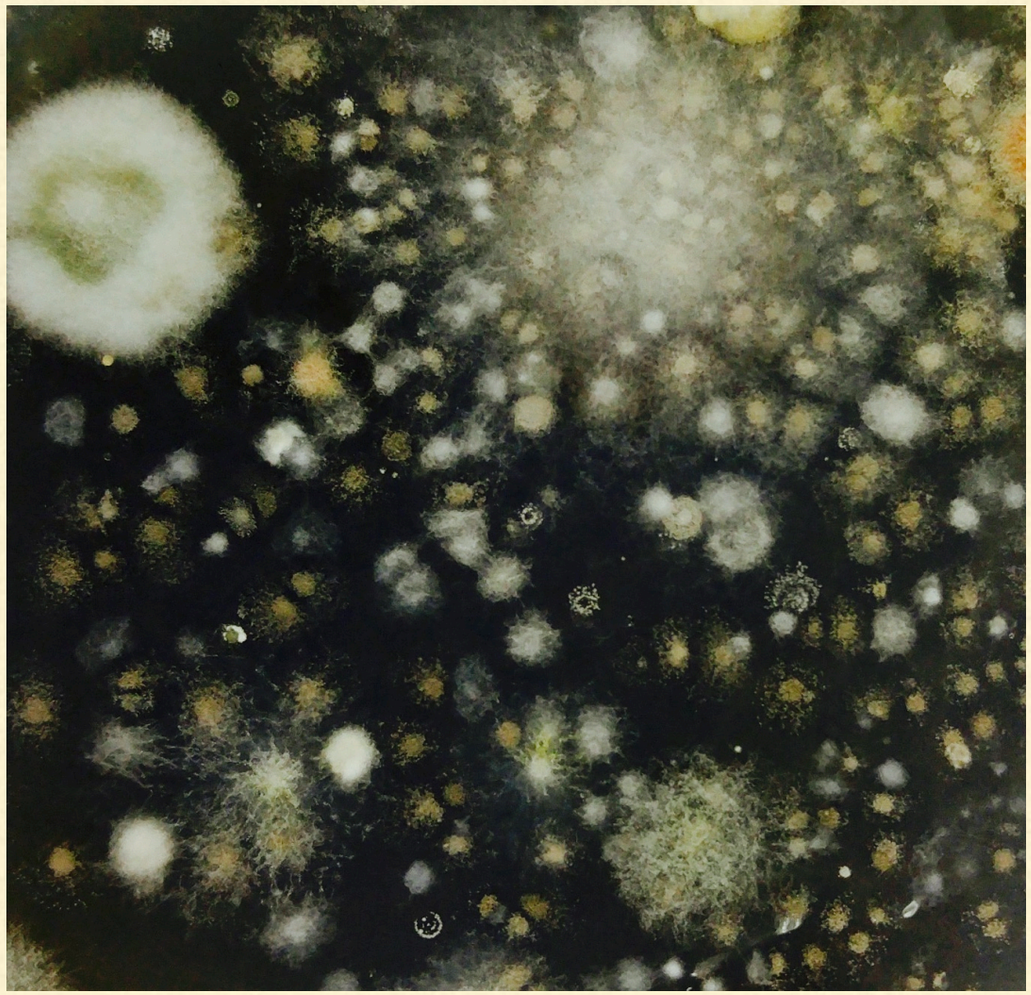
MEASUREMENT ERROR IN MICROBIOME DATA



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Slides available at github.com/adw96/presentations

Photo credit: T.D. Berry, Whitman lab, UW Madison

HIGH-THROUGHPUT SEQUENCING

- High-throughput sequencing (HTS) has given us high-resolution profiles of the genetic/genomic content of microbial communities
- Today's focus:

Are we *interpreting* and *analysing* HTS data correctly?

MICROBIOME DATA FROM HTS

- At the end of the sample preparation + sequencing + bioinformatics pipeline, we often have data as counts or relative abundances

Counts

	G.vaginalis	A.vaginae	L.crispatus	L.iners
s1-1	1	1	13670	0
s1-2	1650	2930	22645	0
s1-3	1514	2073	3	1
s1-4	0	2217	16037	0
s1-5	754	0	3	0
s1-6	1	500	2	9866

Relative abundances

	G.vaginalis	A.vaginae	L.crispatus	L.iners
s1-1	0.0000	0.0000	0.6016	0.0000
s1-2	0.0606	0.1076	0.8316	0.0000
s1-3	0.0782	0.1071	0.0002	0.0001
s1-4	0.0000	0.1117	0.8081	0.0000
s1-5	0.0489	0.0000	0.0002	0.0000
s1-6	0.0001	0.0340	0.0001	0.6718

CURRENT PARADIGM

- *Widely-held belief:*
  - Within a study, we can compare the relative abundance of taxa across samples
    - Directly: DeSeq2, LefSE, corncob…
    - Indirectly: Diversity (Shannon, Simpson, UniFrac), networks…

Michael McLaren (NCSU)

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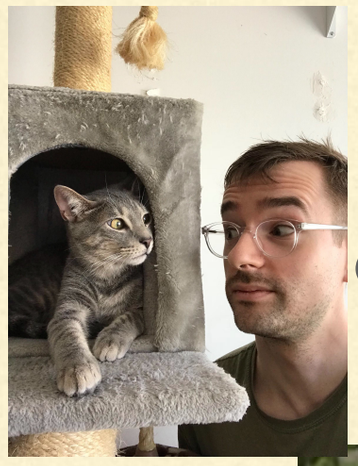
RESEARCH ARTICLE

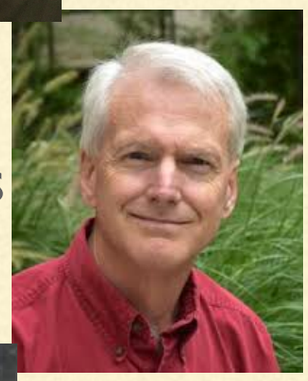
Consistent and correctable bias in metagenomic sequencing experiments

Michael R McLaren[1], Amy D Willis[2], Benjamin J Callahan[1,3]*

Ben Callahan (NCSU)



David Clausen (UW)



Jim Hughes (UW)

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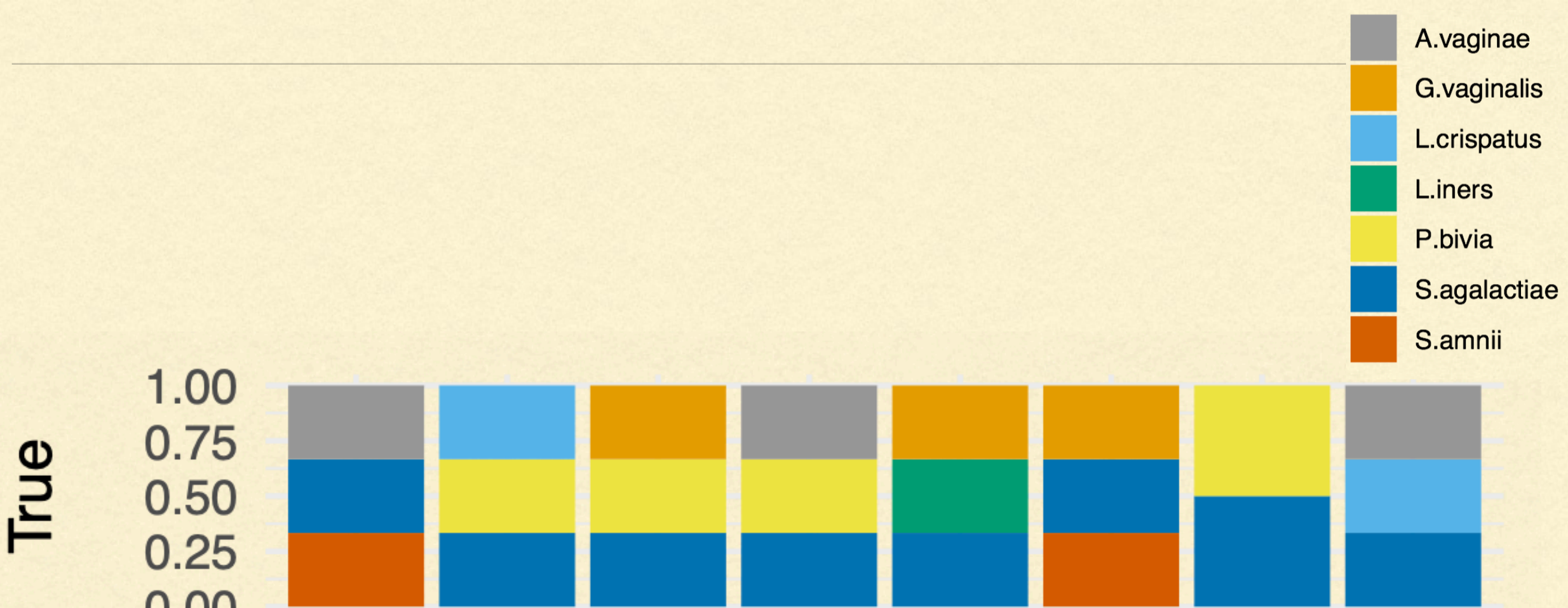
A multi-view model for relative and absolute microbial abundances

Brian D. Williamson, James P. Hughes, Amy D. Willis

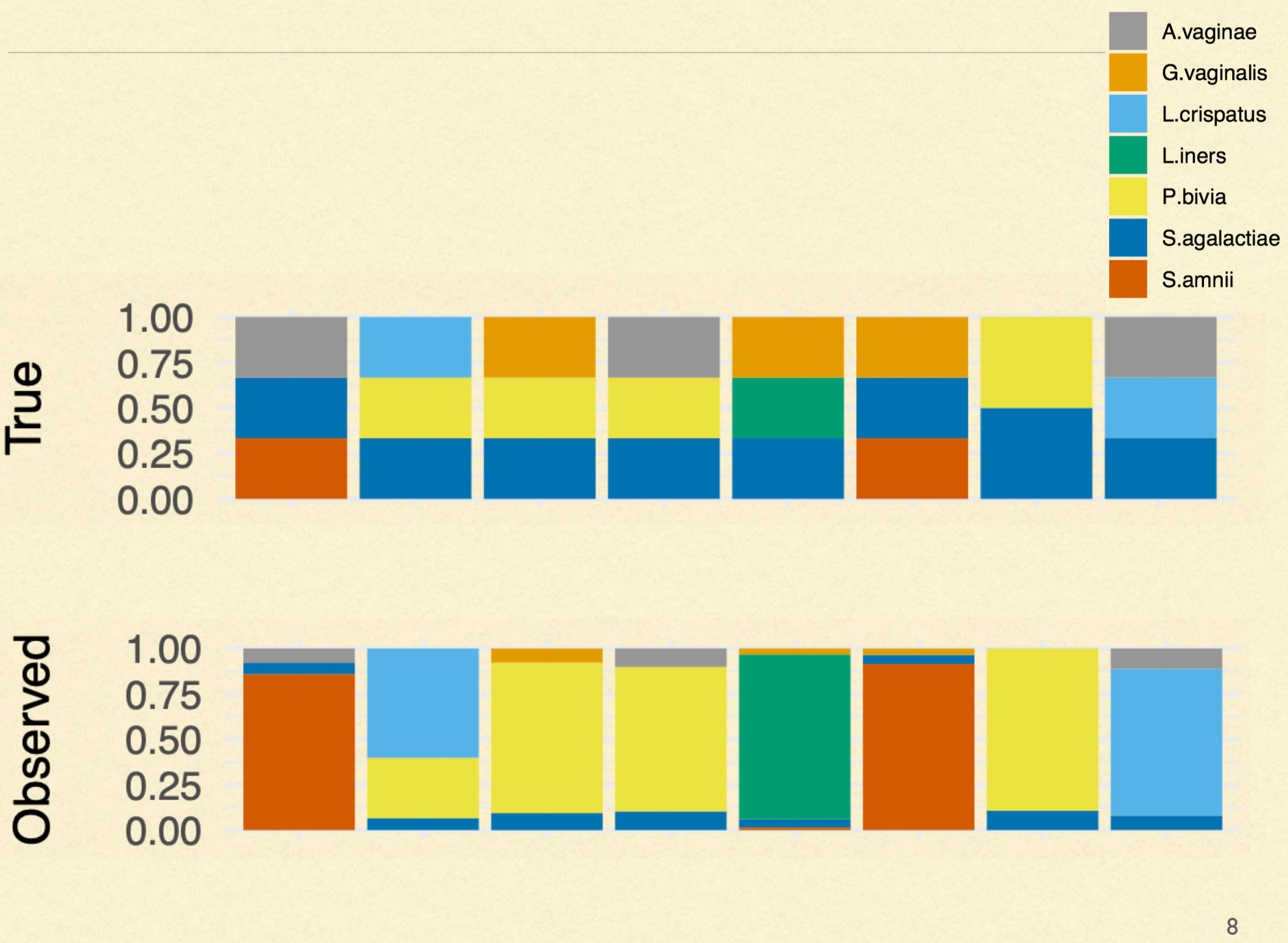
doi: https://doi.org/10.1101/761486

EVALUATING RELATIVE ABUNDANCE DATA

- Mock communities
- Artificially constructed communities of known composition
- Commonly used to benchmark sequencing and bioinformatics pipelines



Data from Brooks *et al* (2015) BMC Microbiology



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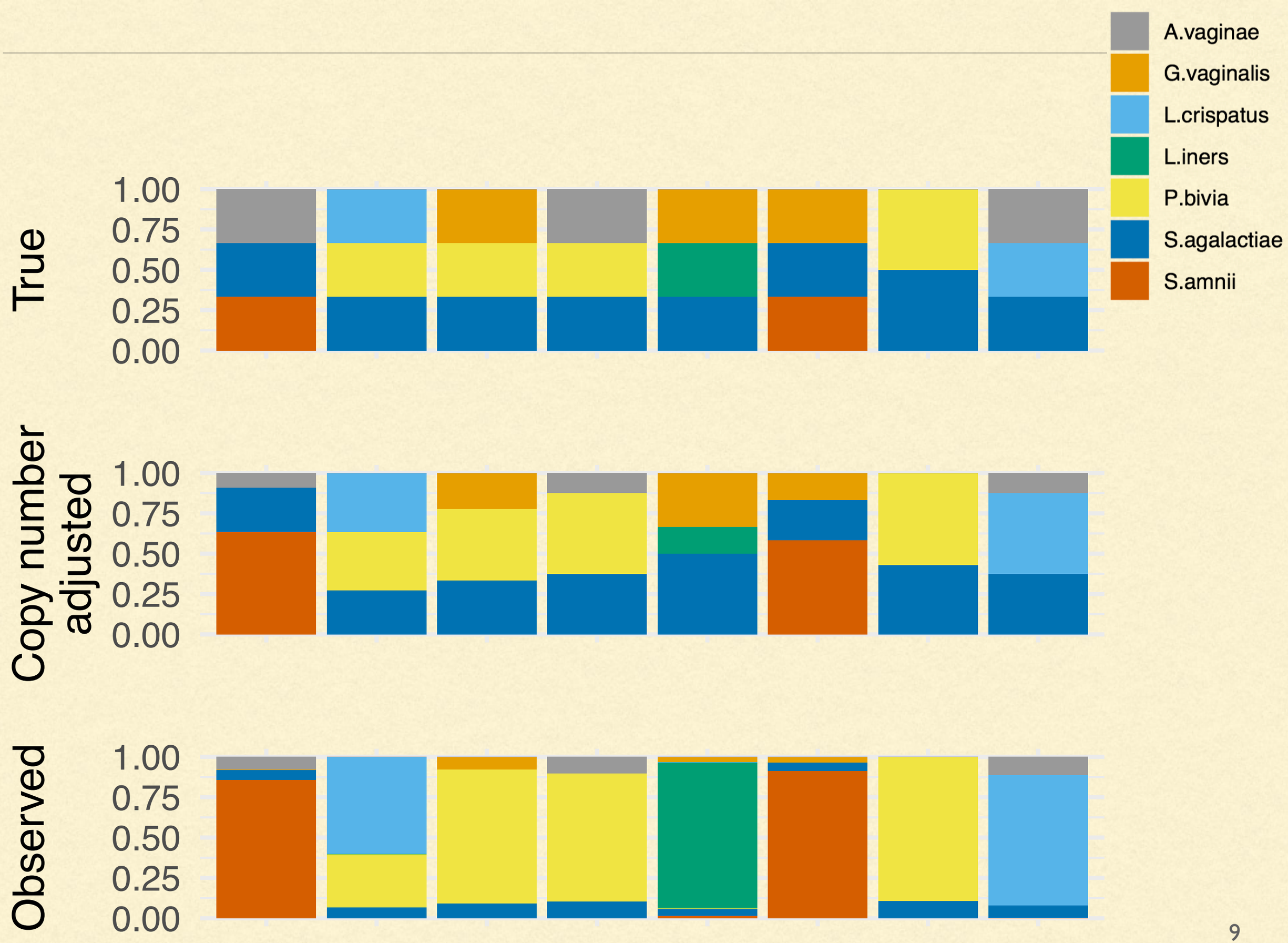

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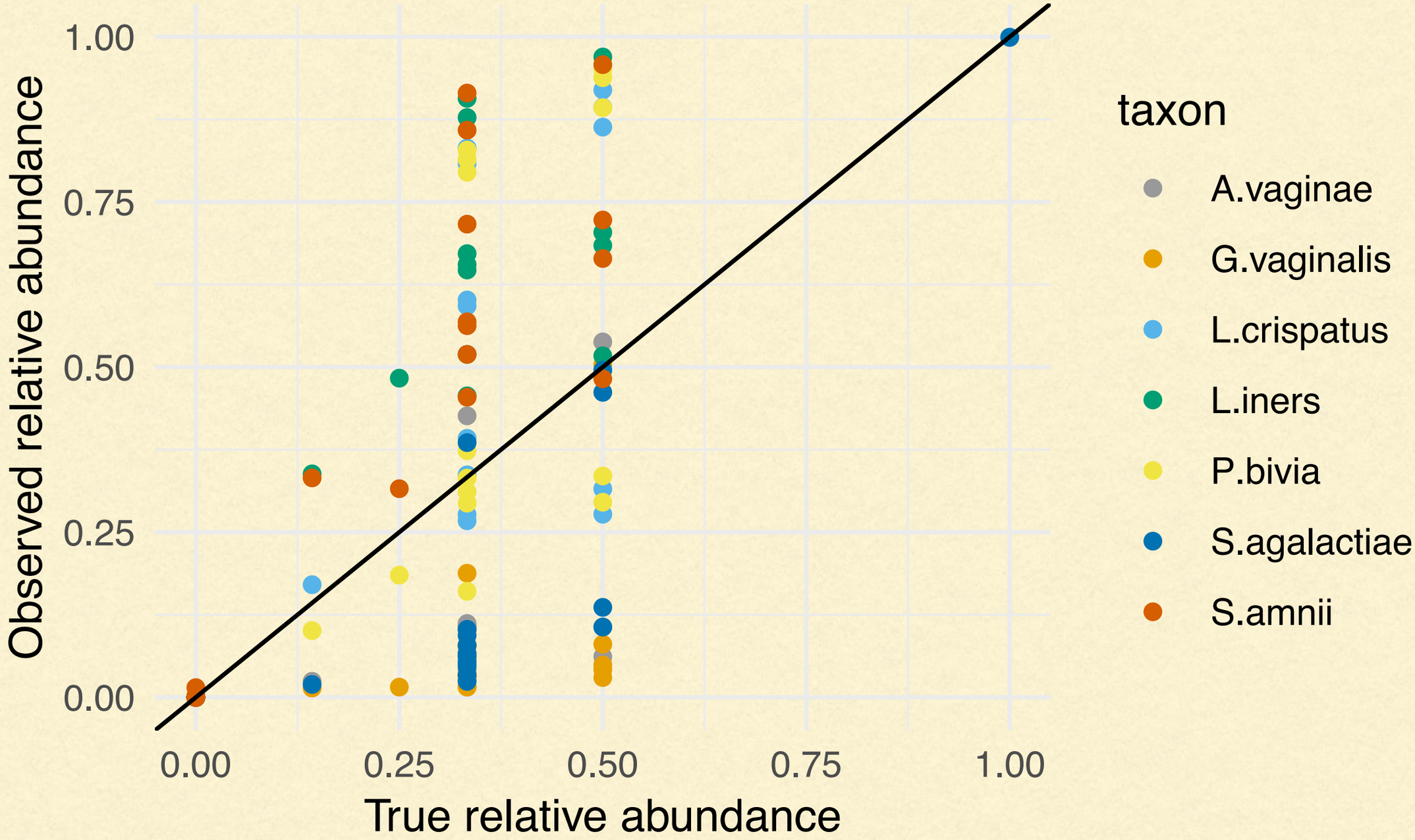
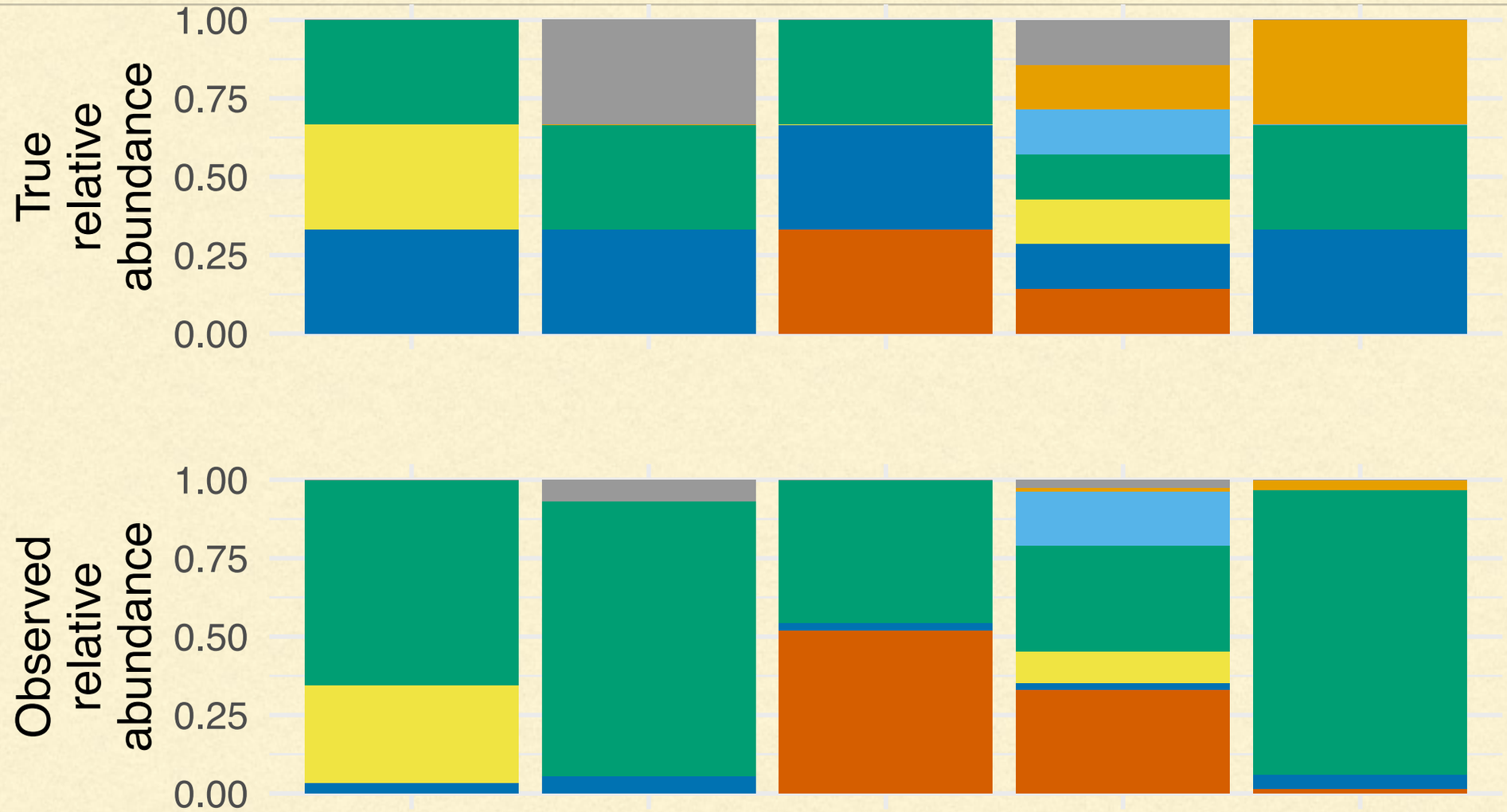
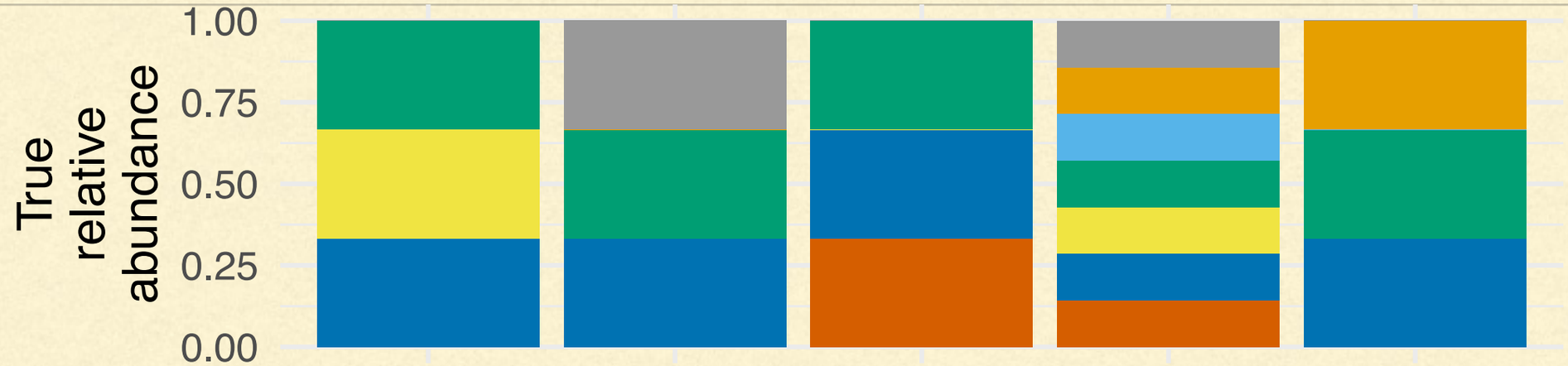
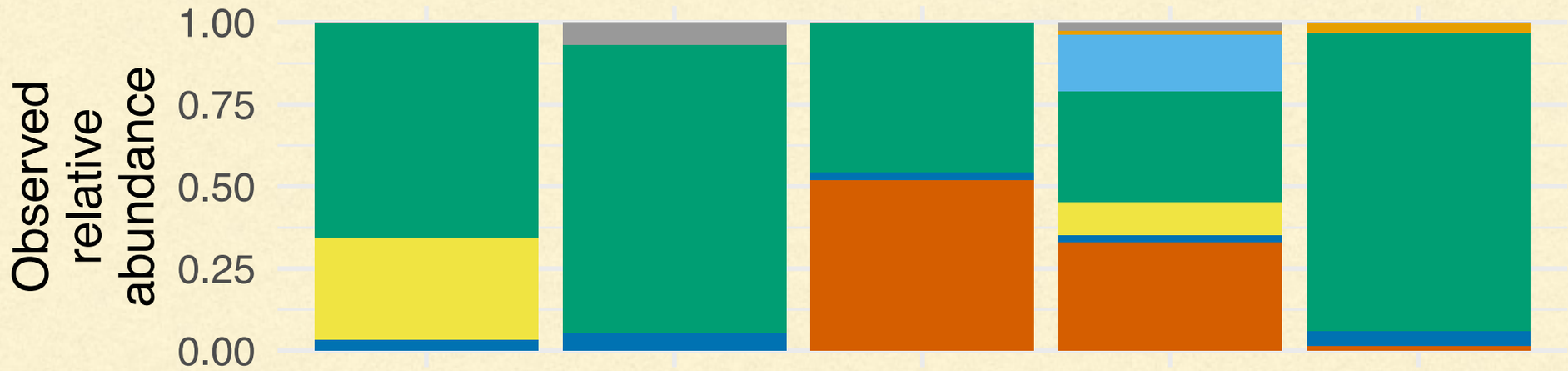

1.00
0.75
0.50
0.25
0.00
Observed relative abundance
0.00
0.25
0.50
0.75
1.00
True relative abundance
taxon
A.vaginae
G.vaginalis
L.crispatus
L.iners
P.bivia
S.agalactiae
S.amnii

WHAT PATTERNS CAN WE FIND?



WHAT PATTERNS CAN WE FIND?





L.iners/S.agalactiae	19.5	16.1	18.9	17.4	19.9

mean = 18.3, std dev = 1.6

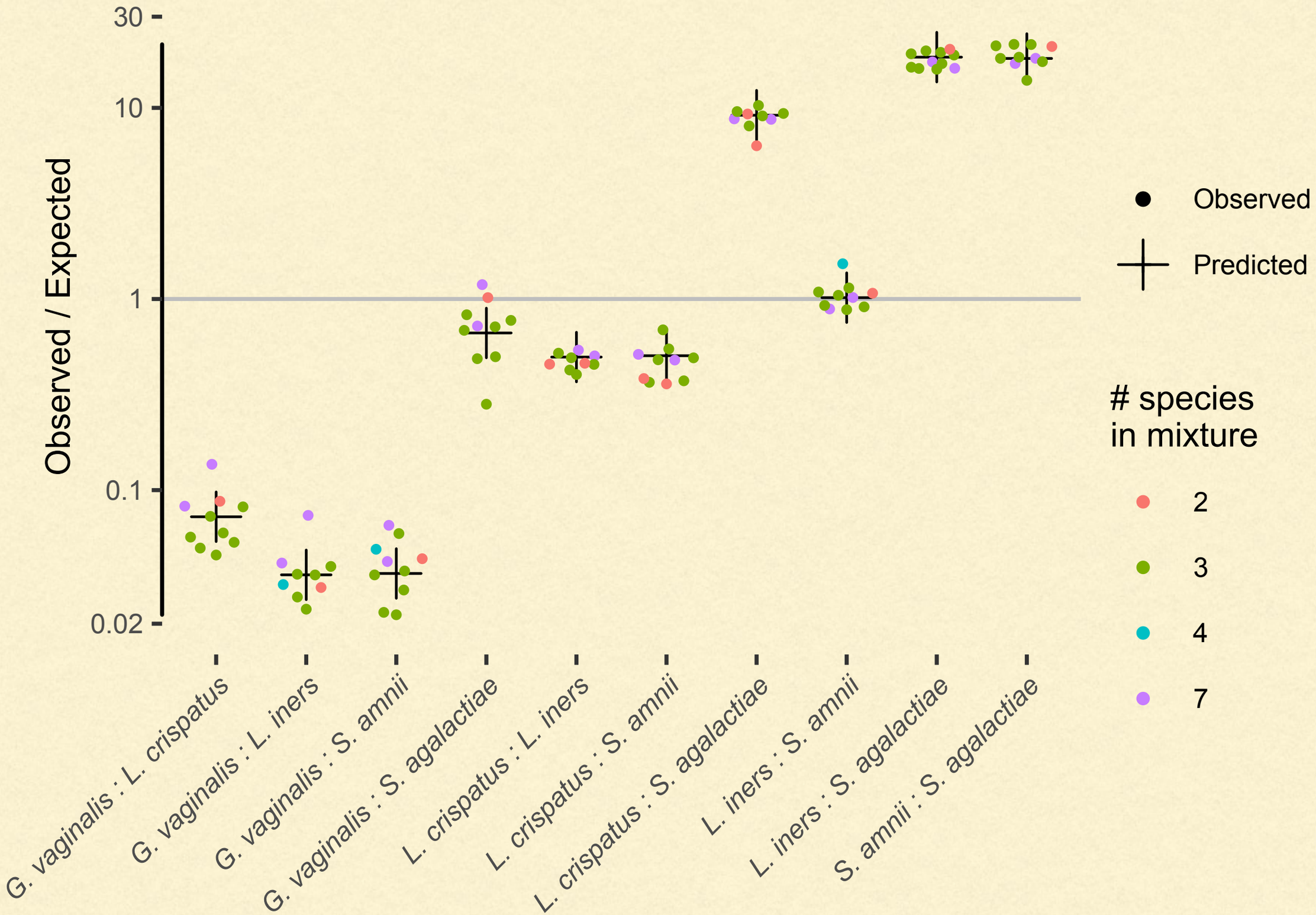


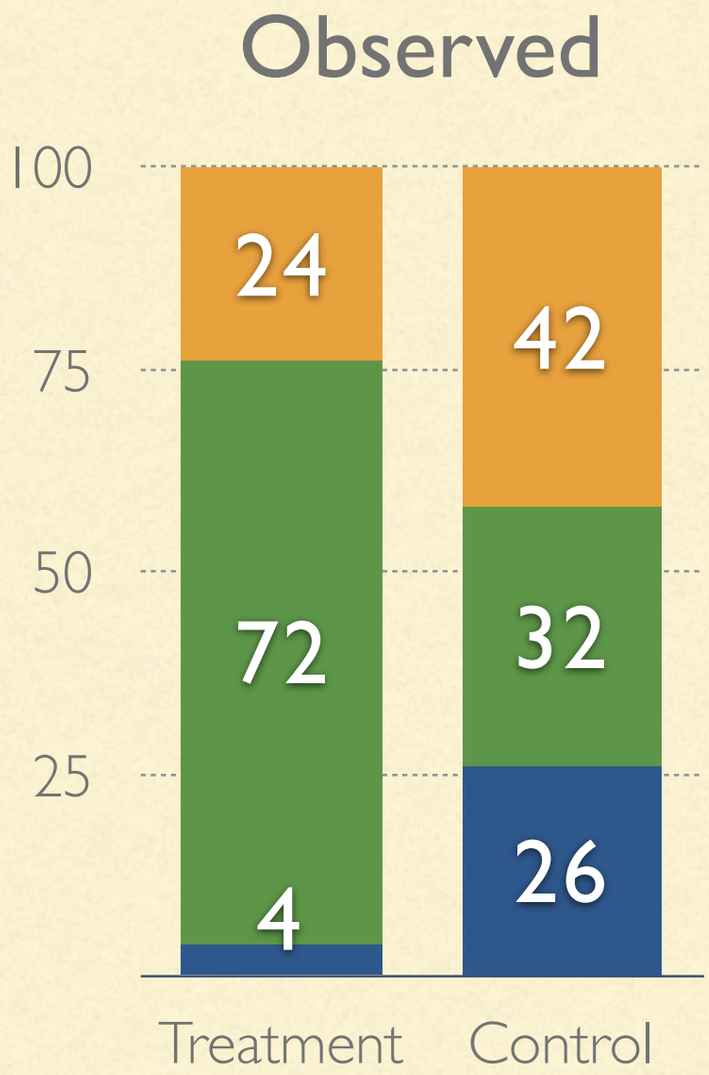
Figure from McLaren et al., 2019, *eLife*

MEASUREMENT ERROR MODEL

- This data suggests the following model:

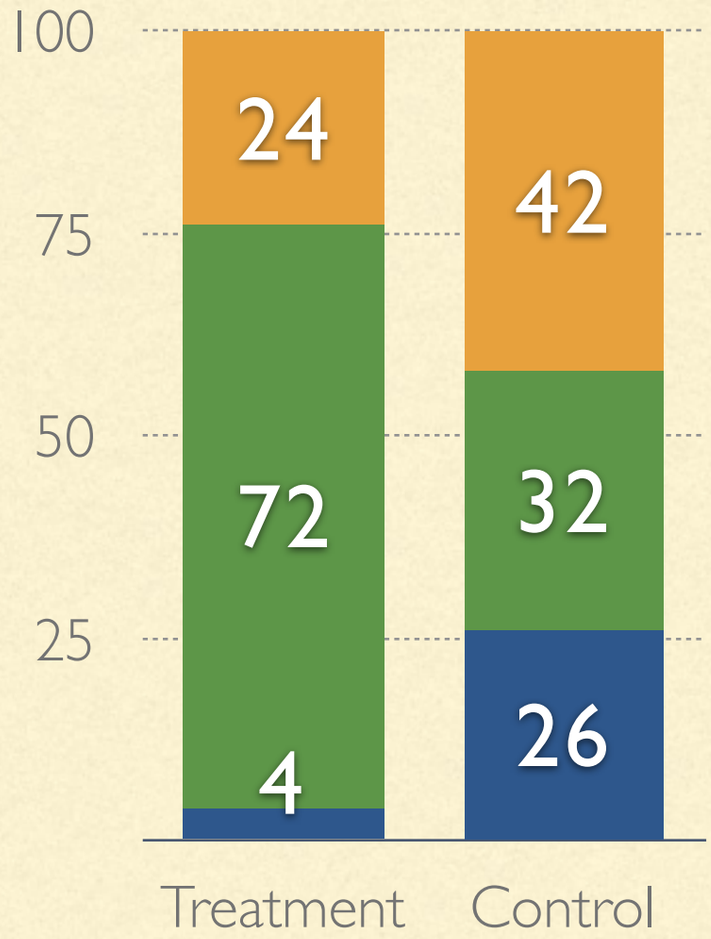
Observed relative abundance $\propto$ True relative abundance $\times$ Taxon-specific efficiencies

Expected value of $\dfrac{W_{ij}}{\sum_k W_{ik}} = \dfrac{p_{ij}e_j}{\sum_k p_{ik}e_k}$

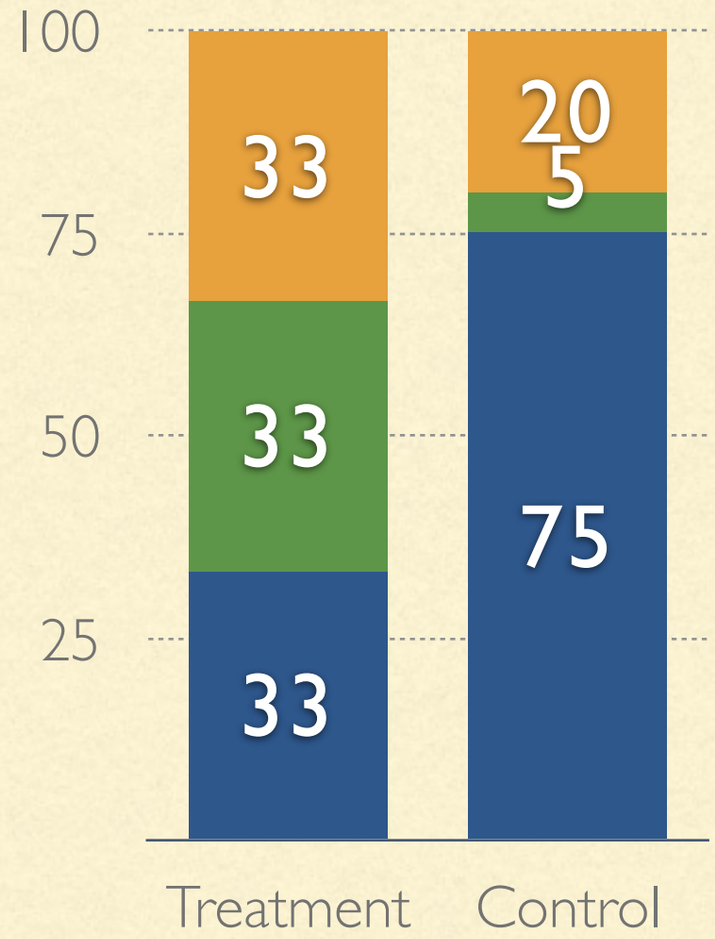


- A tempting conclusion:
  - The relative abundance of **taxon orange** *decreased* in the Treatment sample (left) compared to the Control sample (right)

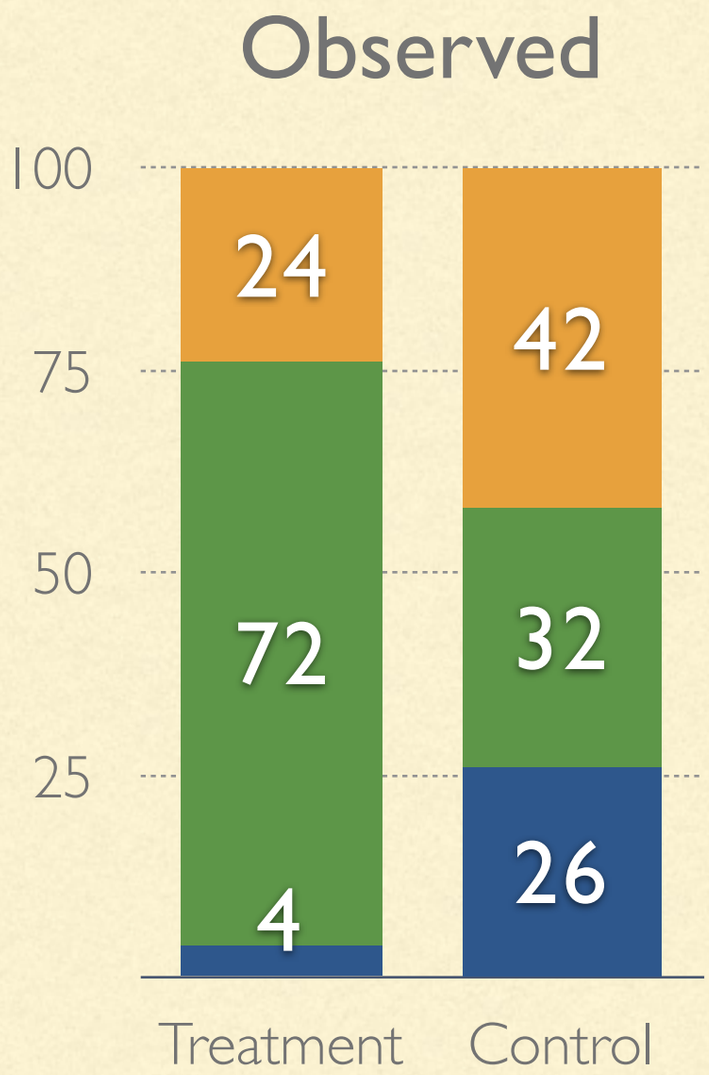
Observed

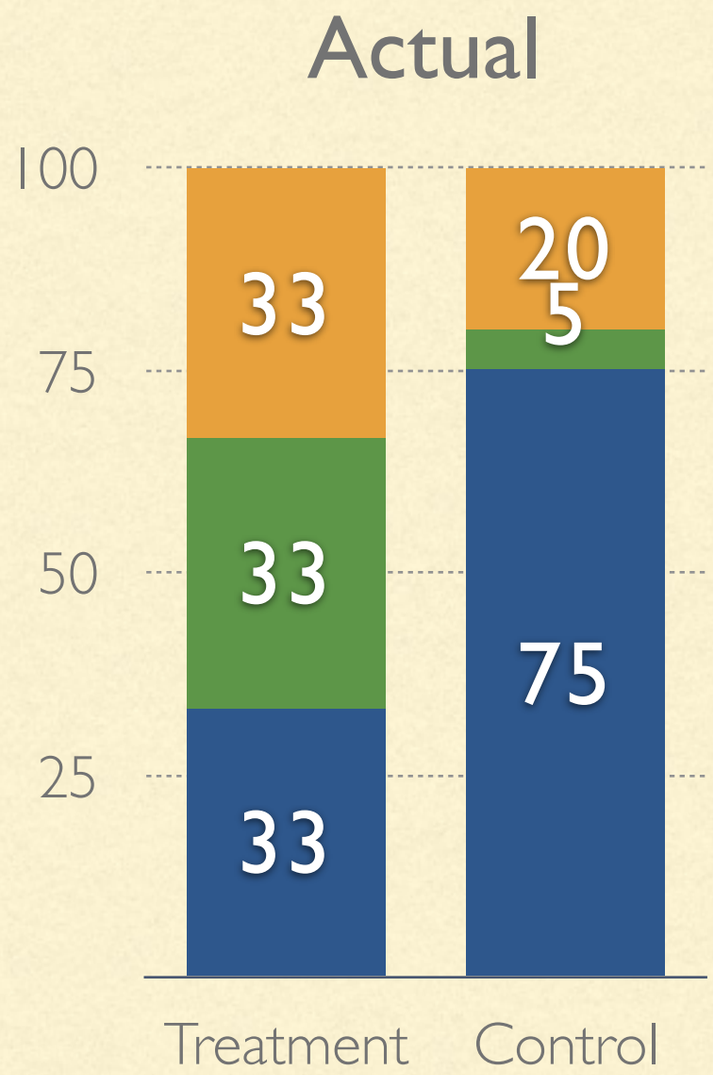


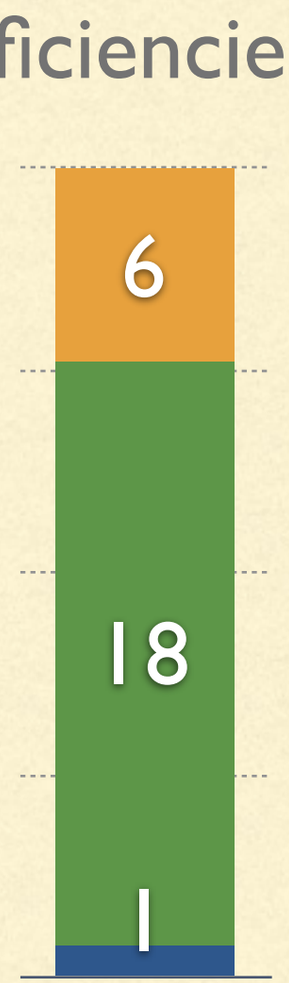
Actual



- In fact, the relative abundance of **taxon orange** *increased* in the Treatment sample compared to the Control sample

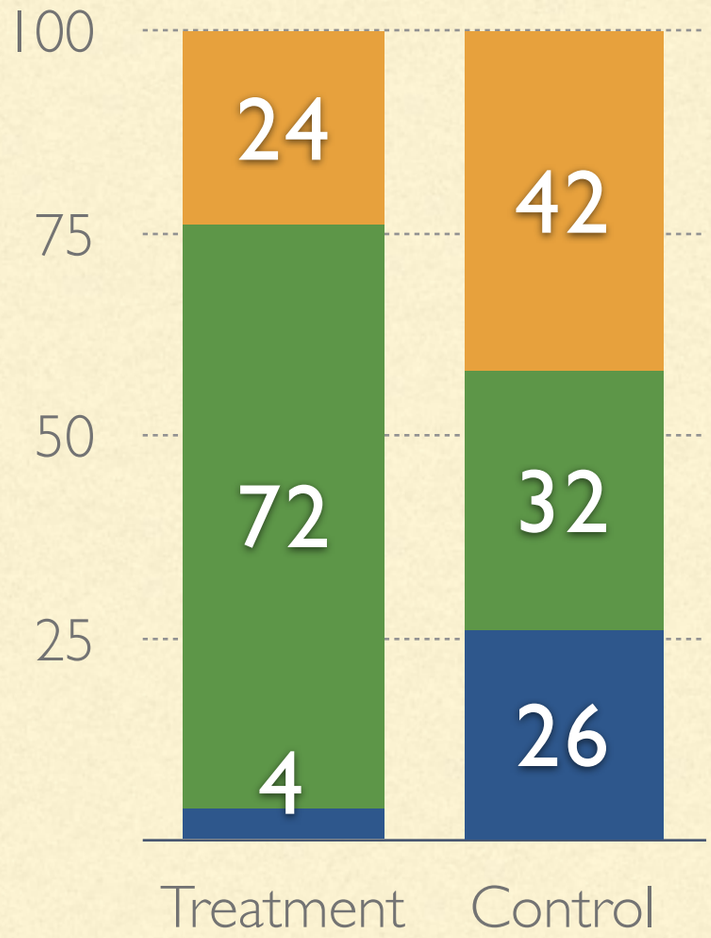




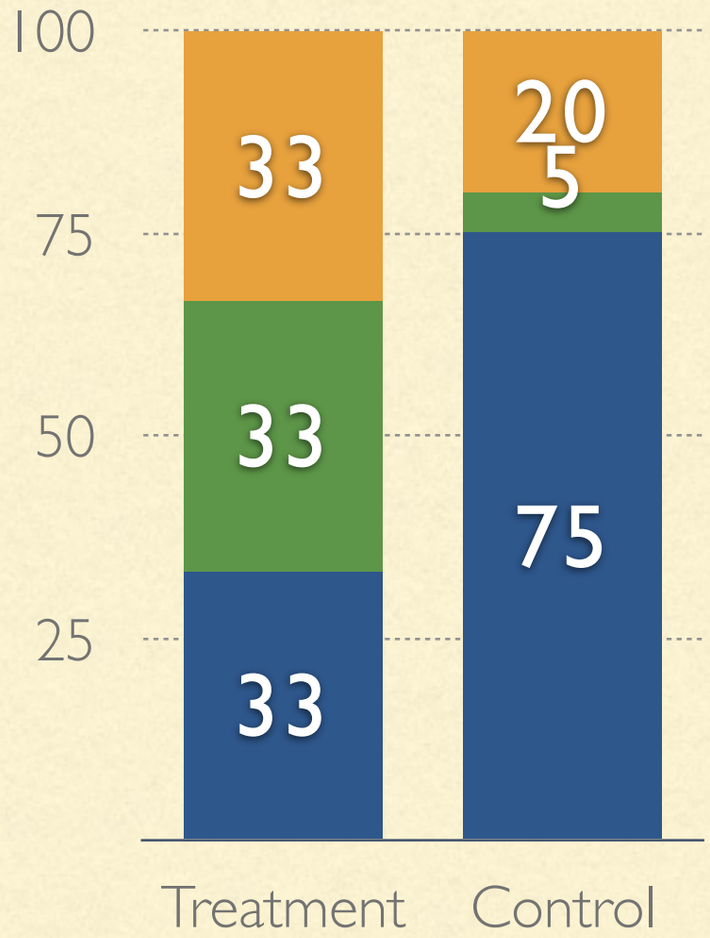


- In fact, the relative abundance of **taxon orange** *increased* in the Treatment sample compared to the Control sample

Observed



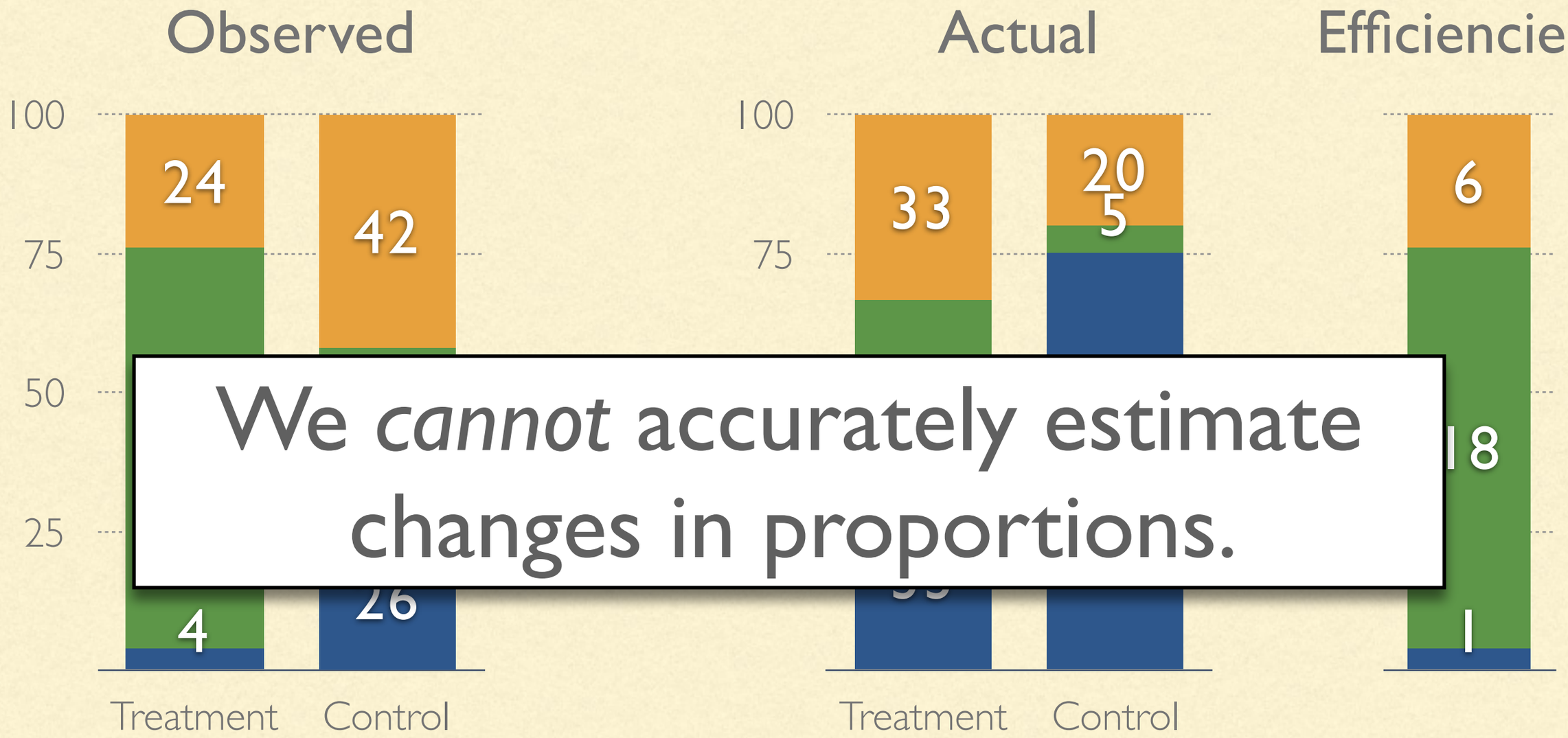
Actual



Efficiencies

- **Taxon green** is high efficiency; its abundance increased (Ctrl vs Tmt). Additionally, **taxon blue** is low efficiency, and its abundance decreased.

- **Taxon orange**'s abundance depends on the abundance of **the other taxa**

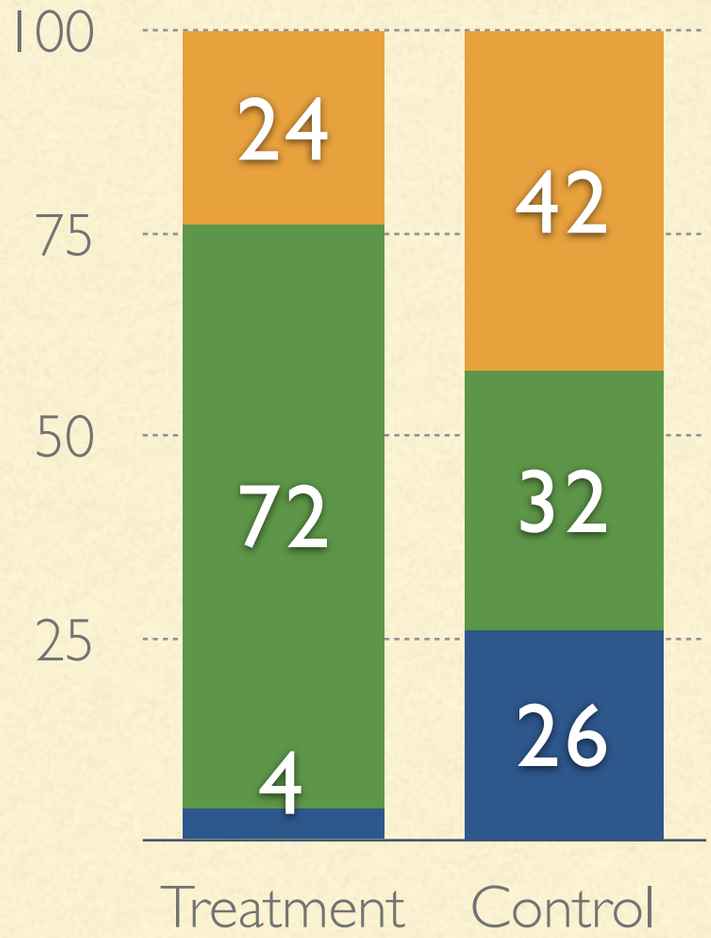


We *cannot* accurately estimate changes in proportions.

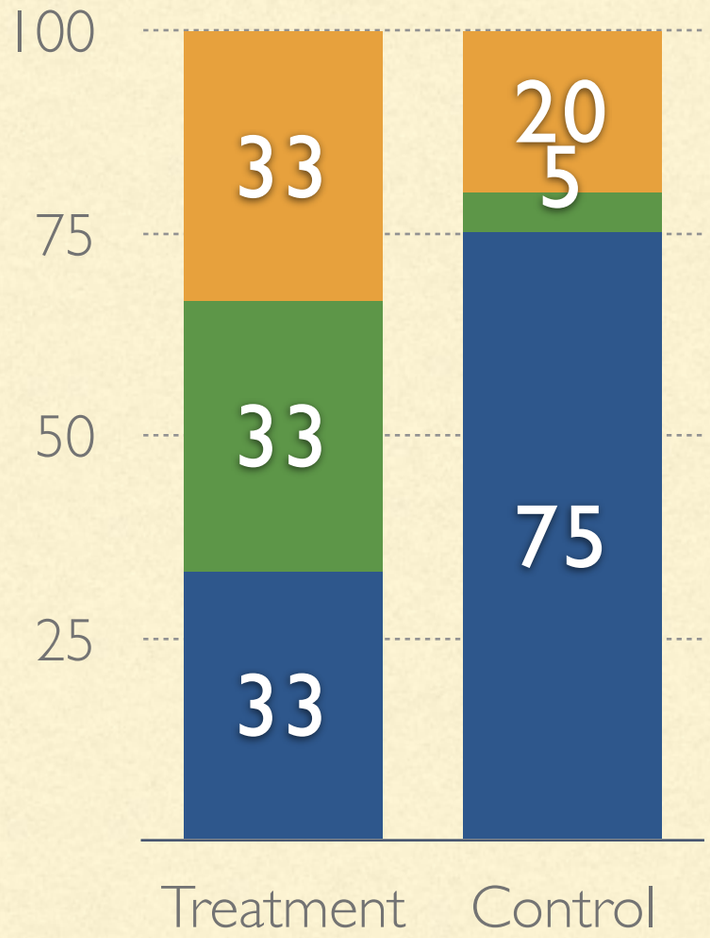
- **Taxon green** is high efficiency; its abundance increased (Ctrl vs Tmt). Additionally, **taxon blue** is low efficiency, and its abundance decreased.

- **Taxon orange**'s abundance depends on the abundance of **the other taxa**

Observed

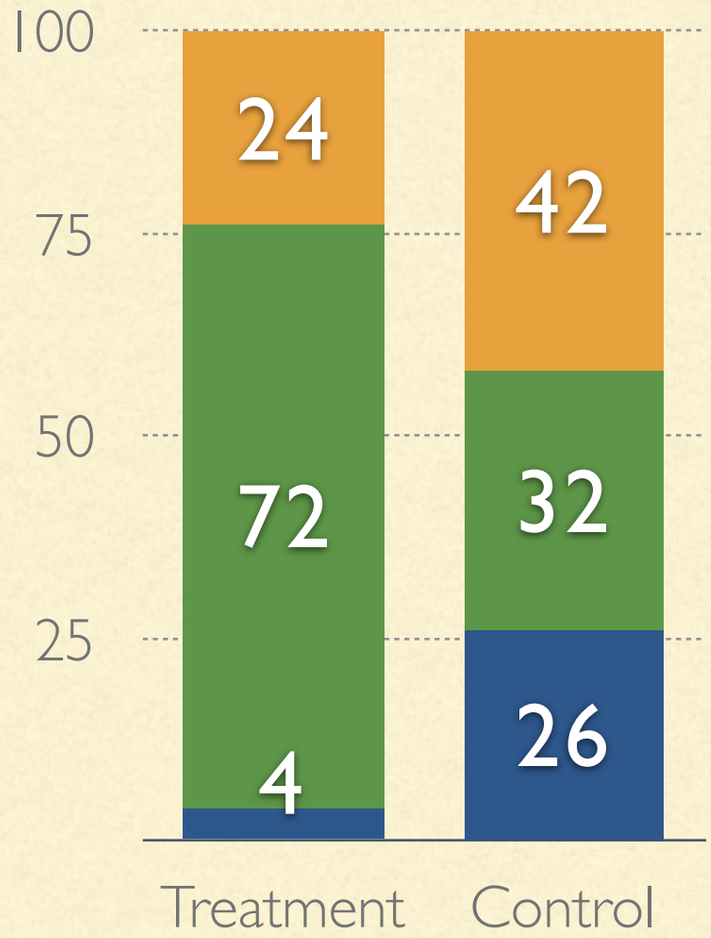


Actual



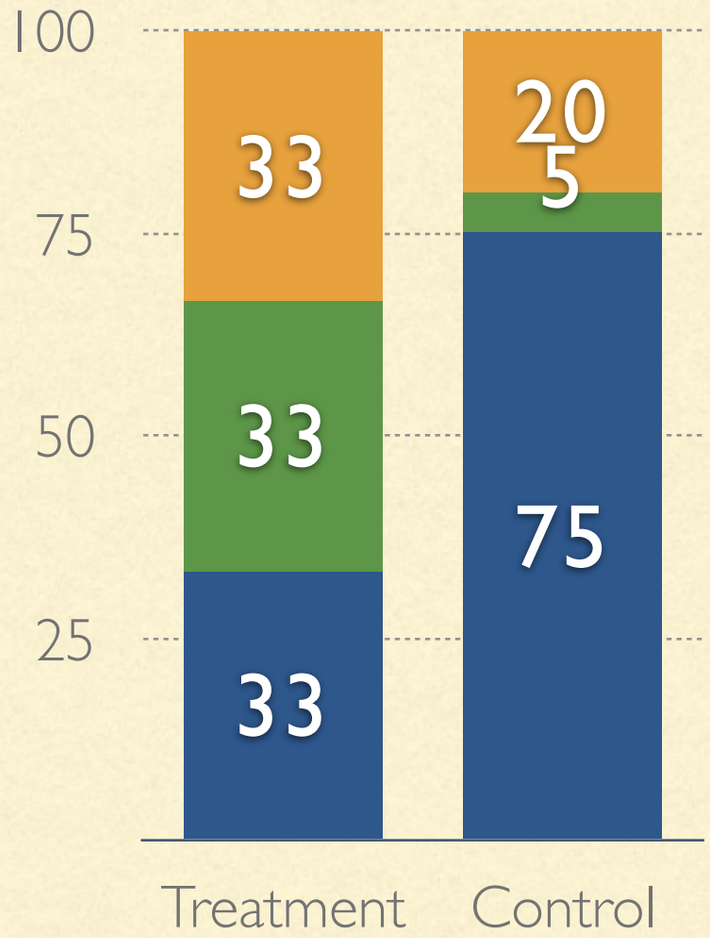
Efficiencies

Observed



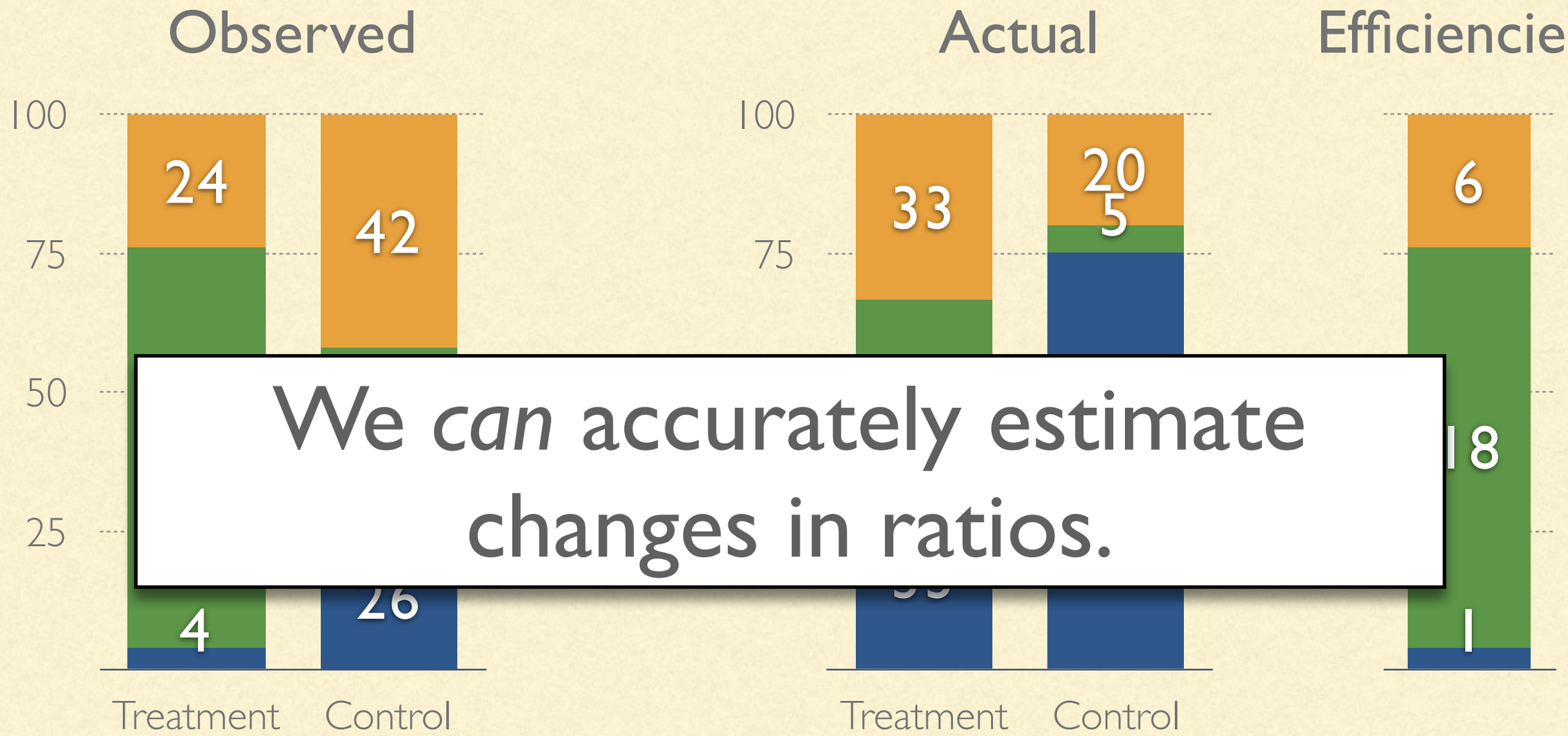
$$\frac{72}{4} \Big/ \frac{32}{26} = 15$$

Actual



$$\frac{33}{33} \Big/ \frac{5}{75} = 15$$

Efficiencies



We *can* accurately estimate changes in ratios.

$$\frac{\frac{72}{4}}{\frac{32}{26}} = 15$$

$$\frac{\frac{33}{33}}{\frac{5}{75}} = 15$$

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RESEARCH ARTICLE

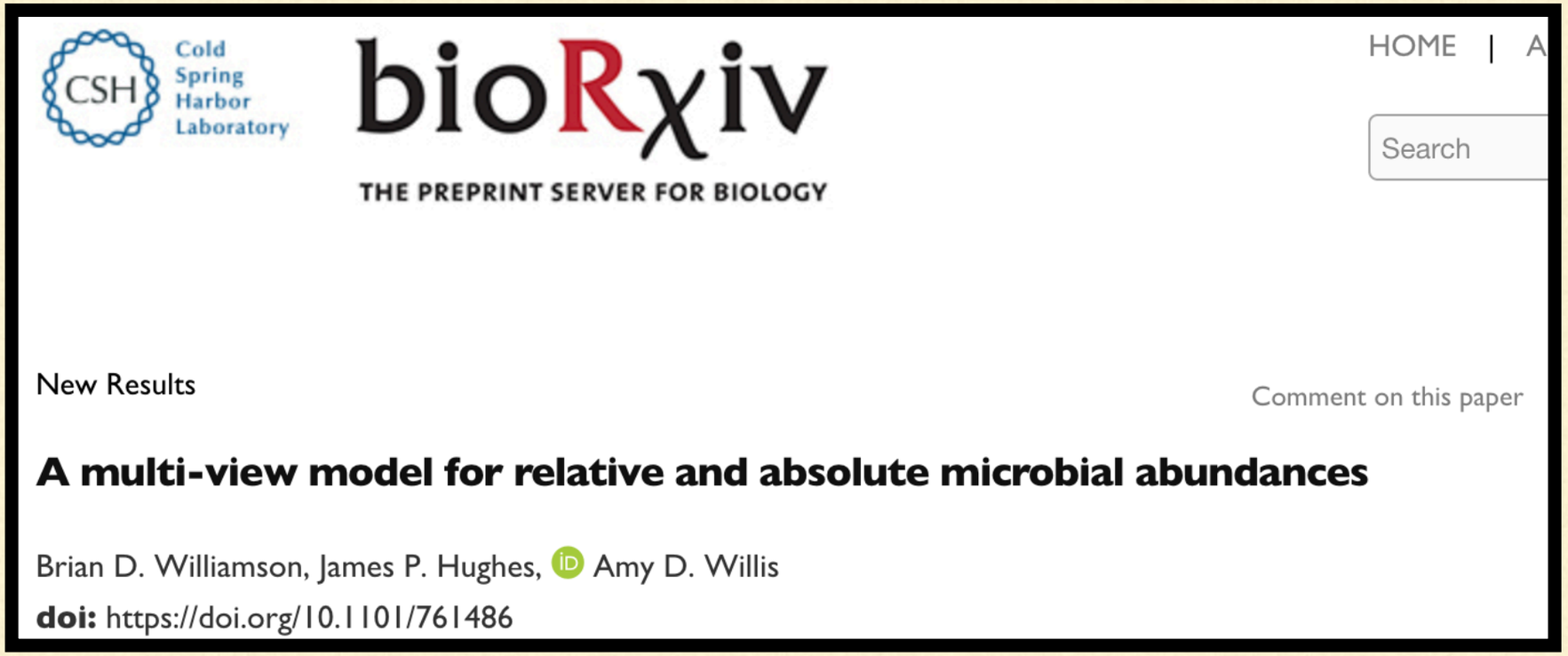
Consistent and correctable bias in metagenomic sequencing experiments

Michael R McLaren[1], Amy D Willis[2], Benjamin J Callahan[1,3]*

Michael McLaren (NCSU)

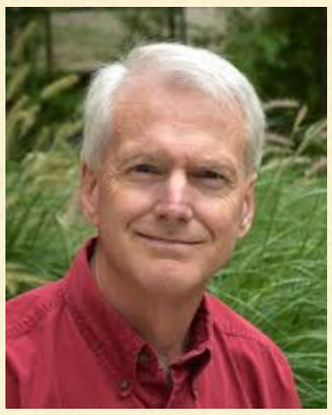
Ben Callahan (NCSU)

- Proposed model: taxon-specific efficiencies ("biases") confound estimation of relative abundance
- Model is validated in 16S & shotgun metagenomic data
  - Shotgun data is *also biased* — see *paper for details*



Brian Williamson (UW)

- Calibration with taxon-specific qPCR + 16S
  - Combing low-throughput + high-throughput
- *Key implication:* Do not multiply total 16S concentration and 16S relative abundance to estimate taxon-specific concentration



Jim Hughes (UW)

IMPLICATIONS

- Analyse ratios, not proportions
  - If you must analyse proportions, focus on largest signals
- Avoid aggregating abundances by taxonomy/phylogeny
- Avoid “quantitative” diversity indices

ONGOING & FUTURE WORK

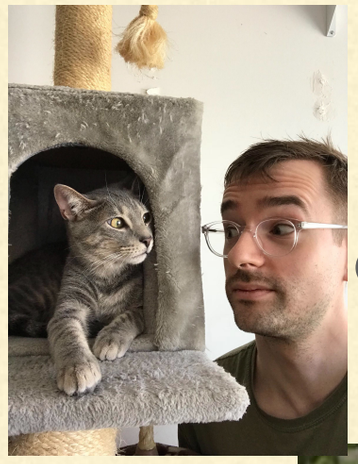
- Robust statistical methods
- Protocol selection
- Optimal experimental design (positive + negative controls)
- Bias prediction from phylogeny
- Calibration & meta-analysis (?)

SUMMARY

- Proposed a mathematical description of bias in sequencing
  - Validated on 16S and shotgun metagenomics data
- Model has serious implications for analysing microbiome data
  - *Proportions can lead to conclusions in the wrong direction*
  - Certain analysis methods are robust to bias; others are not

Michael McLaren (NCSU)

Ben Callahan (NCSU)



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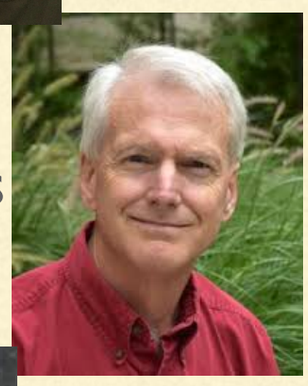

Jim Hughes (UW)

Brian Williamson (UW[2,3])

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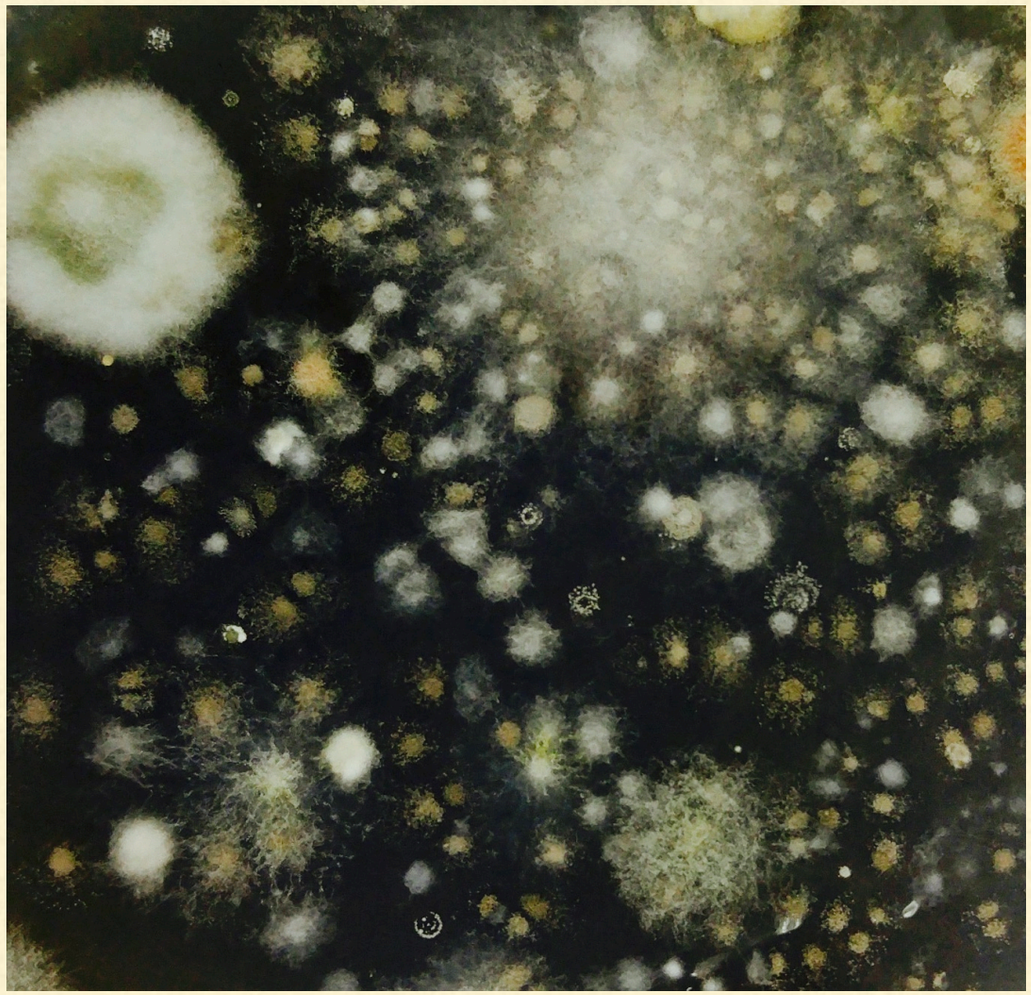

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Slides available at github.com/adw96/presentations

Photo credit: T.D. Berry, Whitman lab, UW Madison

SUPPLEMENTARY SLIDES

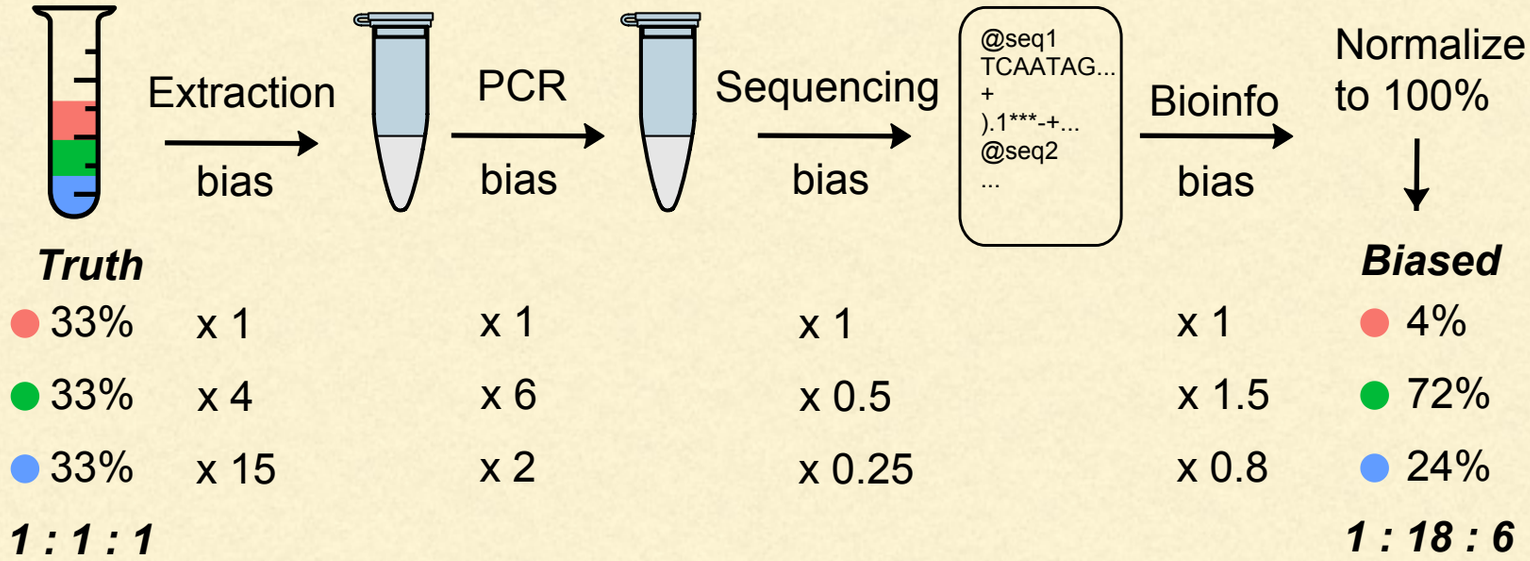

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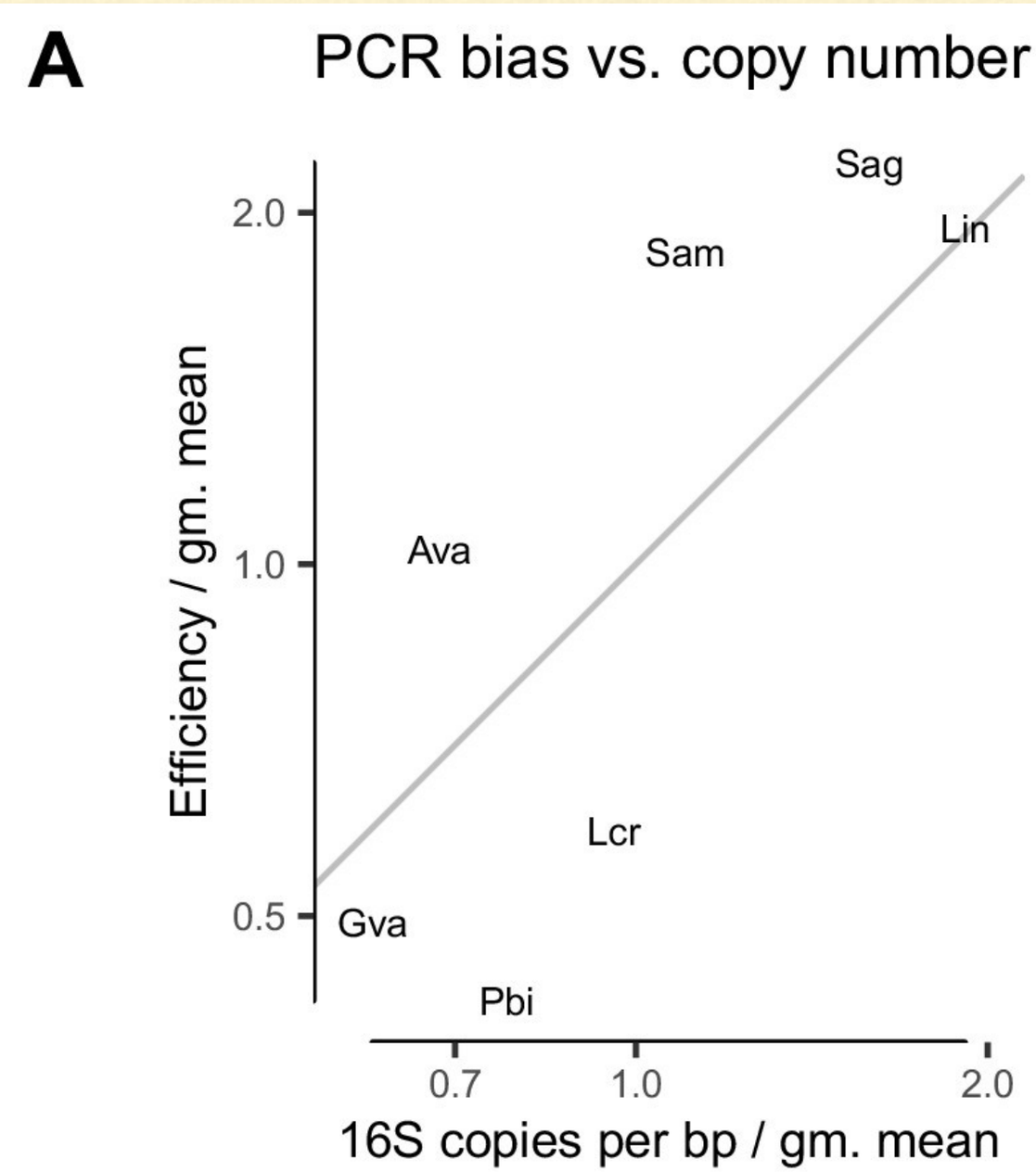
Figure from McLaren et al., 2019, *eLife*



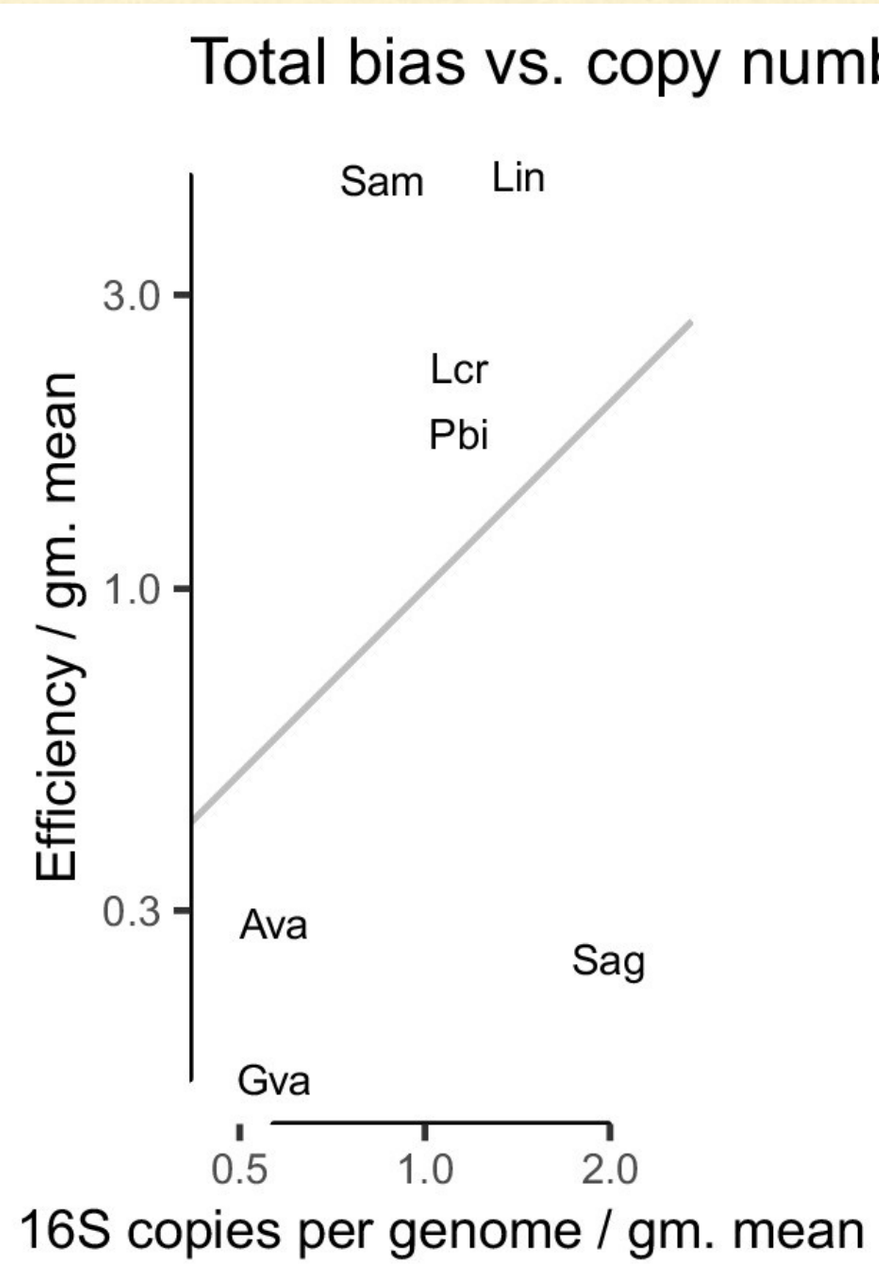
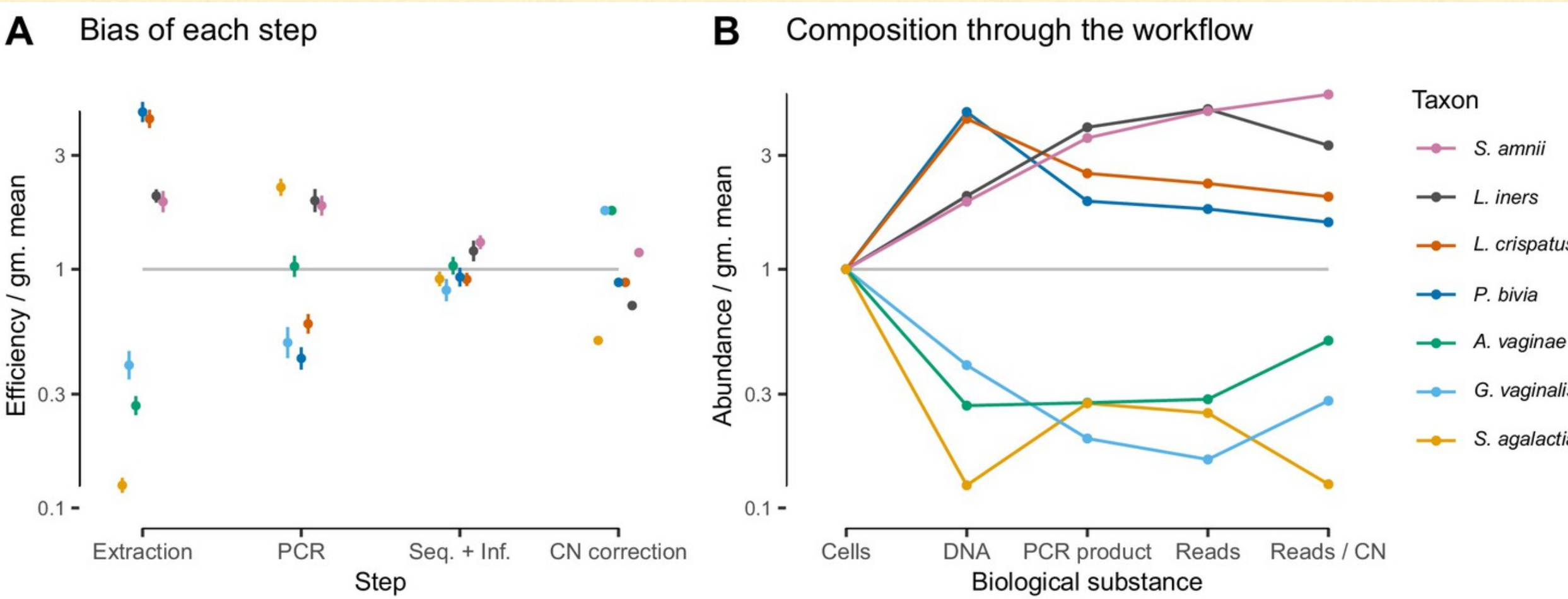


Figure from McLaren et al., 2019, *eLife*



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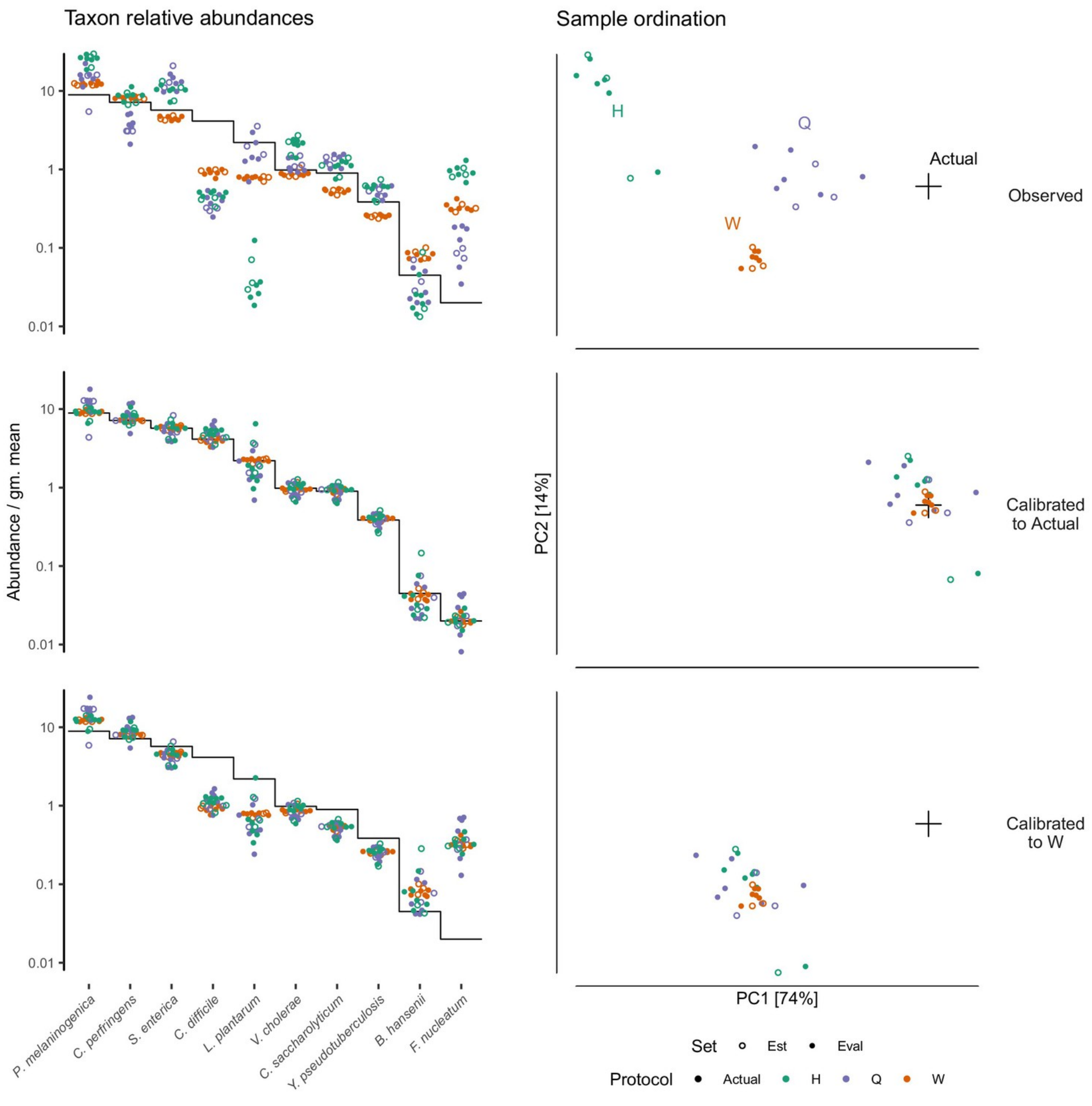
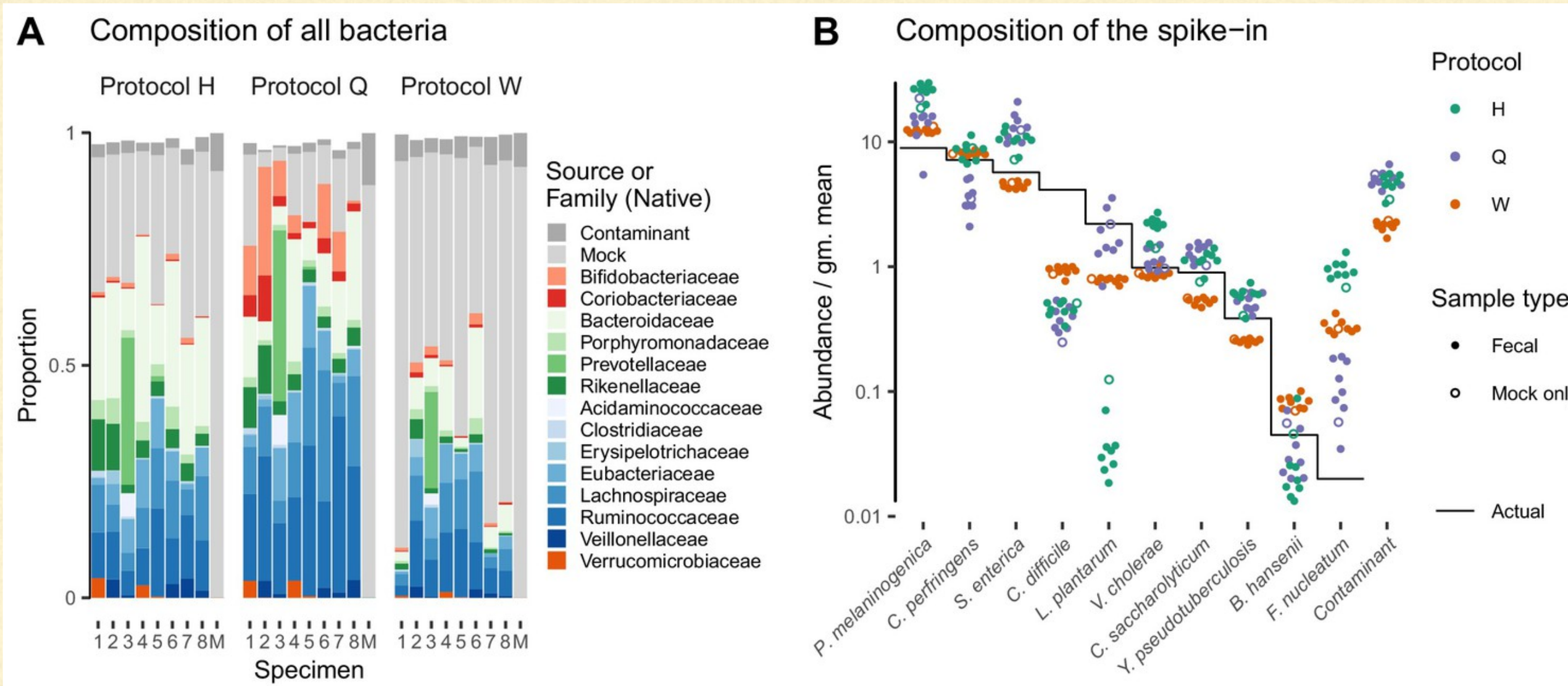

Taxon relative abundances
Sample ordination
Abundance / gm. mean
10
1
0.1
0.01
H
Q
W
Actual
Observed
Calibrated to Actual
Calibrated to W
PC2 [14%]
PC1 [74%]
P. melaninogenica
C. perfringens
S. enterica
C. difficile
L. plantarum
V. cholerae
C. saccharolyticum
Y. pseudotuberculosis
B. hansenii
F. nucleatum
Set Est Eval
Protocol Actual H Q W



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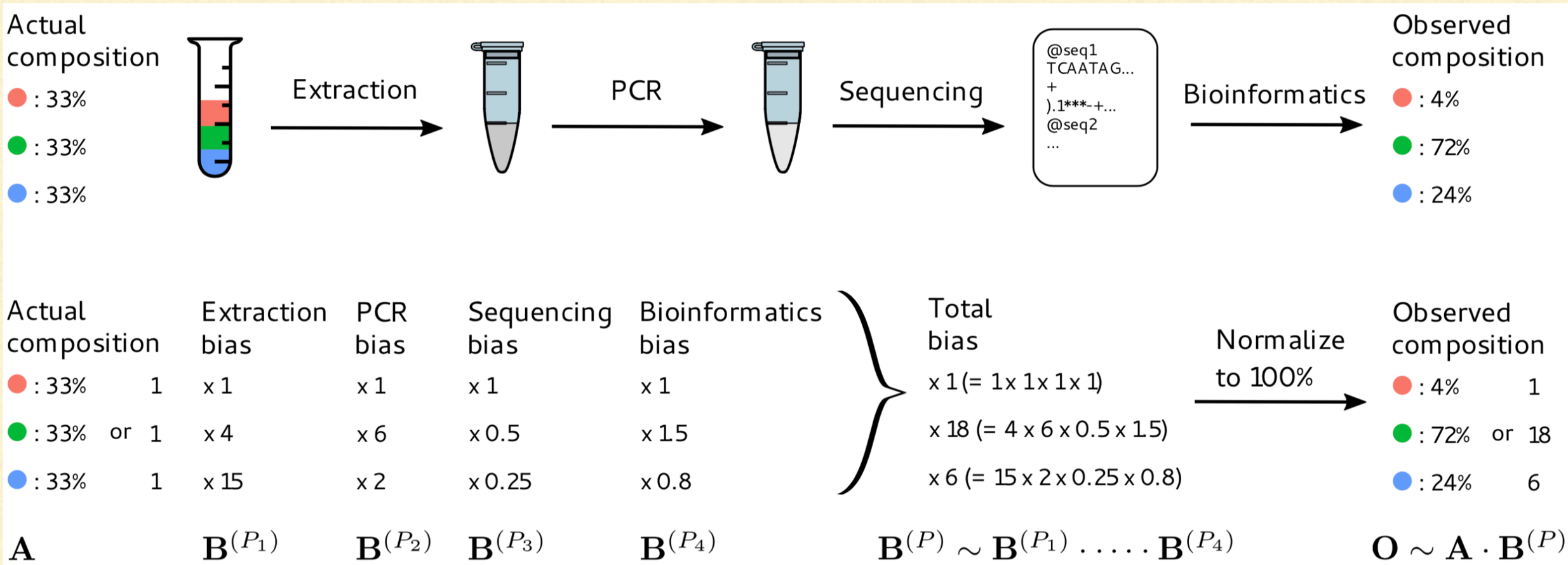

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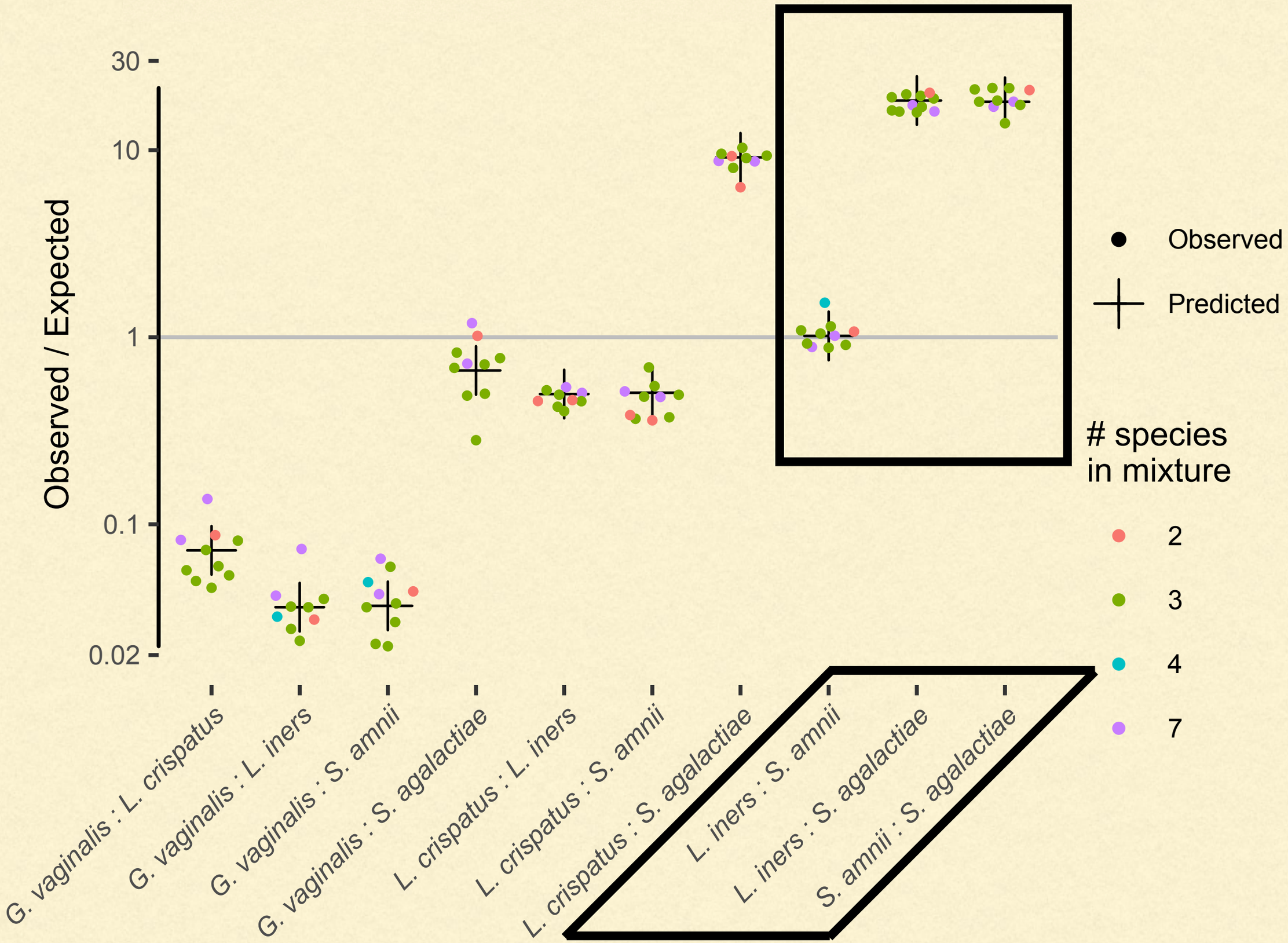

Figure from McLaren et al., 2019, *eLife*

WHY ARE RATIOS OF RATIOS UNAFFECTED BY BIAS?

$$\frac{\frac{p_{11}e_1}{\sum p_{1j}e_j}}{\frac{p_{12}e_2}{\sum p_{1j}e_j}} \Big/ \frac{\frac{p_{21}e_1}{\sum p_{2j}e_j}}{\frac{p_{22}e_2}{\sum p_{2j}e_j}} = \frac{p_{11}e_1}{p_{12}e_2} \Big/ \frac{p_{21}e_1}{p_{22}e_2} = \frac{p_{11}}{p_{12}} \Big/ \frac{p_{21}}{p_{22}}$$

p_{ij} = proportion of taxon j in sample i
e_j = efficiency of taxon j

HOW CONSISTENT IS MICROBIOME DATA WITHIN AND ACROSS LABS?

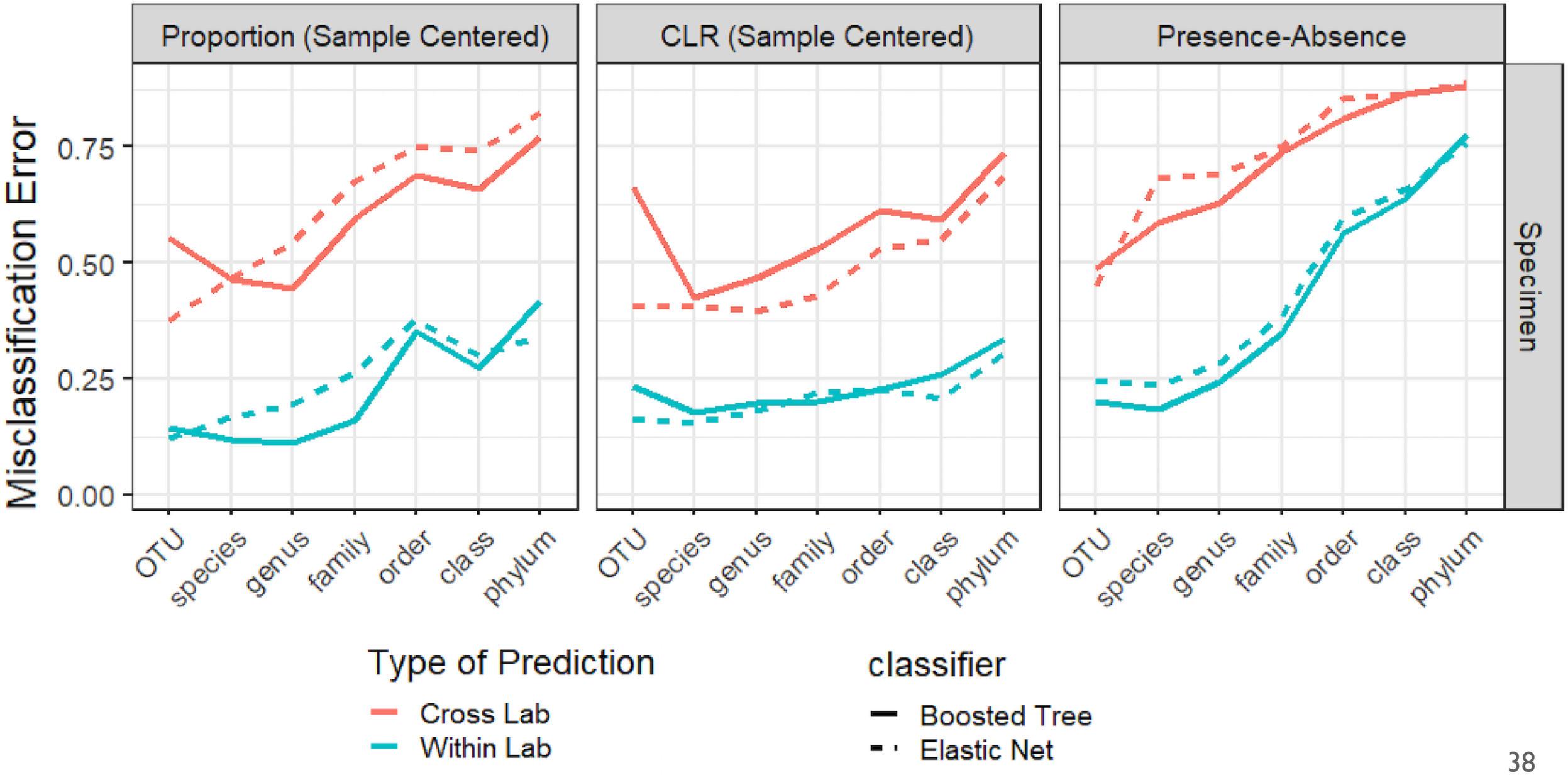

Figure from Clausen & Willis, 2019+