Rev1

>Dear Editor,
>I have revised the manuscript entitled "Quantifying the impact of groundwater fauna and temperature on the ecosystem service of microbial carbon degradation" by Schmidt, Rütz and Marxsen. I found the work very interesting and timely. The study plausibly demonstrates the important ecosystem service provided by microbes and groundwater-dependent fauna, both in terms of carbon degradation and in preventing brownification of groundwater used for human consumption. I recommend acceptance after the authors have considered the points listed below.
>The introduction is well written and supported by robust and up-to-date literature. The rationale is clearly presented and the hypotheses are explicitly stated at the end of the section. Figure S1 is well constructed and informative.

Reply: We thank the reviewer for the comprehensive diligent review and constructive feedback.

>Although I do not consider myself fully qualified to evaluate every aspect of the modelling framework, I carefully followed the methodological steps as presented. The calculations appear internally consistent and the logical structure is coherent. The authors also provide a transparent discussion of the main assumptions and limitations of the model in the final section of the manuscript, which I found very appropriate and commendable. However, it might be helpful to frame the model more explicitly, already in the Methods or early Discussion, as a strongly parameterised proof-of-principle approach.

Reply: We agree with the reviewer that this point needs to be stressed and have included it in the abstract (former line 13 f.), in the introduction (former line 121 f. and 135), and in the "2 Study area and methods" section, former li. 143f..

> Given the number of assumptions and parameter choices, the results are best interpreted in qualitative and comparative terms among scenarios rather than as precise quantitative predictions. Making this perspective explicit earlier in the manuscript would further strengthen its clarity and robustness.

Reply: We completely agree with the reviewer and have additionally to the places named above, also included it in the first line of the discussion, former li. 319f., and picking up in the first line of the conclusion, former li. 530f.:

former li. 319f.: "*This is to our knowledge the first attempt at developing a **process-based proof-of-principle approach** model of the groundwater food web, and it is the first endeavour at comparing scenarios of excluding fauna from the system*."

former li. 530f.:

"*This first **proof-of-principle** study on quantifying the change in ecosystem services at scenarios of losing groundwater fauna, and of increased temperature, showed that the partial loss of ecosystem services (BOC degradation) due to lacking fauna increased remaining BOC up to 660-fold, based on the 3.5-year observation period.*"

> The results are overall convincing and internally coherent. The model reproduces the measured time series reasonably well (Fig. 1b–e), and the agreement between simulated and observed dynamics supports its use for exploring scenarios. Figure 2 clearly shows that differences among scenarios are driven mainly by the presence or absence of fauna rather than by moderate temperature increases, since the "reference" and "+1.5 °C" lines largely overlap across zones. The strong divergence of the "no fauna" scenarios in the "M" and "P" zones convincingly supports the conclusion that faunal grazing substantially influences BOC dynamics and therefore the ecosystem service of carbon degradation.

Reply: We are glad for the feedback that the presentation was overall convincing.

>Some statements, however, would benefit from slightly more cautious wording (see specific comments below).
>I found the discussion particularly interesting where the authors provide a quantitative estimate of the loss of ecosystem services (in terms of BOC degradation) under scenarios combining fauna absence and increased temperature due to climate change. The estimates are convincing and I appreciate the effort, because although it is widely acknowledged that subterranean ecosystems provide important services (see, for instance, Mammola et al., 2026. Subterranean environments contribute to three-quarters of classified ecosystem services. Biol Rev Camb Philos Soc. 2026 Feb 10. doi: 10.1002/brv.70137), their quantitative evaluation is still limited.

Reply: We thank the reviewer for pointing out this recent publication and have added a reference to the Introduction, former li. 24:

"*Ecosystems fulfil functions that humans rely on (Koch et al, 2024b). Groundwater is no exception (Avramov et al., 2010;* ***Mammola et al., 2026****).*"

> In the final part of the manuscript, the authors openly discuss the weaknesses of the model and the study. This transparency is commendable and provides a solid basis for future refinement as new data become available. I found this attempt to model groundwater ecosystem services both courageous and valuable.

Reply: We appreciate the reviewer's feedback that our approach was convincing

>Specific comments

>Abstract

>No major comments.

>Introduction

>Line 24. I suggest expanding the references supporting the statement "Groundwater is no exception (Avramov et al., 2010)" by including the most recent work by Mammola et al. 2026 doi: 10.1002/brv.70137

Reply: Done as suggested; see also above

>Lines 42–44. The term "producers" may not be immediately clear. Consider revising to: "…producers (i.e. the basal microbial community including both autotrophs and heterotrophs sustained by imported organic matter)."

Reply: Done as suggested

>Line 44. Perhaps: "When even the producer biomass becomes substantial…".

Reply: We agree with the reviewer that there might be a temporal aspect to the development of a complex food web, as implied by "when". We were thinking rather of the spatial aspect, because we still lack clear indicators where to expect complex food webs and where not (e.g. Saccò et al. 2024). We have therefore added ", both on temporal and spatial scales," in former line 50 so that the sentence now reads "*Under which circumstances, **both on temporal and spatial (Saccò et al. 2024) scales**, which complexity of the food web emerges, is still largely unknown and has thus been near-impossible to parameterize*.", and we have added "and where" in former line 44 so that the sentence begins with: "***When and** where even the producer biomass becomes substantial, ...* "

>In addition, regarding the term "grazers," I would suggest using a more general term, since groundwater fauna can include both grazers and deposit feeders that ingest sediments and digest the microbial biofilm attached to them.

Reply: We appreciate this important addition and have rephrased the sentence, and added a short explanation: "*When and where even the producer biomass becomes substantial, a further level may be added, i.e. predators preying on grazers **and deposit feeders** (Ercoli et al., 2019; Saccò et al., 2019). **In the following, we will encompass deposit feeders in the notion "grazers"**.*"

>Lines 53–54. This sentence seems slightly disconnected from the surrounding text. It may benefit from relocation to improve the logical flow.

Reply: We see the reviewer's point and have moved the sentence to the following paragraph as the new last line.

>Line 111. Please consider adding: Vaccarelli et al. (2023, One Earth, 6, 1510–1522), which strongly supports your statement.

Reply: Done as suggested

>Line 115. You may consider citing: Iannella et al. (2020, Scientific Reports, 10, 19043) to support this point.

Reply: Done as suggested

>Line 128. Possibly: "in faunal composition (Di Lorenzo et al., 2025)?"

Reply: Thank you for pointing out this oversight – we cite two publications by Di Lorenzo et al. from 2025, and this is the one indicated as 2025a in the reference list; now corrected in the text.

>Results
>Lines 236–238. The statement that event-based recharge "contributed little" seems plausible, but this is not directly demonstrable from the figure alone. It would be safer to write "appeared to contribute little under the model assumptions."

Reply: Changed as suggested

>Lines 255–257. The interpretation that summer temperatures became "lethal" at +1.5 °C is mechanistic and not directly shown in the plots. It would be safer to state that fauna declined seasonally under elevated temperature, without explicitly attributing lethality unless this is independently supported.

Reply: Thank you for spotting this. Even if the interpretation was more substantiated, it should not appear within the results, so we deleted the half sentence about possible lethal effects.

>Discussion
>Lines 353–357. Please clarify this interpretation. The text states that early sudden dips in microbial dry mass occurred in the "A" and "R" zones and might reflect feeding by higher-than-average fauna biomass. However, in Fig. 1d the most pronounced temporary reductions to near-zero microbial dry mass appear in the "M" and "P" zones, and these coincide with peaks in fauna dry mass in Fig. 1e (as mentioned in lines 369–370). Could this be a mislabelling of zones, or am I overlooking a specific pattern in "A" and "R"?

Reply: This was misleading wording. Too much was left out for the sake of brevity. We added sentences and tried to lead more succinctly to the interpretation: "***In the "P" zone, dry mass rose to apparently the carrying capacity, dipped at the beginning of 1979 to low values and then rose again to apparently the carrying capacity. Fauna dry masses were lowest in the "P" zone (see below). In the "M" zone, the microbial dry mass rose very slightly to apparently the carrying capacity and then dropped at the end of 1978 to near-zero and never recovered. Also in the "A" and "R" zone, dry masses dropped to near-zero, already early in 1978, and never recovered.*** *The* ***fact that the dry masses*** *in the "A" and "R" zones* ***dropped so early****, might reflect moments of feeding by higher-than-average fauna biomass (see below).*"

>Lines 389–397. The interpretation that higher microbial dry mass in the "no fauna" scenarios is associated with reduced BOC degradation and attributed to a lack of rejuvenating grazing could be further strengthened. An additional explanation might relate to microbial community structure and activity. For example, Di Lorenzo et al.

(2025, Biogeosciences, 22, 1237–1256) reported a correlation between groundwater-obligate crustacean abundance and low-nucleic-acid (LNA) bacterial cells, suggesting selective feeding interactions. Since LNA cells are generally less metabolically active, the absence of fauna could allow the accumulation of microbial biomass that is not functionally active in terms of carbon uptake. In this perspective, higher microbial dry mass but lower BOC degradation in the "no fauna" scenarios may reflect a shift towards less active microbial fractions rather than more effective processing. Even if the current model does not distinguish active and inactive pools, mentioning this conceptual perspective could provide a biologically grounded explanation.

Reply: These are very important points that we gladly expand upon in the discussion. We added the following sentences: "***In addition to quantitative effects, grazing might also qualitatively change the microbial community (Longnecker et al., 2009) and might thus influence functions, and in the consequence, ecosystem services. As an example, groundwater crustacean abundance correlated with low-nucleic-acid (LNA) bacterial cells (Di Lorenzo et al., 2025a). This may hint to selective feeding (Jochem et al., 2004). HNA cells are often more metabolically active (Longnecker et al. 2005) and might thus be the preferred food source in grazing, leaving over microbial biomass less functionally active which then degrades even less carbon than the same dry mass of an un-grazed community might have done***."

>Line 533. Please compare the BOC values here with those reported in line 492 and line 18 for consistency (6.6 time vs. 660-fold).

Reply: Thank you for spotting this oversight. Now corrected.

>Supplementary File

>Line 78. Please clarify what "L" means in "1419.5 mm $year^{-1}$, i.e. L m^{-2} $year^{-1}$."

Reply: We clarified as follows in the former L. 78: "*… the average precipitation in mm per year was 1419.5 mm $year^{-1}$, i.e. L m^{-2} $year^{-1}$,* ***Liters per square meter and year (Kopáček et al., 2009; note that precipitation is either reported as mm in a rain gauge, or as volume per square meter).***"

>Line 108. Should this read "10^6 cells mL^{-1}"? Please check that "cells" is not missing and verify the exponent (−6). This last point is important because I think it is 10^6 cell/mL

Reply: Thank you for spotting this writing error – of course it is 10^6 cells/mL

>Lines 109–111. It is not clear how dry mass per cell was derived (pg $cell^{-1}$? fg $cell^{-1}$?).

This is not necessarily incorrect, but an important methodological step seems to be missing.

Reply: Indeed, this step was unfortunately missing from the explanations in Marxsen et al. (2021), and thus, was not tractable here. As in Marxsen et al. (2021), we had multiplied cell volumes with a known bacterial wet biomass density of 1.07 g/cm^{-3} (Bakken and Olsen, 1983: Cell, 45, 1188–1195) and multiplied by 0.3, assuming a dry matter content of 30% (Bakken and Olsen, 1983). The prokaryote cell numbers in the River Fulda floodplain had been reported for size classes. When multiplying the average volumes of bacterial cell size classes first listed in Marxsen (1982; Beiträge zur Naturkunde Osthessens - Schlitzer produktionsbiologische Studien, 51, 3–11) and used in Marxsen et al. (2021), yields between 0.016 pg for a <1 µm length rod and 1.9 pg for a > 2 µm cocci, or between 16 fg for a <1 µm length rod and 1926 fg for a > 2 µm cocci (the latter were rare):

dry mass	Rods				
	<1 µm	**1-2 µm**	**2-3 µm**	**3-4 µm**	**>4 µm**
	0.01605	0.0642	0.0963	0.1284	0.1926
here: pg (10^-12 g)					
	Cocci				
	<0,5 µm	**0.5-1 µm**	**1-2 µmm**	**>2 µmm**	
	0.00963	0.08025	0.642	1.926	

We have added this information in the SI, now SI3.

>Line 111. The average prokaryotic biomass of ~200 µg L^{-1} should be explicitly stated as dry mass to avoid confusion with carbon or COD.

Reply: Done as suggested.

>Lines 112–115. Please check this sentence for clarity. A closing parenthesis may be missing.

Reply: Thank you for spotting this oversight. Now corrected: “To calculate a budget from the different types of carbon compounds (prokaryote dry mass, fauna dry mass, DOM = dissolved organic matter, TOM = total organic matter), were translated to mol COD L-1.”

>Line 114. “Converted” may be more appropriate than “translated.”

Reply: Done as suggested.

>Lines 120–122. The reaction appears correct, but I wonder whether COD (acetate) should be expressed as 2 mol O_2 per mol of acetate, rather than "2 mol L^{-1}" in absolute terms. Should this not be linked to acetate concentration?

Reply: Indeed; Done as suggested.

>Lines 166–169. The adopted temperature scaling can be justified as a pragmatic modelling choice. However, it should be stated more clearly that it relies on a linear regression derived from optimal growth rates of a limited number of bacterial species over a warm temperature range (14–44 °C), and that applying it to typical groundwater temperatures, often below this range, represents an extrapolation beyond the original domain of validity. Explicitly stating this assumption would improve transparency.

Reply: We agree with the reviewer and have added the following sentences after the 14–44 °C range: "***Such temperature range is warmer than Middle European average groundwater temperatures, and thus, the derived regression might not apply for Middle European aquifers. Until further data are available, this is the best estimate at hand***."

>Line 253. I believe you are referring to S5 rather than S3.

Reply: Yes, thank you for spotting this. Now corrected.