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 #2
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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 K0.5 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 pCO_2 increased $K_{0.5}$ (Table 2) although the increase at the higher P level was not statistically significant.