

RC1:

This manuscript presents an interesting experimental and modelling study of O₂ dynamics in microphytobenthos-inhabited muddy intertidal sediments, examining responses to light, temperature, and immersion-emersion conditions. The combination of high-resolution microsensor measurements with reaction–transport modelling is potentially valuable, and I particularly found the experimental dataset and the observed responses across the different environmental treatments interesting.

However, in its current form, I think the manuscript places too much emphasis on the predictive and mechanistic interpretation of the model. The model contains a relatively large number of parameters, many of which are fitted to the experimental observations rather than independently constrained, while several important quantities show substantial variability or cannot be directly verified. This makes some of the mechanistic conclusions derived from the model difficult to evaluate independently. I therefore recommend refocusing the manuscript more strongly on the experimental observations, with the model primarily serving as a framework for interpreting these observations and identifying processes and hypotheses for future investigation. In fact, I think one of the important conclusions emerging from the study is that we are still far from a robust mechanistic understanding of the processes controlling the MPB associated O₂ dynamics in intertidal sediments. Such a refocusing would also provide an opportunity to substantially shorten and streamline the manuscript, which I believe would make the main findings clearer and the study more accessible and appealing to a broader readership.

Response:

*We thank the reviewer for this thoughtful and constructive assessment and suggestions. We are fully aware that several processes and parameters represented in the model cannot be independently constrained by the measurements presented in this study, and that model-derived interpretations need to be distinguished from direct experimental observations. In the original manuscript, we therefore paid special attention to present our conclusions as **suggestions** derived from model-aided interpretation of the experimental data rather than a robust “final answer”. In response to the reviewer’s comments, we have gone through the text again and revised the relevant passages such that this general tone is clearer, in particular by more explicitly stating the intended role and limitations of the model (see below).*

As shown in Table S3, most of the model parameters were estimated through fitting to the experimental observations. Where literature information was available, such as for parameters describing the P–I relationship and photorespiration, we used reported values

to constrain the parameter ranges as much as possible. Other parameters, including those describing the vertical distribution of MPB biomass and several process rates, cannot be independently measured with the experimental setup used in this study and therefore necessarily have to be inferred from model–data fitting. We agree with the reviewer that, consequently, the model cannot independently demonstrate every mechanism represented in it or provide unique mechanistic attribution for all observed patterns.

*However, developing a model in which every represented process and parameter can be independently constrained was **not** the objective of this study. Rather, we see the value of the reaction–transport model in providing a quantitative framework that (i) connects the high-resolution experimental observations with candidate processes, (ii) allows us to estimate the impact of processes that cannot be inferred from O₂ profiles alone, (iii) and translates the observed patterns into quantitative and testable hypotheses. From this perspective, we are fully aligned with the reviewer’s suggested framing that the model should primarily serve as “a framework for interpreting these observations and identifying processes and hypotheses for future investigation.”*

To make this intended role of the model clearer, we have revised the manuscript throughout to distinguish more explicitly between quantities directly constrained by the experimental observations and interpretations derived from the model. Rather than shifting the focus away from the modeling framework, these revisions clarify the level of inference supported by the different model components and make the assumptions, parameter constraints, and limitations more explicit. The specific concerns regarding individual model components, parameter constraints, and interpretations are addressed in our responses to the reviewer’s comments below.

Major comments

1. **Model claims.** The model is technically sound and nicely designed, but the difficulty is that the model goes substantially beyond what the measurements independently constrain. It has 34 parameters, with most estimated by fitting the same experimental dataset that the model is subsequently used to interpret. Some particularly influential parameters are weakly constrained. For example, light attenuation and the vertical MPB distribution cannot be independently determined from the measurements, so several of these parameters are fitted simultaneously to the O₂-production profiles. Deep ODU concentrations vary by about an order of magnitude between cores but were not measured directly. Photorespiration was introduced because simulations without it systematically overestimated measured O₂- concentrations. That does not mean photorespiration is

unimportant, but the present data do not independently demonstrate that it is the process responsible for the discrepancy. Also the parameterization based on on industrial studies focusing on microalgae culturing. Similarly, the model requires a fivefold change in the ODU reoxidation parameter between dark and light conditions, from which the manuscript then infers depth-dependent differences in reoxidation. These are useful hypotheses, but I would be cautious about presenting them as mechanistically demonstrated results.

Response:

As mentioned earlier, we did cautiously formulate the broader conclusions of our study in terms of suggestions or hypotheses, rather than as mechanistically demonstrated results. Apparently, this intention was not fully clear to the reviewer. We reworded the relevant parts of the manuscript to make this clearer.

1.1 Regarding light attenuation and the vertical MPB distribution: This is an important limitation of the model parameterization that we were aware of when developing the model. This limitation is already communicated in the manuscript. The measured O₂ production profiles reflect the combined effects of the vertical distribution of active MPB biomass and the light available at depth. Because the vertical MPB biomass distribution was not independently measured and the light measurements showed substantial spatial variability, we were unable to constrain these two components independently by our present dataset. Consequently, the parameter estimates describing MPB distribution are not intended to represent unique estimates of the actual biomass distribution. To ensure that this distinction is sufficiently clear to the reader, we have strengthened the corresponding discussion in Sec. 4.2 and now state more explicitly that the inferred MPB distributions represent model-derived distributions that are consistent with the observed O₂ production profiles under the assumed light attenuation.

1.2 Regarding Deep ODU concentrations and ODU reoxidation parameter: The substantial variability in fitted ODU concentrations among cores and the multi-fold difference in the fitted reoxidation rate coefficient between dark and light conditions are already reported and discussed in the manuscript. These fitted differences were not intended to be interpreted as evidence showing differences in actual ODU concentrations or intrinsic reoxidation kinetics. To avoid any ambiguity on this point, we have further clarified their interpretation in the revised manuscript.

The representation of ODU in our model is deliberately simplified. A range of reduced compounds can contribute to O₂ consumption through reoxidation in sediments, but

explicitly representing each compound and its associated reactions would require many additional state variables and parameters. We therefore aggregate these reduced compounds into a single ODU state variable, leaving dissolved O₂ and ODU as the two principal chemical state variables in the model. Consequently, the fitted ODU concentration and reoxidation rate coefficient should be regarded as effective parameters representing the aggregate O₂ demand associated with unresolved reduced compounds.

We have strengthened the wording in Sec. 4.3 to make this existing interpretation more explicit. The approximately order-of-magnitude variability in fitted deep ODU concentrations and the approximately five-fold light–dark difference in the fitted reoxidation coefficient are therefore described explicitly as model-derived patterns rather than direct evidence of changes in ODU pools or intrinsic reoxidation kinetics. As already discussed in this section, several unresolved mechanisms could produce these apparent differences, including transient ODU dynamics, solid-phase reduced compounds not represented in the model, or pH-dependent changes in the speciation and reoxidation of reduced compounds. These mechanisms remain hypotheses rather than uniquely resolved explanations, and we have strengthened this distinction where necessary. Distinguishing among the other possible mechanisms would require independent measurements of ODU components and further model development.

1.3 Regarding photorespiration: The manuscript already treats photorespiration as a model-based interpretation rather than an independently demonstrated process. Nevertheless, the reviewer’s comment indicates that the distinction can be made more prominent. Demonstrating the contribution of photorespiration directly would require dedicated physiological and ecological experiments that quantify this process in the studied MPB community. To our knowledge, comparable quantitative formulations and parameterizations of photorespiration are currently not available for MPB systems. We therefore adopted the formulation developed for microalgal cultures and used parameter ranges reported in these studies to constrain the model. We have strengthened the discussion of this limitation and now make still more explicit that modeled photorespiration represents a plausible contribution to the observed O₂ consumption rather than a uniquely identified mechanism.

When revising our interpretation and the role of photorespiration, we identified yet another plausible process that could produce a similar apparent O₂ loss at high levels of irradiance: ebullition. Bubble formation and release can remove dissolved O₂ when

photosynthetic production results in strong O₂ supersaturation and could therefore account for part of the discrepancy attributed entirely to photorespiration in the original version of our manuscript. We have now included this process in the model and revised the corresponding results and discussion accordingly.

Importantly, distinguishing between photorespiration and ebullition primarily affects the modeled partitioning of O₂ consumption under high-irradiance, high-O₂ conditions. It does not affect the estimated O₂ production rates or the model's ability to reproduce the measured O₂ profiles. We therefore now present photorespiration and ebullition as alternative – and potentially co-occurring – mechanisms for the additional O₂ loss inferred under these conditions, rather than attributing this loss uniquely to photorespiration.

2. Sediment characterization. Given the considerable variability observed among replicate cores, I find the characterization of the experimental sediments rather limited, and I do not consider references to previous studies sufficient in this context. Important sediment properties that could directly affect O₂ dynamics and model parameterization, including porosity, grain size, organic matter content, and the concentration and distribution of reduced compounds, are either assumed, inferred through model fitting, or referred to previous studies. This makes it difficult to determine whether the observed differences among cores primarily reflect physiological responses of the MPB community or variability in sediment properties. I therefore suggest that the authors provide all available sediment characterization for the experimental cores and discuss this limitation more explicitly.

Response:

Sediment heterogeneity was already considered in the original manuscript as a plausible contributor to the variability observed among replicate cores. To substantiate this interpretation with additional experimental evidence, we have now included the available sediment characterization data from the same study site in the Supplementary Information.

Our experimental work was conducted in two phases. During a preliminary phase, six sediment cores collected from the same muddy ZK1 site were characterized for porosity, organic carbon and total nitrogen content, C:N ratio, and porewater concentrations of NH₄⁺, NO₂⁻, NO₃⁻/NO_x, PO₄³⁻ and dissolved Si. These measurements revealed clear vertical gradients in sediment properties, including generally decreasing porosity, organic carbon and total nitrogen content with depth and increasing concentrations of several porewater

nutrients. We have now included all these available measurements in the Supplementary Information (Fig. S6) and added their description to the Methods. We did not do this in the original manuscript in order not to overwhelm the reader, but now we acknowledge that this was not a good idea.

Importantly, these measurements were performed on separate cores from those subsequently used for the PI, TR and IE microsensor experiments. We have made this distinction explicit in the revised manuscript. The new data therefore provide direct characterization of sediment collected from the same study site, but they cannot be used to quantify sediment heterogeneity among the experimental cores studied by microsensors. They nevertheless demonstrate appreciable variability in sediment geochemistry among cores collected from ZK1, supporting sediment heterogeneity as a plausible contributor to the variability observed in the microsensor measurements. Grain-size distributions and the concentrations and distributions of the reduced compounds represented collectively by the modeled ODU pool were also not measured in those individual cores.

We have also strengthened the discussion of how sediment heterogeneity affects the interpretation of differences among replicate cores in the section “Model assumptions and uncertainties”. Because sediment properties were not characterized in the exact same PI, TR and IE cores, the observed differences among these cores cannot be attributed uniquely to differences in MPB biomass distribution or physiology. Variability in sediment geochemistry, particularly organic matter and reduced compounds contributing to sediment O₂ demand, provides an additional plausible source of this variability. In this context, fitted differences in parameters such as the deep ODU concentration should be interpreted as model-derived representations of differences in sediment O₂ demand rather than as direct measurements of the underlying reduced compounds. Resolving the relative contributions of MPB variability and sediment heterogeneity would require sediment characterization and microsensor profiling to be performed on the exact same cores.

3. Microphytobenthos Physiology. The physiology of the microphytobenthos is represented in an overly simplified manner. To give just a few examples, microphytobenthos can assimilate substantial amounts of nitrate and ammonium and, together with their vertical migration and DNRA, drive a dynamic redistribution of ODUs within the sediment. This can shift hotspots of metabolic activity, and thus oxygen consumption, vertically over time. Relatedly, it is unclear what happens when cells experience, for example, extreme temperatures or prolonged emersion, potentially leading to physiological stress or cell lysis. I would not necessarily expect such processes to be explicitly represented if the model were more clearly framed as a simplified model

applicable to a specific range of conditions, rather than as a broadly applicable model of oxygen dynamics in intertidal sediments.

Response:

The physiological and biogeochemical representation of MPB in O2DIA is deliberately simplified. The aim of the model was not to explicitly represent all processes that may influence O₂ dynamics in natural intertidal sediments, but rather to develop a parsimonious framework that captures the dominant O₂-producing and O₂-consuming processes under the conditions investigated here. Adding processes such as inorganic nitrogen assimilation and transformation, DNRA, physiological stress, or cell lysis would require additional state variables, process formulations and parameters, many of which cannot be independently constrained by the measurements available in the present study. Such additions would substantially increase model complexity without necessarily improving its identifiability.

For this reason, we restricted the model to two state variables, O₂ and ODU, and formulated the represented processes, wherever possible, in terms of environmental controls that can be – directly or indirectly – related to O₂ dynamics. The ODU pool is itself a deliberate simplification that aggregates the O₂ demand associated with multiple reduced compounds rather than explicitly resolving their individual production, transport and consumption pathways.

We agree, however, that the original manuscript did not sufficiently communicate the resulting limits of model applicability. O2DIA should not be interpreted as a broadly applicable mechanistic representation of all processes governing O₂ dynamics in intertidal sediments. Rather, it is a deliberately simplified model developed and evaluated for the range of environmental conditions and timescales covered by the present experiments. Processes omitted from the model, including those highlighted by the reviewer, may become important outside this range or over longer timescales.

We have revised the manuscript throughout to make this modeling philosophy and its implications more explicit. In particular, we now clarify the intended scope of O2DIA in the model description and expand the section “Model assumptions and uncertainties” to discuss the omitted processes and the conditions under which they may limit model applicability.

4. Microscale heterogeneity. Recent and older studies have shown that microscale heterogeneity can play an important role in intertidal sediments, including processes and structures occurring at spatial scales smaller than those resolved by the microsensors used here. Examples include localized organic matter accumulations and associated

anoxic hotspots, porosity variability, but also small-scale heterogeneity during emersion, such as changes in the light field caused by scattering around water droplets. These processes and their potential implications for the interpretation of the measurements and model results should at least be discussed.

Response:

We have expanded the section “Model applicability and future development” to explicitly consider heterogeneity occurring at spatial scales comparable to or smaller than those resolved by the microsensor measurements. We now discuss localized organic matter accumulations and associated O₂ depletion or anoxic microniches, microscale variability in sediment structure and porosity, and heterogeneous surface wetness and light exposure during emersion. These unresolved features may contribute to variability among individual microsensor profiles and consequently to uncertainty in the fitted model parameters. We have also added relevant references supporting these microscale effects.

5. Advective terms. The authors mention in several places that the model does not apply to permeable, advection-dominated sediments. I consider this an important limitation that should be reflected more clearly in the title, abstract, and conclusions. Although this limitation is acknowledged in the manuscript, it can easily get lost, particularly in some of the broader concluding statements. The applicability of the model is therefore restricted to some intertidal sediments in which molecular diffusion dominates solute transport, rather than intertidal sediments in general.

Response:

We have made the domain of applicability more explicit throughout the manuscript. The title now specifies diffusion-dominated intertidal sediments, while the Abstract, Discussion and Conclusions also clarify that the current model is primarily applicable to cohesive, diffusion-dominated sediments. We also state explicitly that application to permeable sediments, where advective porewater transport becomes important, would require further model development to represent advective transport. Broader statements implying applicability to intertidal sediments in general have been revised accordingly.