

# BIOL334 Lecture 23:

# Metagenomics

---

**Tim Ghaly**

Environmental and Molecular Microbiological Analysis (EMMA) Lab

# Metagenomics

What is metagenomics?

- How does it work?
- What sort of analyses does it allow?
- What kind of questions can you answer?

What are the applications of metagenomics from a conservation/sustainability viewpoint?

# Metagenomics

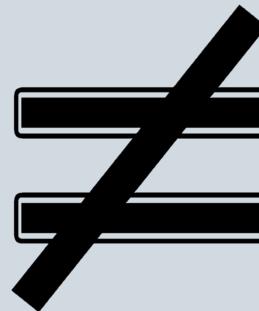
The study of total DNA recovered directly from environmental / host samples.

- Used to study assemblages of microbial communities and the genes that they carry

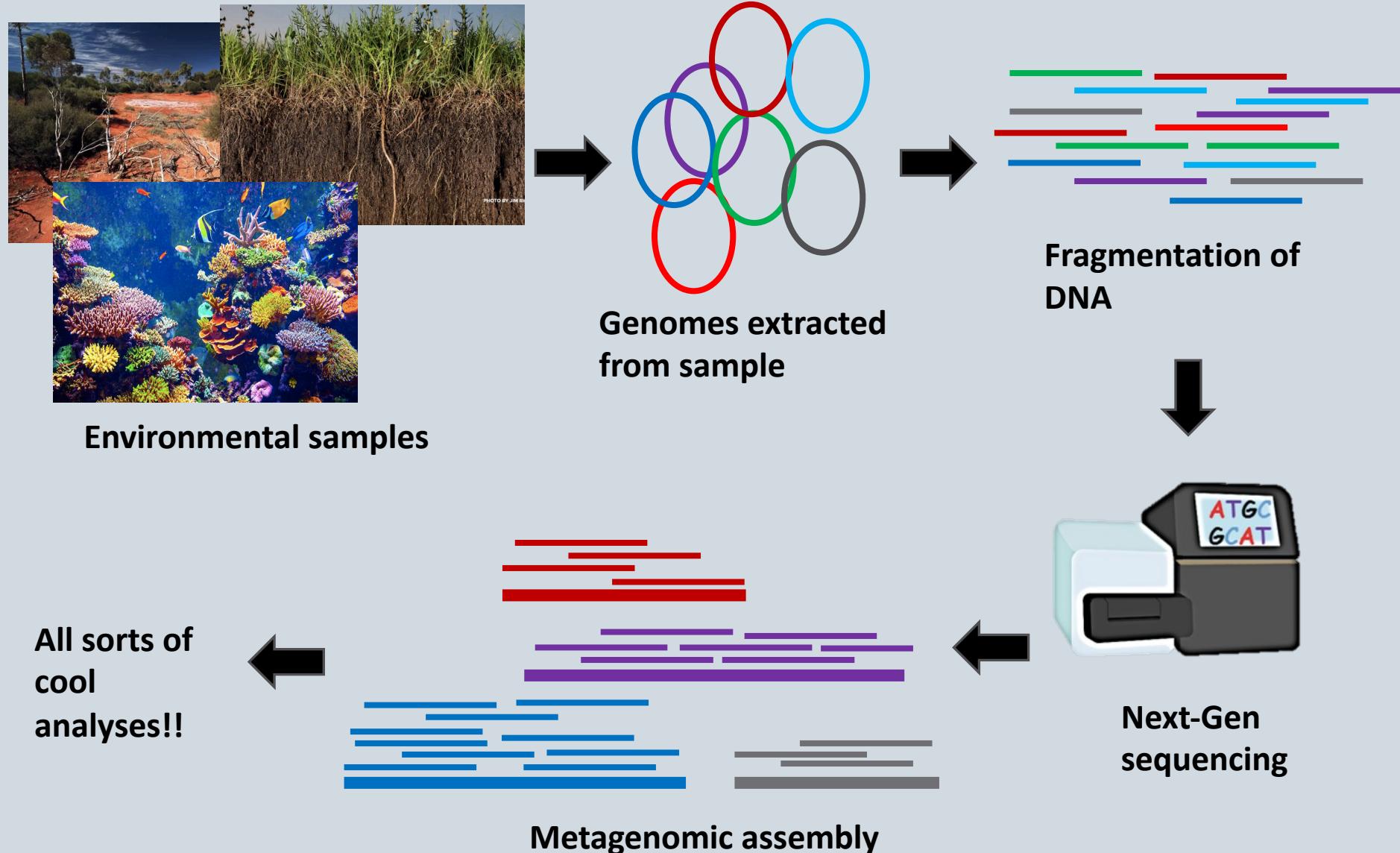
# Metagenomics

The study of total DNA recovered directly from environmental / host samples.

- Used to study assemblages of microbial communities and the genes that they carry
- Overcomes the limitations of pure culture genomics

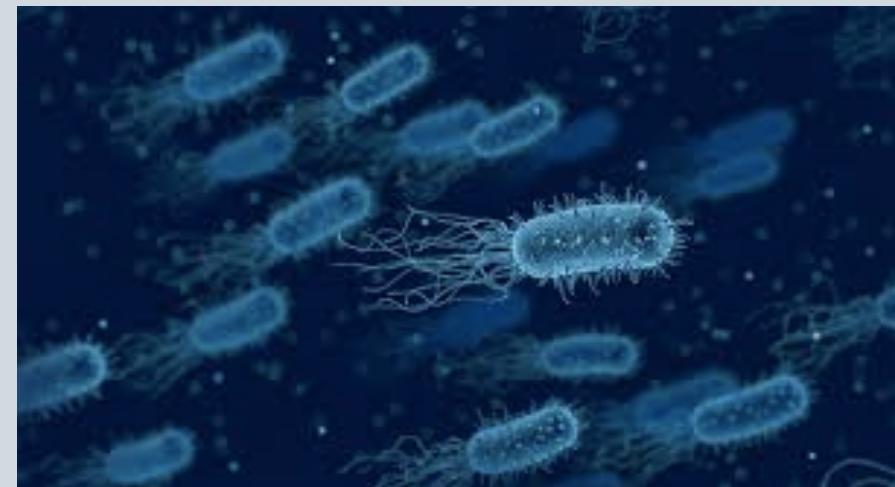


# Metagenomics



# Metagenomic Analyses

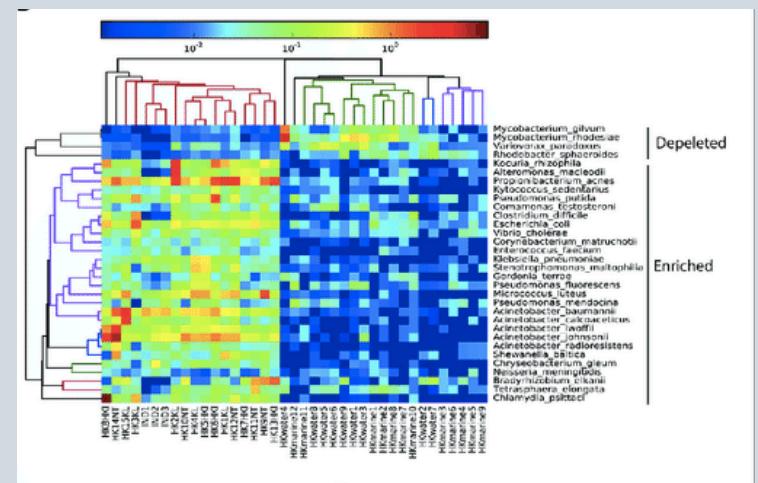
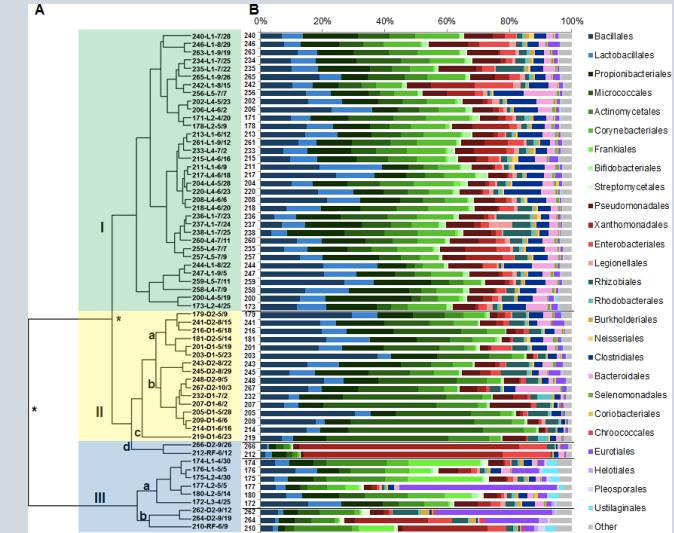
- Prediction of genes and gene functions
- Analysis of richness and abundance within and among species
- Reconstruction of metabolism of ‘uncultivable’ organisms
- Identification of novel gene functions and their ecological impacts
- Comparison of the above data between different environments
- Linking ecosystem functions with community composition



# Role of metagenomics in understanding microbes

Revealing species composition  
of any microbial community

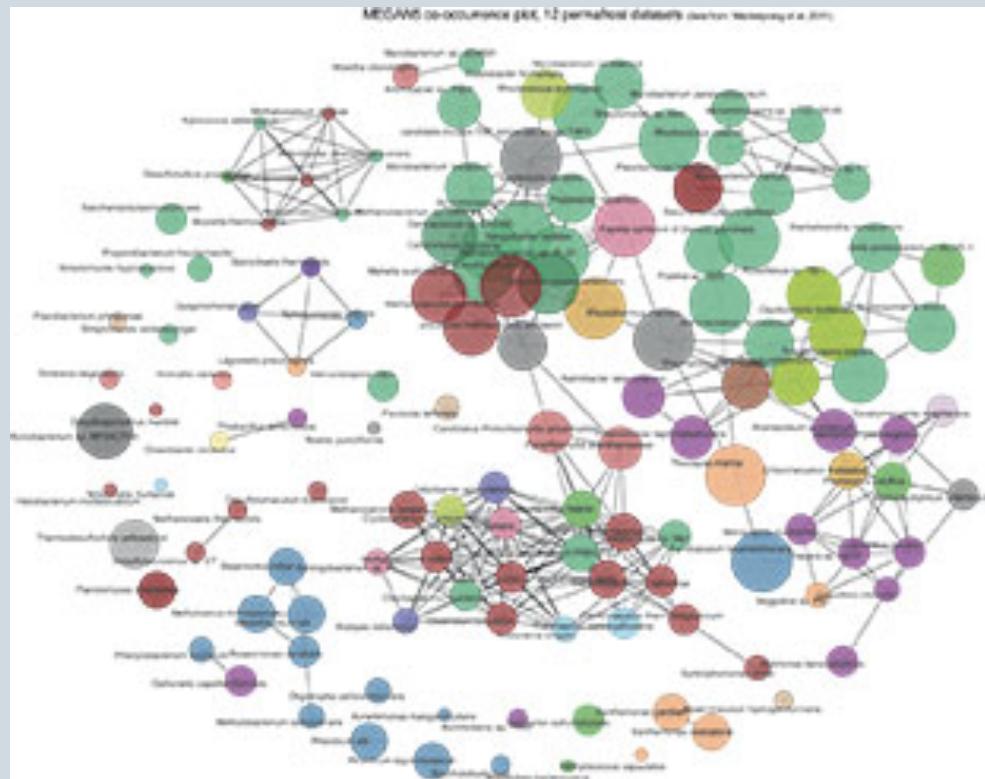
Compare relative  
abundances of species  
between treatments



# Role of metagenomics in understanding microbes

Determine which species have a higher probability of occurring together

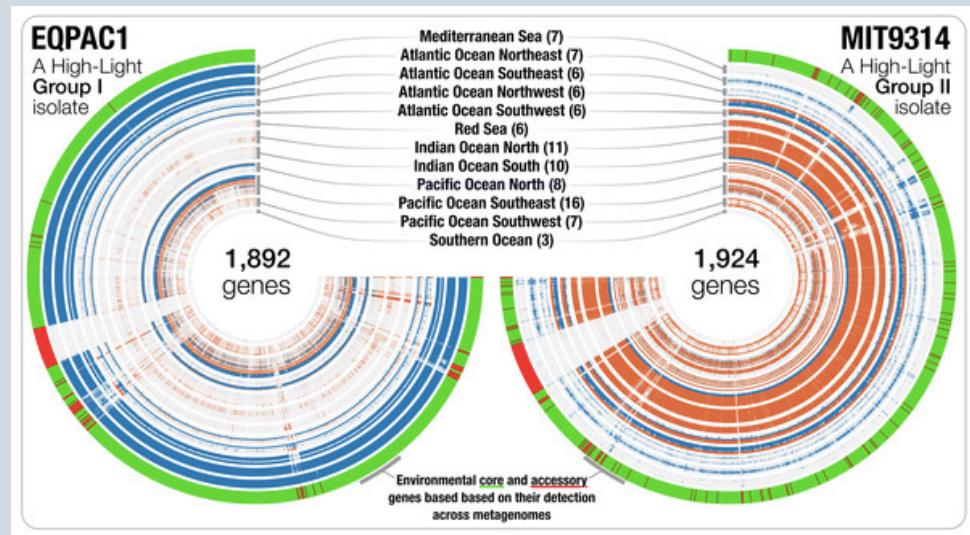
- Infer species interactions
- Such interactions can have dramatic effects on ecosystem functions or host fitness



# Role of metagenomics in understanding microbes

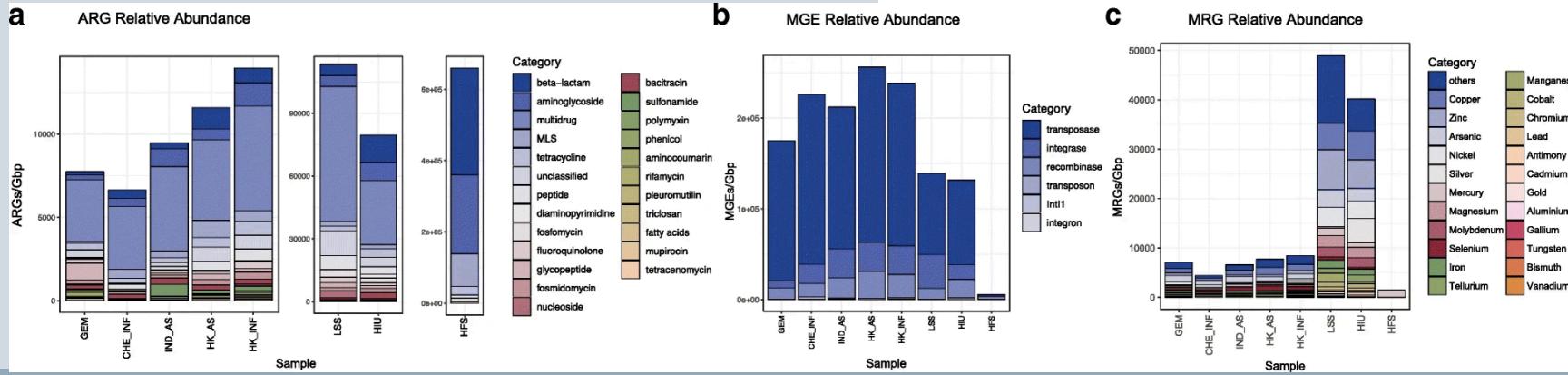
Examine core genes that exist within a given environment type.

Associate bacterial genes with ecosystem/host function



# Role of metagenomics in understanding microbes

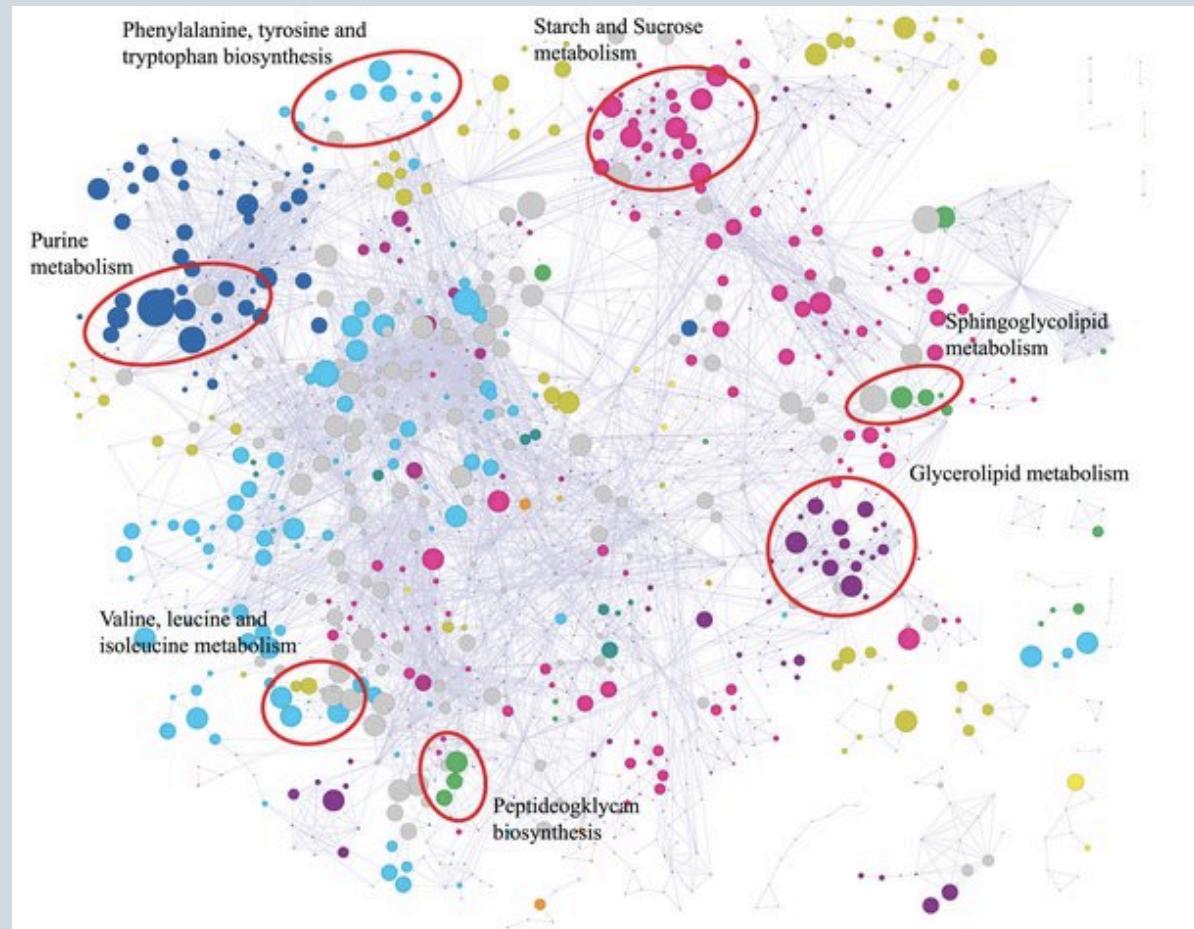
Studying relative  
abundances,  
prevalences and co-  
occurrences of gene  
families of interest



# Metatranscriptomics

The study of gene expression (RNA) recovered directly from environmental / host samples.

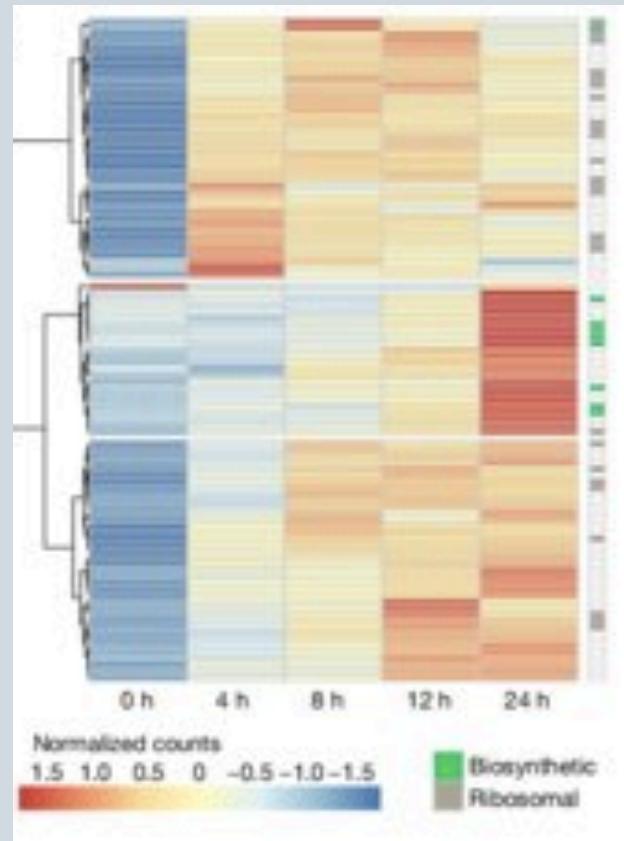
Used to study whole gene expression profiles of complex microbial communities



# Metatranscriptomics

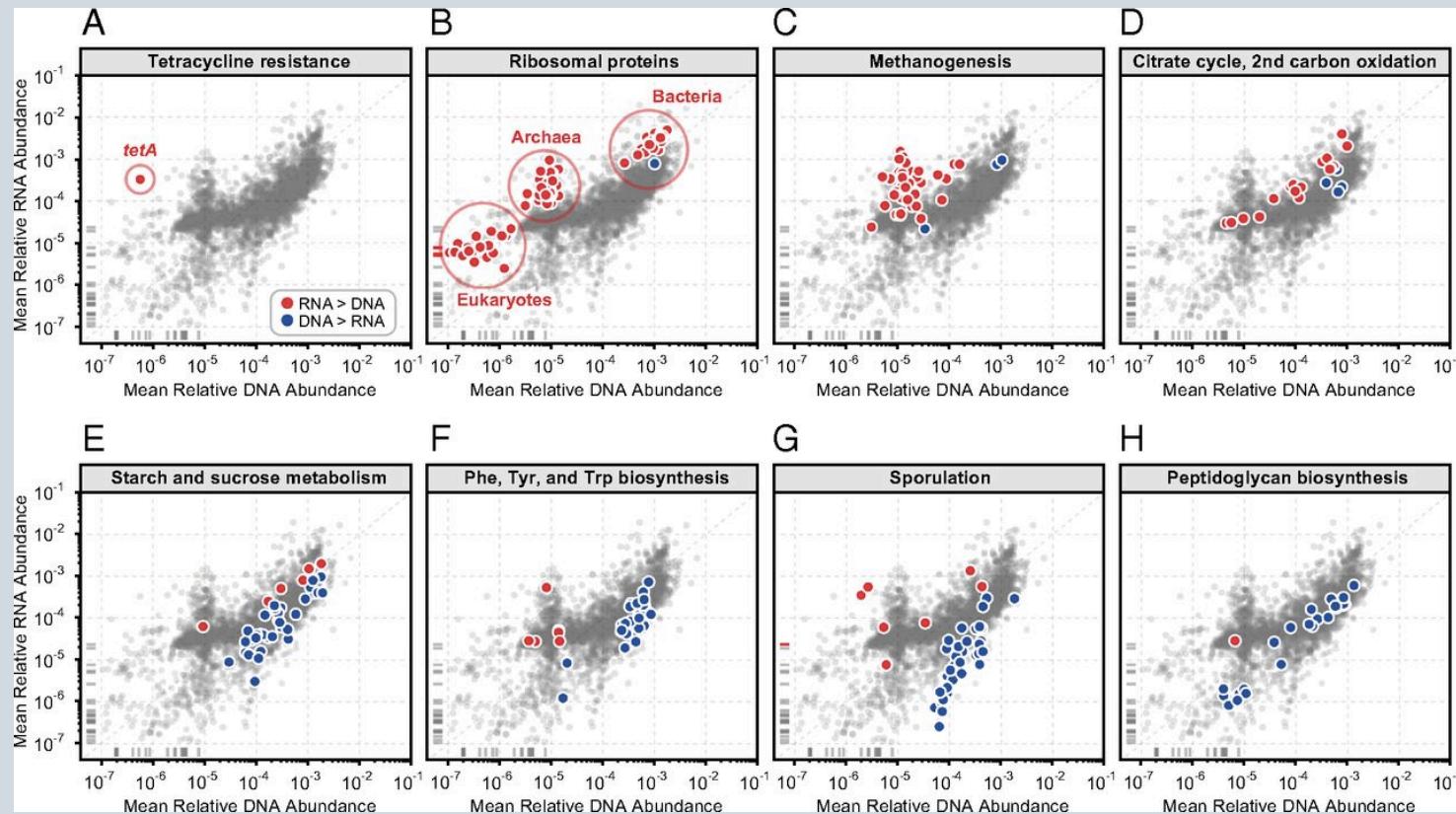
Which genes show the highest change in expression between different conditions?

What is the most important functionality in a particular environment?



# Linking Metagenomics with Metatranscriptomics

What genes are overexpressed in a given environment / treatment group?

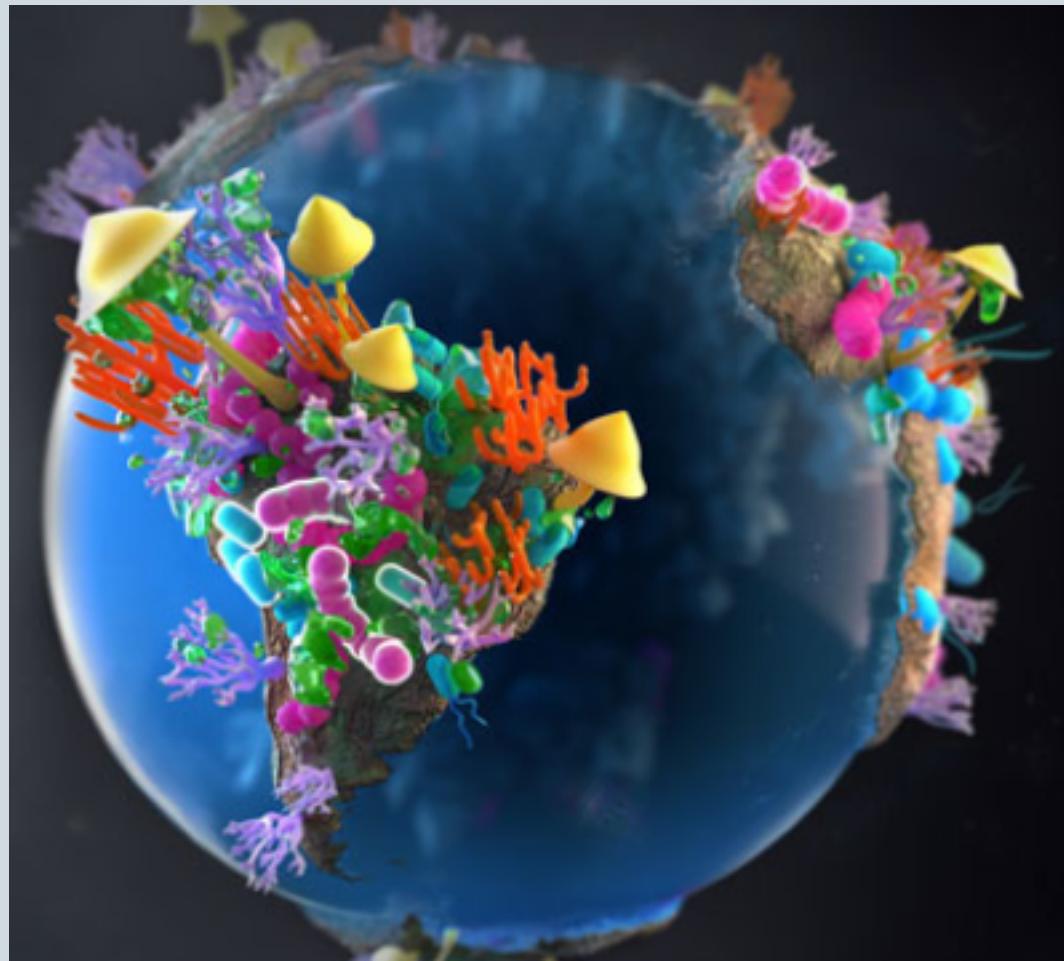


# Exploring the unseen majority

Soil microbial biomass  
= above ground  
biomass of plants and  
animals

(>1,000 kg of microbial  
biomass per hectare of  
soil)

A gram of soil can  
contain tens of  
thousands of species

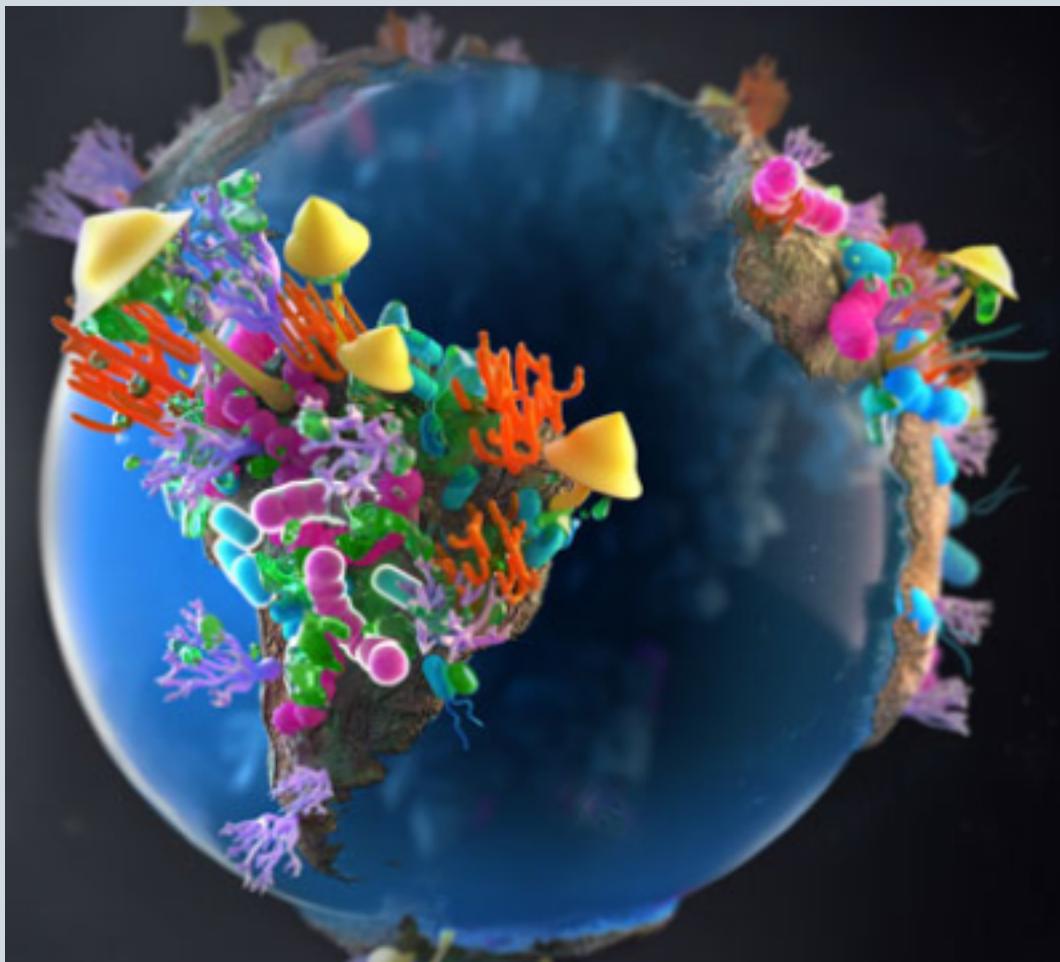


# Exploring the unseen majority

Estimated diversity of plants and animals:

- ~7.77 million animal spp.
- ~300K plant spp.

Mora *et al.* 2011. *PLoS Biology*, e1001127



# Exploring the unseen majority

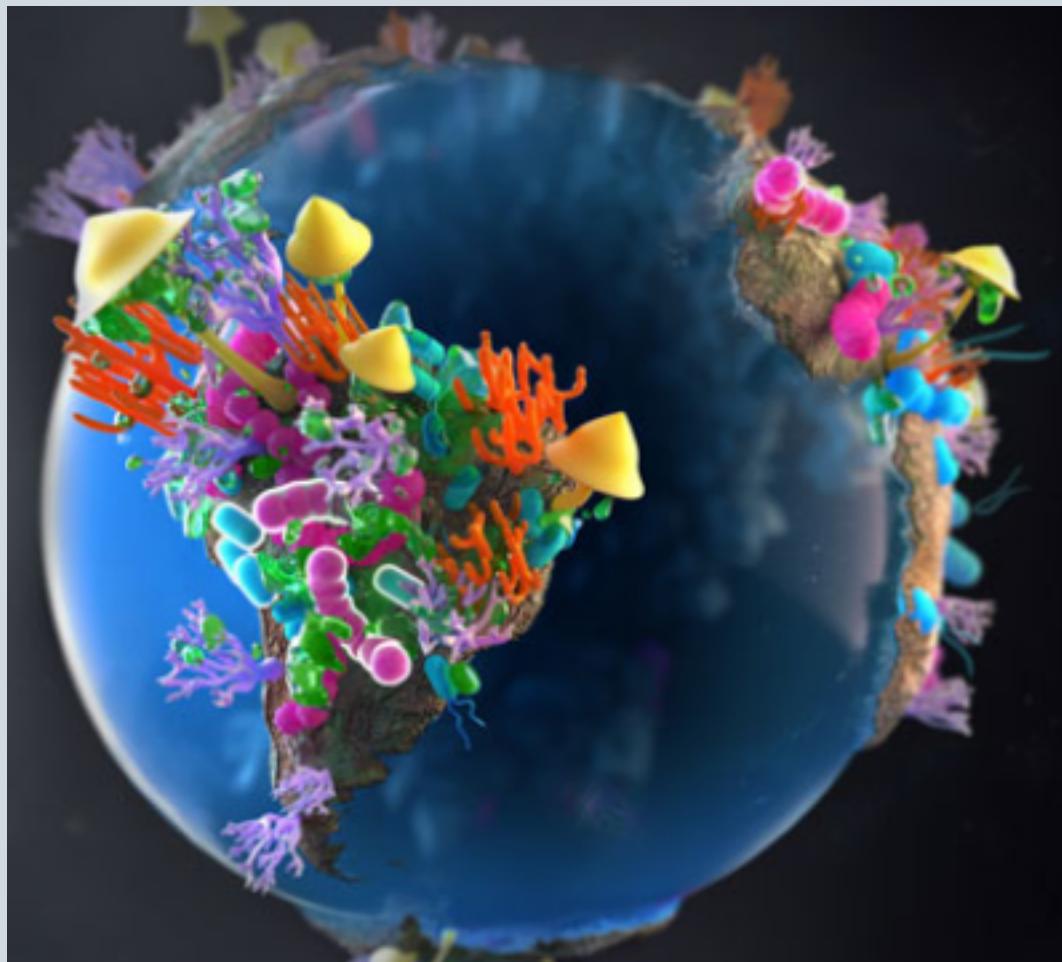
Estimated diversity of plants and animals:

- ~7.77 million animal spp.
- ~300K plant spp.

Mora *et al.* 2011. *PLoS Biology*, e1001127

- **~1 trillion ( $10^{12}$ ) bacterial spp.**

Locey *et al.* 2016. *PNAS*, **113**: 5970-5975.



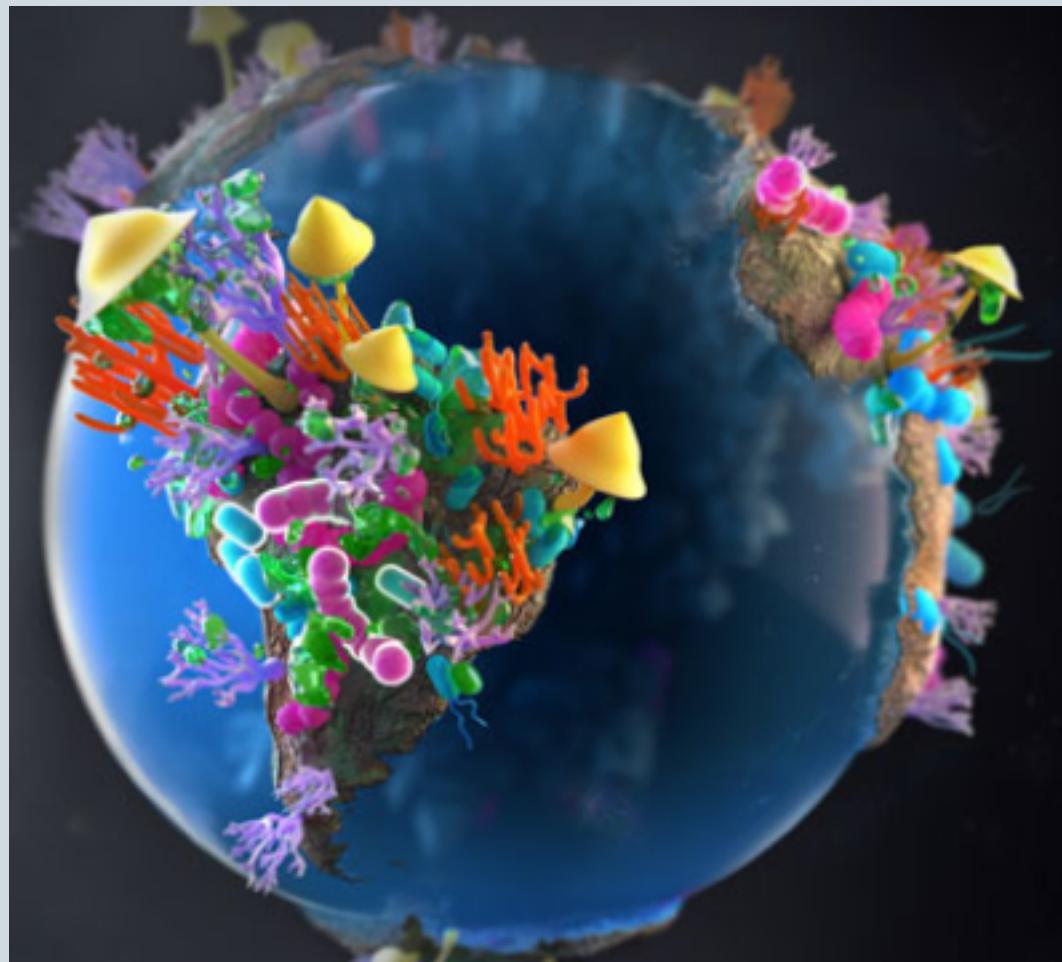
# Exploring the unseen majority

Estimated diversity of plants and animals:

- ~7.77 million animal spp.
- ~300K plant spp.
- **~1 trillion ( $10^{12}$ ) bacterial spp.**

Mora *et al.* 2011. *PLoS Biology*, e1001127

Locey *et al.* 2016. *PNAS*, **113**: 5970-5975.



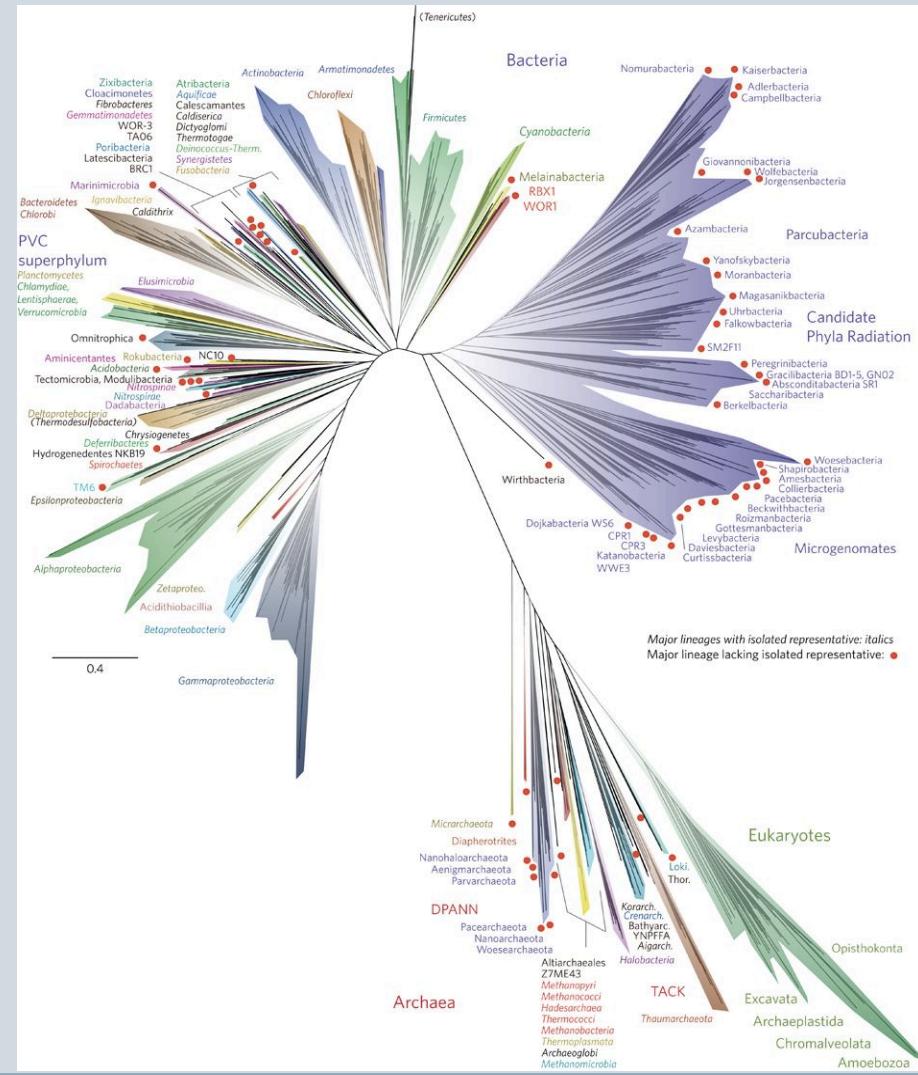
Microorganisms support the existence of all higher trophic life forms

# Maintaining genetic diversity

- Majority of genetic diversity on the planet is within bacteria
- Within animal microbiomes, the number of microbial genes is much larger than the number of host genes

e.g. Human microbiome has ~150 X more genes than the human genome

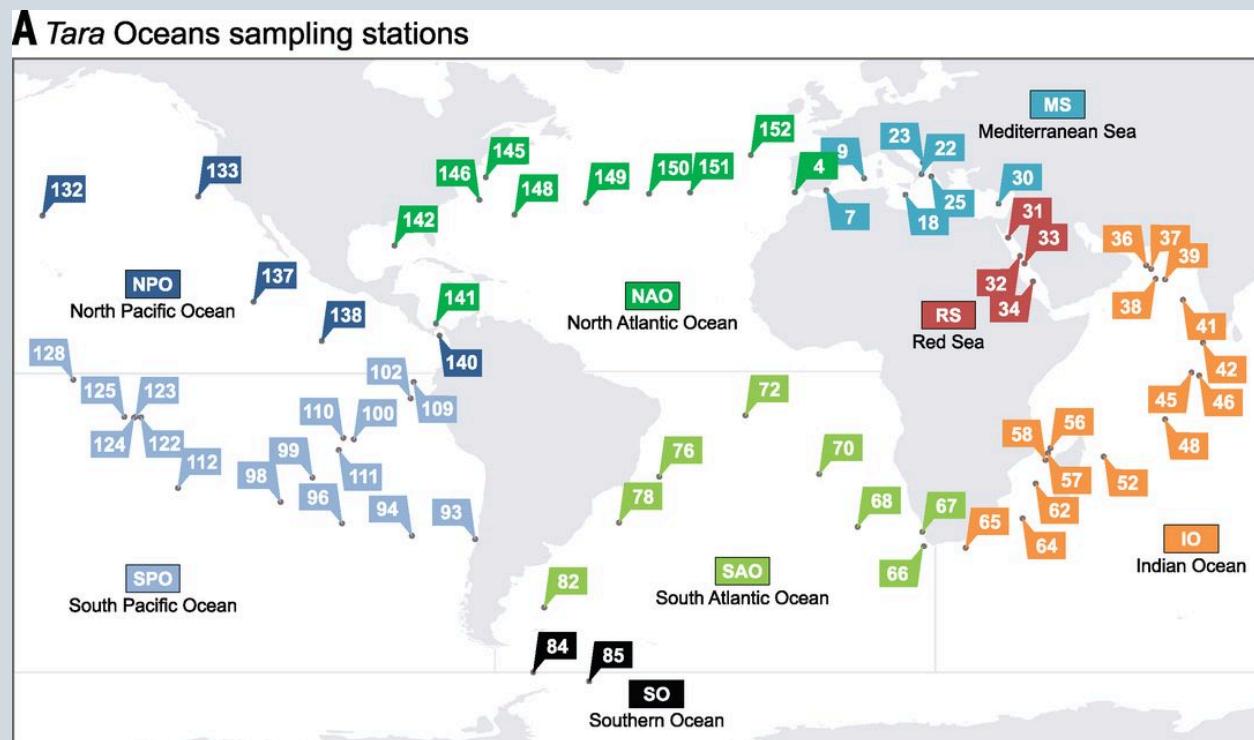
Qin et al. 2010. *Nature*, 490: 55-60



Hug et al. 2016. *Nature Microbiology*, 1: 16048

# Maintaining genetic diversity

- 243 samples from 68 sites:
  - Sequenced 111.5 million genes (40M unique genes)



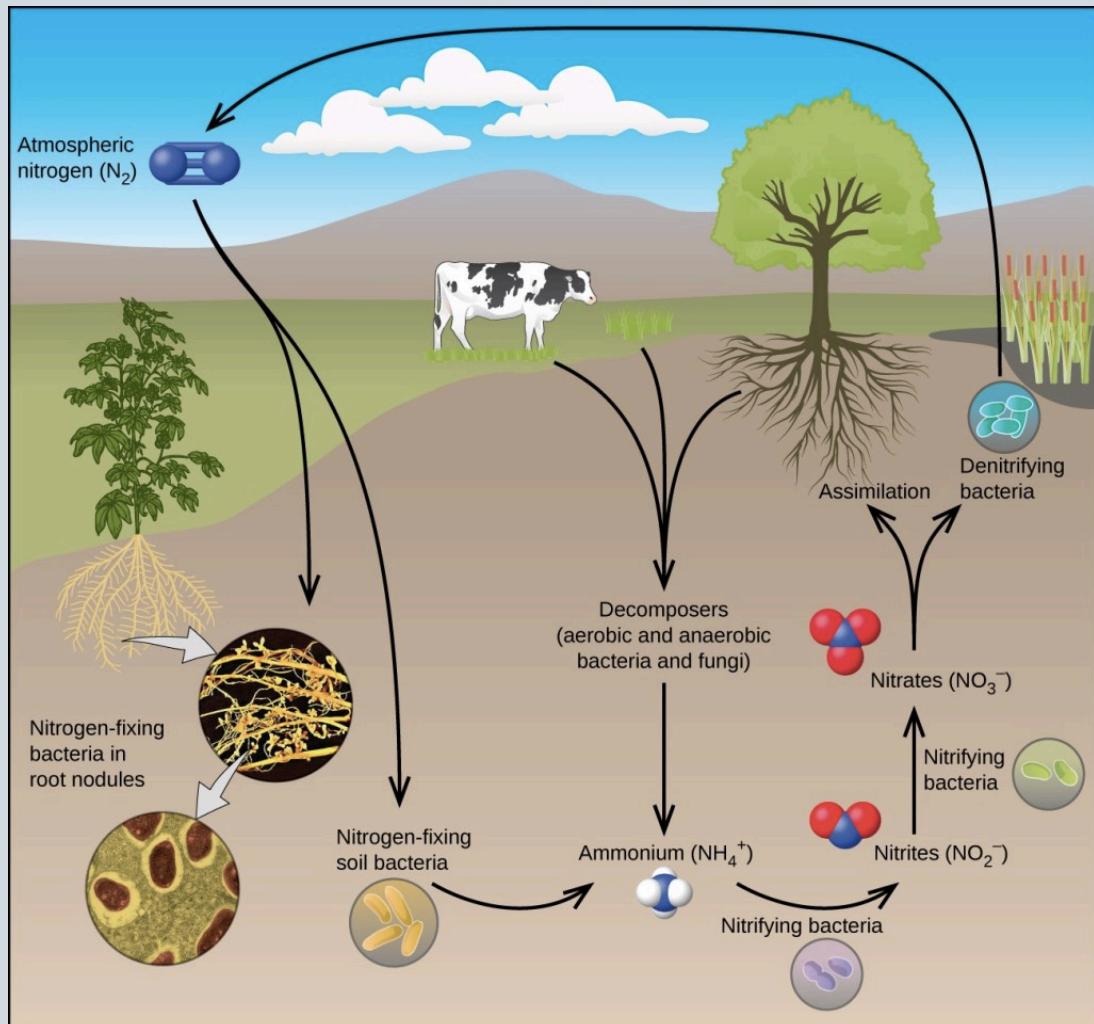
Sunagawa *et al.* 2015. *Science*, **348**: 1261359

- ~160,000 catalogued genes among insects (the most diverse group of animals) *Yin et al. 2015. Nucleic Acids Research*, **44**: D801-D807

# Ecosystem services

## Nitrogen cycle:

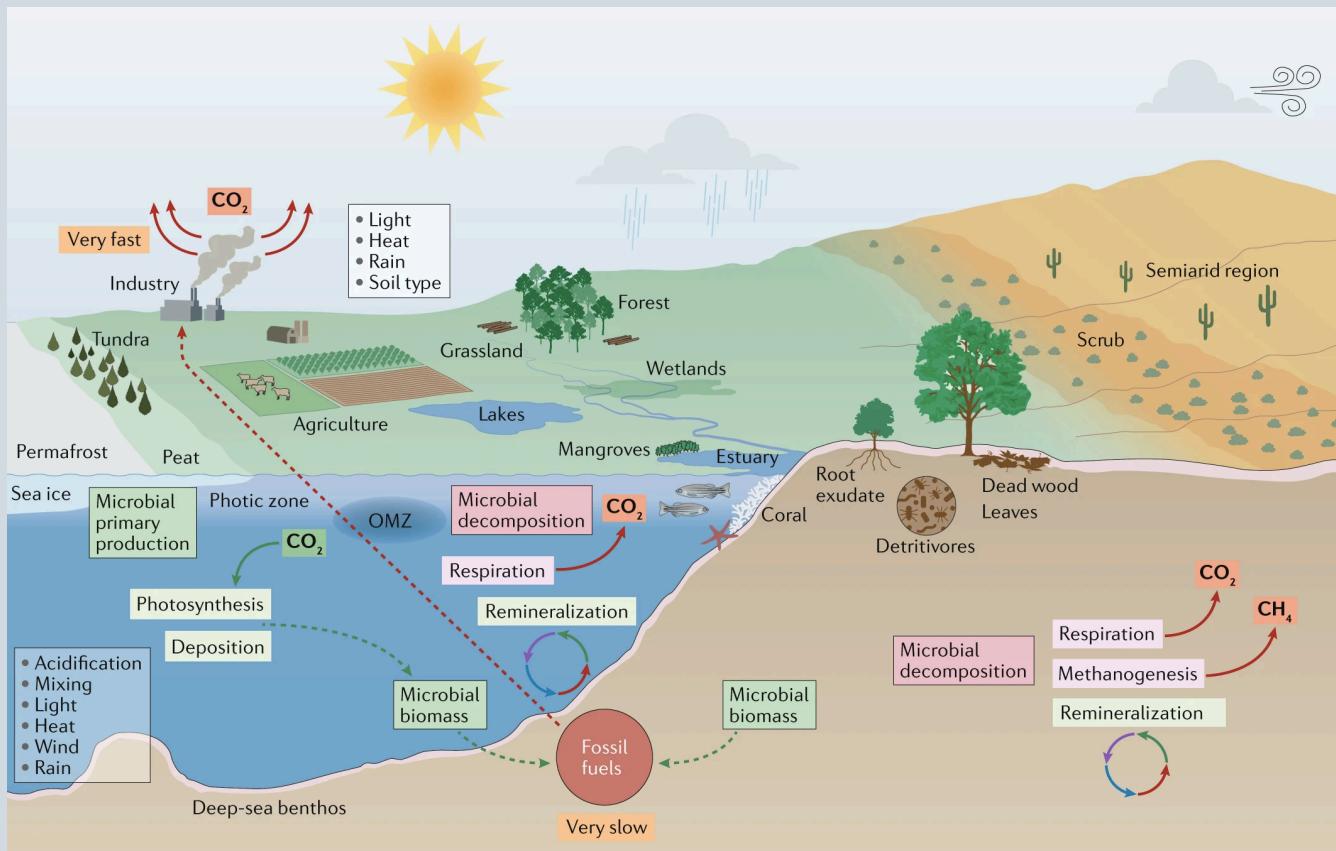
**~120 million tonnes**  
of nitrogen gas is  
made biologically  
available per year



# Ecosystem services

## Photosynthesis (Carbon cycle)

Marine bacteria produce half of the world's oxygen.

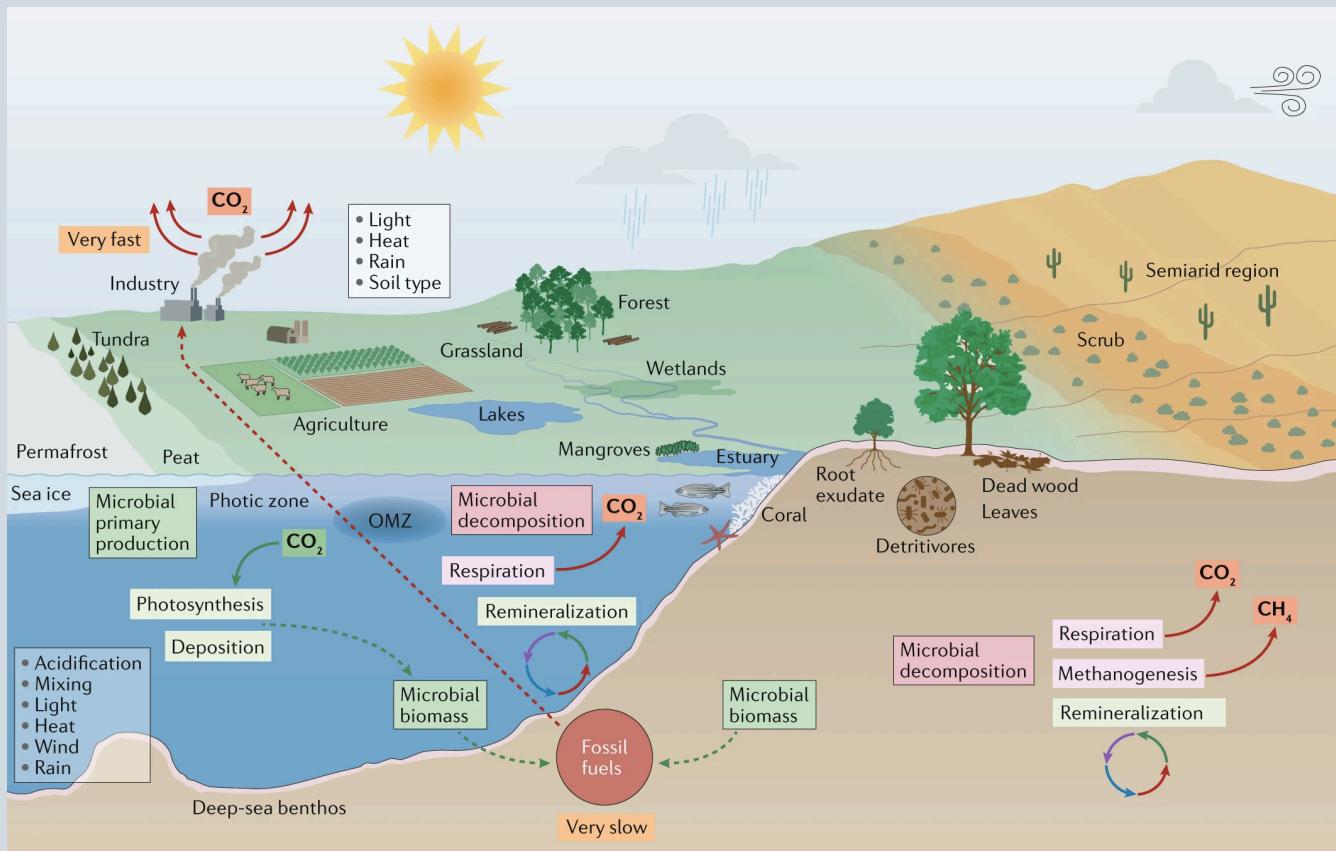


Much of the CO<sub>2</sub> removed from the atmosphere is driven by marine bacteria

# Ecosystem services

## Decomposition (Carbon cycle)

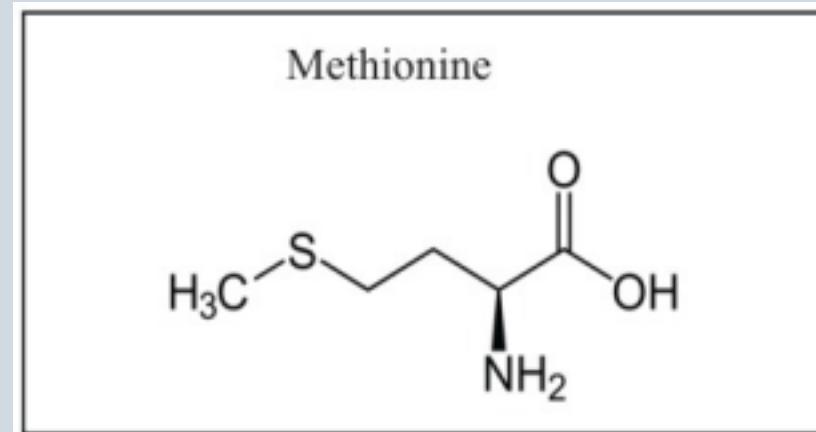
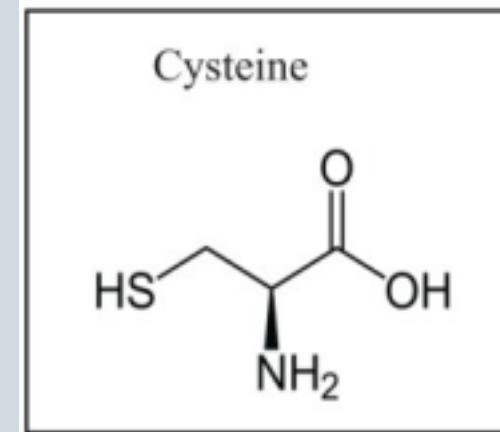
Release of  
nutrients and  
 $\text{CO}_2$



# Ecosystem services

## Sulfur cycle:

- Sulfur is an essential element for the macromolecules of all living organisms.
  - Needed for several amino acids
  - Needed for several vitamins

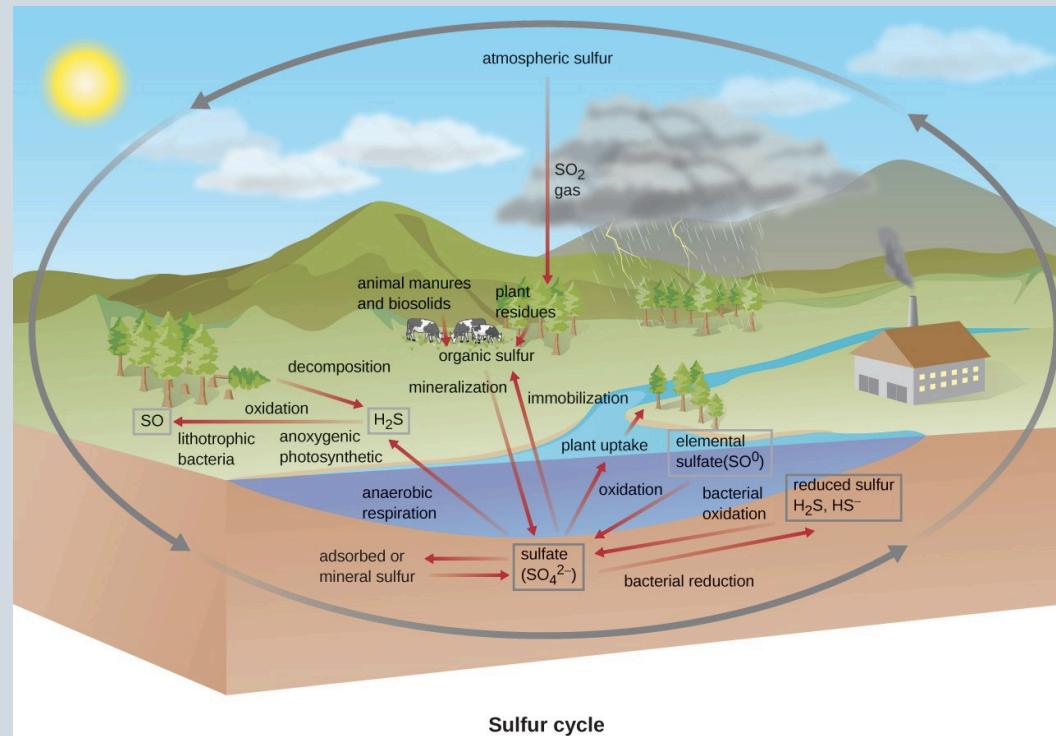


# Ecosystem services

## Sulfur cycle:

Anoxygenic  
photosynthetic bacteria /  
Chemoautotrophic  
bacteria:

- Oxidise inorganic hydrogen sulfide into sulfate that is available for the rest of the biosphere



# Ecosystem services

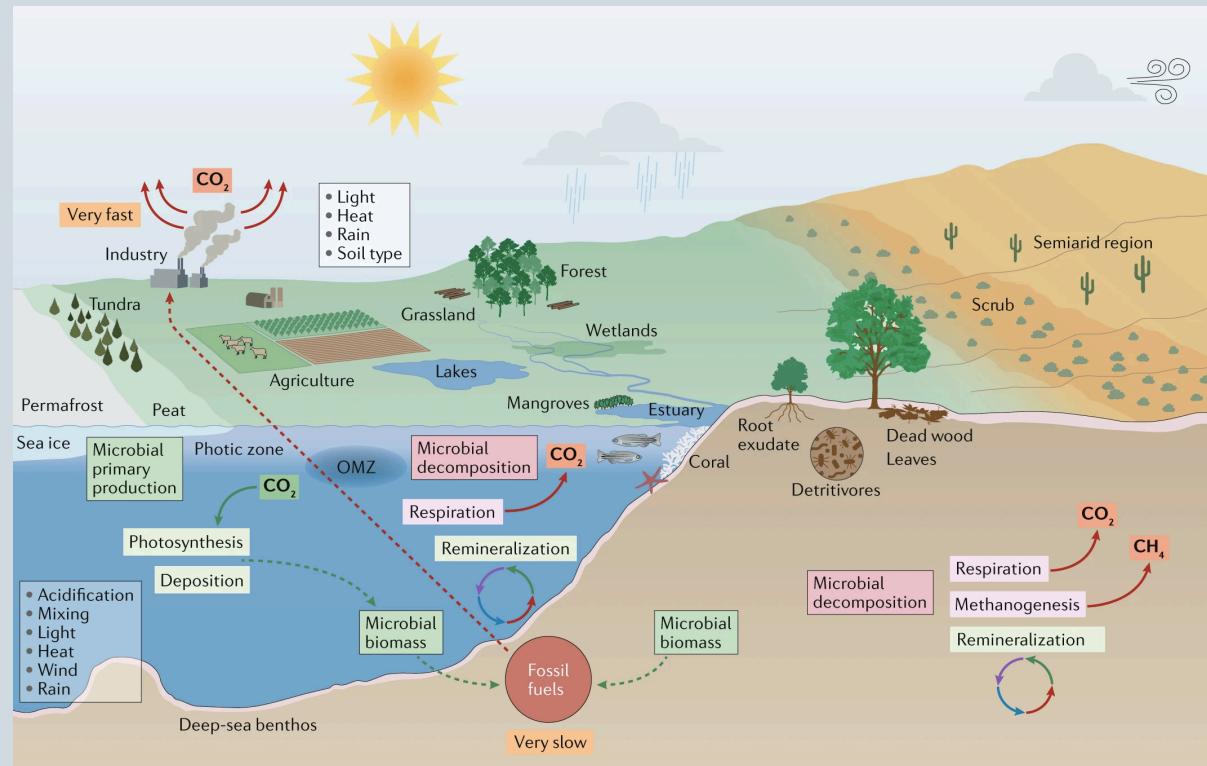
## Symbioses:



# What can ‘omics’ tell us about microbial ecosystem services?

What are the ‘key’ species involved in such ecological functions?

How do interactions between species affect whole ecosystems?

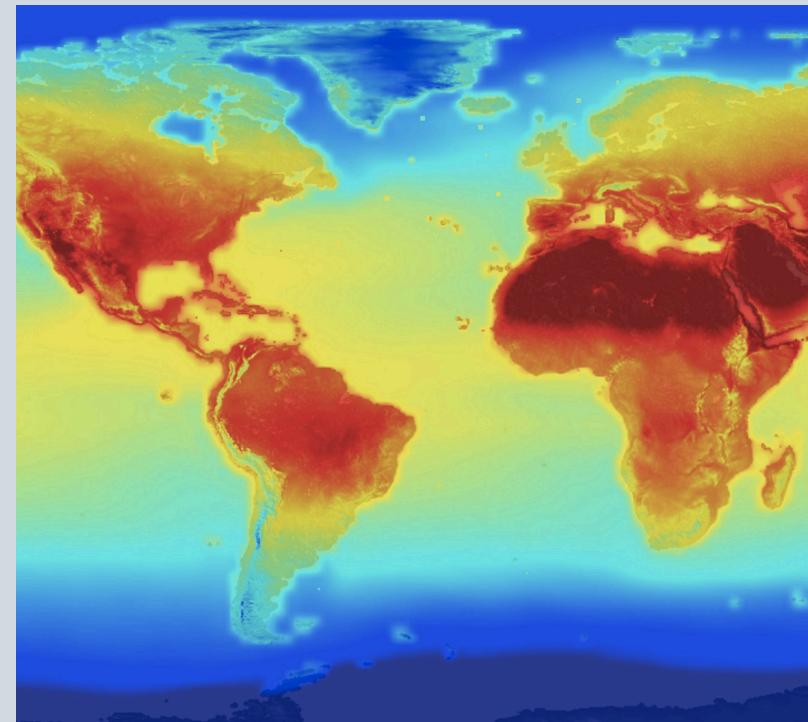


Caviccholi et al. 2019. *Nature Rev. Micro.* **17**, 569-586

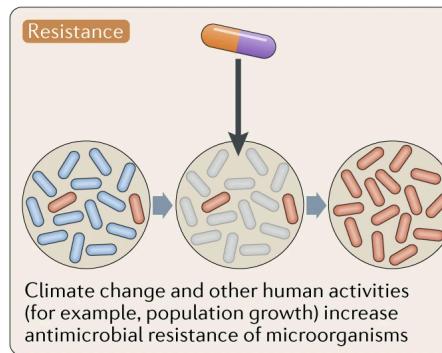
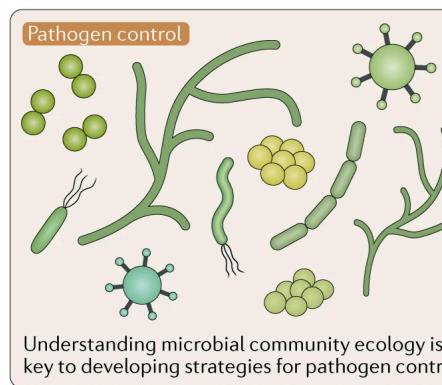
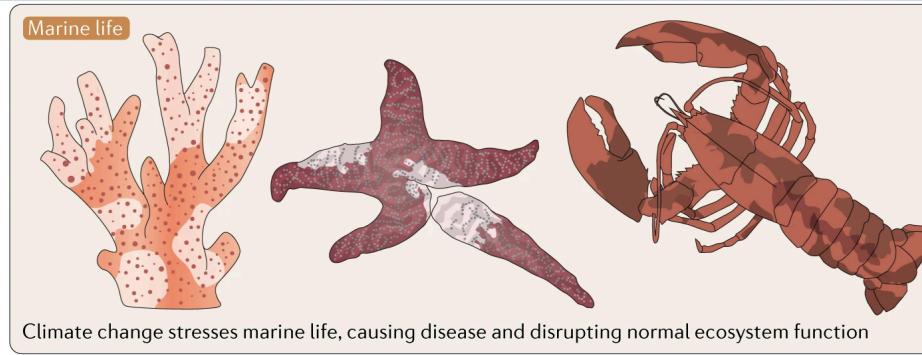
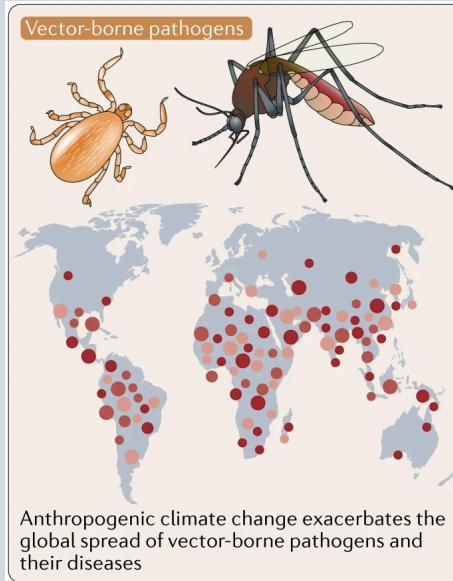
How do we as humans not mess this up...further?

# Microorganisms contribute to climate change

- Microbes are a neglected component of climate change
  - They contribute to both the production and consumption of greenhouse gases
  - Marine microbes fix as much CO<sub>2</sub> as all terrestrial plants
- As we alter the ratio of carbon uptake relative to release, we drive positive feedbacks by promoting microbial production of greenhouse gasses



# Climate change and disease



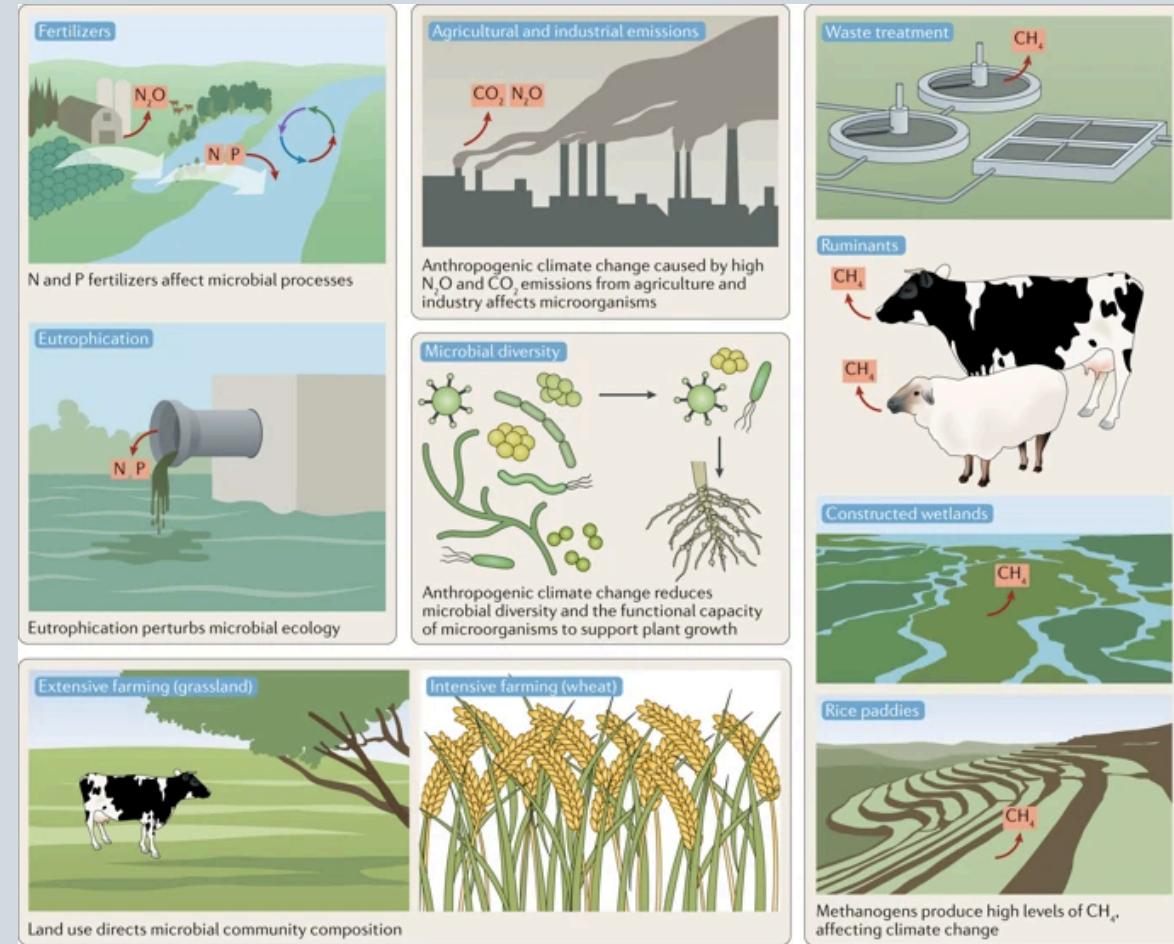
# Microorganisms can mitigate climate change

- Microbes may also be harnessed to mitigate climate change
  - Carbon sequestration
  - Improved agricultural outcomes
  - Production of biofuels
  - Remediation of pollution



# Key questions remaining: Microorganisms and climate change

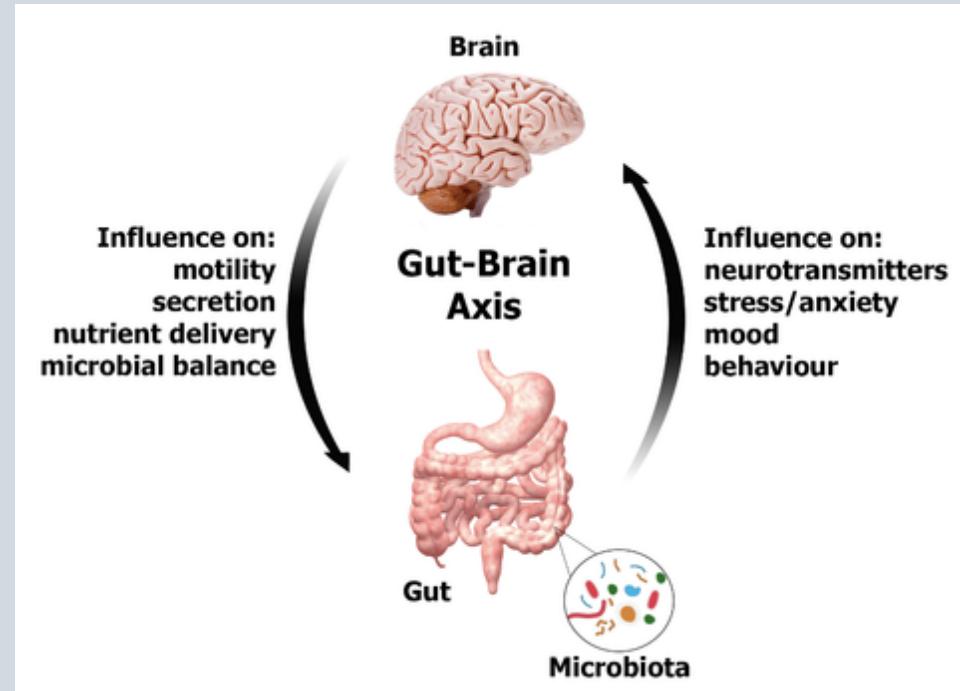
Metagenomics can reveal changes to microbial communities that can either positively or negatively affect climate change.



Caviccholi et al. 2019. *Nature Rev. Micro.* **17**, 569-586

# Microbiota of animals: The extended genotype

- Microorganisms can influence macroorganism health, brain function and behavior: **Microbiome-Gut-Brain Axis**
- Behavioural influences range from courtship and reproductive behavior to kin and mate recognition



# Animals live in a chemosensory world

- Infochemicals provide information about:
  - Health and breeding status
  - Group membership
  - Kinship
  - Competitors
  - Predators
- Microbes can influence olfactory communication via the diverse set of secondary metabolites they produce



# Microbes and animal olfactory communication

- Microbiota living in hyena scent glands produce infochemicals used to communicate social information to other hyenas
- Similar cases have been found, not just in mammals, but in birds and insects and other taxa.



Ozenwa & Williams. 2014. *BioEssays*, 36: 847-854

# Microbiota of animals raised in captive breeding programs

- Captive-bred animals will have reduced microbial diversity
  - Animals are removed from the natural area and the microbes endemic to that region.
  - Do not obtain microbes from their natural diets
  - Sanitised conditions prevent natural acquisition of important microbes



Carthey *et al.* 2018 *Trends in Ecology & Evolution*, 33: 885-894

# Microbiota of animals raised in captive breeding programs

- Problematic for carnivores in captivity:
  - Lack the gut contents of prey
  - Lack meat in various stages of decomposition
- Can significantly reduce exposure to important microorganisms



Carthey *et al.* 2018 *Trends in Ecology & Evolution*, 33: 885-894

# Microbiota of animals raised in captive breeding programs

- Given microbes play a crucial role in animal olfactory communication:
  - A decline in microbial diversity has serious implications for social cohesion and potentially even for reproduction



Carthey *et al.* 2018 *Trends in Ecology & Evolution*, 33: 885-894

# Microbiota of animals raised in captive breeding programs

- ~ 26 billion animals from over 10,000 species kept in captivity around the world.
- A significant proportion of animals in zoos fail to reproduce

# Microbiota of animals raised in captive breeding programs

- ~ 26 billion animals from over 10,000 species kept in captivity around the world.
- A significant proportion of animals in zoos fail to reproduce

May be a case of ‘miscommunication’ due to loss of diversity within their microbiota.



Carthey *et al.* 2018 *Trends in Ecology & Evolution*, 33: 885-894

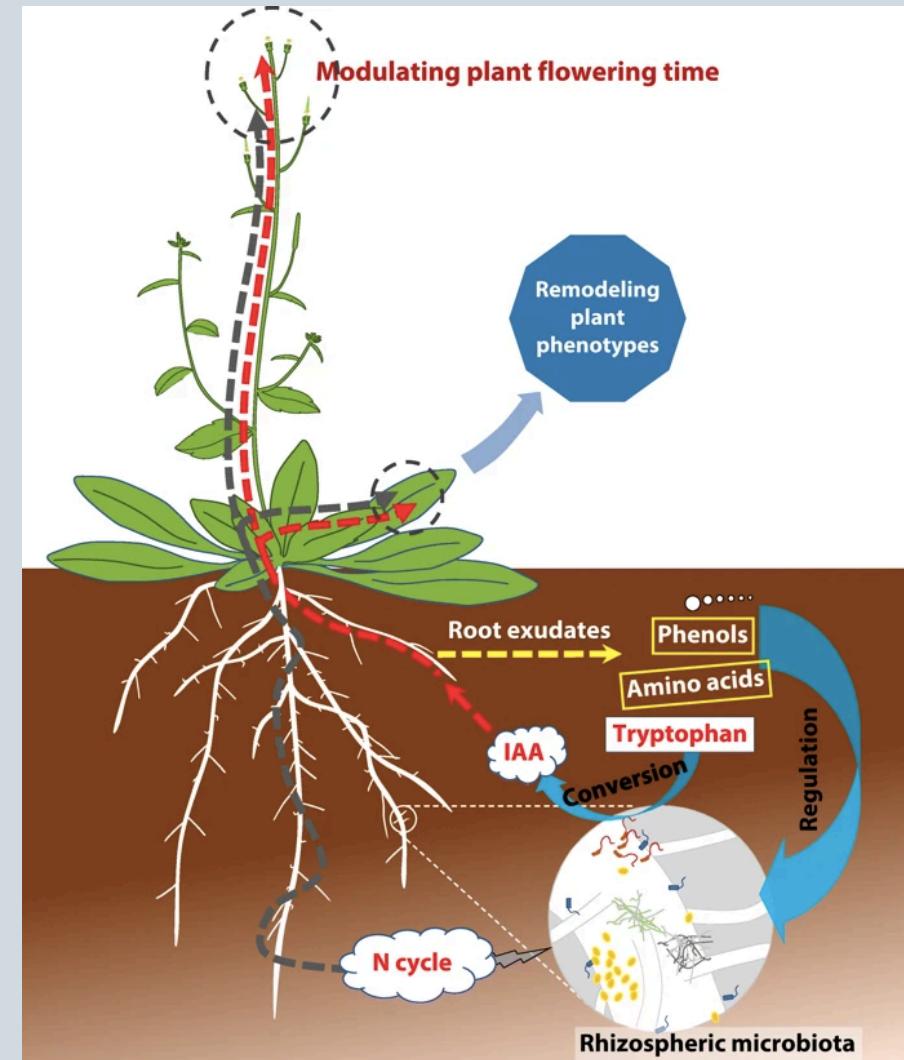
# Plants and microbes

- Microorganisms have a huge impact on plant productivity and fitness
  - Bioavailability of nutrients
  - Timing of flowering
  - Disease resistance
  - Tolerance to abiotic stresses



# Timing of Flowering

- Root microbes (rhizosphere) can influence the timing of plant flowering
  - The amount of N made available to plant will influence its expression of genes that trigger flowering
  - Opt for further plant growth -> delays flowering

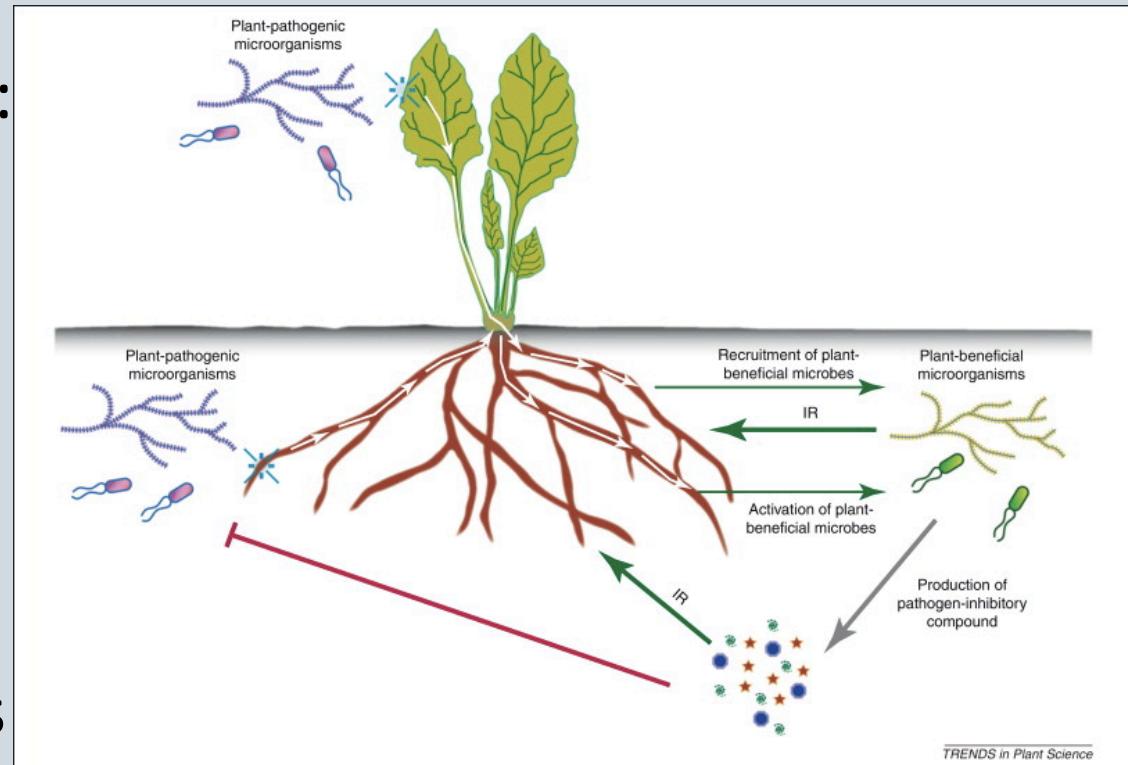


Lu et al. 2018 *Microbiome*, 6: 231

# Plant disease resistance

Beneficial microbes can:

- Induce resistance directly within the plant (They can prime the immune system of the plant).
- Produce pathogen-inhibitory compounds
- Outcompete plant pathogens

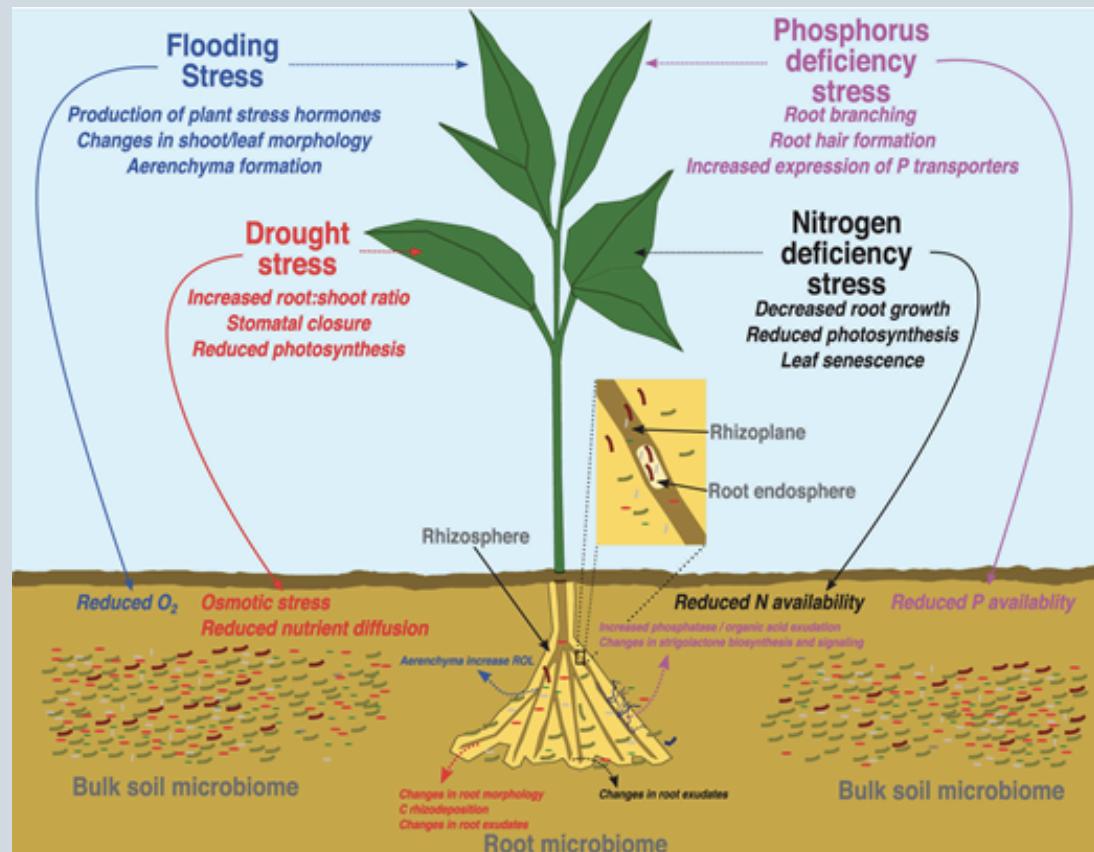


Berendsen *et al.* 2012 *Trends in Plant Science*, 17: 478-486

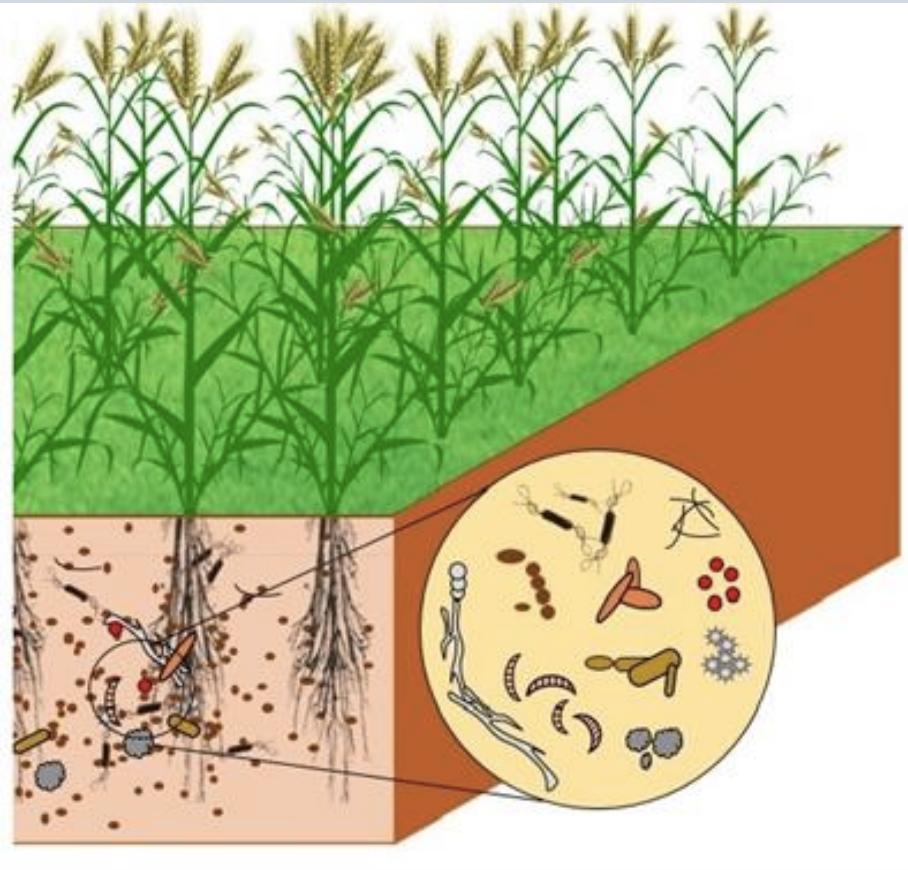
TRENDS in Plant Science

# Plant tolerance to abiotic stress

Feedback loops between plant and microbes can trigger biochemical cascades that help alleviate abiotic stress on plant

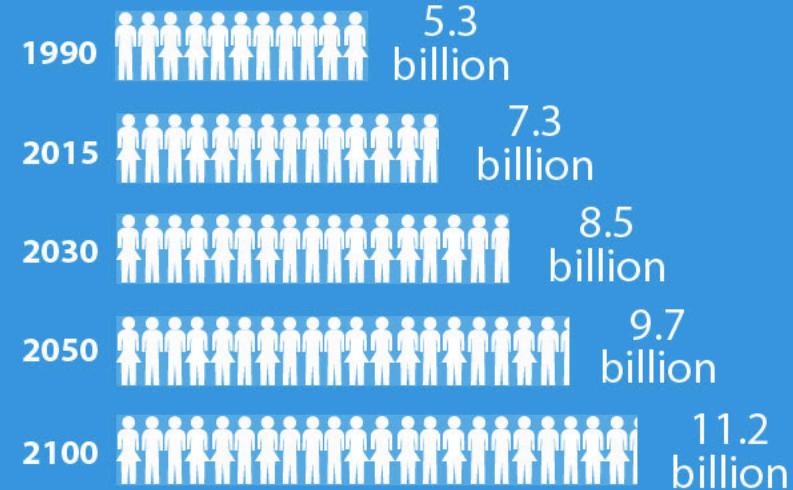


# Using microbes for plant conservation and sustainable agriculture



## World Population

*Projected world population until 2100*



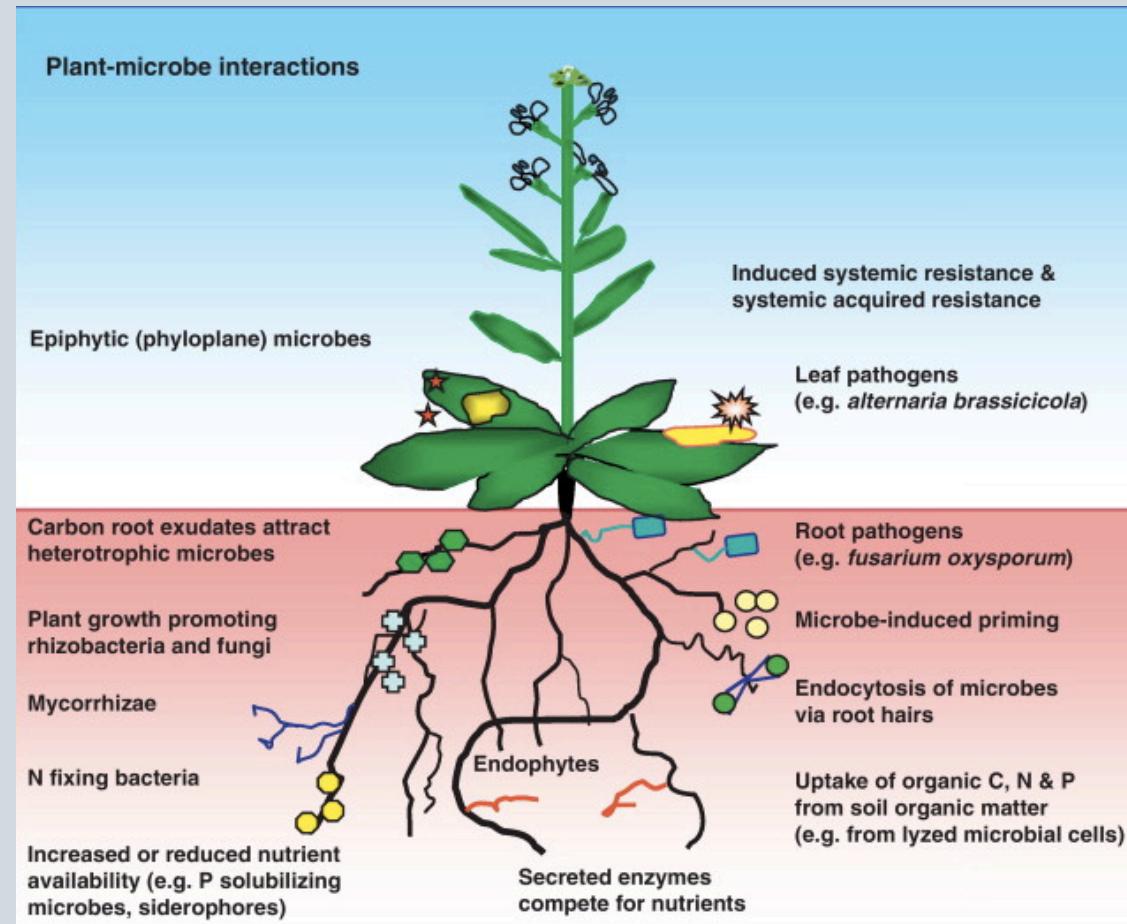
Source: United Nations Department of Economic and Social Affairs,  
Population Division, *World Population Prospects: The 2015 Revision*  
Produced by: United Nations Department of Public Information



# 'omics' and plant productivity

**Metagenomics** can link microbial gene pathways with desired plant traits.

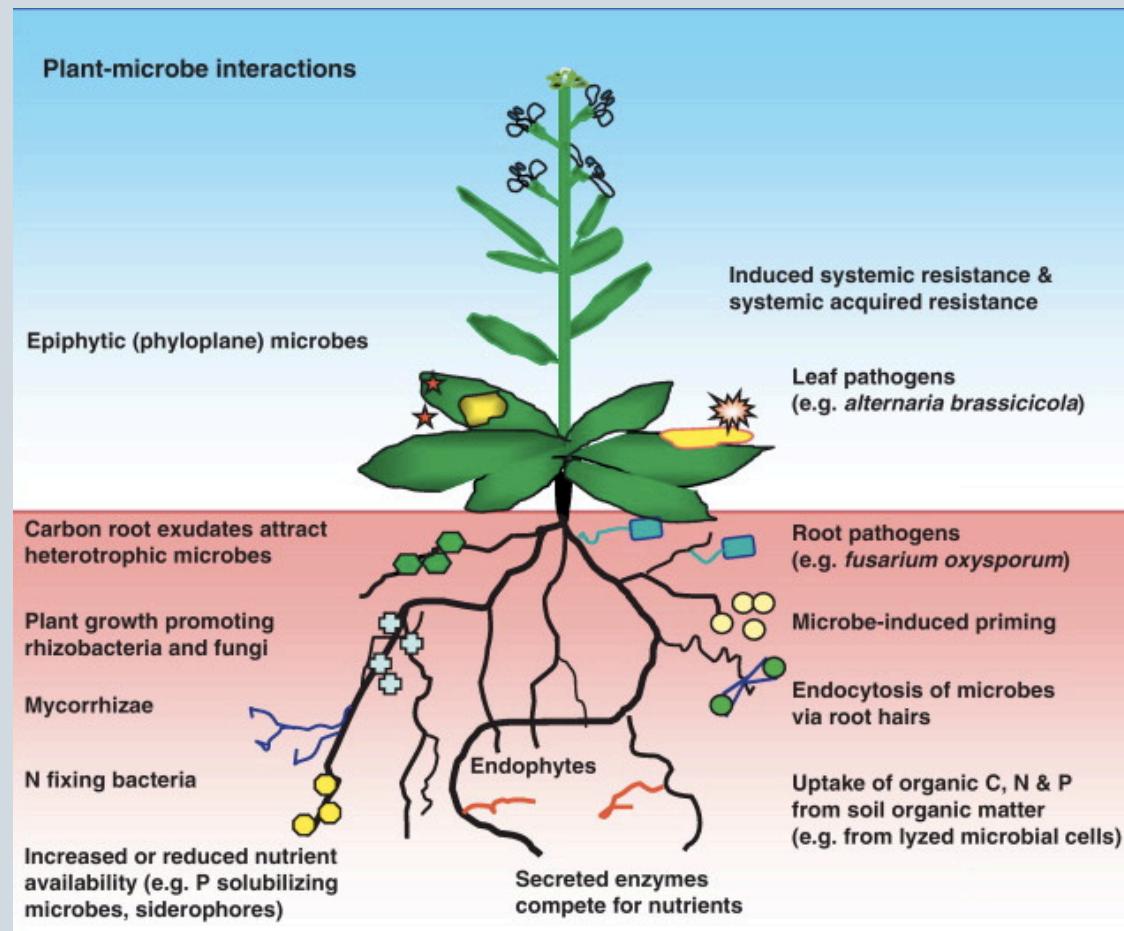
- Which species promote plant growth?
- Which microbial genes are linked to increased plant productivity?



# 'omics' and plant productivity

**Transcriptomics** can link the expression of bacterial genes with plant fitness under different environmental conditions.

- Hugely important for sustainable agriculture
- Synthetic biology can be key

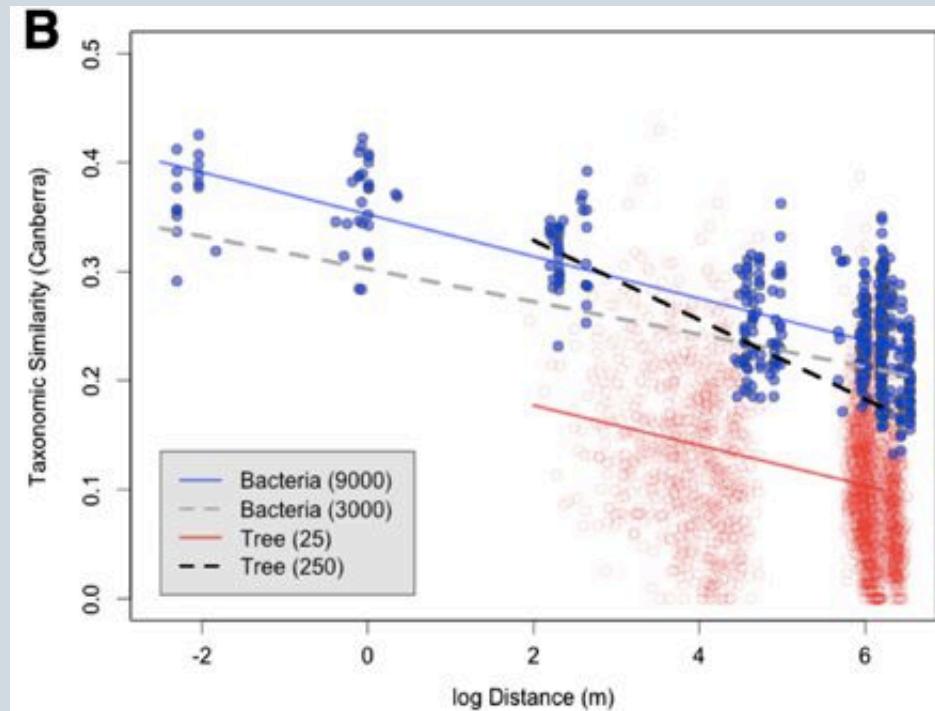


# Do microbes go extinct?

- Old way of thinking in microbiology: “*Everything is everywhere*”
  - Suggests that microbes do not exhibit biogeographic structure (i.e. a homogenous distribution)

# Do microbes go extinct?

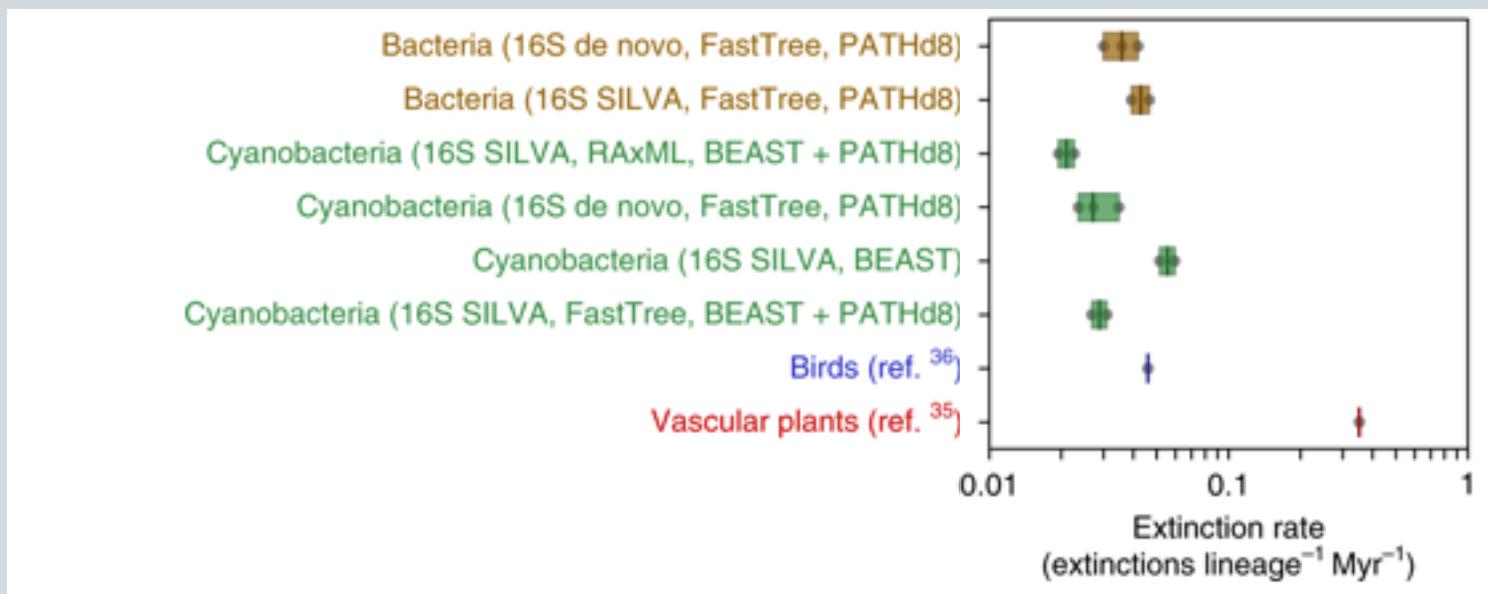
- Old way of thinking in microbiology: “*Everything is everywhere*”
  - Suggests that microbes do not exhibit biogeographic structure (i.e. a homogenous distribution)
- This is **NOT** true. Based on metagenomic data, we now know that bacteria exhibit the same biogeographic pattern as plants and animals.



Meyer *et al.* 2018. *The ISME Journal*, **12**: 1404-1413

# Do microbes go extinct?

- Phylogenetic techniques have been used to estimate 45,000 to 95,000 bacterial extinctions in the last million years alone
  - Disproves the idea that bacteria are unlikely to go extinct

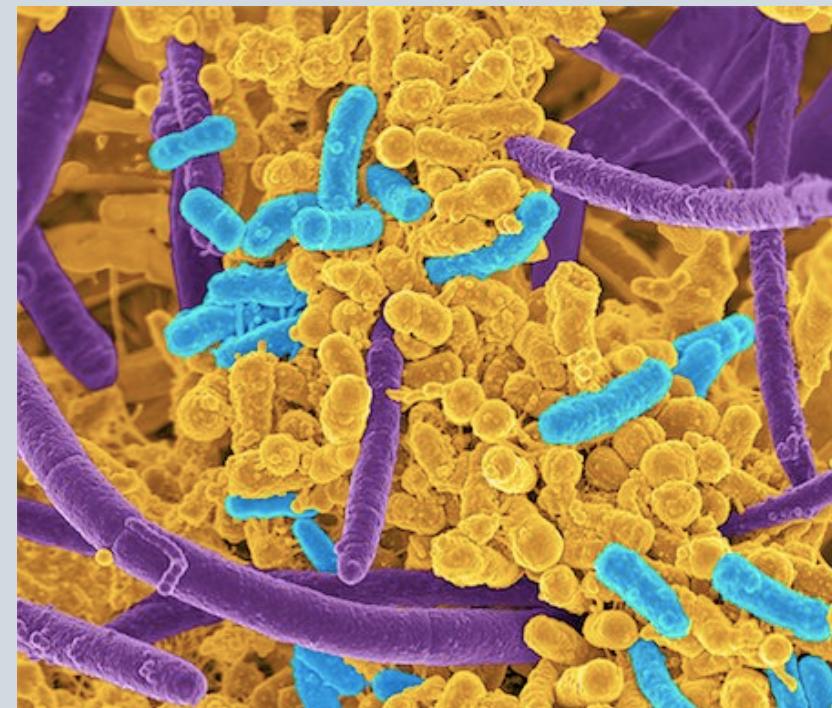


Louca *et al.* 2018. *Nature Ecology & Evolution*, **2**: 1458-1467

# What are our impacts?

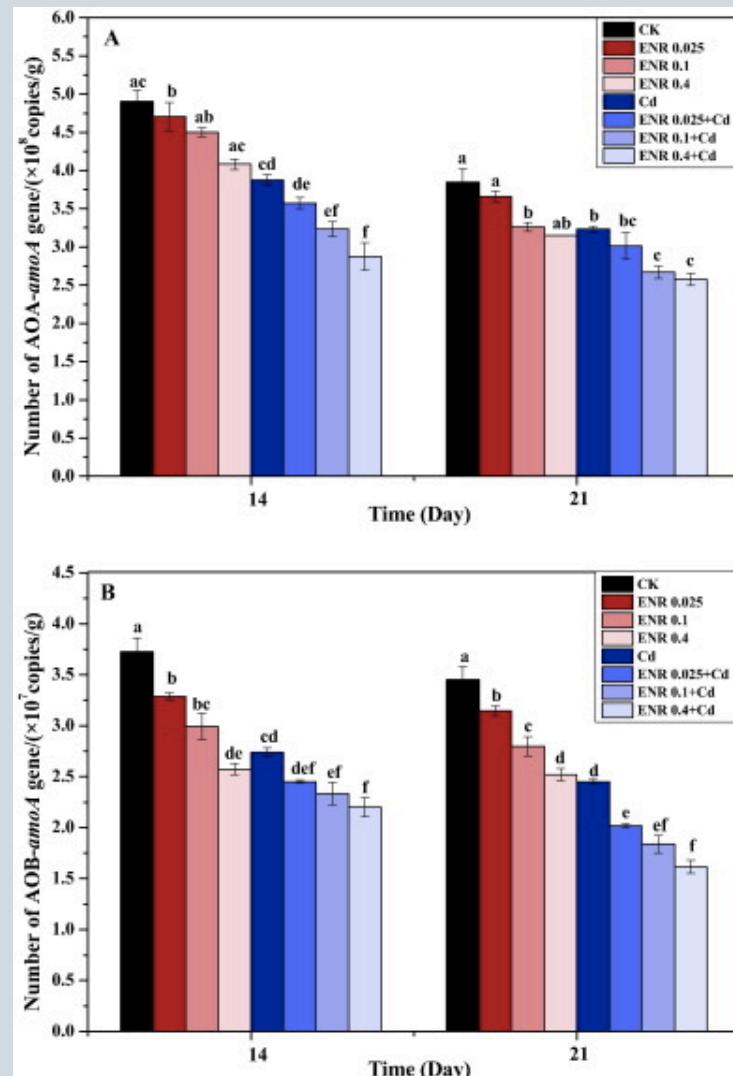
Using metagenomics, we can now ask how microbial communities respond to:

- Heavy metal pollution
- Increased global temperatures
- Ocean acidification
- Loss of plant/animal hosts



# Heavy metal pollution

Heavy metal pollution leads to a reduction in expression of ammonia oxidation genes AOA-*amoA* and AOB-*amoA*



# Increased global temperatures

Warming by 5°C altered the relative abundances of forest soil bacteria

DeAngelis *et al.* 2015. *Frontiers in Microbiology*, 6: 104

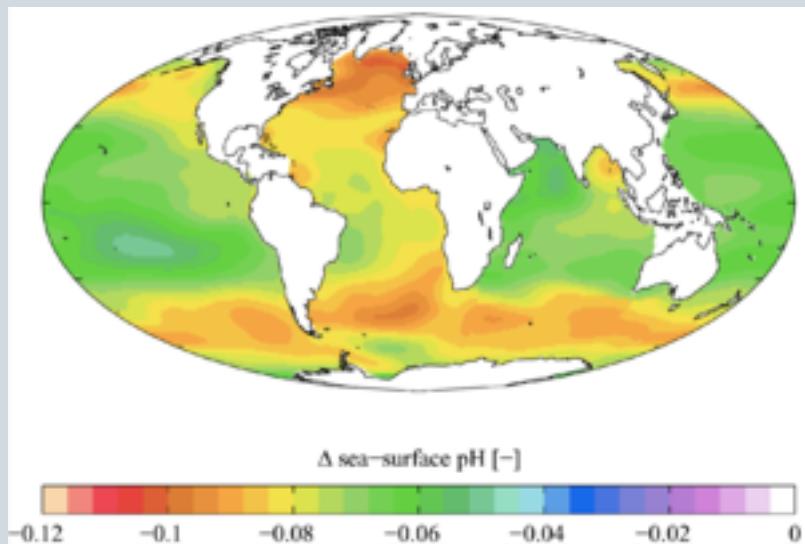
Not only global extinctions are of concern, but regional community changes are important as well.

Shifts in microbial community composition are likely to lead to changes in ecosystem function



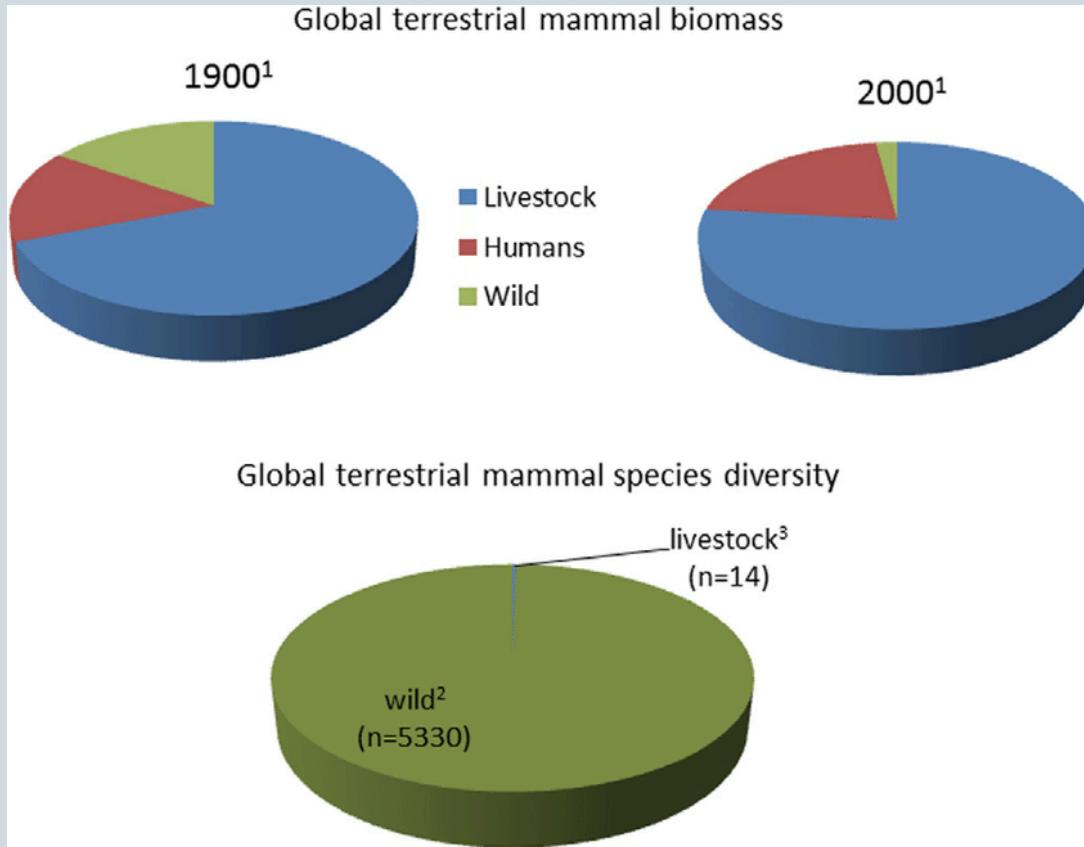
# Ocean acidification

- Marine bacteria when exposed to acidification significantly shift their metabolism.
- Increased energy expenditure on pH homeostasis
- Less resources for nitrogen fixation and degradation of organic matter
  - Could have significant effects on the health of ocean ecosystems



Change in pH between the 1700s and the 1990s.

# Loss of animal hosts



Zeller et al. 2017. *Global Ecology and Conservation*, **10**: 114-125.

- Not only mammals.
  - Biomass of domestic poultry is three times higher than that of all species of wild birds

Bar-On *et al.* 2018. *PNAS*, **115**: 6506-6511

- Many microbes are endemic to their animal hosts

# What can we do?

- Only now starting to survey microbial diversity, which will provide us with a baseline of microbial community structure.
  - Need continued monitoring to track changes over space and time.
  - Greater controlled manipulative studies
- A greater understanding of our impacts on microbial communities.
- Help inform policy

# A call for microbial conservation

**“if the last blue whale  
choked to death on the last  
panda, it would be  
disastrous but not the end  
of the world. But if we  
accidentally poisoned the  
last two species of  
ammonia-oxidizers, that  
would be another matter. It  
could be happening now  
and we wouldn’t even  
know...”**

Tom Curtis, 2006

