

## ***Interactive comment on “Effects of *in situ* CO<sub>2</sub> enrichment on structural characteristics, photosynthesis, and growth of the Mediterranean seagrass *Posidonia oceanica*” by T. E. Cox et al.***

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This manuscript on the *in situ* CO<sub>2</sub> enrichment of *Posidonia oceanica* by Cox et al is an ambitious attempt to conduct a long-term manipulative F.O.C.E. experiment simulating high CO<sub>2</sub> / low pH conditions. In theory, F.O.C.E. systems have the potential to simulate at least some aspects of climate change precisely, in discrete areas. This system is technologically-advanced and appears to have functioned well over a considerable period of time – no small accomplishment! The authors are to be commended for attempting such an ambitious experiment.

Unfortunately, this system seems to suffer from a lack of true replication, an issue inherent in many F.O.C.E. system designs. I am not a statistician but, like the authors,

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I have struggled with similar challenges of replication of in situ F.O.C.E. experiments for some time. It is indeed difficult to manage true replication in the field, with limited resources. I am sympathetic.

The following is my understanding of the statistical issues. I would encourage further discussion of these issues, which would hopefully include experts more qualified than I. Such a discussion would help to move the field forward considerably, as the issues are common challenges in most (but not all) F.O.C.E. experiments.

In this case the system is limited by having only a single control chamber (N=1) to compare to a single treatment chamber (N=1). As a result, plants sampled from each chamber are pseudo-replicates. The variation reported as standard errors, for example in the figures, would presumably reflect only the variation within a given chamber, underestimating natural variation and leading statistical test to mistakenly indicate treatment differences where there may be none. Also, the design does not allow for sampling from randomly-interspersed replicate plots, an assumption of most statistical tests. Thus, the experiment is vulnerable to "random demonic instructions", unknown events could impact every one of the treatment or controls samples, without one's knowledge, over the entire course of the experiment. This vulnerability is amplified by the clonal nature of the seagrasses – simply sampling one may affect others in the same chamber, or in nearby chambers. For these reasons, the application of ANOVAs and other similar statistical analyses seems questionable. The authors may have solved these issues (certainly, they spent considerable time planning the experiments and running the analyses) but, if so, I didn't see that explained fully in the manuscript.

The authors address some of these concerns in the section "Accounting for pseudo-replication". They acknowledge that their experimental design and statistical analyses includes pseudo-replication that "inflates Type I error". The justification that follows may be appropriate but is difficult to follow. How are "some tested parameters" "considered true replicates" while others are not, of they are all made from one of two chambers? I'm not sure why statistical tests were run both with pseudo-replication (correctly?)

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and without pseudo-replication (incorrectly?). The authors state that results were the same but this seems very counter-intuitive. How could the results of statistical tests assuming replication and those not assuming replication, which must differ dramatically in statistical power, yield the same results? If they do, why not just run the statistics properly and report those data?

These are important questions, not only for these authors but for the field. Some F.O.C.E. systems, including some studying seagrasses, have managed true replication but have had to make other compromises – such as shorter experimental periods or less control of environmental conditions. The authors mention some of the later in the works of Campbell et al. and Arnold et al. (full acknowledgment, I was obviously involved in some of these studies). The various approaches all have limitations that can be criticized. In general F.O.C.E. systems focusing on seagrass responses have been either technologically-advanced and precisely controlled or appropriately replicated, but not both. This is one reason why the field has benefited from the use of multiple approaches involving F.O.C.E. systems, natural high CO<sub>2</sub> vents, and controlled laboratory experiments.

In short, I have struggled with this issue as well and am sympathetic. However, a lack of replication in F.O.C.E. experiments significantly limits the appropriate use of statistical analyses and reduces the ability to draw conclusions from the data. If the authors have developed a solution to this common problem they would have done the field a great service - and this in itself should be a focus of the manuscript.

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