

***Interactive comment on* “Mechanisms for the suppression of methane production in peatland soils by a humic substance analog” by R. Ye et al.**

Anonymous Referee #2

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The manuscript “Mechanisms for the suppression of methane production in peatland soils by a humic substance analog” by Ye and co-authors describes laboratory batch incubation experiments with two peatland soils incubated at different temperatures and with the amendment of different organic substrates for microbial degradation processes including the humic substance analog AQDS. The concentrations of several substrates for anaerobic microbial metabolism and the end products CO₂ and CH₄ were measured for 45 days. Furthermore, the concentrations of AHQDS, the reduced form of the applied humic substance analog, were analysed.

The manuscript is well written and deals with an important topic, important to better understand the role of organic matter as an external electron acceptor for microbial organic matter degradation and the regulation of methane production in different anoxic

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environments. However, I have some objections on the presentation and interpretation of the results.

An important topic of the manuscript is the role of humic substances as potential terminal electron acceptors (TEA) in soils. Unfortunately, the concentrations of further TEAs (e.g. nitrate, ferrous iron, sulphate) in the incubated soils were not measured and their role in the incubations remains speculative. However, at least CO₂ concentrations were measured but pore water CO₂ concentrations are reported neither. It seems that the authors do not consider CO₂ as an important inorganic TEA (see abstract or first paragraph of the discussion) although CO₂ concentrations in anoxic peat soils are generally in the mM range and CO₂ is the inorganic TEA for hydrogenotrophic methanogenesis. Hence, CO₂ and not AQDS seems to me the most important TEA in their incubation studies.

A second general issue is the calculation of Q₁₀ values from the experimental data. The authors measured concentrations of important substrates for CO₂ production (glucose, acetate) and methane production (acetate, hydrogen) at six different time points over an incubation period of 45 days (Fig 2-4) at three different temperatures. Furthermore, they measured over the same time period CO₂ and CH₄ production rates (Fig. 5 and 6) which change over time as do the substrate concentrations. The authors are certainly aware that microbial CO₂ and CH₄ production rates are strongly affected by the concentration of available substrates and not only by temperature. However, they use the different CO₂ and methane production and AQDS reduction rates at the different time points, characterised by very different substrate concentrations at the different temperatures, to calculate Q₁₀ values. Q₁₀ gives the sensitivity of e.g. microbial process rates on temperature and may only be calculated from microbial process rates if temperature is the only variable affecting these microbial rates, e.g. if steady state conditions or substrate saturation may be assumed. This is not the case in the presented experiments. Hence, the presented data seem to me not suitable for calculating Q₁₀ values. The impact of substrate concentrations on the measured process rates is most

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likely the explanation for the strong and rapid shifts in the calculated Q10 values (Fig. 7) and not a rapid shift in the active microbial community composition. Hence, also the elaborate discussion on the dynamics of Q10 values is to my understanding not to the point since the presented data do not meet the criteria for being suitable for Q10 value calculations. Therefore, I suggest omitting the whole part on the temperature adaptation of the different processes.

A further obstacle for the interpretation of the presented data is a lack in clarity on how the microbial process rates were calculated. The authors state that they were calculated from the cumulative production of CO₂ and CH₄ but it is unclear which time period and how many data points they used for the calculation of microbial rates.

The authors produced a substantial data set but it remained somewhat unclear to me, what the new findings are. The authors should make very clear what the novelty of their results is, especially in the discussion and the abstract. Also the presentation of the data may be improved. Despite presenting seven very similar graphs with the measured values they should rather focus on the new findings of the presented study.

Minor comments:

P1740 I23ff: The GWP of methane is 25 times that of CO₂ or 24 times higher than that of CO₂. Please clarify that GWP is calculated on a weight not on a molar basis

P1741 I5ff: There is a much wider variety of low molecular weight end products of fermentation, not only acetate and hydrogen that can be respired by microorganisms under anaerobic conditions.

P1744 I5ff: Please give soil T during sampling and MAT of the two sites.

P1745 I26: Did you also consider the gas pressure in the closed vials? This is essential for calculating gas production.

P1746, I3: Please give the acceleration in g not rpm.

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P1746, I16: How did you measure pH?

P1746, I22ff: Which data points did you use for calculating the rates? What was the time period for the calculation? How many data points did you use for the calculations? How did you calculate rates at day 2 from cumulative production?

P1747, I21: Please indicate at which time the difference was significant.

P1748, I7: see above

P1750, I3ff: The measured data seem to me not suited for calculating Q10 values because the rates are not only affected by temperature but also by the different substrate concentrations in the vials at the same time but different temperatures.

P1751 I20ff: I do not follow this conclusion. CO₂ concentrations will have been most likely high during the experiment (data were measured) and to my understanding CO₂ is a TEA in the process of hydrogenotrophic methanogenesis. Hence CO₂ was the most abundant natural TEA probably in higher concentrations than AQDS.

P1755 I21: CO₂ is a TEA (see above)

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