Sampling – the good, the bad, and the ugly

Considerations for designing a microbiome research project

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Getting Started
put the "Re" in Research

The Good: General Considerations

- **Question or hypothesis:** before you dive into sampling, it is important to revisit your hypotheses.
- This will help you set the right objectives and the right expectations for sampling.
- Ask yourself what you would need to see in the data you receive in order to support or reject your hypothesis.

Objectives & Scientific Contribution

- **Objectives:** Now that you have envisioned possible outcomes for your study, what are your primary and secondary objectives?
- **Note:** secondary objectives find evidence for forming a follow-up hypothesis, or it may be to gain more detailed mechanistic understanding of the research topic.
- **Scientific Contribution:** is it a novel study, who will benefit from this research, how will this question contribute to the corpus of knowledge?

Sampling Considerations

- **Type of study:** another very important consideration that will help you better design your study. Is this an **observational study,** which would bring up a set of new factors to consider, such as:
  - Stratification by potential confounders – examples include age, sex, diet, lifestyle factors, medications
- By contrast**, interventional studies**:
  - evaluate the effect of medications, probiotics or other interventions on the microbiome.

To Model…or not to Model?

Type of model: a key consideration is whether you are working with an animal, plant, or microrganism model.

If so, how will the findings **translate** to human or other microbiomes? If you are working with a plant model, how will it translate to other species?

No model organism? Just remember that this path is one of discovery…and can have its own challenges.

http://thecircular.org/
www.mayo.edu/research/labs/zebrafish-genetics/
Arabidopsis – Getty Images https://www.gettyimages.com/detail/photo/interior-of-a-fitotron-with-three-handles-of-royalty-free-image/840131416?adppopup=true

Sampling Ethics

- **Ethics:** there are multiple regulatory rules that need to be considered. For example, if it's a human study, how will the personal data be stored and is there an infrastructure in place to protect patient privacy?
- **Institutional Review Board's (I.R.B)** approvals are a common step in study planning as well.
- If it is an animal study, other regulations such as **American Association for Laboratory Animal Science (IACUC)** approval may be required. If you are working with soil or plant samples, field experiments will often have applicable regulations.

https://www.shutterstock.com/image-photo/wooden-signpost-code-ethics-concept-accountability-537942472

Sampling Ethics: the Bad & Ugly – Take Caution

- What are your motivations to sample in a particular region?
- "Fortune and glory, kid…"

OCTOBER 10, 2006

BACK FROM YET ANOTHER GLOBETROTTING ADVENTURE, INDIANA JONES CHECKS HIS MAIL AND DISCOVERS THAT HIS BID FOR TENURE HAS BEEN DENIED

by ANDY BRYAN

https://www.cnet.com/news/steven-spielberg

https://www.mcsweeneys.net/articles/

Sample Collection gone awry

- Criticisms of Dr. Jones ranged from "possessing a perceptible methodological deficiency" to "practicing archaeology with a complete lack of, disregard for, and colossal ignorance of current methodology, theory, and ethics" to "unabashed grave-robbing." Given such appraisals, perhaps it isn't surprising to learn that several Central and South American countries recently assembled to enact legislation aimed at permanently prohibiting his entry.

- Moreover, no one on the committee can identify who or what instilled Dr. Jones with the belief that an archaeologist's tool kit should consist solely of a bullwhip and a revolver." – McSweeny's

Sampling Ethics cont.

- Bioprospecting (searching) versus biopiracy (taking):

  "Biopiracy happens when researchers or research organisations take biological resources without official sanction, largely from less affluent countries or marginalised people."

- Biopiracy is not limited to drug development. It also occurs in agricultural and industrial contexts.

- Permits to sample on federal, state, county or city land

- Permission from subjects and stakeholders

Many rubber trees derive from seeds Henry Wickham took from Brazil.

Good read: Biopiracy started with a bounce- Nature 2008 – Henry Wickham 1876 & *Hevea* seeds

https://theconversation.com/biopiracy-when-indigenous-knowledge-is-patented-for-profit-55589#

Communicating the promise, risks, and ethics of large-scale, open space microbiome and metagenome research

Daria Shamarina, Iana Stoyantcheva, Christopher E. Mason, Kyle Bibby & Eran Elhaik

Microbiome **5**, Article number: 132 (2017) | Cite this article

Am J Public H

Integrating Biodiversity Management and Indigenous Biopiracy Protection to Promote Environmental Justice and Global Health

Tim K. Mackey, MAS and Bryan A. Liang, MD, PhD, JD

Research Article

When nature goes digital: routes for responsible innovation

Koen Bruynseels

Received 20 Jun 2018, Accepted 14 May 2020, Published online: 19 Jun 2020

 https://doi.org/10.1080/23299460.2020.1771144

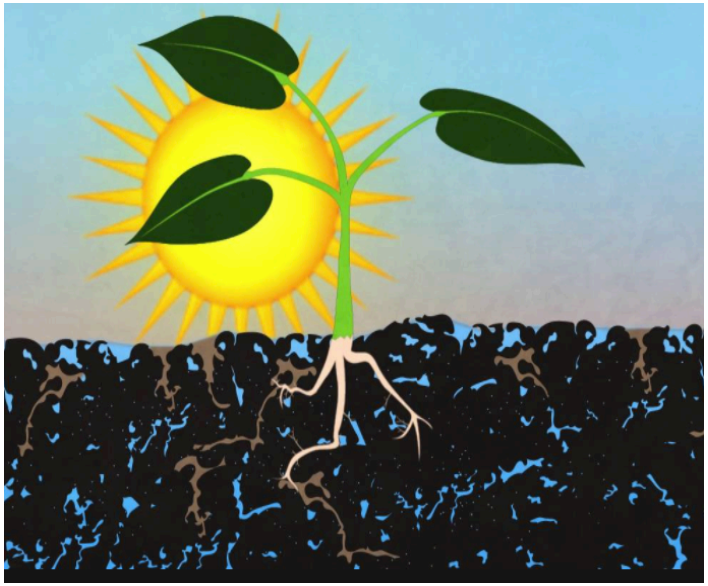
Sample Types for Microbiome Projects

- Fecal Samples – non invasive, snapshot of the gut microbiome, other well studied systems are oral/ urine microbiomes

https://microbenotes.com/collection-and-transport-of-stool-specimens/

Sample Types for Microbiome Projects

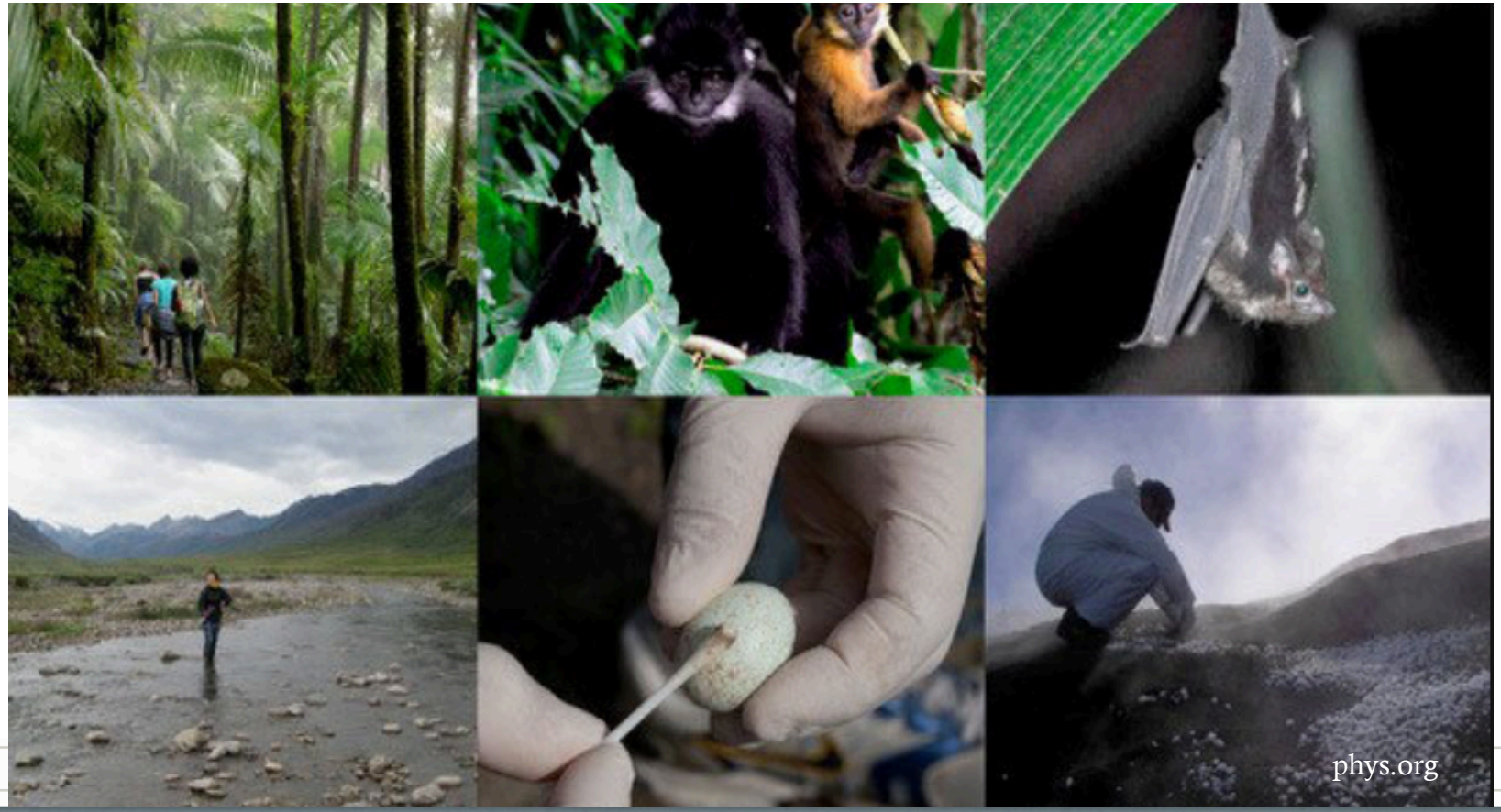
- **Soil, Plant, Water**
- Soils can also present high spatial variability in the form of microenvironments, thus replication is very important when designing soil microbiome studies.
- **A plant's rhizosphere** is important: the microorganisms (including mycorrhizal fungi) live at the interface of the plant's root system and the surrounding soil. To the plant they provide fixed nitrogen, access to micronutrients, and protection from pathogens in the soil; in turn, the plant roots release nutrients (for example, sugars and amino acids) that sustain the microbes.
- Soil microbes are an important component of many biogeochemical cycles including Carbon, Nitrogen, Oxygen, Sulfur cycles.



soil 388-OSU.edu

Environmental Samples

- Earth Microbiome Project



Toward a unified sampling protocol for sampling methods?

Open Access | Published: 01 November 2017

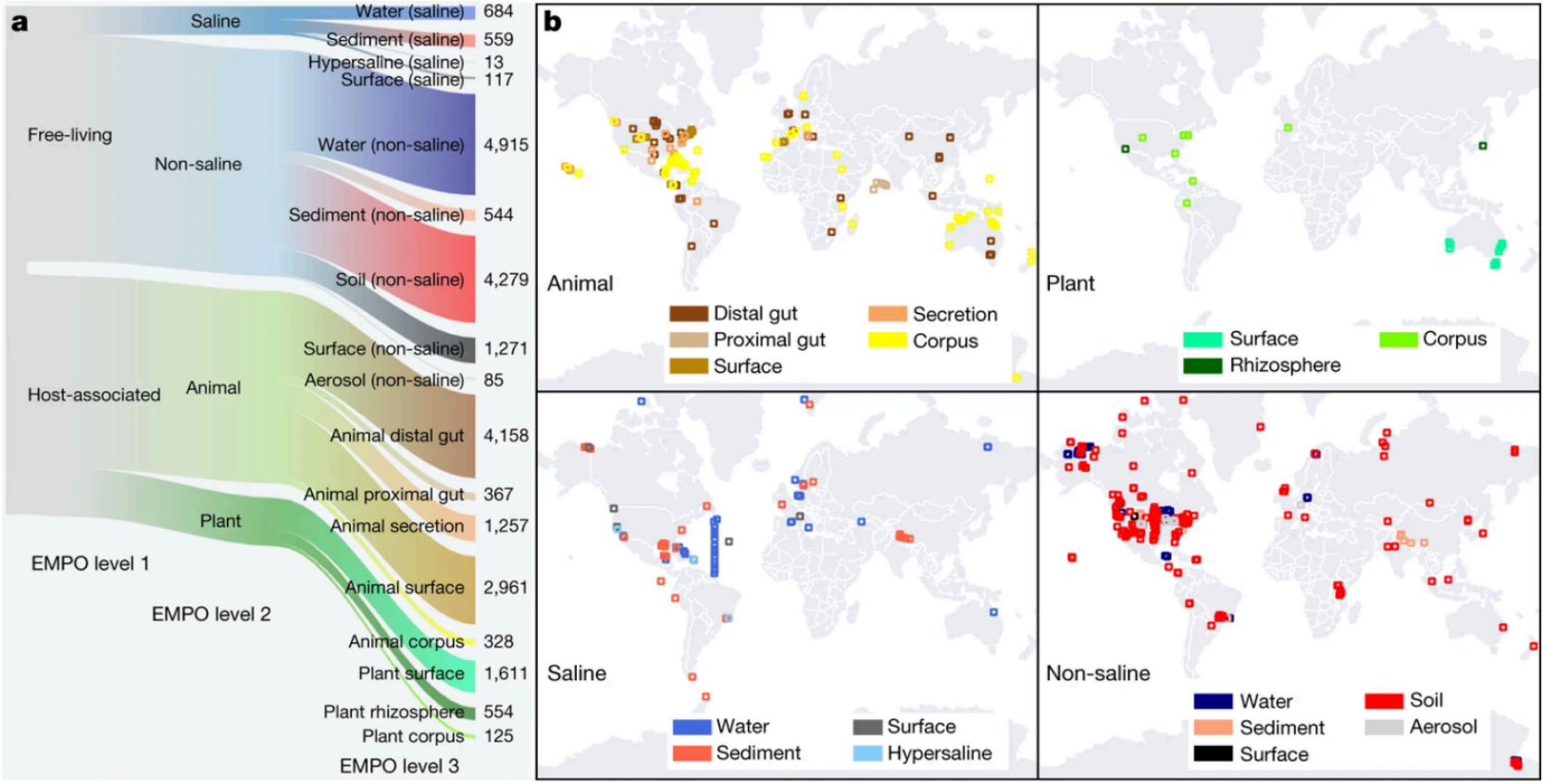
A communal catalogue reveals Earth's multiscale microbial diversity

Luke R. Thompson, Jon G. Sanders, [...] The Earth Microbiome Project Consor

Nature **551**, 457–463(2017) | Cite this article

Figure 1: Environment type and provenance of samples.

From: A communal catalogue reveals Earth's multiscale microbial diversity



a, The EMP ontology (EMPO) classifies microbial environments (level 3) as free-living or host-associated (level 1) and saline or non-saline (if free-living) or animal or plant (if host-associated) (level 2). The number out of 23,828 samples in the QC-filtered subset in each environment is provided. EMPO is described with examples at http://www.earthmicrobiome.org/protocols-and-standards/empo. **b**, Global scope of sample provenance: samples come from 7 continents, 43 countries, 21 biomes (ENVO), 92 environmental features (ENVO), and 17 environments (EMPO).

L R Thompson *et al. Nature* 1–9 (2017) doi:10.1038/nature24621

Search project website for sampling and processing protocols

Aug 22, 2018

Bookmark

Run

Copy / Fork

Earth Microbiome Project (EMP) high throughput (HTP) DNA extraction protocol

Forked from EMP DNA Extraction Protocol

Lisa Marotz1[1], Tara Schwartz[1], Luke Thompson[2], Greg Humphrey[1], Grant Gogul[1], James Gaffney[1], Amnon Amir[1], Rob Knight[1]

[1]UC San Diego; [2]National Oceanic and Atmospheric Administration (NOAA)

1 *Works for me*

dx.doi.org/10.17504/protocols.io.pdmdi46

Earth Microbiome Project

Lisa Marotz
UCSD

Sample Info: Metadata

Sample	Tree_Sample	Phytophthora	Pcinn	Type	T
ITS_ 1_BANT1A_ITS1_S3	BANT1	positive	negative	Root	R
ITS_ 2_BANT1B_ITS1_S33	BANT1	negative	negative	Root	R
ITS_ 3_BANT2A_ITS1_S63	BANT2	negative	negative	Root	R
ITS_ 4_BANT2B_ITS1_S93	BANT2	negative	negative	Root	R
ITS_ 5_BANT3A_ITS1_S123	BANT3	negative	negative	Root	R

Farm	Location	Root_vs_Soil_Phy	Salinity_dS_per_
BANT	South	No_data	1_0.5_to_1
BANT	South	No_data	1_0.5_to_1
BANT	South	No_data	1_0.5_to_1
BANT	South	No_data	1_0.5_to_1
BANT	South	No_data	1_0.5_to_1

geome-db.org

GEOME

GETTING STARTED QUERY WORKBENCH

Genomic Observatories MetaDatabase (GEOME)

The Genomic Observatories Meta-Database (GEOME) is a web-based database that captures the who, what, where, and when of biological samples and associated genetic sequences. GEOME helps users with the following goals:

- Ensure the metadata from your biological samples is findable, accessible, interoperable, and reusable
- Improve the quality of your data and comply with global data standards
- Integrate with R, ease publication to NCBI's sequence read archive, and work with an associated LIMS

Getting Started

Getting started and additional information about GEOME

Query

Search and download sequence files and associated metadata

Workbench

View and manage project specific data

Sample Collection, Storage, & Shipment

- **Who** – authorized / trained people to collect samples
- **What** – needs to be collected, in what containers, using what sterile technique. What is the spatio-temporal scale of sampling?
- **When** – can samples degrade quickly? Timing is important
- **Where** – will the samples be processed? Will they be mailed, do then need to on dry ice?
- **How** – how will the samples make it from point a to b? Do you have the permits to ship?
- **Why** – are you collecting, storing and shipping this way? Always reflect on your sampling method and figure out if there is an efficient way to do this, perhaps other more seasoned scientists have advise for you

Sampling Design

- Depends on your research question! Sometimes preliminary studies are good – smaller n, not as expensive
- **Tips**: Think about what you are measuring. If you are measuring microbial diversity…think about how you might analyze this downstream. What is a sampling size with replicates that will
- Think carefully about what is a replicate. When can /should you pool samples?
- Graphical Hypotheses – sketch out what you think your results might look like
- Then work backwards…how might I sample to test this hypothesis?

Sampling Cost

- Materials for collection, shipping storage
- Processing DNA extraction, purification, library prep (in-house cheaper)
- Sequencing Run & Analysis (
- Microbiome Analysis software (GUI software CLC Genomics or command line, R, etc.)
- Example: Amplicon-based NGS sequencing soil per sample:

$1 falcon tube/gloves x $1 shipping/ice x 1 soil sample x library prep reagents, primers, adapters for 16S $50 = ~ $50 per sample

Obtain a quote to help you plan for sampling design

PennState
Huck Institutes of the Life Sciences

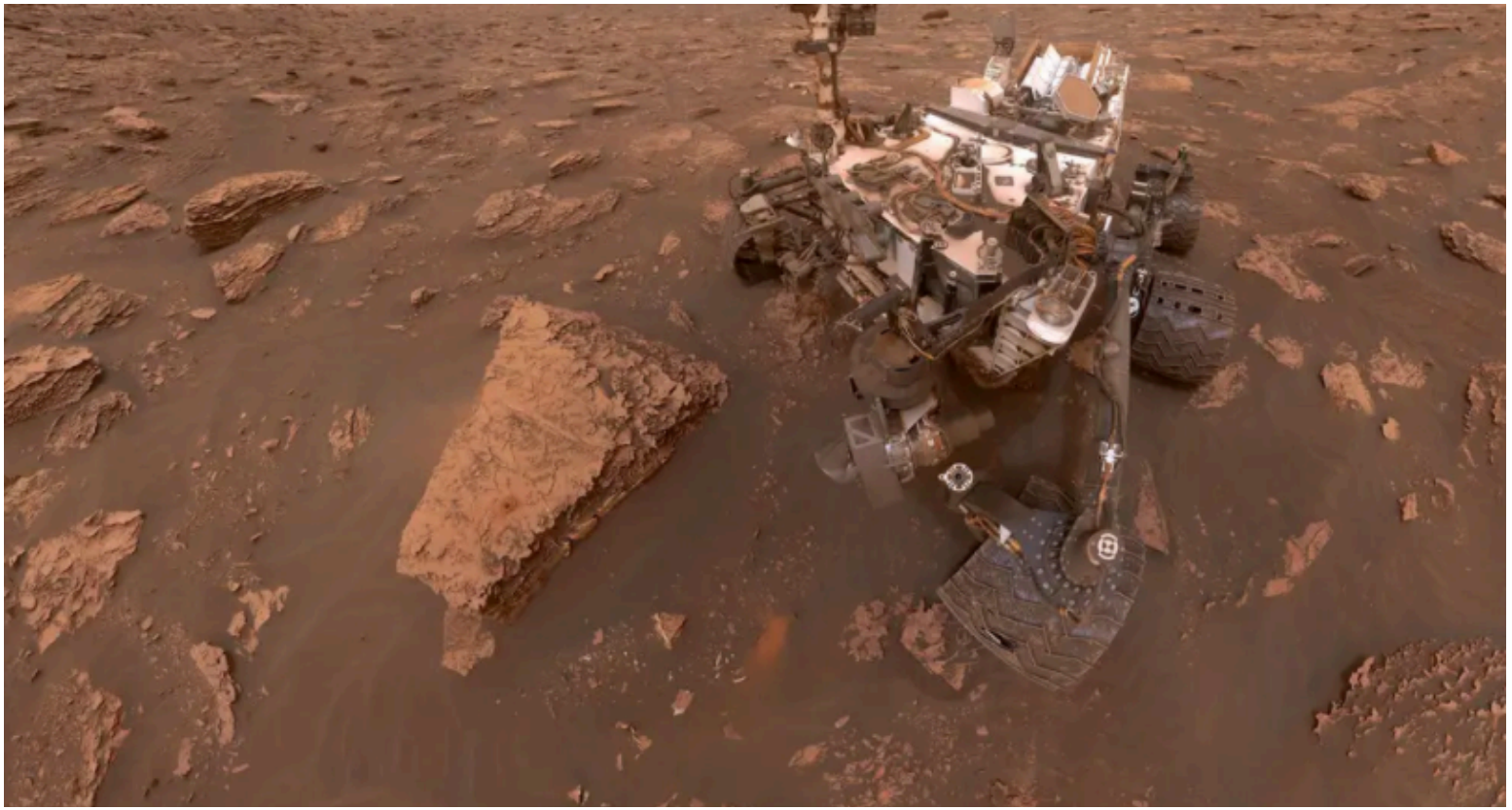
Example
Graphical (Sketched) Hypothesis

- **Hypothesis:** the abundance of soil microbes will be higher in undisturbed vs. disturbed field sites.
- **On your own:** try to sketch out a bar graph based on this hypothesis on a piece of paper. Label your axes!
- Think about what **type of samples** you'd need to test this hypothesis? List them next your sketch. This is a good starting point by thinking about a statistical measure first.

grist.org

Breakout groups:
Space Force Scenario!

Your Mission – Propose the best way to sample the Martian Microbiome



NASA/JPL-Caltech via AP

Sampling Scenario Priors

1. Yes, life has been discovered on Mars on a previous rover mission! Boots on ~~Moon~~ Mars!
2. Life forms are microbial and carbon based.
3. Money is NOT an issue. The sky is not the limit, space - maybe.
4. There is water under the soil surface, Mars also has a thin atmosphere with less gravity than Earth. It is considered an "extreme" environment to live in.

Sampling Teams:

Breakout Group Questions

It is your mission to propose the best way to sample the Martian microbiome. (10-15 minutes)

1. Give yourself a team name: Space Force _____

2. How will you go about sampling on Mars?

Tip: Get creative, don't get too hung up on super technical details. Design a thoughtful sampling regime.

3. What do you need to consider to make this mission successful in order to better understand the diversity, structure, and function of the Martian microbiome?

4. Extend these sampling questions to your own research project idea!

Additional References

- Kim, D., Hofstaedter, C.E., Zhao, C. et al. Optimizing methods and dodging pitfalls in microbiome research. Microbiome 5, 52 (2017). https://doi.org/10.1186/s40168-017-0267-5
- "How to plan and conduct a microbiome study" 2020 – A Guide from Microbiome Insights - pdf online

Thank you!