

Detailed responses to referees' comments

Dear Editors and Reviewers:

Thank you for your letter and the reviewers' comments concerning our manuscript titled "Microbial response of Fe and Mn biogeochemical processes in hyporheic zone affected by groundwater exploiting along riverbank" (water-3936516) Those comments are extremely valuable and helpful for revising and improving our paper. They also hold significant guiding importance for our research. We have carefully studied the comments and made revisions, hoping that they will meet with approval. The main corrections in the paper and our responses to the reviewers' comments are as follows:

Responds to the reviewer's comments:

Reviewers 1#:

Response to overall comments:

Thank you very much for taking your valuable time to review this article. We are also very pleased to have your recognition and many valuable suggestions that will help improve the quality of the article. In response to your suggestions, we have done our best to make changes and add some content to make the article more scientific and reasonable. Next, we will respond to your questions one by one:

1) The paper, titled "Microbial response of iron manganese biogeochemical processes in the subsurface flow zone to water intake from rivers," addresses an important topic for the hydrological community and an interesting area of research. However, at this stage, it requires significant corrections and/or additions. Some sections of the text require additional commentary. Many sections of the paper (e.g., Chapter 2.1.1) are written in an overly confusing manner, contain terminological and factual errors, and are generally difficult for the reader to understand. Perhaps it would be worthwhile to simplify these chapters somewhat and write them more concisely, making them less confusing for the reader? I strongly suggest proofreading by a native speaker of the natural sciences.

Response: Thank you for your comments and suggestions. I have reviewed and

revised the terminology in the article, hoping the revised version will be clearer and enhance your reading experience.

2) Line 29: I am not convinced that the term "phreatic zone" refers to the zone of contact between surface and groundwater, as the Authors suggest. In my opinion, this is a broader concept and simply describes the subsurface region where all pores and fissures in the soil or rock are completely filled with water and lateral water flow dominates (also known as the saturation zone). In essence, this is simply part of the aquifer system. I suggest reconsidering the terminology used. This also applies to terms such as "observation holes" used by the Authors.

Response: Thank you for your suggestion regarding line 29. I have revised relevant terms, such as changing "phreatic zone" to "hyporheic zone" and "observation holes" to "observation well".

3) Lines 62-67: "...To investigate ..." – This sentence is too long and unclear.

Response: Thank you for your suggestions regarding lines 62-67. I have revised this sentence, and the revised version is as follows: Exploring the co-evolutionary relationship between the microbial community structure and function in hyporheic zone affected by groundwater exploiting along riverbank, as well as the groundwater chemical composition, It is possible to deeply explore the biochemical processes and migration transformation rules of Fe-Mn transformation in hyporheic zone, and Reveal the biogeochemical processes and microbial ecological processes of Fe-Mn during the infiltration of river water. It provides certain references and basis for the water supply safety of drinking water sources.

4) Line 65: "submerged zone" – an unclear term. What does this actually mean?

Response: Thank you for your suggestions regarding line 65. I have revised terms such as changing "submerged zone" to "hyporheic zone".

5) Line 78: Were any studies conducted on the physicochemical parameters of groundwater and river water? The Authors repeatedly mention the correlation of microbiological and physicochemical test results in the manuscript.

Response: Thank you for your suggestions regarding line 78. For the study of physical and chemical parameters, the detection indicators include:

Metal ions: Fe^{2+} (mg/kg), Mn^{2+} (mg/L);

Environmental factors: DO (Dissolved Oxygen, mg/L), Eh (Oxidation-Reduction Potential, mV), pH, DOC (Dissolved Organic Carbon, mg/L), SO_4^{2-} (mg/L).

6) Line 85: "...water dynamics" – Again, an unclear term. What does this actually mean?

Response: Thank you for your suggestions regarding line 85. I have revised terms such as changing "water dynamics" to "dynamic water". And modify this paragraph to: to provide a reference for understanding the migration and transformation processes of iron and manganese in the hyporheic zone under the dynamic water conditions of riverbank filtration.

7) Lines 91 and later: "The main recharge mode of groundwater is the lateral recharge of surface water, and the main seepage path of river water..." This sentence is too long and incomprehensible. Furthermore, much of the information presented here may be misleading.

Response: Thank you for your suggestions regarding lines 91 and later. The revised content is as follows: The main replenishment method of groundwater is lateral replenishment of surface water, and the main seepage path of river water groundwater can be generalized into two layers: deep and shallow. The water circulation depth of the shallow seepage path is less than 10m, and its range is from 0 to 17.0m away from the riverbank. The shallow rock type particles are finer, so the permeability coefficient is small and the infiltration rate is slow. Chemical components such as iron manganese and ammonia nitrogen have sufficient time for biochemical reactions.

8) Lines 93-95: On what basis did the Authors determine the groundwater flow paths described here? Is this based on hydrodynamic analysis, flow modeling (ModFlow or other tools), or other types of research?

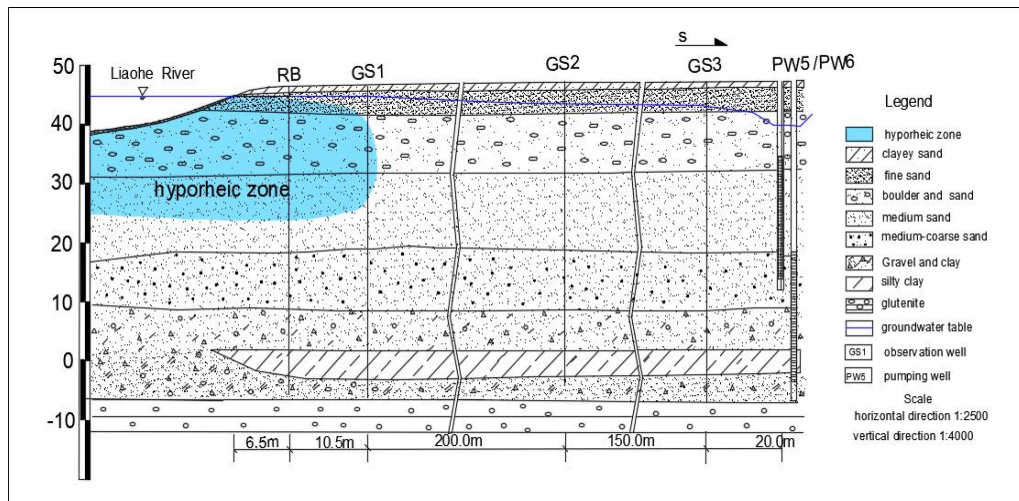
Response: Thank you for your suggestions regarding lines 93-95. Based on the data released by the Water Conservancy Bureau on its official website, existing research literature, and the water levels measured on-site during each period.

9) Line 97: "...so it takes place at a more adequate time." - an unclear term. What does this actually mean?

Response: Thank you for your suggestions regarding line 97. This part has been re-edited in the original text. The core message here is that chemical components such as iron, manganese, and ammonia nitrogen have ample time for biochemical reactions. I apologize for any unpleasant reading experience this may have caused you.

10) Line 99: How can river water infiltrate within 350 meters of the riverbank?

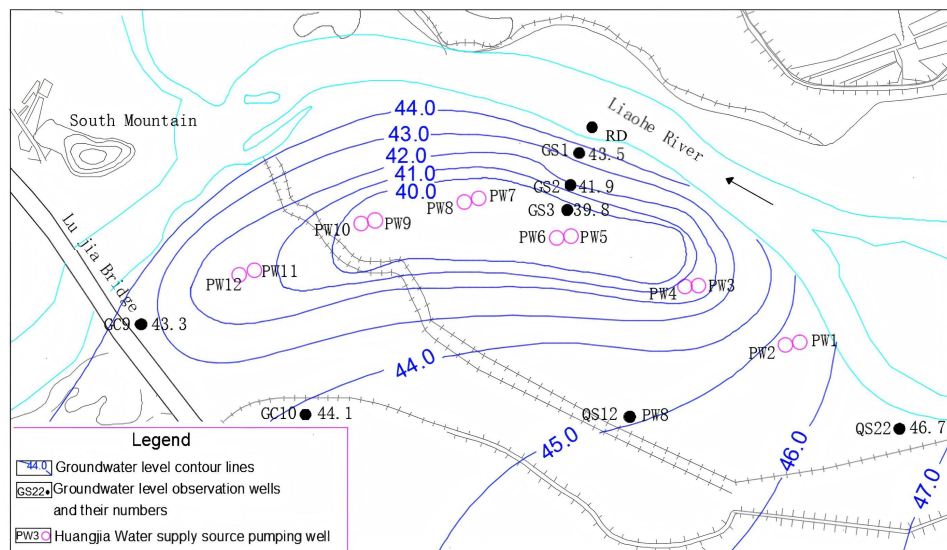
Response: Thank you for your suggestions regarding line 99. The study area is the Huangjia Water Source in Shenyang. After exploitation, groundwater is recharged by river water, and a series of water supply wells have been drilled perpendicular to the river. The production wells PW5 and PW6 are approximately 387 meters away from the riverbank.



11) The description of groundwater circulation conditions is very confusing, and in many parts, I seriously doubt its substantive accuracy. Overall, the description of the hydrogeological conditions of the study area is, in my opinion, overly simplified. This is quite surprising, since the work concerns groundwater. There is no groundwater table map of the aquifer(s), which could reflect the hydrodynamic conditions of the study area. The current nature of water flow/interaction between surface water and the groundwater system is unclear. A groundwater table map would be useful for a better understanding of the problem. In fact, the hydrogeological cross-

section of the study area should be included in this chapter, not as Fig. 4 in Chapter "Discussion of Results." I believe this information is worth presenting in the manuscript because it represents the main focus of the authors' research. This is crucial for data analysis and interpretation.

Response: Thank you for your suggestions. We have made the corresponding revisions and added a water level chart to the original text.



12) Line 101: The authors indicate that samples were collected from the hyporheic zone, but the issues surrounding this zone and its importance in surface water/groundwater interactions are completely omitted from the manuscript. Overall, the review of scientific research on surface water/groundwater interactions is rather sparse. I believe the introduction (or elsewhere) could provide a more extensive discussion of the hyporheic zone, or more generally, surface water-groundwater interactions. Generally speaking, the key issue for surface water-groundwater interactions is the hydraulic relations between the river and the aquifer. It is worth noting that a broad approach is used to assess the extent of the riverwater-groundwater mixing zone, or the hyporheic zone itself. For example, Harvey et al. (1996) indicate that the river-groundwater mixing zone also includes "groundwater that has not reached the riverbed." Hyporheic flow, however, is usually distinguished from groundwater flowing into river channels due to its bidirectional nature— infiltration of river water into the bed and its return as a result of drainage as a

mixture with groundwater (Boano et al., 2014). The mixing zone of surface water and groundwater can be characterized by complex flow and mixing conditions. For example, studies conducted using gaseous tracers (CFCs, SF₆, noble gases) allowed for a better understanding of the nature of groundwater and surface water inflow and mixing in the hyporheic zone and an assessment of the dynamics of this process under natural and conditions disturbed by groundwater abstraction (Kotowski et al., 2023). These studies allowed, among other things, to determine the ratio of surface water to groundwater in the hyporheic zone and the extent of the hyporheic exchange zone. The water mixture in this zone may differ in terms of the content of abiotic/biotic components, both from river water and groundwater.

Response: Thank you for your suggestions regarding line 101. We have made corresponding modifications to the question you raised. I have referred to the literature you provided and revised the first paragraph of the introduction in the original manuscript. The revised content is as follows:

The hyporheic zone is a critical transition area for the interaction between surface water and groundwater[1], serving as the core hub for material exchange and energy transfer between the two. The key issue in the interaction between surface water and groundwater lies in the hydraulic relationship between rivers and aquifers. As the central region for their interaction, the delineation of the hyporheic zone's scope and its flow characteristics hold significant research value. Harvey, Boano, and others have proposed that the surface water-groundwater mixing zone includes "groundwater not reaching the riverbed," while hyporheic zone flow, characterized by bidirectionality, differs from groundwater discharging into the river—after infiltrating the riverbed, surface water mixes with groundwater and recharges the river. This area exhibits active solute transport and transformation as well as intense biogeochemical processes. By regulating microbial nutrient supply and energy acquisition, it shapes a unique transitional biological community[2,3]. The flow and mixing conditions in the surface water-groundwater mixing zone are complex. Kotowski pointed out that gaseous tracers (CFCs, SF₆, noble gases) can better help understand the inflow and mixing characteristics of surface water and groundwater in the hyporheic zone, assess

dynamic changes under natural and exploitation disturbances, and determine the mixing ratio of the two and the scope of the hyporheic exchange zone. Additionally, the abiotic/biotic component contents of the mixed water in this area may differ from those of river water and groundwater[4]. Its core importance is reflected in maintaining hydrological connectivity, purifying water pollutants, and regulating regional water quality. It acts not only as a "buffer zone" for mutual recharge between surface water and groundwater but also as an "active engine" driving element cycling and supporting ecosystem stability, playing an irreplaceable role in maintaining watershed hydrological and ecological balance.

Changes in the biological community structure directly or indirectly cause changes in the hydrochemical composition of groundwater and affect the migration and transformation pathways of substances such as iron and manganese[5,6]. Research by Park et al. has shown that when river water infiltrates to recharge groundwater, the microbial community structure is an important influencing factor in biogeochemical processes, and differences in the activity of dominant and functional bacteria affect the migration behavior of pollutants in groundwater[7]. So Jeong et al., focusing on the riparian zone of the Geum River in South Korea, found that IRB (Iron-Reducing Bacteria) exhibit spatial heterogeneity. Redox zonation and acid-base conditions influence their species distribution—strong iron reduction zones (near the river) are dominated by the genus *Geobacter*, while weak iron reduction zones (far from the river) are dominated by the genus *Rhodospirillum rubrum*[8]. Research by Medihalla et al. on the infiltration scenario of the North Saskatchewan River in Canada showed that the structure and function of groundwater microbial communities have significant spatial heterogeneity, with environmental changes such as groundwater exploitation being the main influencing factors. The area near the river is dominated by iron and manganese reducing bacteria, while the area 1-2 meters around pumping wells is dominated by iron and manganese oxidizing bacteria[9].

13) Line 128: „Samples of microorganisms in groundwater were collected from the river and ...” - ??

Response: Thank you for your suggestions regarding line 128. The meaning of this sentence is that the sampling object is "microbial samples from river water and various groundwater observation holes", not "collecting microorganisms from groundwater in rivers". The original text is modified to: A total of 20 microbial samples were collected in this study, covering river water and various groundwater observation wells.

14) Lines 129 and further: I also have a few questions regarding the groundwater sampling and used methodology. It is unclear whether the samples originated from one or more aquifer systems. It is not known at all what depth the samples were collected from or at what depth the well's screen is located. The authors mention that sampling was conducted from observation wells. Were these wells pumped and flushed before sampling?

Response: Thank you for your suggestions on the content starting from Line 129. Samples were collected from multiple aquifers. The groundwater flow paths in the study area are divided into two layers: the shallow layer (0–17.0 meters from the riverbank, with a depth of less than 10 meters) and the deep layer (17.0–350 meters from the riverbank, with a depth ranging from 10 meters to 45 meters). These two layers correspond to the aquifer media of the shallow and deep flow paths respectively, and the sampling covers both types of aquifers. Prior to water collection, these wells were pumped and flushed.

15) Line 179 and further: The information presented here regarding microorganisms is very extensive, but most of it is not used in further interpretation of the Authors' research results. I believe that the entire chapter could be significantly shortened without compromising the quality of the scientific communication.

Response: Thank you very much for your suggestion. It is true that while the content on microorganisms is detailed, its relevance to the research findings can be further strengthened. I will streamline the content in line with your idea, ensuring that scientific rigor is not compromised while making the overall expression more concise and focused.

16) Lines 260-267: How did the Authors determine the geochemical evolution of groundwater during river water infiltration in the study area? And how does this relate to the structure and function of microbial communities? Are these conclusions based on the Authors' own observations, or perhaps the observations/conclusions of other authors? (citation issue?).

Response: Thank you for your suggestions regarding lines 260-267. Based on self-observation and support from existing literature: self-observation includes sampling analysis, 16S rRNA sequencing, detection of hydrochemical indices, and RDA analysis; meanwhile, 20 relevant literatures are cited to confirm the association between the functions of bacterial genera and the iron-manganese biogeochemical processes.

17) Lines 266-267: "Therefore, microorganisms in the environment have a certain response pattern to the redox subzone" – Again. What is the basis for this statement?

Response: Thank you for your suggestions regarding lines 266-267. This claim is based on the following: Spatial distribution correlation: There are significant differences in dominant bacterial genera across different redox subzones (including O_2/NO_3^- reduction zone, Mn (IV) reduction zone, Fe (III) reduction zone, etc.). For instance, the Mn (IV) reduction zone is dominated by *Geobacter*, while the Fe (III) reduction zone is mainly composed of *Pseudomonas* and *Geothrix*.

Abundance matching: The total abundance of dominant bacteria corresponds to redox subzones (the total abundance of dominant bacteria in redox subzones of the shallow seepage path is 67.86%, and that of the deep seepage path is 56.90%). Additionally, the functions of bacterial genera are consistent with the geochemical processes of the subzones (e.g., the abundance of iron-reducing bacteria is positively correlated with the Fe^{2+} content in groundwater).

18) Line 278: "...a good correlation" - ?? What is the R or R² value of this correlation?

Response: Thank you for your suggestions regarding line 278. This study verified the correlations through RDA (Redundancy Analysis): the first and second axes

cumulatively explained 89.4%-97.5% of the variations in microbial composition, and the cumulative correlation coefficient between species and environmental factors reached 91.6%-94.8%. This indicates an extremely strong overall correlation between environmental factors such as Fe, Mn, DO, and Eh and the composition of dominant bacterial species.

19) Lines 306-307: "As analyzed earlier, the effective form of manganese content in deep water containing medium was lower than that of iron, so the Fe²⁺ content in deep groundwater was relatively high." - Have the Authors conducted studies on the physicochemical parameters of groundwater and river water? If so, where are the results of these studies and a description of the sampling/measurement methodology?

Response: Thank you for your suggestions regarding lines 306-307. A study on physical and chemical parameters was conducted, with the tested indicators including:

Metal ions: Fe²⁺ (mg/kg), Mn²⁺ (mg/L);

Environmental factors: DO (Dissolved Oxygen, mg/L), Eh (Oxidation-Reduction Potential, mV), pH, DOC (Dissolved Organic Carbon, mg/L), SO₄²⁻ (mg/L).

Redox-sensitive indicators such as dissolved oxygen (DO), oxidation-reduction potential (Eh), pH, electrical conductivity (EC), temperature, water level, and total dissolved solids (TDS) were measured using a Hach electrode (HQ40d; Colorado, USA); meanwhile, ions including Fe²⁺, Mn²⁺, and SO₄²⁻ in water samples were determined with a Hach spectrophotometer (HQ2800; Colorado, USA).

20) Line 326: "The environmental factors (DO, Eh, pH) ..." – Again. See my previous comment.

Response: Thank you for your suggestions regarding line 326. Revisions have been made to the entire text regarding this issue.

21) The Conclusion chapter contains virtually no conclusions, merely a repetition of information presented earlier. It is largely a superficial summary of the research conducted, rather than any conclusions drawn from it. Some statements are also completely incomprehensible and do not directly follow from the content of the paper and the presented research results. In light of the above comments, I believe the

Summary chapter should be thoroughly revised, and I am not addressing the content of this chapter at this time.

Response: Thank you for your comments and suggestions. I have revised the conclusion section. The revised content is as follows: This study took the Huangjia water source area in the Shenyang section of the main stream of the Liaohe River as the research object to explore the synergistic evolution relationship between hyporheic zone microorganisms and groundwater hydrochemistry under the influence of riverbank filtration. The results showed that the dominant bacterial phyla in the study area were Proteobacteria, Actinobacteria, etc. Proteobacteria contained a variety of functional bacteria, which were involved in the cycles of C, N, S, Fe and Mn. The spatial distribution of microbial communities was heterogeneous and related to redox zonation, and there were differences in the abundance and types of dominant bacteria between shallow and deep seepage paths. RDA (Redundancy Analysis) showed that the dominant bacteria had a good correlation with environmental factors such as Fe, Mn, DO (Dissolved Oxygen), and Eh (Oxidation-Reduction Potential). Their functions and activities evolved synergistically with the hydrochemical environment. This not only provides a microbiological theoretical basis for redox zonation but also offers a reference for understanding the migration and transformation of iron and manganese in the hyporheic zone.