Bayesian Nonparametric Mixed Effects Models in Microbiome Data Analysis

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Abstract

Detecting associations between microbial composition and sample characteristics is one of the most important tasks in microbiome studies. Most of the existing methods apply univariate models to single microbial species separately, with adjustments for multiple hypothesis testing. We propose a Bayesian nonparametric analysis for a generalized mixed effects linear model tailored to this application. The marginal prior on each microbial composition is a Dirichlet Processes, and dependence across compositions is induced through a linear combination of individual covariates, such as disease biomarkers or the subject’s age, and latent factors. The latent factors capture residual variability and their dimensionality is learned from the data in a fully Bayesian procedure. We propose an efficient algorithm to sample from the posterior and visualizations of model parameters which reveal associations between covariates and microbial composition. The proposed model is validated in simulation studies and then applied to analyze a microbiome dataset for infants with Type I diabetes.
1 Introduction

Large scale studies of the human microbiome have become increasingly common thanks to advances in next generation sequencing (NGS) technologies (Human Microbiome Project Consortium, 2012; Qin et al., 2010). A relevant task in these studies is to measure the association between a sample’s microbial composition and individual characteristics, such as biomarkers and aspects of the sample’s environment, which might elucidate the mechanism through which microbiome perturbations affect a range of conditions (Turnbaugh et al., 2009; Quince et al., 2013; Kostic et al., 2015). The abundance of microbial taxa is measured by assigning DNA reads to reference genomes. Some experiments target specific genes, such as the the 16S rRNA gene, while others sample the entire bacterial genome. In all cases, the resulting count data for a collection of samples are organized into a contingency table known as the operational taxonomic unit (OTU) table.

Several methods for association studies with microbial data apply ideas from RNA-seq and other high-throughput genomic experiments (Robinson et al., 2010; Anders and Huber, 2010; Morgan et al., 2012; Paulson et al., 2013). These methods use raw or transformed counts of microbial species to test the association of a single species with relevant covariates. Typically, these tests are carried out one species at a time by using generalized linear models (GLMs) combined with families of distributions that are over-dispersed and zero-inflated (Xu et al., 2015) to accommodate well-known characteristics of microbial abundance data (Li, 2015). The major drawback of this approach is that it models species independently. This does not take into account correlations across microbial species and does not allow borrowing of information across species.

The outlined limitation has prompted the introduction of joint models of microbial abundance (Chen and Li, 2013; Xia et al., 2013; Wadsworth et al., 2017; Grantham et al., 2017). These methods model the counts of microbial species \( n_{i,j}; i = 1, \ldots, I \), of a specific sample \( j \), say a saliva sample, with a multinomial distribution. To account for the over-dispersion these methods assume the multinomial parameter \( P_j = (P_{j1}, \ldots, P_{jI}) \) is random and distributed according to a parametric model. For example, in Chen and Li (2013) and Wadsworth et al. (2017), \( P_j \)'s follow independent Dirichlet distributions and in Xia et al. (2013) and Grantham et al. (2017), the \( P_j \)'s follow multivariate logistic normal distributions. To associate the covariates to the microbial compositions, all models link the parameters
of each distribution of $P^j$ to covariates of sample $j$ via a regression function. Inference on the regression coefficients indicates whether a covariate is associated with the abundance of a species or not. Although these two joint models overcome limitations of separate modeling of single species, the assumed distributions of the $P^j$’s in these methods are restrictive. For instance $P^j_i$ is strictly positive for all $i$ and $j$. This does not reflect the fact that some species can be completely absent in the sample $j$. In addition, the variation of $P^j$’s across samples might be mainly associated to some latent characteristics that are not observed. In this case, the methods which link the model parameters exclusively to covariates cannot capture underlying patterns of residual variation.

Bayesian nonparametric methods that jointly model the compositions $P^j$ offer flexible alternatives. A widely used class of nonparametric models stems from the Hierarchical Dirichlet process (Teh et al., 2006). In its simplest form, the Hierarchical Dirichlet process (HDP) assumes samples are exchangeable and the compositions $P^j$ are identically distributed. The exchangeability assumption in the HDP does not capture potential association between $P^j$’s and covariates. Nonparametric models with covariates explicitly embedded are ideal candidates for modeling dependence of compositions $P^j$ on covariates. A relevant class of nonparametric models embedding covariates utilize the Chinese restaurant processes representation (Caron et al., 2007; Johnson et al., 2013). A second class of such models utilizes completely random measures (Lijoi et al., 2014). A third class of models follow the idea in MacEachern (2000). Among these, Dunson and Park (2008), Dunson and Xing (2009), Rodriguez and Dunson (2011), Müller et al. (2011), Griffin et al. (2013), and Arbel et al. (2016) construct dependent random measures using stick-breaking processes with atoms and weights specified through covariate-indexed stochastic processes. This third class of models can capture a wide range of dependence structures between microbial compositions and covariates and is computationally attractive.

Recently, a Bayesian nonparametric model for microbiome data specified through sample-specific latent factors has been discussed in Ren et al. (2016). This construction induces a marginal Dirichlet Process prior for each composition $P^j$, and introduces dependences across samples by associating microbial compositions $P^j$ to linear combinations of latent factors. A shrinkage prior on the latent factors is used in this model to produce parsimonious estimates that concentrate on a low-dimensional space. Approximating matrix data with low-dimensional linear factors has found theoretical justification in Udell and Townsend (2017).
This manuscript extends the model of Ren et al. (2016), linking the microbial composition \( P^j \) to covariate effects as well as to the latent factors. By estimating coefficients for a linear combination of relevant covariates, we infer whether a given covariate is associated with the microbial compositions or not. Our model preserves all the merits discussed for the construction in Ren et al. (2016) and can additionally perform association studies for microbiome data, without discernible increase in the computational cost. We discuss the interpretation of model parameters and propose approaches to visualize covariate effects, which may vary from sample to sample due to the compositional nature of the data.

The paper is organized as follows. In Section 2 we propose the Bayesian model and discuss the identifiability of relevant model parameters. Section 3 is dedicated to computational aspects and provides an overview of the sampling algorithm for posterior inference. Section 4 presents simulation studies and in Section 5 we discuss an application of the model to a study of Type I diabetes mellitus which collected longitudinal microbiome data from a cohort of infants. Section 6 concludes and discusses possible extensions of the proposed model.

2 Prior model

In this section, we first describe the construction of the Dependent Dirichlet processes in Ren et al. (2016), and then provide a new version of the model which incorporates covariates. We also discuss the identifiability of the model parameters, including the parameters that correspond to the covariates’ effects. The model will be used in the next sections to analyze the OTU table \( \mathbf{n} = (n_{i,j}; i \leq I, j \leq J) \), where \( n_{i,j} \) is the observed count of the microbial species \( i \) in sample \( j \). \( I \) and \( J \) are the total number of species and samples respectively. We want to extract from the OTU table information on the relationships between microbial composition and observed sample characteristics.

2.1 Dependent Dirichlet processes

In Table 1, we illustrate an example of OTU table with six samples, where half are Russian (RUS) subjects and half are Finnish (FIN) subjects (Vatanen et al., 2016). The table records the counts of 10 microbial species in all six samples based on 16S rRNA sequencing. Let \( Z_i \) be the \( i \)th observed species (e.g. \( Z_1 \) is the Bifidobacterium longum)
and \( n_{i,j} \) be the observed counts of OTU \( Z_i \) in samples \( j \). For example, \( n_{1,1} = 0 \) refers to the counts of Bifidobacterium longum in the RUS1 sample.

For the sample \( j \), we assume the vector \((n_{1,j}, \ldots, n_{I,j})\) follows a multinomial distribution with unknown parameters. Our analyses extend easily to the case in which the counts \( n_{i,j} \) are Poisson random variables with unknown means. The sequencing depth \( n_j = \sum_{i=1}^{I} n_{i,j} \) and the sample-specific multinomial probabilities \((P_{1}^{j}, \ldots, P_{I}^{j})\) determine the distribution of \((n_{i,j}; i \leq I)\). The probabilities \((P_{1}^{j}, \ldots, P_{I}^{j})\) represent the microbial composition of the sample \( j \). We use \( P^j(\{Z_i\}) = P_i^j \) to denote the relative abundance of \( Z_i \) in the sample \( j \). With \( J \) samples we expect variations in the corresponding compositions \( P^j \). This variability is associated to heterogeneity of either measured or unknown characteristics of the \( J \) samples. For instance, in Table 1 the MLE of \( P_1^j \) tends to be higher in Russian samples than in the Finnish ones. In this case the evidence of different \( P_1^j \) parameters across samples could be, at least in part, attributable to the variation of nationality within the collection of samples [Vatanen et al. 2016].

Microbiome studies aim to identify and quantify associations of samples characteristics with microbial compositions.

We describe a Bayesian model for the unknown compositions \( P^j, j = 1, \ldots, J \). Let \( Z_i \in Z, i \geq 1 \) be a sequence of distinct species. We do not constrain \textit{a priori} the number of species present in our \( J \) samples. We specify the probability mass assigned to a group of OTUs \( A \subset Z \) as

\[
P^j(A) = \frac{M^j(A)}{M^j(Z)},
\]

\[
M^j(A) = \sum_{i=1}^{\infty} \mathbb{I}(Z_i \in A)\sigma_i(x_i, y_j)^{\frac{1}{2}},
\]

where \( \sigma_i \in (0, 1) \) and \( x_i, y_j \in \mathbb{R}^K \). \( \mathbb{I}(\cdot) \) is the indicator function and \( x^+ = x \times \mathbb{I}(x > 0) \). We use \( \langle \cdot, \cdot \rangle \) to denote
the standard inner product in \( \mathbb{R}^K \). We define \( Q_{i,j} = \langle X_i, Y_j \rangle \). In addition, \( \boldsymbol{\sigma} = (\sigma_i; i \geq 1), \boldsymbol{X} = (X_i; i \geq 1), \boldsymbol{Y} = (Y_j; j \leq J) \) and \( \boldsymbol{Q} = (Q_{i,j}; i \geq 1, j \leq J) \).

We can interpret \( \sigma_i > 0 \) as a summary of the overall abundance of species \( i \) across samples. We call \( \boldsymbol{X}_i \) and \( \boldsymbol{Y}_j \) species vector and sample vector respectively. The \( k \)-th components of \( \boldsymbol{X}_i \) and \( \boldsymbol{Y}_j \) are denoted as \( X_{k,i} \) and \( Y_{k,j} \). \( \boldsymbol{X}_i \) and \( \boldsymbol{Y}_j \) are latent components of the probability model. Differences across compositions \( P^j \) are determined by the \( \boldsymbol{Y}_j \) latent vectors. The vectors \( \boldsymbol{Y}_j \) can be viewed as latent characteristics of the samples that affect their microbial compositions. For example, an unobserved feature of the subject’s diet, such as vegetarianism, could affect the abundance of certain species. We assume that there are \( K \) latent characteristics, and \( \boldsymbol{X}_i \) has the same dimensionality as \( \boldsymbol{Y}_j \). The \( k \)-th entry of the species vector \( \boldsymbol{X}_i \) can be interpreted as the effect of a latent characteristic \( Y_{k,j} \) on the abundance of the species \( Z_i \).

We also note that the angle \( \phi_{j,j'} \) between \( \boldsymbol{Y}_j \) and \( \boldsymbol{Y}_{j'} \) determines the degree of similarity between compositions \( P^j \) and \( P^{j'} \). Specifically, small \( \phi_{j,j'} \) indicates that \( P^j \) and \( P^{j'} \) are similar. When \( \phi_{j,j'} = 0 \), compositions \( P^j \) and \( P^{j'} \) are identical. Symmetrically, the angle \( \varphi_{i,i'} \) between \( \boldsymbol{X}_i \) and \( \boldsymbol{X}_{i'} \) can be viewed as a measure of similarity between species \( Z_i \) and \( Z_{i'} \). When \( \varphi_{i,i'} \) decreases towards zero, the correlation between \( (P^j(\{Z_i\}; j \leq J)) \) and \( (P^{j'}(\{Z_{i'}\}; j \leq J)) \) increases to one.

We specify a prior distribution. First \( \sigma_1 > \sigma_2 > \sigma_3 \ldots \), are a priori ordered points from a Poisson process on \((0, 1)\) with intensity \( \nu(\sigma) = \alpha \sigma^{-1} (1 - \sigma)^{-1/2} \). Second the \( X_{k,i} \) random variables are independent Gaussian \( \mathcal{N}(0, 1) \), \( i = 1, 2, \ldots, k = 1, 2, \ldots, K \). We can assume for the moment that the \( \boldsymbol{Y}_j \)'s are fixed.

The resulting marginal prior distribution on the composition \( P^j \) is a Dirichlet process [Ren et al., 2016]. To provide some intuition on this construction of Dependent Dirichlet processes we consider a similar model with \( I < \infty \) species. For simplicity we set \( \|\boldsymbol{Y}_j\| = 1 \), where \( \|\cdot\| \) is the Euclidean norm of a real vector. The prior on \( \boldsymbol{X}_i \)'s induces a standard normal distribution on \( (Q_{1,j}, \ldots, Q_{I,j}) \). The prior distribution of \( Q_{i,j}^{+2} \) is therefore a mixture of a point mass at zero and a Gamma\((1/2, 1/2)\) distribution, because the square of a standard normal random variable is gamma distributed. Assume \( (\sigma_1, \ldots, \sigma_I) \) are independent Beta\((\alpha/I, 1/2 - \alpha/I)\) variables, it follows that the products \( (\sigma_i Q_{i,j}^{+2}, i = 1, \ldots, I) \) are distributed according to a mixture with a point mass at zero and a Gamma\((\alpha/I, 1/2)\)
component. The normalized vector \( \frac{\sigma_i Q_{i,j}^2}{\sum_j \sigma_i Q_{i,j}^2}, i = 1, \ldots, I \), conditioned on \( (I(Q_{1,j} > 0), \ldots, I(Q_{1,j} > 0)) \) follows a Dirichlet distribution with weights proportional to \( I(Q_{1,j} > 0) \). If \( I \to \infty \), we then verify that the \( (\sigma_1, \ldots, \sigma_I) \) converges in distribution to the Poisson process with intensity \( \nu \). And \( \frac{\sigma_i Q_{i,j}^2}{\sum_j \sigma_i Q_{i,j}^2}, i = 1, \ldots, I \) becomes a Dirichlet process (Ferguson, 1973). This holds also when \( \|Y_j\| \neq 1 \) because the distribution of \( (\sigma_i Q_{i,j}^2 / \sum_j \sigma_i Q_{i,j}^2, i = 1, \ldots, I) \) does not depend on \( \|Y_j\| \).

For inferential and visualization purposes it is desirable that the \( Y_j \) latent vectors concentrate approximately on a low dimensional space. Our goal is to identify parsimonious \( Y_j \) latent vectors that are representative of the variability of observed species abundances across samples. To this end, we apply the prior studied in Bhattacharya and Dunson (2011),

\[
Y_j \sim \mathcal{N}(0, \text{diag}\{\gamma_1, \ldots, \gamma_K}\)),
\]

where \( \gamma_k \) rapidly decrease with \( k \). The prior formalizes the requirement that norm \( \|Y_j\| \) is mostly driven by the first few components of \( Y_j \), say the first three components \( (Y_{1,j}, Y_{2,j}, Y_{3,j}) \), and the rest of the components, \( (Y_{4,j}, \ldots, Y_{K,j}) \), vanish with negligible values. In different words, only a small set of \( Y_j \) entries—three in the example—are relevant. We preferred this approach to an hyper-prior on the \( Y_j \) dimensionality mainly for computational convenience.

### 2.2 Fixed effects

The goal of this subsection is to model relationships between microbial compositions and samples’ characteristics. For example, in studies of Inflammatory Bowel Disease (IBD) (Morgan et al., 2012; Greenblum et al., 2012; Gevers et al., 2014), researchers were interested in identifying microbes that correlate with the onset of IBD to develop therapeutic hypotheses. These analyses typically utilize regression models where the outcomes coincide with OTU abundances. Following a similar strategy, we expand the model in Subsection 2.1.

Assume there are \( L \geq 1 \) observed covariates. We use \( w_j = (w_{l,j}; l = 1, \ldots, L) \) to denote the covariates’ values for sample \( j \). The effects of this set of covariates on species \( i \) are \( v_i = (v_{l,i}; l = 1, \ldots, L) \). The collection of all \( w_j \) and \( v_i \) are \( w = (w_1, \ldots, w_J) \) and \( v = (v_1, \ldots, v_I) \). Our extended model directly modifies the random variables...
$Q_{i,j}$'s in (1) by adding a linear function of $w_j$ and an error term:

$$Q_{i,j} = \langle X_i, Y_j \rangle + \langle v_i, w_j \rangle + \epsilon_{i,j},$$  

where $\epsilon_{i,j} \overset{iid}{\sim} N(0,1)$ is the error term. The inner product $\langle v_i, w_j \rangle$ represents the fixed effects of our model, whereas $\langle X_i, Y_j \rangle$ represents the random effects. We fix the variance of the errors to one since the model for $P_j$ is invariant if we rescale all $Q_{i,j}$ variables by a fixed multiplicative term.

In this construction, $v_i$ and $w_j$ can be viewed as additional dimensions of $X_i$ and $Y_j$ respectively. The angle between $(w_j, Y_j)$ and $(w_{j'}, Y_{j'})$, denoted as $\tilde{\phi}_{j,j'}$, measures the similarity between the microbial compositions $P_j$ and $P_{j'}$. As in model (1), one can verify that the correlation $\text{cor}(P_j(A), P_{j'}(A))$ is monotone with respect to $\tilde{\phi}_{j,j'}$. Similarly, the angle between $(v_i, X_i)$ and $(v_{i'}, X_{i'})$, $\tilde{\phi}_{i,i'}$, is representative of the correlation between abundances of species $i$ and $i'$ across samples. A small $\tilde{\phi}_{i,i'}$ value makes the correlation between vectors $(P_j(\{Z_i\}); j \leq J)$ and $(P_j(\{Z_i'\}); j \leq J)$ close to one.

The coefficients $v_i$ are a priori independent normal random variables with mean zero and variance one. When the latent factors $Y$ are fixed, and the prior for $X_i$ and $\sigma_i$ remains the same as in Subsection 2.1, the microbial composition $P_j$, for each $j = 1, \ldots, J$, retains a marginal Dirichlet Process distribution. More precisely, $P_j$ is a Dirichlet process with concentration parameter $\alpha$. This can be shown using the same argument as in Subsection 2.1.

It is important not to misinterpret the coefficients $v_i$. The species abundances are not linear functions of the covariates (see Figure 1). In certain cases, the relationship between the covariates and the species abundances is not monotone. Consider a single covariate $w_{1,j}$ and assume $Q_{i,j} = v_{1,i}w_{1,j} + \langle X_i, Y_j \rangle + \epsilon_{i,j}$, where $v_{1,1} = 5$, $v_{1,2} = 1$ and $v_{1,i} = 0$ when $i > 2$. For simplicity, assume in addition $\sigma_i$ variables all equal to 0.5. When $w_{1,j}$ is positive and small, say $w_{1,j} \in (0, 0.5)$, the abundances of species 1 and 2 increases with $w_{1,j}$. However, as $w_{1,j}$ gets larger, say $w_{1,j} > 5$, species 1 will dominate all other species and the abundance of species 2 decreases to zero.
Figure 1: Effect of a single covariate $w_{1,j}$ on microbial species abundances. We illustrate the expected abundances of 10 species when $w_{1,j}$ varies (Left) and the observed microbial abundances in one simulated dataset as $w_{1,j}$ changes (Right). We focus on a single sample $j$ and fix the random effects $\langle X_i, Y_j \rangle$ in all simulations. Only the value of $w_{1,j}$ and the error terms $\epsilon_{i,j}$'s vary. The expected abundances are calculated by averaging over 1000 simulation replicates. We consider the cases where $Q_{i,j} = v_{1,i}w_{1,j} + \langle X_i, Y_j \rangle + \epsilon_{i,j}$ with $v_{1,1} = 5$, $v_{1,2} = -5$ and $v_{1,i} = 0$ for $i > 2$ (Top) and $Q_{i,j} = v_{1,i}\sin(w_{1,j}) + \langle X_i, Y_j \rangle + \epsilon_{i,j}$ with $v_{1,1} = 5$ and $v_{1,i} = 0$ for $i > 1$ (Bottom). The covariate $w_{1,j}$ varies from $-5$ to $5$ with 0.1 increments.
2.2.1 Fixed effects and longitudinal data

In our analyses we considered longitudinal data with repeated measurements over time for each subject. Assume samples $j = 1, \ldots, J$ are partitioned into $U$ groups, i.e. $U$ distinct individuals. We use $u_j$ to identify the individual associated to the sample $j$. We enforce the samples $j$ and $j'$ from the same individual $u (u_j = u_{j'} = u)$ to share common latent factors $Y_u$. The longitudinal version of model (2) utilizes $Q_{i,j} = \langle X_i, Y_{u_j} \rangle + \langle v_i, w_{j} \rangle + \epsilon_{i,j}$. The rationale for this model is that samples derived from the same subject tend to be similar to each other. The covariates $w_{j}$ will include time information (e.g. individual age) for each sample $j$. This version of the model is tailored towards longitudinal data and studies with repeated measurements, and it allows one to visualize time trends of microbial compositions.

2.2.2 Truncated model

We also consider a truncated version of model (2) in data analyses, which we call the finite-species model. With $I < \infty$ species the finite-species model is defined by

$$Q_{i,j} = \langle X_i, Y_j \rangle + \langle v_i, w_{j} \rangle + \epsilon_{i,j},$$

$$P^j(\{Z_i\}) = \frac{\sigma_i Q_{i,j}^{+2}}{\sum_{i'=1}^{I} \sigma_{i'} Q_{i',j}^{+2}}, \quad i = 1, \ldots, I. \quad (3)$$

The prior for $X_i$ and $Y_j$ remain identical. The $\sigma_i$’s become $\sigma_i \sim \text{Beta}(\alpha/I, 1/2 - \alpha/I)$.

2.3 Identifiability

In this subsection we consider the identifiability of model (2). Since the model is invariant under simultaneous rotations of the vectors $Y_j$ and $X_i$, we cannot learn $Y$ from the data. We discuss the identifiability of the correlation matrix $S$ associated to $\Sigma = Y^\top Y + I$, where $I$ is the $J \times J$ identity matrix. Similarly, since the composition $P^j$ is invariant to scale transformation of $\sigma$ we will discuss identifiability of the ratios $\sigma_i/\sigma_{i'}$ for $i \neq i'$. We assume that the number of samples, $J > L$, is finite and that covariates $w_{j}$’s are independent with $E(w_{j} w_{j}^\top)$ of full rank.
We proceed assuming initially that \((P^j(\{Z_i\}); i \geq 1, j \leq J)\) are observable. Recall that

\[
(Q_{i,j}; j \leq J) \mid v_i, Y, w \sim \mathcal{N}(w^T v_i, \Sigma).
\]

Since we assume that \(P^j(\{Z_i\})\) is observable, we have that \(P^j(\{Z_i\}) = 0\) implies \(Q_{i,j} \leq 0\). Consider a set of new random variables, denoted as \((\tilde{P}^j(\{Z_i\}); i \leq I, j \leq J)\), where \(\tilde{P}^j(\{Z_i\}) = \mathbb{I}(P^j(\{Z_i\}) > 0)\). The distribution of \((\tilde{P}^j(\{Z_i\}); i \geq 1, j \leq J)\) is

\[
p(\tilde{P}^j(\{Z_i\}), i \geq 1, j \leq J | \sigma, Y, v, w)
= \prod_i \left[ \int_{A_i} (2\pi)^{-J/2} |\Sigma|^{-1/2} \times \exp \left( -\frac{1}{2} (Q_i - \mu_i)^T \Sigma^{-1} (Q_i - \mu_i) \right) dQ_i \right].
\]

(4)

Here \(Q_i = (Q_{i,1}, \ldots, Q_{i,J})\), \(\mu_i = w^T v_i\), \(A_i = \bigcup_{j=1}^J A_{i,j}\) and \(A_{i,j} = (-\infty, 0]\) if \(\tilde{P}^j(\{Z_i\}) = 0\), while \(A_{i,j} = [0, \infty)\) when \(\tilde{P}^j(\{Z_i\}) = 1\). To illustrate the identifiability of the parameters \((\sigma_i/\sigma_{i'}; i \neq i')\), \(S\) and \(v\), we start with two simplified cases and then give a proposition.

1. **Without random effects** \((Y = 0)\). We first note that conditioning on \(w\), for a fixed \(i\), \((\tilde{P}^j(\{Z_i\}); j \leq J)\) are samples from a standard probit model [Albert and Chib 1993], where \(v_i\) serves as the regression coefficient and the sample covariate is \(w_j\). Based on the theory of generalized linear models \(v_i\) is identifiable when \(E(w_jw_j^T)\) is of full rank.

We then consider \((\sigma_i/\sigma_{i'}; i \neq i')\). By construction,

\[
P^j(\{Z_i\}) = \frac{\sigma_i Q_{i,j}^2}{\sigma_{i'} Q_{i',j}^2}.
\]

Here we use the convention that the ratio is zero whenever the denominator is zero. To illustrate the identifiability of \((\sigma_i/\sigma_{i'}; i \geq 1, j \leq J)\), we want to show that if

\[
(P^j(\{Z_i\}); i \geq 1, j \leq J), w \mid v, \sigma \overset{d}{=} (P^j(\{Z_i\}); i \geq 1, j \leq J), w \mid v', \sigma',
\]

then \(\sigma_i/\sigma_{i'} = \sigma_i'/\sigma_{i'}'\) for all \(i \neq i'\). Using the identifiability of \(v_i\), the above equality in distribution implies \(v_i = v_i'\).
and in turn the equality of the conditional distributions \( p((Q_{i,j}^{+2}, Q_{i,j}^{+2}), w_j | v, \sigma) \) and \( p((Q_{i,j}^{+2}, Q_{i,j}^{+2}), w_j | v', \sigma') \).

This directly implies \( \sigma_i / \sigma_{i'} = \sigma_i' / \sigma_{i'}' \) for all \( i \neq i' \).

2. **Without fixed effects** \((v_i = 0)\). We consider \( \sigma \) and \( S \). The distribution of \((\tilde{P}^j(\{Z_i\}), \tilde{P}^j(\{Z_i\}))\) is

\[
p(\tilde{P}^j(\{Z_i\}), \tilde{P}^j(\{Z_i\}) | \sigma, Y) = \frac{1}{2\pi} \int_{A_{i,j} \times A_{i,j}'} (1 - S_{j,j'})^{-1/2} \exp \left( -\frac{1}{2} q' S_{j,j'}^{-1} q \right) dq,
\]

where \( S_{j,j'} \) is the correlation between \( Q_{i,j} \) and \( Q_{i,j'} \), and \( S_{j,j'} \) is the correlation matrix of \((Q_{i,j}, Q_{i,j'})\). \( A_{i,j} = (-\infty, 0] \) if \( \tilde{P}^j(\{Z_i\}) = 0 \), while \( A_{i,j} = [0, \infty) \) if \( \tilde{P}^j(\{Z_i\}) = 1 \). Corollary 3.12 in [Ledoux and Talagrand (2013)] shows that \( p(\tilde{P}^j(\{Z_i\}), \tilde{P}^j(\{Z_i\}) | v_i, Y) \) is monotone with respect to \( S_{j,j'} \). This implies that \( S_{j,j'} \) is identifiable.

Using the same arguments as in the case where no random effect is present, one can show that the ratios \((\sigma_i / \sigma_{i'}; i \neq i')\) remain identifiable.

We proceed to the general case with a proposition for the identifiability of the model parameters. The proof of this proposition is in the appendix.

**Proposition 1.** Assume that \( w_j, j = 1, \ldots, J \), are independently distributed with \( \mathbb{E}(w_j w_j') \) of full rank. Consider two sets of parameters \((v, Y, \sigma)\) and \((v', Y', \sigma')\) having \( \text{trace}(\Sigma) = \text{trace}(\Sigma') \), where \( \Sigma = Y' Y + I \) and symmetrically \( \Sigma' = (Y')' Y' + I \). If \([v, Y, (\sigma_i / \sigma_{i'}; i \neq i')]\) and \([v', Y', (\sigma_i' / \sigma_{i'}'; i \neq i')]\) are different, then there exists an integer \( n_0 > 0 \), such that when \( n_j \geq n_0, j = 1, \ldots, J \), under the two sets of parameters, the joint distributions of \([n_{i,j}; i \geq 1, j \leq J, w]\) are different.

We note that the requirement \( \text{trace}(\Sigma) = \text{trace}(\Sigma') \) is not restrictive in data analysis. This is because the model is invariant to scale transformation of \( Q \). By scaling \( Q \) we can make \( \text{trace}(\Sigma) \) to be equal to any pre-specified value.
3 Posterior simulations and visualization of covariates’ effects

In this section we focus on posterior inference and computational aspects. In Subsection 3.1 we introduce an algorithm for posterior simulations with the model described in Subsection 2.2. Then, in Subsection 3.2 we propose graphical visualizations to illustrate associations of microbial compositions and covariates. These representations are relevant for the analysis of microbial abundances because, as we mentioned in Subsection 2.2, a positive (or negative) element of the vector \( v_i \), say the \( l \)-th element, does not imply a monotone relation between the \( l \)-th covariate and the abundances of species \( i \). To illustrate the relation between the \( l \)-th covariate and species \( i \), we estimate how the abundance of species \( i \) would vary at hypothetical values of the \( l \)-th covariate.

3.1 Posterior simulations

We proceed with the finite-species model (3). The likelihood function is

\[
p(n|Q, \sigma) \propto \prod_{j=1}^{J} \prod_{i=1}^{I} (\sigma_i Q_{i,j}^{-2})^{n_{i,j}} \times \prod_{j=1}^{J} \left( \sum_{i=1}^{I} \sigma_i Q_{i,j}^{-2} \right)^{-n_j},
\]

and

\[
p(\sigma, Q, X, Y, v|n, w) \propto \prod_{j=1}^{J} \prod_{i=1}^{I} (\sigma_i Q_{i,j}^{-2})^{n_{i,j}} \times \prod_{j=1}^{J} \left( \sum_{i=1}^{I} \sigma_i Q_{i,j}^{-2} \right)^{-n_j} \times 
\pi(\sigma, Q|X, Y, v, w)\pi(X, Y, v),
\]

where \( \pi \) indicates the prior. By introducing positive latent random variables \( T = (T_1, \ldots, T_J) \) as in James et al. (2009), we rewrite the conditional distribution,

\[
p(\sigma, Q, X, Y, v|n, w) \propto \int \pi(\sigma, Q, X, Y, v|w) \times 
\prod_{j=1}^{J} \left\{ \left( \prod_{i=1}^{I} (\sigma_i Q_{i,j}^{-2})^{n_{i,j}} \right) T_j^{n_j-1} \exp \left( -T_j \sum_{i} \sigma_i Q_{i,j}^{-2} \right) \right\} dT.
\]

We use a Gibbs sampler to perform posterior simulations. The algorithm iteratively samples \( \sigma, T, Q, X, Y \) and \( v \) from the full conditional distributions. We describe the two components of the algorithm.
1. The first component samples $\sigma, T$ and $Q$ from the full conditional distributions. We note that $\sigma_1, \ldots, \sigma_I$, given the remaining variables, are conditionally independent. The sampling of $(\sigma_1, \ldots, \sigma_I)$ from the full conditional distribution is identical as in Ren et al. (2016). The random variables $T_1, \ldots, T_J$, given $(Q, n, \sigma)$, are conditionally independent with Gamma distributions. These random variables can be straightforwardly generated from the full conditional distribution. To complete this part of the algorithm we can write

\[ p(Q_{i,j} | n, Q_{-i,-j}, T, \sigma, X, Y, w, v) \propto \]

\[ Q_{i,j}^{+2n_{i,j}} \times \exp(-T_j \sigma_i Q_{i,j}^{-2}) \times \exp\left(-\frac{(Q_{i,j} - \langle X_i, Y_j \rangle - \langle v_i, w_j \rangle)^2}{2}\right), \tag{7} \]

where $Q_{-i,-j}$ is identical to $Q$ with the only exception that it does not include $Q_{i,j}$. The density (7) indicates that the $Q_{i,j}$’s random variables are conditionally independent. We also note that the density in (7) is log-concave. We use these arguments to sample $Q$ from the full conditional distribution.

2. The second component considers the sampling of $Y, X$ and $v$ from the full conditional distributions. Using expression (6) we write

\[ p(X | n, \sigma, T, Q, Y, v, w) \propto \exp\left(-\sum_{i,j} (Q_{i,j} - \langle X_i, Y_j \rangle - \langle v_i, w_j \rangle)^2 / 2\right) \times \pi(X). \]

Recall that the $X_i$’s are a priori independent normal random variables. Therefore the full conditional distribution of $X_i$ coincides with the conjugate posterior distribution in a standard linear model (Lindley and Smith 1972). Sampling of $Y$ and $v$ from the full conditional distributions follows identical arguments. Indeed the prior model studied in Bhattacharya and Dunson (2011), which we use for $Y$, is conditionally conjugate.

3.2 Visualization of covariate effects

We consider the partial derivatives

\[ \frac{\partial P^j(\{Z_i\})}{\partial w_{i,j}} := \partial \left[ \frac{\sigma_i(\langle X_i, Y_j \rangle + \langle v_i, w_j \rangle + \epsilon_{i,j})^{+2}}{\sum_{i'} \sigma_{i'}(\langle X_{i'}, Y_j \rangle + \langle v_{i'}, w_j \rangle + \epsilon_{i',j})^{+2}} \right] / \partial w_{i,j}. \]
The derivative $\partial P^j({\{Z_i\}})/\partial w_{l,j}$ quantifies the abundance variation of species $i$ in sample $j$ in response to an infinitesimal increment of the $l$th component of $w_j$. We can estimate these derivatives from the data using the posterior approximation obtained by the algorithm in Subsection 3.1. We use the estimates $E(\partial P^j({\{Z_i\}})/\partial w_{l,j}|n,w)$.

For example, the top row of Figure 3 summarises the posterior distributions of $\partial P^j({\{Z_i\}})/\partial w_{l,j}$, $j = 1, \ldots, 300$, for three species. In this case $l = 1$. In species 1, the estimates of the derivatives are positive for the majority of the samples and tend to be large when $w_{1,j} > 0$. We also note that the estimates of $\partial P^j({\{Z_i\}})/\partial w_{l,j}$ are larger for samples in the subgroup $w_{2,j} = 1$ than in the subgroup $w_{2,j} = 0$. These results indicate that, for any $j = 1, \ldots, 300$, if we could increase (decrease) the value of $w_{1,j}$ while holding $w_{2,j}$ fixed, then one would expect an increase (decrease) of the relative abundances of species 1, and this trend appears more pronounced in those samples with $w_{1,j} > 0$ and $w_{2,j} = 1$.

We define $P^j({\{Z_i\}}; w_0) := \sigma_i (\langle X_i, Y_j \rangle + \langle v_i, w_0 \rangle + \epsilon_{i,j})^2 / \sum_{i'} \sigma_{i'} (\langle X_{i'}, Y_j \rangle + \langle v_{i'}, w_0 \rangle + \epsilon_{i',j})^2$; it is the abundance of species $i$ if the covariates values of sample $j$ could be enforced to be equal to $w_0$. When estimating the effect of a binary covariate $w_{l,j} \in \{0,1\}$ on microbial compositions, we replace derivatives by differences:

$$\frac{\Delta P^j({\{Z_i\}})}{\Delta w_{l,j}} := P^j({\{Z_i\}}; w^1_{l,j}) - P^j({\{Z_i\}}; w^0_{l,j}),$$

(8)

here $w^1_{l,j}$ is identical to $w_j$ with the exception that the $l$-th component $w_{l,j}$ is set to be one and symmetrically $w^0_{l,j}$ is specified with $w_{l,j}$ equal to zero. Therefore $\Delta P^j({\{Z_i\}})/\Delta w_{l,j}$ is the variation of $P^j({\{Z_i\}})$ that one would observe by changing the value of a binary covariate.

We also consider the population-level associations between microbial compositions and a specific covariate, say the $l$-th covariate, when adjusting for all other covariates. To this end, we first define $\bar{P}({\{Z_i\}}; w_0)$, the population average abundance of species $i$ at a covariate value $w_0$, by

$$\bar{P}({\{Z_i\}}; w_0) := \frac{1}{J} \left( \sum_{j=1}^{J} P^j({\{Z_i\}}; w_0) \right),$$
which quantifies the average abundance of species $i$ when all $J$ samples in the study have the same hypothetical covariates values $\mathbf{w}_0$. We estimate $\mathbb{P}(\{Z_i\}; \mathbf{w}_0)$ from the data with $\mathbb{E}\left(\mathbb{P}(\{Z_i\}; \mathbf{w}_0) | \mathbf{n}, \mathbf{w}\right)$.

To illustrate the association between the abundance of species $i$ and the $l$-th covariate, we visualize the variation of $\mathbb{P}(\{Z_i\}; \mathbf{w}_0)$ as $w_{l,0}$ (the $l$th entry of $\mathbf{w}_0$) varies and all other covariates remain fixed at $\mathbf{w}_{-l,0}$. This visualization is obtained by plotting the estimated $\mathbb{P}(\{Z_i\}; \mathbf{w}_0)$ against $w_{l,0}$. We call the resulting curve the *population trend* of species $i$ with respect to the $l$-covariate at $\mathbf{w}_{-l,0}$. In Figure 3, bottom row, we illustrate population trends of three species with respect to the first covariate at $w_{2,0} = 0$ and at $w_{2,0} = 1$.

Interactions terms for pairs of covariates, and more generally functions of the covariates, can be included in the proposed model. We specify a function $f : \mathbb{R}^L \rightarrow \mathbb{R}^{L'}$ for interactions terms. One example is $f(\mathbf{w}_j) = w_{1,j}w_{2,j}$. The definition of $Q_{i,j}$ in (2) when interactions are incorporated becomes

$$Q_{i,j} = \langle \mathbf{X}_i, \mathbf{Y}_j \rangle + \langle \mathbf{v}_i, (\mathbf{w}_j, f(\mathbf{w}_j)) \rangle + \epsilon_{i,j},$$

where $\mathbf{v}_i \in \mathbb{R}^{L+L'}$. In these cases variations of the $l$-th coordinate of $\mathbf{w}_j$ affect $f(\mathbf{w}_j)$ and translate into compositional variations equal to $\partial P^i(\{Z_i\})/\partial w_{l,j}$ or $\Delta P^i(\{Z_i\})/\Delta w_{l,j}$.

### 4 Simulation study

In this section we focus on the model in Subsection 2.2 and illustrate that we can produce estimates of the parameters of interest. This verifies numerically our results on model identifiability. We also illustrate that we can transform the model parameters into interpretable results on the relationship between covariates and microbial compositions.

In our simulation study we included $I = 100$ species and $J = 300$ samples. The 300 samples are taken from $U = 50$ individuals (see Subsection 2.2.1). Each individual is measured six times. The read-depth of each sample is $n_j = 10^5$. We simulate $\sigma$ using independent Beta densities with mean 0.2 and variance 0.1. As we discussed in Subsection 2.1, $\sigma_i$ represents the average abundance of species $i$ across all samples. We included in the simulation a continuous covariate $w_{1,j}$, generated from independent $\mathcal{N}(0,1)$ distributions, and a binary covariate $w_{2,j}$, generated
Table 2: Specification of \( v \) in the simulation study.

| Species (\( i \)) | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | \ldots | 100 |
|-------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|\ldots |    |
| \( v_{1,i} \) (for \( w_{1,j} \)) | 5  | 5  | 5  | 5  | 5  | 5  | -5 | -5 | -5 | -5 | -5 | -5 | -5 | -5 | -5 | -5 | 0  | \ldots | 0   |
| \( v_{2,i} \) (for \( w_{2,j} \)) | 5  | 5  | 5  | -5 | -5 | -5 | 5  | 5  | 5  | -5 | -5 | -5 | -5 | 0  | \ldots | 0   |
| \( v_{3,i} \) (for \( w_{1,j} \cdot w_{2,j} \)) | 10 | -5 | -5 | -10| 10 | -5 | -5 | -10| -10| 5  | 5  | 10 | 10 | 0  | \ldots | 0   |

from independent Bernoulli(0.5). We also use the interaction term \( w_{1,j} \times w_{2,j} \) to specify scenarios where effects of \( w_{1,j} \) differ in the groups \( w_{2,j} = 0 \) and \( w_{2,j} = 1 \). We will later discuss in Section 5 this type of interaction in a microbiome study for Type I diabetes at early infancy.

For the latent factors \( Y \) we assumed \( Y_u \in \mathbb{R}^4 \). For the first half of the individuals, \( u = 1, \ldots, 25 \), we set \( Y_{3,u} = Y_{4,u} = 0 \) while for the other half, \( u = 26, \ldots, 50 \), we set symmetrically \( Y_{1,u} = Y_{2,u} = 0 \). The non-zero components in \( Y_u \) were simulated independently from a \( \mathcal{N}(0,1) \) density. This specification of \( Y \) makes the correlation matrix \( S \) block diagonal (see Figure 2(b)).

We simulate the first eight species with positive \( v_{1,i} \)'s and the following eight species (\( i = 9, \ldots, 16 \)) with negative \( v_{1,i} \)'s. As detailed in Table 2 the first 16 species abundances correlates with \( w_{2,j} \). Moreover, we make the assumption that some of the trends with respect to \( w_{1,j} \) are either amplified or reversed when we contrast the two groups \( w_{2,j} = 1 \) and \( w_{2,j} = 0 \). All other species (\( i > 16 \)) have the corresponding \( v_i \) coefficients equal to 0 (Table 2).

We further examine the robustness of our method by checking its performances when model (2) is misspecified. We apply our method to data simulated using the following specification of \( (P^j(\{Z_i\}); i \leq I, j \leq J) \),

\[
P^j(\{Z_i\}) = \frac{\sigma_i Q_{i,j}^+}{\sum_{i'} \sigma_{i'} Q_{i',j}^+}.
\]

The specification of \( \sigma, v, Y \) and \( w \) remains the same as described in the previous paragraphs.

4.1 Estimating species and sample parameters \( v \) and \( S \)

We first consider estimation of \( v \) and \( S \) between individuals when the model is correctly specified. Recall, from Proposition 1, \( v \) is identifiable when trace(\( \Sigma \)) is assumed fixed at a constant value. We assume trace(\( \Sigma \)) = 1 and
compute the posterior distribution of $v / \sqrt{\text{trace}(\Sigma)}$. The performance of the estimate of $S$ is measured by the RV-coefficient [Robert and Escoufier, 1976], which is bounded between zero and one, between the estimated $S$ and the actual value of $S$. Larger RV-coefficient indicates that the estimate is close to the actual $S$.

In Figure 2(a) we illustrate the estimates of $v_i$, $i = 1, \ldots, 16$, in one simulation. The posterior means of $v_i$ for the first 16 species are in general close to the corresponding actual values. One exception is species 16, whose average relative abundance is the lowest ($8.1 \times 10^{-5}$) among the first 16 species. In the left panel of Figure 2(b), we illustrate the posterior mean of $S$ between individuals in one simulation. The estimate is close to the actual value of $S$ with RV coefficient between them at 0.98, although the estimate indicates weak correlation between two independent subgroups (subject 1-25 and subject 26-50).

When the model is misspecified, the estimates of $v$ are not comparable to the corresponding actual values. However, this result does not discourage the application of model [2] when estimating effects of covariates on microbial compositions. The model can still capture the derivatives and population trends (see Subsection 4.2), which directly describe the covariates’ effects. The estimate of $S$, on the other hand, is only minimally affected by model misspecification and preserves its closeness to the actual value of $S$ (Figure 2(b), right panel). The RV coefficient between the estimate and the actual value of $S$ is 0.96 in this case.

We further repeat the simulation for 50 times under the correctly specified model as well as the misspecified model to verify the observed accuracy levels. We fix across all simulation replicates the values of $\sigma$. When the model is correctly specified, the mean square errors (MSEs) between the posterior means of scaled $v_i$ and the actual values across the 50 simulation replicates are comparable across species. The smallest average MSE across 50 replicates is $4.3 \times 10^{-6}$ for species 18 ($sd = 5.8 \times 10^{-6}$) and the largest average MSE is $5.2 \times 10^{-3}$ for species 14 ($sd = 2.3 \times 10^{-2}$). The RV coefficients between the posterior means of $S$ and the actual value of $S$ across 50 replicates are close to one, whether the model is correctly specified or not. When the model is correctly specified, the mean and the standard deviation of the RV-coefficients are 0.964 and 0.009. When the model is misspecified, the mean and the standard deviation are 0.960 and 0.012.
Figure 2: Estimates of $v_i$, $i = 1, \ldots, 16$, and $S$ between individuals. (a) Posterior distributions of $v_i/\sqrt{\text{trace}(\Sigma)}$, $i = 1, \ldots, 16$. The posterior distributions are visualized by boxplots. The corresponding actual values of $v_i/\sqrt{\text{trace}(\Sigma)}$ are indicated by dots. (b) Posterior mean of the correlation matrix $S$ between individuals (values above the main diagonal) compared to the truth (values below the main diagonal) in one simulation when the model is correctly specified (Left) and misspecified (Right).
4.2 Visualizing the relationship between covariates and microbial compositions

As we mentioned in Subsection 2.2, the values of $v$ do not directly express the sign and the magnitude of the covariates’ effects on microbial compositions. Recall for example that a positive $v_{l,i}$ might correspond to a decreasing trend with respect to the covariate $w_{l,j}$. This can happen when the $v_{l,i'}$ of another species $i'$ is larger than $v_{l,i}$. The goal of this Subsection is to evaluate if we can estimate responses of species abundances to variations of covariates of interest.

We consider the visualization approaches described in Subsection 3.2. We first focus on the estimates of the derivatives $\partial P^j(\{Z_i\})/\partial w_{l,j}$. These provide, at the individual level, estimates of the microbial abundance variations that would correspond to an infinitesimal increment of a specific covariate $w_{l,j}$, while the other covariates remain fixed. The results for three representative species are summarized in the top panels of Figure 3. The X-axes indicate the value of $w_{l,j}$ and the Y-axes the value of $\partial P^j(\{Z_i\})/\partial w_{l,j}$. Each point in these figures is the posterior mean of $\partial P^j(\{Z_i\})/\partial w_{l,j}$, and the associated error bar indicates the 95% credible interval. We also calculate the actual values of $\partial P^j(\{Z_i\})/\partial w_{l,j}$ using the $\sigma, X, Y$ and $v$ that generated the data. These actual values are marked with crosses in the figures. We link the size of the points and the crosses to the observed abundance of species.

We then focus on the population level estimates of covariates’ effects by visualizing the population trend of species $i$ with respect to a given covariate (see Subsection 3.2). Population trends of three representative species with respect to $w_{1,0}$ at different values of $w_{2,0}$ are summarized in Figure 3 bottom panels. The X-axes indicate the value of $w_{1,0}$ and the Y-axes the population average abundance $\bar{P}(\{Z_i\}; w_0)$. The shaded areas indicate the point-wise 95% credible bands of population trends.

From the top panels of Figure 3, we observe that the estimated derivatives of species abundances with respect to $w_{l,j}$ are accurate. The credible intervals cover the actual derivatives for most of the samples and tend to be wider for samples with smaller observed species abundance. The bottom panels of Figure 3 show that the posterior credible bands cover the actual population trends well for all three species.

When the model is misspecified, the comparisons of estimated derivatives and population trends to the truth are shown in Figure 4. When calculating the actual derivatives and population trends, we use the specification of
Figure 3: Posterior estimates of individual- and population-level relationship between $w_{1,j}$ and relative abundances when the model is correctly specified. (Left) Increasing trend for the group $w_{2,j} = 0$ and the group $w_{2,j} = 1$. (Middle) Increasing trend for the group $w_{2,j} = 0$ and non-monotone trend for the group $w_{2,j} = 1$. (Right) Decreasing trend for the group $w_{2,j} = 0$ and the group $w_{2,j} = 1$. We visualize the posterior distributions of derivatives (Top) and the posterior distribution of population trends (Bottom).

$P^j(\{Z_i\})$ in [9]. From the top panels of Figure 4, we observe that the estimates of derivatives capture the sign of the actual values correctly for most of the samples. However, the estimates are not as close to the actual values of the derivatives as in the case where the model is correctly specified. The magnitudes of the errors tend to be larger when $|w_{1,j}|$ is large, and the credible intervals in several cases do not cover the actual values. This result is expected as we erroneously assume that $P^j(\{Z_i\})$ depends on $Q_{i,j}^+$ instead of $Q_{i,j}$. Bottom panels of Figure 4 illustrate that the estimated population trends follow the actual trend closely but deviate when $|w_{1,j}|$ is large. The posterior credible bands do not cover the truth as in the previous example, where the model is correctly specified.

We repeat the simulation for 50 times under the correctly specified model as well as under the misspecified model. For each species $i$, we use MSE between the posterior mean of $(P^j(\{Z_i\}); j \leq J)$ and its actual value. In Supplementary Figure B.1 (top panel), we plot the distributions of MSEs across simulation replicates. This figure confirms the results in Figure 3 and Figure 4. The estimates of derivatives in the correctly specified model are closer to the truth compared to the estimates with the misspecified model. For both, correctly specified and misspecified
models, the mean \( \text{MSE} \) across replicates reaches its maximum for species 9, with value \( 8.7 \times 10^{-4} \) under the correctly specified model and \( 3.8 \times 10^{-2} \) under the misspecified model.

We then consider the estimates of population trends in the 50 replicates. In Supplementary Figure B.1 middle and bottom panels, we illustrate the estimated population trends in three species, when the model is correctly specified and misspecified. When the model is misspecified, the overall shape of each band still mirror the actual trend but fails to cover the actual trend in several intervals of \( w_{0,1} \).

The code for replicating the simulation studies are available from the Github repository [https://github.com/boyuren158/DirFactor-fix](https://github.com/boyuren158/DirFactor-fix). We also include the scripts in the Supplementary Materials.

### 5 Microbiome analyses for Type I diabetes in early infancy

We utilize the longitudinal model in Subsection 2.2.1 to evaluate associations between gut microbiome compositions, clinical variables and demographic characteristics of infants in the DIABIMMUNE project [Vatanen et al. (2016)](https://doi.org/10.1093/obq/obw061). The
DIABIMMUNE project collected longitudinal microbiome data in 157 infants over a period up to 1600 days after birth. Infants were enrolled from Finland, Estonia and Russia. Dietary information has been collected from each participant. The main goal of this project is to examine the relationship between Type I diabetes (T1D) associated autoantibody seropositivity (seroconverted), which is an indicator of T1D onset, and the infants’ gut microbiome. In this project, seven out of 157 infants are seroconverted.

The dataset contains a total of 55 microbial genera and 762 samples from 157 infants. A large collection of potential associations between relative abundances of microbial taxa and covariates has been previously discussed in (Vatanen et al., 2016). Among these associations, the most significant ones link nationality and age to 44 microbial genera. Due to moderate sample size, only limited evidence of variations of the microbiome profile associated with seroconversion has been reported.

We present analyses based on the proposed Bayesian model. The set of covariates is composed by nationality, age, seroconversion and the interaction between age and nationality. We want to verify consistency of our posterior inference with the results discussed in the literature. Additionally, we want to quantify the uncertainty of the estimated relationship between seroconversion and microbial compositions in human gut.

5.1 Estimating the effects of age

We estimated the effects of age on microbial compositions using the visualization approaches in Subsection 3.2. In the top panels of Figure 5 we illustrate the estimated derivatives of microbial abundances with respect to age, $\partial P^i(\{Z_i\})/\partial w_{3,j}$, for two genera, Bifidobacterium and Bacteroides. We only plot the $\partial P^i(\{Z_i\})/\partial w_{3,j}$’s for 150 randomly selected samples for visual clarity. We show the 95% credible intervals for derivatives with bars, and the sizes of points are proportional to the observed abundances.

In the bottom panels of Figure 5 we plot the estimated population trends of the same genera with respect to age. We consider the population trends for Estonian, Finnish and Russian infants and assume that the infants are not seroconverted. Posterior credible bands for the population trends are visualized by shaded areas. The observed abundances of Bifidobacterium and Bacteroides in all samples are illustrated by scatter plots together with the
estimated population trends.

Figure 5: (Top) Estimated \( \partial P_j(\{Z_i\})/\partial w_{3,j} \) for two genera. Each point represents a sample. Colors indicate nationalities and the sizes of points are proportional to the observed abundances. The error bars indicate 95\% credible intervals. We only plot 150 randomly selected samples. (Bottom) Estimated population trends of Bifidobacterium and Bacteroides for Estonian, Finnish and Russian infants. The infants are assumed to be nonseroconverted. Curves represent the estimated population trends and the shaded areas illustrate point-wise 95\% credible bands. Points indicate the observed abundance of Bifidobacterium or Bacteroides in all samples. We use colors to indicate nationalities.

The estimated derivatives with respect to age for Bifidobacterium are significantly smaller than zero in most of the samples, indicating that the abundances of Bifidobacterium in infants’ gut microbiome tend to decrease with age. This is to some extent expected, since bacteria from this genus are associated with breastfeeding [Fanaro et al., 2003]. The results on derivatives are consistent with the estimated population trends. In all three populations (Estonian, Finnish and Russian), Bifidobacterium is estimated to have a decreasing population trend with respect to age. The trends for Finnish and Estonian infants are similar, while for Russian infants the decrease is faster for infants that are less than 600 days old.
The association between genus Bacteroides and age is less pronounced. The derivatives of Bacteroides tend to be positive in samples taken before 300 days. When the infants get older the derivatives become slightly negative in Estonian and Finnish infants but remain positive in the Russian group. The population trends in this case are also consistent with the estimated derivatives. For nonseroconverted Estonian and Finnish infants, the estimated population abundances of Bifidobacterium increase with age when the infants are less than 450 days old and start to decrease slowly afterward. In Russian infants, the initial increasing trend is more pronounced with a narrower confidence band than the other two populations until 900 days. After 900 days, the population average abundance reaches a plateau and the credible band widens.

5.2 Estimating effects of nationalities and seroconversion

We make inference about the associations between the gut microbial compositions and nationalities using the differences $\Delta P^j(\{Z_i\})/\Delta w_{l,j}$ defined in (8). For each sample, we estimate $\Delta P^j(\{Z_i\})/\Delta w_{1,j}$, which is the difference associated to the change of nationality from Finland (FIN) to Estonia (EST), as well as $\Delta P^j(\{Z_i\})/\Delta w_{2,j}$, the difference associated to the change from Finland (FIN) to Russia (RUS). We consider the averages of $\Delta P^j(\{Z_i\})/\Delta w_{1,j}$ and $\Delta P^j(\{Z_i\})/\Delta w_{2,j}$ in each of five consecutive age groups. The posterior distributions of these population averages (Figure 6) illustrate the effect of nationality. In both panels of Figure 6, the X-axis identifies age groups and the Y-axis indicates the value of $\Delta P^j(\{Z_i\})/\Delta w_{1,j}$ and $\Delta P^j(\{Z_i\})/\Delta w_{2,j}$. Each box-plot approximates, using posterior simulations, the posterior distribution of the average $\Delta P^j(\{Z_i\})/\Delta w_{l,j}, l = 1, 2$. These averages are defined by integration within a specific age group.

There is an increase of Bifidobacterium abundance when we compare FIN to RUS nationalities. This increase diminishes with age. In the last age group (670-1160 days), the posterior distribution of $\Delta P^j(\{Z_i\})/\Delta w_{2,j}$ indicates that the abundances of Bifidobacterium in samples collected from infants older than 670 days remain comparable across nationalities. In the second comparison of nationalities, FIN to EST, only minor changes of Bifidobacterium abundance levels are observed.

We also explored associations between microbial compositions and seroconversion status. In this case we
again examine the posterior distributions of average \( \Delta P^j(\{Z_i\})/\Delta w_{1,j} \) in five consecutive age groups. We do not find evidence in our analyses of any genus associated to seroconversion, due to high uncertainty of the estimated average \( \Delta P^j(\{Z_i\})/\Delta w_{4,j} \) in all age groups.

### 5.3 Similarities of microbial genera

In this subsection, we focus on similarities between microbial genera. We first consider the simple approach where the similarity of two genera is measured by the correlation between their observed relative abundances across all samples. The result of this approach is a correlation matrix, denoted by \( S_{\text{raw}} = (S_{\text{raw}}(i, i'); i, i' \leq I) \), where \( S_{\text{raw}}(i, i') = \text{cor}[(n_{i,j}/n_j; j \leq J), (n_{i',j}/n_j; j \leq J)] \). We then consider two approaches which utilize the proposed model. The first approach uses the cosine of the angle between \( \mathbf{v}_i \) and \( \mathbf{v}_{i'} \) to quantify the similarity of genera \( i \) and \( i' \), whereas the second approach uses the cosine of the angle between \( \mathbf{X}_i \) and \( \mathbf{X}_{i'} \). The results of these two approaches are normalized Gram matrices, denoted as \( S_{\mathbf{v}} \) and \( S_{\mathbf{X}} \) respectively. In the top panels of Figure 7 we illustrate the estimates of \( S_{\text{raw}}, S_{\mathbf{v}} \) and \( S_{\mathbf{X}} \) by heat-maps. Each row or column of the heat-map represents a specific genus and the color of each tile represents the estimated similarity of two genera.

We then focus on examining the concordance of \( S_{\text{raw}}, S_{\mathbf{v}} \) and \( S_{\mathbf{X}} \) to the phylogenetic relations of the observed
genera. To this end, we compare the phylogenetic tree of the observed genera published in Segata et al. (2013) to the heat-maps. If an estimated correlation matrix indicates clusters of genera that share similarities with the phylogenetic tree, then we conclude that the estimate is consistent with phylogenetic relations.

From the figures we can find that $S_{raw}$ indicates little between-genera similarity and does not recover phylogenetic relations of the observed genera. On the other hand, both $S_X$ and $S_V$ indicate clusters of genera that are consistent with the phylogenetic tree. For instance, the cluster in the middle of the heat-map of $S_X$ and $S_V$ corresponds to 13 genera from phylum Firmicutes (Clostridium, Ruminococcus, etc). These results suggest that both $S_X$ and $S_V$ capture the phylogenetic relations of the observed genera. The ordination plot of genera based on $S_X$ in the bottom panel of Figure 7 further confirms this conclusion. We generate the ordination plot using the method in Ren et al. (2016), which represents each genus by a region instead of a single point. In the ordination plot we find that genera from the same cluster in $S_X$ or $S_V$ are close to each other.

We also verify quantitatively the consistency of $S_X$ and $S_V$ to the phylogenetic relations. We first calculate the pair-wise phylogenetic distance matrix of the observed genera using unweighted-Unifrac dissimilarity (Lozupone et al. 2011). We then convert this distance matrix into a normalized Gram matrix $S_{unifrac}$ by Torgerson Classical Scaling (Borg and Groenen 2005) and compare $S_{unifrac}$ to $S_{raw}$, $S_X$ and $S_V$. The estimated $S_X$ and $S_V$ are both similar to $S_{unifrac}$ with RV-coefficients 0.66 and 0.76 respectively, while the RV-coefficient between $S_{raw}$ and $S_{unifrac}$ is 0.32.

6 Discussion

We proposed a Bayesian mixed effects regression model to perform multivariate analyses for microbiome data. This regression analysis estimates the effects of covariates on microbial composition while allowing for correlations of the residuals. We illustrate that the model parameters are identifiable. This result is consistent with our simulation study. The model allows to infer the relationship between covariates and microbial compositions with two visualization approaches. In simulations we showed that both the individual-level and the population-level relationships between covariates and microbial compositions can be accurately estimated. We finally applied the model to a longitudinal
Figure 7: Estimated similarities of genera. (Top) Estimates of $S_X$, $S_v$ and $S_{raw}$. Each row or column in the heat-maps correspond to a specific genus. The color of each entry is determined by the estimated pair-wise similarity. The rows and columns in heat-map are reordered so that adjacent rows or columns correspond to genera that are close phylogenetically. The phylogenetic tree for these genera are plotted at the right side of the figure. (Bottom) Ordination of genera based on $S_X$. The contour lines indicate uncertainty regions in the ordination configuration. The contour line of a genus is colored accordingly to the phylum of the genus.
microbiome dataset and compared our results with those previously reported in the literature.

The current posterior computation is implemented with a Gibbs-sampler. This can be inefficient when the number of parameters is large. The computation time increases approximately linearly with the number of samples and, similarly, with the number of microbial species. For the longitudinal microbial dataset that we analyzed the computation time of one chain with $10^5$ iterations is around 90 minutes. A possible substantial improvement in computation time can probably be obtained with Hamiltonian Monte Carlo or variational Bayes methods.

In the future we would also like to investigate appropriate variable selection techniques for the fixed effects. This is particularly helpful in settings with large collections of covariates. A more flexible model for the fixed effects is also desirable. Currently, the relationship between abundances and covariates are depicted by linear functions of the samples characteristics, possibly augmented by pre-specified transformations of the covariates. Finally, the current prior specification ignores potential relationship across regression vectors $v_i$ associated to similar microbial species. A systematic way to incorporate such information would involve the specification of a prior distribution on $v$ that mirrors the phylogeny of microbial species.

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A Proof of Proposition 1

We first show that for parameter values \((\sigma, Y, v)\) and \((\sigma', Y', v')\), the equality, under the two sets of parameters, of the joint distribution of \([P_j({\{Z_i\}}); i \geq 1, j \leq J], w\)] implies

\[
\frac{\sigma_i}{\sigma_{i'}} = \frac{\sigma'_i}{\sigma'_{i'}}, i \neq i', \quad S = S', \quad v_i = v'_i, i \geq 1.
\] (10)

1. Denote \(\bar{P}_j({\{Z_i\}}) = \mathbb{I}(P_j({\{Z_i\}}) > 0),
\[
p(\bar{P}_j({\{Z_i\}})|\sigma, v, w_j, Y) f(w_j) = \Phi((1 - 2\bar{P}_j({\{Z_i\}}))v_i^Tw_j/\sqrt{\Sigma_{j,j}}) f(w_j),
\]
where \(\Phi\) is the CDF of the standard normal distribution and \(f(w_j)\) is the density of \(w_j\). If the joint distribution of \([P_j({\{Z_i\}}); i \geq 1, j \leq J], w\)] is identical under the two sets of parameters,

\[
[(P_j({\{Z_i\}}); i \geq 1, j \leq J], w) \mid v, \sigma, Y \overset{d}{=} [(P_j({\{Z_i\}}); i \geq 1, j \leq J], w) \mid v', \sigma', Y',
\] (11)

it follows that \(v_i^Tw_j\Sigma_{j,j}^{-1/2} = (v'_i)^Tw_j(S'_{j,j})^{-1/2}\) almost surely. With the assumption that \(E(w_jw_j^T)\) is of full rank, we get \(v_i\Sigma_{j,j}^{-1/2} = v'_i(S'_{j,j})^{-1/2}\) for \(i \geq 1\) and \(j = 1, \ldots, J\).

If \(v = 0\), it is straightforward to verify that \([11]\) implies \(v = v'\). If \(v_i \neq 0\), since we know that for any \(j \neq j'\),

\[
v_i\Sigma_{j,j}^{-1/2} = v'_i(S'_{j,j})^{-1/2}, \quad v_i\Sigma_{j',j'}^{-1/2} = v'_i(S'_{j',j'})^{-1/2},
\]

we have \(\Sigma_{j,j}/\Sigma_{j',j'} = (\Sigma'_{j,j})/(\Sigma'_{j',j'})\). This equality, combined with the assumption that \(\text{trace}(\Sigma) = \text{trace}(\Sigma')\), implies that \(\Sigma_{j,j} = \Sigma'_{j,j}\) for all \(j \leq J\), and therefore \(v_i = v'_i\) for all \(i \geq 1\) if \([11]\) holds.

2. We then prove that \([11]\) implies \(S = S'\). We write

\[
f(w_j)f(w_j') p(\bar{P}_j({\{Z_i\}}), \bar{P}_j'({\{Z_i\}})|w, \sigma, v, Y) = 
f(w_j)f(w_j') \int \frac{1}{2\pi} (1 - S_{j,j}^2)^{-1/2} \exp \left(-\frac{1}{2}q^T S_{j,j}^{-1} q\right) dq,
\] (12)
where \( \mathcal{A} = \mathcal{A}_j \times \mathcal{A}_{j'} \). \( \mathcal{A}_j = (-\infty, v_i^\top w_j \Sigma^{-1/2}_{j,j}] \) if \( \tilde{P}^j(\{Z_i\}) = 0 \) and \( \mathcal{A}_j = [v_i^\top w_j \Sigma^{-1/2}_{j,j}, \infty) \) if \( \tilde{P}^j(\{Z_i\}) = 1 \). Using Corollary 3.12 in [Ledoux and Talagrand (2013)], we know that the probability in (12) is monotone with respect to \( S_{j,j'} \). Based on the previous paragraphs, \( \mathcal{A} = \mathcal{A}' \) for every \( w \). Therefore (11) implies that \( S_{j,j'} = S'_{j,j'} \) for all \( j, j' \leq J \). When \( v \neq 0 \), we further get \( \Sigma = \Sigma' \).

3. Finally, we prove that (11) implies \( \sigma_i / \sigma_{i'} = \sigma'_i / \sigma'_{i'} \) for all \( i \neq i' \). By construction,

\[
\frac{P^j(\{Z_i\})}{P^j(\{Z_{i'}\})} = \frac{\sigma_i Q_{i,j}^{+2}}{\sigma_{i'} Q_{i',j}^{+2}}.
\]

We use the convention that the ratio is zero whenever the denominator is zero. By combining (11) and the previous paragraphs, the joint distribution of \( (Q_{i,j}^{+2} / Q_{i',j}^{+2}, w_j) \) remains the same when the parameters values change from \( (\sigma, v, Y) \) to \( (\sigma', v', Y') \). This directly implies that \( \sigma_i / \sigma_{i'} = \sigma'_i / \sigma'_{i'} \) for all \( i \neq i' \) if (11) holds.

If (10) does not hold, then the joint distribution of \( [(P^j(\{Z_i\}); i \geq 1, j \leq J), w] \) is different under the two sets of parameters. By de Finetti’s theorem [Hewitt and Savage (1955)], the joint distribution of the observable variables \( (n_{i,j}; i \geq 1, j \leq J) \) and \( w \) is uniquely determined by the mixing distribution, which in our case is the law of \( (P^j(\{Z_i\}); i \geq 1, j \leq J)|w, v, \sigma, Y, \) and vice versa. This fact completes the proof.

B Derivatives and population trends in 50 simulation replicates
Figure B.1: Summary of the estimates of derivatives and population trends in 50 simulation replicates. (Top) We consider the distributions of MSEs of the estimated derivatives for the first 16 species, when the model is correctly specified and misspecified. (Middle) The bands which include 94% of the simulation-specific estimates of the population trends along with the actual trends when the model is correctly specified. (Bottom) The same figures of population trends for the misspecified model.