

## Explicit: graphical user interface software for metadata-driven management, analysis and visualization of microbiome data

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### ABSTRACT

**Summary:** Studies of the human microbiome, and microbial community ecology in general, have blossomed of late and are now a burgeoning source of exciting research findings. Along with the advent of next-generation sequencing platforms, which have dramatically increased the scope of microbiome-related projects, several high-performance sequence analysis pipelines (e.g. QIIME, MOTHUR, VAMPS) are now available to investigators for microbiome analysis. The subject of our manuscript, the graphical user interface-based Explicit software package, fills a previously unmet need for a robust, yet intuitive means of integrating the outputs of the software pipelines with user-specified metadata and then visualizing the combined data.

**Availability and Implementation:** Explicit is implemented in C++ via the Qt framework and supported in native code on all major operating systems (Windows, Macintosh, Linux). The source code, documents and tutorials are freely available under an open-source license at [www.explicit.org](http://www.explicit.org).

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### 1 INTRODUCTION

The scope of microbial ecology studies has increased significantly over the past decade, driven in part by the realization that microbial communities are critical mediators of human and ecosystem health. Furthermore, a 10 000-fold decrease in the cost of DNA sequencing ([www.genome.gov/sequencingcosts](http://www.genome.gov/sequencingcosts)) has resulted in a commensurate increase in the volume of data to analyze. In response, many software systems (e.g. 16S pipelines) have been created to deal with the resulting glut of data (Frank 2008, 2009; Frank and Robertson, 2011; Giongo *et al.*, 2010; Hartman *et al.*, 2010; Kuczynski *et al.*, 2012; Schloss *et al.*, 2009; [vamps.mbl.edu](http://vamps.mbl.edu)). At present, software tools for microbiome analysis often require some sophistication in computer skills to perform even basic analyses. Because tools using graphical user interfaces provide more facile interactivity for data exploration,

we have developed Explicit (Latin for 'explanation'), which consumes the output from existing sequence analysis pipelines and greatly expedites metadata-driven management, analysis and visualization of sequence classification results.

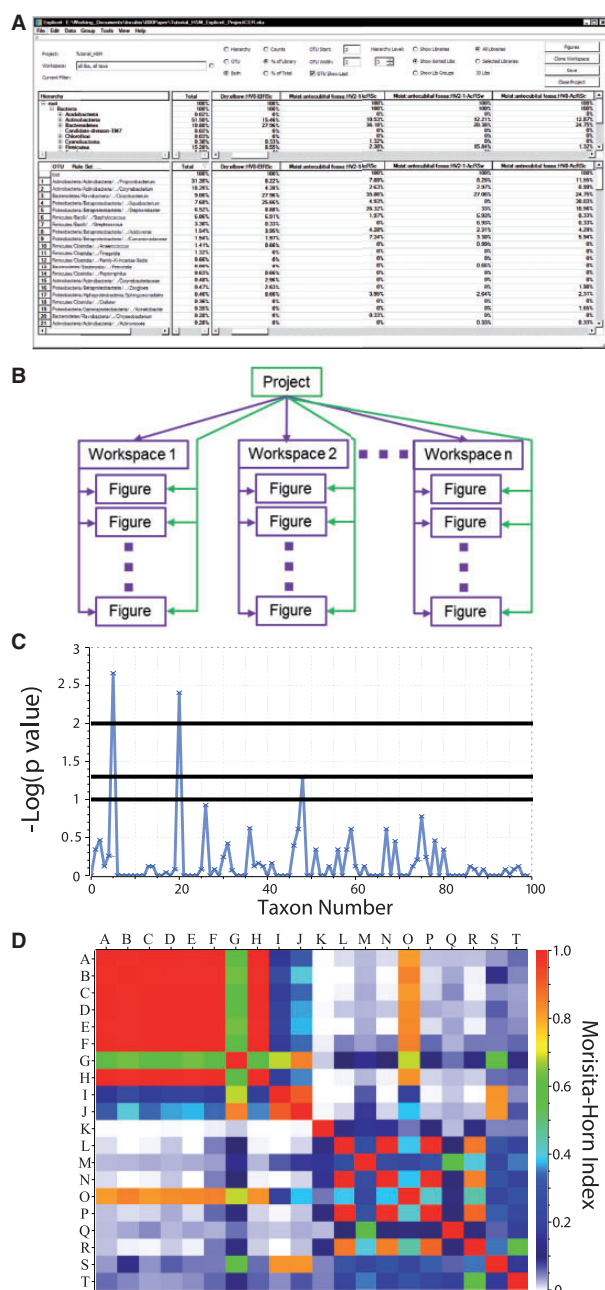
### 2 OVERVIEW OF EXPLICIT

Explicit is a scalable (laptop to server) open-source software package implemented in C++ using the Qt cross platform application framework ([qt-project.org](http://qt-project.org)) and the plotting methods from Qwt ([qwt.sourceforge.net](http://qwt.sourceforge.net)). As such, the software is provided as native code on multiple operating systems (Windows, Macintosh, Linux). Performance of the primary display window and file size are  $O$  [number of samples  $\times$  number of operational taxonomic units (OTUs)], yielding good performance with modest compute capability.

Explicit is compatible with any upstream sequence analysis pipeline that produces OTU table data, which Explicit displays in a spreadsheet-like window (Fig. 1A). Metadata, for instance environmental or clinical attributes, are imported through tab-delimited or comma separated value formatted flat files. OTU data and metadata are managed within a single file as an Explicit Project. Projects may contain one or more user-defined Workspaces, which allow bioinformatic experiments to be performed on metadata-derived subsets of Project samples or OTUs (or both), (e.g. samples from males aged >40 years and only the OTUs belonging to the phylum Firmicutes). Workspaces store user-generated Figures displaying descriptive data or Explicit computations. Workspaces and Figures are persistent and available for user reference or revision during the analysis life cycle (Fig. 1B).

Explicit provides several tools for metadata-driven analysis of microbiome datasets. Basic distributions of OTUs in samples or categories of samples (defined by user-supplied metadata) can be displayed via stacked bar plots, pie charts or heatmaps. OTUs that differ in abundance and/or prevalence between user-defined groups of samples can be identified by Wilcoxon, two-proportion and two-part statistical tests (Wagner *et al.*, 2011) and graphically portrayed as Manhattan plots (Fig. 1C). Ecological alpha and beta diversity indices (Schloss and Handelsman, 2007) can

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**Fig. 1.** Explicit windows displaying a portion of a human skin microbiome dataset (Grice *et al.*, 2009). (A) Explicit workspace window showing the row/column data displays characteristics of an OTU file. The upper pane displays the taxonomic hierarchy of the dataset and individual samples. The lower pane shows the full OTU table perspective on the data. (B) Relationships between data management entities in Explicit. (C) Manhattan plot of a Two-Part (Wagner *et al.*, 2011) non-parametric statistical comparison of samples, indicating that two taxa differ significantly between sample types. (D) Heatmap of Morisita–Horn similarity between samples

be calculated through resampling and rarefaction; results are presented as line plots, heatmaps and data tables (Fig. 1D). All tabulated data are exportable as delimited text files for import into other tools (e.g. R or SAS), whereas Figures are exported as editable PDFs.

### 3 CONCLUSIONS

Explicit fills a critical need for a robust, yet intuitive means of integrating the outputs of sequence processing and classification pipelines with user-specified metadata and then visualizing the combined data. Although designed initially for 16S analysis, Explicit can be applied to any dataset organized through a hierarchical classification scheme (e.g. other genes or metagenomes). For bioinformaticians, Explicit offers a powerful means of rapidly evaluating and segmenting large microbial ecology datasets. For non-bioinformaticians, a familiar easy-to-use mouse-driven software application softens the training focus away from software logistics and toward the microbiology and statistical methods that underpin microbial ecology. Practical use of Explicit in several laboratory settings has been found to reduce the requirements for personnel with bioinformatics/computational expertise (Hara *et al.*, 2013; Markle *et al.*, 2013; Robertson *et al.*, 2013). Consequently, the analysis of complex microbiome datasets is now much more accessible to the growing number of investigators who wish to bring a microbial ecological perspective to their fields of interest, especially in medicine and the environmental sciences.

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