Supplementary information for

CRAmed: A conditional randomization test for high-dimensional mediation analysis in sparse microbiome data

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Contents

I. Technical details

A. Derivation of the average natural direct effect and average natural indirect effect

Using the notation in the main text, we can derive the average natural direct effect (NDE) and average natural indirect effect (NIE) as follows:

$$
\begin{aligned}
\text{NDE} &= E\{Y(1,\boldsymbol{M}(0)) - Y(0,\boldsymbol{M}(0)) \mid \boldsymbol{X}=\boldsymbol{x}\} \\
&= E\{Y(1,\boldsymbol{M}(0)) \mid \boldsymbol{X}=\boldsymbol{x}\} - E\{Y(0,\boldsymbol{M}(0)) \mid \boldsymbol{X}=\boldsymbol{x}\} \\
&= \sum_{m} E\{Y(1,\boldsymbol{m}) \mid \boldsymbol{M}(0)=\boldsymbol{m},\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{x}) \\
&\quad -\sum_{m} E\{Y(0,\boldsymbol{m}) \mid \boldsymbol{M}(0)=\boldsymbol{m},\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{x}) \\
&\overset{\textbf{A4}}{=} \sum_{m} E\{Y(1,\boldsymbol{m}) \mid \boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\quad -\sum_{m} E\{Y(0,\boldsymbol{m}) \mid \boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\overset{\textbf{A1}}{=} \sum_{m} E\{Y(1,\boldsymbol{m}) \mid T=1,\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\quad -\sum_{m} E\{Y(0,\boldsymbol{m}) \mid T=0,\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\overset{\textbf{A2}}{=} \sum_{m} E\{Y(1,\boldsymbol{m}) \mid T=1,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\quad -\sum_{m} E\{Y(0,\boldsymbol{m}) \mid T=0,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\overset{\textbf{A3}}{=} \sum_{m} E\{Y(1,\boldsymbol{m}) \mid T=1,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid T=0,\boldsymbol{X}=\boldsymbol{x}) \\
&\quad -\sum_{m} E\{Y(0,\boldsymbol{m}) \mid T=0,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid T=0,\boldsymbol{X}=\boldsymbol{x}) \\
&= \sum_{m} E\{Y \mid T=1,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid T=0,\boldsymbol{X}=\boldsymbol{x}) \\
&\quad -\sum_{m} E\{Y \mid T=0,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid T=0,\boldsymbol{X}=\boldsymbol{x}) \\
&= \left[\beta_0+\beta_1+\sum_{j=1}^{p}\beta_{mj}E\{M_j \mid T=0,\boldsymbol{X}=\boldsymbol{x}\}+\boldsymbol{\beta}_x^{\top}\mathbf{X}\right] - \left[\beta_0+\sum_{j=1}^{m}\beta_{mj}E\{M_j \mid T=0,\boldsymbol{X}=\boldsymbol{x}\}+\boldsymbol{\beta}_x^{\top}\mathbf{x}\right] \\
&= \beta_1.
\end{aligned}
$$

$$\begin{aligned}
\text{NIE} &= E\{Y(1,\boldsymbol{M}(1)) - Y(1,\boldsymbol{M}(0)) \mid \boldsymbol{X}=\boldsymbol{x}\} \\
&= E\{Y(1,\boldsymbol{M}(1)) \mid \boldsymbol{X}=\boldsymbol{x}\} - E\{Y(1,\boldsymbol{M}(0)) \mid \boldsymbol{X}=\boldsymbol{x}\} \\
&= \sum_{m} E\{Y(1,\boldsymbol{m}) \mid \boldsymbol{M}(1)=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(1)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\quad - \sum_{m} E\{Y(1,\boldsymbol{m}) \mid \boldsymbol{M}(0)=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\overset{\textbf{A4}}{=} \sum_{m} E\{Y(1,\boldsymbol{m}) \mid \boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(1)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\quad - \sum_{m} E\{Y(1,\boldsymbol{m}) \mid \boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\overset{\textbf{A1}}{=} \sum_{m} E\{Y(1,\boldsymbol{m}) \mid T=1,\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(1)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\quad - \sum_{m} E\{Y(1,\boldsymbol{m}) \mid T=1,\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\overset{\textbf{A2}}{=} \sum_{m} E\{Y(1,\boldsymbol{m}) \mid T=1,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(1)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\quad - \sum_{m} E\{Y(1,\boldsymbol{m}) \mid T=1,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid \boldsymbol{X}=\boldsymbol{x}) \\
&\overset{\textbf{A3}}{=} \sum_{m} E\{Y(1,\boldsymbol{m}) \mid T=1,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(1)=\boldsymbol{m} \mid T=1,\boldsymbol{X}=\boldsymbol{x}) \\
&\quad - \sum_{m} E\{Y(1,\boldsymbol{m}) \mid T=1,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid T=0,\boldsymbol{X}=\boldsymbol{x}) \\
&= \sum_{m} E\{Y \mid T=1,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(1)=\boldsymbol{m} \mid T=1,\boldsymbol{X}=\boldsymbol{x}) \\
&\quad - \sum_{m} E\{Y \mid T=1,\boldsymbol{M}=\boldsymbol{m},\boldsymbol{X}=\boldsymbol{x}\} P(\boldsymbol{M}(0)=\boldsymbol{m} \mid T=0,\boldsymbol{X}=\boldsymbol{x}) \\
&= \left(\beta_0 + \beta_1 + \sum_{j=1}^{m} \beta_{mj} E\{M_j \mid T=1,\boldsymbol{X}=\boldsymbol{x}\} + \boldsymbol{\beta}_x^\top \boldsymbol{x}\right) \\
&\quad - \left(\beta_0 + \beta_1 + \sum_{j=1}^{m} \beta_{mj} E\{M_j \mid T=0,\boldsymbol{X}=\boldsymbol{x}\} + \boldsymbol{\beta}_x^\top \boldsymbol{x}\right) \\
&= \sum_{j=1}^{m} \beta_{mj} \times [E\{M_j \mid T=1,\boldsymbol{X}=\boldsymbol{x}\} - E\{M_j \mid T=0,\boldsymbol{X}=\boldsymbol{x}\}] \\
&= \sum_{j=1}^{m} \beta_{mj} \times \left\{ \frac{\exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x})}{1+\exp(\gamma_{0j}+\gamma_{1j}+\boldsymbol{\gamma}_{xj}^\top \boldsymbol{x})} - \frac{\exp(\alpha_{0j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x})}{1+\exp(\gamma_{0j}+\boldsymbol{\gamma}_{xj}^\top \boldsymbol{x})} \right\}.
\end{aligned}$$

B. Decomposition for the average natural indirect effect

To identify the path specific effects NIEP_j and NIEA_j mentioned in main text, we extend the preceding assumptions **A1-A4** to the two ordered mediators model case. And the assumptions is given as:

B1: No unmeasured confounding of the $(T, Z_j, M_j) - Y$ relationship: $Y(t, z_j, m_j) \perp\!\!\!\perp T \mid \boldsymbol{X} = \boldsymbol{x}$ for all levels of t, z_j, m_j, and $\boldsymbol{x}$, $Y(t, z_j, m_j) \perp\!\!\!\perp Z_j \mid T = t, \boldsymbol{X} = \boldsymbol{x}$ for all levels of t, z_j, m_j, and $Y(t, z_j, m_j) \perp\!\!\!\perp M_j \mid T = t, Z_j = z_j, \boldsymbol{X} = \boldsymbol{x}$ for all levels of t, z_j, m_j;

B2: No unmeasured confounding of the $T - Z_j$ or $(T, Z_j) - M_j$ relationships: $Z_j(t) \perp\!\!\!\perp T \mid \boldsymbol{X} = \boldsymbol{x}$ for all levels of t, $M_j(t, z_j) \perp\!\!\!\perp Z_j \mid T = t, \boldsymbol{X} = \boldsymbol{x}$ for all levels of t, z_j, and $M_j(t, z_j) \perp\!\!\!\perp T \mid \boldsymbol{X} = \boldsymbol{x}$ for all levels of t, z_j.

Using the notation in the main text, we can write $E\{Y(1, Z_j(1), M_j(1, Z_j(1)))|\boldsymbol{X} = \boldsymbol{x}\}$ as

$$\begin{aligned}
&E\{Y(1, Z_j(1), M_j(1, Z_j(1)))|\boldsymbol{X} = \boldsymbol{x}\} \\
&= \sum_{m_j} E\{Y(1,1,m_j) \mid Z_j(1)=1, M_j(1,Z_j(1)))=m_j,\boldsymbol{x}\} p\{M_j(1,Z_j(1))=m_j \mid Z_j(1)=1,\boldsymbol{x}\} p\{Z_j(1)=1|\boldsymbol{x}\} \\
&+ \sum_{m_j} E\{Y(1,0,m_j) \mid Z_j(1)=0, M_j(1,Z_j(1)))=m_j,\boldsymbol{x}\} p\{M_j(1,Z_j(1))=m_j \mid Z_j(1)=0,\boldsymbol{x}\} p\{Z_j(1)=0|\boldsymbol{x}\}. \quad (1)
\end{aligned}$$

Given above assumptions, it is straightforward to demonstrate the identifiability of the first term in equation (1), as illustrated

$$
\begin{aligned}
&E\{Y(1,1,m_j) \mid Z_j(1)=1, M_j(1,Z_j(1)))=m_j, \boldsymbol{X}=\boldsymbol{x}\} \\
&\overset{\textbf{B1}}{=} E\{Y(1,1,m_j) \mid T=1, Z_j(1)=1, M_j(1,Z_j(1))=m_j, \boldsymbol{X}=\boldsymbol{x}\} \\
&= E\{Y \mid T=1, Z_j=1, M_j=m_j, \boldsymbol{X}=\boldsymbol{x}\}.
\end{aligned}
$$

Similarly, we can derive $E\{Y(1,0,m_j) \mid Z_j(1)=0, M_j(1,Z_j(1)))=m_j, \boldsymbol{X}=\boldsymbol{x}\}$, where

$$
\begin{aligned}
&E\{Y(1,0,m_j) \mid Z_j(1)=0, M_j(1,Z_j(1)))=m_j, \boldsymbol{X}=\boldsymbol{x}\} \\
&\overset{\textbf{B1}}{=} E\{Y(1,0,m_j) \mid T=1, Z_j(1)=0, M_j(1,Z_j(1))=m_j, \boldsymbol{X}=\boldsymbol{x}\} \\
&= E\{Y \mid T=1, Z_j=0, M_j=m_j, \boldsymbol{X}=\boldsymbol{x}\}.
\end{aligned}
$$

For the second term of equation (1), we can easily derive $p\{M_j(1,Z_j(1))=m_j \mid Z_j(1)=1,\boldsymbol{x}\}$ and $p\{M_j(1,Z_j(1))=m_j \mid Z_j(1)=0,\boldsymbol{x}\}$, where

$$
\begin{aligned}
&p\{M_j(1,Z_j(1))=m_j \mid Z_j(1)=1, \boldsymbol{X}=\boldsymbol{x}\} \\
&= p\{M_j(1,1)=m_j \mid Z_j(1)=1, \boldsymbol{X}=\boldsymbol{x}\} \\
&\overset{\textbf{B2}}{=} p\{M_j(1,1)=m_j \mid T=1, Z_j(1)=1, \boldsymbol{X}=\boldsymbol{x}\} \\
&= p\{M_j=m_j \mid T=1, Z_j=1, \boldsymbol{X}=\boldsymbol{x}\},
\end{aligned}
$$

and

$$
\begin{aligned}
&p\{M_j(1,Z_j(1))=m_j \mid Z_j(1)=0, \boldsymbol{X}=\boldsymbol{x}\} \\
&= p\{M_j(1,0)=m_j \mid Z_j(1)=0, \boldsymbol{X}=\boldsymbol{x}\} \\
&\overset{\textbf{B2}}{=} p\{M_j(1,0)=m_j \mid T=1, Z_j(1)=0, \boldsymbol{X}=\boldsymbol{x}\} \\
&= p\{M_j=m_j \mid T=1, Z_j=0, \boldsymbol{X}=\boldsymbol{x}\}.
\end{aligned}
$$

Therefore,

$$
\begin{aligned}
&E\{Y(1,Z_j(1),M_j(1,Z_j(1)))|\boldsymbol{X}=\boldsymbol{x}\} \\
&= \sum_{m_j} E\{Y \mid T=1, Z_j=1, M_j=m_j, \boldsymbol{X}=\boldsymbol{x}\} P(M_j=m_j \mid T=1, Z_j=1, \boldsymbol{X}=\boldsymbol{x}) P(Z_j=1 \mid T=1, \boldsymbol{X}=\boldsymbol{x}) \\
&\quad + \sum_{m_j} E\{Y \mid T=1, Z_j=0, M_j=m_j, \boldsymbol{X}=\boldsymbol{x}\} P(M_j=m_j \mid T=1, Z_j=0, \boldsymbol{X}=\boldsymbol{x}) P(Z_j=0 \mid T=1, \boldsymbol{X}=\boldsymbol{x}) \\
&= \left\{\beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj} E(M_j \mid T=1, Z_j=1, \boldsymbol{X}=\boldsymbol{x})\right\} P(Z_j=1 \mid T=1, \boldsymbol{X}=\boldsymbol{x}) \\
&\quad + \left\{\beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}++\beta_{mj} E(M_j \mid T=1, Z_j=0, \boldsymbol{X}=\boldsymbol{x})\right\} P(Z_j=0 \mid T=1, \boldsymbol{X}=\boldsymbol{x}) \\
&= \beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj}\exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) P(Z_j=0 \mid T=1, \boldsymbol{X}=\boldsymbol{x}).
\end{aligned}
$$

Similarly, we can easily derive $E\{Y(1,Z_j(0),M_j(1,Z_j(0)))|\boldsymbol{X}=\boldsymbol{x}\}$ and $E\{Y(1,Z_j(0),M_j(0,Z_j(0)))|\boldsymbol{X}=\boldsymbol{x}\}$, where

$$
\begin{aligned}
&E\{Y(1,Z_j(0),M_j(1,Z_j(0)))|\boldsymbol{X}=\boldsymbol{x}\} \\
&= \sum_{m_j} E\{Y \mid T=1, Z_j=1, M_j=m_j, \boldsymbol{X}=\boldsymbol{x}\} P(M_j=m_j \mid T=1, Z_j=1, \boldsymbol{X}=\boldsymbol{x}) P(Z_j=1 \mid T=0, \boldsymbol{X}=\boldsymbol{x}) \\
&\quad + \sum_{m_j} E\{Y \mid T=1, Z_j=0, M_j=m_j, \boldsymbol{X}=\boldsymbol{x}\} P(M_j=m_j \mid T=1, Z_j=0, \boldsymbol{X}=\boldsymbol{x}) P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x}) \\
&= \left\{\beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj} E(M_j \mid T=1, Z_j=1, \boldsymbol{X}=\boldsymbol{x})\right\} P(Z_j=1 \mid T=0, \boldsymbol{X}=\boldsymbol{x}) \\
&\quad + \left\{\beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj} E(M_j \mid T=1, Z_j=0, \boldsymbol{X}=\boldsymbol{x})\right\} P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x}) \\
&= \beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj}\exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x}),
\end{aligned}
$$

and

$$
\begin{aligned}
&E\{Y(1, Z_j(0), M_j(0, Z_j(0)))|\boldsymbol{X}=\boldsymbol{x}\} \\
&= \sum_{m_j} E\{Y \mid T=1, Z_j=1, M_j=m_j, \boldsymbol{X}=\boldsymbol{x}\} P(M_j=m_j \mid T=0, Z_j=1, \boldsymbol{X}=\boldsymbol{x}) P(Z_j=1 \mid T=0, \boldsymbol{X}=\boldsymbol{x}) \\
&\quad + \sum_{m_j} E\{Y \mid T=1, Z_j=0, M_j=m_j, \boldsymbol{X}=\boldsymbol{x}\} P(M_j=m_j \mid T=0, Z_j=0, \boldsymbol{X}=\boldsymbol{x}) P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x}) \\
&= \left\{\beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj} E(M_j \mid T=0, Z_j=1, \boldsymbol{X}=\boldsymbol{x})\right\} P(Z_j=1 \mid T=0, \boldsymbol{X}=\boldsymbol{x}) \\
&\quad + \left\{\beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj} E(M_j \mid T=0, Z_j=0, \boldsymbol{X}=\boldsymbol{x})\right\} P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x}) \\
&= \beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj} \exp(\alpha_{0j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x}),
\end{aligned}
$$

Based on the above equations, we can derive the indirect effect NIEP$_j$ and NIEA$_j$, respectively, where

$$
\begin{aligned}
\text{NIEP}_j &= E\{Y(1, Z_j(1), M_j(1, Z_j(1)))|\boldsymbol{X}=\boldsymbol{x}\} - E\{Y(1, Z_j(0), M_j(1, Z_j(0)))|\boldsymbol{X}=\boldsymbol{x}\} \\
&= \left\{\beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj} \exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) P(Z_j=0 \mid T=1, \boldsymbol{X}=\boldsymbol{x})\right\} \\
&\quad - \left\{\beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj} \exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x})\right\} \\
&= \beta_{mj} \exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) P(Z_j=0 \mid T=1, \boldsymbol{X}=\boldsymbol{x}) - \beta_{mj} \exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x}) \\
&= \beta_{mj} \exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) \left\{P(Z_j=0 \mid T=1, \boldsymbol{X}=\boldsymbol{x}) - P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x})\right\} \\
&= \beta_{mj} \left\{ \frac{\exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x})}{1+\exp(\gamma_{0j}+\gamma_{1j}+\boldsymbol{\gamma}_{xj}^\top \boldsymbol{x})} - \frac{\exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x})}{1+\exp(\gamma_{0j}+\boldsymbol{\gamma}_{xj}^\top \boldsymbol{x})} \right\},
\end{aligned}
$$

and

$$
\begin{aligned}
\text{NIEA}_j &= E\{Y(1, Z_j(0), M_j(1, Z_j(0)))|\boldsymbol{X}=\boldsymbol{x}\} - E\{Y(1, Z_j(0), M_j(0, Z_j(0)))|\boldsymbol{X}=\boldsymbol{x}\} \\
&= \left\{\beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj} \exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x})\right\} \\
&\quad - \left\{\beta_0+\beta_1+\boldsymbol{\beta}_x^\top \boldsymbol{x}+\beta_{mj} \exp(\alpha_{0j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x})\right\} \\
&= \beta_{mj} P(Z_j=0 \mid T=0, \boldsymbol{X}=\boldsymbol{x}) \left\{\exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x}) - \exp(\alpha_{0j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x})\right\} \\
&= \beta_{mj} \left\{ \frac{\exp(\alpha_{0j}+\alpha_{1j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x})}{1+\exp(\gamma_{0j}+\boldsymbol{\gamma}_{xj}^\top \boldsymbol{x})} - \frac{\exp(\alpha_{0j}+\boldsymbol{\alpha}_{xj}^\top \boldsymbol{x})}{1+\exp(\gamma_{0j}+\boldsymbol{\gamma}_{xj}^\top \boldsymbol{x})} \right\}.
\end{aligned}
$$

With NIEP$_j$ and NIEA$_j$ defined, we have the decomposition: NIE$_j$ = NIEP$_j$ + NIEA$_j$, so that the overall natural indirect effect decomposes into the sum of the effect through Z_j and not through Z_j.

II. Additional figures for simulation studies

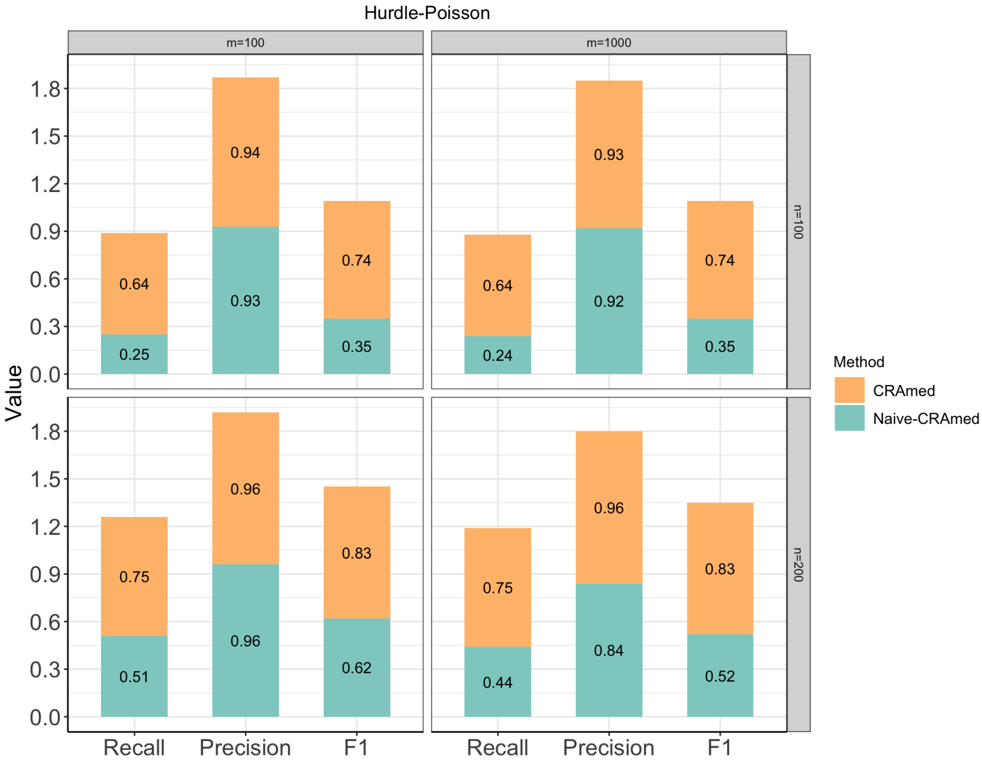


Figure S1: Comparison of Recall, Precision, and F1 score for the Naive-CRAmed and CRAmed methods using microbiome data generated from the hurdle Poisson model. Sample size $n \in \{100, 200\}$ and number of taxa $m \in \{100, 1000\}$.

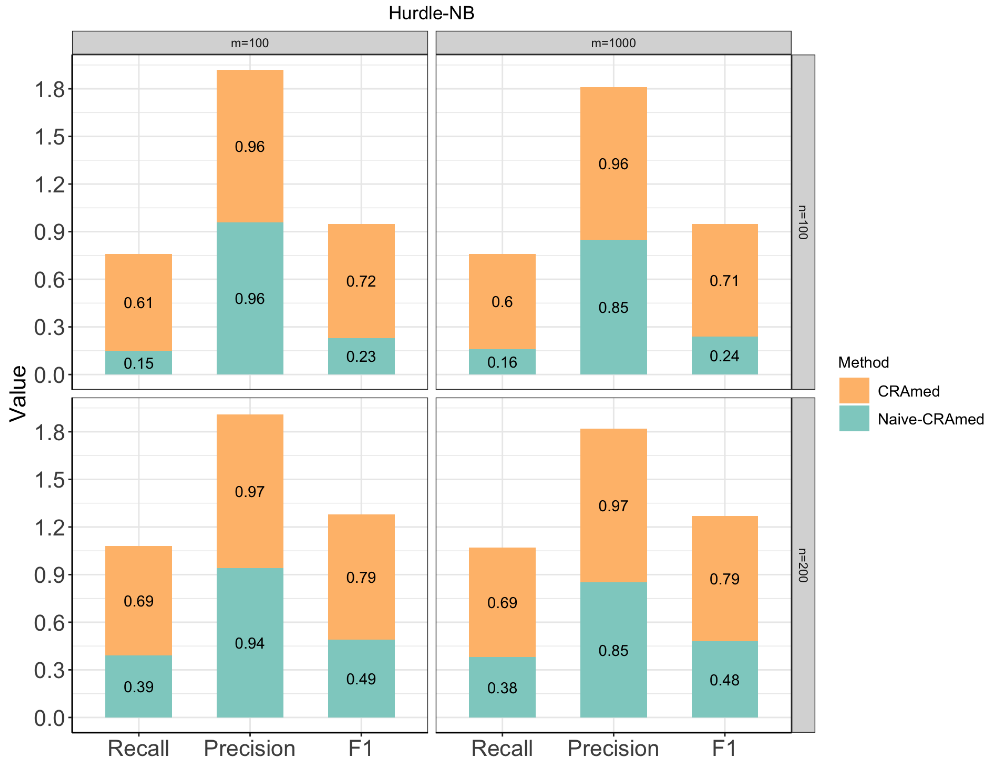


Figure S2: Comparison of Recall, Precision, and F1 score for the Naive-CRAmed and CRAmed methods using microbiome data generated from the hurdle NB model. Sample size $n \in \{100, 200\}$ and number of taxa $m \in \{100, 1000\}$.

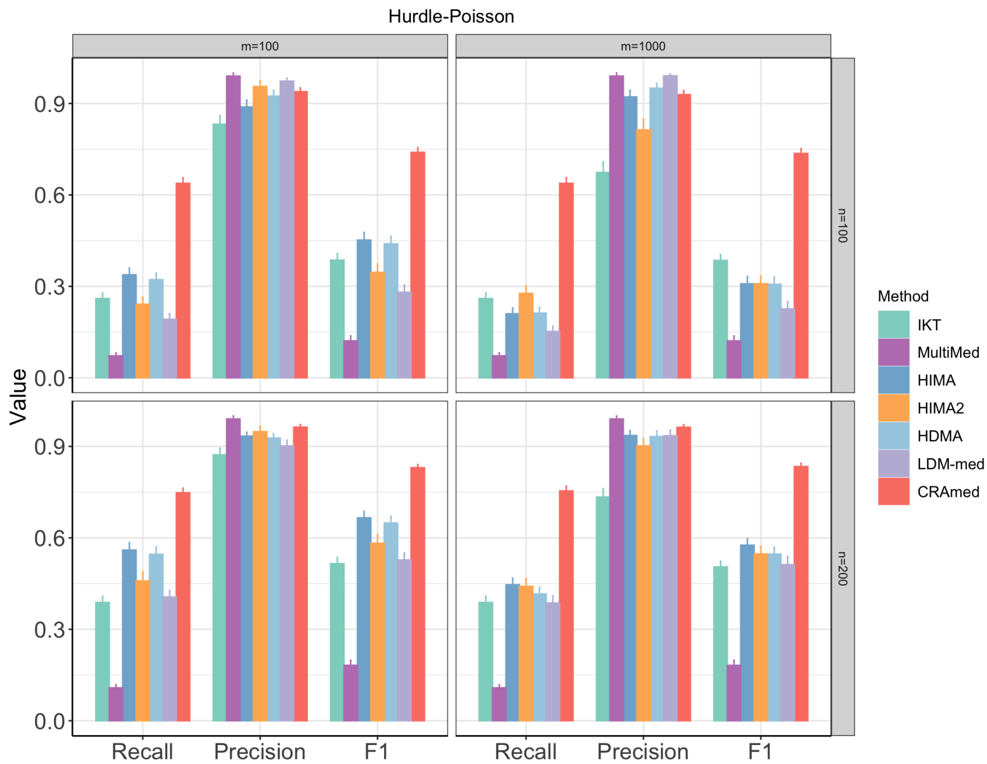


Figure S3: Comparison of Recall, Precision, and F1 score for different mediation analysis methods using microbiome data generated from the hurdle Poisson model. Sample size $n \in \{100, 200\}$ and number of taxa $m \in \{100, 1000\}$.

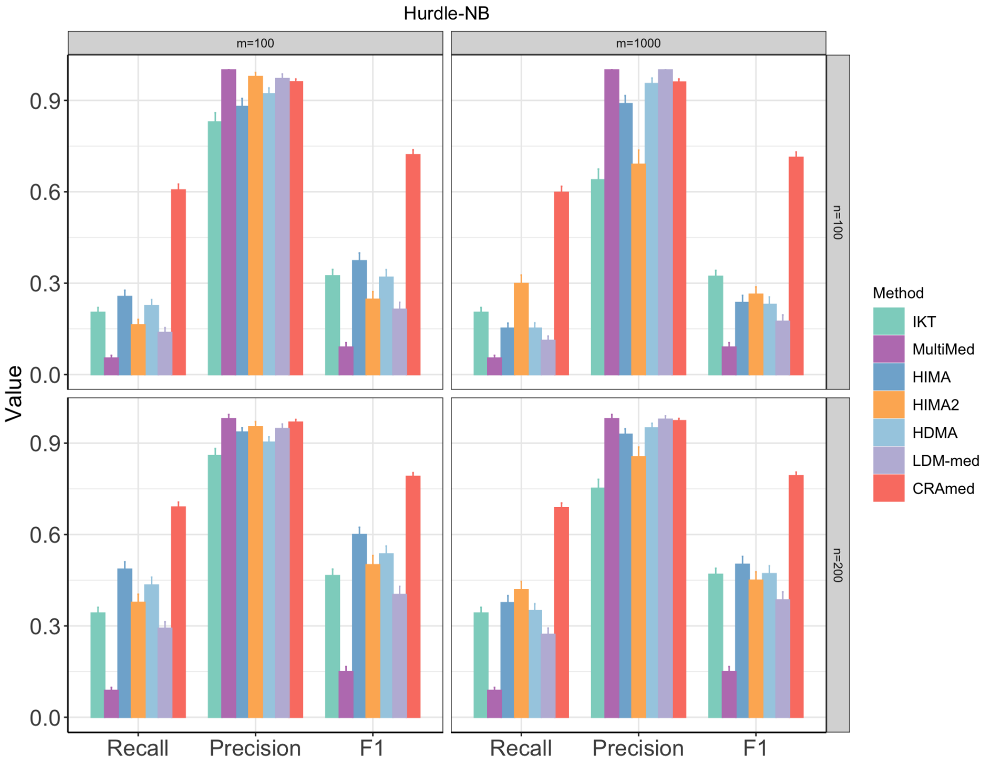


Figure S4: Comparison of Recall, Precision, and F1 score for different mediation analysis methods using microbiome data generated from the hurdle NB model. Sample size $n \in \{100, 200\}$ and number of taxa $m \in \{100, 1000\}$.

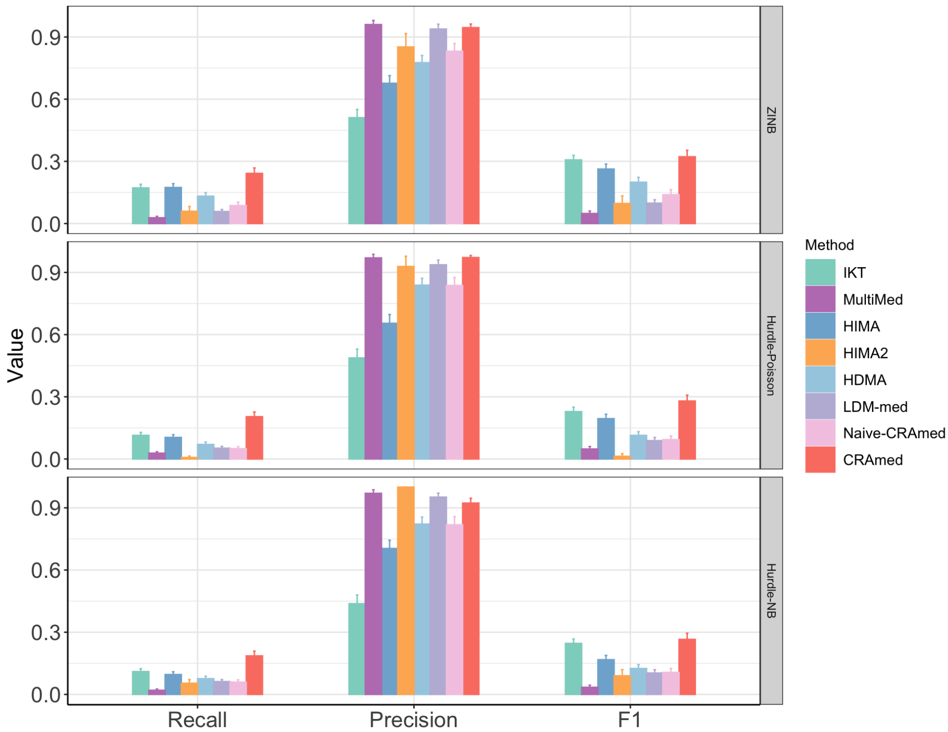


Figure S5: Comparison of Recall, Precision, and F1 score for sensitivity analysis using microbiome data generated from either the ZINB model, or the hurdle Poisson or NB model, with unobserved confounders. Sample size $n = 100$ and number of taxa $m = 100$.

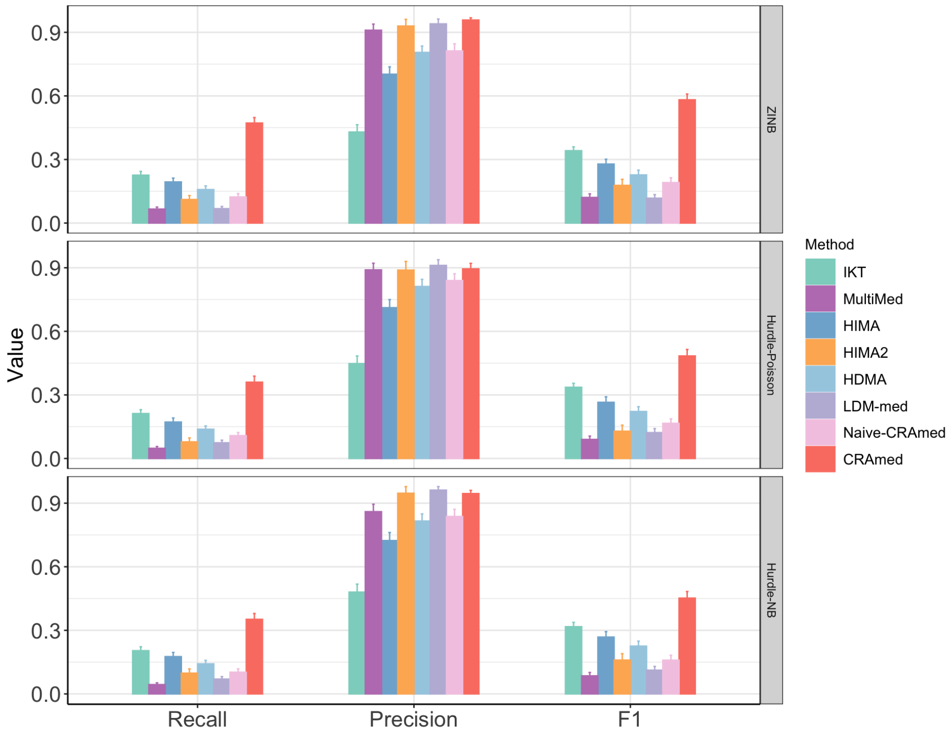


Figure S6: Comparison of Recall, Precision, and F1 score for sensitivity analysis using microbiome data generated from either the ZINB model, or the hurdle Poisson or NB model, with unobserved confounders. Sample size $n = 200$ and number of taxa $m = 100$.

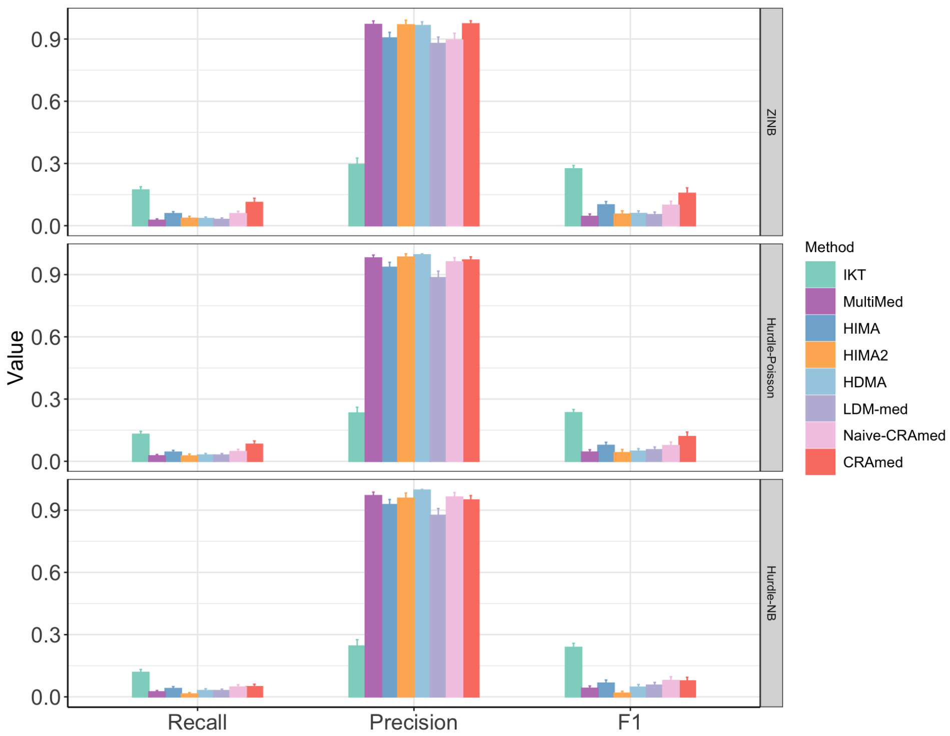


Figure S7: Comparison of Recall, Precision, and F1 score for sensitivity analysis using microbiome data generated from either the ZINB model, or the hurdle Poisson or NB model, with unobserved confounders. Sample size $n = 100$ and number of taxa $m = 1000$.

III. Preprocessing and additional results for the application studies in Section 3

A. *Identification of microbial mediators of weight under different modes of delivery*

We utilize a publicly available gut microbiome dataset from a previous study (Yassour et al, 2016), encompassing clinical examinations and gut microbiome data from 1098 infants. Our investigation centers on the outcome variable weight, aiming to explore whether the gut microbiome mediates the relationship between delivery mode and the infants' weight.

After filtering out taxa with a prevalence of less than 10%, we obtained a dataset with 876 taxa and 1098 samples. Figures S8 (a) and (b) depict distinct microbial profiles between the C-section and vaginal groups. Regarding alpha diversity, the vaginal group exhibits higher microbial richness and evenness, compared to the C-section group. In terms of Beta diversity, Bray–Curtis dissimilarity reveals that the C-section group has distinct community structures compared to the vaginal group. These community-level findings indicate that the microbiome may mediate the relationship between the mode of delivery and infants' weight.

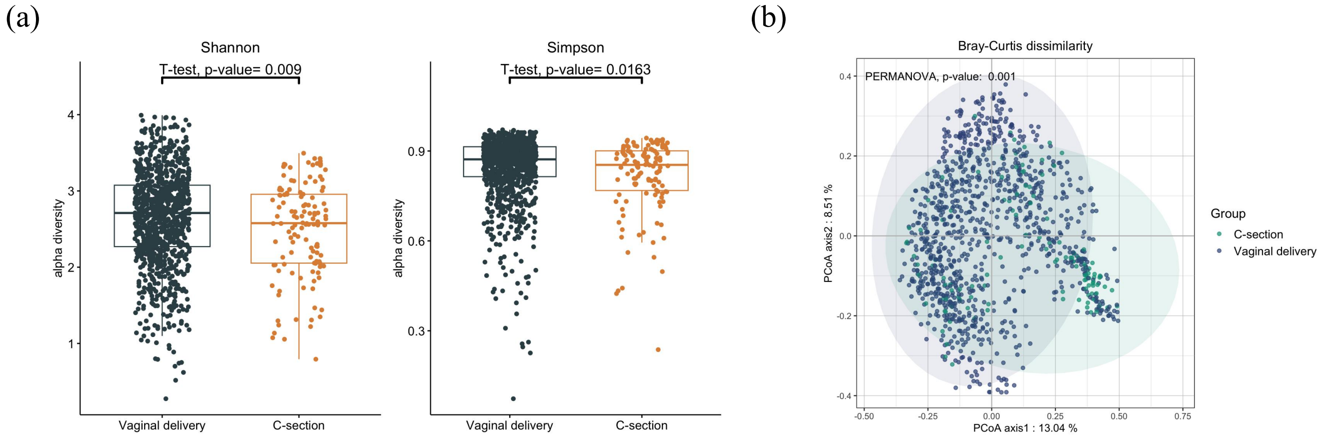


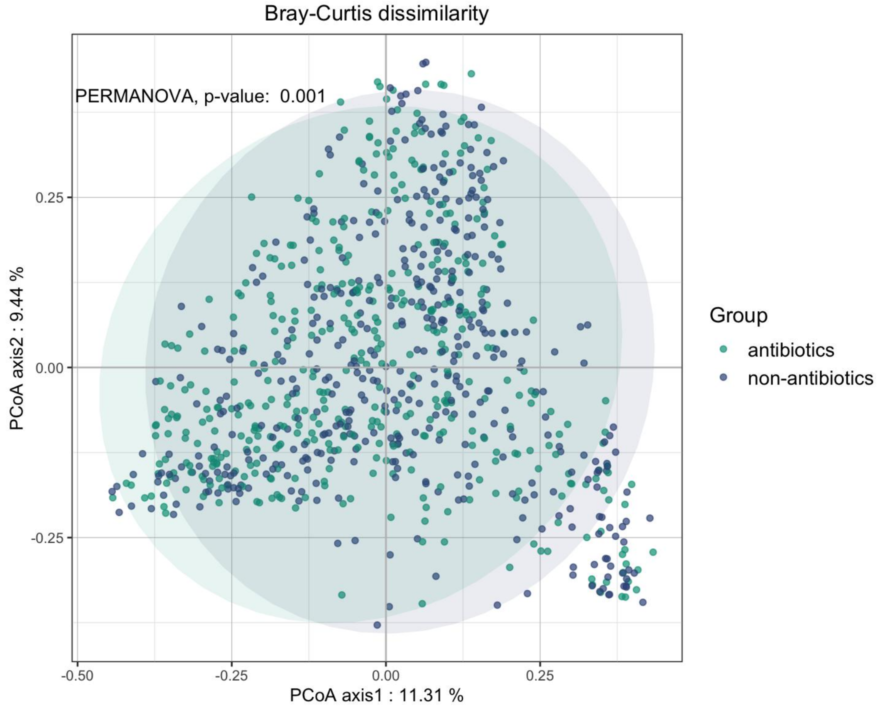
Figure S8: Alpha and beta diversity analyses for the DIABIUMMUNE dataset. (a) Box plots of alpha diversities (Shannon and Simpson indices) between the C-section and vaginal delivery groups. (b) PCoA plots using the Bray–Curtis dissimilarity.

B. *Identification of microbial mediators of BMI and waist circumference under antibiotic treatment*

We employ a dataset from the Guangdong Gut Microbiome Project (GGMP), a large community-based cross-sectional cohort conducted between 2015 and 2016, comprising 7009 participants with high-quality gut microbiome data (He et al, 2018). Notably, the taxa in the microbiome dataset are sparse and high-dimensional. Before proceeding, we filtered taxa with prevalence less than 10%. Due to the computational burden imposed by the large sample size, we randomly sampled an equal number of samples from non-antibiotics individuals as antibiotics individuals, resulting in 944 taxa across 894 samples.

Figure S9(a) demonstrates that the antibiotics and non-antibiotics groups exhibit distinct microbial profiles in terms of community diversity. From Figure S9(b), it is evident that the microbiome community is significantly correlated with BMI and waist circumference (WC). Hence, we posit that the microbiome may play a mediating role between antibiotics and phenotypes.

(a)



(b)

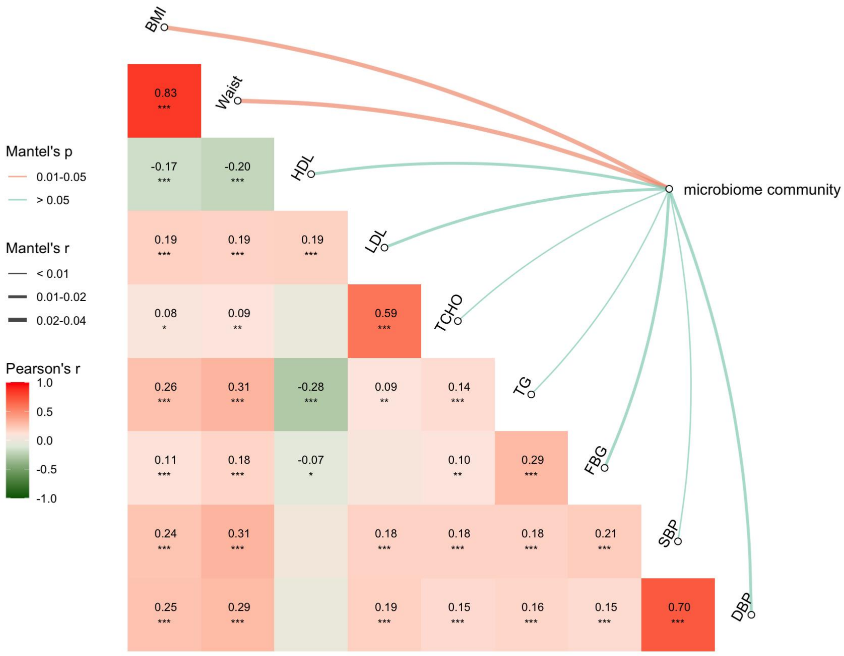


Figure S9: Association analyses for the GGMP dataset. (a) PCoA plots using the Bray–Curtis dissimilarity. (b) Mantel test examining the relationship between gut microbiota and CMD-related risk factors (BMI, WC, HDL, LDL, TCHO, TG, FBG, SBP, and DBP). For subplot (b), $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

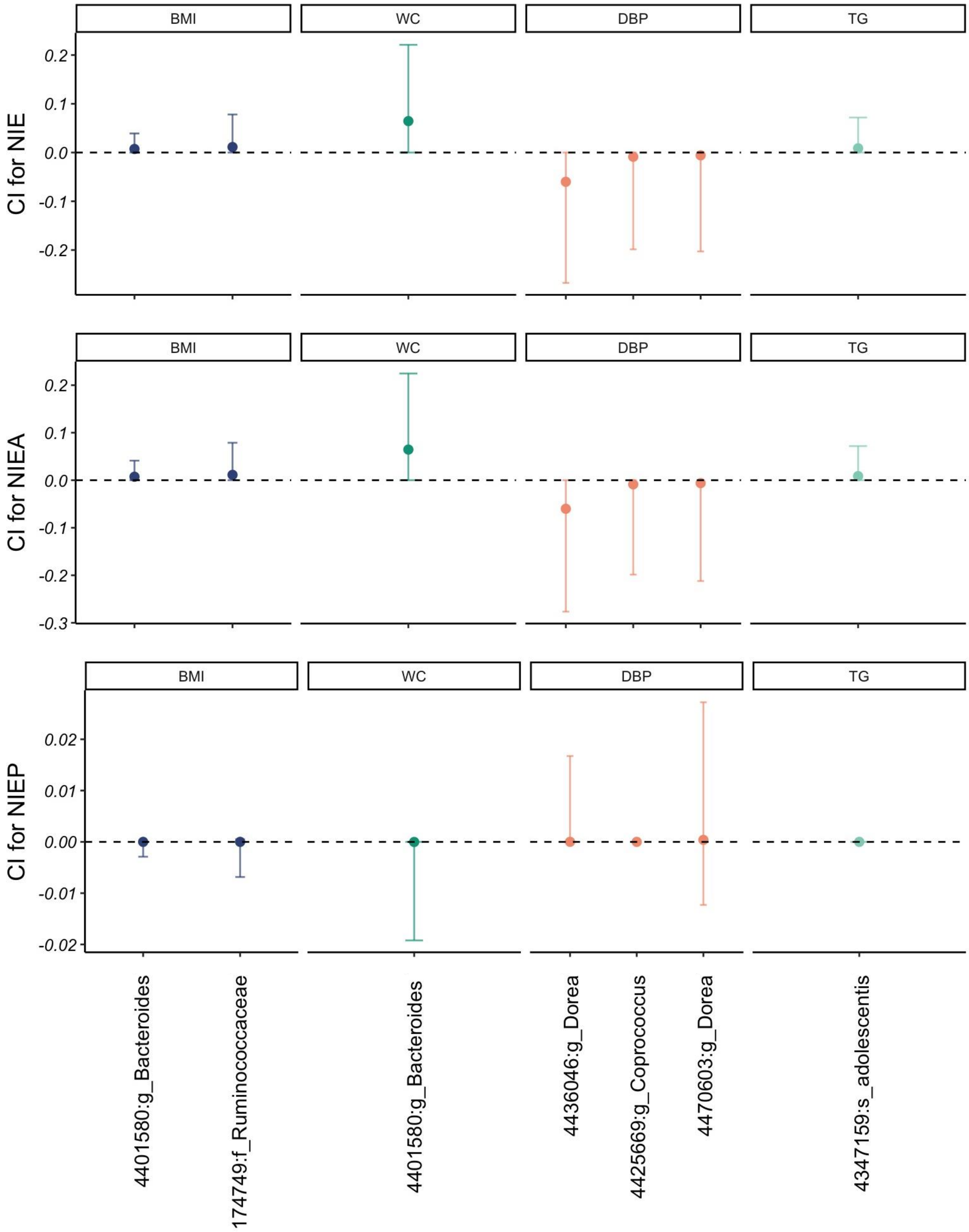


Figure S10: Point and 95% CI estimates of NIE, NIEA, and NIEP for CRAmed-identified OTUs for the GGMP dataset mediating the effect of antibiotics on CMD-related risk factors. 95% CI estimates of NIE, NIEA, and NIEP were calculated based on the permutation strategy with 1000 repetitions.

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