

Global long-term agricultural experiments reveal consequences of mineral fertilization for soil microbiomes

Corresponding Author: Professor Huaiying Yao

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This study presents a meta-analysis based on a comprehensive dataset (501 experiments) to investigate the effects of long-term (>5 years) mineral fertilization on soil carbon, nitrogen, crop yield, pH, and microbial composition. The authors report that mineral fertilization decreases soil pH while increasing soil carbon. Notably, the study provides global-scale evidence for shifts in soil microbial composition in response to long-term mineral fertilization.

Overall, this is an interesting and well-executed study. The design is robust, and the findings are novel, timely, and important. However, I have several concerns that should be addressed before the manuscript can be considered for publication. Below, I outline the major points for revision.

General comments:

1 The authors should be cautious in using the term “trade-off” to describe the simultaneous increase in soil organic carbon and decrease in soil pH under mineral fertilization. While both patterns are observed, there is no direct evidence demonstrating that these two responses are mechanistically linked in a trade-off relationship. As such, this remains speculative. Presenting this as the first key result may give it undue emphasis.

2 the term “long-term experiment” should be clearly defined early in the Abstract or Introduction, rather than only in the Methods section.

3 The current introduction focuses primarily on the effects of mineral fertilization on soil microbial communities, while largely overlooking other key soil functions such as nutrient dynamics, organic carbon storage, and pH regulation, all of which are also analyzed in the results.

4 I agree that the manuscript has high novelty with its focus on long-term (>5 yrs) mineral fertilization effects on soil microbial composition, but the authors should more thoroughly situate their contribution within the existing literature. A considerable number of previous meta-analyses have explored fertilization effects on soil microbial diversity or composition. The authors should explicitly cite these and then clearly articulate how their work differs to justify the claimed novelty.

5 It is weird to put the conclusion before results (Line 104-118)

6 Several parts of the discussion are overly speculative. In addition, some statements are not supported by citation. See specific comments for detail.

7 The statistical approach requires clarification. It is not clearly stated which variables were used in multivariate model. In addition, the rationale for selecting the specific predictors (pH, SOC, MAT, MAP, and N addition rate) for mapping should be provided. Whether multicollinearity among these predictors was assessed?

Specific comments:

Line 75-77 The two sentences in this section appear to be repetitive

Line 133-134 citation?

Line 139-143 The authors state that mineral N fertilization more effectively promotes the accumulation of microbially derived SOC. However, increased microbial biomass is a downstream effect of greater plant productivity. If so, why would mineral N fertilization preferentially enhance microbial-derived SOC over plant-derived SOC?

Line 146 what is balanced, how to define this, be clear

Line 165 citation?

Line 166 Do all compound fertilization treatments include P? Were there any treatments that included only N and K, without P? Be clear

Line 183 not SOC, greater crop inputs should be the main reason

Line 188-190 too speculative

Line 205-206 statistic approach need to use account for variation in sequencing depth and sampling effort

Line 260 I do not see trade-off

Line 263-265 The negative association described here is not surprising, as it is a mathematical consequence of the log response ratio calculation. Specifically, if $c = \ln(a/b)$, then c will always be negatively correlated with b .

Early career researcher comments:

This study integrates data from 11 long-term fertilization trials across China and previous long-term experiments and reports a global trend in which mineral fertilization induces soil acidification, increases soil organic carbon accumulation, microbial biomass, and nitrogen- and phosphorus-acquisition enzymes without decreasing microbial richness or promoting fungal pathogens. The study involves substantial and careful work and presents important large-scale findings. However, the manuscript would benefit from better organization in the Introduction and a more in-depth Discussion of the results.

Abstract

Lines 50-52: The abstract does not sufficiently emphasize the key conclusions. For example, the sentence “We found major shifts in dominant soil taxa with the relative abundance of Proteobacteria increasing and that of Firmicutes declining with acidification” is descriptive but lacks interpretation. It is suggested to explicitly state what this pattern indicates in terms of ecological processes or implications. Or maybe just delete it in the abstract if it is not the main result.

Introduction

It would be better to move the importance of soil microbes forward. The first paragraph could be improved by addressing the importance of soil microbes in regulating ecosystem processes and linking this background more directly to the study's objectives.

Figures

Figures are somewhat difficult to locate without labeling next to the figure panels.

Fig. 4c: Please clarify what “S1–S11” represents.

Results and discussion

The responses of soil properties, microbial communities, and functions appear to differ between single mineral N and compound mineral N fertilization treatments. This difference seems noteworthy. Please consider briefly addressing it in the Conclusion or Abstract.

Line 106: The phrase “trade-offs in soil biochemistry and microbial communities” is somewhat confusing. The analysis presented does not clearly demonstrate real trade-offs; please consider clarifying or revising.

Line 130: Please provide the corresponding figure or table reference (e.g., Supplementary Fig. 1?).

Lines 137-139: Again, please specify the corresponding figure (perhaps Supplementary Fig. 1?).

Lines 168-173: The explanation for the independence of C-mineralizing enzyme activity from mineral fertilization is insufficient. Why is there an apparent decoupling between increased N- and P-acquisition enzyme activities and unchanged C-mineralization enzymes? Conceptually, wouldn't abundant nutrients promote C-mineralization enzyme activity, as microbes have sufficient nutrients to produce enzymes that decompose carbon substrates, given that the original resources are limited?

Lines 181-182: The sentence “This is expected as croplands are characterized by frequent disturbance and nutrient availability that favor bacterial dominance” seems incomplete. In addition, the logic is unclear — how does bacterial dominance in croplands explain an increase in bacterial PLFAs but not fungal PLFAs? Dominance in the baseline community does not necessarily lead to that. Please clarify this reasoning.

Lines 197-220: This section initially attributes the absence of fertilization effects on microbial richness and pathogenic fungi merely to data consistency and interpretability. The latter part largely restates the results without deeper analysis. A more critical discussion of the mechanisms or ecological implications underlying these patterns would strengthen the manuscript.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)

This is a very comprehensive meta-analysis of the effects of mineral fertilizers on soil microbiomes and soil chemistry, including carbon content. The combination of field surveys and literature synthesis comprises a significant effort. It is unfortunate that soil sampling was conducted only in China, given that this is an international paper (though I understand that this was led by Chinese institutions).

Some of the results are expected and well known, particularly acidification, though there is nuance here in that the greatest pH change was found in neutral soils. There are many interesting observations in the results, including the changes in soil viral communities. Data analyses are robust and comprehensive throughout. The Supplementary Materials are informative. Long term carbon storage in soils is dependent on formation of recalcitrant compounds. Do the data collated here enable differentiation between labile and recalcitrant fractions?

I was not fully convinced by the a priori hypothesis that mineral fertilization promotes plant pathogenic fungi, and therefore was not surprised that this hypothesis was rejected by the analysis. The review paper cited in support of this hypothesis (Wang et al., 2025) itself draws primarily on two empirical studies, one conducted in grasslands and the other on the abandonment of arable fields. Neither study directly addresses the specific question of whether mineral fertilization in actively managed croplands favours plant pathogenic fungi. In this context, the absence of a consistent fertilization effect on pathogen prevalence in the present synthesis appears entirely plausible.

If there is a weakness here, it is that the obvious comparison (reported in many other studies in several meta-analyses) is with the effects of organic fertilizers, particularly manure. Organic fertilizers result in substantially greater microbial biomass carbon than mineral fertilizers (Lori et al., 2017) and improved nutrient cycling (Luo et al., 2018). Mineral fertilizer production is the most important contributor of agricultural greenhouse gas emissions; if we want to reduce these emissions, then switching to organic fertilizer (along with more efficient use of mineral fertilizer) is the way. All agriculture utilizes fertilization to enhance crop growth, and more crop growth result in increased carbon flow to the rhizosphere and hence greater microbial biomass. The question of what changes in soils when mineral fertilizer is added is therefore somewhat academic, though still mechanistically interesting. Approximately the same number of people are supported by organic and mineral fertilizer globally. Hence, a brief discussion comparing the results in this paper, with results published elsewhere on organic/mineral comparison, would be helpful.

Lori, M., Symnack, S., Mäder, P., Deyn, G.D., Gättinger, A., 2017. Organic farming enhances soil microbial abundance and activity—A meta-analysis and meta-regression. *PLOS ONE* 12, e0180442. <https://doi.org/10.1371/journal.pone.0180442>

Luo, G., Li, L., Friman, V.-P., Guo, J., Guo, S., Shen, Q., Ling, N., 2018. Organic amendments increase crop yields by improving microbe-mediated soil functioning of agroecosystems: A meta-analysis. *Soil Biology and Biochemistry* 124, 105–115. <https://doi.org/10.1016/j.soilbio.2018.06.002>

Wang, G., van der Putten, W.H., Klironomos, J., Zhang, F., Zhang, J., 2025. Steering plant-soil feedback for sustainable agriculture. *Science* 389, eads2506. <https://doi.org/10.1126/science.ads2506>

(Remarks on code availability)

I was not able to access the code at that URL (403 Forbidden).

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

We have reviewed this manuscript before (Referee 1&2), so I will take it from there.

It is good to see this revised submission and the detailed responses to my earlier comments. Thank you for addressing those points. The manuscript has clearly improved, and my major previous concerns have largely been resolved. However, I still have a few minor concerns.

Lines 72 to 73: Please delete “plant-soil feedbacks,” as I do not think this study directly addresses plant-soil feedbacks.

Lines 70 to 73: It is unclear what “these changes” refers to. Does it mean changes in soil microbial communities? More importantly, the routine use of mineral fertilizers can itself strongly affect soil functions, including carbon sequestration through increased inputs, rather than only indirectly through shifts in soil microbial communities.

Comment 4: My earlier suggestion was to highlight the differences between the current study and previous studies in order to better demonstrate the novelty of this work, rather than to emphasize how it complements previous work.

Comment 7: Some predictors, such as MAT and MAP, as well as N addition rate, and pH, are intuitively likely to be strongly correlated, which raises concerns about multicollinearity. Did the authors test models with these predictors entered separately? A VIF of 5.28 is still quite high.

Line 210: Please clarify how sequencing depth and sampling effort were accounted for in the Methods. Also, in my earlier comment, I meant that the authors should account for soil sampling depth, for example 0 to 5 cm versus 5 to 20 cm, in the model.

In addition, the statistical methods are not described clearly enough. Please report all packages in the appropriate section and provide a clearer description of how the models were fitted and used in the analysis.

Co-reviewer

Thank the authors for their careful revisions. The manuscript has improved, particularly in the reorganization of the Introduction and the expanded Discussion, and most of my previous comments have been addressed satisfactorily. I still have one concern regarding the interpretation of enzyme responses.

Lines 163-168: The proposed explanation for the decoupling between increased N- and P-acquisition enzymes and unchanged C-mineralization enzymes is plausible, but it appears largely inferred rather than directly supported by measurements in this study. In particular, if fertilization alleviates nutrient limitation, it remains unclear why N- and P-acquisition enzymes increased rather than decreased. Framing this pattern in terms of microbial stoichiometric balancing may provide a clearer interpretation.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)

The authors addressed my previous comments.

(Remarks on code availability)

I had a quick look at the R code for the meta-analysis, it appears to be well formulated but I did not run it.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Thank you. We do not have any further comments.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

(Remarks on code availability)

Open Access This Peer Review File is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

Authors' Response to Reviewer #1

General Comments. This study presents a meta-analysis based on a comprehensive dataset (501 experiments) to investigate the effects of long-term (>5 years) mineral fertilization on soil carbon, nitrogen, crop yield, pH, and microbial composition. The authors report that mineral fertilization decreases soil pH while increasing soil carbon. Notably, the study provides global-scale evidence for shifts in soil microbial composition in response to long-term mineral fertilization. Overall, this is an interesting and well-executed study. The design is robust, and the findings are novel, timely, and important. However, I have several concerns that should be addressed before the manuscript can be considered for publication. Below, I outline the major points for revision.

Response: We thank you for your careful and positive evaluation of our study. We aimed to address all your concerns below. Statements that explicitly or implicitly implied a “trade-off” were rephrased, ambiguous wording was clarified, missing citations were added, overly speculative discussion was removed, and the statistical analyses were corrected or refined where needed. Detailed point-by-point responses to your comments are provided below. We appreciate your comments, which helped improve the clarity and rigor of the manuscript.

Comment 1

The authors should be cautious in using the term “trade-off” to describe the simultaneous increase in soil organic carbon and decrease in soil pH under mineral fertilization. While both patterns are observed, there is no direct evidence demonstrating that these two responses are mechanistically linked in a trade-off relationship. As such, this remains speculative. Presenting this as the first key result may give it undue emphasis.

Response: We fully agree with your point that, while both patterns are observed, there is no direct evidence demonstrating that these two responses are a “trade-off” relationship. We have therefore revised the wording in the Abstract, Results and Discussion to remove the use of “trade-off”. The revised manuscript no longer contains any use of the term “trade-off”. Please see our detailed responses below.

Comment 2

The term “long-term experiment” should be clearly defined early in the Abstract or Introduction, rather than only in the Methods section.

Response: We appreciate the suggestion. We have now defined the “long-term experiment” in the Introduction (L82) as “long-term experiments (LTEs; ≥ 5 years since establishment)” and shortly mentioned in the Abstract (L47) as “a global dataset of 501 long-term agricultural experiments (≥ 5 years, median duration 25 years)”.

Comment 3

The current introduction focuses primarily on the effects of mineral fertilization on soil microbial communities, while largely overlooking other key soil functions such as nutrient dynamics, organic carbon storage, and pH regulation, all of which are also analyzed in the results.

Response: Thank you. Your suggestion strengthened our Introduction. In line with Reviewer #2, Comment 2, we balanced the statements of soil microbial communities and other key soil functions, and revised the following two statements accordingly:

L67-73: Routine use of mineral fertilizers has transformed agricultural soils, as intermittent inputs of water-soluble nutrients and sustained increases in plant productivity have jointly shaped distinctive belowground microbial communities³. These changes are increasingly associated with functions central

to fertilized agroecosystems, including the decomposition of organic matter, the sequestration of organic carbon, and plant-soil feedbacks⁴.

Comment 4

I agree that the manuscript has high novelty with its focus on long-term (>5 yrs) mineral fertilization effects on soil microbial composition, but the authors should more thoroughly situate their contribution within the existing literature. A considerable number of previous meta-analyses have explored fertilization effects on soil microbial diversity or composition. The authors should explicitly cite these and then clearly articulate how their work differs to justify the claimed novelty.

Response: Thank you for the suggestion. The effects of mineral fertilization on soil microbial biomass and activities¹, as well as on bacterial community composition², have been addressed in two relevant studies (also classified as hot papers by WoS). We now explicitly cite both papers and clearly articulate how our work differs from them at distinct points, thereby clarifying the novelty of our study.

1. Geisseler, D. & Scow, K. M. Long-term effects of mineral fertilizers on soil microorganisms – a review. *Soil Biol. Biochem.* **75**, 54-63 (2014).
2. Dai, Z. *et al.* Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of *Actinobacteria* and *Proteobacteria* in agro-ecosystems across the globe. *Glob. Change Biol.* **24**, 3452-3461 (2018).

L200-202 (Cite the *Soil Biol. Biochem.* paper): Our study complements previous work by showing that increases in microbial biomass under long-term mineral fertilization are primarily driven by bacterial components and depend on agroecosystem type³.

L261-264 (Cite the *Glob. Change Biol.* paper): Building on previous reports of increased *Proteobacteria* under mineral fertilization¹², our results indicate that

soil acidification is the main cause of shifts in bacterial taxa, with virulent bacteriophages potentially contributing to microbial turnover.

Comment 5

It is weird to put the conclusion before results (Line 104-118).

Response: Thank you. We agree that this section was redundant, and we have therefore removed it. The Conclusions subsection sufficiently covers this content.

Comment 6

Several parts of the discussion are overly speculative. In addition, some statements are not supported by citation. See specific comments for detail.

Response: We have revised the statements you considered overly speculative and added the missing citations accordingly. Please see our detailed responses to the comments below.

Comment 7

The statistical approach requires clarification. It is not clearly stated which variables were used in multivariate model. In addition, the rationale for selecting the specific predictors (pH, SOC, MAT, MAP, and N addition rate) for mapping should be provided. Whether multicollinearity among these predictors was assessed?

Response: We agree that a clarification of statistical approach was needed here. Predictor selection was hypothesis-driven. Soil pH and soil organic carbon were included as proximal soil regulators reflecting fertilization-induced acidification and carbon accumulation. Mean annual temperature and precipitation were selected to account for large-scale climatic constraints on microbial activity, and the annual rate of mineral N addition was included to quantify fertilization intensity. We tested for collinearity among environmental covariates (predictors)

using variance inflation factor (VIF) analysis, which indicated no severe collinearity according to a general rule of thumb¹.

1. Kim, J. H. Multicollinearity and misleading statistical results. *Korean J Anesthesiol* **72**, 558-569 (2019).

VIF analysis results:

pH	SOC	MAT	MAP	N addition rate
2.303	1.170	3.473	5.275	1.091

And we clarify this in the Methods:

L566-574: Each GAM followed the structure: $\ln RR = s(\text{pH}) + s(\text{Soil organic C}) + s(\text{Mean annual temperature}) + s(\text{Mean annual precipitation}) + s(\text{Annual rate of mineral N addition}) + s(\text{Longitude, Latitude})$. The term $s()$ denotes a smooth function capturing the non-linear influence, and $s(\text{Longitude, Latitude})$ is a two-dimensional thin-plate spline accounting for spatial autocorrelation. Soil pH and soil organic C represent proximal soil regulators, mean annual temperature and precipitation capture large-scale climatic constraints, and the annual rate of mineral N addition quantifies fertilization intensity. Multicollinearity among predictors was assessed using variance inflation factors (VIFs), which ranged from 1.09 to 5.28, indicating no severe multicollinearity.

Comment 8

Line 75-77 The two sentences in this section appear to be repetitive.

Response: Thank you for pointing out this repetitive writing. We have replaced the two sentences as follows:

L84-88: Many LTEs, such as the iconic Broadbalk experiment at Rothamsted, were established to address production-oriented questions (*e.g.*, crop yield and fertilizer performance) and follow regional agronomic practices, making them

robust proxies for commercial farming systems across thousands of hectares^{10,11}.

Comment 9

Line 133-134 citation?

Response: We apologize for the omission of a reference here. The missing citation has now been added in the revised manuscript (now Ref. 18).

18. Minasny, B. *et al.* Soil carbon 4 per mille. *Geoderma* **292**, 59-86 (2017).

Comment 10

Line 139-143 The authors state that mineral N fertilization more effectively promotes the accumulation of microbially derived SOC. However, increased microbial biomass is a downstream effect of greater plant productivity. If so, why would mineral N fertilization preferentially enhance microbial-derived SOC over plant-derived SOC?

Response: Thank you, this is a good point. We totally agree that increased microbial biomass can be a downstream consequence of higher plant productivity. That is, the use of mineral fertilizers on croplands may improve crop productivity, causing increased plant-derived C inputs to soil, thereby indirectly stimulating soil microbial biomass C. We are sorry that our original wording was ambiguous and could be interpreted as implying a preferential enhancement of microbial-derived SOC. Our intention was not to suggest that microbial biomass C itself, nor that microbial-derived C preferentially, constitutes a major fraction of SOC, and we have replaced the previous misleading statement as follows :

L131-137: Compound mineral fertilizers (*e.g.*, NP, NK, or NPK) supported greater SOC accumulation than sole N application ($p < 0.001$, Supplementary Fig. 1). This increase was associated with higher crop productivity (lnRR = 0.97) and microbial biomass (lnRR = 0.13) under compound fertilization regimes

compared with sole N fertilizer application ($p < 0.001$, Supplementary Figs. 1 and 3). These concurrent increases suggest that nutrient inputs from compound fertilizers may be more effective than N fertilizer alone at offsetting SOC losses in intensively managed croplands^{19,20}.

Comment 11

Line 146 what is balanced, how to define this, be clear.

Response: Sorry for the unclear definition. Here, we have now clarified the meaning of “balanced” and revised the text as follows:

L139: While not the focus of our analysis, these co-benefits reinforce the role of balanced mineral fertilization (N with P and/or K) in sustaining agroecosystem productivity in the long term.

Comment 12

Line 165 citation?

Response: Thank you for pointing this out. We inadvertently omitted a reference here, which has now been added in the revised manuscript (now Ref. 24).

24. Guo, L. *et al.* Unveiling pervasive soil microbial P limitation in terrestrial ecosystems worldwide. *Ecol. Lett.* **27**, e70011 (2024).

Comment 13

Line 166 Do all compound fertilization treatments include P? Were there any treatments that included only N and K, without P? Be clear

Response: Thank you for raising this important point. Not all compound fertilization treatments in our dataset included P; a very small subset received NK treatments only. However, it is important to note that in the enzyme activity dataset shown in Supplementary Fig. 2, only 4 data points originate from NK

treatments, because enzyme measurements were largely unavailable for NK treatments. We have replaced sentences as follows:

L159-162: When only N is supplied, other nutrients, particularly P, may become limiting and restrict microbial growth and enzymatic activity²². In contrast, compound fertilization (NP or NPK) provides additional nutrients (P and/or K), which can alleviate nutrient constraints, stimulate microbial activity and nutrient demand, and thereby increase investment in nutrient-acquisition enzymes²⁵.

Also, we conducted a sensitivity analysis excluding NK (4 data points in dehydrogenase and phosphatase), and results were consistent (please see table below). We hope this addresses the concerns.

Enzyme	Single minN			Compound minN			Single minN			Compound minN (-NK)		
	estimate	CI.lb	CI.ub	estimate	CI.lb	CI.ub	estimate	CI.lb	CI.ub	estimate	CI.lb	CI.ub
BG	0.07	-0.17	0.32	0.30***	0.13	0.47	No NK treatments included					
NAG	0.16	-0.19	0.52	0.44***	0.25	0.63	No NK treatments included					
LAP	-0.10	-0.41	0.20	0.30**	0.09	0.50	No NK treatments included					
DHA	0.24	-0.09	0.58	0.39**	0.10	0.67	0.23	-0.09	0.57	0.39**	0.10	0.68
PHO	0.11	-0.08	0.31	0.24***	0.13	0.36	0.09	-0.10	0.29	0.25***	0.14	0.37

Abbreviations: BG, β -D-glucosidase; NAG, N-acetylglucosaminidase; LAP, leucine aminopeptidase; DHA, dehydrogenase; PHO, phosphatase; CI.lb, lower bound of confidence interval; CI.ub, upper bound of confidence interval.

Comment 14

Line 183 not SOC, greater crop inputs should be the main reason

Response: We agree and have revised the wording to clarify that increased bacterial PLFAs are primarily linked to greater crop-derived carbon inputs under long-term fertilization, with SOC serving as an integrative indicator. We have changed the sentence to say:

L192-195: The increase in bacterial PLFAs was associated with enhanced crop-derived C inputs under long-term fertilization, with elevated SOC coinciding

with increased substrate availability and a higher abundance of copiotrophic *Gammaproteobacteria* (Fig. 4)^{36,37}.

Comment 15

Line 188-190 too speculative.

Response: Yes, we now realize that this statement was reaching a little too far, and we have therefore removed this speculative statement from the revised manuscript.

Comment 16

Line 205-206 statistic approach need to use account for variation in sequencing depth and sampling effort.

Response: Thank you for pointing this out. We agree and have revised the sentence into:

L210: Estimators based on discrete counts need to account for variation in sequencing depth and sampling effort to ensure comparability across studies⁴¹.

Comment 17

Line 260 I do not see trade-off.

Response: Yes, we have removed the term “trade-off” throughout the manuscript, and the corresponding sentence here has been revised to:

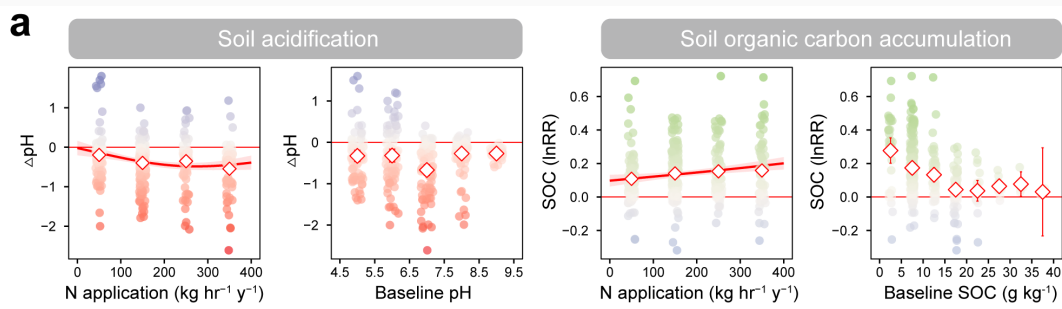
L271-274: We showed that fertilizer-induced soil acidification and SOC accumulation independently contributed to shifts in bacterial phyla and increases in microbial biomass (Fig. 4). We therefore examined whether the responses of soil acidification and SOC accumulation to mineral fertilization were consistent across LTEs.

Comment 18

Line 263-265 The negative association described here is not surprising, as it is a mathematical consequence of the log response ratio calculation. Specifically, if $c = \ln(a/b)$, then c will always be negatively correlated with b .

Response: Thank you for bringing the ambiguity here to our attention. We agree this was a clumsy sentence and have therefore deleted it. We also recognized the potential for misinterpretation in the baseline panels and revised them by removing the GAM trends. These panels now show only binned group means with 95% confidence intervals, presenting the results descriptively rather than implying functional relationships.

Figure 5a:



(a) The magnitude of soil acidification (ΔpH) and soil organic carbon (SOC) accumulation ($\ln\text{RR}$) increased with higher fertilization intensity, and the strongest average responses were observed in soils with neutral baseline pH and low baseline SOC, respectively. Red diamonds denote bin-averaged means with 95% confidence intervals. For the panels showing N application, curves with shaded bands represent generalized additive model fits to all data points.

Concluding Response to Reviewer #1. We sincerely thank you for the positive and careful evaluation of our manuscript. In response, we (i) removed all references to “trade-offs” and now describe soil acidification and SOC accumulation as separate processes; (ii) clarified definitions, including “balanced” fertilization and “long-term experiment”; (iii) rebalanced the first paragraph of the Introduction to better integrate microbial processes with

other key soil functions; (iv) strengthened the articulation of novelty and dataset breadth through explicit comparison with existing studies; (v) corrected missing citations and revised or removed overly speculative or repetitive statements; (vi) revised the interpretation of SOC increases to emphasize greater crop-derived carbon inputs; and (vii) corrected an error in Fig. 5a and added missing statistical details in the multivariate analyses. Your constructive comments have helped us substantially improve the manuscript.

Authors' Response to Reviewer #2 (co-reviewed)

General Comments. This study integrates data from 11 long-term fertilization trials across China and previous long-term experiments and reports a global trend in which mineral fertilization induces soil acidification, increases soil organic carbon accumulation, microbial biomass, and nitrogen- and phosphorus-acquisition enzymes without decreasing microbial richness or promoting fungal pathogens. The study involves substantial and careful work and presents important large-scale findings. However, the manuscript would benefit from better organization in the Introduction and a more in-depth Discussion of the results.

Response: Thank you for the positive evaluation of our work and for recognizing the substantial effort and value of the dataset and analyses. This study represents nearly two years of work to compile and analyze data from long-term experiments. We have carefully revised the manuscript in response to your suggestions by improving the organization of the Introduction and substantially strengthening the Discussion (please see detailed responses below). We are grateful for your comments, which have helped to improve the clarity and depth of the manuscript.

Comment 1

Abstract Lines 50-52: The abstract does not sufficiently emphasize the key conclusions. For example, the sentence “We found major shifts in dominant soil taxa with the relative abundance of *Proteobacteria* increasing and that of *Firmicutes* declining with acidification” is descriptive but lacks interpretation. It is suggested to explicitly state what this pattern indicates in terms of ecological processes or implications. Or maybe just delete it in the abstract if it is not the main result.

Response: Thank you. Yes, this sentence was overly descriptive. In line with the suggestion of Reviewer #1, we have removed all sentences that explicitly or

implicitly referred to a “trade-off”. The effects of soil organic carbon accumulation and soil acidification are now described separately. We have also revised the relevant sentences to clarify that acidification exerts a stronger influence on bacterial communities than on fungal communities, while avoiding the introduction of speculative language in the Abstract. We have rephrased the Abstract as follows:

L44-62: Mineral fertilizers have sustained food security for decades, yet the long-term impacts on soil microbial communities underpinning soil health remain virtually unknown. We combined standardized field surveys and literature synthesis to assemble a global dataset of 501 long-term agricultural experiments (≥ 5 years, median duration 25 years) to evaluate the impacts of sustained mineral fertilization on soil properties and microbial communities. Long-term mineral fertilization increased soil organic carbon by 14% and decreased soil pH by 0.31 units on average relative to unfertilized controls. Soil organic carbon accumulation largely explained increased microbial biomass carbon and living bacterial biomass. Mineral fertilizer-induced acidification primarily reshaped dominant bacterial taxa, with the relative abundance of *Proteobacteria* increasing and *Firmicutes* declining, whereas fungal community composition remained stable. Virulent bacteriophages increased in association with shifts in bacterial hosts. Microbial activities revealed a decoupling of more nitrogen- and phosphorus-acquisition enzymes from the unchanged production of carbon-mineralization enzymes. Microbial communities were taxonomically reorganized without reducing richness or promoting fungal pathogens. These responses were evaluated in the context of agroecosystem type, fertilization regime, and cropping regime. Our findings provide global-scale evidence for the consequences of long-term mineral fertilization on soil health, which is integral to guiding fertilizer management for sustainable agriculture.

Comment 2

Introduction: It would be better to move the importance of soil microbes forward. The first paragraph could be improved by addressing the importance of soil microbes in regulating ecosystem processes and linking this background more directly to the study's objectives.

Response: Thank you for the suggestion. We have revised the first paragraph of the Introduction to foreground the relevance of soil microbial communities to long-term mineral fertilization. Specifically, we added text highlighting that repeated mineral fertilization can shape distinctive belowground microbial communities and is associated with functions central to fertilized agroecosystems, thereby linking microbial responses more directly to the objectives of this study (please also refer to Reviewer #1 Comment 3). We hope this revision improves the balance and clarity of the paragraph.

L64-80: Mineral fertilizers have revolutionized global agriculture since the 1950s, fueling unprecedented increases in crop yields that now sustain more than half of the world's population^{1,2}. While mineral fertilizers are calibrated to support crop productivity and quality, the nutrients also impact soil properties, microbiomes, and functions³. Routine use of mineral fertilizers has transformed agricultural soils, as intermittent inputs of water-soluble nutrients and sustained increases in plant productivity have jointly shaped distinctive belowground microbial communities³. These changes are increasingly associated with functions central to fertilized agroecosystems, including the decomposition of organic matter, the sequestration of organic carbon, and plant-soil feedbacks⁴. Adding mineral forms of nitrogen (N), phosphorus (P), and other nutrients overcomes the natural limitations to soil microbial growth. However, excessive fertilization may lead to long-term outcomes such as soil acidification and biodiversity loss^{4,5}. Mineral fertilizers may also increase the prevalence of fungal pathogens, a risk factor for plant disease⁶. While such outcomes are widely hypothesized and raise legitimate concerns, the

consequences of long-term mineral fertilization for microbial responses linked broadly to soil health have not yet been comprehensively assessed at the global scale^{7,8}.

Comment 3

Figures: Figures are somewhat difficult to locate without labeling next to the figure panels. Fig. 4c: Please clarify what “S1–S11” represents.

Response: Thank you for pointing this out. We have added labels to both main Figures 2 and 3 and revised the corresponding figure references in the Results and Discussion accordingly. The labels “S1–S11” in Fig. 4c refer to our standardized sampling sites, which is now clarified in the figure legend. In addition, Supplementary Table 1 has been updated to provide the correspondence between site codes and site names. To maintain figure clarity, we did not add site names directly to the figure and hope this is understandable.

Fig. 4. (c) Heatmap from standardized field surveys shows that most sites (S1–S11) exhibited increases in abundance of *Proteobacteria* and virulent phages and decreases in *Firmicutes* at the class level. Corresponding site names are provided in Supplementary Table 1. Colors denote log₂ fold change (mineral-fertilized relative to unfertilized), truncated at ± 0.5 .

Comment 4

Results and discussion: The responses of soil properties, microbial communities, and functions appear to differ between single mineral N and compound mineral N fertilization treatments. This difference seems noteworthy. Please consider briefly addressing it in the Conclusion or Abstract.

Response: We agree, thanks. We realized that the distinction between single N fertilization and compound fertilization was not sufficiently reflected in the original Abstract. We have therefore revised the Abstract to briefly introduce this

distinction and to signal the subgroup analyses by adding the sentence “These responses were evaluated in the context of agroecosystem type, fertilization regime, and cropping regime.”, while keeping the Abstract concise and free of detailed results.

Comment 5

Line 106: The phrase “trade-offs in soil biochemistry and microbial communities” is somewhat confusing. The analysis presented does not clearly demonstrate real trade-offs; please consider clarifying or revising.

Response: We fully agree. Consistent with the comment from Reviewer #1, we have removed all expressions related to “trade-off” in the revised manuscript. This paragraph has now been removed (as suggested by Reviewer #1 Comment 5).

Comment 6

Line 130: Please provide the corresponding figure or table reference (*e.g.*, Supplementary Fig. 1?).

Response: Sorry for this omission. We have now added the figure reference.

L123: Acidification was more pronounced in upland than in paddy soils ($p < 0.05$), due to greater H^+ production via nitrification and base cation leaching (Supplementary Fig. 1)¹⁷.

Comment 7

Lines 137-139: Again, please specify the corresponding figure (perhaps Supplementary Fig. 1?).

Response: We are sorry for it. We have added the figure reference.

L131: Compound mineral fertilizers (*e.g.*, NP, NK, or NPK) supported greater SOC accumulation than sole N application ($p < 0.001$, Supplementary Fig. 1).

Comment 8

Lines 168-173: The explanation for the independence of C-mineralizing enzyme activity from mineral fertilization is insufficient. Why is there an apparent decoupling between increased N- and P-acquisition enzyme activities and unchanged C-mineralization enzymes? Conceptually, wouldn't abundant nutrients promote C-mineralization enzyme activity, as microbes have sufficient nutrients to produce enzymes that decompose carbon substrates, given that the original resources are limited?

Response: Thank you for this comment. We agree that increased nutrient availability could, in principle, stimulate C-mineralizing enzyme production. As clarified in the revised text, recent evidence suggests that extracellular enzyme activities largely reflect microbial resource allocation under stoichiometric constraints rather than overall decomposition capacity. Under long-term fertilization, increased inputs of readily assimilable plant-derived carbon may reduce the incentive for investment in C- mineralization enzymes, while microbial allocation is toward N and P acquisition to alleviate remaining nutrient imbalances. This interpretation is consistent with the observed decoupling between enhanced N and P acquisition and unchanged C-mineralizing enzyme activities. We have revised and clarified this section as follows:

L163-180: Recent evidence suggests that extracellular enzyme activities primarily reflect microbial resource allocation under nutrient stoichiometric constraints rather than a general increase in decomposition capacity²⁶. In long-term fertilized croplands, microbes may preferentially invest in N and P acquisition to alleviate nutrient limitation, without increasing investment in C mineralizing enzymes. The suppression of C-acquiring activities over the N and P acquisition may be explained by the fact that under N enrichment C is no longer the primary limiting source²⁷. This is because long-term N fertilization generally increases plant biomass and is therefore often associated with higher plant-derived carbon inputs to soils, including increased root exudation²⁸. Root

exudates consist largely of low-molecular-weight, water-soluble compounds that are directly assimilable by microorganisms, reducing microbial reliance on complex organic C compounds that must undergo extracellular enzymatic decomposition²⁹. In such circumstances, abundant N could generate a source of simple plant C substrates, resulting in less production of C-mineralizing enzymes. Overall, such nutrient-driven C flow to the soil increases microbial growth efficiency and promotes the incorporation of C into microbial biomass, which produces byproducts and cellular debris that accumulate in SOC pools under compound mineral fertilization^{30,31}.

26 Li, S. *et al.* Phosphorus limitation regulates the responses of microbial carbon metabolism to long-term combined additions of nitrogen and phosphorus in a cropland. *Soil Biol. Biochem.* **200**, 109614 (2025).

27 Shao, J. *et al.* Long-term n fertilization decreases soil multifunctionality and decouples its relationships with the microbial community. *Appl. Soil Ecol.* **214**, 106371 (2025).

28 Tang, S. *et al.* Soil carbon sequestration enhanced by long-term nitrogen and phosphorus fertilization. *Nat. Geosci.* **18**, 1005-1013 (2025).

29 Jones, D. L., Hodge, A. & Kuzyakov, Y. Plant and mycorrhizal regulation of rhizodeposition. *New Phytol.* **163**, 459-480 (2004).

30 Averill, C. & Waring, B. Nitrogen limitation of decomposition and decay: How can it occur? *Glob. Change Biol.* **24**, 1417-1427 (2018).

31 Ramirez, K. S., Craine, J. M. & Fierer, N. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Glob. Change Biol.* **18**, 1918-1927

Comment 9

Lines 181-182: The sentence “This is expected as croplands are characterized by frequent disturbance and nutrient availability that favor bacterial dominance” seems incomplete. In addition, the logic is unclear — how does bacterial dominance in croplands explain an increase in bacterial PLFAs but not fungal PLFAs? Dominance in the baseline community does not necessarily lead

to that. Please clarify this reasoning.

Response: Thanks for your kind reminder. The term “bacterial dominance” used previously may have been imprecise. Regular disturbance in croplands (mainly tillage, but also harvesting operations, residue management, fertilization and fungicide application, irrigation) disrupt fungal hyphae but have less effect on soil bacteria. Also, larger N availability by fertilization is of advantage for fast-growing bacteria compared to their slower-growing fungal counterparts. Therefore, the microbial community composition in cropland soils shifted towards bacteria (increase in bacterial PLFAs but not of the fungal PLFAs) as compared to natural ecosystems. We have revised the text to make this logic clearer.

L186-192: Total bacterial PLFAs increased under long-term mineral fertilization ($p < 0.05$), whereas fungal PLFAs remained unchanged (Fig. 2c). Croplands are subject to regular disturbances (*e.g.*, tillage, harvesting, residue management and fungicide application), which disproportionately disrupt filamentous fungal hyphal networks, whereas bacteria are less affected³⁴. In addition, long-term fertilization increases mineral N availability, which generally favors fast-growing bacteria over fungi due to their slower growth and longer generation times³⁵.

Comment 10

Lines 197-220: This section initially attributes the absence of fertilization effects on microbial richness and pathogenic fungi merely to data consistency and interpretability. The latter part largely restates the results without deeper analysis. A more critical discussion of the mechanisms or ecological implications underlying these patterns would strengthen the manuscript.

Response: Thanks. We agree that the original version of this section placed too much emphasis on methodological considerations and insufficiently discussed the ecological implications underlying these patterns. We have revised this section as follows:

L204-243: *Long-term fertilization maintains microbial richness without promoting pathogenic fungi*

A widely held assumption is that long-term mineral fertilization, via soil acidification or ammonium oxidation (which releases H^+ ions), inevitably reduces microbial α -diversity⁴⁰. However, our analysis revealed no consistent decline in α -diversity, challenging the generality of this assumption in croplands (Fig. 2d). Interpretations of α -diversity metrics require caution. Estimators based on discrete counts need to account for variation in sequencing depth and sampling effort to ensure comparability across studies⁴¹. Estimators such as Chao1 and ACE are also prone to inflation by singletons and sequencing artifacts⁴². While our within-study effect sizes controlled for methodological heterogeneity through paired observations, they do not resolve the interpretational limitations of α -diversity metrics. Apparent α -diversity increases or decreases should not be overinterpreted as (un)favorable microbial consequences of long-term mineral fertilization⁴³. To complement α -diversity patterns, we assessed microbial community similarity based on relative abundance data, which capture variation in both composition and taxon dominance. Long-term mineral fertilization consistently shifted microbial community composition across sites (*i.e.*, centroid shift), while preserving within-treatment similarity (*i.e.*, unchanged β -dispersion) and α -diversity (Fig. 2d and Supplementary Fig. 5)⁴⁴. The stability of α -diversity and β -dispersion despite a clear centroid shift points to turnover among dominant taxa without erosion of overall diversity. This pattern is consistent with trophically structured, niche-differentiated soil microbial communities that accommodate nutrient enrichment through compositional reweighting rather than stress-induced attrition of specific groups⁴⁵. Together, these findings demonstrate that long-term mineral fertilization induces structured shifts in soil microbial composition by reshaping dominant taxa, yet when it is precisely

calibrated within well-managed croplands it remains compatible with the maintenance of biodiversity⁸.

We further examined fungal guilds to determine whether long-term mineral fertilization alters plant-microbe trophic interactions in croplands. A global synthesis in grassland ecosystems reported that mineral fertilization favors pathogenic over mutualistic fungi, yet this pattern did not emerge in our cropland LTE dataset (Fig. 3b)⁴⁶. Although some site-level studies have reported pathogen increases, the absence of a general trend across LTEs likely reflects differences in cropping systems, crop rotations, and baseline pathogen prevalence⁴⁷. While our analyses reveal compositional patterns of fungal guilds, a recent large-scale cropland study has complemented such approaches with soil suppressiveness assays to assess pathogen activity beyond community composition⁴⁸. Overall, mineral fertilization alone does not explain variation in pathogenic fungal guild abundance in croplands, underscoring that pathogen risk cannot be inferred from fertilization history or community composition alone.

Concluding Response to Reviewer 2. We thank you for your careful review of our manuscript. In response to your comments, we (i) carefully checked all figure references and reassigned the panel labels in main Figures 2 and 3 to improve clarity, and (ii) substantially expanded the relevant Results and Discussion sections to deepen the interpretation of underlying mechanisms and ecological implications where these aspects were previously insufficient. We hope these revisions address your concerns, and we thank you again for your constructive feedback.

Authors' Response to Reviewer #3

General Comments. This is a very comprehensive meta-analysis of the effects of mineral fertilizers on soil microbiomes and soil chemistry, including carbon content. The combination of field surveys and literature synthesis comprises a significant effort. It is unfortunate that soil sampling was conducted only in China, given that this is an international paper (though I understand that this was led by Chinese institutions). Some of the results are expected and well known, particularly acidification, though there is nuance here in that the greatest pH change was found in neutral soils. There are many interesting observations in the results, including the changes in soil viral communities. Data analyses are robust and comprehensive throughout. The Supplementary Materials are informative.

Response: We very much appreciate the positive and supportive comments and are pleased you find our data valuable. We also thank you to stimulate an interesting discussion here. We understand your concern about new data coming from only in China. Because soil sample importation is generally not permitted in China (see FAO guidance on soil import legislation¹), we established a coordinated sampling campaign across national long-term experiments (LTEs) and integrated these harmonized data with a global synthesis of published studies to identify consistent responses. Importantly, our dataset is not limited to sites sampled exclusively by our team in China, nor to data extracted only from the published literature. We also obtained original datasets directly from collaborators managing long-term experiments outside China (*e.g.*, Lelystad in the Netherlands, Gembloux in Belgium and Hamerstorf in Germany), which further improves the geographic coverage and representativeness of the overall dataset. Some components generated through our standardized sampling, such as large-scale sequencing data (*e.g.*, soil viral communities), are reported here for the first time. We sincerely hope that these newly generated datasets will help update current understanding and support future synthesis and hypothesis-driven research,

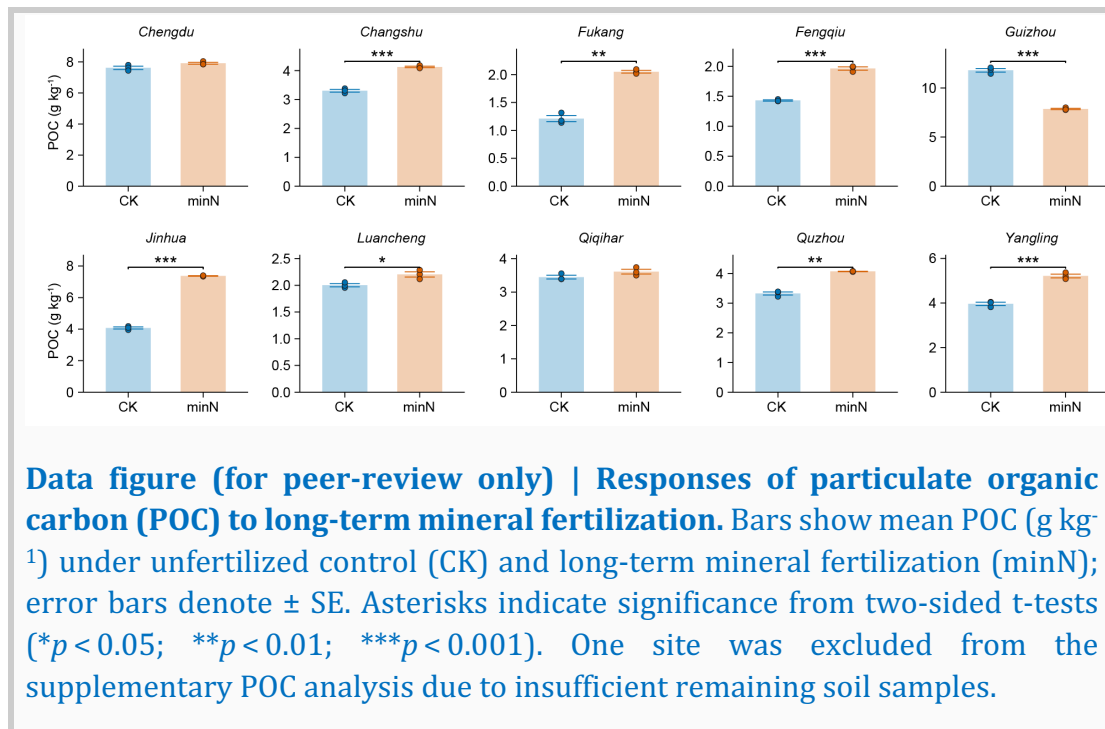
highlighting the long-term value in LTEs and the principles of slow science². We also wish to acknowledge the enduring commitment of those who have established, maintained and curated these long-term experimental sites.

1. <https://www.fao.org/global-soil-partnership/glosolan-old/simple-soil-import-legislation/custom-control-procedure-database/country/China/en/>
2. Owens, B. Long-term research: Slow science. *Nature* **495**, 300–303 (2013).

Comment 1

Long term carbon storage in soils is dependent on formation of recalcitrant compounds. Do the data collated here enable differentiation between labile and recalcitrant fractions?

Response: Thank you for your interest in the labile and recalcitrant fractions of SOC. We carefully re-examined all 225 primary studies included in our synthesis but identified only three that reported SOC fractionation data¹⁻³. During the revision, we therefore conducted additional new experiments and supplemented our dataset with newly generated measurements of the proportion of particulate organic carbon (POC). Although SOC fractionation is not a central focus of this study, we hope that these new results, based on our standardized sampling, help to address this question. We also noted a recently published meta-analysis that may interest you, reporting that N fertilization alone increased POC by 30%⁴. Our standardized sampling across experimental sites further showed that mineral fertilization increased POC (lnRR = 0.19), corresponding to an approximate 21% increase (please see Data figure below). Here, POC was determined using the fractionation method of Cambardella and Elliott⁵.



1. Liu, B. *et al.* 14 year applications of chemical fertilizers and crop straw effects on soil labile organic carbon fractions, enzyme activities and microbial community in rice-wheat rotation of middle China. *Sci. Total Environ.* **841**, 156608 (2022).
2. Hao, X., Han, X., Wang, S. & Li, L.-J. Dynamics and composition of soil organic carbon in response to 15 years of straw return in a Mollisol. *Soil Till. Res.* **215**, 105221 (2022).
3. Yang, F. *et al.* Functional soil organic matter fractions, microbial community, and enzyme activities in a Mollisol under 35 years manure and mineral fertilization. *J. Soil Sci. Plant Nutr.* **19**, 430-439 (2019).
4. Zhou, R. *et al.* Particulate and mineral-associated organic matter in cropland soils: Meta-analysis of management effects. *Agric. Ecosyst. Environ.* **400**, 110218 (2026).
5. Cambardella, C. A. & Elliott, E. T. Methods for physical separation and characterization of soil organic matter fractions. *Geoderma* **56**, 449-457 (1993).

Comment 2

I was not fully convinced by the a priori hypothesis that mineral fertilization promotes plant pathogenic fungi, and therefore was not surprised that this hypothesis was rejected by the analysis. The review paper cited in support of this hypothesis (Wang *et al.*, 2025) itself draws primarily on two empirical studies, one conducted in grasslands and the other on the abandonment of

arable fields. Neither study directly addresses the specific question of whether mineral fertilization in actively managed croplands favours plant pathogenic fungi. In this context, the absence of a consistent fertilization effect on pathogen prevalence in the present synthesis appears entirely plausible.

Response: We appreciate your recognition that rejecting this hypothesis is reasonable. Indeed, the idea that mineral fertilization (sometimes described as inorganic fertilization) promotes plant pathogenic fungi has become widespread in the literature and is often stated in introductions, despite limited empirical support from cropland systems. We are not entirely certain how this assumption became so pervasive, but it seems to have been largely accepted from studies conducted in grasslands, forests, and abandoned arable fields¹⁻³. Further, this narrative is sometimes reinforced by contrasts with organic fertilization⁴. Our study specifically targets well managed croplands and focuses on mineral fertilization as a management practice. Within this context, the hypothesis was not supported by our synthesis. While further work is certainly needed, we hope that our findings contribute to a more balanced interpretation of the evidence and help avoid overstating the ecological risks of mineral fertilizers in managed agroecosystems. Long-term inputs of mineral N fertilizer are integral to modern agriculture, and our goal is to evaluate the evidence pragmatically so future fertilizer management is guided by the science facts⁵.

1. Lekberg, Y., Arnillas, C.A., Borer, E.T. *et al.* Nitrogen and phosphorus fertilization consistently favor pathogenic over mutualistic fungi in grassland soils. *Nat. Commun.* **12**, 3484 (2021).
2. Hannula, S. E. *et al.* Shifts in rhizosphere fungal community during secondary succession following abandonment from agriculture. *ISME J.* **11**, 2294-2304 (2017).
3. Wu, Y. *et al.* Long-term nitrogen and sulfur deposition increased root-associated pathogen diversity and changed mutualistic fungal diversity in a boreal forest. *Soil Biol. Biochem.* **155**, 108163 (2021).
4. Wang, G., van der Putten, W. H., Klironomos, J., Zhang, F. & Zhang, J. Steering plant-soil feedback for sustainable agriculture. *Science* **389**, eads2506 (2025).

5. Nakweya, G. Lack of mineral fertilizer worsens crop nutrient depletion. *Nat. Africa*. **620**, 2023. <https://www.nature.com/articles/d44148-023-00312-y>.

Comment 3

If there is a weakness here, it is that the obvious comparison (reported in many other studies in several meta-analyses) is with the effects of organic fertilizers, particularly manure. Organic fertilizers result in substantially greater microbial biomass carbon than mineral fertilizers (Lori *et al.*, 2017) and improved nutrient cycling (Luo *et al.*, 2018). Mineral fertilizer production is the most important contributor of agricultural greenhouse gas emissions; if we want to reduce these emissions, then switching to organic fertilizer (along with more efficient use of mineral fertilizer) is the way. All agriculture utilizes fertilization to enhance crop growth, and more crop growth result in increased carbon flow to the rhizosphere and hence greater microbial biomass. The question of what changes in soils when mineral fertilizer is added is therefore somewhat academic, though still mechanistically interesting. Approximately the same number of people are supported by organic and mineral fertilizer globally. Hence, a brief discussion comparing the results in this paper, with results published elsewhere on organic/mineral comparison, would be helpful.

Response: We agree and thank you for your wise suggestion. We agree that comparisons between organic and mineral fertilization are widely discussed in the literature, and we acknowledge that organic fertilizers/amendments often enhance microbial biomass, soil organic stocks and nutrient cycling¹⁻². There is ongoing debate over the relative merits of organic and mineral fertilization, with organic inputs often associated with benefits for environmental impact, while mineral fertilizers play a central role in sustaining crop yields and global food production³. Thus, we recognize the value of placing our findings within the broader fertilization literature. We have therefore added a brief discussion comparing our results with existing studies on organic versus mineral fertilization.

The text added to the Discussion is as follows, supported by additional references (Refs. 57-61):

L337-356: Our findings are complementary to other literature debating the ecological consequences of organic and mineral fertilization strategies⁵⁶. Previous meta-analyses comparing the two types of fertilization have reported higher microbial biomass and activity under organic fertilization, commonly attributed to enhanced carbon stocks, lower soil acidification, and additional organic inputs⁵⁷⁻⁵⁹. Our results show that long-term mineral fertilization alone can also increase microbial biomass and activity, while maintaining microbial diversity without changing the occurrence of plant pathogenic fungi. Thus, our synthesis provides complementary evidence that sustained mineral fertilization reshapes soil microbiomes in croplands in ways that are not necessarily detrimental to key aspects of soil biological integrity. Combining organic inputs with mineral fertilization may help to sustain crop productivity while preserving soil biodiversity^{60,61}. Addressing remaining uncertainties will require global coordination in the integration of long-term data, as well as a greater emphasis on temporal resolution in microbial monitoring. One way to achieve this would be through collaborative networks such as the Global Long-Term Agricultural Experiment Network (GLTEN)^{9,62}. Despite these limitations, our synthesis leverages the unique temporal depth of long-term agricultural experiments to provide rare global evidence of how the belowground microbiome is reshaped by decades of mineral fertilization. This offers critical insights for assessing soil health and managing fertilizers sustainably⁶³.

57 Luo, G. *et al.* Organic amendments increase crop yields by improving microbe-mediated soil functioning of agroecosystems: A meta-analysis. *Soil Biol. Biochem.* **124**, 105-115 (2018).

58 Lori, M., Symnaczik, S., Mäder, P., De Deyn, G. & Gattinger, A. Organic farming enhances soil microbial abundance and activity—a meta-analysis and meta-regression. *PLoS One* **12**, e0180442 (2017).

59 Gattinger, A. *et al.* Enhanced top soil carbon stocks under organic farming. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 18226-18231 (2012).

60 Seufert, V., Ramankutty, N. & Foley, J. A. Comparing the yields of organic and conventional agriculture. *Nature* **485**, 229-232 (2012).

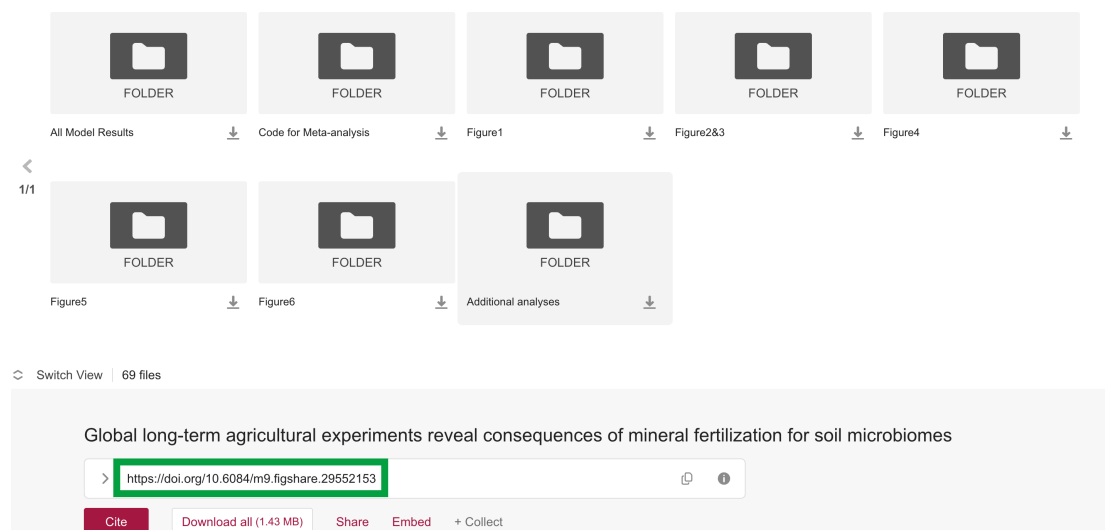
61 Wei, W. *et al.* Effects of combined application of organic amendments and fertilizers on crop yield and soil organic matter: An integrated analysis of long-term experiments. *Agric. Ecosyst. Environ.* **225**, 86-92 (2016).

1. Seufert, V., Ramankutty, N. & Foley, J. Comparing the yields of organic and conventional agriculture. *Nature* **485**, 229–232 (2012).
2. Gattinger, A. *et al.* Enhanced top soil carbon stocks under organic farming. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 18226-18231 (2012).
3. Reganold, J., Dobermann, A. Comparing apples with oranges. *Nature* **485**, 176–177 (2012).

Comment 4

Code availability: I was not able to access the code at that URL (403 Forbidden).

Response: Thank you for the reminder. It may be because the domain prefix “https://doi.org/” was not included (Or could it be that your institution has a firewall?). Please try the following link¹; it opens correctly on our end.



1. <https://doi.org/10.6084/m9.figshare.29552153>

Concluding Response to Reviewer 3. Thank you for your positive evaluation of our work. During the revision, within the limited time available, we (i) re-measured the proportion of particulate organic carbon (POC) in our samples in response to your interest in SOC fractions, and (ii) strengthened the discussion by more explicitly comparing our results with existing studies on organic versus mineral fertilization. We are grateful again for your insightful and constructive comments.

Responses to Referees for Manuscript NCOMMS-25-80403A

**Global long-term agricultural experiments reveal consequences of mineral
fertilization for soil microbiomes**

Addressed Comments for Publication to

Nature Communications

By

Shengwen Xu, Manuel Delgado-Baquerizo, Yongxiang Yu, Haoxin Fan, Limei
Zhang, Xiaoyan Tang, Zhi Zhang, Shuai Wu, Nataliya Bilyera, Yakov Kuzyakov,
Christoph C. Tebbe, Graeme W. Nicol, Joann Whalen, Huaiying Yao

Authors' Response to Reviewer #1

General Comments. We have reviewed this manuscript before (Referee 1&2), so I will take it from there. It is good to see this revised submission and the detailed responses to my earlier comments. Thank you for addressing those points. The manuscript has clearly improved, and my major previous concerns have largely been resolved. However, I still have a few minor concerns.

Response: Thank you for your careful evaluation of our revised manuscript. We are grateful to both reviewers (#1 & #2) for your time, effort, and helpful comments. We have carefully considered the remaining minor concerns you raised in this round and provide detailed responses below. We hope you will find this revised version really improved and suitable for publication.

Comment 1

Lines 72 to 73: Please delete “plant-soil feedbacks,” as I do not think this study directly addresses plant-soil feedbacks.

Response: Agreed, thanks for pointing this out and done.

Comment 2

Lines 70 to 73: It is unclear what “these changes” refers to. Does it mean changes in soil microbial communities? More importantly, the routine use of mineral fertilizers can itself strongly affect soil functions, including carbon sequestration through increased inputs, rather than only indirectly through shifts in soil microbial communities.

Response: Thanks for your kind reminding. We agree that the phrase “these changes” was ambiguous here. We have therefore revised this sentence to clarify that long-term mineral fertilization can affect soil functions not only through shifts in belowground microbial communities, but also through direct changes in soil

physicochemical properties and plant-derived carbon inputs associated with increased crop productivity. The revised text now reads:

L67-75: Routine use of mineral fertilizers has transformed agricultural soils, as intermittent inputs of water-soluble nutrients and sustained increases in plant productivity have altered soil properties and shaped distinctive belowground microbial communities³. These changes in soil properties and microbial communities are increasingly associated with functions central to fertilized agroecosystems, including the decomposition of organic matter and the sequestration of organic carbon⁴. Adding mineral forms of nitrogen (N), phosphorus (P), and other nutrients overcomes the natural limitations to soil microbial growth and influences associated soil functions.

Comment 3

Comment 4: My earlier suggestion was to highlight the differences between the current study and previous studies in order to better demonstrate the novelty of this work, rather than to emphasize how it complements previous work.

Response: Thanks, and we agree that the novelty of the present study should be stated more clearly. We now emphasize that our analysis distinguishes responses across agroecosystem types, revealing that microbial biomass increases under long-term mineral fertilization are primarily driven by bacterial components in upland systems, whereas no clear response is observed in paddy soils. This distinction helps clarify patterns that were not resolved when cropland systems were considered together in previous studies. We also highlight the drivers of shifts in dominant bacterial taxa, showing that soil acidification is a primary driver of these changes, with virulent bacteriophages potentially contributing to microbial turnover. Accordingly, we replaced phrases such as “complements previous work” and “building on previous reports” with statements that directly emphasize the patterns identified in this study:

L206: Our study demonstrates that increases in microbial biomass under long-term mineral fertilization are primarily driven by bacterial components and depend on agroecosystem types³.

L267: Consistent with previous reports of increased *Proteobacteria* under mineral fertilization¹², our results further indicate that soil acidification is a primary driver of shifts in bacterial taxa, with virulent bacteriophages potentially contributing to microbial turnover.

Comment 4

Comment 7: Some predictors, such as MAT and MAP, as well as N addition rate, and pH, are intuitively likely to be strongly correlated, which raises concerns about multicollinearity. Did the authors test models with these predictors entered separately? A VIF of 5.28 is still quite high.

Response: Thank you again for emphasizing the issue of collinearity. Yes, we have considered model selection. To evaluate whether multicollinearity among predictors influenced the GAM results, we conducted a sensitivity analysis by fitting a series of GAM models in which each predictor was removed in turn (drop-one analysis). Removing covariates including MAT, soil pH and SOC substantially reduced model performance ($\Delta AIC = 8-15$; R^2 decreased by 0.08–0.17), indicating that these variables provide independent explanatory power. In contrast, removing MAP resulted in only minor changes in model performance ($\Delta AIC = 0.3$; R^2 decreased by ~ 0.02). This pattern suggests that MAP shares part of its climatic signal with MAT, which likely contributes to its moderately elevated VIF value. Importantly, results remained consistent across alternative model specifications; models excluding either MAT or MAP were statistically indistinguishable from the full model according to the Burnham-Anderson criterion ($\Delta AIC < 2$)¹. The results

are shown in the table below. We have further clarified this sensitivity analysis in the Methods section and corresponding Supplementary Table 4.

Microbial biomass C response:

Model	AIC	R ²	Deviance explained
^αFull model	79.2	0.33	0.42
-s(pH)	87.3	0.25	0.33
-s(Soil organic C)	87.0	0.23	0.31
-s(Mean annual temperature)	94.7	0.16	0.25
-s(Mean annual precipitation)	79.5	0.31	0.39

Proteobacteria response:

Model	AIC	R ²	Deviance explained
^αFull model	-57.4	0.56	0.66
-s(pH)	-42.3	0.47	0.59
-s(Soil organic C)	-48.5	0.50	0.60
-s(Mean annual temperature)	-58.1	0.56	0.65
-s(Mean annual precipitation)	-58.2	0.56	0.65

Firmicutes response:

Model	AIC	R ²	Deviance explained
^αFull model	96.3	0.54	0.66
-s(pH)	96.5	0.51	0.61
-s(Soil organic C)	97.7	0.49	0.59
-s(Mean annual temperature)	93.8	0.56	0.66
-s(Mean annual precipitation)	96.3	0.55	0.68

^αlnRR = s(pH) + s(Soil organic C) + s(Mean annual temperature) + s(Mean annual precipitation) + s(Annual rate of mineral N addition) + s(Longitude, Latitude).

1. Burnham, K. & Anderson, D. Model selection and multimodel inference. *A Practical Information-theoretic Approach* (2004).

In Methods (Spatial prediction of microbial responses to mineral fertilization):

Because the highest VIF occurred for mean annual precipitation, we further evaluated its potential influence by comparing alternative models excluding either mean annual temperature or precipitation. As these models showed

comparable performance, the full model was retained for prediction to preserve the complete set of relevant predictors (Supplementary Table 4).

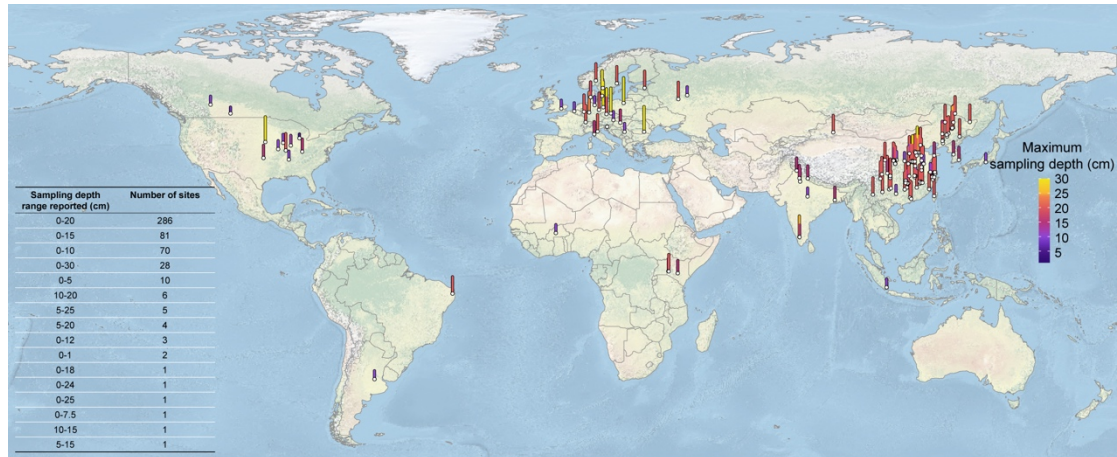
Comment 5

Line 210: Please clarify how sequencing depth and sampling effort were accounted for in the Methods. Also, in my earlier comment, I meant that the authors should account for soil sampling depth, for example 0 to 5 cm versus 5 to 20 cm, in the model.

Response: We apologize for misunderstanding your comment in the previous round. We have rephrased this sentence to clarify how differences in sequencing depth were accounted for in our study. With respect to soil sampling depth, the studies included in our synthesis were restricted to topsoil samples collected within the cultivated layer, where mineral fertilizers are typically applied¹. According to FAO guidelines, this layer generally corresponds to the upper soil horizon (commonly 0–20 cm, occasionally extending to 30 cm depending on soil type)². Consistent with this definition, most sites (~97%, 484 out of 501) reported composite samples collected across the cultivated layer (see figure below). We agree that distinguishing soil sub-layers (*e.g.*, 0–5 cm versus 5–20 cm) can be important for understanding vertical heterogeneity. However, because most samples were typically obtained as composite cores spanning the full sampling interval, only a small proportion of sites (~3%, 17 out of 501) reported stratified sampling that distinguished sub-layers³. Consequently, soil sampling depth could not be robustly incorporated as a model factor. Our dataset was therefore defined at the data inclusion stage to include only samples collected within the cultivated topsoil layer. This clarification has now been added to the Methods section. In addition, we have updated the Source Data to include the reported soil sampling depth ranges for each site for reference.

1. Jin, H. *et al.* Evaluation of the quality of cultivated-layer soil based on different degrees of erosion in sloping farmland with purple soil in China. *Catena* 198, 105048 (2021).

2. p65, Guidelines for Soil Description, FAO, 2006 (<http://www.fao.org/3/a-a0541e.pdf>).
3. <https://lgpress.clemson.edu/publication/precision-agriculture-based-soil-sampling-strategies/>



Data figure (for peer-review only) | Global distribution of soil sampling sites and sampling depths. Bars indicate the maximum reported sampling depth (cm) at each site. The table in the lower-left shows the sampling depth ranges reported in the primary studies and frequencies.

L216: Estimators based on discrete counts are sensitive to sequencing depth, which can complicate comparisons across studies⁴². In our synthesis, effect sizes were derived from paired observations within individual studies, thereby minimizing biases associated with differences in sequencing depth. Nevertheless, estimators such as Chao1 and ACE remain susceptible to inflation by singletons and sequencing artifacts⁴³. Apparent α -diversity increases or decreases should not be overinterpreted as (un)favorable microbial consequences of long-term mineral fertilization⁴⁴.

In Methods (Inclusion and exclusion criteria):

(vi) Only bulk topsoil samples collected within the cultivated (plough) layer were included, typically corresponding to sampling depths within 0-20 cm

(occasionally extending to 30 cm depending on mollic horizon). Studies involving soil replacement or deeper layers were excluded.

Comment 6

In addition, the statistical methods are not described clearly enough. Please report all packages in the appropriate section and provide a clearer description of how the models were fitted and used in the analysis.

Response: Thank you for pointing this out. In the revised manuscript, we have expanded the description of the statistical models and packages used in the Methods (Statistical analysis) section. The procedures for model fitting are described in detail in the corresponding methodological sections, and the analysis scripts already include detailed annotations to ensure transparency. We appreciate your help again, which improved the clarity of the manuscript.

In Methods (Statistical analysis):

All statistical analyses were performed in R v4.4.2 (R Core Team, 2024). Meta-analyses were conducted using the R packages *metafor*, *metagear*, *corpcor*, *orchaRd*, and *multcomp*^{85,86}. The *metafor* package was used to fit multilevel meta-analytic models with variance-covariance matrices and random effects. Missing standard deviations were imputed using *metagear*, and variance-covariance matrices were adjusted to ensure positive definiteness using *corpcor*. Subgroup contrasts were evaluated using *multcomp*, and orchard plots were generated using *orchaRd* to visualize effect sizes and confidence intervals. Package *tidyverse* was used for data processing, and *tidyplots* was used for basic plotting⁸⁷. Co-occurrence networks of host-associated virulent phage vOTUs were inferred using Spearman correlations implemented in the R package *ggClusterNet*, with abundance data organized using *phyloseq*. Spatial raster processing was performed using *tidygeocoder*, *terra*, and *sf*. Generalized additive models were implemented using the *mgcv* package, including

smoothing terms for environmental predictors and a two-dimensional spline for geographic coordinates to account for spatial autocorrelation. The fitted models were applied to global gridded environmental layers to generate spatial predictions. Predictor multicollinearity was evaluated using variance inflation factors. Predictor importance was further assessed using drop-one model comparisons by excluding each covariate and comparing model performance. Partial effects of predictors were visualized from the fitted GAMs. Inverse-variance weighted regressions were used to examine relationships between statistically significant changes in soil physicochemical properties and microbial response variables, with weights equal to the inverse of the sampling variance.

Authors' Response to Reviewer #2 (co-reviewed)

General Comments. Thank the authors for their careful revisions. The manuscript has improved, particularly in the reorganization of the Introduction and the expanded Discussion, and most of my previous comments have been addressed satisfactorily. I still have one concern regarding the interpretation of enzyme responses.

Response: We thank you for your time and effort in reviewing our manuscript. We hope that the revised interpretation of enzyme responses is much clearer now. Thank you again for your help!

Comment 1

Lines 163-168: The proposed explanation for the decoupling between increased N- and P-acquisition enzymes and unchanged C-mineralization enzymes is plausible, but it appears largely inferred rather than directly supported by measurements in this study. In particular, if fertilization alleviates nutrient limitation, it remains unclear why N- and P-acquisition enzymes increased rather than decreased. Framing this pattern in terms of microbial stoichiometric balancing may provide a clearer interpretation.

Response: We appreciate your suggestion. We have revised the relevant text to clarify the interpretation of this pattern and now frame the discussion within the context of microbial stoichiometric balancing. Our manuscript greatly benefited from your comments.

L164: Recent evidence suggests that extracellular enzyme activities primarily reflect microbial resource allocation under nutrient stoichiometric constraints rather than a general increase in decomposition capacity²⁶. Long-term mineral fertilization enhances crop production and increases the N:P ratio of plant litter,

which can aggravate P limitation for microorganisms²⁷. Higher crop yield results in higher C input to the soil, which increases microbial biomass C across all LTEs (Fig. 2c). As microbial biomass C increases under nutrient imbalances, microbes allocate more resources to N- and P-acquisition enzymes to maintain their internal C:N:P stoichiometric balance. Higher basal respiration under long-term fertilization may indicate greater energy input to microbial metabolism, which could be associated with increased microbial growth rates or nutrient imbalance (Fig. 2b). This is consistent with studies showing that higher microbial growth rates are associated with increased production of N- and P-acquisition enzymes rather than C-acquiring enzymes²⁶. In contrast, C-acquiring enzyme activities remained unchanged, suggesting that C availability was not the primary constraint on microbial metabolism under N enrichment²⁸. This may be because long-term N fertilization increases plant biomass and plant-derived C inputs to soils, including greater root exudation²⁹. Root exudates consist largely of low-molecular-weight, water-soluble compounds that are directly assimilable by microorganisms, reducing microbial reliance on complex organic C compounds that must undergo extracellular enzymatic decomposition³⁰. Overall, such nutrient-driven C flow to the soil increases microbial growth efficiency and promotes the incorporation of C into microbial biomass, which produces byproducts and cellular debris that accumulate in SOC pools under compound mineral fertilization^{31,32}.

Added supporting references:

26 Li, S. *et al.* Phosphorus limitation regulates the responses of microbial carbon metabolism to long-term combined additions of nitrogen and phosphorus in a cropland. *Soil Biol. Biochem.* 200, 109614 (2025).

27 Luo, M. *et al.* Nitrogen loading enhances phosphorus limitation in terrestrial ecosystems with implications for soil carbon cycling. *Funct. Ecol.* 36, 2845-2858 (2022).

Authors' Response to Reviewer #3

General Comments. (Remarks to the Author): The authors addressed my previous comments. Reviewer #3 (Remarks on code availability): I had a quick look at the R code for the meta-analysis, it appears to be well formulated but I did not run it.

Response: We are very grateful for the time and effort you made to make clear suggestions to improve the manuscript. We learned a great deal from your thoughtful comments.

Responses to Referees for Manuscript NCOMMS-25-80403B

**Global long-term agricultural experiments reveal consequences of mineral
fertilization for soil microbiomes**

Addressed Comments for Publication to

Nature Communications

By

Shengwen Xu, Manuel Delgado-Baquerizo, Yongxiang Yu, Haoxin Fan, Limei
Zhang, Xiaoyan Tang, Zhi Zhang, Shuai Wu, Nataliya Bilyera, Yakov Kuzyakov,
Christoph C. Tebbe, Graeme W. Nicol, Joann Whalen, Huaiying Yao

Authors' Response to Reviewer #1

General Comments. Thank you. We do not have any further comments.

Response: Thank you for your careful evaluation of our revised manuscript.

Authors' Response to Reviewer #2 (co-reviewed)

General Comments. I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We thank you for your time and effort in reviewing our manuscript.