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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 is well structured and written, and consequently easy to read and understand. Thematically, it falls into a highly interesting and timely field of research. The understanding of the environmental regulation of methane emissions from such important sources still is limited, but on the other hand will have important consequences for the determination of global greenhouse gas budgets. The combination of microbiological as well as geochemical methods applied here is sufficient to tackle scientific questions on the environmental regulation of methane production in the selected environments. The experiments presented provide a solid piece of laboratory work, well planned and conducted. A weakness of the manuscript is the lack of additional data, for example carbon stable isotopic signatures would have been good to

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get a better impression of the importance of the different methanogenic pathways for the different systems. Second, a molecular biological tool, like cloning or a fingerprinting assay, RNA or SIP, would be important. This would provide important and more detailed information on active microorganisms, metabolic processes and their possible regulation and relationships. The figures all look very similar. The authors should consider to provide an illustrative "summary figure / sketch" presenting the most important findings on relationships and regulatory factors at-one-glance.

R1. We appreciate the thoughtful comments of the reviewer and have endeavored to fully address them. While we believe that our work thoroughly examines a number of the key biogeochemical processes related to the effect of humic substances on anaerobic decomposition in peatland soils, we acknowledge that there is still more work to do in this important area. Work exploring methanogenic pathways and microbial community structure was beyond the scope of the current project, but is certainly fertile ground for future research. Indeed, much of our recent and ongoing research program has focused on experiments investigating the importance/significance of TEAs, anaerobic processes, and methanogenic pathways to anaerobic decomposition from the two sites that are the focus of this manuscript (as well as several others) with biogeochemical and isotope-tracing approaches. A Ph.D. student has additionally done extensive methanogen community analysis in these sites and related it to the predominance of the two CH4 pathways, but that work is not yet published. However, for this study we intended to quantify the significance of humic substances in the sense of anaerobic decomposition and CH4 production. As pointed out by the reviewer, the methods applied here are sufficient to answer the questions posed in this manuscript. We now cite this previous work in lines 146-151 as providing context for the current manuscript, and cite it elsewhere as appropriate. We appreciate the helpful comment about the utility of a summary figure, but we have made a summary table (Table 2) that we think serves that purpose better than would a figure.

Q2. Please state more clearly what the new findings of your study are, and what the

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important implications for global greenhouse gas budgets.

R2. The objective of this study was to investigate the importance of humic substances to anaerobic decomposition and CH4 production and their responses to warming (L.126-130), but not directly related to greenhouse gas budgets. However, we do now state that the net effect of humic substances is to inhibit CH4 production, regardless of mechanisms (L. 30-32). We do not believe that our data allow us to infer any more specific effect on greenhouse gas budgets. Our finding that humic analog inhibited CH4 production in different types of peatlands with different mechanisms is new (P2, L. 23-27; 30-32). Furthermore, to our knowledge, it is the first time that the effects of humic substances on the temperature sensitivity of CH4 production have ever been reported, and we have discussed/highlighted the importance of this finding (L. 33-35; 519-524; 540-545).

Q3. Concerning acetate and peatlands, there presumably is more literature to be cited (e.g. by H Drake, K Küsel, SH Zinder, J Parkes and colleagues) which could be valuable for the interpretation of the presented results.

R3. We did not specifically discuss acetate production in peatlands in this study. However, the work by Drake, Zinder, and their colleagues was cited and discussed in our recent paper (Ye et al., 2014), which focused on acetate dynamics in peatland soils.

Q4. Please add in the introduction an explanation and comparison of “bog” and “fen” for the less expert / non-native readers.

R4. We briefly define bog and fen in lines L. 121-122 now. We provided more detailed information to distinguish the two in the site description section.

Q5. Page 10, lines 193ff: Can you exclude any chemical effects caused by the H2 present in the glove box?

R5. We conducted the chemical analysis in the anaerobic box trying to avoid O2 effects or re-oxidations of the reduced AQDS. We are not aware of any H2 effects on the stabil-

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ity of reduced AQDS. Furthermore, all the standard series and samples were prepared similarly in the box, which can cancel any H2 interference, if it did exist. The actual samples were flushed with N2 after treatment additions and were then destructively sampled, so the H2 in the glove box should not have affected anaerobic pathways.

Q6. Page 17, lines 346f: Was the absence of "endogeneous inorganic and organic TEAs" checked experimentally, via direct measurements or determination of microbial activities?

R6. Our previous study (Ye et al. 2012) suggested that inorganic TEAs were minimal in these peat soils (now cited, L.356-362). A pre-incubation of 2 weeks is sufficient enough to consume all of them. We were unable to directly exclude organic TEAs. However, we did observe the production of CH4 after a lag period during the pre-incubation, which suggested that the endogenous organic and inorganic TEAs were exhausted. This assumption and its reasoning are discussed in lines 356-360. Moreover, even if all organic TEAs were not reduced, it would not change our interpretation of the results and conclusions about the dual roles of AQDS in the two peats.

Q7. Page 19, lines 388f: What might be the mechanisms behind the inhibition, what the affected microbial groups?

R7. Unraveling the precise mechanism for this inhibition and the microbial groups involved was beyond the scope of the current project. At this point, we only know that the observed inhibition affected the fermenting bacteria and methanogenic archaea as a whole. However, we cite two papers that give an extended discussion of the potential mechanisms for the toxicity effects of AQDS (L. 392).

Q8. Page 21, lines 440ff: Also it will be very important in the future to reveal the respective microbial communities, their relationships and regulations.

R8. We totally agree with this statement and have an active research program in this area.

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Q9. Page 25, lines 522: Please point out more clearly what is really new in this study, what re the “take home messages” / rules in the system?

R9. We have deleted the text that unintentionally suggested that our findings are redundant with the literature (L. 531-534). In general, while there is ample published evidence that humic substances can have both toxic effects and act as TEAs, we are unaware of any previous studies that examined the relative importance of these two mechanisms in peatlands as they relate to anaerobic carbon cycling. Our resulted demonstrated 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.

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