Inverse Probability Weighting-based Mediation Analysis for Microbiome Data

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Abstract

Mediation analysis is an important tool to study causal associations in biomedical and other scientific areas and has recently gained attention in microbiome studies. Using a microbiome study of acute myeloid leukemia (AML) patients, we investigate whether the effect of induction chemotherapy intensity levels on the infection status is mediated by the microbial taxa abundance. The unique characteristics of the microbial mediators—high-dimensionality, zero-inflation, and dependence—call for new methodological developments in mediation analysis. The presence of an exposure-induced mediator-outcome confounder, antibiotic use, further requires a delicate treatment in the analysis. To address these unique challenges in our motivating AML microbiome study, we propose a novel nonparametric identification formula for the interventional indirect effect (IIE), a measure recently developed for studying mediation effects. We develop the corresponding estimation algorithm using the inverse probability weighting method. We also test the presence of mediation effects via constructing the standard normal

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bootstrap confidence intervals. Simulation studies show that the proposed method has good finite-sample performance in terms of the IIE estimation, and type-I error rate and power of the corresponding test. In the AML microbiome study, our findings suggest that the effect of induction chemotherapy intensity levels on infection is mainly mediated by patients’ gut microbiome.

**Keywords:** causal inference; confounder; high-dimensional mediators; interventional indirect effect

## 1 Introduction

The importance of the human microbiome has been increasingly recognized in biomedicine, due to its association with many complex diseases, such as obesity (Turnbaugh et al., 2009), cardiovascular disease (Koeth et al., 2013), diabetes (Qin et al., 2012; Dobra et al., 2019; Ren et al., 2020), liver cirrhosis (Qin et al., 2014), inflammatory bowel disease (Halfvarson et al., 2017), psoriasis (Tett et al., 2017), and colorectal cancer (Zackular et al., 2016), as well as its noteworthy response to cancer immunotherapy (Frankel et al., 2017; Gopalakrishnan et al., 2018; Zitvogel et al., 2018).

Advances in high-throughput next generation sequencing technologies (e.g., 16S ribosomal RNA [rRNA] sequencing, shotgun sequencing) make it possible to fully characterize the human microbiome, better understand the risk factors (e.g., clinical, genetic, and environmental factors) that shape the human microbiome, and decipher the function and impact of the microbiome profile on human health and diseases (Li, 2015; Chen and Li, 2016; Zhu et al., 2017; Zhang et al., 2018; Reyes-Gibby et al., 2020; Sun et al., 2020; Wang et al., 2020b). An in-depth understanding of the role of the microbiome underlying human health and diseases will provide key information (e.g., treatment effect, disease progression) to help develop new strategies for clinical prevention or intervention, and to treat health issues or diseases, by potentially modifying the relevant microbiota (Faith et al., 2013; Le Chatelier et al., 2013; Zhang et al., 2018).

Recent studies in human microbiomes have revealed the potentially complex interplay among the risk factors, microbiome, and human health and diseases. For example, studies in cancer patients have shown that during allogeneic hematopoietic stem cell transplantation, the diversity and stability of the intestinal flora are disrupted, resulting in bacterial domination that is associated with subsequent infection (Taur et al., 2012). Such an observation suggests that changes in the microbiome profile may play a mediation role in the causal pathway between the allogeneic
hematopoietic stem cell transplantation and subsequent infection. Other examples include the potential mediation effect of the microbiome on the association between dietary intake and immune response or chronic diseases (Wu et al., 2011; Sivan et al., 2015; Koslovsky et al., 2020), and the potential modulatory effect of the microbiome on the association between genetic variants and diseases (Snijders et al., 2016).

Motivated by a unique acute myeloid leukemia (AML) microbiome study conducted at The University of Texas MD Anderson Cancer Center (MD Anderson), in this paper we are interested in studying the potential mediating roles of microbiome features in the causal effect of induction chemotherapy (IC) type on the infection status in AML patients undergoing IC. Since most infections in patients with cancer are caused by commensal bacteria (Montassier et al., 2013), infection control is an area of patient care that is likely to be profoundly influenced by investigations of the microbiome (Zitvogel et al., 2015). AML patients receiving intensive IC are highly susceptible to infections that generally arise from their commensal microbiota (Bucaneve et al., 2005; Gardner et al., 2008). Infection is a substantial cause of therapy-associated morbidity and mortality and represents a frequent cause of treatment withdrawal in this specific patient population (Cannas et al., 2012). About 77% of the febrile episodes occurring in AML patients are microbiologically or clinically documented infections. A preliminary data analysis of 34 AML patients undergoing IC at MD Anderson showed that the baseline microbiome α-diversity was associated with infection during IC; the change in the α-diversity during IC might be related to subsequent infection in the 90 days after discharge (Galloway-Peña et al., 2016, 2020). These findings suggest potential mediating roles of microbiome features in the effect of treatment option (e.g., IC type) on clinical response (e.g., infection status) in AML patients.

Mediation analysis helps researchers understand how and why a causal effect arises. Traditionally, in the social and health sciences, mediation analysis has been formulated and understood within the linear structural equation modeling framework (e.g., Baron and Kenny, 1986; Shrout and Bolger, 2002; MacKinnon, 2008; Wang et al., 2010; Taylor and MacKinnon, 2012). Similar approaches have recently been adopted to study the mediation effect of the microbiome in human health and diseases (Zhang et al., 2018, 2019, 2021). Under this framework, definitions of mediation effects are model-driven, and hence by construction, they may not be easily generalized beyond linear models. In particular, they are not suitable for answering our question of interest here
as the infection status (i.e., outcome) is binary. Instead, modern causal mediation analyses are built upon the nonparametric definition and identification of mediation effects. Robins and Greenland (1992) provided nonparametric definitions of direct and indirect effects, while Pearl (2001) showed that these effects might be nonparametrically identifiable under a set of nonparametric structural equation models with independent errors. Along this line, Sohn and Li (2019) proposed a sparse compositional mediation model based on the algebra for compositional data in the simplex space, along with bootstrap for tests of total and component-wise mediation effects. Wang et al. (2020a) proposed a rigorous sparse microbial causal mediation model to deal with the high-dimensional and compositional features of microbiome data using linear log-contrast and Dirichlet regression models, as well as regularization techniques for variable selection to identify significant microbes. Li et al. (2020) developed a mediation analysis method that focuses on mediators with zero-inflated distributions.

However, none of the aforementioned methods can be directly applied to test the mediation effect of microbiome features in our AML microbiome study. A major challenge in our study is to address the confounding effect of an intermediate variable (i.e., antibiotic use) which confounds the relationship between the mediators (i.e., microbiome profile) and the outcome (i.e., infection status), and can also be affected by the exposure variable (i.e., IC type). This is a common problem in microbiome studies but has largely been overlooked by previous mediation studies for microbiome data. To deal with a similar problem in a different context, VanderWeele et al. (2014) introduced an alternative notion called interventional indirect effect and showed that it could be nonparametrically identified in the presence of exposure-induced mediator-outcome confounders. They also developed a weighting-based method to estimate the interventional indirect effect. However, their estimation method involves modeling the conditional distributions of mediators, which is difficult in our problem as the microbial mediators are high-dimensional, zero-inflated, and dependent (Martin et al., 2020). To address this challenge, we develop a novel identification formula for the interventional indirect effect. Our identification formula does not involve conditional distributions of mediators, thereby circumventing the need to model the complex mediators. Instead, our approach requires modeling the conditional expectation of binary infection status, as well as the two conditional distributions of binary antibiotic use status. As the microbial mediators are high-dimensional, we adopt the sparsity-induced regularization to model the binary infection sta-
We test the presence of the interventional indirect effect via constructing the standard normal bootstrap confidence interval (Efron and Tibshirani, 1994).

The remainder of this paper is organized as follows. We provide a detailed description of the motivating AML microbiome study in Section 2. In Section 3, we introduce our mediation model and related estimation procedures. We assess the performance of our proposal through simulation studies in Section 4 and apply the proposed method to the AML microbiome study in Section 5. We end with a discussion in Section 6. We provide the technical proofs, implementation details of bagging with the optimal subset of deep neural networks, and additional results for the AML microbiome study in the Supplementary Material.

2 The motivating study

Our analysis is motivated by the AML microbiome study conducted at MD Anderson, which is among the first-in-human studies in its subject field. This study seeks to understand how the microbiome influences the care of patients being treated for AML, with a particular focus on infectious toxicity. It is the largest longitudinal microbiome study to date for hematologic malignancy patients during intensive treatment (Galloway-Peña et al., 2020).

The study included 97 adult patients with newly diagnosed AML undergoing IC treatment at MD Anderson from September 2013 to August 2015 (Galloway-Peña et al., 2016, 2017, 2020). Fecal specimens were collected from each patient at baseline (prior to starting IC), and continued approximately every 96 hours over the IC course, resulting in a total of 566 samples. DNA was extracted from patient fecal specimens and the 16S rRNA V4 region was sequenced on the Illumina MiSeq platform. 16S rRNA gene sequences were assigned into operational taxonomic units (OTUs) based on a 97% similarity cutoff at the genus level. An OTU table was generated for downstream analyses, and contained the number of sequences (abundance) that were observed for each taxon in each sample.

In our investigation, we are concerned with exploring the causal associations among IC type, microbiome features, and infection status, where the microbiome features are relatively high-dimensional, zero-inflated, and dependent. This is best answered within the framework of mediation analysis, which was first proposed in the social sciences (Baron and Kenny, 1986; MacKinnon,
2008) and further developed in the causal inference literature (Robins and Greenland, 1992; Pearl, 2001; VanderWeele et al., 2014). Figure 1 illustrates the conceptual model of interest. Under the framework of mediation analysis, we aim to elucidate the roles of the microbiome features (i.e., mediators) and IC type (i.e., exposure variable) in causing infection (i.e., outcome) following treatment, specifically, the mediation effect of microbiome features during the AML treatment on the causal relationship between the IC type and infection status. The mediation analysis is further complicated by the administration of various antibiotics during the AML treatment, which is commonly prescribed to prevent and treat infections. It is known that the use of antibiotics will lead to changes in the composition of gut microbiota (Donnat et al., 2018; Fukuyama et al., 2019; Schulfer et al., 2019; Zhang and Chen, 2019; Xavier et al., 2020). Therefore, the effect of the gut microbiome on infection status may be confounded by the administration of antibiotics.

![Figure 1: A conceptual model for the AML microbiome study.](image)

In the conceptual model, the exposure variable is the binary IC type, with one indicating high-intensity regimens and zero indicating low-intensity regimens. In particular, high-intensity regimens included fludarabine-containing regimens and high-intensity non-fludarabine-containing regimens. Low-intensity regimens included hypomethylator-based combinations, including decitabine and azacitidine, and low-dose cytarabine in combination with omacetaxine or cladribine (Galloway-Peña et al., 2017). We consider the gut microbiome profile (abundance of taxa) as the mediator, based on AML patient samples collected immediately prior to the development of infection or at the last
sampling time point for patients without infection. The outcome of interest is the binary infection status during IC, which is defined microbiologically or clinically as described previously (Galloway-Peña et al., 2016, 2017). For antibiotic use, we focus on the use of broad-spectrum antibiotics between the initiation of IC and the development of infection. In addition to antibiotic use, we also adjust for baseline covariates, including age and gender.

3 Methodology

3.1 The preamble

Let $Z$ be a binary exposure variable taking values 0 or 1, $Y$ be the outcome of interest, $M$ be a potentially high-dimensional mediator, $L$ be an exposure-induced mediator-outcome confounder, and $X$ be a set of baseline covariates. Suppose we observe independent and identically distributed samples from the joint distribution of $(Z, Y, M, L, X)$. Following the potential outcome framework, let $M(z)$ denote the value of the mediator that would have been observed had the exposure $Z$ been set to level $z$, and $Y(z, m)$ denote the value of the outcome that would have been observed had $Z$ been set to level $z$, and $M$ been set to $m$. We also use $Y(z)$ to denote $Y(z, M(z))$. The observed data can be related to the potential counterparts under the following consistency assumption, which we maintain throughout this paper. We refer interested readers to Cole and Frangakis (2009) for a discussion of this assumption.

Assumption 1 (Consistency). $M = M(z)$ when $Z = z; Y = Y(z, m)$ when $Z = z$ and $M = m$.

The total effect of $Z$ on $Y$ is defined as $\text{TE} = E\{Y(1)\} - E\{Y(0)\}$. We are interested in how this effect is mediated through $M$. One classical approach is to decompose the total effect into the natural direct effect (NDE) and natural indirect effect (NIE), which are respectively defined as follows (Robins and Greenland, 1992; Pearl, 2001):

$$\text{NDE} = E[Y \{1, M(0)\}] - E[Y \{0, M(0)\}];$$
$$\text{NIE} = E[Y \{1, M(1)\}] - E[Y \{1, M(0)\}].$$

Based on this definition, the NIE can be used to measure the mediation effect. The NDE and NIE may be identified through the following so-called mediation formula.
Proposition 1. (Mediation formula, Pearl, 2001) Suppose that Assumption 1 and the following assumptions hold:

Assumption 2 (No unmeasured $Z - Y$ confounding). For all $z, m$, $Z \perp Y(z, m) \mid X$;

Assumption 3 (No unmeasured $Z - M$ confounding). For all $z$, $Z \perp M(z) \mid X$;

Assumption 4 (No unmeasured $M - Y$ confounding). For all $z, m$, $M \perp Y(z, m) \mid \{Z, X\}$;

Assumption 5 (No effect of $Z$ that confounds the $M - Y$ relationship). For all $m$, $M(0) \perp Y(1, m) \mid X$.

Then the NDE and NIE are identifiable. If $X$ and $M$ are discrete, then

$$\text{NDE} = \sum_{x,m} \{E(Y \mid z_1, m, x) - E(Y \mid z_0, m, x)\} P(m \mid z_0, x)P(x);$$

$$\text{NIE} = \sum_{x,m} E(Y \mid z_1, m, x) \{P(m \mid z_1, x) - P(m \mid z_0, x)\} P(x),$$

where we use the shorthand that $E(Y \mid z_1, m, x) = E(Y \mid Z = 1, M = m, X = x), E(Y \mid z_0, m, x) = E(Y \mid Z = 0, M = m, X = x), P(m \mid z_1, x) = pr(M = m \mid Z = 1, X = x), P(m \mid z_0, x) = pr(M = m \mid Z = 0, X = x), P(x) = pr(X = x)$, following the convention in the mediation analysis literature.

Under the following nonparametric structural equation models (NPSEM):

$$X = f_X(\epsilon_X), \quad Z(x) = f_Z(x, \epsilon_Z), \quad M(x, z) = f_M(x, z, \epsilon_M), \quad Y(x, z, m) = f_Y(x, z, m, \epsilon_Y).$$

(1)

Assumptions 2–5 can be derived from the independent error (IE) assumption that $\epsilon_X \perp \perp \epsilon_Z \perp \perp \epsilon_M \perp \perp \epsilon_Y$. Figure 2 provides the causal diagram associated with the NPSEM in (1).

![Causal diagram](image)

Figure 2: A causal diagram associated with the NPSEM in (1).
3.2 Development in the presence of confounders

As described in Section 2, there is an exposure-induced mediator-outcome confounder in the AML microbiome study (see Figure 1), resulting in violation of Assumption 5. When Assumption 5 is potentially violated, VanderWeele et al. (2014) proposed to study the following interventional direct effect (IDE) and interventional indirect effect (IIE):

\[
\text{IDE} = E[Y\{1, G(0 \mid X)\}] - E[Y\{0, G(0 \mid X)\}];
\]
\[
\text{IIE} = E[Y\{1, G(1 \mid X)\}] - E[Y\{1, G(0 \mid X)\}],
\]

where \(G(z \mid X)\) denotes a random draw from the distribution of the mediator \(M\) with exposure status fixed to \(z\) conditional on covariates \(X\). The IDE and IIE both can be identified without making Assumption 5.

**Proposition 2.** (VanderWeele et al., 2014) Suppose that Assumptions 1 – 3, and the following assumption hold:

**Assumption 4a** (No unmeasured \(M \rightarrow Y\) confounding). For all \(z, m, Y(z, m)\not\perp M \mid \{Z, L, X\}\).

Then the interventional effects IDE and IIE are identifiable. If \(X, L,\) and \(M\) are all discrete, then

\[
\text{IDE} = \sum_{x,l,m} \{E(Y \mid z_1, l, m, x)P(l \mid z_1, x) - E(Y \mid z_0, l, m, x)P(l \mid z_0, x)\} P(m \mid z_0, x)P(x);
\]
\[
\text{IIE} = \sum_{x,l,m} E(Y \mid z_1, l, m, x)P(l \mid z_1, x) \{P(m \mid z_1, x) - P(m \mid z_0, x)\} P(x),
\]

where we use the shorthand that \(E(Y \mid z_1, l, m, x) = E(Y \mid Z = 1, L = l, M = m, X = x)\), \(E(Y \mid z_0, l, m, x) = E(Y \mid Z = 0, L = l, M = m, X = x)\), \(P(l \mid z_1, x) = \text{pr}(L = l \mid Z = 1, X = x)\), \(P(l \mid z_0, x) = \text{pr}(L = l \mid Z = 0, X = x)\), and other meanings of notations are the same as those in Proposition 1.

Note that (2) can be extended to accommodate continuous \(X, L,\) and \(M\) by replacing the summation with integration. Assumptions 2, 3, and 4a hold under the causal diagram in Figure 1. They would also hold if the causal association between \(L\) and \(Y\) was confounded by some unmeasured factors.
Remark 1. Assumption 5 is a “cross-world” independence assumption (Robins and Richardson, 2010), in the sense that it cannot be established by any randomized experiment on the variables in Figure 2. In contrast, all the assumptions in Proposition 2 are “single-world” and can be guaranteed under randomization of \(Z\) and \(M\).

Remark 2. If \(L\) is empty, then the identification formulas for the IDE and IIE reduce to the identification formulas for the NDE and NIE.

3.3 Estimation of the interventional direct and indirect effects

In this section, we elaborate the estimation method for the interventional effects IDE and IIE. It’s worth noting that our method is specifically tailored to address the unique challenges in the AML microbiome study, including the high-dimensional, zero-inflated, and dependent mediators \(M\) (microbiome features) and the binary confounder \(L\) (antibiotic use).

VanderWeele et al. (2014) suggested estimating the IIE based on the following formula:

\[
\text{IIE} = E \left\{ \frac{ZY}{\text{pr}(Z = 1 \mid X)} \frac{\text{pr}(M \mid Z = 1, X)}{\text{pr}(Z = 1, L, X)} \right\} - E \left\{ \frac{ZY}{\text{pr}(Z = 0 \mid X)} \frac{\text{pr}(M \mid Z = 1, X)}{\text{pr}(Z = 1, L, X)} \right\}. \tag{3}
\]

Estimation based on (3), however, involves modeling \(\text{pr}(M \mid Z, X)\) and \(\text{pr}(M \mid Z, L, X)\). This can be challenging in the AML microbiome study, as the potential mediators \(M\) are high-dimensional and zero-inflated, and it can be difficult to model the dependence among them.

To circumvent the need to model the conditional distributions of \(M\), we note that according to (2), \(\text{IIE} = \theta_1 - \theta_2\), where

\[
\theta_1 = \sum_{x,l,m} E(Y \mid z_1, l, m, x) P(l \mid z_1, x) P(m \mid z_1, x) P(x);
\]

\[
\theta_2 = \sum_{x,l,m} E(Y \mid z_1, l, m, x) P(l \mid z_1, x) P(m \mid z_0, x) P(x).
\]

Take \(\theta_2\) as an example. If we re-weight the population by the ratio of \(E(Y \mid z_0, l, m, x) P(l \mid z_0, m, x)\) and \(E(Y \mid z_1, l, m, x) P(l \mid z_1, x)\), then \(\theta_2\) in the re-weighted population is

\[
\theta_2^* = \sum_{x,l,m} E(Y \mid z_0, l, m, x) P(l \mid z_0, m, x) P(m \mid z_0, x) P(x) = E \left\{ \frac{(1 - Z)Y}{\text{pr}(Z = 0 \mid X)} \right\}. \tag{4}
\]

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To estimate the last term in (4), one only needs to model the so-called propensity score, \( \text{pr}(Z = 1 \mid X) \), or \( 1 - \text{pr}(Z = 0 \mid X) \). Furthermore, the weight applied to the population here does not depend on the conditional distributions \( \text{pr}(M \mid Z, X) \) or \( \text{pr}(M \mid Z, L, X) \), hereby avoiding the need to model the conditional distribution of \( M \) in the resulting estimation procedure. Finally, \( \theta_2 \) can be obtained by re-scaling \( \theta_2^* \) back from the re-weighted population to the original population. This result is formalized in Theorem 1.

**Theorem 1.** Suppose that Assumptions 1–3, 4a, and the following assumption hold:

**Assumption 6** (Positivity). For \( z = 0, 1 \) and all \( x \), \( \text{pr}(Z = z \mid X = x) > 0 \); for all \( l, m \), and \( x \), \( E(Y \mid Z = 0, L = l, M = m, X = x) > 0 \); and for \( z = 0, 1 \) and all \( l, m \), and \( x \), \( \text{pr}(L = l \mid Z = z, M = m, X = x) > 0 \).

Then we have

\[
\text{IDE} = E \left\{ \frac{(1 - Z)Y}{\text{pr}(Z = 0 \mid X)} E(Y \mid Z = 1, L, M, X) \text{pr}(L \mid Z = 1, X) \right\} - E \left\{ \frac{(1 - Z)Y}{\text{pr}(Z = 0 \mid X)} \text{pr}(L \mid Z = 0, X) \right\} \tag{5}
\]

\[
\text{IIE} = E \left\{ \frac{Z Y}{\text{pr}(Z = 1 \mid X)} \text{pr}(L \mid Z = 1, X) \right\} - E \left\{ \frac{(1 - Z)Y}{\text{pr}(Z = 0 \mid X)} \frac{E(Y \mid Z = 1, L, M, X) \text{pr}(L \mid Z = 1, X)}{E(Y \mid Z = 0, L, M, X) \text{pr}(L \mid Z = 0, M, X)} \right\} \tag{6}
\]

The proofs of Proposition 1, Proposition 2, and Theorem 1 are deferred to the Supplementary Material S1–S3. We can further simplify the estimation of the IDE and IIE by considering only a subset of \( M \) that is conditionally dependent on the outcome \( Y \) given \( Z \), \( L \), and \( X \), as shown in Corollary 1.

**Corollary 1.** If there exists \( M^{(1)} \) and \( M^{(2)} \) such that \( M^{(1)} \cup M^{(2)} = M \), \( M^{(1)} \cap M^{(2)} = \emptyset \), \( M^{(1)} \not\perp \perp Y \mid \{Z, L, X\} \), and \( M^{(2)} \perp \perp Y \mid \{Z, L, X\} \), then under Assumptions 1–3, 4a, and the following assumption:

**Assumption 6a** (Positivity). For \( z = 0, 1 \) and all \( x \), \( \text{pr}(Z = z \mid X = x) > 0 \); for all \( l, m^{(1)} \), and \( x \), \( E(Y \mid Z = 0, L = l, M^{(1)} = m^{(1)}, X = x) > 0 \); and for \( z = 0, 1 \) and all \( l, m^{(1)} \), and \( x \), \( \text{pr}(L = l \mid Z = z, M^{(1)} = m^{(1)}, X = x) > 0 \).
we have

\[
\text{IDE} = E \left\{ \frac{(1 - Z)Y}{pr(Z = 0 | X)} \frac{E(Y \mid Z = 1, L, M(1), X)pr(L \mid Z = 1, X)}{E(Y \mid Z = 0, L, M(1), X)pr(L \mid Z = 0, M(1), X)} \right\} - E \left\{ \frac{(1 - Z)Y}{pr(Z = 0 | X)} \frac{pr(L \mid Z = 0, X)}{pr(L \mid Z = 0, M(1), X)} \right\}; \quad (7)
\]

\[
\text{IIE} = E \left\{ \frac{ZY}{pr(Z = 1 | X)} \frac{pr(L \mid Z = 1, X)}{pr(L \mid Z = 1, M(1), X)} \right\} - E \left\{ \frac{(1 - Z)Y}{pr(Z = 0 | X)} \frac{E(Y \mid Z = 1, L, M(1), X)pr(L \mid Z = 1, X)}{E(Y \mid Z = 0, L, M(1), X)pr(L \mid Z = 0, M(1), X)} \right\}. \quad (8)
\]

Furthermore, we can estimate the IDE and IIE based on (7) and (8). In the AML microbiome study, since \(Z\) and \(L\) are binary variables, we assume logistic regression models for \(pr(Z = 1 \mid X; \alpha)\) and \(pr(L = 1 \mid Z, X; \gamma)\). Estimation of \(\alpha\) and \(\gamma\) can be obtained by maximizing the corresponding likelihood functions. Since \(Y\) is a binary variable and \(M(1)\) is unknown in practice, we use the penalized logistic regression method to estimate \(pr(Y = 1 \mid Z, L, M, X; \beta) = pr(Y = 1 \mid Z, L, M(1), X)\) with the constraint that the resulting model includes the covariates \(Z, L, X\) and at least one mediator. Note that at least one mediator being included in the model of \(Y\) posterior to variable selection would make it practically meaningful to study the mediation effect. Specifically, let \(\beta = (\beta_0, \beta_Z, \beta_L, \beta_M^T, \beta_X^T)^T\) and \(q\) be the dimension of \(M\). For \(j = 1, \ldots, q\) and a fixed value of tuning parameter \(\lambda_j\), let

\[
\hat{\beta}_j(\lambda_j) = \arg \min_{\beta} \left\{ -\log \{L_n(\beta)\} \right\} + \lambda_j \sum_{k \neq j} |\beta_{Mk}|,
\]

where \(L_n(\beta)\) is the likelihood function corresponding to the logistic regression model for \(Y\), and \(\beta_{Mk}\) is the \(k\)th element of \(\beta_M\). The tuning parameter \(\lambda_j\) is selected by minimizing the extended Bayes information criterion (Chen and Chen, 2008):

\[
\hat{\lambda}_j = \arg \min_{\lambda_j} \text{eBIC}(\lambda_j) = \arg \min_{\lambda_j} \left[ -2 \log[L_n(\hat{\beta}_j(\lambda_j))] + \nu(\lambda_j) \log(n) + 2 \log \{\tau(\lambda_j)\} \right],
\]

where \(\nu(\lambda_j)\) is the number of non-zero values in \(\hat{\beta}_j(\lambda_j)\) except the intercept and \(\tau(\lambda_j)\) is \((\text{dim}(\beta) - 1)\). The estimated value of \(\beta\) is taken as \(\hat{\beta} = \hat{\beta}_{\text{index}}(\hat{\lambda}_{\text{index}})\), where \(\text{index} = \arg \min_{\lambda_j} \text{eBIC}(\lambda_j)\). The corresponding set of selected mediators is denoted as \(\hat{M}(1)\). Based on Corollary 1, we still need to estimate \(pr(L \mid Z, \hat{M}(1), X)\). Recall that we have assumed a logistic regression model for \(pr(L =
To avoid model incompatibility issues, we estimate \( \text{pr}(L \mid Z, \hat{M}(1), X) \) using bagging with the optimal subset of DNNs (Mi et al., 2019), rather than using the maximum likelihood estimator by assuming a logistic regression model for \( \text{pr}(L \mid Z, \hat{M}(1), X) \). Note that the method of bagging with the optimal subset of DNNs can model complex non-linear relationships and reduce overfitting. The implementation details of this method in the simulation studies and real data analysis can be found in the Supplementary Material S4. After getting all the estimates, we just need to plug the above estimates into the formulas of (7) and (8) and use the empirical means as the estimated values of the IDE and IIE.

Algorithm 1 summarizes the proposed procedure for the estimation of the IIE based on Corollary 1. The algorithm for the estimation of the IDE is similar, and we omit it here to save space. It is worth mentioning that we make the above model assumptions based on types of the AML microbiome data. For different types of data, we can make different model assumptions.

**Algorithm 1** Proposed inverse probability weighting approach to estimate the IIE

1. Fit logistic regression models for \( \text{pr}(Z = 1 \mid X; \alpha) \) and \( \text{pr}(L = 1 \mid Z, X; \gamma) \) using the maximum likelihood estimation. Let \( \hat{\text{pr}}(Z = 1 \mid X) = \text{pr}(Z = 1 \mid X; \hat{\alpha}) \) and \( \hat{\text{pr}}(L \mid Z = 1, X) = \text{pr}(L \mid Z = 1, X; \hat{\gamma}) \), where \( \hat{\alpha} \) and \( \hat{\gamma} \) are the maximum likelihood estimates of \( \alpha \) and \( \gamma \), respectively.

2. Estimate \( E(Y \mid Z = z, L, M^{(1)}, X) \), \( z = 0, 1 \) using the penalized logistic regression method described earlier. Denote the set of selected mediators as \( \hat{M}(1) \) and the estimated value of \( E(Y \mid Z = z, L, M^{(1)}, X) \) as \( \hat{E}(Y \mid Z = z, L, \hat{M}(1), X) \).

3. Estimate \( \text{pr}(L \mid Z = z, \hat{M}(1), X), z = 0, 1 \) using bagging with the optimal subset of DNNs. Denote the estimate as \( \hat{\text{pr}}(L \mid Z = z, \hat{M}(1), X) \).

4. The estimated value of the IIE is

\[
\hat{\text{IIE}} = \mathbb{P}_n \left\{ \frac{Z Y}{\hat{\text{pr}}(Z = 1 \mid X)} \frac{\hat{\text{pr}}(L \mid Z = 1, X)}{\hat{\text{pr}}(L \mid Z = 1, \hat{M}(1), X)} \right\} - \mathbb{P}_n \left\{ \frac{(1 - Z) Y}{\hat{\text{pr}}(Z = 0 \mid X)} \frac{\hat{E}(Y \mid Z = 1, L, \hat{M}(1), X) \hat{\text{pr}}(L \mid Z = 1, X)}{\hat{E}(Y \mid Z = 0, L, \hat{M}(1), X) \hat{\text{pr}}(L \mid Z = 0, \hat{M}(1), X)} \right\}, \tag{9}
\]

where \( \mathbb{P}_n \) denotes the empirical mean operator.
3.4 Hypothesis testing

In the AML microbiome study, an important question to be addressed is whether the microbiome features mediate the effect of IC type on the infection status in AML patients. According to the definition of the IIE in Section 3.2, the IIE can be used to measure the mediation effect. Therefore, transforming this question into a statistical language, we can test on $H_0: \text{IIE} = 0$ versus $H_a: \text{IIE} \neq 0$, that is, whether the IIE is significant or not at a significance level of $\alpha$. To solve this question, we propose to first construct the $100(1-\alpha)\%$ standard normal bootstrap confidence interval for the IIE (Efron and Tibshirani, 1994). Then we will reject the null hypothesis of $H_0: \text{IIE} = 0$ if zero does not fall into the obtained confidence interval with $\alpha = 0.05$; otherwise not. This hypothesis testing method is easy to implement in practice, and the computation time can be greatly reduced by parallel computing.

4 Simulation studies

In this section, we perform simulation studies to evaluate the finite sample performance of the proposed method. We implement the following steps to generate the data. First, we simulate $X = (X_1, X_2)^\top$ by sampling age and gender with replacement from the AML microbiome data; age is divided by 100 so that it is on a similar scale as gender. Given $X$, we then generate $Z$ and $L$ from the following logistic regression models, respectively: $\text{pr}(Z = 1 \mid X) = \expit(\alpha_0 + \alpha_X^\top X)$ and $\text{pr}(L = 1 \mid Z, X) = \expit(\gamma_0 + \gamma_Z Z + \gamma_X^\top X)$, where $\expit(x) = \exp(x)/\{1 + \exp(x)\}$. The mediators $M = (M_1, \ldots, M_q)^\top$ are then generated from the following models:

$$f(M_1 \mid Z, L, X) = \pi_{01} I(M_1 = 0) + (1 - \pi_{01}) I(M_1 > 0) \Gamma(\eta_{01}, \theta_{11}(Z, L, X)),$$

$$f(M_k \mid Z, L, X, M_{k-1}) = \pi_{0k} I(M_k = 0) + (1 - \pi_{0k}) I(M_k > 0) \Gamma(\eta_{0k}, \theta_{1k}(Z, L, X, M_{k-1})), k = 2, \ldots, q,$$

where $\theta_1(Z, L, X) = \exp(\theta_{01} + \theta_Z Z + \theta_L L + \theta_X^\top X)/\eta_{01}$ and $\theta_k(Z, L, X, M_{k-1}) = \exp(\theta_{0k} + \theta_Z Z + \theta_L L + \theta_X^\top X + \theta_{M,k-1}^\top M_{k-1})/\eta_{0k} (k = 2, \ldots, q)$. Finally, the outcome $Y$ is generated from the logistic regression model $\text{pr}(Y = 1 \mid Z, L, M, X) = \expit(\beta_0 + \beta_Z Z + \beta_L L + \beta_M^\top M + \beta_X^\top X)$.

In the simulation studies, we let $\alpha_0 = 0.2$, $\alpha_X = (-1, 1)^\top$, $\gamma_0 = 0.2$, $\gamma_X = (0.5, -0.5)^\top$.\vspace{0.5cm}
\( \theta_X = (0.5, -0.5)^T, \beta_0 = 8, \beta_Z = 1, \beta_L = 8, \beta_M = (-8, -8, 0, \ldots, 0)^T \) with \( q = 62 \), and \( \beta_X = (1, 1)^T \). We independently draw \( \{\pi_{0k}, k = 1, \ldots, q\} \) from Uniform \((0.3, 0.9)\), \( \{\theta_{0k}, k = 1, \ldots, q\} \) from Uniform \((1.3, 2)\), and \( \{\eta_{0k}, k = 1, \ldots, q\} \) from Uniform \((4, 4.5)\); the values of \( \pi_{0k}, \theta_{0k}, \) and \( \eta_{0k}, k = 1, \ldots, q \) remain the same for all Monte Carlo replications. We consider the following two dependence structures of mediators:

- **Structure 1 (Conditionally independent mediators):** for \( k \geq 2 \), \( \theta_{M,k-1} = 0 \);

- **Structure 2 (Conditionally dependent mediators):** for \( k \geq 2 \), \( \theta_{M,k-1} = -0.3 \).

Under Structure 1, all the mediators are conditionally independent given \( Z, L, \) and \( X \), while under Structure 2, \( M_k \) is conditionally dependent on \( M_{k-1} \) \((k = 2, \ldots, 62)\) given \( Z, L, \) and \( X \). Let \( \gamma_Z \) be 0 or 0.5, corresponding to zero effect or non-zero effect of the path \( Z \rightarrow L \). Let \( \theta_Z \) be 0, \(-1.2\) or \(-1.5\), and \( \theta_L \) be 0, 0.1 or 0.2, corresponding to zero effect, weak effect or strong effect of the paths \( Z \rightarrow M \) and \( L \rightarrow M \), respectively. Note that under our simulation settings, the true value of the IIE is zero when \( \theta_Z = \gamma_Z = 0 \) and non-zero when \( \theta_Z \neq 0 \). We consider sample size \( n = 70 \) or 200. All simulation results are based on 500 Monte Carlo replications. The number of bootstrap replications is 500.

We remark that the parameters are chosen so that the simulated data reflect the characteristics of the real AML microbiome data. In particular, when \( \gamma_Z = 0.5, \theta_z = -1.2, \theta_L = 0.1, \) and the mediators are conditionally independent (i.e., Structure 1), the marginal distributions of \( Z, L, Y, \) and \( \Pr(M_j = 0) \) are close to those in the AML microbiome study.

For comparison purposes, in addition to the proposed method, we also implement a method that estimates the NIE and ignores information on the exposure-induced mediator-outcome confounder \( L \). We detail the procedure for estimating the NIE in Appendix A. We compare them using two measures: (1) type-I error rate or power for testing the hypothesis that \( H_0 : \) mediation effect = 0 versus \( H_a : \) mediation effect \( \neq 0 \); a significance level of \( \alpha = 0.05 \) is assumed; (2) bias for estimating the mediation effect.

Table 1 shows the bias and standard deviation (SD) for the two estimators of mediation effect, as well as the type-I error rate for testing \( H_0 : \) mediation effect = 0 versus \( H_a : \) mediation effect \( \neq 0 \) when \( \theta_Z = \gamma_Z = 0 \) so that both NIE and IIE equal zero and can be identified from the observed data. For both methods, the type-I error rates are close to the nominal level of 0.05 even when \( n =

15
70, suggesting good small-sample performance. As the sample size becomes larger, in general, the type-I error rate becomes closer to the nominal level of 0.05. The absolute value of the bias of the proposed method is slightly larger than that of the NIE-based method. This may be because compared with the NIE-based method, the proposed method involves modeling the conditional distributions of $L$, and thus inducing more variability. Nevertheless, the differences become smaller as the sample size increases, and all the biases are small relative to SDs.

Table 2 shows the true value of the IIE, and bias and SD for the estimators of mediation effect, as well as the power for testing $H_0: \text{mediation effect} = 0$ versus $H_a: \text{mediation effect} \neq 0$ when mediation effect $\neq 0$ and the effect of $L \rightarrow M$ is weak ($\theta_L = 0.1$). Note that for the scenarios considered in Table 2, since Assumption 4 and/or Assumption 5 fail, the NIE can not be identified. In this case, the IIE is still identifiable. We regard the true value of the IIE as the true value of mediation effect. Simulation results show that the absolute value of the bias of the NIE-based method can be much larger than that of the proposed method. In addition, the power of the NIE-based method can also be substantially lower than that of the proposed method when the sample size is 70. One can also find that the absolute value of the true value of the IIE increases with the strength of $Z \rightarrow M$ and the strength of $Z \rightarrow L$. As expected, the power increases with the absolute value of the true value of the IIE. The power of the proposed method is fairly large at $n = 70$. When the sample size increases to 200, the powers of both methods are very close to 1.

We also conduct sensitivity analyses for the no-unmeasured-confounding assumptions (i.e., Assumptions 2, 3, and 4a) needed for the identification of interventional effects. To investigate the sensitivity of the estimators considered here to the presence of unmeasured confounding among $Z, Y,$ and $M$, we consider further simulations where we independently generate a latent confounder $U$ from $N(0, 1)$. The exposure variable $Z$ is generated from $\text{pr}(Z = 1 \mid X, U) = \expit(\alpha_0 + \alpha_X^\top X + \alpha_U U)$. The mediators $M = (M_1, \ldots, M_{100})^\top$ are then generated from the following models:

$$f(M_1 \mid Z, L, X, U) = \pi_{01} I(M_1 = 0) + (1 - \pi_{01})I(M_1 > 0)\text{Gamma}\{\eta_{01}, \theta_1(Z, L, X, U)\},$$

$$f(M_k \mid Z, L, X, U, M_{k-1}) = \pi_{0k} I(M_k = 0) + (1 - \pi_{0k})I(M_k > 0)\text{Gamma}\{\eta_{0k}, \theta_k(Z, L, X, U, M_{k-1})\}, k = 2, \ldots, 62,$$

where $\theta_1(Z, L, X, U) = \exp(\theta_{01} + \theta_Z Z + \theta_L L + \theta_X^\top X + \theta_U U)/\eta_{01}$ and $\theta_k(Z, L, X, U, M_{k-1}) =$
Table 1: Bias $\times 100$ and standard deviation (SD) $\times 100$ for the estimators of mediation effect, and type-I error rate for testing $H_0 : \text{mediation effect} = 0$ versus $H_a : \text{mediation effect} \neq 0$ at the significance level of $\alpha = 0.05$ when mediation effect $= 0$ ($\theta_Z = \gamma_Z = 0$)

| Dependence sample size $L \rightarrow M$ | Proposed method | NIE-based method |
|----------------------------------------|-----------------|-----------------|
| Bias $\times 100$ | SD$\times 100$ | type-I error rate | Bias $\times 100$ | SD$\times 100$ | type-I error rate |
| 70 | zero | 1.71 | 10.17 | 0.056 | 1.31 | 9.87 | 0.038 |
|  | weak | 1.51 | 10.39 | 0.044 | 1.25 | 9.94 | 0.046 |
|  | strong | 1.45 | 10.22 | 0.034 | 1.16 | 9.66 | 0.036 |
| 200 | zero | 0.41 | 6.32 | 0.048 | 0.18 | 6.14 | 0.058 |
|  | weak | 0.37 | 6.31 | 0.050 | 0.15 | 6.13 | 0.056 |
|  | strong | 0.23 | 6.27 | 0.056 | 0.05 | 6.09 | 0.056 |
| 70 | zero | 1.69 | 10.52 | 0.064 | 1.31 | 10.11 | 0.042 |
|  | weak | 1.62 | 10.41 | 0.052 | 1.23 | 10.09 | 0.042 |
|  | strong | 1.64 | 10.16 | 0.038 | 1.12 | 9.89 | 0.032 |
| 200 | zero | 0.40 | 6.41 | 0.046 | 0.16 | 6.24 | 0.056 |
|  | weak | 0.30 | 6.41 | 0.054 | 0.11 | 6.27 | 0.052 |
|  | strong | 0.24 | 6.33 | 0.056 | 0.03 | 6.23 | 0.054 |
Table 2: True value (Truth) $\times 100$ of the IIE, bias $\times 100$ and standard deviation (SD) $\times 100$ for the estimators of mediation effect, and power for testing $H_0: \text{mediation effect} = 0$ versus $H_a: \text{mediation effect} \neq 0$ at the significance level of $\alpha = 0.05$ when $\text{mediation effect} \neq 0$ and $\theta_L = 0.1$.

| Dependence sample size | $Z \rightarrow L$ | $Z \rightarrow M$ | Truth $\times 100$ | Bias $\times 100$ | SD $\times 100$ | power |
|-------------------------|-------------------|-------------------|-------------------|--------------|-------------|-------|
| **Structure 1**         |                   |                   |                   |              |             |       |
| 70                      | zero weak         | 30.76             | 0.36              | 11.21        | 0.704       | -1.45  |
|                         | zero strong       | 43.21             | 0.15              | 11.20        | 0.900       | -3.26  |
|                         | non-zero weak     | 32.61             | 0.82              | 11.17        | 0.746       | -1.60  |
|                         | non-zero strong   | 45.20             | 0.47              | 11.02        | 0.924       | -3.22  |
| 200                     | zero weak         | 30.76             | 0.01              | 6.77         | 0.990       | -0.63  |
|                         | zero strong       | 43.21             | 0.16              | 6.48         | 1.000       | -0.96  |
|                         | non-zero weak     | 32.61             | 0.32              | 6.86         | 0.994       | -0.45  |
|                         | non-zero strong   | 45.20             | 0.54              | 6.61         | 1.000       | -0.76  |
| **Structure 2**         |                   |                   |                   |              |             |       |
| 70                      | zero weak         | 32.60             | 0.62              | 10.99        | 0.778       | -0.95  |
|                         | zero strong       | 45.56             | 0.60              | 10.32        | 0.944       | -2.71  |
|                         | non-zero weak     | 34.71             | 0.71              | 11.32        | 0.812       | -1.26  |
|                         | non-zero strong   | 47.78             | 0.78              | 10.30        | 0.952       | -2.69  |
| 200                     | zero weak         | 32.60             | -0.10             | 6.80         | 0.992       | -0.68  |
|                         | zero strong       | 45.56             | 0.28              | 6.34         | 1.000       | -0.79  |
|                         | non-zero weak     | 34.71             | 0.24              | 6.91         | 0.994       | -0.51  |
|                         | non-zero strong   | 47.78             | 0.54              | 6.59         | 1.000       | -0.61  |
\[ \exp(\theta_0k + \theta_ZZ + \theta_LL + \theta_X^TX + \theta_UU + \theta_{M,k-1}M_{k-1})/\eta_{0k} \] (\(k = 2, \ldots, 62\)). The outcome \(Y\) is generated from \(\text{pr}(Y = 1 \mid Z, L, M, X, U) = \expit(\beta_0 + \beta_ZZ + \beta_LL + \beta_M^TM + \beta_X^TX + \beta_UU)\).

In the following, we let \(\gamma_Z = 0.5, \theta_Z = -1.5, \theta_L = 0.1, n = 70\), and the dependence structure of mediators be Structure 1. We consider three scenarios: (I) \(U\) confounds the \(Z - Y\) relationship, i.e., \(\alpha_U \neq 0, \beta_U \neq 0, \) and \(\theta_U = 0\); (II) \(U\) confounds the \(Z - M\) relationship, i.e., \(\alpha_U \neq 0, \theta_U \neq 0, \) and \(\beta_U = 0\); and (III) \(U\) confounds the \(M - Y\) relationship, i.e., \(\beta_U \neq 0, \theta_U \neq 0, \) and \(\alpha_U = 0\).

To investigate how the results change with the effects of the confounder \(U\) on \(Z, M,\) and \(Y\), we vary the values of \(\alpha_U, \theta_U, \) and \(\beta_U,\) respectively. Specifically, let \(\alpha_U\) range from -0.9 to 0.9 with a step size of 0.3, \(\theta_U\) range from -0.3 to 0.3 with a step size of 0.1, and \(\beta_U\) range from -6 to 6 with a step size of 2. Figure 3 shows the absolute value of the bias of the proposed method and NIE-based method in various scenarios. One can see that the absolute value of the bias of the proposed method is relatively small for the parameter values considered here, indicating that it is relatively robust with respect to violations of the no-unmeasured-confounding assumptions (i.e., Assumptions 2, 3, and 4a).

### 5 Analysis of the AML microbiome data

We use the AML microbiome data to investigate the mediation role of the gut microbiome in the causal pathway from IC treatment type to infection status in AML patients during IC, taking into account the confounder antibiotic use and baseline covariates age and gender (Figure 1) as described in Section 2. For the mediation analysis, we exclude patients without any microbiome samples collected between the initiation of IC and the development of infection, resulting in 70 patients with 440 stool samples. The average age of the study population was 56.2 years old with a standard deviation of 15.2; 37 of them were female. In our analysis, we normalize the OTU counts by rarefying based on the rarefaction curves so that all samples have the same total count (McMurdie and Holmes, 2014; Weiss et al., 2017). After normalization, the ratio of the relative abundances for two taxa is the same as the ratio of OTU counts for these two taxa. Therefore, OTU counts for different taxa are comparable, and we do not further transform the OTU count of each taxon to relative abundance. Taxa with low abundance are excluded from the analysis (Chen and Li, 2016; Zhang et al., 2017; Lu et al., 2019). Specifically, we focus on taxa presenting...
Figure 3: The absolute value of the bias (|Bias|) of the proposed method and NIE-based method when one of the parameters in \{\alpha_U, \beta_U, \theta_U\} varies. The solid line (in red) represents the results for the proposed method, while the dashed line (in blue) represents the results for the NIE-based method. Here \alpha_U represents the effect of \(U\) on \(Z\) on the logistic scale, \beta_U represents the effect of \(U\) on \(Y\) on the logistic scale, and \theta_U encodes the effect of \(U\) on \(M\). The first row corresponds to the case of unmeasured confounding between \(Z\) and \(Y\), the second row corresponds to the case of unmeasured confounding between \(Z\) and \(M\), and the bottom row corresponds to the case of unmeasured confounding between \(M\) and \(Y\).
in at least 10% of all samples (Lu et al., 2019), then the remaining taxa no longer have the compositional structure. The filtering process yields data from 70 patients with 62 bacterial genera for mediation analysis.

In the AML microbiome study, 46 patients received the high-intensity regimens, while the others received the low-intensity regimens. In the high-intensity regimen group, 39 of patients used at least one broad-spectrum antibiotic, and 15 of them developed infections. In contrast, in the low-intensity regimen group, 14 of patients used at least one broad-spectrum antibiotic, and 8 of them developed infections. We estimate the average treatment effect (ATE) of IC type on infection status using the Horvitz-Thompson estimator (Horvitz and Thompson, 1952) adjusted for age and gender, with a logistic regression model for the propensity score \( \Pr(Z = 1|X) \). Analysis results show that after adjusting for age and gender, the high-intensity regimen is associated with 23.5% (95% confidence interval: \([-7.7\%, 54.7\%]\)) increase in infection rate; here the confidence interval is chosen to be the standard normal bootstrap confidence interval, and the number of bootstrap replications is 500.

To investigate whether the effect of IC type on infection status is mediated through the gut microbiome features, we apply the proposed methods in Sections 3.3 and 3.4 to estimate and test the mediation effect. Table 3 shows the estimated values and the 95% standard normal bootstrap confidence intervals for the IIE and IDE. These results suggest that the effect of IC type on infection status is mainly mediated through the changes in the gut microbiome profile, as the IIE is significantly different from zero at the significance level of \( \alpha = 0.05 \), whereas the IDE is not. For comparison, we also show the estimated values and the 95% standard normal bootstrap confidence intervals for the NIE and NDE in Table 3, from which we can get similar conclusions. It is not surprising to observe such results since most infections in patients during treatment are caused by commensal bacteria.

We also find that there is an important bacterial genus that mediates the effect of IC type on infection status, that is OTU unc05eti (genus name was not assigned) from the Ruminococcaceae family. Interestingly, the Ruminococcaceae family has been shown in the literature to be associated with antibiotic usage and cancer. Specifically, Ruminococcaceae family is one of the most abundant bacterial families found in the human gut, contributing to 16-27% of total fecal bacteria in healthy adults (Ishiguro et al., 2018). Studies on humans (Panda et al., 2014) and weaned piglets
Table 3: Estimated values and the 95% standard normal bootstrap confidence intervals for the IIE, IDE, NIE, and NDE. The number of bootstrap replications is 500

|          | Estimated value | 95% confidence interval |
|----------|-----------------|-------------------------|
| IIE      | 0.286           | [0.019, 0.554]          |
| IDE      | -0.024          | [-0.149, 0.101]         |
| NIE      | 0.281           | [0.007, 0.555]          |
| NDE      | -0.046          | [-0.198, 0.105]         |

(Hu et al., 2020) both showed a short-term reduction in the abundance of fecal *Ruminococcaceae* after administration of antibiotics. Several recent clinical studies investigating the relationship between the composition of the human gut microbiome and cancer therapy outcomes have found that the *Ruminococcaceae* family plays an important role. Studies have also shown that increased abundance of *Ruminococcaceae* and other bacterial families under the phylum of *Firmicutes* is associated with the beneficial clinical response to the anti-PD-1 therapy and higher levels of effector CD4+ and CD8+ T cells in the systemic circulation in patients with metastatic melanoma (Chaput et al., 2017; Gopalakrishnan et al., 2018). Similar results were found in another study on advanced non-small cell lung cancer (Routy et al., 2018). Taken together, disruption in *Ruminococcaceae* abundance due to cancer therapy and antibiotic use during treatment may increase the chance of infection, thereby acting as a mediator of the effect of cancer therapy on the development of infection.

The above findings may suggest that intervening in the abundance of OTU *unc05eti* or *Ruminococcaceae* family at specific time points during the course of treatment may help in the prevention and/or intervention of developing infections during IC for AML patients. Additional studies might be needed to further validate this result.

We conclude with a note on the computational cost. Our real data analysis is implemented on a 48-core node with an Intel Platinum 8160F Skylake (2.1GHz) CPU. We use 20 cores for parallel computing. The total computation time for getting the estimates of the IIE and IDE and the associated confidence intervals is 95.1 seconds, while that for the NIE and NDE is 38.3 seconds.
6 Discussion

In this paper, we study the causal relationships among the IC treatment type, infection status, and on-treatment gut microbiome profile, using data from the AML microbiome study conducted at MD Anderson. To account for the exposure-induced antibiotic use that may confound the relationship between the gut microbiome and infection status, we adopt the interventional indirect effect framework. To circumvent the challenging characteristics of the microbial mediators in the study, including high-dimensionality, zero-inflation, and dependence, we propose novel identification formulas and associated estimation methods for the interventional effects. In particular, we adopt the sparsity-induced regularization for parameter estimation associated with the high-dimensional microbiome variables. We also test the presence of mediation effects through the microbial variables via constructing the standard normal bootstrap confidence intervals. Simulation studies demonstrate satisfactory performance of the proposed method in terms of the mediation effect estimation, and type-I error rate and power of the corresponding test. Analysis of the AML microbiome data reveals that most of the effect of IC type on infection status is mediated by a particular genus from the *Ruminococcaceae* family.

In the current investigation, we have restricted our attention to the microbiome measurements at a single time point that is deemed clinically interesting. However, the AML microbiome study contains multiple measurements of the microbiome profile during the IC treatment. It would be desirable to consider all the measurements in the analysis. Associated with this, however, is the increased complexity and difficulty of mediation analysis. We will pursue this direction in our future research.

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A Estimation and hypothesis testing methods for the natural effects

Following the idea of the proposed estimation method for the interventional effects, we can estimate the natural effects based on Theorem 2, which can avoid modeling the conditional distributions of $M$.

**Theorem 2.** If there exists $M^{(1)}$ and $M^{(2)}$ such that $M^{(1)} \cup M^{(2)} = M$, $M^{(1)} \cap M^{(2)} = \emptyset$, $M^{(1)} \not\perp \perp Y \mid \{Z, X\}$, and $M^{(2)} \perp \perp Y \mid \{Z, X\}$, then under Assumptions 1–5,

\[
\text{NDE} = E \left\{ \frac{(1 - Z)Y}{pr(Z = 0 \mid X)} E(Y \mid Z = 1, M^{(1)}, X) \right\} - E \left\{ \frac{(1 - Z)Y}{pr(Z = 0 \mid X)} \right\}; \quad (A1)
\]

\[
\text{NIE} = E \left\{ \frac{ZY}{pr(Z = 1 \mid X)} \right\} - E \left\{ \frac{(1 - Z)Y}{pr(Z = 0 \mid X)} \frac{E(Y \mid Z = 1, M^{(1)}, X)}{E(Y \mid Z = 0, M^{(1)}, X)} \right\}. \quad (A2)
\]

As a result, we can follow the similar procedure described in Section 3.3 to estimate the NDE and NIE based on (A1) and (A2), except that we do not need to model $L$ and we need to estimate $E(Y \mid Z = z, M^{(1)}, X), z = 0, 1$ instead of $E(Y \mid Z = z, L, M^{(1)}, X), z = 0, 1$. To estimate $E(Y \mid Z = z, M^{(1)}, X), z = 0, 1$, we can use a penalized logistic regression method similar to that in Section 3.3, except that we do not consider $L$ in the model for $Y$.

We can also test $H_0 : \text{NIE} = 0$ versus $H_a : \text{NIE} \neq 0$ at the significance level of $\alpha$, based on similar ideas to those for the IIE in Section 3.4.

We refer to the estimation and hypothesis testing methods for the NDE described above as NDE-based methods, and the estimation and hypothesis testing methods for the NIE as the NIE-based methods.
Supplementary Material for
“Inverse Probability Weighting-based Mediation Analysis for Microbiome Data"

Abstract

In this Supplementary Material, we provide proofs of Proposition 3.1, Proposition 3.2, and Theorem 3.3 in Section 3 of the main paper. We also provide the implementation details of bagging with the optimal subset of deep neural networks (DNNs), and additional results for the AML microbiome study.

S1 Proof of Proposition 3.1

Proof. If Assumptions 1–5 hold, then

\[ \text{NDE} = E[Y \{1, M(0)\}] - E[Y \{0, M(0)\}] \]

\[ = \sum_{x, m} E\{Y(1, m) \mid M(0) = m, X = x\} \text{pr}\{M(0) = m \mid X = x\} \text{pr}(X = x) \]

\[ - \sum_{x, m} E\{Y(0, m) \mid M(0) = m, X = x\} \text{pr}\{M(0) = m \mid X = x\} \text{pr}(X = x) \]

\[ = \sum_{x, m} E\{Y(1, m) \mid X = x\} \text{pr}\{M(0) = m \mid X = x\} \text{pr}(X = x) \]

\[ - \sum_{x, m} E\{Y(0, m) \mid X = x\} \text{pr}\{M(0) = m \mid X = x\} \text{pr}(X = x) \]

\[ = \sum_{x, m} E\{Y(1, m) \mid Z = 1, X = x\} \text{pr}\{M(0) = m \mid Z = 0, X = x\} \text{pr}(X = x) \]

\[ - \sum_{x, m} E\{Y(0, m) \mid Z = 0, X = x\} \text{pr}\{M(0) = m \mid Z = 0, X = x\} \text{pr}(X = x) \]

\[ = \sum_{x, m} E\{Y(1, m) \mid Z = 1, M = m, X = x\} \text{pr}(M(0) = m \mid Z = 0, X = x) \text{pr}(X = x) \]

\[ - \sum_{x, m} E\{Y(0, m) \mid Z = 0, M = m, X = x\} \text{pr}(M(0) = m \mid Z = 0, X = x) \text{pr}(X = x) \]

\[ = \sum_{x, m} E(Y \mid Z = 1, M = m, X = x) \text{pr}(M = m \mid Z = 0, X = x) \text{pr}(X = x) \]

\[ - \sum_{x, m} E(Y \mid Z = 0, M = m, X = x) \text{pr}(M = m \mid Z = 0, X = x) \text{pr}(X = x) \]
\[
\sum_{x, m} \left[ \{E(Y \mid Z = 1, M = m, X = x) - E(Y \mid Z = 0, M = m, X = x)\} \times \text{pr}(M = m \mid Z = 0, X = x) \text{pr}(X = x) \right].
\]

\[\text{NIE} = E[Y\{1, M(1)\}] - E[Y\{1, M(0)\}]
\]
\[
= \sum_{x, m} E\{Y(1, m) \mid M(1) = m, X = x\} \text{pr}\{M(1) = m \mid X = x\} \text{pr}(X = x)
\]
\[- \sum_{x, m} E\{Y(1, m) \mid M(0) = m, X = x\} \text{pr}\{M(0) = m \mid X = x\} \text{pr}(X = x)
\]
\[
= \sum_{x, m} E\{Y(1, m) \mid X = x\} \text{pr}\{M(1) = m \mid X = x\} \text{pr}(X = x)
\]
\[- \sum_{x, m} E\{Y(1, m) \mid X = x\} \text{pr}\{M(0) = m \mid X = x\} \text{pr}(X = x)
\]
\[
= \sum_{x, m} E\{Y(1, m) \mid Z = 1, X = x\} \text{pr}\{M(1) = m \mid Z = 1, X = x\} \text{pr}(X = x)
\]
\[- \sum_{x, m} E\{Y(1, m) \mid Z = 1, X = x\} \text{pr}\{M(0) = m \mid Z = 0, X = x\} \text{pr}(X = x)
\]
\[
= \sum_{x, m} E\{Y(1, m) \mid Z = 1, M = m, X = x\} \text{pr}\{M(1) = m \mid Z = 1, X = x\} \text{pr}(X = x)
\]
\[- \sum_{x, m} E\{Y(1, m) \mid Z = 1, M = m, X = x\} \text{pr}\{M(0) = m \mid Z = 0, X = x\} \text{pr}(X = x)
\]
\[
= \sum_{x, m} E(Y \mid Z = 1, M = m, X = x) \text{pr}(M = m \mid Z = 1, X = x) \text{pr}(X = x)
\]
\[- \sum_{x, m} E(Y \mid Z = 1, M = m, X = x) \text{pr}(M = m \mid Z = 0, X = x) \text{pr}(X = x)
\]
\[
= \sum_{x, m} \left[ E(Y \mid Z = 1, M = m, X = x)
\right.
\]
\[
\left. \times \{\text{pr}(M = m \mid Z = 1, X = x) - \text{pr}(M = m \mid Z = 0, X = x)\} \text{pr}(X = x) \right].
\]
\[
\]
**S2 Proof of Proposition 3.2**

*Proof.* If Assumptions 1–3 and 4a hold, then

\[
\text{IDE} = E[Y\{1, G(0 \mid X)\}] - E[Y\{0, G(0 \mid X)\}]
\]

\[
= \sum_{x,m} E\{Y(1, m) \mid G(0 \mid x) = m, X = x\} \text{pr}\{G(0 \mid x) = m \mid X = x\} \text{pr}(X = x)
\]

\[
- \sum_{x,m} E\{Y(0, m) \mid G(0 \mid x) = m, X = x\} \text{pr}\{G(0 \mid x) = m \mid X = x\} \text{pr}(X = x)
\]

\[
= \sum_{x,m} E\{Y(1, m) \mid X = x\} \text{pr}\{M(0) = m \mid X = x\} \text{pr}(X = x)
\]

\[
- \sum_{x,m} E\{Y(0, m) \mid X = x\} \text{pr}\{M(0) = m \mid X = x\} \text{pr}(X = x)
\]

\[
= \sum_{x,m} \left[ E\{Y(1, m) \mid Z = 1, X = x\} \text{pr}\{M(0) = m \mid Z = 0, X = x\} \text{pr}(X = x) \right]
\]

\[
- \sum_{x,l,m} \left[ E\{Y(0, m) \mid Z = 0, L = l, X = x\} \text{pr}\{L = l \mid Z = 0, X = x\} \text{pr}(X = x) \right]
\]

\[
= \sum_{x,l,m} \left[ E\{Y(1, m) \mid Z = 1, L = l, M = m, X = x\} \text{pr}\{L = l \mid Z = 1, X = x\} \text{pr}(X = x) \right]
\]

\[
- \sum_{x,l,m} \left[ E\{Y(0, m) \mid Z = 0, L = l, M = m, X = x\} \text{pr}\{L = l \mid Z = 0, X = x\} \text{pr}(X = x) \right]
\]

\[
= \sum_{x,l,m} \left[ E(Y \mid Z = 1, L = l, M = m, X = x) \text{pr}(L = l \mid Z = 1, X = x) \text{pr}(X = x) \right]
\]

\[
- \sum_{x,l,m} \left[ E(Y \mid Z = 0, L = l, M = m, X = x) \text{pr}(L = l \mid Z = 0, X = x) \right]
\]

\[
- \sum_{x,l,m} \left[ E(Y \mid Z = 0, L = l, M = m, X = x) \text{pr}(L = l \mid Z = 0, X = x) \right]
\]
\[ \times \Pr(M = m \mid Z = 0, X = x)\Pr(X = x) \]

\[ = \sum_{x, l, m} \left[ \{E(Y \mid Z = 1, L = l, M = m, X = x)\Pr(L = l \mid Z = 1, X = x) \right. \]

\[ - E(Y \mid Z = 0, L = l, M = m, X = x)\Pr(L = l \mid Z = 0, X = x) \}

\[ \left. \times \Pr(M = m \mid Z = 0, X = x)\Pr(X = x) \right\} . \]

\[ \mbox{HIE} = E[Y\{1, G(1 \mid X)\}] - E[Y\{1, G(0 \mid X)\}] \]

\[ = \sum_{x, m} E\{Y(1, m) \mid G(1 \mid x) = m, X = x\}\Pr\{G(1 \mid x) = m \mid X = x\}\Pr(X = x) \]

\[ - \sum_{x, m} E\{Y(1, m) \mid G(0 \mid x) = m, X = x\}\Pr\{G(0 \mid x) = m \mid X = x\}\Pr(X = x) \]

\[ = \sum_{x, m} E\{Y(1, m) \mid X = x\}\Pr\{M(1) = m \mid X = x\}\Pr(X = x) \]

\[ - \sum_{x, m} E\{Y(1, m) \mid X = x\}\Pr\{M(0) = m \mid X = x\}\Pr(X = x) \]

\[ = \sum_{x, m} \left[ E\{Y(1, m) \mid Z = 1, X = x\}\Pr(L = l \mid Z = 1, X = x) \right. \]

\[ \times \Pr\{M(1) = m \mid Z = 1, X = x\}\Pr(X = x) \]

\[ - \sum_{x, l, m} \left[ E\{Y(1, m) \mid Z = 1, L = l, X = x\}\Pr(L = l \mid Z = 1, X = x) \right. \]

\[ \times \Pr\{M(0) = m \mid Z = 0, X = x\}\Pr(X = x) \]

\[ = \sum_{x, l, m} \left[ E\{Y(1, m) \mid Z = 1, L = l, M = m, X = x\}\Pr(L = l \mid Z = 1, X = x) \right. \]

\[ \times \Pr\{M(1) = m \mid Z = 1, X = x\}\Pr(X = x) \]

\[ - \sum_{x, l, m} \left[ E\{Y(1, m) \mid Z = 1, L = l, M = m, X = x\}\Pr(L = l \mid Z = 1, X = x) \right. \]

\[ \times \Pr\{M(0) = m \mid Z = 0, X = x\}\Pr(X = x) \]
\begin{align*}
&= \sum_{x,l,m} \left[ E(Y \mid Z = 1, L = l, M = m, X = x) \text{pr}(L = l \mid Z = 1, X = x) \\
&\quad \times \text{pr}(M = m \mid Z = 1, X = x) \text{pr}(X = x) \right] \\
&- \sum_{x,l,m} \left[ E(Y \mid Z = 1, L = l, M = m, X = x) \text{pr}(L = l \mid Z = 1, X = x) \\
&\quad \times \text{pr}(M = m \mid Z = 0, X = x) \text{pr}(X = x) \right] \\
&= \sum_{x,l,m} \left[ E(Y \mid Z = 1, L = l, M = m, X = x) \text{pr}(L = l \mid Z = 1, X = x) \\
&\quad \times \{\text{pr}(M = m \mid Z = 1, X = x) - \text{pr}(M = m \mid Z = 0, X = x)\} \text{pr}(X = x) \right].
\end{align*}

\(\square\)

**S3  Proof of Theorem 3.3**

**Proof.** Based on Proposition 3.2 in Section 3.2, under Assumptions 1–3 and 4a,

\[
\text{IDE} = \sum_{x,l,m} \left[ \{ E(Y \mid z_1, l, m^{(1)}, x) P(l \mid z_1, x) - E(Y \mid z_0, l, m^{(1)}, x) P(l \mid z_0, x) \} \\
\quad \times P(m^{(1)} \mid z_0, x) P(x) \right];
\]

\[
\text{IIE} = \sum_{x,l,m} E(Y \mid z_1, l, m^{(1)}, x) P(l \mid z_1, x) \{ P(m^{(1)} \mid z_1, x) - P(m^{(1)} \mid z_0, x) \} P(x).
\]

Let

\[
\eta_1 = \sum_{x,l,m} E(Y \mid z_1, l, m^{(1)}, x) P(l \mid z_1, x) P(m^{(1)} \mid z_0, x) P(x);
\]

\[
\eta_2 = \sum_{x,l,m} E(Y \mid z_0, l, m^{(1)}, x) P(l \mid z_0, x) P(m^{(1)} \mid z_0, x) P(x);
\]

\[
\eta_3 = \sum_{x,l,m} E(Y \mid z_1, l, m^{(1)}, x) P(l \mid z_1, x) P(m^{(1)} \mid z_1, x) P(x).
\]

First, we show that

\[
\eta_1 = E \left\{ \frac{I(Z = z_0) Y}{\text{pr}(Z = z_0 \mid X)} \frac{E(Y \mid Z = z_1, L, M^{(1)}, X) \text{pr}(L \mid Z = z_1, X)}{E(Y \mid Z = z_0, L, M^{(1)}, X) \text{pr}(L \mid Z = z_0, M^{(1)}, X)} \right\}. \tag{S2}
\]

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RHS of (S2)
\[
= E_{X,L,M^{(1)},Z} \left\{ \frac{I(Z = z_0)Y}{\text{pr}(Z = z_0 \mid X)} \frac{E(Y \mid Z = z_1, L, M^{(1)}, X) \text{pr}(L \mid Z = z_1, X)}{E(Y \mid Z = z_0, L, M^{(1)}, X) \text{pr}(L \mid Z = z_0, M^{(1)}, X)} \right\} X, L, M^{(1)}, Z
\]
\[
= \sum_{x,l,m^{(1)}} \left[ E \left\{ \frac{Y}{\text{pr}(Z = z_0 \mid X = x)} \frac{E(Y \mid z_1, l, m^{(1)}, x) P(l \mid z_1, x)}{E(Y \mid z_0, l, m^{(1)}, x) P(l \mid z_0, m^{(1)}, x)} \right\} x, Z = z_0, l, m^{(1)} \right] \times P(x) P(z_0 \mid x) P(l, m^{(1)} \mid z_0, x)
\]
\[
= \sum_{x,l,m^{(1)}} E(Y \mid x, z_0, l, m^{(1)}) P(m^{(1)} \mid z_0, x) P(x) P(l \mid z_1, x)
\]
=LHS of (S2).

Second, we show that
\[
\eta_2 = E \left\{ \frac{I(Z = z_0)Y}{\text{pr}(Z = z_0 \mid X)} \frac{E(Y \mid Z = z_0, L, M^{(1)}, X) \text{pr}(L \mid Z = z_0, X)}{E(Y \mid Z = z_0, L, M^{(1)}, X) \text{pr}(L \mid Z = z_0, M^{(1)}, X)} \right\}.
\] (S3)

RHS of (S3)
\[
= E_{X,L,M^{(1)},Z} \left\{ \frac{I(Z = z_0)Y}{\text{pr}(Z = z_0 \mid X)} \frac{E(Y \mid Z = z_0, L, M^{(1)}, X) \text{pr}(L \mid Z = z_0, X)}{E(Y \mid Z = z_0, L, M^{(1)}, X) \text{pr}(L \mid Z = z_0, M^{(1)}, X)} \right\} X, L, M^{(1)}, Z
\]
\[
= \sum_{x,l,m^{(1)}} \left[ E \left\{ \frac{Y}{\text{pr}(Z = z_0 \mid X = x)} \frac{E(Y \mid z_0, l, m^{(1)}, x) P(l \mid z_0, x)}{E(Y \mid z_0, l, m^{(1)}, x) P(l \mid z_0, m^{(1)}, x)} \right\} x, Z = z_0, l, m^{(1)} \right] \times P(x) P(z_0 \mid x) P(l, m^{(1)} \mid z_0, x)
\]
\[
= \sum_{x,l,m^{(1)}} E(Y \mid x, z_0, l, m^{(1)}) P(m^{(1)} \mid z_0, x) P(x) P(l \mid z_0, x)
\]
=LHS of (S3).

Third, we prove that
\[
\eta_3 = E \left\{ \frac{I(Z = z_1)Y}{\text{pr}(Z = z_1 \mid X)} \frac{\text{pr}(L \mid Z = z_1, X)}{\text{pr}(L \mid Z = z_1, M^{(1)}, X)} \right\}.
\] (S4)
RHS of (S4)

\[
E_{X, L, M^{(1)}} \sum_{x, l, m^{(1)}} \left[ E \left\{ \frac{I(Z = z_1)Y}{\text{pr}(Z = z_1 | X)} E(Y | Z = z_1, L, M^{(1)}, X) \text{pr}(L | Z = z_1, X) \right\} \right.
\]

\[
\left. \times P(x) P(x | x) P(l, m^{(1)} | z_1, x) \right\}
\]

\[
= \sum_{x, l, m^{(1)}} E(Y | x, z_1, l, m^{(1)}) E(Y | z_1, l, m^{(1)}, x) P(l | z_1, x) P(x) P(z_1 | x) P(l, m^{(1)} | z_1, x)
\]

\[
= \sum_{x, l, m^{(1)}} E(Y | x, z_1, l, m^{(1)}) P(m^{(1)} | z_1, x) P(x) P(l | z_1, x)
\]

\[
= \text{LHS of (S4)}.
\]

Therefore, based on (S1),

\[
\text{IDE} = \eta_1 - \eta_2
\]

\[
= E \left\{ \frac{I(Z = z_0)Y}{\text{pr}(Z = z_0 | X)} E(Y | Z = z_0, L, M^{(1)}, X) \text{pr}(L | Z = z_0, M^{(1)}, X) \right\}
\]

\[
- E \left\{ \frac{I(Z = z_0)Y}{\text{pr}(Z = z_0 | X)} \text{pr}(L | Z = z_0, M^{(1)}, X) \right\} ;
\]

\[
\text{IIE} = \eta_3 - \eta_1
\]

\[
= E \left\{ \frac{I(Z = z_1)Y}{\text{pr}(Z = z_1 | X)} \text{pr}(L | Z = z_1, M^{(1)}, X) \right\}
\]

\[
- E \left\{ \frac{I(Z = z_0)Y}{\text{pr}(Z = z_0 | X)} E(Y | Z = z_0, L, M^{(1)}, X) \text{pr}(L | Z = z_0, M^{(1)}, X) \right\} .
\]
S4 The implementation details of bagging with the optimal subset of deep neural networks

To implement the method of bagging with the optimal subset of deep neural networks (DNNs), we follow the idea of Mi et al. (2019) and use their R package deepTL. The introduction of R package deepTL can be found in https://github.com/SkadiEye/deepTL.

In the simulation studies and real data analysis, we use 3-hidden-layer feedforward DNNs with 30 nodes per layer. The activation function is set to be the rectified linear unit (ReLU), where \( \text{ReLU}(t) = \max(0, t) \). The tuning parameter \( \lambda \) for the \( L_1 \) penalty function is set to \( 10^{-4} \). The batch size for the mini-batch stochastic gradient descent algorithm is set to 50. The maximum number of epochs is set to 100. The adaptive learning rate adjustment method is chosen to be Adam (Kingma and Ba, 2014). The number of DNNs in the ensemble is first set to 100. Then the optimal subset of DNNs utilized by the ensemble is chosen based on the criterion in Mi et al. (2019). Simulation studies show that simulation results are not very sensitive to the choice of above tuning parameters.

S5 Additional results for the AML microbiome study

In this section, we provide some additional results for the AML microbiome study. The Pearson’s correlation coefficient between the selected mediator \( M_{46} \) and \( Y \) is \(-0.235\) (95% CI: \([-0.348, -0.081]\)), suggesting that \( M_{46} \) is negatively associated with \( Y \); here the confidence interval is chosen to be the nonparametric bias-corrected and accelerated (BCa) bootstrap confidence interval (DiCiccio and Efron, 1996) and the number of bootstrap replications is 500. Table S1 shows the Pearson’s correlation coefficients between the selected mediator \( M_{46} \) (i.e., OTU unc05eti) and all other elements of \( M \). It can be found that some mediators are positively associated with \( M_{46} \), such as \( M_{62} \); some mediators are negatively associated with \( M_{46} \), such as \( M_{19} \).
Table S1: Pearson’s correlation coefficients between the selected mediator \( M_{46} \) and all other elements of \( M \)

|       | \( M_1 \) | \( M_2 \) | \( M_3 \) | \( M_4 \) | \( M_5 \) | \( M_6 \) | \( M_7 \) | \( M_8 \) | \( M_9 \) | \( M_{10} \) | \( M_{11} \) |
|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|----------|
| \( M_{12} \) | 0.114   | -0.019  | -0.035  | -0.104  | -0.107  | -0.110  | 0.311   | 0.026   | 0.141   | 0.134    | 0.060    |
| \( M_{13} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{14} \) | 0.226   | 0.263   | -0.066  | -0.089  | -0.123  | -0.047  | 0.049   | -0.243  | -0.070  | -0.191   | -0.109   |
| \( M_{15} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{16} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{17} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{18} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{19} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{20} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{21} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{22} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{23} \) | 0.059   | -0.003  | -0.043  | -0.138  | -0.077  | -0.097  | -0.011  | -0.103  | -0.072  | -0.012   | -0.101   |
| \( M_{24} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{25} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{26} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{27} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{28} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{29} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{30} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{31} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{32} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{33} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{34} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{35} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{36} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{37} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{38} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{39} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{40} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{41} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{42} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{43} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{44} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{45} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{46} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{47} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{48} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{49} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{50} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{51} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{52} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{53} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{54} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{55} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{56} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{57} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{58} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{59} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{60} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{61} \) |        |         |         |         |         |         |         |         |         |          |          |
| \( M_{62} \) |        |         |         |         |         |         |         |         |         |          |          |