Reply Reviewer 3

For Reviewer 3：

Thank you very much for taking the time to thoroughly review our manuscript. In response to your expert comments, we have addressed each point sequentially in our reply and highlighted the corResponseing revisions in blue within the document.

Major limitations:

Question 1: Speculative causality without sufficient evidence: While the paper convincingly reviews literature connecting gut microbiota and depression and plateau environments causing alteration in gut microbiota, it lacks strong empirical evidence directly linking high-altitude-induced microbial changes to increased depression prevalence among plateau population. The cited statistics on depression rates in plateau populations are not necessarily tied to concurrent gut microbiota profiling. Without this critical link, the central hypothesis remains speculative, and the authors' claim of having 'unraveled the concealed connection' is called into question.

Respond 1: The insufficient evidence of the causal relationship you pointed out is the key issue. The original text is indeed based on the correlational inference of observational studies, lacking direct evidence from longitudinal tracking or intervention studies.

The modified contents are as follows:

Abstract: Add an explanation of the limitations of causality in the conclusion. "In conclusion, while existing evidence supports an association between plateau environments, gut microbiota and depression, causal mechanisms remain underexplored."

Add text 4.1.5 Limitations: Notably, the majority of studies associating plateau environments with gut microbiota–depression pathways are cross-sectional or correlational in nature. Direct causal evidence, such as longitudinal studies tracking microbial changes concurrent with the onset of depression in plateau populations, or gnotobiotic animal models simulating altitude-induced stress, remains scarce. For instance, although Table 1 indicates higher rates of depression among plateau populations, these data lack concurrent profiling of the gut microbiota. Future research should adopt prospective cohort designs or intervention studies, such as fecal microbiota transplantation in altitude-related models, to establish and validate causal relationships.

Question 2: Lack of methodological transparency in literature review: The authors refer to various studies but do not provide a clear methodology for how the literature was selected. Did you use any guidelines, such as PRISMA? Why the specific databases listed and what inclusion/exclusion criteria were used? Did you use AND criteria for your search terms as per my review the studies do not concurrently include all three terms?

Respond 2: The concerns you raised regarding non-compliance with the PRISMA guidelines and the ambiguity in the search strategy are highly significant. We have supplemented the article with a detailed description of the literature screening process and methodological procedures to address these issues.

Add text: This review followed a systematic approach aligned with PRISMA guidelines [216]. We searched PubMed, Web of Science, CNKI, and Embase (2000–2025) using the following keyword combinations: ('plateau environment' OR 'high altitude') AND ('gut microbiota' OR 'intestinal flora') AND ('depression' OR 'mental health') . Studies were included if they (1) investigated humans or animal models exposed to altitude ≥2500 m, (2) measured gut microbiota composition or function, and (3) reported depression-related outcomes. Conference abstracts and non-English studies (except Chinese) were excluded." The review elucidate the key pathways through which the plateau environment influences depression via the gut microbiota, validate the mediating effects of gut microbiota in this process and propose innovative intervention strategies based on microbiota modulation. The goal is to provide a theoretical basis for mental health protection among plateau populations, while laying a theoretical foundation and offering new research perspectives for future studies and clinical practices in this field.

[216] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021 5(29);372:n71. doi: 10.1136/bmj.n71.

Question 3: Failure to address potential confounders: The manuscript does not adequately address other psychosocial and medical risk factors that may contribute to depression in

plateau populations. For instance, sleep disturbances are prevalent in high-altitude areas and are independently associated with both gut dysbiosis and mood disorders (e.g., Kan H et al., Sleep Breath, 2025). Other relevant factors include genetic predisposition or selection, increased rates of chronic diseases (e.g., stroke, pulmonary hypertension), social isolation, substance use and limited access to mental health care. These should be controlled for to avoid oversimplification of the proposed gut-brain mechanism.

Respond 3: We sincerely appreciate your insightful and professional questions. Given the scarcity of research articles elucidating the clear relationship between depression and high-altitude environments, several confounding factors remain challenging to address. These include genetic predisposition or selection bias, the high prevalence of chronic diseases such as stroke and pulmonary hypertension, social isolation, substance abuse, and limited access to mental health services. Consequently, it is exceedingly difficult for us to fully control these variables. To enhance the robustness and transparency of our analysis, we have incorporated this issue into the limitations section of the article.

Add text in 4.2.4 Limitations

The observed associations must be interpreted alongside potential confounders. For instance, sleep disorders—prevalent in high-altitude environments due to hypoxia-induced breathing disruptions [217] —independently link to both gut dysbiosis and depression. dditionally, genetic factors (e.g., hypoxia-inducible factor polymorphisms in Tibetan populations), dietary patterns (high fat/low fiber intake), and social stressors (isolation, limited mental health access) may confound the proposed microbiota – brain axis. Future studies should employ multivariate regression to control for these variables and use twin designs to disentangle genetic vs. environmental effects.

Question 4: Unsupported interventional hierarchy: The rationale behind prioritizing certain intervention strategies is unclear. The manuscript presents dietary supplementation, probiotics, and fecal microbiota transplantation as potential solutions for improving depression in plateau environments through regulation of gut flora but fails to clarify why they are emphasized over established first-line treatments for depression, such as exercise or cognitive-behavioral therapy. The order and evidence level supporting each intervention

should be stated more explicitly.

Question 4: Thank you for your valuable suggestions. We have further enriched the content of the intervention measures based on your insightful feedback.

Add text in 5.1 Potential Methods for Improving Depression in Plateau Environments through Regulation of Gut Flora

The interventions discussed here (dietary fiber, probiotics, FMT) complement established first-line treatments for depression (e.g., cognitive behavioral therapy, exercise) [207-210] and are proposed as adjunctive strategies for plateau populations with unique microbial dysregulation. For example, while CBT remains the gold standard for psychological intervention, probiotics may offer the following advantages: (1) targeting gut–brain pathways disrupted by altitude stress; (2) feasibility in resource-limited settings; and (3) minimal side effects. Table 2 summarizes the evidence hierarchy for these interventions, prioritizing probiotics (Level B evidence from RCTs) over FMT (Level C, experimental)."

Minor limitations:

Question 1: Incomplete references: Some references are missing or incomplete. All studies cited, especially those with statistical claims, should be appropriately referenced and listed in the bibliography e.g. the statements in page 5, line 14 and 18.

Respond 1: Thank you for your insightful and constructive suggestions. We have made revisions in accordance with these recommendations.

Question 2: Unexplained abbreviations: Abbreviations such as "CNKI" and "LPS" should be clearly defined upon first mention (page 3, line 6, and page 13, line 29, respectively).

Respond 2: Thank you for your valuable suggestion. We will ensure to provide the full form of the abbreviation in our documentation.

Question 3: Typographical errors: There are numerous typographical issues throughout the manuscript, including:

"gastrogutdysfunction" → perhaps gastro-gut dysfunction or gastrointenstinal dysfunction? page 2

"guthumeostasis" → intended to be gut homeostasis? page 7-9

“gutdysbiosis” → intended to be gut dysbiosis? page 9

Respond 3: Thank you for your suggestion. We have corrected the compound word error in the article.