Interactive comment on "Physiological response of a golden tide alga (Sargassum muticum) to the interaction of ocean acidification and phosphorus enrichment" by Zhiguang Xu et al.

Anonymous Referee #1

Received and published: 25 October 2016

The present manuscript provides interesting and useful information on the influence of future ocean acidification and eutrophication on a golden tide alga, Sargasssum muticum. The authors suggested that future ocean acidification and eutrophication may promote the growth of S. muticum and thus occurrence of gold tide events however, ocean acidification and eutrophication may not boost the gold tides events synergistically. The authors discussed their results reasonably within a physiological and ecological context. The experiments were reasonably performed and described. The data analysis was satisfactory and the results were clearly presented. The conclusions were sufficiently justified. The figures and tables were all adequate and essential. Therefore, in my opinion, this manuscript is suited for publication in BIOGEOSCIENCES.

Response: We really appreciate these comments.

Anonymous Referee #2

Received and published: 26 October 2016

This is an interesting paper describing the combined effects of elevated CO2 (and hence ocean acidification) and elevated P levels on growth and physiology of Sargassum muticum. The work is well designed and executed and the data presented and discussed thoroughly, although English expression is a little strange in places.

Response: We sincerely thank the anonymous referee for these comments. Thanks to Dr. Douglas A. Campbell, English expression has been improved.

I do though draw the authors attention to a couple of points:

Line 239: It is stated that projected ocean acidification increased pCO2 by 138.29% (LP) and 134.08% (HP) but surely it is the changes in pCO2 that cause OA?

Response: We totally agree with the reviewer. The text has been corrected to "elevated pCO_2 decreased pH by 0.31 unit at both LP and HP, CO_3^{2-} by 45% (LP) and 45% (HP), but increased DIC by 10% (LP) and 9% (HP), HCO_3^- by 14% (LP) and 14% (HP), and CO_2 by 139% (LP) and 134% (HP)." at lines 238-241.

Line 348-9: Here it is stated that "The evidence above indicates that the CO2 in seawater should be carbon limited for marine macroalgae". This is based on the high k0.5 CO2 for Rubisco and the diffusive resistance to CO2 on seawater - that the k0.5 CO2 values for intact thalli are very much lower than those for Rubisco is prima facie evidence that an active CCM is present. More could be made of this and the fact that it appears CCM activity is not down regulated by the high CO2 conditions. The explanation on lines 359-61 that this is "mainly because of increased CO2 availability for Rubisco and depressed photorespiration at the elevated ratio of CO2 to O2" would not apply to P vs DIC curves.

Response: We do agree that most algae have an active CCM, contributing to much lower $K_{0.5}$ values for intact thalli in comparison with those for Rubisco. Meanwhile, we think the CCM was down regulated by increased pCO_2 in the present study based on the increased $K_{0.5}$ that is deemed as a signal of down regulation of CCMs (Giordano et al., 2005, Gao and Campbell, 2014). The lines 359-61 was not used to explain the P vs DIC curves but the decrease of photosynthetic affinity for DIC did not lead to reduced photosynthesis in *S. muticum*. We have clarified it to "But this decrease of photosynthetic affinity for DIC at the higher pCO_2 did not lead to reduced photosynthesis in *S. muticum* compared to that at the lower pCO_2 in the present study, mainly because of increased CO_2 availability for Rubisco and depressed photorespiration at the elevated ratio of CO_2 to O_2, which has been confirmed in red seaweed *Lomentaria articulate* (Kübler et al., 1999)." at lines 358-362.

Gao, K. and Campbell, D. A.: Photophysiological responses of marine diatoms to elevated CO_2 and decreased pH: a review, Funct. Plant Biol., 41, 449-459, 2014.

Giordano, M., Beardall, J. and Raven, J. A.: CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annu. Rev. Plant Biol., 56: 99-131, 2005.

Kübler, J. E., Johnston, A. M., and Raven, J. A.: The effects of reduced and elevated CO_2 and O_2 on the seaweed *Lomentaria articulata*, Plant Cell & Environment, 22, 1303-1310, 1999.

The authors suggest in several places (e.g. lines 388-91) that the HC conditions may have down-regulated CCMs in S. muticum, but there is no evidence for this in their

data
(Fig 3, Table 2).

Response: In a review (Gao and Campbell, 2014), it states: "Downregulation of CCMs can include decreased CO_2 affinity resulting in an increased requirement for pCO_2 to support photosynthesis, inhibition of carbonic anhydrase activity, depressed HCO_3^- transport, and downregulation of PEPCase and PEPCKase (Reinfelder et al. 2000; Giordano et al. 2005; Roberts et al. 2007a, 2007b; Raven 2010; Reinfelder 2011)." Giordano et al. (2005) also thought that high CO_2 could down regulate the CCM by suppressing expression of a high-affinity DIC state. Therefore, we think the increased $K_{0.5}$ could be considered as a hint for the down regulated CCM. In our study, the higher $p$$CO_2$ increased $K_{0.5}$ (Table 2) although the increase at the higher P level was not statistically significant.

Gao, K. and Campbell, D. A.: Photophysiological responses of marine diatoms to elevated CO_2 and decreased pH: a review, Funct. Plant Biol., 41, 449-459, 2014.
Giordano, M., Beardall, J. and Raven, J. A.: CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annu. Rev. Plant Biol., 56: 99-131, 2005.

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Received and published: 31 October 2016

The authors grew an invasive Sargassum species under an ecophysiologically reasonable matrix of pCO2 and [phosphate]. They analyzed the growth rate, photosynthetic rates, nitrate uptake and reduction rates and composition of the algae. They show interactive effects of pCO2 and [phosphate].

The study is well designed and potentially interesting. The current discussion spends words on entirely speculative interpretations that might well be true, but which are not directly supported by the data presented. On the other hand, intriguing ratios and discrepancies in the presented results are not discussed. For example, how can algal nitrate uptake rates exceed measured nitrate reduction rates? Does the tissue store NO3- differentially depending upon conditions? Are there variable rates of denitrification in the media?

Response: We appreciate these comments. We believe our manuscript has been improved by answering the reviewer’s queries. Please see the following response for details.

What happens to the environmental effects upon photosynthesis if it is normalized to chlorophyll rather than fresh weight?

Response: The reviewer raised a valuable point. We have normalized photosynthesis rate to chl *a*. The net photosynthetic rates under different treatments were 135.4 ± 27.0 (LCLP), 142.2 ± 6.5 (LCHP), 161.1 ± 4.4 (HCLP), and 193.0 ± 7.6 (HCHP) μmol O_2 mg^{-1} chl *a* h^{-1} respectively. The higher pCO_2 increased the net photosynthetic rate by 35% at HP and the higher P increased it by 20% at HC.

I offer some suggestions below for the authors. best regards, Doug Campbell

Abstract: ’the development of golden tides...’ (not ’evolvement’)

Response: Corrected.

39.31% etc. over precision. It is not possible to report such values to 1 part per 10,000 but that is what is implied by 39.31%

Response: It has been changed to 39%.

Introduction: ’...it originates from Japan..." (not ’it origins...’)

Response: Corrected.

Materials & Methods line 155: units for total alkalinity?

Response: We presume the reviewer meant the unit for salinity here. The unit for salinity has been developing. The Practical Salinity Scale (PSS) was defined in 1978 and later promulgated by the UNESCO/ICES/SCOR/IAPSO Joint Panel on Oceanographic Tables and Standards in Sidney, BC, Canada, 1-5 September 1980. Because it makes no sense to say the salinity is, for example, 35 PSS, the term Practical Salinity Unit (PSU) was introduced. However, the use of PSU is discouraged because salinity is by definition a dimensionless parameter. For now, most oceanographers follow the recommendation of the Scientific Committee for Oceanic Research (SCOR) that salinity be represented by a unitless number, as it's a unitless ratio and its measurement is now based on conductivity instead of the long time gone determination of evaporated mass.

Line 195: Decrease in NO3- in the media could result from microbial denitrification? A cross check would be whether nitrate reductase activity matched 1;1 with decrease in NO3-2 in the media?

Response: The reviewer raised a point worthy of discussion. We agree that nitrate reductase activity should match 1:1 with decrease in NO_3^- in the media, in theory. However, the undoupling between them is not uncommon and could be found in both microalgae (Collos 1982; Blasco et al., 1984) and macroalgae (Gordillo et al., 2001; Zou, 2005). One possible cause that leads to the NO_3^- uptake from the media exceeding NO_3^- reductase activity in the present study may be the intercellular NO_3^- storage (Collos 1982; Viaroli et al., 1996). It has been reported that the NO_3^- reductase activity (NRA) peak was 11-fold less than the NO_3^- uptake rate in *Ulva* sp. , suggesting that the reduction of NO_3^- reductase to nitrite NO_2^- by nitrate reductase was the rate-limiting step in NO_3^- assimilation (Lartigue and Sherman, 2005). Another reason might be the underestimation of NRA as the NO_2^- release may be limited not only by NRA, but also by the diffusion rates of NO_3^- into the cells and NO_2^- out of the cells in the assay used in the present study (Lartigue and Sherman, 2002). As for the microbial denitrification, we presume there is less possibility that the additional decrease of NO_3^- was caused by it. As far as we know, denitrification only takes place in anoxic environments while our cultures were aerated by ambient or CO_2 enriched air. Apparently, we do not have evidence to support these specific interpretations. To minimize the content of speculation, we would like to add one sentence to the text “It is worth noting that the nitrate uptake rates were commonly higher than the corresponding reduction rates of NO_3^- to nitrite NO_2^- by nitrate reductase in the present study, which might be due to the intercellular nitrate storage (Collos, 1982; Lartigue and Sherman, 2005) and the underestimation of RNA measured by the in situ assay (Lartigue and Sherman, 2002).” at lines 416-420.

Blasco, D., MacIsaac, J. J., Packard, T. T, and Dugdale, R. C.: Relationship between nitrate reductase and nitrate uptake in phytoplankton in the Peru upwelling region, Limnol. Oceanogr., 29, 275-286, 1984.

Collos Y.: Transient situations in nitrate assimilation by marine diatoms. III. Short-term uncoupling of nitrate uptake and reduction, J. Exp. Mar. Bio. Ecol., 62, 285-295, 1982.

Gordillo, F. J. L., Niell, F. X., and Figueroa, F. L.: Non-photosynthetic enhancement of growth by high CO_2 level in the nitrophilic seaweed *Ulva rigida* C. Agardh (Chlorophyta), Planta, 213, 64-70, 2001.

Lartigue, J., and Sherman, T. D.: Field assays for measuring nitrate reductase activity in *Enteromorpha* sp. (Chlorophyceae), *Ulva* sp. (Chlorophyceae), and *Gelidium* sp. (Rhodophyceae), J. Phycol., 38, 971-982, 2002.

Lartigue, J., and Sherman, T. D.: Response of *Enteromorpha* sp. (Chlorophyceae) to a nitrate pulse: nitrate uptake, inorganic nitrogen storage and nitrate reductase activity, Mar. Ecol. Prog. Ser., 292,147-157, 2005.

Viaroli, P., Naldi, M., Bondavalli, C. and Bencivelli, S. Growth of the seaweed *Ulva rigida* C. Agardh in relation to biomass densities, internal nutrient pools and external nutrient supply in the Sacca di Goro lagoon (Northern Italy), Hydrobiologia, 329, 93–103, 1996.

Zou, D.: Effects of elevated atmospheric CO_2 on growth, photosynthesis and nitrogen metabolism in the economic brown seaweed, *Hizikia fusiforme* (Sargassaceae, Phaeophyta), Aquaculture, 250, 726-735, 2005.

Fig. 3: There is an inhibition response in HCLP that is not apparent in other treatments.

Response: It appears that the last two points are lower than the two points before them but there are no statistical differences between these four points.

Fig. 4: Would a renormalization of photosynthetic rates (fig. 2) to chlorophyll content (fig. 4) eliminate some of the differences among treatments? I think maybe yes. Then some of the photosynthetic data can be explained by nutrient effects on content of photosynthetic units.

Response: The reviewer raised a valuable point. We have normalized photosynthesis rate to chl *a*. The net photosynthetic rates under different treatments were 135.4 ± 27.0 (LCLP), 142.2 ± 6.5 (LCHP), 161.1 ± 4.4 (HCLP) and 193.0 ± 7.6 (HCHP) µmol O_2 mg^{-1} chl *a* h^{-1} respectively. The higher pCO_2 increased the net photosynthetic rate by 35% at HP and the higher P increased it by 20% at HC. Compared to the results normalized to fresh weight, it does eliminate the differences at LC or LP. We would say this renormalization could partially explain the effects of pCO_2 and P on photosynthetic rate. Meanwhile, to the best of our knowledge, the photosynthesis rate of macroalgae in most studies is normalized to fresh weight/dry weight. We hope we can keep the current results to compare our study with others'.

Fig. 5, Fig 6 There is a discrepancy. NO3- uptake from the media cannot exceed NO3- reductase rates, unless the tissue is storing NO3-.

Response: Yes. We think it is mainly because of the intercellular nitrate storage as explained in the above response.

Fig 2 vs. Fig 8 dark respiration = _1/2 of photosynthetic rates?

Response: We realize that this ratio may be a little higher, particularly compared to microalgae. However, it might not be surprising for macroalgae. For instance, the ratio of respiration to photosynthesis varies between 0.14 and 0.54 in *Gracilaria lemaneiformis* (Zou and Gao, 2013), around 0.2–0.7 in *Hizikia fusiform* (Zou et al., 2011) and it could even be close to 1 in *Gracilaria tikvahiae* (Lapointe and Tenore, 1984), depending on different culture conditions.

Lapointe, B. E., Tenore, K. R.: Dawes C J. Interactions between light and temperature on the physiological ecology of *Gracilaria tikvahiae* (Gigartinales: Rhodophyta). Mar. Biol., 80, 161-170, 1984.

Zou, D., Gao, K.: Thermal acclimation of respiration and photosynthesis in the marine macroalga *Gracilaria lemaneiformis* (Gracilariales, Rhodophyta), J. Phycol., 49, 61-68, 2013.

Zou D, Gao K, Luo H: Short‐and long‐term effects of elevated CO_2 on photosynthesis and respiration in the marine macroalga *Hizikia fusiformis* (Sargassaceae, Phaeophyta) grown at low and high n supplies, J. Phycol., 47, 87-97, 2011.

Results Lines 237-241 Over precision in reporting of results to 1 part in 10,000. This is a problem throughout.

Response: It has been revised to 1 part in 100 throughout the text.

Discussion Lines 428 to 440 are entirely speculative. They might be true, but there is no evidence supporting these specific interpretations, in this paper.

Response: We agree with the reviewer. The length of speculation needs to be reduced, although it can supply a direction for future research. It has been shortened to seven lines and it reads now "The increased soluble protein and decreased NRA at the condition of higher pCO_2 and higher P suggest some H^+ transport-related protein, such as plasma membrane H^+-ATPase, might be synthesized to counteract the acid–base perturbation caused by increased pCO_2 and H^+. The additional production of H^+ transport-related protein like plasma membrane H^+-ATPase could competitively decrease the synthesis of nitrate reductase. This hypothesis needs further experimental evidence to stand even though it could explain the results in the present study." at lines 431-440.