

Biogeosciences Discuss., referee comment RC1  
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## Comment on bg-2021-72

Anonymous Referee #1

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Referee comment on "Predicting the impact of spatial heterogeneity on microbially mediated nutrient cycling in the subsurface" by Swamini Khurana et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-72-RC1>, 2021

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### General comments

The knowledge on factors controlling the fate of carbon and nutrients in subsurface porous aquatic systems (aquifer sediments) is very limited. One reason is without doubt the technical limitations in studying aquifer systems in situ in an appropriate spatial and temporal resolution. In fact, most of the experience has been obtained from lab experiments by means of flow-through sediment column or microcosm experiments. In consequence, modelling exercises are very useful for testing different hypotheses and possible scenarios. The submission by Khurana et al. in this respect is an interesting contribution. However, in its current form the manuscript is not ready for publication. Major issues are, as will explained in more detail below, an obvious lack of expert knowledge in microbiology, wrong assumptions as input parameters, and a discussion that avoids critical reflection of the overall outcome. Considerable reworking of the MS is needed before it can be accepted for publication in Biogeosciences.

### Detailed comments:

#### Introduction

P1-L18: 'undertook'

P1-L21: In biology we have a clear nomenclature. Conditions are either 'oxic' or 'anoxic',

Organisms and processes are 'aerobic' or 'anaerobic'. I suggest to use this nomenclature concisely throughout the MS.

P2-L37: Here and at many other spots

P2-L49: Papers of potential interest for the authors: Zhou et al. (2012) FEMS Microbiol. Ecol. 81: 230-242, Hofmann et al. (2020) Front. Microbiol. 11: 543567

P2-L50: Papers of potential interest for the authors: McGuire et al. (2000) Chem Geol 169: 471-485, McGuire et al. (2005) Ground Water 43: 518-530

In the Introduction section important issues such as the discrimination between 'active' and 'inactive' as well as 'mobile' and 'immobile' cells are not picked out as central points.

## Methods

As highlighted in the first paragraph, the conceptual model is simplistic. While the focus is to test for spatial heterogeneity and flow velocity as steering factors with respect to carbon, nutrient and microbial dynamics, frame conditions for the model simulations are non-dynamic with steady-state flow and constant inflow concentrations of dissolved species. In this respect, I would expect an in-depth discussion of the model output. Are the set frame conditions sensitive factors? How may the results change with respect to transient input concentrations of carbon and nutrients.

The authors mention the 'use of geochemical and geomicrobial observations from a common study site' as basis of the conceptual model. However, I could not find any sources (papers cited) with respect to 'values'. The concentrations of TOC, DOC, NH<sub>4</sub>, NO<sub>3</sub>, O<sub>2</sub>, prokaryotic cells (active and inactive) have been selected and based on which studies and sites.). In P6-L182 you say: 'The concentrations of the reactive species mimicked conditions observed in the subject site'. However, in the discussion it is mentioned that there were two orders of magnitude difference in prokaryotic cell numbers. I ask the authors to carefully consider input values. Is it true that a prokaryotic concentration of mobile prokaryotic cells in groundwater of 10<sup>9</sup> have been found in the field? Seems very high to me.

The concept of the reaction network is simplistic, which is ok. But it should be as realistic

as possible. Does all  $\text{NH}_4$  originate from DOM? Isn't there direct input of  $\text{NH}_4$  from the surface? . As mentioned in Table A.4.2. there is a constant input of  $60\mu\text{M}$  of  $\text{NH}_4$  (about  $1\text{mg/L}$ ) that cannot originate from the  $10\text{mg}$  of DOC ( $800\mu\text{M}$ ). I recall that the studied Hainich Critical Zone sites are partially located in areas with agriculture. To which of the Hainich sites does the conceptual model refer to?

I know that it is hard to collect reliable information from the literature with respect to microbial features in shallow aquifers. Having this in mind, one need to carefully select values for 'rate constants', 'yield coefficients', ... The values summarized in Table A.4.1 originate from field studies and lab studies very different in nature, i.e. values derived from lab experiments with pure bacterial cultures. Are the chosen values sufficiently representative for the Critical Zone in Hainich and shallow aquifers in general? This at least needs to be critically discussed.

With respect to DOC, an constant input concentration of  $800\mu\text{M}$  has been chosen. DOC degradation in soil and in groundwater is determined not only by its concentration but more likely by its quality (degradability). Has this been considered.

There is dynamics in many aspects, including flow velocity, water retention time, activity and biomass of microbes, DOM concentration and transformation, N transformation, ... Only a subset of parameters, i.e. spatial heterogeneity and flow velocity (related to residence time) has been tested. This needs to be clearly mentioned already in the Introduction section.

Captions of Tables are generally on top of the tables, not below. See all tables.

P11-L297: Is there evidence for a parallel reduction of nitrate and oxidation of ammonium?

P12-L321: Is there any evidence that the portions of active and inactive cells/species are realistic? In particular when these ratios are calculated for individual physiological guilds (nitrate reducers, ammonium oxidizers, ...). See also table 4.

P15-L386:  $\log_{10}\text{Da}$ ?

P15-L394: Dissolved oxygen (DO) is not a nutrient.

P16-L411: Provide a citation that supports this statement.

## Discussion

P19-L459: The 'available' process knowledge, does it refer to the Hainich study site?

P19-L474: from carbon concentration and carbon content per cell one will not end up with gene copies per volume but cells per volume. Quantification of cell numbers in groundwater and aquifers by means of molecular tools quantifying 16S rDNA gene copies is only a rough estimation of cell abundance.

P19-L474: Does 100times less cells equal 100times less microbial activity and 100times less transformation of C and N? Please comment on that.

P20-L490 & L516: There is techniques and reports available on high-resolution sampling in aquifers. The reader should not get the impression one cannot get spatially more resolved in sampling. E.g. Ronen et al. 1987 J. Hydrol. 92, 173–178, Báez-Cazull et al. 2007 Appl. Geochem. 22, 2664–2683, Smith et al. 1991

Contam. Hydrol. 7, 285–300, Anneser et al. 2008 Appl Geochem 23:1715–1730.

P20-L501: Have the authors considered that there is different growth rates with different physiological groups within the microbes, i.e. aerobes may grow faster than nitrate reducers and sulfate reducers are extremely slow. Did I miss this information?

P20-Fig. 6: What do you mean with 'oxic cells'. Please change.

P21-L528: Consider the review paper of Smith et al. 2018 FEMS Microb. Ecol. 94: fiy191

P22-L565: I fully agree with this statement. In many cases the contribution of the mobile fraction of microbes can be neglected in terms of 'transformation processes'.

Findings from other studies (like the one already cited Grösbacher et al. 2018) are not discussed in comparison to the model outcome.

Summary and conclusion

P24-L630: mention at which spatial scale.

P24-L640: Can this be visualized?