**Time-Informed Dimensionality Reduction for Longitudinal Microbiome Studies**

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**Abstract**

Complex dynamics of microbial communities underlie their essential roles in health and disease, but our understanding of these dynamics remains incomplete. To bridge this gap, longitudinal microbiome data are being rapidly generated, yet their power is limited by technical challenges in design and analysis, such as missing temporal samples, complex correlation structure, and high dimensionality. Here, we present TEMPoral TEnsor Decomposition (TEMPTED), the only time-informed dimensionality reduction method that extracts the underlying microbial dynamics while overcoming the statistical challenges posed by this type of data. TEMPTED facilitates beta-diversity analysis at both sample- and subject-level and enables the transfer of the learned low-dimensional representation from training data to unseen test data. In data-driven

simulations, TEMPTED enables host phenotype classification at 90% accuracy compared to random using existing methods. In real data we show that TEMPTED deconvolutes the dynamics of the vaginal microbiome during pregnancy, allowing for the detection of microbial signatures associated with term and preterm births that are reproducible across datasets.

## Main Text

### Introduction

Given the highly dynamic and complex nature of microbial communities, identifying and predicting their time-dependent patterns is crucial to understanding their structure and function. The collection of longitudinal microbiome samples provides a unique opportunity to capture the dynamics of microbial communities and their associations with host phenotypes. However, the nature of longitudinal microbiome data poses several analytical challenges. First, microbiome data is high-dimensional, making dimensionality reduction key in guiding analysis and interpretation. Second, the pattern of intra-host variation may change over time and vary across hosts, making it challenging to extract robust temporal patterns of microbial features[1]. Third, due to inherent practical limitations of longitudinal studies (e.g., missed patient follow-ups or inconsistent sample collection), multiple hosts often have missing temporal samples which translates into irregular temporal sampling across hosts[2–5].

To model longitudinal microbiome data, many studies first use dimensionality reduction methods, such as principal coordinate analysis (PCoA); however, this method analyzes data at the sample level and does not utilize or account for temporal structures. In recent years, methods designated to model longitudinal microbiome data have been developed. Unsupervised methods like CTF[1], TCAM[6] and microTENSOR[7] format temporal microbiome data into tabular tensors and apply tensor decomposition to identify low-dimensional structures. Yet, they assume that all hosts have the same sampling time points, which is often unrealistic in clinical settings. In addition, some of these methods do not account for the temporal order of the samples and therefore can not extract the underlying dynamics and can not transfer the learned low-dimensional representation from training data to unseen test data. Another relevant class of models are the multivariate functional models that can depict feature trajectories. However, they are unsuitable for dimensionality reduction or managing unknown structures in hosts[8]. An alternative to analyzing longitudinal microbiome data is by using supervised methods, which are focused on generative models inferring the dynamics of these communities (e.g., generalized Lotka Volterra)[9–12]. Another example is the mixed-effect models which are widely used to quantify intra-host variation, but typically analyze one microbial feature at a time with limited ability to model temporal patterns[13]. While these methods account for the correlation structure induced by repeated measures as well as for sparsity and compositionality, their output does not directly allow clustering of phenotypes by microbial community dynamics.

Here, we introduce TEMPoral TEnsor Decomposition (TEMPTED), an unsupervised dimensionality reduction tool for high-dimensional temporal data with flexible temporal sampling. TEMPTED formats longitudinal microbiome data into an order-3 temporal tensor with subject,

feature, and continuous time as its three dimensions. The tensor is then decomposed into a summation of low-dimensional components, each consisting of a subject loading vector, a feature loading vector, and a temporal loading function (**Fig. 1**). These loadings provide time-informed beta-diversity analysis at both the sample and subject levels, and identify corresponding microbial signatures whose temporal trends can aid in discerning host phenotypes. TEMPTED also enables the transfer of the learned low-dimensional representation from training data to unseen test data. TEMPTED is unique in that it accounts for the temporal order of samples as a continuous variable, without assuming identical temporal sampling across hosts. It is able to retain hosts with missing time points and prevents bias introduced by imputing missing samples or binning time points. It is the only dimensionality reduction method currently available for temporal data that offers this flexibility. These unique properties enable accurate host phenotype classification in data-driven simulations, where otherwise classification was impossible. Further, using TEMPTED we uncover previously undetectable microbial dynamics separating mice with leukemia from healthy ones and pregnancies ending in preterm and term birth.

## Results

We evaluated TEMPTED's ability to perform phenotype discrimination using two data-driven simulations. The first simulation is based on the ECAM dataset[4], which sampled the gut microbiome of infants delivered vaginally versus by C-section during the first two years of life (**Fig. S1**). This dataset was chosen due to previously observed differences in longitudinal trajectories between delivery modes[3,4]. The second simulation is based on Fettweis et. al[6], which longitudinally sampled the vaginal microbiome during pregnancy (**Fig. S3**) in women who delivered term and preterm. This dataset was chosen due to its highly variable temporal sampling across hosts, its use of metagenomics sequencing, and previously observed differences between pregnancies resulting in term and preterm births[6,11]. We evaluated TEMPTED and alternative computational methods on their ability to differentiate host phenotypes based on microbial dynamics at the subject level through classification precision-recall and the sample level through PERMANOVA F-statistic (**Methods**).

These evaluations were performed across random subsets of samples from each subject in order to simulate sparse and unaligned temporal sampling to assess the impact of different sampling densities. First, at the subject level, we evaluated TEMPTED as compared to CTF, since methods like PCoA, including Bray-Curtis, Unifrac, and Weighted Unifrac are limited to sample-level analysis. TEMPTED reduces the precision-recall error of host-phenotype classification from 0.5, a level that is close to classifying at random (equivalent to AUC-PR accuracy=0.5), to 0.1 (equivalent to AUC-PR accuracy = 0.9, **Fig. 2a**). Of note, in performing this classification task, TEMPTED is transfering the learned low-dimensional representation from training to test data and performing out-of-sample prediction, while CTF is only able to perform in-sample inference (**Fig. 2ab; Methods**). Second, at the sample level, we evaluated TEMPTED as compared to existing methods, where TEMPTED enabled a 2-fold improvement in phenotype differentiation across all sampling densities(**Fig. 2b,c**). Furthermore, when

temporal sampling is highly variable across hosts, existing methods did not outperform TEMPTED even when using more time points (**Fig. 2c**).

To examine the performance of TEMPTED on real data, we applied it to three publicly available datasets. First, we used a mice study of Acute Lymphoblastic Leukemia (ALL), the most common form of childhood cancer with high genetic predisposition[3]. Fecal microbiome samples were longitudinally sampled from wildtype and predisposed (Pax5+/1 genotype) mice that were raised in a specific pathogen-free environment and transferred to conventional facilities at early adulthood to resemble children's transition into kindergarten[3] (**Fig. S2**). While existing methods could successfully recover mouse genotype from the microbiome (wild type vs. Pax5+/-), they failed to consistently predict the onset of ALL from microbial profiles (**Fig. S5, S6**). In contrast, TEMPTED was the only method that distinguished healthy mice from those that developed ALL while associating the difference to specific ASVs (**Fig. 3a-c, S7, S8, Table S1, S2, Supp Table 1**). TEMPTED also clearly separated genotypes while associating the difference with microbial features (**Fig. 3c**). As there is currently no reliable biomarker for ALL onset, this finding might facilitate lead time for therapy. Furthermore, TEMPTED could correlate disease onset to just 12 out of 1065 ASVs. Several of the ALL onset associated ASVs have been associated previously with leukemia and other cancers in mice, in particular those classified in the *Acetatifactor* genus[14–16].

Next, we also applied TEMPTED to two independent studies that longitudinally sample the vaginal microbiome during pregnancy: one study sequenced shotgun metagenomics and used metagenome-assembled genomes (MAGs)[4,17] and while the second sequenced 16S rRNA genes and used ASVs[4,5] (**Fig. S3, S4**). Remarkably, TEMPTED consistently captured differences in the vaginal microbiome trajectories of pregnancies that resulted in term vs. preterm birth in both datasets. Notably, this differentiation between term and preterm births is based on the dynamics of 8 leading microbial features in each study with a significant overlap (**Fig. 3e, 3h**), which can potentially serve as a biomarker to detect preterm birth early in pregnancy (**Fig. 3f, 3i**). Of note, the dynamics of these features is required in order to differentiate between term and preterm birth as this separation was not found when performing differential abundance analysis using ALDEx2[18] at any single time point (**Supp Table 2-3; Methods**). The identified leading ASVs and MAGs both associate *Lactobacillus spp.* and specifically *Lactobacillus crispatus* with term birth, and associate *Gardnerella, Megasphaera, and Atopobium vaginae* with preterm birth (**Fig. 3e, 3h**), consistent with existing literature[4,17,19]. Overall, our results highlight the robustness of TEMPTED in multiple studies and sequencing technologies in uncovering microbial dynamics underlying host phenotypes.

## Discussion

TEMPTED was designed to address an important unmet need in the rapidly evolving field of microbiome research – namely unsupervised dimensionality reduction for longitudinal microbiome data that account for the temporal order of samples and accommodates varying temporal sampling schemes and missing values. By leveraging temporal structures shared across hosts, TEMPTED is a computationally efficient tool that facilitates beta-diversity analysis

at both sample and subject levels and allows the transfer of the learned low-dimensional representation from training data to unseen datasets.

We demonstrated the utility of TEMPTED using data-driven simulations and real data applications. First, using data-driven simulations from two different ecosystems (the infant gut microbiome and the vaginal microbiome during pregnancy) we showcase that TEMPTED outperforms existing methods by a large margin in the ability to discern phenotype differences. Second, using three publicly available datasets, we showcase that TEMPTED uncovers biological signals that go undetected by existing methods and performs robustly in multiple data types (e.g. 16S and metagenomic sequencing). The microbial features identified by TEMPTED are strong indicators of phenotype differentiation and thus can be leveraged in the design of microbiome-based biomarkers. Finally, its flexibility in accommodating varying temporal sampling, and ability in transferring the learned low-dimensional representation from training to testing data, make it a highly practical tool for microbiome research, promoting research reproducibility.

Despite its many strengths, users of TEMPTED should also be aware of several limitations. Like other unsupervised dimensionality reduction methods such as PCA, PCoA, and CTF, TEMPTED does not guarantee the capture of the phenotype of interest in its leading components or a single component. Nevertheless, TEMPTED has a flexible model setting that accommodates a wide range of high-dimensional temporal data, making it a valuable and powerful tool for research beyond longitudinal microbiome studies.

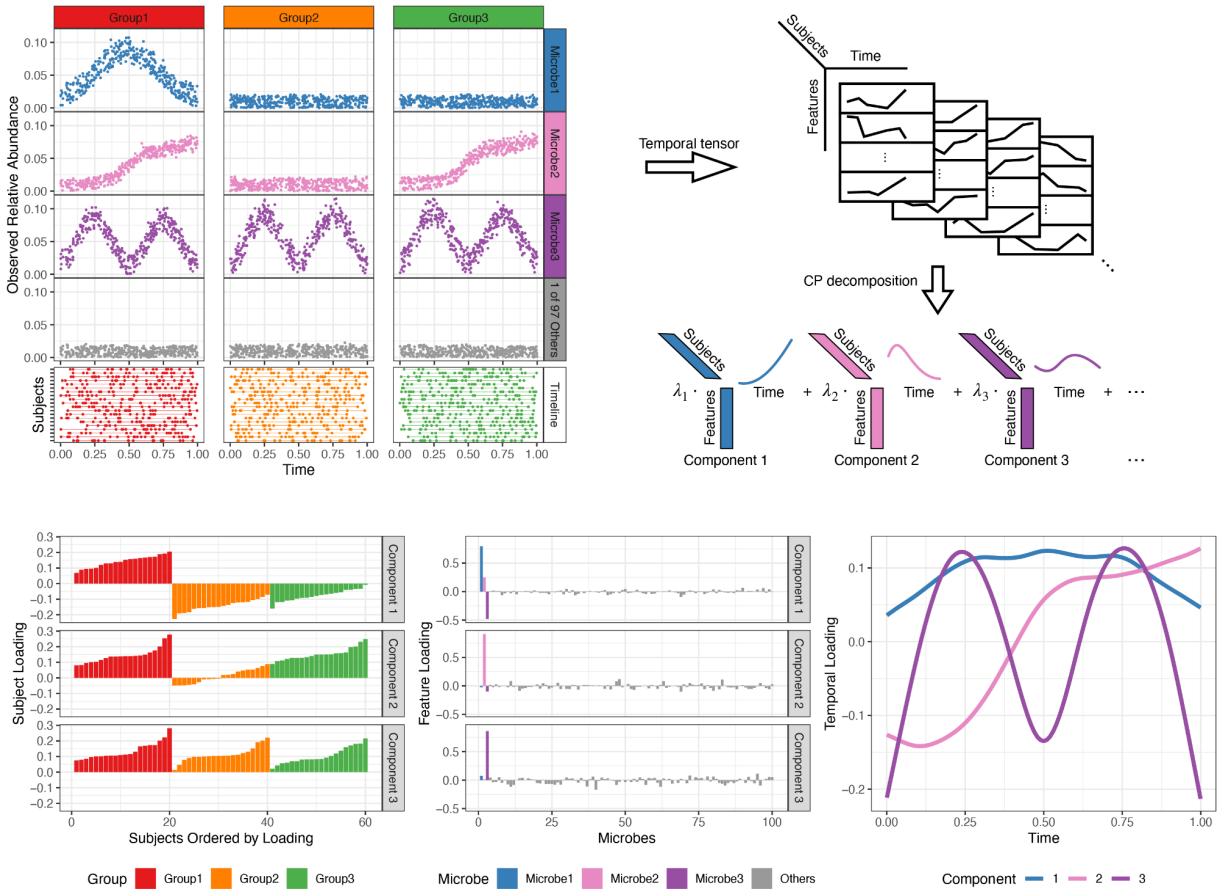

**Figure 1. Overview of the TEMPTED algorithm for analyzing multi-subject multi-feature temporal microbiome data. (a)** Simulated microbiome count data is transformed into relative abundance and plotted for four representative taxa and three groups of hosts. Taxon 1 has a unique temporal pattern for Group 1, Taxon 2 has a temporal pattern shared by Groups 2 and 3, Taxon 3's temporal pattern is shared by all groups, and Taxa 4-100 have no temporal patterns. Sampling time points are uniformly distributed and used as is without binning. **(b)** The observed multi-subject multi-feature temporal data is formatted into a temporal tensor with three modes representing subject, feature, and time, respectively. TEMPTED reduces the dimension of the temporal tensor by decomposing it into a small number of components, each of which contains a subject loading vector, a feature loading vector, and a temporal loading function. **(c-e)** TEMPTED loadings of the first three components from the simulated data. The first component captures the bell-shaped trend of Taxon 1 and separates Group 1 from Groups 2 and 3. The second component captures the increasing trend of Taxon 2 and separates Group 2 from Groups 1 and 3. The third component captures the m-shaped trend of Taxon 3 and does not separate any of the groups. Taxa 4-100 have low feature loadings in all components.

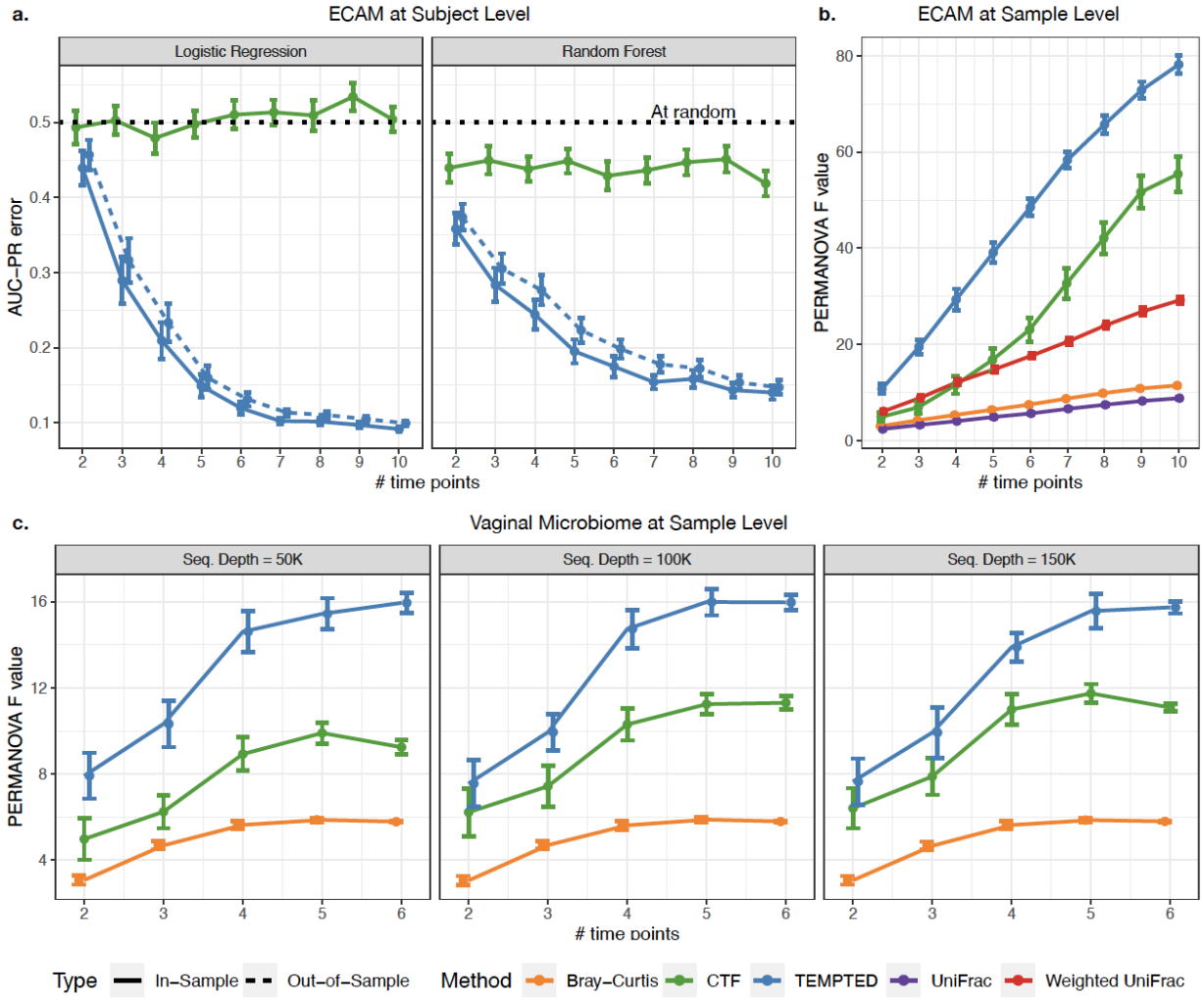


**Figure 2. TEMPTED outperforms CTF and PCoA in identifying group structures using popular distance metrics.** Subject-level group classification error is quantified by 1-the area under the precision-recall curve of logistic regression and random forest. TEMPTED reduces the precision-recall error of host-phenotype classification from 0.5, a level that is close to classifying at random (equivalent to AUC-PR accuracy=0.5), to 0.1 (equivalent to AUC-PR accuracy=0.9) **(a)**. Sample-level group discriminatory power is quantified by PERMANOVA pseudo F-statistic (**b, c**). Dimension reduction is performed in-sample and out-of-sample respectively, and group labels are predicted using leave-one-out for logistic regression and out-of-bag for random forest. We evaluate the performance of these methods on two datasets: the ECAM infant fecal microbiome data **(a, b)**, which distinguishes between infants delivered vaginally (N-subject=24) and by cesarean section (N-subject=18); and the vaginal microbiome dataset **(c)**, which distinguishes between pre-term (N-subjects=35) and term (N-subject=111) births. Error bars represent 1.96 standard errors of the mean. Time points are not identical across subjects for both studies, and are treated as is for TEMPTED, while CTF bins them to months. The first two components of CTF and TEMPTED were used. We obtain different numbers of time points per subject by randomly choosing from existing samples, and different sequencing depths by resampling reads in each sample.

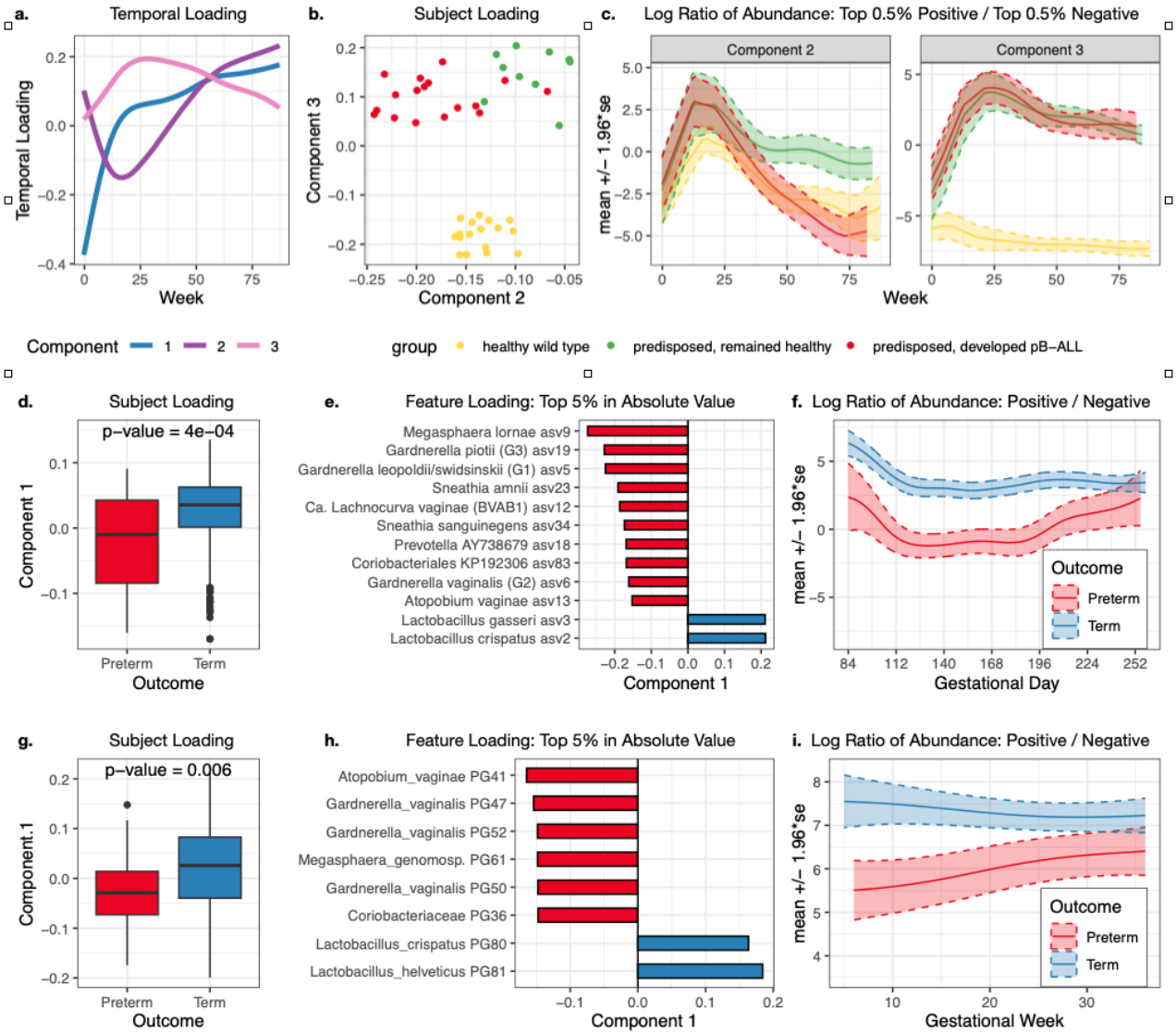


**Figure 3. TEMPTED reveals latent trajectories distinguishing health phenotypes. (a)-(c)** show the results of applying TEMPTED to a mouse study on leukemia (17 wild-type mice, 27 Pax5 mice of which 17 developed leukemia)[3]. **(a)** Temporal loadings capture major temporal patterns in the central-log-ratio-transformed microbial abundance. **(b)** Subject loadings separate genotypes in Component 3 and disease statuses in Component 2. **(c)** Log ratio of the total abundance of the top 0.5% ASVs over the bottom 0.5% ASVs, where 1065 ASVs are ranked by feature loadings of Components 2 (left) and Component 3 (right), respectively. The log ratio from Component 2 diverges between disease statuses for Pax5 mice at around 40 weeks after antibiotic treatment. The log ratio from Component 3 shows distinct temporal patterns between genotypes. **(d-f)** Results of applying TEMPTED to ASVs in the vaginal microbiome during pregnancy (141 term and 49 preterm)[4,5]. **(g-i)** Results of applying TEMPTED to MAGs in the

vaginal microbiome during pregnancy (111 term and 35 preterm)[4,17]. **(d,g)** Subject loadings separating pregnancies resulting in term and preterm deliveries in Component 1. P-value is from Wilcoxon rank sum test. **(e,h)** Top ASVs and MAGs ranked by the absolute value of feature loadings of Component 1. Features with negative and positive loadings are associated with preterm birth and term birth, respectively. **(f,i)** Log ratio of the abundance of the ASVs with top positive loadings and top negative loadings in (e,h), separating pregnancies resulting in term and preterm deliveries.

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# Methods

## Data Preprocessing

Let $i = 1, \ldots, n$ denote subjects, $j = 1, \ldots, p$ denote features, and $\boldsymbol{\mathcal{Y}}_{ijt}$ denote the observed read count of feature $j$ from subject $i$ at time points $t \in T_i = \{t_{i1}, \ldots, t_{im_i}\}$, where $T_i$ is a subset of interval $T$ and can be different across subjects. `TEMPTED` allows users to choose their own preferred data normalization and transformation. Here, to account for the variation in sequencing depth across samples and the highly skewed distribution of microbiome sequencing data, we apply the centered-log-ratio (CLR) transformation to read counts added by .5 [1, 7]

$$\tilde{\boldsymbol{\mathcal{Y}}}_{ijt} = \log\left(\frac{\boldsymbol{\mathcal{Y}}_{ijt} + .5}{\left(\prod_{\tilde{j}=1}^{p}(\boldsymbol{\mathcal{Y}}_{i\tilde{j}t} + .5)\right)^{1/p}}\right).$$

Because some bacteria taxa are much more abundant than others across most time points, when such a time-invariant structure is not of interest, we offer an optional step to extract and remove this structure from $\tilde{\boldsymbol{\mathcal{Y}}}$ to improve the efficiency of our algorithm. Specifically, we average $\tilde{\boldsymbol{\mathcal{Y}}}_{ijt}$ across $t$, to form matrix $\bar{\boldsymbol{\mathcal{Y}}} = (Y_{ij\cdot})$, calculate the first singular component of matrix $\bar{\boldsymbol{\mathcal{Y}}}$, and subtract the resulting rank-1 approximation of $\bar{\boldsymbol{\mathcal{Y}}}$ from $\tilde{\boldsymbol{\mathcal{Y}}}_t$ for each $t$. This optional step reduces the effect of highly abundant bacteria taxa and other time-invariant factors that may confound downstream analysis.

## `TEMPTED` algorithm

We adopt the model setting proposed in [5], which decomposes the temporal tensor formed by $\tilde{\boldsymbol{\mathcal{Y}}}_{ijt}$ using an approximately CP low-rank structure. However, while [5] assumes $T_i$ to be identical across all subjects, TEMPTED's algorithm can accommodate the more common scenario of varying temporal sampling $T_i$ across subjects. Our objective is to minimize the following function:

$$\min_{a_i, b_j, \xi} \sum_{i=1}^{n} \sum_{j=1}^{p} \sum_{t \in T_i} \left(\tilde{\boldsymbol{\mathcal{Y}}}_{ijt} - \sum_{\ell=1}^{r} \lambda_\ell \hat{a}_i^{(\ell)} \hat{b}_j^{(\ell)} \hat{\xi}^{(\ell)}(t)\right)^2$$

$$\text{subject to } \sum_{i=1}^{n} (a_i^{(\ell)})^2 = \sum_{j=1}^{p} (b_j^{(\ell)})^2 = \int \left(\xi^{(\ell)}(t)\right)^2 \mathrm{d}t = 1, ||\xi^{(\ell)}(t)||_{\mathcal{H}} \leq C_\xi,$$

where $r$ is the number of low-rank components to approximate the data tensor $\tilde{\boldsymbol{\mathcal{Y}}}$, $a^{(\ell)} = (a_1^{(\ell)}, \ldots, a_n^{(\ell)})$ are subject loadings for subject-level beta analysis, $b^{(l)} = (b_1^{(l)}, \ldots, b_p^{(l)})$ are feature loadings that can be used to construct sample-level beta analysis and to identify features contributing to the beta analysis, $\xi^{(l)}(t)$ is the temporal loading that captures the shared temporal patterns among subjects and features, and $\lambda$ quantifies the contribution of

each component. Here, $\|\xi(t)\|_{\mathcal{H}^2}$ is the *reproducing kernel Hilbert space (RKHS) norm* of function $\xi(t)$ with the rescaled Bernoulli polynomial as the reproducing kernel,

$$\mathbb{K}(s,t) = 1 + k_1(s)k_2(t) + k_2(s)k_2(t) - k_4(|s-t|),$$

where $k_1(s) = s - .5$, $k_2(s) = (k_1^2(s) - 1/12)/2$, and $k_4(s) = (k_1^4(s) - k_1^2(s)/2 + 7/240)/24$. This kernel guarantees $\xi(t)$s to be absolutely continuous and squared-integrable in its second-order derivative (see [5] for details). $C_\xi$ is a tuning parameter to control the smoothness of $\xi(t)$s.

For each component $\ell = 1, \ldots, r$ sequentially, we perform the following Steps 1 to 3 to estimate $a, b$, and $\xi(t)$.

Step 1: (Initialization) Initialize $\hat{a} = (1/\sqrt{n}, \ldots, 1/\sqrt{n})$. Set $\hat{b}$ as the first left singular vector of mode-2 matricization of $\tilde{\boldsymbol{\mathcal{Y}}}$: i.e., the $p$-by-$(\sum_{i=1}^n |T_i|)$ matrix with $\{\tilde{\boldsymbol{\mathcal{Y}}}_{i\cdot}(t) \in \mathbb{R}^p\}_{1=i\ldots,n,t\in T_i}$ as its columns.

Step 2: (Estimation of loadings) To estimate the loadings, we minimize the following function by iteratively updating $\hat{\xi}$, $\hat{a}$, and $\hat{b}$ respectively until convergence:

$$\sum_{i=1}^n \sum_{j=1}^p \sum_{t\in T_i} \left(\tilde{\boldsymbol{\mathcal{Y}}}_{ijt} - \eta a_i b_j \xi(t)\right)^2 + C_\lambda \|\xi\|_{\mathcal{H}}^2. \tag{1}$$

(a) Update $\hat{\xi}$ by applying kernel ridge regression [5] to solve

$$\hat{\xi} = \arg\min_{\xi\in\mathcal{H}} \sum_{i=1}^n \sum_{j=1}^p \sum_{t\in T_i} \left(\tilde{\boldsymbol{\mathcal{Y}}}_{ijt} - \hat{a}_i \hat{b}_j \xi(t)\right)^2 + C_\lambda \|\xi\|_{\mathcal{H}}^2, \tag{2}$$

and normalize $\hat{\xi}$ to $\hat{\xi} = \hat{\xi} / \left(\int \hat{\xi}(t)^2 \mathrm{d}t\right)^{1/2}$.

(b) Update $\hat{a} = (\hat{a}_1, \ldots, \hat{a}_n)$ by

$$\hat{a}_i = \frac{\sum_{j=1}^p \sum_{t\in T_i} \tilde{\boldsymbol{\mathcal{Y}}}_{ijt} \hat{b}_j \hat{\xi}(t)}{\sum_{t\in T_i} \hat{\xi}(t)^2}, \quad \text{and } \hat{a} = \hat{a}/\|\hat{a}\|_2. \tag{3}$$

(c) Update $\hat{b} = (\hat{b}_1, \ldots, \hat{b}_p)$ by

$$\hat{b}_j = \frac{\sum_{i=1}^n \sum_{t\in T_i} \tilde{\boldsymbol{\mathcal{Y}}}_{ijt} \hat{a}_i \hat{\xi}(t)}{\sum_{i=1}^n \sum_{t\in T_i} (\hat{a}_i \hat{\xi}(t))^2}, \quad \text{and } \hat{b} = \hat{b}/\|\hat{b}\|_2. \tag{4}$$

Step 3: (Subtracting previous components) Update $\tilde{\boldsymbol{\mathcal{Y}}}$ by

$$\tilde{\boldsymbol{\mathcal{Y}}}_{ijt} = \tilde{\boldsymbol{\mathcal{Y}}}_{ijt} - \hat{\eta} \hat{a}_i \hat{b}_j \hat{\xi}(t),$$

where $\hat{\eta} \in \mathbb{R}$ is obtained by solving the following least squares problem:

$$\arg\min_{\eta} \sum_{i=1}^n \sum_{j=1}^p \sum_{t\in T_i} \left(\tilde{\boldsymbol{\mathcal{Y}}}_{ijt} - \eta \hat{a}_i \hat{b}_j \hat{\xi}(t)\right).$$

Step 4: $\lambda = (\lambda_1, \ldots, \lambda_r)$ is estimated via the following least squares problem:

$$\widehat{\lambda} = \underset{\lambda}{\operatorname{argmin}} \sum_{i=1}^{n} \sum_{j=1}^{p} \sum_{t \in T_i} \left( \boldsymbol{\mathcal{Y}}_{ijt} - \sum_{\ell=1}^{r} \lambda_\ell \hat{a}_i^{(\ell)} \hat{b}_j^{(\ell)} \hat{\xi}^{(\ell)}(t) \right)^2 .$$

## Sample-Level Beta Diversity and Subject-Specific Trajectories

The feature loading $b_j^{(\ell)}$ quantifies the contribution of Feature $j$ to Component $\ell$. They can be used as the weights vector to aggregate the trajectory of all $p$ features into the following subject-specific trajectory corresponding to Component $\ell$:

$$\boldsymbol{\mathcal{B}}_{it}^{(\ell)} = \sum_{j=1}^{p} b_j^{(\ell)} \tilde{\boldsymbol{\mathcal{Y}}}_{ijt}. \tag{5}$$

Here, the vector $\left(\boldsymbol{\mathcal{B}}_{it}^{(1)}, \cdots, \boldsymbol{\mathcal{B}}_{it}^{(r)}\right)$ also serves as an $r$-dimensional representation of Sample $t$ from Subject $i$, which can be used for sample-level beta analysis.

For microbiome studies specifically, subject-specific trajectories can be constructed using the log-ratio abundance of top over bottom ranking features sorted by feature loading:

$$\boldsymbol{\mathcal{B}}_{it}^{(\ell)} = \log \left( \left( 0.5 + \sum_{j: b_j^{(\ell)} \text{ in top quantile}} \boldsymbol{\mathcal{C}}_{ijt} \right) / \left( 0.5 + \sum_{j: b_j^{(\ell)} \text{ in bottom quantile}} \boldsymbol{\mathcal{C}}_{ijt} \right) \right).$$

where $\boldsymbol{\mathcal{C}}_{ijt}$ is the read count of Feature $j$ in the $t$th sample of Subject $i$.

## Transfer of Dimension Reduction to New Data

Let $\boldsymbol{\mathcal{Y}}_{\text{train}}$ and $\boldsymbol{\mathcal{Y}}_{\text{test}}$ be two datasets consisting of the same features measured within the same time frame $[0, T]$. Suppose that `TEMPTED` decomposes $\boldsymbol{\mathcal{Y}}_{\text{train}}$ into $r$ components, where the $\ell$th component has subject loading $a_{\text{train}}^{(\ell)}$, feature loading $b_{\text{train}}^{(\ell)}$, and temporal loading $\xi_{\text{train}}^{(\ell)}$. Assuming that $\boldsymbol{\mathcal{Y}}_{\text{train}}$ and $\boldsymbol{\mathcal{Y}}_{\text{test}}$ share the same feature loading and temporal loading, we can estimate the subject loading of $\boldsymbol{\mathcal{Y}}_{\text{test}}$ (i.e., $a_{\text{test}}^{(\ell)}$) through one iteration of Step 2 of the TEMPTED algorithm, with $b_{\text{train}}^{(\ell)}$ and $\xi_{\text{train}}^{(\ell)}$ plugged in. Since the dimension reduction is performed without any knowledge of $\boldsymbol{\mathcal{Y}}_{\text{test}}$, classifiers trained by $a_{\text{train}}^{(\ell)}$ have no information from the testing data. The prediction of phenotypes for the testing data by such classifiers is purely out-of-sample, and its accuracy is evaluated through data-driven simulation (Figure 2 dashed lines).

## Case Study: Pax5 Mice Leukemia Data

The Pax5 dataset was published by [8] and deposited at `https://qiita.ucsd.edu/study/description/11953`, artifact ID 75878. The dataset we used here consists of fecal samples

collected from 17 wild type and 27 Pax5 heterozygous mice that were treated with antibiotics at the beginning of the experiment, and 17 Pax5 mice developed leukemia during the study. Samples were subjected to 16S V4 short-read Illumina sequencing. Raw reads are deposited at ENA with accession PRJEB34720. Please refer to [8] for detailed sequence processing methods. Note that the original publication contains further amplicon sequence data and PacBio long read data not used here. Samples with $< 35000$ reads are removed. Mice with $< 2$ time points after filtering are removed. Time points were recorded as days from the end of antibiotic treatment and were divided by 7 to obtain weeks. `TEMPTED` uses ASVs appearing in $> 5\%$ of all samples and `CTF` uses ASVs appearing in $> 10\%$ of all samples.

## Case Study: Shotgun Metagenomic Vaginal Data

The dataset used in this study was collected and published by [4] and deposited under dbGaP (study no. 20280; accession ID phs001523.v1.p1). It comprises a total of 705 vaginal samples obtained from 175 pregnant women visiting maternity clinics in Virginia and Washington. Among them, 40 women had preterm pregnancies, and the remaining 135 women had term pregnancies. To ensure data quality, subjects with less than two time points and MAGs that appeared in less than 5% of all the samples were removed from the analysis. For the time-specific analysis we used ALDEx2 with gestational weeks 5-36. A test was performed on weeks with at least 2 term and 2 preterm subjects.

## Case Study: 16S Vaginal Data

The 16S data used in this study were published by [3] and [4], and were deposited under BioProjects PRJNA393472 (subjects enrolled at the University of Alabama, Birmingham) and PRJNA821262 (subjects enrolled at Stanford University). Preprocessed data were obtained from `https://purl.stanford.edu/pz745bc9128`. We focus specifically on the second- and third-trimester pre-delivery vaginal samples from 141 term pregnancies and 49 pre-term pregnancies. Samples with $< 40000$ reads were removed, and ASVs (amplicon sequence variants) appearing in $\leq 5\%$ of all the samples were removed as well. For time-specific analysis using ALDEx2, to ensure enough sample size at each time point, gestational days were floored to gestational weeks. No test was performed on Week 36 because it only contains three preterm subjects.

## Data Driven Simulation

The ECAM dataset was published by [2] (Qiita ID 10249). Preprocessed data were obtained from `https://codeocean.com/capsule/6494482/tree/v1`. Time points were recorded in days, used as is for `TEMPTED`, and rounded into months for analysis using the `CTF` method. Months 15 and 19 were removed due to their small number of samples for `CTF`. Operational taxonomic units (OTUs) appearing in $\leq 5\%$ of the remaining samples were removed, as were samples with $< 2000$ reads. Subjects with only one time point were also removed, leaving

24 vaginally delivered infants and 18 Cesarean-delivered infants in the analysis. TEMPTED removes OTUs appearing in $\leq 5\%$ of the remaining samples in its analysis.

The vaginal microbiome dataset used for the simulation is the same as the metagenomic vaginal microbiome data in the case study. TEMPTED removes metagenome-assembled genomes (MAGs) appearing in $\leq 5\%$ of the samples in its analysis.

## Software Usage

PERMANOVA was implemented using R package `vegan`. Logistic regression and random forest were implemented using R package `stats` and `randomForest`, respectively. AUC-ROC was calculated using R package `PRROC`. ALDEx2 was performed using R package `ALDEx2` (1.32.0). CTF was performed using Python plugin gemelli (0.0.8).

## Code Availability

TEMPTED is compiled into an R package that can be installed from `https://github.com/pixushi/tempted`. All data analysis and simulation are performed using R and Python scripts through R package `reticulate`. All processed data and codes used for this paper are available on `https://github.com/pixushi/TEMPTED_paper`.

# Supplementary

## Details of kernel ridge regression to update $\xi(\mathbf{t})$

By the presenter theorem [6], the solution of (2) must be a linear combination of $\mathbb{K}(\cdot, s)$ for $s \in \cup_{i=1}^{n} T_i$, and the weight of this linear combination can be solved by kernel ridge regression. Specifically, we introduce the concatenated observation vector and kernel matrix:

$$\tilde{y} = (y_{1,1,T_1}, y_{2,1,T_2}, \ldots, y_{n,1,T_n}, \ldots, y_{1,p,T_1}, y_{2,p,T_2}, \ldots, y_{n,p,T_n})^\top \in \mathbb{R}^{p(|T_1|+\cdots+|T_n|)};$$

$$\tilde{K} = \begin{bmatrix} \mathbb{K}(T_1, T_1) & \cdots & \mathbb{K}(T_1, T_n) \\ \vdots & \ddots & \vdots \\ \mathbb{K}(T_n, T_1) & \cdots & \mathbb{K}(T_n, T_n) \end{bmatrix} \in \mathbb{R}^{(|T_1|+\cdots+|T_n|)\times(|T_1|+\cdots+|T_n|)};$$

$$\widetilde{AB} = \begin{bmatrix} b_1^{(t)} \cdot diag\left(\overbrace{a_1^{(t)}}^{|T_1|\text{times}}, \ldots, \overbrace{a_n^{(t)}}^{|T_n|\text{times}}\right) \\ b_2^{(t)} \cdot diag\left(\overbrace{a_1^{(t)}}^{|T_1|\text{times}}, \ldots, \overbrace{a_n^{(t)}}^{|T_n|\text{times}}\right) \\ \vdots \\ b_p^{(t)} \cdot diag\left(\overbrace{a_1^{(t)}}^{|T_1|\text{times}}, \ldots, \overbrace{a_n^{(t)}}^{|T_n|\text{times}}\right) \end{bmatrix} \in \mathbb{R}^{p(|T_1|+\cdots|T_n|)\times(|T_1|+\cdots+|T_n|)}.$$

Minimizing (1) is equivalent to

$$\min_{\alpha \in \mathbb{R}^{|T_1 \cup \cdots \cup T_n|}} \left(\tilde{y} - \widetilde{AB}\tilde{K}\alpha\right)^2 + \lambda \alpha^\top \tilde{K}\alpha,$$

which can be solved by

$$\hat{\alpha} = (\widetilde{AB}^\top \widetilde{AB}\tilde{K} + \lambda I)^{-1} \widetilde{AB}^\top \tilde{y}, \quad \hat{\xi}(t) = \alpha^\top \left(\mathbb{K}(T_1, s), \ldots, \mathbb{K}(T_n, s)\right)^\top.$$

Here, $I$ is the identity matrix, and $b$ will cancel out in $\widetilde{AB}^\top \widetilde{AB}$ because $||b||_2 = 1$.

## Toy Example

We simulated a dataset with 100 features and three groups of subjects, each containing 20 subjects. For each subject, we randomly selected 20 time points from uniform distribution between 0 and 1. We considered three temporal trends: $f_1(t) = \frac{1}{2}\sin(2\pi t - \frac{1}{2}\pi) + \frac{1}{2}$, $f_2(t) = \text{atan}(10t - 5)/\pi + \text{atan}(5)/\pi$, and $f_3(t) = \frac{1}{2}\sin(4\pi t - \frac{1}{2}\pi) + \frac{1}{2}$. For Group 1, we simulated the absolute abundance of the first feature from $f_1(t)$ perturbed by a uniform distribution

on (0, 0.5). For Groups 1 and 3, we simulated the absolute abundance of the second feature from $f_2(t)$ perturbed by a uniform distribution on (0, 0.25). For Groups 1, 2, and 3, we simulated the absolute abundance of the third feature from $f_3(t)$ perturbed by a uniform distribution on (0, 0.5). The absolute abundance of the remaining features was simulated from a uniform distribution on (0, 0.25). We then calculated the relative abundance and used them as proportion parameters in a multinomial distribution to generate counts. The sequencing depth was generated from an exponentially transformed normal distribution with a mean of 10 and standard deviation of 0.5, resulting in minimum, median, and maximum depths of 5K, 22K, and 113K, respectively.

## Temporal Sampling of Studies

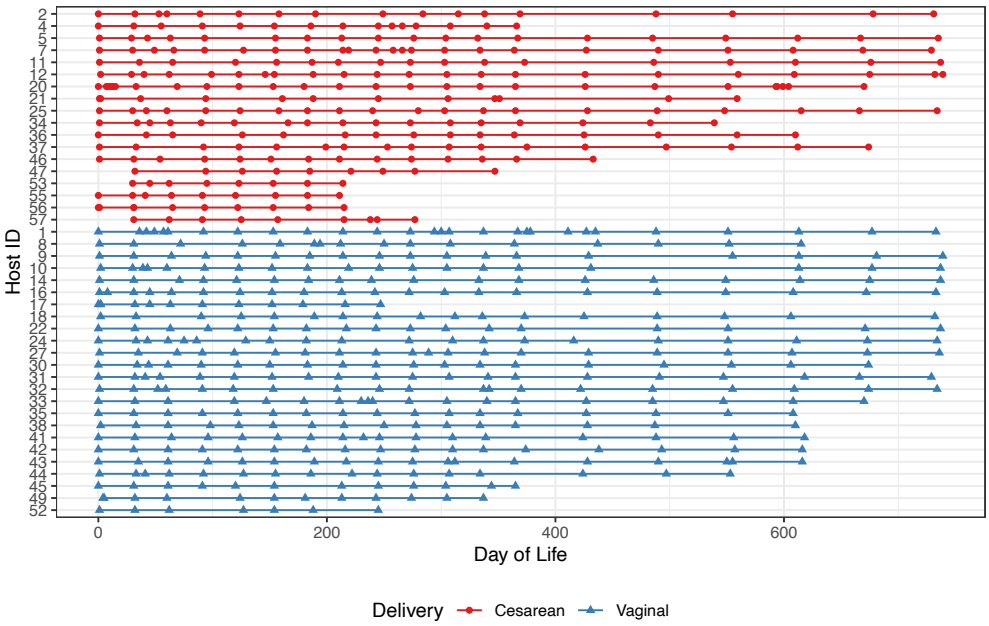


Figure 1: Temporal sampling of ECAM infant gut microbiome data

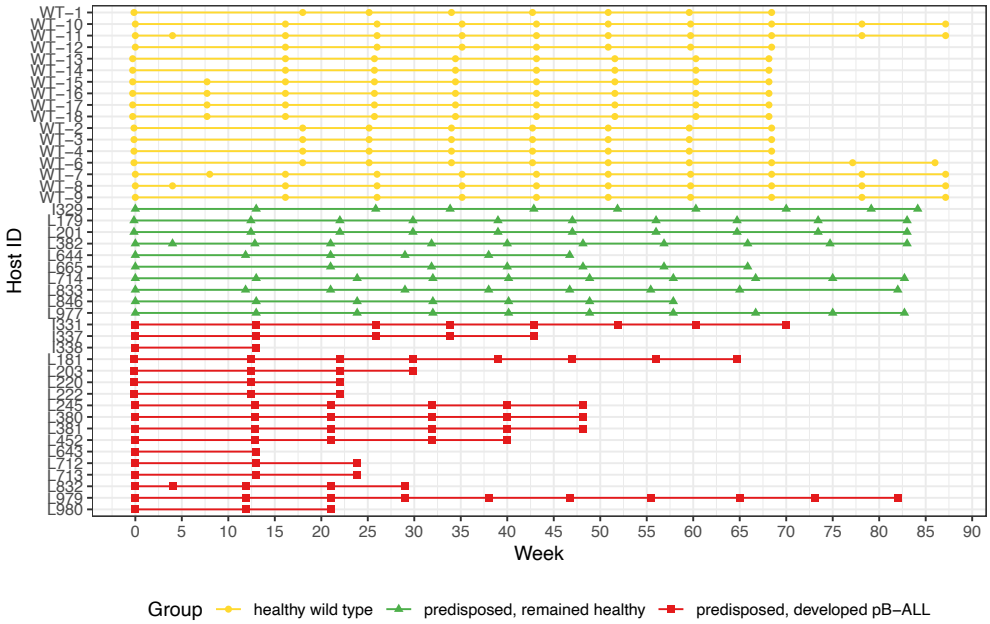


Figure 2: Temporal sampling of Pax5 mice leukemia data

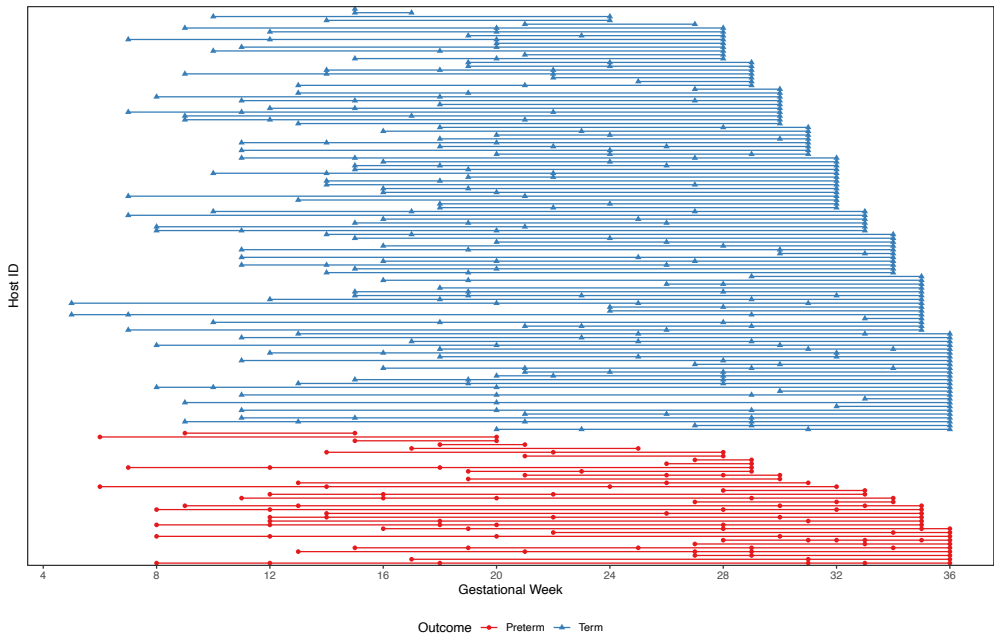


Figure 3: Temporal sampling of metagenomic vaginal microbiome data.

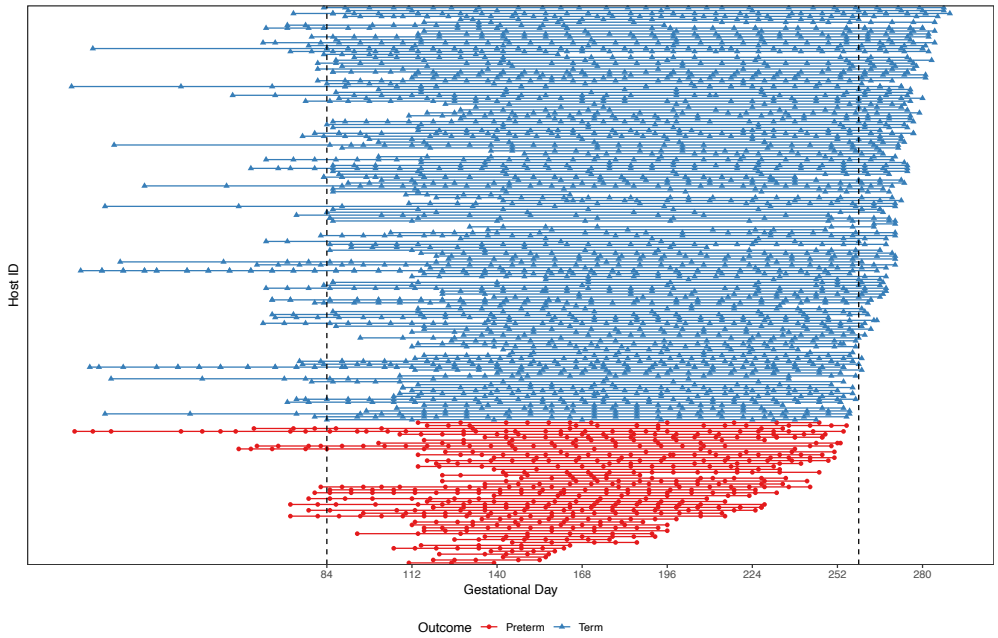


Figure 4: Temporal sampling of 16S vaginal microbiome data. Only second and third trimester samples, i.e. samples between the two dashed vertical lines, were used.

## Analysis of Pax5 Mice Leukemia Data with Other Methods

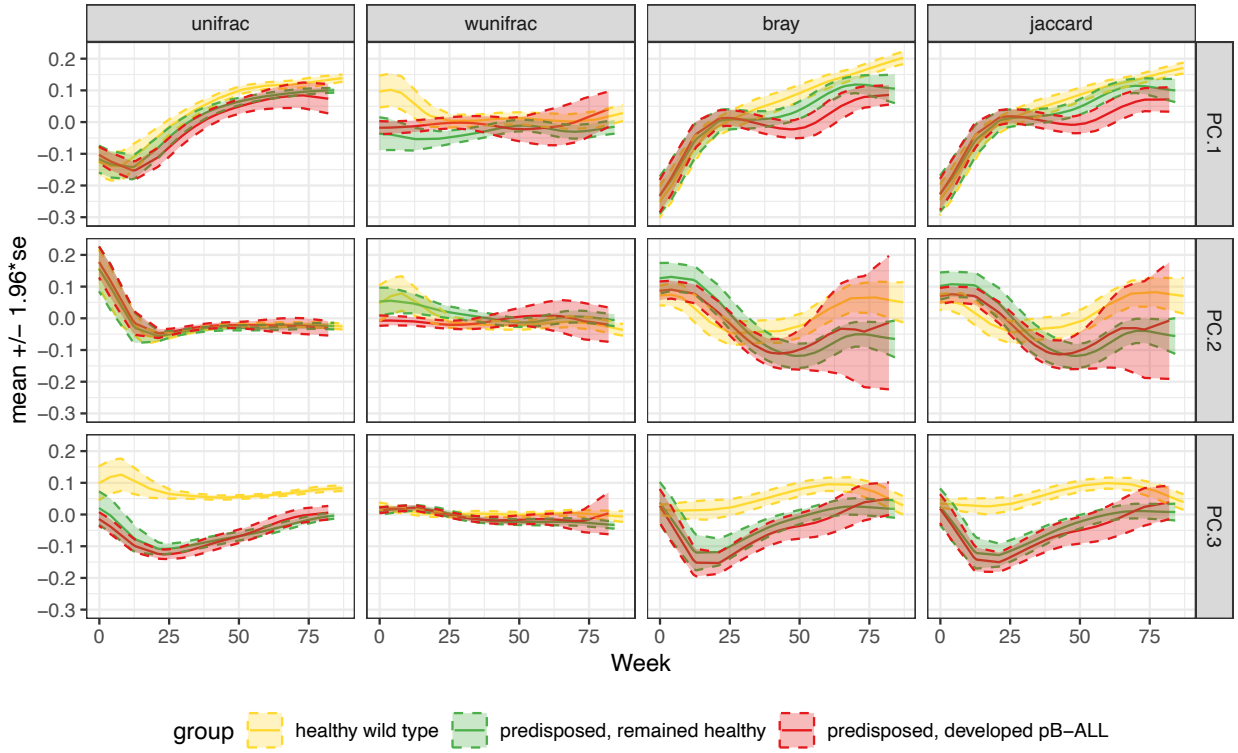


Figure 5: Trajectories of first three PCoA components for various distance metrics on the Pax5 mice leukemia data. Mean and error bands representing 1.96 standard errors of the mean are smoothed using kernel regression.

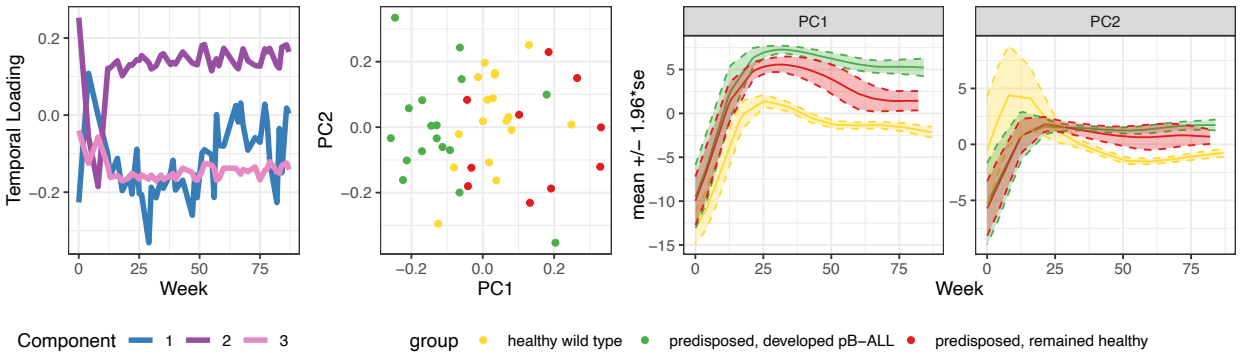


Figure 6: Results from `CTF` on the Pax5 mice leukemia data.

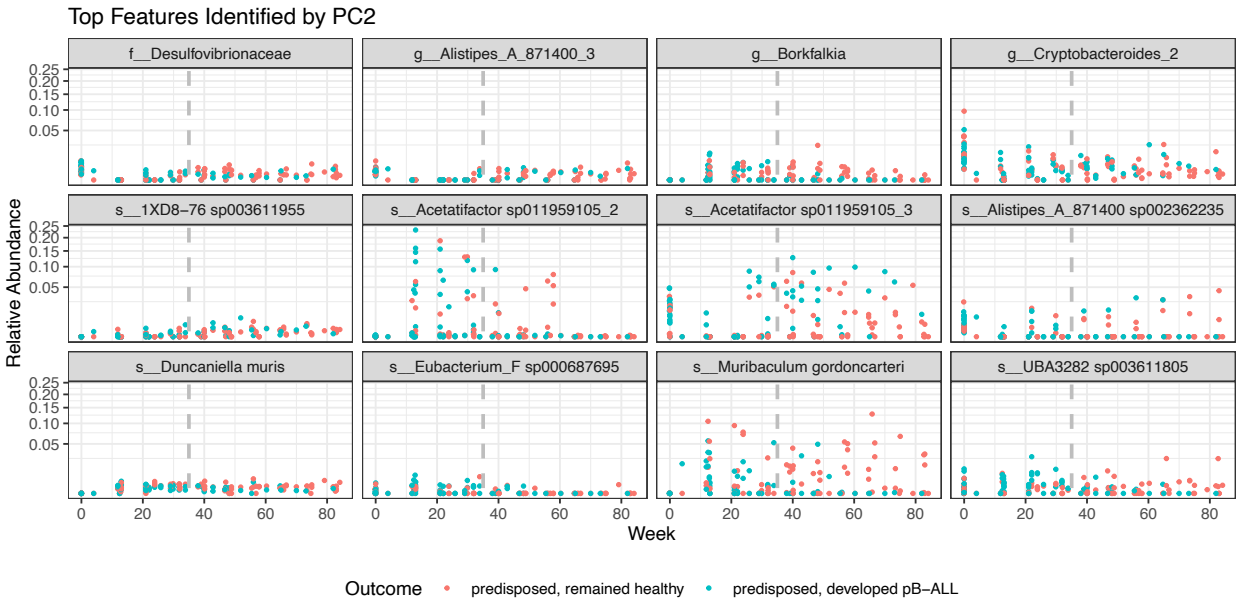


Figure 7: Relative abundance top ASVs identified by the second component of TEMPTED.

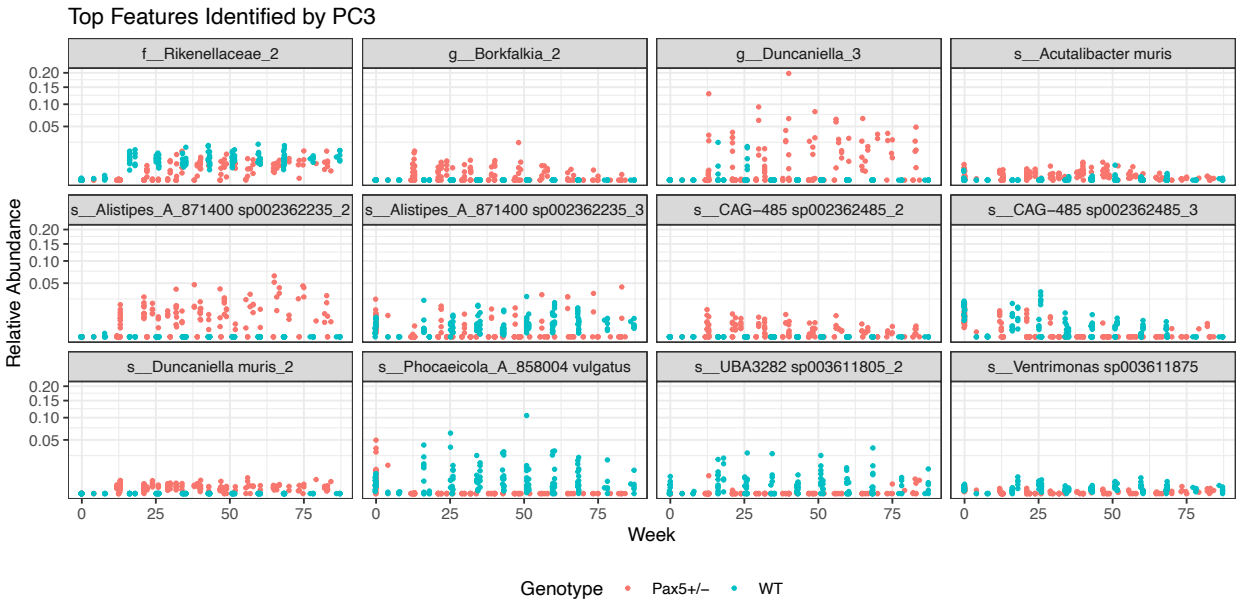


Figure 8: Relative abundance of top ASVs identified by the third component of TEMPTED.

| | bray | jaccard | unifrac | wunifrac | TEMPTED | CTF |
|---|---|---|---|---|---|---|
| p-value | 0.143 | 0.143 | 0.342 | 0.798 | 0.001 | 0.032 |
| F-value | 1.996 | 1.930 | 0.956 | 0.145 | 6.922 | 3.707 |

Table 1: PERMANOVA test between leukemia outcomes for 35-70 week samples among Pax5+/− mice

| Component | bray | jaccard | unifrac | wunifrac | TEMPTED | CTF |
|---|---|---|---|---|---|---|
| 1 | 0.007 | 0.006 | 0.135 | 0.672 | | 0.029 |
| 2 | 0.613 | 0.523 | 0.131 | 0.613 | 1.7e-5 | |
| 3 | 0.491 | 0.430 | 0.849 | 0.759 | | |

Table 2: Wilcoxon test between leukemia outcomes for 35-70 week samples among Pax5+/− mice for each component

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