

Interactive comment on “Effects of *in situ* CO₂ enrichment on structural characteristics, photosynthesis, and growth of the Mediterranean seagrass *Posidonia oceanica*” by T. E. Cox et al.

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This paper by Cox et al. is a pioneering attempt to use the FOCE approach in coastal waters, and it was set up to mimic natural variability in abiotic factors (e.g. diurnal and seasonal changes in light, temperature and CO₂ levels) which is a major advance on much of the work that has been carried out to date.

As such this paper is novel and publishable by Biogeosciences, especially given the meticulous way in which the project methods are described and the results reported. This will be of great interest to the marine carbon research community worldwide, as well as those specialising in seagrasses and/or ocean acidification. There are clearly potential advantages with the FOCE approach (greater realism than lab work, greater

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CO₂ dose control than volcanic seeps) and it is important that we obtain multiple lines of evidence to reduce uncertainties about how increased CO₂ will affect coastal habitats such as seagrass beds.

The lack of true replicates in this study is problematic when using formal statistical tests of differences between the three experimental treatments, especially as *Posidonia* is a clonal organisms. However, there are others more qualified/interested in the statistical design of scientific experiments than me that should scrutinize and offer constructive advice about this aspect of the study.

My main cause for concern is that the two enclosures that were used seem to have had a major adverse effect on the seagrass, since their growth and biomass were much reduced compared to the natural open seagrass plot and so this result should be mentioned in the abstract. Caution must always be used in interpreting experiments where the ‘control’ organisms seem to be stressed by the test conditions.

The abstract mentions speculations about the potential for increased CO₂ levels to confer resistance to thermal stress, yet there is no reference any published work on this in the text. Either remove it, or explain the basis of this speculation backed up with references.

Line 67 states that variability in CO₂ prevents the determination of a reliable dose-response relationship at seeps. This was true a few years ago but more recent work has been able to assess the CO₂ dose more accurately (Boatta et al. at Vulcano, Fabricius et al. in PNG, Kroeker et al. off Ischia). So replace ‘prevent’ with ‘hampers’.

Line 69: work has been carried out using the FOCE approach in Chesapeake Bay by Tom Arnold; I do not know if this has been published so this is worth checking.

The authors have not mentioned an *in situ* study of the effects of increased CO₂ levels on several seagrass species by Russell et al. (2013) Mar Poll Bull 73, 463-469 which I think would augment the introduction and discussion sections, especially as

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this investigates net primary production and respiration alongside biometrics.

I found the result provided on line 401 interesting and wonder if the authors could elaborate on what they think drove the seasonal change in seawater pH in the Discussion.

Line 441 confused me a little; were the plot quadrats not placed haphazardly? Please clarify.

Lines 444-451: when I read this section I began to understand perhaps why the findings of this study (little or no discernible effect of CO₂ on seagrass in the test and control plots) differ from findings at various CO₂ seeps. *Posidonia oceanica* in Italy, for example, tend to be heavily encrusted by Corallinaceae. At multiple Italian CO₂ seeps this grass has much reduced calcareous epiphytic cover which presumably helps the *Posidonia*, as competitors for light and nutrients are removed. This may explain why seagrass is so abundant at CO₂ seeps around the world. The results obtained in the high CO₂ FOCE chamber in the current study may not be representative of what would be found in a more typical stand of *Posidonia* with its attendant coralline algal flora (see Martin et al. 2008 *Biology Letters*). Please consider this possibility in the Discussion section.

The lower below ground biomass and stunted growth rates in the FOCE chambers are additional reasons to be cautious about presuming that the results from a 1.7m³ treatment area show that *Posidonia* will not benefit from ocean acidification (as long as other factors, such as temperature, are conducive – see below). The *Posidonia* appear to not to have been thriving in the enclosed treatments and so the stress of enclosure may have cancelled any potential boost by CO₂. So I would tone down assertions such as Line 551 e.g. by replacing 'support the conclusion of limited stimulation' with "shows that ocean acidification may have no effect on increasing the resilience of *Posidonia* to stressors". This point could then tie in with the very important point about the imminent threat that warming could lead to the extinction of *Posidonia* which is worth emphasising in the introduction and discussion – ie that rising CO₂ levels could kill off

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Posidonia due to warming even though they are highly resilient to the effects of ocean acidification.

Line 558 has some discussion of the effects of increased CO₂ on plant mechanical strength. Recent work by Newcombe et al. (2015) in *Biology Letters* showing that increased CO₂ can weaken *Acetabularia* might be worthy of inclusion here.

Line 569 unclear meaning 'discredits need for'?

Line 617 I don't think this paper should be drawing upon unpublished data, so the discussion of carbohydrates and carbon content can be left out for a future publication.

Line 631 'are mixed in support' meaning unclear

Line 643 For the reasons set out above I do not think that this paper provides a "major advancement in our understanding of the response of *Posidonia* to ocean acidification" at all. It is a major advance in the use of the FOCE approach and can be presented as such, as in a methods paper.

Line 680 – what speculation, where? Delete, or refer to published work on this.

Line 688 'amendable'? unclear and I think 'potentially powerful' is closer to the truth, given the difficulties of doing this sort of work and the limited sets of results to date.

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