Supporting Information for "Modeling complex measurement error in microbiome experiments"

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1 Weighted Likelihood

In section 3 of the main text, we introduce a weighted Poisson log likelihood with weight for the likelihood contribution of W_{ij}, the observation in taxon j for sample i given by

$$\hat{w}_{ij} = \frac{\hat{\mu}_{ij} + 1}{\hat{\sigma}^2_{ij} + 1}$$

where $\hat{\mu}_{ij}$ is the fitted mean for W_{ij} given parameters $\hat{\theta}$ estimated under a Poisson likelihood and read depth $W_{i\cdot}$ arising from a model fit to $\mathbf{W}$ via a Poisson likelihood (without reweighting) and $\hat{\sigma}^2_{ij}$ is a fitted value from a monotone regression of squared residuals $(W_{ij} - \hat{\mu}_{ij})^2$ on fitted means $\hat{\mu}_{ij}$ (with $i = 1, \ldots, n$ and $j = 1, \ldots, J$). In other words, $\hat{\sigma}^2_{ij}$ is an estimate of $\text{var}(W_{ij}|W_{i\cdot}, \theta)$.

To motivate why this reweighting is reasonable, we consider the case in which θ is in the interior of the parameter space Θ. In this setting we can express the Poisson MLE as a solution to the

following score equations:

$$\begin{cases} \sum_{i,j} \frac{1}{\mu_{ij}} [\frac{\partial}{\partial \theta_1} \mu_{ij}](W_{ij} - \mu_{ij}) &= 0 \\ \vdots \\ \sum_{i,j} \frac{1}{\mu_{ij}} [\frac{\partial}{\partial \theta_L} \mu_{ij}](W_{ij} - \mu_{ij}) &= 0 \end{cases}$$

Equivalently, we can write

$$\sum_{i,j} \frac{1}{\mu_{ij}} \mathbf{g}_{ij}(\theta) = 0$$

letting $\mathbf{g}_{ij} = [\frac{\partial}{\partial \theta^T} \mu_{ij}](W_{ij} - \mu_{ij})$. Hence, we can view this system of equations as a weighted sum of zero expectation terms $\mathbf{g}_{ij}$ with weights given by $\frac{1}{\mu_{ij}}$ — that is, one over a model-based estimate of $\mathrm{Var}(W_{ij} - \mu_{ij})$.

In this setting, if the Poisson mean-variance relationship holds and the score equations have a unique solution, we expect the estimator given by this solution to be asymptotically efficient (McCullagh, 1983), whereas when a different mean-variance relationship holds, in general we expect to lose efficiency.

When the Poisson mean-variance relationship does not hold, we expect to be able to improve efficiency by reweighting the score equations with a more flexible estimator of $\mathrm{Var}(W_{ij} - \mu_{ij})$. To accomplish this, we use a consistent estimator of θ, the Poisson MLE $\hat{\theta}$, to estimate $\boldsymbol{\mu}$ and $\mathrm{Var}(W_{ij}|\mu_{ij})$.

Specifically, we estimate $\sigma^2(\hat{\mu}_{ij}) := \mathrm{Var}(W_{ij}|W_{i\cdot}, X_i, Z_i, \tilde{Z}_i, \boldsymbol{\beta}, \mathbf{p}, \tilde{\mathbf{p}}, \tilde{\boldsymbol{\gamma}})$ under the assumption that $\sigma^2()$ is an increasing function via a centered isotonic regression of $(W_{ij} - \hat{\mu}_{ij})^2$ on $\hat{\mu}_{ij}$. Weighting log likelihood contribution of W_{ij} $l_{ij} := W_{ij}\log(\mu_{ij}) - \mu_{ij}$ by a factor of $\frac{\hat{\mu}_{ij}}{\hat{\sigma}^2_{ij}}$ then yields reweighted score equations

$$\sum_{i,j} \frac{\hat{\mu}_{ij}}{\hat{\sigma}_{ij}^2} \frac{1}{\mu_{ij}} \mathbf{g}_{ij}(\theta) = \sum_{i,j} \frac{\hat{\mu}_{ij}}{\mu_{ij}} \frac{1}{\hat{\sigma}_{ij}^2} \mathbf{g}_{ij}(\theta)$$

in which each $\mathbf{g}_{ij}$ is, up to a factor of $\frac{\hat{\mu}_{ij}}{\mu_{ij}} \xrightarrow{p} 1$, weighted by one over a flexible estimate of $\mathrm{Var}(W_{ij}|\mu_{ij})$

In practice, however, the weighting above may be unstable when $\hat{\mu}_{ij}$ and $\hat{\sigma}_{ij}^2$ are small. Hence, we weight instead by $\frac{\hat{\mu}_{ij}+1}{\hat{\sigma}_{ij}^2+1}$ preserves behavior of weights when the estimated mean and variance are both large (where reweighting is typically most important) and stabilizes them when these quantities are small.

2 Proofs

2.1 Notation

$\mathbf{W}_i = (W_{i1}, \ldots, W_{iJ})$: as in main text, a measured outcome of interest in sample i across taxa $j = 1, \ldots, J$; we also use $\mathbf{W}$ without subscript i where this does not lead to ambiguity

$\mathbf{X}_i$ here corresponds to covariates $(Z_i, X_i, \tilde{Z}_i)$ described in the main text

$\mathcal{W}$: the support of $\mathbf{W}$

$\mathcal{X}$: the support of $\mathbf{X}$

$\mathcal{W}.$: the support of $W. := \sum_{j=1}^{J} W_j$

v: a weighting function from $\mathcal{W}. \times \mathcal{X}$ into $\mathbf{R}_{>0}^J$; for simplicity of notation, we frequently suppress dependence on $W_{i.}$ and $\mathbf{X}_i$ and write v_{ij} to indicate $v_j(W_{i.}, \mathbf{X}_i)$

$\hat{v}_n$: an empirical weighting function estimated from a sample of size n

θ: unknown parameters $(\mathbf{p}, \boldsymbol{\beta}, \tilde{\mathbf{p}}, \tilde{\boldsymbol{\gamma}})$; we denote the true value with θ_0

$\boldsymbol{\mu}_\theta$: a parametrization of the mean model given in equation (5) in main text; $\mathbb{E}[\mathbf{W}|\mathbf{X}, \gamma, \theta] = \exp(\gamma)\boldsymbol{\mu}_\theta(\mathbf{X})$; we typically suppress dependence on $\mathbf{X}$

$M_n^v(\theta)$: profile log likelihood under weighting function v, evaluated at θ on a sample of size n

$M^v(\theta)$: expected profile log likelihood under weighting function v, evaluated at θ

2.2 Assumptions

(A) We draw triples $(\mathbf{W}, \mathbf{X}, \Gamma) \overset{\text{iid}}{\sim} P_{\theta_0}$ (of which we observe only $(\mathbf{W}, \mathbf{X})$) where $\mathbf{W}$ has bounded support $\mathcal{W} \subset \mathbb{R}_{\geq 0}^J$, $\mathbf{X}$ has bounded support $\mathcal{X} \subset \mathbf{R}^p$, Γ has bounded support $\mathcal{G} \subset \mathbb{R}$

(B) Letting θ denote $(\mathbf{p}, \boldsymbol{\beta}, \tilde{\mathbf{p}}, \tilde{\boldsymbol{\gamma}})$, for a set of known functions from $\mathcal{X}$ to $\mathbb{R}_{\geq 0}^J$ $\{\boldsymbol{\mu}_\theta : \theta \in \Theta\}$ we have that $\mathbb{E}[\mathbf{W}|\Gamma, \mathbf{X}] = \exp(\Gamma)\boldsymbol{\mu}_{\theta_0}(\mathbf{X})$ and $\mathbb{E}[\mathbf{W}|\Gamma, \mathbf{X}, \mathbf{W}.] = \mathbf{W}.\frac{\boldsymbol{\mu}_{\theta_0}(\mathbf{X})}{\mu_{\theta_0.}(\mathbf{X})}$, with $\mu_\theta(x)$ differentiable in θ for all $x \in \mathcal{X}$ and for each fixed $\theta \in \Theta$, $\mu_\theta(x)$ a bounded function on $\mathcal{X}$.

(C) For almost all $\mathbf{x} \in \mathcal{X}, \gamma \in \mathcal{G}$, $\mathrm{P}([\sum_{j=1}^J W_j] > \epsilon | \mathbf{X} = x, \Gamma = \gamma) = 1$ for some $\epsilon > 0$

2.3 Form of profile log likelihood

We first derive the form of a log likelihood in which Γ_i, treated as a fixed effect γ_i, has been profiled out and characterize its population analogue.

Estimating Γ_i as a fixed effect via maximum likelihood under a Poisson likelihood for $\mathbf{W}$ with (positive) weight v_{ij} given to the likelihood contribution of W_{ij}, we obtain the following profile log likelihood:

$$M_n^v(\theta) := \frac{1}{n} \sum_{i=1}^n \sup_{\gamma_i \in \mathbb{R}} \Big[\sum_{j=1}^J v_{ij} \Big(W_{ij} \log[\exp(\gamma_i)\mu_{\theta j}(X_i)] - \exp(\gamma_i)\mu_{\theta j}(X_i) \Big) \Big] \tag{1}$$

$$= \frac{1}{n} \sum_{i=1}^n \sum_{j=1}^J \Big[v_{ij} \Big(W_{ij} \log[\frac{\mathbf{v}_i \cdot \mathbf{W}_i}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta}\mu_{\theta j}] - \frac{\mathbf{v}_i \cdot \mathbf{W}_i}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta}\mu_{\theta j} \Big) \Big] \tag{2}$$

4

where we suppress dependence on $\mathbf{X}_i$ for simplicity in the second row. We derive the profile likelihood in the second row via differentiation with respect to γ_i; the optimum is unique by convexity of $ay - b\exp(y)$ in y when $a, b > 0$. We use $\mathbf{v}_i \cdot \mathbf{W}_i$ to denote $\sum_{j=1}^{J} v_{ij} W_{ij}$ and similarly for $\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta$.

We now allow weights $\mathbf{v}_i = (v_{i1}, \ldots, v_{iJ})$ to be given as a (bounded positive) function of $\mathbf{X}_i$ and $\mathbf{W}_{i\cdot} := \sum_{j=1}^{J} W_{ij}$ and examine the population analogue $M^v(\theta)$ of of the weighted profile log likelihood $M_n^v(\theta)$.

$$M^v(\theta) = \mathbb{E}_{\mathbf{W},\mathbf{X},\Gamma}\left[\sum_{j=1}^{J} v_{ij}\left(W_{ij}\log[\frac{\mathbf{v}_i \cdot \mathbf{W}_i}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta}\mu_{\theta j}] - \frac{\mathbf{v}_i \cdot \mathbf{W}_i}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta}\mu_{\theta j} \right) \right] \tag{3}$$

$$\mathbb{E}_{\mathbf{W}_\cdot,\mathbf{X},\Gamma}\mathbb{E}_{\mathbf{W}|\mathbf{W}_\cdot,\mathbf{X},\Gamma}\left[\sum_{j=1}^{J} v_{ij}\left(W_{ij}\log[\frac{\mathbf{v}_i \cdot \mathbf{W}_i}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta}\mu_{\theta j}] - \frac{\mathbf{v}_i \cdot \mathbf{W}_i}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta}\mu_{\theta j} \right) \right] \tag{4}$$

$$= \mathbb{E}_{\mathbf{W},\mathbf{X},\Gamma}\left[\sum_{j=1}^{J} v_{ij} W_{ij}\log\mathbf{v}_i \cdot \mathbf{W}_i \right] \tag{5}$$

$$+ \mathbb{E}_{\mathbf{W}_\cdot,\mathbf{X},\Gamma}\left[\sum_{j=1}^{J} v_{ij}\left(W_{i\cdot}\frac{\mu_{\theta_0 j}}{\mu_{\theta_0 \cdot}}\log\frac{\mu_{\theta j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta} - W_{i\cdot}\frac{\mathbf{v}_i \cdot \boldsymbol{\mu}_{\theta_0}}{\mu_{\theta_0 \cdot}}\frac{\mu_{\theta j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta} \right) \right] \tag{6}$$

$$= C + \mathbb{E}_{\mathbf{W}_\cdot,\mathbf{X},\Gamma}\left[W_{i\cdot}\frac{\mathbf{v}_i \cdot \boldsymbol{\mu}_{\theta_0}}{\mu_{\theta_0 \cdot}} \sum_{j=1}^{J} v_{ij}\left(\frac{\mu_{\theta_0 j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_{\theta_0}}\log\frac{\mu_{\theta j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta} - \frac{\mu_{\theta j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta} \right) \right] \tag{7}$$

We note that the term in line 5 above depends on θ_0 but not θ; accordingly, we represent it with constant C on line 7.

2.4 Optimizer of profile likelihood

We now show that, under a suitable identifiability condition, a weak condition on $\mathbf{W}$, and a condition on weighting function v, that if $\mathbb{E}[\mathbf{W}|\mathbf{X},\Gamma] = \exp(\Gamma)\boldsymbol{\mu}_{\theta_0}(\mathbf{X})$, then the unique optimizer of population criterion $M^v(\theta)$ is θ_0.

The additional conditions we need are as follows:

(D) For all $\theta, \theta' \in \Theta$, we have that, for any $a \in \mathbb{R}^+$, $\theta \neq \theta' \Rightarrow \boldsymbol{\mu}_\theta(\mathbf{x}) \neq a\boldsymbol{\mu}_{\theta'}(\mathbf{x})$ holds for all

$\mathbf{x} \in A \subset \mathcal{X}$ with $P_{\mathbf{X}}(A) > 0$.

(E) Weighting function $v : \mathcal{X} \times \mathcal{W}. \to \mathbb{R}^J_{>0}$, where $\mathcal{W}.$ is the support of $W.$, is continuous and bounded.

We will use the following simple lemma:

Lemma 1: For every $a \geq 0$, the function defined by $f_a(b) := a\log(b) - b$ is uniquely maximized at $b = a$ (defining $0\log 0 := 0$ and letting $a\log 0 = -\infty$ for every $a > 0$).

Proof: First consider the case $a > 0$. Since $f_a(0) = -\infty$ in this case and f_a is finite for all $b > 0$, the optimum cannot occur at $b = 0$. Over $b \in \mathbb{R}^+$, $\frac{\partial^2}{\partial b^2} f = -\frac{a}{b^2} < 0$, so f_a is strictly convex over $\mathbb{R}^+$ and hence takes a unique optimum. Setting $\frac{\partial}{\partial b} f = \frac{a}{b} - 1 = 0$ gives us that the optimum occurs at $b = a$.

When $a = 0$, $f_a(b) = \begin{cases} 0 & \text{if } b = 0 \\ -b & \text{if } b > 0 \end{cases}$, so f_a is optimized at 0 since $-b < 0$ when $b > 0$.

Theorem 1

Suppose that conditions (A) - (D) are met. Then for any weighting function satisfying (E), the criterion $M^v(\cdot)$ defined above is uniquely optimized at θ_0.

Proof

From above we have the form of the population criterion $M^v(\theta)$:

$$M^v(\theta) = C + \mathbb{E}_{\mathbf{W}.,\mathbf{X},\Gamma}\left[W.\frac{\mathbf{v} \cdot \boldsymbol{\mu}_{\theta_0}}{\mu_{\theta_0}.} \sum_{j=1}^{J} v_{ij}\left(\frac{\mu_{\theta_0 j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_{\theta_0}}\log\frac{\mu_{\theta j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta} - \frac{\mu_{\theta j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta}\right)\right] \tag{8}$$

For each fixed $\mathbf{x} \in \mathcal{X}$,

$$h_j^v(x, \theta; \theta_0) := \frac{\mu_{\theta_0 j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_{\theta_0}}(\mathbf{x})\log\frac{\mu_{\theta j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta}(\mathbf{x}) - \frac{\mu_{\theta j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta}(\mathbf{x}) \tag{9}$$

6

is maximized when $\frac{\mu_{\theta j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_\theta}(\mathbf{x}) = \frac{\mu_{\theta_0 j}}{\mathbf{v}_i \cdot \boldsymbol{\mu}_{\theta_0}}(\mathbf{x})$ by lemma 1.

Before proceeding, we show that $-\infty < M^v(\theta_0) < \infty$. By definition, we have

$$M^v(\theta_0) = \mathbb{E}_{\mathbf{W},\mathbf{X},\Gamma}\sup_{\gamma\in\mathbb{R}}\sum_{j=1}^{J} v_j\left(W_j\log\left[\exp(\gamma)\frac{\mathbf{v}\cdot\mathbf{W}}{\mathbf{v}\cdot\boldsymbol{\mu}_{\theta_0}}\mu_{\theta_0 j}\right] - \exp(\gamma)\frac{\mathbf{v}\cdot\mathbf{W}}{\mathbf{v}\cdot\boldsymbol{\mu}_{\theta_0}}\mu_{\theta_0 j}\right) \tag{10}$$

$$\geq \mathbb{E}_{\mathbf{W},\mathbf{X},\Gamma}\sum_{j=1}^{J} v_j\left(W_j\log\left[\frac{\mathbf{v}\cdot\mathbf{W}}{\mathbf{v}\cdot\boldsymbol{\mu}_{\theta_0}}\mu_{\theta_0 j}\right] - \frac{\mathbf{v}\cdot\mathbf{W}}{\mathbf{v}\cdot\boldsymbol{\mu}_{\theta_0}}\mu_{\theta_0 j}\right) \text{ (setting } \gamma = 0) \tag{11}$$

$$= \mathbb{E}_{\mathbf{W},\mathbf{X},\Gamma}\sum_{j=1}^{J} v_j W_j\log\mathbf{v}\cdot\mathbf{W} \tag{12}$$

$$+ \mathbb{E}_{W.,\mathbf{X},\Gamma}\frac{\mathbf{v}\cdot\boldsymbol{\mu}_{\theta_0}}{\mu_{\theta_0 .}}W.\sum_{j=1}^{J}\left[\frac{\mu_{\theta_0 j}}{\mathbf{v}\cdot\boldsymbol{\mu}_{\theta_0}}\log\frac{\mu_{\theta_0 j}}{\mathbf{v}\cdot\boldsymbol{\mu}_{\theta_0}} - \frac{\mu_{\theta_0 j}}{\mathbf{v}\cdot\boldsymbol{\mu}_{\theta_0}}\right] \tag{13}$$

The term in line (12) is equal to $\mathbb{E}_{\mathbf{W},\mathbf{X},\Gamma}\mathbf{v}\cdot\mathbf{W}\log\mathbf{v}\cdot\mathbf{W}$, which as the integral of a bounded

function over a bounded domain is finite. By assumption (C), we must have $\sum_j \mu_{\theta_0 j}(\mathbf{X}) > 0$

almost surely, so the front term in line (13) is almost surely bounded by boundedness of μ_θ and

v. Inside the sum in this line, we have terms of the form $a\log a - a$, which is a bounded function

on any bounded set in $\mathbb{R}_{\geq 0}$. Hence line (13) is an integral of a bounded function over a bounded

domain and so is also finite, so $M^v(\theta_0) > -\infty$.

Similarly,

$$M^v(\theta_0) = \mathbb{E}_{\mathbf{W},\mathbf{X},\Gamma}\sup_{\gamma\in\mathbb{R}}\sum_{j=1}^{J} v_j\left(W_j\log\left[\exp(\gamma)\frac{\mathbf{v}\cdot\mathbf{W}}{\mathbf{v}\cdot\boldsymbol{\mu}_{\theta_0}}\mu_{\theta_0 j}\right] - \exp(\gamma)\frac{\mathbf{v}\cdot\mathbf{W}}{\mathbf{v}\cdot\boldsymbol{\mu}_{\theta_0}}\mu_{\theta_0 j}\right) \tag{14}$$

$$\leq \mathbb{E}_{\mathbf{W},\mathbf{X},\Gamma}\sum_{j=1}^{J} v_j\left(W_j\log W_j - W_j\right) \text{ by lemma 1} \tag{15}$$

$$< \infty \text{ since } \sum_{j=1}^{J} v_j\left(W_j\log W_j - W_j\right) \text{ is bounded on } \mathcal{W}\times\mathcal{X}\times\mathcal{G} \tag{16}$$

Hence $-\infty < M^v(\theta_0) < \infty$, which guarantees that the difference in the following argument is

not of the form $\infty - \infty$.

7

Now, for any $\theta \in \Theta$ with $\theta \neq \theta_0$, we have

$$M^v(\theta) - M^v(\theta_0)$$

$$= \int_A g(\mathbf{x}) \int \int w \frac{\mathbf{v} \cdot \boldsymbol{\mu}_{\theta_0}}{\mu_{\theta_0}} \sum_{j=1}^J \left[h_j^v(\mathbf{x}, \theta; \theta_0) - h_j^v(\mathbf{x}, \theta_0; \theta_0) \right] dP_{W.|\Gamma, \mathbf{x}}(w) dP_{\Gamma|\mathbf{x}}(\gamma) dP_{\mathbf{x}}(\mathbf{x})$$

$$+ \int_{A^C} \int \int w \frac{\mathbf{v} \cdot \boldsymbol{\mu}_{\theta_0}}{\mu_{\theta_0}} \sum_{j=1}^J \left[h_j^v(\mathbf{x}, \theta; \theta_0) - h_j^v(\mathbf{x}, \theta_0; \theta_0) \right] dP_{W.|\Gamma, \mathbf{x}}(w) dP_{\Gamma|\mathbf{x}}(\gamma) dP_{\mathbf{x}}(\mathbf{x})$$

$$\leq \int_A \int \int w \frac{\mathbf{v} \cdot \boldsymbol{\mu}_{\theta_0}}{\mu_{\theta_0}} \sum_{j=1}^J \left[h_j^v(\mathbf{x}, \theta; \theta_0) - h_j^v(\mathbf{x}, \theta_0; \theta_0) \right] dP_{W.|\Gamma, \mathbf{x}}(w) dP_{\Gamma|\mathbf{x}}(\gamma) dP_{\mathbf{x}}(\mathbf{x}) \quad (\star)$$

$$< 0$$

The first inequality is a result of θ_0 maximizing (not necessarily uniquely) h_j^v; i.e., $h_j^v(\mathbf{x}, \theta; \theta_0) - h_j^v(\mathbf{x}, \theta_0; \theta_0) \leq 0$. Strict inequality holds in the last line because the integrand in $(\star)$ is strictly negative, since $h_j^v(\mathbf{x}, \theta; \theta_0) - h_j^v(\mathbf{x}, \theta_0; \theta_0) < 0$ for $\theta \neq \theta_0$ on A, and the front term $w \frac{\mathbf{v} \cdot \boldsymbol{\mu}_{\theta_0}}{\mu_{\theta_0}}$ is a.s. strictly positive by assumption (C) and positivity of $\mathbf{v}$. Hence $M^v(\theta_0) > M^v(\theta)$ for all $\theta \neq \theta_0$ in Θ, so θ_0 is the unique maximizer of the population criterion $M^v(\cdot)$.

2.5   Consistency of M-estimators

We apply theorem 5.14 of van der Vaart (1998) to show consistency of maximizers $\hat{\theta}_n^v$ of M_n^v for θ_0. We also show consistency of estimators $\hat{\theta}_n^{\hat{v}_n}$, where $\{\hat{v}_n\}$ is a sequence of random continuous positive bounded weighting functions converging uniformly in probability to v.

We first require the following assumption on v and $\hat{v}_n$:

(F) $\sup_{t \in \mathcal{X} \times \mathcal{W}.} |v(t) - \hat{v}_n(t)| \xrightarrow{p} 0$ and every $\hat{v}_n$ is positive and bounded.

Theorem 2

Suppose conditions (A) through (F) are satisfied. Then $P(d(\hat{\theta}^v, \theta_0) > \epsilon) \to 0$ for all $\epsilon > 0$ and $P(d(\hat{\theta}^{\hat{v}_n}, \theta_0) > \epsilon) \to 0$ for all $\epsilon > 0$ where $d(\cdot, \cdot)$ is given by $d(s, t) := \sum_k |\arctan s_k - \arctan t_k|$.

Proof

We first compactify our parameter space Θ to obtain $\bar{\Theta}$ by allowing elements of unconstrained Euclidean parameters to take values in the extended reals.

A necessary condition for theorem 5.14 is that we have $P\sup_{\theta\in U}m^v_\theta < \infty$ for $m^v_\theta(\mathbf{w}) :=$ $\sum_{j=1}^J v_j\left(w_j\log\left[\frac{\mathbf{v}\cdot\mathbf{w}}{\mathbf{v}\cdot\mu_\theta}\mu_{\theta_j}\right] - \frac{\mathbf{v}\cdot\mathbf{w}}{\mathbf{v}\cdot\mu_\theta}\mu_{\theta_j}\right)$ and a sufficiently small ball $U \in \Theta$. By lemma 1, $\sup_{\theta\in U}m^v(\mathbf{w}) \le$ $\sum_{j=1}^J v_j\left(w_j\log w_j - w_j\right)$, which is bounded above since v is bounded. Hence by assumption (A), $P\sup_{\theta\in U}m^v_\theta \le P\sum_{j=1}^J v_j\left(w_j\log w_j - w_j\right) < \infty$. We also require $M^v_n(\hat{\theta}^v) = M^v_n(\boldsymbol{\theta_0}) + o_P(1)$ which is trivially satisfied since $\hat{\theta}^v$ maximizes M^v_n.

Then letting compact set $K = \bar{\Theta}$, we can directly apply theorem 5.14 to obtain $P(d(\hat{\theta}^v, \theta_0) \ge \epsilon) \to 0$ for any $\epsilon > 0$.

To apply theorem 5.14 to $\hat{\theta}^{\hat{v}_n}$, we only need in addition to the above that $M^v_n(\hat{\theta}^{\hat{v}_n}) = M^v_n(\theta_0) + o_P(1)$.

For any fixed $\hat{v}$, we have

$$M^{\hat{v}}_n(\hat{\theta}^{\hat{v}}_n) = M^{\hat{v}}_n(\theta_0) + o_P(1) \tag{17}$$

$$= M^v_n(\theta_0) + o_P(1) + (M^v_n(\theta_0) - M^{\hat{v}}_n(\theta_0)) \tag{18}$$

However, the term $(M^v_n(\theta_0) - M^{\hat{v}}_n(\theta_0)) = o_P(1)$ if we let $\hat{v} = \hat{v}_n$ since

$$|M^{\hat{v}_n}_n(\theta_0) - M^v_n(\theta_0)| = \left|\frac{1}{n}\sum_{i=1}^n\sum_{j=1}^J(\hat{v}_{n,ij} - v_{ij})l_{ij}(\theta_0)\right| \tag{19}$$

$$\le \sup_{t\in\mathcal{X}\times\mathcal{W}}|\hat{v}_n(t) - v(t)|\frac{1}{n}\sum_{i=1}^n\sum_{j=1}^J|l_{ij}(\theta_0)| \xrightarrow{p} 0 \tag{20}$$

since $\sup_{t\in\mathcal{X}\times\mathcal{W}}|\hat{v}_n(t) - v(t)| \xrightarrow{p} 0$.

Hence $M^v_n(\hat{\theta}^{\hat{v}_n}) = M^v_n(\theta_0) + o_P(1)$, so $P(d(\hat{\theta}^{\hat{v}_n}, \theta_0) \ge \epsilon) \to 0$ for any $\epsilon > 0$.

2.6 Convergence in distribution of M-estimators

In this section we provide a brief sketch of convergence results for $\sqrt{n}(\hat{\theta}^v_n - \theta_0)$.

9

Letting $\mathbb{P}_n$ denote the empirical distribution function of n independent pairs $(\mathbf{W}_i, \mathbf{X}_i)$, with each pair taking values in some subset of $\mathbb{R}^d$ and with P the true distribution for $(\mathbf{W}, \mathbf{X})$, we have

$$\sqrt{n}(\mathbb{P}_n - P) \rightsquigarrow \mathbb{G}_P \tag{21}$$

in $l^\infty(P)$ for Gaussian process $\mathbb{G}_P$.

Letting $g(P) := \arg\max_{\theta \in \Theta} P m_\theta^v$, if g has Hadamard directional derivative $\dot{g}$ at P, then we can apply the infinite dimensional delta method (Römisch, 2004) to obtain

$$\sqrt{n}(g(\mathbb{P}_n) - g(\mathbb{P})) = \sqrt{n}(\hat{\theta}_n^v - \theta_0) \rightsquigarrow \dot{g}(\mathbb{G}_P) \tag{22}$$

Using weights $\hat{v}_n$ estimated from data does not affect convergence, as we have

$$\sqrt{n}(M_n^{\hat{v}_n}(\theta) - M^{\hat{v}_n}(\theta)) \tag{23}$$

$$= \sqrt{n}(M_n^v(\theta) - M^v(\theta) + \left[[M_n^{\hat{v}_n}(\theta) - M_n^v(\theta)] - [M^{\hat{v}_n}(\theta) - M^v(\theta)]\right]) \tag{24}$$

$$= \sqrt{n}(M_n^v(\theta) - M^v(\theta)) + \sqrt{n}(\mathbb{P}_n - P)m_\theta^{\hat{v}_n - v} \tag{25}$$

$$= \sqrt{n}(M_n^v(\theta) - M^v(\theta)) + o_P(1) \tag{26}$$

since

$$\sqrt{n}(\mathbb{P}_n - P)m_\theta^{\hat{v}_n - v} = \sqrt{n}\frac{1}{n}\sum_{i=1}^n \sum_{j=1}^J (\hat{v}_{n;ij} - v_{ij})l_{ij}(\theta) \tag{27}$$

$$\leq \sqrt{n}\frac{1}{n}\sum_{i=1}^n \sum_{j=1}^J |l_{ij}(\theta)| \sup_{t \in \mathcal{W} \times \mathcal{X}} |\hat{v}_n(t) - v(t)| \tag{28}$$

$$\xrightarrow{p} 0 \tag{29}$$

Note that $\arg\max_{\theta \in \Theta} M^{\hat{v}_n}(\theta) = \arg\max_{\theta \in \Theta} M^v(\theta) = \theta_0$ by theorem 1.

3 Optimization

3.1 Reparametrization of barrier subproblem

Letting $\theta^\star$ indicate the unknown parameters in our model under the above reparametrization of relative abundance, we now have the following unconstrained minimization problem:

$$\arg\min_{\theta^\star} f_n(\theta^\star) + \frac{1}{t^{(r)}} \Bigg[\sum_k \left(\sum_{j=1}^{J-1} -\rho_{kj} + J\log\left[1 + \sum_{j=1}^{J-1} \exp(\rho_{kj})\right]\right) \tag{30}$$

$$\sum_{\tilde{k}} \left(\sum_{j=1}^{J-1} -\tilde{\rho}_{\tilde{k}j} + J\log\left[1 + \sum_{j=1}^{J-1} \exp(\tilde{\rho}_{\tilde{k}j})\right]\right) \Bigg] \tag{31}$$

3.2 Barrier algorithm

Barrier Algorithm

1. Initiate with value of penalty parameter t set to starting value $t^{(0)}$ and values of parameters $\theta^\star$ equal to $\theta^{\star(0)}$. Set iteration $r = 0$.

2. Using current value $t^{(r)}$ of t and starting at parameter estimate $\theta^{\star(r)}$, solve barrier subproblem r given above via Fisher scoring. Denote the solution of this subproblem $\theta^\star_{(r+1)}$ and set $t_{(r+1)} = at_{(r)}$ for a prespecified $a > 1$.

3. If $t_{(r+1)} > t_{\max}$ for prespecified $t_{\max}$, return $\theta^\star_{(r+1)}$. Otherwise set iteration $r = r + 1$ and return to step 2.

3.3 Constrained Newton within Augmented Lagrangian Algorithm

We calculate update steps from $\mathcal{L}_k$ given in section 3 of the main text as follows:

Constrained Newton within Augmented Lagrangian Algorithm

Initiate with initial values $\nu^{(0)}$ and $\mu^{(0)} > 0$ of penalty coefficients ν and μ

1. Calculate proposed update $\mathbf{p}_k^{\text{update}}$ via nonnegative least squares on $\mathcal{L}_k$ using current values of ν and μ

2. If $|\sum_j^J p_{kj}^{\text{update}} - 1| < \delta$ for some prespecified tolerance $\delta > 0$, set update direction $\mathbf{s}_k := \mathbf{p}_k - \mathbf{p}_k^{\text{update}}$ and proceed to step (3). Otherwise update ν and μ via algorithm given in Bazaraa (2006) (p. 496) and return to step (1).

3. Perform a line search in direction $\mathbf{s}_k$ to determine updated parameter value $\mathbf{p}_k^{\text{updated}} := \mathbf{p}_k + \epsilon \mathbf{s}_k$ that decreases $f_n(\mathbf{p}_k)$ for some $0 < \epsilon \leq 1$.

3.4 Quadratic approximation to $f_n(\mathbf{p}_k)$.

In section 3 of the main text, we specify $\mathcal{L}_k$ in terms of a quadratic approximation $Q_k^{(t)}$ to objective $f_n(\mathbf{p}_k)$. In practice we construct $Q_k^{(t)}$ as a slightly modified Taylor expansion of $f_n()$ around the current value of $\mathbf{p}_k^{(t)}$. We use the gradient of f_n with respect to $\mathbf{p}_k$ in the first order term, and in the second order term, and in place of the Hessian, we use (-1 times) the Fisher information matrix in $\mathbf{p}_k$ regularized (for numerical stability) by addition of magnitude of the gradient times an identity matrix.

4 Costea (2017) Analysis

4.1 Details of model specification

Costea et al. (2017) published two flow cytometric readings for every species in the synthetic community with the exception of *V. cholerae*, for which only one reading was published. In all taxa save *V. cholerae*, we take the mean reading as our observation, and we include the resulting vector of readings augmented by the single reading for *V. cholerae* as a row in $\mathbf{W}$. We anticipate that our use of mean readings represents a fairly small loss of information, as flow cytometric readings did not vary substantially within taxon. However, in a similar setting where multiple sets of flow cytometric readings across all taxa were available, we could include each set as a row of $\mathbf{W}$ to capture variability in these measurements.

To estimate detection effects relative to flow cytometry measurements, we specify $X_1 = \mathbf{0}$. For $i \geq 2$, $\mathbf{X}_{i\cdot} = [1 \; \mathbf{1}_Q \; \mathbf{1}_W]$ where $\mathbf{1}_Q$ is an indicator for sample i being processed according to protocol Q, and similarly for $\mathbf{1}_W$.

4.2 Cross-validation design

We construct folds for our 10-fold cross-validation on Costea et al. (2017) data so that, with the exception of samples A and B, which we grouped together in a single fold, each fold included all observations for a given specimen. For each fold, we fit a model in which all observations in all other folds, along with flow cytometry readings, were treated as arising from a common specimen (as in fact they do, save for flow cytometry readings, which were taken on specimens mixed to create the mock spike-in). We model each sample in the held-out fold as arising from a distinct specimen of unknown composition to allow our model to estimate a different relative abundance profile for distinct samples processed according to different protocols.

4.3 Model summaries

Table 1: Point estimates and 95% bootstrap confidence intervals for protocol-specific detection effects $\boldsymbol{\beta}$ (with reference taxon Y. pseudotuberculosis) estimated from Costea (2017) data

Taxon	Protocol H	Protocol Q	Protocol W
B. hansenii	-1.61 (-2.00 − -1.16)	-1.55 (-1.75 − -1.31)	-0.08 (-0.16 − 0.00)
C. difficile	-0.18 (-0.30 − 0.01)	-0.57 (-0.79 − -0.41)	1.23 (1.18 − 1.28)
C. perfringens	3.38 (3.27 − 3.57)	2.48 (2.31 − 2.62)	4.05 (4.03 − 4.07)
C. saccharolyticum	-0.19 (-0.23 − -0.16)	-0.01 (-0.12 − 0.1)	-0.10 (-0.13 − -0.06)
F. nucleatum	2.37 (2.28 − 2.44)	0.14 (-0.16 − 0.42)	2.11 (2.05 − 2.16)
L. plantarum	-2.62 (-2.96 − -2.12)	0.72 (0.60 − 0.93)	0.60 (0.56 − 0.63)
P. melaninogenica	4.17 (4.12 − 4.2)	3.88 (3.82 − 4.04)	4.25 (4.23 − 4.27)
S. enterica	2.49 (2.45 − 2.51)	2.74 (2.64 − 2.79)	2.48 (2.46 − 2.51)
V. cholerae	1.54 (1.50 − 1.56)	0.90 (0.78 − 0.99)	1.48 (1.44 − 1.50)

Table 1 provides point estimates and marginal 95% confidence intervals for the detection effects for each of protocols H, Q, and W estimated via the full model described above. This model was fit with reference taxon *Y. pseudotuberculosis* (i.e., under the constraint that the column of $\boldsymbol{\beta}$ corresponding to this taxon consists of 0 entries). Hence we interpret estimates in this table in terms of degree of over- or under-detection relative to *Y. pseudotuberculosis* – for example, we estimate that, repeated measurement under protocol H of samples consisting of 1:1 mixtures of *B. hansenii* and *Y. pseudotuberculosis*, the mean MetaPhlAn2 estimate (under the settings used by McLaren et al. (2019)) of the relative abundance of B. hansenii will be $\exp(-1.61) \approx 0.20$ as large as the mean estimate of the relative abundance of *Y. pseudotuberculosis*.

5 Karstens (2019) Analysis

5.1 Preprocessing

We process raw read data reported by Karstens et al. (2019) using the DADA2 R package (version 1.20.0) (Callahan et al., 2016). We infer amplicon sequence variants using the *dada* function with option 'pooled = TRUE' and assign taxonomy with the *assignSpecies* function using a SILVA v138 training dataset downloaded from https://benjjneb.github.io/dada2/training.html (Quast et al., 2012).

5.2 Model Specification

We conduct a three-fold cross-validation of a model containing both contamination and detection effects. For each held-out fold r, if we let $\mathbf{e}_r := (\mathbf{1}_{[\text{sample 1 in fold } r]}, \ldots, \mathbf{1}_{[\text{sample n in fold } r]})^T$, $\mathbf{d} = (3^0, \ldots, 3^8)^T$, and $\circ$ indicate element-wise multiplication then we specify the model for this fold with

$$\mathbf{Z} = \begin{bmatrix} \mathbf{1} - \mathbf{e}_r & \mathbf{e}_r \end{bmatrix}$$

$$\tilde{\mathbf{Z}} = \begin{bmatrix} \mathbf{d} \circ (\mathbf{1} - \mathbf{e}_r) \end{bmatrix} + \exp(\tilde{\alpha}) \begin{bmatrix} \mathbf{d} \circ \mathbf{e}_r \end{bmatrix}$$

$$\mathbf{X} = \vec{\mathbf{1}}$$

$$\tilde{\mathbf{X}} = 0$$

The relative abundance matrix $\mathbf{p}$ consists of two rows, the first of which is treated as fixed and known and contains the theoretical composition of the mock community used by Karstens et al. (2019). The second row is to be estimated from observations on samples in the held-out fold. $\tilde{\mathbf{p}}$ consists of a single row, the first 247 elements of which we treat as unknown. We fix $\tilde{p}_{248} = 0$

as an identifiability constraint – identifiability problems arise here because all samples sequenced arise from the same specimen, we lack identifiability over, for any choice of fixed $\tilde{\mathbf{p}}^\star$ and $\mathbf{p}$, the set $\{\tilde{\mathbf{p}} = a * \tilde{\mathbf{p}}^\star + (1-a)\mathbf{p} : 0 \leq a \leq 1\}$. Briefly, we do not consider the assumption $\tilde{p}_{248} = 0$ unrealistic; while in general distinguishing between contaminant and non-contaminant taxa is challenging, it is fairly frequently the case that choosing a single taxon *unlikely* to be a contaminant is not difficult. Moreover, we anticipate that in most applied settings, more than one specimen will be sequenced and this identifiability problem will hence not arise.

$\boldsymbol{\beta}$ consists of a single row, the first $J-8 = 240$ elements of which (corresponding to contaminant taxa) are treated as fixed and known parameters equal to 0, as we cannot estimate detection efficiencies in contaminant taxa. The following 7 elements of β are treated as fixed and unknown (to be estimated from data), and the 248th element of $\boldsymbol{\beta}$ is set equal to 0 as an identifiability constraint. $\tilde{\boldsymbol{\gamma}}$ is specified as a single unknown parameter in $\mathbb{R}$

The full model fit without detection efficiencies $\boldsymbol{\beta}$ is specified by treating $\boldsymbol{\beta}$ as fixed and known with all elements equal to 0. We treat all samples as arising from the same specimen, so $\mathbf{Z} = \mathbf{1}$, $\tilde{\mathbf{Z}} = \mathbf{d}$, and $\mathbf{p}$ consists of a single row treated as an unknown relative abundance. Specifications of $\mathbf{X}$, $\tilde{\mathbf{X}}$, $\tilde{\mathbf{p}}$, and $\tilde{\gamma}$ do not differ from above.

5.3 Additional summaries

Table 2: Entries of $\hat{\boldsymbol{\beta}}$ (reference taxon L. fermentum) estimated from Karstens (2019) data

Taxon	Estimate
P. aeruginosa	-1.29
E. coli	-0.20
S. enterica	-0.48
E. faecium	-2.09
S. aureus	-3.05
L. monocytogenes	-1.60
B. halotolerans	-0.74

For each taxon for which β_j is identifiable (i.e., taxa in the mock community), our model produces a point estimate $\hat{\beta}_j$, as show in table 2 above. (The reference taxon, *L. fermentum*, for which we enforce identifiability constraint $\beta_j = 0$, is excluded.) On the basis of this model, we estimate that in an equal mixture of *E. coli* and our reference taxon, *L. fermentum* sequenced by the method used by Karstens et al. (2019), we expect on average to observe $\exp(-0.20) \approx 0.82$ *E. coli* reads for each *L. fermentum* read. In an equal mixture of *S. aureus* and *L. fermentum* similarly sequenced, we expect on average to observe $\exp(-3.05) \approx 0.047$ reads for each *L. fermentum* read. As only two samples were treated as known in our analysis, we do not attempt to quantify uncertainty around these point estimates.

6 Brooks (2015) simulation

6.1 Identifiability

In our simulation using Brooks et al. (2015) data, we repeatedly selected sets of 3, 5, 10, or 20 samples from each of two plates of 40 samples sequenced by Brooks et al. (2015) to treat as known. For each randomly selected subset of samples to be treated as known, we required that $\boldsymbol{\beta}$ be identifiable on the basis of the taxa present in all known samples on each plate (i.e., identifiable from either group of samples). Briefly, this amounts to requiring that the graph whose nodes are the 7 taxa under consideration with edges between two nodes if the taxa those nodes correspond to are present in the same known sample. When a randomly selected set of samples failed this requirement, we redrew sets of samples until we found one that satisfied it.
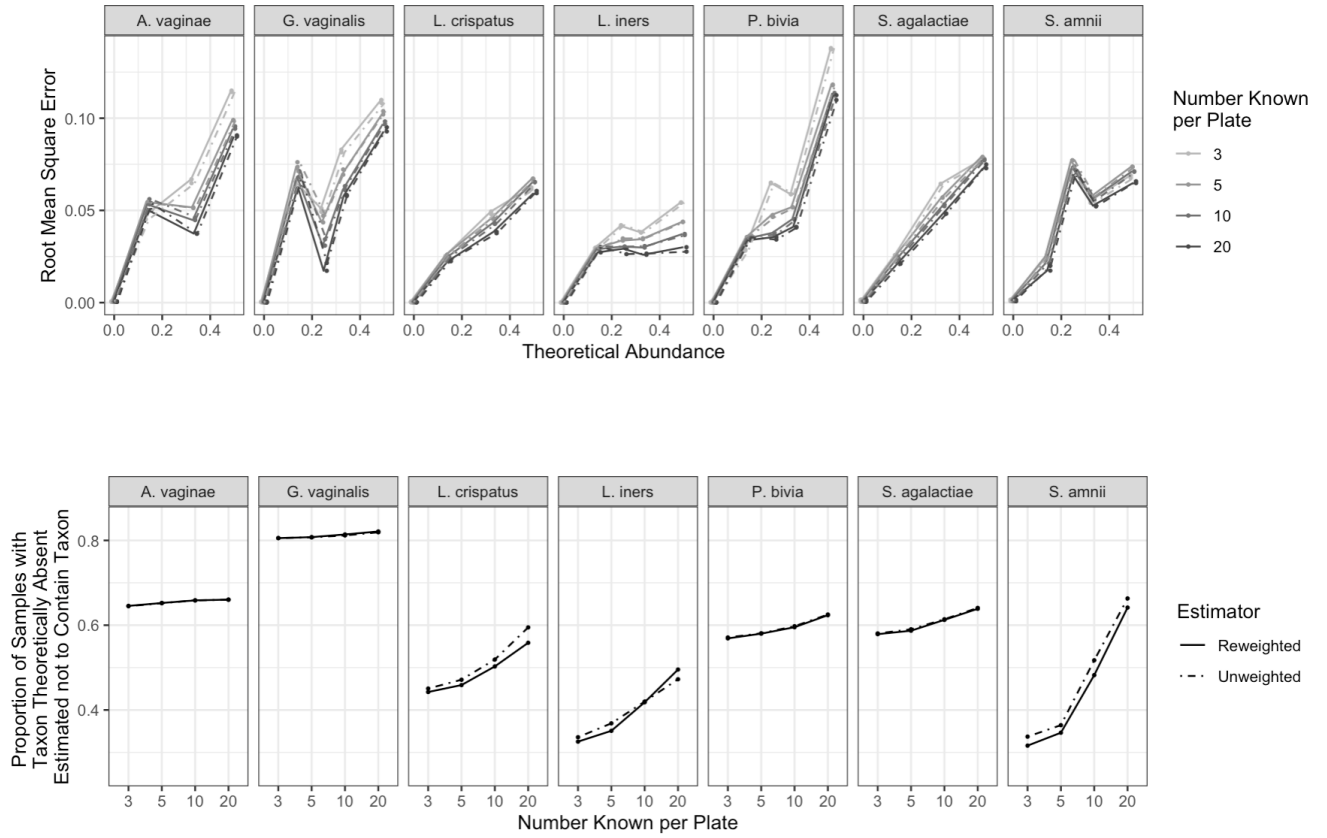
6.2   Figures

Figure 1: Predictive performance of models fit on Brooks et al. (2015) data. The upper row includes root mean square error of relative abundance estimates by estimator, taxon, number of samples treated as known per plate, and true relative abundance. True relative abundance is given on the x-axis and root mean square error is plotted on the y-axis; for concision, true relative abundances equal to 1 are plotted at 0. Each column pane contains estimates for a different taxon, estimator is indicated with line type (solid for Poisson and dashed for weighted Poisson), and number of samples known per plate is indicated by color. In the lower row, proportion of elements of $\mathbf{P}$ truly equal to zero estimated to be equal to zero is plotted on the y-axis of each pane, and the x-axis gives number of samples per plate treated as known. Taxon and estimator are represented as in the upper row, and the proportion of nonzero elements of $\mathbf{W}$ corresponding to zero elements of $\mathbf{P}$ for each taxon is plotted as a dotted horizontal line.

References

Bazaraa, M.S. (2006). Nonlinear programming: theory and algorithms.

Brooks, J.P. et al (2015). The truth about metagenomics: quantifying and counteracting bias in 16s rrna studies. *BMC Microbiology 15*(1), 1–14.

Callahan, B.J. et al (2016). DADA2: high-resolution sample inference from illumina amplicon data. *Nature Methods 13*(7), 581.

Costea, P.I. et al (2017). Towards standards for human fecal sample processing in metagenomic studies. *Nature Biotechnology 35*(11), 1069.

Karstens, L. et al (2019). Controlling for contaminants in low-biomass 16s rrna gene sequencing experiments. *MSystems 4*(4), e00290–19.

McCullagh, P. (1983). Quasi-likelihood functions. *The Annals of Statistics 11*(1), 59–67.

McLaren, M.R., Willis, A.D. and Callahan, B.J. (2019). Consistent and correctable bias in metagenomic sequencing experiments. *eLife 8*.

Quast, C. et al (2012). The silva ribosomal rna gene database project: improved data processing and web-based tools. *Nucleic acids research 41*(D1), D590–D596.

Römisch, W. (2004). Delta method, infinite dimensional.