

RNA sequencing of microbial communities

Braden Tierney

April 30th, 2024



Reality check

True or False: Most bacteria, when ingested, will make you sick

In 1 pound of stool, ____ % is bacterial biomass

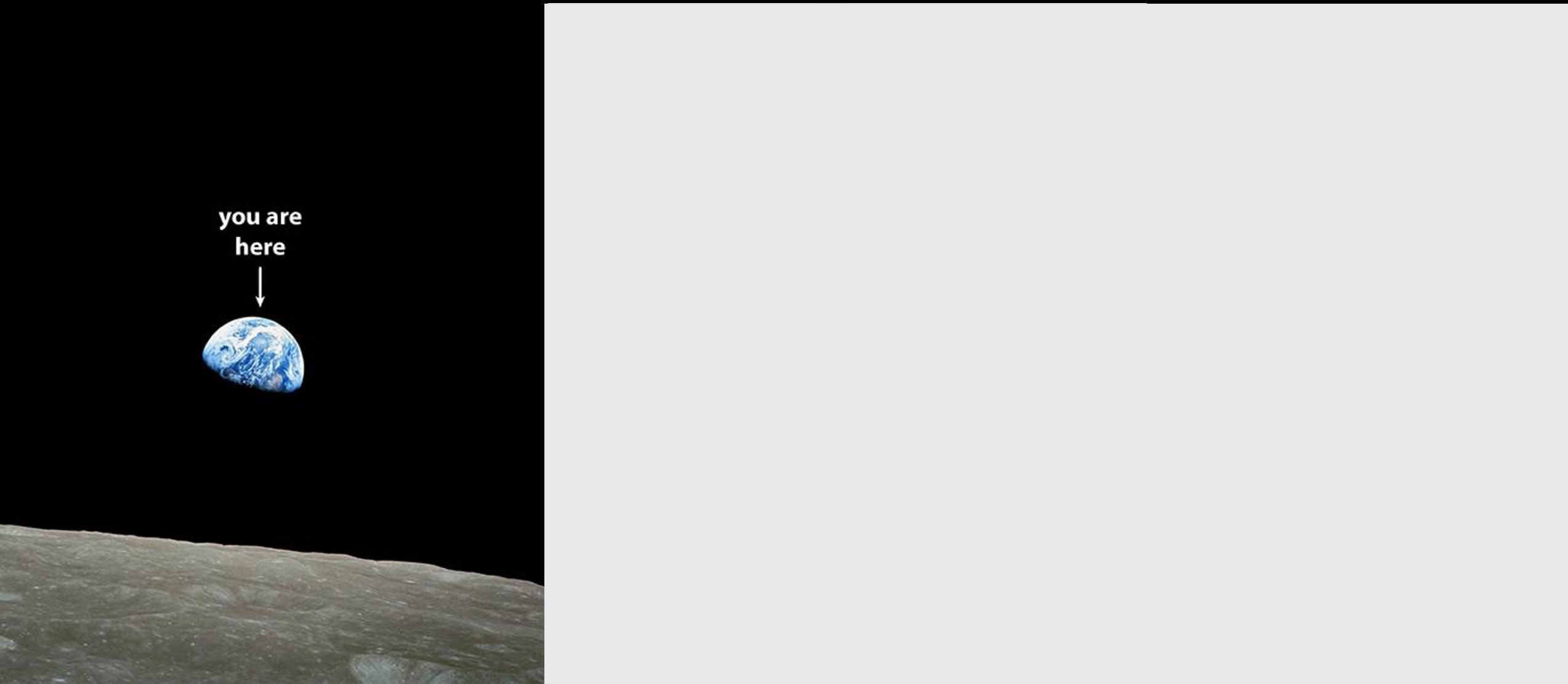
The ratio of unique human genes to gut microbial genes is ____.

Humans release 29 gigatons of CO₂ per year. Microbes process ___ gigatons naturally.

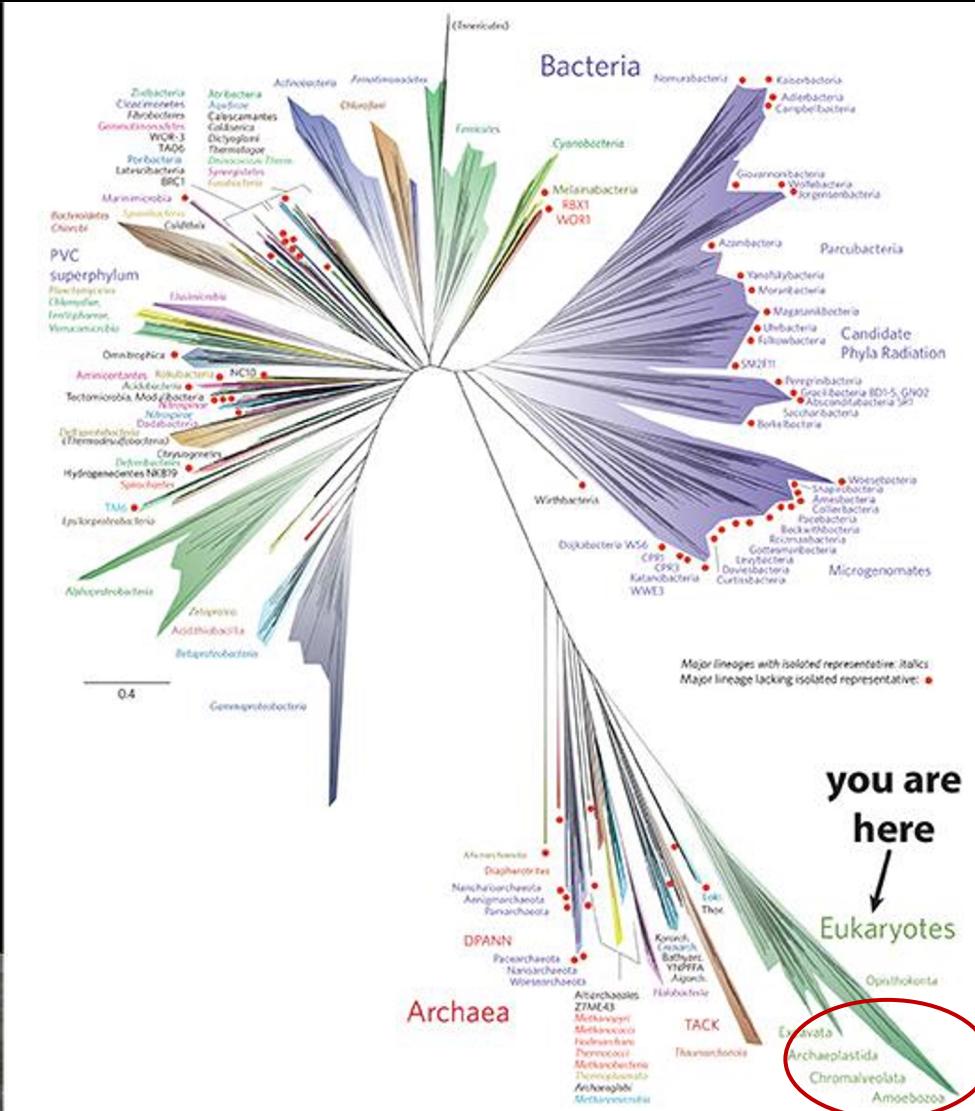
About ___ % of organisms on earth either breathe oxygen or have chlorophyll.



We live in a microbial world



We live in a microbial world



Composed of an immense number of organisms and genes, most totally unknown.



All organisms visible to the eye



We live in a microbial world



100,000,000 microbial cells
in 10s o thousands of species



What is a Microbiome?



Microbiome:

a community of different microorganisms that occupies a particular environment [including the environment of a host macroorganism] and how they interact with each other and their surrounding environmental conditions.



What is a Microbiome?

Microbiome:

a community of different microorganisms [resident to a host macroorganism] and their

Feeling lonely?



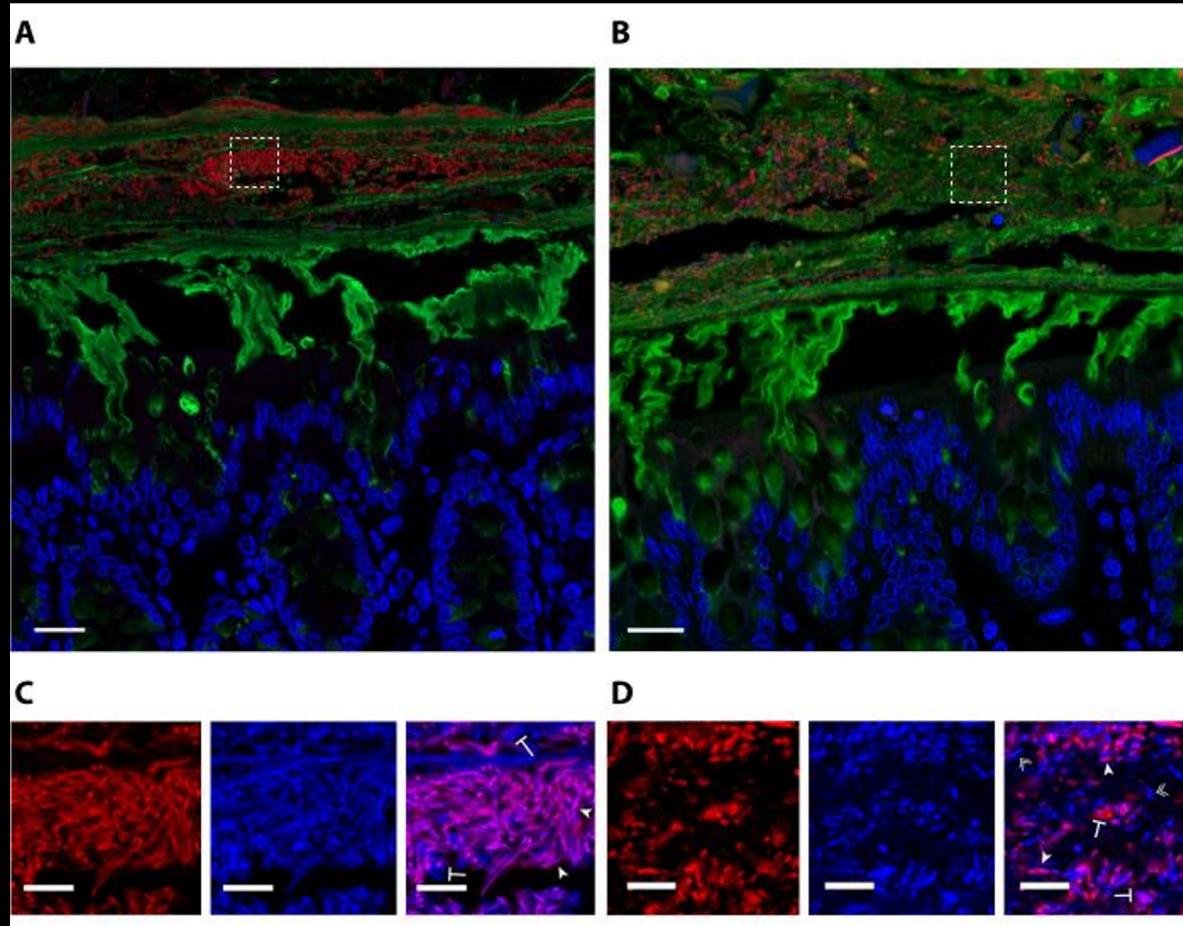
Just remember,
you're not alone.



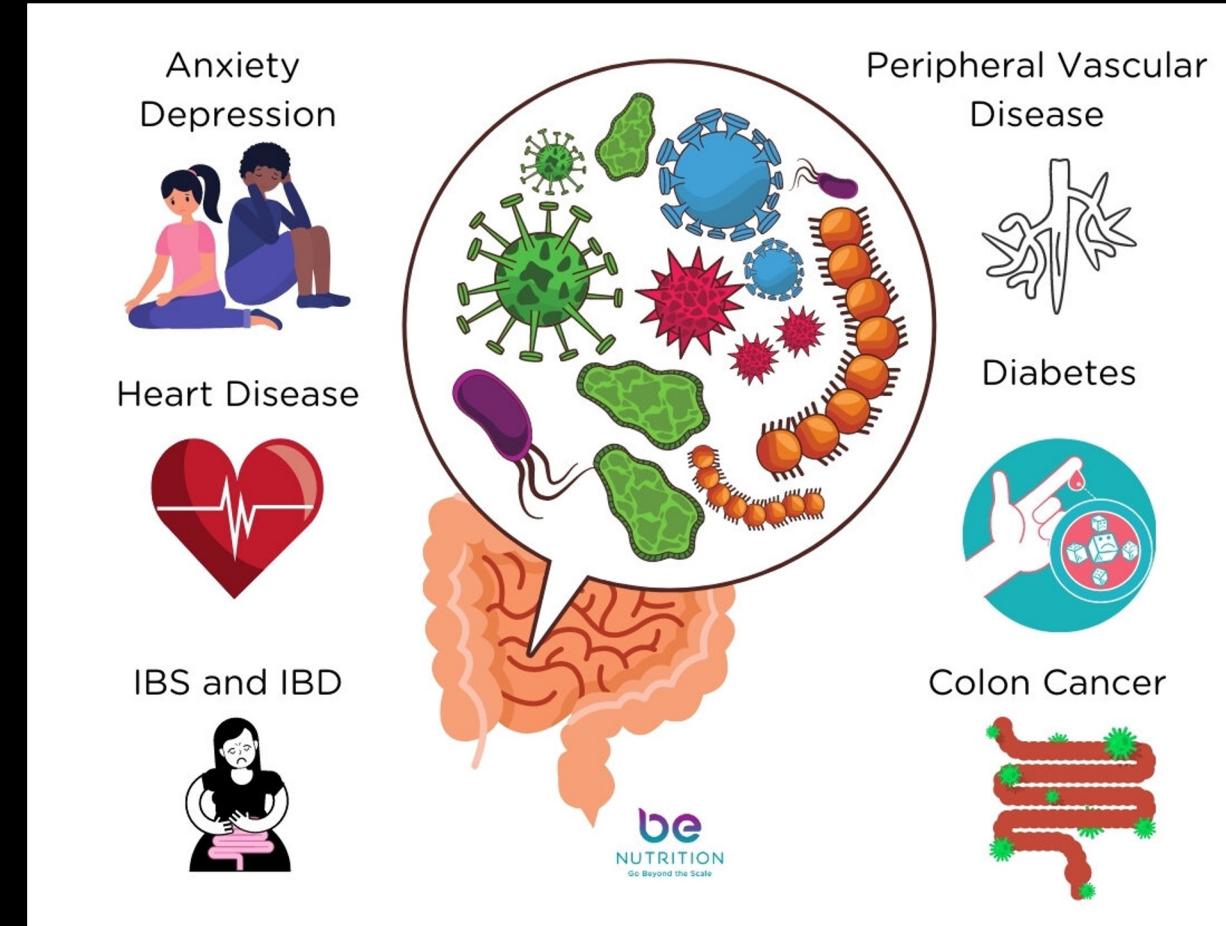
YOU ARE NEVER ALONE.



The gut microbiome!



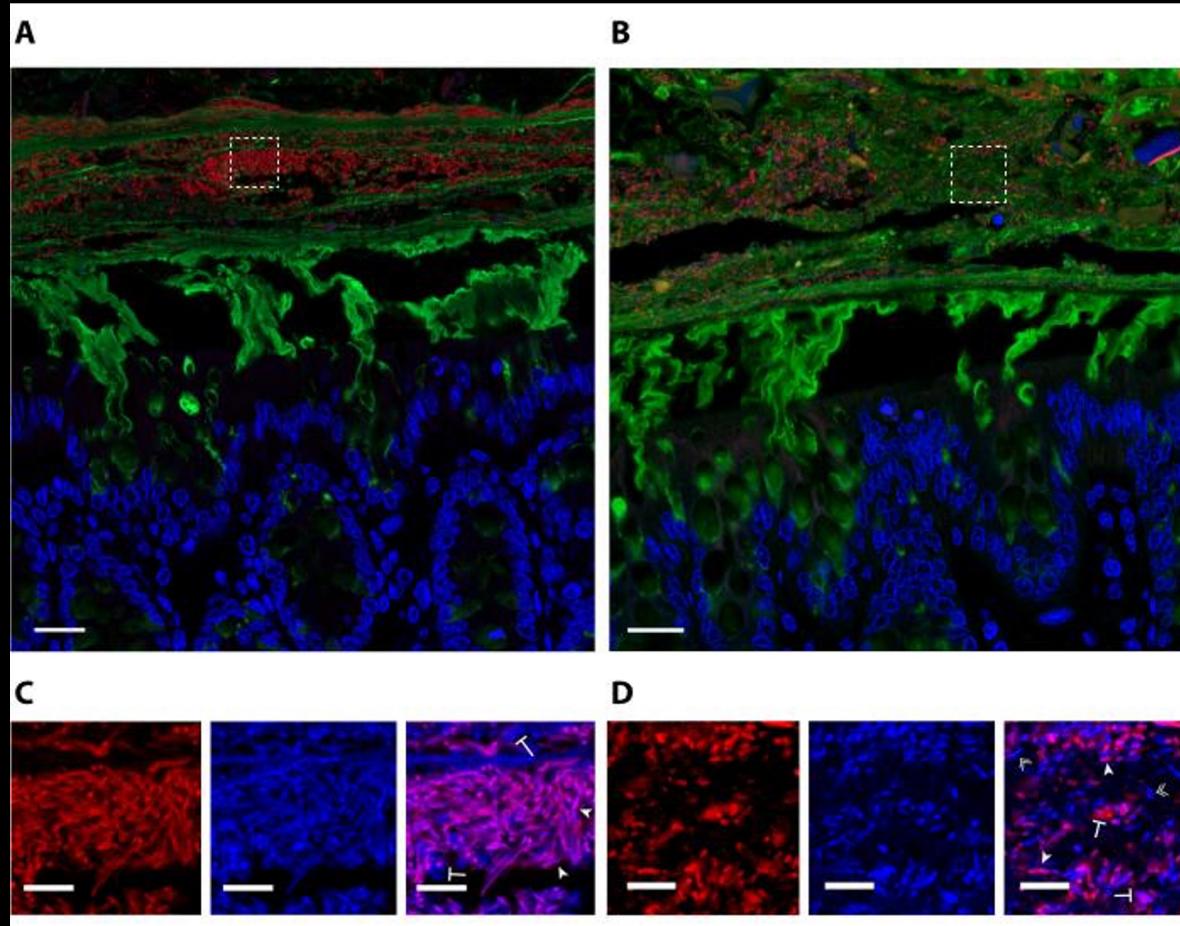
<https://app.jove.com/t/62646/visualization-of-gut-microbiota-host-interactions-via-fluorescence-in-situ-hybridization-lectin-staining-and-imaging>



<https://benutritionco.com/blog/2020/1/gut-healthandchronic-disease>



The gut microbiome!



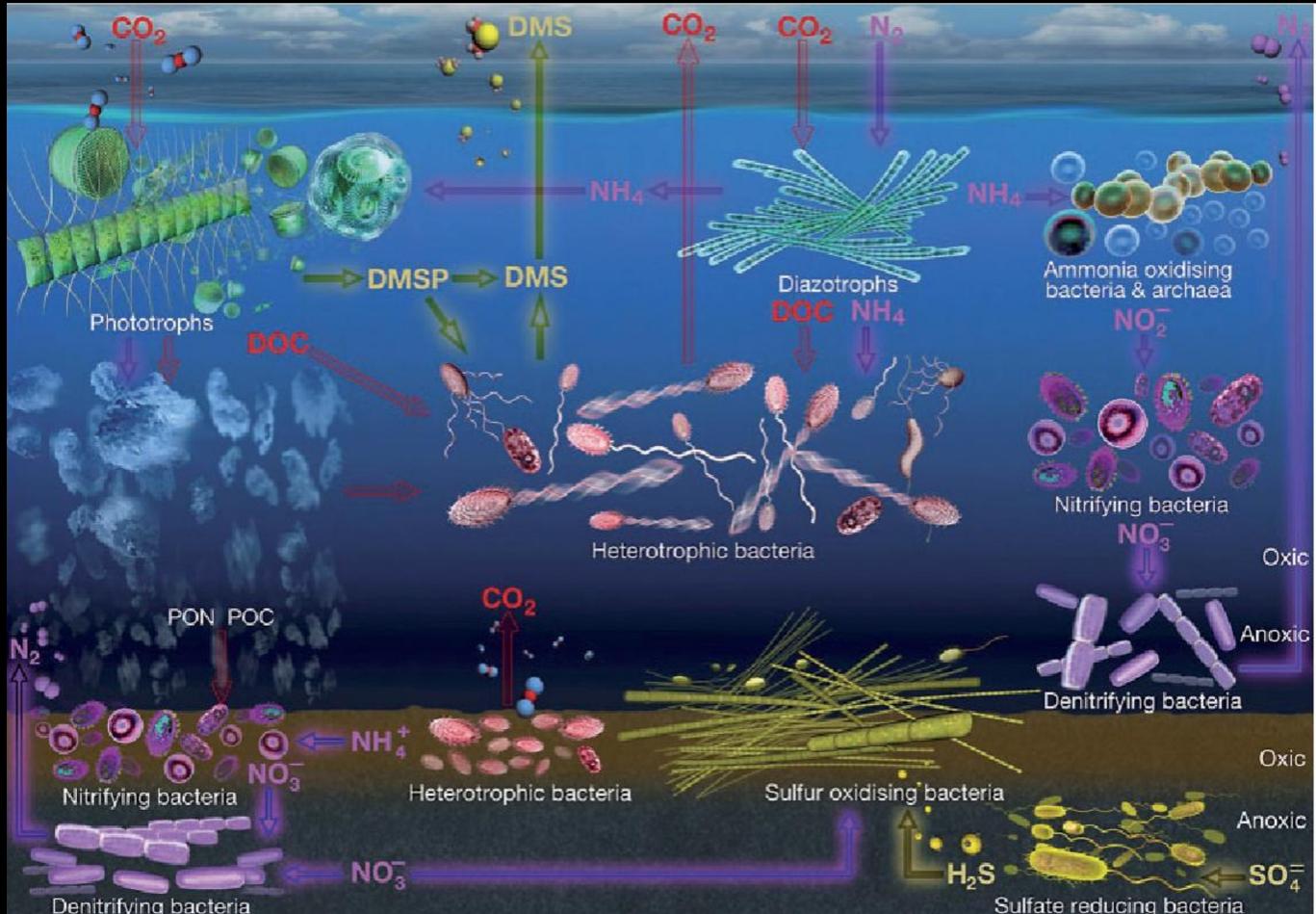
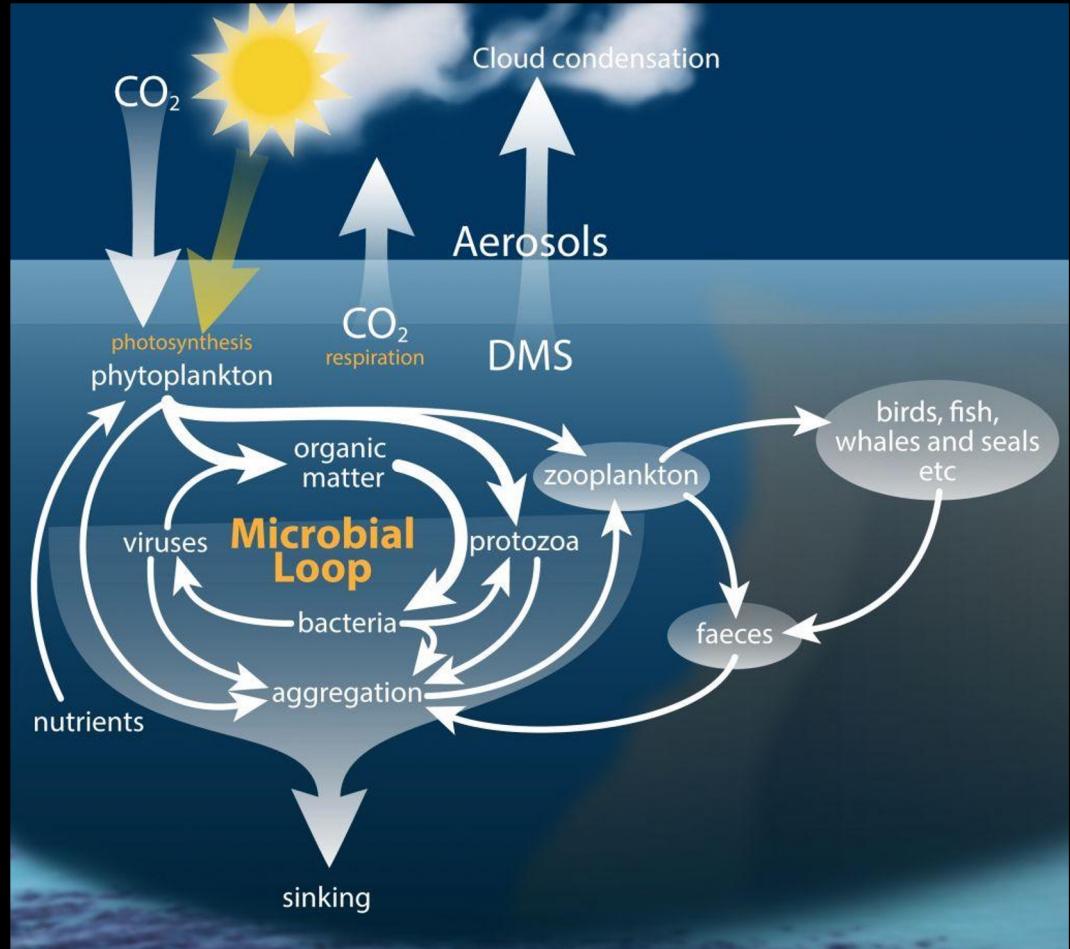
The gut microbiome can:

1. Digest our food
2. Affect GI issues (bloating, constipation)
3. Reduce/increase gut inflammation
4. Inhibit/modify different therapeutics (cancer treatment, diabetes drugs)
5. Alter Body Mass Index
6. Drive (in part) cardiovascular disease
7. Produce/stimulate the production of serotonin

<https://app.jove.com/t/62646/visualization-of-gut-microbiota-host-interactions-via-fluorescence-in-situ-hybridization-lectin-staining-and-imaging>

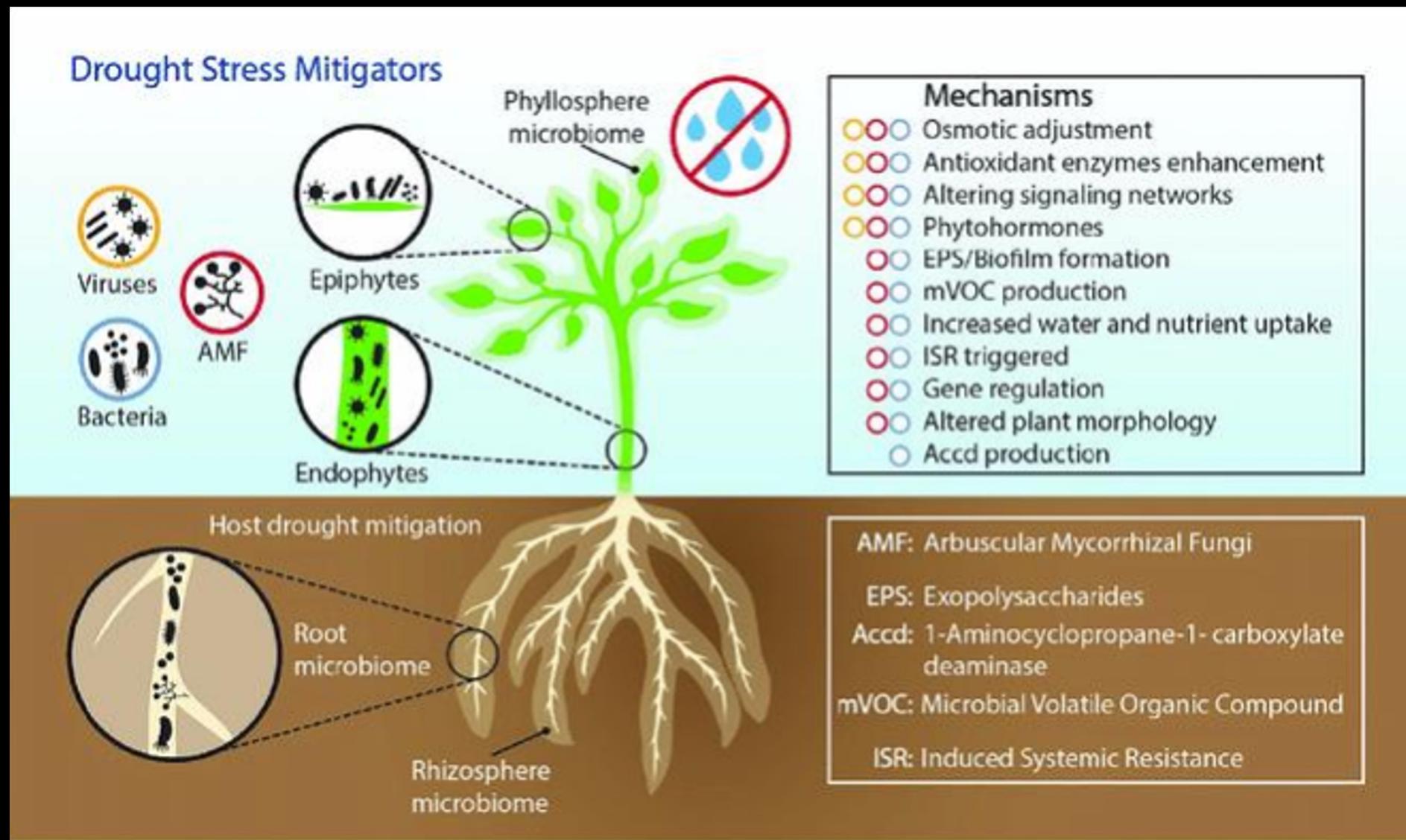


The carbon cycle!



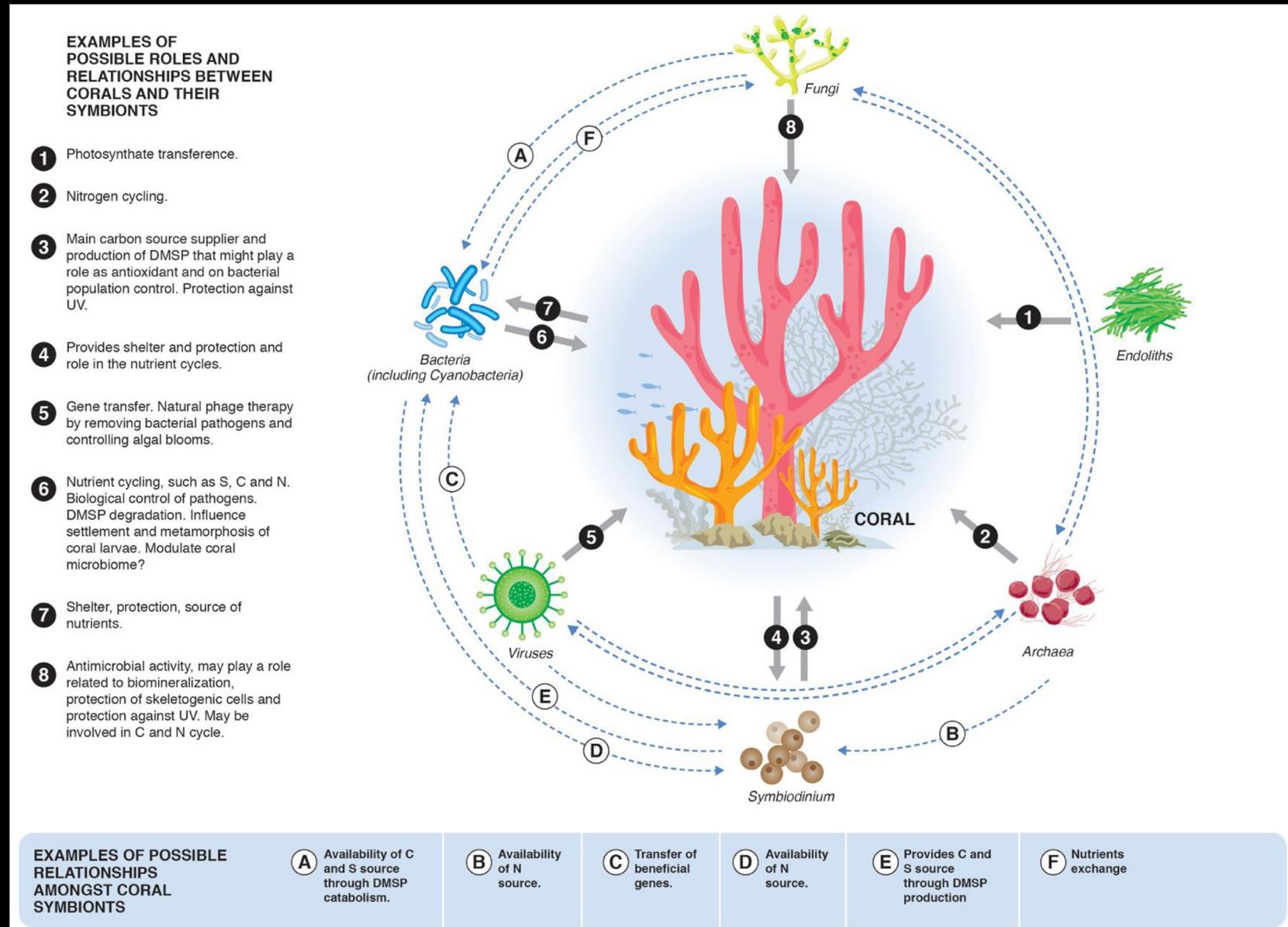


Agriculture!





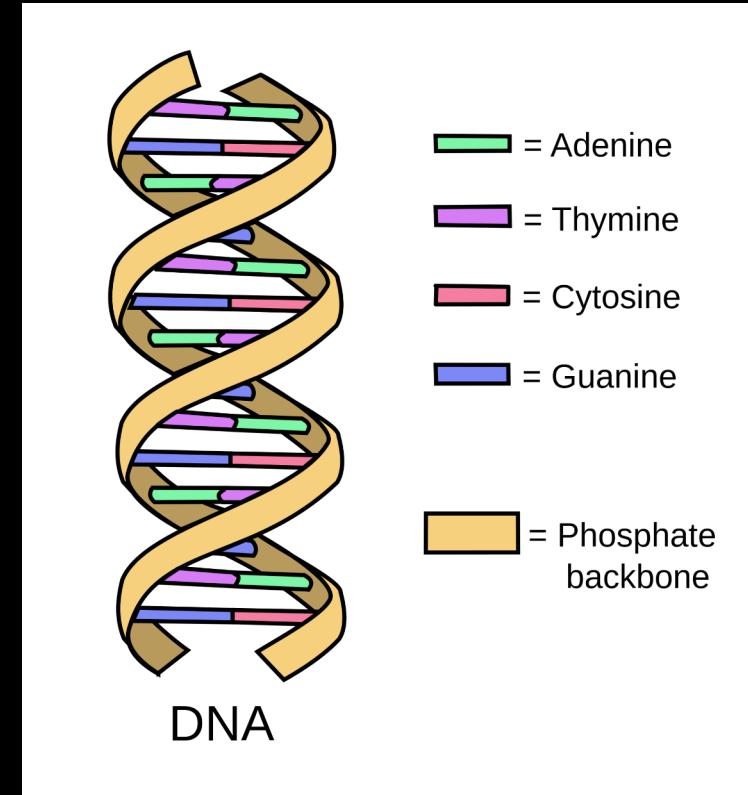
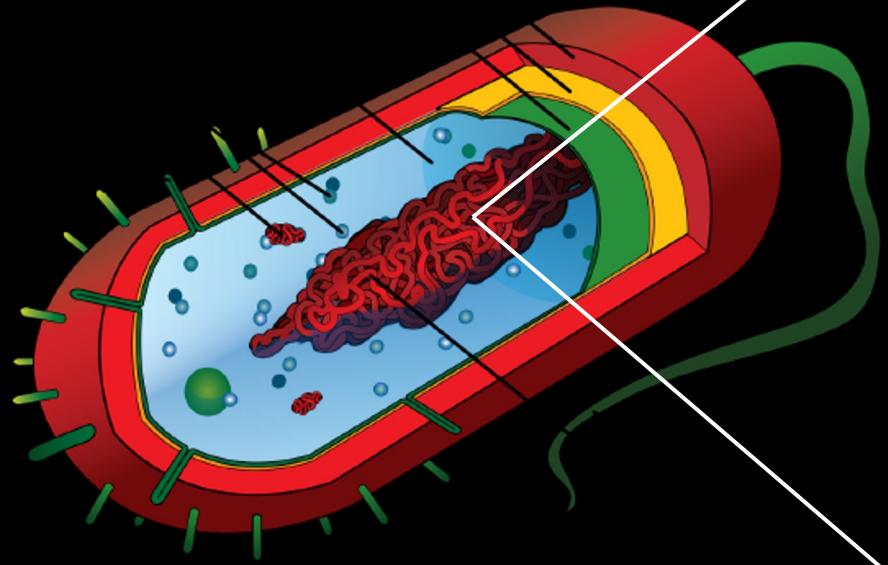
Coral reefs!



But how do we study microbial communities?

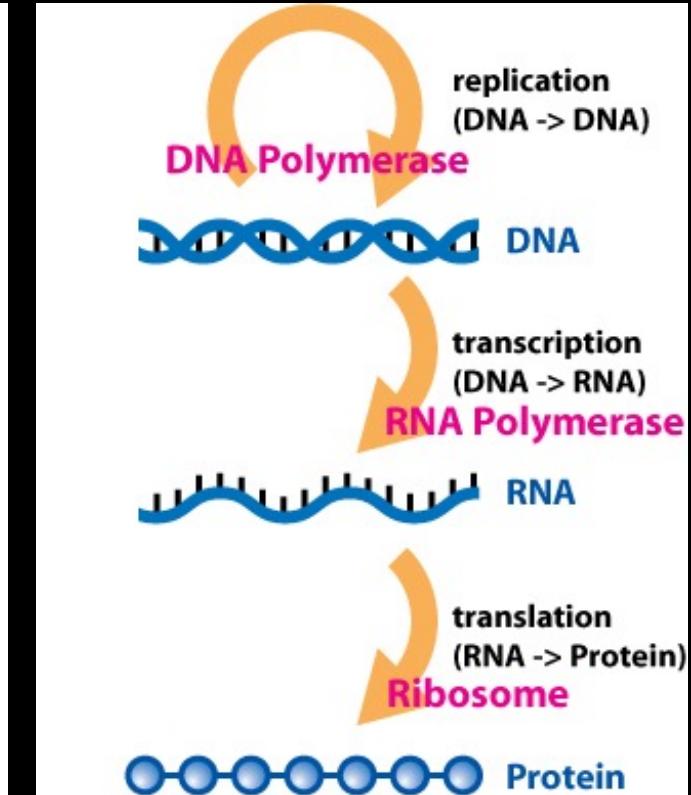
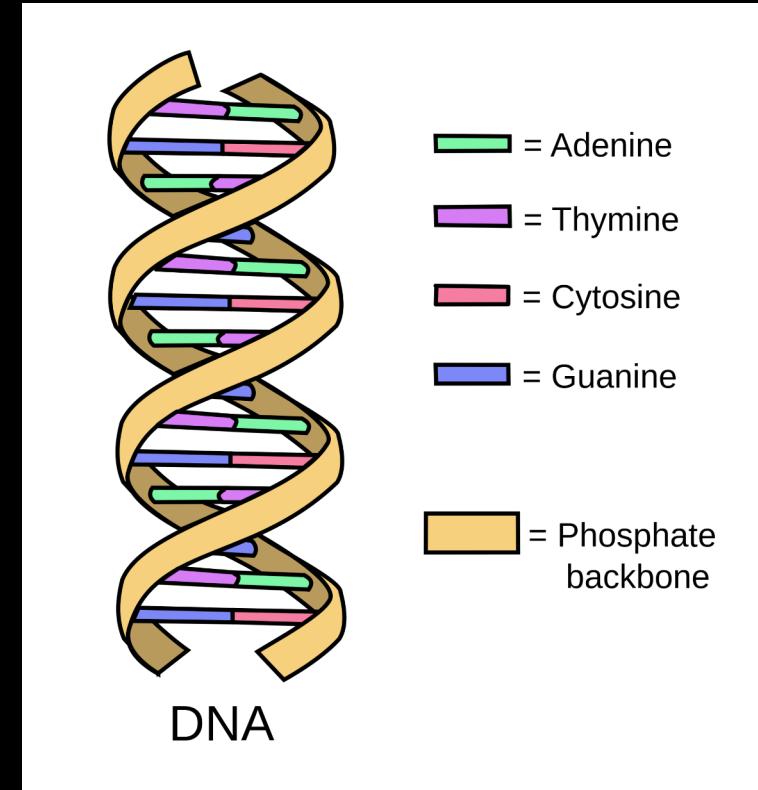
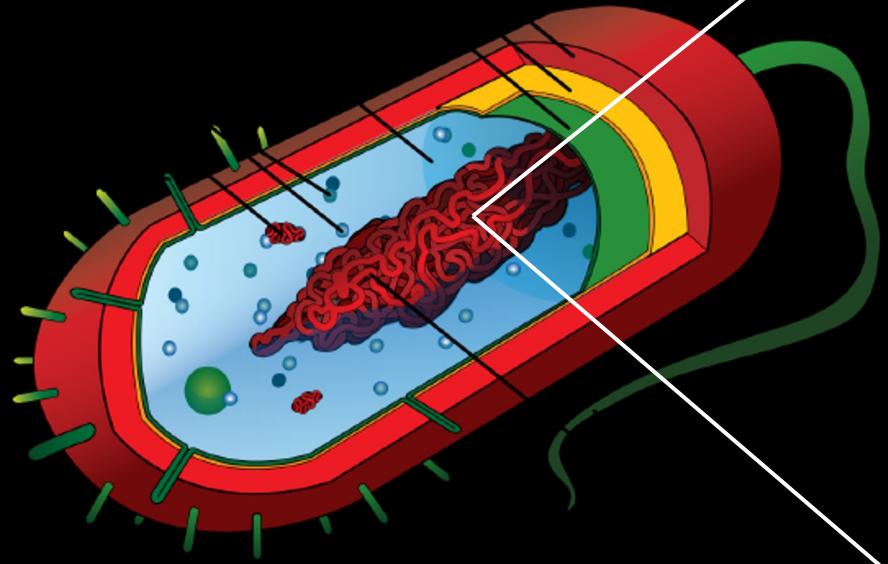


The universe of **microbiomes** are a vast unseen natural resource written in DNA



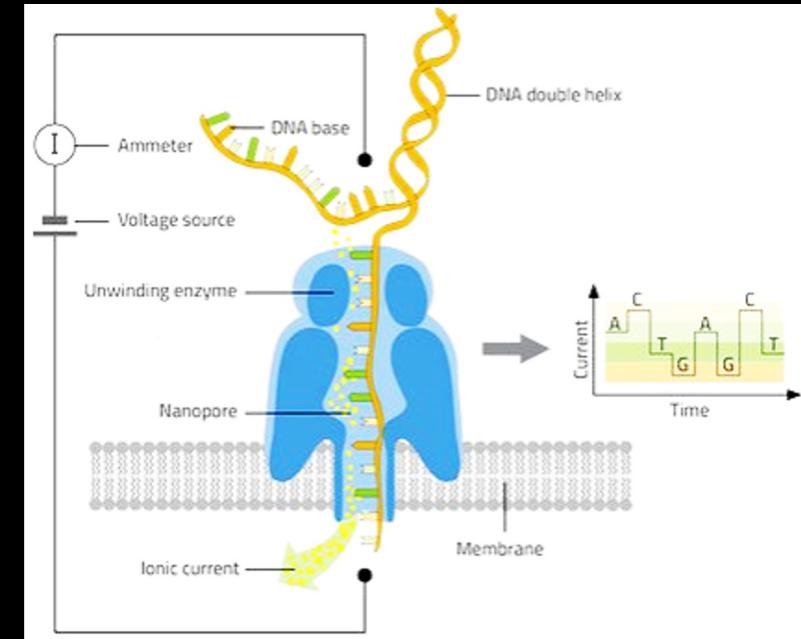
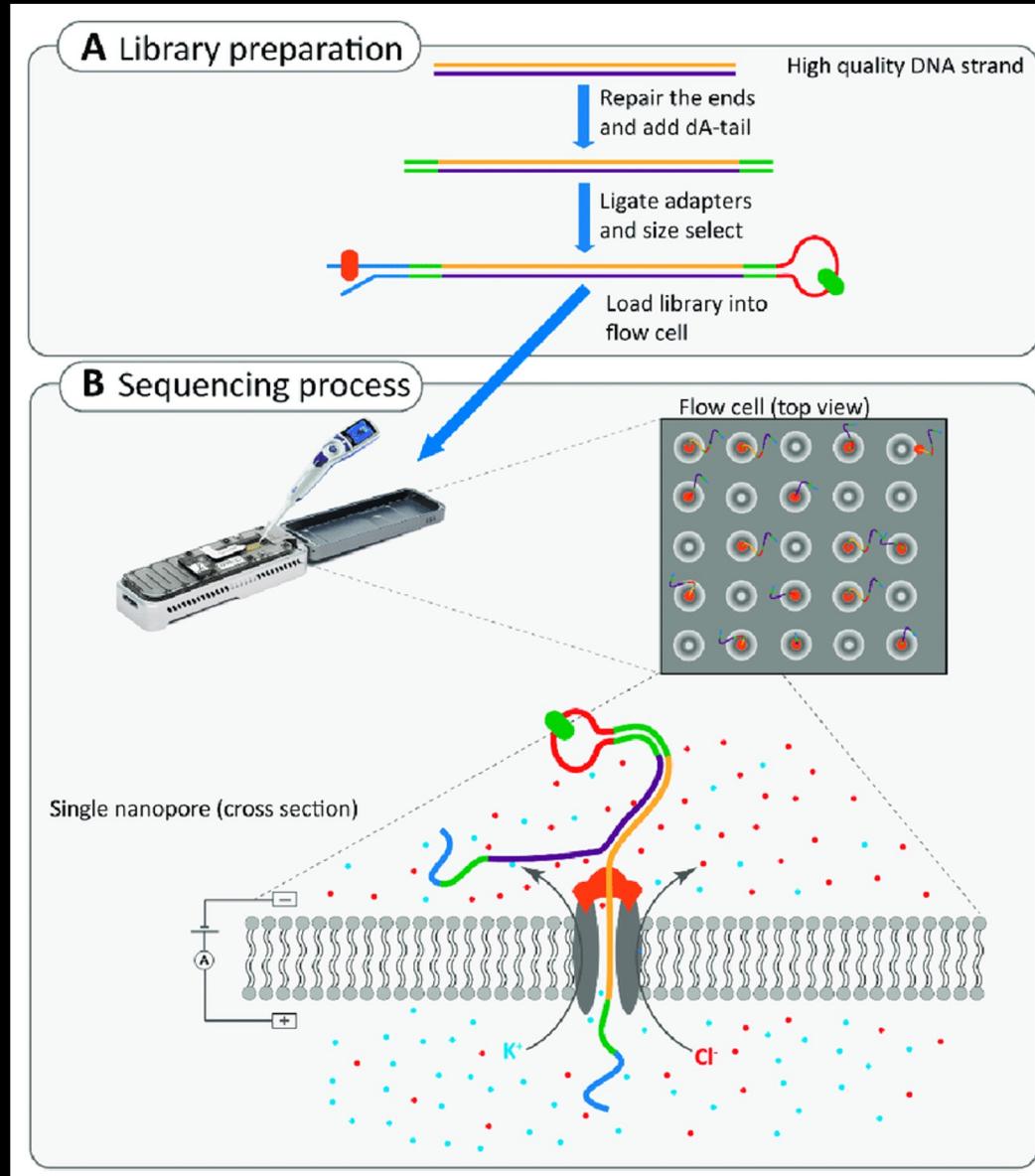


The universe of **microbiomes** are a vast unseen natural resource written in DNA



....but as we know, DNA is transcribed to **RNA**, which is translated to **protein**

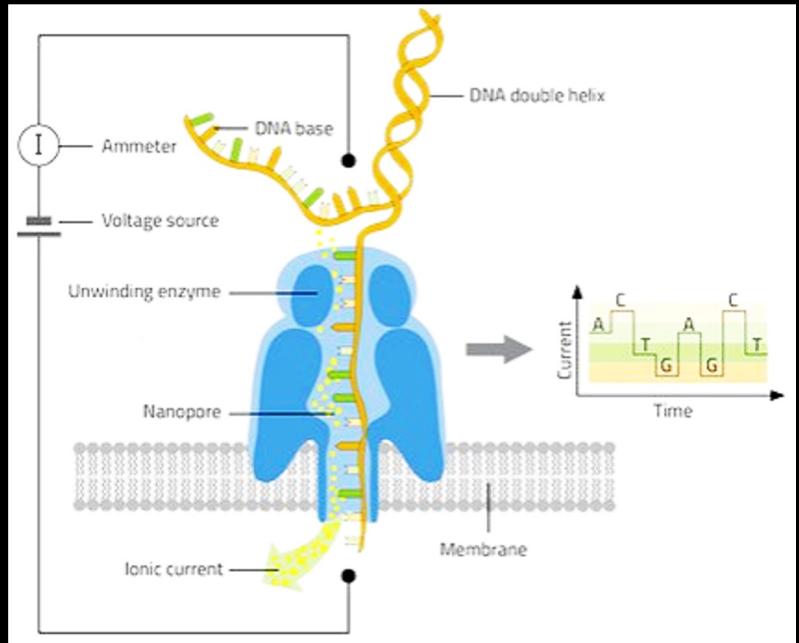
We can sequence both DNA and RNA -- but what does that actually mean?



3rd generation
Long-read technology



Bioinformatics



CCTCTTCCTCTCTGATCTT
TTGATCTTCTCCAGAAATG
CAGAAATGTCCAGAAAAAT
AGAAAATCCAACGTGCCTC
CGATCCCAAATCCAAGTGC
AAGTGCCTCAGAGTCTACT
TCTACTTGATCTTCTCT
TTCTCGATAACCAAAATCC
AAATCCAAGTGCCTCAGAT

“Ctrl - F” searching for signatures of known bacterial genomes

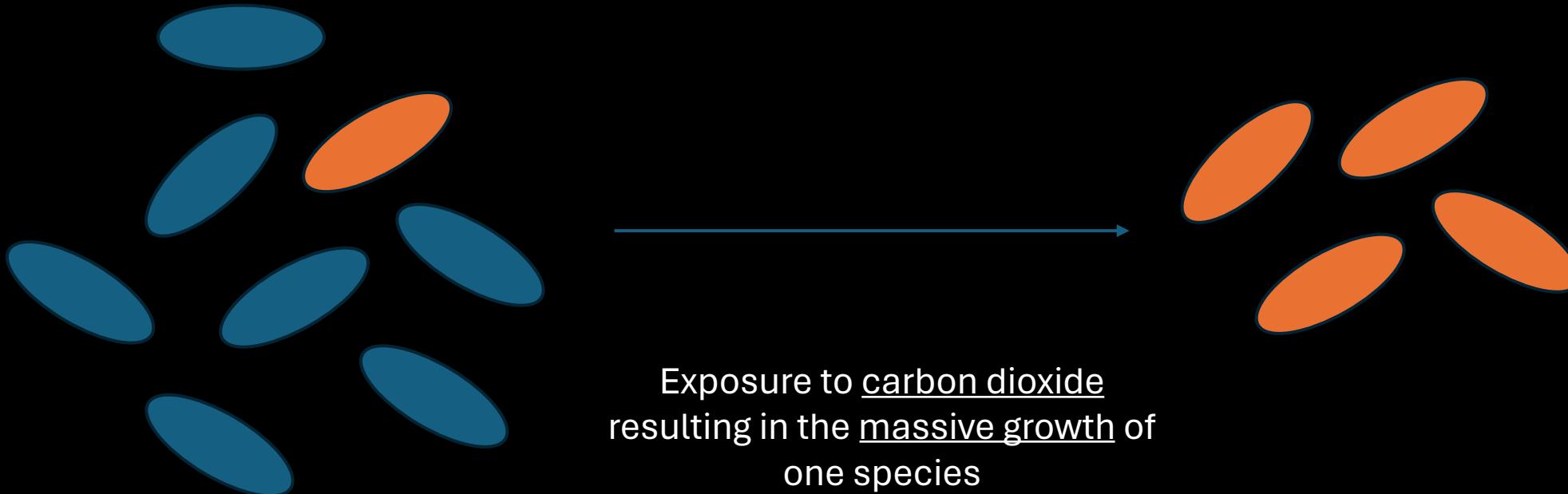
Found match:

Cyanobacterium aponium var vulcano
DNA sequence for CO₂ capture gene

TGTCCAGAAATATCCA **AGAAAATCCAACGTGCCTC** TTCCCTCTTGATCGGCTCCAGAAATGTCCA
GAAATATCCA **AGAAAATCCAACGTGCCTC**

We can sequence both DNA and RNA -- but what's the biological interpretation of doing so?

Let's consider a simple experiment



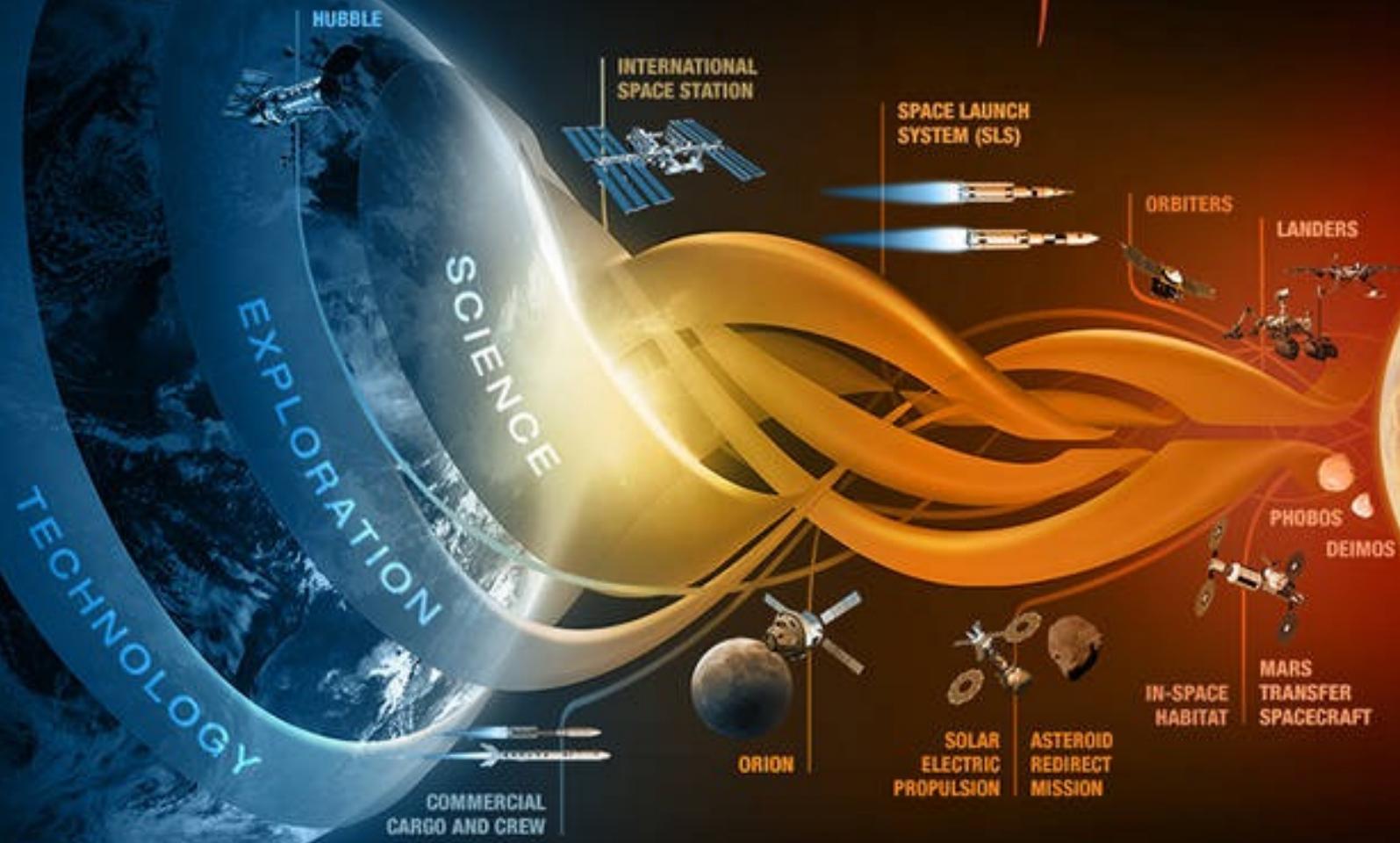
What would you expect to see in DNA vs. RNA sequencing?

Two brief vignettes applying RNA sequencing in actual studies

1. Astronaut microbiota
2. Wastewater Sequencing



JOURNEY TO MARS



Mammals were not made for spaceflight....



Spaceflight dramatically alters human physiology

“My skin had not touched anything in 340 days...
anything it touched, it felt like it was on fire.”

“rashes and discoloration anywhere I had contact.”

“flu-like symptoms”

“wanted to go to the emergency room”

-Captain Scott Kelly

**Long-term survival in space requires understanding and
mitigating these changes**

8.30.2021

Inspiration4 Crew Will Conduct Health Research to Further Human Exploration of Space



Space Omics and
Medical Atlas (SOMA)
IRB Protocol

		Subject Location During Study									
		Space-Earth			In-Flight				Post-Flight		
		Pre-Flight			FD 1-3				Post-Flight		
Sample	Analysis	June 14th	July 5th	July 26th	Sept 13th	R+ 0-7	R+ 14	R+ 30	R+ 6m	R+ 1y	R+ 2y
4 Vials & 1 Card	PBMCs	●	●	●	●	●	●	●	○	●	○
	Epigenome	●	●	●	●	●	●	●	○	●	○
	scRNA/scATAC	●	●	●	●	●	●	●	○	●	○
	Sorted Blood (CD4+, CD8+, CD19+, LD)	●	●	●	●	●	●	●	○	●	○
	Epigenome	●	●	●	●	●	●	●	○	●	○
	RNA-seq	●	●	●	●	●	●	●	○	●	○
	TCR	*	●	●	●	●	●	●	○	●	○
	Telomeres	●	●	●	●	●	●	●	○	●	○
	Plasma	●	●	●	●	●	●	●	○	●	○
	Biochemistry **	●	●	●	●	●	●	●	○	●	○
2 Vials	Cytokines **	●	●	●	●	●	●	●	○	●	○
	Metabolomics **	●	●	●	●	●	●	●	○	●	○
	Proteomics **	●	●	●	●	●	●	●	○	●	○
	cfDNA/cfRNA **	●	●	●	●	●	●	●	○	●	○
	Exosomes	●	●	●	●	●	●	●	○	●	○
1 Sample	Stool	●	●	●	○	●	●	●	○	●	○
	Metagenomics	●	●	●	○	●	●	●	○	●	○
1 Sample	Urine	●	●	●	○	●	●	●	○	●	○
	cfDNA	●	●	●	○	●	●	●	○	●	○
	Exosomes	●	●	●	○	●	●	●	○	●	○
1 Sample	Saliva/Buccal	●	●	●	●	●	●	●	○	●	○
	RNA-seq	●	●	●	●	●	●	●	○	●	○
	Metagenomics	●	●	●	●	●	●	●	○	●	○
1 Skin Punch 10 Swabs	Skin	●	○	●	●	●	●	●	○		
	Functional	●	○	●	●	●	●	●	○		
	Metagenomics ***	●	●	●	●	●	●	●	○	○	○
1 Sample	Reproductive Tissue	○	○	○	●	○	○	○	○	○	○
	WGS	○	○	○	●	○	○	○	○	○	○
	WGBS	○	○	○	●	○	○	○	○	○	○
Tests	RNA-seq	○	○	○	●	○	○	○	○	○	○
	Cognition	●	●	●	●	●	●	●	●	●	
10 Swabs	Environment	Metagenomics				●	●	●			

Is there a microbiome-immune axis during spaceflight?

Overview of the i4 microbiome project

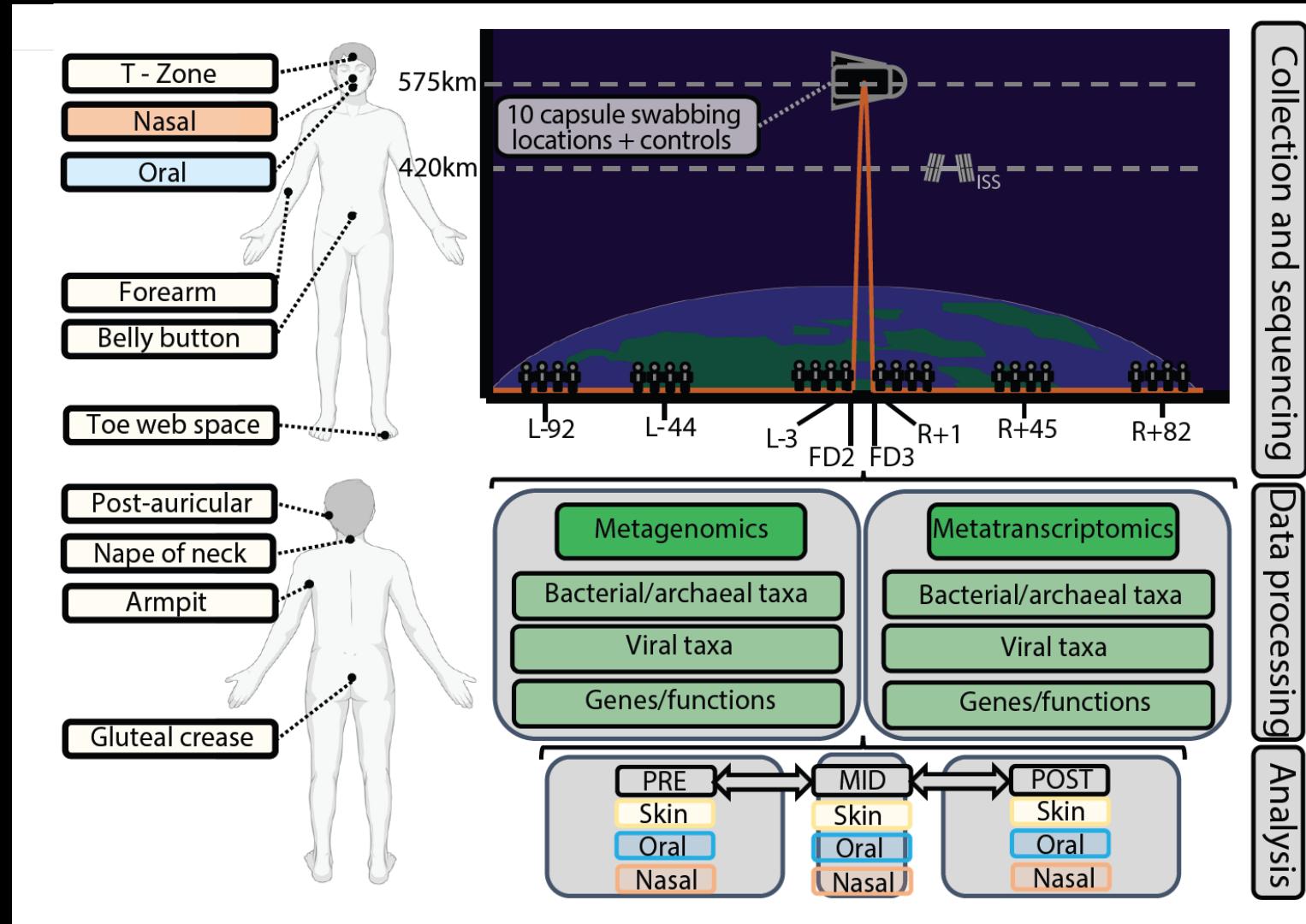
Low biomass sites!



Require extensive extraction + amplification optimization prior to sequencing



As well as extensive use of negative controls in bioinformatic analysis



Tierney and Kim et al., *in press*, *Nature Microbiology*

750 samples in total

+ integration with single cell RNA seq for immune cell profiling

Kim and Tierney et al, in revision

Three questions:

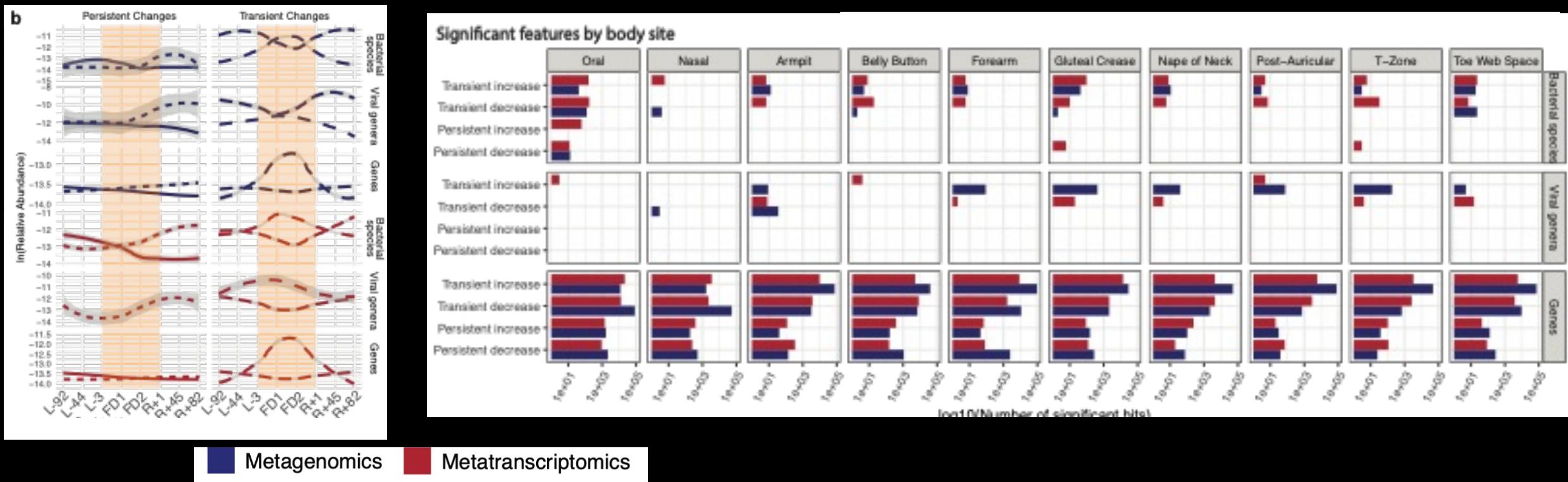
Are metagenomic and metatranscriptomic changes
in the same features?

What are the sources of these microbiome shifts?

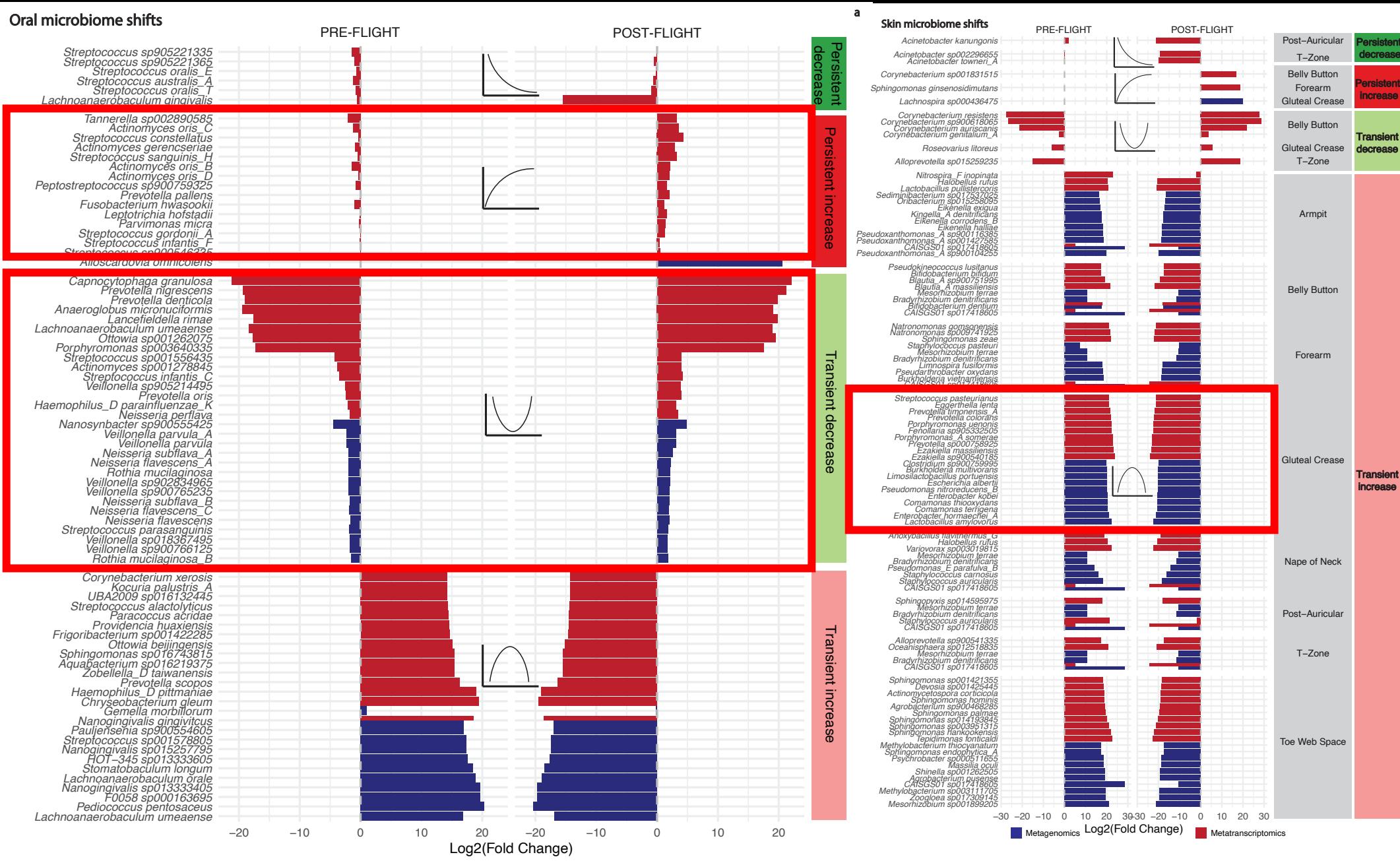
What is the relationship between microbiome shifts
and host immune cell gene expression?

The microbiome “architecture” dramatically shifts during spaceflight, with varying degrees of permanence

Taxa/genes were grouped into four categories: transiently increased, transiently decreased, persistently increased, persistently decreased

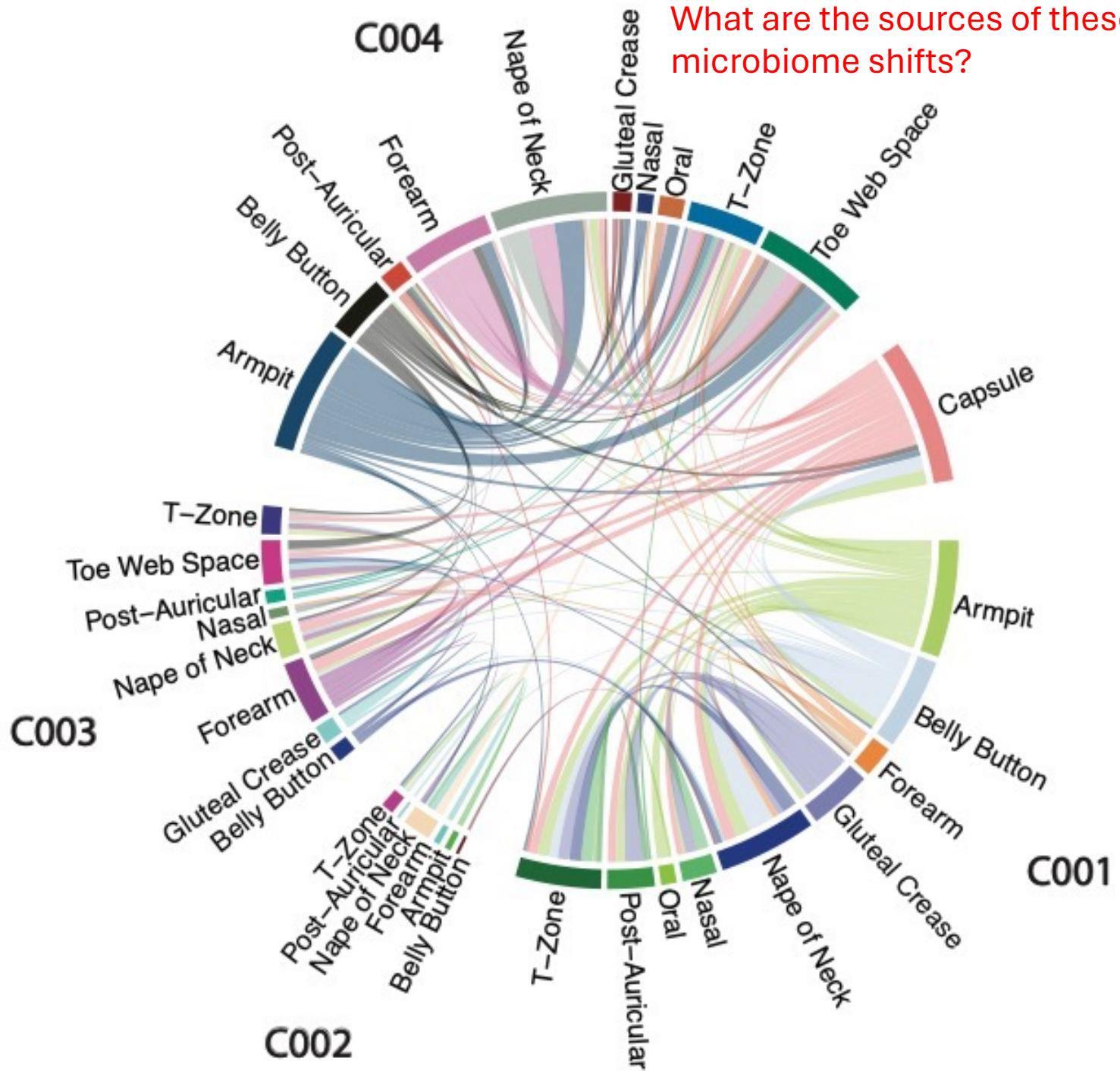
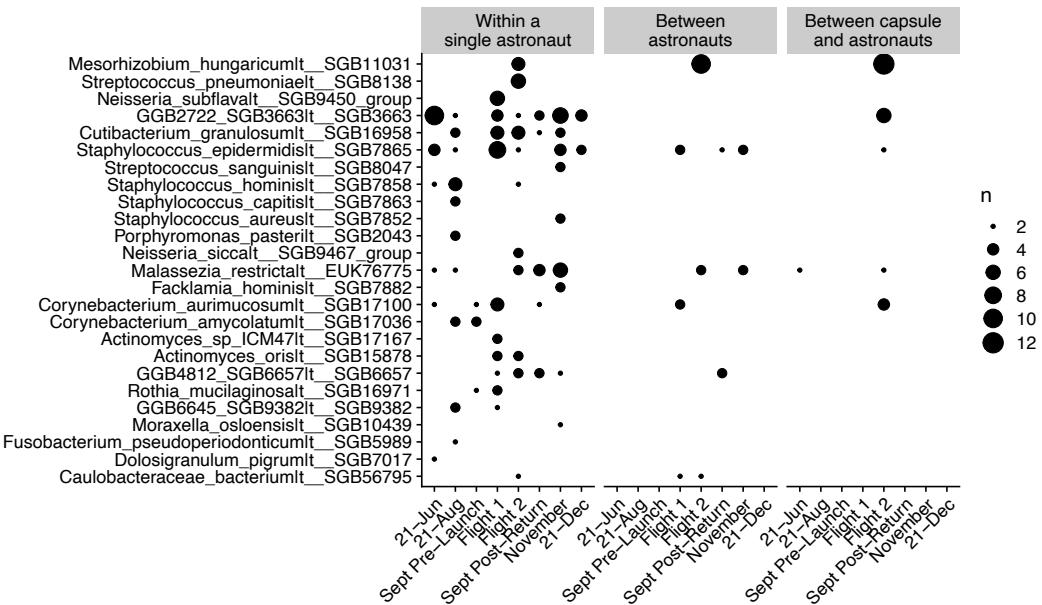
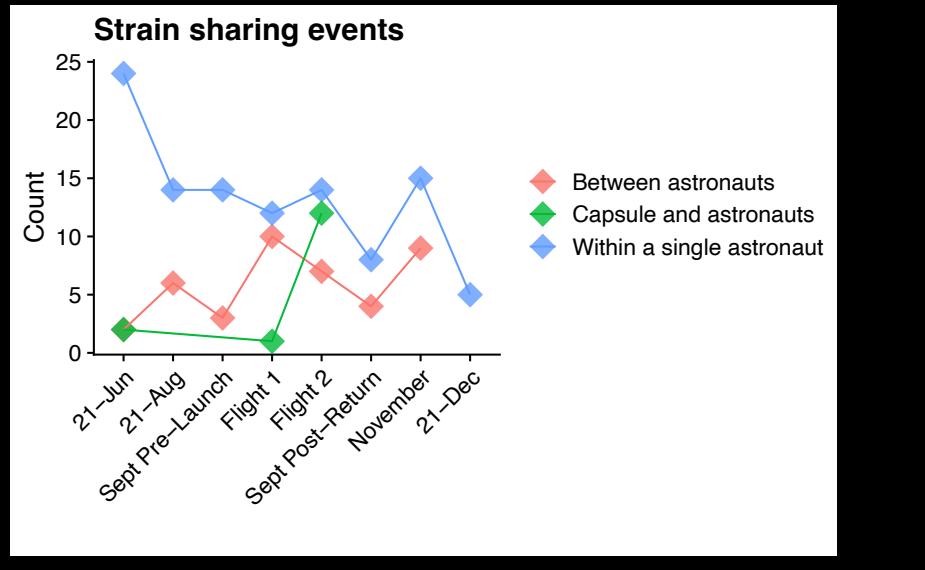


Are metagenomic and metatranscriptomic changes in the same features? No!

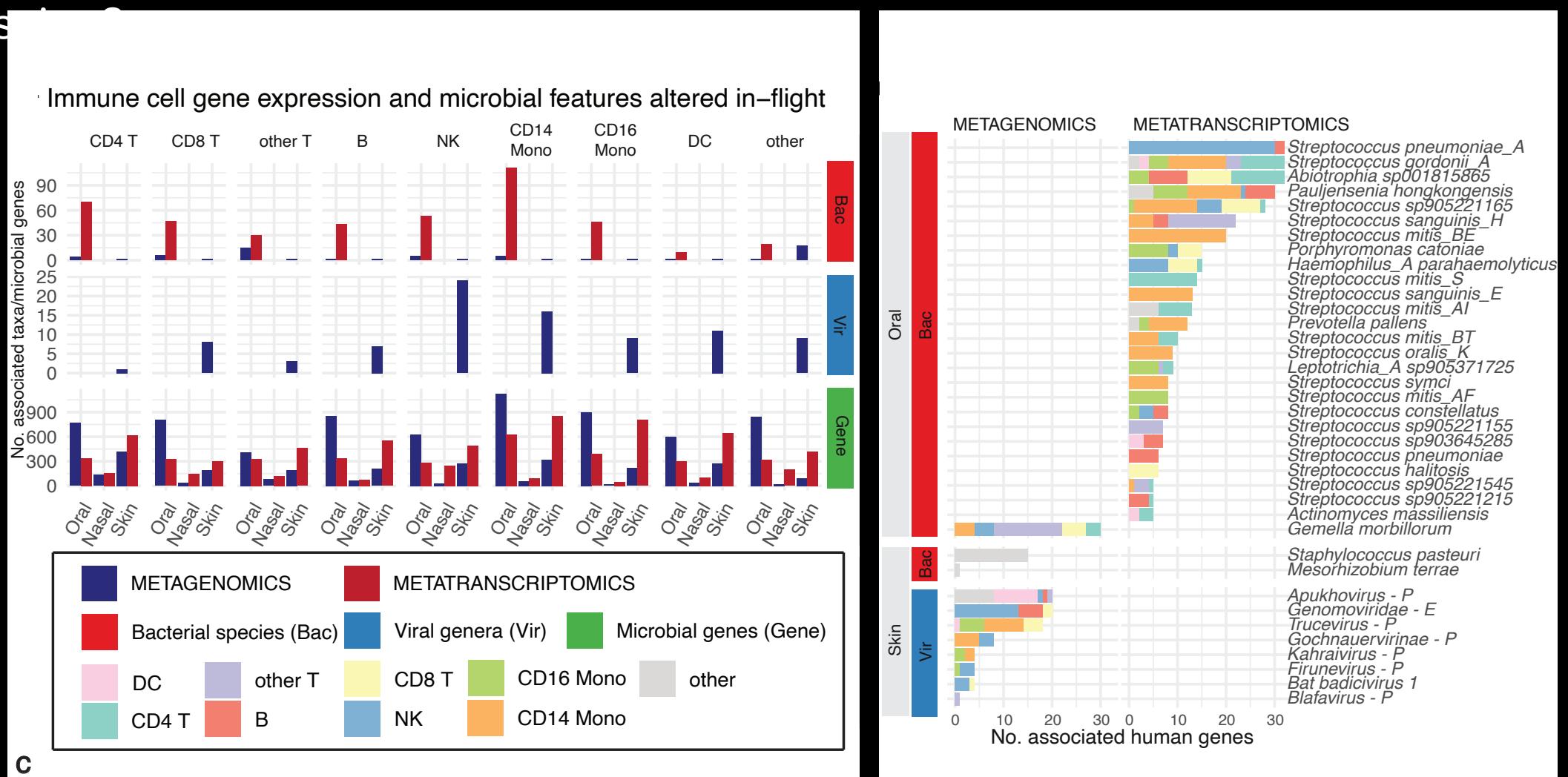


Is strain exchange
driving the
metagenomic
changes?

Skin alterations are, in-part driven by strain sharing



What is the relationship between microbiome shifts and host immune cell gene expression?



Metatranscriptomics dominates associations with immune changes

Exchanged strains are **not** associated with immune alterations

Immune alterations are correlated to expression changes in native commensals, not exchanged strains

Future directions

1. Evaluation of the microbe-immune axis in Earth-based settings
2. Drug repositioning for spaceflight according to microbial/genetic/immune signatures
3. Further data collection and analysis on future flights to validate these findings...

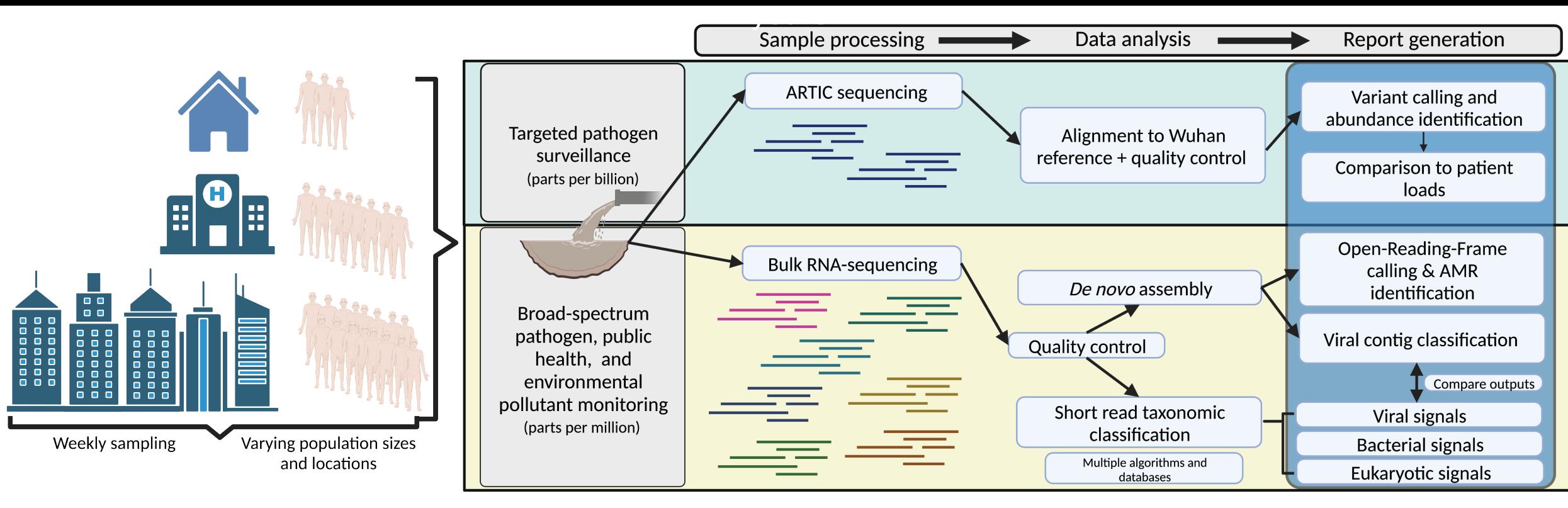
Vignette #2!

RNA sequencing of wastewater enables you to see RNA virus diversity AND bacterial transcriptional activity....

“Metatranscriptomic architectures beyond the human ecosystem: wastewater for public health surveillance”

Wastewater as a tool for public health surveillance

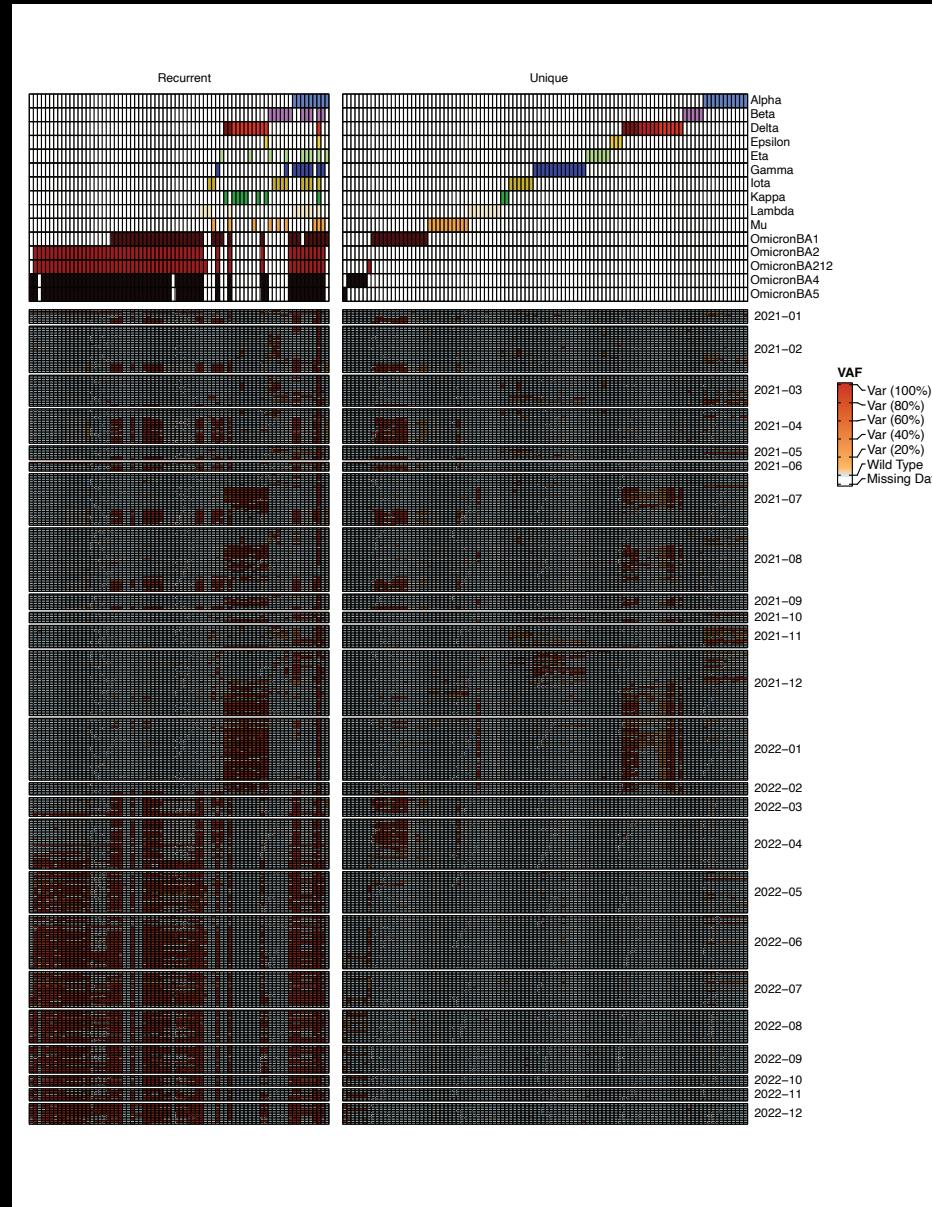
1000 targeted SARS-CoV-2 sequencing wastewater samples + 1200 bulk metatranscriptomic samples across 2



26 sites: dorms, hospitals, wastewater treatment plants, entire university campus basins

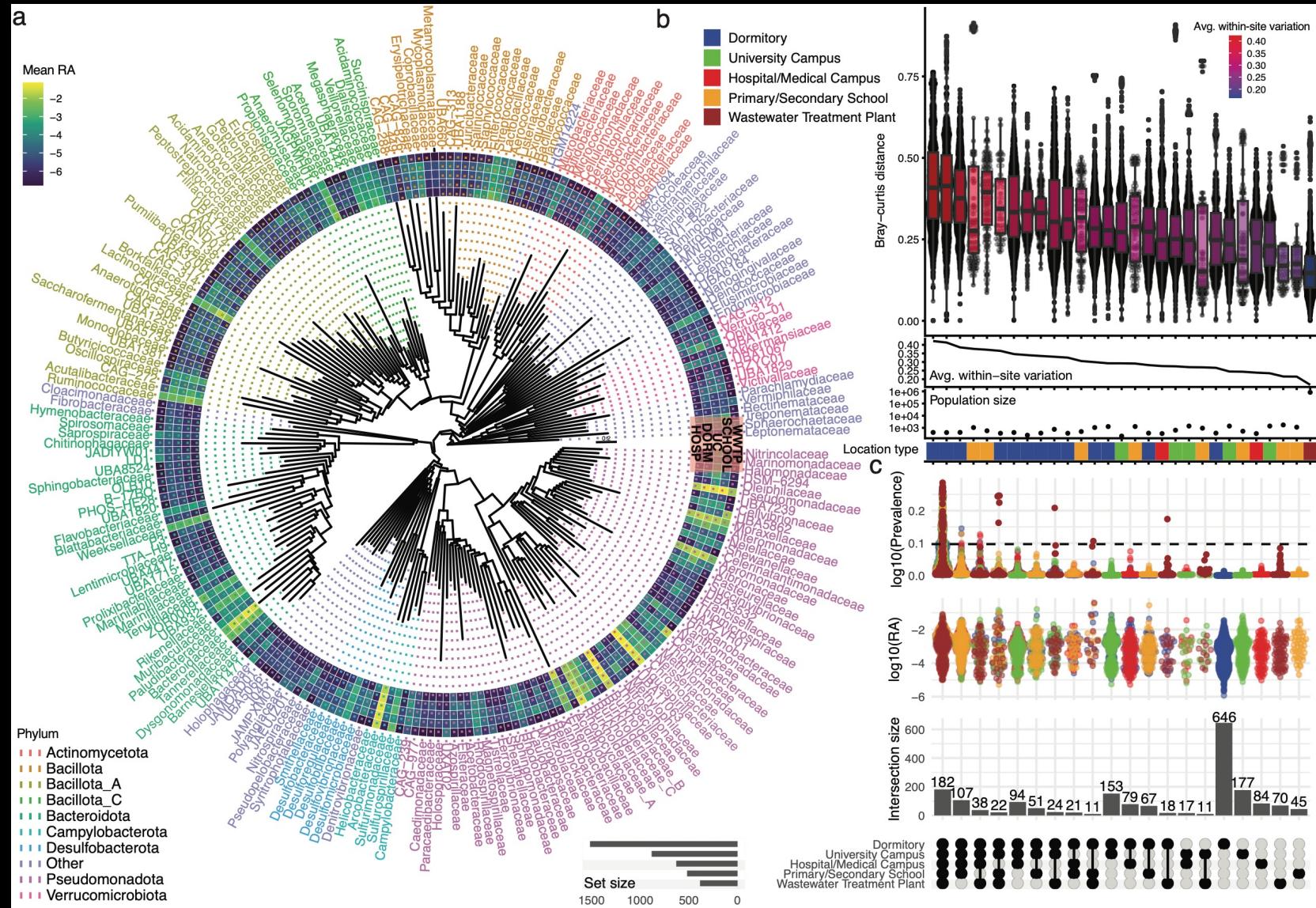
Tierney, Braden T., Jonathan Foox, Krista A. Ryon, Daniel Butler, Namita Damle, Benjamin G. Young, Christopher Mozsary, et al. 2023. "Geospatially-Resolved Public-Health Surveillance via Wastewater Sequencing." *medRxiv : The Preprint Server for Health Sciences*, June. <https://doi.org/10.1101/2023.05.31.23290781>. [in revision]

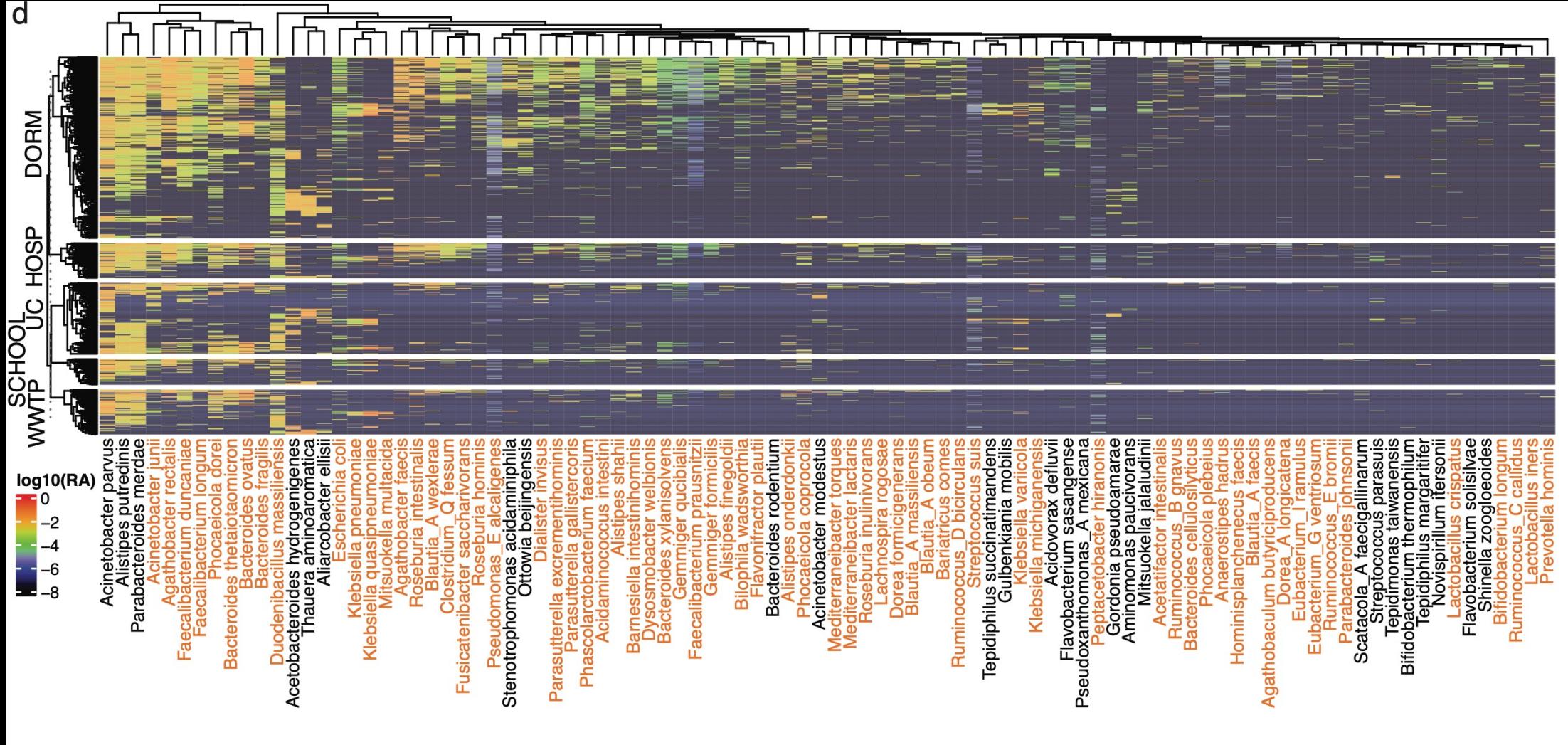
Wastewater as a tool for geospatially-stratified public health surveillance

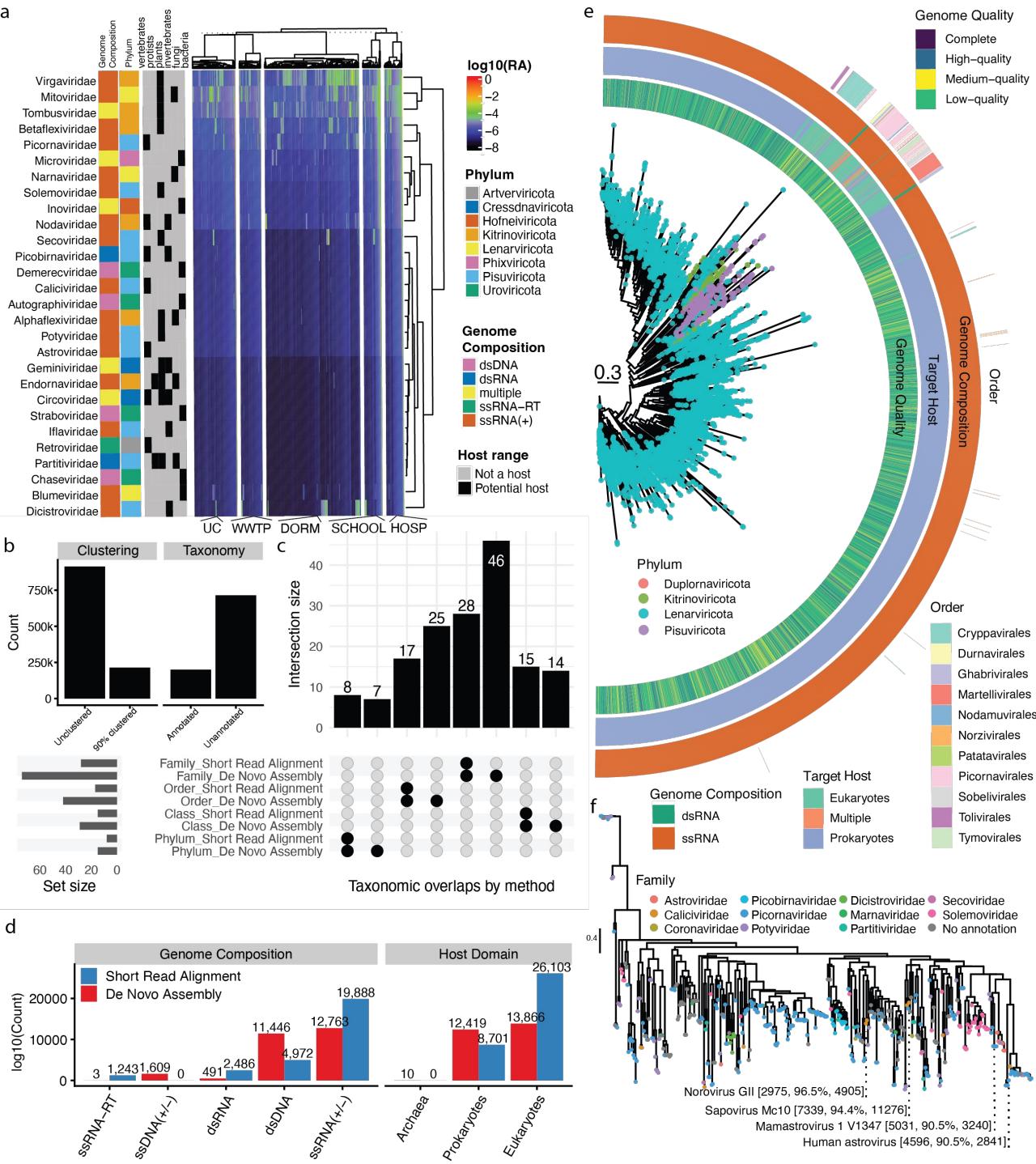


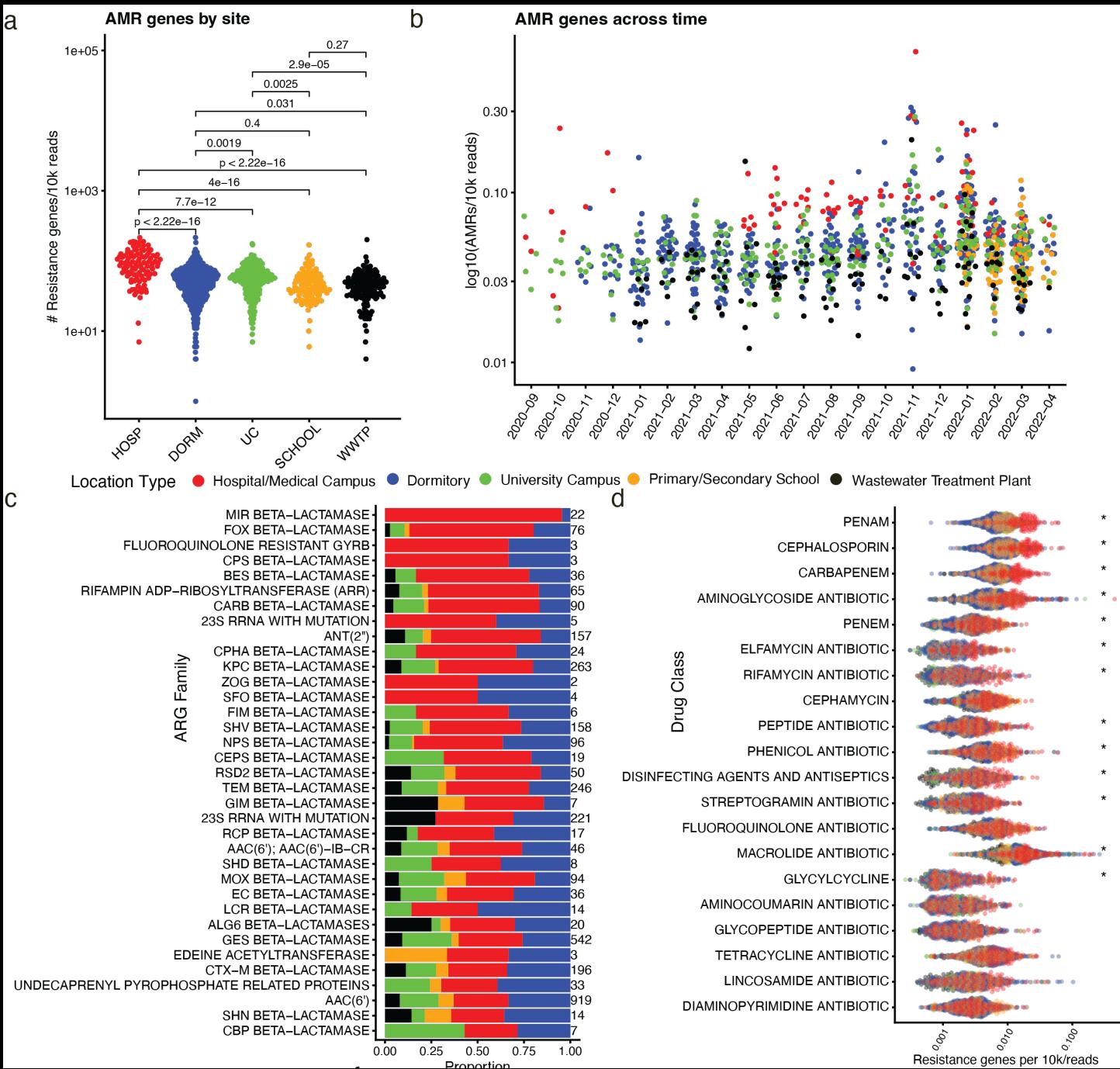
As expected, SARS-CoV-2 waves can be observed over time, often before peaks in the clinic

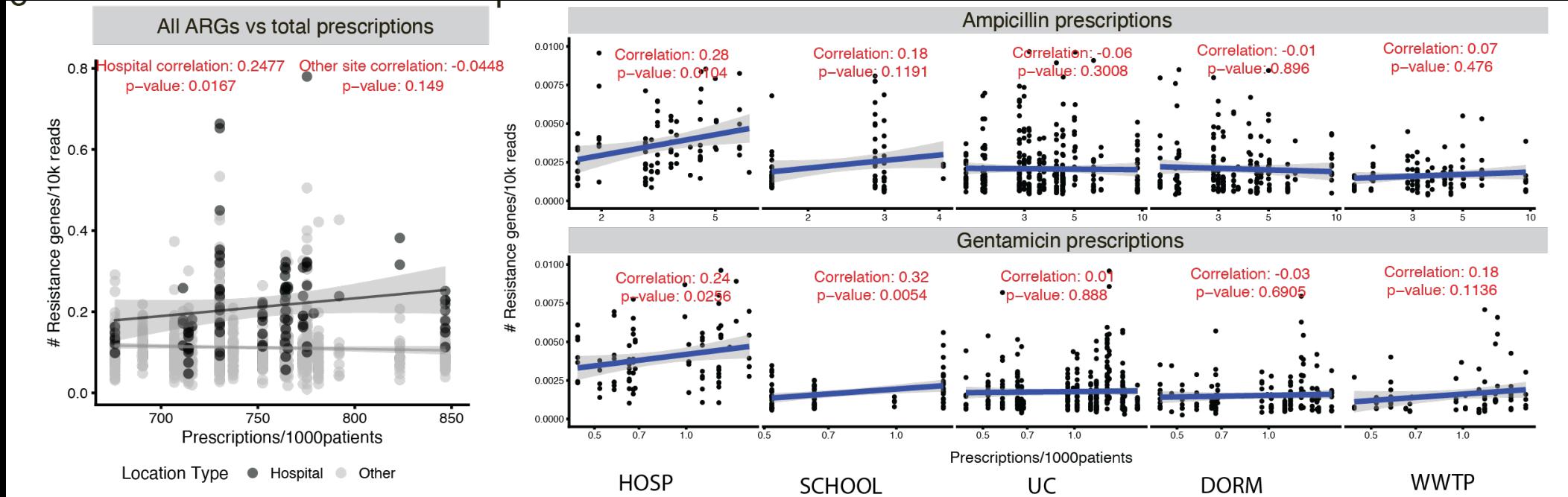
However, bulk metatranscriptomics reveals a diverse, human-relevant microbial architecture of wastewater











Wrapping up....

1. Microbial communities hold all kinds of secrets that are useful for humans
2. RNA tells a very different story than DNA in microbial communities – important to look at both!



2FRONTIERS PROJECT



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