Review report:

“Reviews and syntheses: Methane biogeochemistry in Sundarbans mangrove ecosystem, NE coast of India; a box modeling approach” by M.K. Dutta and S.K. Mukhopadhyay

General comment: Mangrove ecosystems have recently been identified as one of the highest carbon rich ecosystems on earth; however, are one the mostly threatened tropical ecosystems as well. Mangrove ecosystems are also significant source of potential green house gases like \( \text{CH}_4 \) and hence proper quantification and measurements are highly required. Moreover, Sundarbans is the largest mangrove forest in single block and represents almost 50% of mangrove coverage in India. Thus, the present study is very much essential in the present context of climate change. The manuscript is well written and the flow is good. It includes all components of the mangrove ecosystem: sediment - water, water - atmosphere and sediment – atmosphere to explain the observed methane fluxes. Photo-oxidation of methane has also been addressed. However, there are certain points which should be clarified by the authors and they are as given bellow:

- Figures are very badly presented. Uniformity is lacking in the figures. Not a single panel has been places properly. For example in Fig. 3, the last number of Y axis has been cropped and overlapped with the axis and so on. Resolution is too poor. Fig. 8 presenting the methane budget looks too bright with many colours.

- The title of the paper states “review and synthesis: methane biogeochemistry in sundarbans……………..”

I am not sure if the manuscript has been written in the form of a “review and synthesis”. The paper has been presented as a complete independent research article with all data set on methane dynamics collected during June 2010 to Dec 2012. The data set collected during June 2010 –Dec 2011 has been published in the first two references given bellow and the data set collected during Jan 2011 –Dec 2012 are published in the third reference.


This shows that the data set presented here has already been published elsewhere. Hence, presenting the same dataset with pretty similar illustrations, results and explanations does not sound like a synthesis of the published results.
• The scheme for methane photo-oxidation (Fig. 1) is from the published paper Dutta et al., 2015 in Agricultural and Forest Meteorology, however has not been mentioned below the diagram.

• The same box model approach has been used in all these three articles. Structuring of the manuscript is also very much alike. After going through these articles, I miss the novelty of the present work.

• In the methodology section, (page 7 line 147-153) the authors mentioned that methane oxidation was studied in-vitro and 6ml of sediment water mixture was filled in a glass vial with rubber septum and incubated for 4 days at ambient temperature. I would like to know what does it mean by **ambient temperature**? Does it mean that the authors incubated the flasks in-situ where the sediment was collected or brought back to the laboratory? Were those 60ml flasks incubated under natural day and light? Or under dark incubator at fixed temperature? No reference has been cited for this method. It could also happen that the spiked methane leaked from the vials during these 4 days incubation. What guarantee that the methane concentration in the head space decreased due to oxidation? Moreover, whenever the methane has been injected into the headspace it must create some concentration gradient and there will always be some chances to leak from the vial. Was there any dark control to show that in the absence of light there was no methane oxidized and the concentration remained same as the initial? This would also support the concept that the spiked methane did not leak.

• I have the same concern in the methodology of methane production measurements (page 7, line 132-138). The vials 1.2cmx10cm are quite small and I am curious how the vials were made air free and kept air free for 24 hours. This is a kind of tricky job to make anything air free or oxygen free. The authors said that nitrogen was used to flush out air from the bottles. It is not really clear for me which way it has been done. In that case a special cap would be required with two parallel apertures, one for nitrogen in and another for air out. Incubation cannot be continued with this type of cap and it must have been changed. But the question is how to change the cap, since it would immediately be contaminated with air. It is only possible to do within a nitrogen environment inside a “glove Box”. The author can please clarify this point preferably with photograph or schematic presentation. Here also the meaning of ambient temperature should be clarified.

• Page 9-line 196-200: Samples for chlorophyll was kept under ice before filtration. I am not sure if chlorophyll samples keeping on ice which could give a temperature of 4°C is enough for preservation. The author followed a spectrophotometric method for chlorophyll analysis where minimum detection limit is 0.5µgL⁻¹. I wonder if the authors have also done phaeopigments? In that case I am sure the amount of phaeopigments would be higher than Chla. Please explain in details the methodology followed from filtration, type of filter paper, extraction media and the formula used to calculate the final Chla concentration.
Page 10-line 200-202: Productivity and community respiration in the estuarine surface waters was measured in situ by dissolved oxygen light–dark bottle method. I am curious to know three things here:

1. What time the samples were collected, during high or low tide? Because in a dynamic mangrove dominated estuary, the rate of both photosynthesis and community respiration would vary widely depending on the time of sampling. During high tide, relatively clearer water with more phytoplankton and less organic carbon would usually show a high productivity rate over respiration. However during low tide, high turbidity, organic carbon and low phytoplankton would definitely show a high respiration rate over photosynthesis.

2. How long the samples were incubated in situ? Since, this estuary experiences almost 5m tidal amplitude, incubation in situ may not be possible for longer time and it must be done where water level remains even after low tide. Please clarify this point.

3. Data presented in table 4 does not mention what is the sample size (n). However, the standard deviation of community respiration value during monsoon (102±116) indicates large variation in the data set and also indicates during monsoon months the rate of respiration could be very low or high at a certain point. This point should be clarified. While writing “bottom” water, which is not surely collected from the bottom, “subsurface” would be a better term instead. Please mention the average depth.

The methodology to measure soil organic carbon has not been given anywhere in the method section.

Result and discussion:

• page 11: The author used the term “methane production potential” many times in the beginning of the result and discussion session. However, this terminology has not been defined anywhere in the text before. To me it sounds like methane production rate. Can they be used as synonyms?

• In table 1 “AVS” has been used and also many other places in the text, however, the abbreviation has been used without introducing the detail terminology in the text.

• Page 12-line 248-250: The statement made here that the reduced % of soil organic carbon with depth is due to anaerobic organic matter mineralization. This is definitely not a correct statement. In every forest type this is a general profile for soil organic carbon that the % decreases with depth and this is mainly because of the reason the input is more at the surface. One example is given as follows:
However, the % of organic carbon depletion depends on the amount of litter fall, the type of vegetation and also on the types of soil. In the case of perforated soil the % of mineralization would be faster than the waterlogged soil like mangrove.

- The organic carbon data presented in Fig. 3c are single point measurements or average of duplicates?
  - Value of ammonia given in page 15, line 323, are quite high (1.01 - 3.31µM) and considering that mangrove are nitrogen limited, I am not sure such high ammonia concentration can occur in the soil of high microbial activity. Reduced form of nitrogen would be readily taken up by the heterotrophs.
  - Line 274-279: This study has been done only at one location (Lothian Island) and extrapolating this to the entire islands (almost 200) could largely over or under estimate the scenario.
  - Page 26: Line 557 -560: the quantitative methane budget sounds very strong extrapolation to me. Particularly, where the author is considering that in situ methane production is equal everywhere in the other islands and hence the single data has been extrapolated over 200 islands. I am not sure if this is convincing.
  - Fig. 7 and table 4 present the physicochemical and biological parameters of the estuarine waters. During June –September, there is a steady decline in the Salinity values reaching almost 15 psu and secchi disk depth data corroborates nicely with this drop. However, I am really surprised to see that the productivity values increased linearly from July to October, which is unlikely to happen in turbid estuarine water.
  - Moreover, the pH values never went bellow 8, though the salinity reached almost 15 psu. This is very difficult to believe.
  - This again surprises me that the community respiration values were the lowest during the monsoon months (June –Sept) and, I am not sure how
the large standard deviation occurred for this data set (102±116 mg C m$^{-2}$ hr$^{-1}$). From Fig. 7 it is likely that the premonsoon months should show minimum productivity. Dissolved oxygen values also do not show much variation during monsoon months, though productivity increased. Author should check the data set.

- Page 20, line 429-436: “Influences of dissolved inorganic nitrogen (DIN) concentration on microbial CH4 oxidation had been reported previously in Lake 227 (Rudd and Hamilton 1979). According to their observation in the presence of O2 concentrations > 31 µM bacterial CH$_4$ oxidation was inhibited when DIN concentration was low (< 3 µM) as methanotrophs can fix nitrogen under low DIN conditions (< 3µ M). The nitrogen fixation is disrupted by high concentrations of O2 but not inhibited when DIN concentration reaches to 20µ M”.

This discussion part on nitrogen fixation, DIN and methanotrophy is not clear to me and neither is sound relevant to the text. Nitrogen fixation takes place in well oxygenated water and the organisms, either bacteria or cyanobacteria they either separate the time of fixing nitrogen and carbon (like in $Trichodesmium$) or make a separate cell for fixing nitrogen (diazocyte) or some other mechanism to protect the enzyme nitrogenase from oxygen. This happens inside the cell. Moreover, there are many literature available showing the inhibitory impacts of dissolved inorganic nitrogen source on nitrogen fixation. Since, nitrogen fixation is a high energy consuming process, until the cell is critically suffering from nitrogen limitation they would not spare so much of energy. So when DIN is 20µM it is unlikely that the organism fixes nitrogen.

I would suggest the authors that if they really wish to make a proper synthesis and review of methane dynamics in this mangrove system, they should collect and include all previous published papers from the same system and make a trend analysis over the years of sampling. For example there is couple of papers provided in the reference list:


I am really skeptical about the novelty of this paper and hence would like to suggest including the suggestions and may submit a revised version for further consideration in this journal.