Dear Editor,

We would like to thank you, and the reviewers for the contributions to this manuscript. The constructive feedback has been extremely helpful. We have accepted all the changes suggested and made the appropriate changes to the study. We believe that the manuscript is considerably clearer and more impactful as a result.

Attached please find our point-by-point responses to the reviewer’s comments.

We thank you for your consideration and hope you will find this version suitable for publication in *Earth System Science Data*.

Best regards,

Zhiqiang Wang, and on behalf of all co-authors

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Response to reviewer's comments

<u>Responses to the Reviewer's comments</u>

The paper presents an excellent and timely study, offering a comprehensive global-scale analysis of the contributions of fungal and bacterial necromass carbon (FNC and BNC) to soil organic carbon (SOC) across agricultural and natural ecosystems. The manuscript is well-written, methodologically rigorous, and addresses a topic of significant importance in soil biogeochemistry. The findings provide valuable insights into microbial-mediated carbon stabilization mechanisms in terrestrial ecosystems. I suggest this study highly suitable for publication in ESSD, however, some questions should be resolved before final acceptance.

Response: We sincerely thank the reviewer for the positive and encouraging comments on our manuscript. We particularly appreciate the reviewer's recognition of the global-scale analysis, methodological rigor, and significance of our study to the field of soil biogeochemistry.

We have carefully considered all the points raised by the reviewer. In the sections below, we provide a point-by-point response to the specific questions and have revised the manuscript accordingly to address them. We believe that these revisions have further strengthened the quality and clarity of our work.

Major concerns
In Section 2.1, The authors should justify the use of interpolated data (e.g., for MAT, MAP, and soil properties) obtained from public databases. Please address the potential uncertainties and describe any steps taken to validate these values against site-specific conditions or to quantify the associated error in the analysis.

Response: Thanks for this important suggestion. We agree that acknowledging and addressing the potential uncertainties associated with these datasets is crucial for the robustness of our global-scale analysis. Below, we provide a justification for their use and describe the steps we took to mitigate potential issues.

The primary rationale for employing globally interpolated datasets (e.g., WorldClim, SoilGrids) was to ensure consistent, continuous, and spatially complete coverage of environmental variables across all 486 globally distributed sites. The original publications from which microbial necromass data were extracted frequently did not report the full suite of climatic and soil variables required for our unified analysis. By using these standardized, high-resolution global datasets, we maintained methodological consistency and mitigated potential biases arising from missing data.

We acknowledge that interpolated data inherently contain uncertainties. To address this, we took the following steps:

(1) We exclusively used globally recognized and widely cited databases (e.g., WorldClim v2.1 with a 30-arc second resolution, SoilGrids 2.0), which represent the current state-of-the-art in global spatial interpolation and are extensively used in global ecological and biogeochemical studies (e.g., Lu et al., 2022; Ren et al., 2024; Shi et al., 2025; Zhou et al., 2025).

(2) After retrieving missing value from gridded data, we typically calibrate them against field-reported values via a field-anchored bias correction (i.e., a site- or region-specific "delta" adjustment) to minimize errors introduced by gridded data.

(3) Our statistical approach inherently accounts for data uncertainty. The performance metrics of our models (e.g., the R^2 values ranging from 23% to 66% in our Boosted Regression Tree analysis, as shown in Figure 4) already reflect the unexplained variance, which partly incorporates the measurement and interpolation errors of all input variables. The fact that we still identified strong and significant drivers suggests that the signals we detected are robust enough to overcome the background noise, including potential errors from interpolation.

In response to the reviewer's comment, we have revised the Section 2.1 (Data collection) in the revised manuscript to explicitly address this point. The added text reads:

"We supplemented missing climatic and soil variables using high-resolution, globally interpolated datasets to ensure consistent spatial coverage across all sites. After retrieving missing value from gridded data, we typically calibrate them against field-reported values via a field-anchored bias correction (i.e., a site- or region-specific "delta" adjustment) to minimize errors introduced by gridded data. While the use of such data introduces inherent uncertainties, these databases are widely adopted in global-scale ecological analyses and provide the most feasible approach for a unified assessment."
For further detail, please see Lines 156–163 of the revised manuscript.

Section 3.2 presents a highly detailed and, at times, repetitive description of the results. This level of minutia can obscure the key findings for the reader. To improve clarity and impact, I strongly recommend that the authors streamline this section. The text should be condensed to focus on the primary results, avoiding a minute description of every statistical outcome. Reorganizing the content into clearer thematic paragraphs would also significantly enhance its readability.

Response: Thank you for this constructive comment. As recommended, we have reorganized the Section 3.2 as followings:

"Soil physicochemical factors were the most important influence on the contributions of FNC and BNC to SOC across both ecosystem types (Figures 3a–d, 4a–d). Specifically, they explained 16% and 17% of the variance in the contributions of FNC and BNC to SOC in agricultural ecosystems, respectively (Figures 3a, c), and 20% and 24% in natural ecosystems (Figures 3b, d). BRTs corroborated this pattern, with soil physicochemical factors showing the highest relative influence (51% for FNC, and 44%

for BNC) in agricultural systems and 44% in natural systems (Figures 4a–d). All BRT models were significant (P < 0.001), with explained variance 36–66%. While soil factors dominated overall, responses to individual variables differed between ecosystems. In detail, in agricultural systems, the C/N ratio ranked third for FNC after clay and SOC (Figure 4a), whereas C/N was the top predictor for FNC in natural systems and for BNC in both ecosystems (Figures 4b–d). Consistently, linear models showed declines in the contributions of FNC and BNC with increasing C/N in both ecosystems (Figures S5g, S6g). SEMs yielded convergent results, indicating both direct and indirect pathways (Figures 5a–d, 6a–d). Notably, the direct and total effects of soil physicochemical factors on FNC were negative in agricultural but positive in natural ecosystems (Figures 5a, b, 6a, b), whereas the effects on BNC were negative in both ecosystem types (Figures 5c, d, 6c, d).

Our results indicated that geographical factors were the most important contributors to explain the FNC/BNC ratio in both agricultural and natural ecosystems, accounting for 21% and 10% of the explained variance in the FNC/BNC ratio, respectively (Figures 3e, f). The results of the BRTs suggested that geographical factors played a similar role in explaining the FNC/BNC ratio (Figures 4e, f). In the BRT models, geographical factors emerged as the primary influencers of the FNC/BNC ratio in agricultural and natural ecosystems, accounting for 32% and 44% of the variance in each case, respectively (Figures 4e, f). To be more specific, elevation was the most significant geographical factors influencing the FNC/BNC ratio in both ecosystems (Figures 4e, f). Moreover, the FNC/BNC ratio in agricultural and natural ecosystems show significantly increased with an increase elevation (Figure S7a). The results of SEMs also indicated that geographical factors were the most influential factors for the FNC/BNC ratio in agricultural and natural ecosystems, exerting both direct and indirect effects on this ratio (Figures 5e, 6e), with the standardized total effect being positive (Figures 5f, 6f).".

For further details, please see Lines 235–267 of the revised manuscript.

Meanwhile, I suggest the authors separately describe the effects of driving factors on the contributions with agricultural and natural ecosystems. Also, in the section 4.2, the authors should better discuss it separately about agricultural and natural ecosystems.

Response: Thanks for the constructive comment. As recommended, we have separately described the effects of driving factors on the contributions with agricultural and natural ecosystems as followings:

"While soil factors dominated overall, responses to individual variables differed between ecosystems. In detail, in agricultural systems, the C/N ratio ranked third for FNC after clay and SOC (Figure 4a), whereas C/N was the top predictor for FNC in natural systems and for BNC in both ecosystems (Figures 4b–d). Consistently, linear models showed declines in the contributions of FNC and BNC with increasing C/N in both ecosystems (Figures S5g, S6g). SEMs yielded convergent results, indicating both direct and indirect pathways (Figures 5a–d, 6a–d). Notably, the direct and total effects of soil physicochemical factors on FNC were negative in agricultural but positive in

natural ecosystems (Figures 5a, b, 6a, b), whereas the effects on BNC were negative in both ecosystem types (Figures 5c, d, 6c, d).".
For further details, please see Lines 242–252 of the revised manuscript.

In addition, we have reorganized and discussed it separately about agricultural and natural ecosystems in the Section 4.2. The revised text now reads:
"In agricultural ecosystems, high soil N levels primarily result from fertilization (Chen et al., 2020). In contrast, natural ecosystems experience minimal anthropogenic disturbance, N often acts as the key limiting factor for microbial activity (Elser et al., 2007). Under N-limited conditions, microbes (both fungi and bacteria) allocate more energy and C resources to the synthesis of N-acquiring enzymes (e.g., proteases and chitinases). This shift in metabolic strategy reduces the C allocated to biomass synthesis, thereby diminishing the amount of C ultimately converted into microbial necromass (Mooshammer et al., 2014; Liu et al., 2024). Thus, although microbial community composition differs between natural and agricultural ecosystems, the regulatory role of soil C/N ratio in shaping their structure and function remains consistent (Han et al., 2024). In our study, soil clay content was identified as the predominant factor governing the contribution of FNC to SOC in agricultural ecosystems (Figure 4a), with this contribution increasing concomitantly with clay content (Figure S5d). This suggests that soils with higher clay and silt contents generally accumulate greater amounts of microbial residues, particularly those derived from fungi, which can be attributed to the promotion of stable organo-mineral complex formation by abundant fine soil particles (Six et al., 2006 and Liang et al., 2017). Furthermore, although agricultural management practices often disturb soil structure, they simultaneously enhance clay enrichment and aggregate formation, thereby providing effective physical protection for the long-term stabilization of fungal-derived C (Chen et al., 2020; Mou et al., 2021; Zhou et al., 2023).".
For further details, please see Lines 344–365 of the revised manuscript.

The Discussion would benefit from a sharper focus on the novelty of this study. Currently, the overemphasis on aligning with previous findings (e.g., Lines 305–306, 340–341) detracts from highlighting the new insights. This is apparent in Section 4.1, where the interpretation of results, such as the elevated FNC and BNC in agricultural ecosystems, needs more mechanistic depth. The authors should use their own analytical evidence (e.g., from BRT and SEM on C/N ratio and clay content) to explain these patterns, rather than merely stating them. The discussion should use prior literature to frame the study's unique conclusions, not just to confirm them.

Response: Following the constructive comment, we have reorganized and revised some parts of Section 4.1 in the manuscript. The updated text now reads:
"Although this general pattern has been reported in previous studies (Liang et al., 2019; Wang et al., 2021a; Zhang et al., 2023; Ding et al., 2024), the systematic differences in the magnitude of these contributions between agricultural and natural ecosystems—and their underlying drivers—have remained poorly understood. Our study not only

confirms the broad pattern but also elucidates these ecosystem-level disparities and their environmental determinants. Consistent with our finding that the contribution of fungal necromass carbon (FNC) to SOC exceeded that of bacterial necromass carbon (BNC) in both ecosystem types (Table 1), the predominance of fungal necromass may be attributed to its more recalcitrant cell wall composition (e.g., chitin) and slower decomposition rate (Wang et al., 2021a). Our BRT and SEM analyses further identified soil clay content and C/N ratio as key drivers of FNC accumulation (Figs. 4a, 5a), reinforcing the importance of organo-mineral associations in the stabilization of fungal-derived carbon.".

For further details, please refer to Lines 275–287 of the revised manuscript.

Furthermore, we have reorganized and revised the specific paragraph (contained the content in Lines 340–341 of the original manuscript), strengthening the support for our findings by integrating relevant pre-existing literature. The updated text now reads:

"Furthermore, nutrient-rich conditions prevalent in agricultural systems (e.g., due to fertilization) often select for bacterial-dominated communities, as many bacteria exhibit *r*-strategist traits that support rapid growth under high resource availability. In contrast, natural ecosystems—characterized by lower nutrient availability and greater resource heterogeneity—tend to favor fungal dominance, since fungi often function as *K*-strategists with higher efficiency in decomposing complex organic matter under resource-limited conditions (Strickland & Rousk, 2010; Yu et al., 2022). This shift in microbial community composition is reflected in our results, which show a significantly higher FNC/BNC ratio in natural ecosystems across our global dataset (Figure 2c, Table 1). A high FNC/BNC ratio signifies a fungal-dominated decomposition pathway. Fungal necromass—rich in recalcitrant compounds such as chitin—is more resistant to decay, and fungal hyphae play a key role in the formation of stable soil aggregates that physically protect organic matter from degradation (Lenardon et al., 2007). This pathway promotes the formation of stable, long-turnover SOC pools essential for long-term carbon sequestration (Six et al., 2006; Lehmann et al., 2020). Furthermore, fungi generally exhibit higher carbon use efficiency than bacteria, meaning a larger proportion of assimilated carbon is allocated to biomass production (and subsequently necromass) rather than being respired as CO_2 (Wang & Kuzyakov, 2024). Thus, the fungal-driven pathway characteristic of natural ecosystems represents a highly efficient conversion of plant litter into persistent soil organic matter (Kallenbach et al., 2016; Malik et al., 2016). Conversely, the lower FNC/BNC ratio observed in agricultural ecosystems reflects a bacterial-dominated pathway, accelerated by practices such as tillage and nutrient amendments. This pathway is associated with faster carbon cycling and greater carbon loss through respiration. Although microbial necromass can accumulate under these conditions—sometimes contributing more significantly to a reduced total SOC pool—the resulting carbon is often less stabilized (Zhou et al., 2023). Therefore, the FNC/BNC ratio serves not merely as a descriptive metric, but as a functional biomarker that elucidates fundamental differences in the stability and persistence of SOM between managed agricultural systems and natural ecosystems.".

For further details, please refer to Lines 306–335 of the revised manuscript.

Minor concerns
Line 21: Delete this sentence.
Response: Done.

Lines 78–81: Suggest change into "Previous studies indicated that the contributions of FNC and BNC to SOC depended on the type of ecosystems (Wang et al., 2021a; Cao et al., 2023; Xu et al., 2024)."
Response: Thanks. We have rewritten in the revised manuscript (Lines 77–79).

Lines 126–127: Natural ecosystems include grasslands and forests. What habitats does the agricultural ecosystem consist of? Please clarify this carefully.
Response: Thanks. We have explicitly classified the agricultural ecosystem into dry land, irrigated cropland, and submerged paddy. For further details, please refer to Lines 123–124 of the revised manuscript.

Lines 182–183: Why is the threshold for the variance inflation factor set at 3.3 instead of the more common 5 or 10 that we commonly used?

Response: Thank you for this insightful and important comment. The choice of a more conservative Variance Inflation Factor (VIF) threshold of 3.3, as opposed to the more commonly cited values of 5 or 10, was a deliberate decision to ensure the robustness and reliability of our models by more rigorously minimizing multicollinearity.

The detailed justification for selecting this specific threshold of 3.3 as following:

(1) Conventional Thresholds and Their Implications. (a) VIF < 10: This is a very lenient standard, more common in earlier statistical applications. It implies that 90% of an independent variable's variance can be explained by the other independent variables (since 1 - 1/10 = 0.9). In modern research demanding higher model precision, this threshold is often considered too permissive and may fail to effectively eliminate problematic collinearity. (b) VIF < 5: This is a moderate and frequently used standard, deemed acceptable in many fields. It indicates that up to 80% of a variable's variance is explained by others. This threshold often provides a reasonable balance in many situations.

(2) Rationale for a Stricter Threshold (VIF < 3.3). Our reference to Kock (2015) is pivotal here. This literature advocates for and substantiates the necessity of a stricter VIF threshold, primarily based on the following points: (a) although this study ultimately uses BRTs and SEM, the threshold proposed by Kock (2015) was initially developed within the context of Partial Least Squares Structural Equation Modeling (PLS-SEM) for comprehensive collinearity assessment. This concept has since been adopted by numerous researchers and applied to a wider range of multivariate statistical models as a gold standard for ensuring predictor independence; (b) A variable with a VIF of 5 still has 20% of its variance inflated by other variables in the model. This remains a non-negligible

proportion that can distort regression coefficient estimates, making them unstable and difficult to interpret. Setting the threshold at 3.3 ensures that no more than approximately 30% of any predictor's variance is explained by others ($1 - 1/3.3 \approx 0.7$). This more effectively guarantees that the influence of each predictor on the response variable is relatively independent, leading to more reliable and trustworthy model outcomes; (c) In ecology and environmental sciences, many environmental drivers (e.g., temperature, precipitation, soil nutrients) are inherently correlated. Employing a strict VIF threshold proactively addresses these issues during the variable selection stage. This ensures that the "most important factors" subsequently identified in the Boosted Regression Trees and Structural Equation Models are genuinely influential, not merely appearing significant due to high correlations with other excluded variables. This significantly strengthens the robustness of the study's conclusions.

Therefore, our selection of the threshold value of 3.3 was not an arbitrary choice, but was grounded in established literature and driven by our commitment to more stringent criteria for data integrity and model robustness.
We sincerely hope this clarification adequately addresses your concern.

Lines 230–233: Suggest delete this sentence. Just provide an objective description of the result, without delving into other details.
Response: Thanks. We have deleted the sentence, and revised the respective section to provide a more objective description of the results. For further details, please refer to Lines 235–252 of the revised manuscript.

Lines 286–296: This section contains too much overlap with the introduction and results sections. Suggest delete it.
Response: Done.

Lines 300–302: Delete this sentence.
Response: Done.

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