

Microbial carbon metabolism is linked to organic matter chemistry across soil systems

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

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I appreciate the work and expertise that went into the current study. The dataset is extensive and cutting-edge in many regards. The figures are informative and very well composed. However, the current text as written creates many points of confusion that will need resolved prior to publication. I feel that the strengths of the current work will be better highlighted after a large amount of clarification. I would also ask the authors if they really feel that the work associated with the previous manuscript "11" is a necessary component of the current manuscript. As currently written, the inclusion of this dataset seems to create confusion, and it's not clear what it is adding to the important messages of the more current work.

The inclusion of data/models from publication "11" is very confusing. The current work never describes exactly what publication "11" measured. For example, how are the publication "11" measurements of growth, respiration, and CUE different from those measured more recently? What were the models that produced these "residual" datasets, and why wouldn't the authors just start with new models for the current publication? The presentation of the R2 values for the "residual" models adds another layer of confusion. What were the original R2 values of the publication "11" models?

Where did the microbial community composition data come from? If it didn't come from the current study then how can it be appropriate to use it in the models associated with the current study?

Two major methods questions:

1) How representative is incubation data (biomass, respiration, community composition) for conditions in the field? I acknowledge that all soil measurements are just proxies or metrics to some degree, but the level of manipulation undergone during the current work's incubation seems extreme. Microbial communities in the field would have significant proportions of mycorrhizal fungi, wouldn't they?

2) Would KCl-extractable (salt-extractable) organic matter include a bunch of compounds from cell lysis? I understand that microbes do recycle their own necromass over and over, but does a KCl extraction run the risk of over emphasizing the contribution of microbial necromass to the microbially-available organic matter pool? How would the inclusion of a large necromass component influence the interpretation of the model outputs?

Line 35: What is a biomass-normalized rate of growth? Is it the relative increase in biomass over time rather than the absolute increase? I understand that biomass-normalized rate of respiration is an expression of carbon use efficiency, but I don't understand that biomass-normalized growth is.

Line 39: Is the "growth" mentioned here the biomass-normalized rate of growth mentioned above or some other measurement of biomass? In the sentence above, you link growth to certain compound classes which have specific oxidation states (usually reduced? ~0?), but here you are saying the opposite.

Line 61: Please explain what "potential" microbial carbon metabolism is. Maximum rates under ideal conditions?

Line 64: CUE is not previously defined, and it isn't clear in the abstract whether respired C is the numerator or the denominator - are we talking about "specific respiration" or "carbon use efficiency"? I was under the impression that specific respiration and CUE were direct complements of each other - the numerator and denominator were just switched. This sentence implies otherwise.

After looking up the terms, I see what the difference between CUE and qCO2 are. Please state these differences explicitly in the introduction or methods.

Line 72: What is "microbial carbon turnover"? Is this the death and recycling of microbial biomass?

Line 89: Please provide a reference for KCl-extractable SOM being a proxy for microbially-available SOM.

Line 124: "in situ rates". Laboratory incubations exclude mycorrhizal fungi which likely do most of the C processing in these

soils. Unless these grass species don't have mycorrhizal associations... please discuss.

Lines 138-140: What justifies the assumption of a stable amount of microbial biomass during incubation? One would think that the biomass would increase until substrate limitations were encountered and then decrease.

Line 211: The analysis of residuals from a previous study is confusing. The data from the previous publication - was it from laboratory incubations? Field measurements? If it's the same variables (CUE, biomass, etc.), how is it different than the "new" data measured for the current paper?

Line 263: Was the microbial community data in publication "11" from field soils or incubated soils?

Figure 1: Thank you for figure 1. This figure is really nicely composed and helpful.

Line 309 and throughout: The use of "full" to refer to the new dataset (as opposed to the dataset from publication 11) is confusing as the terms "full" and "reduced" usually refer to models containing all initial fixed effects versus a reduced number of fixed effects - ditto with the use of "residual" to describe the data from paper 11. Would the researchers please choose different adjectives to refer to these two datasets?

Line 315: It's confusing to try to interpret an R² value for a model where the response variable is the residual variation from another model... is this 27% and 43% of the variation in the residuals from the first model? The reader can't really assess the how much of the *overall* variation in the original dataset is now being explained.

Line 379-380: "microbial carbon turnover". The methods never explained what "microbial carbon turnover" is. How are you measuring biomass turnover if that's what this variable represents? Or is it just respiration? It's frustrating that the first paragraph of the discussion centers on this variable when the reader isn't ever told what it is.

Lines 405-407: This is doubly confusing. How are you deducing that substrate chemistry was correlated with the environmental factors? That information isn't in figure 2.

Reviewer #2

(Remarks to the Author)

This study provides a valuable and novel contribution by empirically linking the molecular composition of extractable soil organic matter, characterized by high-resolution mass spectrometry, to metrics of microbial carbon metabolism (growth, respiration, and carbon use efficiency) across a broad geoclimatic gradient. The methodological integration is robust, and the finding that substrate chemistry explains significant variance in microbial physiology, in part independently of other environmental drivers. However, several points require clarification and strengthening to ensure the conclusions are fully supported and the study's implications are clear for the broad readership of CEE.

1. The authors hypothesize that "the dominance of aliphatic compounds would favor higher rates of specific growth and respiration." However, the preceding text does not provide sufficient mechanistic or literature-based reasoning to support this specific prediction.

2. The study uses 1M KCl-extractable organic matter as a proxy for the substrate pool available to microbes. While a practical and common approach, this definition requires a more critical treatment. The authors should more explicitly discuss this limitation in the Discussion.

3. Line 94, The text should explicitly state that the "full" and "residual" variation that was left unexplained in ref. 11

4. Robustness and interpretation of PLS regression models, author reported the positive and negative correlation between microbial carbon metabolism and organic matter chemistry, such as "the full variation of CUE was almost exclusively positively linked with compounds classified as aromatic" (L450-451), I look forward to seeing the key factors identified through the model, and conducting further linear and nonlinear fitting to demonstrate the relationship between them, as part of the supp..

5. The study encompasses 33 grassland soil samples collected across a vast geographical and climatic range. Although the text mentions this geographical and climatic gradient and cites relevant previous studies, I think providing a brief overview of the characteristics of sampling sites in the main text would help readers better understand the research background and enhance the readability of the paper.

6. This paper constitutes an in-depth follow-up to the authors' prior work (in particular Reference 11). In the Discussion section, it is recommended that the authors synthesize the key findings from their earlier research and conduct a comprehensive discussion of the regulatory factors and underlying mechanisms through which the environment, soil microbiome, and organic matter chemistry collectively modulate soil microbial carbon metabolism.

Reviewer #3

(Remarks to the Author)

Reviewer #1: I have now reviewed the paper entitled " Microbial carbon metabolism is linked to organic matter chemistry across soil systems " (COMMSENV-26-0940-T) and below you will find my comments and suggestions. The paper is an interesting work, which intends to answer how the chemical composition of microbially available organic matter affects soil microbial carbon metabolism across soil systems. However, there have some questions in methods section puzzled me. There are a few comments regarding the manuscript that I would like to express.

Introduction

1.Lines 49-51. In aerated soils, heterotrophic respiration is the dominant catabolic process by which microbes extract energy from organic substrates.

What is the difference between the ordinary soil and aerated soil? Please explain clearly the difference in the decomposition of organic matter by microorganisms in ordinary soil and aerated soil.

2.Lines 98-101. Please clearly articulate the hypothesis section of the paper, including the content of Hypothesis 1 and

Hypothesis 2.

Material and Methods

3.Lines111-113. Please specify the meanings of MAP, PET, and MAT, and ensure that their full forms are provided upon first mention in the manuscript.

4.Lines 128-130. Please rewrite this sentence and the logic is not clear.

5.Lines 233-234. What is the 'varImp' in the manuscript of R software—is it a package or something else? This was not clearly specified.

Results

6.Lines 281-282. Why is organic matter fractionated at 0.45 μm and what specific fractions is this method intended to separate?

7.Lines 335-336. The logic of this sentence is unclear

8.Lines 341-343. What is the meaning of "low O"? Please examine the entire manuscript to ensure that the manuscript is clearly comprehensible to readers.

Discussion

9.Lines 401-404. Is it appropriate to rely solely on R^2 to explain the correlation, without considering the significance level (p-value) (Figure 2)?

10.Lines 467-469. This sentence is incomplete; please rewrite it.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I thoroughly appreciate the supporting information and clarifications added to the manuscript during revision. The objectives, hypotheses, and connections to the previous Wasner work are crystal clear and very aptly stated. I enjoyed reading the revised version of this work and look forward to recommending it to colleagues. I have only two small requests for further information.

Line 105: Please state directly what the "full variation" models are. It is implied that these models use only the FT-ICR-MS data as explanatory variables (leaving out the climate and soil physicochemical variables?), but please state directly so there is no chance for confusion.

Line 244: Please list all the variables from the previous models here or elsewhere. Which specific climate and soil variables were used? What were the specific metrics of community composition used in the models? Please remind the reader how community composition was measured.

Reviewer #2

(Remarks to the Author)

I have carefully reviewed the revised manuscript and am satisfied with the authors' revisions in response to the feedback provided. The concerns raised in my initial review have been addressed comprehensively, and no further revisions are required at this stage.

Reviewer #3

(Remarks to the Author)

The authors have made the revisions as required, and I have no other questions. I recommend accepting this manuscript.

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