PathoStat User Manual

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Package

PathoStat 1.4.1

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1 Introduction

Welcome! This is the manual for the PathoStat package. PathoStat is a Shiny App interactive package that will let you explore metagenomic datsets, e.g., microbiome abundance tables, for exploratory data analysis, differential abundance hypothesis testing, and more. PathoStat can take metagenomic abundance data produced by any taxonomic profiling pipeline, however, to get the most out of PathoStat we recommend using PathoScope2. PathoStat is not limited to whole-metagenomic shotgun data but also can take metataxonomic data, i.e., 16S rRNA, ITS, etc. Plots can be exported in vector-based file formats (svg, PDF) for sharing or further editing. For detailed installation instructions check the introductory and advanced vignettes.

```
require(PathoStat)
vignette("PathoStatIntro")
vignette("PathoStatUserManual")
vignette("PathoStatAdvanced")
```

2 Installation and Run

While there are a few functions that can work from the console, PathoStat is designed to be run interactively. From the R console simply type:

```
source("http://bioconductor.org/biocLite.R")
biocLite("PathoStat")
```

If all went well you should now be able to load PathoStat:

require(PathoStat)
runPathoStat()

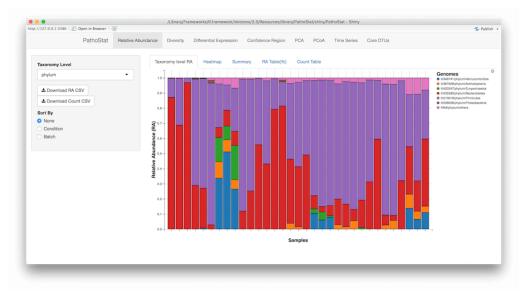
PathoStat functionality is grouped into eight tabs that comprise all analyses and visualizations. Here, we will go over each of those eight tabs and their main features.

3 Relative Abundance

The relative abundance tab is further subdivided into five subtabs where you can explore your data in the form of a stacked bar chart, a heatmap, summary, and searchable relative abundance and count tables. You can download these data both in read counts or in relative proportions.

3.1 Taxonomy level

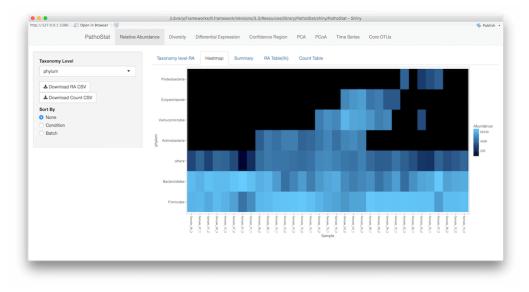
Within the Taxonomy level RA subtab, you can hove over different stacked bars and get the taxonomy membership and relative abundance. In addition you can sort the stacked bars (samples) by different factors in your data and download a svg image for further editing.



The image above shows the user interface of the stacked bar chart. On the left you can select the appropriate taxonomy level to plot, and by clicking on the gear icon on the upper right side of the chart, you can select the file format for your download.

3.2 Heatmap

This subtab shows you read count abundance of selected taxonomic levels by sample. As with the previous subtab, you can select what taxonomic level you wish to plot by simply using the dropdown menu at the left of the user interface.



The legend on the right side of the plot shows the scale and the color gradient.

3.3 Summary

The Summary table shows summary statistics that may come in handy for detecting trends in the data and identifying outliers. As with the previous subtabs, Summary offers redundant information that points to the distribution of the taxonomic composition of your samples.

PathoStat Relative At	undance Diversity Differential Expression Confidence Region PCA PCoA Time Series Core OTUs	
Taxonomy Level	Taxonomy level RA Heatmap Summary RA Table(%) Count Table	
phylum A Download FA CSV A Download Count CSV Sort By Condition Batch	Sample_011 Sample_021 Sample_021 Sample_021 Sample_021 Hrn. 0.0.0000 Hin. 0.0.0000	

3.4 RA Table (%)

This subtab presents a searchable and sortable table of taxa abundance by sample. At user-selected taxonomy levels, you get information about the taxonomy ID (taxid) of the organism as per NCBI's taxonomy database and their relative abundance. All columns are sortable, and at the top right corner of the table you can use the search case to check for specific taxa.

//127.0.0.1:3386 2 Open in Browser G PathoStat Relativ	e Abundance Diversity	Different	tial Expression C	confidence Region	PCA PCoA	Time Series	Core OTUs		😔 Publi
Taxonomy Level	Taxonomy level I	RA He	atmap Summar	y RA Table(%)	Count Table				
phylum -	Show 10 😧 ent	ries						Search:	
	phylum \$	taxid 🕴	Sample_01_1 0	Sample_01_2	Sample_01_3 0	Sample_02_1 0	Sample_02_2	Sample_02_3	Sample_03_1
Download RA CSV Download Count CSV	Firmicutes	515619	12.69	30.84	3.09	70.15	72.34	93.72	28.76
Sort By	Verrucomicrobia	349741	0.00	0.00	0.00	0.00	0.63	0.00	33.70
None	Actinobacteria	367928	0.00	0.00	0.00	0.00	0.00	0.00	10.82
Condition Batch	Euryarchaeota	420247	0.00	0.00	0.00	0.00	0.00	0.00	16.07
O Dater	others	NA	0.22	0.43	0.02	0.19	0.25	1.60	4.02
	Bacteroidetes	435590	87.09	68.73	96.89	28.96	26.47	2.85	6.63
	Proteobacteria	536056	0.00	0.00	0.00	0.69	0.31	1.83	0.00
	Showing 1 to 7 of 7	entries						Previous	1 Next

3.5 Count table

This table is similar to the previous one but it shows abundance data as raw read counts instead of proportions. This is useful when you want to test for differential abundance of taxa between conditions. Statistical models such as those implemented in EdgeR and DESeq2 explicitly require count data to ensure specificity.

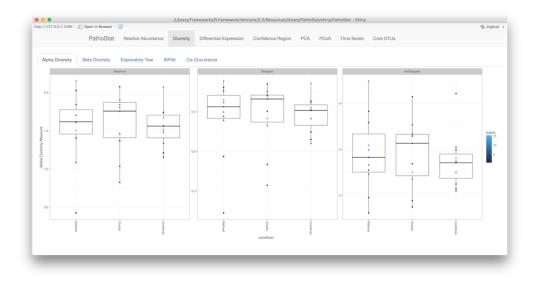
PathoStat Re	lative Abundance	Diversity	Differenti	al Expression C	onfidence Region	PCA PCoA	Time Series	Core OTUs				
Tatiootat		Directory	Differenti	ar Expression 1 0	ormaenee negion	TON TOON		0100103				
Taxonomy Level	Тахог	nomy level F	RA Hea	tmap Summan	RA Table(%)	Count Table						
phylum	• Show	10 👩 enti	ries				Search:					
Lownload RA CSV	phyle	am ()	taxid $\boldsymbol{\varphi}$	Sample_01_1 0	Sample_01_2 0	Sample_01_3 0	Sample_02_1 0	Sample_02_2 0	Sample_02_3 0	Sample_03_1		
& Download Count CSV	Firmic	utes	515619	19047	58672	6474	141663	133928	165268	4441		
Sort By	Verruc	omicrobia	349741	0	0	0	0	1166	0	5204		
None	Actino	bacteria	367928	0	0	0	0	0	0	1670		
Condition Batch	Euryar	chaeota	420247	0	0	0	0	0	0	2481		
	others		NA	332	822	46	389	455	2820	620		
	Bacte	roidetes	435590	130765	130748	203260	58479	49007	5031	1025		
	Protec	bacteria	536056	0	0	0	1401	583	3221			
	Showin	g 1 to 7 of 7	entries						Previous	1 Next		

4 Diversity

The second tab in PathoStat is the Diversity tab. Here, users can obtain estimates of alpha and beta diversity, as well as explore a clustering dendrogram decorated with the abundance of certain taxonomic groups by sample. Also, the Biplot subtab illustrates the relationships between user-selected variables using multidimensional scaling. Finally, the Co-Occurrence subtab shows the relationships among taxa at user-defined distances allowing the exploration of potential biological associations.

4.1 Alpha Diversity

Alpha diversity considers presence and absence of taxonomit units as well as their homogeneity or evenness. In this subtab, you obtain estimates for alpha diversity using the Shannon, Simpson, and Inverse Simpson metrics as a function of a factor.



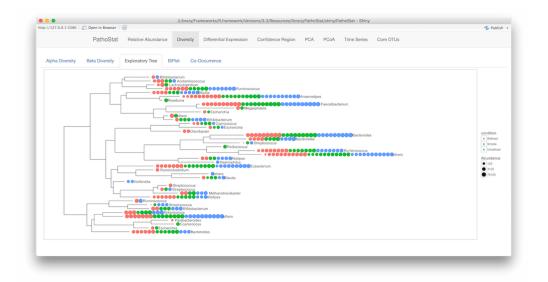
4.2 Beta Diversity

The beta diversity subtab provides the user with a heatmap of sample to sample variation using the Bray-Curtis distance metric. The color key indicates a normalized score (the row z-score) and the dendrogram is constructed using hierarchical clustering.



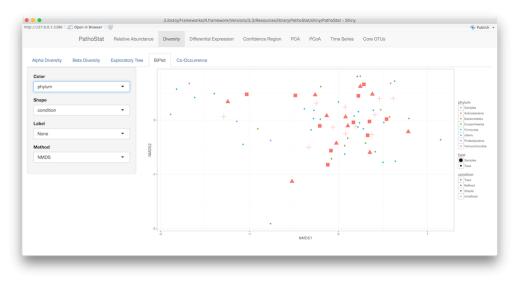
4.3 Exploratory Tree

This subtab shows a dendrogram (tree) among all the samples in your dataset. In the figure below, the tree is decorated with the most abundant taxon and their relative abundance by condition. This tree can reveal not only similarity among samples but also what taxon dominates each sample.



4.4 Biplot

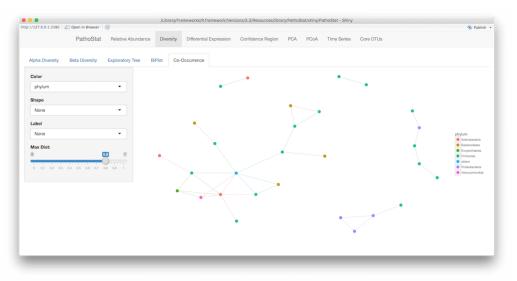
Another useful feature of PathoStat is the biplot. Here, the user can explore potential associations among taxa, and among taxa and factors in the dataset, e.g., condition, treatment, etc. The menu on the left side of the panel lets you select the variables to explore as well as the method to estimate the distance among variables.



4.5 Co-Occurrence

One recurrent question in metagenomics is whether two or more taxa tend to co-occur among a set of samples. In general, one might suspect that if two or more taxa co-occur then there might be a functional relationship among them. In the Co-Occurrence subtab, the user selects

relevant taxonomic levels to be compared and the maximum distance that will connect two taxa together. The result is a network graph where edges are proportional to the strength of co-occurrence.

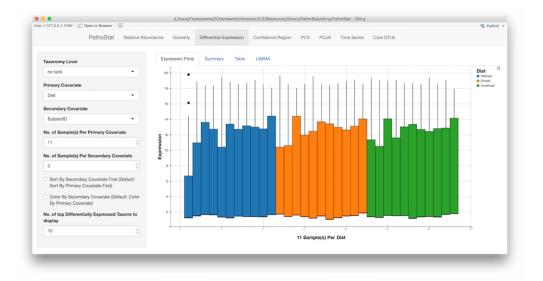


5 Differential Expression

The third tab in PathoStat allows you to test for differential abundance of taxa between conditions. This test is analogous to differential expression of genes in transcriptomic experiments and uses similar statistical models.

5.1 Expression Plots

In the Expression Plot subtab, you first select the taxonomy level of choice for the comparison and then you select the primary and secondary covariates. The results are shown as BoxPlots colored by covariate.



5.2 Summary, Table and LIMMA

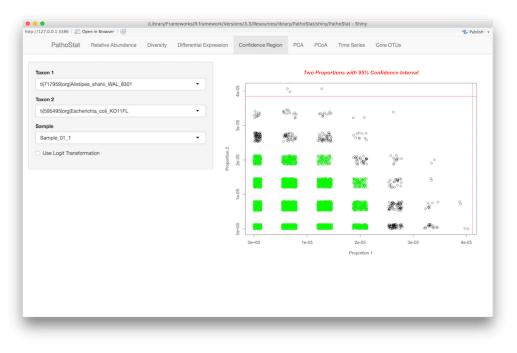
The Summary subtab shows text summary of the data used for the BoxPlots in the Expression Plots subtab. The next two subtabs summarize the results as a table of raw values and as a Fold Change table, respectively.

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Taxonomy Level		Expres	sion Plots	Sun	nmary	Table	LIMMA										
no rank	•	3	6	7	11	14	18	20	22	27	30	31	1	5	9	12	15
Primary Covariate		1.25	1.50	1.70	15.98	15.94	13.77	1.47	14.12	14.74	13.54	1.66	1.74	1.43	1.62	17.19	15.84
		1.25	12.26	1.70	1.64	1.35	1.25	1.47	1.43	1.41	1.39	1.66	1.74	1.43	1.62	1.41	12.60
Diet	•	1.25	1.50	1.70	1.64	1.35	1.25	1.47	1.43	1.41	1.39	1.66	1.74	1.43	1.62	1.41	1.22
Secondary Covariate		8.57	1.50	1.70	1.64	1.35	1.25	1.47	1.43	1.41	1.39	1.66	1.74	1.43	1.62	1.41	1.22
SubjectID	-	1.25	18.87	1.70	18.32	16.92	17.93	18.15	1.43	17.09	16.42	1.66	1.74	18.51	1.62	16.29	16.68
No. of Sample(s) Per Primary		1.25	1.50	18.36	1.64	1.35	1.25	15.88	1.43	1.41	1.39	17.07	1.74	12.62	18.01	1.41	1.22
Covariate		1.25	1.50	16.72	1.64	1.35	1.25	1.47	1.43	1.41	1.39	16.50	1.74	1.43	15.31	1.41	1.22
11	٢	6.68	1.50	1.70	1.64	1.35	1.25	1.47	1.43	1.41	1.39	1.66	11.20	1.43	1.62	1.41	1.22
No. of Sample(s) Per Secondary		1.25	1.50	1.70	1.64	1.35	1.25	1.47	1.43	1.41	1.39	16.88	1.74	1.43	1.62	1.41	1.22
Covariate		1.25	10.95	13.60	12.73	1.35	1.25	14.13	1.43	1.41	14.52	15.25	1.74	13.80	14.78	11.96	1.22
3	٢	1.25	1.50	17.29	1.64	1.35	1.25	15.69	1.43	1.41	1.39	1.66	1.74	1.43	17.79	1.41	1.22
Sort By Secondary Covariate	First	1.25	12.93	1.70	1.64	1.35	1.25	1.47	1.43	1.41	1.39	1.66	1.74	1.43	1.62	1.41	1.22
(Default: Sort By Primary Cov		1.25	14.60	1.70	1.64	1.35	1.25	1.47	1.43	1.41	1.39	1.66	1.74	1.43	1.62	1.41	1.22
First)		1.25	1.50	1.70	1.64	1.35	1.25	1.47	1.43	1.41	1.39	1.66	1.74	1.43	1.62	1.41	1.22
 Color By Secondary Covariat (Default: Color By Primary Co 		1.25	1.50	1.70	1.64	1.35	1.25	1.47	1.43	13.82	1.39	1.66	10.40	1.43	1.62	1.41	1.22
		1.25	1.50	1.70	1.64	1.35	1.25	1.47	1.43	1.41	1.39	1.66	1.74	1.43	1.62	1.41	1.22
No. of top Differentially Expressed Taxons to display		1.25	1.50	1.70	1.64	1.35	1.25	1.47	13.73	1.41	1.39	1.66	1.74	1.43	1.62	1.41	1.22
10	0	1.25	1.50	14.53	16.90	13.42	13.97	13.51	1.43	1.41	16.94	15.32	1.74	11.04	1.62	16.47	13.84
		1.25	1.50	1.70	1.64	1.35	1.25	1.47	1.43	1.41	1.39	1.66	1.74	1.43	1.62	1.41	1.22
		1.25	1.50	1.70	1.64	14.27	1.25	1.47	1.43	13.99	1.39	1.66	14.24	1.43	1.62	1.41	13.75

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PathoStat Relative Abundance	Diversity	Differential Expression	Confide	nce Region	PCA	PCoA	Time Serie	is Ci	ore OTUs		
Taxonomy Level	Expressi	on Plots Summary	Table	LIMMA							
no rank 👻	Primary	Covariate: Simple (logFC)	Primar	y Covariate:	Unrefined	d (logFC)	AveExpr	F	P.Value	adj.P.Val	
Primary Covariate		-0.04				3.73	2.73	5.53	0.01	0.44	
Diet •		-5.98				-2.96	6.57	4.20	0.03	0.57	
		-5.48				-5.22	4.46	3.19	0.06	0.65	
Secondary Covariate		-0.09				-3.79	15.53	3.12	0.06	0.65	
SubjectID -		-5.89				-5.94	6.04	2.45	0.11	0.65	
No. of Sample(s) Per Primary		-1.95				-0.94	13.97	2.43	0.11	0.65	
Covariate		4.65	1.15				2.39	0.11	0.65		
11 0		-0.83				-3.35	3.41	2.22	0.13	0.65	
No. of Sample(s) Per Secondary		-0.04	1.97			2.14	2.00	0.16	0.65		
Covariate		0.75				2.68	2.64	1.99	0.16	0.65	
3											
 Sort By Secondary Covariate First (Default: Sort By Primary Covariate First) 											
Color By Secondary Covariate (Default: Color By Primary Covariate)											
No. of top Differentially Expressed Taxons to display											
10 .											

6 Confidence Region

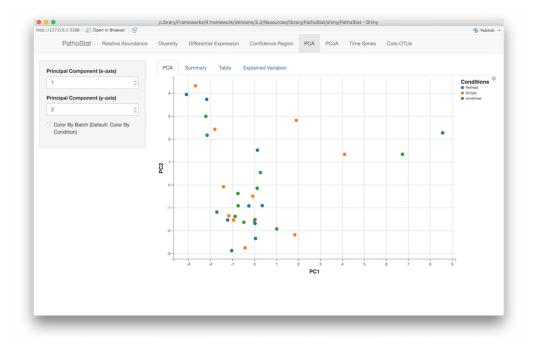
Many times researchers are interested in the accuracy of taxon abundance estimates. In this subtab, we provide a way to compare within-sample taxa in terms of their abundance estimate and 95% confidence interval.



On the left-side menu, you can select both the sample and the taxa to be used in the comparison. The results are plotted as a jitter plot that indicates the 95% confidence interval between the selected taxa.

7 PCA and PCoA

The next two tabs in PathoStat calculate multidimensional scaling using Principal Components and Principal Coordinates Analysis. These tools can help you odentify overall trends in the data. For instance, you can explore whether your samples are related by some biological condition of interest or by technical batch, in which case you would need to denoise the data first using methods such as surrogate variable analysis.



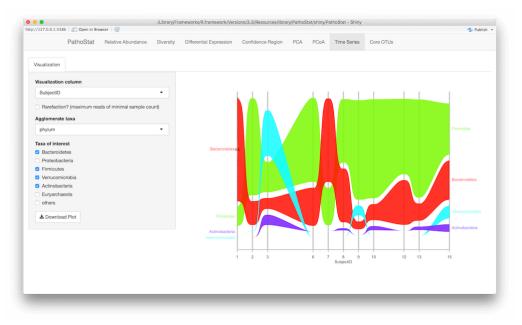
7.1 Explained Variation

An important aspect of PCA analysis is to understand to what extend the resulting vectors can explain the observed multidimensional variation. In this subtab, you can get an idea of that by exploring the different columns in terms of Proportion of Variance, Cumulative Variance and percent variation, among others.

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PathoStat Rela	ative Abundance	Diversity Differe	ntial Expression	Confidence Region PCA	PCoA Tim	PCoA Time Series Core OTUs				
Principal Compone	nt (x-axis)	PCA Sun	imary Table	Explained Variation						
I Principal Component (y-axis)		Proportion of Variance	Cumulative Proportion of	Percent Variation Explained by Either	Percent Variation Explained by	Condition Significance	Percent Variation Explained by	Batch		
		(%)	Variance (%)	Condition or Batch	Condition	(p-value)	Batch	(p-value)		
2	0	14.51	14.51	90.30	0.10	0.93	90.20	0.00		
Color By Batch (D	Default: Color By	10.66	25.17	91.80	0.10	0.89	91.70	0.00		
Condition)		9.15	34.32	19.40	11.20	0.27	8.20	0.99		
		7.67	41.99	75.90	1.00	0.66	74.90	0.00		
		6.43	48.41	70.00	4.00	0.29	66.00	0.00		
		5.56	53.97	72.40	4.20	0.24	68.20	0.00		
		5.33	59.30	70.50	1.40	0.63	69.10	0.00		
		4.82	64.13	66.80	3.60	0.36	63.20	0.01		
		4.33	68.46	43.70	17.30	0.07	26.40	0.52		
		4.05	72.50	61.40	11.10	0.08	50.20	0.03		
		3.82	76.32	31.10	6.80	0.39	24.30	0.71		
		3.20	79.51	42.40	13.50	0.12	29.00	0.47		
		2.82	82.33	37.30	3.00	0.62	34.20	0.41		
		2.80	85.14	22.80	7.50	0.40	15.30	0.93		

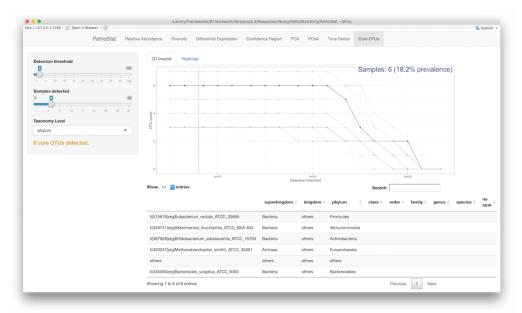
8 Time Series

The Time Series tab allows you to understand variation in relative abundance as a function of time. Strictly speaking, you could use any discrete numerical variable from your dataset for visualization. In the example below, we use SubjectID (not a numerical variable though) and agglomerate the data by Phylum.



9 Core OTUs

Many times we would like to understand what is particular and what is general about a set of metagenomic samples. Analogous to pangenome analysis in comparative genomics, the Core OTU tab allows you to identify shared taxa or core taxa among samples.



You first set up a detection threshold, meaning a minimum abundance proportion a OTU must have in order to be considered in the analysis, and the number of samples selected from your dataset. You can also select the taxonomic level of interest at which you would like to perform the analysis.