

Supplementary Materials

Gut Microbiome Mediates Age- and Sex-Divergent Effects of Environmental Exposures on Human Health

Jieying Ou ¹, Yuwei Tang ¹, Xinghan Wang ¹, Yiwen Yuan ¹, Xi Fu ^{2,*} and Yu Sun ^{1,*}

¹ State Key Laboratory of Swine and Poultry Breeding Industry, Guangdong provincial key laboratory for the development biology and environmental adaptation of agricultural organisms, College of Life Sciences, South China Agricultural University, Guangzhou 510642, China

² Guangdong Provincial Engineering Research Center of Public Health Detection and Assessment, NMPA Key Laboratory for Technology Research and Evaluation of Pharmacovigilance, School of Public Health, Guangdong Pharmaceutical University, Guangzhou 510006, China

* Correspondence: sunyu@scau.edu.cn (Y.S.); fuxi@gdpu.edu.cn (X.F.)

How To Cite: Ou, J.; Tang, Y.; Wang, X.; et al. Gut Microbiome Mediates Age- and Sex-Divergent Effects of Environmental Exposures on Human Health. *Glob. Environ. Sci.* **2026**, *2*(3), 295–311. <https://doi.org/10.53941/ges.2026.100020>

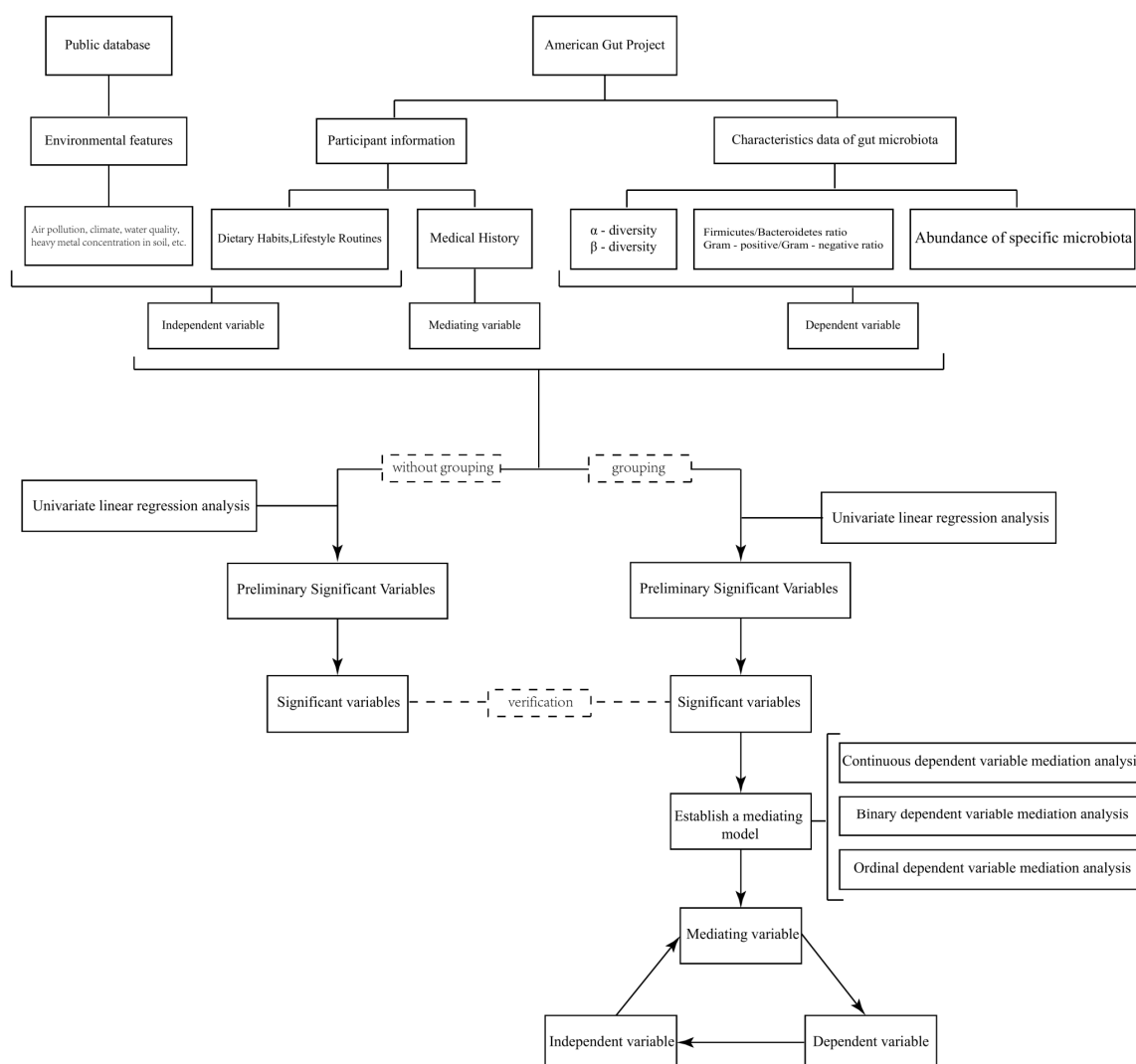


Figure S1. Schematic Overview of the Data Integration and Analysis Workflow. This flowchart illustrates the multi-stage process used in this study. The workflow began with data acquisition from two primary sources: the American Gut Project (AGP) and various Public Databases. 1. Data Curation and Variable Definition: Data from the AGP included participant information (e.g., dietary habits, medical

history) and gut microbiota characteristics (e.g., diversity metrics, specific taxon abundances). Data from public databases provided environmental features (e.g., air pollution, climate data). These data streams were curated and categorized into the three core components of our mediation models: Independent Variables (environmental/personal factors), Mediating Variables (microbial indices), and Dependent Variables (health outcomes). 2. Association Screening: A two-step screening process was performed to identify significant associations. First, Univariate Linear Regression was used to test for associations between independent and dependent variables, both for the entire cohort ('without grouping') and within demographic subgroups. This generated a list of 'Preliminary Significant Variables'. 3. Variable Verification and Model Building: The preliminary variables were then subjected to a 'Verification' step (e.g., stepwise regression, as detailed in the Methods) to identify the final 'Significant Variables' for each subgroup. These robustly associated variables were then used to Establish a Mediating Model. 4. Mediation Analysis: The final step involved formal mediation analysis to test the hypothesis that the effect of an Independent Variable on a Dependent Variable is mediated by the Mediating Variable (the microbiome). This analysis was adapted based on the nature of the dependent variable (continuous, binary, or ordinal).

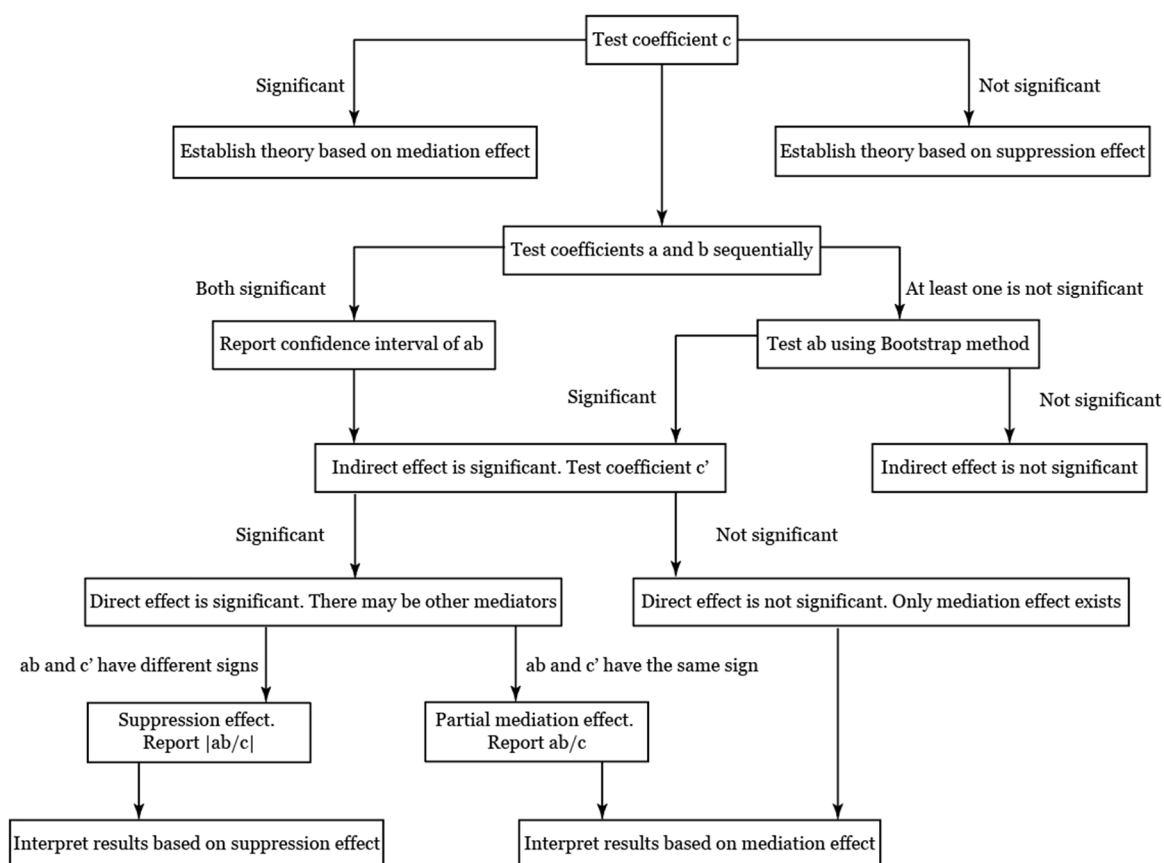


Figure S2. Decision Framework for the Interpretation of Mediation Analysis Results. This flowchart outlines the systematic procedure used to test for and interpret mediation effects, adapted from the causal steps approach. The process begins with assessing the significance of individual path coefficients from the mediation model: c (the total effect of X on Y), a (the effect of X on M), and b (the effect of M on Y). The significance of the indirect effect, quantified as the product ab , is formally tested using a non-parametric bootstrapping method. If the indirect effect (ab) is significant, the direct effect (c' , the effect of X on Y after accounting for M) is then examined. The final interpretation of the model depends on the significance and signs of both the direct (c') and indirect (ab) effects.

 $(R^2 = 0.0059, p = 0.001).$

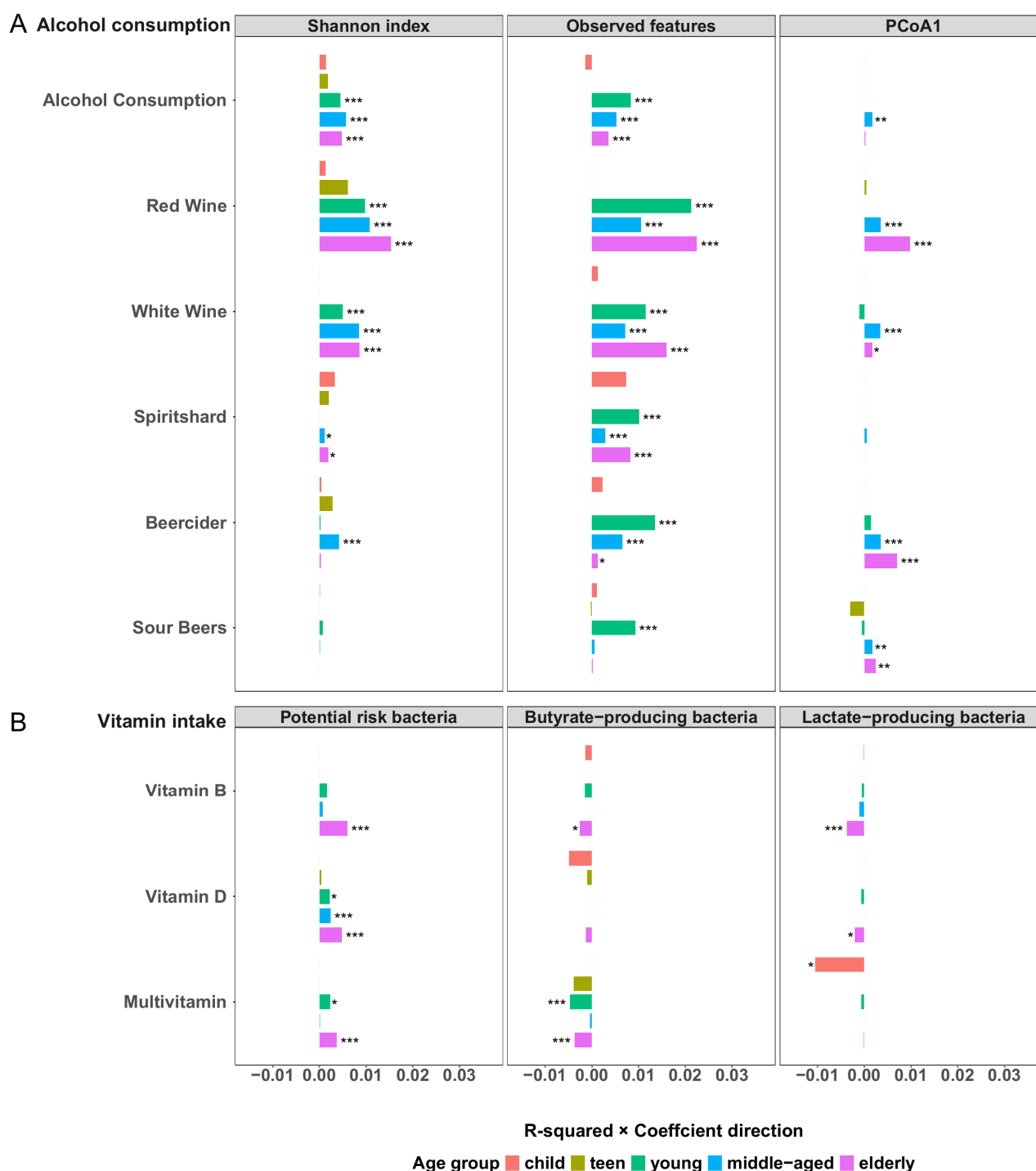


Figure S4. Age-Stratified Effects of Alcohol and Vitamin Consumption on the Gut Microbiome. This figure illustrates the age-stratified associations between alcohol and vitamin intake (y-axis) and select gut microbial indices (panel columns). Bar charts display the results of univariate linear regressions performed separately for each of the five age groups. The length of each bar represents the proportion of variance in the microbial index explained by the factor (Adjusted R^2), and the direction of the bar indicates a positive (right) or negative (left) association. (A) Associations between different types of alcohol consumption and three microbial indices, stratified by age. (B) Associations between vitamin B, D, and multivitamin intake and three functional microbial indices, stratified by age. Asterisks denote the statistical significance of each association after Benjamini-Hochberg correction for multiple tests: * $q < 0.05$, ** $q < 0.01$, *** $q < 0.001$.

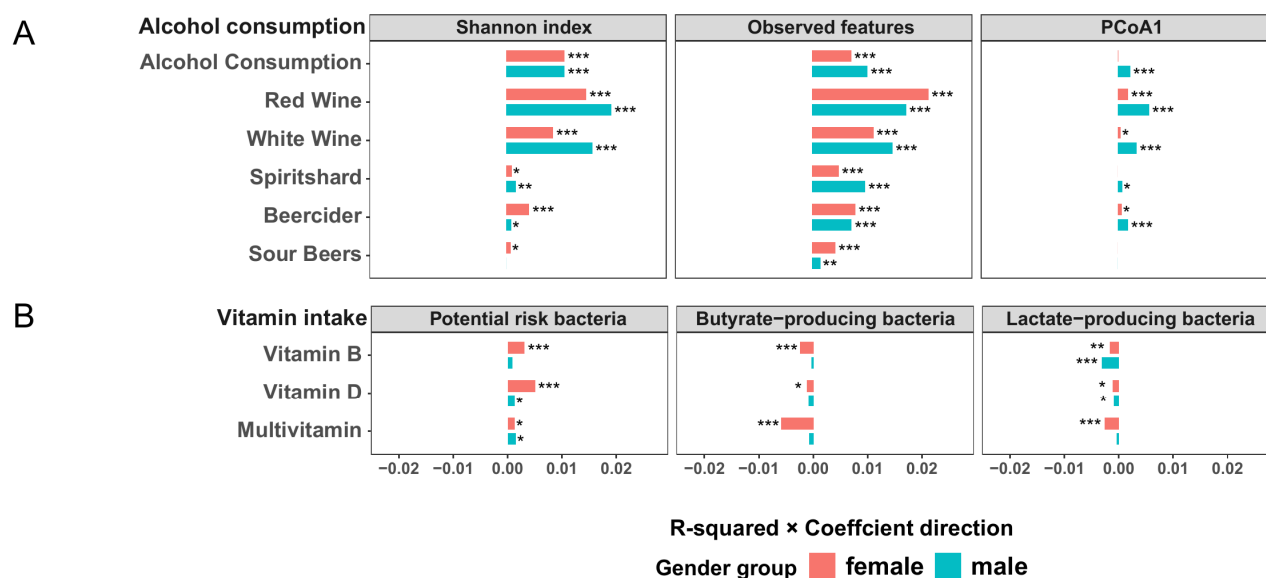


Figure S5. Sex-Stratified Effects of Alcohol and Vitamin Consumption on the Gut Microbiome. This figure illustrates the sex-stratified associations between alcohol and vitamin intake (y-axis) and select gut microbial indices (panel columns). Bar charts display the results of univariate linear regressions performed separately for female and male participants. The length of each bar represents the proportion of variance in the microbial index explained by the factor (Adjusted R^2), and the direction of the bar indicates a positive (right) or negative (left) association. **(A)** Associations between different types of alcohol consumption and three microbial indices, stratified by sex. **(B)** Associations between vitamin B, D, and multivitamin intake and three functional microbial indices, stratified by sex. Asterisks denote the statistical significance of each association after Benjamini-Hochberg correction for multiple tests: * $q < 0.05$, ** $q < 0.01$, *** $q < 0.001$.