Response to reviewer 1

We thank the review for their thoughtful comments which we address below. Our responses are in italics

- The manuscript by Hall et al., performed sediment slurry incubations, headspace methane determinations, and metabolomic analysis to show potential methylotrophic methanogenesis in sandy sediments derived from osmolytes commonly found in seaweeds (e.g., *Ulva*) and coastal plants (e.g., mangroves). They found that degradation of kelp and red filamentous developed low concentrations of methane. Whereas incubations with degrading Ulva biomass led to significant methane development. I believe the work is important, and relevant tot eh scope of Biogeosciences, but as it stands the manuscript has several that must be addressed before recommendation for acceptance/publication.

  Major comments:

  1). The paper needs more citations that are critical, specifically papers that investigate the production of osmolytes and their role in methane production See publications from Wang and Lee, 1994 and 1993 and seminal studies from Aharon Oren.

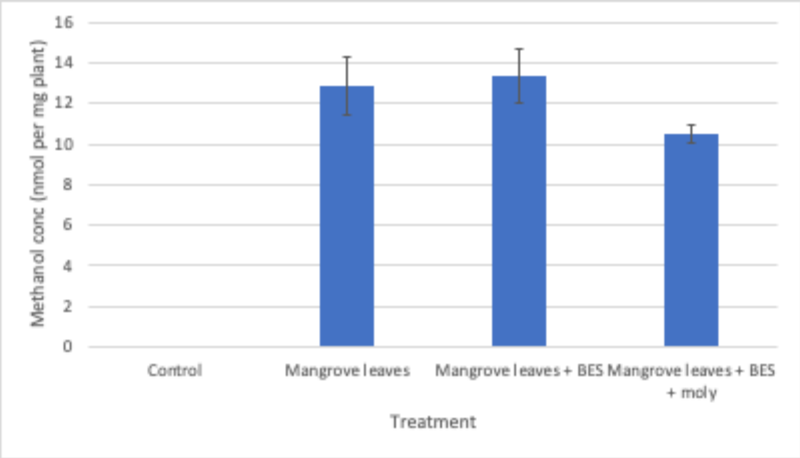
  We agree with the reviewer that these relevant seminal studies have been omitted and will include them in a re-written introduction and discussion. We note that this work contains important context on osmolytes in vascular plants such as spartina that will help substantially improve the framing of the manuscript.

  2). Methanol was a suspected substrate for methanogenesis, why did the incubations not include a methanol control and why was Pectin (the precursor to methanol) not analyzed in any of the host organic matter and during the incubations? Given that the results apparently found little no methanol and with no data to show, it may be worth considering getting rid of that text all together.

  We are currently undertaking follow up studies on the role of pectin in methane production. To address this comment we will reframe the study to focus on the role of the osmolytes we did measure in the plant/algae species for which there are no published data.

  3). The results are not well described and could be organized better, specifically regarding methanol, which seems to be lacking.

  We will add in the experimental data on methanol production as per the plot below. The addition of mangrove leaves to the sediment resulted in methanol production. The addition of the methane production inhibitor BES did not significantly increase methanol production. The addition of the sulfate reduction inhibitor molybdate reduced methanol production, suggesting sulfate reducing bacteria may play a role in its production.



4). The discussion, unfortunately, is the weakest part of the manuscript. Particularly, the interpretations of the data are either contradictory or have no teeth because no data shown (i.e., methanol trends). Moreover, the authors could elaborate more on the broader impacts and significance of the data and interpretations of the study throughout the discussion. I have identified areas that need attention in the in-line comments below. Lastly, the discussion ends rather abruptly. I suggest the authors either add a concluding paragraph or a conclusions chapter that summarizes their findings.

We agree the discussion needs improvement and will alter this as described in response to the specific comments outlined below

In-line comments:

L32: There are many other papers that show methylotrophic methanogenesis activity in coastal environments that you can include such as Xiao et al., 2017; Maltby et al., 2016; Zhuang et al., 2026; Krause et al., 2021 and 2025 and probably several others.

We agree there is a large literature on this, but we focus the current citations towards those relevant to sandy sediments and those influenced by seaweeds.

L50: I would suggest adding citing papers from Oren and Lee and Wang who did some seminal work on production of methylated substrates in coastal environments including coastal wetlands.

We agree these references provide important context and will be added into the revised version

L95-96: How does sieving and homogenizing the 0-5 cm layer provide a seed population of methanogens? Were methanogens added to the sediment? Needs clarification.

As per Hall et al (2025), methylotrophic methanogens are ubiquitous in permeable sediments and respond rapidly to osmolyte inputs. We will clarify this statement to reflect this

L98: Incomplete sentence…

Will be deleted

L100: In this section the authors prepared sediment slurries with different ratios of sediment slurry to kelp biomass. Is there a reason why these ratios are so different? For example, the kelp biomass to sediment is <1% but there is 10% mangrove leaves to sediment. There could be one sentence here to explain why these proportions were chosen. Additionally, there are differences in incubation times for each set of slurries, but no clear explanation as to why.

The ratio of plant and seaweed biomass to sediment was the same for the methane production experiments

For the methanol production experiments we added leaf material because we hypothesized that methanol would be an intermediate (both produced and consumed) so we wanted to maximise our chances of detecting this.

L104-105: I think this sentence could be split into two for clarity.

Will be re-written

L106: How long were samples purged with argon for?

3 minutes will be clarified

L107: If you did do artificial day/night cycles then you need to elaborate more (e.g., duration in the light vs. at night and by what light source).

This refers to the fact that the samples were left in the lab under ambient lighting, rather than an attempt to stimulate photosynthesis, we will clarify this.

L111-117: Were controls prepared for this incubation?

Yes, leaf free controls were run (shown above) as well as an initial experiment with leaves only that detected no methanol in the leaves.

L113-115: Spell out “5”. Numbers in the beginning of sentences should be spelled out. This sentence does not read well; I suggest reorganizing it for clarity.

Sentence will be edited for clarity

L121: Are these the same time points listed in the previous section?

Yes, this information will be placed in the previous section for clarity

L128-132: Issues with this sentence. Spell out “20” if it’s the beginning of the sentence. The long parenthetical could be a separate sentence. There are several grammatical errors that make the sentence hard to understand. I recommend reorganizing for clarity.

Will be edited to improve grammar

L175-176: Citation? What is the theoretical methane production potential based on? Is it based on how many moles of TMA and DMS that were added? Needs clarification.

We will now use the ratio of ¾ methyl groups produce methane

*Ferry JG (1999). Enzymology of one-carbon metabolism in methanogenic pathways. Fems Microbiol Rev **23:** 13-38.*

L201-202: I think this sentence is rather vague and just breezes by some important details in the data. The authors can expand a little more on this result description because although see the DMSP being dominant in the Ulva, one could also see the dominance of Choline in the Mangrove samples. I think 1 or 2 more sentences to cover these important trends would make it stronger.

This will be expanded as suggested

L205: I suggest that the authors be careful with using the word "plants" in their manuscript. Technically, seaweed is not plants, they are algae. I think in colloquial conversations "plants" can be used and understood to mean kelp and other seaweeds but in peer-review journals one should be careful.

We will be more specific when referring to plants/macroalgae throughout

L215: Citation? Is there literature on how DMSP supports methanogenesis not only by providing the methyl group but also the nutrients (i.e., P) for metabolic activity?

There are no references on this, we are speculating here, we will make this clearer in the revised draft

L225: Can the authors elaborate on what "other plant components" are specifically? Isnt it possible that after that 7-10 days the most labile osmolytes are consumed and the system reaches a steady-state or has a tough time breaking down the more recalcitrant organic matter from the seaweed to further produce methane production? What data supports the claim, since there doesn't seem to be a tracking of osmolyte concentration in the batch incubations to suggest exhaustion?

The 'other' plant components are most likely dominated by carbohydrates, and we will be more specific in the revised manuscript. We believe it is unlikely that this organic matter is more recalcitrant and will keep breaking down via fermentation and sulfate reduction. We suggest exhaustion based on the mass balance of osmolytes in the plants and methane produced during the incubations. Quantifying the osmolytes during the incubation is very difficult because of low concentrations and high salt content.

L230-232: I agree that methanogen abundance probably starts low (do you have data to support that?) but two other things need to be considered 1) the system needs to be fully anoxic, which even with the procedure may still have small concentrations; 2) I also think that the algal and plant biomass added to the system still has intact cell structures that contain the osmolytes which need time to break down and release the osmolytes. This would be easy to tell if osmolyte concentrations during the study were monitored overtime. Additionally, I can see how methanogenesis in this case is a transient phenomenon, but can the authors elaborate more on why that might be and the significance of this observation. If methanogenesis is short lived from these organic matter sources, then why should we care?

Hall et al (2025) showed rapid growth of methanococcoides from a small seed population in permeable sediments. We agree there will be traces of oxygen even after purging, but that respiration will consume this within minutes to hours. We agree that osmolyte release is another factor controlling the time lag observed. As per above, analysis of osmolytes in the incubations is technically very difficult. In addition to this, there is likely to be both a dissolved and intracellular pool in the slurries at any given time, further complicating the interpretations of such measurements.

Whether the transient methane pulse is important or not, comes down to the integrated amount over the release period. Indeed the work here allows future research to make estimates of potential form methane release from blooms based on tissue osmolyte content.

We will add in more detail to this section of the discussion to incorporate these points

L240-245: Your data in figure 3A does not really suggest Red Filamentous Algae to be a potent contributor of methane. Only one location saw some methane development but was more than half less than the Ulva condition. Maybe expand here a bit as to why Red Algae don't contribute much methane perhaps Figure 4B has some suggestions. Moreover, this statements sort of contradict your previous section where methane production appears to be short lived. So why do we care in the context of this study if Ulva becomes more abundant with warming climate if methane production is short lived?

We agree this observation was only at one site, and will clarify this and elaborate some more on what might control this with reference to osmolyte tissue content. As per above, while methane production is short lived, it is the integrated amount that is released that is important.

L245-247: I am not really sure what this statement is getting at? Are you trying to imply that Sargassum may lead to methane production when washed up on beaches? If so, I would expand a bit otherwise it doesn't really fit well with the story.

This will be expanded to be more explicit about the deposition of macroalgal blooms more generally including sargassum

L248: This section has no teeth since no methanol data was shown.

Methanol data will be added