Response to reviewer comment 3

Altzitser et al. present a study investigating structural changes in loess following the addition of chia seed mucilage and polyacrylamide through measuring aggregate stability, CO2 respiration, tracer breakthroughs, and spectral induced polarization (SIP). The use of SIP is especially interesting as a non-destructive method for monitoring soil change. The manuscript is generally well-written and the experiment includes an interesting combination of methods. However, I generally share the concerns of the other two reviewers and also think that aspects of the experimental design, statistical analysis, and soil characterization require further clarification. Therefore, I believe major revisions are necessary before publication.

We thank the reviewer for the careful evaluation of our manuscript and the constructive comments. We will revise the manuscript accordingly. Below, we provide detailed responses to each point raised:

Major comments:

1. Interpretation of CO2 measurements

   The authors primarily attribute the loss of mucilage-induced stabilization to microbial degradation of the mucilage. The higher CO2 production in the mucilage treatments could indicate increased microbial activity following labile carbon addition, but I do

not think it directly demonstrates degradation of the mucilage itself. As the authors also acknowledge, it could be influenced by calcite dissolution. Additionally, the CO2 measurements were made in separate incubations under slightly different physical/hydrological conditions, which could influence microbial activity and degradation. As a result, I think the authors should be a bit more cautious using the respiration measurements to explain structural changes observed in the columns, unless there is additional evidence of degradation available.

We thank the reviewer for this comment, and we agree that elevated CO_2 emission indicates stimulated microbial activity but does not directly quantify mucilage degradation, since the residual mucilage in the soil was not measured in this study.

Regarding calcite dissolution, all treatments were prepared from the same calcareous loess, so calcite dissolution is expected to contribute to CO_2 emission in every treatment, including the control. As stated in the manuscript, the CO_2 results were therefore interpreted as differences between treatments relative to the control, rather than as absolute values. On this basis, the excess emission in the mucilage treatments, which reached a 3-fold increase in MH on days 3 and 7 (Fig. 2), is attributed to the added mucilage. This interpretation is consistent with the effluent chemistry of the SIP columns: the control, ML and MH treatments reached similar Ca^{2+} and total cation concentrations by day 20 (Fig. 7), suggesting a similar extent of calcite dissolution among these treatments.

Regarding the separate incubations, the respiration jars and the SIP columns were prepared from the same treated soils, following the same protocol, and both were incubated under static conditions. The jars were kept at a moisture content of 30% (w/w), close to saturation given the porosity of the packed soil (0.41–0.45). We agree, however, that the two systems are not identical. The columns were fully water-saturated and were flushed at packing. The jars retained some air-filled porosity and a headspace, and may therefore have had higher oxygen availability. For this reason, we regard the respiration data as an indicator of the relative biodegradability of the two polymers, rather than a measure of the degradation rate within the columns.

Importantly, the attribution of the transient mucilage effect to microbial degradation does not rest on the respiration data alone. Over the same period, aggregate stability in the mucilage treatments declined (Fig. 3), the ML breakthrough curve converged toward control-like behaviour by day 20 (Fig. 4), and σ" converged between ML and MH (Fig. 5). Together with the well-documented lability of mucilage polysaccharides (Nazari, 2021; Nazari et al., 2022), these independent observations are consistent with microbial degradation as the main driver of the transient stabilization.

2. Statistical analysis and replication

   I could not find a description of the statistical analyses used in the manuscript. For example, in Fig 2, significant differences are shown among the treatments but it is

not stated which test was used or how pairwise differences were determined. Replication is also unclear for some of the other measurements. Although replication is reported for the CO_2 and tracer experiments, the number of replicate columns used for the SIP measurements is also not clear. I think the manuscript would benefit from a statistical analysis section clearly describing tests used and the number of independent replicates for each part of the experiment.

We thank the reviewer for this comment. The same point was raised by Reviewer 1, and we refer the reviewer to our detailed response there (RC1, comment 6). In brief, we will add a statistical analysis section to the Methods specifying the tests used and the number of independent replicates for each experiment, including the SIP columns, which were run in triplicate.

3. Soil characterization

The description of the soil used seems quite limited considering how much of the discussion relies on mineralogy and chemistry. The soil is described mainly as a sandy clay loam collected from an uncultivated loess site, but later interpretations depend on calcite dissolution, surface conductivity, changes in pore-water chemistry, and mineral surface properties. I think more thorough characterization of the soil would add needed support to these interpretations. At minimum, it would be helpful to report pH, carbonate content, SOC, and particle-size distribution, and ideally CEC if

available. If these properties were not measured, I think the authors should acknowledge this limitation and be more cautious in discussion of underlying mechanisms.

We thank the reviewer for this comment. We agree that the soil description should be expanded, since several of our interpretations rely on the soil's mineral composition. The loess soil used in this study consists of 60.5% sand, 17.5% silt and 21.9% clay, with 2.9% organic matter and 19.5% $CaCO_3$. These properties will be added to the Materials section of the revised manuscript.

These data provide direct support for several of the mechanisms discussed in the manuscript. The high carbonate content (19.5% $CaCO_3$) establishes calcite as a major and readily available mineral phase. This is consistent with our attribution of the increase in effluent Ca^{2+} and σ' in the control and mucilage treatments to calcite dissolution, and of the decline in σ" to the loss of reactive mineral surfaces. The clay content (21.9%) supports the relevance of surface conductivity and EDL polarization to the interpretation of the SIP signals.

Minor comments:

1. Lines 124 and 206–207: There seems to be an inconsistency in the reported CO2 sampling dates. The methods state that samples were collected on days 1, 3, 4, 10, 14, 17, and 20, whereas the results refer to a significant difference on day 7. Please check and correct the sampling dates throughout the manuscript.

Thank you for noticing. The sampling day listed as "day 4" in the Methods is a typographical error and should read day 7. The sampling dates will be corrected throughout the revised manuscript to match those presented in Fig. 2 (days 1, 3, 7, 14, 17 and 20).

2. Lines 140–142: The sand fraction is estimated from the control based on the assumption that no highly stable aggregates are present and is then subtracted from all treatments. I think this assumption should be better justified, particularly since the highly stable fraction is important for the subsequent interpretation. It would also be useful to clarify why the sand fraction was not determined independently.

We thank the reviewer for this comment. The control soil received no stabilizer, and its aggregate stability remained low throughout the experiment (Fig. 3). After full chemical dispersion with sodium hexametaphosphate, the material retained on the sieve in the control was therefore assumed to consist of primary sand particles.

The sand fraction was determined from the control rather than taken from the particle-size analysis, because the two do not measure the same quantity. Particle-size analysis reports all sand particles (>50 µm), whereas only particles coarser than the 60-mesh sieve (250 µm) are retained in the wet-sieving procedure. In the control, only a small fraction of the sample mass was retained on the sieve after full dispersion, although sand makes up 60.5% of the soil. This indicates that most of the sand in this loess is finer than 250 µm and passes the sieve, consistent with the fine-grained nature of loess. Subtracting the sand content obtained from

particle-size analysis would therefore greatly overestimate the retained sand mass. The control, processed under identical conditions with the same sieve, sample mass, and dispersion protocol, provides a direct measure of the sand mass retained by this procedure.

Furthermore, because the retained mass in the control was small, the correction has only a minor effect on the calculated stability fractions, and the approach is conservative. If part of the material retained in the control consisted of naturally stable aggregates rather than sand, it would also be subtracted from the amended treatments. The highly stable fraction in these treatments would then be slightly underestimated rather than overestimated.

3. Lines 148–149 and 190–192: It is stated that A-PAMH was excluded from the tracer and SIP experiments because a stable flow could not be maintained due to excessive backpressure and leakage. I think this observation itself is potentially important and should be discussed when generalizing the A-PAML results to A-PAM more broadly.

We thank the reviewer for this comment. We agree that this observation is important in its own right. The inability to maintain stable flow in the A-PAMH columns, owing to excessive backpressure, indicates a substantial reduction in permeability at the higher A-PAM concentration. This is consistent with a strong effect of A-PAM on pore structure, although it may also reflect other processes such as polymer swelling or pore clogging. It also suggests that the transport behaviour observed in A-PAML cannot be directly extrapolated to higher

application rates, and it is in line with previous reports that excessive A-PAM doses can be less beneficial than optimal ones (Soltani et al., 2022).

In the revised manuscript, we will report this observation explicitly in the Results and discuss its implications. We will also specify throughout the Discussion and Conclusions that the tracer and SIP findings refer to A-PAML, rather than generalizing them to A-PAM as a whole.