TITLE: Balances: a new perspective for microbiome analysis

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ABSTRACT

High-throughput sequencing technologies have revolutionized microbiome research by allowing the relative quantification of microbiome composition and function in different environments. One of the main goals in microbiome analysis is the identification of microbial species that are differentially abundant among groups of samples, or whose abundance is associated with a variable of interest. Most available methods for microbiome abundance testing perform univariate tests for each microbial species or taxa separately, ignoring the compositional nature of microbiome data.

We propose an alternative approach for microbiome abundance testing that consists on the identification of two groups of taxa whose relative abundance, or balance, is associated with the response variable of interest. This approach is appealing, since it has direct translation to the biological concept of ecological balance between species in an ecosystem. In this work, we present *selbal*, a greedy stepwise algorithm for balance selection.

We illustrate the algorithm with 16s abundance data from an HIV-microbiome study and a Crohn-microbiome study.

Importance

A more meaningful approach for microbiome abundance testing is presented. Instead of testing each taxon separately we propose to explore abundance balances among groups of taxa. This approach acknowledges the compositional nature of microbiome data.

INTRODUCTION

Human microbiome research, focused on understanding the role in health and disease of microbes living in the human body, has experienced significant growth in the last few years. High-throughput sequencing technologies have revolutionized this field by allowing the quantification of microbiome composition and function in different environments. Large scale projects, like the Human Microbiome Project (1),(2) or MetaHIT (Metagenomics of the Human Intestinal Tract), have established standardized protocols for creating, processing and interpreting metagenomic data (3). However, the analysis of microbiome data for differential abundance or association with sample metadata is still challenging.

Typically, after DNA sequencing, bioinformatics preprocessing and quality control of the sequences, an abundance table with the number of sequences (*reads*) per sample for different microbial species (taxa) is obtained. Total number of sequences for each sample is highly variable, and depends on laboratory sample preparation. Indeed, raw abundances and the total number of reads per sample are non-informative since they depend on physical and technical mechanisms when sequencing the DNA. In order to mitigate the problem of different sampling depth, microbiome data are often normalized previous to differential abundance testing (4),(5).

Working with proportions, that is, relative abundances instead of raw abundances, does not solve the problem since there is a dependence structure in the data that may lead to misleading results such as spurious correlations or incoherent distances (6),(7).

Rarefaction, which consists of random sampling of the same number of sequences for each sample, is similar to working with relative abundances. Though rarefaction might be convenient for richness and diversity analyses and avoids the problem of different sample depth, it supposes a loss of information and the increase of Type I error for differential abundance analyses (4). Other normalization alternatives, developed for RNA-Seq, are also applied in microbiome analysis for dealing with the problem of different number of counts per sample through variance stabilizing transformations (5). However, these RNA-Seq proposals also present problems with the false discovery rate when library sizes are very different among samples (8).

An alternative approach to rarefaction and normalization methods for microbiome analysis is to acknowledge the compositional nature of microbiome data and to use the mathematical theory available for compositional data (CoDa). Compositional data is defined as a vector of strictly positive real numbers carrying relative information. Relative information refers to the fact that the information of interest is contained in the ratios between the components of the composition and the numerical value of each component by itself is irrelevant (9).

As mentioned before, raw microbiome abundances are by itself non-informative since they depend on technical artifacts such as sequencing depth. Thus, microbiome data fits the

84 definition of compositional data except for the fact that microbiome abundance tables
85 contain many zeros. Assuming that observed zeros are rounded zeros, meaning that they
86 correspond to values below the detection limit, they can be replaced by a positive value or
87 pseudo count (10) so that CoDa analysis in terms of relative abundances between groups of
88 microorganisms can be applied.
89
90 Several recent works acknowledge the compositional nature of microbiome abundance
91 data and propose their analysis accordingly (11,12). Most of these approaches consider the
92 *centered log-ratio transformation* (clr) and perform relative abundance testing for each clr
93 transformed component, which is given by the logarithm of the component divided by the
94 geometric mean of all the components in the sample. This allows the identification of clr
95 transformed components that are associated with a specific characteristic of interest.
96 However, the interpretation of such association is not straightforward because the clr
97 transformation involves the abundances of all the taxa in the sample.
98 Instead, we propose to perform microbiome relative abundance testing by identifying two
99 groups of taxa whose relative abundance is associated with the phenotype of interest. For
100 this we use the notion of balance between two groups of components of a composition,
101 which is a central concept in CoDa analysis.
102
103 Mathematically, a balance is defined as follows. Let $X = (X_1, X_2, \dots, X_k)$ be a composition
104 of the number of counts for k different microbial species or taxa. Given two disjoint subsets

of components in X, denoted by X_+ and X_-, indexed by I_+ and I_-, and composed by k_+ and k_- taxa, respectively, the balance between X_+ and X_- is defined as:

$$B = \sqrt{\frac{k_+ \cdot k_-}{k_+ + k_-}} \log \frac{\left(\prod_{i \in I_+} x_i\right)^{1/k_+}}{\left(\prod_{j \in I_-} x_j\right)^{1/k_-}} \ .$$

Expanding the logarithm, the balance is proportional to

$$B \ \propto \ \frac{1}{k_+} \sum_{i \in I_+} \log X_i - \frac{1}{k_-} \sum_{j \in I_-} \log X_j \ ,$$

which is a more familiar expression corresponding to the difference in means of the log-transformed abundances between the two groups.

Balances are in compositional data analysis a key element in the construction of new coordinates through the so called *isometric log-ratio transformation* (ilr) (13) .

The concept of balance, as proposed in the compositional data theory, provides a new and interesting perspective for microbiome data analysis, since this mathematical concept is related to the biological concept of ecological balance in ecosystems.

Recently, some authors have proposed the use of CoDa approaches for microbiome analysis with different objectives such as the differential abundance between groups (14),

differentiation of niches (15), or the inclusion of phylogenetic associations between the components included in the study (16).

In this work, we propose an algorithm for the identification of balances between groups of taxa that are associated with a dependent component of interest. This approach provides a new perspective to differential abundance and microbiome association studies. Starting with the balance composed by only two taxa that is most associated with the response, the algorithm performs a forward selection process and, at each step, a new taxon is added to the existing balance so that the specified association criterion is maximized. The algorithm stops when none of the possible additions improves the current association.

The paper is organized as follows. In the Results and Discussion section, the proposed algorithm is applied to an HIV-microbiome study and to a Crohn's disease-microbiome study. Then these results are analyzed and both the advantages and technical issues of the algorithm when applied to microbiome data sets are discussed. Finally, in Material and Methods we present a detailed explanation of the algorithm.

RESULTS

We illustrate the proposed methodology with a dataset from a cross – sectional HIV – microbiome study conducted in Barcelona (Spain) including both HIV – infected subjects and HIV – negative controls (17). Microbiome information is derived from MiSeq™ 16SrRNA sequence and bioinformatically processed with Mothur. After applying abundance filters and agglomerating taxa to genus level, microbiome abundance is summarized in a matrix of raw abundances for 155 samples and 60 different genera (Bioproject accession number: PRJNA307231, SRA accession number: SRP068240). Below, we present the results for two different analyses, the association of microbiome abundance with HIV status and with the inflammation parameter, sCD14. In the first case, the component of interest is dichotomous while in the second case it is continuous.

We also present the results of a Crohn's disease study (18). Only patients with Crohn's disease (n = 662) and those without any symptom (n = 313) were analyzed. The information was obtained from MiSeq™ 16SrRNA sequence, agglomerated to the genus level, resulting in a matrix with information of 48 genus for 975 samples. In this case, the goal is to identify groups of taxa whose abundance balance is associated with Crohn's disease.

Microbiome and HIV status

The main goal of this analysis is to find a microbiome balance associated with HIV-status, that is, a microbiome balance that is able to discriminate between HIV-positive and HIV-negative individuals. As exposed in Noguera–Julian et al. (17), the HIV risk factor MSM (Men

who have Sex with Men) vs non-MSM should be considered as a possible confounder in any HIV - microbiome study. The proposed algorithm implements a regression model which allows adjustment for other variables. Thus, we applied the algorithm to Y=HIV-status and X=microbiome abundance at genus level, adjusted by Z=MSM factor.

According to the cross-validation (cv) procedure implemented with function *selbal.cv*, the optimal number of components to be included in the balance is 2 (Figure 1). The balance we identified as the most associated with HIV-status is given by X_+, a taxon of the family *Erysipelotrichaceae* and unknown genus and X_-, a taxon of the family *Ruminococcaceae* and unknown genus (Figure 2). HIV-positive status is associated with higher balance scores, that is, larger relative abundances of *Erysipelotrichaceae* with respect to *Ruminococcaceae*. The discrimination accuracy of this balance is moderate, with an AUC of 0.786 on the whole sample and a cross-validation AUC of 0.674. As can be observed in the boxplot in Figure 2, HIV-negative individuals are associated with lower balance values, most of them negative, while HIV-positive individuals have heterogeneous balance values. Figure 3 shows the result of the cross – validation procedure. The balance identified with the whole dataset is the most frequently identified in the cross-validation procedure, appearing 44% of the times, an indicator of robustness for the proposed global balance.

Microbiome and sCD14 inflammation parameter

Acute and chronic inflammations typically occur after HIV infection. Even patients under antiretroviral medications and undetectable viral load present chronic inflammation, which may cause tissue damage and is associated with many chronic diseases. In this context, there is a great interest in defining possible interventions involving modifications of the gut bacterial environment, which may reduce inflammation in HIV patients. This requires a good understanding of the association between gut microbial composition and several inflammation parameters. In this case, we focus on an immune–marker related to the chronic inflammation: the levels of soluble CD14 (sCD14), which was measured for a subset of samples (*n* = 151). The optimal number of components to be included in the model is four, according to the cv-MSE (Figure 4). The balance that is identified as the most associated with sCD14 is composed by two taxa in the numerator, $X_+ =$ {*g_Subdoligranulum, f_Lachnospiraceae_g_unclassified*} and two in the denominator $X_- =$ {*f_Lachnospiraceae_g_Intertae_Sedis, g_Collinsella*}. The association is moderate, with R = 0.53. Figure 5 provides a scatter plot of the balance values and sCD14 values, indicating that higher balance scores are associated with higher sCD14 values. The robustness of the selected balance can be evaluated through the results of the cv-procedure (Figure 6). We see that the proposed global balance is also the one that has been more frequently selected in the cv, 34% of the times. The four taxa defining the global balance correspond to the top 4 most frequently selected in the cross - validation. These results emphasize the robustness of the selected global balance.

Crohn's disease

Crohn's disease is an inflammatory bowel disease (IBD) linked to microbial alterations in the gut (18),(19). We ran *selbal.cv* algorithm with the goal of identifying groups of taxa whose abundance balance can discriminate between individuals with Crohn's disease from those without the disease.

The optimal number of components in the balance is twelve according to the MSE criterion (Figure 7). The groups defining the balance are X_+= {*g_Roseburia, o_Clostridiales_g_, g_Bacteroides, f_Peptostreptococcaceae_g_*} and X_- = {*g_Dialister, g_Dorea, o_Lactobacillales_g_, g_Eggerthella, g_Aggregatibacter, g_Adlercreutzia, g_Streptococcus, g_Oscillospira*}. Cases with Crohn's disease have lower balance scores than controls (Figure 8) which means lower relative abundances of X_+ with respect to X_-. The discrimination value of the identified balance is important, with an AUC = 0.838 and a cv-AUC = 0.819.

The identified global balance is very robust as the results of the cv reveal (Figure 9). The global balance obtained with the whole dataset is also the most frequently identified balance in the cv-procedure, namely 36% of the times. Moreover, the components defining the global balance are also the ones more frequently selected in the cv procedure. The balance identifies *Bacteroides* and *Clostridiales* as part of the denominator of the balance, which have also been identified previously as less abundant in Crohn's disease individuals than in controls (18).

DISCUSSION

The identification of individual microbial species, or taxa, that are differentially abundant among groups of samples is challenging because the change in relative abundance of one taxon affects the relative abundances of the other taxa. As an alternative, we propose the analysis of relative abundances among groups of taxa instead of analyzing each taxon separately. In this work, we present *selbal*, a greedy stepwise algorithm for balance selection that takes into account the compositional nature of microbiome abundance data. The algorithm identifies two groups of taxa whose relative abundance, or balance, is associated with the response variable of interest.

selbal overcomes the problem of differences in sample size that is usually treated with different methods based on count-normalization, rarefaction or transformation into proportions. The only way in which data is altered in *selbal* is at the zero imputation stage required because of the use of logarithms and ratios in the definition of balances. This replacement of zeros by positive numbers is performed under the assumption that observed zeros are rounded zeros, that is, all taxa are present in all the samples but some of them are not detected because of low abundance and insufficient sample depth. However, it is not clear how the imputation method and the presence of structural zeros (absence of the taxa in the sample) may influence the results. Future research will be focused on the treatment of zeros with the aim of more precisely evaluating if zeros are rounded or structural and on selecting the best replacement method.

Due to the computational cost, *selbal* does not explore the whole balance space and the method for selecting the optimal balance is suboptimal and may be improved. Thus, exploring for alternative approaches in the search of the optimal balance is another topic of future research.

In order to improve classification or prediction accuracy of the variable of interest a prediction model with several balances can be obtained by applying *selbal* algorithm sequentially. This sequential search of balances can be performed similarly to partial least squares approach: when the first balance B1 is identified, all variables are deflated by the first balance, that is, each variables is adjusted for the first balance, by regressing the variable on B1 and taking residuals. Then, the second balance is searched on the new orthogonalized data.

Endorsed by the compositional treatment of microbiome abundance data, *selbal* can also be useful for comparing different microbial studies. Since balances are based on relative abundances among groups of taxa, this relative information is likely to remove the noise and biases of each particular study.

MATERIALS AND METHODS

Let $X = (X_1, X_2, \dots, X_k)$ be a composition, that is, a vector of strictly positive real numbers. Given two disjoint subsets of components in X, denoted by X_+ and X_-, indexed by I_+ and I_-, composed by k_+ and k_- components respectively, the balance between X_+ and X_- is defined as:

$$B(X_+, X_-) = \sqrt{\frac{k_+ \cdot k_-}{k_+ + k_-}} \log \frac{\left(\prod_{i \in I_+} x_i\right)^{1/k_+}}{\left(\prod_{j \in I_-} x_j\right)^{1/k_-}} \ .$$

Expanding the logarithm, the balance is proportional to

$$B(X_+, X_-) \ \propto \ \frac{1}{k_+} \sum_{i \in I_+} \log X_i - \frac{1}{k_-} \sum_{j \in I_-} \log X_j = \ M_+ - \ M_- \ ,$$

which corresponds to the difference of the arithmetic means of the log-transformed initial components in the two groups that we denote by M_+ and M_-, respectively. This second expression is preferable from a computational point of view and is the one implemented in the proposed algorithm.

Given Y, a response variable, which can be either numeric or dichotomous, a composition $X = (X_1, X_2, \dots, X_k)$ and additional covariates $Z = (Z_1, Z_2, \dots, Z_r)$, the goal of the algorithm is to determine the sub-compositions of X, X_+ and X_-, indexed by I_+ and I_-, respectively, so that the balance B between X_+ and X_- is highly associated with Y after adjustment for

covariates Z. Depending on the nature of the dependent variable, the association can be defined in several ways.

For a continuous variable Y, the optimization criterion is defined as maximization of the coefficient of determination of the linear regression model:

$$Y = \beta_0 + \beta_1 B + \gamma' Z \ .$$

For a dichotomous variable Y, we fit the logistic regression model

$$logit(Y) = \beta_0 + \beta_1 B + \gamma' Z \ ,$$

and, in this case, we consider three possible optimization criteria: the area under the ROC curve (default option), the maximization of the explained variance (20) or the discrimination coefficient (21).

The main function of the proposed algorithm to detect the most associated balance is called *selbal* and follows these steps:

STEP 0: *Zero replacement*

The initial matrix of counts in a microbiome study, denoted by $\widetilde{\boldsymbol{X}}$, typically contains zeros. In order to apply the mathematical theory of compositional data, the observed zeros are

assumed to be non-structural zeros but a consequence of under detection limit. They are replaced by a positive value using a Bayesian-Multiplicative replacement (10) of count zeros as implemented in function *cmultRep()* of the R package *zCompositions* (22). It is important to remark that this transformation keeps the information contained in the ratios between non-zero components. The resulting matrix without zeros is denoted by $\boldsymbol{X}$ and coincides with $\widetilde{\boldsymbol{X}}$ only if the latter has no null value.

STEP 1: *Optimal balance between two components*

The algorithm evaluates exhaustively the optimization criterion for all possible balances composed by only two components; that is, all the balances of the form:

$$B = \sqrt{\frac{1}{2}}\left(\log(X_i) - \log(X_j)\right)$$

for $i, j \in \{1, \ldots, k\} \quad i \neq j$. We denote by $B^{(1)}$ the optimal two- component balance in terms of maximization of the association value.

For each pair of components (X_i, X_j) there are two options when defining a balance:

$$\sqrt{\frac{1}{2}}\left(\log(X_i) - \log(X_j)\right) \qquad \text{and} \qquad \sqrt{\frac{1}{2}}\left(\log(X_j) - \log(X_i)\right)$$

differenced only by their sign. For dichotomous variables, they will provide the same AUC value; nevertheless *selbal* returns the balance whose coefficient in the regression model is positive.

STEP *s*: *Optimal balance adding a new* component

For $s > 1$ and until the stop criterion is fulfilled, let $B^{(s-1)}$ be

$$B^{(s-1)} \propto M_{+}^{(s-1)} - M_{-}^{(s-1)} = \frac{1}{k_{+}^{(s-1)}} \sum_{i \in I_{+}^{(s-1)}} \log(X_i) - \frac{1}{k_{-}^{(s-1)}} \sum_{j \in I_{-}^{(s-1)}} \log(X_j)$$

where $I_{+}^{(s-1)}$ and $I_{-}^{(s-1)}$ are two disjoint subsets of indices in $\{1, \ldots, k\}$, with $k_{+}^{(s-1)}$ and $k_{-}^{(s-1)}$ elements, respectively.

For each index $p \notin (I_{+}^{(s-1)} \cup I_{-}^{(s-1)})$, the algorithm evaluates the optimization criterion of the balance that is obtained by adding $\log(X_p)$ to $B^{(s-1)}$ including p either in $I_{+}^{(s-1)}$ or in $I_{-}^{(s-1)}$. That is, the algorithm evaluates the optimization criterion for both, $B^{(s+)}$ and $B^{(s-)}$, defined as:

$$B^{(s+)} = \sqrt{\frac{(k_{+}^{(s-1)}+1) \cdot k_{-}^{(s-1)}}{k_{+}^{(s-1)} + k_{-}^{(s-1)} + 1}} \left(\frac{k_{+}^{(s-1)} * M_{+}^{(s-1)} + \log(X_p)}{k_{+}^{(s-1)} + 1} - M_{-}^{(s-1)} \right),$$

$$B^{(s-)} = \sqrt{\frac{k_{+}^{(s-1)} * (k_{-}^{(s-1)}+1)}{k_{+}^{(s-1)} + k_{-}^{(s-1)} + 1}} \left(M_{+}^{(s-1)} - \frac{k_{-}^{(s-1)} * M_{-}^{(s-1)} + \log(X_p)}{k_{-}^{(s-1)} + 1} \right),$$

and selects as $B^{(s)}$ the one that maximizes the optimization criterion. If $B^{(s)} = B^{(s+)}$, the sets of new indices are defined as, $I_{+}^{(s)} = I_{+}^{(s-1)} \cup \{p\}$ and $I_{-}^{(s)} = I_{-}^{(s-1)}$, and similarly for $B^{(s)} = B^{(s-)}$.

STOP criterion. *selbal* function has two parameters to decide the stopping criterion:

- *th.imp,* threshold improvement (default 0). The algorithm stops the iteration process when the improvement in association is lower than the specified threshold improvement.
- *maxV*, maximum number of components. The algorithm stops when the specified maximum number of components has been included in the balance.

Cross-validation: *selbal.cv*

We perform a cross-validation procedure with two goals: (1) to identify the optimal number of components to be included in the balance and (2) to explore the robustness of the global balance identified with the whole dataset.

The cv procedure is implemented in the *selbal.cv* function.

For each cv process, the dataset is divided into K folds (default value, K = 5). K-1 folds are used to obtain the balance (with *th.imp* = 0 as the stop rule) and the remaining fold is used to test the result. The process is repeated M times (default value, M = 10)

373

374 **Optimal number of components**

375

376 For each combination of K and M we perform the *selbal* function on the training dataset
377 and find the optimal balance with c components, $c \in \{2, \ldots, C\}$ (default value, C = 20) and
378 evaluate the mean squared error (MSE) of the model on the test dataset. For each c we
379 obtain $\overline{MSE}_c$, the mean MSE of the different models with c components and the
380 corresponding standard error. The optimal number of components is defined with the 1se
381 rule, as the minimum number of components whose mean MSE is below the minimum $\overline{MSE}$
382 plus its standard error.

383

384 For dichotomous components, the MSE is computed in the same way codifying the two
385 groups as 0 and 1.

386

387 **Robustness of the result**

388

389 Once the optimal number of components k_{opt} has been chosen, all the balances obtained
390 in the cv procedure are reduced to k_{opt} components. Then, a frequency table is built both
391 for balances and for individual components. This information, available in the output of
392 *selbal.cv,* is summarized in a table as those shown in Figure 3, Figure 6 and Figure 9.

393

394 The cv process also provides the association or discrimination value for each balance in the
395 cv which can be used as a more accurate measure of association or discrimination of the
396 global model.
397

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Figure 1: Mean squared error (MSE) as a function of the number of components included in the balance. The optimal number of components is highlighted with a vertical dashed line.

Figure 2: The components defining the selected balance are specified on top of the boxplot that represents the distribution of the balance score for each of the groups. The right part of the figure contains the ROC–curve with its AUC value (0.786) and the density curve for each group.

Figure 3: Cross–validation (cv) results: first column contains the names of the taxa appearing in the most frequently selected balances in the cv procedure, the second column provides the frequency of selection (in percentage), the third column corresponds to the global balance, that is, the balance obtained using all the samples. Columns 4 to 6 represent the most frequent balances identified in the cv procedure. Colored rectangles indicate if the component is in the numerator of the balance (*red*), in the denominator (*blue*) or not included (*white*). The last row provides the proportion of times the balance has been selected as optimal in the cv procedure.

Figure 4: Mean squared error (MSE) as a function of the number of components included in the balance. The optimal number of components is highlighted with a vertical dashed line.

493

494 **Figure 5**. Representation of the balance obtained (*X* axis) for the sCD14 immune-marker
495 values (*Y* axis), the bacteria groups composing it (top of the figure) and the corresponding
496 regression line (*blue*).

497

498 **Figure 6:** Cross – validation (cv) results: first column contains the names of the taxa
499 included in the most frequently selected balances in the cv procedure, the second column
500 provides the frequency of selection (in percentage), the third column corresponds to the
501 global balance, that is, the balance obtained using the whole sample. Columns 4 to 6
502 represent the most frequent balances identified in the cv procedure. Colored rectangles
503 indicate if the component is in the numerator of the balance (*red*), in the denominator
504 (*blue*) or not included (*white*). The last row provides the proportion of times the balance
505 has been the selected in the cv procedure.

506 **Figure 7**: Mean squared error (MSE) as a function of the number of components included
507 in the balance. The optimal number of components is highlighted with a vertical dashed
508 line.

509

510 **Figure 8**: The components defining the selected balance are specified on top of the boxplot
511 which represents the distribution of the balance score for each of the groups. The right part
512 of the figure contains the ROC – curve with its AUC value (0.838) and the density curve for
513 each group.

514

Figure 9: Cross – validation (cv) results: first column contains the names of the taxa appearing in the most frequently selected balances in the cv procedure, the second column provides the frequency of selection (in percentage), the third column corresponds to the global balance, that is, the balance obtained using the whole sample. Columns 4 to 6 represent the most frequent balances identified in the cv procedure. Colored rectangles indicate if the component is in the numerator of the balance (*red*), in the denominator (*blue*) or not included (*white*). The last row provides the proportion of times the balance has been the selected in the cv procedure.

Figure 1

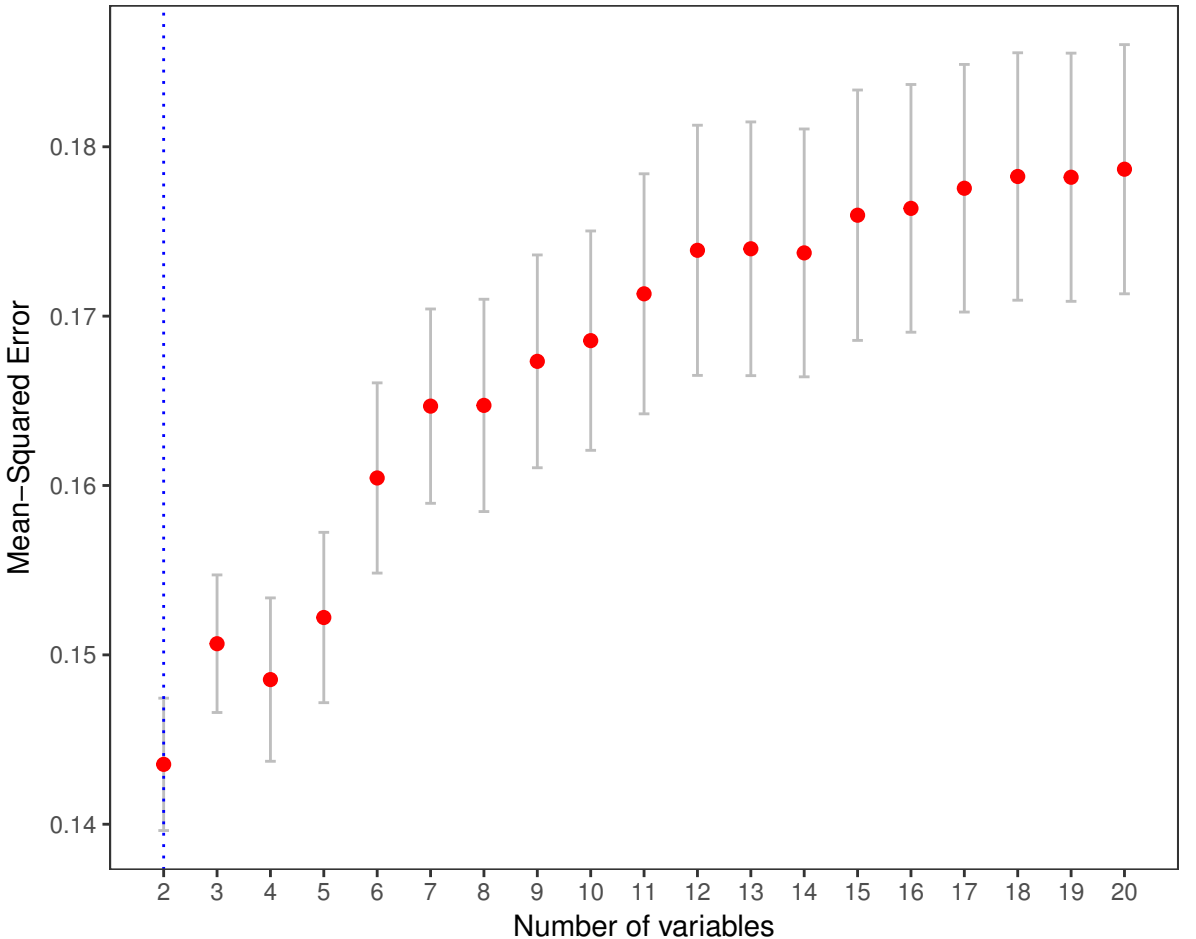
Mean−Squared Error
0.14
0.15
0.16
0.17
0.18
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Number of variables

Figure 2

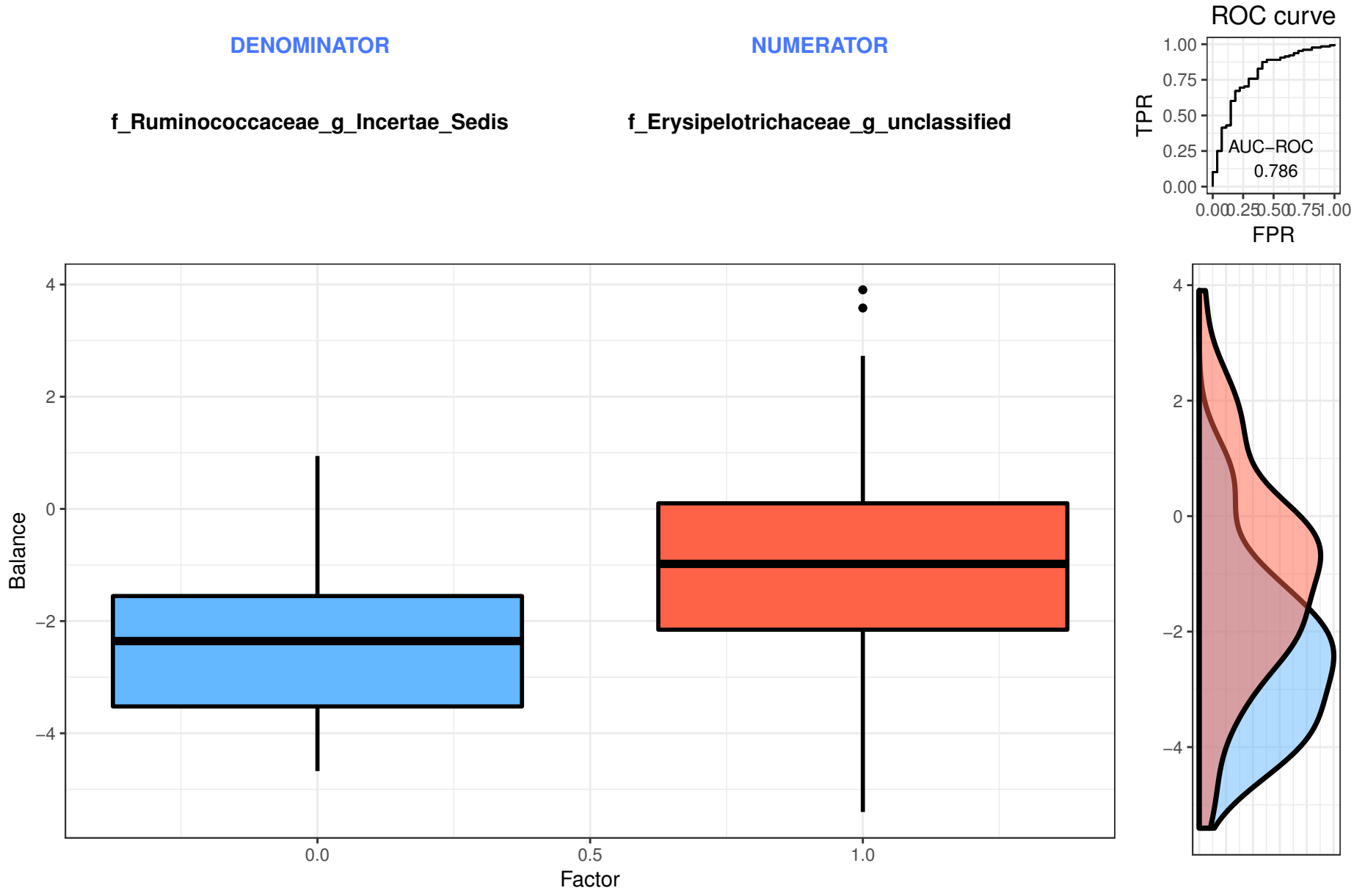
DENOMINATOR
NUMERATOR
f_Ruminococcaceae_g_Incertae_Sedis
f_Erysipelotrichaceae_g_unclassified
ROC curve
TPR
FPR
AUC–ROC
0.786
1.00
0.75
0.50
0.25
0.00
0.00
0.25
0.50
0.75
1.00
Balance
Factor
4
2
0
−2
−4
0.0
0.5
1.0

Figure 3

	%	Global	BAL 1	BAL 2	BAL 3
f_Ruminococcaceae_g_Incertae_Sedis	76				
f_Erysipelotrichaceae_g_unclassified	50				
g_Bacteroides	30				
g_Phascolarctobacterium	12				
FREQ	–	–	0.44	0.24	0.06

Figure 4

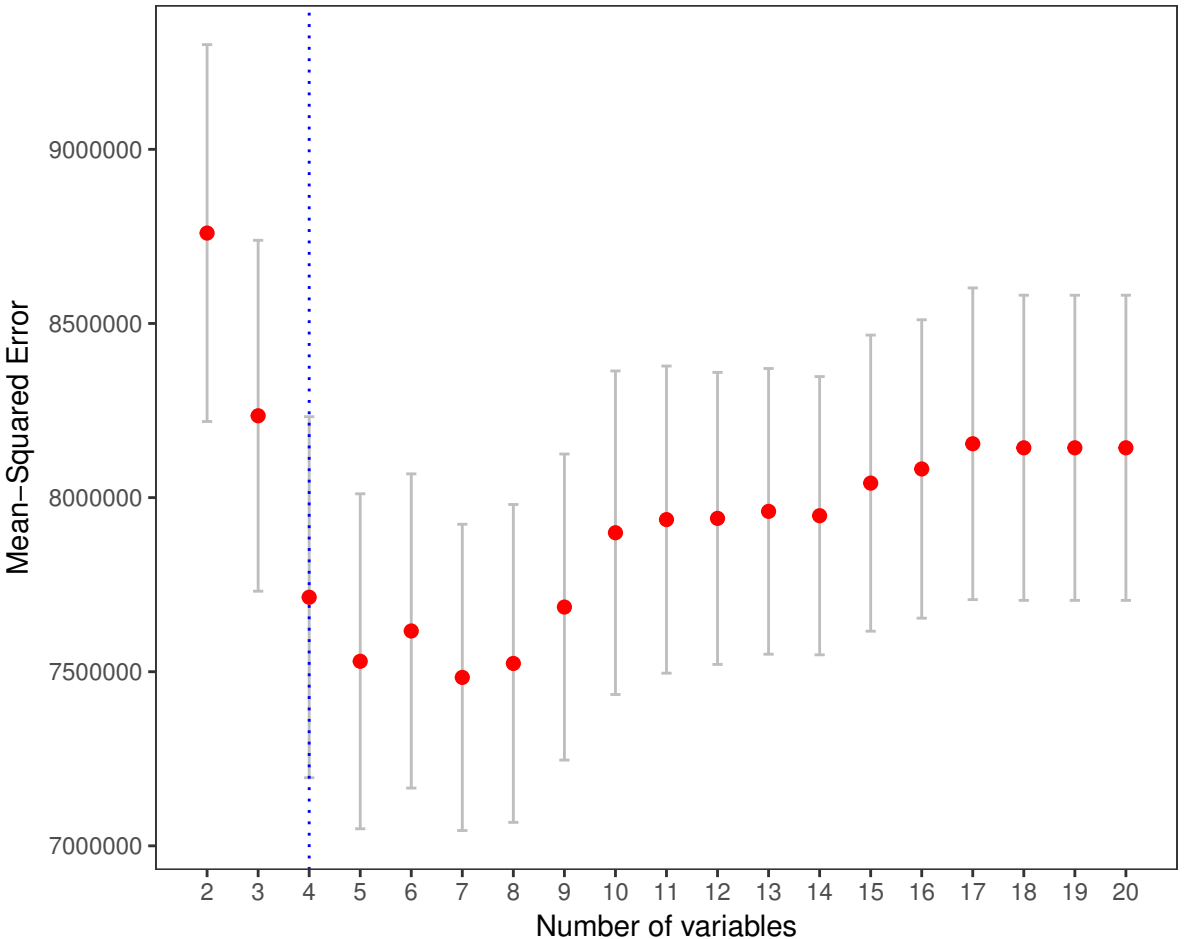
Mean−Squared Error
9000000
8500000
8000000
7500000
7000000
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Number of variables

Figure 5

DENOMINATOR

f_Lachnospiraceae_g_unclassified

g_Collinsella

NUMERATOR

g_Subdoligranulum

f_Lachnospiraceae_g_Incertae_Sedis

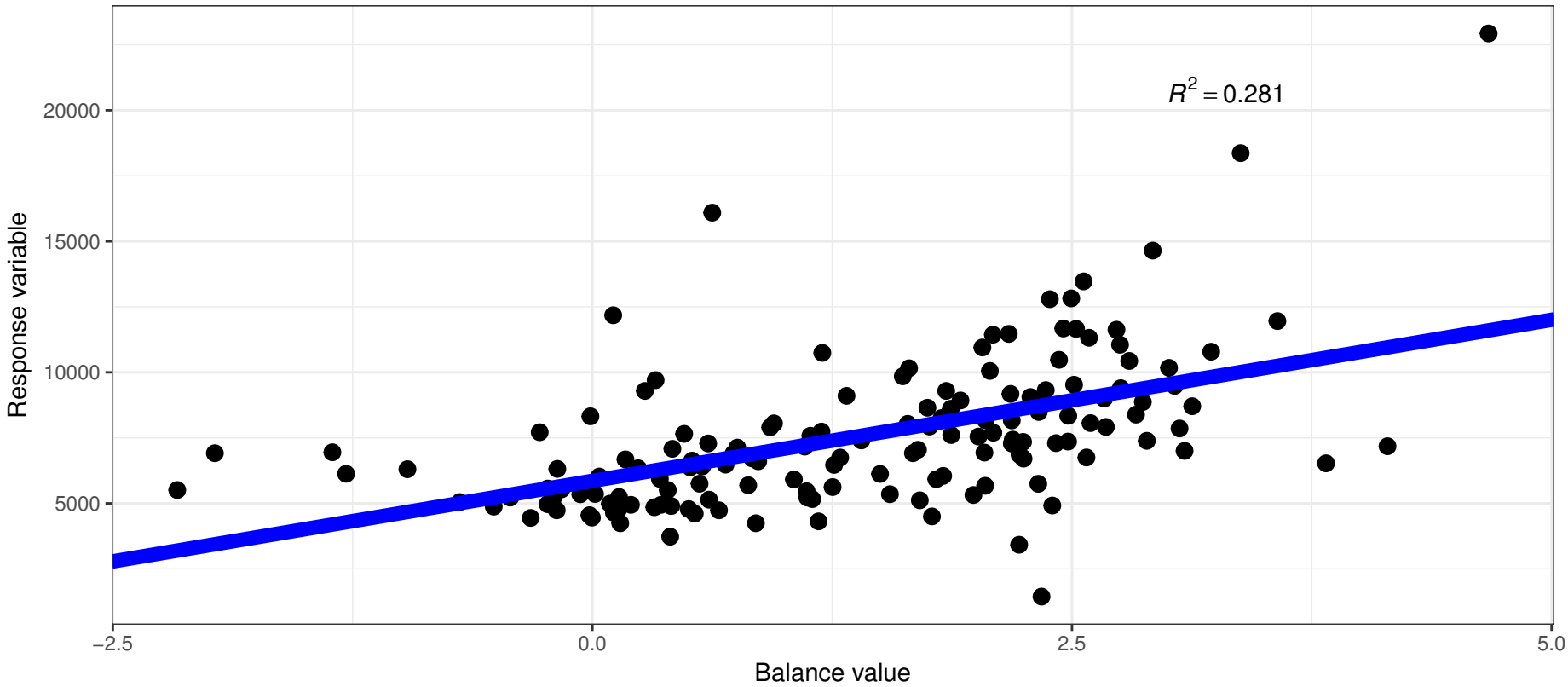

Figure 6

	%	Global	BAL 1	BAL 2	BAL 3
f_Lachnospiraceae_g_unclassified	94				
g_Collinsella	76				
g_Subdoligranulum	72				
f_Lachnospiraceae_g_Incertae_Sedis	54				
g_Thalassospira	50				
g_Bifidobacterium	14				
FREQ	–	–	0.34	0.12	0.08

Figure 7

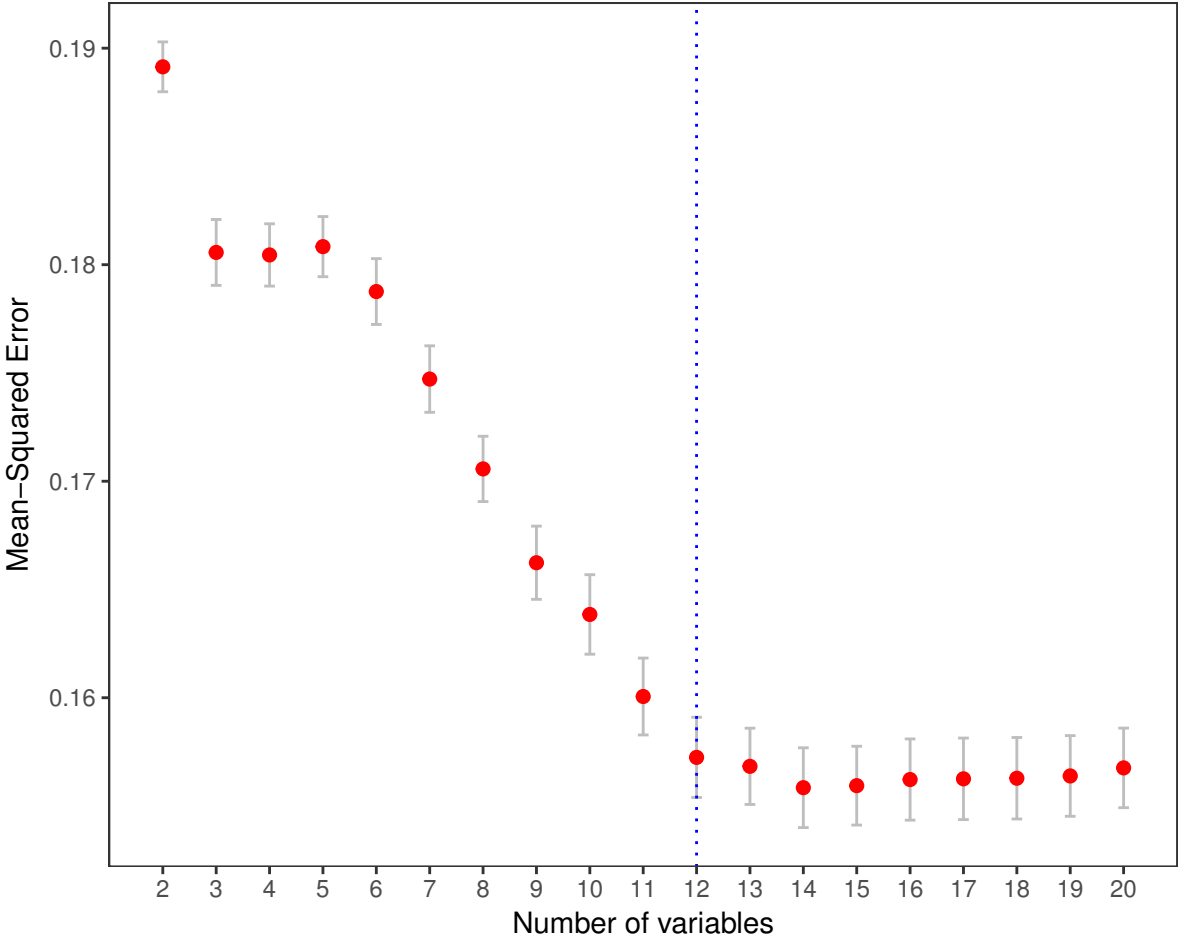
0.19
0.18
0.17
0.16
Mean−Squared Error
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Number of variables

Figure 8

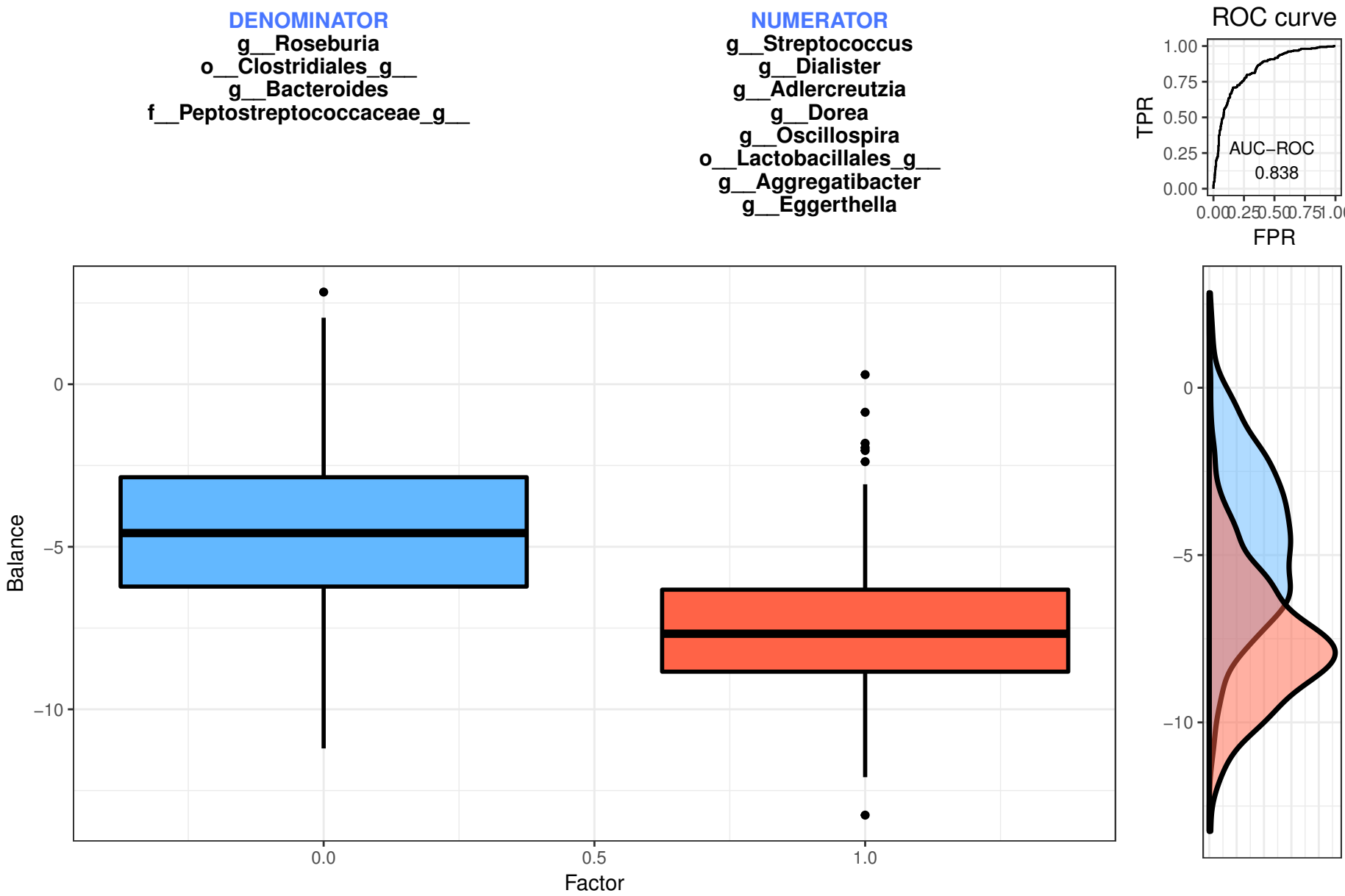
DENOMINATOR
g__Roseburia
o__Clostridiales_g__
g__Bacteroides
f__Peptostreptococcaceae_g__
NUMERATOR
g__Streptococcus
g__Dialister
g__Adlercreutzia
g__Dorea
g__Oscillospira
o__Lactobacillales_g__
g__Aggregatibacter
g__Eggerthella
ROC curve
TPR
FPR
AUC−ROC
0.838
Balance
Factor

Figure 9

	%	Global	BAL 1	BAL 2	BAL 3
g__Dialister	100				
g__Roseburia	100				
o__Clostridiales_g__	98				
g__Bacteroides	98				
g__Dorea	96				
o__Lactobacillales_g__	94				
g__Eggerthella	92				
g__Aggregatibacter	92				
g__Adlercreutzia	90				
f__Peptostreptococcaceae_g__	86				
g__Streptococcus	76				
g__Oscillospira	72				
g__Actinomyces	26				
g__Blautia	24				
FREQ	–	–	0.36	0.1	0.1