





Gene expression

benchdamic: benchmarking of differential abundance methods for microbiome data

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Abstract

Summary: Recently, an increasing number of methodological approaches have been proposed to tackle the complexity of metagenomics and microbiome data. In this scenario, reproducibility and replicability have become two critical issues, and the development of computational frameworks for the comparative evaluations of such methods is of utmost importance. Here, we present *benchdamic*, a Bioconductor package to benchmark methods for the identification of differentially abundant taxa.

Availability and implementation: *benchdamic* is available as an open-source R package through the Bioconductor project at <https://bioconductor.org/packages/benchdamic/>.

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Supplementary information: [Supplementary data](#) are available at *Bioinformatics* online.

1 Introduction

Differential abundance (DA) analysis identifies significant differences in the microbial community composition between groups of samples, providing new insights into the composition of microbial communities and on their associations with the environment. Although many approaches have been proposed for DA analysis, it is widely recognized that the best method (i.e. a method with performances uniformly better than all the others) does not exist and that a careful exploratory data analysis is necessary to address methodological choices (Calgaro *et al.*, 2020; Hawinkel *et al.*, 2019; Nearing *et al.*, 2022; Thorsen *et al.*, 2016; Weiss *et al.*, 2017).

Building on our previous work (Calgaro *et al.*, 2020), we present the *benchdamic* R/Bioconductor package, which provides a computational framework to guide researchers in the selection of the method that best fits their data.

The structure of *benchdamic* can be summarized into four main parts (Fig. 1). Each section is developed to answer specific questions when comparing samples from different experimental groups, namely (i) the ability for a given statistical distribution to successfully fit the input data, with particular focus on sparsity and their count nature; (ii) the ability of the DA methods to control the type I error; (iii) the concordance among methods; and (iv) the accuracy of the findings based on a priori biological knowledge. Altogether, *benchdamic* is a flexible and customizable framework that can be used for the benchmarking of new and existing DA methods.

2 Implementation

benchdamic builds on existing R/Bioconductor infrastructure packages: the primary input of *benchdamic*'s main functions is a *phyloseq* or a *TreeSummarizedExperiment* object (Huang *et al.*, 2021; McMurdie and Holmes, 2013). Ready-to-use normalization and DA methods included in *benchdamic* are based on the *edgeR* (Robinson *et al.*, 2010), *DESeq2* (Love *et al.*, 2014), *limma-voom* (Law *et al.*, 2014; Phipson *et al.*, 2016; Ritchie *et al.*, 2015), *metagenomeSeq* (Paulson *et al.*, 2013), *ALDEx2* (Fernandes *et al.*, 2014, 2013), *corncob* (Martin *et al.*, 2020), *MAST* (Finak *et al.*, 2015), *Seurat* (Butler *et al.*, 2018), *dearseq* (Gauthier *et al.*, 2020), *NOISeq* (Tarazona *et al.*, 2015), *ANCOMBC* (Lin and Peddada, 2020; Mandal *et al.*, 2015) and *zinbwave* (Risso *et al.*, 2018; Van den Berge *et al.*, 2018) packages. Combinations of parameters are possible as well as the inclusion of custom methods (see [Supplementary material Section S3](#)).

In the following sections, we briefly outline the main functionality of the package. See Calgaro *et al.* (2020) for technical details on how these metrics are computed.

2.1 Goodness of fit

DA statistical models are based on different statistical distributions. Five different distributions are available in *benchdamic* for testing the goodness of fit on user-provided data: negative binomial, zero-inflated negative binomial, zero-inflated Gaussian, truncated Gaussian and Dirichlet-multinomial (see [Supplementary material](#)

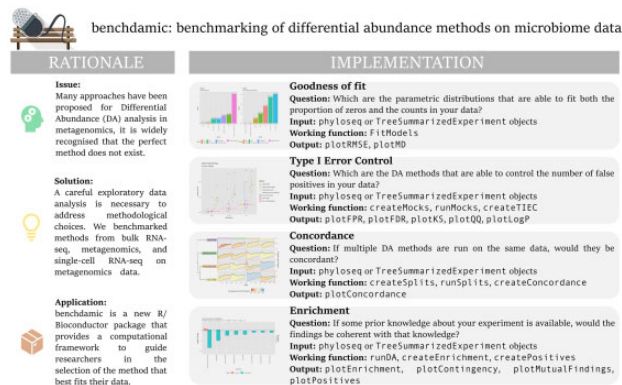


Fig. 1. Graphical abstract. Each box on the right represents a step of the analysis where information about the research question, type of input data, working functions and outputs are reported

Section S2). Goodness of fit is measured by the ability of each method to correctly estimate the average counts and the probability of observing a zero.

2.2 Type I error control

To investigate the Type I error rate control of each DA method (i.e. the probability of the statistical test to call a feature DA when it is not) mock datasets with no true DA are generated starting from the user-provided data (see [Supplementary material Section S4](#)).

Briefly, the dataset is split into two random subsets and DA analysis, based on a chosen list of methods, is performed. The process is repeated N times ($N \geq 1000$ suggested). The performances of each method are then summarized and graphically represented considering the false positive rate, false discovery rate, and departure from uniformity for the P -values distribution.

2.3 Concordance

benchdamic can be used to measure the between-method concordance (BMC), in which a DA method is compared to other methods in the same dataset, and the within-method concordance (WMC), in which a method is compared to itself in two random subsets of the same dataset ([Supplementary material Section S5](#)). Firstly, the dataset is randomly divided in half to obtain two subsets (Subset1 and Subset2) with samples from two or more biological groups, then DA analysis is performed between two groups, independently on each subset. The process is repeated N times ($N \geq 100$ suggested) and average WMC and BMC metrics are computed and summarized using a heatmap representation.

2.4 Enrichment

Enrichment analysis can provide an alternative way of ranking methods in terms of their ability to identify, as significantly different, taxa that are known to be differentially abundant between two groups. DA analysis needs to be performed on a dataset where some a priori knowledge is available ([Supplementary material Section S6](#)). Given the direction of the DA features (over- or under-abundant) and the expected group in which the features should be differentially abundant according to the prior knowledge, several contingency tables are created for each DA method. A Fisher exact test is then performed to test the enrichment and the DA features identified by more than one method are highlighted. Additionally, the users will be able to rank the methods based on the difference between the total number of true positives and false positives for several thresholds (based on P -values, adjusted P -values or other statistics). The same approach can also be used to perform power analysis using simulated data ([Supplementary material Section S6.8](#)).

3 Conclusions

The benchdamic R/Bioconductor package aims to be a support tool for the identification of DA microbial taxa and the benchmarking of new methods. We envision two main uses of our package: (i) for practitioners interested in performing DA analysis on a new dataset, benchdamic can be used to identify the best DA methods among those already in the literature; (ii) for method developers interested in benchmarking their new approach, benchdamic can be used as an impartial tool to evaluate the relative merits of the new method compared to what is already available.

benchdamic is available as an open-source package through the Bioconductor project. The package includes a vignette with a detailed tutorial.

The future of benchdamic is oriented to the addition of new aspects of analysis e.g. new normalization methods and new DA approaches.

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Conflict of Interest: none declared.

References

- Butler, A. et al. (2018) Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat. Biotechnol.*, **36**, 411–420.
- Calgaro, M. et al. (2020) Assessment of statistical methods from single cell, bulk RNA-seq, and metagenomics applied to microbiome data. *Genome Biol.*, **21**, 191.
- Fernandes, A.D. et al. (2013) ANOVA-Like differential expression (ALDEx) analysis for mixed population RNA-Seq. *PLoS One*, **8**, e67019.
- Fernandes, A.D. et al. (2014) Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome*, **2**, 15.
- Finak, G. et al. (2015) MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data. *Genome Biol.*, **16**, 278.
- Gauthier, M. et al. (2020) Dearsq: a variance component score test for RNA-seq differential analysis that effectively controls the false discovery rate. *NAR Genom. Bioinform.*, **2**, lqaa093.
- Hawinkel, S. et al. (2019) A broken promise: microbiome differential abundance methods do not control the false discovery rate. *Brief Bioinform.*, **20**, 210–221.
- Huang, R. et al. (2021) TreeSummarizedExperiment: a S4 class for data with hierarchical structure. *F1000Research*, **9**, 1246.
- Law, C.W. et al. (2014) Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol.*, **15**, R29.
- Lin, H. and Peddada, S.D. (2020) Analysis of compositions of microbiomes with bias correction. *Nat. Commun.*, **11**, 3514.
- Love, M.I. et al. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.*, **15**, 550.
- Mandal, S. et al. (2015) Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microb. Ecol. Health Dis.*, **26**, 27663.
- Martin, B.D. et al. (2020) Modeling microbial abundances and dysbiosis with beta-binomial regression. *Ann. Appl. Stat.*, **14**, 94–115.
- McMurdie, P.J. and Holmes, S. (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, **8**, e61217.
- Nearing, J.T. et al. (2022) Microbiome differential abundance methods produce different results across 38 datasets. *Nat. Commun.*, **13**, 342.

- Paulson, J.N. *et al.* (2013) Differential abundance analysis for microbial marker-gene surveys. *Nat. Methods*, **10**, 1200–1202.
- Phipson, B. *et al.* (2016) Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. *Ann. Appl. Stat.*, **10**, 946–963.
- Risso, D. *et al.* (2018) A general and flexible method for signal extraction from single-cell RNA-seq data. *Nat. Commun.*, **9**, 284.
- Ritchie, M.E. *et al.* (2015) Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.*, **43**, e47.
- Robinson, M.D. *et al.* (2010) edgeR: a bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, **26**, 139–140.
- Tarazona, S. *et al.* (2015) Data quality aware analysis of differential expression in RNA-seq with NOISeq R/bioc package. *Nucleic Acids Res.*, **43**, e140.
- Thorsen, J. *et al.* (2016) Large-scale benchmarking reveals false discoveries and count transformation sensitivity in 16S rRNA gene amplicon data analysis methods used in microbiome studies. *Microbiome*, **4**, 62.
- Van den Berge, K. *et al.* (2018) Observation weights unlock bulk RNA-seq tools for zero inflation and single-cell applications. *Genome Biol.*, **19**, 24.
- Weiss, S. *et al.* (2017) Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, **5**, 27.