SuRF: a New Method for Sparse Variable Selection, with Application in Microbiome Data Analysis

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Abstract

In this paper, we present a new variable selection method for regression and classification purposes. Our method, called Subsampling Ranking Forward selection (SuRF), is based on LASSO penalised regression, subsampling and forward-selection methods. SuRF offers major advantages over existing variable selection methods in terms of both sparsity of selected models and model inference. We provide an R package that can implement our method for generalized linear models. We apply our method to classification problems from microbiome data, using a novel agglomeration approach to deal with the special tree-like correlation structure of the variables. Existing methods arbitrarily choose a taxonomic level a priori before performing the analysis, whereas by combining SuRF with these aggregated variables, we are able to identify the key biomarkers at the appropriate taxonomic level, as suggested by the data. We present simulations in multiple sparse settings to demonstrate that our approach performs better than several other popularly used existing approaches in recovering the true variables. We apply SuRF to two microbiome data sets: one about prediction of pouchitis and another for identifying samples from two healthy individuals. We find that SuRF can provide a better or comparable prediction with other methods while controlling the false positive rate of variable selection.

keywords

Variable selection; Generalised Linear Models; LASSO; SuRF; Microbiome; Forward selection; Stability selection; Identifying biomarkers.
1 Introduction

Traditional statistical methods face lots of challenges when analyzing high-dimensional data. These challenges occur in model fitting, variable selection and model diagnosis. A series of regularized models from the field of data mining have become popular inference approaches for high dimensional data such as gene expression. The most well-known methods include LASSO regression (Tibshirani, 1996), the elastic-net regression model (Zou and Hastie, 2005) and various variations such as group LASSO (Yuan and Lin, 2006), Bayesian LASSO (Park and Casella, 2008), etc. LASSO is based on penalising the model by the sum of the absolute values of coefficients of all variables and hence it is a soft thresholding method so that some variables are eliminated due to a resulting zero coefficient. This has the advantage of selecting sparse models. In addition, it is a suitable method to use for tree-structured data, such as microbiome data, as we discuss in Section 2.3.

There are a few issues with the use of LASSO for microbiome data. The first is inference — LASSO can select a parsimonious model, but it does not provide a direct quantitative assessment of the significance of each variable selected. For scientific and clinical research, it is vital to include these assessments of the significance of variables ($p$-values). There is a method related to this matter (Lockhart et al., 2014) but, in practice, high dimensional data rarely satisfies the weak collinearity assumption needed. The more robust approach of Tibshirani et al. (2016) is only available for Gaussian response variables. Secondly, LASSO provides only a list of variables, with coefficients, but in many cases very strong correlation exists between some variables, either of which might be selected with no indication that the other variables might have an almost equally strong association with the response variable. The choice of which variables are selected can be very unstable.

We are particularly interested in applications to microbiome research. The microbiome is the collection of all bacteria present in a location, e.g. a person’s gut, and one of the main questions of microbiome research is the relationship between phenotypes (e.g., healthy versus disease groups) and the microbiome. The data consist of counts of various types of microbes, classified into Operational Taxonomic Units (OTUs). Due to the vast number of OTUs and the cost of samples, the sample size is always much smaller than the number of OTUs.

We introduce a variable selection method, SuRF, based on regularized regression and subsampling of observations in the generalized linear model setting. This method provides a $p$-value for each variable, and gives information on the stability of the selected variables.

There has been previous work on dealing with the lack of stability in LASSO, such as Zakharov and Dupont (2013) and Grave et al. (2011). A promising recent approach to this issue which has some similarity to our SuRF method is stability selection (Meinshausen and Bühlmann, 2010). We show in our simulations that SuRF gives much better performance in variable selection than stability selection.
2 Subsampling Ranking Forward selection (SuRF)

The framework of SuRF has two main steps: creating a list of ordered predictors that have been selected most frequently by LASSO on subsamples of the observations, and selecting variables by forward selection on the list of ordered variables. The second step consists of determining the significance of the variables in terms of likelihood ratio statistics via sequential permutation tests and implementing ANOVA with forward selection to eliminate or alleviate the issue of surrogate variables. The algorithm pseudo code is provided in Appendix A of the web supplementary material.

2.1 Variable Ranking

The subsampling approach plays an important role in formulating the list of top predictors. This technique is widely used in many recent methods for variable selection and the details were summarised well by Dezeure et al. (2015). In each of the splits, a part of the data is used to select variables by LASSO. We focus on LASSO here because of the tree structure in microbiome data and its empirically better screening performance than other regularized models (Bühlmann and Mandozzi, 2014); however, the subsampling could be applied with other variable selection methods. We recommend about 90% of the data for this purpose when the sample size is extremely limited but otherwise the proportion should make a minimal difference in results (see Web Table 11). We also recommend taking stratified subsamples (subsamples having the same proportion in each class as the true data) in classification problems since if the subsamples are not balanced, this can affect prior probabilities of each class, resulting in worse classification. The sample splitting is repeated in a similar manner a large number, $B$, of times. Some literature suggests that $B = 50$ or $B = 100$ is sufficient (Dezeure et al., 2015), but there is little cost to using larger $B$ to ensure better results. For each subsample, we record the variables selected by LASSO, with the tuning parameter selected by cross-validation over the subsample. We rank variables by the frequency they are selected over the $B$ subsamples. (Ties are broken by reduction of deviance residuals from models containing all higher-ranked variables.) The order of variables can be interpreted as measuring the strength of the association with the response variable.

2.2 Sequential permutation tests with ANOVA forward selection

The forward selection involves sequentially testing the null hypothesis that no variables beyond the currently selected variables are good predictors for the response variable, given the currently selected variables. In forward selection, the order in which variables are added to the model can be important. At each stage, if multiple predictors are significant, we add the one ranked highest by our variable ranking procedure. Because we are testing multiple predictors at each stage, the log-likelihood ratio statistic does not follow a $\chi^2$ distribution.
The predictors are not independent, so Bonferroni correction cannot be used. Instead we calculate the critical value empirically using permutations.

More specifically, we start with a list of candidate variables, containing all variables in the order found using the ranking method from Section 2.1, and a list of selected variables being initially empty. At each step, we generate a random permutation for all observations, and apply it only to the variables in the candidate variable list. This breaks the relationship between the candidate variables and the response variable, but preserves the correlation structure among the candidate variables and between the response variable and the selected variables. Now for each candidate variable, we compute the log-likelihood ratio statistic between the current model and the model with this variable added. We record the largest log-likelihood ratio statistic. We repeat this process for many more permutations (we usually use 200 permutations as a compromise between accuracy and speed), to obtain the null distribution of the maximum log-likelihood ratio statistic. We use the $1 - \alpha$ ($\alpha = 0.05$) percentile of this null distribution as our critical value, denoted as $D_{1-\alpha}$ for the $i$th variable in this forward sequential variable selection procedure. We now return to the original unpermuted data, and for each candidate variable in the ranking order, we calculate the log-likelihood ratio statistic between the current model and the model with this candidate variable added. We select the first candidate variable for which this statistic exceeds $D_{1-\alpha}$, and add this variable to the model (and remove it from the candidate variable list). We then generate a new distribution, with new permutations and repeat the same procedure. When the log-likelihood ratio statistic for each candidate variable is no greater than the critical value, we terminate the algorithm and output the current model as the final model selected.

For each variable added to the model, SuRF has computed a $p$-value based on the comparison of the likelihood ratio statistic with the empirically calculated null distribution. This $p$-value is based on the increase in predictive ability over the variables that were already included in the model. That is, the $p$-value is for the null hypothesis that all true variables are in the current model. A variable which is a surrogate for a variable that has been already selected may not have a significant $p$-value if there is not significant evidence that this variable improves prediction over the surrogate already selected.

### 2.3 Theory

SuRF is designed to combine the best parts of three methods: stability selection, LASSO and forward selection. The advantages and disadvantages of these methods are as follows. Forward Selection provides clear $p$-values at each stage, but heavily depends on the order of variables entering the model. LASSO does well at identifying the correct variables, but does not provide $p$-values and often selects too many variables. Stability selection is robust to outliers, but can “fall between two stools” with surrogates, and offers only limited $p$-values.

Because the final selection in SuRF is using forward selection, we will base our theory on this approach. We want to show that asymptotically, SuRF will
select the true variables. This occurs in two stages. Firstly, the true variables must be ranked highly by the subsampling procedure. This relies on the performance of LASSO at identifying the true variables. It is known that LASSO is consistent provided the irrepresentability condition holds (Zhao and Yu, 2006). In this case, the subsampling is guaranteed to select the true variables before other variables, which overcomes the danger with forward selection that the surrogate will be selected first, preventing the true variables from entering the model. In addition, the subsampling approach offers some robustness, allowing the top variables to be highly ranked even if some outliers might make them less highly ranked in some subsamples.

Secondly, assuming the true variables have been ranked above other variables, the hypothesis test must reject the null model. We show that, asymptotically, even for many cases with \( p \gg n \), this will happen using the following theorems, which are proved in Appendix B of the online supplementary materials.

**Theorem 1.** Suppose we have a true model \( \mathbb{E}(Y|X) = g^{-1}(X\beta) \), where \( Y|X \) follows an exponential family distribution, \( g \) is a link function, and \( \beta_i \) is zero for all but a fixed number \( p_{\text{true}} \) of predictors. Suppose further that we have already selected the set \( S \) of variables, all of which satisfy \( \beta_j \neq 0 \) and that there is at least one variable \( i \notin S \) with \( \beta_i \neq 0 \). Let \( D(k) \) be the log-likelihood ratio statistic for the variable \( k \). Then there is some \( j \notin S \) with \( \beta_j \neq 0 \) such that \( D(j) = O(n) \), where \( n \) is the number of observations.

**Theorem 2.** Suppose \( X \) is an \( n \times p \) matrix and \( Y \) is a random vector (independent of \( X \)) from the exponential family distribution fitted by the model, then the maximum deviance of a single column of \( X \) as a predictor of \( Y \) has survival function bounded by \( S(x) \leq \frac{2pe^{-2x}}{\sqrt{2\pi x^2}} \).

From Theorem 2, the critical value at level \( \alpha \) is bounded above by the solution to \( \frac{2pe^{-2x}}{\sqrt{2\pi x^2}} = \alpha \), which for all reasonable \( p \) and \( \alpha \) satisfies \( x \leq -2 \log \left( \frac{\alpha}{2p} \right) \).

On the other hand, Theorem 1 says the test statistic is asymptotically \( O(n) \) for at least one true variable. Therefore, provided \( \frac{\log(p)}{n} \to 0 \), or equivalently \( p = o(e^{\epsilon n}) \) for any \( \epsilon > 0 \), the true variables must be selected.

**Remark 3.** Note that the bound on the critical value in Theorem 2 is the critical value for a Bonferroni correction for the multiple testing. We use the empirical value in practice because the Bonferroni critical value is too large, since it does not account for correlation between predictors.

### 2.4 Tree Structure

SuRF can be applied to any exponential-family GLM variable selection problem. However, our application of interest is microbiome data. In this section we discuss the particular way we have adapted SuRF to deal with such data.
Microbiome data typically consist of proportions of OTUs present in each sample. OTUs are clusters of DNA sequences, usually clustered at 97% similarity, approximately equivalent to species-level resolution. We are working with a GLM \( g(\mathbb{E}(Y|X)) = \beta_0 + X\beta \), where \( X \) is the column-centralised OTU data matrix with each column representing an OTU variable. The phylogenetic relationships among OTUs provide us with prior knowledge about \( \beta \). Namely, we expect the \( \beta_i \) to be close for closely-related OTUs because of phenotypic similarity. We reflect this prior knowledge via the regularisation of the coefficients. We choose to base this on the taxonomic tree, rather than more detailed phylogenetic trees, because estimation of the taxonomic tree is more robust, and the taxonomic tree is easily available from the output of most pipelines. However it is trivial to use a phylogenetic tree instead.

A common practice is to aggregate variables at an arbitrarily chosen taxonomic level, usually genus or phylum. That is, to replace the original data matrix \( X \) by the aggregated data matrix \( \tilde{X} = XC \), where \( C \) is the clustering matrix at the chosen level. For example,

\[
C_{ij} = \begin{cases} 
1 & \text{if OTU } i \text{ is in phylum } j, \\
0 & \text{otherwise.}
\end{cases}
\]

Now, fitting a model \( g(\mathbb{E}(Y|X)) = \tilde{X}\alpha + \beta_0 \) is equivalent to fitting \( g(\mathbb{E}(Y|X)) = X\beta + \beta_0 \), where \( \beta = C\alpha \). That is, this regularisation consists of the restriction \( \beta = C\alpha \), namely that OTUs from the same phylum have the same coefficients.

While aggregating at a sufficiently high taxonomic level can have the convenient consequence that classical statistical methods can be applied, the aggregated data may lack the resolution to answer the scientific questions, or may lead us to make unsupported or false generalisations. On the other hand, the large noise when analysing at a low taxonomic level may obscure general patterns, and not provide a satisfactory prediction (Hou et al., 2015).

The trouble with aggregation at a certain taxonomic level is that it converts the soft prior expectation that coefficients for OTUs in the same group should be similar into a hard requirement that the coefficients be equal, even if this is disproved by the data. Instead, we penalise the extent to which the coefficients differ. More formally, instead of setting \( \tilde{X} = XC \), we set \( \tilde{X} = X(C, I) \), where \((C, I)\) is a matrix whose first columns are \( C \), and whose remaining columns are the identity matrix. There are now multiple ways to represent a given model in terms of the variables in \( \tilde{X} \) because of the linear dependence between columns. The regularisation means that only a single way to represent the model minimises the penalty. For any coefficient vector \( \beta \) in the model \( g(\mathbb{E}(Y|X)) = X\beta + \beta_0 \), many vectors \( \alpha \) satisfy \( \beta = (C, I)\alpha \). These vectors can all be considered correct models. However they are distinguished by the penalty term \( \|\alpha\|_1 \) (since the loglikelihood is the same based on either \( \beta \) or \( \alpha \)). It can be shown that the penalty term is minimised when for any \( j \) at the higher taxonomic level, \( \alpha_j \) is the median of \( \{0\} \cup \{\beta_i | C_{ij} = 1\} \), and for any \( i \) in cluster \( j \) (i.e. \( C_{ij} = 1 \)) \( \alpha_i = \beta_i - \alpha_j \).

We can apply the same method after constructing similar aggregations at every taxonomic level. The resulting penalty for a particular coefficient vector \( \beta \) is the
additional variables

(a) shows a taxonomic tree relating the OTU variables in the aggregated model are $\alpha_g$ and $\beta_g$. For the coefficient vector $= (0, 1, 2, 2, 5, -2, -1, -0.5)$, we create the combinations $X_1 + X_2 + X_3$ at leaf nodes, these are the coefficient estimates and the values shown at internal nodes are cumulative sums of the branches.

We clarify this with an example:

Figure 1(a) shows a small taxonomic tree containing OTUs $X_1, \ldots, X_6$. In (b), we consider the estimate $Y = X\beta + \beta_0$ where $\beta = (1, 2, 2.5, -2, -1, -0.5)^T$. Similarly, for the coefficient vector $\beta' = (2, 2, 2.5, -2, -1, -0.5)^T$.

The coefficients in the expanded model are shown on the branches of the trees, and the values shown at internal nodes are cumulative sums of the branches above. For leaf nodes, these are the coefficient $\beta$ in the original model.

The most parsimonious total change of coefficients over the taxonomic tree structure. We clarify this with an example:

Figure 1(a) shows a small taxonomic tree containing OTUs $X_1, \ldots, X_6$. We create the combinations $X_7 = X_1 + X_2 + X_3$, $X_8 = X_4 + X_5$, $X_9 = X_4 + X_5 + X_6$, and $X_{11} = X_1 + \cdots + X_6$. We do not consider the combination $X_{10}$ because it is equal to $X_7$. For the coefficient vector $\beta = (1, 2, 2.5, -2, -1, -0.5)^T$, i.e., the model $g(E(Y|X)) = X_1 + 2X_2 + 2.5X_3 - 2X_4 - X_5 - 0.5X_6$, the most parsimonious coefficients in terms of the expanded set of predictors are $\alpha = (0, 1, 1.5, -1, 0, 0, 1, -0.5, -0.5, 0, 0)$ as shown in Figure 1(b). That is, $g(E(Y|X)) = X_2 + 1.5X_3 - X_4 + X_7 - 0.5X_8 - 0.5X_9$ is equivalent to the original estimate, but is given a lower penalty by LASSO. Similarly, for the coefficient vector $\beta' = (2, 2, 2.5, -2, -1, -0.5)^T$, in Figure 1(c), the most parsimonious coefficients in the aggregated model are $\alpha' = (0, 0, 0.5, -1, 0, 0, 2, -0.5, -0.5, 0, 0)$.
In the original model, the penalty assigned to $\beta'$ is $\lambda(|2| + |2| + 2.5| + | − 2| + | − 1| + | − 0.5|) = 10\lambda$, which is larger than the penalty $\lambda(|1| + |2| + 2.5| + | − 2| + | − 1| + | − 0.5|) = 9\lambda$ assigned to $\beta$, whereas, for the aggregated model, the penalty assigned to $\alpha$ is

$$\lambda(|0| + |1| + |0| + |1.5| + | − 0.5| + | − 0.5| + |0| + | − 1| + |0|) = 5.5\lambda$$

and the penalty for $\alpha'$ is

$$\lambda(|0| + |2| + |0| + |0.5| + | − 0.5| + | − 0.5| + |0| + | − 1| + |0|) = 4.5\lambda$$

so the penalty for $\alpha$ is larger. Thus, the aggregation approach uses the same space of models, but a different regularisation, which can affect the selected model. In this aggregated setting, we will often say something like “We select the higher level variable $X_7$” as a shorthand for “We select a model in which variables $X_1$, $X_2$ and $X_3$ are included, but constrained to have equal coefficients.”

Yan and Bien (2018) are independently developing a similar method involving adding aggregated variables to alter the regularisation. Their approach is tailored to text mining problems, and consequently differs from ours in a couple of respects: Firstly, they include an additional penalty for the coefficients at leaf nodes. This does not make sense for OTU data, since leaf nodes are clusters of lower level strains, so should not be treated differently. Furthermore, additional penalty for coefficients at leaf nodes creates a new optimisation problem. Secondly, their method does not scale the variables before regularisation. For LASSO, standardisation makes predictors more comparable, so that penalties are equivalent. For their count data, the counts are already equivalent. For our tree-based LASSO, it is less clear what standardisation means. Further work on fine-tuning the procedure to produce a better penalty that more accurately reflects this is outside the scope of the current paper, which focusses on the SuRF procedure, but is a topic the authors plan to address in future work.

The theory for this augmented version of LASSO is still not developed. It is not possible to apply the standard theory for the augmented set of predictors, because there are many representations of the true model using the augmented predictors. Described in terms of the augmented predictors, even the notion of consistency is challenging to define — there are multiple correct sets of selected augmented variables, and when we convert back to the original variables, it can be challenging to even determine whether or not a given original variable has been selected. Developing a new theory about the consistency of augmented LASSO is beyond the scope of the current paper, but is an interesting area for future work.

There are several ad-hoc methods in the literature to incorporate tree structure into the LASSO model, for example Xiao et al. (2018). However, an advantage of our aggregated LASSO method is that it is trivial to also incorporate covariates which do not fit into the tree structure, simply by not creating aggregated variables for them. This approach can also be applied to multiple hierarchical clustering structures on the same set of variables e.g. all clades
from all gene trees for a given data set. We can do this by simply adding a set of aggregated variables for each clustering.

3 Simulation

3.1 Study 1: Simulation using variables from higher taxonomic levels

Our first simulation is based on the original microbiome data matrix $X$ from the pouch data (afferent limb site) in (Tyler et al., 2013) (see details of the data in Section 4.1). We examine our method under the null case (no variable is significantly associated with the outcome variable) and under various sparse settings using variables from higher taxonomic levels: phylum or class. These settings were chosen to be similar to the results from the real data analysis on that dataset. For each simulated dataset we compare the performance of SuRF with several existing popular variable selection methods: LASSO, VSURF (Genuer et al., 2015) and stability selection. VSURF uses the variable importance from the random forest method to select variables. Stability selection performs LASSO variable selection on a large number of subsamples of the data, and selects the variables that are selected by LASSO for a large proportion of these subsamples.

Due to the sample size, we cannot afford to hold out a test sample, so only in-sample prediction (same predictor matrices for the training and test data) results are available. The penalty parameter $\lambda$ for LASSO is obtained by a 5-fold cross validation procedure and we use the $\lambda$ which gives an error within 1 standard error of the best model. For stability selection, we adopt a range of threshold probabilities recommended in Meinshausen and B"uhlmann (2010), between 0.6 and 0.9 (results for stability with cutoff 0.6 and 0.9 in simulation study 1 and 2 are shown in the Tables 2-4, and results with cutoffs 0.7 and 0.8 in web tables 5-7), and use the default family error rate upper bound parameter of 1. VSURF offers variable selection for different objectives: interpretation and prediction. We compare only the variables selected for prediction, which are always a subset of the variables selected for interpretation.

For assessment of results, we look at both the variables selected and the predictive accuracy. Variable selection can be used either for interpretation or for prediction. For microbiome data, the interpretation can be challenging because of the large number of surrogate variables. We therefore view predictive accuracy as the primary objective of our variable selection, with interpretation a secondary goal. However, selection of the true variables is important for both prediction and interpretation. Therefore, we have included it in the results of our simulations.
3.1.1 Null case

We simulated 200 datasets with binary outcomes randomly generated from a Bernoulli distribution with probability 0.3. In this case, a good variable selection algorithm is the one that selects no variables in most cases and on average includes the least number of noise variables. The mean and standard deviation of number of noise variables selected are summarized in Table 2(b).

3.1.2 Sparse setting

We simulate four scenarios for the true predictors: a single variable (Bacteroidetes) that has a strong surrogate variable (S1 in Table 2); a single variable (Firmicutes) that has no extreme surrogate variable (S2 in Table 2); two unrelated variables (Bacteroidetes and Firmicutes) with equal signal strength (S3 in Table 2); and two variables (Bacilli and Clostridia) that together make up the majority of a single phylum (S4 in Table 2). The same data matrix $X$ is used to simulate the inflammation outcome at different signal-to-noise ratio (SNR) levels under a logistic regression model. The overall SNR is approximately $\frac{\text{Var}(P(Y))}{\text{E}(P(Y))(1-P(Y)))}$.

In the simulation, the $\beta$ coefficient(s) (see Table 1) are chosen so that SNR is approximately 0.7 (weak), 1 (Fair), and 3 (strong). The coefficients that achieve these SNRs depend on a number of factors, such as the distribution and correlation of the predictors. Because OTU abundance is often heavy-tailed, and the response is Bernoulli, these coefficients can be large compared with other datasets. For example, in Scenario 1, the coefficient for Bacteroidetes is $-4.58$ in the low SNR case, $-2.84$ in the medium SNR case, and $-4.58$ in the high SNR case. In all cases, the intercept of the model is set to be the same. The coefficients of Bacteroidetes and Firmicutes are set to be negative and positive respectively according to the relationship from the original data.

Table 1: Coefficients for four different simulation scenarios

| Case                        | SNR | Coefficients of variables ($\beta$) |
|-----------------------------|-----|-------------------------------------|
|                             |     | Bacteroidetes | Firmicutes | Bacilli | Clostridia |
| Single variable             |     |               |            |        |            |
| with one strong surrogate   | High| -4.58         |            |        |            |
| (Case 1)                    | Fair| -2.84         |            |        |            |
|                             | Low | -2.40         |            |        |            |
| Single variable             | High| 5.00          |            |        |            |
| with no extreme surrogate   | Fair| 2.32          |            |        |            |
| (Case 2)                    | Low | 1.85          |            |        |            |
| Two variables               | High| -4.87         | 4.39       |        |            |
| with equal strength         | Fair| -1.82         | 1.64       |        |            |
| (Case 3)                    | Low | -1.42         | 1.28       |        |            |
| Two variables               | High| 4.76          | 4.76       |        |            |
| equivalent to one variable  | Fair| 2.21          | 2.21       |        |            |
| (Case 4)                    | Low | 1.75          | 1.75       |        |            |
The variable selection results for the null case and all four sparse cases are given in Table 2(a,b). For Case 1, Bacteroidetes is one of the major phyla existing in the human gut and this phylum is mainly composed of the class Bacteroidia. The correlation of these two variables in our data is almost one and they are deemed as a pair of strong surrogate variables. Given such a high correlation, the algorithm may identify either of them as the predictor and we deem either case as correct. No variable selection method is able to distinguish between them as predictors. However, we deem the selection of both variables as the inclusion of a noise variable, since once the first variable is included, the second variable does not give additional information.

In Case 2 (S2 in Table 2), Firmicutes is another dominant phylum but there is no class that has a correlation with it as high as that between Bacteroidetes and Bacteroidia. The class Clostridia has fairly high correlation, but we deem Clostridia as an incorrect variable in this case.

In Case 3 (S3 in Table 2), the strengths of the two variables (Bacteroidetes and Firmicutes) are set equal by adjusting the coefficients according to their standard deviation. As in Case 1, we deem Bacteroidia as a correct alternative to Bacteroides, but deem the selection of both to constitute a noise variable.

In Case 4 (S4 in Table 2), two class-level variables Bacilli and Clostridia are the two true predictors, with the same coefficients. The phylum Firmicutes is divided into three major classes including Bacilli, Clostridia and Erysipelotrichi with the former two classes representing the majority of the Firmicutes phylum so that the sum of classes Bacilli and Clostridia is highly correlated with total Firmicutes. SuRF and other methods will often pick up either the Firmicutes phylum as the true variable due to its surrogate status, which is deemed as a correct result, or chose the combination of the Firmicutes phylum and Erysipelotrichi class (or sometimes the Incertae Sedis genus which represents the majority of the class Erysipelotrichi) as an equivalent linear combination to the sum of classes Bacilli and Clostridia, which is an almost perfect result. As the SNR decreases from high to low, preference is often given to Firmicutes as the only true predictor.

The prediction results in these simulations are obtained by fitting a logistic regression model on the selected variables for each method. The results are shown in Table 2(c).

Throughout the simulation study, SuRF performed well at eliminating noise variables. Given the sequential nature of the variable selection, the number of noise variables selected should be a geometrically distributed variable with probability 0.95 (probability that the selection process stops when all remaining variables are noise variables) where we define the geometric random variable as number of failures rather than number of trials, so the average number of noise variables per data set should be $1/0.95 - 1 = 0.0526$. The results from our simulation study are mostly consistent with this, perhaps with slightly higher false positive rate on average. Since the null hypothesis is that all true variables are in the model, additional noise variables can be selected if they are ranked above the true variables. Therefore, the reliability of the $p$-values is better assessed by counting noise variables that are selected after all true variables.
are already included in the model. This is considered in more detail in Appendix D. Other methods perform much worse in terms of false positives, with the exception of stability selection with cut-off 0.9. Note that contrary to the claims of [Meinshausen and Bühlmann (2010), the results of stability selection are sensitive to the choice of cut-off probability.

Looking at false negatives, SuRF performs well in nearly all cases compared with other methods. For cases with a single true variable, no method has a significantly lower false negative rate than SuRF, not even the methods whose false positive rate is an order of magnitude higher than that of SuRF. For the two-variable cases, no method identifies at least one of the true variables significantly more often than SuRF. For Cases 3 and 4, LASSO selects two variables significantly more often than SuRF (except in Case 3 with high SNR). However, given the huge difference in number of noise variables selected, it would be very surprising if LASSO did not select both true variables significantly more often. The only method which had comparable false positive rate to SuRF was stability selection with cut-off 0.9, and this performed much worse than SuRF in terms of false negatives.

From a prediction prospective, SuRF achieves very good in-sample misclassification error rates compared to other methods. In Simulation Study 2 (see Table 3), we find that the other methods show significantly better performance when assessed via in-sample test error, compared with hold-out test data, whereas SuRF actually performs worse on in-sample test data. If a similar pattern applies to this study, then we would expect SuRF to outperform all other methods on classification of test data. Performance on test data is more important than on in-sample test data, because it better reflects the usage — in practice, we want a model that will generalise to new data, which often will not have the same predictor values.

3.2 Study 2: Simulation using variables from lower taxonomic levels

Classification at the lower taxonomic level such as genus or species level is more challenging [Hou et al. 2015]. In this section, we test the ability of SuRF to identify predictors at species level. We base this simulation on the OTU abundance from the moving picture data (see description in Section 4.2) simulating three species-level true predictors. We simulate a binary response variable at three levels of SNR, which are set using the same procedure as described in Study 1. We also simulate a Gaussian response variable with SNR levels set to 1 (Low), 3 (Fair), and 5 (High). For this case, we fix the irreducible error at 1 for all SNRs and adjust signal strength. We compare methods on their true positive rate, false positive rate, and either misclassification error rate (for binary response) or median MSE and $R^2$ (for continuous response). In Simulation study 1, we saw that stability selection had a much higher false positive rate than SuRF. It is possible to control the false positive rate more tightly in stability selection, by setting the family error rate upper bound parameter. For Simulation 2, we attempt to use this parameter to make the false positive rates
Table 2: Simulation study 1

| Scenario | SNR  | SuRF | Stability | VSURF | LASSO |
|----------|------|------|-----------|-------|-------|
|          |      | 0.6  | 0.9       |       |       |
| High     | 100  | 98   | 58        | 82    | 100   |
| Fair     | 98   | 88   | 26        | 79    | 100   |
| Low      | 95   | 81   | 16        | 83    | 95    |
| High     | 100  | 100  | 100       | 100   | 100   |
| Fair     | 100  | 100  | 93        | 93    | 95    |
| Low      | 97   | 99   | 71        | 83    | 87    |
| High     | 100  | 90   | 24 (100)  | 86    | 100 (100) |
| Fair     | 66   | 72   | 3 (63)    | 63    | 94    |
| Low      | 35   | 43   | 1 (46)    | 49    | 93    |
| High     | 19 (100) | 22 (100) | 1 (100) | 8    | 100 (100) |
| Fair     | 66 (100) | 72 (99)  | 3 (63)  | 63 (94) | 88 (99) |
| Low      | 35 (96) | 43 (92)  | 1 (46)  | 49 (93) | 70 (98) |

(b) False positive results: average number of noise variables (SD)

| Scenario | SNR  | SuRF | Stability | VSURF | LASSO |
|----------|------|------|-----------|-------|-------|
|          |      | 0.6  | 0.9       |       |       |
| High     | 0.02 | 4.06 | 0.46 (0.63) | 4.50 | (3.11) | 22.45 (21.62) |
| Fair     | 0.11 | 1.78 | 0.15 (0.46) | 4.76 | (3.13) | 31.46 (37.69) |
| Low      | 0.09 | 1.24 | 0.04 (0.20) | 4.58 | (3.30) | 42.96 (55.03) |
| High     | 0.06 | 0.56 | 0.00 (0.00) | 5.17 | (2.97) | 24.04 (25.64) |
| Fair     | 0.11 | 0.89 | 0.08 (0.34) | 5.26 | (2.87) | 33.92 (37.04) |
| Low      | 0.07 | 0.93 | 0.02 (0.14) | 5.00 | (2.82) | 29.52 (42.46) |
| High     | 0.05 | 0.54 | 0.01 (0.10) | 2.49 | (2.27) | 18.79 (32.51) |
| Fair     | 0.06 | 0.81 | 0.04 (0.20) | 4.15 | (2.76) | 31.57 (43.18) |
| Low      | 0.16 | 1.12 | 0.04 (0.20) | 4.12 | (2.98) | 27.61 (37.27) |
| High     | 0.08 | 0.84 | 0.03 (0.22) | 5.60 | (2.95) | 26.24 (24.94) |
| Fair     | 0.11 | 1.04 | 0.14 (1.51) | 4.30 | (2.52) | 19.56 (29.58) |
| Low      | 0.09 | 0.82 | 0.02 (1.14) | 4.33 | (2.49) | 19.21 (33.25) |

(c) In-sample average misclassification error rate (SD)

| Scenario | SNR  | SuRF | Stability | VSURF | LASSO |
|----------|------|------|-----------|-------|-------|
|          |      | 0.6  | 0.9       |       |       |
| High     | 0.085 | 0.104 | 0.252 (0.194) | 0.168 | (0.093) | 0.126 (0.682) |
| Fair     | 0.094 | 0.219 | 0.416 (0.141) | 0.240 | (0.038) | 0.365 (0.142) |
| Low      | 0.240 | 0.274 | 0.454 (0.107) | 0.276 | (0.027) | 0.418 (0.120) |
| High     | 0.083 | 0.095 | 0.092 (0.008) | 0.122 | (0.018) | 0.224 (0.058) |
| Fair     | 0.173 | 0.178 | 0.187 (0.074) | 0.222 | (0.023) | 0.294 (0.104) |
| Low      | 0.210 | 0.210 | 0.282 (0.142) | 0.266 | (0.024) | 0.368 (0.144) |
| High     | 0.102 | 0.115 | 0.196 (0.056) | 0.124 | (0.015) | 0.228 (0.064) |
| Fair     | 0.204 | 0.192 | 0.316 (0.133) | 0.232 | (0.021) | 0.311 (0.100) |
| Low      | 0.262 | 0.232 | 0.365 (0.139) | 0.265 | (0.026) | 0.342 (0.127) |

1 In Scenarios 1 and 2, the table gives the total number of times the true single variable/surrogate variable is selected. In Scenario 3, the table gives the total number of two true variables selected and the number of times at least one of two true variables selected in the bracket. In Scenario 4, the table gives the number of times two true/surrogate variables are selected (perfect selection) and the number of times the phylum Firmicutes is selected in brackets.

2 The null Simulation is over 200 batches; all other scenarios are 100 batches.
more comparable with SuRF by setting it equal to the average number of noise variables selected by SuRF (i.e. the numbers in the SuRF column of Table 2(b) and Table 3(b)).

The results are shown in Table 3 (binary response) and Table 4 (continuous response). For both binary and Gaussian response, SuRF can identify all three variables much more frequently than any other method at all levels of SNR. Under a similar family error rate, stability selection usually only identifies one true variable. With this setting, the results of stability selection are still sensitive to choice of cut-off probability, but less so than with the default setting. For the binary outcome (see Table 3), SuRF gives the lowest misclassification error rate for both in-sample data and test samples. For the continuous outcome (see Table 4), the median MSE for SuRF is almost identical to the irreducible error of 1. Other methods are much worse. We see the same trend when we use $R^2$ as a performance measure.

3.3 Study 3: Simulation with more true predictors

We also performed a more challenging simulation with 8 true predictors, covering a range of taxonomic levels and rarities of taxa, also with different signal strengths for different taxa. Full details of the simulation are presented in the online supplementary materials. As expected, variables with larger coefficients are more easily selected. However, rarer taxa are selected less often, even when they have relatively high coefficients. These patterns are common to all variable selection methods. Across the range of signal-noise ratios and coefficients, SuRF outperforms stability selection (with default family wise error rate upper bound) in terms of both false positive and false negative rate. SuRF hugely outperforms LASSO in terms of false positive rate, and outperforms LASSO in false negative rate at high SNR, with comparable performance at lower SNR. In terms of misclassification error rate, SuRF is clearly the best method. We did not compare VSURF in this simulation because of its slow running time.

4 Application: the pouchitis and moving picture data

Two published datasets are analyzed using SuRF. The first dataset is from a pouchitis study (Tyler et al., 2013). The second dataset includes samples from four body sites of two individuals over a long time period (Caporaso et al., 2011).

4.1 Pouchitis study

Colectomy with ileal pouch anal anastomosis (IPAA), also referred to as “J-pouch surgery”, is a common surgery for patients who have ulcerative colitis (UC) and those with familial adenomatous polyposis syndrome (FAP) (Shen).
| SNR | No of true variables selected | SuRF | Stability 0.6 | Stability 0.9 | VSURF | LASSO | RF | SVM |
|-----|--------------------------------|------|---------------|---------------|-------|-------|----|-----|
|     |                                |      |               |               |       |       |    |     |
| High|                                | 3    | 0.120         | N/A           | 0.120 | 0.383 | 0.197 |
|     |                                | 2    | 0.120         | N/A           | 0.120 | 0.383 | 0.197 |
|     |                                | 1    | 0.120         | N/A           | 0.120 | 0.383 | 0.197 |
|     |                                | 0    | 0.120         | N/A           | 0.120 | 0.383 | 0.197 |
| Fair|                                | 3    | 0.020         | N/A           | 0.020 | 0.010 | 0.010 |
|     |                                | 2    | 0.020         | N/A           | 0.020 | 0.010 | 0.010 |
|     |                                | 1    | 0.020         | N/A           | 0.020 | 0.010 | 0.010 |
|     |                                | 0    | 0.020         | N/A           | 0.020 | 0.010 | 0.010 |
| Low |                                | 3    | 0.010         | N/A           | 0.010 | 0.010 | 0.010 |
|     |                                | 2    | 0.010         | N/A           | 0.010 | 0.010 | 0.010 |
|     |                                | 1    | 0.010         | N/A           | 0.010 | 0.010 | 0.010 |
|     |                                | 0    | 0.010         | N/A           | 0.010 | 0.010 | 0.010 |

Table 3: Simulation study 2 (Binary outcome)

(a) Frequency of number of true variables selected over 100 simulations

(b) Number of noise variables selected over 100 simulations

(c) Mis-classification error rate in test samples

(d) In-sample mis-classification error rate

| SNR | No of true variables selected | Mean 0.100 | Mean 0.220 | Mean 0.259 |
|-----|--------------------------------|------------|------------|------------|
|     |                                | 0.120      | 0.383      | 0.383      |
|     |                                | 0.120      | 0.383      | 0.383      |
|     |                                | 0.120      | 0.383      | 0.383      |
|     |                                | 0.120      | 0.383      | 0.383      |
| High|                                | 0.100      | 0.220      | 0.259      |
|     |                                | 0.100      | 0.220      | 0.259      |
|     |                                | 0.100      | 0.220      | 0.259      |
|     |                                | 0.100      | 0.220      | 0.259      |
| Fair|                                | 0.220      | 0.383      | 0.383      |
|     |                                | 0.220      | 0.383      | 0.383      |
|     |                                | 0.220      | 0.383      | 0.383      |
|     |                                | 0.220      | 0.383      | 0.383      |
| Low |                                | 0.259      | 0.383      | 0.383      |
|     |                                | 0.259      | 0.383      | 0.383      |
|     |                                | 0.259      | 0.383      | 0.383      |
|     |                                | 0.259      | 0.383      | 0.383      |
Table 4: Simulation study 2 (Continuous outcome)

| SNR | No of true variables | Stability | SuRF | 0.6 | 0.9 | VSURF | LASSO | RF |
|-----|----------------------|-----------|------|-----|-----|-------|-------|----|
|     |                      |           |      |     |     |       |       |    |
|     |                     | High      | 3    | 99  | 0   | 0    | 81    | 35  |
|     |                      |           | 2    | 1   | 0   | 0    | 19    | 65  |
|     |                      |           | 1    | 0   | 100 | 100  | 0     | 0   |
|     |                      |           | 0    | 0   | 0   | 0    | 0     | 0   |
|     |                      | Fair      | 3    | 97  | 0   | 0    | 71    | 12  |
|     |                      |           | 2    | 3   | 0   | 2    | 19    | 81  |
|     |                      |           | 1    | 0   | 100 | 98   | 0     | 7   |
|     |                      |           | 0    | 0   | 0   | 0    | 0     | 0   |
|     |                      | Low       | 3    | 80  | 0   | 0    | 40    | 1   |
|     |                      |           | 2    | 19  | 4   | 2    | 60    | 52  |
|     |                      |           | 1    | 1   | 96  | 98   | 0     | 47  |
|     |                      |           | 0    | 0   | 0   | 0    | 0     | 0   |

(a) Number of true variables selected

| SNR | mean | SD     |     |     |     |     |     |     |
|-----|------|--------|-----|-----|-----|-----|-----|-----|
|     | High | 0.040  | 0.360| 0.090| 15.16| 52.540|
|     | Fair | 0.140  | 0.790| 0.100| 4.334| 29.186|
|     | Low  | 0.540  | 0.700| 0.010| 28.700|

(b) Number of noise variables selected

| SNR | mean | SD     |     |     |     |     |     |     |
|-----|------|--------|-----|-----|-----|-----|-----|-----|
|     | High | 0.040  | 0.360| 0.090| 15.16| 52.540|
|     | Fair | 0.140  | 0.790| 0.100| 4.334| 29.186|
|     | Low  | 0.540  | 0.700| 0.010| 28.700|

(c) Median MSE (IQR) in test samples

| SNR | 1   | 1   | 1   |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     | 1.009| 4.433| 5.555| 2.784| 3.470| 2.977|     |     |
|     | (0.131)| (0.672)| (0.678)| (0.379)| (0.794)| (0.416)|     |     |
|     | 1.004| 2.870| 3.117| 2.083| 2.535| 2.236|     |     |
|     | (0.143)| (0.430)| (0.357)| (0.258)| (0.460)| (0.272)|     |     |
|     | 1.011| 1.691| 1.711| 1.428| 1.644| 1.431|     |     |
|     | (0.176)| (0.267)| (0.237)| (0.232)| (0.499)| (0.224)|     |     |

(d) $R^2$ in test samples

| SNR | 0.503 | 0.508 | 0.515 | 0.515 | 0.515 | 0.515 | 0.515 | 0.515 |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|
|     | High  | 0.801 | 0.367 | 0.356 | 0.460 | 0.324 | 0.417 |       |
|     | Fair  | 0.706 | 0.389 | 0.346 | 0.351 | 0.207 | 0.359 |       |
|     | Low   | 0.455 | 0.439 | 0.274 | 0.253 | 0.215 | 0.127 | 0.211 |

Average Oracle $R^2$ (sd)
Pouchitis is a common complication of J-pouch surgery involving inflammation of the ileal pouch. It is unclear what triggers pouchitis in some patients but not others: pouchitis occurs almost exclusively in patients with inflammatory bowel disease and not in patients with FAP.

Our data come from the study Tyler et al. (2013) which includes microbiome samples from biopses of 71 patients following a J-pouch surgery. Our objective is to classify individuals between the healthy and inflammation group. The inflammation group is composed of the 34 subjects from the “pouchitis” and “CD (Crohn’s disease)-like” groups in the original paper. It includes inflammation in either the pouch or the pre-pouch ileum; and the inflammation may or may not be active at time of biopsy. The healthy group is composed of the 37 subjects in the “FAP” and “no pouchitis” groups from the original study.

Some patients received one or two antibiotic treatments before the biopsy. We include two variables describing antibiotics usage in addition to the proportions of OTUs at each taxonomic rank, making a total of 1781 predictors. The same information was measured at both pouch and afferent limb for each patient.

The mean classification error rate is estimated by averaging the cross-validated classification error across a thousand subsamples. It is about 0.2 and 0.35 for pouch and afferent limb, respectively. At both biopsy sites, the phylum Bacteroidetes is the only variable significant at level 0.05. The agreement on the importance of Bacteroidetes at both biopsy sites suggests this phylum is significantly associated with inflammation. The single bacteroidetes phylum gives a 0.88 and 0.83 AUC (area under the ROC curve shown in Web Figure 3) in the pouch and afferent limb respectively. The ROC curves suggest that bacteriodetes is an effective discriminant variable for differentiating the inflammation condition at both biopsy sites, especially for the pouch data.

Even the non-significant highly ranked variables are potentially interesting variables for future studies. Among the top variables in both sites, there are several common variables, which are Bacteroidetes, Bacteroidia, Erysipelotrichi,and Bacilli. Most of these organisms have been found to be associated with IBD and pouchitis in the literature. Bacteroidia is a major class of the Bacteroidetes phylum. 99.7% of this phylum are Bacteroidia in this dataset. Both taxonomy levels are found to be negatively associated with the disease at both biopsy sites. This is consistent with previous findings from other datasets that there is a decreased abundance and diversity of Bacteroidetes in CD samples (De Palma et al., 2015). Similar findings are also reported in Tyler et al. (2013) using the same dataset we have analysed here. They identified that Bacteroidetes are significantly reduced in the pouchitis and CD-like groups compared to the FAP and no pouchitis groups. Many of the other highly ranked OTUs have been linked with related conditions such as IBD in previous studies. The family Fusobacteriaceae has previously been found to have a higher proportion in patients with UC who underwent pouch surgery (Reshef et al., 2015). Though we know little about the role of Turicibacter in IBD (Jones-Hall et al., 2015), it has been shown significantly decreased in dogs with IBD (Rossi et al., 2014). Bacilli and Erysipelotrichi have been found increased in patients with
UC (Michail et al., 2012) and low counts of *Subdoligranulum* have been found in patients with Crohn’s disease (Thomas et al., 2014).

Stability selection also selects Bacteroidetes for cut-off probability 0.6 for both pouch and afferent limb, but selects no variables at higher cut-off probabilities 0.8 and 0.9. At cut-off probability 0.7, it selects Bacteroidetes for the pouch data, but selects no variables for the afferent limb data. In Table 5, we compare the predictive accuracy of the logistic regression model using the selected variable Bacteroidetes, with other commonly used classification methods for microbiome data, namely Random Forest (RF) and Support Vector Machine (SVM) with a linear kernel (we obtained similar results for other kernels and omitted them from the table). These predictive accuracies are computed using leave-one-out cross-validation with their corresponding tuning parameters chosen by cross-validation within the training data. The predictive accuracy from RF and SVM are comparable to the results using SuRF since the mean test errors are all within one standard deviation.

4.2 Moving picture data

The moving picture data set (Caporaso et al., 2011) recorded a long period of repeated observations from multiple body sites (gut, tongue, left and right palms) of two individuals. This data set has a larger sample size for each body site than the pouchitis data. The number of observations for the gut, tongue, left palm and right palm are respectively 131, 135, 134 and 134 for the first individual, and 336, 373, 365 and 359 for the second individual. We split the dataset for each site into a training and a test sample set with a ratio of 2:1. At each body site, the observations from each individual are ordered by time. The earlier 2/3 of time points from each individual are used as training samples and the rest as test samples.

We train SuRF to classify samples from each body site between the two individuals using the training data. The selected variables are summarised in Web Table 4, and the joint distributions are displayed in Web Figure 4. Table 5(b) shows the misclassification error rate for SuRF and other methods. Between one and four variables are selected at each body site and the prediction errors for the test samples are very low at all sites. SuRF has found a small set of variables that can distinguish two individuals’ microbial environments. For most methods the test error tends to be lowest in the gut and highest for palms. This can be well explained by the fact that the microbiome community is most stable in the gut (Voigt et al., 2015) and least stable for palms because, in contrast to the human gut, the composition of microbial communities from hands, though in the long run relatively stable (Oh et al., 2016) and personalized (Fierer et al., 2010), can change dramatically even from washing hands with some disinfectant cleaning products. Identifying individuals using the palm microbiomes is feasible but more variable than using a more closed environment such as the gut.

We also tested cross-predictions — using models fitted on one body part to predict the owner of samples from another body part. The prediction model
trained on one palm could identify samples from the other palm with low prediction error. The two bacteria selected in the two palm models include the same species-level variable from genus *Deinococcus* and two different unspecified species-level variables from genus *Corynebacterium*. This suggests a similarity between the microbiomes on two palms from a single individual. No other cross-predictions performed significantly better than random guessing.

SuRF and stability selection (using cutoff probability 0.9 and default family error upper bound) were on average comparable in predictive accuracy to Random Forests and significantly better than SVM (see Table 5(b)). Compared to stability selection, we found that SuRF seemed to achieve a lower prediction error, and consistently selected fewer variables.

In the gut data, SuRF chooses one unspecified species from the genus *Bacteroides*, which is one of three variables selected by stability selection with cut-off probability 0.9. With one variable we obtain exactly the same prediction training and test errors as with three variables selected by stability selection. The other two variables selected by stability selection (another unclassified species from the genus *Bacteroides* and the family Porphyromonadaceae) don’t provide additional predictive accuracy for recognizing individuals.

In the tongue data, even using cut-off probability 0.9, stability selection still selects eight variables. SuRF selects only three variables: the most important variable is one species from genus *Neisseria* and the remaining two are unspecified species from the family Lachnospiraceae, and order Sphingobacterales. There are no common variables selected by both SuRF and stability selection. This is the case where SuRF performed less well than other methods, so it is natural to ask whether SuRF might have selected too few variables in this case. However, using only the first two variables chosen by SuRF reduces the test error to 0.03, so the poor performance here is not entirely explained by excessive sparsity.

For the left palm data, both stability selection with the highest cut-off probability and SuRF choose the same set of variables (one unspecified species from the genus *Corynebacterium* and another unspecified species from *Deinococcus*).

For the right palm data, SuRF selects the same species from the genus *Deinococcus* and a different unspecified species from the genus *Corynebacterium*. The former is also selected by stability selection for the right palm model, but the second variable is replaced by the kingdom Bacteria. Both methods choose two variables (using cut-off 0.9 for stability selection), however, SuRF not only provides a smaller prediction error for both training and test data, but also indicates a similarity between two palms within the individual which is not reflected by the variables selected by stability selection.

These two real datasets exemplify the ability of SuRF to select discriminant OTUs at the appropriate taxonomic level. For the pouchitis data, with large within-class variation at lower levels, SuRF identifies a phylum-level variable. For comparing two healthy individuals, the higher-level structure is more similar, so SuRF selects species-level variables.
5 Concluding Remarks

We have developed a very useful variable selection method for GLMs, SuRF, which involves a subsampling based approach to rank variables that may be highly associated with the response variable followed by variable selection with forward ANOVA. This method takes advantage of the sparseness of the model selected by LASSO and chooses variables that appear more frequently and contribute significantly to reducing residual deviances in the forward ANOVA procedure. Due to its high sparseness and stability, SuRF can be particularly useful for microbiome data or any data that is high dimensional and contains many surrogate variables. The method provides a conservative but stable selection of variables that can predict and classify the outcomes. SuRF can also provide a reasonable way to compute $p$-values for all variables according to sequentially calculated empirical distributions, whereas LASSO does not provide $p$-values directly. The forward selection procedure helps to alleviate the phenomenon of including surrogate variables and leads to a highly sparse model for microbiome data. Due to its short list of selected variables SuRF is particularly suitable for identifying biomarkers.

In our simulation studies we saw that in comparison to many competing methods, SuRF is able both to select the true variable more often, and also to select fewer noise variables for both binary and continuous outcomes. This leads to excellent performance in prediction.

In the two real data analyses, we found that no other methods significantly outperformed SuRF in terms of prediction error, but SuRF selects fewer variables than other methods. SuRF was able to adjust the taxonomic levels of the variables selected to suit the individual datasets.

There are many promising avenues for future research into extending the SuRF framework. In this paper, we have presented SuRF based on penalised regression followed by generalised linear models, because that seemed most appropriate to the structure of the microbiome data. However, the core idea is to use subsampling with a simple variable selection method, then use the ensuing ranking in a forward selection method. This core idea could be applied with
any combination of a variable selection method and a family of nested models to be used in forward selection. For example, we could develop a ranking based on Random Forest, and then perform the forward selection based on neural networks. The use of the permutation test for evaluating a variable automatically adjusts to our choice of method. Further research is needed into what combinations of methods work well in this framework.

6 Supplementary Materials

Web Appendices, Tables, and Figures referenced in Sections 2–4 are available with this paper at the Biometrics website on Wiley Online Library.

An R package for applying SuRF is available from Toby Kenney’s website at [www.mathstat.dal.ca/~tkenney/Rpackages/](http://www.mathstat.dal.ca/~tkenney/Rpackages/). The authors also plan to submit this package to CRAN. The package uses the glmnet package for fitting LASSO regression, and can be used for any models that the glmnet package supports.

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