Log-Contrast Regression with Functional Compositional Predictors: Linking Preterm Infant’s Gut Microbiome Trajectories in Early Postnatal Period to Neurobehavioral Outcome

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Abstract

When compositional data serve as predictors in regression, the log-contrast model is commonly applied. A prominent feature of the model is that it complies with the simplex geometry and enables the regression analysis to have various desirable invariance properties. Motivated by the needs in understanding how the trajectories of gut microbiome compositions during early postnatal stage impact later neurobehavioral outcomes among preterm infants, we develop a sparse log-contrast regression with functional compositional predictors. The functional simplex structure is preserved by a set of zero-sum constraints on the parameters, and the compositional predictors are allowed to have sparse, smoothly varying, and accumulating effects on the outcome through time. Through basis expansion, the problem boils down to a linearly constrained group lasso regression, for which we develop an efficient augmented Lagrangian algorithm and obtain theoretical performance guarantees. The proposed approach yields interesting results in the preterm infant study. The identified microbiome markers and the estimated time dynamics of their impact on the neurobehavioral outcome shed lights on the functional linkage between stress accumulation in early postnatal stage and neurodevelopmental process of infants.

Keywords: constrained optimization; functional data; group lasso; longitudinal data; variable selection.

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1 Introduction

Over the past decade, advances in neonatal care have contributed to a dramatic increase in survival among very preterm birth infants (born before 32 weeks’ gestation) from 15% to over 90% (Fanaroff et al., 2003; Stoll et al., 2010). With this cheerful gain in survival, recent research has shifted focus to the investigation of the increase in neurological morbidity and long-term adverse outcomes related to immature neuro-immune systems and stressful early life experience (Mwaniki et al., 2012). In particular, the neonatal intensive care unit (NICU) experience is found to be one of the most crucial factors that drive preterm infant neurodevelopmental and health outcomes. Accumulated infant stress at NICU arises from numerous causes, such as repeated painful procedures, daily clustered care, maternal separation, among others. Mwaniki et al. (2012) showed that these neonatal insults were associated with a much alleviated risk of long-term neurological morbidity, e.g., 39.4% of NICU survivors had at least one neurodevelopmental deficit. However, the onset of the altered neuro-immune progress induced by infant stress/pain is often insidious, and the mechanism of this association, which holds the key for reducing costly health consequences of prematurity, remain largely unclear. On the other hand, expanding research evidence supports that a functional communication exists between the central nervous system and gastrointestinal tract, the brain-gut axis, in which the gut microbiome plays a key role in early programming and later responsivity of the stress system (Dinan and Cryan, 2012).

As such, a central hypothesis is that the stressful early life experience of very preterm neonates is imprinting gut microbiome by the regulation of the brain-gut axis, and consequently, certain microbiome markers are predictive of later infant neurodevelopment. To investigate, a study was conducted in a NICU in the northeast of the U.S., where stable preterm infants were recruited. Infant fecal samples were collected daily when available, during the infant’s first month of postnatal age. Bacterial DNA were isolated and extracted
from each stool sample, and through sequencing and processing, resulted in microbiome compositional data (Bomar et al., 2011; Caporaso et al., 2012; Cong et al., 2017). Gender, delivery type, birth weight, feeding type, among others, were also recorded for each infant. Infant neurobehavioral outcomes were measured when the infant reached 36–38 weeks of post-menstrual age, using the NICU Network Neurobehavioral Scale (NNNS). More details on the study and the data are provided in Section 6. The above scientific hypothesis can then be approached through a statistical analysis, by examining how the microbiome compositions collected over the early postnatal period predict or impact on the later NNNS score, after adjusting for the effects of relevant infant characteristics.

Compositional data analysis is not an unfamiliar territory to statisticians. Data consisting of percentages or proportions of certain composition are commonly encountered in various scientific fields including ecology, biology and geology. One unique attribute of compositional data is the unit-sum constraint, i.e., the components of a composition are non-negative and always sum up to one; this entails that the data live in a simplex and thus renders many statistical methods that comply with Euclidean geometry inapplicable. Much foundational work on the statistical treatment of compositional data was done by John Aitchison (Aitchison, 1982; Aitchison and Bacon-Shone, 1984; Aitchison, 1986); see Aitchison (2003) for a thorough survey on the subject. Of particular interest to us is regression with compositional predictors, for which the log-contrast models have been very popular. A prominent feature of the model is that it enables the regression analysis to obey the so-called principle of subcompositional coherence, i.e., the compositional data should be analyzed in a way that the same results can be obtained regardless of whether we analyze the entire composition or only a subcomposition. Recently, Lin et al. (2014) studied a sparse linear regression model with compositional covariates, extending the log-contrast model to high dimensions. The problem was nicely formulated as a constrained lasso regression (Tib-shirani, 1996), with a zero-sum linear constraint on the regression coefficients. Shi et al.
(2016) further extended the sparse regression model to the case of multiple linear constraints for the analysis of microbiome subcompositions, and a de-biased procedure was adopted to obtain an asymptotically unbiased estimator of the regression coefficients and its asymptotic distribution. However, to our knowledge, regression method on handling high-dimensional compositional trajectories or series is still lacking.

Motivated by the needs in identifying potential microbiome markers and estimating how the trajectories of microbiome compositions along early postnatal stage impact later neurobehavioral outcome, we propose a *sparse log-contrast regression model with functional compositional predictors*. In our approach detailed in Section 2, the compositional predictors are allowed to have smoothly varying, accumulating effects on the outcome through certain continuous domain, e.g., time. A component of the composition is deemed irrelevant when it does not impact the outcome at all throughout the time window, i.e., its corresponding coefficient curve is a zero line. Sparsity-inducing regularized estimation is thus adopted as it is expected that only a few compositional components are associated with the outcome while most of them are irrelevant or having negligible effects. Through a simple yet effective basis expansion step, the proposed setup reduces to a linearly-constrained group lasso regression. For conducting regularized estimation, an augmented Lagrangian algorithm is developed in Section 3. The oracle properties of the resulting sparse estimator of the regression coefficients are established in Section 4. In Section 5 extensive simulation studies showcase the superior performance of the proposed approach over several competing methods. The data analysis of the preterm infant study is presented in Section 6. The identified microbiome markers are justifiable based on existing literature, and further, the estimated dynamic trajectories of their impact on the outcome shed new lights on the functional linkage between the accumulation of prenatal stress and neurodevelopment of infants. Some concluding remarks are provided in Section 7.
2 Log-Contrast Regression with Functional Compositional Predictors

2.1 Linear Log-Contrast Model

Suppose we observed \( n \) independent observations of a response variable \( y_i \in \mathbb{R} \) and a compositional predictor \( x_i = [x_{i1}, \ldots, x_{ip}]^T \) such that \( x_i \in \mathbb{S}^{p-1} = \{[x_1, \ldots, x_p]^T \in \mathbb{R}^p; x_j > 0, \sum_{j=1}^p x_j = 1\} \). Here we use \( \mathbb{S}^{p-1} \) to denote the \((p-1)\)-dimensional positive simplex lying in \( \mathbb{R}^n \). Denote \( \mathbf{y} = [y_1, \ldots, y_n]^T \in \mathbb{R}^n \) as the response vector and \( \mathbf{X} = [\mathbf{x}_1, \ldots, \mathbf{x}_n]^T \in \mathbb{R}^{n \times p} \) as the compositional predictor matrix.

It is apparent that ignoring the simplex structure of \( \mathbf{X} \) would lead to parameter identifiability issue in the linear regression of \( \mathbf{y} \) on \( \mathbf{X} \). One naive “remedy” is to exclude an arbitrary component of the compositional vector in the regression, which, however, leads to a method that is not invariant to the choice of the removed component and consequently makes proper model interpretation and inference difficult. Ever since the pioneer work by John Aitchison \( \text{[Aitchison, 1982; Aitchison and Bacon-Shone, 1984; Aitchison, 1986]} \) on the statistical treatments of compositional data, the so-called log-contrast model has gained much popularity in a variety of regression problems with compositional predictors. The main idea is to perform a log-ratio transformation of the compositional data, such that the transformed data admit the familiar Euclidean geometry in \( \mathbb{R}^{p-1} \). Specifically, for each \( i = 1, \ldots, n \), let \( z_{ij} = \log(x_{ij}/x_{ir}) \), where \( r \in \{1, \ldots, p\} \) is a chosen reference level, and \( j = 1, \ldots, r-1, r+1, \ldots, p \), resulting in \( \tilde{\mathbf{Z}}_r = [\tilde{z}_{ij}] \in \mathbb{R}^{n \times (p-1)} \). The linear log-contrast regression model is then expressed as

\[
\mathbf{y} = \beta_0^* \mathbf{1}_n + \tilde{\mathbf{Z}}_r \beta_r^* + \mathbf{e},
\]

where \( \beta_0^* \) is the intercept, \( \beta_r^* \in \mathbb{R}^{p-1} \) is a subvector of a regression coefficient vector \( \beta^* \in \mathbb{R}^p \)
by removing its $r$th component $\beta_r^*$, and $e \in \mathbb{R}^n$ is the random error vector with zero mean. Interestingly, although it appears that the model in (1) depends on the choice of the reference level, it in fact admits a symmetric form. To see this, let $z_{ij} = \log(x_{ij})$ and $Z = [z_{ij}] \in \mathbb{R}^{n \times p}$. Then model (1) can be equivalently expressed as

$$ y = \beta_0^*1_n + Z\beta^* + e, \quad \text{s.t.} \quad \sum_{j=1}^p \beta_j^* = 0. \quad (2) $$

Consequently, in classical regression setups, the least squares estimation under model (1) is equivalent to the constrained least squares estimation under model (2). However, in high dimensional scenarios, i.e., when $p$ is much larger than $n$, the two model formulations could lead to discrepancies in regularized estimation. For example, the two corresponding lasso criteria (Tibshirani, 1996) are no longer equivalent:

$$ \min_{\beta_0, \beta} \left\{ \frac{1}{2n} \| y - \beta_0 1_n - Z\beta \|_2^2 + \lambda \| \beta \|_1 \right\}, \quad (3) $$

$$ \min_{\beta_0, \beta} \left\{ \frac{1}{2n} \| y - \beta_0 1_n - Z\beta \|_2^2 + \lambda \| \beta \|_1 \right\}, \quad \text{s.t.} \quad \sum_{j=1}^p \beta_j = 0, \quad (4) $$

where $\| \cdot \|, \| \cdot \|_1$ denote the $\ell_2, \ell_1$ norms, respectively, and $\lambda$ is a tuning parameter controlling the amount of regularization. Although (3) is simpler to compute, clearly its solution and hence its variable selection depend on the choice of the reference component. In contrast, (4) remains to be symmetric in all the $p$ compositional components. Lin et al. (2014) proposed and studied (4) and showed that the estimator admits many desirable properties (Aitchison, 1986).
2.2 Sparse Functional Log-Contrast Regression

In many applications including the preterm infant study, the compositional predictors are observed over a continuous domain, e.g., time, and thus they should be treated as functional compositional data. Still let $\mathbf{y} = [y_1, \ldots, y_n]^T \in \mathbb{R}^n$ be the observed outcome/response vector, which is time invariant. For any $t \in \mathbb{T}$, let $\mathbf{x}_i(t) \in \mathbb{S}^{p-1}$ be the compositional vector for the $i$th subject; let $\mathbf{X}(t) = [\mathbf{x}_1(t), \ldots, \mathbf{x}_n(t)]^T \in \mathbb{R}^{n \times p}$ be the matrix of the functional predictors at $t$. Here to focus on the main idea, we assume $\mathbf{X}(t)$ is completely observed for $t \in \mathbb{T}$, and the discussion about handling discrete time data is deferred to Section 3.2. Similar as in Section 2.1, we define $\mathbf{r}_Z(t) \in \mathbb{R}^{n \times (p-1)}$, for $r = 1, \ldots, p$, and $\mathbf{Z}(t) = \log(\mathbf{X}(t)) \in \mathbb{R}^{n \times p}$.

Some time-invariant control variables may also be available, e.g., gender, delivery type, among others, which form $\mathbf{Z}_c \in \mathbb{R}^{n \times p_c}$.

Motivated by model (2), we propose a functional log-contrast regression model,

$$
\mathbf{y} = \beta_0^* \mathbf{1}_n + \mathbf{Z}_c \beta_c^* + \int_{t \in \mathbb{T}} \mathbf{Z}(t) \beta^*(t) dt + \mathbf{e}, \quad \text{s.t.} \quad \mathbf{1}_p^T \beta^*(t) = 0, t \in \mathbb{T}, \quad (5)
$$

where $\beta_0^*$ is the intercept, $\beta_c^*$ is the regression coefficient vector corresponding to the control variables, $\beta^*(t) = [\beta_1^*(t), \ldots, \beta_p^*(t)]^T \in \mathbb{R}^p$ is the functional regression coefficient vector as a function of $t$, and the remaining terms are defined the same as in model (2). The proposed model allows the compositional predictors to have potentially different effects on the response through $\beta^*(t)$, and their aggregated effects on the response is then given by the integral of $\mathbf{Z}(t)$ weighted by $\beta^*(t)$ over time. Following Lin et al. (2014), here we adopt the symmetric form of the log-contrast model, in which the zero-sum constraints preserve the simplex structure over time while all the compositional components are treated on an equal footing.

The merit of the above model lies in imposing some meaningful low-dimensional structures or constraints on the coefficient curves $\beta^*(t)$. Motivated by the preterm infant study,
we consider both sparsity and smoothness of $\beta^*(t)$. First, we assume the true coefficient curves are sparse:

**Assumption 1.**

\[ s^* = |S| \ll p, \quad S = \{ j; \beta_j^*(t) \neq 0, t \in T, j = 1, \ldots, p \}. \]

This assumption is the basis of component selection and is widely applicable, because in many applications only a few compositional components are relevant to the prediction of the outcome, especially when $p$, the number of compositional components, is large. Second, we assume the coefficient curves are smooth, and adopt a truncated basis expansion approach (Ramsay and Silverman, 2005) to bring the infinite dimensional problem to finite dimensions:

**Assumption 2.**

\[ \beta^*(t) = B^* \Phi(t), \]

where $B^* = [b_1^T, \ldots, b_p^T]^T \in \mathbb{R}^{p \times k}$ is a fixed but unknown coefficient matrix, and $\Phi(t) = [\phi_1(t), \ldots, \phi_k(t)]^T \in \mathbb{R}^k$ consists of a set of known basis functions with $J_{\phi\phi} = \int_{t \in T} \Phi(t)\Phi^T(t)dt$ being a positive definite (p.d.) matrix.

Assumption 2 is motivated by the belief that the effects of gut microbiome compositions on preterm infant’s neurodevelopment evolves gradually over the postnatal period. Some discussions on the basis functions are in order. Here for simplicity the same set of basis functions is used in the expansion of each $\beta_j(t)$, $j = 1, \ldots, p$, which usually suffices in practice, and the extension to use different basis for different $\beta_j(t)$ is straightforward. There are many choices of the basis functions, e.g., Fourier basis, wavelet basis, and spline basis; see Ramsay and Silverman (2005) for a detailed account on the truncated basis expansion approaches in functional regression. In classical least squares types of estimation, the choice
of \( k \) usually boils down to a bias-and-variance tradeoff. That is, while larger values of \( k \) can lead to a better in-sample estimation at the risk of potential overfitting, smaller values of \( k \) result in simpler estimators at the expense of missing interesting local oscillations. The issue can be resolved by echoing regularization, i.e., taking a relatively large \( k \) to ensure the flexibility of the model and performing regularized coefficient estimation to avoid overfitting. Henceforth we treat \( k \) as a fixed and known quantity in the derivation of the proposed methodology.

The proposed model in (5) is simplified under Assumptions 1–2. The functional sparsity in \( \beta^*(t) \) now amounts to the row-sparsity of the coefficient matrix \( B^* \). The zero-sum constraint on \( \beta^*(t) \), i.e., \( 1_p^T \beta^*(t) = 0 \) for all \( t \in T \), is now equivalent to \( B^*^T 1_p = 0 \). To see this, note that \( 1_p^T \beta^*(t) = 0 \) leads to

\[
\int_{t \in T} Z(t) 1_p^T \Phi(t) 1_p^T (1_p^T B^*)^T dt = 1_p^T B^* (1_p^T B^*)^T = 0 \text{; it follows that } B^*^T 1_p = 0 \text{ as } J_{\phi\phi} \text{ is p.d.. (The other direction holds trivially.)}
\]

Further, the integral part in the model becomes

\[
\int_{t \in T} Z(t) \beta^*(t) dt = \int_{t \in T} Z(t) B^* \Phi(t) dt = \left\{ \int_{t \in T} Z(t) (I_p \otimes \Phi(t)) dt \right\} \text{vec}(B^*)^T = Z \beta^*,
\]

where we define \( \beta^* = \text{vec}(B^*)^T = [\beta_1^T, \ldots, \beta_p^T]^T \in \mathbb{R}^{pk} \) and

\[
Z = \int_{t \in T} Z(t) (I_p \otimes \Phi(t)) dt = [Z_1, \ldots, Z_p] \in \mathbb{R}^{n \times (pk)}.
\] (6)

Each \( \beta_j^* \in \mathbb{R}^k \) and \( Z_j \in \mathbb{R}^{n \times k} \) correspond to the coefficient vector and the covariate matrix for the \( j \)th compositional component, respectively. We remark that in practice \( Z \) is usually not exactly computed since \( Z(t) \) may not be fully observed; we defer the discussion to Section 3.2.
From the above derivation, the functional model in (5) becomes a constrained sparse linear regression model
\[ y = \beta_0^* 1_n + Z_c \beta_c^* + Z \beta^* + e, \quad \text{s.t.} \quad \sum_{j=1}^p \beta_j^* = 0, \quad (7) \]
where \( \beta^* \) is expected to be sparse accordingly to the row-sparsity of \( B^* \). To enable the selection of the compositional components, we therefore propose to conduct model estimation by minimizing a linearly constrained group lasso criterion (Yuan and Lin, 2006),
\[
\min_{\beta_0, \beta_c, \beta} \left\{ \frac{1}{2n} \| y - \beta_0 1_n - Z_c \beta_c - Z \beta \|^2 + \lambda \sum_{j=1}^p \| \beta_j \| \right\}, \quad \text{s.t.} \quad \sum_{j=1}^p \beta_j = 0, \quad (8)
\]
where \( \lambda \) is a tuning parameter controlling the amount of regularization.

The proposed estimator possesses several desirable invariance properties (Aitchison, 1986; Lin et al., 2014):

- **Scale invariance:** the estimator is invariant to the transformation \( X(t) \to SX(t) \) where \( S = \text{diag}(s) \) is a diagonal matrix with diagonal elements \( s = [s_1, \ldots, s_n]^T \) and all \( s_i > 0 \). That is, it does not matter whether the data vectors are scaled to have a unit sum; the method only cares about the relative proportions. This is simply because \( Z(t)\beta(t) = \{\log(X(t)) + \log(s) 1_n^T\}\beta(t) = \log(X(t))\beta(t) \), due to the zero-sum constraints. In fact, this scale invariance continues to hold when the scaling factor \( s \) changes in time.

- **Permutation invariance:** results of the analysis do not depend on the sequence by which the components are given or labeled.

- **Subcomposition coherence:** if we know in advance that some \( \beta_j(t) \) curves are zero, the analysis is unchanged if we apply the procedure to the subcompositions formed by
the components of $X(t)$ corresponding to the other $\beta_j(t)$ curves. To see this, suppose $\beta_j(t) \equiv 0$ for $j \in S^c$, where $S^c$ is the complement of a set $S$ on $\{1, \ldots, p\}$. Let $s(t) = \{X_S(t)1_{|S|}\}^{-1} \in \mathbb{R}^n$ be a scaling factor in which the inversion is entrywisely applied, so that $\text{diag}(s(t))X_S(t)$ gives the subcompositions formed by the components in $S$. Then we have

$$\log(X(t))\beta(t) = \log(X_S(t))\beta_S(t)$$
$$= \{\log(X_S(t)) + \log(s(t))1_{|S|}^T\}\beta_S(t)$$
$$= \log(\text{diag}(s(t))X_S(t))\beta_S(t).$$

In particular, when there are only two non-zero components, e.g., $\beta_1(t) \neq 0$, $\beta_2(t) \neq 0$ and $\beta_j(t) = 0$ for $j = 3, \ldots, p$, it is necessarily true that $\beta_1(t) = -\beta_2(t)$ due to the zero-sum constraint. We point out that this is neither an unpleasant artifact nor a limitation of the proposed method. This special case can be understood from the above property of subcomposition coherence: the analysis becomes the same as using the subcompositions formed from the first two components of $X(t)$; consequently, the two possible log-ratios are exactly opposite to each other, so do their corresponding coefficient curves. Therefore, this feature is consistent with the simplex structure of the data, since in any two-part compositional data, either part carries exactly the same amount of information.
\section{Computation}

\subsection{Solving Constrained Group Lasso}

To present the computational algorithm, we consider a criterion that is slightly more general than (8),

$$\min_{\beta_0, \beta_c, \beta} \left\{ \frac{1}{2n} \| y - \beta_0 \mathbf{1}_n - Z_c \beta_c - Z \beta \|^2 + \lambda \sum_{j=1}^p \| W_j \beta_j \| \right\}, \quad \text{s.t.} \quad \sum_{j=1}^p A_j \beta_j = b, \quad (9)$$

where each $W_j \in \mathbb{R}^{k \times k}$ is invertable, e.g., a diagonal matrix with positive diagonal elements, and the linear constraints, with choices of conformable $A_j$s and $b$, remain feasible, i.e., \{\beta; \sum_{j=1}^n A_j \beta_j = b\} \neq \emptyset. The problem is convex and can be solved by an augmented Lagrangian algorithm (Boyd \textit{et al.}, 2011).

To derive the algorithm, we first construct the scaled augmented Lagrangian function

$$L(\beta_{0c}, \beta; \alpha, \mu) = \frac{1}{2n} \| y - \beta_0 \mathbf{1}_n - Z_c \beta_c - Z \beta \|^2 + \frac{\mu}{2} \| \sum_{j=1}^p A_j \beta_j - b + \alpha \|^2 + \lambda \sum_{j=1}^p \| W_j \beta_j \|,$$

where $\mu > 0$ is a prespecified penalty parameter, $\alpha \in \mathbb{R}^k$ is the scaled Lagrange multiplier, and $\beta_{0c} = (\beta_0^T, \beta_c^T)^T$ collects the unpenalized coefficients.

The algorithm alternates between two steps, a primal step and a dual step, until convergence. Let $\ell = 0, 1, \ldots$ denote the iteration number. The primal step minimizes $L(\beta_{0c}, \beta; \alpha, \mu)$ with respect to $\beta_{0c}$ and $\beta$ with everything else held fixed,

$$\min_{\beta_{0c}, \beta} \{ L(\beta_{0c}, \beta; \alpha, \mu) \}.$$  

The problem is equivalent to a standard group lasso problem, for which many algorithms
are available (Huang et al., 2012). To see this, consider
\[
\arg\min_\beta \left\{ \frac{1}{2n} \| y - Z\beta \|^2 + \frac{\mu}{2} \sum_{j=1}^{p} A_j \beta_j - b + \alpha^\ell \|^2 + \lambda \sum_{j=1}^{p} \| W_j \beta_j \| \right\}.
\]

Here we have omitted the intercept term and the control variables as they can be treated as a group with zero penalty. Define \( A = (A_1, \ldots, A_p) \), \( \tilde{\beta}_j = W_j \beta_j \), and \( \tilde{\beta} = W\beta = \text{diag}(W_1, \ldots, W_p)\beta \). Then the objective can be expressed in terms of \( \tilde{\beta} \) as
\[
\frac{1}{2n} \tilde{\beta}^T (W^{-1})^T (Z^T Z + n \mu A^T A) W^{-1} \tilde{\beta} - \frac{1}{n} (y^T Z + n \mu (b - \alpha^\ell)^T A) W^{-1} \tilde{\beta} + \lambda \sum_{j=1}^{p} \| \tilde{\beta}_j \|.
\]

The dual step updates \( \alpha \) as
\[
\alpha^{\ell+1} \leftarrow \alpha^\ell + \sum_{j=1}^{p} A_j \beta_j^{\ell+1} - b.
\]

To speed up computation, the penalty term \( \mu \) can be set to slowly increase along the iterations (Boyd et al., 2011).

The optimization procedure for any fixed \( \lambda \) is summarized in Algorithm 1. When the model is fitted for a sequence of \( \lambda \) values, a warm start strategy is adopted, i.e., the solution for the previous \( \lambda \) value is used as the initial value for the next one.

A general way to select the tuning parameters, i.e., the basis dimension \( k \) and the group lasso penalty level \( \lambda \), is the \( K \)-fold cross validation (Stone, 1974), which is based on the predictive performance of the models. However, it is well known that the best model for prediction may not coincide with that for variable selection, and in fact, the former often leads to overselection. This phenomenon under our model is revealed in Section 4, where it is shown that consistent variable selection shall be based on the zero pattern of a thresholded estimator. Following Fan and Tang (2013) and Lin et al. (2014), we thus also experiment with minimizing a generalized information criterion (GIC) for model selection which favors
Algorithm 1

Initialize $\alpha^0 \geq 0, \mu^0 \geq 0$. Choose $\rho > 1$, e.g., $\rho = 1.05$. Choose convergence thresholds $\epsilon_1 > 0$ and $\epsilon_2 > 0$, e.g., $\epsilon_1 = \epsilon_2 = 10^{-4}$. Set $\ell \leftarrow 0$.

**repeat**

(1) Primal step: $(\beta^{\ell+1}_0, \beta^{\ell+1}) \leftarrow \min_{\beta_0, \beta} \{L(\beta_0, \beta; \alpha^\ell, \mu^\ell)\}$.

(2) Dual step: $\alpha^{\ell+1} \leftarrow (\alpha^\ell + \sum_{j=1}^{p} A_j \beta^{\ell+1}_j - b) / \rho$.

$\mu^{\ell+1} \leftarrow \rho \mu^\ell$.

$\ell \leftarrow \ell + 1$.

**until** convergence, i.e., $(\|\beta^{\ell+1}_0 - \beta_0\|^2 + \|\beta^{\ell+1} - \beta\|^2)/(\|\beta^{\ell}_0\|^2 + \|\beta^{\ell}\|^2) \leq \epsilon_1$ and $\|\sum_{j=1}^{p} A_j \beta^{\ell+1}_j - b\|^2 \leq \epsilon_2$.

**return** $\beta_0 = \beta^{\ell}_0$ and $\beta = \beta^{\ell}$.

more sparse models,

$$GIC(\lambda, k) = \log (\hat{s}^2(\lambda, k)) + (s(\lambda, k) - 1) k \log \left( \max\{pk + 1, n\} \right) \frac{\log(\log n)}{n},$$

where $\hat{s}^2(\lambda, k)$ is the mean squared error defined as $\|y - \hat{\beta}_0(\lambda, k)1_n - Zc \hat{\beta}_c(\lambda, k) - Z \hat{\beta}(\lambda, k)\|^2 / n$ with $\hat{\beta}_0(\lambda, k), \hat{\beta}_c(\lambda, k)$ and $\hat{\beta}(\lambda, k)$ being the regularized estimators of regression coefficients, and $s(\lambda, k)$ is the number of nonzero coefficient groups in $\hat{\beta}(\lambda, k)$.

### 3.2 On Discrete Time Observations

So far we have treated the integrated design matrix $Z$ defined in (3) as given. In practical situations, however, the functional compositional predictors are most often not observed continuously but at discrete points, so $Z$ cannot be computed exactly. It is preferable that the induced uncertainty is considered in statistical modeling. In functional regression with a scalar response, Ramsay and Silverman (2005) discussed using truncated basis expansions for both the functional predictor and the functional coefficient curve to convert the infinite dimensional problem to finite dimensional, where truncation can be viewed as a type of regularization. Integrals were approximated by finite Riemann sums with discrete observations. The subsequent methodological development in functional regression has mainly
followed along this general strategy, with various choices of basis functions and associated regularization approaches (Morris, 2013). For example, a functional predictor could be expanded by its eigenbasis via a functional principal component analysis, and the coefficient function could be expanded either by the same eigenbasis or by other functional basis such as wavelet or spline.

Due to the nature of the compositional data, ideally the functional compositions shall be expanded by a multivariate basis that preserves the simplex structure under truncation or other types of regularization, which however, to the best of our knowledge, is not yet available. In essence, a multivariate functional principal component analysis for compositional data, or a joint modeling approach of both the functional compositions and the regression, is needed, which is beyond the scope of the current work.

For the preterm infant study, we take an ad-hoc yet pragmatic way of lifting the discrete-time data to continuous time. In this study, stool sample of each baby was collected daily whenever available; this resulted in a good coverage rate, with on average 12.2 daily samples for each infant over a 24-day study period. Also, biologists believe that the gut microbiome compositions change continuously over time. As such, we simply apply linear interpolation to obtain continuous time compositional curves. It can be readily seen that the linear interpolation approach amounts to compute $Z$ defined in (6) using the trapezoid rule.

Specifically, suppose for each $i = 1, \cdots, n$, we observe $x_i(t) = [x_{i1}(t), \cdots, x_{ip}(t)]^T$ at discrete time points $t_{i,v} \in \mathbb{T} = [T_1, T_2]$, for $v = 1, \cdots, m_i$. That is, different subjects may be observed at different sets of time points in $\mathbb{T}$. Correspondingly, we have

$$z_i(t) = \log(x_i(t)) = [z_{i1}(t), \cdots, z_{ip}(t)]^T, \quad t = t_{i,1}, \cdots, t_{i,m_i}, \quad i = 1, \cdots, n.$$ 

Recall that $Z = \int_{t \in \mathbb{T}} Z(t)(I_p \otimes \Phi(t))^T dt \in \mathbb{R}^{n \times (pk)}$. Let $Z = [Z_1, \cdots, Z_p] \in \mathbb{R}^{n \times (pk)}$ with $Z_j = [z_{ij}]_{n \times k} \in \mathbb{R}^{n \times k}$ for $j = 1, \cdots, p$. Adopting linear interpolation, the entries of $Z$ are
computed using the trapezoid rule as follows,

\[
z_{ijl} = \sum_{v=2}^{m_s} \left( \phi_l(t_{i,v-1})z_{ij}(t_{i,v-1}) + \phi_l(t_{i,v})z_{ij}(t_{i,v}) \right) \frac{t_{i,v} - t_{i,v-1}}{2} + \phi_l(t_{i,1})z_{ij}(t_{i,1} - T_0) + \phi_l(t_{i,m_i})z_{ij}(T_1 - t_{i,m_i}), \quad l = 1, \ldots, k.
\]  

(10)

In what follows, unless otherwise noted, the integrals in the case of discrete data are computed using the above trapezoid rule.

4 Theoretical Properties

Our theory concerns the setting when Assumptions 1–2 hold and the integrated design matrix \( \mathbf{Z} \) is given. For any \( \beta = [\beta_1^T, \ldots, \beta_p^T]^T \in \mathbb{R}^{pk} \), define \( \beta_r \in \mathbb{R}^{(p-1)k} \) as a subvector of \( \beta \) by removing its \( r \)th component \( \beta_r \), for each \( r = 1, \ldots, p \). Let \( \mathcal{J} \subset \{1, \ldots, p\} \) be an index set, and denote \( \beta_{\mathcal{J}} \) be a subvector of \( \beta \) consisting of \( \beta_j, \ j \in \mathcal{J} \). Denote \( \mathcal{J}^c \) as the complement of \( \mathcal{J} \). Recall that \( \mathbf{X}(t) = [\mathbf{x}_1^T(t), \ldots, \mathbf{x}_n^T(t)] \in \mathbb{R}^{n \times p}, \mathbf{Z}(t) = [z_{ij}(t)] \in \mathbb{R}^{n \times p} \) with \( z_{ij}(t) = \log(x_{ij}(t)) \), and \( \tilde{\mathbf{Z}}_r(t) = [\tilde{z}_{ij}(t)] \in \mathbb{R}^{n \times (p-1)} \) with \( \tilde{z}_{ij}(t) = \log(x_{ij}(t)/x_{ir}(t)) \) for each \( r = 1, \ldots, p \). Moreover, due to Assumption 2, we define \( \tilde{\mathbf{Z}}_r = \int_{t \in \mathcal{T}} \tilde{\mathbf{Z}}_r(t)(\mathbf{I}_p \otimes \Phi(t))^T dt \in \mathbb{R}^{n \times (p-1)k} \) and \( \mathbf{Z} = \int_{t \in \mathcal{T}} \mathbf{Z}(t)(\mathbf{I}_p \otimes \Phi(t))^T dt \in \mathbb{R}^{n \times (pk)} \) as in (4). Write \( \tilde{\mathbf{Z}}_r = [\tilde{\mathbf{Z}}_{r,1}, \ldots, \tilde{\mathbf{Z}}_{r,p-1}, \tilde{\mathbf{Z}}_{r,p+1}, \ldots, \tilde{\mathbf{Z}}_{r,p}] \) with each \( \tilde{\mathbf{Z}}_{r,j} \in \mathbb{R}^{n \times k} \). Write \( \mathbf{Z} = [\mathbf{Z}_1, \ldots, \mathbf{Z}_p] \) with each \( \mathbf{Z}_j \in \mathbb{R}^{n \times k} \). Let \( \mathbf{y}_{r,j} = \tilde{\mathbf{Z}}_{r,j}^T \tilde{\mathbf{Z}}_{r,j}/n \), for \( r = 1, \ldots, p, \ j = 1, \ldots, p \) and \( j \neq r \).

It boils down to analyze the constrained linear model with grouped predictors in (7).

For simplicity, we omit the intercept and the control variables, and write the symmetric from of the log-contrast model as

\[
\mathbf{y} = \mathbf{Z}\beta^* + \mathbf{e}, \quad \text{s.t.} \quad \sum_{j=1}^{p} \beta_j^* = 0,
\]

(11)
where $\beta^* = [\beta^*_1, \ldots, \beta^*_p]^T \in \mathbb{R}^{pk}$. Recall that $S = \{ j ; \beta^*_j(t) \neq 0, j = 1, \ldots, p. \} = \{ j ; \beta^*_j \neq 0, j = 1, \ldots, p. \}$, and $s^* = |S| \ll p$.

We study the properties of the constrained group lasso estimator,

$$
\hat{\beta} = \arg \min_{\beta} \left\{ \frac{1}{2n} \| y - Z\beta \|^2 + \lambda \sum_{j=1}^{p} \| \beta_j \| \right\}, \quad \text{s.t. } \sum_{j=1}^{p} \beta_j = 0. \quad (12)
$$

This estimator satisfies that $\hat{\beta}_r = -\sum_{j \neq r} \hat{\beta}_j$. Therefore, it holds true that for any $r = 1, \ldots, p$,

$$
\tilde{\beta}_r = \arg \min_{\beta_r} \left\{ \frac{1}{2n} \| y - Z_r\beta_r \|^2 + \lambda \sum_{j \neq r} \| \beta_j \| + \lambda \sum_{j \neq r} \| \beta_j \| \right\}. \quad (13)
$$

On the other hand, one naive method is the so-called baseline method, which chooses an arbitrary reference component to perform the log-ratio transformation of the compositional predictors and then proceeds with an unconstrained group lasso regression. When the $r$th component is choosing as the reference level, the estimator is given by

$$
\tilde{\beta}_r = \arg \min_{\beta_r} \left\{ \frac{1}{2n} \| y - \tilde{Z}_r\beta_r \|^2 + \lambda \sum_{j \neq r} \| \beta_j \| \right\}. \quad (14)
$$

Our theoretical analysis follows and extends the work by Lounici et al. (2011) on group lasso to the case of the constrained group lasso in (12) arising from the functional compositional data analysis.

**Assumption 3.** The error terms $e_1, \ldots, e_n$ are independently and identically distributed as $N(0,1)$ random variables.
Assumption 4 (Restricted Eigenvalue Condition (RE)). There exists $\kappa > 0$, such that

$$\min \left\{ \frac{\|Z\Delta\|}{\sqrt{n}\|\Delta_{\mathcal{J}}\|} : |\mathcal{J}| \leq s^*, \Delta \in \mathbb{R}^{pk} \neq 0, \sum_{j=1}^{p} \Delta_j = 0, \right. \left. \sum_{j \in \mathcal{J}^c} \|\Delta_j\| + \min_{j} \|\Delta_j\| \leq 3 \sum_{j \in \mathcal{J}} \|\Delta_j\| \right\} \geq \kappa.$$

Due to the linear constraints, the restricted set of $\Delta$ for which the minimum is taken is smaller than that of the regular group lasso estimator. Therefore, the condition for the constrained model becomes weaker.

Theorem 4.1 (Error Bounds). Suppose Assumptions 2–4 hold. Choose $\lambda \geq \min_{r} \max_{j \neq r} \frac{2\sigma}{\sqrt{n}} \sqrt{\text{tr}(\Psi_{r,j}) + 2\sigma_{\max}(\Psi_{r,j})(2q \log(p - 1) + \sqrt{kq \log(p - 1)})}$. Then, with probability at least $1 - 2(p - 1)^{-q}$, the constrained group lasso estimator $\hat{\beta}$ in (12) satisfies that

$$\frac{1}{n} \|Z(\hat{\beta} - \beta^*)\|^2 \leq \frac{16\lambda^2 s^*}{\kappa^2},$$

$$\sum_{j=1}^{p} \|\hat{\beta}_j - \beta_j^*\| + \min_{j} \|\hat{\beta}_j - \beta_j^*\| \leq \frac{16\lambda s^*}{\kappa^2}. \tag{16}$$

It is interesting to compare with the baseline approach in (14). In view of the RE condition and the choice of $\lambda$, the above results reveal that the proposed approach is capable of achieving the best possible performance of the baseline method under a possibly weaker condition.

Assumption 5 ($\beta$-min Condition). Choose the same $\lambda$ as in Theorem 4.1. Assume that

$$\min_{j \in \mathcal{S}} \|\beta_j^*\| \geq \frac{16\lambda s^*}{\kappa^2}.$$
Corollary 4.2 (Selection Consistency). Suppose Assumptions 2-5 hold. Let

\[ \hat{S} = \{ j : \| \hat{\beta}_j \| > \frac{8\lambda s^*}{\kappa^2} \}. \]

Then, with probability at least \(1 - 2(p - 1)^{1-q}\), we have that \(\hat{S} = S\).

Corollary 4.2 reveals the “overselection” phenomenon due to convex penalization; see, e.g., Wei and Huang (2010). That is, the constrained group lasso estimator in general does not miss important variable groups/components, albeit overselecting some irrelevant ones. As such, a thresholding operation is preferred in order to recovery the correct sparsity pattern exactly. However, the theoretical threshold is not available in practice, as it involves unknown quantities such as \(\sigma^2\) and the RE constant \(\kappa\). Nevertheless, the results provide high probability guarantee that using the original (unthresholded) estimator can avoid false negatives at the expense of some false positives, which is quite acceptable in many applications.

5 Simulation

We conduct simulation studies to compare the performance of our proposed sparse functional log-contrast regression via constrained group lasso (CGL) in (8), the baseline approach in the form of (14) via group lasso (BGL) in which the reference level is chosen randomly, and the naive approach of group lasso (GL) in which the zero-sum constraints are ignored in (8),

The compositional data are generated as follows. We first generate \(M\) time points within the interval \([0, 1]\), i.e., \(0 = t_1 < \cdots < t_M = 1\). For inducing dependence between time points, we consider an autoregressive correlation structure, \(\Sigma_T = [\rho_T^{|\mu - \nu|}]_{M \times M}\), where \(1 \leq \mu, \nu \leq M\); for inducing dependence between compositions, we consider a compound symmetry correlation structure, \(\Sigma_X = [\rho_X^{I(j=j')}]_{p \times p}\), where \(1 \leq j, j' \leq p\) and \(I(\cdot)\) is the indicator.
function. The “non-normalized” data for each subject \( i, i = 1, \ldots, n \), are then generated from multivariate normal distribution as \( \mathbf{w}_i = [\mathbf{w}_i(t_1)^T, \ldots, \mathbf{w}_i(t_M)^T]^T \sim N(\mathbf{0}, \sigma_X^2 (\Sigma_T \otimes \Sigma_X)) \), where each \( \mathbf{w}_i(t_\nu) \in \mathbb{R}^p \) for \( \nu = 1, \ldots, M \). Finally, the compositional data are obtained as \( x_{ij}(t_\nu) = \exp(w_{ij}(t_\nu))/\sum_{j=1}^p \exp(w_{ij}(t_\nu)) \), for \( i = 1, \ldots, n \), \( j = 1, \ldots, p \) and \( \nu = 1, \ldots, M \). The regression curves \( \mathbf{\beta}^*(t) \) are generated as \( \mathbf{B}^* \Phi(t) \), where \( \Psi(t) \) is from a set of cubic spline basis computed using the bs function in the R package splines with \( t \in \{t_1, \ldots, t_M\} \) and degrees of freedom set to 5. The first three rows of \( \mathbf{B}^* \) are set as \([1, 0, 1, 0, -0.5], [0, 0, -1, 0, 1] \) and \([-1, 0, 0, 0, -0.5] \), respectively, and the rest are set to zero. The intercept is set to be \( \beta^*_0 = 1 \) and for simplicity we do not consider additional control. The error terms are generated as independent \( N(0, \sigma^2) \) random variables where \( \sigma^2 \) is set to control the signal to noise ratio (SNR). Finally, the response \( y \) is generated according to model (5), where the integral is computed as in (10). We have experimented with model dimensions \( (n, p) \in \{ (50, 30), (100, 30), (100, 100), (100, 200) \} \) and parameter settings \( M = 20, \sigma_X^2 = 9, \rho_T \in \{ 0, 0.6 \}, \rho_X = \{ 0, 0.6 \} \) and SNR = \{ 2, 4 \}. The simulation is repeated 100 times under each setting.

The prediction error (Pred) is measured by \( \|y_{te} - Z_{te} \tilde{\mathbf{\beta}}\|^2/n_{te} \), computed from an independently generated test sample \( (y_{te}; X_{te}(t), t \in \{t_1, \ldots, t_M\}) \) of size \( n_{te} = 500 \). The estimation error (Est) is measured by \( \sum_{j=1}^p (\int_{[0,1]} |\tilde{\beta}_j(t) - \beta^*_j(t)|^2 dt)^{1/2}/p \). For variable selection of the compositional components, we report the false positive rate (FPR) and the false negative rate (FNR), based on the sparsity patterns of \( \tilde{\mathbf{\beta}}(t) \) and \( \mathbf{\beta}^*(t) \). We have experimented with both 10-fold cross validation (CV) and GIC for selecting tuning parameters \( k \) and \( \lambda \). As shown in Corollary 4.2, a thresholding of the estimator is preferred for the purpose of variable selection, although the ideal threshold is not available in practice. Here with the same spirit and based on empirical evidence, we define the selected index set \( \hat{S} \) based on the relative magnitudes of the \( p \) estimated coefficient curves:

\[
\hat{S} = \{ j; \left( \int_{[0,1]} \tilde{\beta}_j^2(t) dt \right)^{1/2}/\left\{ \sum_{j=1}^p \left( \int_{[0,1]} \tilde{\beta}_j^2(t) dt \right)^{1/2} \right\} \geq 1/p, j = 1, \ldots, p \}.
\]

That is, we only
count the components whose relative “energy” exceeds the average $1/p$ as selected.

The simulation results for $(n, p) = (50, 30)$ and $(n, p) = (100, 200)$ with SNR = 4 are reported in Tables 1–2. In general, CGL shows better predictive and selection performance than both GL and BGL, and in some cases the improvement can even be substantial. (We have also tried the unpenalized least squares estimator, which fails miserably in prediction and hence is omitted.) The BGL method performs the worst among the three. The two tuning methods, CV and GIC, show quite different behaviors: the former generally yields larger false positive rates and much smaller false negative rates than the latter. Indeed, this is consistent with the theoretical results in Section 4 that the proposed convex regularized estimation approach has a tendency of over-selection when tuned based on optimizing predictive performance. Nevertheless, the CV-tuned estimators rarely miss important components and performs much better in prediction comparing to their GIC tuned counterparts. Therefore, the CV method may be preferable in practice when one cares more about prediction and can afford to live with some false alarms for the capture of all the relevant signals.

Figures 1–2 show boxplots of prediction errors from CV tuning under various simulation settings. The performance of all methods deteriorates when the signal to noise ratio becomes smaller, the between-component correlation becomes smaller, or the between-time correlation becomes stronger. Small between-component correlation in general causes the presence of a few dominating compositional components, due to the unit-sum constraints of the compositional data, while large between-time correlation makes the functional compositions smooth over time and consequently makes it hard to distinguish the relevant components from the others.
Table 1: Simulation results for \((n, p) = (50, 30)\) and SNR = 4. Reported are the average values over 100 simulation runs, with the standard deviations in parentheses. For better presentation, the values of Est and Pred are multiplied by 10.

| \((\rho_X, \rho_T)\) | Criterion | Method | Est    | Pred    | FPR (%) | FNR (%) |
|----------------------|-----------|--------|--------|---------|----------|----------|
| (0, 0)               | CV        | BGL    | 0.25 (0.01) | 0.39 (0.01) | 28.85 (1.28) | 0.00 (0.00) |
|                      |           | GL     | 0.23 (0.01) | 0.39 (0.01) | 27.48 (1.35) | 0.00 (0.00) |
|                      |           | CGL    | 0.23 (0.01) | 0.34 (0.01) | 29.22 (1.43) | 0.00 (0.00) |
|                      | GIC       | BGL    | 0.33 (0.01) | 1.46 (0.06) | 4.04 (0.19) | 48.00 (3.33) |
|                      |           | GL     | 0.31 (0.01) | 1.44 (0.05) | 0.19 (0.08) | 52.67 (2.69) |
|                      |           | CGL    | 0.29 (0.01) | 1.24 (0.05) | 1.63 (0.24) | 20.00 (2.37) |
| (0, 0.6)             | CV        | BGL    | 0.28 (0.01) | 1.27 (0.04) | 30.70 (1.48) | 0.33 (0.33) |
|                      |           | GL     | 0.26 (0.01) | 1.21 (0.03) | 29.04 (1.40) | 0.00 (0.00) |
|                      |           | CGL    | 0.25 (0.01) | 1.13 (0.03) | 29.67 (1.43) | 0.00 (0.00) |
|                      | GIC       | BGL    | 0.34 (0.01) | 4.61 (0.16) | 3.74 (0.16) | 52.67 (2.60) |
|                      |           | GL     | 0.31 (0.00) | 3.93 (0.12) | 0.11 (0.06) | 51.67 (2.39) |
|                      |           | CGL    | 0.31 (0.01) | 3.91 (0.17) | 1.52 (0.24) | 23.67 (2.19) |
| (0.6, 0)             | CV        | BGL    | 0.25 (0.01) | 0.15 (0.01) | 29.26 (1.35) | 0.00 (0.00) |
|                      |           | GL     | 0.24 (0.01) | 0.16 (0.00) | 29.93 (1.42) | 0.00 (0.00) |
|                      |           | CGL    | 0.23 (0.01) | 0.14 (0.00) | 29.07 (1.22) | 0.00 (0.00) |
|                      | GIC       | BGL    | 0.34 (0.01) | 0.65 (0.02) | 3.81 (0.19) | 56.33 (2.67) |
|                      |           | GL     | 0.32 (0.01) | 0.62 (0.02) | 0.19 (0.08) | 59.33 (2.25) |
|                      |           | CGL    | 0.30 (0.01) | 0.54 (0.02) | 1.63 (0.22) | 22.67 (2.22) |
| (0.6, 0.6)           | CV        | BGL    | 0.29 (0.01) | 0.53 (0.02) | 33.52 (1.38) | 0.33 (0.33) |
|                      |           | GL     | 0.26 (0.01) | 0.49 (0.02) | 30.22 (1.31) | 0.00 (0.00) |
|                      |           | CGL    | 0.25 (0.01) | 0.45 (0.01) | 30.37 (1.44) | 0.00 (0.00) |
|                      | GIC       | BGL    | 0.35 (0.01) | 1.85 (0.06) | 3.81 (0.15) | 53.67 (2.59) |
|                      |           | GL     | 0.32 (0.00) | 1.69 (0.05) | 0.11 (0.06) | 57.67 (2.00) |
|                      |           | CGL    | 0.31 (0.01) | 1.52 (0.06) | 1.74 (0.23) | 25.00 (2.24) |
Table 2: Simulation results for \((n, p) = (100, 200)\) and \(\text{SNR} = 4\). The layout is the same as in Table 1.

| \((\rho_X, \rho_T)\) | Criterion | Method | Est | Pred | FPR (%) | FNR (%) |
|---------------------|-----------|--------|-----|------|---------|---------|
| (0, 0)             | CV        | BGL    | 0.04 (0.00) | 0.31 (0.01) | 15.28 (0.48) | 0.00 (0.00) |
|                    |           | GL     | 0.04 (0.00) | 0.31 (0.01) | 15.27 (0.48) | 0.00 (0.00) |
|                    |           | CGL    | 0.04 (0.00) | 0.29 (0.00) | 15.57 (0.51) | 0.00 (0.00) |
|                    | GIC       | BGL    | 0.05 (0.00) | 1.45 (0.05) | 0.51 (0.01) | 44.00 (3.07) |
|                    |           | GL     | 0.04 (0.00) | 1.33 (0.05) | 0.01 (0.01) | 46.33 (2.88) |
|                    |           | CGL    | 0.04 (0.00) | 1.13 (0.05) | 0.19 (0.03) | 11.67 (1.73) |
| (0, 0.6)           | CV        | BGL    | 0.04 (0.00) | 1.02 (0.02) | 16.26 (0.51) | 0.00 (0.00) |
|                    |           | GL     | 0.04 (0.00) | 0.97 (0.02) | 15.62 (0.52) | 0.00 (0.00) |
|                    |           | CGL    | 0.04 (0.00) | 0.94 (0.02) | 16.32 (0.50) | 0.00 (0.00) |
|                    | GIC       | BGL    | 0.05 (0.00) | 4.15 (0.16) | 0.51 (0.01) | 43.00 (3.01) |
|                    |           | GL     | 0.04 (0.00) | 3.44 (0.12) | 0.01 (0.01) | 42.67 (2.92) |
|                    |           | CGL    | 0.04 (0.00) | 3.57 (0.15) | 0.10 (0.02) | 16.00 (1.92) |
| (0.6, 0)           | CV        | BGL    | 0.04 (0.00) | 0.12 (0.00) | 14.78 (0.49) | 0.00 (0.00) |
|                    |           | GL     | 0.04 (0.00) | 0.12 (0.00) | 15.44 (0.61) | 0.00 (0.00) |
|                    |           | CGL    | 0.04 (0.00) | 0.12 (0.00) | 15.07 (0.55) | 0.00 (0.00) |
|                    | GIC       | BGL    | 0.05 (0.00) | 0.55 (0.02) | 0.53 (0.01) | 39.00 (3.39) |
|                    |           | GL     | 0.04 (0.00) | 0.47 (0.02) | 0.02 (0.01) | 36.33 (3.22) |
|                    |           | CGL    | 0.04 (0.00) | 0.41 (0.02) | 0.15 (0.03) | 9.67 (1.79) |
| (0.6, 0.6)         | CV        | BGL    | 0.04 (0.00) | 0.41 (0.01) | 16.21 (0.50) | 0.00 (0.00) |
|                    |           | GL     | 0.04 (0.00) | 0.40 (0.01) | 15.30 (0.55) | 0.00 (0.00) |
|                    |           | CGL    | 0.04 (0.00) | 0.39 (0.01) | 15.59 (0.50) | 0.00 (0.00) |
|                    | GIC       | BGL    | 0.05 (0.00) | 1.76 (0.06) | 0.52 (0.01) | 48.33 (2.93) |
|                    |           | GL     | 0.04 (0.00) | 1.46 (0.05) | 0.01 (0.01) | 47.33 (2.73) |
|                    |           | CGL    | 0.04 (0.00) | 1.40 (0.06) | 0.15 (0.03) | 17.00 (1.98) |
Figure 1: Boxplots of prediction errors for various simulation settings with SNR = 4. The dark grey, light grey and white colors correspond to three different estimation methods BGL, GL and CGL, respectively.
Figure 2: Boxplots of prediction errors for various simulation settings with SNR = 2. The layout is the same as in Figure 1.

(a) $n = 50, p = 30$

(b) $n = 100, p = 30$

(c) $n = 100, p = 100$

(d) $n = 100, p = 200$
6 Preterm Infant Study

6.1 Data Description

Data were collected at a Level IV NICU in the northeast region of the U.S. (Level IV NICUs provide the highest level, the most acute care.) Fecal samples of preterm infants were collected daily when available, mainly during the infant’s postnatal age (PNA) of 5 to 28 days ($t \in [5, 28]$). Bacterial DNA were isolated and extracted from each stool sample (Bomar et al., 2011; Cong et al., 2017); the V4 regions of the 16S rRNA gene were sequenced using the Illumina platform and clustered and analyzed using QIIME (Cong et al., 2017; Caporaso et al., 2012), resulting in microbiome count data. As the number of sequencing reads varied a lot across samples, the count data were transformed into compositional data with zero count replaced by 0.5, the maximum rounding error (Aitchison, 1986; Lin et al., 2014). The compositional data consisted of $p = 22$ categories at the order level of the taxonomic ranks. (Taxonomic rank is the relative level of a group of organisms in a taxonomic hierarchy in biological classification; the major ranks are species, genus, family, order, class, phylum, kingdom, and domain.) In this study, infants with less than 5 fecal samples were excluded, which resulted in $n = 34$ infants. There were totally 414 fecal samples, so the average number of daily fecal samples collected for each infant was 12.2.

Infant neurobehavioral outcomes were measured when the infant reached 36–38 weeks of post-menstrual age or prior to hospital discharge, using the NICU Network Neurobehavioral Scale (NNNS). The NNNS is a standardized assessment of neonatal neurobehavioral outcomes that provides an appraisal of neurological integrity and behavioral function of the normal and at-risk/preterm infant. In particular, the Stress/Abstinence subscale (NSTRESS) measures signs of stress and includes 50 items. Each sign of stress/abstinence is scored as present or absent during the exam, and the composite NSTRESS score ranges between 0 and 1. A higher NSTRESS score demonstrates a more stressful behavioral performance.
Cong et al. (2017) showed that the composite NSTRESS score is positively associated with early life painful/stressful experience in preterm infants.

Other variables about birth, growth and characteristics of infant included gender, delivery type, premature rupture of membranes (PROM), score for Neonatal Acute Physiology–Perinatal Extension-II (SNAPPE-II), birth weight, and percentage of feeding with mother’s breast milk during the study period (%MBM).

### 6.2 Statistical Modeling and Results

Our main objective is to identify the microbiome markers that are predictive of later infant neurodevelopment as measured by NNNS. This predictive association, if proven true, can provide supporting evidence to the claim that the stressful early life experience of preterm infants is imprinting gut microbiome by the regulation of the brain-gut axis. We tackle the problem with the functional log-contrast regression model in (5), in which the composite NSTRESS score serves as the response variable, the gut microbiome observed during the early postnatal period serves as the functional compositional predictors, and the infant characteristics listed in Table 3 below serve as the time-invariate controls. We apply the proposed CGL approach for model estimation and compositional component selection. The cubic spline basis is used, and the tuning of the degrees of freedom $k$ of the spline as well as the sparsity parameter $\lambda$ is done using cross validation.

Our approach is able to identify four bacteria categories that are associated with the neurobehavioral outcome of infant, after controlling for the effects of several infant characteristics. Before we discuss the selected microbiome markers, let’s first focus on the effects of the control variables. Table 3 shows the estimated coefficients of the control variables along with some descriptive statistics. It is seen that the neurobehavioral outcome is better (i.e., NSTRESS is small) for infants with larger birth weight, smaller SNAPPE-II score and more mother’s breast milk for feeding. Regarding the delivery of infant, vaginal delivery
and the absence of premature rupture of membranes are associated with better neurobehav-
ioral development. These interesting and intuitive results are consistent with existing
literature. The analysis also shows that female infants tend to perform slightly better than
male, after accounting for the effects of other variables including the integrated effects of
the gut microbiome.

Table 3: Descriptive statistics of infant characteristics and their estimated coefficients from fitting the sparse
functional log-contrast regression. Values of estimated coefficient are multiplied by 100.

| Numerical variable       | Mean (sd)   | Estimated coefficient |
|--------------------------|-------------|-----------------------|
| Birth weight (in gram)   | 1451.7 (479.3) | −0.003               |
| SNAPE-II                 | 9.3 (10.6)   | 0.108                 |
| %MBM                     | 61.8 (29.9)  | −9.08                 |

| Binary variable          | Percentage of ones | Estimated coefficient |
|--------------------------|--------------------|-----------------------|
| Gender (female = 1)      | 50.0%              | −0.109                |
| PROM (yes = 1)           | 44.1%              | 2.74                  |
| Delivery type (vaginal =1)| 35.3%              | −5.09                 |

The estimated functional effects of the four selected bacteria categories are shown in
the four panels of Figure 3 respectively. In each panel, the lower part shows the estimated
functional effects of a category over time (between 5 and 28 days of postnatal age), and the
upper part attempts to show directly from raw data how this category changes over time for
infants with high, medium, or low “adjusted” NSTRESS score. Specifically, we construct
smoothed curves of log-compositions of each selected category for three clusters of infants
(using locally weighted scatterplot smoothing). For each category, the clusters are based
on the percentiles of its partial residuals, obtained by subtracting the estimated effects of
the control variables and other selected bacteria categories from the observed NSTRESS
scores. The curve with its 90% confidence band is shown in red for the high group, i.e.,
infants with the upper one third of the adjusted scores, in blue for the medium group, i.e.,
infants with the middle one third of the adjusted scores, and in green for the low group,
i.e., infants with the lower one third of the adjusted scores. As an example, for category 1,
the red curve increases in the beginning to be above the other two curves and then becomes
mostly below them in the later stage. This suggests that the time-varying effect of category 1 on the NSTRESS score is first positive and then negative, which is clearly reflected by the estimated functional effects. Similarly for the other three selected categories, the patterns of the estimated effects agree well with those of the observed data. This verifies visually that our proposed model and the estimation approach yield sensible results.

To access the stability of the results, we have generated 100 bootstrap samples and used the same cross validation procedure to select the best models. The results are show in Figure 4. The signs of the coefficients of the control variables are quite stable, except for the gender and SNAPE-II variables; this shows that these two variables may not have much effect on the outcome when conditioning on other terms in the model. For each control variable, the sign with the higher proportion among its 100 bootstrap estimates agrees with that of the estimate from fitting the original data, except for the gender. Furthermore, the top four categories with the highest proportions of being selected in bootstrap coincide with the categories selected from fitting the original data. Categories 10 and 19 are selected about 90% of the times, while categories 9 and 1 are selected more than 70% and 60% of the times, respectively.

Category 10 consists of Clostridiales, which are an order of bacteria belonging to the phylum Firmicutes. Studies showed that infants fed with mother’s milk had significantly higher abundance in Clostridiales (Cong et al., 2016). Clostridiales are generally regarded as hallmarks of a healthy gut; it can be a sign of infection when their subtypes such as Eubacteria die off in the large intestine. Our results show that controlling for other effects in the model, the effect of Clostridiales on the stress score switches from negative to positive during the postnatal days from 5 to 28. Category 9 consists of Lactobacillales, or lactic acid bacteria (LAB), another order of bacteria belonging to the phylum Firmicutes. These bacteria are usually found in decomposing plants and milk products; they are considered beneficial and produce organic acids such as lactic acid from carbohydrates. Our analysis...
shows that controlling for the other effects in the model, higher LAB proportions are associated with higher stress scores for a period of time during the early postnatal days. Both Clostridia and LAB belong to phylum Firmicutes, which make up the largest portion of the human gut microbiome, and the abundance of Firmicutes has been shown to be associated with inflammation and obesity (Clarke et al., 2012; Boulangé et al., 2016). Category 19 consists of Enterobacteriales, an order of gram-negative bacteria. They are responsible for various infections such as bacteremia, lower respiratory tract infections, skin infections, etc. Category 1 consists of other unclassified bacteria. The functional regression analysis presented here may lead to a better understanding of how the trajectories of gut microbiome during early postnatal stage impact neurobehavioral outcomes of infants through the gut-brain axis.

7 Discussion

We have attempted a functional log-contrast regression approach to identify trajectories of compositional components that are associated with a scalar outcome variable. There are several directions for future research to address the limitations of the current work. The data analysis can benefit from extending the model to consider potential interactions between the control variables and the gut microbiome, as it is possible, for example, that the effects of certain microbiome markers differ for male and female infants. Extensions to binary outcome or mixture model setup are interesting and could be widely applicable; it boils down to consider a more general loss function than the squared error loss. In this work, we essentially assume that the integrals involving the trajectories of the compositional data can be well approximated via simple interpolation. Consequently, our theoretical analysis has focused on the linearly constrained group lasso models. To take into account the uncertainty due to discrete observations, it is urgent to develop smoothing or dimension reduction methods.
Figure 3: Estimated effects of the four selected bacteria category over infant’s postnatal age (PNA) of 5 to 28 days. In each sub-graph, the upper panel shows how this category changes over time for three clusters of infant. The clusters are based on the percentiles of adjusted er selected categories from the observed scores. Red: subjects with upper 33% adjusted scores; green: lower 33%; blue: middle 33%; the lower panel shows estimated coefficient curve for the category.
Figure 4: Selection results from 100 bootstrap samples. (a) Proportions of the signs of the estimated coefficients of the control variables. Proportions of positive signs are shown as black blocks to the right, and those of negative signs are shown as light gray blocks to the left. (b) Proportions of selecting the 22 bacteria categories. The bars of the four selected categories from fitting the original data are colored in black.

such as multivariate functional principal component analysis for compositional data observed discretely over time. A joint modeling approach of both the regression and the functional compositions themselves may also be fruitful.

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Appendix

Proof of Theorem 4.1. For all $\beta = [\beta_1^T, \ldots, \beta_p^T]^T \in \mathbb{R}^{pk}$, $\sum_{j=1}^p \beta_j = 0$, it holds that

$$\frac{1}{n} \|y - Z\hat{\beta}\|^2 + 2\lambda \sum_{j=1}^p \|\hat{\beta}_j\| \leq \frac{1}{n} \|y - Z\beta\|^2 + 2\lambda \sum_{j=1}^p \|\beta_j\|,$$

by the optimality of the constrained group lasso estimator $\hat{\beta}$. Using $y = Z\beta^* + e$, we have that

$$\frac{1}{n} \|Z(\hat{\beta} - \beta^*)\|^2 \leq \frac{1}{n} \|Z(\beta - \beta^*)\|^2 + \frac{2}{n} e^T Z(\hat{\beta} - \beta) + 2\lambda \sum_{j=1}^p (\|\beta_j\| - \|\hat{\beta}_j\|). \quad (17)$$

We first bound the stochastic term $e^T Z(\hat{\beta} - \beta)$. Due to the zero-sum constraints, it is important to realize that for any $r = 1, \ldots, p$,

$$e^T Z(\hat{\beta} - \beta) = e^T Z_r(\hat{\beta}_r - \beta_r).$$

The following tail bound is from Lemma A.1 in Lounici et al. (2011).

Lemma 7.1. Let $v = [v_1, \ldots, v_n]^T \neq 0$, $\eta_v = \sum_{i=1}^n (e_i^2 - 1)v_i/(\sqrt{2}\|v\|)$, and $m(v) = \|v\|_{\infty}/\|v\|$. Then, under Assumption A1 for all $t > 0$,

$$\mathbb{P}(\eta_v > t) \leq 2 \exp \left( -\frac{t^2}{2(1 + \sqrt{2tm(v)})} \right).$$

For any fixed $r$, it can be shown using Lemma 7.1 (Lounici et al., 2011) that if we choose $\lambda \geq \lambda_r$, where

$$\lambda_r = \max_{j \neq r} \frac{2\sigma}{\sqrt{n}} \sqrt{\text{tr}(\Psi_{r,j}) + 2\sigma_{\max}(\Psi_{r,j})(2q \log(p - 1) + \sqrt{kp \log(p - 1)})},$$
then with probability at least \(1 - 2(p - 1)^{1-q}\),

\[
\frac{2}{n} e^T Z(\hat{\beta} - \beta) \leq \lambda \sum_{j \neq r}^p \|\hat{\beta}_j - \beta_j\|.
\]

Therefore, as long as we choose \(\lambda \geq \min_r \lambda_r\), the preceding inequality holds for some \(r\); it then follows that with probability at least \(1 - 2(p - 1)^{1-q}\), we have

\[
\frac{2}{n} e^T Z(\hat{\beta} - \beta) \leq \lambda \max_r \sum_{j \neq r}^p \|\hat{\beta}_j - \beta_j\|.
\]

By (17), we get

\[
\frac{1}{n} \|Z(\hat{\beta} - \beta^*)\|^2 \leq \frac{1}{n} \|Z(\beta - \beta^*)\|^2 + \lambda \max_r \sum_{j \neq r}^p \|\hat{\beta}_j - \beta_j\| + 2\lambda \sum_{j=1}^p (\|\beta_j\| - \|\beta^*_j\|) + 2\lambda \sum_{j=1}^p (\|\beta_j\| - \|\beta^*_j\|) + 2\lambda \sum_{j=1}^p (\|\beta_j\| - \|\beta^*_j\|).
\]

It then follows that

\[
\frac{1}{n} \|Z(\hat{\beta} - \beta^*)\|^2 + \lambda \sum_{j=1}^p \|\hat{\beta}_j - \beta_j\| + \lambda \min_j \|\hat{\beta}_j - \beta_j\|
\]

\[
\leq \frac{1}{n} \|Z(\beta - \beta^*)\|^2 + 2\lambda \sum_{j=1}^p (\|\beta_j\| - \|\beta^*_j\| + \|\hat{\beta}_j - \beta_j\|).
\]

Now take \(\beta = \beta^*\), we get that

\[
\frac{1}{n} \|Z(\hat{\beta} - \beta^*)\|^2 + \lambda \sum_{j=1}^p \|\hat{\beta}_j - \beta^*_j\| + \lambda \min_j \|\hat{\beta}_j - \beta^*_j\| \leq 4\lambda \sum_{j \in S} \min(\|\beta^*_j\|, \|\hat{\beta}_j - \beta^*_j\|). \quad (18)
\]

The inequality in (18) implies that

\[
\lambda \sum_{j=1}^p \|\hat{\beta}_j - \beta^*_j\| + \lambda \min_j \|\hat{\beta}_j - \beta^*_j\| \leq 4\lambda \sum_{j \in S} \|\hat{\beta}_j - \beta^*_j\|,
\]
which is equivalent to

$$\sum_{j \in S^c} \| \hat{\beta}_j - \beta_j^* \| + \min_j \| \hat{\beta}_j - \beta_j^* \| \leq 3 \sum_{j \in S} \| \hat{\beta}_j - \beta_j^* \| .$$

Therefore, by the restricted eigenvalue condition in Assumption 3, we know that

$$\| \hat{\beta}_S - \beta_S^* \| \leq \frac{\| Z(\hat{\beta} - \beta^*) \|}{\kappa \sqrt{n}} . \quad (19)$$

It follows from (18)–(19) that

$$\frac{1}{n} \| Z(\hat{\beta} - \beta^*) \|^2 \leq 4\lambda \sum_{j \in S} \| \hat{\beta}_j - \beta_j^* \|$$

$$\leq 4\lambda \sqrt{s^*} \| \hat{\beta}_S - \beta_S^* \|$$

$$\leq 4\lambda \sqrt{s^*} \frac{\| Z(\hat{\beta} - \beta^*) \|}{\kappa \sqrt{n}} ,$$

which leads to (13). Also,

$$\sum_{j=1}^p \| \hat{\beta}_j - \beta_j^* \| + \min_j \| \hat{\beta}_j - \beta_j^* \| \leq 4 \sum_{j \in S} \| \hat{\beta}_j - \beta_j^* \|$$

$$\leq 4\sqrt{s^*} \| \hat{\beta}_S - \beta_S^* \|$$

$$\leq 4\sqrt{s^*} \frac{\| Z(\hat{\beta} - \beta^*) \|}{\kappa \sqrt{n}}$$

$$\leq 4\sqrt{s^*} \sqrt{\frac{16\lambda^2}{\kappa^2}} \frac{1}{\kappa}$$

$$= \frac{16\lambda s^*}{\kappa^2} ,$$

which leads to (16). This completes the proof. \qed
Proof of Corollary 4.2. Theorem 4.1 implies that
\[ \| \hat{\beta} - \beta^* \|_{2,\infty} \leq \frac{8\lambda s^*}{\kappa^2} = a. \] (20)

If \( \beta^*_j = 0 \), then \( \| \hat{\beta}_j \| \leq a \); so that \( j \notin \hat{S} \). Now consider \( \beta^*_j \neq 0 \). By the \( \beta \)-min condition, i.e., \( \| \beta^*_j \| > 2a \), together with (20), it must be true that \( \| \hat{\beta}_j \| > a \), so that \( j \in \hat{S} \). This completes the proof.

References

Aitchison, J. (1982) The statistical analysis of compositional data. *Journal of the Royal Statistical Society: Series B*, **44**, 139–177.

Aitchison, J. (1986) *The Statistical Analysis of Compositional Data*. London, UK: Chapman & Hall.

Aitchison, J. and Bacon-Shone, J. (1984) Log-contrast models for experiments with mixtures. *Biometrika*, **71**, 323–330.

Bomar, L., Maltz, M., Colston, S. and Graf, J. (2011) Directed culturing of microorganisms using metatranscriptomics. *2*, e00012–00011.

Boulangé, C. L., Neves, A. L., Chilloux, J., Nicholson, J. K. and Dumas, M.-E. (2016) Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Medicine*, **8**, 42.

Boyd, S., Parikh, N., Chu, E., Peleato, B. and Eckstein, J. (2011) *Distributed Optimization and Statistical Learning via the Alternating Direction Method of Multipliers*, vol. 3.

Caporaso, J., Lauber, C. and Walters, W. (2012) Ultra-high-throughput microbial community analysis on the illumina hiseq and miseq platforms. *6*, 1621–1624.
Clarke, S. F., Murphy, E. F., Nilaweera, K., Ross, P. R., Shanahan, F., O’Toole, P. W. and Cotter, P. D. (2012) The gut microbiota and its relationship to diet and obesity: New insights. *Gut Microbes*, **3**, 186–202.

Cong, X., Judge, M., Xu, W. and Diallo, A. (2017) Influence of feeding type on gut microbiome development in hospitalized preterm infants. *Nursing Research*, **66**, 123–133.

Cong, X., Xu, W., Janton, S., Henderson, W. A., Matson, A., McGrath, J. M., Maas, K. and Graf, J. (2016) Gut microbiome developmental patterns in early life of preterm infants: Impacts of feeding and gender. *PLOS ONE*, **11**, 1–19.

Dinan, T. and Cryan, J. (2012) Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. *Psychoneuroendocrinology*, **37**, 1369–1378.

Fan, Y. and Tang, C. Y. (2013) Tuning parameter selection in high dimensional penalized likelihood. *Journal of the Royal Statistical Society: Series B*, **75**, 531–552.

Fanaroff, A., Hack, M. and MC, W. (2003) The nichd neonatal research network: changes in practice and outcomes during the first 15 years. *Seminars in Perinatology*, **27**, 281–287.

Huang, J., Breheny, P. and Ma, S. (2012) A selective review of group selection in high dimensional models. *Statist. Sci.*, **27**, 481–499.

Lin, W., Shi, P., Feng, R. and Li, H. (2014) Variable selection in regression with compositional covariates. *Biometrika*, **101**, 785–797.

Lounici, K., Pontil, M., van de Geer, S. and Tsybakov, A. B. (2011) Oracle inequalities and optimal inference under group sparsity. *Ann. Statist.*, **39**, 2164–2204.

Morris, J. S. (2015) Functional regression. *Annual Review of Statistics and Its Application*, **2**, 321–359.
Mwaniki, M., Atieno, M., Lawn, J. and Newton, C. (2012) Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. *Lancet,* **379,** 445–452.

Ramsay, J. O. and Silverman, B. W. (2005) *Functional Data Analysis.* Springer Series in Statistics. Springer, 2nd edn.

Shi, P., Zhang, A. and Li, H. (2016) Regression analysis for microbiome compositional data. *Ann. Appl. Stat.*, **10,** 1019–1040.

Stoll, B., Hansen, N. and Bell, E. (2010) Neonatal outcomes of extremely preterm infants from the nichd neonatal research network. **126,** 443–456.

Stone, M. (1974) Cross-validation and multinomial prediction. *Biometrika,* **61,** 509–515.

Tibshirani, R. J. (1996) Regression shrinkage and selection via the lasso. *Journal of the Royal Statistical Society: Series B,* **58,** 267–288.

Wei, F. and Huang, J. (2010) Consistent group selection in high-dimensional linear regression. *Bernoulli,* **16,** 1369–1384.

Yuan, M. and Lin, Y. (2006) Model selection and estimation in regression with grouped variables. *Journal of the Royal Statistical Society: Series B,* **68,** 49–67.