

Dear Reviewers,

The manuscript of agronomy-3643415 has been carefully revised according to your opinions. We appreciate the detailed and useful comments and suggestions from you. These suggestions are very helpful for us to improve the manuscript. Our point-to-point responses to the comments and suggestions are listed as below.

Dynamic effects of sodium selenite on the rhizospheric microenvironment, growth, and antioxidative responses of wheat (*Triticum aestivum* L.) by Fang Qin et al addresses a relevant topic and may be of interest to readers and peers in the field. In their revised 2 manuscript, the authors have improved their method description to make it more clear. However, this does not address the original flaw in the conceptual experiment design of a single experiment conducted in May 2019. Replicating each treatment 4X in a single experiment does not warrant reproducibility of the data. To be convincing, with supportive conclusion, at least repeating the expecting the experiment at least twice in time or space of the same treatments are required. Whereas the data collection and acquisitions appear to be well conducted, the experimental design from which the data were collected is flawed for ensuring reproducibility of the data. The reported and discussed data appeared to be only confirmatory and their originality not pointed out. As a second review, I would recommend a final rejection of this manuscript, and recommend to the authors to carry a second experiments to collect growth, roots soil parameters to confirm the current data for drawing strong conclusions and be complement by their current biochemical data..

Specific concerns can be found below.

No.1.1 *TAabstract*

First sentence of abstract not clear

Response: Thank you for your kind suggestion. We have revised this sentence to clarify its meaning as "Soil selenium (Se) speciation characteristics and their influence on the Se enrichment pattern and physiological characteristics of wheat are poorly understood."

No.1.2 *Literature review has been improved*

English quality can be improved throughout the text

Response: Thanks for your comment. Thank you for acknowledging our introduction. Regarding the quality of the english, we have already used the proofreading services on the MDPI platform.

No.1.3 *Use of Rhizobag now better explained*

Data generated from a single experiment although 4 replications/unit were reported – the flaw in the conceptual design remains and not-addressed.

Response: Thanks for your suggestions. Many researchers have conducted rhizobag experiments, and their data and conclusions have been recognized [1-4]. Unlike field experiments, pot experiments are conducted under controlled conditions and usually do not require repeated experiments. We conducted four replicates for each treatment to ensure the authenticity of the data.

References:

- [1] Li, H.; Li, Z.; Long, J.; Fu, J.; Chen, C. Mechanisms of N-Doped Microporous Biochar Decreased Cd Transition in Rhizosphere Soils and Its Impact on Soil Bacterial Community Composition. *Sci. Total Environ.* **2024**, 951, 175873.
- [2] Zheng, H.; Feng, N.; Yang, T.; Shi, M.; Wang, X.; Zhang, Q.; Zhao, J.; Li, F.; Sun, K.; Xing, B. Individual and Combined Applications of Biochar and Pyroligneous Acid Mitigate Dissemination of Antibiotic Resistance Genes in Agricultural Soil. *Sci. Total Environ.* **2021**, 796, 148962.
- [3] Lyu, P.; Li, L.; Zhou, X.; Huang, J.; Ye, J.; Liu, X.; Xie, J.; Wang, Z. Modification of Arsenic and Cadmium Species and Accumulation in Rice Using Biochar-Supported Iron-(oxyhydr)oxide and Layered Double Hydroxide: Insight from Fe Plaque Conversion and Nano-Bioassembly in the Root. *Chem. Eng. J.* **2024**, 494, 152847.
- [4] Cheraghi, M.; Motesharezadeh, B.; Mousavi, S.M.; Basirat, M.; Alikhani, H.A. Phosphorus Bioavailability and Silicon Fractionation in Wheat Rhizosphere Affected by Soil Water Content and Silicon Application. *Rhizosphere* **2025**, 33, 101017.

No.1.4 *Result section*

Data on the figures are well presented, but their contents and validity are contingent to the design they derive from. – How repeatable the experiments can be is not addressed.

Response: Thank you very much for your advice. Thank you for recognizing the quality of our data presentation. Considering the nature of pot experiment, we are considering applying the results to field trials to verify their reliability in a subsequent study.

No.1.5 *Clarity of discussion has improved*

Authors fail to highlight is the originality of their finding. As presented and discussed the data appear to be purely confirmatory.

Response: Thanks for your comments. Thank you for acknowledging our discussion about improvements. Although our results were interpreted using others' perspectives, our research subjects were different. Furthermore, our original contribution lies in studying the soil-wheat system as a whole, whereas previous studies focused on isolated aspects such as soil selenium morphology, plant selenium absorption, or plant enzyme activity. We explored changes in the soil microenvironment under the influence of roots by comparing the rhizosphere and non-rhizosphere, while other studies may not distinguish between rhizosphere and non-rhizosphere soils.

No.1.6 *The statement on lines 496-497 seems not accurate and lacks rationality. If this reviewer follows the author:*

How does Se move from roots to seed then if usually stays in the root? and

Do all Se applied through foliar application stay in the leave if not mobile?

Response: Thanks for your suggestions. We accept the referee's suggestion and have changed this sentence in the revised manuscript as "Guerrero et al. [68] found that only a small portion (10-20%) of selenium (IV) absorbed by the roots was transferred to the aboveground parts. This is because, compared to Se(VI), Se(IV) has lower mobility in xylem transport and can rapidly incorporate into proteins or be metabolized by thiol-containing enzymes in the plastids into volatile products (DMSe/DMDSe) [69]. "

References:

Guerrero, B.; Llugany, M.; Palacios, O.; Valiente, M. Dual Effects of Different Selenium Species on Wheat. *Plant Physiol. Biochem.* **2014**, *83*, 300-307.

Li, H.F.; McGrath, S.P.; Zhao, F.J. Selenium Uptake, Translocation and Speciation in Wheat Supplied with Selenate or Selenite. *New Phytol.* **2008**, *178*, 92-102.

No.1.6 Conclusions.

Major concerns remain (1) the reproducibility and (2) the originality of the data which are not clear at this point with the presented design, data, and discussions

Response: Thanks for your suggestions. We believe our data is true and valid. All of our data was obtained from our experiments, which guarantees its originality. Our experimental design refers to previous researchers who recognized the rhizobag pot experiment [1-5].

References:

- [1] Li, H.; Li, Z.; Long, J.; Fu, J.; Chen, C. Mechanisms of N-Doped Microporous Biochar Decreased Cd Transition in Rhizosphere Soils and Its Impact on Soil Bacterial Community Composition. *Sci. Total Environ.* **2024**, *951*, 175873.
- [2] Zheng, H.; Feng, N.; Yang, T.; Shi, M.; Wang, X.; Zhang, Q.; Zhao, J.; Li, F.; Sun, K.; Xing, B. Individual and Combined Applications of Biochar and Pyroligneous Acid Mitigate Dissemination of Antibiotic Resistance Genes in Agricultural Soil. *Sci. Total Environ.* **2021**, *796*, 148962.
- [3] Lyu, P.; Li, L.; Zhou, X.; Huang, J.; Ye, J.; Liu, X.; Xie, J.; Wang, Z. Modification of Arsenic and Cadmium Species and Accumulation in Rice Using Biochar-Supported Iron-(oxyhydr)oxide and Layered Double Hydroxide: Insight from Fe Plaque Conversion and Nano-Bioassembly in the Root. *Chem. Eng. J.* **2024**, *494*, 152847.
- [4] Cheraghi, M.; Motesharezadeh, B.; Mousavi, S.M.; Basirat, M.; Alikhani, H.A. Phosphorus Bioavailability and Silicon Fractionation in Wheat Rhizosphere Affected by Soil Water Content and Silicon Application. *Rhizosphere* **2025**, *33*, 101017.
- [5] Zeng, T.; Khaliq, M.A.; Li, H.; Jayasuriya, P.; Guo, J.; Li, Y.; Wang, G. Assessment of Cd Availability in Rice Cultivation (*Oryza sativa*): Effects of

Amendments and the Spatiotemporal Chemical Changes in the Rhizosphere and Bulk Soil. *Ecotoxicol. Environ. Saf.* **2020**, *196*, 110490.

We appreciate the time and effort you put into our manuscripts. We sincerely hope that this revised manuscript has addressed all your comments and suggestions. We appreciated for your warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

Sincerely,

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