

# Journal Club

Preservation methods differ in fecal microbiome stability, affecting suitability for field studies

August 11<sup>th</sup>, 2016

Marc Sze

# Introduction



Mike Cox  
@MikeyJ



Following

Organism with optimal growth temp of 65 degrees associated with disease or perhaps there's a problem here...



RETWEETS 3 LIKES 11

3:19 AM - 7 Aug 2016

Reply Retweet Like



Elisabeth Bik  
@MicrobiomDigest



Following

Bead-beating artefacts in the Bacteroidetes to Firmicutes ratio of the human stool metagenome - Heidi C. Vebø  
[sciencedirect.com/science/article...](http://sciencedirect.com/science/article...)

RETWEETS 23 LIKES 15

3:00 AM - 5 Aug 2016

Reply Retweet Like



Reply to @MicrobiomDigest



Elisabeth Bik @MicrobiomDigest · Aug 5

Oh, that's not good:  
"2 different bead-beating instruments from same producer gave 3-fold difference in B:F ratio."  
FastPrep-24 &-96



Pat Schloss  
@PatSchloss



Following

@alyxschubert because the idea of a "standard" is naive

LIKES 2



8:56 AM - 9 Aug 2016

Reply Retweet Like



Mick Watson  
@BioMickWatson



Following

I am trying to crowdsource "bias in microbiome" studies: [opiniomics.org/the-unbearable...](http://opiniomics.org/the-unbearable...) please comment on blog, mail me or reply to \*this\* tweet

## The unbearable madness of microbiome

This is my attempt to collate the literature on how easy it is to introduce bias into microbiome studies. I hope to crowd-source papers and add them below under each category. PLEASE G...  
[opiniomics.org](http://opiniomics.org)

RETWEETS 44 LIKES 38



10:57 AM - 6 Aug 2016

Reply Retweet Like

## The unbearable madness of microbiome

6TH AUGUST 2016 / BIOMICKWATSON / 13 COMMENTS

This is my attempt to collate the literature on how easy it is to introduce bias into microbiome studies. I hope to crowd-source papers and add them below under each category. **PLEASE GET INVOLVED.** If this works well we can turn it into a comprehensive review and publish 😊 Add new papers in the comments or Tweet me 😊

Special mention to these blogs:

1. Microbiomdigest's page on [Sample Storage](#)
2. Microbe.net's [Best practices for sample processing and storage prior to microbiome DNA analysis freeze? buffer? process?](#)
3. The-Scientist.com's [Spoiler Alert](#)

\*\*\*\*\*

UPDATE 7th August 2016

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For prosperity the original blog post is below, but I am now trying to manage this through a [ZOTERO GROUP LIBRARY](#). Please continue to contribute – join the group, get involved! I think you can join the group [here](#). I am trying to avoid just listing software papers BTW I would prefer to focus on papers that specifically demonstrate sources of bias.

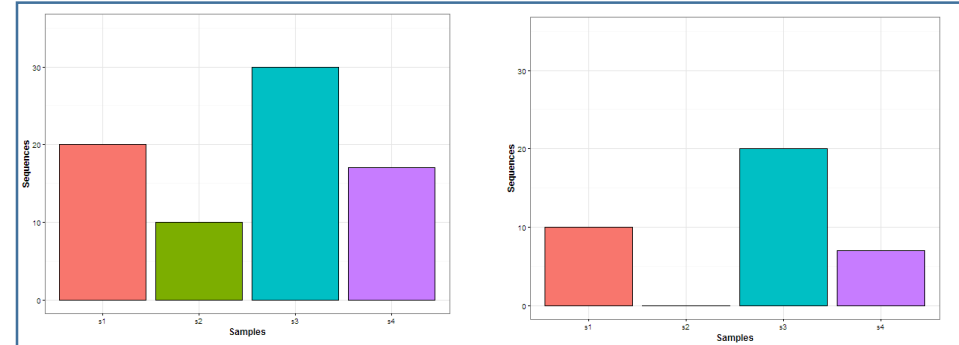
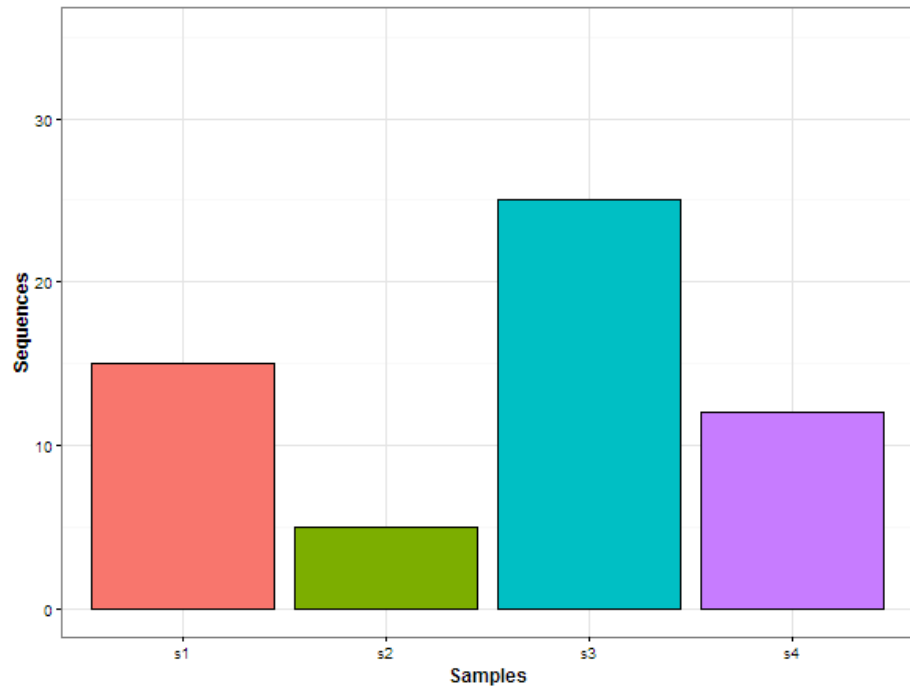
I won't be updating the text below.

# Introduction

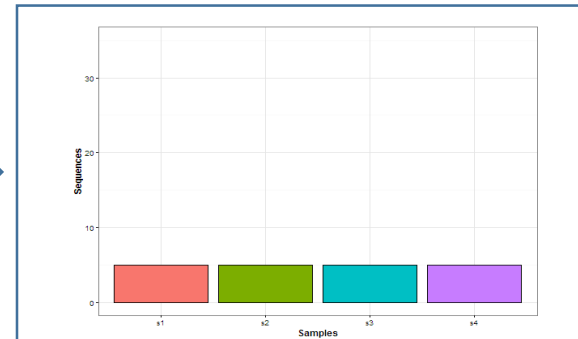
- So why not forget about it and let everyone do their own thing?
  - Weakens a powerful tool we have in pooling data
  - May be problematic to correct for these differences using bioinformatics
  - Can introduce bias in results
  - May make reproducibility difficult
    - E.g. a specific Taq or storage reagent is discontinued

# Introduction

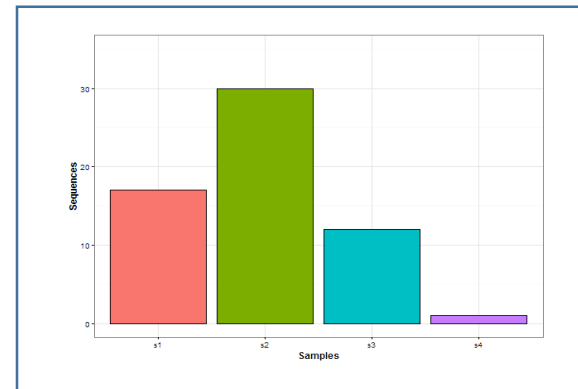
Actual Values



Reduction or increase by a fixed amount

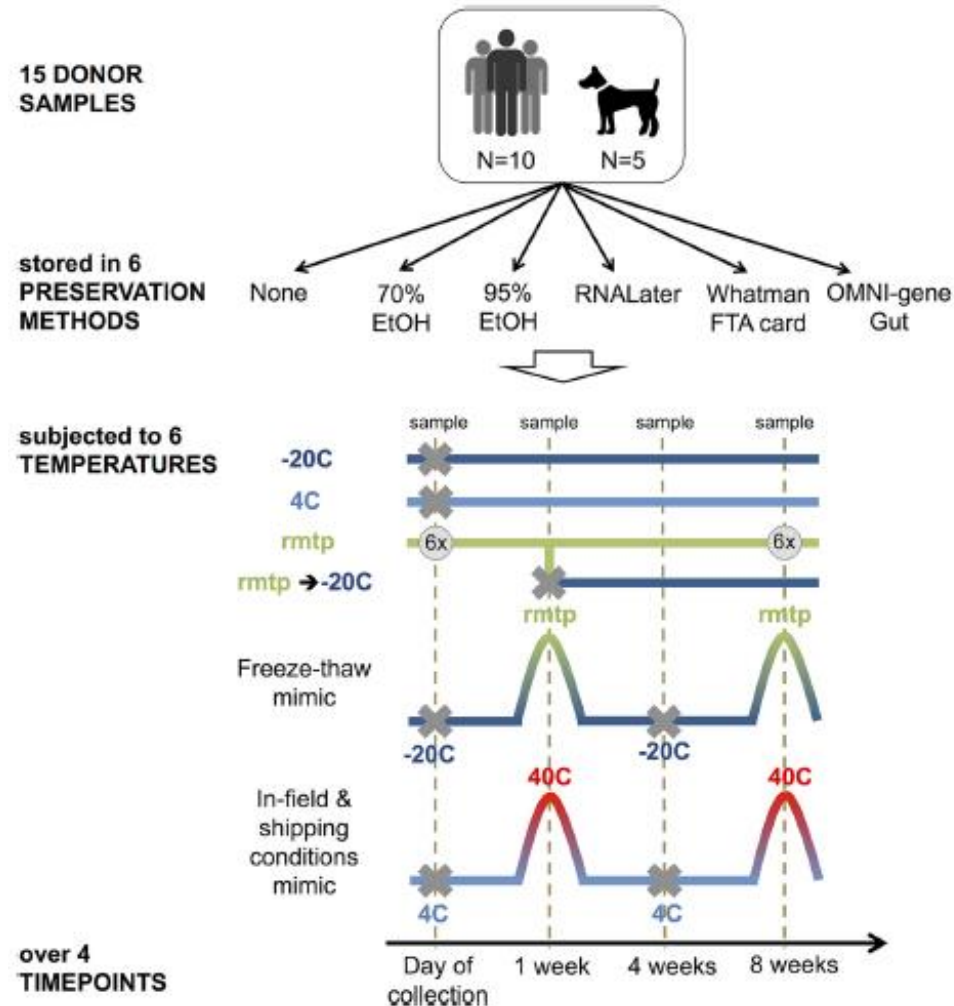


Set threshold or amount sampled



Combination or Random bias/changes

# Study Overview

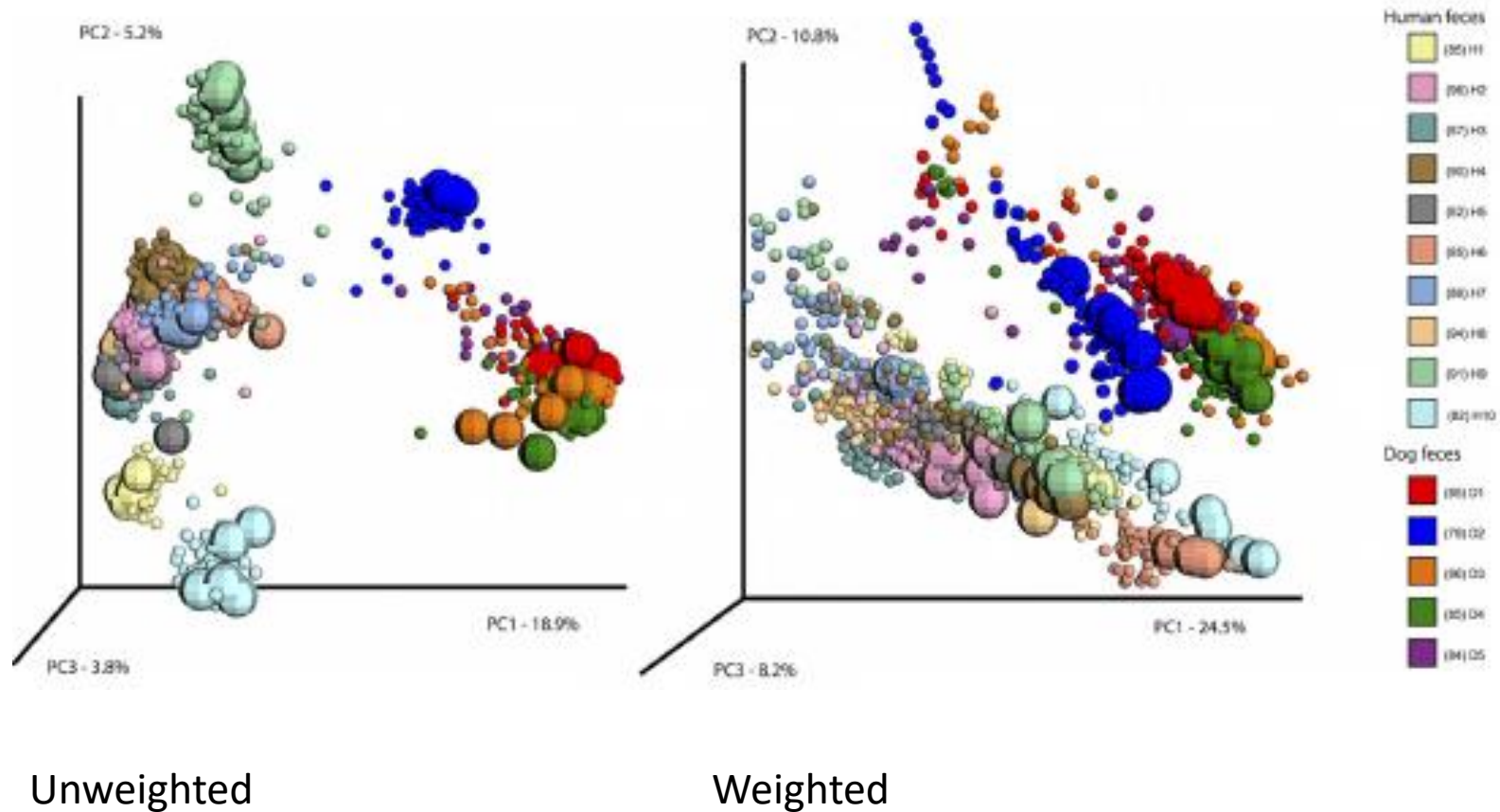


- **Sample Breakdown**
  - 360 samples from 4 time points
    - 15 x 6 preservation methods (90)
    - 90 x 4 time points
  - 540 samples from 4°C and -20°C
    - 90 x 2 storage temperatures (180)
    - 180 x 3 time points
  - 180 samples from RT to -20°C
    - 90 x 2 (3wks or 7wks)
  - 150 sample from mimics
    - 75 change temp wait 1 week
    - 75 change temp wait 8 weeks
    - No Whatman FTA cards used
  - 1230 total + 109 extraction blanks + 15 FTA controls + 180 technical replicates = **1534**
  - **Total kept 1127 ~ 73.4%**
    - 171/180 technical replicates kept (~95%)

# Some Caveats from Data generation

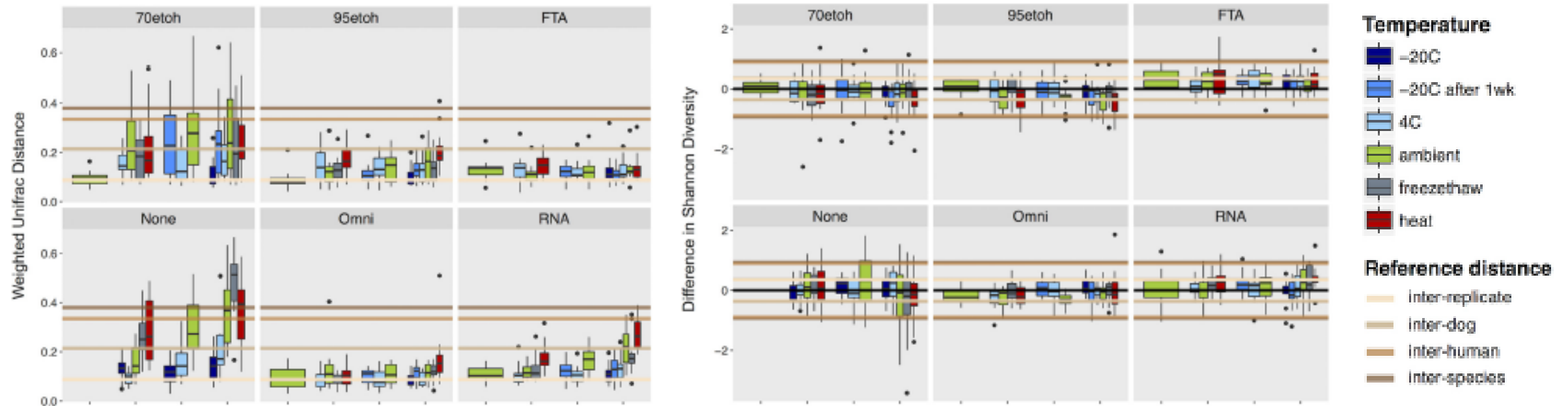
- Used Earth Microbiome Project Protocol
  - PowerSoil with 20 mins @ setting of 20
  - Pooled 3 PCR reaction together per sample
  - Sequencing using HiSeq (125nt read length used)
    - Unique barcode only in Forward direction
    - Samples failed on HiSeq were re-sequenced on MiSeq
  - OTU generation used an Open Reference approach
  - Diversity Measures
    - Unweighted UniFrac
    - Weighted UniFrac
    - Switched to Bray-Curtis for detrending

# Results: Weighted and unweighted UniFrac



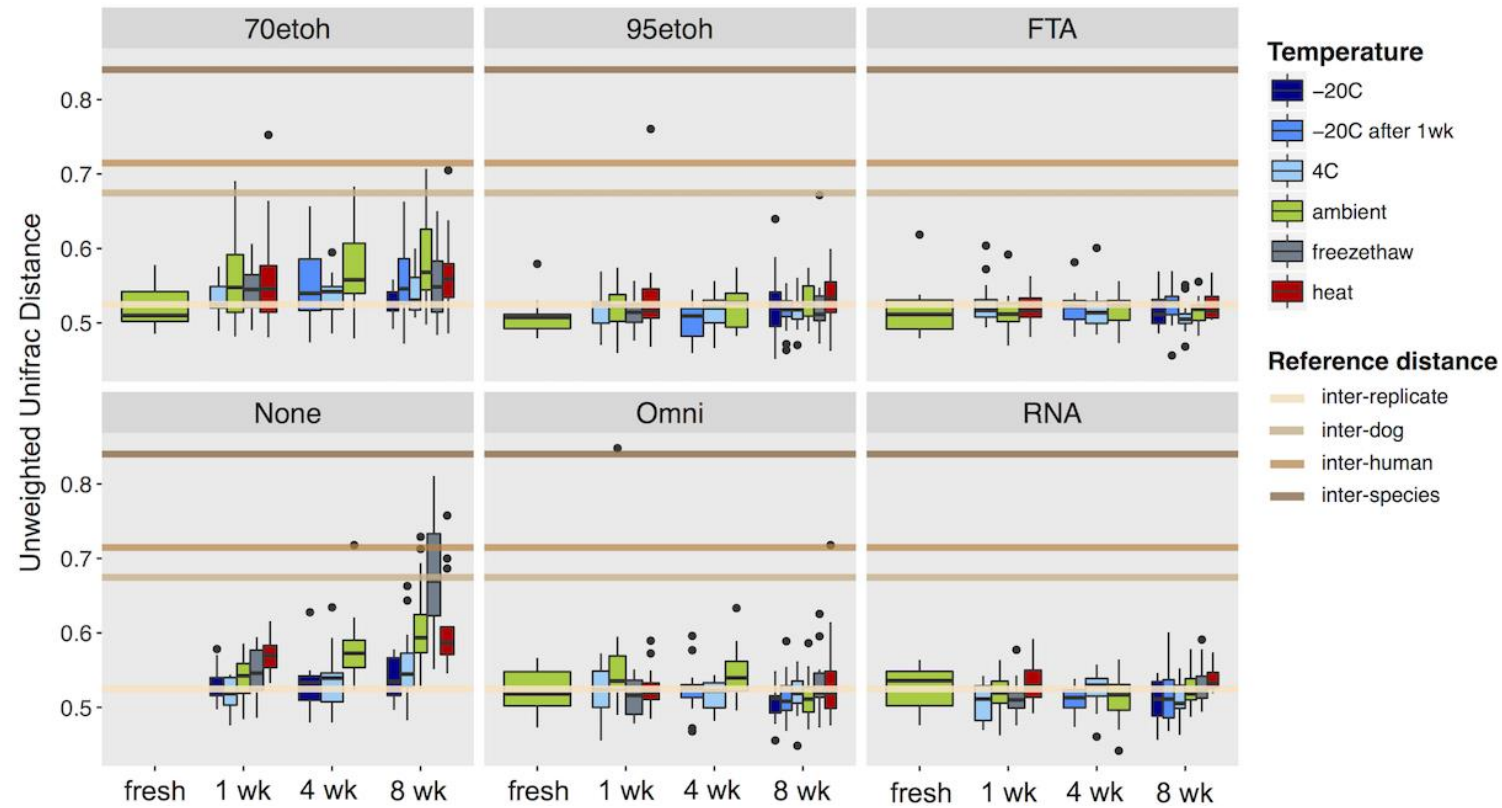
Fresh Samples are the larger circles.

# Results: Sample Variability



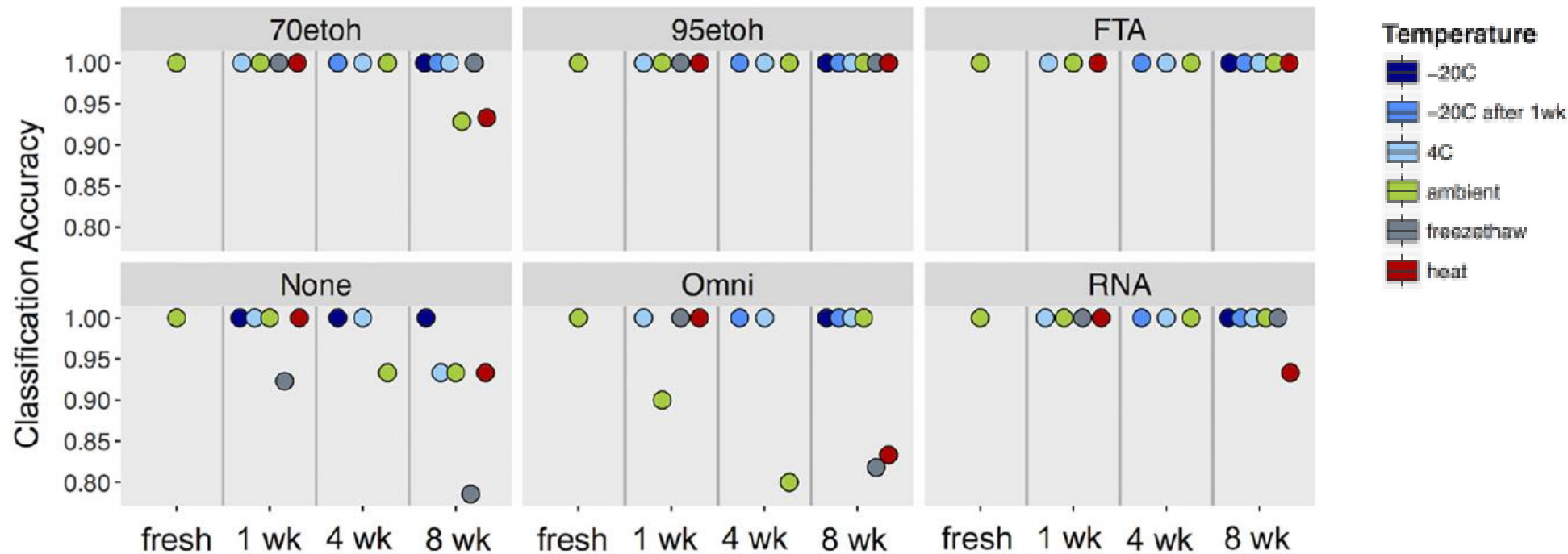


# Results: Sample Variability Cont.

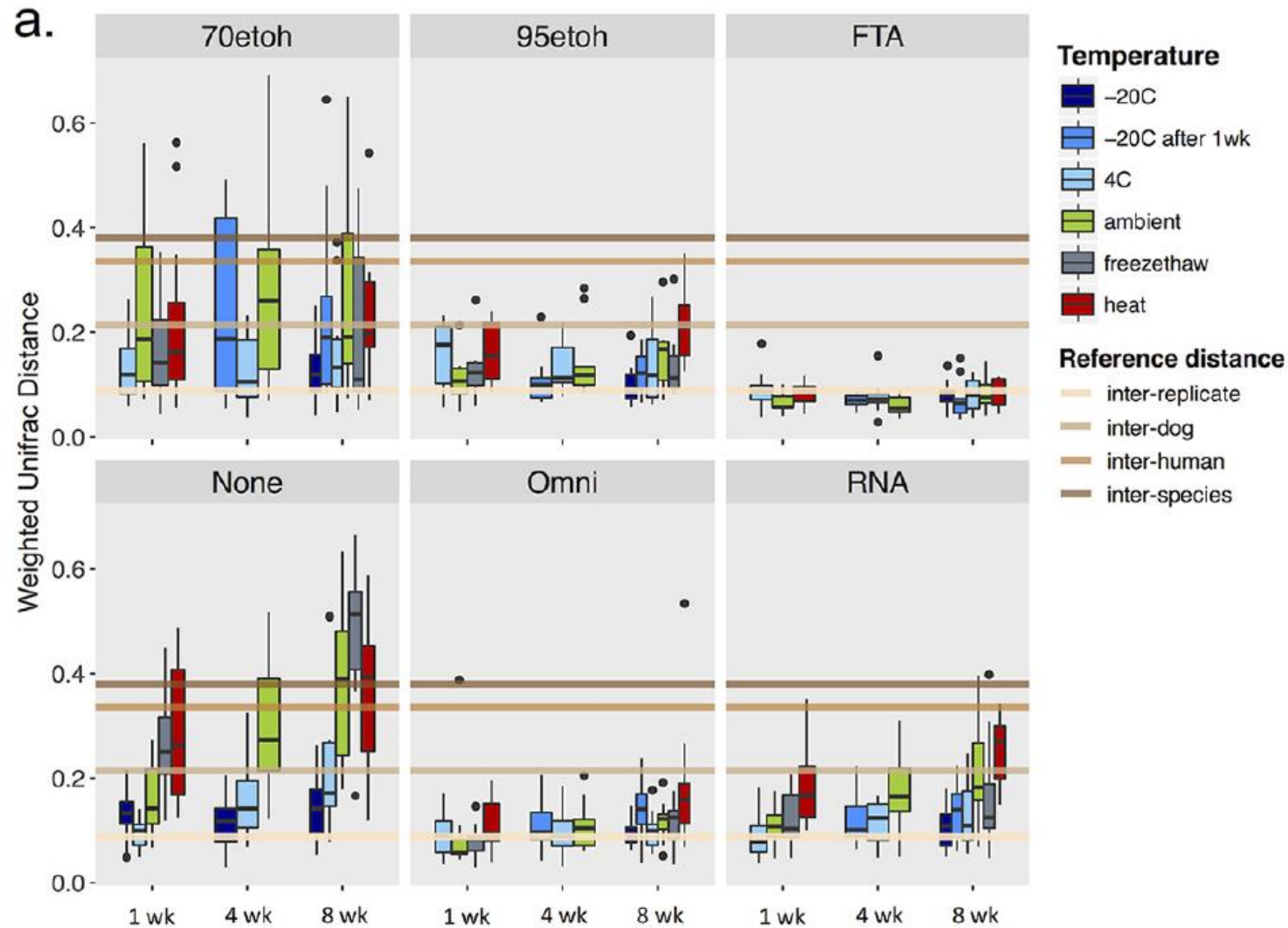


# Results: Classification Accuracy

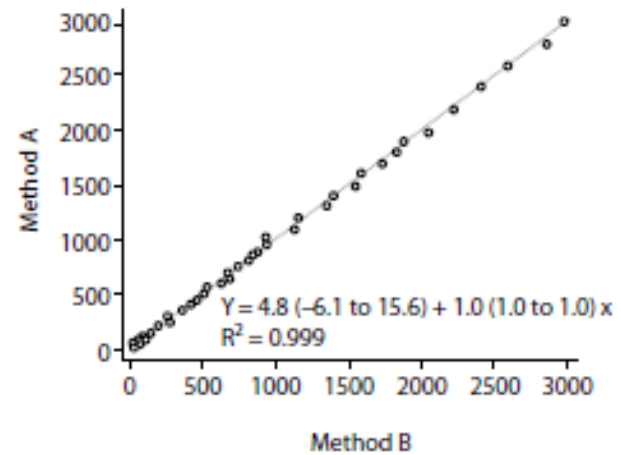
- Train on 0 hours test on other timepoints
  - random forest and caret (<http://topepo.github.io/caret/index.html>)
  - Predict individual regardless of treatment



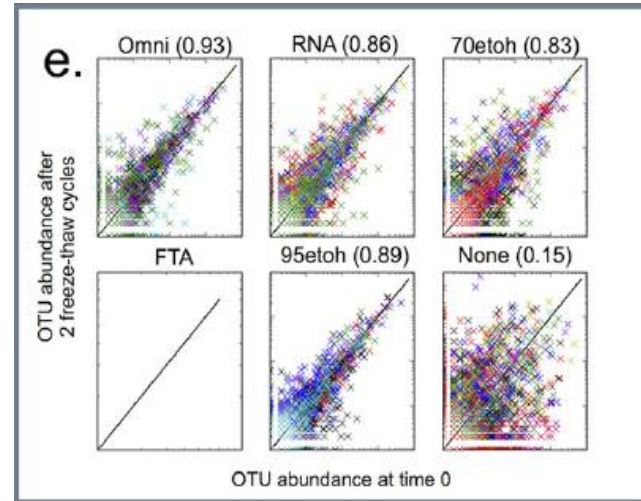
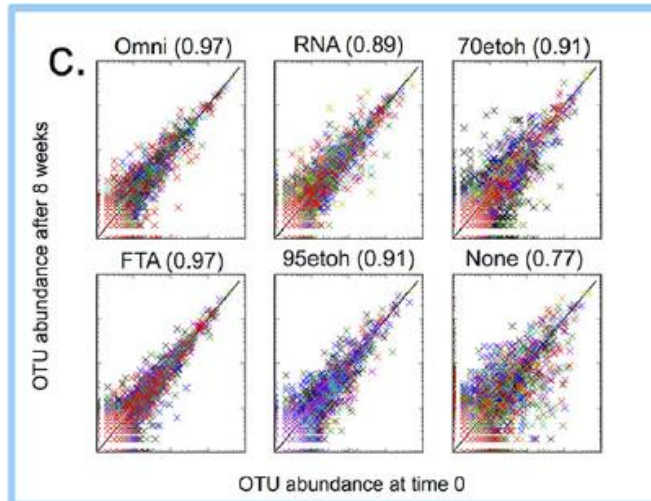
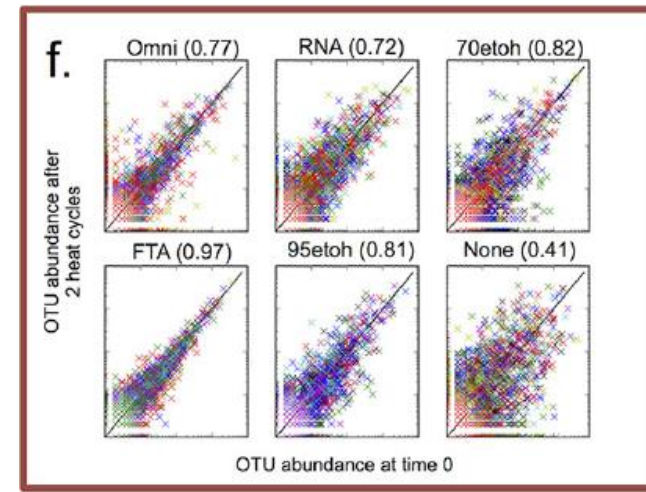
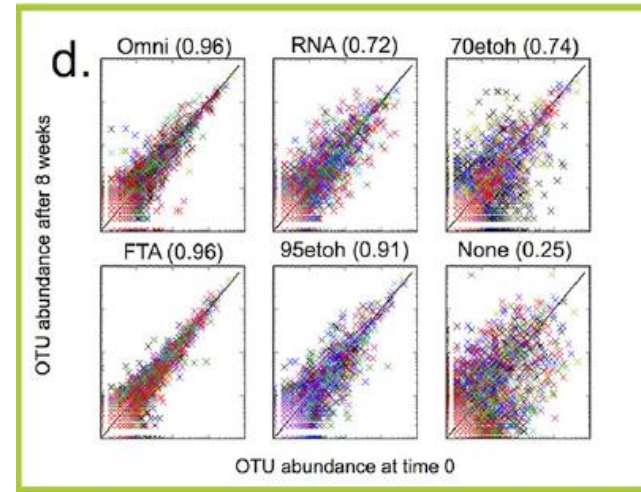
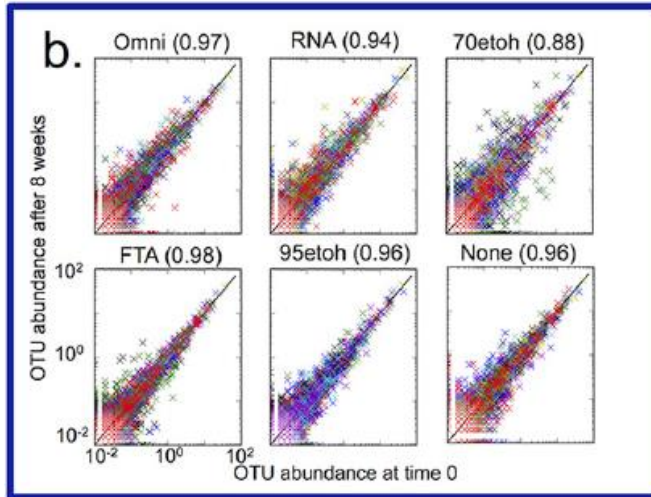
# Results: Temperature Treatments



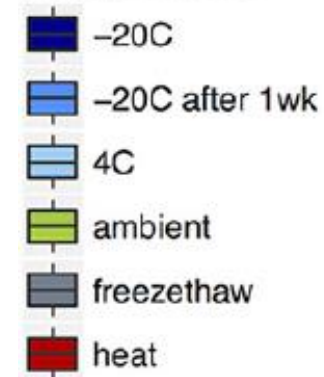
# Results: Bias



# Results: Bias

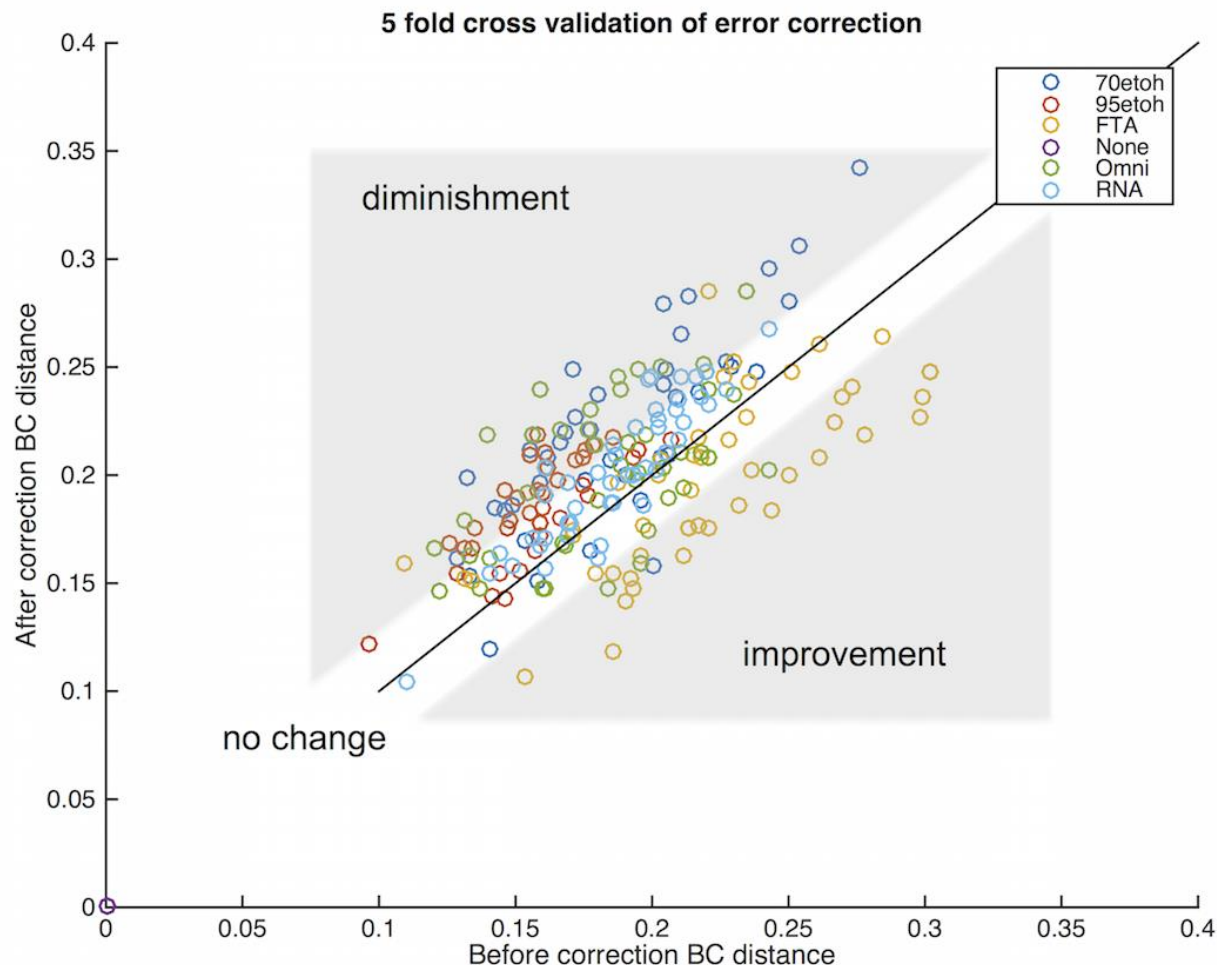


## Temperature





# Results: Detrending



- Not sure if this graph really represents what authors wanted it to.
  - Shows FTA cards might be influencing Bray-Curtis distances.
  - Shows others do not have such a relationship.

Thank you for your time and attention!!!