

Interactive comment on “The response of seagrass (*Posidonia oceanica*) meadow metabolism to CO₂ levels and hydrodynamic exchange determined with aquatic eddy covariance” by Dirk Koopmans et al.

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We thank the referee for their thoughtful consideration of our manuscript and for their valuable suggestions for its improvement. The referee's suggestions are included below in italic.

*The authors report metabolic measurements in *Posidonia oceanica* meadows using the technique of eddy-covariance of O₂. This is the first publication of such technique*

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*in *P. oceanica* meadow and confirm that this marine community is highly productive as previously shown by numerous studies during the last three decades. The authors also made primary production of *P. oceanica* measurements at a CO₂ vent site that are compared to a reference site, in the context of ocean acidification.*

*They conclude that the primary production of *P. oceanica* is lower at the CO₂ vent site and attribute this to phosphate binding to iron emitted from the vent. While this interpretation might make sense (although in contradiction with studies of *Cymodocea nodosa* and other seagrasses at similar CO₂ vent sites), I'm uncomfortable about drawing conclusions on the comparison of a single measurement at a single CO₂ vent site with a single measurement at a single reference site.*

*It would have been convincing if there was replication of different sites, as there are numerous reasons why two “snapshot” primary production measurements between 2 sites might differ. Carefully replicated measurements might have shown a different result for such a comparison. Even at a single site over a *Posidonia oceanica* meadow, strong variations of primary production over an order of magnitude occur through a range of temporal scales (from day-to-day to year-to-year).*

We address the strength of our experimental design, and present ideas for improving its presentation, in four brief sections below.

1. Accounting for diurnal variability

We agree with the referee that even at a single site ‘strong variations of primary production over an order of magnitude occur through a range of temporal scales.’ These variations are particularly important to address in a study that relies on a single

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series of diurnal oxygen flux measurements at an experimental site. We regret that our manuscript does not more clearly convey how we addressed the dominant factors that cause diurnal variability in seagrass primary production. Nor does the manuscript explain the importance of the replication of our measurements at multiple reference sites and over multiple days at each site.

Seagrass primary production is driven primarily by season and irradiance, but also by water temperature, nutrient availability, water velocity, and CO₂ concentration (Introduction, lines 26 through 30). All of these factors were accounted for in our experimental design and were measured concurrently with ecosystem metabolism. For example, to address seasonal variation in primary production, all measurements were made during the same two-week period (13 May to 27 May) of the calendar year. To minimize differences in irradiance between meadows, all meadows were at the same water depth.

2. Reproducibility of our results

We also regret that our manuscript does not do a better job of conveying the reproducibility of our results. Unlike flux chamber measurements, eddy covariance measurements of oxygen flux are independent in time. As environmental variables change, the effect of these changes on ecosystem metabolism can be quantified. One-hundred and eighty independent measurements of ecosystem oxygen fluxes were made for this study. As a result, we can report the first ecosystem-scale photosynthesis-irradiance curves for *P. oceanica* meadows.

Ecosystem metabolism measurements were replicated at reference meadows exposed to differing water velocities, and measurements were replicated from day-to-day

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at each of the reference meadows. Because of the large footprint of eddy covariance, measurements are comparable to replicated benthic chamber measurements (e.g., Reimers et al., 2012; Berg et al., 2013).

To examine the reproducibility of our results, we made measurements at two different reference meadows and replicated measurements over two days at each of the two meadows. If the factors that drive variability in seagrass primary production were similar from day-to-day, one would expect similar net ecosystem metabolism from day-to-day. We measured changes in the factors that drive primary production and found them to be small. Diel differences in net ecosystem metabolism were also small. The factors that drive primary production were similar at the CO₂ vent. The time-of-year, water depth, irradiance, and water temperature were all close to identical to the reference meadows. N-nutrients, CO₂(g), and water velocities were elevated at the CO₂ vent. These factors are associated with increases in primary production.

3. Experimental design

Generally speaking, we understand the referee's underlying concern for designing a study based on a single experimental treatment — in this case a CO₂ vent — but this design is not unconventional. For example, the referee recommended a highly useful study by Apostolaki et al., (2014) who rely on measurements at a single reference site and a single CO₂ vent.

Additional examples cited within this manuscript include Cox et al., (2006) and Barrón et al., (2006). Each study relied on a single experimental treatment. A common factor among these studies is that ecosystem metabolic fluxes were quantified. These require 24 h for measurements of net productivity, reducing the time available for

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replication. Other highly influential studies also rely on measurements across carefully selected, but unreplicated sites. For example, Hall-Spencer et al., (2008) observed a doubling of seagrass shoot density at a single, low-pH site.

4. Changes to the manuscript

To address these shortcomings in our manuscript we will make the strength of our experimental design explicit. Specifically, we will explain how we minimized and accounted for the factors that drive diurnal variability in seagrass meadow primary production and respiration, and we will include the evidence from reference meadows that the results are reproducible over time.

To do this, we will present the experimental design as the first section of the methods. In this section we will describe our approach to minimize and account for the factors that drive day-to-day variability in primary production, and our replication of measurements to examine reproducibility over time.

We will also alter Table 2 to include the primary factors that drive seagrass primary production and respiration — cumulative diurnal irradiance, nutrient concentrations, water temperature, CO₂ concentration, and water velocity. These will appear alongside seagrass respiration and photosynthesis. The reader can then evaluate whether the factors that drive diel variations in primary production were adequately addressed.

We will also include an evaluation of the reproducibility of measurements in the discussion. This includes a description of the independence of eddy covariance measurements over time and the spatial averaging of the technique. Importantly, this section will discuss our success at minimizing the variability in the factors that

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cause diurnal variation in seagrass meadow primary production. It will also include the day-to-day reproducibility of measurements at the reference sites.

*L261-262: For a meaningful comparison between the CO₂ vent site and the reference site, the GPP values should have been normalized by *P. oceanica* biomass (productivity). Biomass could be lower due other factors such as hydrodynamics (exposed vs sheltered) or substrate or different grazing pressure. Also, biomass strongly changes seasonally and with depth. It is unclear if the measurements in the CO₂ vent site and the reference site are really comparable with regards to the timing of the seasonal cycle, and also with regards to other factors affecting primary production such as light availability. Yet on a normalized basis by mass of *P. oceanica*, the GPP could be equivalent or even higher at the CO₂ vent site.*

If we assume that biomass is proportional to meadow height, the gross primary production normalized for biomass at the CO₂ vent is similar to that of the open-water meadow. However, it would be arbitrary to normalize primary production for biomass without also normalizing respiration. Therefore, net ecosystem metabolism would remain marginal, in stark contrast to the reference sites.

The cause of the short meadow height is directly relevant, and it would be a valuable contribution to the discussion to constrain it. The referee includes hydrodynamics, substrate limitation, grazing pressure, depth, season, and light as drivers of changes in biomass. Our study investigated the effects of hydrodynamics, while controlling for season, depth, and light availability. This leaves substrate limitation, contaminants, and grazing pressure as likely causes of reduced biomass at the CO₂ vent. The manuscript discussion includes substrate limitation and contaminants, but we have not addressed grazing. Bite marks are not elevated at the site, but they are present, suggesting that grazing may cause enhanced erosion (Guilini et al., 2017).

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*It might be worth mentioning that *Cymodocea nodosa* biomass (Mishra et al. 2018) and productivity (Apostolaki et al. 2014) seem higher at the vicinity of Mediterranean volcanic CO₂ seeps. This seems to also be the case of a variety of seagrass species (*Cymodocea serrulata*, *Cymodocea rotundata*, *Halodule uninervis*, *Halophila ovalis*, *Thalassia hemprichii*, and *Syringodium isoetifolium*) in volcanic CO₂ vents in Papua New Guinea (Takahashi et al. 2016).*

Indeed, the study by Apostolaki et al., (2014) is directly relevant to our results. We thank the referee for introducing it to us. This study was conducted at another CO₂ vent in the Aeolian islands (Vulcano) where *Cymodocea nodosa* biomass was also reduced but productivity was surprisingly enhanced. These observations were not supported at a CO₂ vent in Papua New Guinea where increases in productivity follow increases in biomass at a high CO₂ site (Russell et al., 2013). These studies, and the study by Takahashi et al., (2016), are a valuable contribution to our discussion. The study by Mishra et al., (2018) is not yet published.

Minor comments L161: Specify detection limits, precision and accuracy for nutrient analysis.

The detection limits of phosphate, nitrate, and ammonium were 0.158, 0.016, and 0.2 $\mu\text{mol L}^{-1}$, respectively. As an estimate of the precision and accuracy of the technique we will present the standard deviation of replicate measurements of a known concentration. These were 0.018, 0.044, and 0.37 $\mu\text{mol L}^{-1}$, respectively.

L163: Specify the estimated accuracy and precision of TA and DIC measurements.

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The precision and accuracy of alkalinity was 8 $\mu\text{mol L}^{-1}$. The precision and accuracy of DIC was 20 mmol L^{-1} . Both estimates are calculated from the standard deviation of replicate measurements. These will be included in the manuscript.

L165: Do the actual TA measurements show that the values were invariant in time as assumed in the computation of DIC from pH?

The TA measurements were made at the beginning and end of the deployments, so they do not include diel variation. However, we examined the potential for diurnal variations in alkalinity to affect our calculations of DIC concentration. According to Frankignoulle (1986) diel alkalinity changes in seagrass meadows can be 15 mmol L^{-1} , or 0.6% of seawater alkalinity. This small change in alkalinity has an insignificant effect on DIC calculation from pH (Lewis et al., 2008).

L165: How well did the computed DIC compare to the measured DIC?

Measured DIC concentrations were $2220 \pm 21 \text{ mmol L}^{-1}$ at Elba and $2244 \pm 34 \text{ mmol L}^{-1}$ at Panarea (line 94). The mean calculated concentrations were 2215 mmol L^{-1} at Elba and 2220 mmol L^{-1} at Panarea. The high similarity between calculated concentrations at the sites is due to a very similar mean pH.

L221: Can you put the "importance of seagrass for CO₂ sequestration" in perspective with regards to the global carbon cycle? The global estimate of seagrass net community production is 21-101 TgC/yr as given by the synthesis of Duarte et al. (2010). This number is negligible compared the global anthropogenic CO₂ emission of 10,000 TgC/yr (as given by the latest IPCC report).

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This is an important point and we concede it to the referee. It is inaccurate to argue that seagrass carbon sequestration occurs at a rate that is significant compared to anthropogenic emissions. We will revise our statements. Instead, we will make the point that the relatively high rate of carbon storage by seagrasses is locally significant.

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