July 4, 2026

REBUTTAL LETTER

Dear reviewer,

We would like to thank the reviewer for the careful and detailed evaluation of our manuscript. We greatly appreciate the time and expertise devoted to providing constructive comments and suggestions. Many of the observations have helped us identify aspects of the manuscript that require clarification, a more balanced discussion, and improved organization. We are confident that addressing these comments will substantially strengthen the manuscript, and we are grateful for the opportunity to improve our work.

1. The manuscript contains an excessive number of paragraphs, but each with very limited content, resulting in the article being extremely fragmented. It is suggested to further integrate the entire text and merge relevant paragraphs. In addition, many viewpoints expressed are not supported by corresponding references, and some of the cited references have the problem of being unsearchable. Therefore, the overall presentation of this manuscript lacks sufficient rigor. A thorough revision of the entire text is recommended.

Thank you for your comments. We appreciate your observations regarding the organization and overall presentation of the manuscript. We acknowledge that the current version contains short paragraphs, and in the revised manuscript we will improve the flow by merging related paragraphs and making the logical connections between sections. We will also carefully review the entire manuscript to ensure that all statements are appropriately supported by the reference and verify the accuracy and accessibility of every citation. The manuscript was written by the authors, and the language was professionally edited by a scientific English editing service and reviewed by a native English speaker. We are confident that these revisions will address the concerns raised and improve the overall clarity, and scientific rigor of the manuscript. We would be pleased to provide any additional clarification if required.

2. The title of Chapter 2 is not sufficiently precise. It should be clarified whether it refers to carbon sequestration in dryland ecosystems or specifically dryland soil carbon sequestration. Furthermore, the logic of this section is disorganized. The authors mention soil microbial carbon sequestration in drylands without clearly specifying which processes are included. Autotrophic microbial groups fixing atmospheric CO_2 constitute a net carbon sequestration process. Although heterotrophic microorganisms decompose organic carbon and release CO2, if the organic carbon substrate originates from outside the soil itself, this also represents a carbon sequestration process from the soil's perspective. At the beginning of this chapter, the author failed to provide a clear definition of the above information.

Thank you for the observation. After read again this topic, we agree that the distinction between microbial carbon fixation, carbon sequestration, and broader carbon cycling processes should be more clearly defined. Our intention was to focus primarily on microbial carbon fixation through autotrophic pathways and its contribution to carbon sequestration in dryland ecosystems. However, we recognize that the current organization may not clearly distinguish these concepts. In the revised manuscript, we will clarify the scope of this section by explicitly defining the processes discussed, distinguishing autotrophic CO2 fixation from heterotrophic carbon transformations, and explaining how these microbial processes contribute to soil carbon sequestration and the broader carbon cycle. We will also revise the section title, if appropriate, to better reflect its content and improve the overall organization of the discussion.

3. L75 what do the authors mean by "microbial GPP"? I have reviewed the cited reference, it did not provide a clear definition of this term. For an ecosystem, GPP refers to the total organic carbon fixed by organisms through photosynthesis per unit time. Does the "microbial GPP" mentioned here refer to the total organic carbon produced by photoautotrophic microorganisms using light energy? If so, what is the rationale for considering only photoautotrophic microorganisms? Why are chemoautotrophic and heterotrophic microorganisms not considered?

Thank you for this important comment. The term microbial GPP (gross primary productivity) cited in our review refers to the estimate reported by Chen et al. (2021a), who used this term to describe the contribution of soil microbial primary producers to CO2 fixation in drylands, particularly photoautotrophic microorganisms such as Cyanobacteria. However, we recognize that this terminology may lead to confusion, particularly because our review addresses microbial carbon fixation through both photoautotrophic and chemoautotrophic pathways. In the revised manuscript, we will clarify the meaning of this term and explain the scope of the original study. We will also explicitly distinguish between the different microbial metabolic strategies involved in carbon fixation.

4. L92-93 What do the authors want to express? Is it to say that heterotrophic microorganisms can also fix atmospheric CO_2? Although some heterotrophic microorganisms can perform dark carbon fixation during metabolic processes, the overall metabolic outcome should still be net CO_2

We thank the reviewer for highlighting the need for clarification. Our intention was to emphasize that genes associated with CO2 fixation pathways in dryland soils are not exclusively restricted autotrophic microorganisms, and that microbial communities exhibiting diverse metabolic strategies may contribute to carbon transformations in these environments. We agree that the wording may be misleading, particularly regarding the inclusion of heterotrophic microorganisms. In the revised manuscript, we will clarify this statement by distinguishing the autotrophic CO2-fixing organisms, including chemoautotrophs, from heterotrophic microorganisms that may only indirectly contribute

to carbon cycling through anaplerotic or auxiliary metabolic processes. We will revise the text to avoid any implication that heterotrophic microorganisms perform net atmospheric CO2 fixation.

5. L122 The cited reference pertains to desert ecosystems. However, drylands also include other types of ecosystems, such as farmlands. Please provide additional references to substantiate this claim.

Thank you for your observation. We agree that drylands include a wide range of ecosystems beyond deserts. In the revised manuscript, we will incorporate additional references to better support this statement and more represent the diversity of dryland environments.

6. L126-127 What evidence can support the claim that "the WL pathway can contribute significantly"? To my knowledge, compared with other carbon fixation pathways, the abundance of the WL cycle can be very low in dryland soils.

We thank the reviewer for this comment. We intended to emphasize that in water-limited environments, where plant photosynthesis activity is reduced, microbial communities may play a relatively more important role in carbon cycling through diverse metabolic strategies, including chemolithoautotrophy and anaerobic carbon fixation pathways. In this context, we also aimed to highlight the potential metabolic relevance of the WL pathway, given its high energy efficiency compared to the CBB cycle and its suitability for anaerobic or microaerophilic microenvironments. We agree that current evidence does not allow us to generalize its abundance or to quantify its contribution as “significant” at the ecosystem scale across drylands. Therefore, in the revised manuscript, we will rephrase this statement to more accurately reflect its potential functional relevance in specific niches, rather than implying a dominant role in dryland carbon sequestration. We will also further emphasize the role of microbial metabolic diversity in sustaining carbon fixation under conditions of water limitation and reduced plant productivity.

7. L132-134 What do the authors mean by "a network of complementary metabolic routes"? The cited reference merely correlated carbon fixation processes with microbial carbon fixation genes, without further analyzing how these pathways operate as a network. Therefore, the results of the cited reference do not support the inference of "a network of complementary metabolic routes." Moreover, from a genetic perspective, the operation of some pathways is not accomplished independently. For example, the rTCA cycle shares enzymes with the 3-HP cycle and the DC/4-HB cycle, and the pathways (rTCA, 3-HP, 3-HP/4-HB, and DC/4-HB) may inherently operate jointly. Whether these pathways operate in soil habitats under natural conditions, and in what network form, remain to be demonstrated and clarified. However, the authors provide no evidence for the issue. Additionally, I do not understand what is meant by "a single dominant pathway." In terms of abundance, the rTCA cycle is typically the dominant carbon fixation pathway in dryland soils, which highlights its potential dominant role. From a genetic perspective,

the WL cycle and the Calvin cycle may be capable of operating independently; however, both pathways typically exhibit very low abundance in dryland soils.

Thank you for your comment. Our intention was not to imply that Liu et al. (2018) experimentally demonstrated the existence of an integrated metabolic network among carbon fixation pathways. Rather, we intended to emphasize that their metagenomic and 13CO2-labeling analyses identified the genetic potential for all six known autotrophic carbon fixation pathways in desert soils, and several of these pathways, including the rTCA, 3-HP, and WL pathways, were significantly associated with microbial CO2 incorporation. These findings support the view that multiple carbon fixation pathways may collectively contribute to microbial carbon fixation in dryland soils. We agree, however, that the term network of complementary metabolic routes may overstate the available evidence. Some carbon fixation pathways, such as the 3-HP/4-HB and DC/4-HB cycles, share some enzymes and metabolic intermediates, however there is currently no experimental evidence demonstrating that a single microorganism simultaneously employs more than one carbon fixation pathway. In the revised manuscript, we will avoid implying functional integration among these pathways and instead emphasize that multiple carbon fixation pathways coexist in dryland microbial communities and may collectively contribute to microbial carbon fixation.

8. L135 Please provide supporting evidence. I have not found any relevant reference that clearly substantiates this inference. Additionally, the reference of Huang et al., 2022 is unsearchable in Soil Biology and Biochemistry.

Thank you for your observation. The title of this article is incorrect in our manuscript. It was published by Huang et al. (2022) in the same journal (Soil Biology and Biochemistry, Volume 172), but the correct title is Metabolic pathways of CO2 fixing microorganisms determined C-fixation rates in grassland soils along the precipitation gradient. We will correct this reference and check all the references in the revised manuscript.

9. L148-151 The authors should provide specific examples of typical species present in dryland soils.

Thank you for your valuable suggestion. We agree that including representative examples would improve this section. In the revised manuscript, we will explore representative examples of thermophilic microorganisms reported in dryland environments and discuss their potential role in carbon fixation.

10. L155-156 This suggestion is too vague. The authors need to supplement the specific (or general) methods of integration to make it operational.

Thank you for your observation. After revisiting this sentence, we agree that it can be better elaborated. In the revised manuscript, we will provide more specific examples of how microbial carbon fixation processes could be incorporated into terrestrial carbon-cycle models.

11. L161-163 This sentence is logically incoherent.

Thank you for your observation. We agree that the sentence is not connected as currently written. In the revised manuscript, we will improve the logical flow and clearly distinguish between carbon exchange and carbon storage while emphasizing the importance of soils as long-term carbon reservoirs.

12. L182-183 This section explores the carbon cycle dynamics in drylands. Why do the authors discuss energy sources here?

Thank you for your comment. We understand that the transition in this section may not be sufficiently clear. Our intention was not to shift the focus from carbon cycling to microbial energy metabolism, but rather to explain that the oxidation of atmospheric trace gases such as H2 and CO provides energy that supports chemolithoautotrophic carbon fixation under carbon and nutriente limited conditions typical of dryland soils. In the revised manuscript, we will clarify this connection to better explain the relevance of these energy sources to microbial carbon fixation and the carbon cycle in dryland ecosystems.

13. L190-192 Similarly, the suggestion is too vague.

Thank you for your observation. We will carefully review the entire manuscript to remove redundancies, eliminate generic statements, and improve the overall flow of the text.

14. L207 This is highly unrigorous.

Thank you for your observation. We agree that this statement may overstate the current evidence. In the revised manuscript, we will revise this sentence to more accurately reflect the available data.

15. L211-213 The study of the cited reference is a short-term and plot-scale research, rather than long-term or ecosystem-scale investigations. Such simplistic scale extrapolation is scientifically unsound.

Thank you for this important comment. We agree that the cited study is based on a short-term, plot-scale experiment and does not directly demonstrate long-term or ecosystem-scale carbon sequestration. Our intention was to highlight the authors scaled-up estimates of the potential contribution of microbial inoculation to carbon sequestration. To avoid overinterpretation, we will revise the text to clearly distinguish between experimentally measured plot-scale responses and the projected estimates reported in the study. We will also emphasize that further long-term and ecosystem-scale investigations are required to validate these projections.

16. L214-221 In this paragraph, the authors should discuss specific case studies of microbial inoculant application and its role in soil carbon fixation, rather than making

vague prospective statements. Additionally, the sentences (Lines 216–219) lack supporting references.

Thank you for your suggestion. In the revised manuscript, we will incorporate representative examples of microbial inoculation and their effects on soil carbon fixation in dryland. We will also add appropriate references to support the statements. In addition, we will improve the paragraphs conclusion throughout the manuscript to better summarize the key findings of each section, improve the flow, and clearly distinguish evidence-supported conclusions from future perspectives.