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

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Q1. 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 environments. However, I have some objections on the presentation and interpretation of the results.

R1. We thank the reviewer for their comments. Yet, we respectfully disagree with the major objections and have endeavored to fully address the comments.

Q2. 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.

R2. Our previous study (Ye et al. 2012) suggested that inorganic TEAs were minimal in these peat soils. A pre-incubation of 2 weeks is sufficient enough to consume all of them. We did observe the production of CH4 after a lag period during the pre-incubation, which clearly suggested that the endogenous TEAs were exhausted. We have also provided references to support our statements at the beginning of the Discussion (L. 351-353; 356-360). The reviewer is correct that CO2 can act as a TEA in chemoautotrophic reactions. However, our discussion of TEAs in this paper is focused on the reduction of TEAs as a source of CO2 and is relevant in regard to the role of AQDS as a TEA in respiratory reactions. Accordingly, we now explicitly state that we use the term TEAs in this more restricted sense in lines 57-59. We previously have considered the role of hydrogenotrophic methanogenesis in electron transfer relative to other TEAs in peatlands in Keller and Bridgham (2007) and Vile et al. (2003b), as well as the role of homoacetogenesis relative to hydrogenotrophic methanogenesis in consumption of H2 in peatlands in Ye et al. (2014). Furthermore, the reported

CO_2 production in the present study actually included both gas and liquid species (L. 188-191).

Q3. A second general issue is the calculation of Q10 values from the experimental data. The authors measured concentrations of important substrates for CO_2 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 CO_2 and CH_4 production rates (Fig. 5 and 6) which change over time as do the substrate concentrations. The authors are certainly aware that microbial CO_2 and CH_4 production rates are strongly affected by the concentration of available substrates and not only by temperature. However, they use the different CO_2 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 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.

R3. We disagree with the statement that "the calculations of the Q10 do not meet the criteria for being suitable for Q10 value calculations." The temperature sensitivity of organic decomposition can be "intrinsic" or "apparent," as is widely acknowledged in the literature (e.g., van Hulzen et al., 1999; Davidson and Janssens, 2006; Conant et

al., 2011). The intrinsic temperature sensitivity is the theoretical sensitivity determined by the inherent kinetic properties of enzymes, while the apparent temperature sensitivity is the observed response to temperature under environmental constraints caused by heterogeneous environmental conditions. We explicitly discuss these two different interpretations of temperature sensitivity in lines 507-519. Apparent Q10s have been very widely reported in the literature for many different processes, and in terms of a global warming response, the apparent Q10 is the more relevant measure of temperature processes. We agree that substrate availability affects the Q10, and so do other environmental variables, e.g. TEA concentrations. This environmental complexity can explain differences between inherent and apparent Q10s, but has been largely neglected in the past. It is important to understand how changes in substrate availability and other environmental constraints will influence ecosystem response to temperature in the sense of decomposition and CH_4 production. Our results provide such information to understand how peatlands will respond differently to climate warming.

Q4. 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 stated that they were calculated from the cumulative production of CO_2 and CH_4 but it is unclear which time period and how many data points they used for the calculation of microbial rates.

R4. The rates were measured at several time points during the incubation with 4 replicates. They were calculated by dividing the cumulative production up to a measurement time point by the duration of the incubation in days up to that time point, which is now clearly stated in lines L. 216-219.

Q5. 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.

R5. As mentioned above (in response to Reviewer 1), we have made a new table (Now Table 2) that summarizes the main results from the figures. In the present study, we clearly demonstrated the significance of humic substances in anaerobic decomposition, with sets of supporting data from different aspects of anaerobic decomposition. The individual data collectively suggested that humic substance can inhibit CH4 production with different mechanisms in 2 different types of peatland soils (L. 23-27; 30-33), which is a new finding. The enzyme latch hypothesis has become a paradigm in understanding soil carbon accumulation in peatlands, but it has traditionally has mainly focused on aerobic decomposition and not considered CH4 production (L. 443-453). Furthermore, it is for the first time, to our knowledge, that the effects of humic substances on the temperature sensitivity of anaerobic processes and CH4 production have ever been reported. Our discussion is centered on the important findings. For instance, for the finding that humic analog inhibited anaerobic decomposition and CH4 production, we briefly described the results and compared our findings to documented literatures (L. 389-400; L. 416-443), followed by explaining the difference between our results and others' with reasonable references and hypothesis (L401-403). We also discuss the new findings from our experiments and their contribution to the existing literatures (L. 443-456). However, we have attempted to make additional changes in our abstract and conclusion to further highlight the new findings and contribution of this study.

Q6. P1740 l23ff: 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

R6. Revisions were made as suggested (L. 40)

Q7. P1741 l5ff: 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.

R7. Revisions were made as suggested (L. 47-51).

Q 8. P1744 l5ff: Please give soil T during sampling and MAT of the two sites.

R8. This information is now given in lines 157-159.

Q9. P1745 l26: Did you also consider the gas pressure in the closed vials? This is essential for calculating gas production.

R9. Yes. Idea Gas Law was applied to calculate the gas production. We have added language to explicitly acknowledge that we accounted for headspace pressure (L. 190).

Q10. P1746, l3: Please give the acceleration in g not rpm.

R10. This information is now given in lines 196.

Q11. P1746, l16: How did you measure pH?

R11. The pH was directly measured on porewater with a pH meter.

Q12. P1746, l22ff: 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?

R12. See R4.

Q13. P1747, l21: Please indicate at which time the difference was significant.

R13. The reference line is not right so we cannot provide any response.

Q14. P1748, l7: see above

R14. The reference line is not right so we cannot provide any response.

Q15. P1750, l3ff: 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.

R15. See R3 for our responses on this issue.

Q16. P1751 l20ff: 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.

R16. See R2 for our responses on this issue.

Q17. P1755 l21: CO2 is a TEA (see above)

R17. See R2 for our responses on this issue.

Interactive comment on Biogeosciences Discuss., 11, 1739, 2014.