The Phylogenetic LASSO and the Microbiome

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Abstract

Scientific investigations that incorporate next generation sequencing involve analyses of high-dimensional data where the need to organize, collate and interpret the outcomes are pressingly important. Currently, data can be collected at the microbiome level leading to the possibility of personalized medicine whereby treatments can be tailored at this scale. In this paper, we lay down a statistical framework for this type of analysis with a view toward synthesis of products tailored to individual patients. Although the paper applies the technique to data for a particular infectious disease, the methodology is sufficiently rich to be expanded to other problems in medicine, especially those in which coincident ‘omics’ covariates and clinical responses are simultaneously captured.

Key Words and Phrases: Bacteriology; Bioinformatics; Clostridium difficile Infection; Faecal Microbiota Transplantation; LASSO; Optimization; Oracle Properties; Phylogeny; Public Health; 16S rRNA.

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1 Introduction

The incorporation of the microbiome as a covariate in response to biological or clinical outcomes is becoming medically and scientifically important especially in understanding etiology and treatment of specific diseases. In addition, this aids in improved understanding of functional pathways such as the gut-brain axis, see for example [22] and [4]. Through sophisticated data capture methods, such as next generation sequencing, researchers are able to rapidly map the complex microbiome [20]. Nevertheless this line of research is challenging due to the shear high-dimensional nature of the microbiome, as well as the ultra-high $p$ and small $n$ problem. An added complexity is the manner in which the covariates are associated with each other. In terms of the ‘tree-of-life’ schematic, bacterial groups at different taxon levels have an associated phylogeny. Recent works incorporating taxonomic information use various methods to identify important taxonomic features, [6], [23], [32], [13], and [24]. Common to all these papers is the incorporation of the underlying microbiology to establish sophisticated computational schemes over and above the already demanding computational methods required to obtain the microbiome data. Due to the complexity of these methods, validation is established mainly through simulations. In this paper we profile a variable selection method that also incorporates the tree-of-life schema, similar in scope to the above papers, but different in terms of how the regularization is carried out. Extensive simulations are performed but we also obtain theoretical oracle properties for our method, which to our knowledge, is very novel for microbiome research.

We now provide a summary of the paper. As motivation, Section 2 provides the microbiology background to this paper. Although we are addressing metagenomics through next generation sequencing of the 16S rRNA gene, the methodology is sufficiently rich to incorporate other ‘-omics’ structures. In Section 3 we provide the development of our penalization of the phylogenetic tree. An application to an infectious disease is discussed in Section 4. We include a detailed discussion because this infectious disease is currently a very important public health concern, and because it is the main motivation behind the development of the methods in this paper. Further detailed simulation results are presented in Section 5 with comparisons made to other variable selection procedures. This is followed by Section 6 which establishes the main theoretical properties with all of the proofs provided in Appendix A.
2 A microbiology primer

Metagenomics, like the other ‘-omics’, bears a structural relationship between covariates. Bacteria exhibit a tree-like relationship with each other, often violated via lateral gene transfer. As a result, their systematic taxonomy is constantly in flux; see for instance the paraphyletic Clostridium \[28, 17\]. Thus their OTU (operational taxonomic unit) proxies, typically the 16S rRNA gene, also exhibit a tree-like structure with patterns of cycles at deep taxon classification. Our resolution depends on the length of the 16S rRNA region, the degree of lateral gene transfer, and the reliability of the reads.

In many problems, it is important to develop a means of selecting the OTUs having dominating roles in the microbial systems at hand. The relationship between OTUs is such that some of them may represent the same type of bacteria. Alternatively, some spurious OTUs are artifacts of the laboratory sequencing protocol. Rather than select OTUs on their individual merits, we want to select them based on their group affiliations.

Precisely identifying and characterizing bacteria currently requires genetic and metabolic analysis of clonal colonies in vitro. In studying the composition of microbiomes in vivo, we require a general descriptor. Absent of molecular characterization, there is no general descriptor of bacterial groups, a property which constrained bacterial research for decades. A broader analysis of mixed bacterial communities is available, sacrificing the resolution of clonal studies. This involves the collection and sequencing of genes common to all bacteria, or to the group of interest. This sequencing may be scaled up to generate billions of sequences in tandem. We refer to this as MPS (massively parallel sequencing). For an in depth description from a mathematical perspective, see \[19\].

In the present paper, we consider MPS data targeting the V3-V5 region of the 16S rRNA gene, whose merits we discuss in \[9\]. Crucially, this is a gene common to all bacteria with sufficient evolutionarily conserved variability to provide a basis for defining bacterial groups \[29\], albeit not the sole defining descriptor. MPS technologies are currently incapable of providing the full 16S sequence, so a hypervariable region is targeted, of which there are nine. Hypervariable is understood as evolutionarily conserved but more variable than the average over the entire sequence. The various hypervariable regions provide differing resolutions in different bacterial groups. An analysis of the precision of each region for identifying pathogenic bacteria is presented in \[3\]. The V3-V5 regions provide estimates similar to the full 16S gene in terms of species richness \[30, 33\], and it has been shown
to provide accurate community structure and low bias estimates of some taxa \[1, 26\]. A limitation is its poor species level resolution, as found in \[3\].

To facilitate comparison between microorganisms, sequences are grouped into OTUs according to some similarity criterion. This is a data-driven proxy to species delineation; we use OTUs in an operational manner. OTUs consist of sequences which are phylogenetically close. Phylogenetic proximity between OTUs is determined by the inter-OTU phylogenetic divergence of the constituent sequences. This provides a structural relationship between the OTUs. One manner of presenting this structure is to assign phylotypes to the OTUs, sequence-based consensus taxonomical classification of the clusters. This induces a rooted tree hierarchy between the OTUs. This structure is exploited in the present article.

3 Generalized linear models and the Φ–LASSO

In this section, we provide details of what we call the phylogenetic LASSO (least absolute shrinkage and selection operator). As motivation, let us review the hierarchial H-LASSO, presented in \[34\] in the context of penalized least-squares. Consider the linear model

\[ Y = X\beta + \epsilon, \]  

for response \( Y \), covariates \( X \), parameter vector \( \beta \in \mathbb{R}^p \), and error \( \epsilon \). Suppose the covariates \( X \) may be assigned to \( k \) mutually exclusive groups \( K_j, j = 1, \ldots, k \). Let \( \beta_j \in \mathbb{R}^{p_j} \) be the subvector of \( \beta \) whose coefficients correspond to the covariates in \( K_j \), \( |K_j| = p_j \), where \(|\cdot|\) denotes set cardinality. We have \( p = \sum_{j=1}^{k} p_j \).

We can decompose \( \beta \) by \( \beta_j = d_j \alpha_j \) where \( d_j \geq 0 \) and \( \alpha_j \in \mathbb{R}^{p_j} \). Clearly this decomposition is not unique, but this does not ultimately matter, see Lemma \[6.2\] below. Let \( d = (d_1, \ldots, d_k) \in \mathbb{R}^k \), \( \alpha = (\alpha_1, \ldots, \alpha_k) \in \mathbb{R}^p \), and define \( \varphi \) as the mapping given by \( (d, \alpha) \mapsto \beta \).

We define the H-LASSO estimator \( \hat{\beta} \) of \( (3.1) \) via \( \hat{\beta} = \varphi(\hat{d}, \hat{\alpha}) \) where \( (\hat{d}, \hat{\alpha}) \) maximizes the penalized least squares function

\[
-\frac{1}{2} \sum_{i=1}^{n} (Y_i - X_i \cdot \varphi(d, \alpha))^2 - \lambda_1 \sum_{j=1}^{k} d_j - \lambda_2 \sum_{j=1}^{k} ||\alpha_j||_1,
\]

where the tuning parameters \( \lambda_1, \lambda_2 > 0 \) are fixed, \( \cdot \) denotes dot product, and \( ||\cdot||_q \) is the usual \( l_q \)-norm for \( q > 0 \). Here we penalize the groups \( K_j \) by the middle term \( \lambda_1 \sum_{j=1}^{k} d_j \) and the individual coefficients by the third term \( \lambda_2 \sum_{j=1}^{k} ||\alpha_j||_1 \).
It is shown in [34] that the penalty values $\lambda_1, \lambda_2$ redistribute geometrically so that we may replace them by a common coefficient $\lambda = \sqrt{\lambda_1 \lambda_2}$. This leads to the result that their regularization is equivalent to maximizing

$$-\frac{1}{2} \sum_{i=1}^{n} (Y_i - X_i \cdot \beta)^2 - 2\lambda \sum_{j=1}^{k} \sqrt{||\beta_j||_1}. $$

The H-LASSO is thus a nonconcave variant of the group LASSO, [31].

While [34] directly treats least squares, their results generalize readily to arbitrary likelihood functions which possess an attractive oracle property, see Theorem 2, [34]. Below, we provide the generalization to accommodate increased depth to the hierarchy by framing it in terms of a taxonomy.

### 3.1 The $\Phi$–LASSO

Our goal now is to create a hierarchical penalization scheme where there are multiple competing ways of grouping covariates. If these groupings have a nesting property, we can represent this as a tree, otherwise the graphical representation has cycles. In light of the above discussion, we use the convex log-likelihood function $\ell$ in lieu of least squares in the sequel. We frame variable selection in terms of OTU selection in metagenomic analysis, but stress that the method easily accommodates other ‘-omic’ data forms, be they from metabolomics, proteomics, and transcriptomics. Further, as life betrays the ability to transmit genetic information horizontally, we incorporate the option to use taxonomies as degenerate as the tree-of-life itself. In particular, this allows us to consider overlapping classifications of the variables, extending analysis to, say, active metabolic pathways of microbial systems or human tissues. We introduce some terminology and notation to help bridge the mathematics and systematics at hand.

**Definition 3.1** Let $X_1, ..., X_d$ be the $d$ column vectors of the design matrix $X \in \mathbb{R}^{n \times p}$. A taxon $\tau$ (plural taxa) is a subset of the indices $I = \{1, ..., p\}$ and $X_\tau = [X_j]_{j \in \tau}$ is the corresponding submatrix of $X$. A taxon level is a collection of pairwise disjoint taxa $\tau_k, k = 1, ..., K$ whose union is $I$.

Consider Table 1 below, as an example. We find that taxa Bacilli = $\{3, 4, 5, 6, 7, 8\}$ and Enterococcaceae = $\{3, 4, 5\}$. The collection $\{$Actinobacteria, Bacilli, Clostridia$\}$ is a taxon level. In general, we subdivide the indices into taxa at $T + 1$ taxon levels. We denote the $k$-th taxon of the $t$-th taxon level by $\tau^t_k$ or $\tau_{tk}^t$. 
| Index | Phylum          | Class          | Order           | Family          | OTU     |
|-------|----------------|----------------|-----------------|-----------------|---------|
| 1     | Actinobacteria | Actinobacteria | Bifidobacteriales | Bifidobacteriaceae | OTU_1   |
| 2     | Actinobacteria | Actinobacteria | Bifidobacteriales | Bifidobacteriaceae | OTU_2   |
| 3     | Firmicutes     | Bacilli        | Lactobacillales | Enterococcaceae  | OTU_3   |
| 4     | Firmicutes     | Bacilli        | Lactobacillales | Enterococcaceae  | OTU_4   |
| 5     | Firmicutes     | Bacilli        | Lactobacillales | Enterococcaceae  | OTU_5   |
| 6     | Firmicutes     | Bacilli        | Lactobacillales | Lactobacillaceae | OTU_6   |
| 7     | Firmicutes     | Bacilli        | Lactobacillales | Lactobacillaceae | OTU_7   |
| 8     | Firmicutes     | Bacilli        | Lactobacillales | Lactobacillaceae | OTU_8   |
| 9     | Firmicutes     | Clostridia     | Clostridiales   | Clostridiaceae   | OTU_9   |
| 10    | Firmicutes     | Clostridia     | Clostridiales   | Clostridiaceae   | OTU_10  |
| 11    | Firmicutes     | Clostridia     | Lachnospirales  | Lachnospiraceae  | OTU_11  |
| 12    | Firmicutes     | Clostridia     | Lachnospirales  | Lachnospiraceae  | OTU_12  |
| 13    | Firmicutes     | Clostridia     | Lachnospirales  | Lachnospiraceae  | OTU_13  |

Table 1: An example taxonomy generated for thirteen OTUs using five taxon levels.

**Definition 3.2** Suppose we have a collection $\mathcal{T}$ of $(T+1)$ taxon levels, where the $(T+1)$-th taxon level consists of singletons, $\{\{j\}\}_{j=1}^d$. We call $\mathcal{T}$ a taxonomy.

There will be times where we wish to refer to those indices that belong to specific taxa at each level. We have:

**Definition 3.3** Let $L = (\tau^1, ..., \tau^T)$ be a $T$-tuple where taxon $\tau^t$ belongs to taxon level $t$. We refer to $L$ as a lineage, with associated indices $J = \cap_{t=1}^T \tau^t$. We write $X_L$ in lieu of $X_J$ and define $|L| := |J|$. When we wish to make it clear we are referring to a lineage’s taxon at a particular taxon level, we write $L^t$, so that $L$ may be re-written $L = (L^1, ..., L^T)$.

In our example, the lineages may be read directly off Table 1, of which there are five. Figure 1 presents the taxonomy more intuitively. Each branch of the tree represents a taxon while each horizontal row represents a taxon level, numbered 1 through 5. Each lineage is a directed path from the root to a branch.

Consider a generalized linear model with a known link function $g$, so that $E(Y) = g^{-1}(X\beta)$. We decompose $\beta$ by $\beta_L = d_L\alpha_L$, where $\alpha_L \in \mathbb{R}^{|L|}$ as before, but now $d_L = \prod_{t=1}^T d_{L^t}$, $d_{L^t} \geq 0$ for $t = 1, ..., T$. We write this decomposition as $(D, \alpha)$. Let $\varphi$ be the map $(D, \alpha) \mapsto \beta$. This decomposition is illustrated in parentheses in Figure 1 below: the coefficients $d_L$ are recovered by multiplying the terms in a lineage. We extend the optimization criterion to:

$$\ell(\varphi(D, \alpha); Y, X) - \sum_{t=1}^T \lambda_t \sum_{k=1}^{K_t} d_{L^t_k} - \lambda_{T+1} ||\alpha||_1$$

(3.2)

where as usual $\ell$ is the log-likelihood, and $\lambda_t > 0$ for $t = 1, ..., T + 1$. The centre double sum is the groups penalty and addresses the increased depth of our taxonomy in contrast.
Figure 1: A graphical representation of the taxonomy as well as an illustration of the decomposition of the parameters $\beta$ following the taxonomy in Table 1.

to $[34]$ and $K^t$ is the number of taxa in taxon level $t$. When the lineage consists of a single taxon level $T = 1$, this reduces to the criterion in $[33]$.

We appear to suffer an affluence of tuning parameters. Lemma 6.1 below, shows that criterion (3.2) is equivalent to the single tuning parameter criterion

$$
\ell(D, \alpha; Y, X) - \sum_{t=1}^{T} \sum_{k=1}^{K^t} d_k^t - \lambda ||\alpha||_1 .
$$

(3.3)

Let $d^t$ denote the vector of groups coefficients of the $t$-th taxon level. Intuitively, as the tuning parameters $\lambda_t$ are redistributed, $D$ and $\alpha$ change in geometric response due to the relationship $\varphi(D, \alpha) = \beta$. Choosing $\lambda = \lambda_t$, $t = 1, ..., T + 1$, we need only concern ourselves with one tuning parameter.

### 3.2 $\Phi$–LASSO algorithm

The algorithm used to obtain the $\Phi$–LASSO estimate relies on iterative adaptive reweighting, deriving from the reformulation of the $\Phi$–LASSO in (3.3).

Let $\psi : \beta \mapsto (D, \alpha)$ be the map from $\beta$ to the unique maximizer of (3.3) over $\varphi^{-1}(\beta)$. In this way $D$ and $\alpha$ may be viewed as projections onto the first and second elements of $\varphi^{-1}(\beta)$. Let $\Lambda$ be a positive collection of tuning parameters $\lambda$. The pseudo-code is spelled out below. We remark that convergence is typically achieved quickly, so that the bottlenecks are the weighted LASSO problem and calculation of $w$. 
Algorithm 1 $\Phi-$LASSO

procedure phylasso
  for each $\lambda \in \Lambda$ do
    obtain initial LASSO estimate $\hat{\beta}^0(\lambda)$
    for $k \in \mathbb{N}$ do
      $w = \varphi(D(\hat{\beta}^{(k-1)}(\lambda), 1))$
      solve weighted LASSO for $\hat{\beta}^k(\lambda)$ with weights $w^{-1}$
      break if $\Delta \hat{\beta}^\lambda < \text{threshold}$
    end for
  end for
end procedure

4 Application to Clostridium difficile Infection

Clostridium difficile (C. difficile) infection (CDI) is the most frequent cause of healthcare-associated infections and its rates are growing in the community [14, 2]. One of the major risk factors for developing CDI is use of antibiotics. The healthy and diverse bacteria which reside within the colon are the major defense against the growth of C. difficile. Antibiotics kill these bacteria and allow C. difficile to multiply, produce toxins and cause disease. The available treatments for this infection are the antibiotics: metronidazole, vancomycin and fidaxomicin. The efficacy of these antibiotics is limited with high recurrence rates, [7].

An alternative to antibiotic therapy for CDI, in particular for recurrent and refractory diseases, is to infuse healthy gut bacteria directly into the colon of infected patients to combat C. difficile by a procedure known as faecal microbiota transplantation (FMT). FMT is a process in which a healthy donor’s stool is administered to an affected patient. This can be performed using a colonoscope, nasogastric tube, or enema. FMT serves to reconstitute the altered colonic flora, in contrast to treatment with antibiotic(s), which can further disrupt the establishment of key microbes essential in preventing recurrent CDI. The literature reveals a cumulative clinical success rate of over 90% in confirmed recurrent CDI cases, [7].

There is a growing interest in the microbiome of CDI patients following an FMT [22, 8, 25, 27, 21]. It is noted that there are vast differences in: the route of administration with all forms covered; donor selection criteria, some used family members, while others used universal donors; sample sizes, although all studies had small sample sizes; and sequencing procedures and equipment. Despite these differences, there are two fundamental points of agreement across all studies. The first is that CDI patients have low diversity in their microbiome, pre-FMT, and that after receiving an FMT(s), their diversity increased. The
second is CDI patients who were successfully cured with FMT undergo changes in their microbiome which initially have similarities to that of their donors, see [22].

We will examine the microbiome data coming from a subset of the CDI-patients treated with FMT by the second author [10], covering the period 2008–2012.

From the 17 selected patients, we note that 13 of these patients responded to a single FMT. We used a taxonomy describing phylum, class, order, family, and genus. The predictors consisted of the relative abundances of 220 pre-FMT OTUs and 347 post-FMT OTUs. Here we consider logistic regression to model the response for two scenarios. First we wish to know whether the pre-FMT microbiome could predict a clinical response to an FMT. Second, we wish to know whether the composition of the post-FMT microbiome could anticipate the need for additional FMTs. To select the tuning parameter for the $\Phi$-LASSO, we perform leave-one-out cross-validation (LOO-CV). The optimal tuning parameter was selected by AUC (area under the curve) and BS (Brier score). This is the same dataset where phylum interaction was investigated, [15].

We are challenged by a sparse predictor matrix as well as a small sample size. Since AUC tends to assign a perfect score to the null model by the way it handles ties, we incorporate BS to compensate for this affect. The results of logistic regression using the pre-FMT OTUs as covariates are captured in Figure 2. Using the globally optimal BS of 0.208, the corresponding tuning parameter is $\lambda = 0.0067$. Relative to the BS, the locally optimal AUC value is 0.846, with the corresponding tuning parameter of $\lambda = 0.0049$. The globally optimal AUC value is 0.865 which would be associated with the null model. Below the figure in Table 2, we display the family and genus of the selected OTUs. Using LOO-CV, the frequency along with the averaged estimate and LOO-CV standard errors are reported. Due to the small sample size the variability in the parameter estimates are large which is to be expected. Concentrating on the frequency using LOO-CV we notice that OTU 7 associated with the family Lactobacillaceae and the corresponding genera Lactobacillus seem to have some positive predictability. This appears to be consistent with some earlier findings that the last author was involved in using a different FMT patient cohort, [22]. We note that some family/genera identification appear to be the same for different OTUs. This in interpreted to mean differences at the species taxon.

For the post-FMT OTUs, we obtain a globally optimal BS of 0.386, $\lambda = 0.0056$, with corresponding AUC value 0.923, $\lambda = 0.0065$. The globally optimal AUC is 0.961. This is displayed in Figure 3. In Table 3 we display the family and genus of the selected OTUs. Using LOO-CV, the frequency along with the averaged estimate and LOO-CV standard
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Table 2: The selected OTUs for pre-FMT microbiomes at the tuning parameters selected by AUC ($\lambda = 0.0049$) and BS ($\lambda = 0.0067$) leave-one-out cross-validation on relative abundances.

| OTU | Family      | Genus  | Frequency | $\hat{\beta}$ (SE) | Frequency | $\hat{\beta}$ (SE) |
|-----|-------------|--------|-----------|---------------------|-----------|---------------------|
| 1   | Enterobacteriaceae | Klebsiella | 0.24 | 10.8 (41.6) | 0.35 | -4.65 (14.7) |
| 2   | Enterobacteriaceae | Escherichia/Shigella | 0.99 | -49.4 (122.7) | 0.88 | -107 (143) |
| 3   | Streptococcus | Streptococcus | 0.92 | -304 (122.7) | 0.76 | -295 (143) |
| 4   | Lachnospiraceae | Blautia | 0.82 | 239 (281) | 0.18 | 20.4 (66.6) |
| 5   | Enterococaceae | Enterococcus | 0.41 | -94.9 (137.1) | 0.65 | -164 (264) |
| 6   | Lactobacillaceae | Lactobacillus | 0.53 | -15.5 (25.2) | 0.82 | -29.9 (29.6) |
| 7   | Lactobacillaceae | Lactobacillus | 0.88 | 349 (335) | 0.88 | 357 (415) |
| 11  | Lachnospiraceae | unclassified | 0.53 | -60.9 (77.5) | 0.59 | -68.6 (85.1) |
| 13  | Veillonellaceae | Veillonella | 0.29 | 78.5 (127) | - | - |
| 24  | Veillonellaceae | unclassified | 0.65 | -108 (141) | - | - |
| 30  | Veillonellaceae | Veillonella | 0.76 | 176 (258) | - | - |
| 33  | Veillonellaceae | Veillonella | - | - | 0.53 | 55.6 (10) |

Errors are reported. Again due to the small sample size the variability in the parameter estimates are large. Concentrating on the frequency using LOO-CV we notice that OTU 5 associated with the family Enterococaceae and the corresponding genera Enterococcus, as well as OTU 17 corresponding to the family Bacteroidaceae and genera Bacteroides appear to have some positive predictability. This again is consistent with some earlier results, [22].

We realize that the interpretation of these results from a bacteriological perspective is well beyond the scope of this medium, and the purpose of this section is to present a portrayal of the type of statistical analysis that comes from looking at the microbiome as a covariate. We highlight above some of the more obvious interpretations that is consistent with what has been observed in other studies. Indeed, results of this work will interest researchers and companies working toward refinement of FMT, via establishment of central stool banks or creation of synthetic stool. With results obtained using larger sample sizes it will be possible to select species that come from the genera identification and culture them in a laboratory biochemistry setting. Thus one could strategically pool samples to achieve a desired composition, to supplement stool with specific microorganisms or to be able to prepare recipient-specific synthetic stool as per the RePOOPulate project, [16]. At this point however, we will end this discussion with the comment that a more in-depth metageonomic analysis of a recent clinical trial, [11], is under investigation.
Figure 2: AUC and BS obtained by leave-one-out cross-validation for pre-FMT OTUs. Labeled are the tuning parameters by (a) AUC ($\lambda = 0.0049$) and (b) BS ($\lambda = 0.0067$).
Figure 3: AUC and BS obtained by leave-one-out cross-validation for post-FMT OTUs. Labeled are the tuning parameters by (a) AUC ($\lambda = 0.0065$) and (b) BS ($\lambda = 0.0056$).

| OTU | Family     | Genus       | Frequency $\hat{\beta}$ (SE) | Frequency $\hat{\beta}$ (SE) |
|-----|------------|-------------|------------------------------|------------------------------|
| 1   | Enterobacteriaceae | Klebsiella | 0.94 7.79 (28.8)             | 0.82 8.05 (28.9)             |
| 2   | Enterobacteriaceae | Escherichia/Shigella | 0.82 -17.6 (80.7)            | 0.94 -17.1 (80.9)            |
| 3   | Streplococcaceae | Streptococcus | 0.88 -22.6 (42.9)            | 0.94 -52.4 (82.4)            |
| 5   | Enterococcaceae | Enterococcus | 0.94 -98.5 (201)             | 0.94 -147 (275)              |
| 17  | Bacteroidaceae | Bacteroides | 0.94 109 (183)                | 0.94 111 (183)               |
| 21  | Acidaminococcaceae | Acidaminococcus | 0.65 -1.34 (1.85)            | 0.71 -15.6 (33.9)            |

Table 3: The selected OTUs for post-FMT microbiomes at the tuning parameters selected by AUC ($\lambda = 0.0065$) and BS ($\lambda = 0.0056$) leave-one-out cross-validation on relative abundances.
5 Simulations

In this section, we report on simulations that approximate the covariance structure we may see in practice with respect to phylogenetic proximity of the OTUs. We will consider $T = 5$ taxon levels where each level is balanced and grows according to $4^k$, $k = 0, 1, 2, 3, 4, 5, 6$. In taxonomy language we will consider a balanced taxonomy where we have a single ‘phylum’ ($k = 0$) followed by ‘class’ ($k = 1$), ‘order’ ($k = 2$), ‘family’ ($k = 3$), ‘genus’ ($k = 4$), and ‘species’ ($k = 5$). This will then lead to 4096 OTUs ($k = 6$).

To each taxon we introduce taxon-wise covariation. This covariance structure is presented in Figure 4 for a single ‘class’ (1024 OTUs) using a 10,000 point sample. The true parameters in the simulation are represented by two classes, with one class dominating the other 3:1.

We consider random Gaussian data generated from a 4096 covariate sparse linear model where 32 covariates have corresponding parameter $\beta_j = 2$ and the rest being zero. We present the corresponding pruned tree in Figure 5.

The species information is deliberately lost to simulate uncertainty in species assignment to OTUs, which mimics the current technology limitation in 16s rRNA sequencing, see [3]. Thus the deepest taxon level used in the fit is the genus level. We introduce a class, order, family, genus, and species-wise covariation $W_c$, $W_o$, $W_f$, $W_g$, and $W_s$, samples from normal distributions $N(0, 0.5^2)$, $N(0, 1^2)$, $N(0, 2^2)$, $N(0, 3^2)$, $N(0, 4^2)$, respectively, where $N(0, \sigma^2)$
denotes a normal distribution with mean 0 and variance $\sigma^2$. The predictors used are then defined as $X = (Z + W_p + W_c + W_f + W_g + W_s)/\sqrt{55.25}$ where $Z \sim N_{4096}(0, 5^2I_{4096})$ is a 4096 multivariate normal distribution with mean vector 0 and covariance matrix $I_{4096}$, the $4096 \times 4096$ identity matrix.

5.1 Tuning parameter selection

To select the tuning parameter for each $(n, \sigma)$ pair, we replicate 100 data sets $Y_i \sim N(X_i\beta, \sigma)$, $1 \leq i \leq n$. We select the tuning parameter $\lambda^{(n,\sigma)}$ minimizing across all models the MSPE (mean squared prediction error) for an independent 10,000 data point validation set generated from the same distribution. We also fit SCAD models using the same datasets, using the same validation set to select the appropriate tuning parameter.

5.2 Performance

To evaluate performance of our models, we consider four measures for 100 replicates: SSE (sum of squared error), MSPE, ‘recall’, and ‘precision’. Recall is defined as the proportion of covariates correctly selected relative to true parameters, $tp/(tp+fn)$, and precision is defined as the proportion of covariates correctly selected relative to total covariates selected, $tp/(tp+fp)$, where $tp$ is true positive, $fn$ is false negative and $fp$ is false positive.

We compare the performance of the $\Phi$-LASSO to SCAD. We also consider OLS (ordinary least squares) for the oracle model where the exact $\beta_j = 0$ is known. MSPE for performance is evaluated using a new 10,000 data point validation set generated independently of the tuning validation set.

5.3 Results: Tuning

Figure 6 presents recall and precision against the tuning parameters. Also displayed is the MSPE curve, scaled to the unit interval $[0, 1]$. Note that the selected parameters $\lambda$ lie in the middle of a relatively flat MSPE region. Perturbations of $\lambda$ would not overly affect
predictive performance. The same holds with respect to SSE (not shown). The shapes of the MSPE curves agree in large measure, aside from the null models, which have lower SSE than the sparsest estimators. Recall and precision are at odds, where for low sample size one must be sacrificed against the other. The selection of $\lambda$ favours higher recall over precision; the coefficients for false positives may be very small and hence affect prediction in a minor way, but false negatives are a complete loss of a structural signal. There is an interesting dip in recall for sample sizes $n \geq 100$ in the region preceding the low, stable MSPE region where the estimators overfit the data. The clear separation of these two regions reflects well on the $\Phi$-LASSO stability.

5.4 Results: Performance

The results for SSE and MSPE are presented in Table 5. SCAD struggles with the covariance structure, performing poorly in estimation. Incredibly, it appears to perform well in MSPE. While SCAD struggles to recall the correct OTUs due to high correlation within species, to its credit it is able to select some related taxa, where the covariance structure leads to similar predictive performance but more flexibility in fitting the model. The $\Phi$-LASSO exhibits none of the SCAD’s difficulties. It quickly converges to agreement with the oracle estimator in estimation and prediction.

The $\Phi$-LASSO quickly approaches near perfect recall as sample size increases whereas SCAD becomes stalled at 25%. The $\Phi$-LASSO consistently improves in precision, dropping false positives. For SCAD, after an initial improvement in precision, it drops as it selects incorrect but related OTUs. When we present the $\Phi$-LASSO and SCAD estimates with a validation set consisting of uncorrelated covariates, there is negligible change in $\Phi$-LASSO performance, while the SCAD’s predictive performance matched its poor estimation performance.

5.5 Additional simulations

We comment on some additional simulations. In the low dimensional, $p < n$, regime with simple sample-wise covariation structure, SCAD performs moderately better than the $\Phi$-LASSO, with the adaptive LASSO trailing behind. This is consistent across sample size and noise level. For alternative $p > n$ scenarios ($p = 1000$), the $\Phi$-LASSO performs well, although experiences some difficulty with precision for $n = 250$. This is qualitatively different than the issue with SCAD in the previous section, as recall is nearly perfect. The false positives generally correspond to relatively small coefficients, so that it appears to result from early exit from the fitting algorithm.
Figure 6: Median recall (solid) and precision (dashed) for $\Phi$-LASSO against $\log(\lambda)$ for sample sizes $n = 50, 100, 150$. Included is the median MSPE curve scaled by largest median (dotted). The ‘+’ indicates the chosen (log) tuning parameter.
Table 4: Estimation and prediction error for the oracle estimator (OLS), Φ-LASSO, and SCAD. Presented are the mean error (standard error).

| n  | SSE  | OLS    | Φ-LASSO | SCAD   | OLS    | Φ-LASSO | SCAD   |
|----|------|--------|---------|--------|--------|---------|--------|
| 50 | 88.10(46.2) | 167.00(62.50) | 621.00(230.00) | 3.20(1.21) | 14.80(17.2) | 167.00(162.00) |
| 100| 19.80(6.05)  | 37.70(12.00)  | 384.00(5.28)  | 1.47(0.14) | 2.43(0.48) | 9.24(0.56)  |
| 150| 11.10(3.69)  | 15.90(6.14)   | 383.00(4.47)  | 1.25(0.07) | 1.49(0.18) | 8.66(0.38)  |
| 200| 8.05(2.48)   | 10.70(4.29)   | 379.00(4.35)  | 1.18(0.05) | 1.29(0.10) | 8.40(0.24)  |
| 250| 6.25(1.77)   | 7.54(2.45)    | 380.00(3.36)  | 1.13(0.03) | 1.19(0.06) | 8.27(0.20)  |

Table 5: Recall and precision for the Φ-LASSO and SCAD. Presented are the mean (standard error).

| n  | Recall | Precision |
|----|--------|-----------|
|    | Φ-LASSO | SCAD      | Φ-LASSO | SCAD      |
| 50 | 0.64(0.11) | 0.18(0.07) | 0.52(0.12) | 0.50(0.34) |
| 100| 0.92(0.04) | 0.25(0.00) | 0.71(0.09) | 0.93(0.12) |
| 150| 0.98(0.02) | 0.25(0.00) | 0.86(0.07) | 0.75(0.16) |
| 200| 0.99(0.01) | 0.25(0.00) | 0.92(0.06) | 0.54(0.11) |
| 250| 0.99(0.01) | 0.25(0.00) | 0.95(0.04) | 0.56(0.11) |

6 Theoretical Results

In this section, we consider the general objective function,

\[ Q^*(\lambda_1, \ldots, \lambda_{T+1}, D, \alpha) = \ell(\varphi(D, \alpha)) - \sum_{t=1}^{T} \lambda_t ||D_t||^q_q - \lambda_{T+1} ||\alpha||^q_q \]

where \( \ell \) is the known but arbitrary log-likelihood, the remainder is the penalization, and \( || \cdot ||^q_q \) is the \( l_q \)-norm, \( q > 0 \). We will also make use of the notation ‘\(^\prime\)’ for matrix transpose, ‘\( \ll \)’, ‘\( \ll_p \)’ to mean ‘big oh’ and ‘big oh in probability’, respectively, \( o, o_p \) to mean ‘little oh’ and ‘little oh in probability’, respectively, ‘\( \asymp \)’ to mean the ratio of two sequences converge to a positive constant, ‘\( \sim \)’ to mean convergence in distribution, and ‘\( \wedge \)’ to mean minimum.

All proofs are provided in Appendix A.

The first result allows us to consider a single penalty parameter, that is, set \( \lambda_t = 1 \) for all \( t = 1, \ldots, T \). Let \( Q_1 = Q^*(\lambda_1, \ldots, \lambda_{T+1}, \cdot, \cdot) \), \( Q_2 = Q^*(1, \ldots, 1, \lambda_{T+1} \prod_{t=1}^{T} \lambda_t^{1/q}, \cdot, \cdot) \).

Lemma 6.1 (Equivalence of optimization) Let \( \lambda_t > 0, t = 1, \ldots, T + 1 \) be fixed. Then \( (D^1, \alpha^1) \) is a local maximizer of \( Q_1 \) if and only if \( (D^2 = (\lambda_t^{1/q} d_t^1), \alpha^2 = \alpha^1 / \prod_{t=1}^{T} \lambda_t^{1/q}) \) is a local maximizer of \( Q_2 \) and hence \( \varphi(D^1, \alpha^1) = \varphi(D^2, \alpha^2) \).
The proof is similar to that of Lemma 1 in [34], where least-squares with $l_1$-regularization is considered.

In the sequel we assume that $\lambda_t = \lambda$ for all $t = 1, ..., T + 1$. This next result provides a relationship between the individual effects parameters and the group coefficients. Intuitively, the mass distributes itself geometrically to minimize the penalty terms.

**Lemma 6.2 (Mass Equilibrium)** Let $(D, \alpha) \in \varphi^{-1}(\beta)$. Then $(D, \alpha)$ is a global maximizer of $Q^*$ over $\varphi^{-1}(\beta)$ if and only if $d_{\tau_k} = \sum_{L: L' = \tau_k} \|\alpha_L\|_q^q = \|\alpha_{\tau_k}\|_q^q$.

This conservation of mass result immediately leads to the following.

**Corollary 6.3** The relationship $d_L = \sum_{L: L' = \tau} \|\alpha_L\|_q^q$ from Lemma 6.2 identifies the unique maximizer of $Q^*$ over $\varphi^{-1}(\beta)$.

Thus $\beta_j$ is an order $T + 1$ polynomial in terms of $\alpha_1, ..., \alpha_p$.

**Definition 6.4** Let $\psi$ be the map $\beta \mapsto (D, \alpha)$ where $(D, \alpha)$ is the unique optimizer of $Q^*|_{\varphi^{-1}(\beta)}$. We refer to $\psi$ as the partial inverse.

**Corollary 6.5** Suppose the taxonomy has two levels, $T = 1$. Then the partial inverse $\psi(\beta) = (D, \alpha)$ is characterized by: if $\beta_L = 0$ then $d_L = 0$ and $\alpha_L = 0$, if $\beta_L \neq 0$ then $d_L = \sqrt{\|\beta_L\|_q}$ and $\alpha_L = \beta_L / \sqrt{\|\beta_L\|_q}$.

Corollary 6.5 is a generalization of Theorem 1 in [34].

### 6.1 LAN conditions and the Oracle

To ensure local asymptotic normality of the MLE for diverging number of parameters, we adopt the regularity conditions from [12] as in [34]:

(A1) For all $n$, the observations $(X_i, Y_i), i = 1, ..., n$, are independently and identically distributed according to the density $f_n(X_i, Y_i; \beta_n)$, where $f_n$ has common support and the model is identifiable. Further,

$$
E_{\beta_n} \left[ \frac{\partial \log f_n}{\partial \beta_{nL_j}} \right] = 0 \quad \forall L, j = 1, ..., |L|
$$

$$
\mathcal{I}_{L_iK_k}(\beta_n) = E_{\beta_n} \left[ \frac{\partial}{\partial \beta_{nL_j}} \log f_n \cdot \frac{\partial}{\partial \beta_{nK_k}} \log f_n \right] = -E \left[ \frac{\partial^2}{\partial \beta_{nL_j} \partial \beta_{nK_k}} \log f_n \right].
$$
(A2) The Fisher information matrix $I(\beta_n) = (I_{L_j K_k}(\beta_n))$ is positive definite with bounds

$$0 < C_1 < \min \sigma(I(\beta)) \leq \max \sigma(I(\beta)) < C_2 < \infty$$

where $\sigma(X)$ is the point spectrum of a matrix $X$,

$$E_{\beta_n} \left[ \frac{\partial}{\partial \beta_n L_j} \log f_n \cdot \frac{\partial}{\partial \beta_n K_k} \log f_n \right]^2 < C_3 < \infty$$

$$E_{\beta_n} \left[ \frac{\partial^2}{\partial \beta_n L_j \partial \beta_n K_k} \log f_n \right]^2 < C_4 < \infty.$$

(A3) There exists an open subset $\omega_n \subset \Omega_n \subset \mathbb{R}^{P_n}$, $P_n$ the number of parameters, containing the true parameter point $\beta_n$ such that for almost all $(X_i, Y_i)$, $\frac{\partial^3 f_n}{\partial \beta_{L_j} \partial \beta_{K_k} \partial \beta_{J_l}}$ is defined for all $\beta \in \omega_n$. Further, there exist functions $M_{nL_j K_k J_l}$ such that

$$\left| \frac{\partial^3 f_n}{\partial \beta_{L_j} \partial \beta_{K_k} \partial \beta_{J_l}} \right| \leq M_{nL_j K_k J_l}(X_i, Y_i)$$

for all $\beta \in \omega_n$ and

$$E_{\beta_n} \left[ M_{nL_j K_k J_l}^2(X_i, Y_i) \right] < C_5 < \infty.$$

We call the conditions (A1)-(A3) as the LAN conditions and note that they are not excessively restrictive and falls within the usual framework of this type of analysis.

Consider the parameters $\beta_n \in \mathbb{R}^{p_n}$ where $p_n$ grow with $n$, and their partial inverse $\psi(\beta_n) = (D_n, \alpha_n)$. Let $w_j(\beta_n) = 1$ if $\phi_j(D_n(\beta_n), 1) = 0$, $\phi_j(D_n(\beta_n), 1)$ otherwise. Then assuming the relationship from Lemma 6.2 we have the following equivalent expression,

$$Q_n(\lambda_n, \beta_n) = Q^*(n\lambda_n/(T + 1), ..., n\lambda_n/(T + 1), D_n, \alpha_n)$$

$$= \ell_n(\beta_n) - n\lambda_n \sum_L \frac{||\beta_nL||}{w_{L1}}. \tag{6.1}$$

For convenience we will write $D_n(\beta_n)$ for the projection of $\psi(\beta_n)$ onto $D_n$.

We have the following consistency results for the $\Phi-LASSO$.

**Theorem 6.6 (Consistency)** Assume that the distribution satisfies the LAN conditions. If $p_n^4 = o(n)$ and $\lambda_n \ll n^{-1/2}$, then there exists a $\gamma_n$-consistent local maximizer $\hat{\beta}_n$ of $Q_n$, where $\gamma_n = \sqrt{p_n(n^{-1/2} + \lambda_n)}$.

A simple choice is $\lambda_n \approx n^{-1/2}$. We find this provides us with taxon selection consistency.

Define $\delta_j : \mathbb{R}^{P_j} \to \{0, 1\}$ by $\delta_j(x) = 0$ if $x = 0$ and 1 otherwise. Let $\delta = (\delta_1, ..., \delta_k)$.
Definition 6.7 Let $\hat{\beta}_n$ be an estimator for $[6.1]$. Then $\hat{\beta}_n$ is said to be consistent in group selection if $\mathbb{P}\{\delta(\hat{\beta}_n) = \delta(\beta_n)\} \to 1$ as $n \to \infty$. Moreover, if $p_j = 1$ for all $j$, the estimator is model selection consistent.

Consider $\beta_0^n\left(\hat{\beta}_n\right)$ the true parameter (estimator) in $[6.1]$. Suppose $A_n = \{\beta_{0,n,kj} \neq 0\}$ and define $\beta_0^{n,A_n}\left(\hat{\beta}_{n,A_n}\right)$ to be the restriction of $\beta_0^n\left(\hat{\beta}_n\right)$ to the set $A_n$.

Definition 6.8 Let $\hat{\beta}_n$ be a model selection consistent estimator of $\beta_0^n$. Suppose $\hat{\beta}_n$ satisfies
\[
\sqrt{n}A_nI_n^{1/2}(\beta_0^{n,A_n})(\hat{\beta}_{n,A_n} - \beta_0^{n,A_n}) \sim N(0, \Sigma)
\]
where $A_n$ is an $r \times |A_n|$ matrix such that $A_nA_n' \to \Sigma$, a positive semidefinite matrix, and $I_n(\beta_0^n)$ is the Fisher information evaluated at $\beta_0^n$, as $n \to \infty$. Then $\hat{\beta}_n$ is said to have the oracle property.

We have the following.

Theorem 6.9 (Taxon selection consistency) Assume that the distribution satisfies the LAN conditions. If $p_n(T+2)^4 = o(n)$ and $\lambda_n \asymp n^{-1/2}$, then there exists a $\sqrt{n}/p_n$-consistent local maximizer $\hat{\beta}_n$ of $[6.1]$ so that $\lim_{n \to \infty} \mathbb{P}\{\delta(\hat{\beta}_nL) = \delta(\beta_0^nL)\} = 1$, the group sparsity property.

For the case of two taxon levels, $T = 1$, the above result is Theorem 2, [34]. Using an adaptive modification to their loss function analogous to [35], [34] obtains the full oracle property. We obtain the oracle property in an alternative manner, by a simple modification of the taxonomy of the variables. The full proofs are quite involved and are relegated to Appendix A. Since we have now shown that consistent taxon selection holds for arbitrary number of taxon levels, we have the full oracle property in the following result using a simple modification of our taxonomy. This is possible as we are able to incorporate $T$, arbitrary, taxon levels.

Theorem 6.10 (Oracle Property) In addition to the LAN conditions, suppose that the taxonomy contains an additional taxon level identical to the singleton level and $p_n^5 = o(n)$. Then $\hat{\beta}_n$ is a $\sqrt{n}/p_n$-consistent local maximizer of $[6.1]$ satisfying

(i) the sparsity property $\mathbb{P}\{\delta(\hat{\beta}_{n,A_n}) = \delta(\beta_0^{n,A_n})\} \to 1$ as $n \to \infty$,
(ii) asymptotic normality

\[ \sqrt{n}A_n I_n^{1/2}(\beta_0^{A_n})(\hat{\beta}_{nA_n} - \beta_0^{A_n}) \rightsquigarrow N(0, \Sigma), \]

where \( A_n \) is an \( r \times A_n \) matrix such that \( A_n A_n' \to \Sigma \), a positive semidefinite matrix, and \( I_n(\beta_0^n) \) is the Fisher information matrix evaluated at \( \beta_0^n \), as \( n \to \infty \).

A simple consequence of Theorem 6.10 is that all estimators obtained with an \( l_1/q \) penalty, \( q \in \mathbb{N}, q > 1 \), have the oracle property.

**Corollary 6.11** Consider the estimator \( \hat{\beta}_n = \arg \max_{\beta} \{ \ell_n(\beta) - \lambda_n ||\beta||_{1/T+1} \} \). Then \( \hat{\beta}_n \) has the oracle property.

This result follows immediately if we consider a taxonomy in which all taxa are singletons.

**Remark 6.12** Alternatively, we can obtain the oracle property from the following modification of the objective function,

\[ \ell(\varphi(D, \alpha); Y, X) - \sum_{t=1}^{T} \sum_{k=1}^{K_t} d_k^t - \lambda ||\alpha||_{1/2}^2, \]

where we have replaced the LASSO penalty on \( \alpha \) by a Bridge penalty with \( \gamma = 1/2 \). The \( l_{1/2} \)-penalty yields an estimator equivalent to that obtained in Theorem 6.10.

### A Appendix: Proofs

In this appendix we provide all proofs.

**Proof of Lemma 6.1** Clearly

\[ Q_1((d_{\tau t}), \alpha) = Q_2((\lambda_t^{1/2} d_{\tau t}), \alpha'_{I_{t=1}^T \lambda_t^{1/2}}). \]  

(\( \implies \)) Let \( (D^1, \alpha^1) \) be a local maximizer of \( Q_1 \). Thus there exists a \( \delta > 0 \) such that if \( (D', \alpha') \) satisfies \( \sum_t ||d_t' - d_t||_p + ||\alpha' - \alpha||_q < \delta \), then \( Q^*(D', \alpha') \leq Q^*(D^1, \alpha^1) \). By the identity [A.1],

\[ Q_2(D'' = (\lambda_t^{1/2} d_{\tau t}''), \alpha'' = \alpha'_{I_{t=1}^T \lambda_t^{1/2}}) = Q_1(D', \alpha') \leq Q_1(D^1, \alpha^1) \leq Q_2(D^2, \alpha^2). \]

As this holds for \((D'', \alpha'')\) in a neighbourhood of \((D^2, \alpha^2)\), \((D^2, \alpha^2)\) is a local maximizer of \( Q_2 \).

(\( \Longleftarrow \)) The converse is similar. \( \square \)

From hereon, we assume \( \lambda_t = \lambda, t = 1, ..., T + 1 \). Before proving Lemma 6.2, we recall the Karush-Kuhn-Tucker (KKT) conditions from convex analysis, here taken from [IS].
Definition A.1 Let $C \neq \emptyset$ be a convex subset of $\mathbb{R}^n$. Let $f_i : C \to \mathbb{R}$ be convex functions on $C$ for $0 \leq i \leq r$ and affine functions on $C$ for $r + 1 \leq i \leq m$. Consider the following problem,

$$\begin{align*}
(P) \quad \text{minimize} & \quad f_0(x) \\
\text{subject to} & \quad f_i(x) \leq 0 \quad (1 \leq i \leq r) \\
& \quad f_i(x) = 0 \quad (r + 1 \leq i \leq m)
\end{align*}$$

We call $(P)$ an ordinary convex program.

Lemma A.2 (KKT Conditions [18]) Let $(P)$ be an ordinary convex program. Let $\mu \in \mathbb{R}^m, x \in \mathbb{R}^n$. In order for $\mu$ to be a KKT vector for $(P)$ and $x$ an optimal solution to $(P)$, it is necessary and sufficient that $(\mu, x)$ be a saddle-point of the Lagrangian of $(P)$. Moreover, this condition holds if and only if $x$ and the components $\mu_i$ of $\mu$ satisfy

(i) $\mu_i \geq 0$, $f_i(x) \leq 0$, and $\mu_i f_i(x) = 0 \quad (1 \leq i \leq r)$;

(ii) $f_i(x) = 0 \quad (r + 1 \leq i \leq m)$;

(iii) $0 \in [\partial f_0(x) + \sum_{i=1}^m \mu_i \partial f_i(x)]$, the subgradient of the Lagrangian at $x$.

Proof of Lemma 6.2. Note that the log-likelihood component of the loss function depends on $(D, \alpha)$ through $\varphi(D, \alpha) = \beta$, so that it remains constant over $\varphi^{-1}(\beta)$. Without loss of generality, assume $\alpha > 0$, since if a component is negative, we can multiply the variable in question by -1, and if it is zero we can exclude it from the current analysis, as it remains fixed. We further assume $D_\tau > 0$ since if it is zero, then again, it is fixed.

We have the following optimization program,

$$\begin{align*}
(P_1) \quad \text{minimize} & \quad \sum_t ||d_t||_q^q + ||\alpha||_q^q \\
\text{subject to} & \quad -d_\tau < 0 \\
& \quad (\Pi L_t) \alpha_{L_t} = \beta_{L_t} \quad (\forall \tau, L_t).
\end{align*}$$

Let $x = (x_\tau) = (\ln d_\tau), y = (y_{L_t}) = (\ln \alpha_{L_t})$, and $B_{L_t} = \ln \beta_{L_t}$. Then $(P_1)$ is equivalent to the following ordinary convex program,

$$\begin{align*}
(P_2) \quad \text{minimize} & \quad f(x, y) = \sum_{\tau} e^{q x_\tau} + \sum_{L_t} e^{q y_{L_t}} \\
\text{subject to} & \quad f_{L_t}(x, y) = y_{L_t} - B_{L_t} + \sum_t x_{L_t} = 0 \quad (\forall L_t)
\end{align*}$$

where we have omitted $f_{\tau}(x, y) = -e^{x_\tau} < 0 \quad (\forall \tau)$ since it is trivially satisfied.

Consider now the Lagrangian of $(P_2)$,

$$\Lambda(x, y, (\mu_{L_t})) = f(x, y) + \sum_{L_t} \mu_{L_t} f_{L_t}(x, y).$$
We remark that KKT conditions (i) and (ii) are immediately satisfied, (i) trivially and (ii) by construction. We need only consider (iii). By the Karush-Kuhn-Tucker theorem, \((x, y)\) is an optimal solution of \((P_2)\) if and only if \(\nabla \Lambda(x, y, (\mu_{L_j})) = 0\), with gradient with respect to \((x, y)\), since the subgradient is unique when the function is differentiable.

We have the following derivatives of the Lagrangian \(\Lambda\),

\[
\frac{\partial \Lambda}{\partial x^u} = q e^{q x^u} + \sum_{L: L^u = \tau^u} \mu_{L_j}, \tag{A.2}
\]

\[
\frac{\partial \Lambda}{\partial y_{L_j}} = q e^{q y_{L_j}} + \mu_{L_j}, \tag{A.3}
\]

\(\Rightarrow\) Assume \((x, y)\) is a local minimum for \((P_2)\). Then the KKT conditions are satisfied, and we can find \(\mu\). By (iii) the derivatives (A.2), (A.3) are zero, so that we obtain

\[
e^{q x^u} = \sum_{L: L^u = \tau^u} e^{q y_{L_j}} > 0. \tag{A.4}
\]

A simple calculation yields

\[
d^u = \sum_{L: L^u = \tau^u} ||\alpha_L||_q^2.
\]

\(\Leftarrow\) Assume \(d^u = \sum_{L: L^u = \tau} ||\alpha_L||_q^2\) for all \(\tau\). This defines an optimal solution if we can find \(\mu\) such that the KKT conditions are satisfied. But we derived these above. □.

### A.1 Proof of Theorem 6.6

We show that \(\mathbb{P}(\sup |u| = c) Q_n(\beta_0^0 + \gamma_n u) < Q_n(\beta_0^0) \geq 1 - \epsilon\), where \(\gamma_n = \sqrt{n}(1/\sqrt{n} + \lambda_n)\), \(c > 0\) constant. Let \(w_L(x) = 1\) if \(\varphi_{L_1}(D(\beta_0^0 + xu), 1) = 0\), \(\varphi_{L_1}(D(\beta_0^0 + xu), 1)\) otherwise. Then

\[
\Delta Q = Q_n(\beta_0^0 + \gamma_n u) - Q_n(\beta_0^0)
\]

\[
= \ell_n(\beta_0^0 + \gamma_n u) - \ell(\beta_0^0) - n\lambda_n \sum_{L} \left( \frac{||\beta_0^0 + \gamma_n u||}{w_L(\gamma_n)} - \frac{||\beta_0^0||}{w_L(0)} \right)
\]

\[
= \Delta \ell_n - n\lambda_n \Delta N.
\]

By third order Taylor series expansion,

\[
\Delta \ell = \gamma_n u^t \nabla \beta_n \ell(\beta_0^0) + u^t \nabla \beta_n^2 \ell_n(\beta_0^0) u\gamma_n^2 / 2 + u^t \nabla \beta_n (u^t \nabla \beta_n (\ell_n \beta_n^*) u) \gamma_n^3 / 6
\]

\[
= I_1 + I_2 + I_3.
\]

We look at each term individually.
By regularity condition (A2),

\[ |I_1| = |\gamma \nabla^\ell_{\beta_n} \ell(\beta^0_n)|u| \]
\[ \leq \gamma_n \|\nabla^\ell_{\beta_n} \ell(\beta^0_n)\| \cdot |u| \]
\[ \ll_p \gamma_n \sqrt{n p_n} |u| - \gamma_n (\sqrt{p_n / n} + \sqrt{p_n \lambda_n / 2 \sqrt{c_1}} n) |u| \]
\[ \ll_p \gamma_n^2 n |u|. \]

For the second term,

\[ I_2 = \frac{1}{2} u^t \left( \frac{1}{n} \nabla^2_{\beta_n} \ell(\beta^0_n) + I_n(\beta^0_n) \right) \cdot \gamma_n \]  
\[ + \frac{1}{2} u^t I_n(\beta^0_n) \gamma_n. \tag{A.5} \]

We find by the Chebyshev inequality,

\[ P \left( \left\| \frac{1}{n} \nabla^2_{\beta_n} \ell(\beta^0_n) + I_n(\beta^0_n) \right\| \geq \frac{\epsilon}{p_n} \right) \leq \frac{p_n^2}{n^2 \epsilon^2} E \left( \sum_{i=1}^{p_n} \sum_{j=1}^{p_n} \left( \frac{\partial^2 \ell_n(\beta^0_n)}{\partial \beta_n i \partial \beta_n j} - E \left( \frac{\partial^2 \ell_n(\beta^0_n)}{\partial \beta_n i \partial \beta_n j} \right) \right)^2 \right) \]
\[ = \frac{p_n^2}{n^2 \epsilon^2} \sum_{i=1}^{p_n} \sum_{j=1}^{p_n} E \left( \frac{\partial^2 \ell_n(\beta^0_n)}{\partial \beta_n i \partial \beta_n j} \right)^2 - E^2 \left( \frac{\partial^2 \ell(\beta^0_n)}{\partial \beta_n i \partial \beta_n j} \right) \]
\[ < \frac{p_n^2}{n^2 \epsilon^2} \sum_{i=1}^{p_n} \sum_{j=1}^{p_n} c_4 \]
\[ = \frac{p_n^4}{n} \cdot \frac{c_4}{n \epsilon^2} \ll \frac{p_n^4}{n^2} = o(1) \]

so that \( I_2 = -u^t I_n(\beta^0_n) \gamma_n^2 + o_p(1) \).

For the third term,

\[ I_3 = \frac{1}{6} \sum_{L=1}^{\lfloor L/2 \rfloor} \sum_{K=1}^{\lfloor K/2 \rfloor} \sum_{J=1}^{\lfloor J/2 \rfloor} \sum_{L'}^{\lfloor L'/2 \rfloor} \sum_{K'}^{\lfloor K'/2 \rfloor} \sum_{J'}^{\lfloor J'/2 \rfloor} \partial^3 \ell_n(\beta^*) \]
\[ \leq \frac{1}{6} \sum_{i=1}^{n} \sum_{L=1}^{\lfloor L/2 \rfloor} \sum_{K=1}^{\lfloor K/2 \rfloor} \sum_{J=1}^{\lfloor J/2 \rfloor} \sum_{L'}^{\lfloor L'/2 \rfloor} \sum_{K'}^{\lfloor K'/2 \rfloor} \sum_{J'}^{\lfloor J'/2 \rfloor} M_{n L K J} \gamma_n^2 \gamma_n^2 \gamma_n^2 \]
\[ \ll_p n p_n^3 |u| 3 \gamma_n^3 \]
\[ \ll_p p_n^3 \gamma_n^3 \ll_p p_n^3 (\lambda_n + 1 / \sqrt{n}) |u|^3 n \gamma_n^2 \]
\[ \ll_p p_n^3 (\lambda_n + 1 / \sqrt{n}) |u|^3 n \gamma_n^2 \]
\[ = o_p (n \gamma_n^2) |u|^3, \]

since \( p_n^4 / n \rightarrow 0 \) and \( p_n^2 \lambda_n \rightarrow 0 \) by hypothesis. Here \( L, K, J \) run over all lineages.

Thus \( \Delta \ell \ll_p \gamma_n^2 |u| - u^t I_n(\beta^0_n) \gamma_n^2 / 2 + o_p (n \gamma_n^2) |u|^3 \). Choosing \( c \) sufficiently large, \( I_2 \) dominates \( I_1 \) uniformly on \( |u| = c \), then choosing \( n \) sufficiently large, \( I_2 \) dominates \( I_3 \) uniformly on \( |u| = c \).
We next turn to $\Delta N$. We have $\Delta N = \sum_L \Delta N_L$ where $\Delta N_L = \frac{||\beta^0_{nL} + \gamma_n u_L||}{w_L(\gamma_n)} - \frac{||\beta^0_{nL}||}{w_L(0)}$.

Suppose $\beta^0_{nL} \neq 0$. Then

$$|\Delta N_L| = \left| \frac{||\beta^0_{nL} + \gamma_n u_L||}{w_L(\gamma_n)} - \frac{||\beta^0_{nL}||}{w_L(0)} \right| \leq \left| \frac{||\beta^0_{nL} + \gamma_n u_L|| - ||\beta^0_{nL}||}{\min\{w_L(\gamma_n), w_L(0)\}} \right|.$$ 

For $n$ large enough, $w_L(\gamma_n) \geq (1 - \xi)w_L(0)$, $\xi > 0$ small, so

$$|\Delta N_L| < \left| \frac{||\beta^0_{nL} + \gamma_n u_L|| - ||\beta^0_{nL}||}{(1 - \xi)w_L(0)} \right| \leq \frac{\gamma_n ||u_L||}{(1 - \xi)w_L(0)} \leq \frac{\gamma_n ||u_L||^{1/(T+1)}}{1 - \xi}.$$ 

We have

$$n\lambda_n \sum_L \gamma_n ||u_L||^{1/(T+1)} \leq \sqrt{n}||u||^{1/(T+1)}$$ 

where $L$ runs over all lineages such that $\beta^0_{nL} = 0$. From this it follows that $I_2$ dominates $\sum_L |\Delta N_L|$.

Suppose now $\beta^0_{nK} = 0$ for lineage $K$. Then

$$\Delta N_K = \frac{||\beta^0_{nK} + \gamma_n u_K||}{w_K(\gamma_n)} - \frac{||\beta^0_{nK}||}{w_K(0)} = \frac{\gamma_n ||u_K||}{w_K(\gamma_n)} > 0.$$ 

Therefore the term $(I_2 - n\lambda_n \sum_K \Delta N_K) < 0$ dominates in $\Delta Q_n$, where $K$ runs over all true sparse lineages, $\beta^0_{nK} = 0$, and we have convergence in probability. Therefore $||\hat{\beta}_n - \beta^0_n|| \ll_p \gamma_n$, which completes the proof of consistency. □

A.2 Proof of Theorem 6.9

We turn now to the sparsity property. Recall that $\beta_{nL_i}$ is a signed polynomial in $|\alpha_{nK_i}|$, $k = 1, ..., |K|$, over lineages $K$. Consider the polynomial map $f : \mathbb{R}^n \to \mathbb{R}^n$ given by $\alpha_n \mapsto \beta_n$, where $\alpha_n$ is the projection onto the second entry of $\psi(\beta_n) = (D_n, \alpha_n)$. When $\hat{\alpha}_n$ (equivalently $\hat{\beta}_n$) is non-sparse, the tangent map $\partial_{\alpha_n} f : T_{\hat{\alpha}_n}(\mathbb{R}^n) \to T_{\hat{\beta}_n}(\mathbb{R}^n)$ is non-singular. Since the entries $\frac{\partial \beta_{K_i}}{\partial \alpha_{L_i}}$ are themselves polynomials, they are continuous and hence $\partial_{\alpha_n} f$ is continuous with respect to $\alpha_n$ in a neighbourhood of $\hat{\alpha}_n$.

We have

$$\partial_{\alpha_n} f \frac{\partial}{\partial \beta_n} Q_n = \partial_{\alpha_n} f \frac{\partial \ell_n}{\partial \beta_n} - n\lambda_n \partial_{\alpha_n} f \frac{\partial}{\partial \beta_n} \sum_L ||\beta_L|| \frac{w_L}{w_L(\gamma_n)}$$

$$= \partial_{\alpha_n} f \frac{\partial \ell_n}{\partial \beta_n} - n\lambda_n \frac{\partial}{\partial \alpha_n} ||\alpha_n||$$

$$= \partial_{\alpha_n} f \frac{\partial \ell_n}{\partial \beta_n} - n\lambda_n \text{sign}(\alpha_n)$$

where sign$(\alpha_n)$ is understood as the vector of signs $(\text{sign}(\alpha_{n1}), ..., \text{sign}(\alpha_{np_n}))$.

Assume hereon that $||\beta^0_n - \beta_n|| \ll_p \sqrt{p_n/n}$. 

By second order Taylor expansion, we have

\[
\frac{\partial \ell_n}{\partial \beta_{L_1}} = \frac{\partial \ell_n}{\partial \beta_{L_k}} \bigg|_{\beta_n^0} + \sum_{K} \sum_{k=1}^{[K]} \frac{\partial^2 \ell_n}{\partial \beta_{K_k} \partial \beta_{L_1}} \bigg|_{\beta_n^0} (\beta_{nK_k} - \beta_{nK_k}^0) \\
+ \sum_{J} \sum_{j=1}^{[J]} \sum_{H} \sum_{h=1}^{[H]} \frac{\partial^3 \ell_n}{\partial \beta_{H_h} \partial \beta_{J_j} \partial \beta_{K_k}} \bigg|_{\beta_n^0} (\beta_{nJ_j} - \beta_{nJ_j}^0)(\beta_{nK_k} - \beta_{nK_k}^0)
\]

\[= G_1 + G_2 + G_3 \]

where \(J, K\) run over all lineages and \(\beta_n^*\) lies between \(\beta_n^0\) and \(\beta_n\).

We have from the proof of consistency that \(G_1 \ll_p \sqrt{npn}\). Bounds for \(G_2\) and \(G_3\) are provided in [E], which we derive here for completeness. For \(G_2\), we have that

\[
G_2 = \sum_{J} \sum_{j=1}^{[J]} \sum_{H} \sum_{h=1}^{[H]} \frac{\partial^3 \ell_n}{\partial \beta_{H_h} \partial \beta_{J_j} \partial \beta_{K_k}} \bigg|_{\beta_n^0} (\beta_{nJ_j} - \beta_{nJ_j}^0)(\hat{\beta}_{nK_k} - \beta_{nK_k}^0)
\]

By Cauchy-Schwarz inequality and \(||\hat{\beta}_n - \beta_n^0|| \ll_p \sqrt{p/n}\),

\[
|F_2| = \left| n \sum_{J} \sum_{j=1}^{[J]} I_{n,J,K_k}(\beta_{nJ_j} - \beta_{nJ_j}^0) \right| \ll_p n(\sqrt{p/n}) \left( \sum_{J} \sum_{j=1}^{[J]} I_{n,J,K_k}(\beta_n^0) \right)^{1/2}
\]

By the regularity condition (A2) on the eigenvalues of \(I_n\), we have \(\sum_{J} \sum_{j=1}^{[J]} I_{n,J,K_k} \ll 1\), so that \(|F_2| \ll_p \sqrt{npn}\). For \(F_1\), by the Cauchy-Schwarz inequality, we have

\[
|F_1| \leq ||\hat{\beta}_n - \beta_n^0|| \left( \sum_{J} \sum_{j=1}^{[J]} \frac{\partial^2 \ell_n}{\partial \beta_{nJ_j} \partial \beta_{nK_k}} \bigg|_{\beta_n^0} - \mathbb{E} \left\{ \frac{\partial^2 \ell_n}{\partial \beta_{nJ_j} \partial \beta_{nK_k}} \bigg|_{\beta_n^0} \right\} \right)^{1/2}
\]

so that by regularity condition (A2), \(|F_1| \ll_p \sqrt{p/n} \sqrt{npn} \ll_p npn\). Thus \(|G_2| \ll_p \sqrt{npn}\).

For \(G_3\),

\[
2G_3 = \sum_{J} \sum_{j=1}^{[J]} \sum_{H} \sum_{h=1}^{[H]} \frac{\partial^3 \ell_n}{\partial \beta_{H_h} \partial \beta_{J_j} \partial \beta_{K_k}} (\hat{\beta}_{nJ_j} - \beta_{nJ_j}^0)(\hat{\beta}_{nK_k} - \beta_{nK_k}^0)
\]

\[= \sum_{J} \sum_{j=1}^{[J]} \sum_{H} \sum_{h=1}^{[H]} \left( \frac{\partial^3 \ell_n}{\partial \beta_{H_h} \partial \beta_{J_j} \partial \beta_{K_k}} - \mathbb{E} \left\{ \frac{\partial^3 \ell_n}{\partial \beta_{H_h} \partial \beta_{J_j} \partial \beta_{K_k}} \bigg|_{\beta_n^0} \right\} \right)(\hat{\beta}_{nJ_j} - \beta_{nJ_j}^0)(\hat{\beta}_{nK_k} - \beta_{nK_k}^0)
\]

\[+ \sum_{J} \sum_{j=1}^{[J]} \sum_{H} \sum_{h=1}^{[H]} \mathbb{E} \left\{ \frac{\partial^3 \ell_n}{\partial \beta_{H_h} \partial \beta_{J_j} \partial \beta_{K_k}} \bigg|_{\beta_n^0} \right\}(\hat{\beta}_{nJ_j} - \beta_{nJ_j}^0)(\hat{\beta}_{nK_k} - \beta_{nK_k}^0)
\]

\[= F_3 + F_4.
\]
Suppose that $\beta$. From equation (A.6), we obtain
\[
\beta_{out}^\tau \text{ and hence } n \leq p \left( \frac{p_n}{n^2} \right) = o_p(n_p)
\]
and
\[
|F_4| \leq C_5^{1/2} n_p \|\hat{\beta}_n - \beta_0\|^2 \ll_p \left( \frac{p_n^2}{n^2} \right) = o_p(\sqrt{n_p}),
\]
hence $G_3 \ll_p \sqrt{n_p}$ and $G_1 + G_2 + G_3 \ll_p \sqrt{n_p}$.

Consider now the elements of the tangent map $D_{\alpha_n} f, \frac{\partial \beta_{nL}}{\partial \alpha_{nK}}$. We may explicitly write out $\beta_{nL}$ in terms of $\alpha_n$,
\[
\beta_{nL} = \alpha_{nL} \cdot \prod_{t=1}^T \sum_{K:K^t=L} \| \alpha_K \|.
\]
From equation (A.6), we obtain
\[
\frac{\partial \beta_{nL} \partial_{\alpha_{nK}}}{\partial_{\alpha_{nK}}} = \begin{cases} 
0 & \text{ if } L^t \neq K^t \forall t \\
\alpha_{nL} \cdot \text{sign}(\alpha_{nL}) \cdot \sum_{u=1}^T \prod_{u=1, u \neq t}^T \sum_{J:J^t=L^t} \| \alpha_{nJ} \| \\
+ \text{sign}(\alpha_{nL}) \cdot \prod_{t=1}^T \sum_{J:J^t=L^t} \| \alpha_{nJ} \| & \text{ if } L_t = J_j \\
\alpha_{nL} \cdot \text{sign}(\alpha_{nK}) \cdot \sum_{t \in S} \prod_{u=1, u \neq t}^T \sum_{J:J^t=L^t} \| \alpha_{nJ} \| & \text{ otherwise, with } S = \{ t : L^t = K^t \}.
\end{cases}
\]
Suppose that $\beta_{n0}^t = 0$ for some taxon $t$ of taxon level $t$. Then on inspection for $L^t = \tau^t$, we find $\frac{\partial \beta_{nL} \partial_{\alpha_{nK}}}{\partial_{\alpha_{nK}}} \approx_p \left( \frac{p_n}{n^2} \right)^{1/(2T+2)}$. From $(D_{\alpha_n} f)^{-1}(D_{\alpha_n} f) = I_{p_n}$, we obtain the relationship
\[
\sum_{j=1} \frac{\partial \alpha_{nL}}{\partial \alpha_{nJ}} \frac{\partial \alpha_{nJ}}{\partial \alpha_{nI}} = \frac{p_n}{n^2} \left( \frac{p_n}{n^2} \right)^{1/(2T+2)} \sum_{j=1} \frac{\partial \alpha_{nI}}{\partial \alpha_{nJ}} \frac{\partial \alpha_{nJ}}{\partial \alpha_{nI}},
\]
so that $(n/p_n)^{1/(2T+2)} \ll_p \sum_{j=1} \frac{\partial \alpha_{nJ}}{\partial \alpha_{nJ}} \frac{\partial \alpha_{nJ}}{\partial \alpha_{nI}}$.

Without loss of generality, assume $\alpha_n > 0$, since we can always transfer the sign from the coefficients to the covariates at every $n$. Evaluated at our $\sqrt{n/p_n}$-consistent estimate $\hat{\beta}_n$, we have
\[
0 = (D_{\alpha_n} f)^{-1}(D_{\alpha_n} f) \nabla_{\alpha_n} \ell_n(\hat{\beta}_n) - n \lambda_n (D_{\alpha_n} f)^{-1} \text{sign}(\alpha_n)
\]
\[
= \nabla_{\beta_n} \ell_n(\hat{\beta}_n) - n \lambda_n (D_{\alpha_n} f)^{-1} \text{sign}(\alpha_n).
\]
Thus for a sparse taxon $L_t \in \tau$,
\[
\nabla_{\beta_n} \ell_n(\hat{\beta}_n) = n \lambda_n (D_{\alpha_n} f)^{-1} \text{sign}(\alpha_n)
\]
\[
n \lambda_n (n/p_n)^{1/(2T+2)} \ll_p n \lambda_n \sum_{j=1} \frac{p_n}{\partial \alpha_{nJ}} \frac{\partial \alpha_{nI}}{\partial \alpha_{nJ}} \ll_p \sqrt{n_p}
\]
and hence $n \ll_p p_n^{T+2}$, a contradiction. Therefore $\lim_{n \to \infty} P(\hat{\beta}_{n\tau} = \delta(\beta_{n\tau})) = 1$ for all taxa $\tau$. $\Box$
A.3 Proof of Theorem 6.10

(i) The proof of model consistency is immediate from Theorem 6.9.

(ii) Let \( A_n \subseteq \{1, \ldots, p_n\} \) be the subset of indices for nonzero parameters. We have that there exists a \( \sqrt{n/p_n} \) maximizer \( \hat{\beta}_n = (\hat{\beta}_{n,A_n}, 0) \) of \( Q_n \). We take to writing \( Q_n(\beta_{n,A_n}) = Q_n(\beta_{n,A_n}, 0) \) through abuse of notation. The following argument concerning the log-likelihood \( \ell_n \) follows [34].

By Taylor expansion of \( \nabla_{\beta_n} Q_n \) about \( \beta_{n,A_n}^0 \), we find
\[
\frac{1}{n} \left( \nabla_{\beta_n}^2 \ell_n(\beta_{n,A_n}^0) \nabla_{\beta_n} \ell_n(\beta_{n,A_n}) - \nabla_{\beta_n} p_\lambda(\hat{\beta}_{n,A_n}) \right) \\
= -\frac{1}{n} \left( \nabla_{\beta_n} \ell_n(\beta_{n,A_n}) + \frac{1}{2} (\hat{\beta}_{n,A_n} - \beta_{n,A_n}^0)^T \nabla_{\beta_n}^2 \ell_n(\hat{\beta}_{n,A_n}) (\hat{\beta}_{n,A_n} - \beta_{n,A_n}^0) \right)
\]
from the relationship \( 0 = \nabla_{\beta_n} Q_n(\hat{\beta}_n) \).

By Cauchy-Schwarz inequality,
\[
\left| \frac{1}{2n} (\hat{\beta}_{n,A_n} - \beta_{n,A_n}^0)^T \nabla_{\beta_n}^2 \ell_n(\hat{\beta}_{n,A_n}) (\hat{\beta}_{n,A_n} - \beta_{n,A_n}^0) \right|^2 \\
\leq \frac{1}{n^2} \sum_{i=1}^n ||\hat{\beta}_{n,A_n} - \beta_{n,A_n}^0||^4 \sum_{j_1 \in A_n} \sum_{j_2 \in A_n} \sum_{j_3 \in A_n} M_{nj_1,j_2,j_3}^3 (X_{ni}, Y_{ni}) \\
\ll_p \frac{p^3}{n^2} = o_p(1/n)
\]

By Lemma 8 in [5], obtain
\[
\left| \left( \frac{1}{n} \nabla_{\beta_n}^2 \ell_n(\beta_{n,A_n}^0) + \mathcal{I}_n(\beta_{n,A_n}^0) \right) (\hat{\beta}_{n,A_n} - \beta_{n,A_n}^0) \right| = o_p(1/\sqrt{n}).
\]

We turn now to the penalty \( p_\lambda \). Since \( ||\beta_n|| \) is a polynomial in \( \alpha_n \) of degree greater than 1, we have \( \frac{1}{n} \nabla_{\beta_n} ||\alpha_n|| = o_p(\frac{1}{n} ||\nabla_{\beta_n} ||\beta_n||||) = o_p(p_n/n) \).

Therefore from the Taylor expansion of \( \nabla_{\beta_n} Q \), we have
\[
\mathcal{I}_n(\beta_{n,A_n}^0)(\hat{\beta}_{n,A_n} - \beta_{n,A_n}^0) + o_p(p_n/n) = \frac{1}{n} \nabla_{\beta_n} \ell_n(\beta_{n,A_n}^0) + o_p(1/\sqrt{n}).
\]

Following [5],
\[
\sqrt{n} A_n \mathcal{I}_n^{-1/2}(\beta_{n,A_n}^0) \left( \mathcal{I}_n(\beta_{n,A_n}^0)(\hat{\beta}_{n,A_n} - \beta_{n,A_n}) + o_p(p_n/n) \right) \\
= \sqrt{n} A_n \mathcal{I}_n^{-1/2}(\beta_{n,A_n}^0) \left( \frac{1}{n} \nabla_{\beta_n} \ell_n(\beta_{n,A_n}^0) + o_p(1/\sqrt{n}) \right)
\]
\[
\sqrt{n} A_n \mathcal{I}_n^{1/2}(\beta_{n,A_n}^0)(\hat{\beta}_{n,A_n} - \beta_{n,A_n}) \sim \sqrt{n} A_n \mathcal{I}_n^{-1/2}(\beta_{n,A_n}^0) \frac{1}{n} \nabla_{\beta_n} \ell_n(\beta_{n,A_n}^0) \\
\sim N(0, \Sigma)
\]
where \( A_n \) and \( \Sigma \) are as described in the statement of the theorem. □
References

[1] GT Bergmann, ST Bates, KG Eilers, CL Lauber, JG Caporaso, WA Walters, R Knight, and N Fierer. The under-recognized dominance of Verrucomicrobia in soil bacterial communities. *Soil Biol Biochem*, 43(7):1450–5, 2011.

[2] LJ Brandt, OC Aroniadis, M Mellow, A Kanatzar, K Kelly, T Park, N Stollman, F Fohlike, and C Surawicz. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent *Clostridium difficile* infection. *Am J Gastroenterol*, 107(3):1079–87, 2012.

[3] S Chakravorty, D Helb, M Burday, N Connell, and D Alland. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *J Microbio Meth*, 69:330–339, 2007.

[4] SM Collins, M Surette, and P Bercik. The interplay between the intestinal microbiota and the brain. *Nat Rev Microbiol*, 10(11):735–742, 2012.

[5] J Fan and H Peng. Nonconcave penalized likelihood with a diverging number of parameters. *Ann Statist*, 32(3):928–961, 2004.

[6] TP Garcia, S Müller, RJ Carroll, and RL Walzem. Identification of important regressor groups, subgroups and individuals via regularization methods: application to gut microbiome data. *Bioinformatics*, 30(6):831–7, 2014.

[7] E Gough, H Shaikh, and AR Manges. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent *Clostridium difficile* infection. *Clin Infect Dis*, 53(10):994–1002, 2011.

[8] MJ Hamilton, AR Weingarden, T Unno, A Khoruts, and MJ Sadowsky. High-throughput DNA sequence analysis reveals stable engraftment of gut microbiota following transplantation of previously frozen fecal bacteria. *Gut Microbes*, 4(2):125–133, 2013.

[9] PT Kim, S Pinder, and STA Rush. Fréchet analysis and the microbiome. *J Stat Plan Inference*, 145(4):37–41, 2014.

[10] CH Lee, JE Bellanger, Z Kassam, M Smiejka, D Higgins, G Broukhanski, and PT Kim. The outcome of long-term follow-up of patients with recurrent and refractory *Clostridium difficile* infection using multiple fecal microbiota transplants via retention enema: A case series of 94 patients. *Eur J Clin Microbiol Infect Dis*, 33(8):1425–1428, 2014.

[11] CH Lee, T Steiner, EO Petrof, M Smieja, D Roscoe, A Nemataliah, JS Weese, S Collins, P Moayyedi, M Crowther, MJ Ropeleski, P Jayaratne, D Higgins, Y Li, NV Rau, and PT Kim. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent *Clostridium difficile* Infection: A Randomized Clinical Trial. *JAMA*, 315(2):142–149, 2016.

[12] EL Lehman and G Casella. *Theory of Point Estimation*. Springer, 2 edition, 2003.
[13] B Liquet, PL de Micheaux, BP Hejblum, and R Thiébaut. Group and sparse group partial least square approaches applied in genomics context. Bioinformatics, 32(1):35–42, 2016.

[14] VG Loo, L Poirier, MA Miller, M Oughton, MD Libman, S Michaud, AM Bourgault, T Nguyen, C Frenette, M Kelly, A Vibien, P Brassard, S Fenn, K Dewar, R Hudson TJ, Horn, P René, Y Monczak, and A. Dascal. A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. N Engl J Med, 353(20):2442–9, 2005.

[15] DHD Martínez, CH Lee, PT Kim, and W Mio. Probing the Geometry of Data with Diffusion Fréchet Functions. arXiv:1605.04955v1, 2016.

[16] EO Petrof, GB Gloor, SJ Vanner, SJ Weese, D Carter, MC Daigneault, EM Brown, K Schroeter, and E Allen-Vercoe. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: ‘RePOOPulating’ the gut. Microbiome, 1(3):1–12, 2013.

[17] FA Rainey, BJ Hollen, and A Small. Genus I. Clostridium. In P DeVos, GM Garrity, D Jones, NR Krieg, W Ludwig, FA Rainey, KH Schleifer, and WB Whitman, editors, Bergey’s Manual of Systematic Bacteriology: The Firmicutes, volume 3, pages 738–828. Springer-Verlag, New York, 2 edition, 2009.

[18] RT Rockafellar. Convex Analysis. Princeton University Press, Princeton, NJ, 1970.

[19] S Rush, S Pinder, M Costa, and PT Kim. A microbiology primer for pyrosequencing. QBS, 31(2):53–81, 2012.

[20] PD Schloss, SL Westcott, T Ryabin, JR Hall, M Hartmann, EB Hollister, RA Lesniewski, BB Oakley, DH Parks, CJ Robinson, JW Sahl, B Stres, GG Thallinger, DJV Horn, and CF Weber. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology, 75(23):7537–7541, 2009.

[21] AM Schubert, MAM Rogers, C Ring, J Mogle, JP Petrosino, VB Young, DM Aronoff, and PD Schloss. Microbiome data distinguish patients with Clostridium difficile infection and non-Clostridium difficile-associated diarrhea from healthy controls. mBio, 5(3):1–9, 2014.

[22] D Shahinas, M Silverman, T Sittler, C Chiu, P Kim, E Allen-Vercoe, S Weese, A Wong, DE Low, and DR Pillai. Toward an understanding of changes in diversity associated with fecal microbiome transplantation based on 16S rRNA gene deep sequencing. mBio, 63(5):e00338–12, 2012.

[23] J Shakar, S Szpakowski, NV Solis, S Mounaud, H Liu, L Losada, WC Nierman, and SG Filler. A systematic evaluation of high-dimensional ensemble-based regression for exploring large model spaces in microbiome analyses. BMC Bioinformatics, 16(31), 2015.
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[24] P Shi, A Zhang, and H Li. Regression analysis for microbiome compositional data. arXiv:1603.00974v1, 2016.

[25] Y Song, S Garg, M Girotra, C Maddox, EC von Rosenvinge, A Dutta, S Dutta, and WF Fricke. Microbiota dynamics in patients treated with fecal microbiota transplantation for recurrent Clostridium difficile infection. Plos ONE, 8(11):e81330, 2013.

[26] C Vilo and Q Dong. Evaluation of the RDP classifier accuracy using 16S rRNA gene variable regions. Metagenomics, 1:Article ID 235551, 2012.

[27] AR Weingarden, C Chen, A Bobr, D Yao, Y Lu, VM Nelson, MJ Sadowsky, and A Khoruts. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. Gastronint Liver Physiol, 306(4):310–319, 2014.

[28] J Wiegel, R Tanner, and FA Rainey. An introduction to the family Clostridiaceae. In M Dworkin, S Falkow, E Rosenberg, KH Schleifer, and E Stackebrandt, editors, The Prokaryotes: Archa. Bacteria: Firmicutes, Actinomycetes, volume 3, pages 654–78. Springer-Verlag, New York, 2006.

[29] CR Woese. How we do, don’t, and should look at bacteria and bacteriology. In E Rosenberg, EF DeLong, F Thompson, S Lory, and E Stackebrandt, editors, The Prokaryotes, volume 1, pages 3–20. Springer-Verlag, Berlin Heidelberg, 4 edition, 2013.

[30] N Youssef, CS Sheik, LR Krumholz, FZ Najar, BA Roe, and MS Elshahed. Comparison of species richness estimates obtained using nearly complete fragments and simulated pyrosequencing-generated fragments in 16S rRNA gene-based environmental surveys. Appl Environ Microbiol, 75(16):5227–36, 2009.

[31] M Yuan and Y Lin. Model selection and estimation in regression with grouped variables. JRSS B, 68(1):49–67, 2006.

[32] Q Zhang, H Abel, A Wells, P Lenzini, F Gomez, MA Province, AA Templeton, GM Weinstock, NH Salzman, and IB Borecki. Selection of models for the analysis of risk-factor trees: leveraging biological knowledge to mine large sets of risk factors with application to microbiome data. Bioinformatics, 31(10):1607–1613, 2015.

[33] L Zhao, G Wang, P Siegel, C He, H Wang, W Zhao, Z Zhai, F Tian, J Zhao, H Zhang, Z Sun, W Chen, Y Zhang, and H Meng. Quantitative genetic background of the host influences gut microbiomes in chickens. Scientific Reports, 3:1163, 2013.

[34] N Zhou and J Zhu. Group variable selection via a hierarchical lasso and its oracle property. Stat Interface, 3:557–74, 2010.

[35] H Zou. The adaptive lasso and its oracle properties. JASA, 101(476):1418–29, 2006.