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Peer Review File

Gut microbiota composition outperforms chronological age in predicting systemic inflammation and incident disease risk

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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)
The authors have satisfactorily addressed my previous comments and the manuscript has been substantially strengthened. I have no further major concerns. However, I encourage the authors to further temper causal interpretations of the mediation analyses and to explicitly discuss limitations related to residual confounding (particularly diet) and the comparison of explained variance between age and microbiota components.

Aug 23, 2026

Dear Reviewer #1,

We sincerely thank you for your thoughtful and constructive feedback on our manuscript (NCOMMS-26-039975-T) entitled *"Gut microbiota composition outperforms chronological age in predicting systemic inflammation and incident disease risk"*. We greatly appreciate the time and expertise invested in evaluating our work.

Below, we provide a detailed point-by-point response, with reviewer comments in plain text and our responses in blue.

Reviewer #1 (Remarks to the Author):

The authors have satisfactorily addressed my previous comments and the manuscript has been substantially strengthened. I have no further major concerns. However, I encourage the authors to further temper causal interpretations of the mediation analyses and to explicitly discuss limitations related to residual confounding (particularly diet) and the comparison of explained variance between age and microbiota components.

Answer: We thank the reviewer for the overall evaluation. We have revised the manuscript to further temper causal interpretations of the mediation analyses. Specifically, we now refer to the mediation results as suggesting that inflammatory cytokines account for part of the association between the Bacteroides 2 enterotype and future disease risk, rather than implying definitive causal mediation. We have also softened the relevant Results section heading and concluding sentence, as follows:

Abstract, line 46-47 now reads: "*This enterotype was associated with increased risk of future incident disease across multiple organ systems (hazard ratio 1.21), with mediation analyses suggesting that inflammatory cytokines accounted for part of this association.*"

Header, line 179-181 now reads: "*Pro-inflammatory cytokines account for part of the association between the Bacteroides 2 enterotype and future disease risk*".

Line 209-212 now reads: "*Together, these findings suggest that the early-adult pro-inflammatory phenotype observed in B2 individuals may partly explain their increased incident disease risk up to nine years later.*"

Concluding sentence, line 309-310: "*while underscoring the need for longitudinal and interventional studies to establish causality*".

In addition, we have added text to explicitly acknowledge residual confounding, particularly by dietary factors that may influence both gut microbiota composition and systemic inflammation.

Discussion, line 280-284: "*As in any observational microbiome study, residual confounding cannot be excluded, particularly from dietary factors that may influence both gut microbiota composition and systemic inflammation. Broad dietary information was available in DanFunD, but more detailed dietary assessment would be needed to fully address this potential source of confounding.*"

Finally, we have clarified that the comparison of explained variance between chronological age and microbiota composition should be interpreted in light of the multidimensional nature of microbiota profiles, which may capture inter-individual differences in inflammatory tone beyond chronological age.

Discussion, line 247-249: "*This greater explained variance likely reflects the capacity of a multidimensional microbiota profile to capture inter-individual differences in inflammatory tone beyond chronological age.*"