixation in *Trichodesmium* are strains–specific, and~~/or~~ are affected ~~by~~ on the time scale of acclimation process.

In the oceans, *Trichodesmium* and other pelagic phytoplankton are often exposed to ~~acute~~ abrupt temperature changes due to strongly disturbed weather conditions, such as tropical cyclones, and marine heat waves. Global warming has been predicted to increase both tropical cyclone intensities, and the frequency of the most intense tropical cyclones (Elsner et al. 2008; Knutson et al., 2010; Wehner et al., 2018). Upper ocean temperature declines prior and during cyclone event~~s~~, and then increases abruptly afterwards (Li et al., 2009), accompanied by strong variations of surface solar radiation and stratification (Sriver and Huber, 2007). ~~The E_a, E_h and T_{opt} values for N_2 fixation of *Trichodesmium* IMS 101 obtained in this work for the cells acclimated to different temperatures and light levels can, to some extent, be useful in understanding its responses to stochastic and abrupt temperature changes.~~ These abrupt temperature changes occurring in nature are not as acute as those in our experiment. For example, temperature changes caused by cyclone and heat waves are on the scale of 0.5 - 1 °C per day (Babin et al., 2004; Beca-Carretero et al., 2018). Nonetheless, these temperature changes occur within one generation of *Trichodesmium* because of its low growth rate, leaving not enough time for full acclimation. Therefore, the values of E_a, E_h, T_h and T_{opt} provided in this study can likely serve as proxies for some types of abrupt natural temperature increases.

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Code/Data availability

All data obtained in this study are in Supplement.

Author contribution

KG and XY designed the experiment. XY carried out the experiment. XY, FXF, DH and KG analysed the data and wrote the manuscript.

Competing interests

The authors declare no competing of interest.

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590 **Table 1** Results of two-way ANOVA for specific growth rate, N_2 fixation rate, effective quantum yield and parameters derived from rETR light curve with interactions between “Temperature” and “Light”. See Supplementary Table S2 for results of pair-wise comparisons.

	Parameter	Effect	d.f	F value	P value
	Specific growth rate	**Temperature**	**2,12**	**22.0**	**< 0.001**
		Light	**1,12**	**662.7**	**< 0.001**
		Temperature*Light	**2,12**	**18.0**	**< 0.001**
	N_2 fixation rate (N_{growth})	**Temperature**	**2,12**	**3912.3**	**< 0.001**
		Light	**1,12**	**149.9**	**< 0.001**
		Temperature*Light	**2,12**	**112.7**	**< 0.001**
	Effective quantum yield	**Temperature**	**2,12**	**22.1**	**< 0.001**
		Light	**1,12**	**233.2**	**< 0.001**
		Temperature*Light	2,12	1.8	0.211
rETR light curve	alpha	**Temperature**	**2,12**	**24.5**	**< 0.001**
		Light	**1,12**	**10.6**	**< 0.01**
		Temperature*Light	2,12	0.2	0.815
	E_k	**Temperature**	**2,12**	**5.0**	**< 0.05**
		Light	**1,12**	**6.0**	**< 0.05**
		Temperature*Light	2,12	1.0	0.394
	$rETR_{max}$	**Temperature**	**2,12**	**31.2**	**< 0.001**
		Light	**1,12**	**139.8**	**< 0.001**
		Temperature*Light	**2,12**	**8.1**	**< 0.01**

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595 **Table** ~~1~~ 2 The light harvesting efficiency (α), relative election transport rate maximum ($rETR_{max}$) and light saturation point (E_k), derived from the ~~rapid~~ light curves (Fig. 2), for *Trichodesmium* grown at different temperature and light intensity levels; values represent the means ~~and~~ ± standard deviations of biological replicates (n=3); ~~error bars for the standard deviations of biological replicates (n=3);~~ superscripts with different letters represent significant difference (Turkey's test, more details in Supplementary Table S2; $p<0.05$;) among the treatments. The units of E_k and $rETR_{max}$ are μmol quanta m^{-2} s^{-1}
600 and arbitrary unit, respectively.

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	~~Acclimation~~Growth conditions					
	~~HL~~Light-saturating			~~LL~~Light-limiting		
	23 °C	27 °C	31 °C	23 °C	27 °C	31 °C
α	0.25 ± 0.01[ac]	0.24 ± 0.03[a]	0.28 ± 0.01[c]	0.30 ± 0.03[bc]	0.28 ± 0.03[b]	0.35 ± 0.03[b]
E_k	316 ± 22[ab]	322 ± 45[ab]	371 ± 16[a]	270± 17[b]	319 ± 38[ab]	329 ± 21[ab]
$rETR_{max}$	78 ± 3[a]	72 ± 3[a]	105 ± 2[b]	80 ± 6[a]	90 ± 2[c]	115 ± 4[b]

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605 **Table 3** Model parameters of thermal responses for N_2 fixation. The structure of the fixed effect is: $N(T_c)$ ~ Temperature * Light; E_a ~ Temperature; E_h ~ Light + Temperature; T_h ~ Light + Temperature. "+" and "*" represent additive and interactive effects, respectively.

Parameter	Light	Temperature(°C)	Estimate	S.E.M	CI(95%)
$N(T_c)$ (μmol N_2 mg Chl-a^{-1} h^{-1})	Light-saturating	23	38.3	1.0	[36.4, 40.3]
		27	39.1	1.0	[37.1, 41.1]
		31	41.3	1.5	[38.2, 44.3]
	Light-limiting	23	19.5	0.8	[18.0, 21.1]
		27	15.0	0.8	[13.4, 16.6]
		31	20.3	1.1	[18.2, 22.5]
E_a (eV)	No Light effect	23	0.49	0.04	[0.41, 0.57]
		27	0.91	0.05	[0.80, 1.01]
		31	1.07	0.04	[0.98, 1.16]
E_h (eV)	Light-saturating	23	4.49	0.51	[3.47, 5.51]
		27	3.99	0.31	[3.36, 4.61]
		31	1.47	0.14	[1.18, 1.75]
	Light-limiting	23	7.51	0.68	[6.15, 8.87]
		27	7.01	0.60	[5.82, 8.21]
		31	4.49	0.49	[3.50, 5.48]
T_h (°C)	Light-saturating	23	32.6	0.1	[32.3, 32.9]
		27	32.4	0.2	[32.1, 32.8]
		31	31.8	0.2	[31.3, 32.3]
	Light-limiting	23	31.1	0.1	[30.9, 31.4]
		27	31.0	0.1	[30.7, 31.2]
		31	30.3	0.2	[29.9, 30.6]
T_{opt} (°C)	Light-saturating	23	28.7	0.2	[28.2, 29.1]
		27	29.5	0.2	[29.2, 29.8]
		31	30.0	0.3	[29.5, 30.6]
	Light-limiting	23	28.2	0.2	[27.9, 28.6]
		27	28.6	0.2	[28.3, 28.9]
		31	27.8	0.2	[27.4, 28.2]
N_{max} (μmol N_2 mg Chl-a^{-1} h^{-1})	Light-saturating	23	16.0	0.3	[15.3, 16.6]
		27	19.3	0.4	[18.5, 20.1]
		31	14.6	0.4	[13.9, 15.4]
	Light-limiting	23	8.3	0.3	[7.6, 8.9]
		27	7.4	0.4	[6.6, 8.2]
		31	8.6	0.4	[7.8, 9.4]

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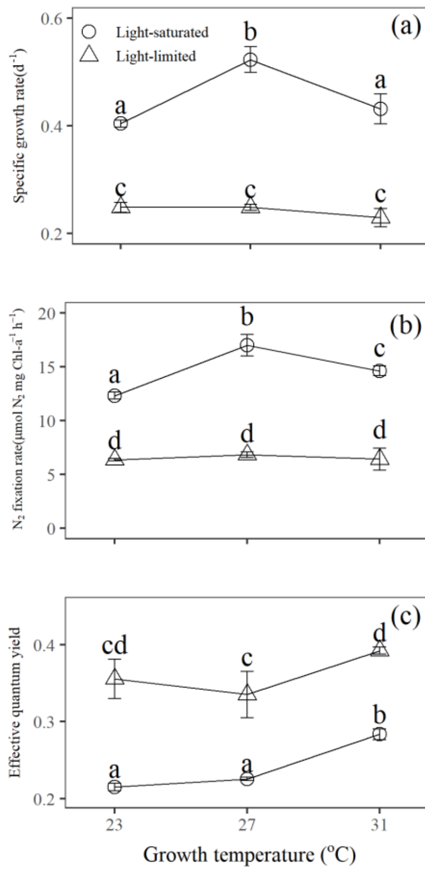

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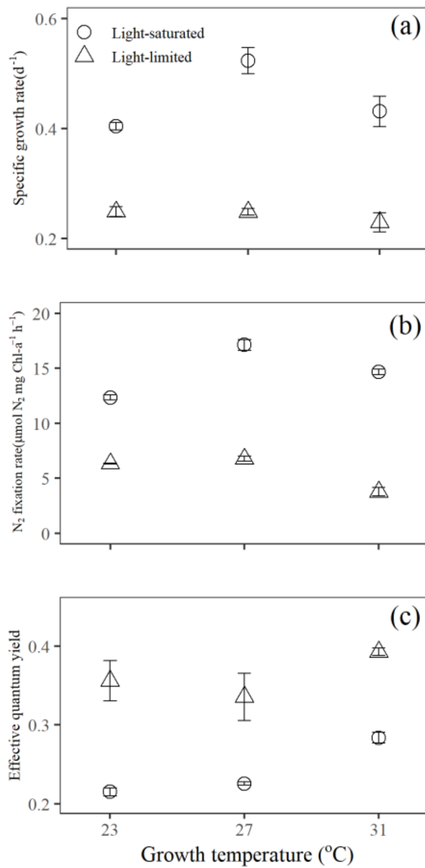

615 **Figure 1** *Trichodesmium* responses of (a) growth, (b) N_2 fixation rate and (c) effective quantum yield to temperature and light availability interactions; values represent the means ± the standard deviations of biological replicates(n=3).values represent the means and error bars for the standard deviations of biological replicates(n=3); points marked with different letters are significantly different from each other (p<0.05).

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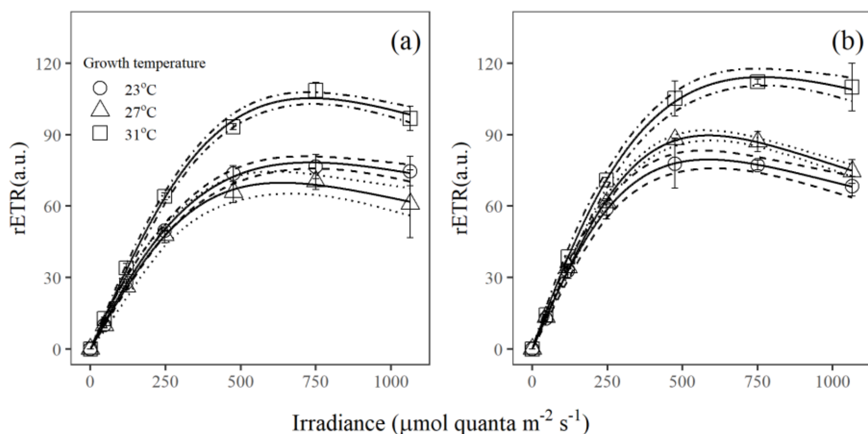

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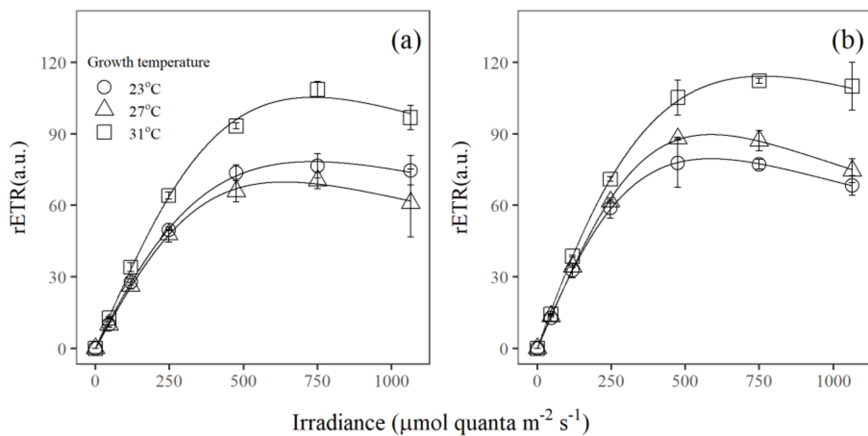

625 **Figure 2** Light response curves of rETR in *Trichodesmium* populations grown under (a) light–saturating and (b) light–limiting conditions; values represent the means ± standard deviations of biological replicates(n=3); Solid lines illustrate the best fit to Eq. (4) with 95% confidence intervals as dashed lines. ~~values represent the means and error bars for the standard deviations of biological replicates(n=3); fitted lines are based on mean parameters at each treatment across replicates (n=3) derived from non–linear least squares regression using the Eilers–Peeters model (Eq. (2)).~~

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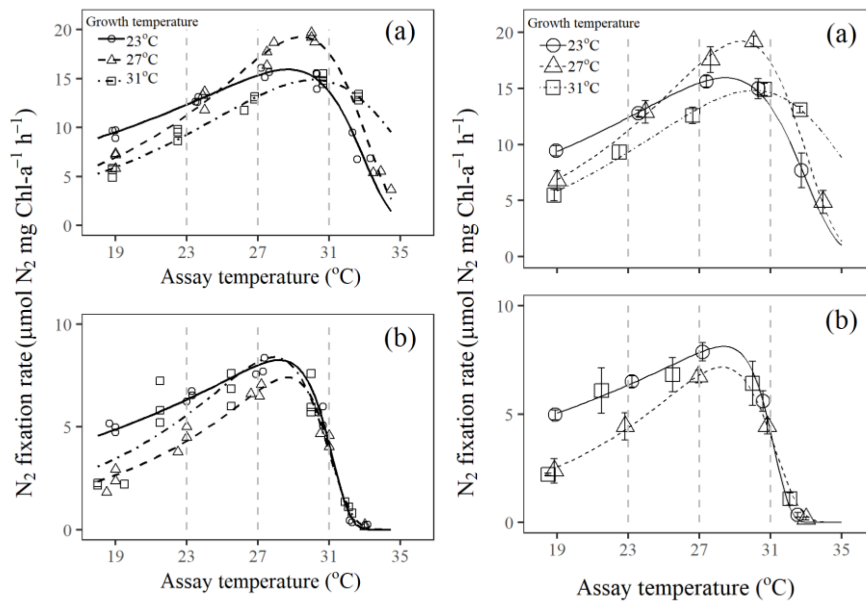

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Figure 3 Short–term thermal response curves for N_2 fixation rate in *Trichodesmium* cultures grown under (a) light–saturating and (b) light–limiting conditions; fitted lines are based on fixed effect coefficients of the nonlinear mixed effects model fitting to Eq. (2); vertical dotted lines mark the assay temperatures 23 °C, 27 °C and 31 °C.

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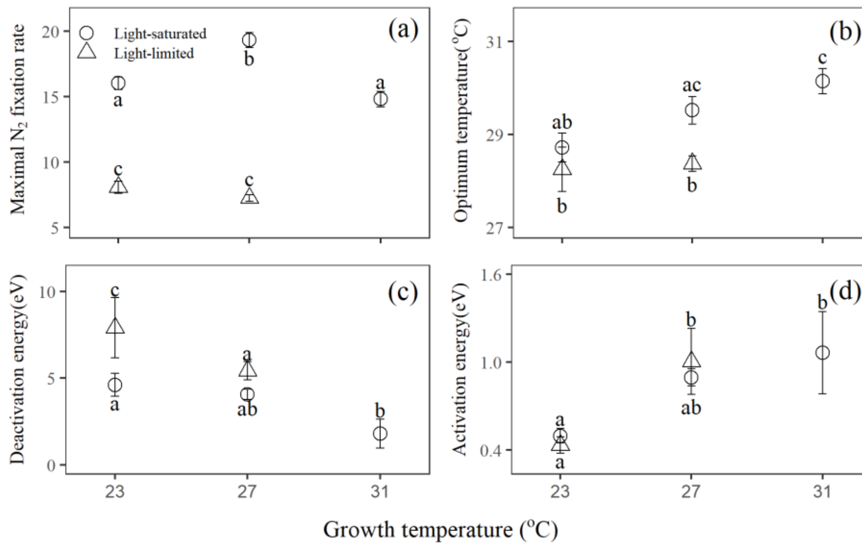
Figure 3 Short–term temperature norms of N_2 fixation rate in *Trichodesmium* populations grown under (a) light–saturating and (b)
635 **light–limiting conditions; values represent the means and error bars for the standard deviations of biological replicates(n=3); fitted lines are based on mean parameters at each treatment across replicates (n=3) derived from non–linear least squares regression using the modified Sharpe–Schoolfield model (Eq. (1)); vertical dotted lines mark the assay temperatures 23 °C, 27 °C and 31 °C.**



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~~**Figure 4 The interactions of temperature and light on (a) maximal N_2 fixation rate, (b) optimum temperature, (c) deactivation energy and (d) activation energy for N_2 fixation in *Trichodesmium*; values represent the means and error bars for the standard deviations of biological replicates(n=3); points marked with different letters are significantly different from each other ($p<0.05$); the unit for maximal N_2 fixation rate is μmol N_2 mg Chl–a^{-1} h^{-1}.**~~

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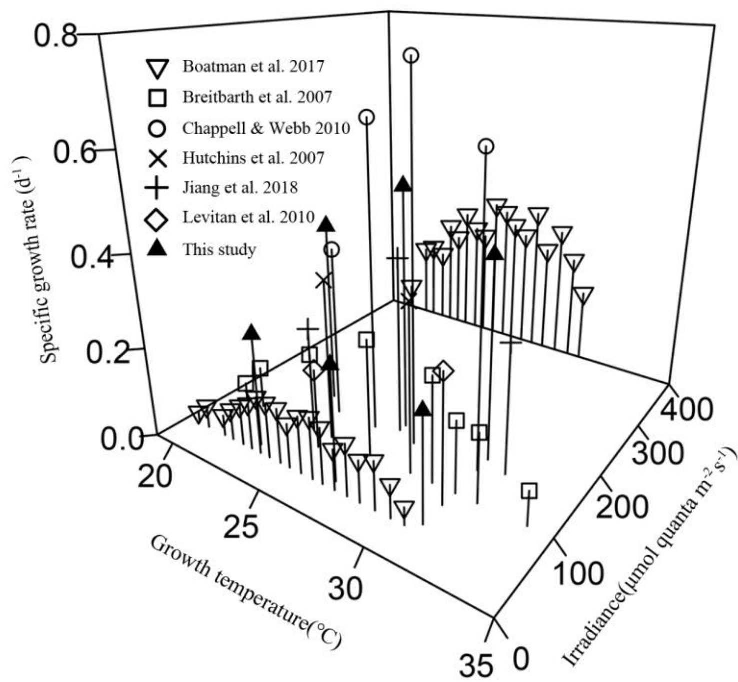

Figure 4 The combined effects of temperature and light intensity on the specific growth rate in *Trichodesmium* IMS 101; data from published literatures involving at least two growth temperatures and this study.

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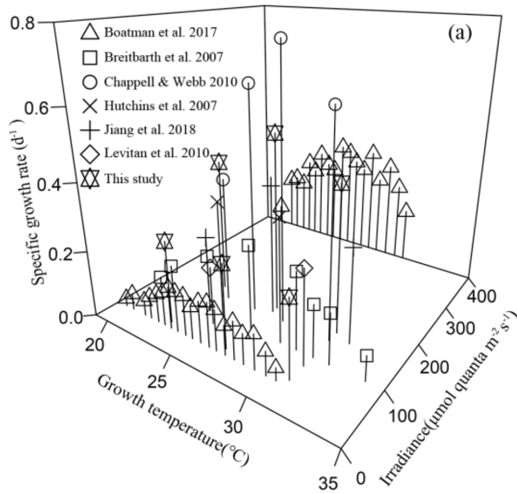

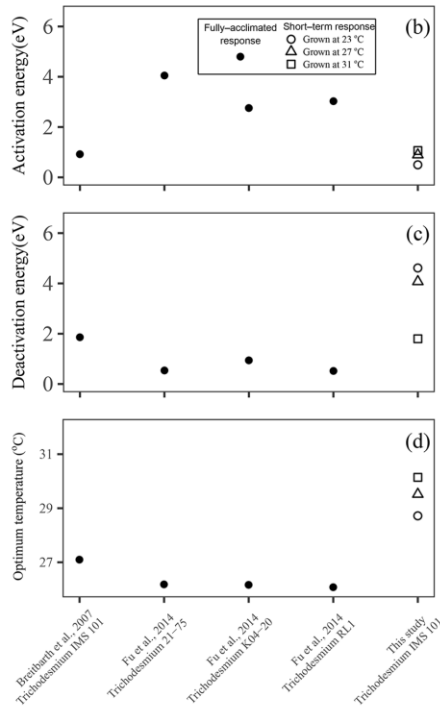

~~Figure 5 (a) The combined effects of temperature and light intensity on the specific growth rate in *Trichodesmium* IMS 101; data from published literature involving at least two growth temperatures and this study. (b) Activation energy, (c) deactivation energy and (c) optimum temperature for N_2 fixation rate in *Trichodesmium*; data are calculated from published literature and this study; in the column of "This study", only light- saturated cultures are presented because of the similarity of growth light intensity to other two studies.~~

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