Response to Reviewers – *Biogeosciences*

Liquid Polymer Enhances Methanogenesis and Restructures Prokaryotic Communities in Freshwater Sediments (Feckler et al.; egusphere-2026-1216)

We thank the Editor and the Reviewers for their efforts and comments that greatly improved the quality of the manuscript. We have addressed all comments in a point-by-point manner below (in blue) and hope that the manuscript is now suitable for publication in *Biogeosciences*. Please find the detailed changes and corresponding line numbers in the revised manuscript as well as our responses to the comments below.

Reviewer 2

The manuscript evaluates the methane production potential during a 56-day anoxic incubation of sediments after amendment of polyvinylpyrrolidone (PVP). PVP was used as a representative of synthetic hydrophilic polymers (SHP), which are increasingly used and released into the environment. Results indicated the presence of PVP increase the CH4 production potential and influences the microbial community. I am less qualified to evaluate technical molecular biology details and the microbial community aspects, so this review focuses on the other parts of the manuscript. I find the focus on SHP interesting, and in general found this study well-executed and the manuscript well-written. However, I have some questions that require clarifications.

We also thank Reviewer 2 for this positive comment and the time and efforts invested to improve the quality of the manuscript.

How much SHP/PVP can be expected to reach sediments? Why are sediments a key environment to evaluated effects of SHP/PVP in?

We thank the reviewer for this question. To our knowledge, there are currently no studies that directly quantify PVP concentrations in sediments or provide robust estimates of the fraction of environmentally released PVP that ultimately reaches sediment compartments. The environmental distribution of PVP is expected to depend on several factors, including polymer molecular weight, sediment characteristics, salinity, pH, and the abundance and type of clay minerals. Available studies indicate that PVP can strongly adsorb to clay particles, with reported adsorption capacities ranging from 0.061 to 0.88 g g^{-1} depending on the clay species, polymer characteristics, and test conditions (Israel et al., 2001; Teepakakorn et al., 2018). Similar observations have been reported for polyethylene glycol (PEG), another nonionic superabsorbent polymer, for which adsorption strongly depended on sediment composition and clay content (Podoll et al., 2002). Collectively, these findings suggest that sediment association may represent an important environmental sink for PVP. However, the currently available evidence does not permit a reliable quantitative estimate of how much PVP ultimately reaches sediments under environmental conditions. Because such an estimate would be highly speculative, we did not incorporate additional statements into the manuscript.

The reasoning behind sediments as a hotspot to study the effects of SHPs in general and PVP in particular has already been included in the Introduction (lines 35-37), stating that sediments are sinks for SHPs and are sites at which fundaments biogeochemical processes occur.

Israel, L.; Güler, Ç.; Yilmaz, H.; Güler, S. The Adsorption of Polyvinylpyrrolidone on Kaolinite Saturated with Sodium Chloride. Journal of Colloid and Interface Science 2001, 238 (1), 80–84. https://doi.org/10.1006/jcis.2001.7465.

Teepakakorn, A. (Pleng); Hayakawa, T.; Bureekaew, S.; Ogawa, M. Removal of Water-Soluble Polymers from an Aqueous Solution by Adsorption onto an Acidic Clay. Clays and Clay Minerals 2018, 66 (2), 96–103. https://doi.org/10.1346/CCMN.2018.064079.

Podoll, R. Thomas.; Irwin, K. C.; Brendlinger, Sheryl. Sorption of Water-Soluble Oligomers on Sediments. Environ. Sci. Technol. 2002, 21 (6), 562–568. https://doi.org/10.1021/es00160a006.

L55. How can PVP be chemically stable if it has clear effects on sediment carbon cycling in the experiment?

We agree that chemical stability and ecological effects are distinct concepts. In this context, the term "chemically stable" refers to the resistance of PVP to chemical degradation under environmental conditions, which contributes to its persistence and widespread occurrence in aquatic ecosystems. However, chemical stability does not imply ecological inertness. Many environmentally persistent compounds can influence ecosystem processes through interactions with dissolved organic matter, sediment constituents, or microorganisms without undergoing rapid degradation themselves. Similarly, PVP may affect microbial activity and carbon cycling through such interactions while remaining chemically stable. Because our wording may have led to this misunderstanding, we have revised the text in lines 53-57 to clarify that chemical stability refers specifically to environmental persistence:

“PVP represents a suitable model SHP because it is highly water soluble and highly resistant to chemical degradation under environmental conditions (Julinová et al., 2012; Luo et al., 2021). This persistence, together with its widespread use in industrial and pharmaceutical applications, contributes to its frequent detection in aquatic environments, such as surface waters downstream of wastewater treatment plants, with measured environmental concentrations of up to 7 mg L^{-1} (Antić et al., 2011).”

Please express sediment carbon content including the leaf powder addition and PVP with comparable units so it is possible to relate all components with the sediment carbon budget and the CH4 formation.

We agree that expressing all carbon sources in comparable units would facilitate a direct assessment of their relative contributions to the sediment carbon budget and potential CH_4 production. Unfortunately, we are unable to retrospectively quantify the total sediment carbon content, including the leaf amendment, because insufficient sample material remained for

additional analyses. However, a previous study using the same sediment source and leaf amendment procedure quantified that the added leaf material contributed approximately 12% of the total sediment organic carbon (Bollinger et al., 2021). We have therefore added this information to the manuscript in lines 71-74 to provide context for the relative contribution of the leaf amendment to the overall sediment carbon pool:

"Adding organic substrates (e.g., leaf litter and glucose) to the test system is common practice when mechanistically addressing methane production (e.g., Bhullar et al., 2013; Bollinger et al., 2025) to ensure sufficient substrate availability for microbial metabolism (including methanogenesis) during incubation. In a previous study using the same approach, the added leaf material contributed approximately 12% of the total sediment organic carbon (Bollinger et al., 2021)."

We note that PVP was added at concentrations substantially lower than the total sediment organic carbon content.

L77-78: Why are wastewater treatment plants increasing the PVP concentrations 8-70-fold compared to sewage water?

Thank you for pointing this out. We agree that our original wording was imprecise and may have caused confusion. The concentration of 0.1 mg L^{-1} was reported for river water receiving municipal wastewater discharges, whereas the substantially higher concentrations (0.9–7 mg L^{-1}) were measured directly in wastewater treatment plant effluents (Antić et al., 2011). Thus, the higher concentrations do not result from wastewater treatment processes increasing PVP concentrations, but rather reflect the difference between undiluted effluent and receiving surface waters. To avoid this misunderstanding, we have revised the manuscript in lines 79-82 as follows:

"The selected concentration range reflected environmentally relevant levels reported for European rivers receiving municipal sewage discharges (up to 0.1 mg L^{-1}) and wastewater treatment plant effluents (0.9-7 mg L^{-1}; Antić et al., 2011), whereas the higher concentrations were selected to assess concentration-dependent effects."

L81. pH adjustment: Please tell more about this pH sensitivity. How sensitive would PVP degradation or other experimental results be to pH? What are the implications of this for the results? How valid are the results in situ t other pH levels?

Our intention was not to imply that PVP degradation itself is strongly pH-dependent, but rather that anaerobic organic matter degradation and methanogenesis are known to be sensitive to pH. Because microbial activity and methane production typically show maximum activity near neutral pH and may be inhibited under more acidic or alkaline conditions, we adjusted all test solutions to neutral pH to minimize the impact of a potential confounding factor (i.e., change in pH due to PVP dissolution) and ensure comparable conditions across treatments. Consequently, any observed treatment effects cannot be attributed to differences in pH among

treatments. We did not specifically investigate the influence of pH on PVP degradation or PVP-mediated effects. Furthermore, we acknowledge that extrapolation to natural systems should be made with caution, as environmental pH varies among aquatic ecosystems and our study was conducted as a controlled laboratory proof-of-concept experiment. We have clarified this rationale in lines 83-86:

“Because anaerobic organic matter degradation is highly sensitive to pH (Wang et al., 1993; Zhang et al., 2010), we adjusted the test solutions to neutral pH after PVP application using NaOH. This standardization minimized pH as a confounding factor among treatments and ensured that observed treatment effects could be attributed to PVP exposure rather than differences in pH.”

L95-97 and Table1: It seems the two lowest levels of PVP amendment (0,5 and 50 mg/L) are below LOQ (70 mg/L), and initial measurements at 50 and 5000 mg/L were <80% of the amendments. Please explain why there is such a large discrepancy. How robust is the method? What was the background PVP level in the original sediment?

We thank the reviewer for spotting this. We double-checked the measurements and calculations of our LOD and LOQ. The LOD was estimated from blank samples in accordance with German DIN 32645, while the LOQ was defined as three times the LOD. In addition, we found a calculation error that resulted in the overestimation of the LOQ by a factor of 10. We added the corrected LOD and LOQ to the manusript in lines 99-100:

“The limits of detection (LOD) and quantification (LOQ) were 2.2 mg $L^{-1}$and 6.5 mg L^{-1}, respectively.”

As explained in our response to a comment by Reviewer 1, the initial deviation between nominal and measured PVP concentrations may have resulted from adsorption of PVP to the glass walls of the storage vials. The more pronounced decline in measured concentrations during later stages of the experiment may additionally reflect interactions of PVP with sediment constituents, including organic matter, as PVP has been shown to associate with humic and fulvic substances. However, to address these differences in nominal and measured PVP concentrations, we have revised lines 102-106:

“The initial deviation between nominal and measured PVP concentrations may have resulted from adsorption of PVP to the glass walls of the storage vials, as PVP is known to adsorb to silica-based surfaces (Al-Harbi et al., 2016). The more pronounced decline in measured concentrations during later stages of the experiment may additionally reflect interactions of PVP with sediment constituents, including organic matter, as PVP has been shown to associate with humic and fulvic substances (Watanabe & Kuwatsuka, 1992).”

Unfortunately, we did not measure the PVP concentrations in the original sediment. We will keep this in mind as an important measurement for future studies.

Eq 1. The final addition of xe is unclear. What does this mean? Why is it needed? Perhaps I misunderstand, but in later equations the number of moles in the headspace and the water is calculated separately and summed to derive the total amount, and that should take care of all partitioning between headspace and water, and a mole fraction correction as expressed in Eq1 seems strange?

We agree that the role of Equation 1 was not sufficiently explained. The purpose of Equation 1 is not to correct for partitioning of CH_4 or CO_2 between the headspace and the aqueous phase. Rather, it corrects the concentration measured by the gas analyzer for dilution occurring during the analytical procedure. Specifically, the analyzer measures the equilibrium mole fraction (x_e) that results after mixing the injected sample gas with the gas already present in the analyzer loop (see Wilkinson et al., 2018; now cited in the manuscript). Equation 1 reconstructs the true headspace mole fraction (x_h) from this measured equilibrium concentration by accounting for the loop volume (V_l), injected sample volume (V_i), and background mole fraction (x_0). The distribution of gases between the headspace and water phase is subsequently accounted for in subsequent equations, where the amounts of gas in the headspace and dissolved phase are calculated separately and then summed. To clarify this distinction, we revised the description of Equation 1 in lines 124-128:

"To determine the true mole fraction in the headspace (x_h), we corrected the equilibrium mole fraction (x_e, ppm) measured by the analyzer after equilibration of the injected sample gas with the gas initially present in the analyzer loop (Wilkinson et al. 2018). The correction accounts for dilution caused by the loop volume (V_l; calibrated using certified CH_4 and CO_2 reference gases; Messer Industriegase, Germany), the injected gas sample (V_i), and the background mole fraction (x_0) assuming ambient pressure in the microcosms (Eq. 1)"

Results and Fig. 1: So, over a PVP-addition range from 0 to 5000, the CH4 production potential increase 20% (1.2-fold). 30% (approx.) of this increase occur happen when the PVP addition increased from 0 to 0.5. The rest happens when increasing PVP 50000-fold. Accordingly, although the results are significant, the response on CH4 is rather modest in relation to the very large change in PVP concentrations. Please discuss this and what it could mean in the manuscript. Is it possible that this effect is linked to something else than PVP given the experimental setup, or can that be excluded? Are that any alternative interpretations of the results?

Thank you for this thoughtful comment. We agree that the observed increase in CH_4 production was relatively modest compared to the large range of PVP concentrations tested. Indeed, a substantial proportion of the response occurred already at the lowest PVP concentration (0.5 mg L^{-1}), whereas further increases in PVP concentration resulted in comparatively smaller gains in CH_4 production. This pattern suggests a non-linear response with an early saturation of the underlying mechanism. We consider it unlikely that the observed effects were caused by factors other than PVP, as all incubations were established using the same sediment, pond water, equipment, and incubation conditions, and all treatments were handled identically (including pH adjustment to avoid secondary influences) except for PVP addition. While we

attribute the observed differences in CH_4 dynamics to PVP exposure, we agree that the mechanisms driving the response remain uncertain. As this study was designed as a proof-of-concept experiment in a largely unexplored research area, we were unable to directly test all potential mechanisms. Instead, we discuss several non-exclusive hypotheses that may explain the response. The apparent saturation of the CH_4 response at higher PVP concentrations may indicate that the affected process was already largely alleviated at environmentally relevant concentrations, although this interpretation requires dedicated mechanistic investigations. We have expanded the Discussion in lines 264-279 accordingly to better acknowledge the relatively modest effect size and the current uncertainty regarding its mechanistic basis:

“Notably, the response magnitude was relatively modest compared with the large PVP concentration range tested. Moreover, the response did not follow a simple concentration-dependent pattern. A substantial proportion of the observed effect occurred already at the environmentally relevant concentration of 0.5 mg L^{-1}, whereas further increases in PVP concentration caused comparatively small additional stimulation. This pattern was particularly evident for τ and r_{max}, indicating that the lowest exposure treatment already accounted for a considerable fraction of the total response observed across the entire concentration gradient. The absence of a proportional increase in methanogenesis across four orders of magnitude in PVP concentration suggests that the affected process became saturated at relatively low exposure levels. Rather than reflecting a continuously increasing effect of PVP concentration, the observed response appears more consistent with a threshold-like alleviation of a constraint on methanogenesis. Once this constraint was sufficiently reduced, additional PVP may have produced only limited further stimulation. Although the identity of this constraint remains unknown, the findings indicate that environmentally realistic concentrations may already be sufficient to induce measurable changes in methane-production dynamics. Despite the comparatively modest increase in total CH_4 production, PVP consistently accelerated CH_4 formation, with all exposure concentrations reaching the phase of maximum CH_4 accumulation earlier than the control. Because all incubations were established and maintained under identical conditions except for PVP addition, alternative experimental explanations for the observed differences appear unlikely. The concomitant increase in both CO_2 and CH_4 production further suggests that PVP exposure enhanced overall anaerobic carbon turnover.”