

Patterns and drivers of soil microbial carbon use efficiency across soil depths in forest ecosystems

Corresponding Author: Professor Jinqun Li

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

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)

Review of "Depth dependent soil microbial carbon use efficiency and underlying mechanisms across forest ecosystems"
The authors have turned the focus of the MS from (i) potential changes in CUE-SOC relations with soil depth in forests, to (ii) changes in controls of microbial CUE with soil depth. This has largely dealt with the main critiques and shifted the focus to an important topic that has been little studied thus far. Though there are several expectations on how CUE controls might change from topsoils to deep subsoils, little experimental tests have checked those. The authors undertook such supplementary experiments, to test changes in controls of aggregate stabilization, C availability and microbial community structure between topsoils and subsoils. Excellent. To me the MS therefore can be accepted after minor revisions. I do not have any major critiques, and some minor corrections are indicated below:
Line 32 and elsewhere: to my mind it should read "physicochemical" (factors, protection, processes) i.e. combined physical and chemical, and not physio-chemical which would mean combined physiological and chemical!!
Line 64: rewrite to "...demands, and lower water availability is expected to...".
Line 71, 73-74, 91, and elsewhere: specify the factors influencing CUE (potentially) as "substrate availability instead of "substrate" and "microbial community structure" instead of "microbial community"
Line 77: "change" instead of "vary" with depth.
Line 171: substitute "outlook on" by "insight into"
Line 186: what is meant with microbial utilization efficiency? Microbial substrate use efficiency? Please clarify.
Line 199: exchange "mechanisms" by "changes in controls"
Line 225: exchange "influenced" by "promoted", and delete "while".
Line 233: add "changes in" microbial CUE

Reviewer #2

(Remarks to the Author)

The authors have changed their manuscript about microbial carbon use efficiency in three soil depths sections in different climate zones of China. However, several of the points that have been criticized by the reviewers previously can still be found in the manuscript. The authors still try to infer conclusions about SOC sequestration from their analysis of microbial CUE, for example, in lines 169/170 (despite thanking all three reviewers for pointing out that microbial CUE is not directly related to soil C sequestration). In addition, the authors still try to infer causality from correlative relationships, which is not exactly sound.

The study suffers from ill-posed research questions and the absence of hypotheses. The authors have no hypotheses. The first research question "What are the general patterns..." is very wide and imprecise. The second question "What are the regulatory mechanisms..." cannot be answered based on a correlative study.

In the revised version of the manuscript, the authors included incubation experiments in which they incubated crushed soil aggregates or inoculated soil with microorganisms from other soil depths sections. I have large doubts whether short-term

incubation experiments are suitable for understanding the differences between top- and subsoil. Crushing soil aggregates typically leads to a large short-term increase in soil respiration, but this effect has little to do with conditions in the soil. Further, it is well known that microorganisms that are transferred to a different soil usually do not survive there. Therefore, inoculation experiment should provide evidence that the transferred microorganisms are alive and active in the soil to which they have been transferred.

The authors have added information about the molecular analysis to the manuscript. However, it is still not clear to me what is shown in Supplementary Fig. 11. What is the unit of variations in bacterial and fungal diversity?

L. 41 Again, CUE says nothing about how much C is taken up by microorganisms nor how long this C stays in the soil (as biomass or necromass). Microbial CUE is a physiological variable that characterizes the efficiency with which microbes use C for building biomass. However, it does not capture the balance between C inputs and C outputs of a soil.

Lines 169/170 Microbial CUE is a physiological variable that characterizes the efficiency with which microbes use C for building biomass. However, it says nothing about the balance between C inputs and C outputs of a soil.

L. 180 This seems to be a misconception. Microorganisms do not sequester C. Microorganisms build up biomass which then decomposes in soils.

L. 234 Again, microorganisms do not sequester a lot of carbon. Microbial C is only about one per mille of soil organic C.

L. 125 I guess the authors mean “factors most strongly correlated with CUE”. As mentioned previously, this is an observational study. Therefore, the authors should be more cautious when drawing conclusions about the correlative relationships they observe.

L. 148/150 Again, this is an observation study and the authors should not talk about “effects” but rather about relationships.

The most interesting results are actually in Supplementary Figs 2 and 3. These correlations seem useful for understanding microbial CUE.

In conclusion, the authors should improve their manuscript by formulating testable hypotheses or answerable research questions. Further, they should concentrate on discussing CUE without trying to stretch their results too much and make statements about soil C sequestration. In addition, they should be more cautious about the inference of causality from correlative relationships.

Further comments

L. 26 Not clear what „its mechanisms” refers to

L. 29 “manipulative experiments” is tautological.

L. 41 not clear what “these processes” refers to.

L. 103 It is not clear what these pairs of numbers refer to.

L.134/135 This sentence is not clear.

L. 136 What is meant by “bacterial and fungal diversity”? Simpson index?

L. 138 What is meant by “substrate availability and quality” and how was this measured?

Reviewer #3

(Remarks to the Author)

The MS reported the microbial CUE of topsoil, subsoil, and deep soils across 60 sites spanning tropical to boreal forests. The current findings suggest that topsoil microbial CUE was primarily regulated by microbial diversity, whereas deep soil microbial CUE was predominantly driven by soil physio-chemical protections. Overall, this is an interesting and important study for both experimental and modelling communities. I did not participate in the first round of the review. When I reviewed the response letter, I think the authors addressed the concerns of three reviewers and provided novel insights into soil carbon cycling. I just have some moderate suggestions to improve the quality of MS for publication.

Particularly, I fully agree that the MS deleted the original conclusion of CUE-SOC correlation, because this relationship lacks the support of mechanism. Following the Y-A-S life history (Malik et al., 2020), a high SOC is the reason for a higher of CUE. However, in the revised manuscript, some of the points still highlight the positive effect of CUE on SOC (like in L179-181). Overall, I suggest that the MS should provide a deep discussion of the debatable of the CUE-SOC correlation, which would provide a robust understanding of CUE-SOC correlation.

I also appreciate the authors conducting a lot of additional experiments, like aggregate disruption, microbial reciprocal transplantation, and glucose addition, to comprehensive validate the conclusions from spatial patterns.

The MS provides a unique and impressive dataset for microbial CUE. Despite previous studies had reported the large-scale pattern of CUE across China’s forest, I think the discussion of the large pattern of CUE along climate gradients is also important.

I suggest that the authors provide the dataset openly for scientists.

Other specific comments:

L24-25: the background should include the soil depth

L41-41: incorporating microbial CUE into ecosystem models

L53-55: please revise this sentence for fluency

L63-64: clarified the influence of temperature on microbial CUE clearer. Replaced the comma by full point after the

temperature.

L94-96: the description of the validation experiments should be presented before the statistical analysis, the role of the statistical analysis is display the information of the data.

Fig 1a, the point size in the figure can show the relative size of microbial CUE

In Fig. 4, I suggest that the relative contribution of climate, plant biomass carbon, pH, substrate, microbial diversity and physio-chemical protection in shaping microbial CUE should be displayed by specific proportions after standardization. In addition, actually, this conceptual diagram did not provide sufficient "conceptual idea" beyond the relative contributions of factors, which had been displayed in previous figures.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

The manuscript has improved during the review process. However, a few comments arise from the last revision.

- 1) There seems to be a grammar mistake in the new title ("with" does not seem to fit any longer after other changes have been made)
- 2) The authors conclude that CUE is primarily influenced by microbial community structure in the topsoil. However, the correlation in Fig. 2 and the path coefficients in Fig. 3 shows that microbial CUE is more strongly related to the SOC content (or DSOC) than to microbial community composition in the topsoil. The authors should mention this in the discussion and conclusion.
- 3) The results shown Fig. 4 do not help to support the interpretation the authors suggest (as explained in the previous round of comments). Therefore, I suggest to move this figure to the Supplement.

Reviewer #3

(Remarks to the Author)

The authors had improved the quality of the manuscript substantially and addressed all of my previous comments. This large-scale experimental study would provide critical understanding for microbial controlling SOC dynamics. At this stage, I have just a very minor comment regarding the classification of boreal forests, which are found exclusively in the Greater Khingan Mountains. The authors should verify the classification of forest biomes in China, despite some inconsistencies in the ecotone.

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Response to Reviewer #1

[Comment 1] The authors have turned the focus of the MS from (i) potential changes in CUE-SOC relations with soil depth in forests, to (ii) changes in controls of microbial CUE with soil depth. This has largely dealt with the main critiques and shifted the focus to an important topic that has been little studied thus far. Though there are several expectations on how CUE controls might change from topsoils to deep subsoils, little experimental tests have checked those. The authors undertook such supplementary experiments, to test changes in controls of aggregate stabilization, C availability and microbial community structure between topsoils and subsoils. Excellent. To me the MS therefore can be accepted after minor revisions.

I do not have any major critiques, and some minor corrections are indicated below.

[Response] Thanks so much for the reviewer's positive assessments. Detailed responses are listed as follows.

[Comment 2] Line 32 and elsewhere: to my mind it should read "physicochemical" (factors, protection, processes) i.e. combined physical and chemical, and not physio-chemical which would mean combined physiological and chemical!!

[Response] Thanks so much for the insightful comment. We have replaced the term "physio-chemical" with the form "physicochemical" throughout the revised manuscript.

[Comment 3] Line 64: rewrite to "...demands, and lower water availability is expected to..."

[Response] Done as suggested (please see page 3, line 61).

[Comment 4] Line 71, 73-74, 91, and elsewhere: specify the factors influencing CUE (potentially) as "substrate availability instead of "substrate" and "microbial community structure" instead of "microbial community".

[Response] We appreciate the reviewer's comment. To improve clarity, we have revised the manuscript by specifying the term "substrate" as "substrate availability" or "substrate quality," and referring to the "microbial community" as "microbial community structure" where appropriate (please see such as page 4, lines 68, 71, and 89).

[Comment 5] Line 77: “change” instead of “vary” with depth.

[Response] Done as suggested (please see page 4, line 74).

[Comment 6] Line 171: substitute “outlook on” by “insight into”.

[Response] Done as suggested (please see page 8, line 169).

[Comment 7] Line 186: what is meant with microbial utilization efficiency? Microbial substrate use efficiency? Please clarify.

[Response] Thanks for the comment. The terms “microbial utilization efficiency” and “microbial substrate use efficiency” were both intended to refer to microbial carbon use efficiency, and we have revised the manuscript accordingly for consistency and clarity (please see page 9, line 182).

[Comment 8] Line 199: exchange “mechanisms” by “changes in controls”.

[Response] Done as suggested (please see page 9, line 195).

[Comment 9] Line 225: exchange “influenced” by “promoted”, and delete “while”.

[Response] Done as suggested (please see page 11, line 222).

[Comment 10] Line 233: add “changes in” microbial CUE.

[Response] Done as suggested (please see page 11, line 241).

Response to Reviewer #2

[Comment 1] The authors have changed their manuscript about microbial carbon use efficiency in three soil depths sections in different climate zones of China. However, several of the points that have been criticized by the reviewers previously can still be found in the manuscript. The authors still try to infer conclusions about SOC sequestration from their analysis of microbial CUE, for example, in lines 169/170 (despite thanking all three reviewers for pointing out that microbial CUE is not directly related to soil C sequestration). In addition, the authors still try to infer causality from correlative relationships, which is not exactly sound.

[Response] We sincerely thank the reviewer for the positive assessment and insightful comments, which have significantly improved the quality of our manuscript. In the revised version, we have removed statements that infer SOC sequestration directly from microbial CUE. Additionally, we have revised the text to avoid implying causality based on correlative relationships. To better reflect the scope and content of the revised manuscript, we have also modified the title from “Depth-dependent soil microbial carbon use efficiency and underlying mechanisms across forest ecosystems” to “Patterns and drivers of soil microbial carbon use efficiency with soil depth across forest ecosystems.” (please see page 1, lines 1-2).

Other detailed responses are provided below.

[Comment 2] The study suffers from ill-posed research questions and the absence of hypotheses. The authors have no hypotheses. The first research question “What are the general patterns...” is very wide and imprecise. The second question “What are the regulatory mechanisms....” cannot be answered based on a correlative study.

[Response] Thanks so much for these insightful comments. In response, we have added specific hypotheses to the revised manuscript. Additionally, we have replaced the term “regulatory mechanisms” with “predictive power” or “drivers” to more accurately reflect the correlational nature of our analyses.

Please see page 4, lines 81-84: “The overall aim of this study is to reveal the general patterns and drivers of microbial CUE with soil depth. We hypothesize that: (1) CUE is higher at greater

soil depth, and (2) substrate availability and microbial diversity have a larger predictive power of CUE in surface soil while physicochemical protection factors are more important in deep soil.”

[Comment 3] In the revised version of the manuscript, the authors included incubation experiments in which they incubated crushed soil aggregates or inoculated soil with microorganisms from other soil depths sections. I have large doubts whether short-term incubation experiments are suitable for understanding the differences between top- and subsoil. Crushing soil aggregates typically leads to a large short-term increase in soil respiration, but this effect has little to do with conditions in the soil. Further, it is well known that microorganisms that are transferred to a different soil usually do not survive there. Therefore, inoculation experiment should provide evidence that the transferred microorganisms are alive and active in the soil to which they have been transferred.

[Response] Thank you for raising this important point. We acknowledge that short-term incubations of crushed soil aggregates may induce transient respiration bursts due to structural disturbance. To minimize such effects prior to CUE measurements, we conducted a 3-week preincubation period. This clarification has been added to the revised manuscript.

Please see page 15, lines 326-328: “After a 3-week preincubation to minimize the possible disturbances (e.g. soil crushing and packing) and activate microorganisms, microbial CUE was then measured as described above.”

Regarding the inoculation experiments, we agree that the survival of transferred microbes is an important concern. Although we did not directly assess microbial viability post-transfer, the observed functional responses—such as measurable microbial respiration—indicate at least partial microbial activity. We measured respiration rates as they are essential for calculating microbial CUE.

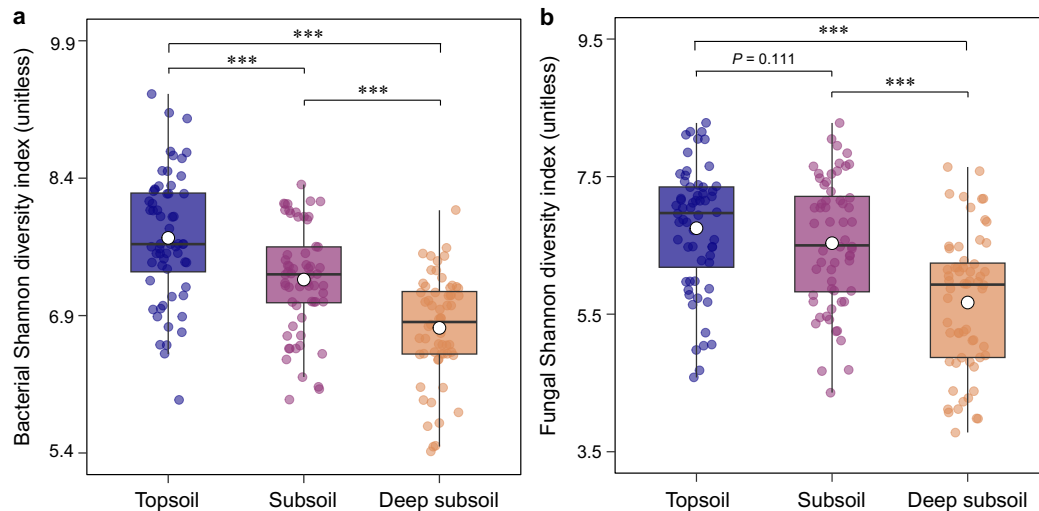
Nonetheless, we believe these limitations do not compromise our main conclusion that microbial CUE and its drivers exhibit clear depth-dependent patterns. These limitations have been acknowledged in the revised manuscript.

Please see page 11, lines 228-239: “Although our study provides empirical evidence for the depth-dependent drivers of soil microbial CUE, several limitations should be acknowledged. First, short-term incubation experiments were employed to assess the direct effects of physicochemical protection and microbial community composition on CUE. Despite a 3-week preincubation designed to reduce potential disturbance effects, the disruption of soil aggregates may have caused a transient increase in soil respiration, potentially influencing short-term CUE estimates. Future studies involving longer-term incubations, in both laboratory and field settings, with controlled aggregate disruption, are warranted to improve our understanding of these processes. Second, while the observed microbial respiration indicates the activity and viability of the transferred microbial communities to some extent, additional direct evidence—such as measurements of enzyme activity—would more robustly confirm microbial viability. Nevertheless, these limitations do not undermine our principal conclusion that microbial CUE and its drivers are depth-dependent.”

[Comment 4] The authors have added information about the molecular analysis to the manuscript. However, it is still not clear to me what is shown in Supplementary Fig. 11. What is the unit of variations in bacterial and fungal diversity?

[Response] Thank you for the comment. In the original Supplementary Fig. 11, microbial diversity referred to alpha diversity, calculated using the Shannon index—a dimensionless metric that captures both richness and evenness of bacterial and fungal communities. We have clarified this in the figure legend (please see the new Supplementary Fig. 12), and we have added this statement in the main text in the revised manuscript.

Please also see page 17, lines 374-375: “Bacterial and fungal diversity was indicated by alpha diversity, which was quantified using the Shannon index.”



Supplementary Fig. 12 | Variations in bacterial and fungal diversity across different soil horizons. The ends of the boxes represent the first and third quartiles, while the whiskers extend to the interquartile range from these quartiles. The horizontal lines inside the boxes indicate the median values. White dots within the box plots represent the mean values, and colored dots correspond to individual data points ($n = 60$). *** $P < 0.001$.

[Comment 5] L. 41 Again, CUE says nothing about how much C is taken up by microorganisms nor how long this C stays in the soil (as biomass or necromass). Microbial CUE is a physiological variable that characterizes the efficiency with which microbes use C for building biomass. However, it does not capture the balance between C inputs and C outputs of a soil.

[Response] Thanks for the comment. In the revised manuscript, we have removed previous statement of “..., serves as an integrative metric reflecting the balance between these processes. Growing evidence suggests that incorporating microbial CUE largely improves SOC storage predictions at regional and global scales, underscoring the need to understand its variability and underlying mechanisms.”

[Comment 6] Lines 169/170 Microbial CUE is a physiological variable that characterizes the efficiency with which microbes use C for building biomass. However, it says nothing about the balance between C inputs and C outputs of a soil.

[Response] Thanks so much for the comment. Based on the comments here and above (**[Comment 1]** and **[Comment 5]**), we have removed this sentence in the revised manuscript.

[Comment 7] L. 180 This seems to be a misconception. Microorganisms do not sequester C. Microorganisms build up biomass which then decomposes in soils.

[Response] Thanks for the comment. Based on the comments here and above (*[Comment 1]*, *[Comment 5]*, and *[Comment 6]*), we have removed these related sentences in the revised manuscript.

[Comment 8] L. 234 Again, microorganisms do not sequester a lot of carbon. Microbial C is only about one per mille of soil organic C.

[Response] In response to the reviewer's suggestion, we have removed the statement referring to the role of microorganisms in carbon sequestration from the revised manuscript.

[Comment 9] L. 125 I guess the authors mean "factors most strongly correlated with CUE". As mentioned previously, this is an observational study. Therefore, the authors should be more cautious when drawing conclusions about the correlative relationships they observe.

[Response] Thanks so much for the comment. Yes, we have revised it.

Please see page 6, lines 123-124: "Classification and regression tree (CART) analysis was firstly conducted to determine the factors most strongly correlated with CUE (Supplementary Figs. 5–6)."

[Comment 10] L. 148/150 Again, this is an observation study and the authors should not talk about "effects" but rather about relationships.

[Response] We appreciate the comment and have revised the sentence by replacing the original wording with "prediction" in the revised manuscript.

Please see page 7, lines 147-149: "Among all tested factors, substrate characteristics and microbial properties had the highest prediction power of CUE variation in the topsoil and subsoil, whereas in the deep subsoil, physicochemical protection had the highest prediction power (Fig. 3d–f)."

[Comment 11] The most interesting results are actually in Supplementary Figs 2 and 3. These

correlations seem useful for understanding microbial CUE.

[Response] We appreciate the comment. As suggested, the previous Supplementary Fig. 2 has been incorporated into the main text as Fig. 2 in the revised manuscript (please see the new Fig. 2).

[Comment 12] *In conclusion, the authors should improve their manuscript by formulating testable hypotheses or answerable research questions. Further, they should concentrate on discussing CUE without trying to stretch their results too much and make statements about soil C sequestration. In addition, they should be more cautious about the inference of causality from correlative relationships.*

[Response] Thank you very much for these insightful comments. In the revised manuscript: (1) we have added explicit hypotheses (please see page 4, lines 81-84); (2) we have carefully avoided making claims or implications regarding the effect of microbial CUE on SOC sequestration; and (3) we have refrained from using terms such as “mechanisms” or “effects” when interpreting our results, opting instead for more appropriate terms such as “predictive power,” “correlation,” and “driver.”

Further comments

[Comment 13] *L. 26 Not clear what “its mechanisms” refers to.*

[Response] Previously, we intended to refer to the mechanisms underlying depth-related changes in microbial CUE. We have revised the sentence to: “Microbial carbon use efficiency (CUE) serves as a key indicator of microbial control over soil carbon turnover, yet its general patterns and drivers across soil depths remain poorly understood.” (please see page 2, lines 24-26).

[Comment 14] *L. 29 “manipulative experiments” is tautological.*

[Response] We have removed “manipulative” in the revised manuscript.

Please see page 2, lines 28-30: “Using multiple analytical and statistical approaches complemented by experiments, we further identified depth-dependent drivers of microbial CUE.”

[Comment 15] L. 41 not clear what “these processes” refers to.

[Response] Previously, we intended to refer to the balance between soil carbon sequestration and decomposition processes. However, in response to the comment above (*[Comment 5]*) and to avoid implying a direct link between microbial CUE and SOC storage, we have removed this sentence from the revised manuscript.

[Comment 16] L. 103 It is not clear what these pairs of numbers refer to.

[Response] The pairs of values denote the minimum and maximum of microbial CUE, and we have clarified this in the revised manuscript (please see page 5, line 101).

[Comment 17] L.134/135 This sentence is not clear.

[Response] We have made it clear in the revised manuscript.

Please see page 6, lines 132-134: “Partial correlation analysis was further performed to determine the relationships of CUE with all individual factors when controlling other groups of factors that do not include this factor (Supplementary Figs. 7–9).”

[Comment 18] L. 136 What is meant by “bacterial and fungal diversity”? Simpson index?

[Response] This refers to the Shannon diversity index in our study, and we have clarified this in the revised manuscript (please see page 7, line 135).

[Comment 19] L. 138 What is meant by “substrate availability and quality” and how was this measured?

[Response] Previously, we intended to refer to substrate availability and substrate quality—specifically, carbon availability and carbon quality. In this study, substrate availability is represented by the carbon availability index, while substrate quality is indicated by SOC decomposability (please see page 17, lines 361-367).

Response to Reviewer #3

[Comment 1] The MS reported the microbial CUE of topsoil, subsoil, and deep soils across 60 sites spanning tropical to boreal forests. The current findings suggest that topsoil microbial CUE was primarily regulated by microbial diversity, whereas deep soil microbial CUE was predominantly driven by soil physio-chemical protections. Overall, this is an interesting and important study for both experimental and modelling communities. I did not participate in the first round of the review. When I reviewed the response letter, I think the authors addressed the concerns of three reviewers and provided novel insights into soil carbon cycling. I just have some moderate suggestions to improve the quality of MS for publication.

[Response] Thanks so much for the reviewer's positive assessments.

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[Response] Thanks for the insightful comment. In response to this and related comments from Reviewer #2 (e.g., *[Comment 1]* and *[Comment 7]*), we have removed the relevant statements referring to the sequestration effect of microbial CUE on SOC from the revised manuscript.

[Comment 3] I also appreciate the authors conducting a lot of additional experiments, like aggregate disruption, microbial reciprocal transplantation, and glucose addition, to comprehensive validate the conclusions from spatial patterns.

[Response] Thanks for the reviewer's positive assessments.

[Comment 4] The MS provides a unique and impressive dataset for microbial CUE. Despite previous studies had reported the large-scale pattern of CUE across China's forest, I think the

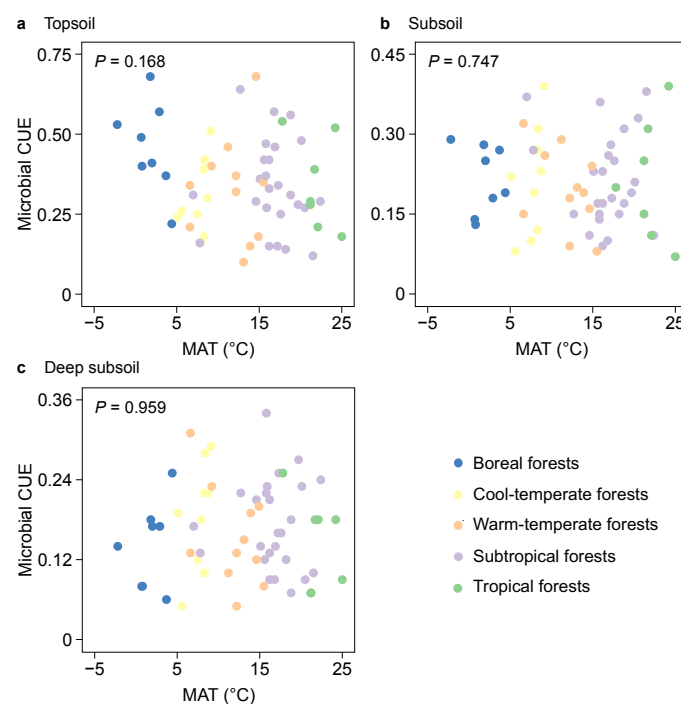
discussion of the large pattern of CUE along climate gradients is also important.

[Response] Thanks so much for the comment. As our findings indicated that microbial CUE was independent of climatic factors such as MAT and MAP, we did not discuss large-scale CUE patterns in the previous version. In response to the comment, we have now included the results on the relationships between CUE and MAT/MAP in the revised manuscript.

Please see page 5, lines 102-104: “However, correlation analyses indicated that CUE was not significantly associated with mean annual temperature (MAT; Supplementary Fig. 2) or mean annual precipitation (MAP; Supplementary Fig. 3).”

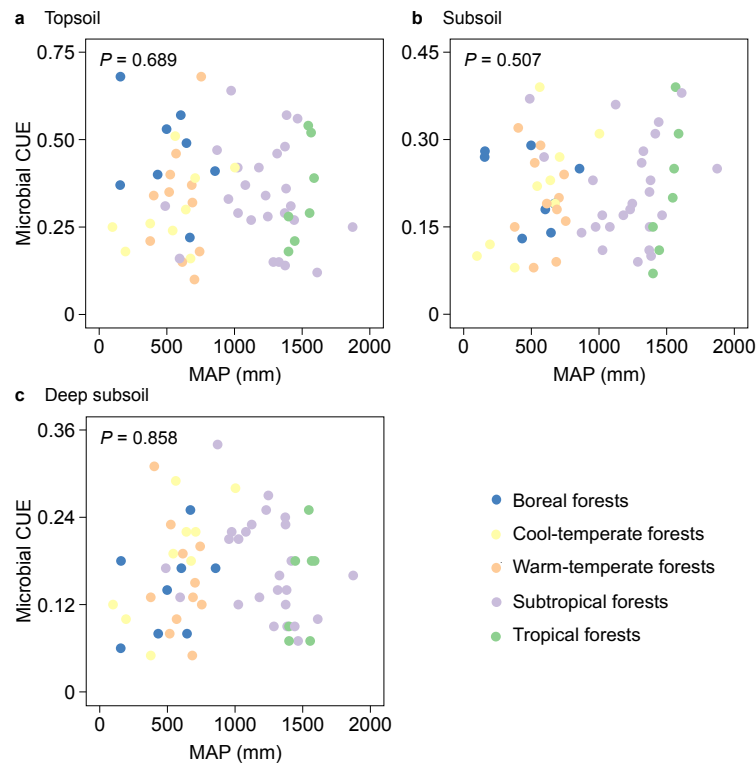
Additionally, we have expanded the discussion to include the relationships between microbial CUE and climate variables.

Please see page 8, lines 170-173: “As in previous cross-scale studies (Wang et al., 2021; Tao et al., 2023; Pei et al., 2024; Ren et al., 2024), our results show that microbial CUE exhibited substantial spatial variability (Fig. 1a). However, CUE was independent of climatic factors such as MAT and MAP (Supplementary Figs. 2–3), which contrasts with earlier findings from forests in China (Wang et al., 2021; Ren et al., 2024) but aligns with results from a recent global-scale synthesis (Hu et al., 2025).”



Supplementary Fig. 2 | Relationships between microbial CUE and MAT. a–c, Linear

regressions depicting the relationship between CUE and MAT in the topsoil (0–10 cm, **a**), subsoil (35–50 cm, **b**) and deep subsoil (70–100 cm, **c**). The dots correspond to individual values ($n = 32, 50$ and 47 for topsoil, subsoil and deep subsoil, respectively). CUE, carbon use efficiency; MAT, mean annual temperature.



Supplementary Fig. 3 | Relationships between microbial CUE and MAP. a–c, Linear regressions depicting the relationship between CUE and MAP in the topsoil (0–10 cm, **a**), subsoil (35–50 cm, **b**) and deep subsoil (70–100 cm, **c**). The dots correspond to individual values ($n = 32, 50$ and 47 for topsoil, subsoil and deep subsoil, respectively). CUE, carbon use efficiency; MAT, mean annual precipitation.

[Comment 5] *I suggest that the authors provide the dataset openly for scientists.*

[Response] The data have been made publicly available for the scientific community. Please refer to the Data Availability Statement in the revised manuscript for details.

Other specific comments:

[Comment 6] *L24-25: the background should include the soil depth*

[Response] Done as suggested.

Please see page 2, lines 24-26: “Subsoils below 30 cm store more than half of global soil carbon. Microbial carbon use efficiency (CUE) serves as a key indicator of microbial control over soil carbon turnover, but the general patterns and drivers of microbial CUE with soil depth remain poorly understood.”

[Comment 7] L41-41: incorporating microbial CUE into ecosystem models

[Response] In response to the comment above (*[Comment 2]*) and related comments from Reviewer #2 (e.g., *[Comment 1]* and *[Comment 7]*), we have removed the sentence “*Growing evidence suggests that incorporating microbial CUE largely improves SOC storage predictions at regional and global scales*” which implies a sequestration effect of CUE on SOC, from the revised manuscript.

[Comment 8] L53-55: please revise this sentence for fluency

[Response] In response to the comment above (*[Comment 2]*), we have removed the sentence “*Microbial CUE plays a pivotal role in determining the extent to which soil carbon in general, and subsoil carbon specifically, regulates climate change*” as it implies a sequestration effect of CUE on SOC, from the revised manuscript.

[Comment 9] L63-64: clarified the influence of temperature on microbial CUE clearer. Replaced the comma by full point after the temperature.

[Response] Thanks for the comment. We have revised the sentence in the manuscript to: “For example, rising temperatures typically reduce CUE (Zhang et al., 2022) due to increased metabolic energy demands (Manzoni et al., 2012), and lower water availability is expected to decrease CUE by elevating osmoregulatory costs and limiting substrate supply to microbial cells (Domeignoz-Horta et al., 2020).” (please see page 3, lines 60-62)

[Comment 10] L94-96: the description of the validation experiments should be presented before the statistical analysis, the role of the statistical analysis is display the information of the data.

[Response] Thanks for the comment. In the revised manuscript, we have repositioned the description of the validation experiments to appear before the description of statistical analyses (please see page 5, lines 90-94).

[Comment 11] Fig 1a, the point size in the figure can show the relative size of microbial CUE

[Response] Done as suggested (Please see the new Fig. 1).

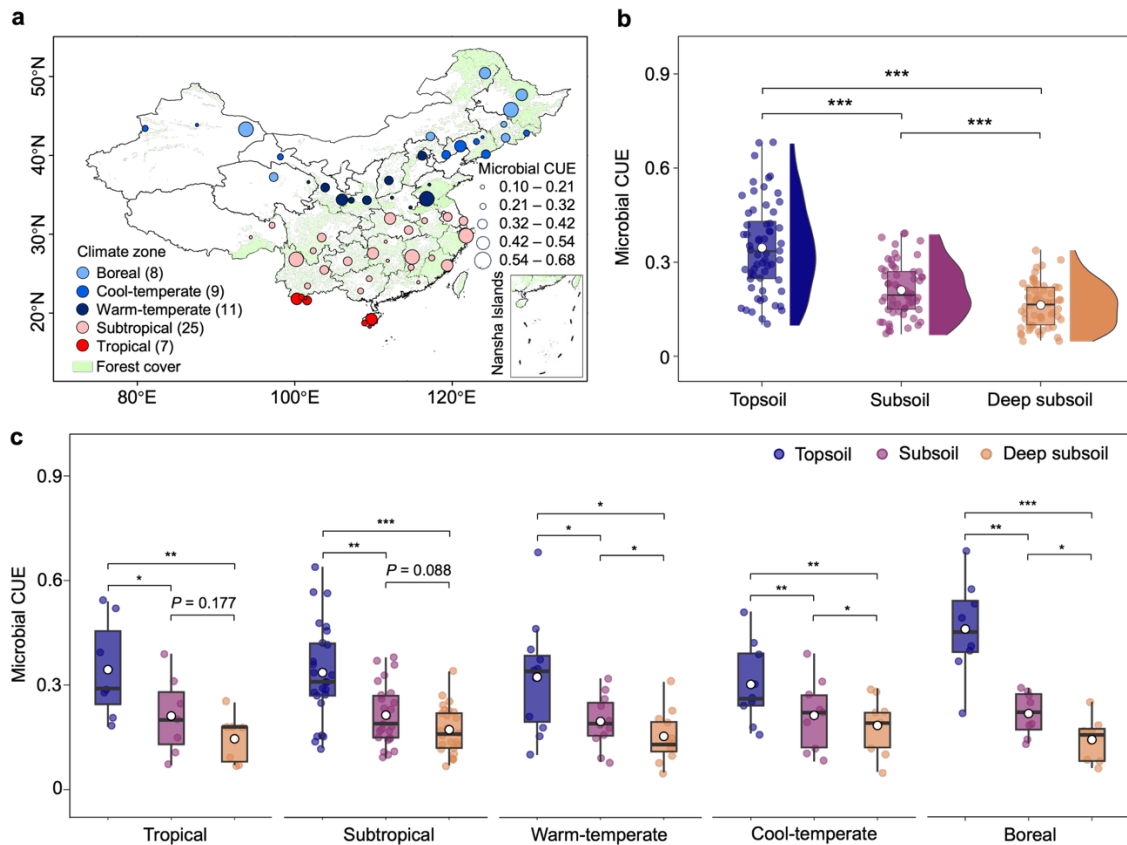


Fig. 1 | Site distribution and the depth-dependent microbial carbon use efficiency (CUE).

a, The 60 soil sampling sites spanning forest ecosystems, with soil samples collected to a depth of one meter. Numbers in parentheses represent the number of sampling sites for each forest type. The geographic variation in CUE indicates values from the topsoil. **b–c**, Box plots illustrating microbial CUE among soil horizons across all sites ($n = 60$; **b**) and in different forest types ($n = 7, 25, 11, 9$ and 8 for tropical, subtropical, warm-temperate, cool-temperate and boreal forests, respectively; **c**). Box edges represent the first and third quartiles, whiskers extend within the interquartile range, and horizontal lines inside the boxes indicate the median. White dots denote mean values, while colored dots represent individual data points. Topsoil: 0–10 cm, subsoil: 35–50 cm and deep subsoil: 70–100 cm; * $P < 0.05$, ** $P < 0.001$, *** $P < 0.001$.

[Comment 12] In Fig. 4, I suggest that the relative contribution of climate, plant biomass carbon, pH, substrate, microbial diversity and physio-chemical protection in shaping microbial CUE should be displayed by specific proportions after standardization. In addition, actually, this conceptual diagram did not provide sufficient “conceptual idea” beyond the relative contributions of factors, which had been displayed in previous figures.

[Response] Thank you very much for the comment. We have redrawn the conceptual diagram in the revised Fig. 5. The relative contributions of climate, plant biomass carbon, pH, substrate, microbial diversity, and physicochemical protection in driving microbial CUE are now displayed as standardized proportions, based on the results from the structural equation models in Fig. 3. Additionally, we have incorporated the regulatory pathways of microbial diversity and physicochemical protection on microbial CUE into the revised diagram (please see the new Fig. 5).

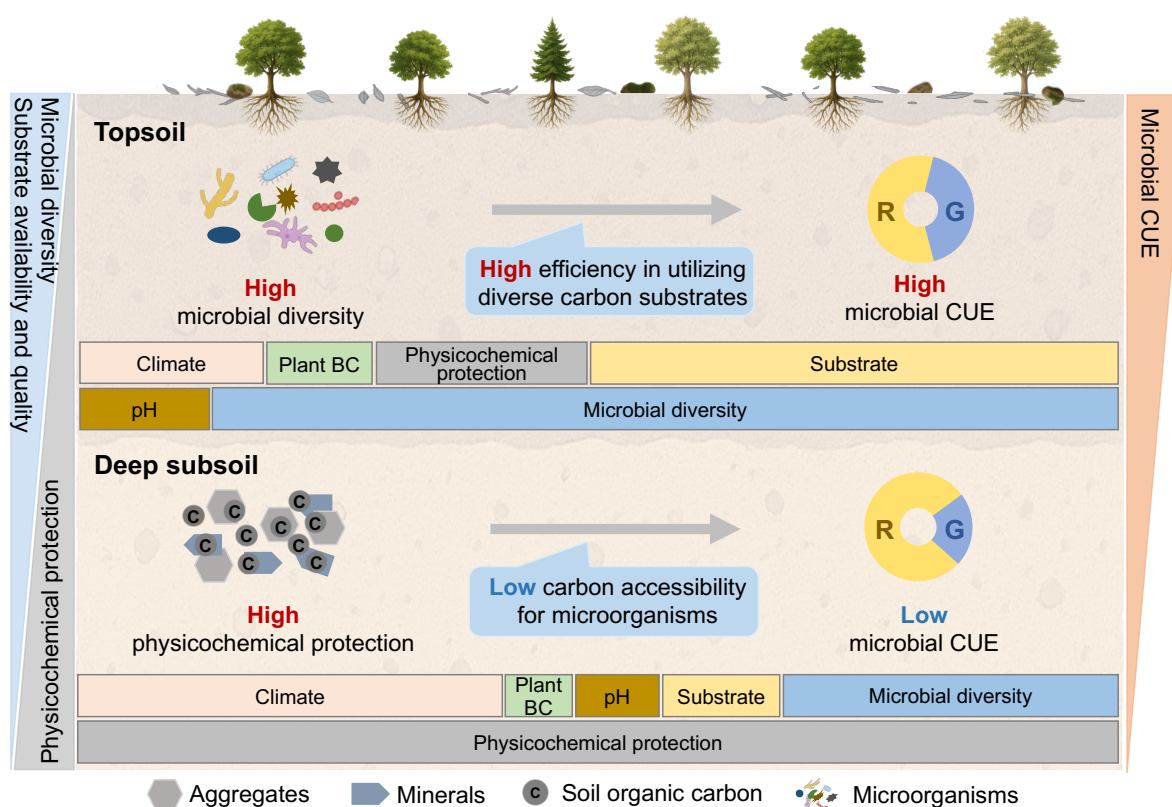


Fig. 5 | Conceptual diagram illustrating the distinct drivers of microbial carbon use efficiency (CUE) in topsoil and deep subsoil. Microbial CUE declines with increasing soil depth (shown on the right), driven by reduced microbial diversity and substrate availability or

enhanced physicochemical protection (shown on the left). In the topsoil, microbial CUE is primarily regulated by microbial diversity, where higher microbial diversity leads to higher CUE. In contrast, in deep subsoil, CUE is predominantly controlled by physicochemical protection, with stronger protection resulting in lower CUE. The relative importance of climate, plant biomass carbon, pH, substrate, microbial diversity and physicochemical protection in shaping microbial CUE in the topsoil or deep subsoil is shown on the bottom; the length of each rectangular bar indicates the relative importance for the corresponding factor, based on structural equation modeling results presented in Figure 3. R, microbial respiration; G, microbial growth; plant BC, plant biomass carbon.

Overall, we sincerely appreciate the insightful comments provided by all three reviewers. These suggestions prompted us deeper reflection on the role of microbial CUE in carbon turnover and emphasized the importance of precise terminology—for example, distinguishing between “mechanisms” and “drivers”. We believe that addressing these comments has significantly improved the quality and clarity of the revised manuscript. Thank you again for your valuable feedback.

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