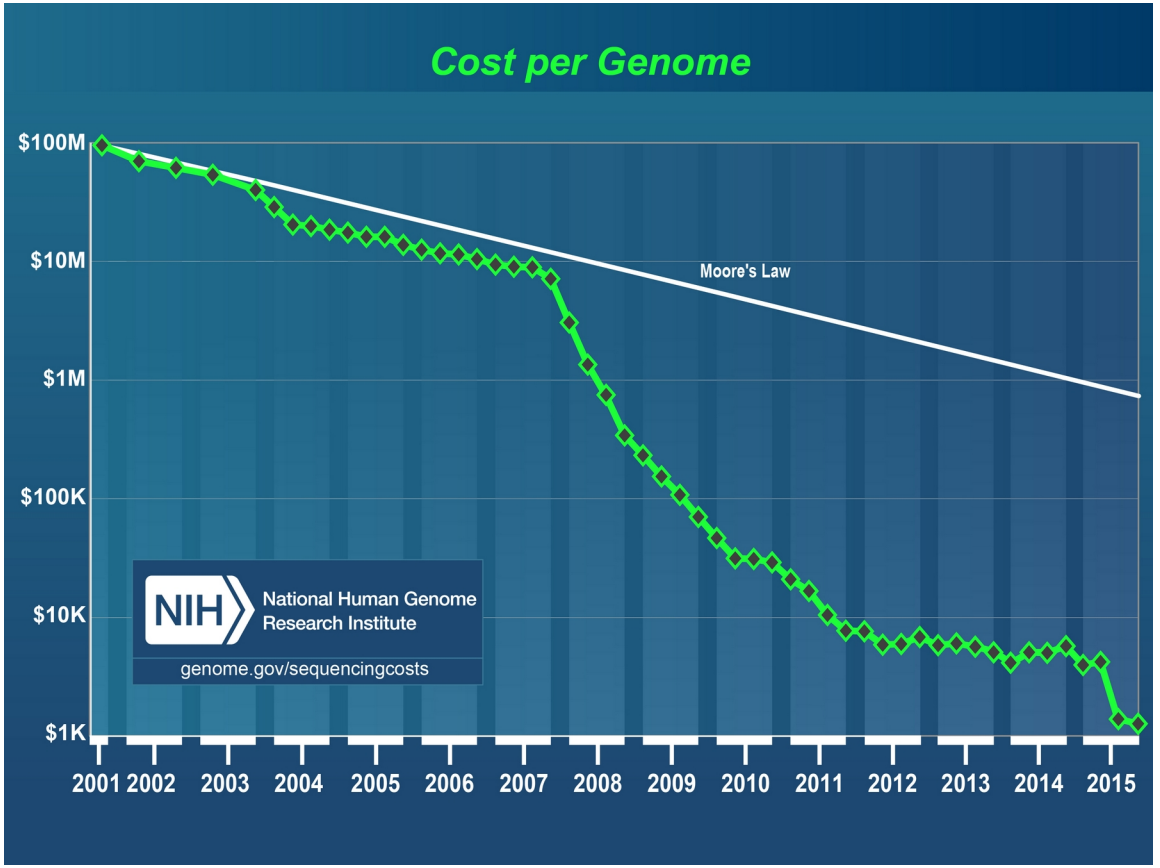
PERSPECTIVE ARTICLE

Front. Microbiol., 22 February 2021 | https://doi.org/10.3389/fmicb.2021.635781

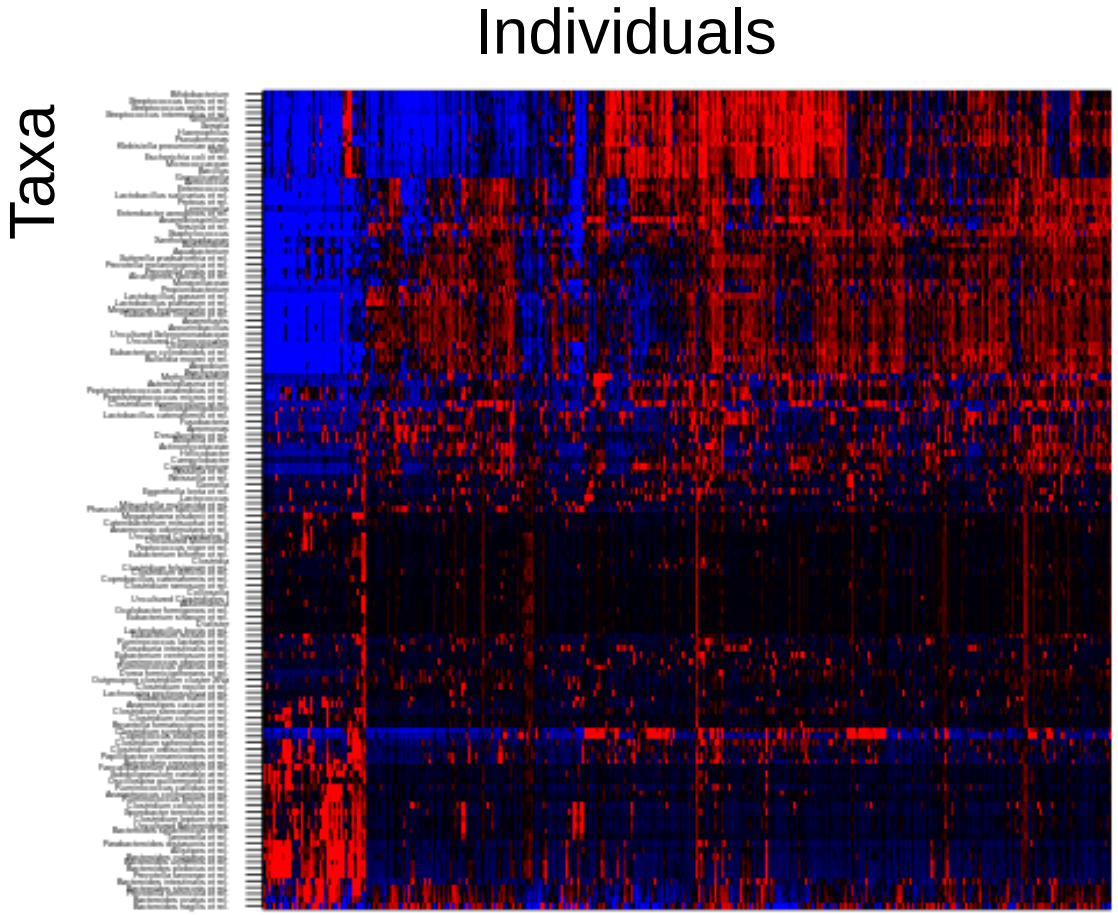
Statistical and Machine Learning Techniques in Human Microbiome Studies: Contemporary Challenges and Solutions

 Isabel Moreno-Indias[1,2*],  Leo Lahti[3], Miroslava Nedyalkova[4], Ilze Elbere[5], Gennady



Human Intestinal Tract (HIT)Chip Atlas: 100+ genera ~ 10,000+ samples

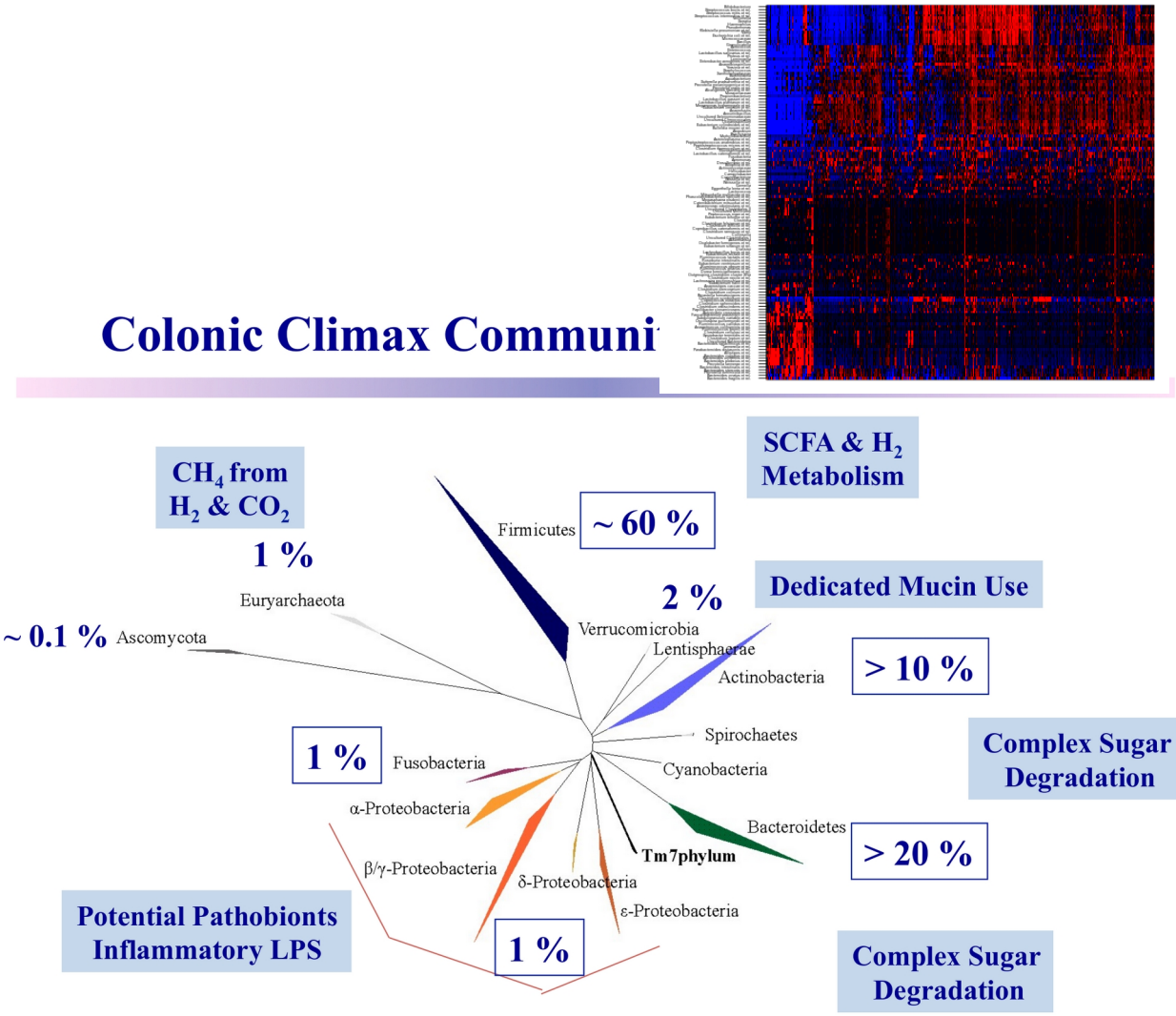
Gut microbiota: 1000 western adults (Lahti *et al.* Nature Comm. 2014)



Standardized – cost efficient – accurate at 0.1% relative abundance
Rajilic-Stojanovic et al. Env. Microbiol. 2009

Special properties of microbiome data

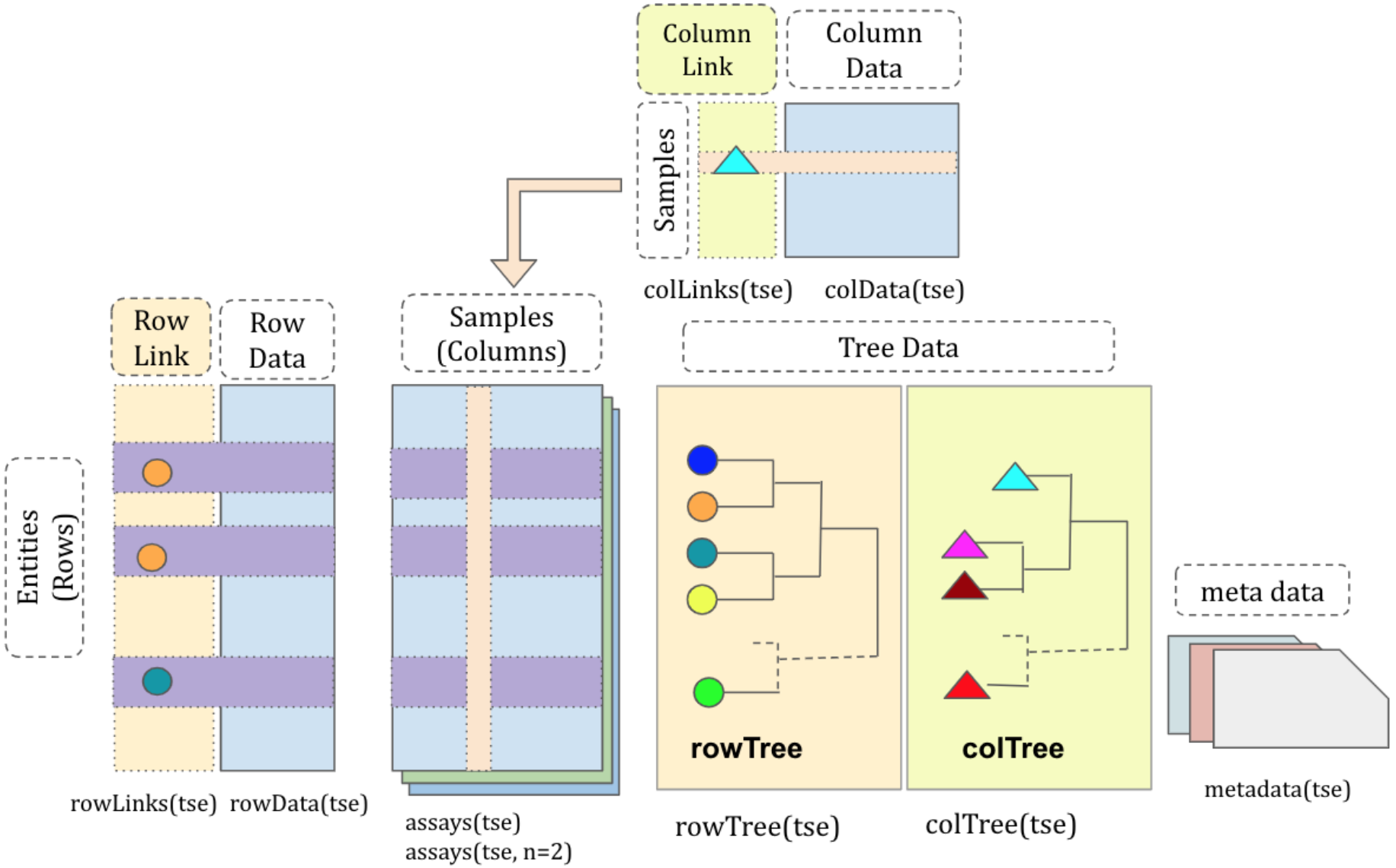
- Sparse
- Compositional
- Non-Gaussian
- Overdispersed
- Discrete
- Complex
- Stochastic
- Multi-level



Zoetendal EG, EE Vaughan & WM de Vos (2006) Mol Microbiol 59: 1639

Lay C, L Rigottier-Gois, K Holmstrom, M Rajilic, EE Vaughan, WM de Vos, MD Collins, R Their, P Namsolleck, M Blaut & J Dore (2005) AEM 71: 4153

Anatomy of TreeSummarizedExperiment

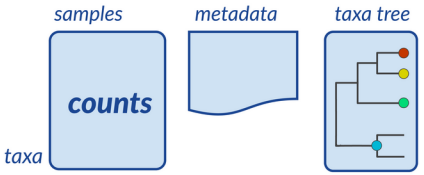


Example workflow

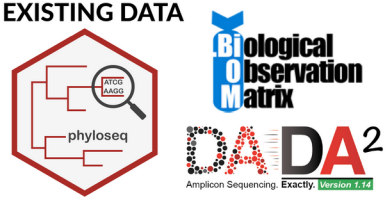
Import Data

This workflow starts with either raw data directly from relative abundance estimation or taxonomic classification OR pre-existing data objects from widely used software.

RAW DATA

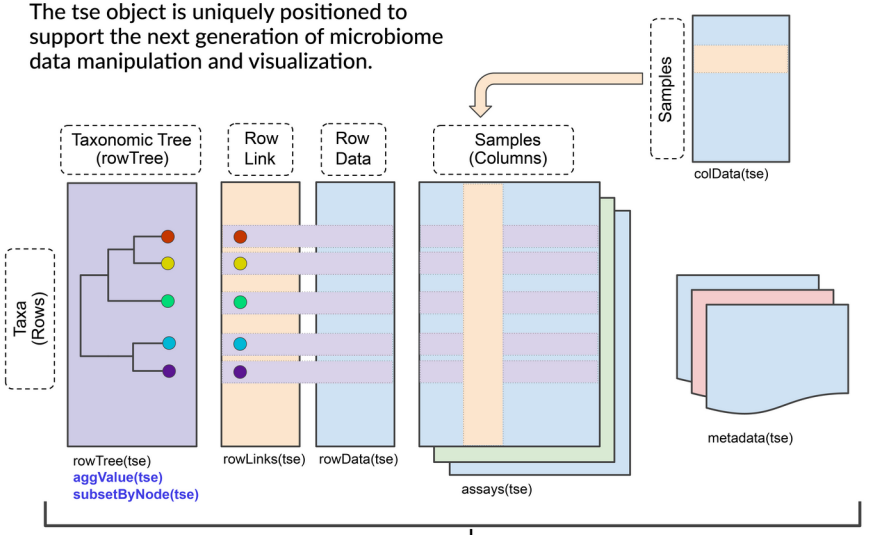


EXISTING DATA



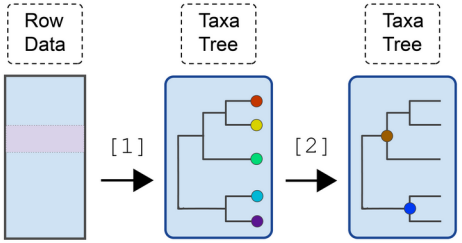
The `TreeSE` object

The tse object is uniquely positioned to support the next generation of microbiome data manipulation and visualization.

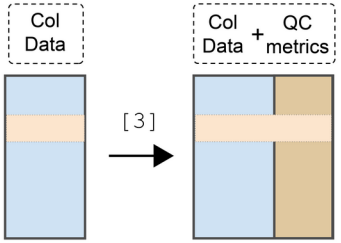


The `mia` Pipeline

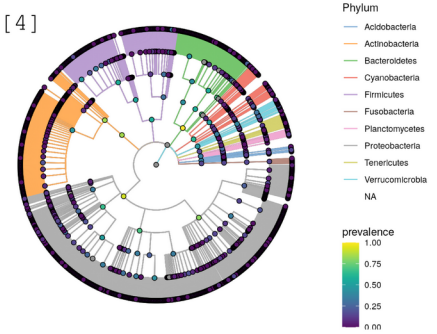
Accessing Taxonomic Info.



Quality Control



Visualizing with `miaViz`




```
[1] mia::addTaxonomyTree(tse)
[2] TreeSE::aggValue(tse)
[3] scatter::addPerCellQC(tse)
[4] miaViz::plotRowTree(tse)
```

Check the poster
F1000 / EuroBioC!

Typical study designs

Case-control studies

Interventions

Cross-sectional population cohorts

Prospective follow-ups

Longitudinal time series

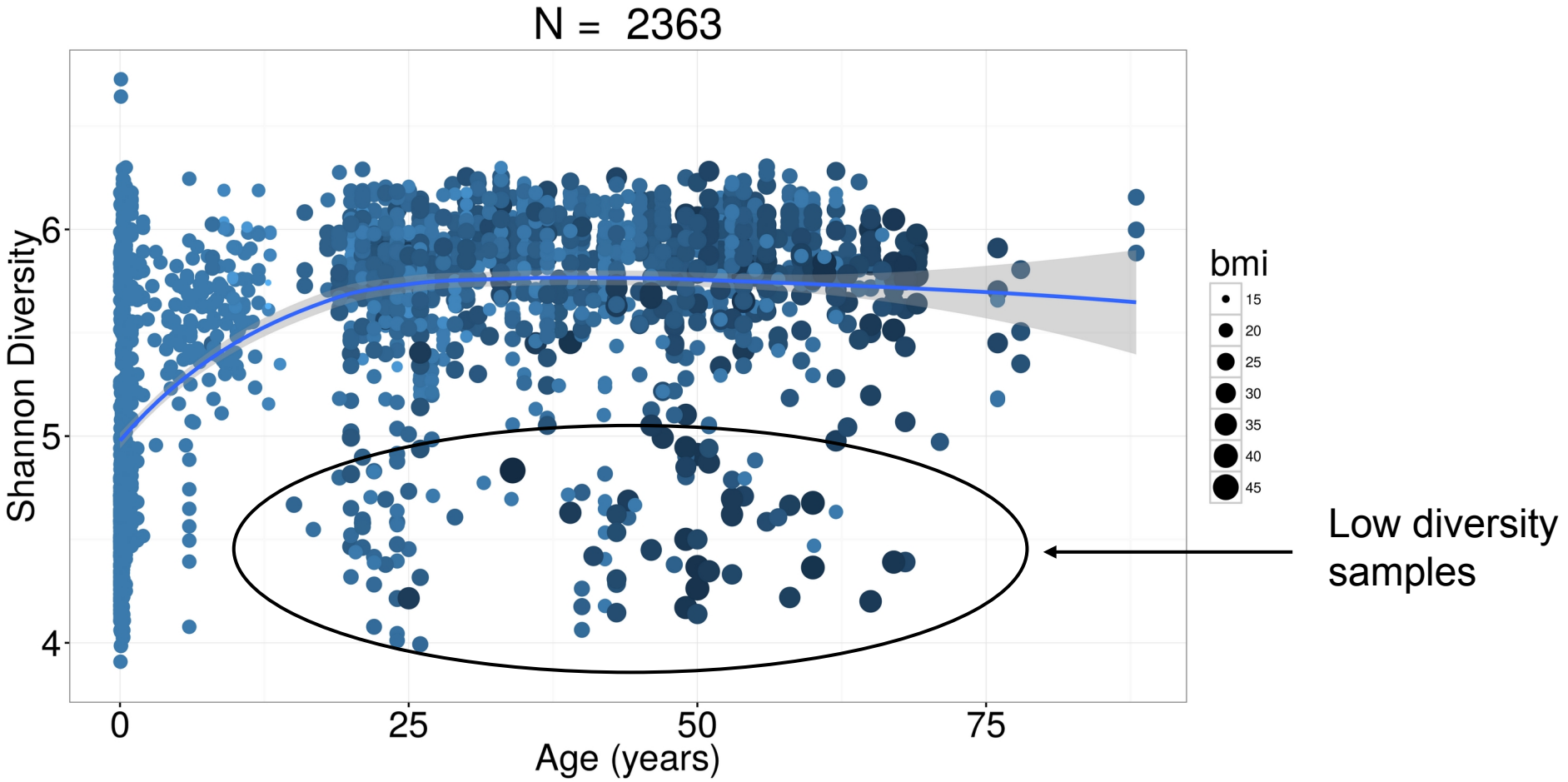
Multi-omics

Lecture: Key concepts

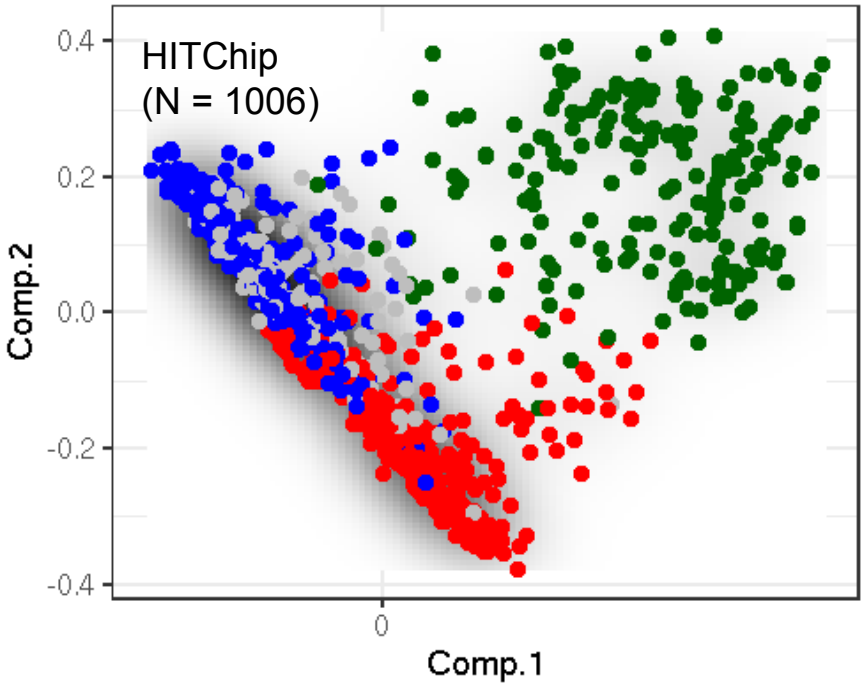
- special properties of microbiome data
- data science workflows

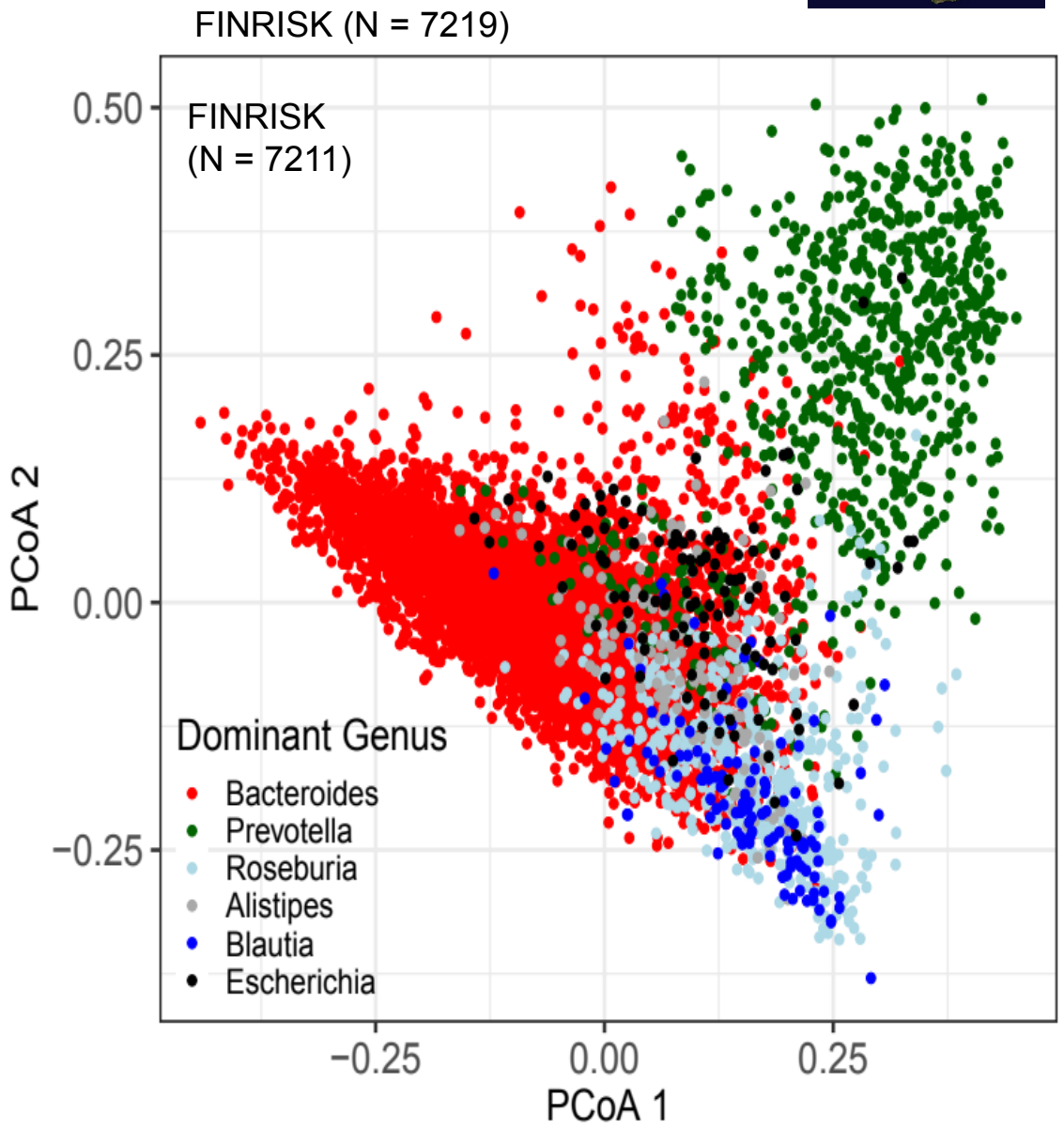
- alpha diversity
- beta diversity
- differential abundance

Alpha diversity & aging
healthy & normal obese subjects



Beta diversity & population landscape



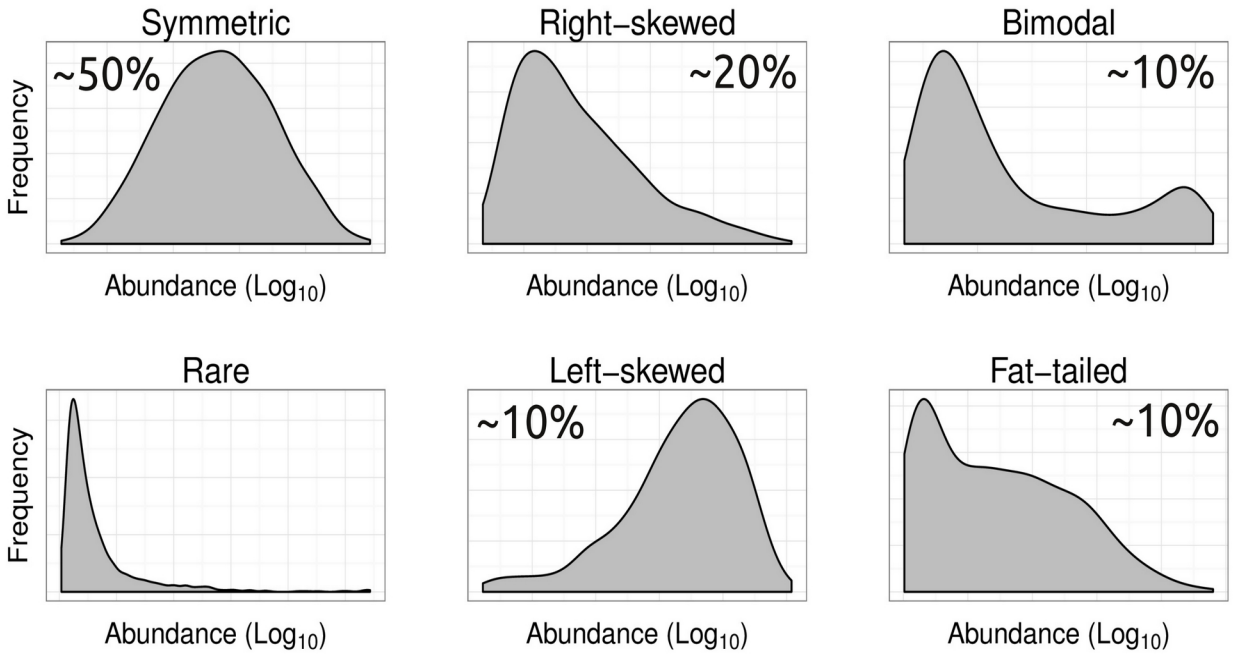


Differential abundance

Standard t-test for two-group comparison?

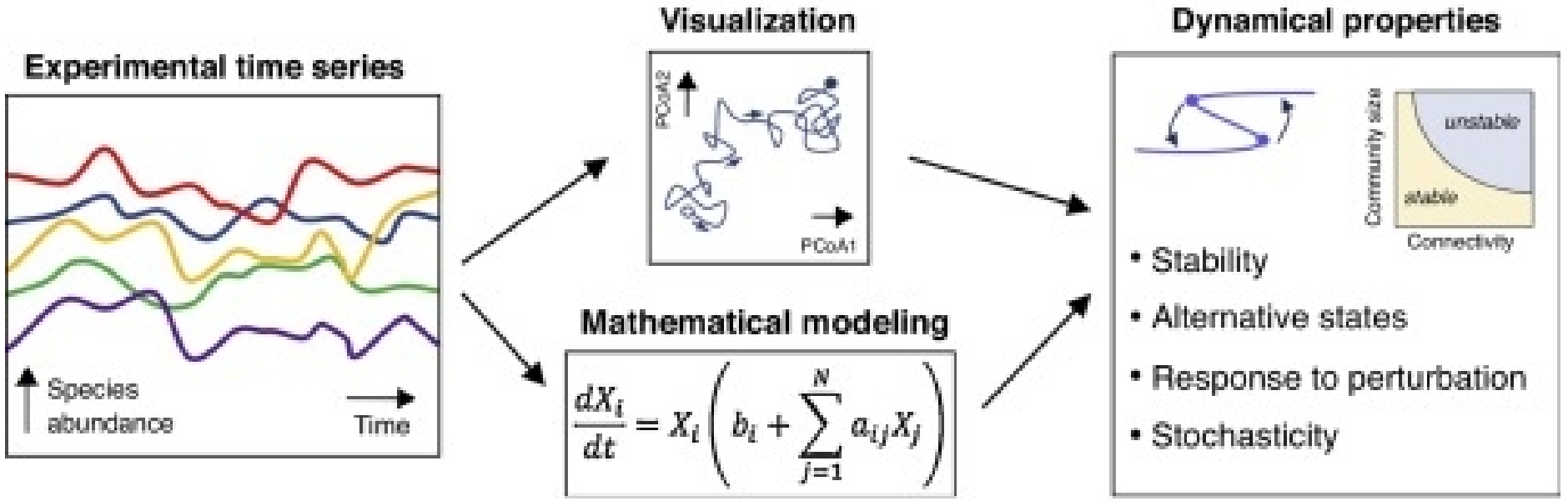
Problems:

- Few replicates
- Non-gaussian, discrete, positive, skewed..
- Multiple testing



Microbial communities as dynamical systems

Didier Gonze [1, 2] ✉, Katharine Z Coyte [3, 4], Leo Lahti [5, 6, 7], Karoline Faust [5] ✉



Overview of generic* metagenomics workflow

*This is generic; specific workflows can vary on the order of steps here and how they are done.

When working with your own data you should never follow any pipeline blindly. There can be critical differences based on your data.

sequencing facility → fastq files → demultiplex (split samples by barodes) → quality filter/trim (remove adapters/primers) → fasta files

might be done by sequencing facility

fastqc/multiqc

@HISEQ2500:282:1:1101:1220:1944 1
ATCGGATCG...
+
<G.<G<AGGII...

Some tools:
- sabre
- fastx_demux (usearch/vsearch)
- idemp
- fastx barcode splitter (fastx-toolkit)

Some tools:
- trimmomatic
- bbduk.sh (bbtools suite of tools)

>HISEQ2500:282:1:1101:1220:1944 1
ATCGGATCG...

no-assembly path

read-based analysis

Some tools:
- TIPP/SEPP
- metaphlan2
- humann2
- sourmash
- kraken

assembly path

consider testing assemblies with and w/o digital normalization

Some tools:
- bbnorm
- diginorm

Count Table

	Sample_A	Sample_B	...
obj_1	0	428	...
obj_2	306	323	...
obj_3	217	1	...
...	...	...	...

MetaQUAST is a great tool for comparing assemblies

Analysis

Some tools:
- phyloseq
- Breakaway
- DivNet
- CORNCOB
- SpiecEasi
- MaAsLin
- DESeq2

(co)-assembly

map individual sample reads to (co)-assembly

Generate coverage information (mapping)

Some tools:
- bowtie2
- bwa

Some assemblers and tools:
- Megahit (assembler)
- SPAdes (assembler)
- idba-ud (assembler)
- MetAMOS (assembler and analysis pipeline)
- MetaCompass (reference-guided)
- MetagenomeScope (visualize assembly graphs)

Gene calling Functional/taxonomic profiling

Some tools:
- prodigal (identifies open reading frames)
- prokka (runs prodigal and performs annotations)
- GHOSTKOALA (web-hosted KEGG annotations)
- BLAST (protein nr db/refseq/COGs)

Recovering genomes from metagenomes

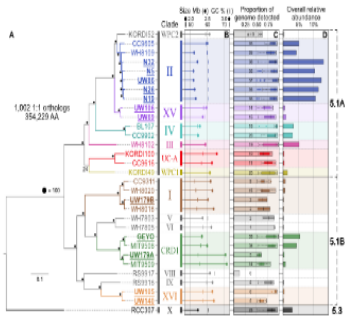
A note on MAGs:
MAGs (metagenome-assembled genomes) are not the same thing as isolate genomes. They are composite representative genomes of closely related genomic lineages.

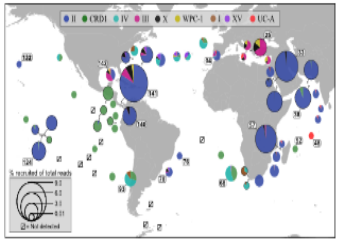
Some tools:
- anvi'o (interactive manual curation of bins; and much more)
- CONCOCT (kmer-based and coverage-based binning; also incorporated in anvi'o)
- COCACOLA (kmer-based, coverage-based, and incorporates paired-read linkage of contigs)
- MetaBAT2 (kmer-based and coverage-based binning tool)
- BinSanity (primarily coverage-based, optional second round kmer-based binning tool)
- checkm (genome-level taxonomy; and much more)
- DASTool (a tool for evaluating bins recovered by different methods)
- DESMAN (tool aimed at resolving strains)

Some common genomics stuff

Phylogenomics
Comparative genomics
Pangenomics
Env. distributions

Some tools:
- anvi'o (integrated HMMs for common single-copy gene sets; integrated pangenomic workflow for identifying orthologs via OrthoMCL)
- PanOCT (identifies orthologs utilizing synteny information)
- StrainPhlAn/PanPhlAn (tools for strain-level analyses)
- MUSCLE (alignment software)
- FastTree (very fast, pseudo-maximum likelihood tree builder)
- RAxML (maximum likelihood tree builder)
- Mauve (whole-genome alignment)





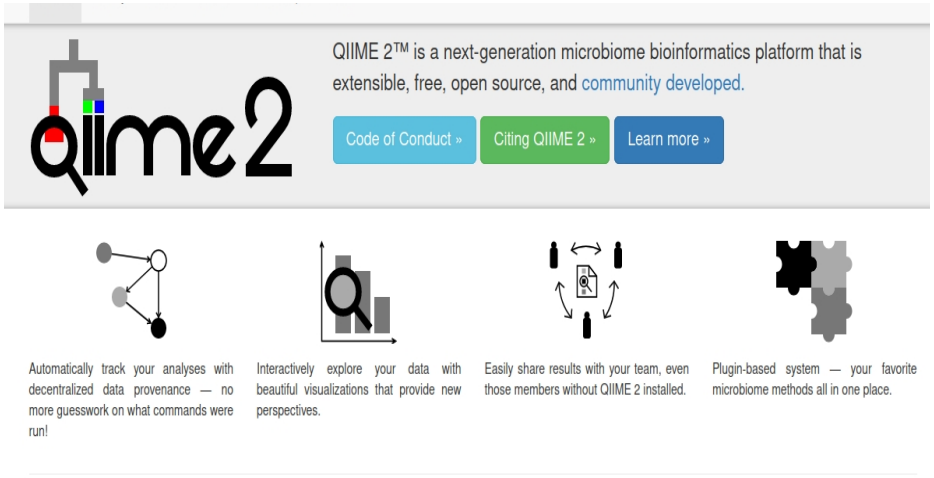
astrobiomike.github.io

Happy Belly Bioinformatics
JOSE 10.21105/jose.00053

AstrobioMike
Orcid: 0000-0001-7750-9145

Lee, (2019). Happy Belly Bioinformatics: an open-source resource dedicated to helping biologists utilize bioinformatics. Journal of Open Source Education, 4(41), 53, https://doi.org/10.21105/jose.00053

open data science ecosystems



Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

PeerJ ›

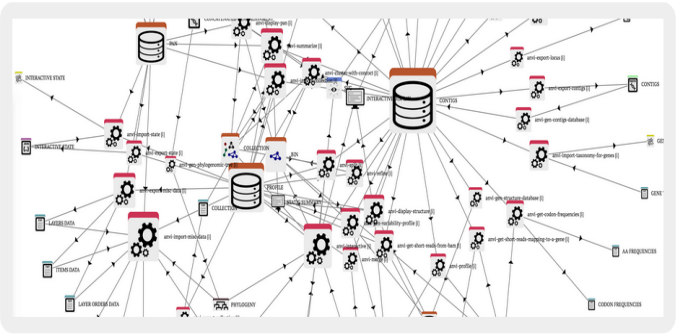
Anvi'o: an advanced analysis and visualization platform for 'omics data

Research article | Bioinformatics | Biotechnology | Computational Biology | Genomics | Microbiology

A. Murat Eren [1,2], Özcan C. Esen [1], Christopher Quince [3], Joseph H. Vineis [1], Hilary G. Morrison [1], Mitchell L. Sogin [1], Tom O. Delmont [1]

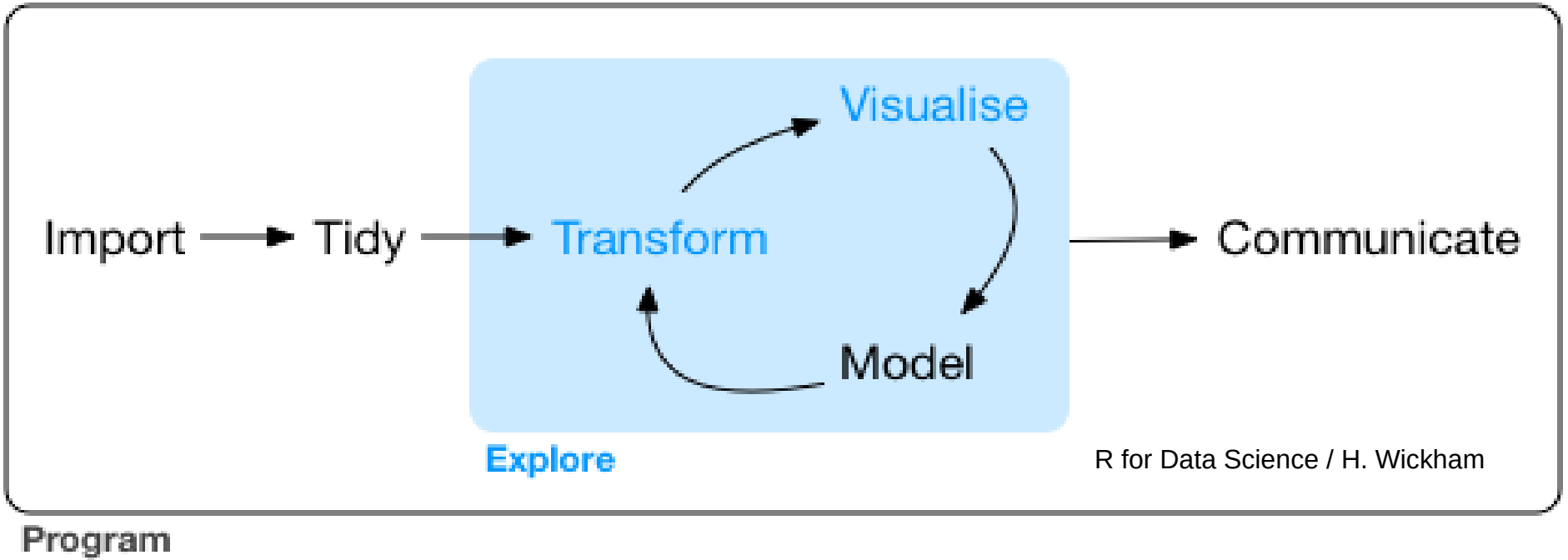
blished October 8, 2015

Anvi'o in a nutshell



Anvi'o is an open-source, community-driven analysis and visualization platform for 'omics data.

Data science workflow

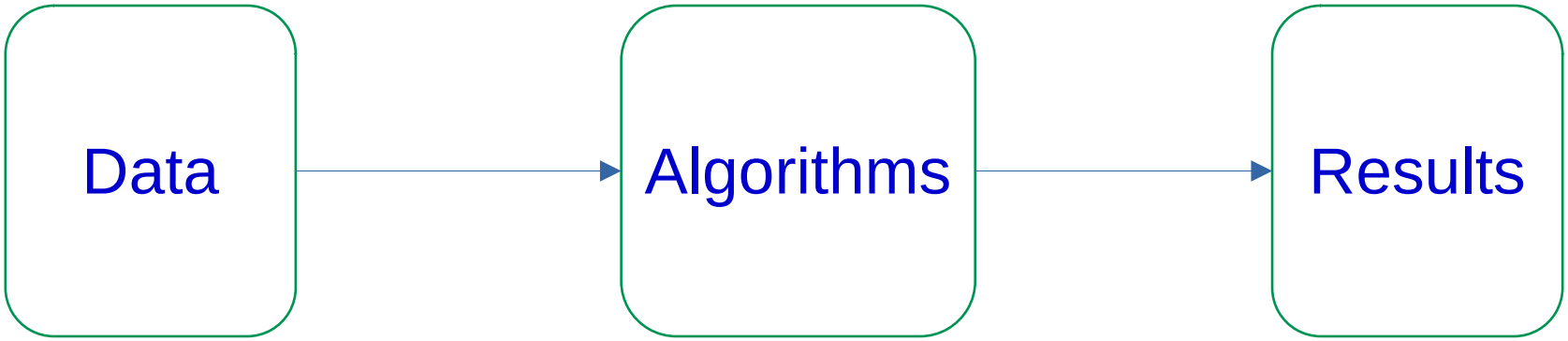


R for Data Science / H. Wickham

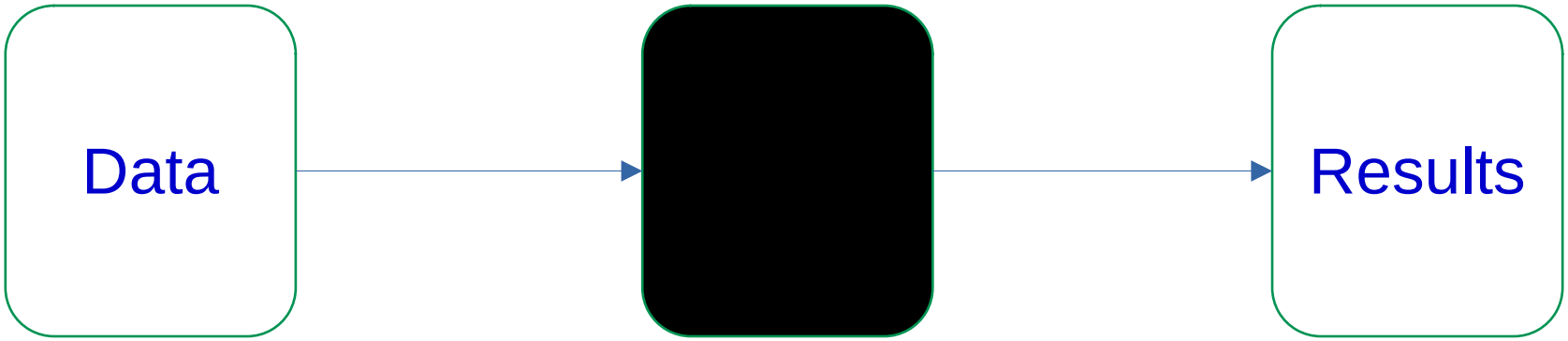
REVISED **Bioconductor Workflow for Microbiome Data Analysis: from raw reads to community analyses [version 2; peer review: 3 approved]**

Ben J. Callahan[1], Kris Sankaran[1], Julia A. Fukuyama[1], Paul J. McMurdie[2], Susan P. Holmes

This article is included in the Bioconductor gateway.


Data
Algorithms
Results

?



RESEARCH PRIORITIES

Shining Light into Black Boxes

A. Morin[1], J. Urban[2], P. D. Adams[3], I. Foster[4], A. Sali[5], D. Baker[6], P. Sliz[1,*]

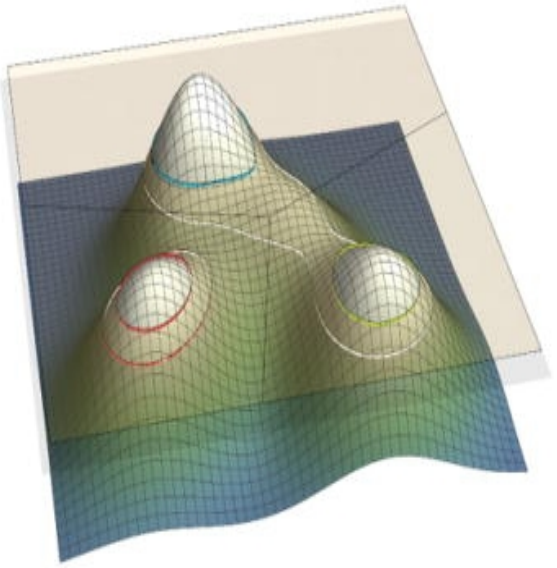
The influence of hidden researcher decisions in applied microeconomics

Nick Huntington-Klein ✉, Andreu Arenas, Emily Beam, Marco Bertoni, Jeffrey R. Bloem, Pralhad Burli, Naibin Chen, Paul Grieco, Godwin Ekpe, Todd Pugatch, Martin Saavedra, Yaniv Stopnitzky

First published: 22 March 2021
https://doi.org/10.1111/ecin.12992

Researchers make hundreds of decisions about data collection, preparation, and analysis in their research. We use a many-analysts approach to measure the extent and impact of these decisions. Two published causal empirical results are replicated by seven replicators each. We find large differences in data preparation and analysis decisions, many of which would not likely be reported in a publication. No two replicators reported the same sample size. Statistical significance varied across replications, and for one of the studies the effect's sign varied as well. The standard deviation of estimates across replications was 3–4 times the mean reported standard error.

How to choose a correct model?
→ a community typing example



Enterotypes in the landscape of gut microbial community composition. Costea *et al*. Nature 2018.

$$2 \times 6^6 = 93312$$

Taxonomic level
- Phylum
- Family
- Order
- Genus
- Species
- Strain..

Filtering
- None
- Prevalent
- Core
- Excl. outliers
- High variance
- Custom

Normalization
- None
- TSS
- CSS
- ILR/ALR/CLR
- phILR
- Hellinger

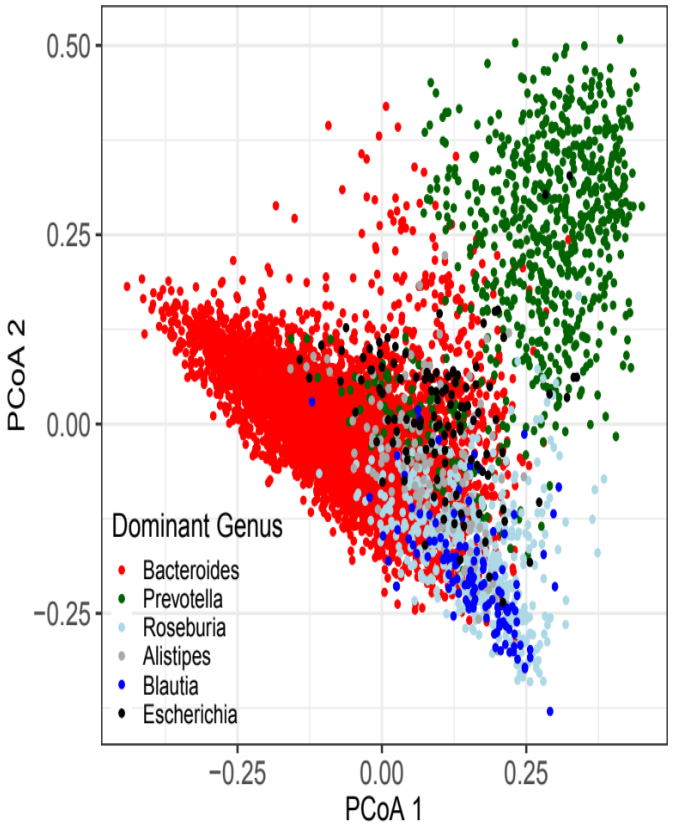
(Dis)similarity
- Eulidean
- Aitchison
- Bray-Curtis
- Jaccard
- weighted Unifrac
- unweighted Unifrac

Clustering method
- Hierarchical / Ward
- Hierarchical / Complete
- Gaussian mixture
- DMM
- PAMR
- K-means

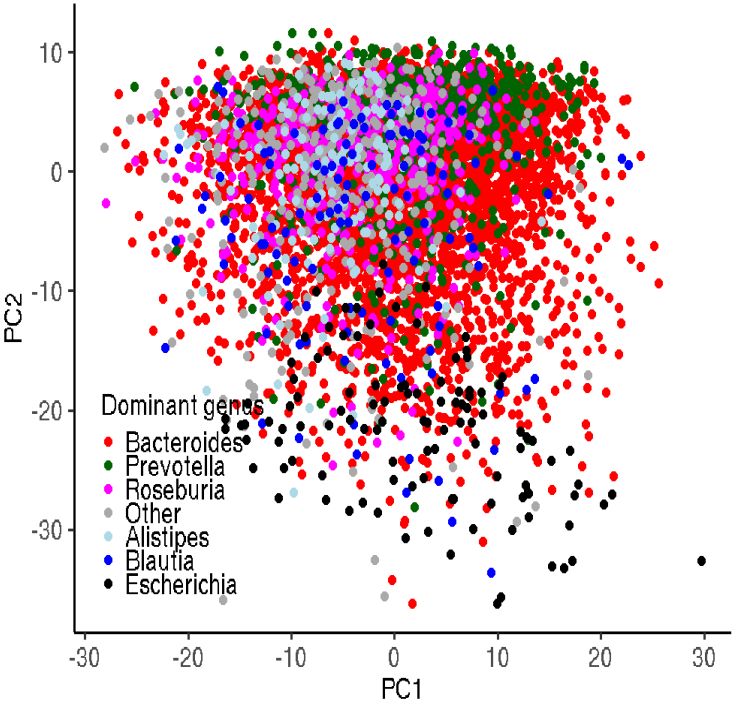
Regulation
- Calinski-Harabasz
- Dirichlet Process
- Silhouette Index
- AIC
- BIC
- DIC

Walk-through example in R/Bioc by Holmes & McMurdie
http://statweb.stanford.edu/~susan/papers/EnterotypeRR.html

PCoA + Bray-Curtis



PCA + Aitchison



Reproducible Research: Enterotype Example

Susan Holmes and Joey McMurdie

http://statweb.stanford.edu/~susan/papers/EnterotypeRR.html

Taxonomic Signatures of Long-Term Mortality Risk in Human Gut Microbiota

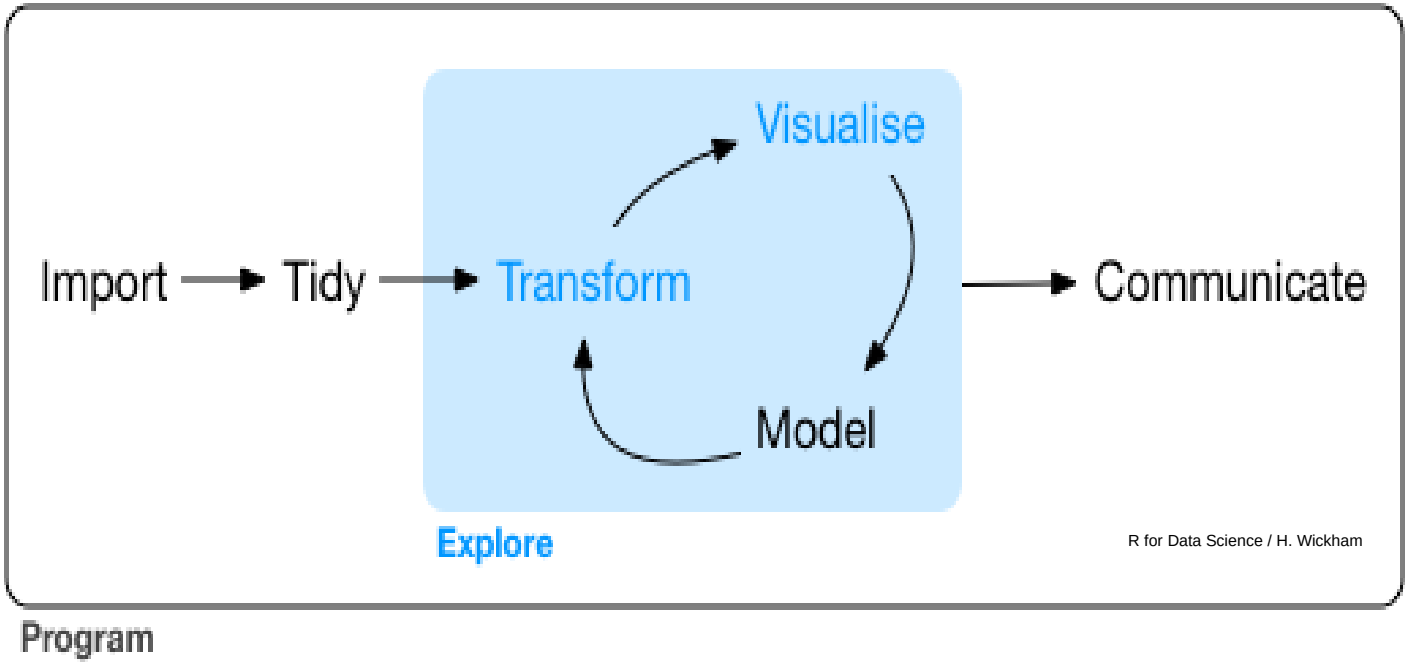
Aaro Salosensaari, Ville Laitinen, Aki Havulinna, Guillaume Meric, Susan Cheng, Markus Perola, Liisa Valsta, Georg Alfthan, Michael Inouye, Jeramie D. Watrous, Tao Long, Rodolfo Salido, Karenina Sanders, Caitriona Brennan, Gregory C. Humphrey, Jon G. Sanders, Mohit Jain, Pekka Jousilahti, Veikko Salomaa, Rob Knight, Leo Lahti, Teemu Niiranen

doi: https://doi.org/10.1101/2019.12.30.19015842

"*I have begun to think that no one ought to publish biometric results, without lodging a well arranged and well bound manuscript copy of all his data, in some place whereit should be accessible, under reasonable restrictions, to those who desire to verify his work.*"

Francis Galton (1901), *Biometrika* 1:1, pp. 7-10.

Reproducible workflows improve transparency and robustness



Taxonomic level?
- Phylum
- Family
- Order
- Genus
- Species
- Strain..

Normalization
- None
- TSS
- CSS
- ILR/ALR/CLR
- phILR
- Hellinger

(Dis)similarity?
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Regulation
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- AIC
- BIC
- DIC

Clustering
- Hierarchical / Ward
- Hierarchical / Complete
- Gaussian mixture
- DMM
- PAMR
- K-means

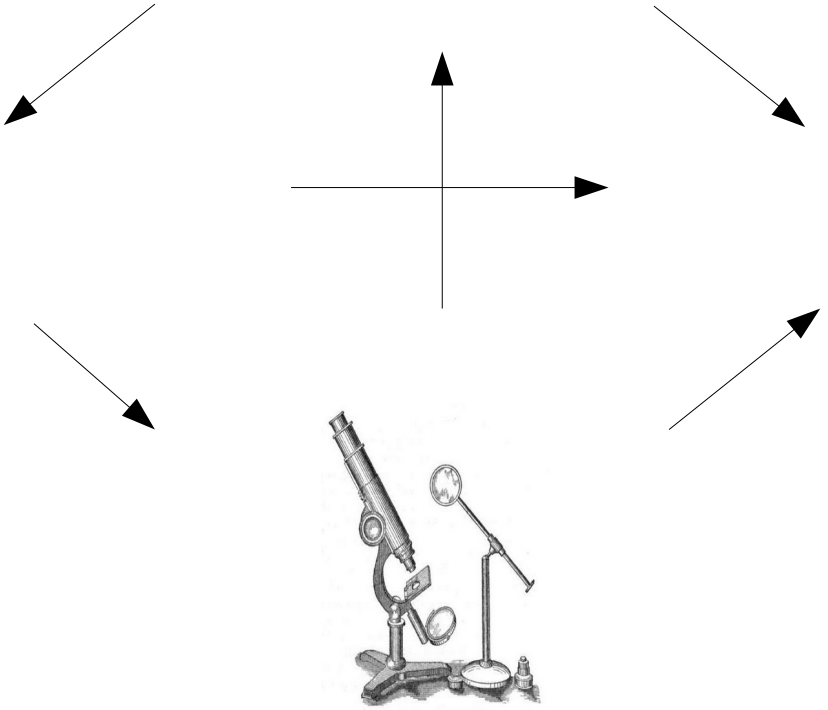
Hypothesis testing vs. hypothesis discovery?

?

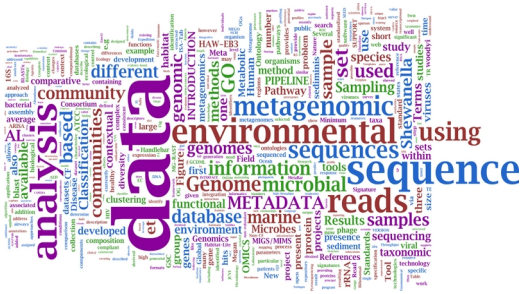
Hypothesis

Method

$$\begin{cases} X = W_x \mathbf{z} + \varepsilon_x \\ Y = W_y \mathbf{z} + \varepsilon_y \end{cases}$$



Tools



Data

PLOS | MEDICINE

Browse | For Authors | Abou

OPEN ACCESS

ESSAY

898,944
VIEWS

Why Most Published Research Findings Are False

John P. A. Ioannidis

Published: Aug 30, 2005 • DOI: 10.1371/journal.pmed.0020124

A scanning electron micrograph of bacteria in human faeces, in which 50% of species originate from the gut.

Microbiome science needs a healthy dose of scepticism

To guard against hype, those interpreting research on the body's microscopic communities should ask five questions, says **William P. Hanage**.

Comment August 2014 Nature

How to Make More Published Research True

John P. A. Ioannidis

Published: October 21, 2014 • DOI: 10.1371/journal.pmed.1001747

```
int getRandomNumber()
{
    return 4;  // chosen by fair dice roll.
               // guaranteed to be random.
}
```

http://web.stanford.edu/class/cs109l/unrestricted/images/

RESEARCH PRIORITIES

Shining Light into Black Boxes

A. Morin[1], J. Urban[2], P. D. Adams[3], I. Foster[4], A. Sali[5], D. Baker[6], P. Sliz[1,*]

The demise of alchemy provides further evidence, if further evidence were needed, that what marks out modern science is not the conduct of experiments (alchemists conducted plenty of experiments), but the formation of a *critical community capable of assessing discoveries and replicating results*. Alchemy, as a clandestine enterprise, could never develop a community of the right sort. Popper was right to think that science can flourish only in an open society.

The Invention of Science: A New History of the Scientific Revolution, by David Wootton

A family of alchemists at work, an engraving by Philip Galle, after a painting by Pieter Bruegel the Elder, published by Hieronymus Cock, *c.*1558.

Data silo


Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

What is Bioconductor ?

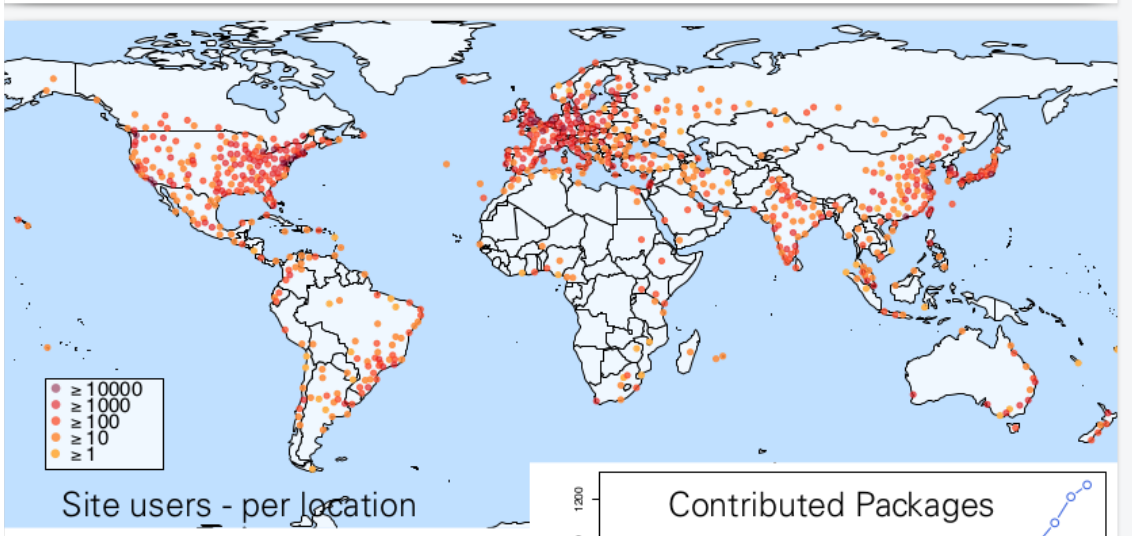
Started 2001 as a platform for analysis & understanding of microarray data

More than 1,600 packages. Domains of expertise:

- Sequencing (RNASeq, ChIPSeq, single-cell, called variants, ...)
- Microarrays (methylation, expression, copy number, ...)
- Flow cytometry
- Proteomics
- Multi-Omics data integration

Important themes

- Reproducible research
- Interoperability between packages & workflows
  … even from different authors
- Usability



World largest bioinformatics project
10,000s users
>18,000 papers in PubmedCentral

What is Bioconductor ?

OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

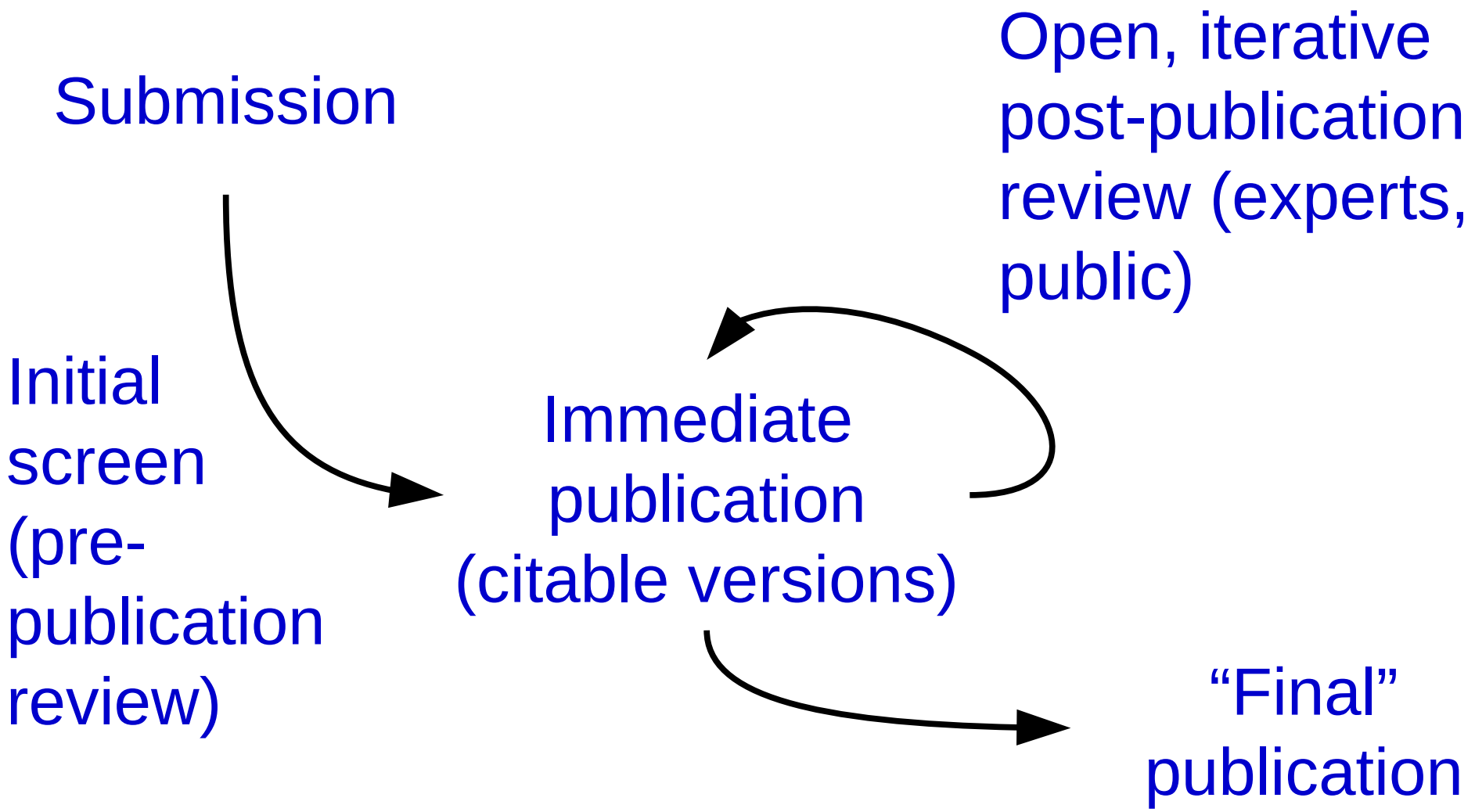
Principally a collaborative software development project

But it is also:

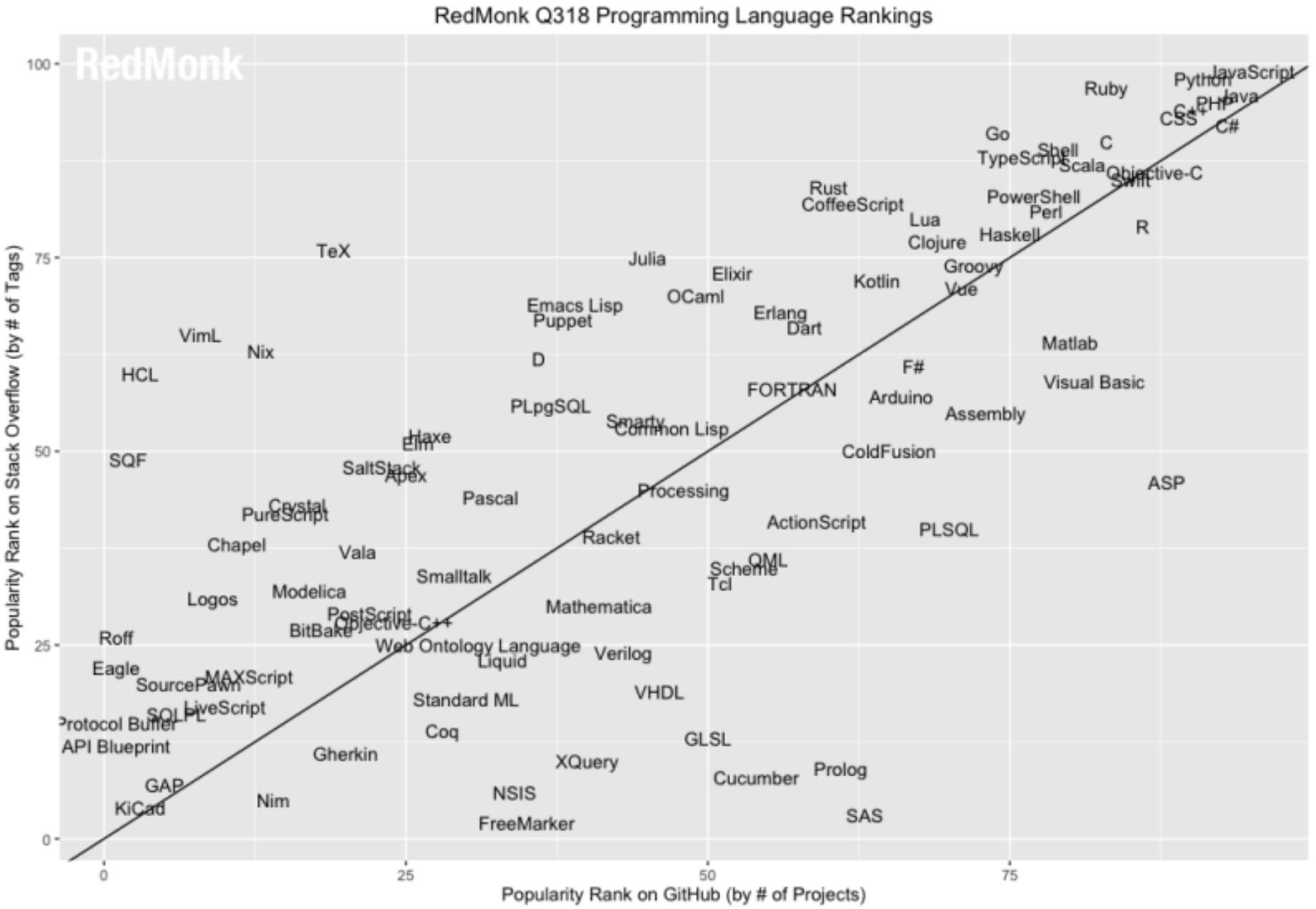
- a software repository
- a bioinformatics support site
- data repository
- publisher for supplementary materials
- source for tutorials and instructional documentation

Managed and maintained by a core team of ~6 people, with contributions coming from all over the world

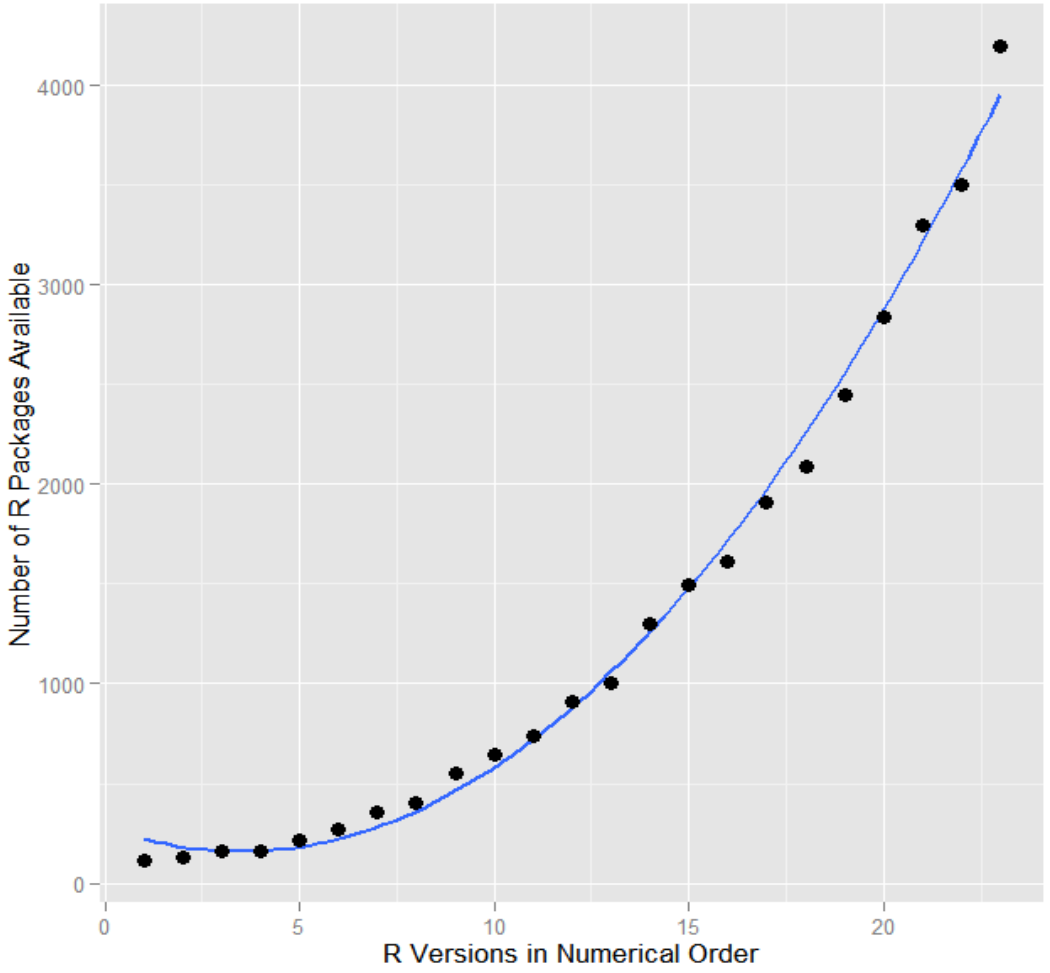
Pre- vs. post-publication review



Varying cultures of open collaboration



Number of open analysis tools has grown exponentially



github SOCIAL CODING

R

rOpenGov

Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

ROpenSci

Value of data can increase through sharing & use

PLOS COMPUTATIONAL BIOLOGY

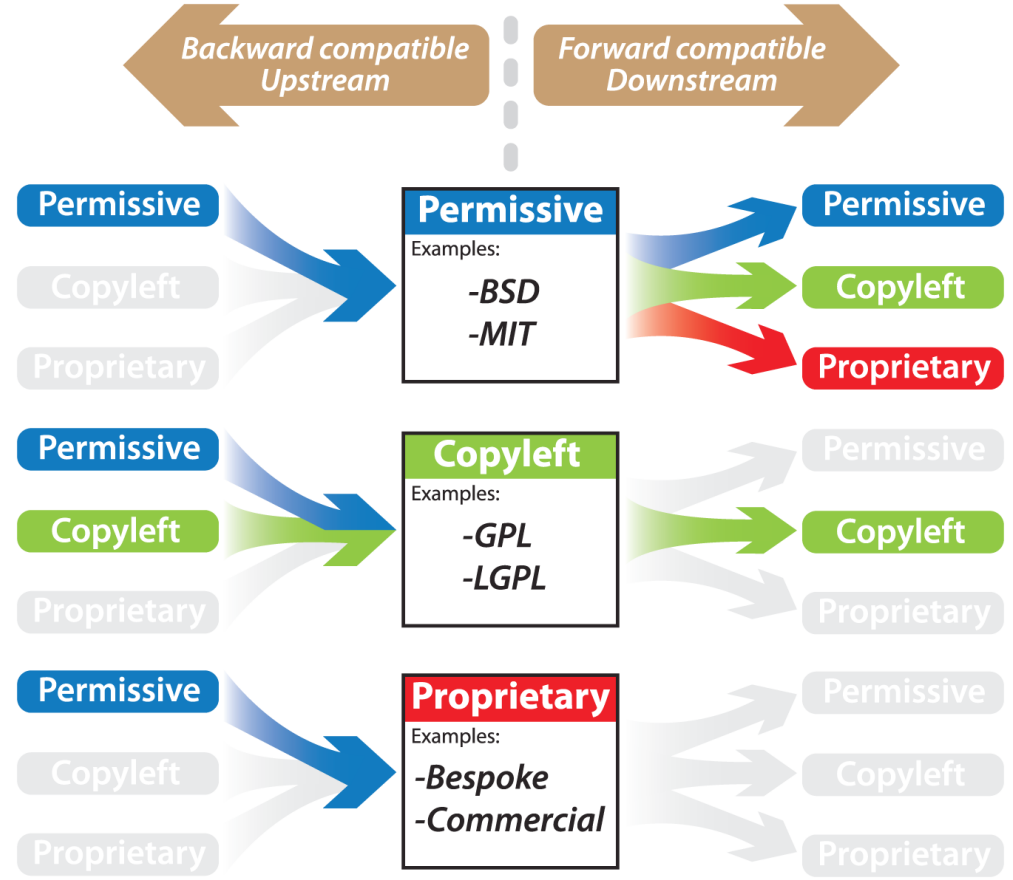
OPEN ACCESS

EDUCATION

A Quick Guide to Software Licensing for the Scientist-Programmer

Andrew Morin, Jennifer Urban, Piotr Sliz

Published: July 26, 2012 • https://doi.org/10.1371/journal.pcbi.1002598



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Software citation principles

Arfon M. Smith[1,*], Daniel S. Katz[2,*], Kyle E. Niemeyer[3,*]
FORCE11 Software Citation Working Group

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[3] School of Mechanical, Industrial, and Manufacturing Engineering, Oregon Sta Corvallis, Oregon, United States
* These authors contributed equally to this work.

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Open reporting and communication were part of academic culture since the early days

BJHS 45(2): 165–188, June 2012. © British Society for the History of Science 2012
doi:10.1017/S0007087412000064 First published online 20 March 2012

Openness versus secrecy? Historical and historiographical remarks

KOEN VERMEIR*

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Alchemy & algorithms: perspectives on the philosophy and history of open science

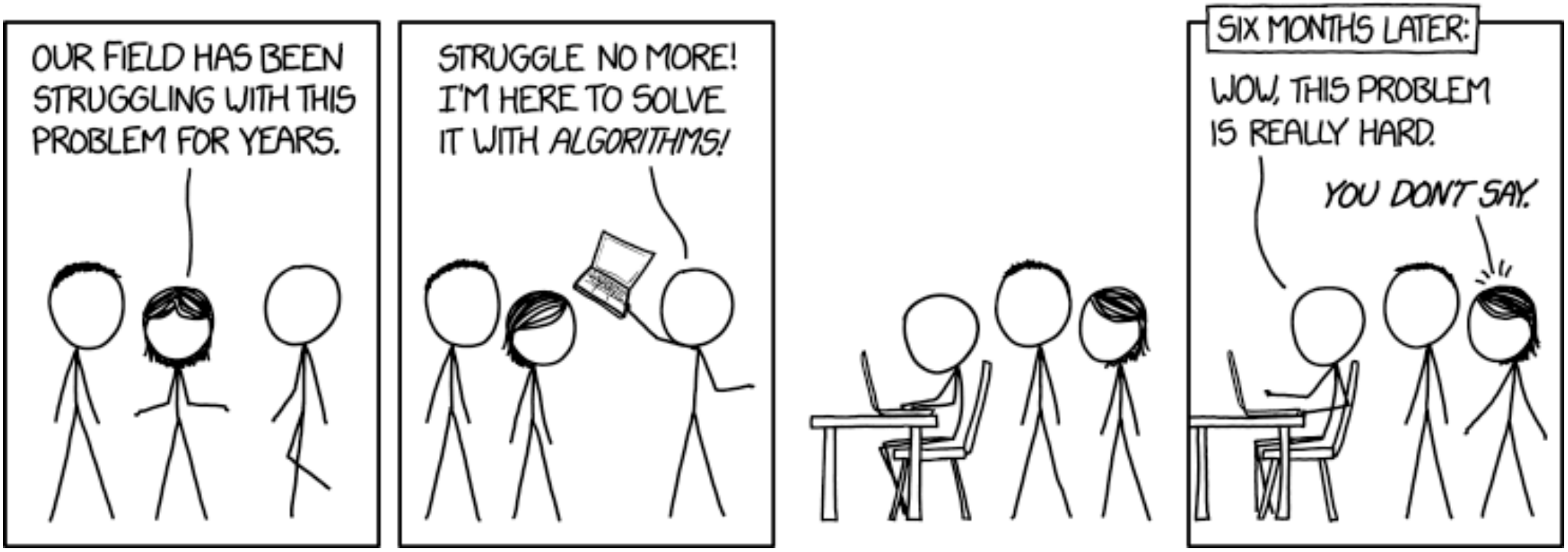
Research Ideas and Outcomes 3:e13593, 2017

Leo Lahti, Filipe da Silva, Markus Petteri Laine, Viivi Lähteenoja, Mikko Tolonen

Beyond Open Access - The Changing Culture of Producing and Disseminating Scientific Knowledge

Heidi Laine Leo Lahti Anne Lehto

Proceedings of the 19th International Academic Mindtrek Conference 2015. ACM New York, NY, USA. ISBN: 978-1-4503-3948-3


OUR FIELD HAS BEEN STRUGGLING WITH THIS PROBLEM FOR YEARS.
STRUGGLE NO MORE! I'M HERE TO SOLVE IT WITH ALGORITHMS!
SIX MONTHS LATER:
WOW, THIS PROBLEM IS REALLY HARD.
YOU DON'T SAY.

Task

Create a clear reproducible report of the example data, including the following aspects:

- Data import
- Data exploration & summaries
- Alpha diversity
- Beta diversity
- Differential abundance analysis
- **Conclusions**