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Interactive comment on "Ocean acidification modulates expression of genes and physiological performance of a marine diatom" *by* Y. Li et al.

Anonymous Referee #2

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General comments: In this manuscript, unialgal cultures of a diatom, Phaeodactylum tricornutum, were used to study the effect of ocean acidification and light conditions on physiological parameters and mRNA levels of genes involved in carbon concentrating, nitrogen metabolism, photosynthesis, and respiration. Although similar experiments have been performed in the past, the authors indicated that a factorial treatment of light and CO2 levels would reveal new insights about responsive mechanisms in this diatom. In such a study, it is important to differentiate whether the cultures are in a transient or an acclimated state. According to the not-so-clear description provided in the manuscript, I am assuming that the cultures were maintained under Low CO2 condition before hour 0 (p. 15813, line 6). As a result, immediately after the onset of the incubation, cells in the Low CO2 condition were fully acclimated while cells in the High CO2

condition were in a transient state. At this time, a fair comparison was unachievable between High and Low CO2 conditions. During the 8-day incubation period (indoor, low light), only 4 days were sampled. This low sampling frequency causes difficulties for a reader to evaluate the results, especially for those measurements that showed large differences between day 1 and day 8 (e.g. Fig. 4B). The situation was getting more serious when incubation reached the second half. A sudden switch from indoor to outdoor illumination would surely put the cultures in a transient state. After the switch, the incubation lasted for another 5 days (day 9 – 13), yet only 3 days were sampled. This created two questions. The authors should explain in what way this operation simulated an ocean acidification situation so that an understanding of the transient changes would be essential. In addition, with vastly different pretreatment and sampling schedules between the indoor and the outdoor incubations, it would be difficult to find statistical procedures to reveal the influences of light intensity and fluctuation. In conclusion, I think highly of the motivation of this research, but am deeply concerned about the experimental design. The sporadic sampling schedule and undefined transient states caused difficulties in quantitative comparisons, especially between the indoor and the outdoor incubations. I think such obtained results are incapable of demonstrating the true effects of CO2 and light on diatom gene expression.

Specific comments: Page 15812 Line 18. Please provide the following information: the dilution rate (% per day) and the time of day when dilution was performed. Page 15813 Line 12. The outdoor incubations apparently had a different sampling schedule. Please describe in details. Line 22. Here, the growth rate was measured on a daily basis. However, growth rates in Fig. 1 were presented as the mean value of triplicate containers (n=3). Please explain the growth rates on which day (or days) were used to generate the mean. Page 15814 Line 22. Please provide the following information: during the incubation period, on which few days that the measurement of P-C curves were performed. Page 15815 Line 24. Please provide the following information: expression level at which sampling point in the Low CO2 group was used as the calibrator. Page 15816 Line 5. In addition to CO2 concentrations and indoor/outdoor incubations,

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light:dark cycle and the progress of acclimation also affect gene expressions in this study. More descriptions are needed to explain how to set up statistical procedures that can properly identify the sources of variations. Line 18. Please identify the interval that represented the acclimated status with increased Fv/Fm. Line 21. I am not sure about the result of similar rETR between indoor illumination and fluctuating sunlight. In Fig. 2B, the rETR obviously decreased on the last day under fluctuating sunlight. Page 15817 Line 13. By comparing data points in Figs. 2, 4, and 5, two data points for mRNA expression were missing for all genes measured. Why? Line 17. I did not see higher expression for Lhcf 3 under high/fluctuating sunlight in Fig. 4B. Page 15819 Line 12. I did not see evidence for high CO2 stimulating RbcL expression in Fig. 4C. Page 15820 Line 10. If the authors want to conclude that an increase in NiR expression was caused by acidification, the time course of pH values during the incubation period should be shown. Line 17. The expression levels of both Lhcf 3 and mATP increased at hour 32. I do not think the expression was totally suppressed in the initial phase of high CO2. In addition, I do not understand how "6 generations" is defined. Page 15821 Line 4. The induction of beta-CA lasted for only one day. I do not think this result should be viewed as a permanent effect of ocean acidification. Line 9. I do not understand in what ways the gene expressions were linked to photo-protection and energy balance. Another question is why these expression patterns appeared only for one day.

Technical corrections: Page 15817, Line 24. Fig. 4C should be cited here. Figs 2, 4, 5. Labels on the x-axis should be in multiples of 24.

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