Response to reviewer 2

We thank the reviewer for their throughful and constructive comments. Our response to their comments are in italics

The manuscript by Hall et al. investigates how different types of marine plant and seaweed biomass influence methane production in sandy sediments collected from a bay in Australia. The authors incubated sandy sediments with biomass from seaweeds and coastal vegetation, including mangroves, seagrasses, and saltmarsh plants, and monitored methane concentrations over time. In addition, they quantified the concentrations of choline, DMSP, TMAO, and TMA in the various biomass types. Because these compounds are known methane precursors, the authors multiplied their measured concentrations by the number of methyl groups contained in each compound to estimate their potential contribution to total methane production.

The most interesting and novel finding of the study is the variation in methane production among the different biomass additions. In particular, Ulva and mangrove biomass stimulated higher methane production than the other biomass types. These findings raise important questions about how future shifts in the abundance and distribution of marine plants and seaweeds may influence methane emissions under ongoing environmental change.

In my opinion, the study is an important addition to the growing literature on the importance and drivers of methylotrophic methanogenesis in coastal sediments. However I identified a couple of concerns and can not recommend it for publication in its current form.

Main comments:

1. Calculating sum of methyl groups

I have some concerns about the way the methane production potential of the different plant types was estimated based on the number of methyl groups of the quantified osmolytes. First, the authors appear to assume that all methyl groups of a given osmolyte are available for methanogenesis and can therefore be reduced to methane. However, it is unclear what electron donor would support the reduction of all methyl groups. Is it hydrogen? I actually think that electron are rather derived from the oxidation of additional methyl groups to CO2, in which case not all methyl groups are in fact reduced. I think this needs to be taken into account and may require revisions of some of the conclusions of this study.

We have reviewed the stoichiometry of the reaction and this is typically regarded as ¾ methyl groups produce methane (Ferry 1999 as cited in the response to reviewer 1). This will not change our conclusions markedly, but the revised manuscript will incorporate this point.

Second, how confident are the authors that indeed all methylated compounds that could serve as methanogenic substrates have been quantified? One notable omission is glycine betaine, which has been identified as a direct substrate for methanogenesis. The exclusion of glycine betaine and its breakdown products could influence the estimates of the relative contributions of the measured osmolytes to methane production.

We have unpublished data to show that adding glycine betaine to these sediments did not stimulate methane production. We will elaborate on this omission and possibility of it producing methane in the discussion.

2. Role of pectin and methanol

In mangrove incubations, the amount of methane produced could not be fully explained by the amount of osmolyte quantified and the authors instead link this methane production to methanol which forms during the breakdown of pectin. However, there is no actual data presented that would support these statements, even though methanol has apparently been measured. I think either data on methanol measurements have to be added or the conclusions about the role of methanol has to be revisited.

As per above, we will remove the unwarranted speculation about pectin and methanol and add this to future work. We are currently undertaking work on this, as part of another student project.

3. Figure 1

I think Figure 1, in its current form, is misleading. Figure 1 presents a universal pathway of methane production in coastal sediment that is more like a summary of the existing literature or shows assumptions that are not supported, like the role of pectin. The figure actually implies that there is a universal pathway by which methane is formed in vegetated sediments irrespective of the type of vegetation which seems to contradict the findings of this study. The novel findings of this study, the comparison of different biomass types, is not shown at all. In my opinion, this figure needs to be substantially revised or removed.

We will modify this figure to focus on the compounds we measured and the variation of osmolytes in the different seaweed species.

4. Results part

I think the results part is very short and misses some key results. I suggest to include data on the absolute osmolyte content of the different biomass types as well as any data available from the methanol analyses.

We will include the absolute osmolyte concentrations as suggested in the revised version.

Line-specific comment:

Line 20: What does “despite low osmolyte levels” refer to? If I look at your dataset 2, mangroves, seagrasses and saltmarshes seem to have the highest levels of choline and trimethylamine. Please clarify.

We agree this is not necessarily the case. We will clarify this when we add in a new figure with the absolute concentrations of osmolytes.

Line 96: Was this done under anoxic conditions? If not, could it be that exposure of the sediments to oxygen could have inhibited the methanogens which could have led to the lag phase at the beginning of the incubation?

We did not do the initial processing under oxic conditions. We have previously shown methanogenesis in permeable sediments is carried out by oxygen tolerant methanogens (Hall et al 2025). They lag phase is most likely growth from their small initial population. We will add in text to methods to clarify this.

Line 98: Sentence is incomplete

Sentence will be deleted

Line 103: How much biomass was added for incubations with plant material?

The same as seaweeds, we will clarify this in the revised version

Line 111: Please include data on methanol analyses here and throughout the manuscript.

Will be added in

Line 112: Why was the experimental setup for the methanol determination different than for the other slurry incubations (less sand and seawater, more plant biomass)?

This separate experiment was undertaken as a follow up, and more biomass was added to maximise our chances of observing methanol

Line 122: Was the collected gas sample injected into the 3 ml exetainer with or without releasing the overpressure?

Yes, the exetainers were over pressurised, this will be clarified

Line 145: It is not clear what "all measured concentrations fell within the calibration range" refers to. In Table 1 LOD and LOQ are shown for four measured compounds but it is not stated what LOD and LOQ were used for in the interpretation of the data? Were all measured compounds above the LOQ for all measured samples? If so, then why are some of the compounds reported as zero in dataset 2? I think this needs clarification. Also, please add units of data shown in datasets 1 and 2.

We will condense this to report only the LOQ, and use units consistent with those for the reported osmolyte concentrations. Although many values appear to be zero, they were actually detected, but in very low concentrations. This will be clarified in the revised manuscript.

Line 160: How did you get to the dry weight of added biomass in the slurries?

The moisture content of the samples was measured by drying. This will be clarified in the revised manuscript

Line 165: Please see my main comment above about the number of methyl groups available to methanogens.

Addressed above

Line 189: the authors state that DMSP was the dominant substrate, as close to 100% of methyl groups were converted to methane. Looking at Figure 3, all seaweed types show that methane production stopped after around 200 hours. Does this mean that by that time all DMSP had been converted to methane? If so, I find it surprising that the same was observed for the control experiment that contained high (80µM) DMS and was probably not limiting.

We agree that the osmolyte is not likely limiting in this case and we speculate that there is a lack of nutrients limiting growth. This point will be added to the manuscript.

Line 235: Terms seaweeds and marine plants are mixed here

We will use more consistent terminology as suggested

Line 265: Without presenting any data on methanol this statement has to be removed.

We will add methanol data as suggested