Quantification of bacterial replication rates (PTRs): A new perspective to study the dynamic role of microbes in IBD?

IBIRI LAB MEETING

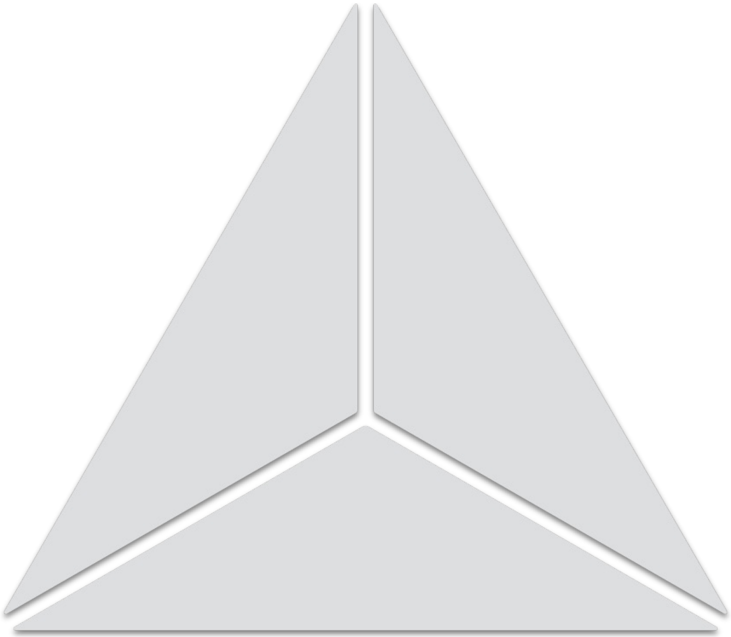
Ashok Kumar Sharma

Oct 21st, 2022

Casero and Devkota Lab
The Inflammatory Bowel and Immunobiology Research Institute (IBIRI)
Department of Gastroenterology
Cedars Sinai Medical Center

Microbial analysis

Limitations in microbial analysis



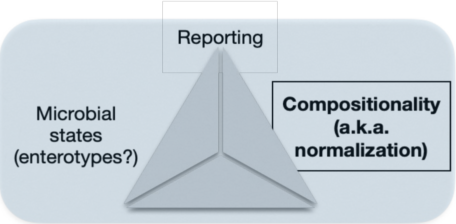
Reporting

Microbial states
(enterotypes?)

Compositionality
(a.k.a. normalization)

Microbial analysis

Bacterial replication rates



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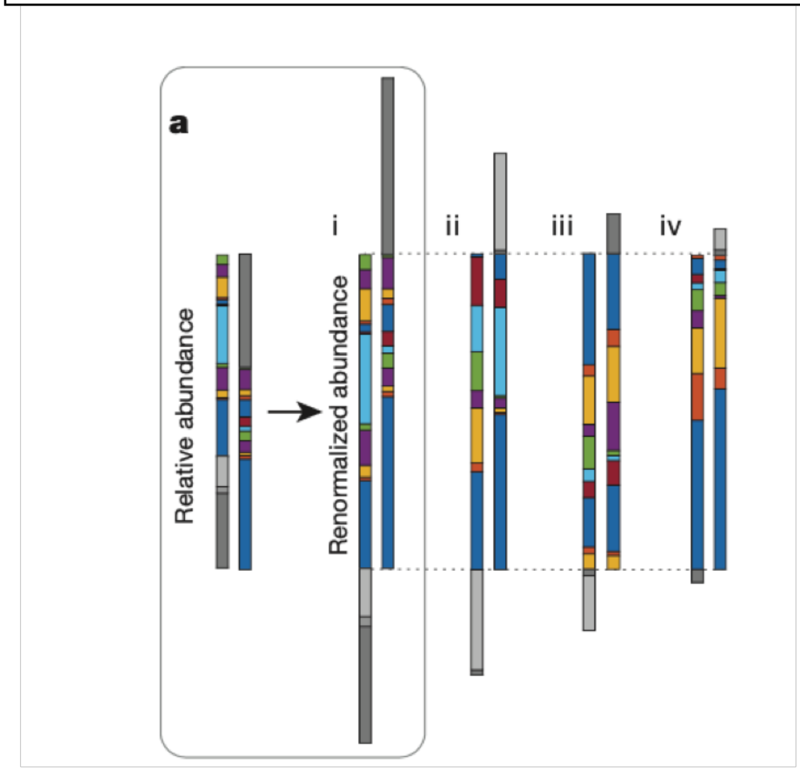
LETTER

doi:10.1038/nature18301

Universality of human microbial dynamics

Amir Bashan[1], Travis E. Gibson[1], Jonathan Friedman[2], Vincent J. Carey[1], Scott T. Weiss[1], Elizabeth L. Hohmann[3] & Yang-Yu Liu[1,4]



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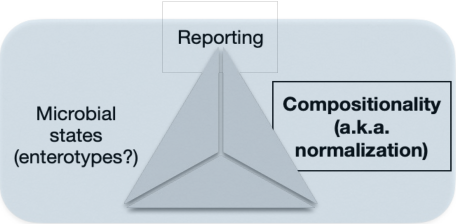
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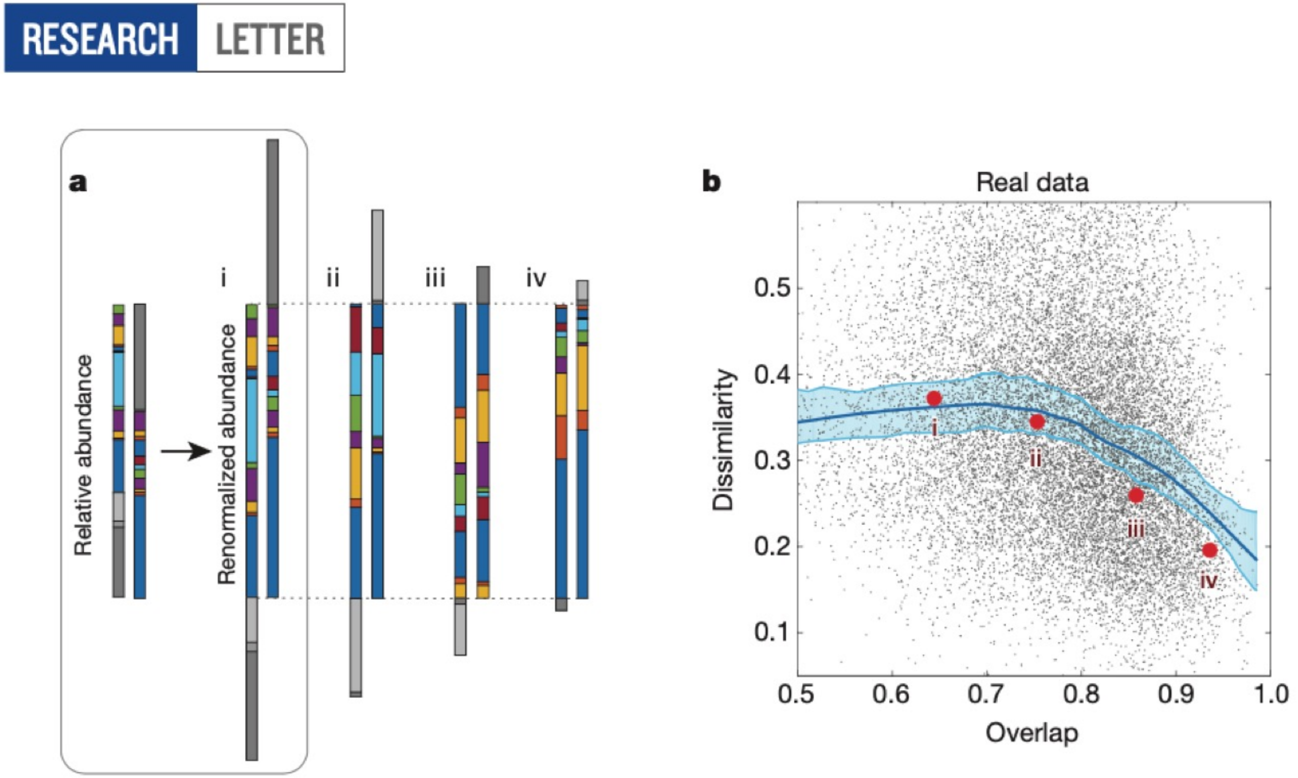

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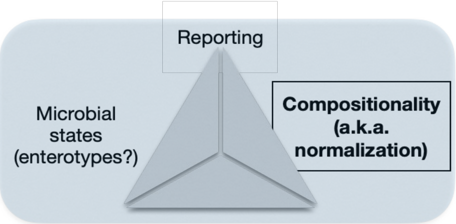
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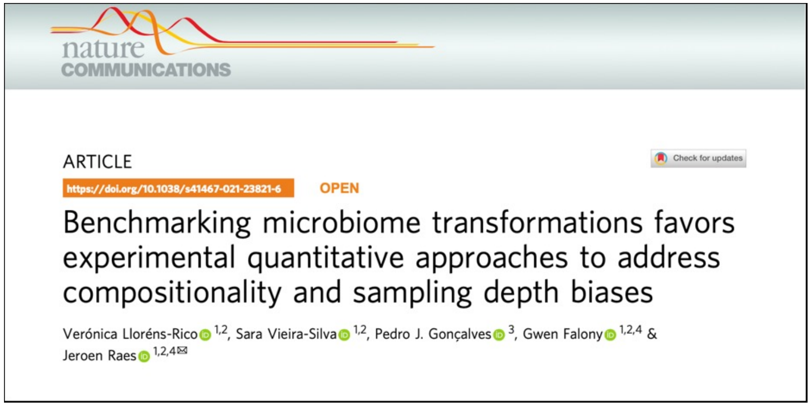
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ARTICLE

https://doi.org/10.1038/s41467-021-23821-6 OPEN

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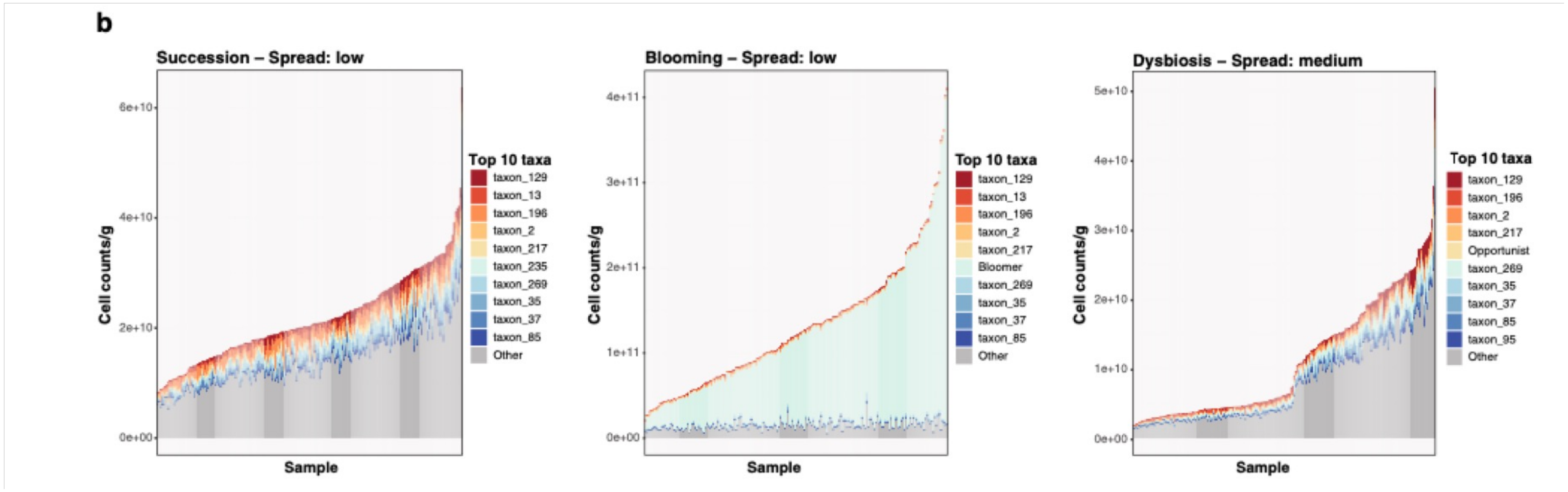
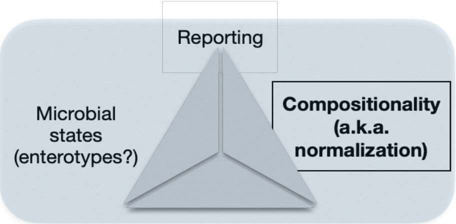
Benchmarking microbiome transformations favors experimental quantitative approaches to address compositionality and sampling depth biases

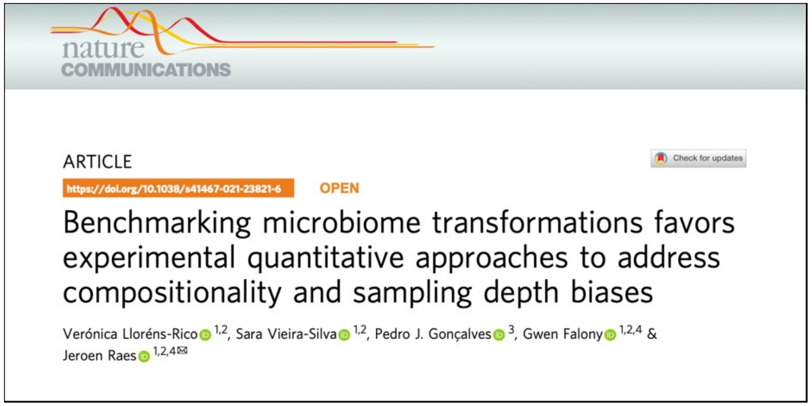
Verónica Lloréns-Rico[1,2], Sara Vieira-Silva[1,2], Pedro J. Gonçalves[3], Gwen Falony[1,2,4] & Jeroen Raes[1,2,4]✉



Microbial analysis

Bacterial replication rates





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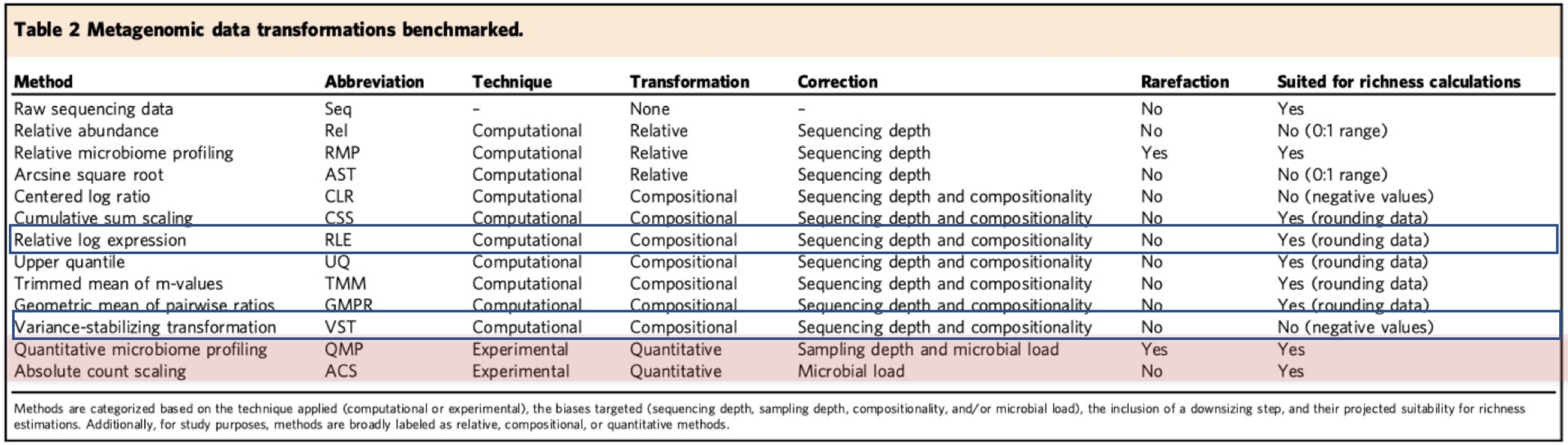


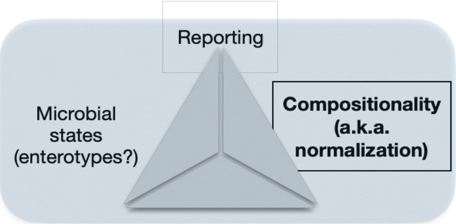
Table 2 Metagenomic data transformations benchmarked.

Method	Abbreviation	Technique	Transformation	Correction	Rarefaction	Suited for richness calculations
Raw sequencing data	Seq	-	None	-	No	Yes
Relative abundance	Rel	Computational	Relative	Sequencing depth	No	No (0:1 range)
Relative microbiome profiling	RMP	Computational	Relative	Sequencing depth	Yes	Yes
Arcsine square root	AST	Computational	Relative	Sequencing depth	No	No (0:1 range)
Centered log ratio	CLR	Computational	Compositional	Sequencing depth and compositionality	No	No (negative values)
Cumulative sum scaling	CSS	Computational	Compositional	Sequencing depth and compositionality	No	Yes (rounding data)
Relative log expression	RLE	Computational	Compositional	Sequencing depth and compositionality	No	Yes (rounding data)
Upper quantile	UQ	Computational	Compositional	Sequencing depth and compositionality	No	Yes (rounding data)
Trimmed mean of m-values	TMM	Computational	Compositional	Sequencing depth and compositionality	No	Yes (rounding data)
Geometric mean of pairwise ratios	GMPR	Computational	Compositional	Sequencing depth and compositionality	No	Yes (rounding data)
Variance-stabilizing transformation	VST	Computational	Compositional	Sequencing depth and compositionality	No	No (negative values)
Quantitative microbiome profiling	QMP	Experimental	Quantitative	Sampling depth and microbial load	Yes	Yes
Absolute count scaling	ACS	Experimental	Quantitative	Microbial load	No	Yes

Methods are categorized based on the technique applied (computational or experimental), the biases targeted (sequencing depth, sampling depth, compositionality, and/or microbial load), the inclusion of a downsizing step, and their projected suitability for richness estimations. Additionally, for study purposes, methods are broadly labeled as relative, compositional, or quantitative methods.

Microbial analysis

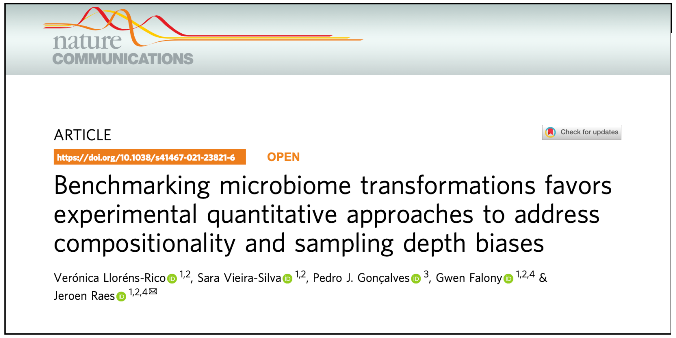
Bacterial replication rates



Reporting

Microbial states (enterotypes?)

Compositionality (a.k.a. normalization)



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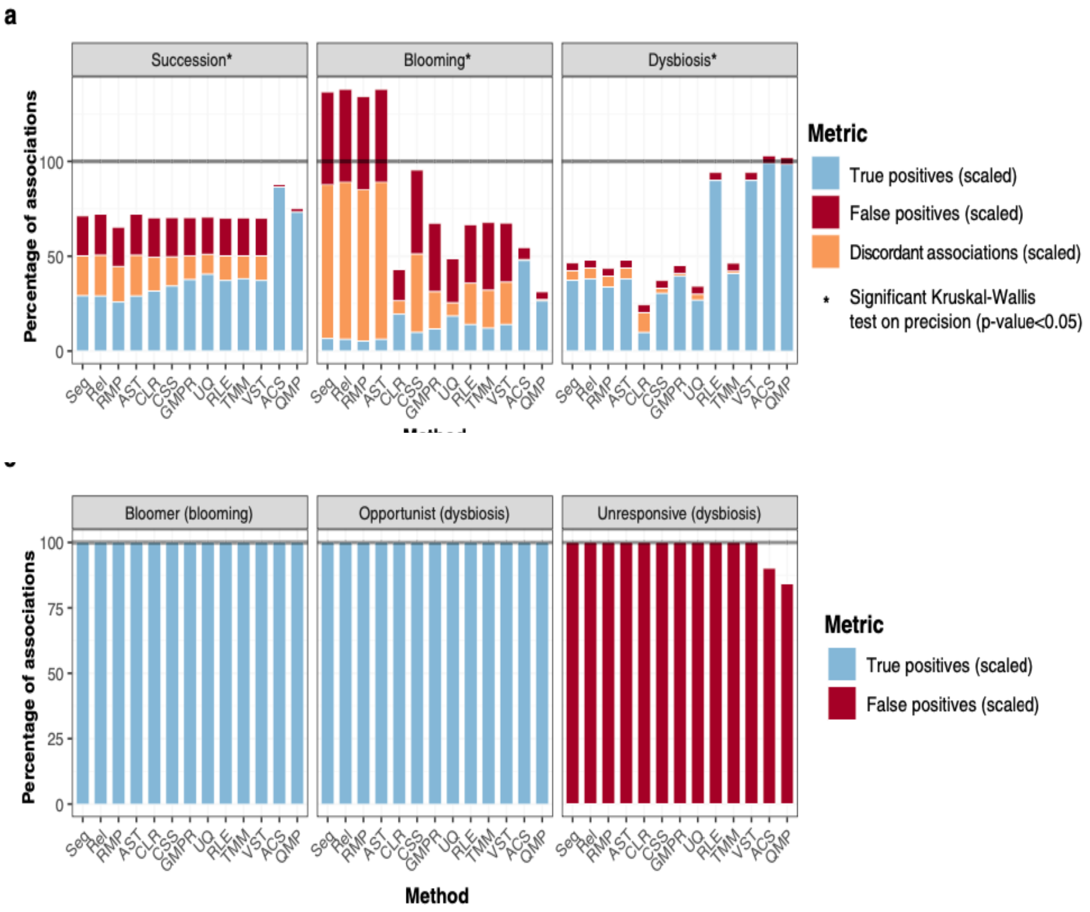

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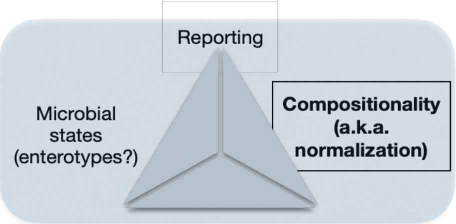

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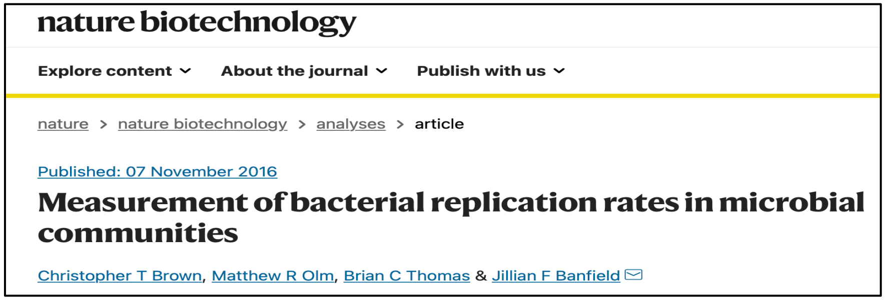

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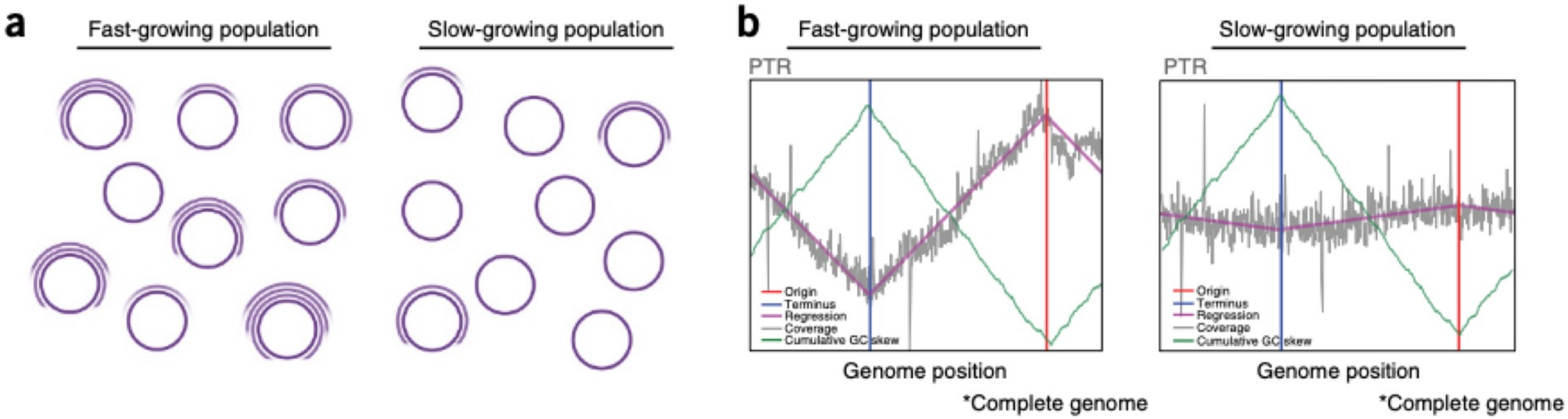
Measurement of bacterial replication rates in microbial communities

Christopher T Brown, Matthew R Olm, Brian C Thomas & Jillian F Banfield



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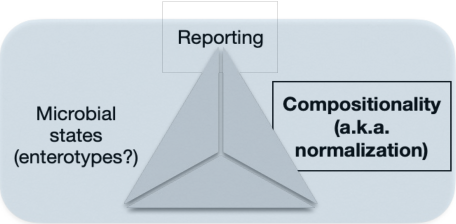

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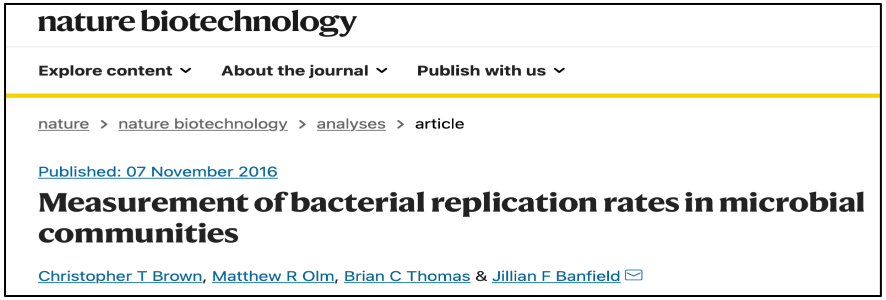

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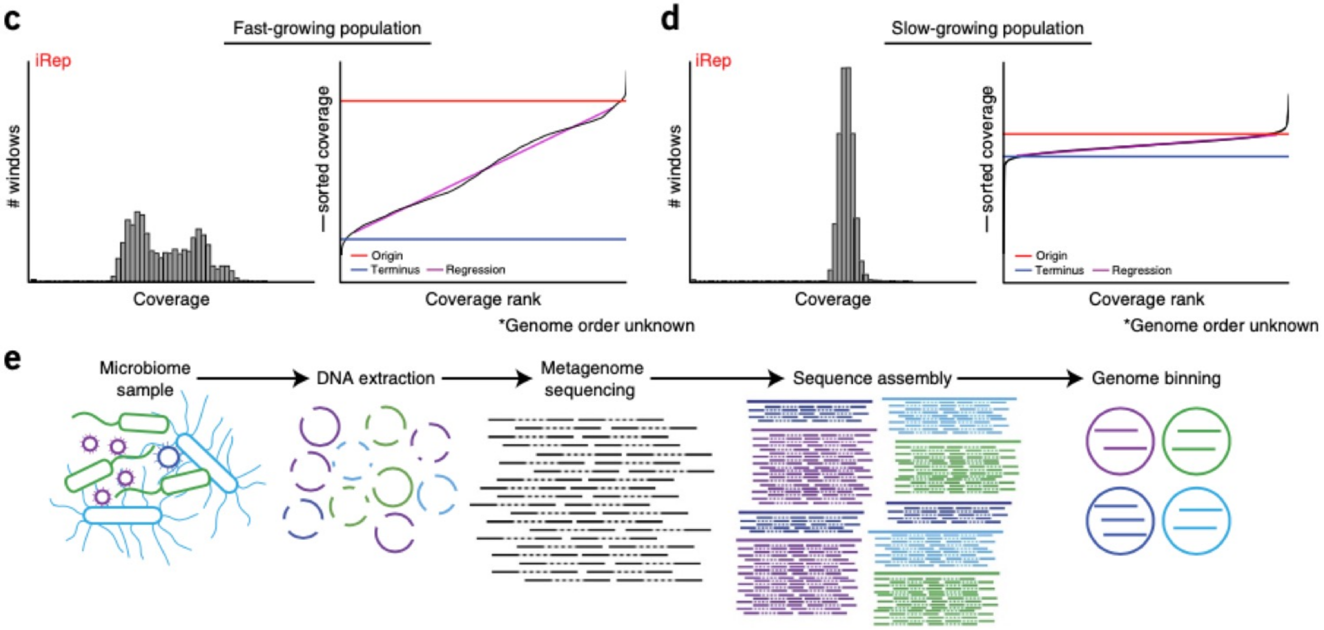

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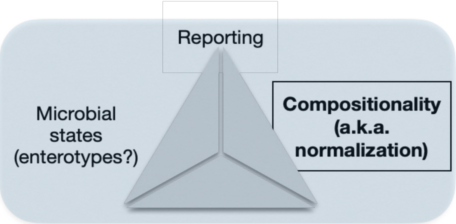
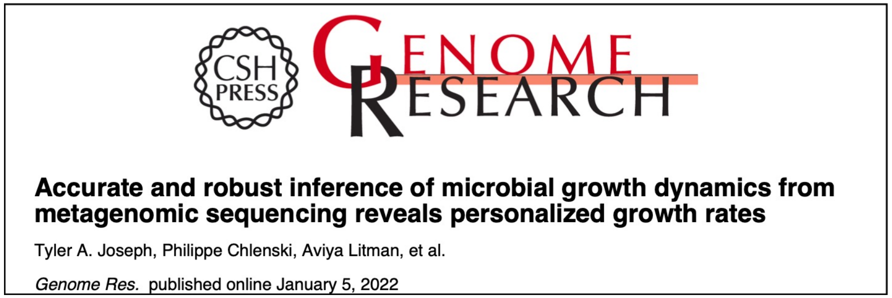


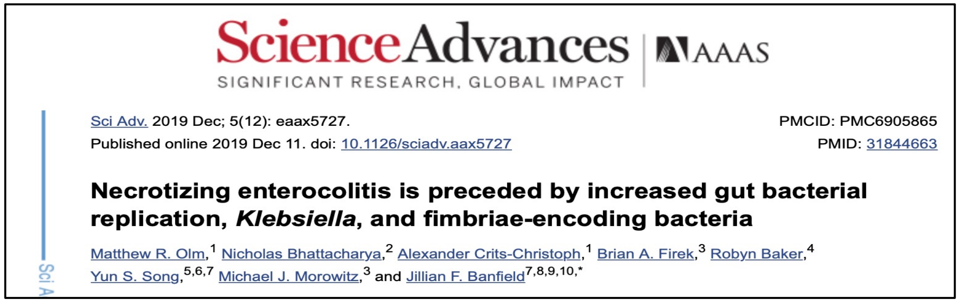


CSH PRESS GENOME RESEARCH

Accurate and robust inference of microbial growth dynamics from metagenomic sequencing reveals personalized growth rates

Tyler A. Joseph, Philippe Chlenski, Aviya Litman, et al.

Genome Res. published online January 5, 2022



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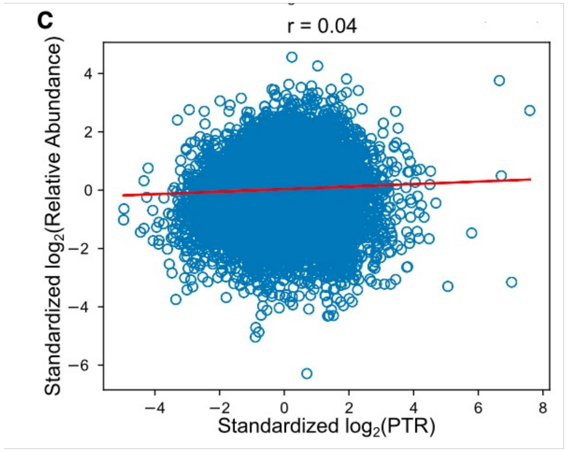
Sci Adv. 2019 Dec; 5(12): eaax5727. PMCID: PMC6905865
Published online 2019 Dec 11. doi: 10.1126/sciadv.aax5727 PMID: 31844663

Necrotizing enterocolitis is preceded by increased gut bacterial replication, *Klebsiella*, and fimbriae-encoding bacteria

Matthew R. Olm,[1] Nicholas Bhattacharya,[2] Alexander Crits-Christoph,[1] Brian A. Firek,[3] Robyn Baker,[4] Yun S. Song,[5,6,7] Michael J. Morowitz,[3] and Jillian F. Banfield[7,8,9,10,*]



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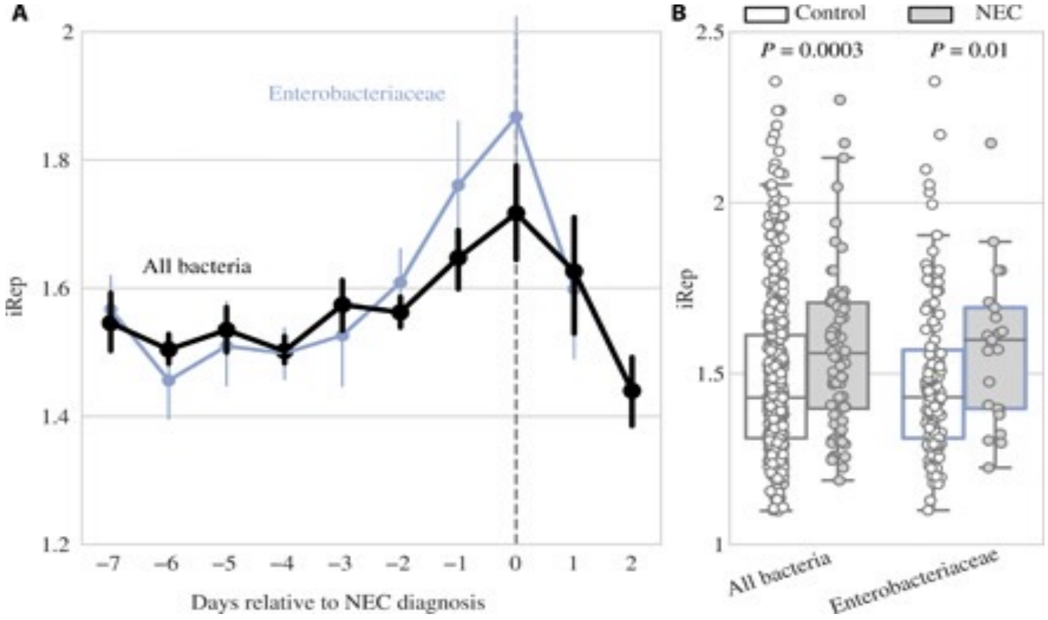

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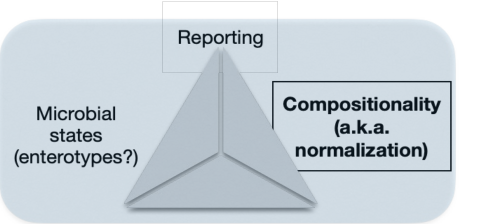

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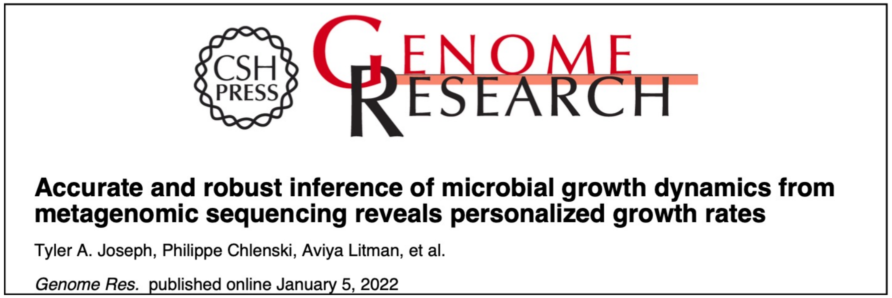

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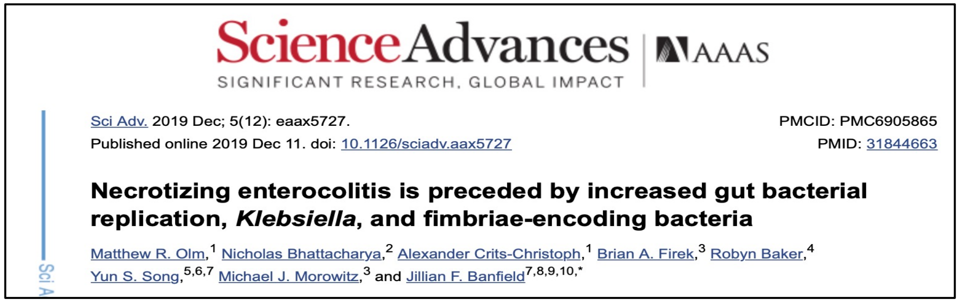

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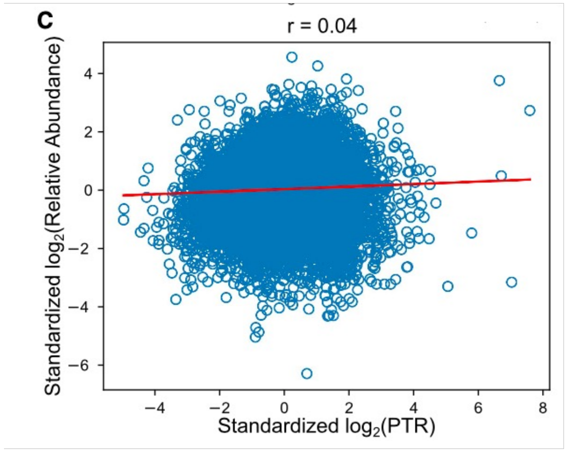

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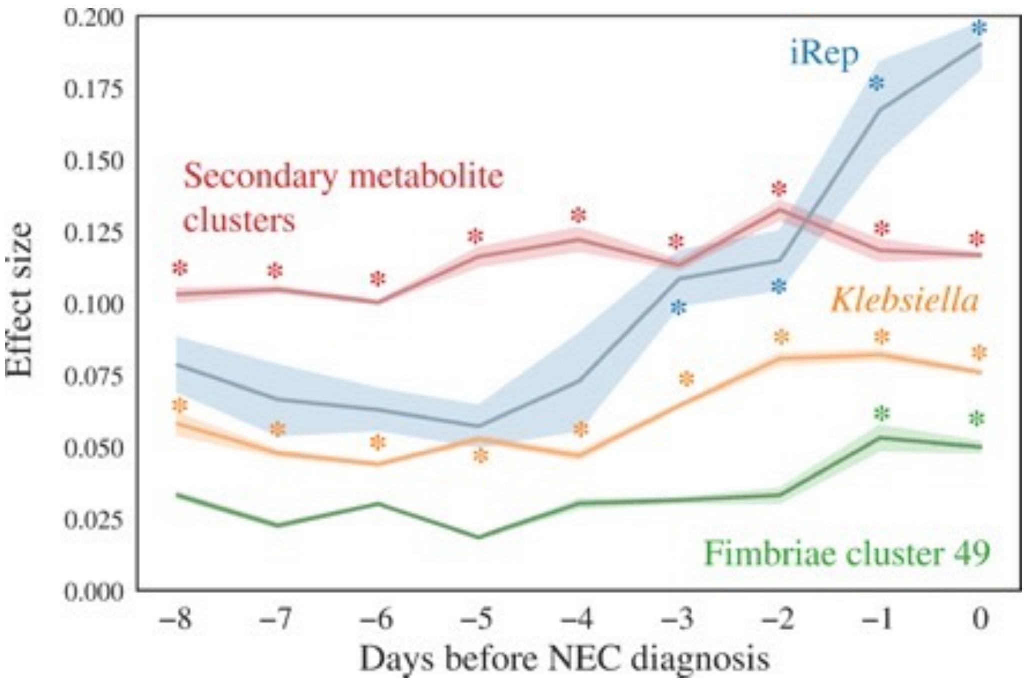

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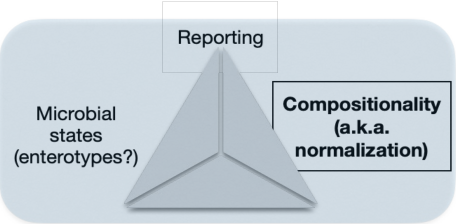

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ARTICLE

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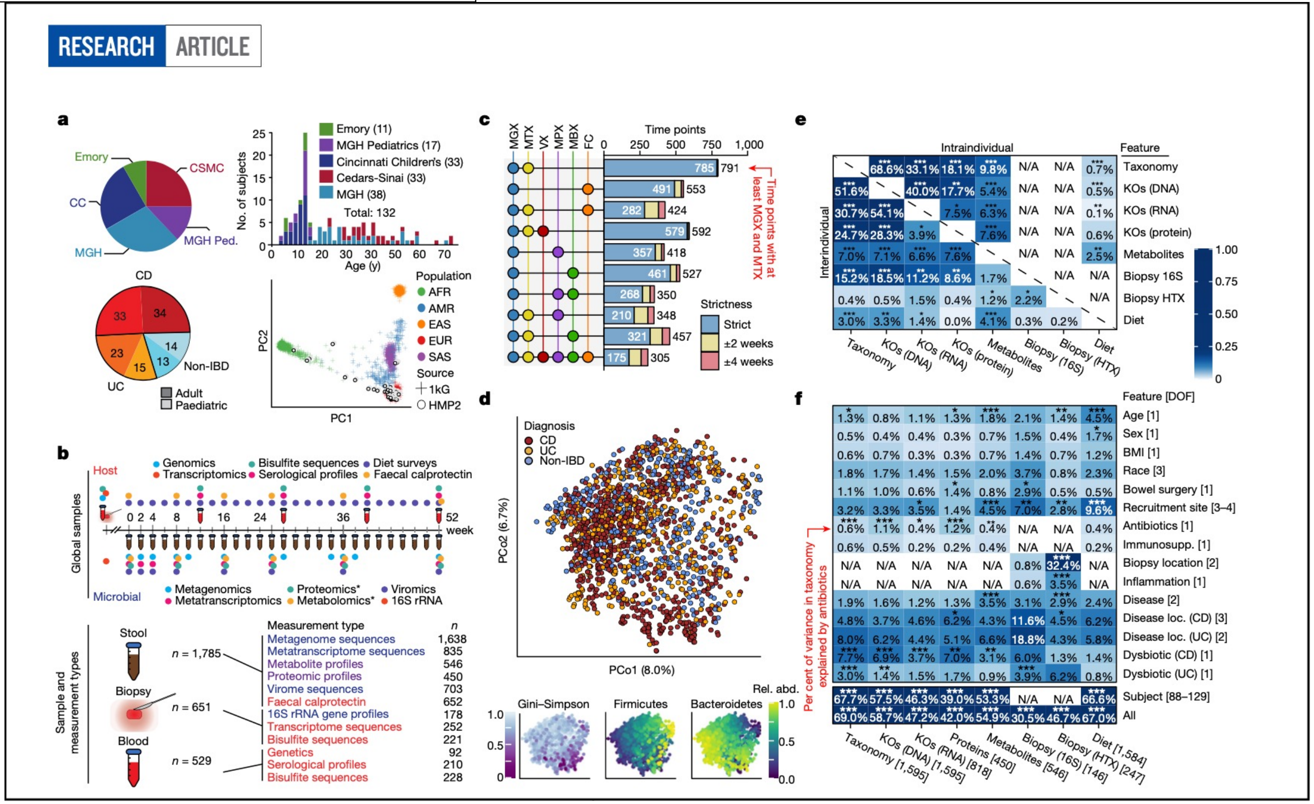
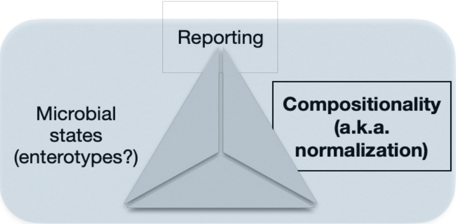
https://doi.org/10.1038/s41586-019-1237-9

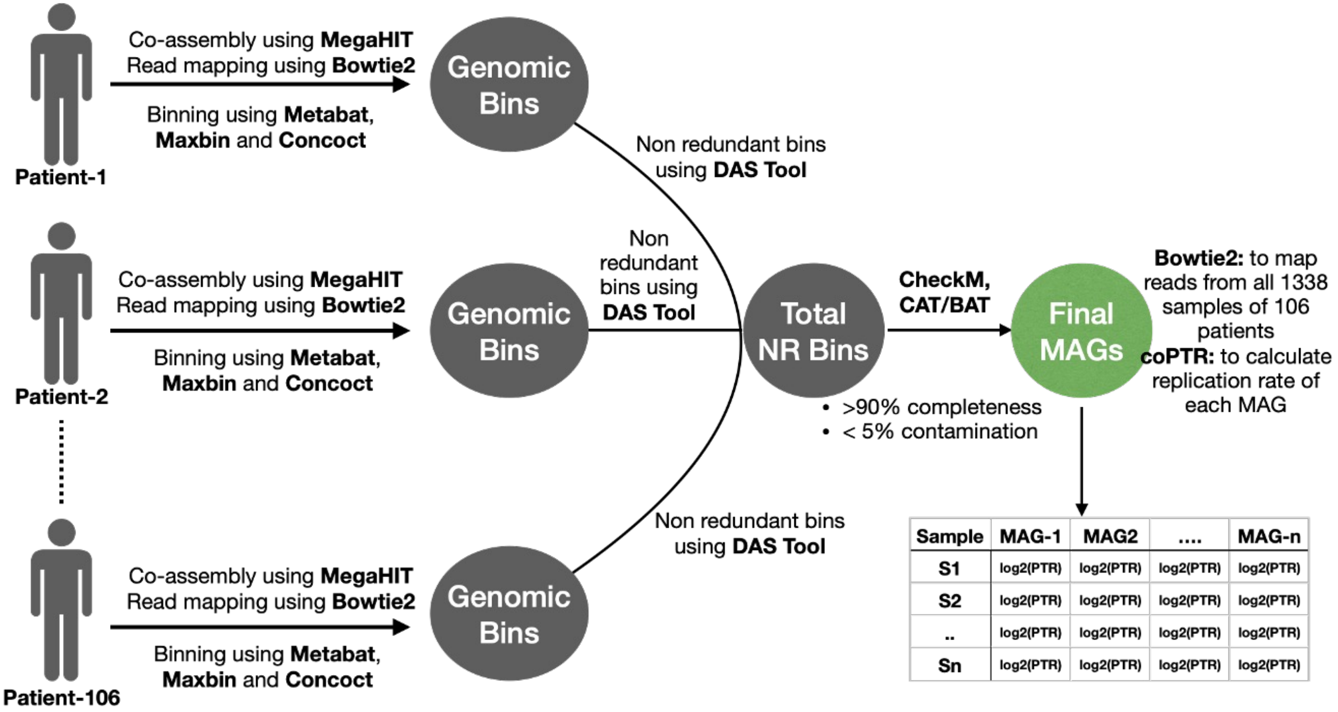
Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases

Jason Lloyd-Price[1,2], Cesar Arze[2], Ashwin N. Ananthakrishnan[3], Melanie Schirmer[1,3], Julian Avila-Pacheco[4], Tiffany W. Poon[1], Elizabeth Andrews[3], Nadim J. Ajami[5], Kevin S. Bonham[1,2], Colin J. Brislawn[6], David Casero[7], Holly Courtney[3], Antonio Gonzalez[8], Thomas G. Graeber[9], A. Brantley Hall[1], Kathleen Lake[10], Carol J. Landers[11], Himel Mallick[1,2], Damian R. Plichta[1], Mahadev Prasad[12], Gholamali Rahnavard[1,2], Jenny Sauk[13], Dmitry Shungin[1,14], Yoshiki Vázquez-Baeza[15,16], Richard A. White III[6], IBDMDB Investigators[17], Jonathan Braun[7], Lee A. Denson[10,18], Janet K. Jansson[6], Rob Knight[8,16,19], Subra Kugathasan[12], Dermot P. B. McGovern[11], Joseph F. Petrosino[5], Thaddeus S. Stappenbeck[20], Harland S. Winter[21,22], Clary B. Clish[4], Eric A. Franzosa[2], Hera Vlamakis[1], Ramnik J. Xavier[1,3,23,24] & Curtis Huttenhower[1,2,24]*





Methodology- to construct metagenome-assembled genomes (MAGs) and compute PTR



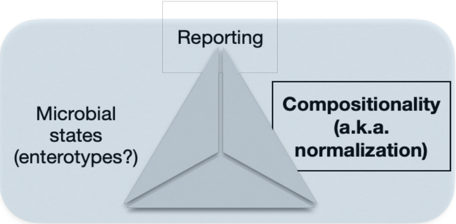
- >90% completeness
- < 5% contamination

Bowtie2: to map reads from all 1338 samples of 106 patients
coPTR: to calculate replication rate of each MAG

Sample	MAG-1	MAG2	....	MAG-n
S1	log2(PTR)	log2(PTR)	log2(PTR)	log2(PTR)
S2	log2(PTR)	log2(PTR)	log2(PTR)	log2(PTR)
..	log2(PTR)	log2(PTR)	log2(PTR)	log2(PTR)
Sn	log2(PTR)	log2(PTR)	log2(PTR)	log2(PTR)

Microbial analysis

Bacterial replication rates


Reporting

Microbial
states
(enterotypes?)

Compositionality
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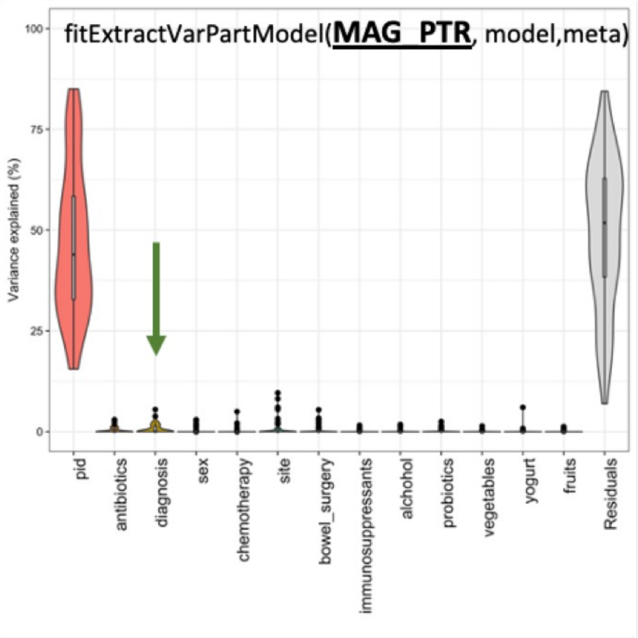
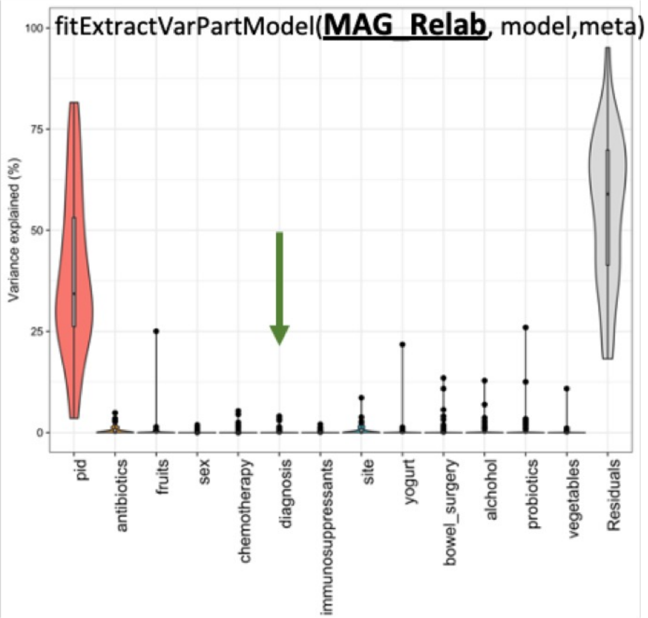
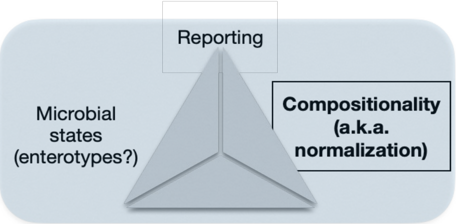
PCoA and Variance partition analysis: Replication rates vs Relative abundances of MAGs

```
model <- ~
(1|diagnosis)+(1|sex)+(1|site)+(1|alchohol)+(1|antibiotics)+(1|immunosuppressants)+(1|chemotherapy)+(1|bowel_surgery)+(1|
vegetables)+(1|probiotics)+(1|yogurt)+(1|fruits)+(1|pid)
```


fitExtractVarPartModel(**MAG_PTR**, model,meta)

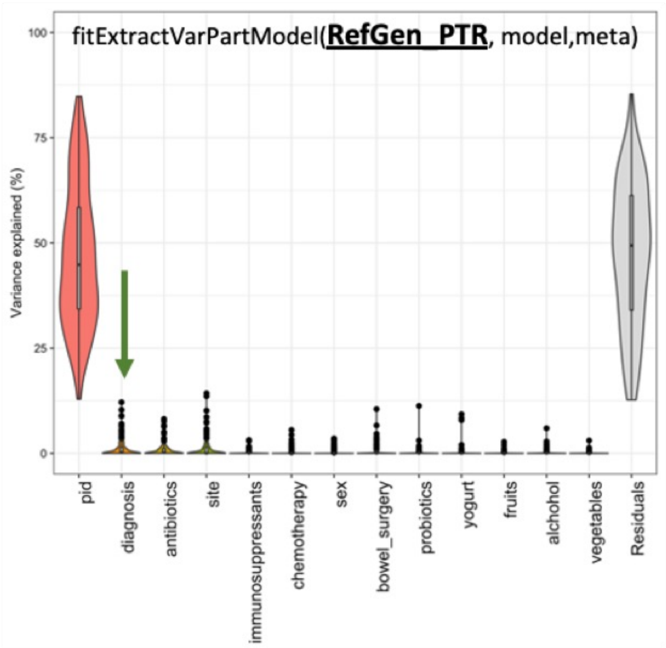

fitExtractVarPartModel(**MAG_Relab**, model,meta)

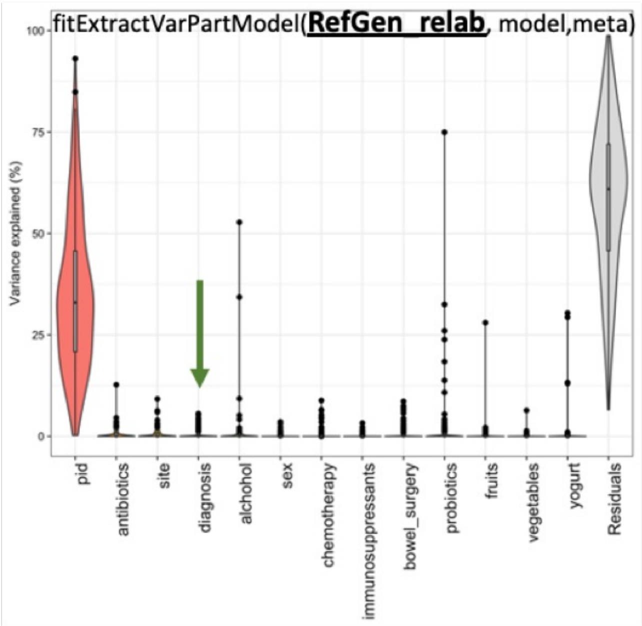


PCoA and Variance partition analysis: Replication rates vs Relative abundances of Reference genomes

model <- ~
(1|diagnosis)+(1|sex)+(1|site)+(1|alchohol)+(1|antibiotics)+(1|immunosuppressants)+(1|chemotherapy)+(1|bowel_surgery)+(1|vegetables)+(1|probiotics)+(1|yogurt)+(1|fruits)+(1|pid)

PTRs (Replication rates)

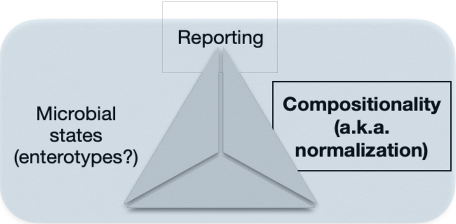

fitExtractVarPartModel(**RefGen_PTR**, model,meta)


fitExtractVarPartModel(**RefGen_relab**, model,meta)

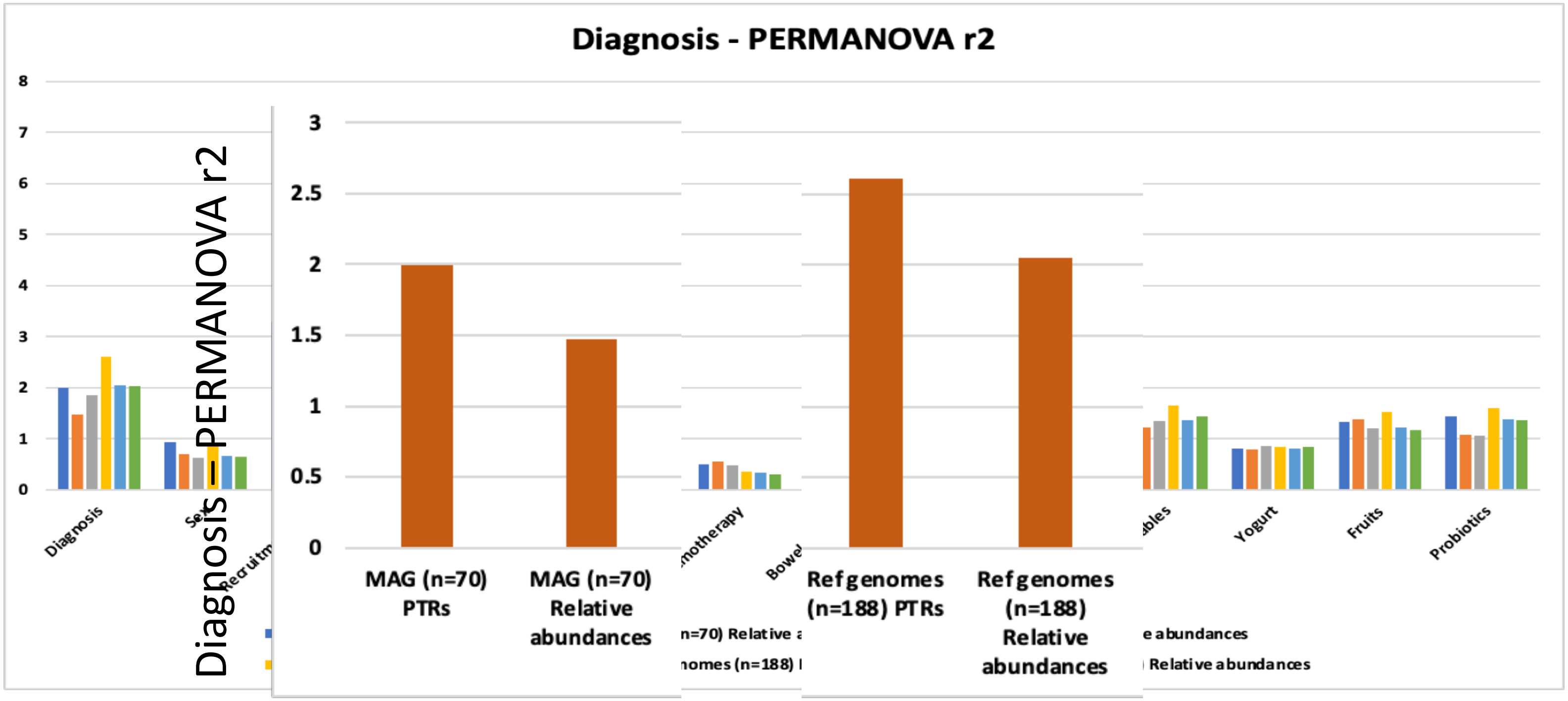
Relative Abundances

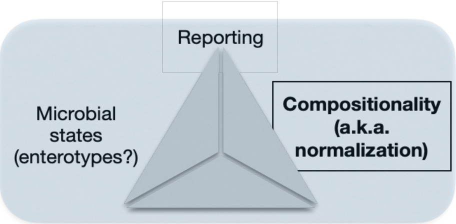
Microbial analysis

Bacterial replication rates



PTR estimates showed better association with clinical phenotypes as compared to relative abundances





Top ten Reference Genomes associated with disease phenotype

PTRs

Gid	Taxa	Diagnosis
ERS473051_14	Lachnospiraceae sp.	0.12
X742821.3	Sutterella wadsworthensis	0.10
ERS537236_4	Alistipes sp.	0.09
ERS608495_69	UBA11524 sp.	0.09
X445970.5	Alistipes putredinis	0.07
SRS077849_38	Lachnospiraceae sp.	0.07
ERS473414_25	DTU089 sp.	0.07
ERS537221_53	Lachnospira sp.	0.07
X349741.6	Akkermansia muciniphila	0.06
ERS473219_31	Lachnospiraceae sp.	0.06

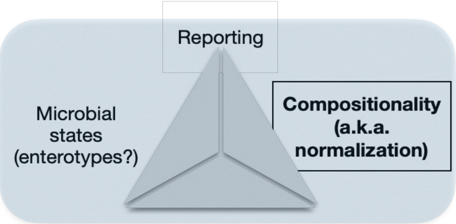
Relative abundances

Gid	Taxa	Diagnosis
ERS473051_14	Lachnospiraceae sp.	0.05
ERS473414_25	DTU089 sp.	0.05
ERS537236_4	Alistipes sp.	0.06
ERS608495_69	UBA11524 sp.	0.05
SRS475574_7	D16 HGM13112	0.04
X1034346.3	Dielma sp.	0.04
X1262951.3	Blautia sp.	0.04
X59620.57	Lachnospiraceae sp.	0.06
X665951.3	Ruminococcus torques	0.04
X742821.3	Sutterella wadsworthensis	0.04

- 4/10 reference genomes were found by both PTR and Relative abundances which shows significant association to Diagnosis
- For each genome PTRs shows higher variance as compared to relative abundances

Microbial analysis

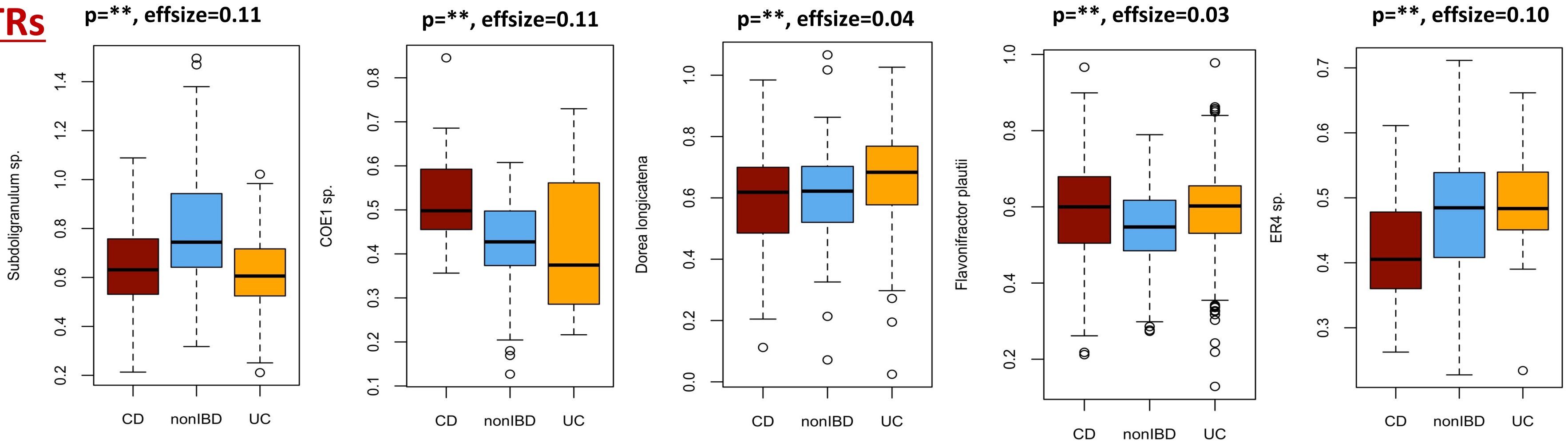
Bacterial replication rates


Reporting

Microbial states (enterotypes?)

Compositionality (a.k.a. normalization)

PTRs


p=, effsize=0.11**

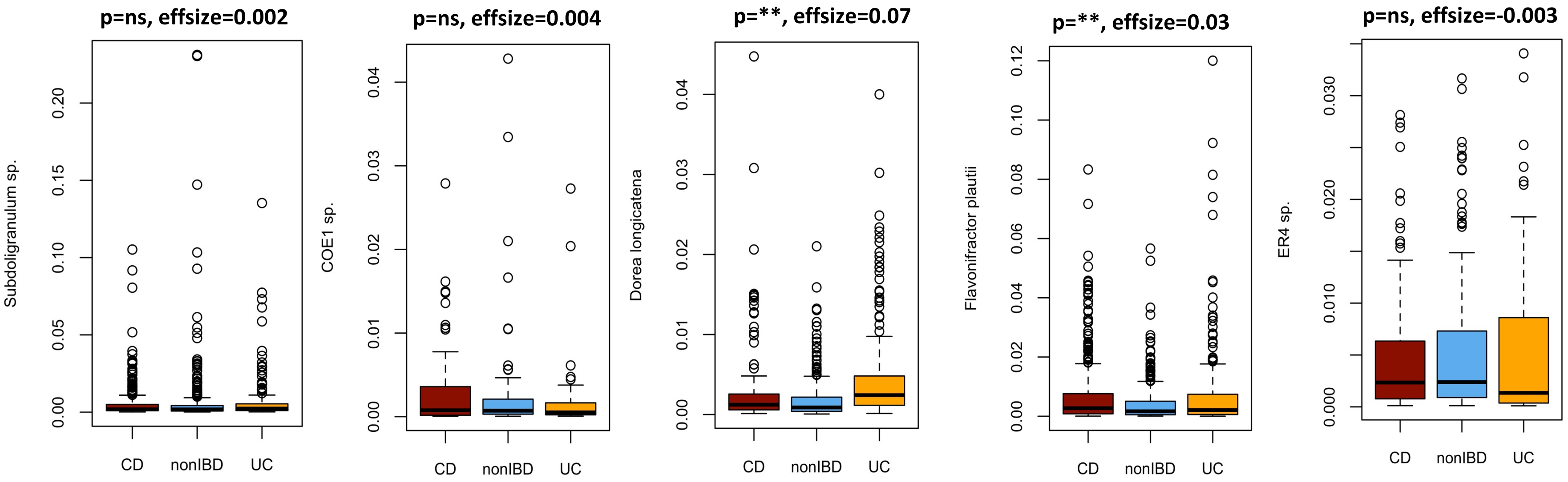
p=, effsize=0.11**

p=, effsize=0.04**

p=, effsize=0.03**

p=, effsize=0.10**

Relative abundances


p=ns, effsize=0.002

p=ns, effsize=0.004

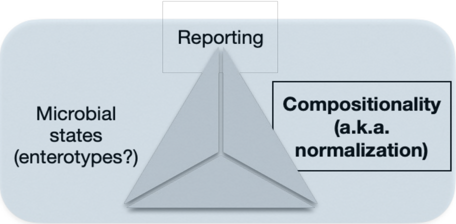
p=, effsize=0.07**

p=, effsize=0.03**

p=ns, effsize=-0.003

Microbial analysis

Bacterial replication rates



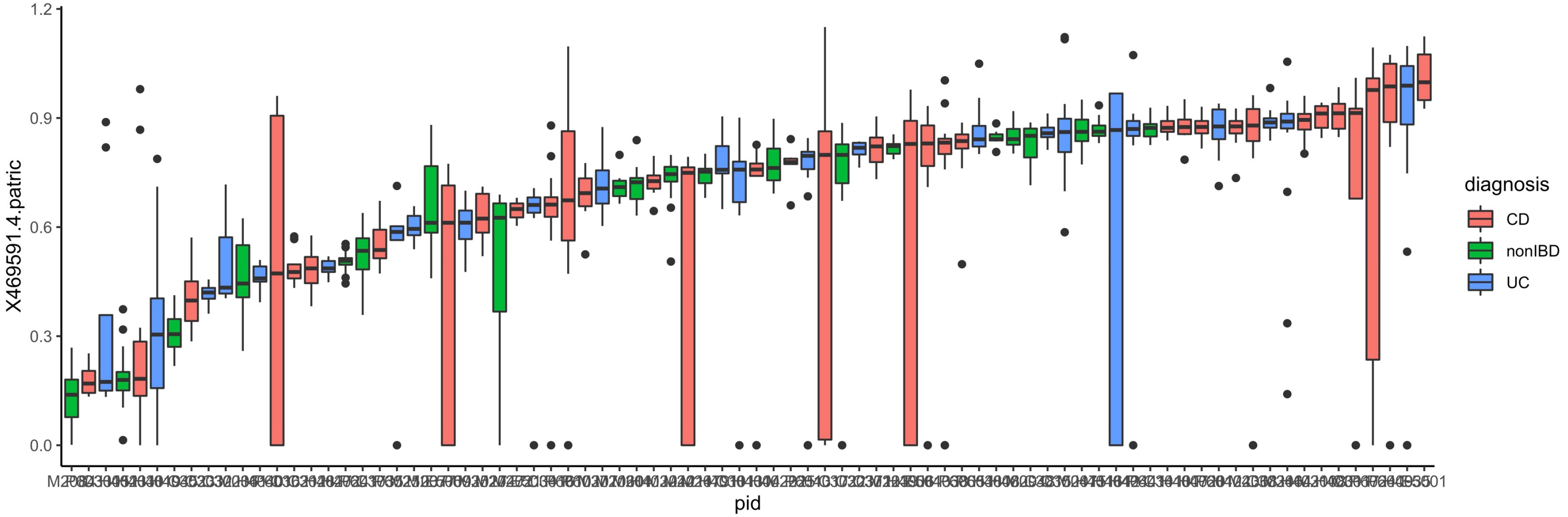
Reporting

Microbial states (enterotypes?)

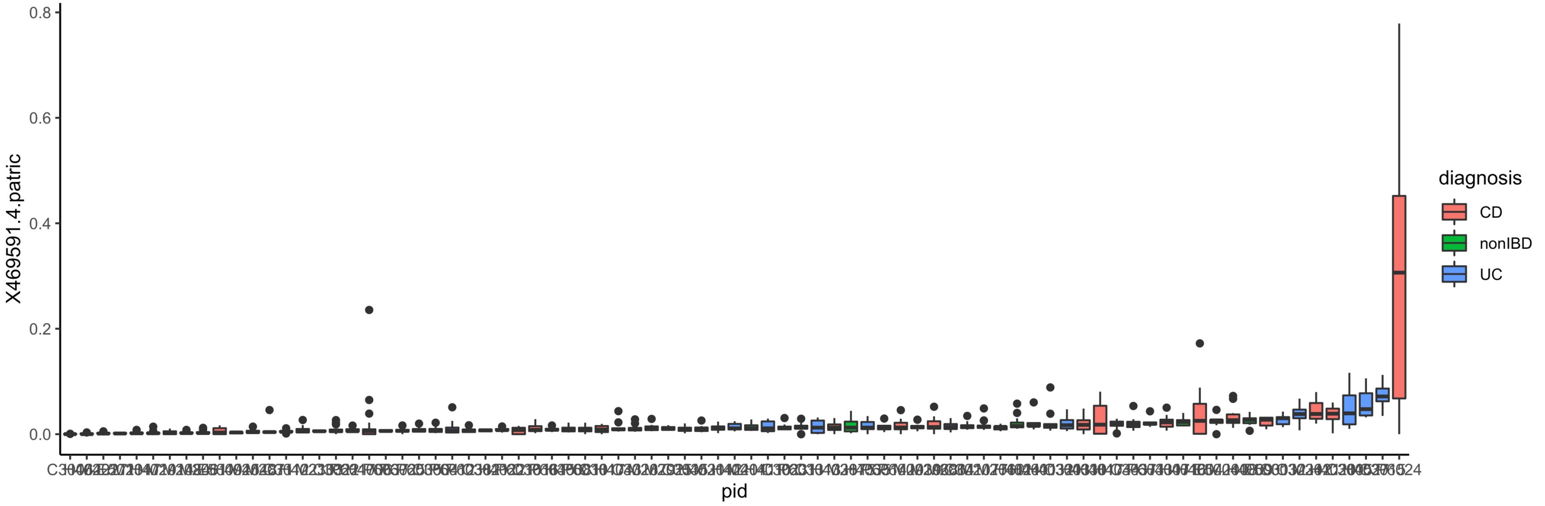
Compositionality (a.k.a. normalization)

Parabacteroides distasonis

PTRs

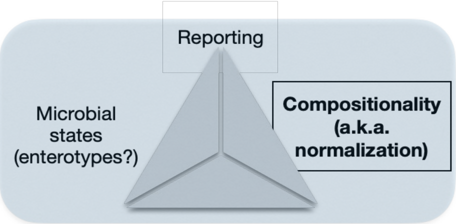


Relative abundances

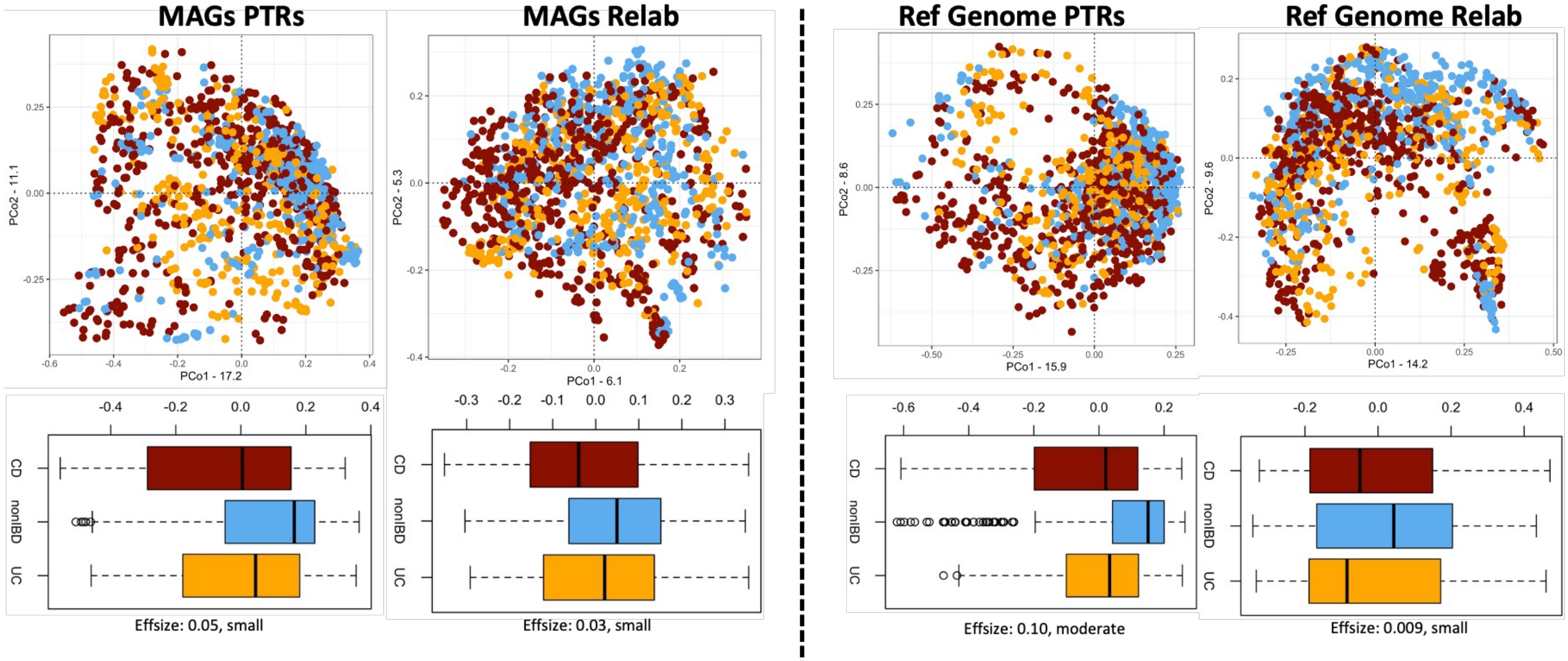


Microbial analysis

Bacterial replication rates



PCoA: PTRs vs Relative abundances



Effsize: 0.05, small

Effsize: 0.03, small

Effsize: 0.10, moderate

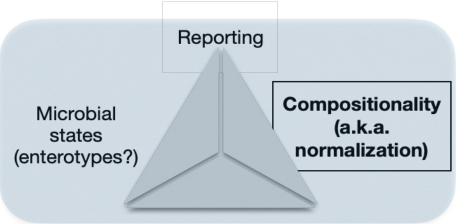
Effsize: 0.009, small

diagnosis

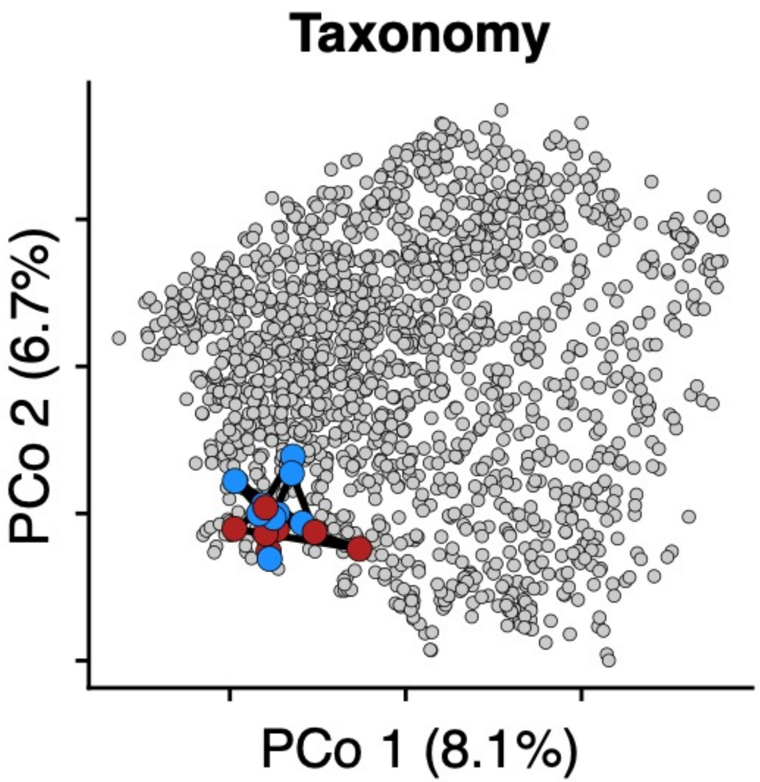
- ● CD
- ● nonIBD
- ● UC

Microbial analysis

Bacterial replication rates



MetaPhln relative abundances



Jason Lloyd-Price et. Al. 2019, Nature

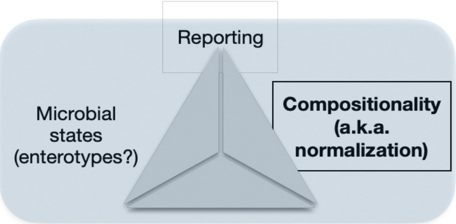
A ‘reference set’ of samples was constructed from **non-IBD subjects** by taking all samples

The **dysbiosis score** of a given sample was then defined as the median Bray–Curtis dissimilarity to this **reference sample set**

Divergent samples were identified – using a **dysbiotic threshold** at the 90th percentile of this score for non-IBD samples

Microbial analysis

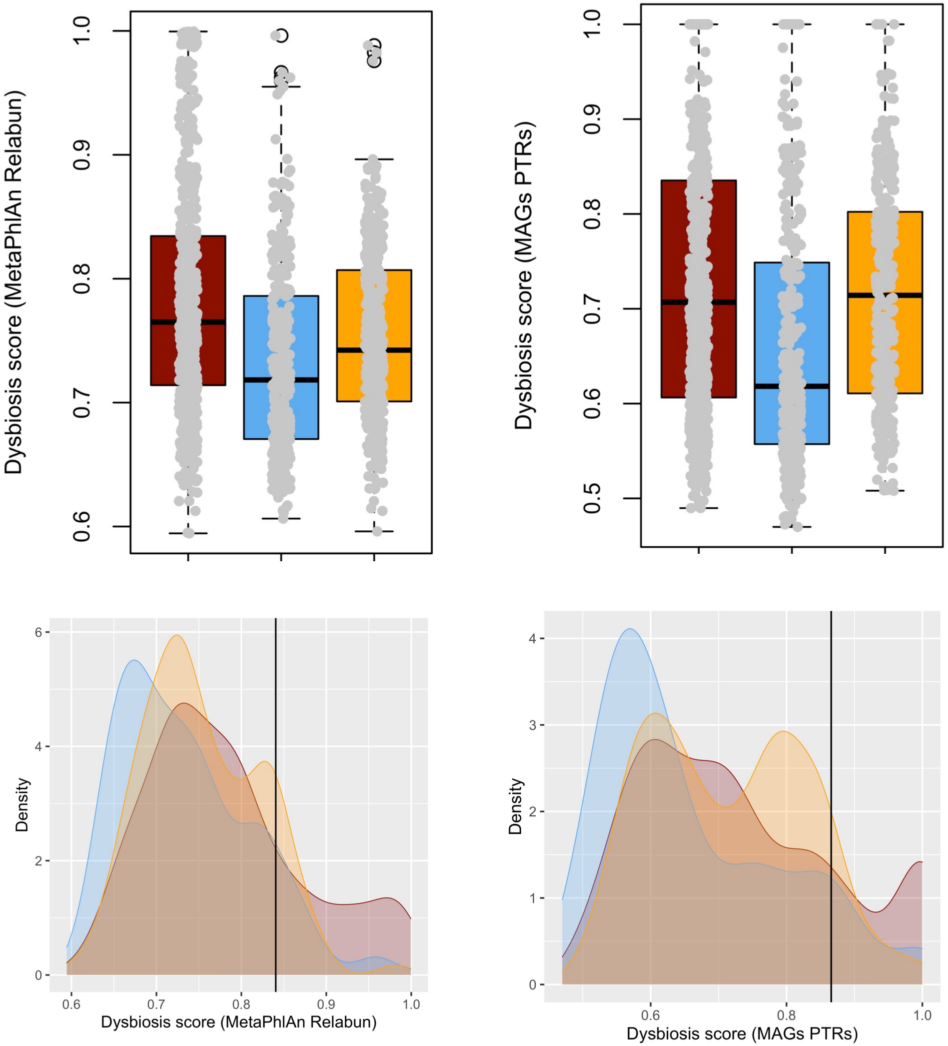
Bacterial replication rates



Reporting

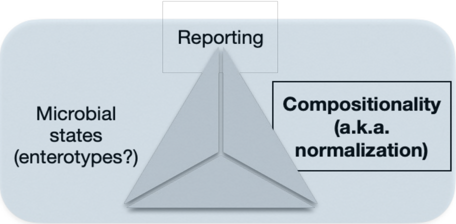
Microbial states (enterotypes?)

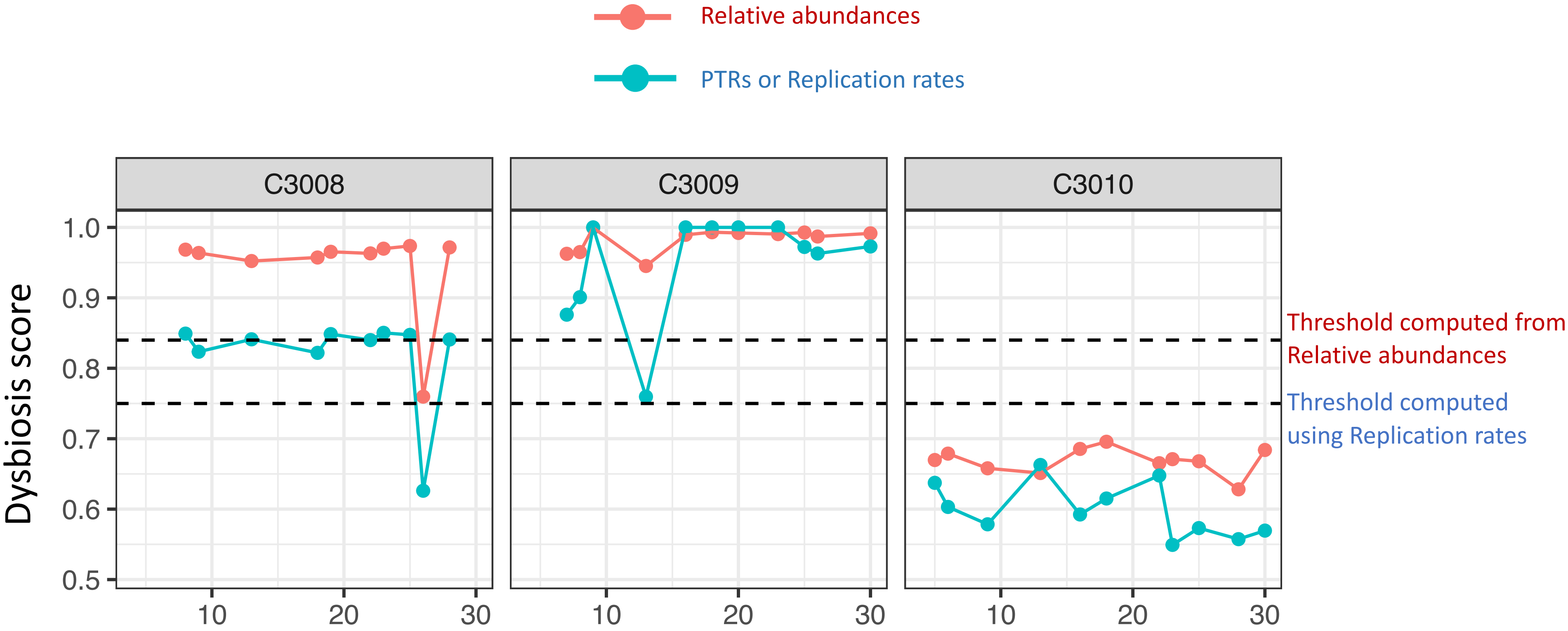
Compositionality (a.k.a. normalization)



Microbial analysis

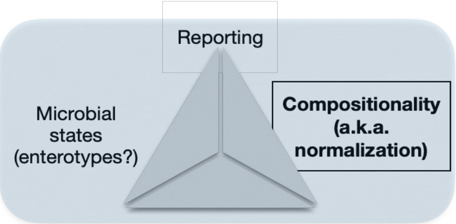
Bacterial replication rates





Microbial analysis

Bacterial replication rates


Reporting

Microbial states (enterotypes?)

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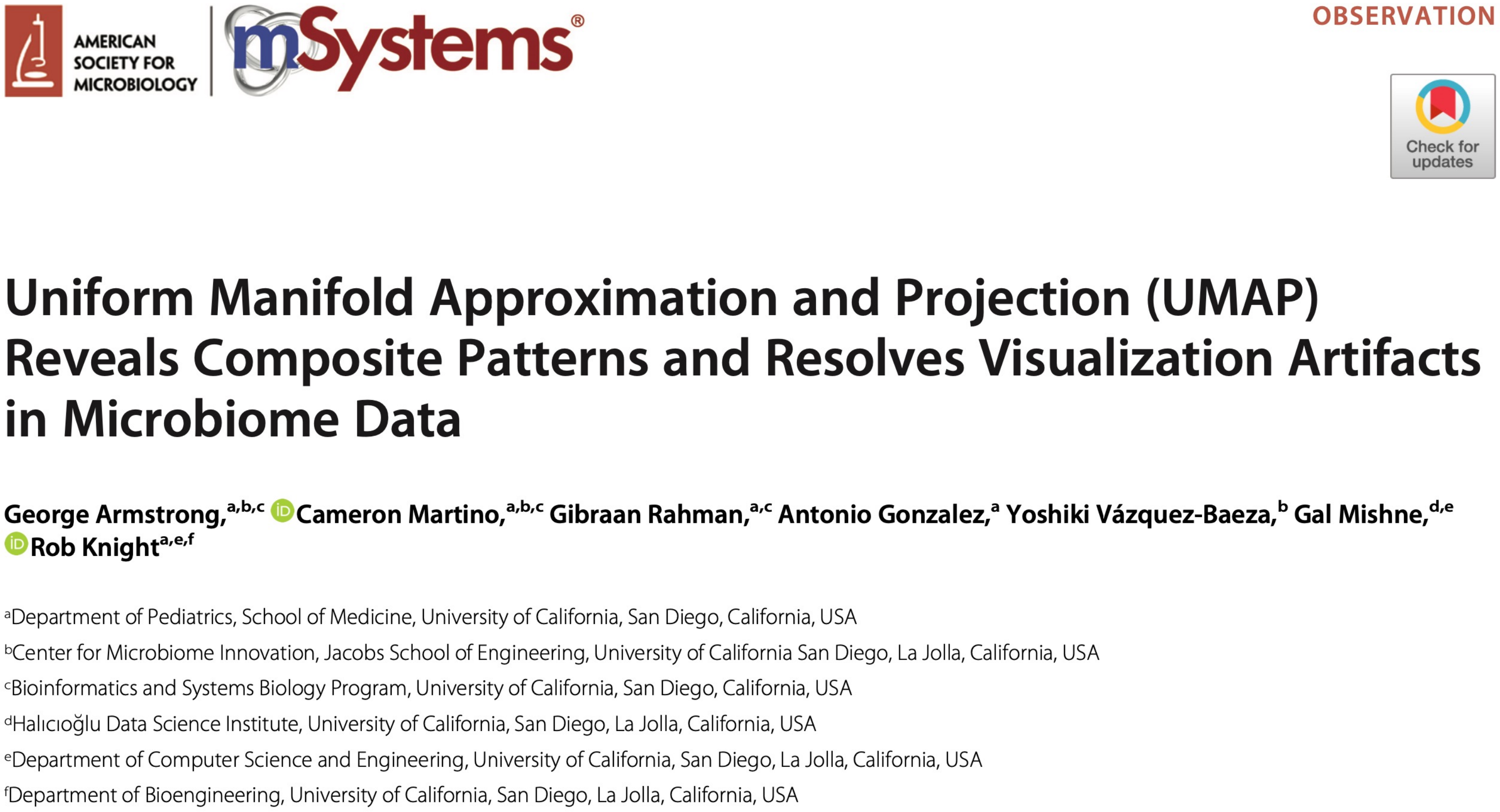
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OBSERVATION

Check for updates

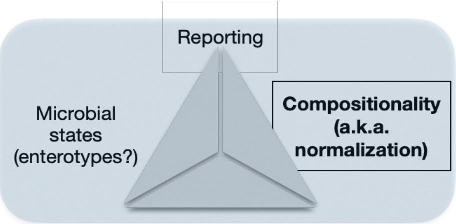
Uniform Manifold Approximation and Projection (UMAP) Reveals Composite Patterns and Resolves Visualization Artifacts in Microbiome Data

George Armstrong,[a,b,c] Cameron Martino,[a,b,c] Gibraan Rahman,[a,c] Antonio Gonzalez,[a] Yoshiki Vázquez-Baeza,[b] Gal Mishne,[d,e] Rob Knight[a,e,f]

[a]Department of Pediatrics, School of Medicine, University of California, San Diego, California, USA
[b]Center for Microbiome Innovation, Jacobs School of Engineering, University of California San Diego, La Jolla, California, USA
[c]Bioinformatics and Systems Biology Program, University of California, San Diego, California, USA
[d]Halıcıoğlu Data Science Institute, University of California, San Diego, La Jolla, California, USA
[e]Department of Computer Science and Engineering, University of California, San Diego, La Jolla, California, USA
[f]Department of Bioengineering, University of California, San Diego, La Jolla, California, USA

Microbial analysis

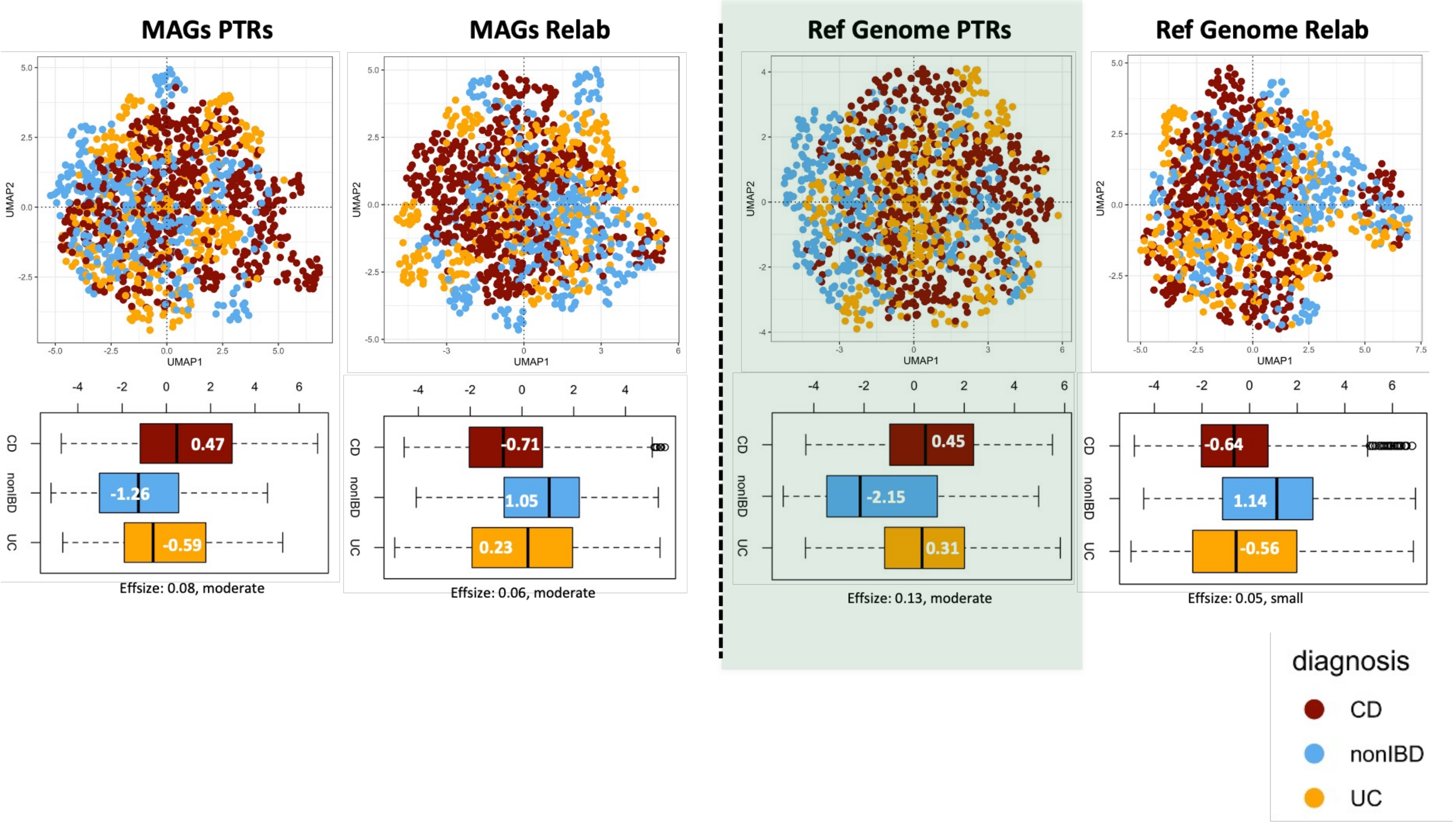
Bacterial replication rates



Reporting

Microbial states (enterotypes?)

Compositionality (a.k.a. normalization)



MAGs PTRs

Effsize: 0.08, moderate

MAGs Relab

Effsize: 0.06, moderate

Ref Genome PTRs

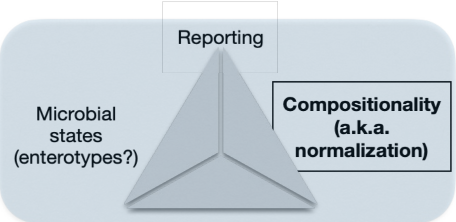
Effsize: 0.13, moderate

Ref Genome Relab

Effsize: 0.05, small

Microbial analysis

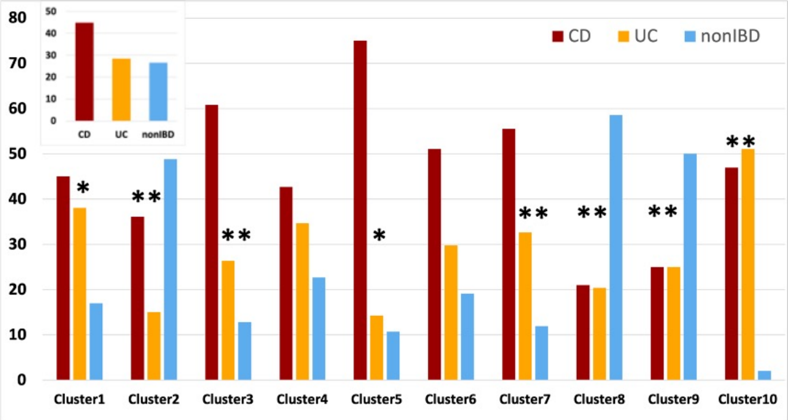
Bacterial replication rates

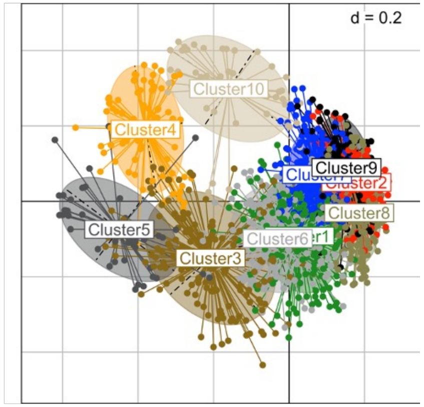

Reporting

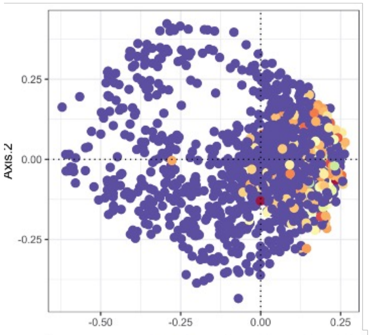
Microbial states (enterotypes?)

Compositionality (a.k.a. normalization)

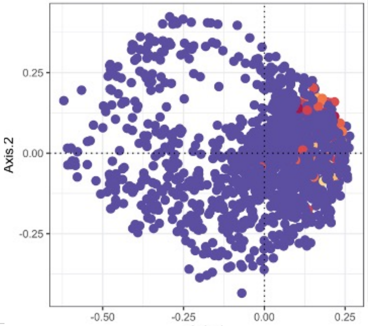
Unsupervised Clustering – Ref Genomes PTRs





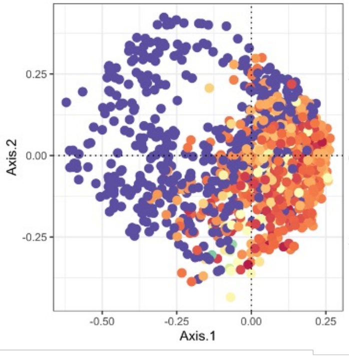


Cluster8- Oscillospiraceae sp.

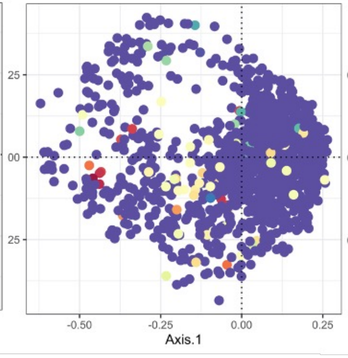


Cluster9- Phascolarctobacterium succinatutens

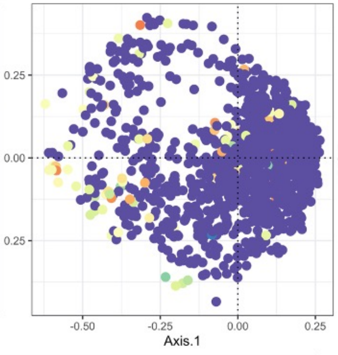
Cluster2- Bacteroides rodentium



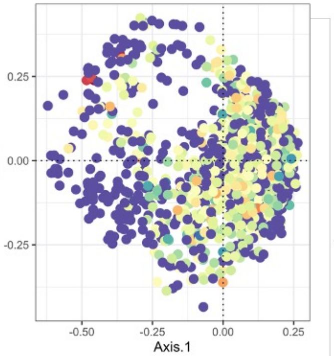
Cluster3- Bacteroides fragilis

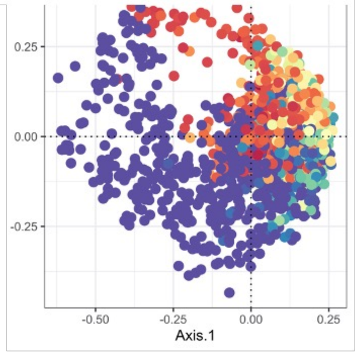


Cluster5- Klebsiella pneumoniae

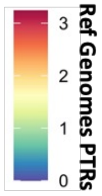


Cluster7- Oscillibacter sp.



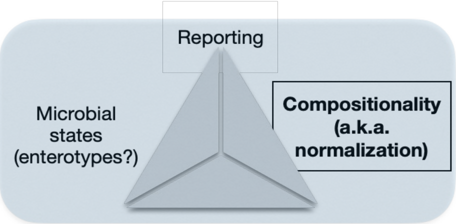


Cluster10- Faecalibacterium



Microbial analysis

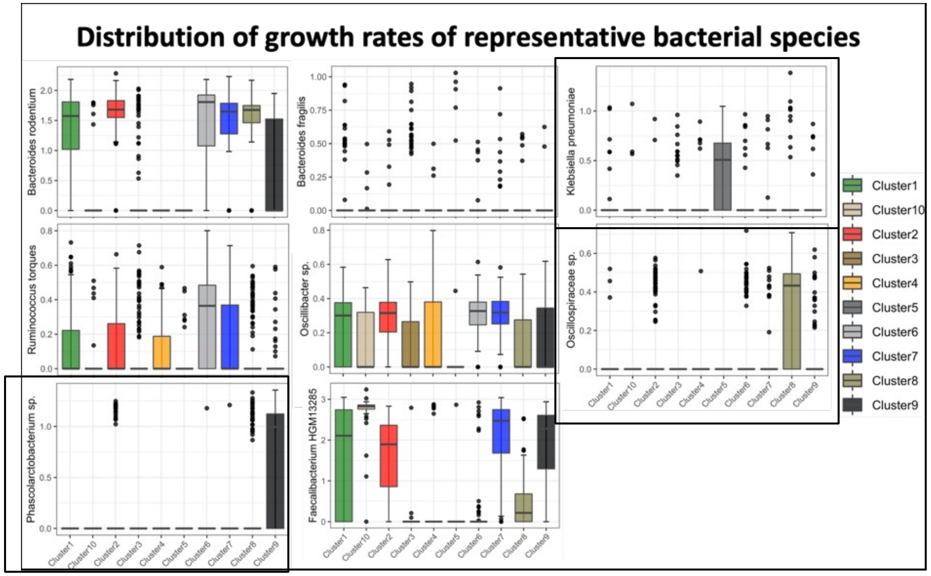
Bacterial replication rates



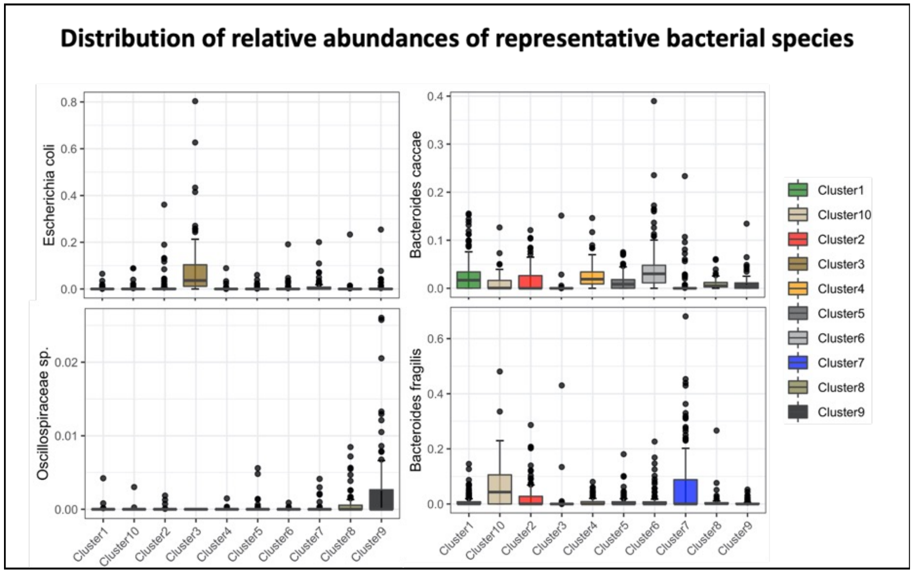
Reporting

Microbial states (enterotypes?)

Compositionality (a.k.a. normalization)



Distribution of growth rates of representative bacterial species

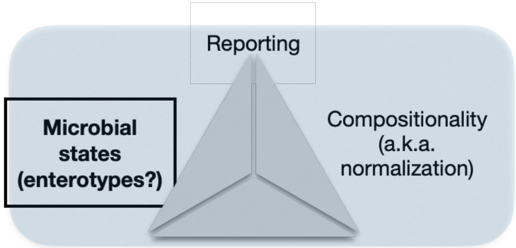


Distribution of relative abundances of representative bacterial species

Association of cluster specific taxonomic markers with disease phenotype – **Ongoing**

Microbial analysis

Microbial states – trajectory analysis



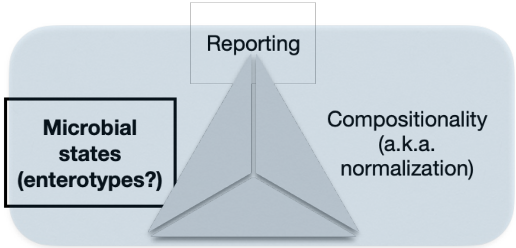
Reporting

Microbial states (enterotypes?)

Compositionality (a.k.a. normalization)

Microbial analysis

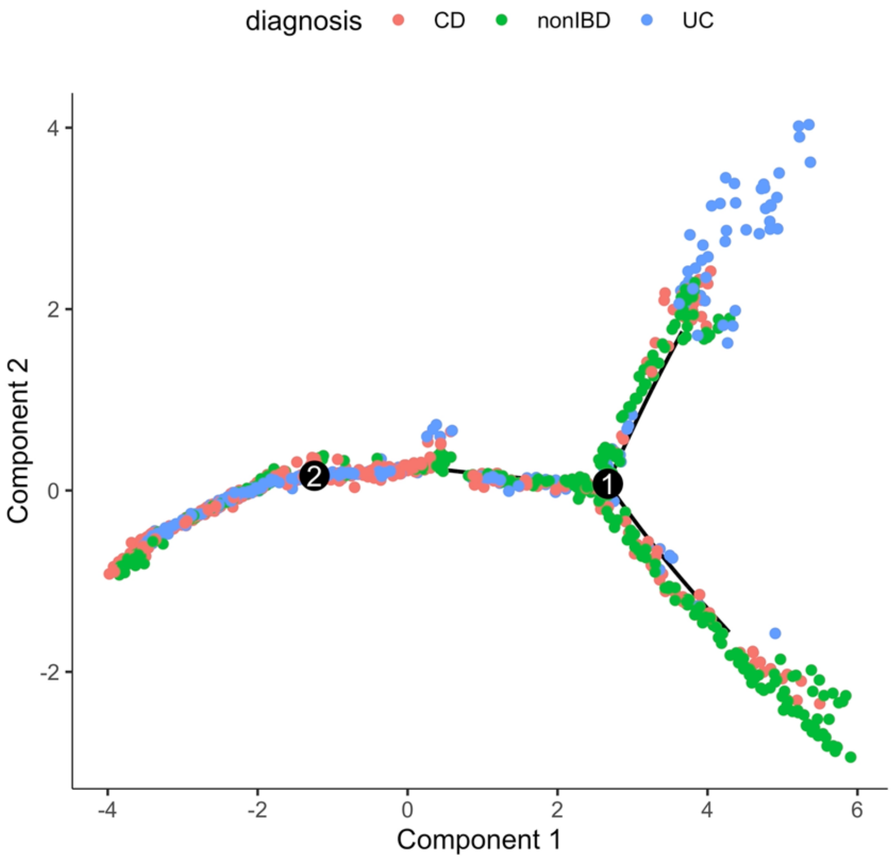
Microbial states – trajectory analysis

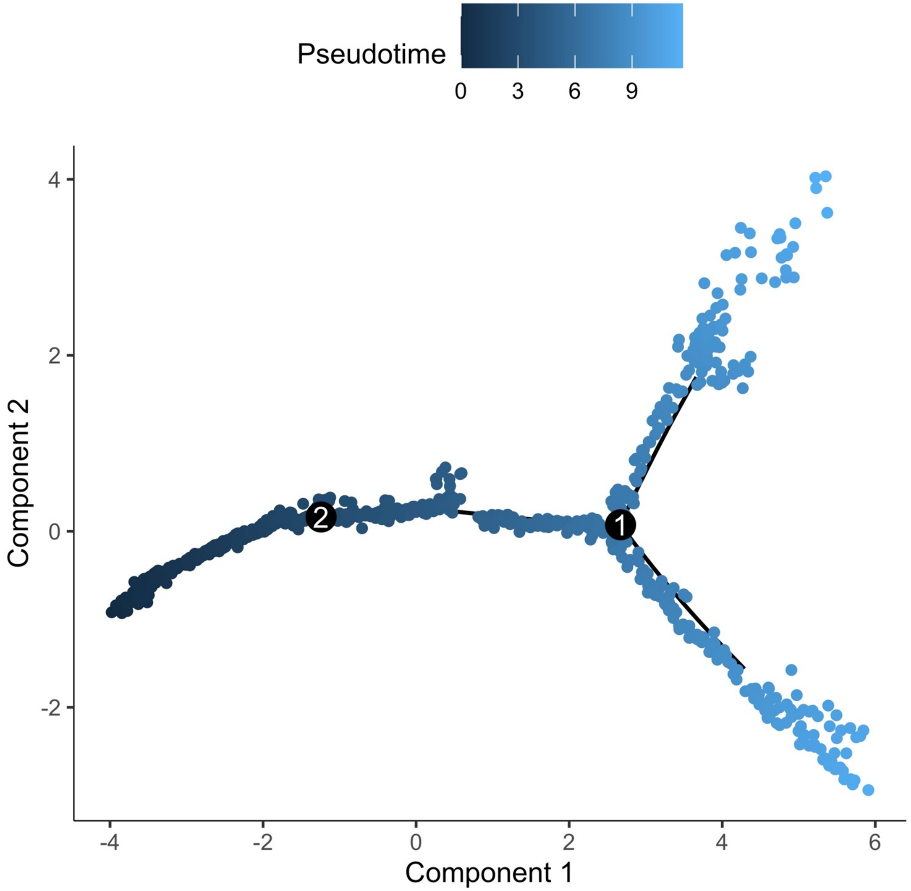


Reporting

Microbial states (enterotypes?)

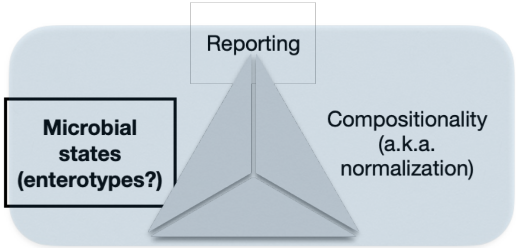
Compositionality (a.k.a. normalization)

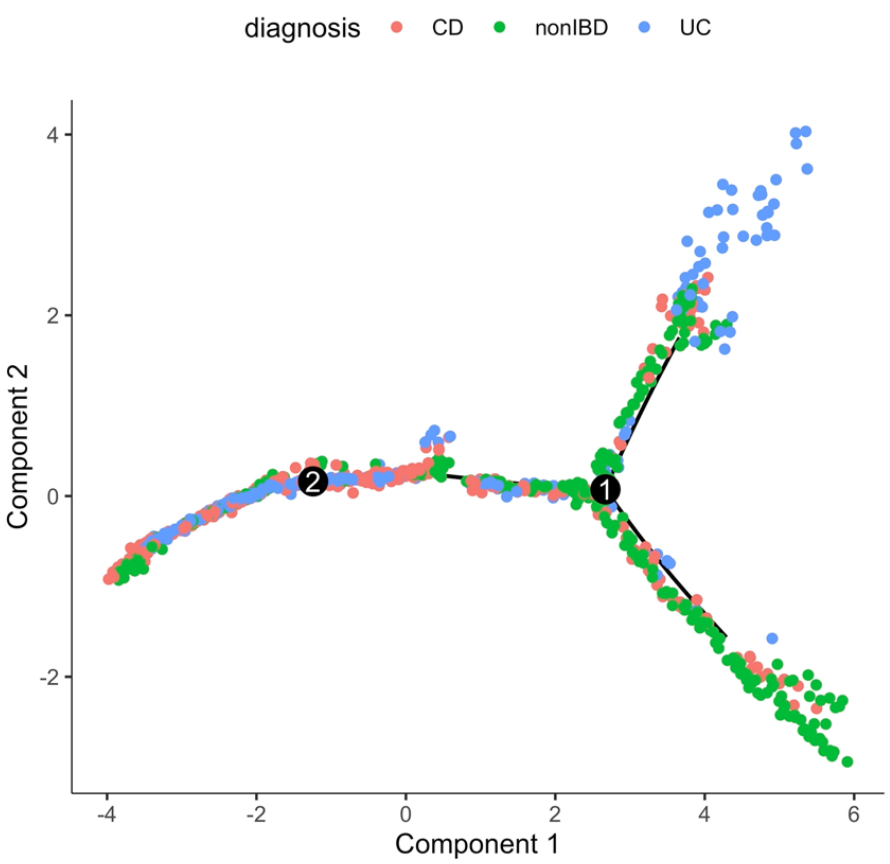


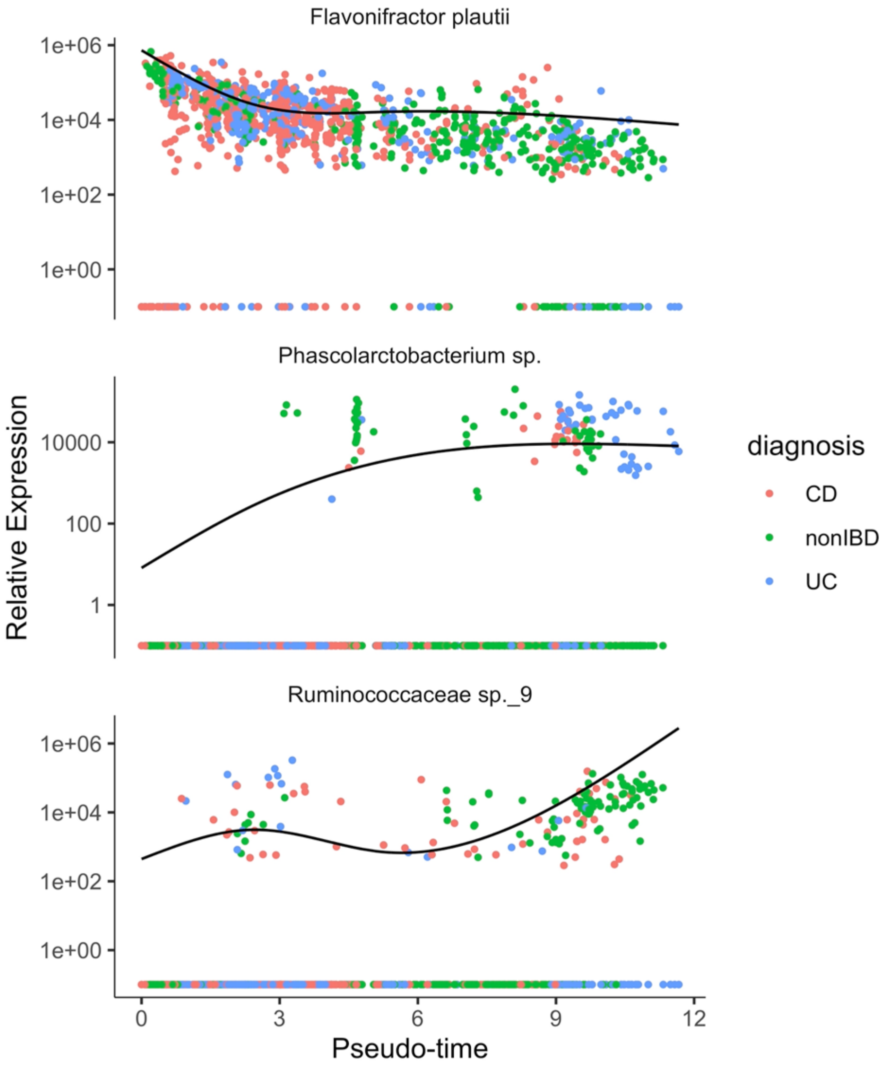


Microbial analysis

Microbial states – trajectory analysis

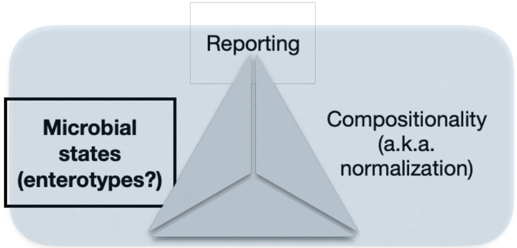


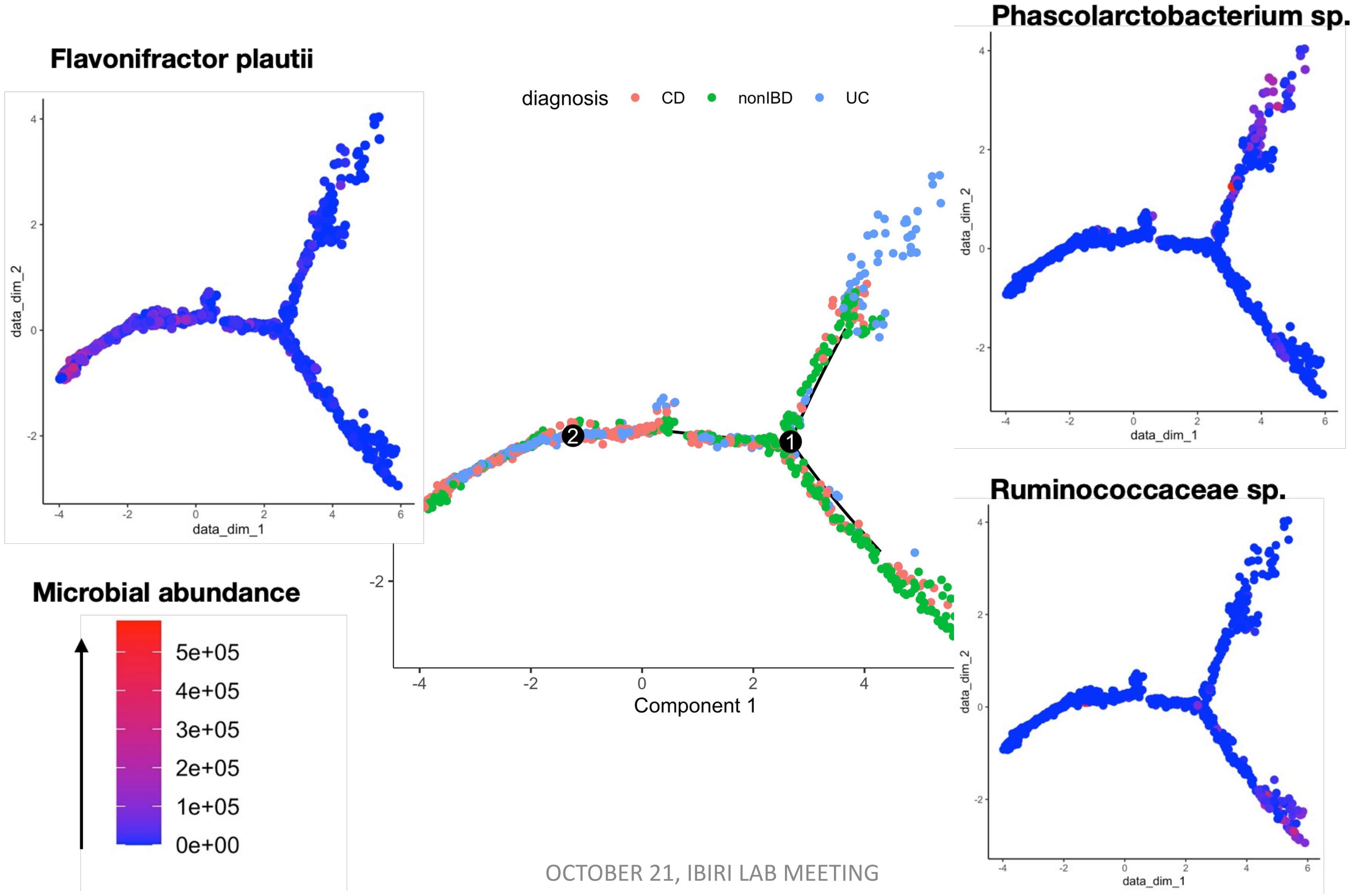




Microbial analysis

Microbial states – trajectory analysis





Flavonifractor plautii

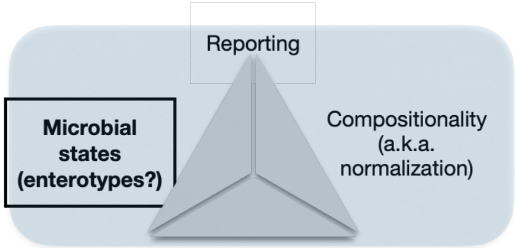
Phascolarctobacterium sp.

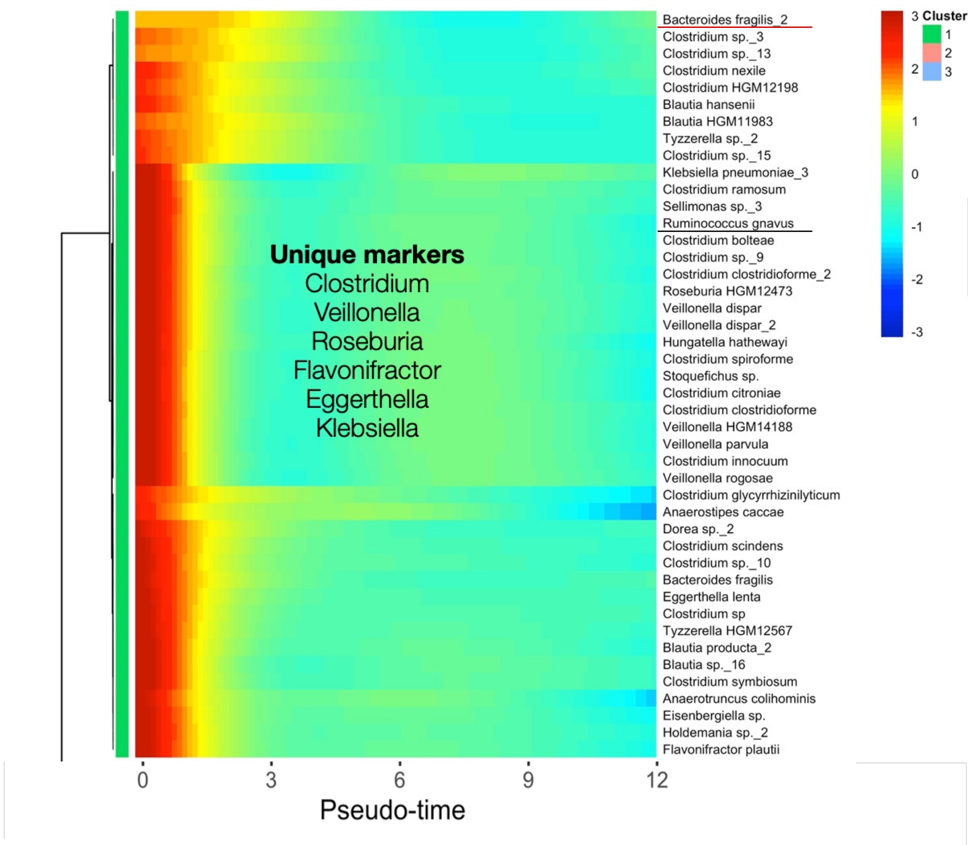
Ruminococcaceae sp.

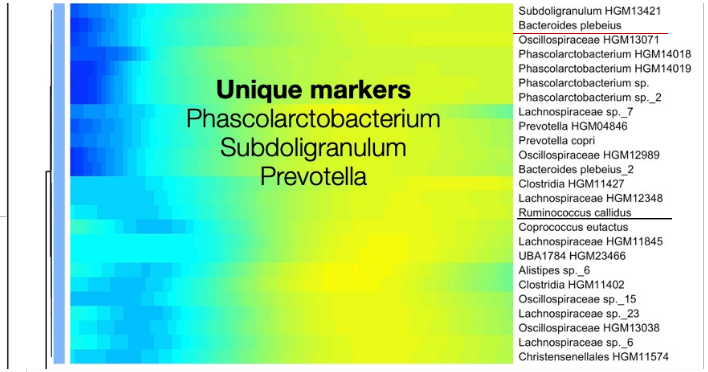
Microbial abundance

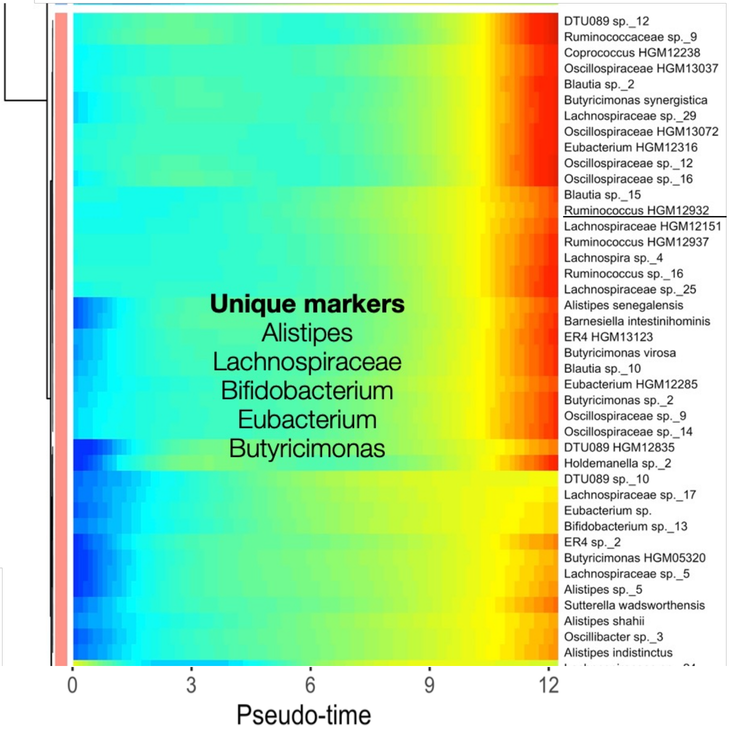
Microbial analysis

Microbial states – trajectory analysis



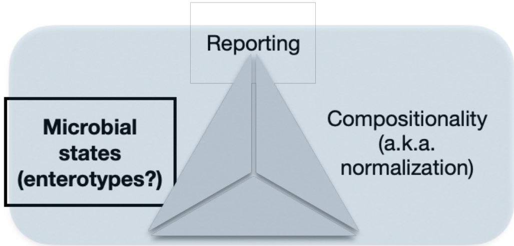






Microbial analysis

Microbial states – trajectory analysis



Summary

- It is possible to identify informative bacterial features under the constrains of low prevalence
- Identified features allow the definition of continuous enterotypes

Ongoing

- Longitudinal analyses of replication rates (association with phenotypes/treatments)
- More data (RISK, SPARC-IBD, 1000IBD, population data)

Acknowledgements

Dr. David Casero
Dr. Suzanne Devkota
Dr. Jonathan Braun
Dr. Ivan Vujkovic-Cvijin

Inflammatory Bowel & Immunobiology Research Institute Team (IBIRI)

Cedars Sinai