

15 May 2025





## Wheat genotype-driven recruitment of rhizosphere microbiome to improve nitrogen use efficiency

Dr. Maria C. Hernandez-Soriano

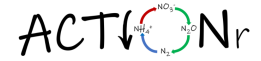
 @looking-at-soil.bsky.social



## Learning objectives:

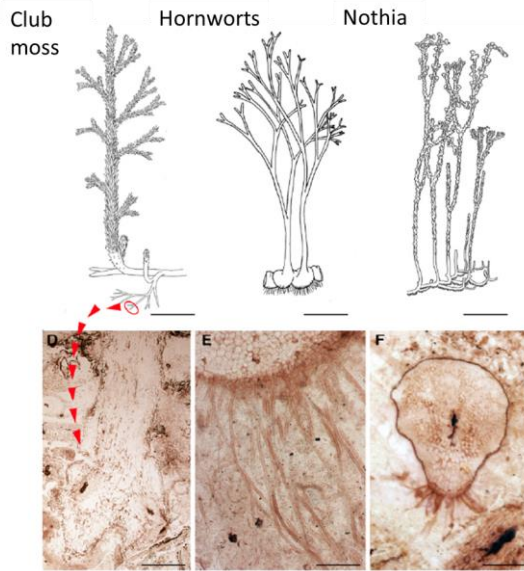
-  Root and rhizosphere traits - plant-microbe interactions
-  Root traits for microbial recruitment, nitrogen cycling, and overall soil health
-  **WISH-ROOTS: Wheat Improving Soil Health - ROOT traits**
-  Potential applications for sustainable agriculture

~500 million years ago ...



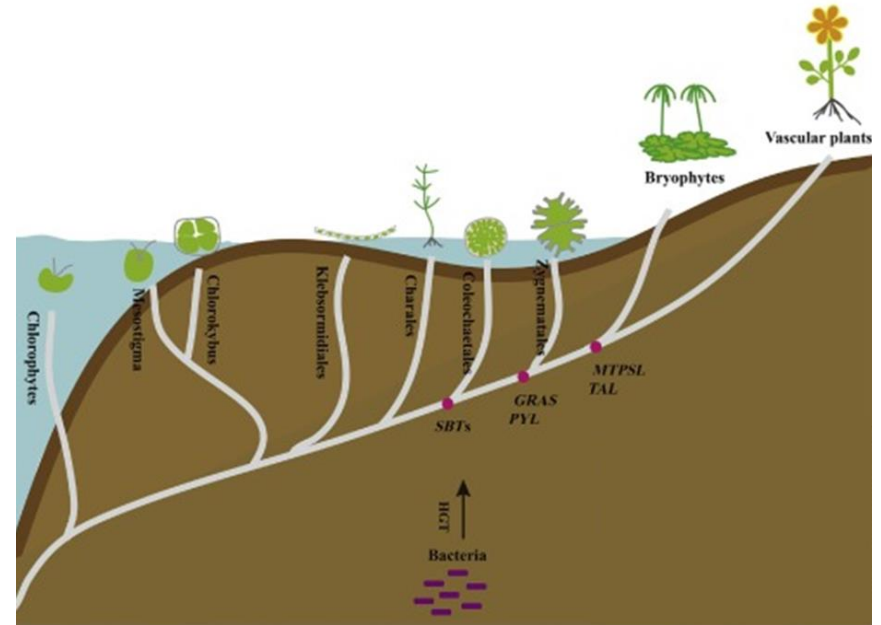
## Plants colonized the land

plants engaged in symbiotic associations with arbuscular mycorrhizal fungi as early as 400 million years ago



Rhizoids – fossils of rooting systems  
Rhynie Chert, Scotland

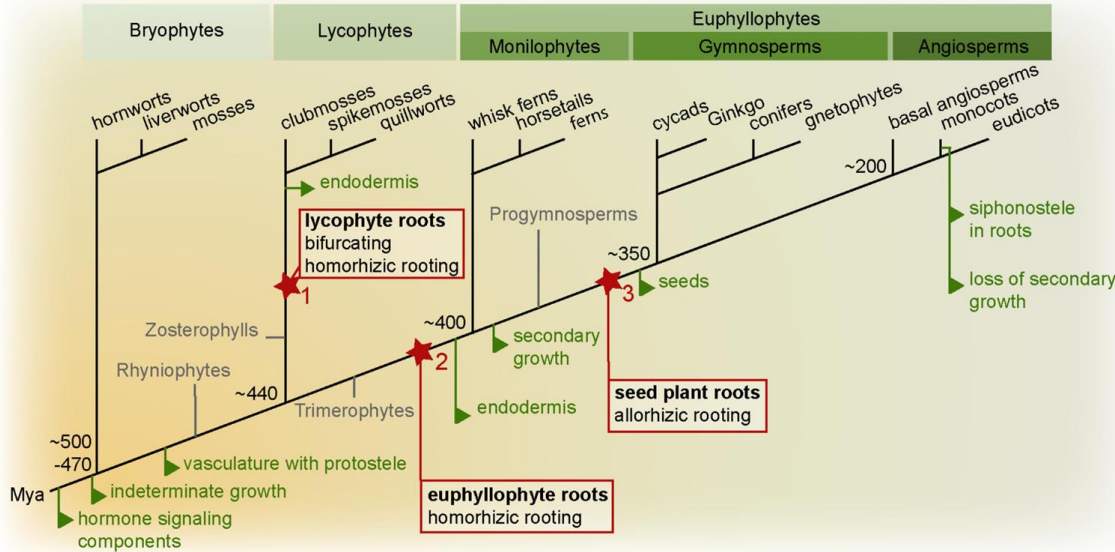
Adapted from Kenrick et al. **Plant Physiol.** 2014



Adapted from Ma et al. **Trends Plant Sci** 2020



# Root plasticity is crucial for plants to adapt to changes in the environment



Different plants + different soils =  
different roots

## Evolutionary adaptation:

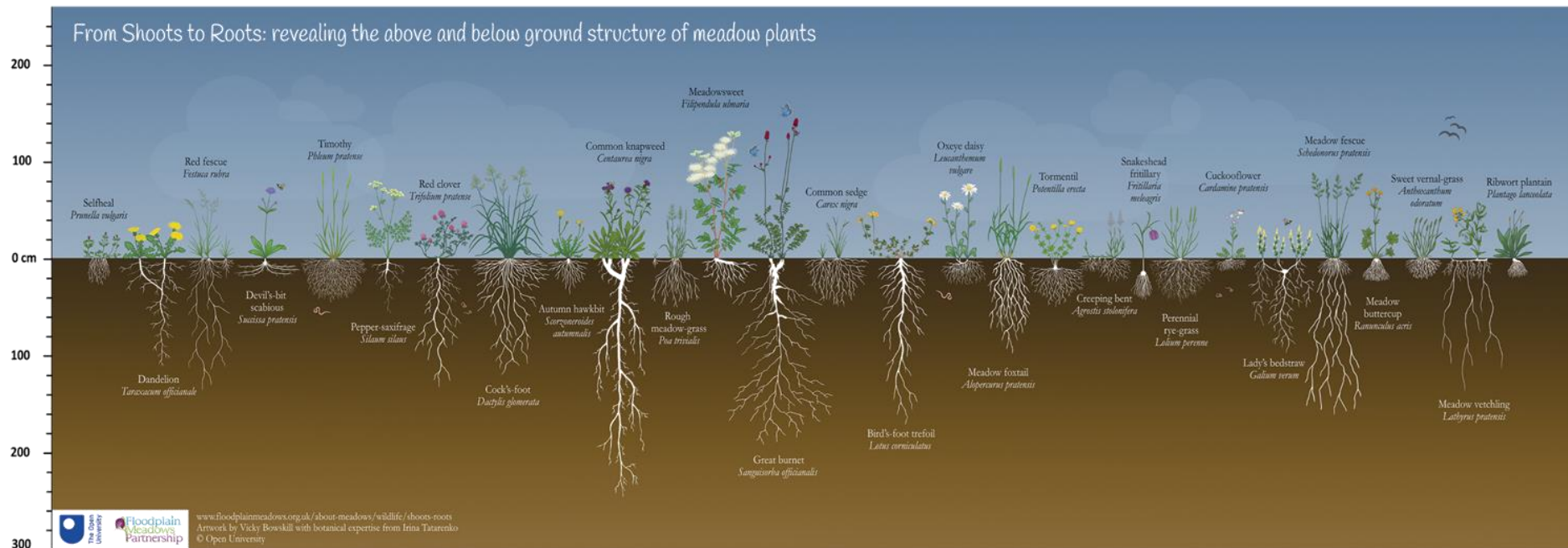
Plant roots have evolved to adapt to different environmental conditions (including soil type and health status). Their plasticity involves traits to keep adapting to emerging challenges.

Zhang, Y. *et al.* 2019. Evolution of fast root gravitropism in seed plants. **Nat Commun** 10, 3480.

Shekhar V, *et al.* 2019. The role of plant root systems in evolutionary adaptation. **Curr Top Dev Biol.** 131:55-80

Peter R. Ryan *et al.* 2016. Plant roots: understanding structure and function in an ocean of complexity. **Annals of Botany** 118(4): 555–559





# Roots today – diversity in root morphology and architecture



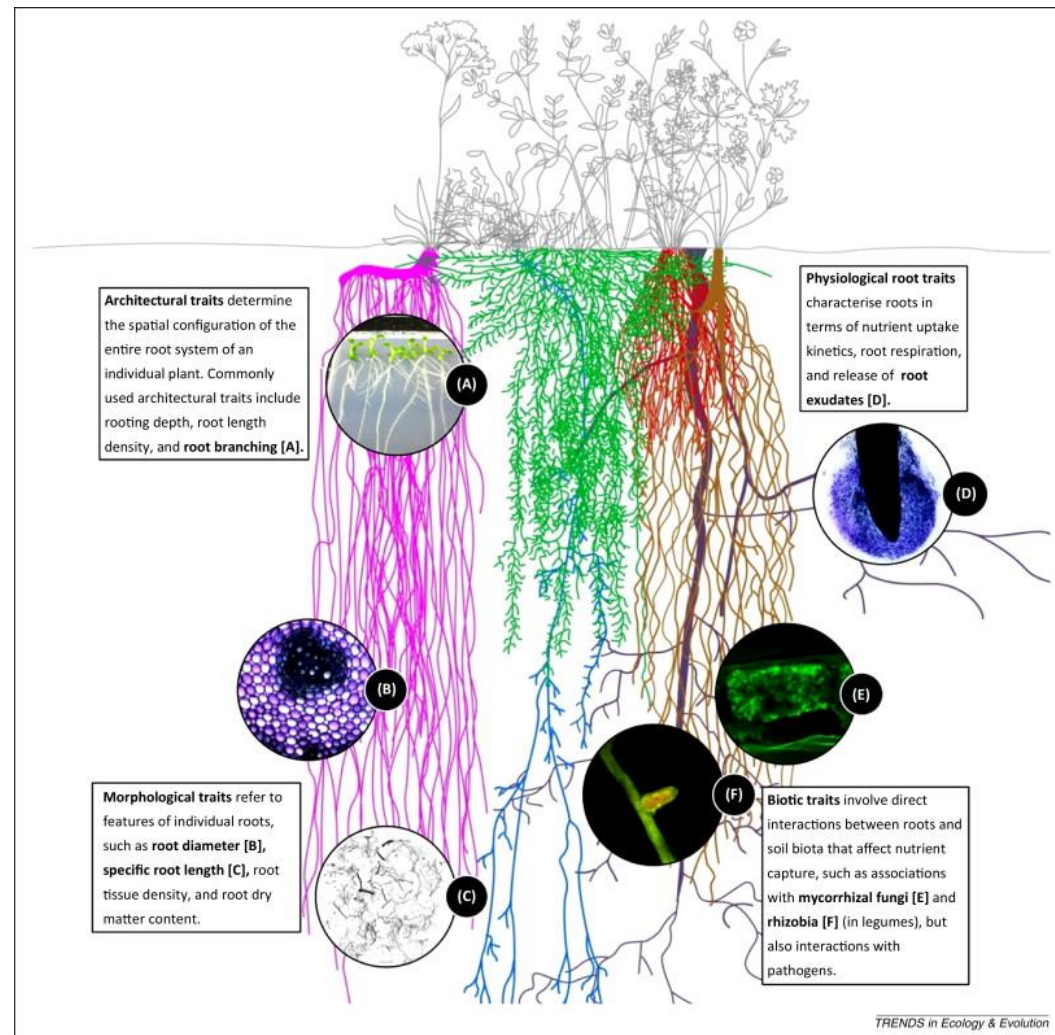
# Target root traits to secure crop productivity and resilience to climate change

## Root traits are important drivers of key ecosystem processes:

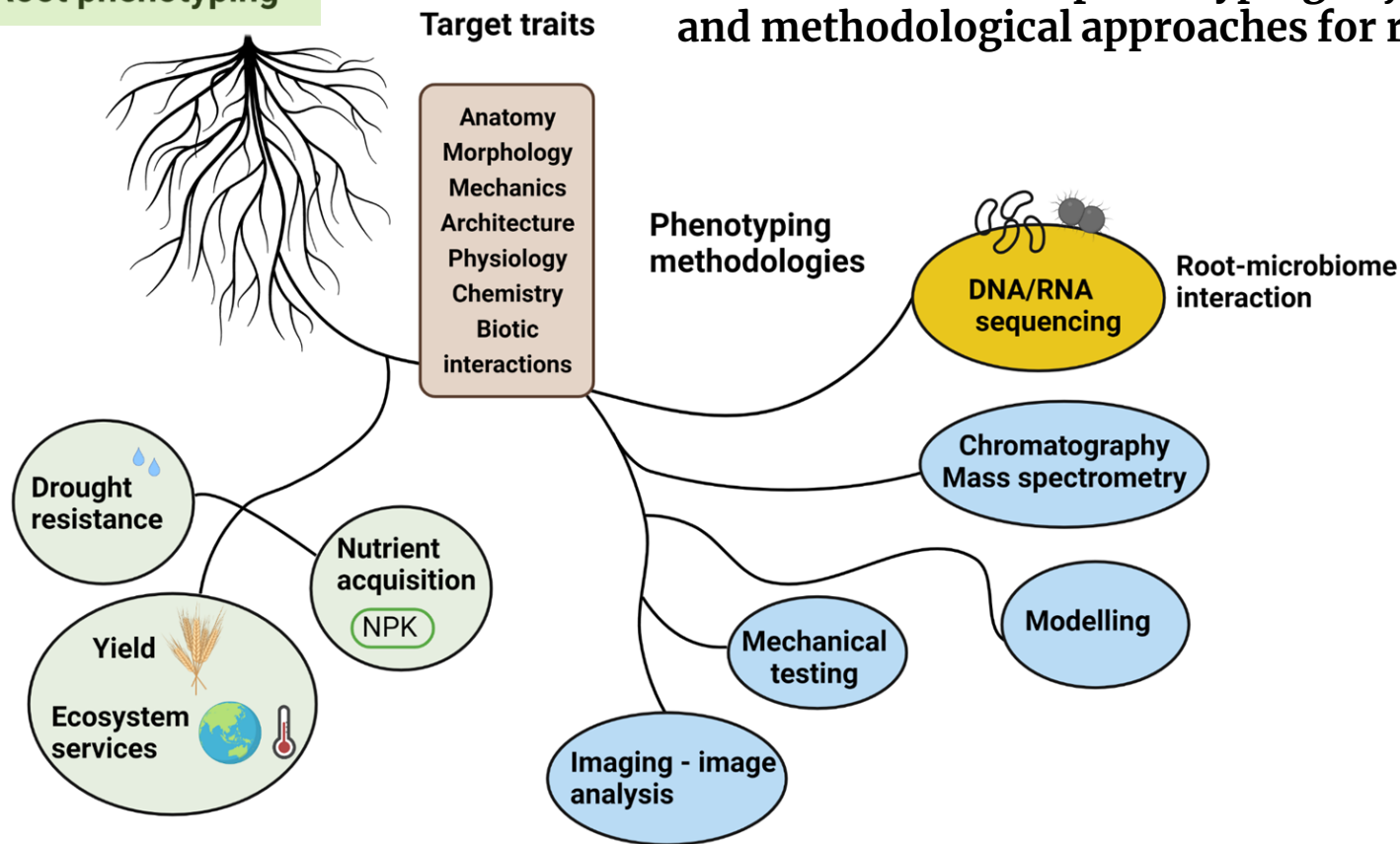
- carbon (C) and nutrient cycling
- the formation and structural stability of soil

-  Architectural traits
-  Morphological traits
-  Physiological traits
-  Biotic traits

Bardgett RD et al. 2014. Going underground: root traits as drivers of ecosystem processes. *Trends in Ecology & Evolution*, 29(12), 692-699







## An overview of root phenotyping objectives, target traits and methodological approaches for root phenotyping

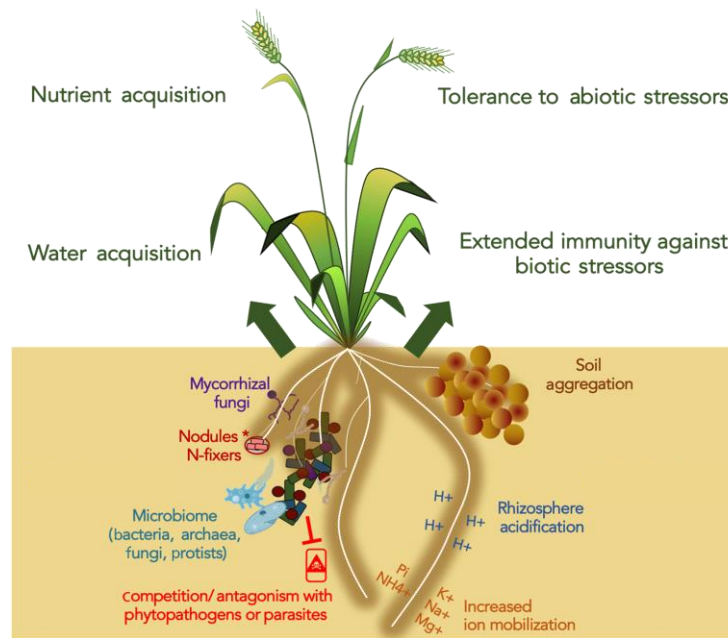




# Root functions: above and below ground

## Root functions – commonly known

-  Anchoring of the plant to the soil.
-  Absorption of water and nutrients from the soil.
  - Transport to the stem
  - Storage of food
-  Vegetative reproduction
-  Competition with other plants



Adapted from de la Fuente Cantó C. *et al.* 2020. *The Plant Journal* 103.3: 951

## Specialized root functions



### Response to abiotic stresses:

nutritional limitations,  
toxicity, waterlogging,  
physical constraints, heat,  
cold, drought, flood



### Response to biotic stresses:

pathogens, commensals,  
herbivorous



### Support of soil health:

structure (pores, air, water,  
carbon) and biodiversity

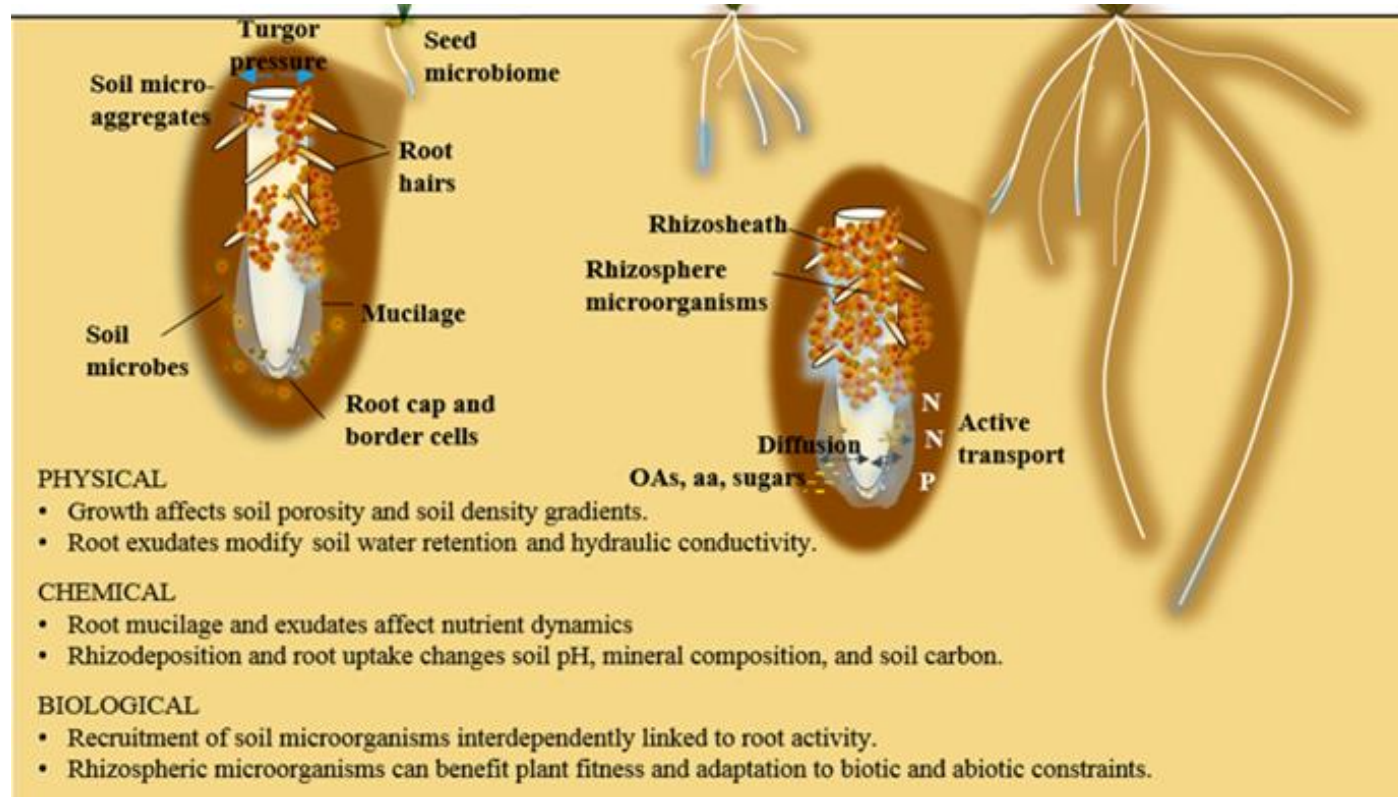


### Plant-microbe cross-talk

- Tracy SR *et al.* 2020. Crop improvement from phenotyping roots: highlights reveal expanding opportunities. *Trends in Plant Science* 25.1: 105-118
- Freschet, GT *et al.* 2021. Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytologist* 232.3: 1123-1158



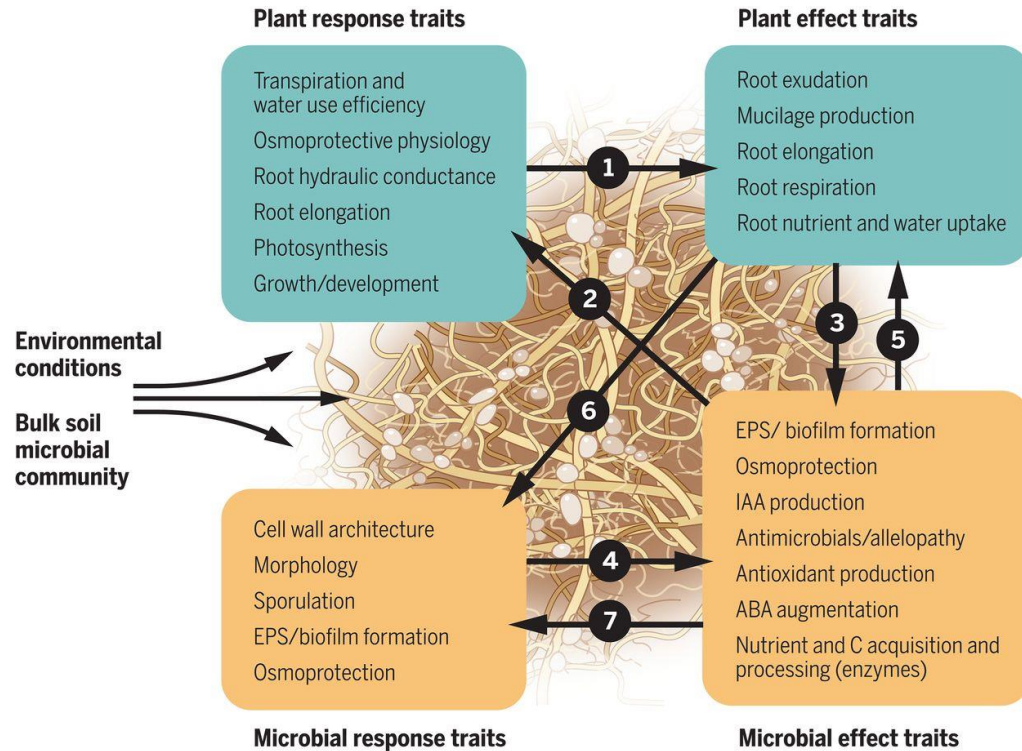
Specialized functions are directly linked with the physical, chemical and biological interaction of roots with soil at different developmental stages.





## Example:

# Relationships among plant and microbial drought response and effect traits.



Drought response traits determine the direct response of plants and microbes to drought.

These traits have a hypothesized link with drought effect traits (arrows 1 and 4), which determine the effect of drought on the plant.

Plant and microbial effect traits can feed back to each other (arrows 3 and 5) and determine plant and microbial response to drought (arrows 2 and 6).

Microbial effect traits can also feedback to influence microbial response to drought (arrow 7).

All traits are affected by environmental conditions and bulk soil microbial communities.

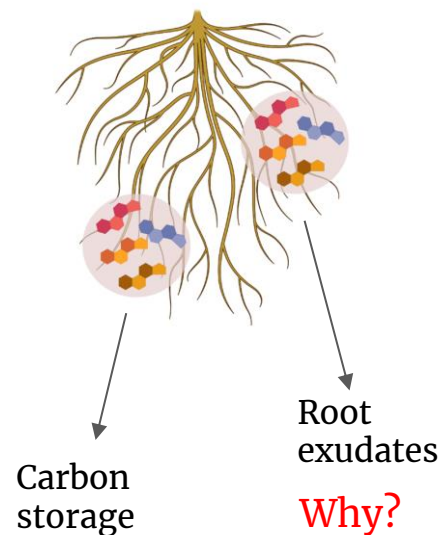
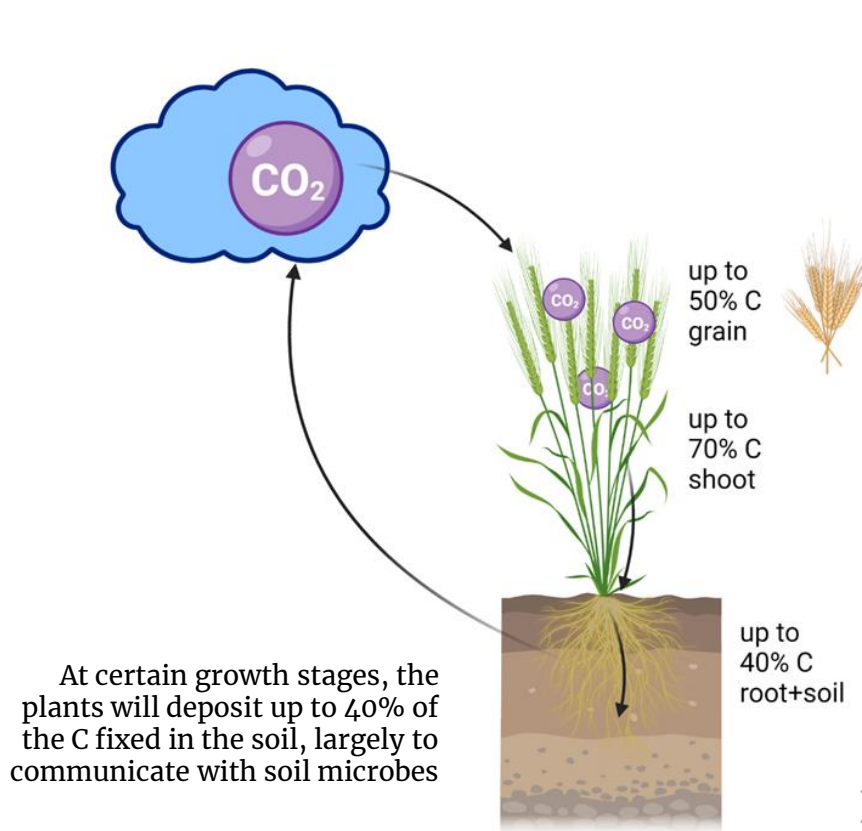
Morphology refers to filamentous hyphal growth of fungi.

EPS, exopolysaccharide

ABA, abscisic acid

IAA, indole acetic acid.

# Up to 40% of the C fixed by photosynthesis can be released in the soil through carbon deposits and exudates



## Release of root exudates: plant-microbes cross-talk

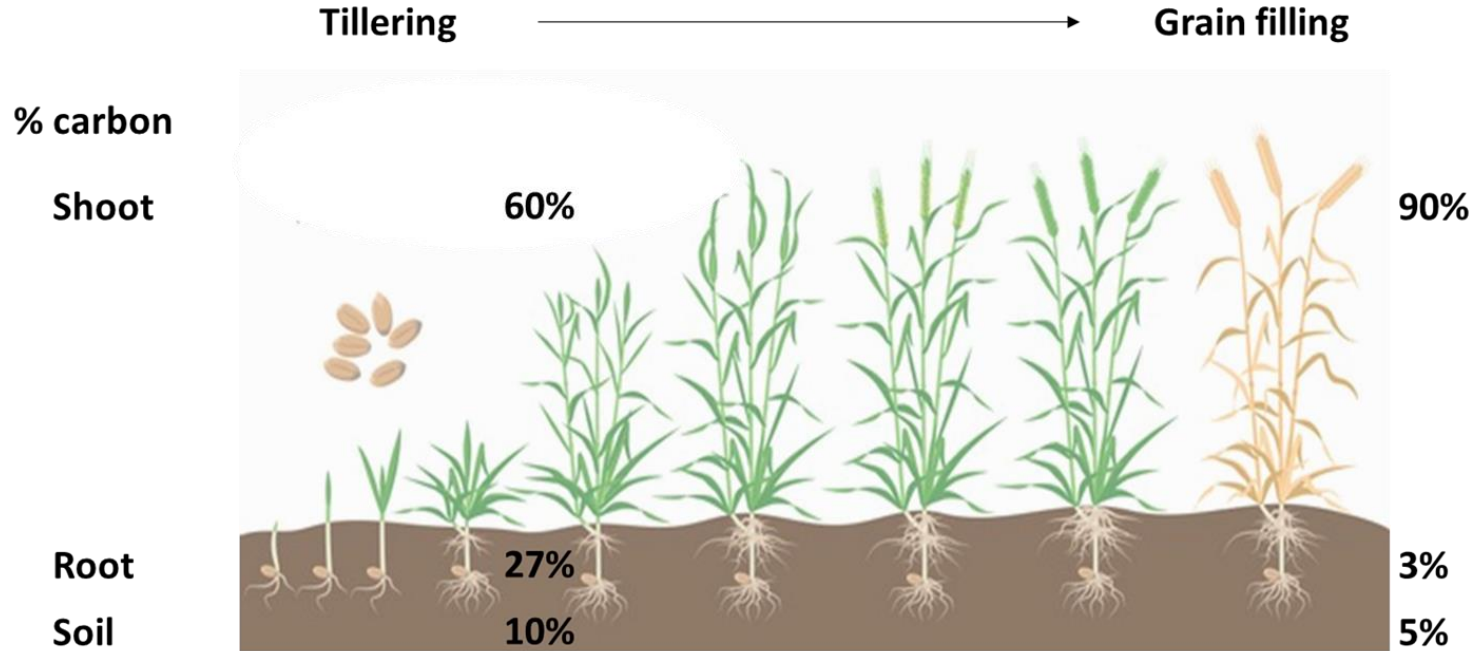
Freschet GT et al. 2021. Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytologist* 232.3: 1123-1158

Philippot L et al. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology* 11.11: 789-799.

# Nitrogen requirements and carbon deposits vary for the different Feekes Growth Stages

When is N more needed - key sampling time points?

Less carbon deposits in soil = less exudates?

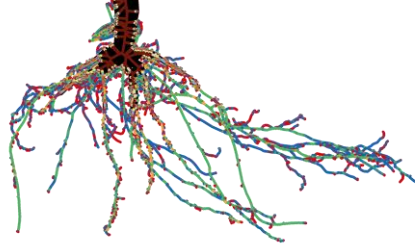


<https://agcrops.osu.edu/newsletter/corn-newsletter/what-meaning-feekes-growth-stages-wheat>

<https://www.extension.purdue.edu/extmedia/id/id-422.pdf>

Sun, Zhaoan, et al. 2018. Allocation of photosynthesized carbon in an intensively farmed winter wheat–soil system as revealed by  $^{14}\text{CO}_2$  pulse labelling. **Scientific Reports** 8.1: 1-10.

# Composition of the wheat root exudates



Root exudates are complex cocktails







- Lipids
- Siderophores/chelators
- Carbohydrates
- Mucilages
- Aminoacids, and other nitrogen compounds
- Phenolics
- **Terpenoids**
- Hormones
- Enzymes

- **Terpenoids - secondary metabolism**  
Wide range of effects on microbiome functionality

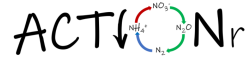
**Microbial guilds:** a group of bacteria that show consistent co-abundant behavior and likely to work together to contribute to the same ecological function

## Learning objectives:

-  Root and rhizosphere traits - plant-microbe interactions ✓
-  Root traits influence microbial communities, nitrogen cycling, and overall soil health
-  **WISH-ROOTS: Wheat Improving Soil Health - ROOT traits.**
-  Potential applications in sustainable agriculture.



## Rhizosphere, rhizoshead and rhizobiome

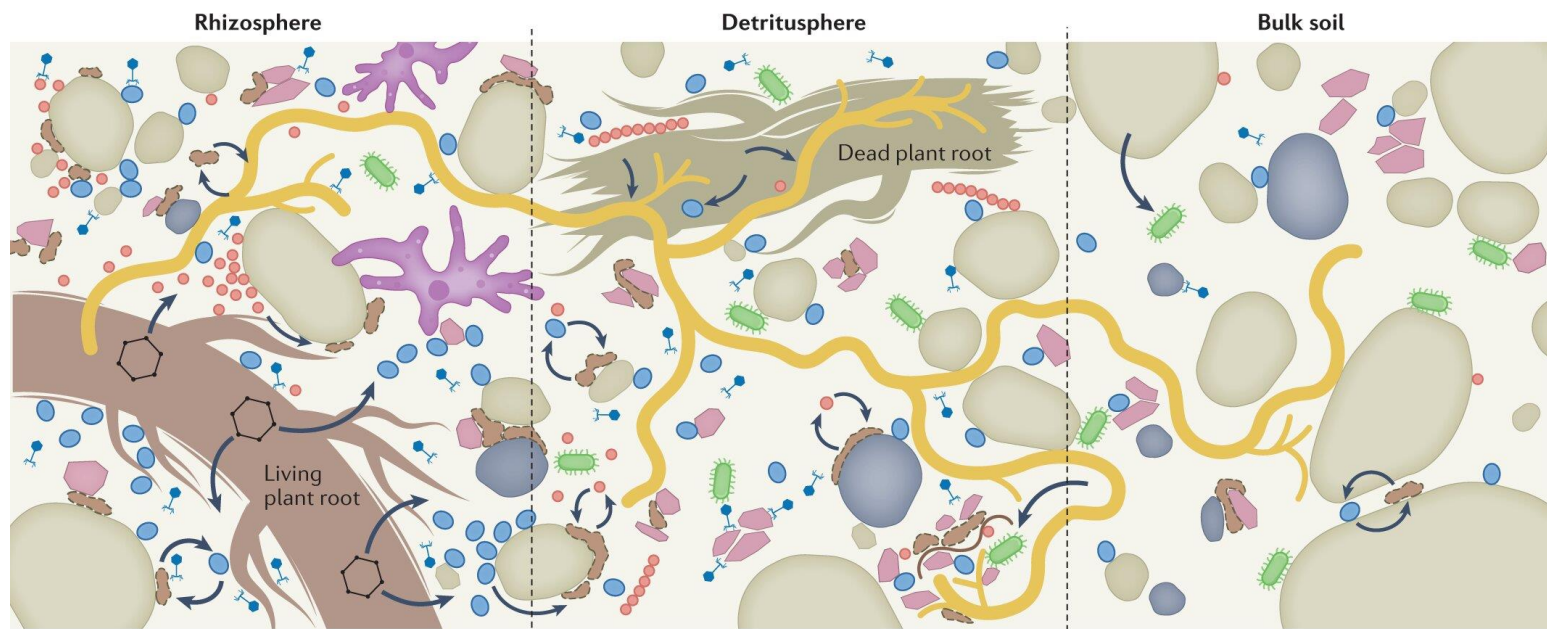


- Julius Sachs, 1862: 'soil binds to the youngest parts of the root system of wheat (*Triticum aestivum*), forming a rhizosheath'
- Lorenz Hiltner, 1904: first coined the term "rhizosphere":  
'the area around a plant root that is inhabited by a unique population of microorganisms influenced by the chemicals released from plant roots'

**Root microbiome  
or rhizobiome**



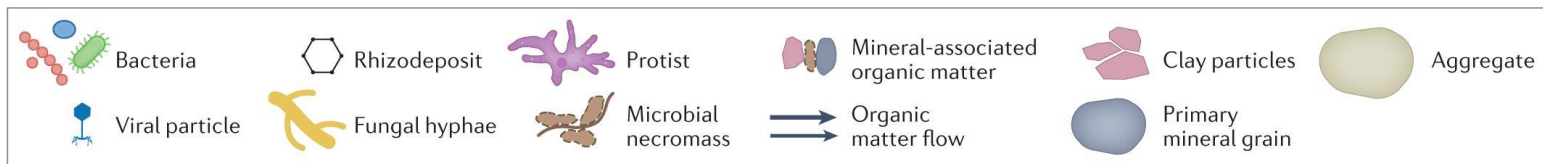




- Organic matter inputs from living root (rhizodeposits)
- Higher microbial biomass and activity
- Lower microbial diversity
- Fast biomass turnover; high rates of organic matter flow
- Increased predation

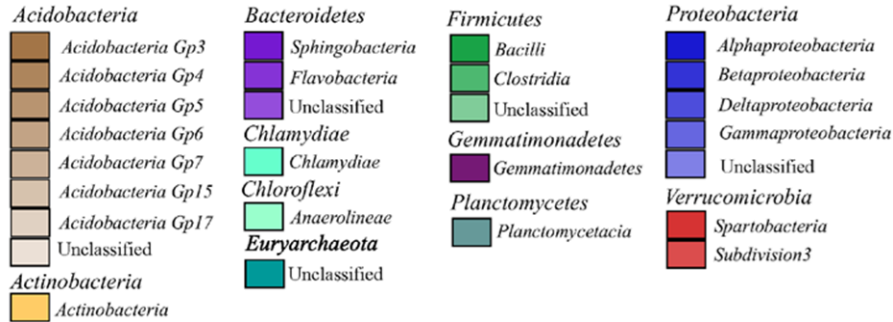
- Organic matter inputs from dead litter
- Higher microbial biomass and activity
- Higher prevalence of saprotrophic fungi
- High rates of organic matter flow

- Lower microbial biomass and activity
- Higher microbial diversity
- Slower biomass turnover and rates of organic matter flow

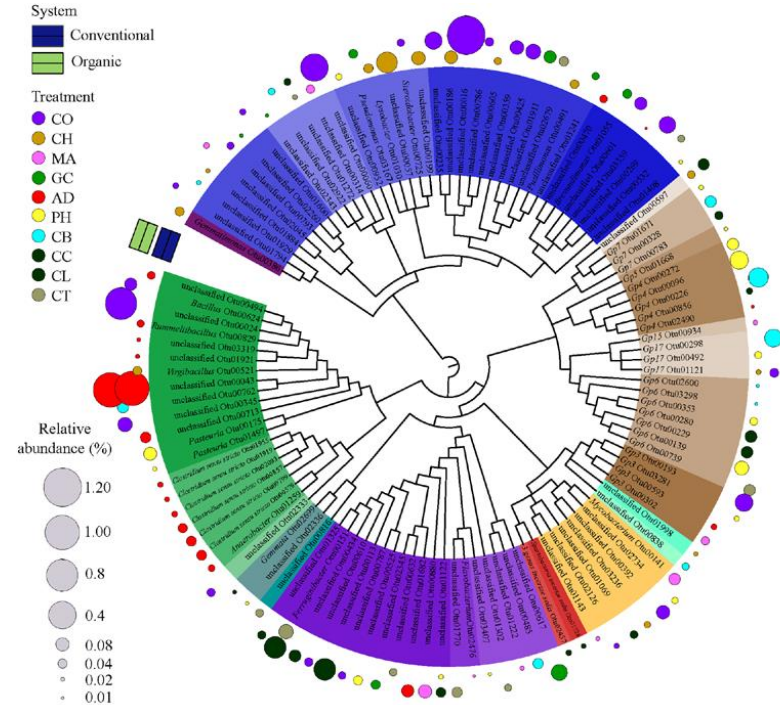


# An overview of the microbiome composition in agricultural soils.

The authors reported higher heterogeneity in organic than in conventional farming system



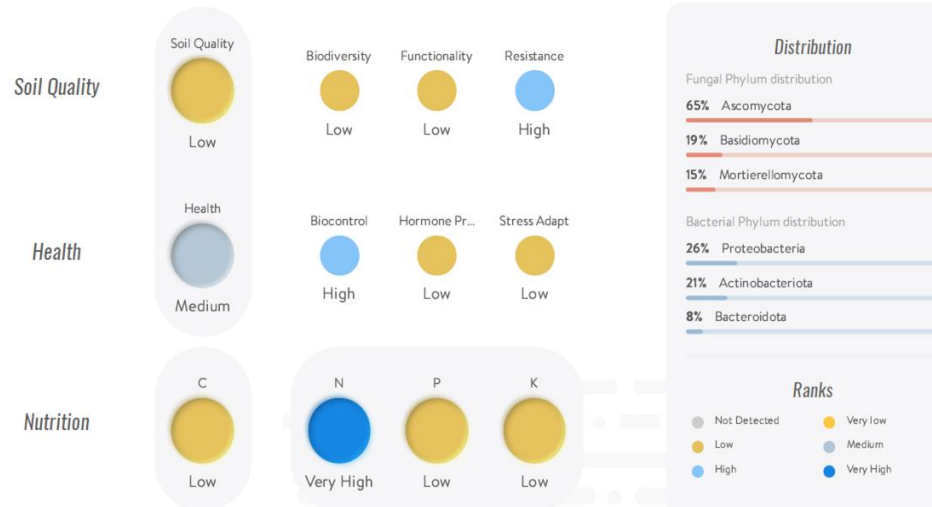
- **Acidobacteria & Bacteroidetes** – decomposers of **organic matter**, promote **plant growth**
- **Firmicutes** – **plant growth** promotion, **biocontrol** of plant pathogens
- **Proteobacteria** – **nitrification and denitrification**
- **Actinobacteria** - improve the **availability of nutrients** and minerals for plants, **inhibit plant pathogens**.
- **Gemmatimonadetes**- **carbon cycling**, reduce  $N_2O$  emissions.
- **Cyanobacteria** – **nitrogen fixation**
- **Planctomycetes** - Anaerobic ammonia-oxidizing (**anammox**)
- **Thaumarchaeota (Nitrososphaerota)** - **ammonia-oxidizing** archaea
- **Verrucomicrobia** - role in **nutrient cycling** through ammonia oxidation and nitrite reduction. Improves plant health. Symbiosis with nematodes. Regulate **methane emissions** by oxidizing methanol in soil
- **Eukaryota/Ascomycota** – **soil stability**, turnover of minerals/organic compounds
- **Nitrospirae** – **nitrite-oxidising** bacterium
- **Chloroflexi** – **degrades organic compounds**
- **Desulfubacterota, Clostridiales** – **degradation of organic matter**



Lupatini, Manoeli, et al. 2017. Soil microbiome is more heterogeneous in organic than in conventional farming system. **Frontiers in microbiology** 7: 2064.

# Ecological functions of soil microbes what do they do?

Microbial activities balance soil ecosystems, including functions like nutrient release, biocontrol, phytohormone production, and promotion of plant stress tolerance.



## 4 Health:

The role of microorganisms in plant health and yield is defined by the balance between levels of soilborne pathogens, biocontrol, hormone production, and stress adaptation.

**BIOCONTROL**  
VERY LOW

**HORMONE PRODUCTION**  
LOW

**STRESS ADAPTATION**  
MEDIUM

## 5 Nutrition:

The potential of soil microorganisms to cycle nutrients and to increase the bioavailability of nutrients for plants and sequester carbon in the soil. This section is divided into macronutrients and micronutrients.

**C**  
Carbon  
MEDIUM

**N**  
Nitrogen  
MEDIUM

**P**  
Phosphorus  
MEDIUM

**K**  
Potassium  
MEDIUM

**BIOME  
MAKERS**

<https://biomemakers.com/>

Imam, Nabeel, et al. 2021. Local Network Properties of Soil and Rhizosphere Microbial Communities in Potato Plantations Treated with a Biological Product Are Important Predictors of Crop Yield. **Mosphere** 6.4: e00130-21.

# Functional analysis helps understanding how we can improve soil health and plant productivity

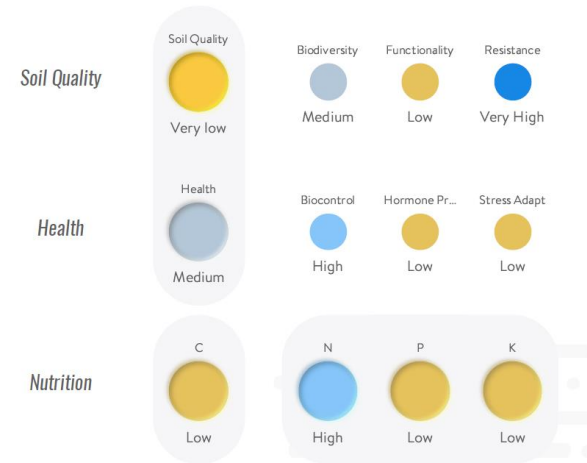
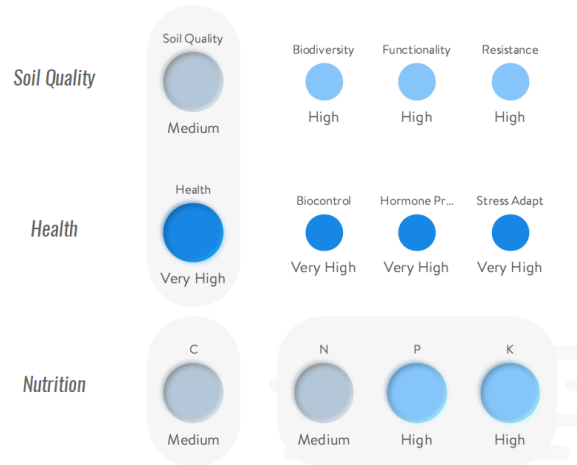
Phacelia



Bare soil



Elite wheat

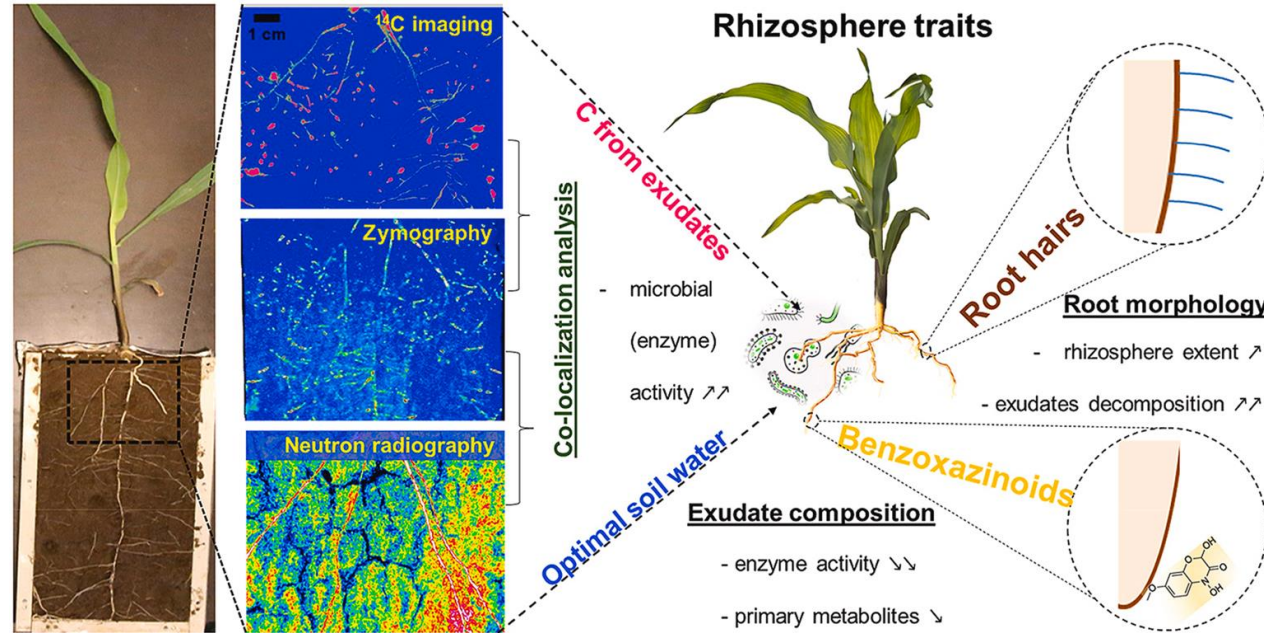


Phacelia (catch crop) - **effective at preventing nitrogen leaching and suppressing weeds.**

<https://biomemakers.com/>



Traits linked to the release of exudates include nutrient acquisition and tuning the rhizobiome composition and associated functionality.



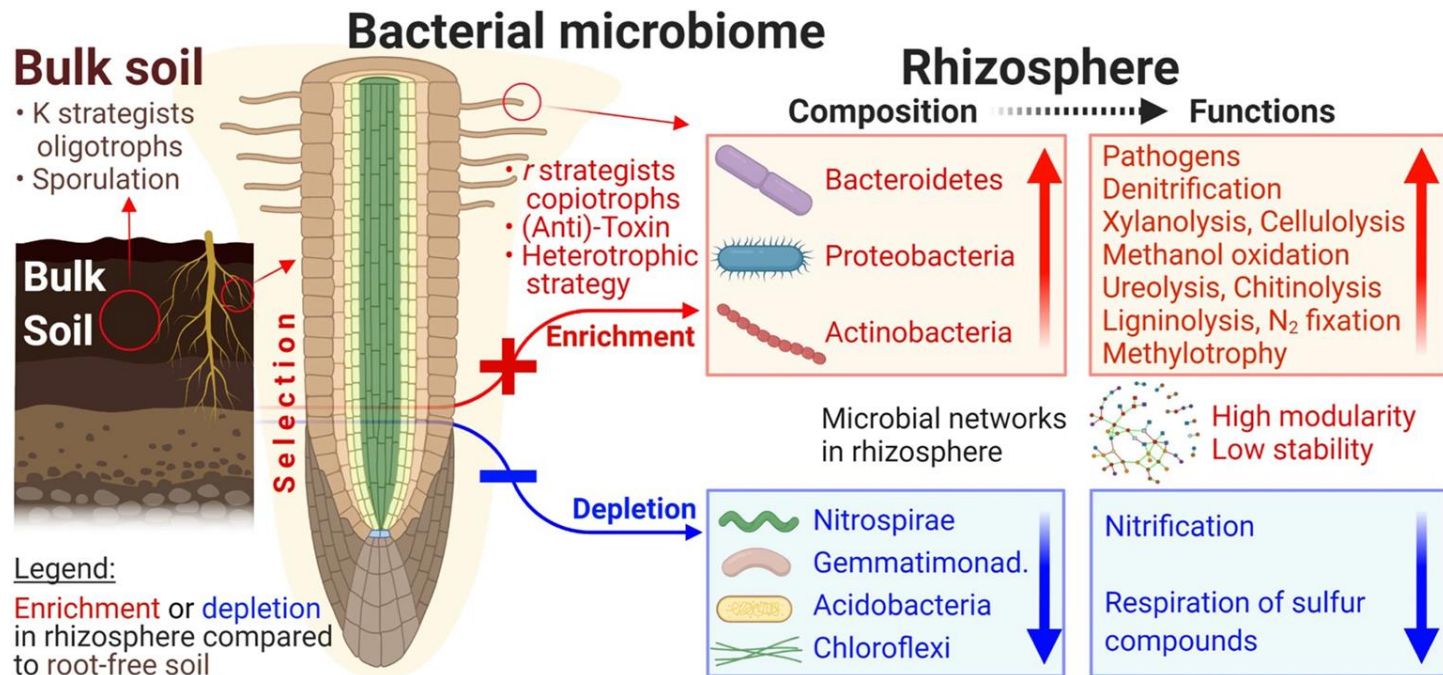
Zymography,  $^{14}\text{C}$  imaging, and neutron radiography coupled to localize rhizosphere processes.

Artificial selection of microbiota opens new avenues for improving plants.

Jacquioud S. et al. 2022. Artificial selection of stable rhizosphere microbiota leads to heritable plant phenotype changes. *Ecology letters* **25.1**: 189-201

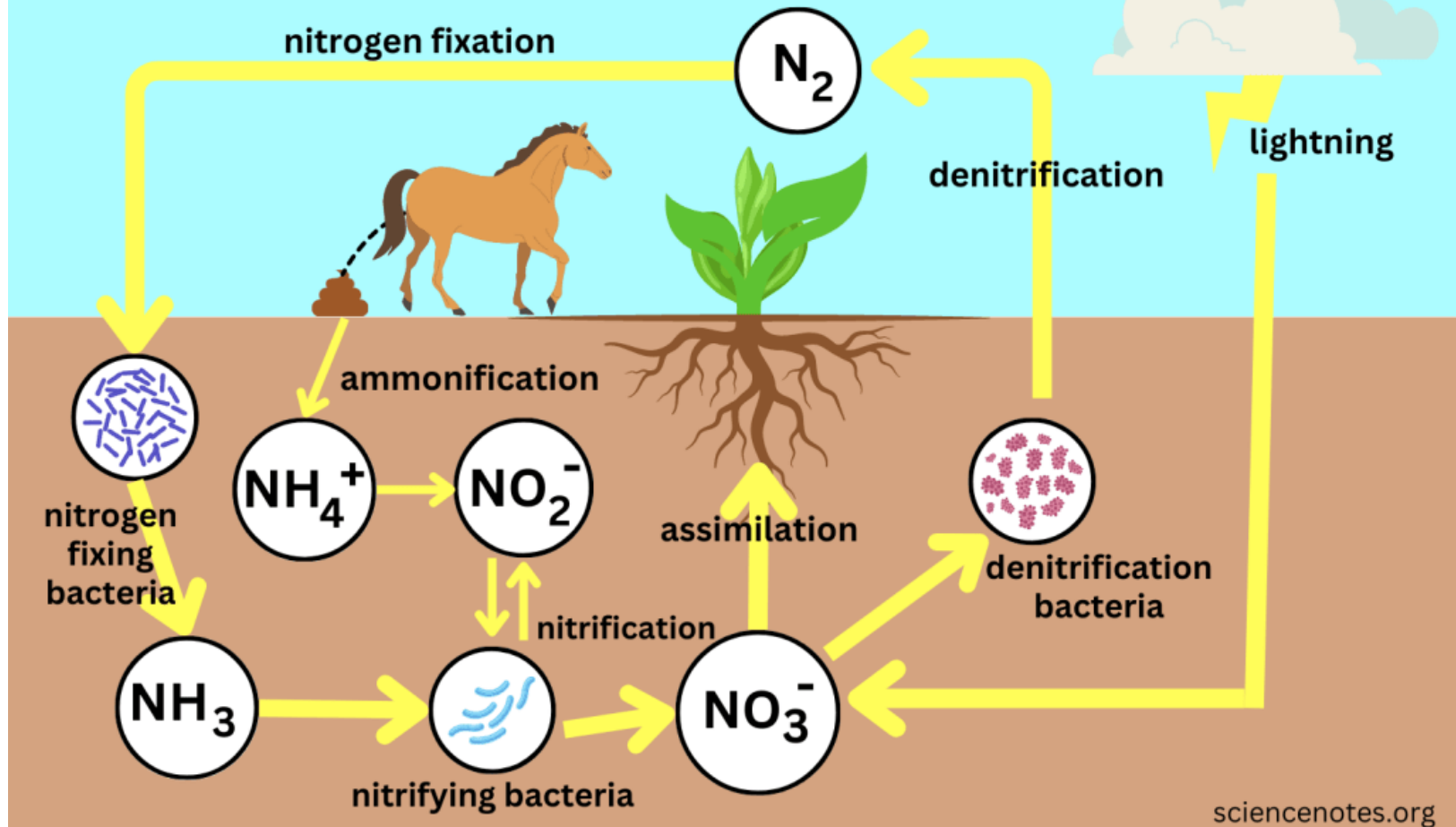
Bilyera N. et al. 2021. Maize genotype-specific exudation strategies: an adaptive mechanism to increase microbial activity in the rhizosphere. *Soil Biology and Biochemistry* **162**: 108426

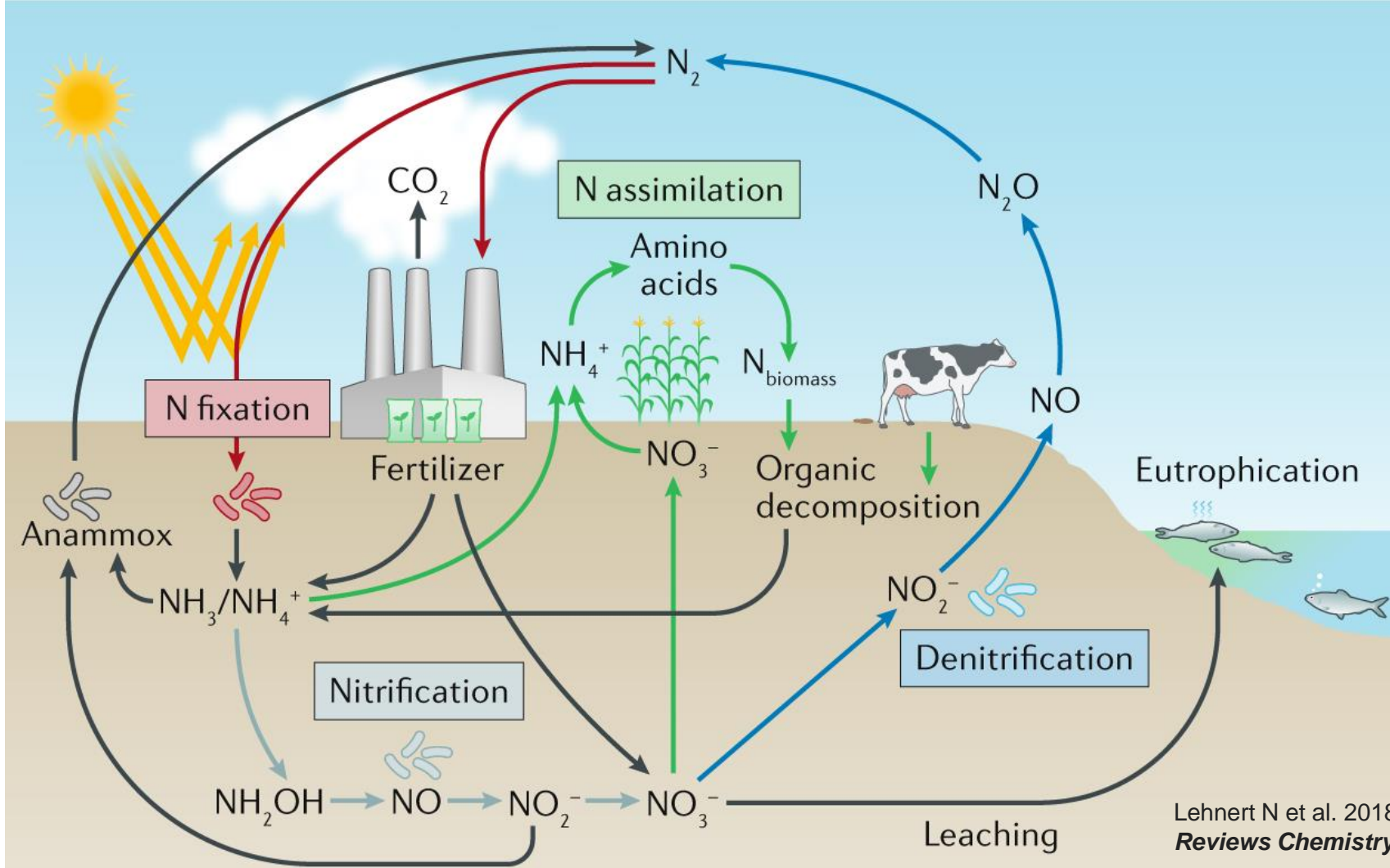
# Enrichment (red) and depletion (blue) of bacterial community taxa and functions in the rhizosphere relative to bulk soil





# Nitrogen Cycle







a solution?

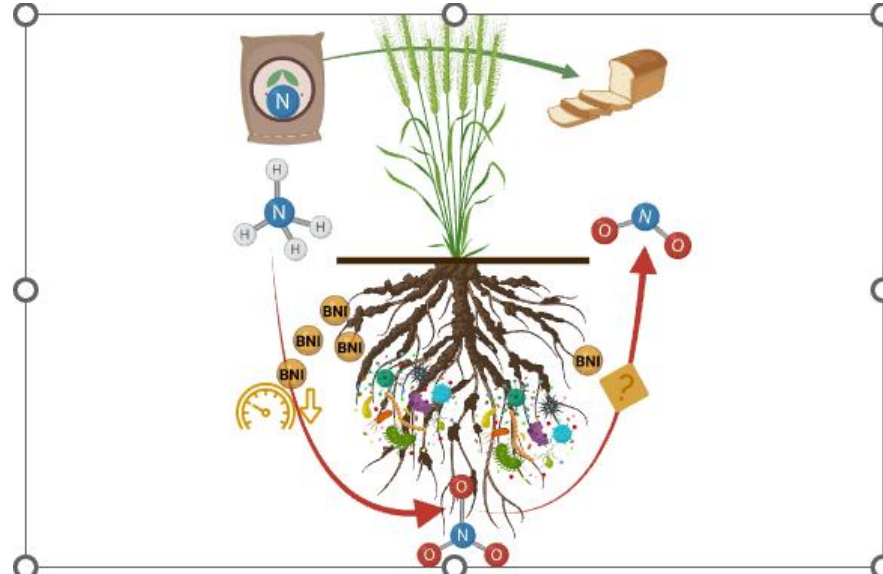
The  
problem



- ✓ Approximately half of the world's food production is supported by the use of N fertilizers.
- X globally, ~50% (70%) of the N-fertilizer applied to crops is lost to the environment by leaching or gaseous emissions.

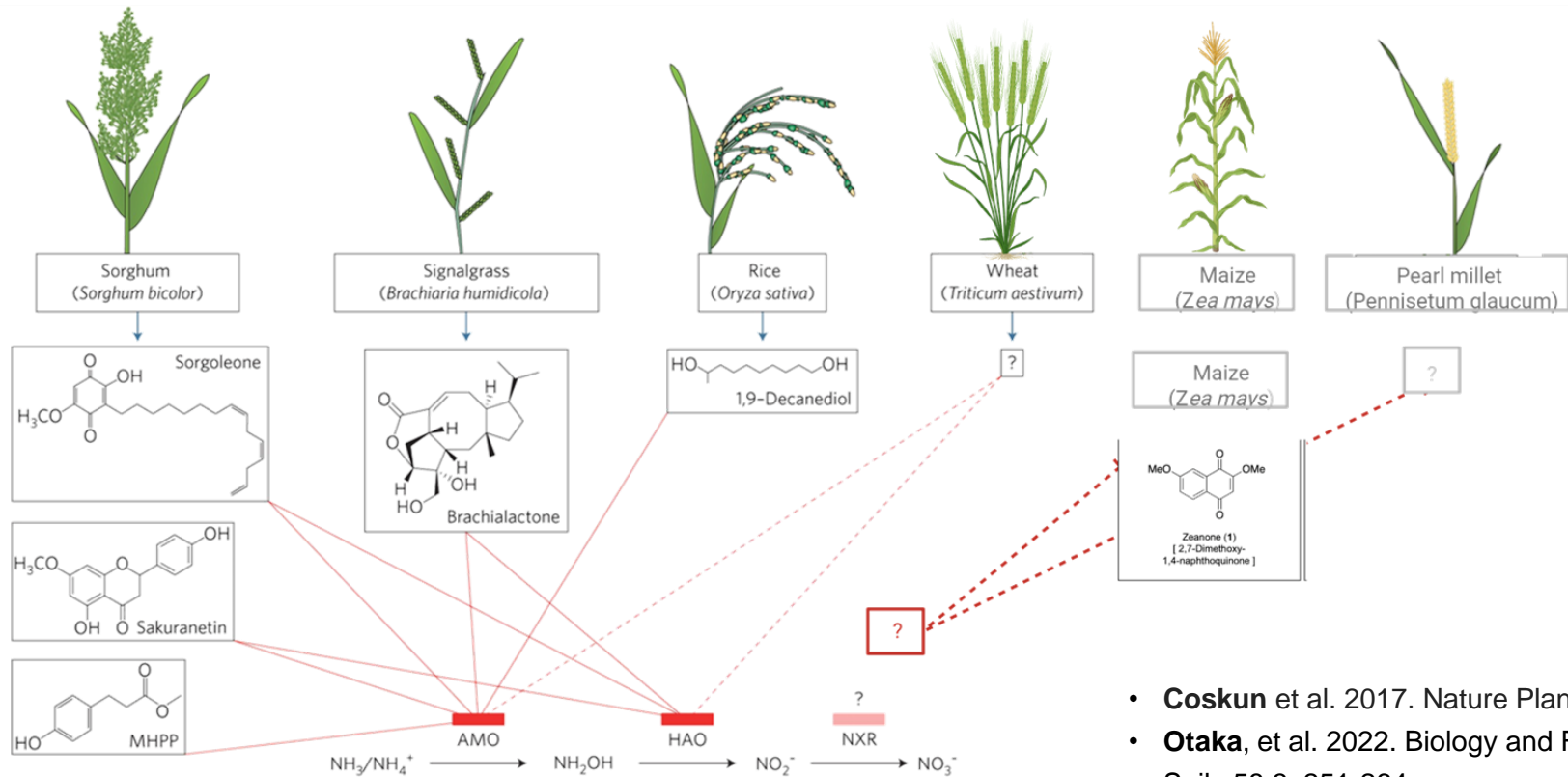
N-fertiliser is usually applied as  $\text{NH}_4^+$  but rapidly transformed to soluble  $\text{NO}_3^-$  and easily lost to the environment

Biological nitrification inhibitors (BNI) present in the root exudates of certain plants can slow down microbial transformation of  $\text{NH}_4^+$  into  $\text{NO}_3^-$  and  $\text{N}_2\text{O}$



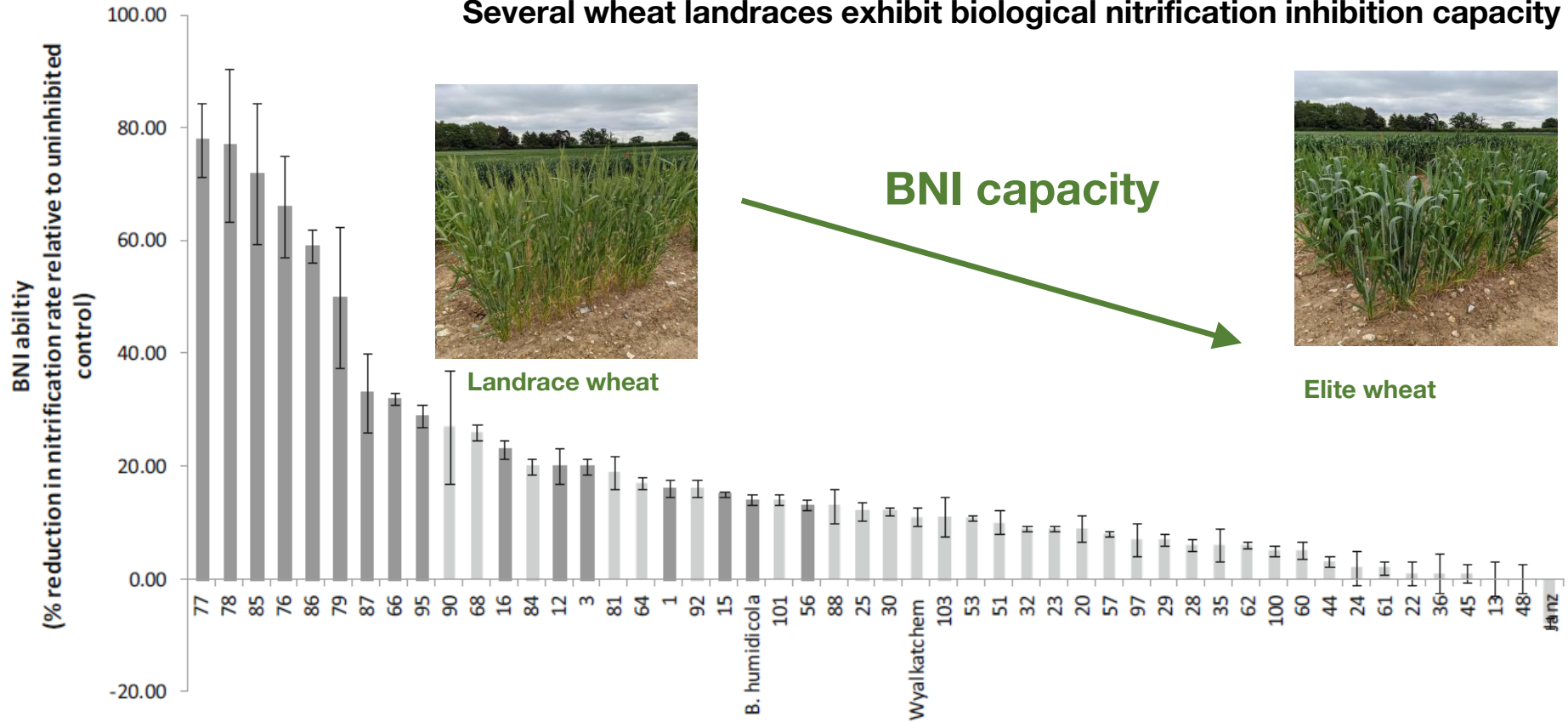
Can you think of some economic and environmental implications? Which form of N does the plant prefer?  
Which root traits are important for N management and cycling in the rhizosphere?

Biological nitrification inhibitors (BNI) are present in the root exudates of certain plants



- **Coskun** et al. 2017. Nature Plants 3.6: 1-10.
- **Otaka**, et al. 2022. Biology and Fertility of Soils 58.3: 251-264.
- **Ghatak** et al. 2022. Biology and Fertility of Soils. 58.3: 291-306.

# Several wheat landraces exhibit biological nitrification inhibition capacity



Results from bioassay -  
*Nitrosomonas europaea*

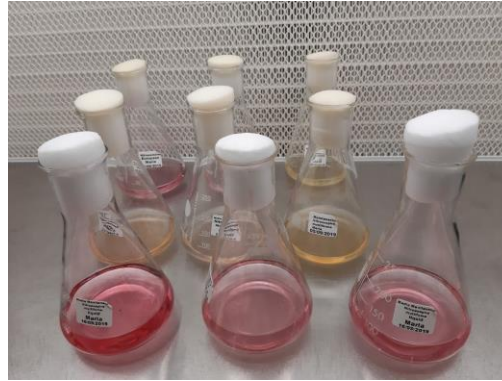
O'Sullivan, CA., et al. 2016. Identification of several wheat landraces with biological nitrification inhibition capacity. **Plant and Soil** 404.1: 61-74.

# A simple approach to phenotyping the trait (biological nitrification inhibition capacity (BNI): Bioassay of a single strain - *Nitrosomonas europaea*

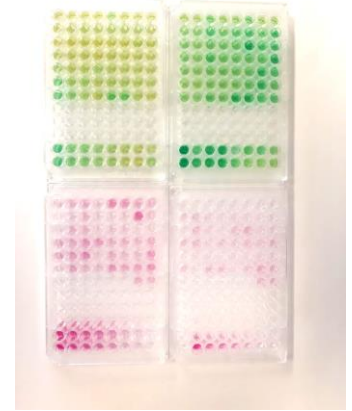
Collection of exudates from  
plants grown in hydroponics



*N. europaea* +  $\text{NH}_4^+$  media +  
exudates - 6h



Colorimetric assay -  
measure remaining  $\text{NH}_4^-$   
and  $\text{NO}_3^-$  produced







## Limitations?

- young plants, only 4 weeks old (is that a good sampling time point?)
- plants will behave differently in soil
- only allows to measure BNI effect on a bacterial strain
- high variability
- *N. europaea* is not often found in soil?



## Learning objectives:

-  Root and rhizosphere traits - plant-microbe interactions ✓
-  Root traits influence microbial communities, nitrogen cycling, and overall soil health ✓
-  **WISH-ROOTS: Wheat Improving Soil Health - ROOT traits.**
-  Potential applications in sustainable agriculture.



# **WISH-ROOTS:** **W**heat **I**mproving **S**oil **H**ealth - **ROOT** traits

2022-2025

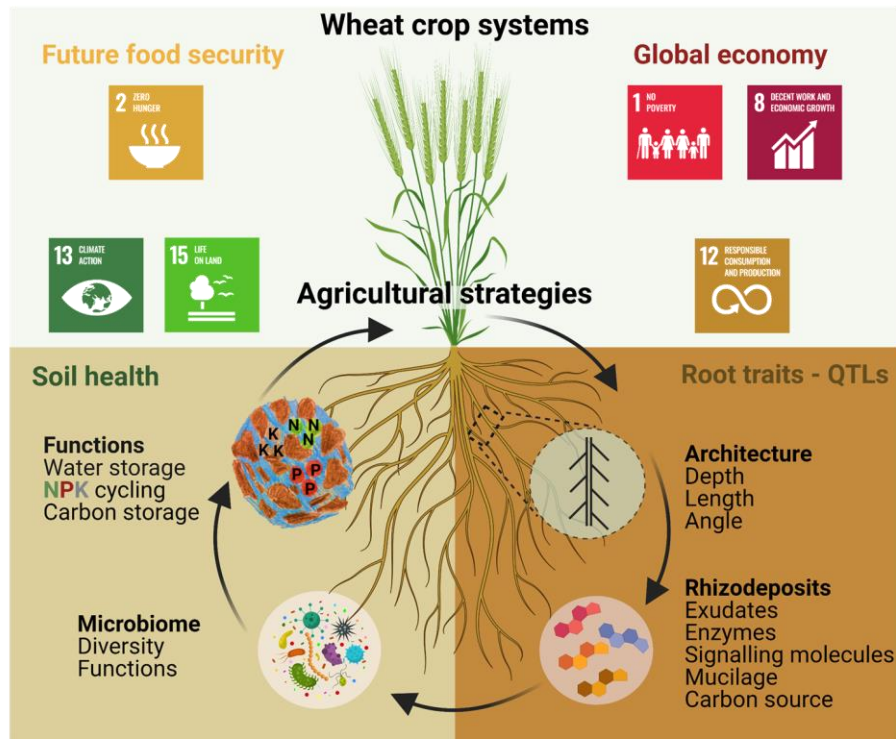
# WISH-ROOTS: **W**heat **I**mproving **S**oil **H**ealth through **ROOTS** traits

## Our objective:

To enhance the potential beneficial effects of wheat cultivation on soil health through the identification of **root traits** that can optimize **nitrogen (N) cycling** and improve **soil structure**.



WISH-ROOTS consortium: plant genetics, soil science, microbial ecology

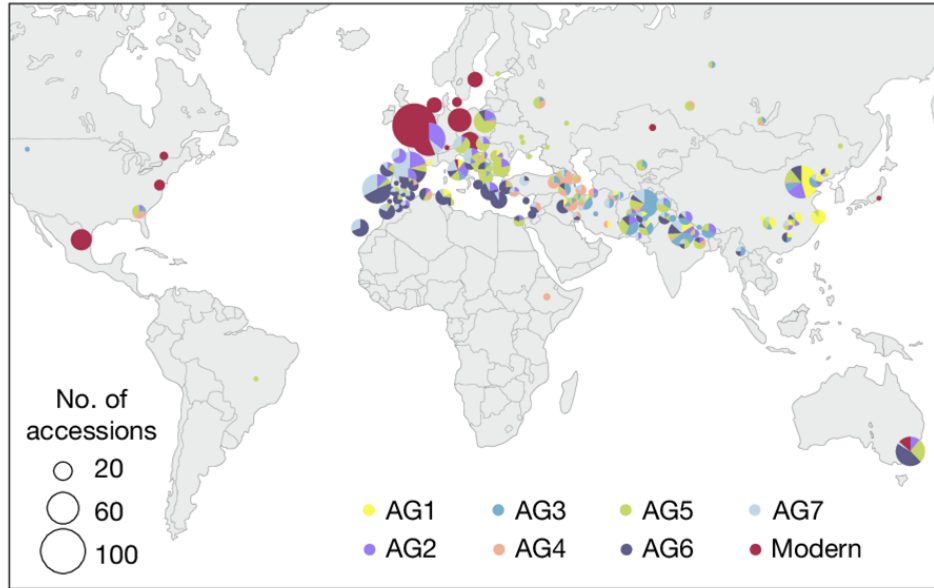


Making soil health a wheat breeding target.

EJP Soil WISH-ROOTS Consortium. Review in preparation.

## The A.E. Watkins Landrace Collection of Bread Wheat: a unique resource to identify wheat root traits

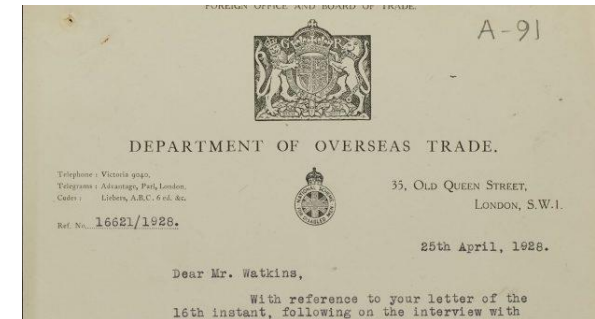
- wheat landraces cultivars acquired by A.E. Watkins in the 1920s from 32 different countries in Asia, Europe and Africa (currently 1291 accessions)
- collected before modern plant breeding and the green revolution - source of genetic variability for novel agronomic trait discovery



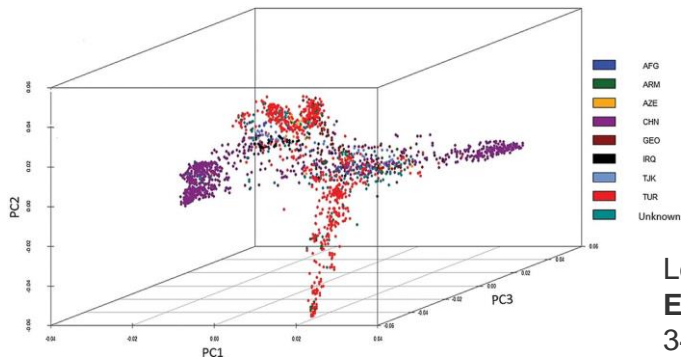
Cheng, S., Feng, C., Wingen, L.U. *et al.* 2024. Harnessing landrace diversity empowers wheat breeding. **Nature** 632, 823–831



<https://www.jic.ac.uk/research-impact/germplasm-resource-unit/>

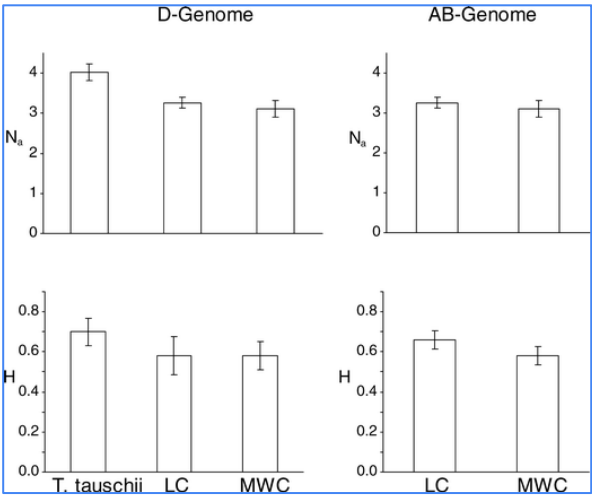


# Wide genetic diversity in wheat landraces: what did we lose through breeding?



10 000 genotype-by-sequencing (GBS) markers run in a total of 3300 wheat landraces

Lopes et al. 2015 **Journal of Experimental Botany** 66 (12): 3477–3486



Standardized number of alleles per locus ( $N_a$ ) and gene diversity ( $H$ ) of 11 *T. tauschii* accessions, 119 LC, and 123 MWC genotypes.

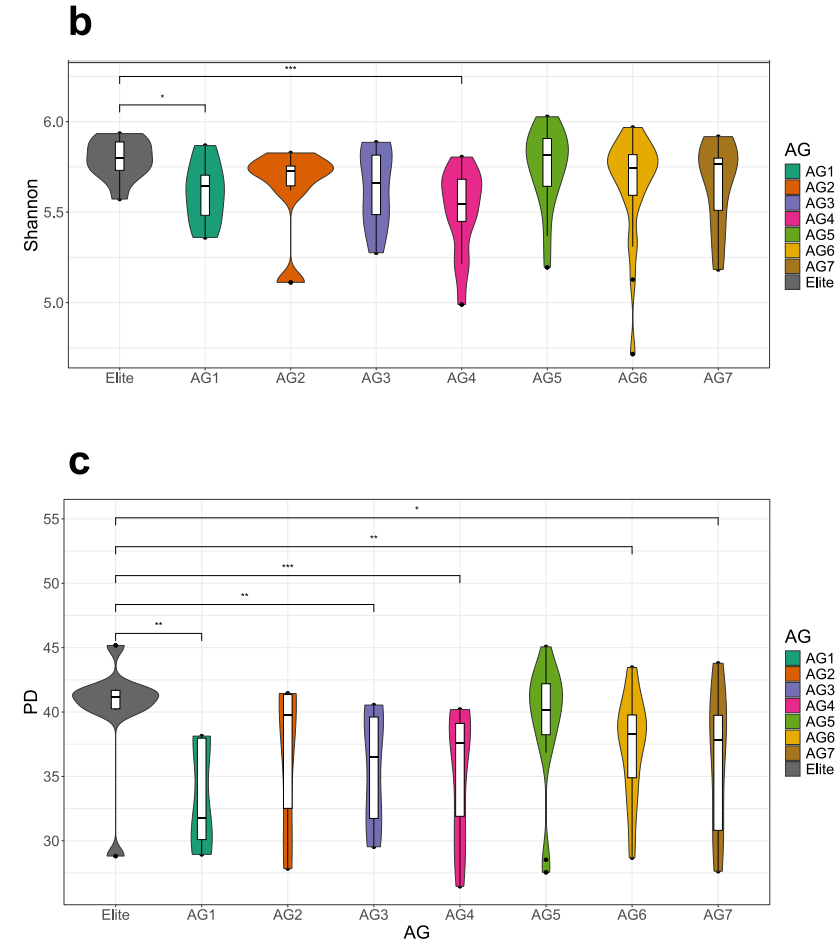
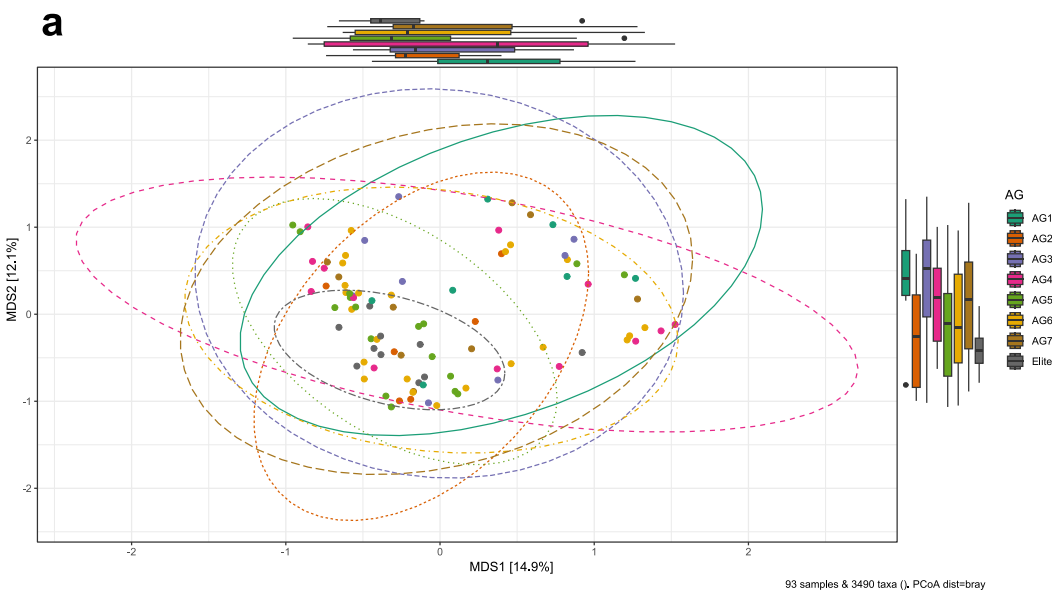
Reif. et al. 2005 **Theor Appl Genet** 110, 859–864





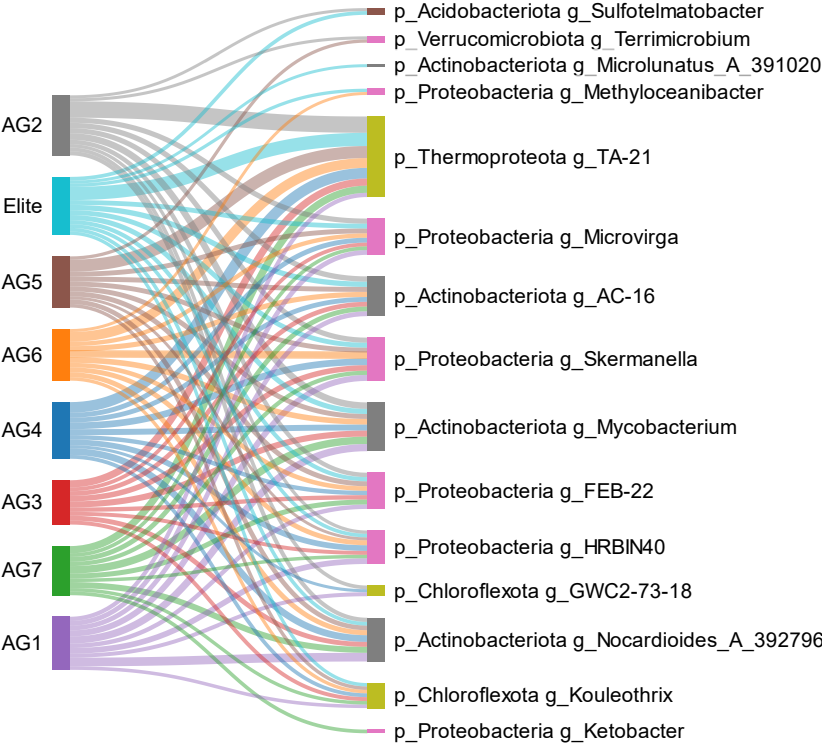
# Microbiome recruited by bread wheat landraces and modern cultivars:

## Biodiversity

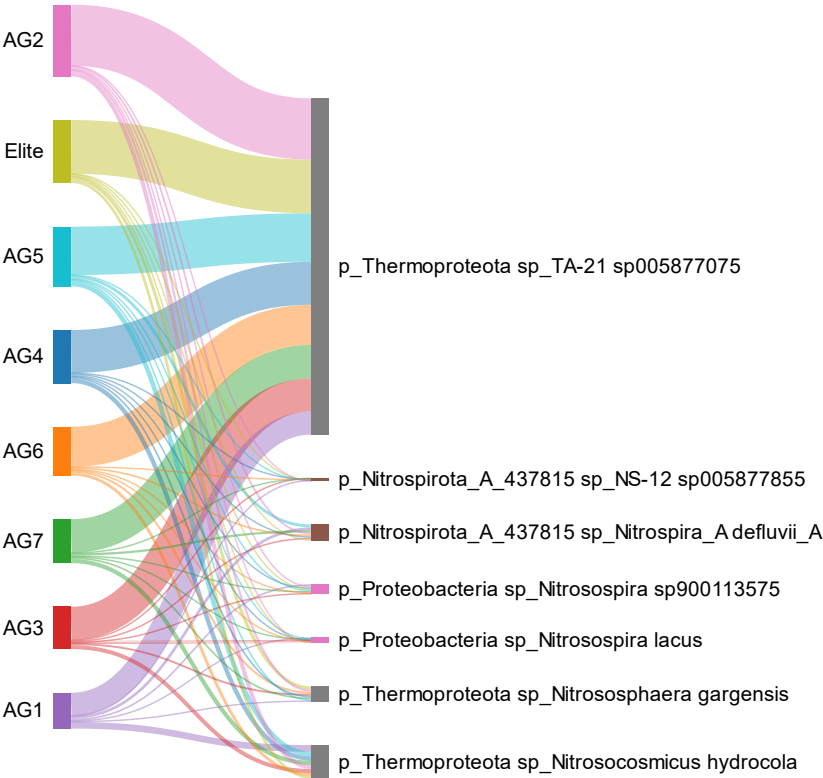


# Microbiome recruited by bread wheat landraces and modern cultivars:

## Core microbiome

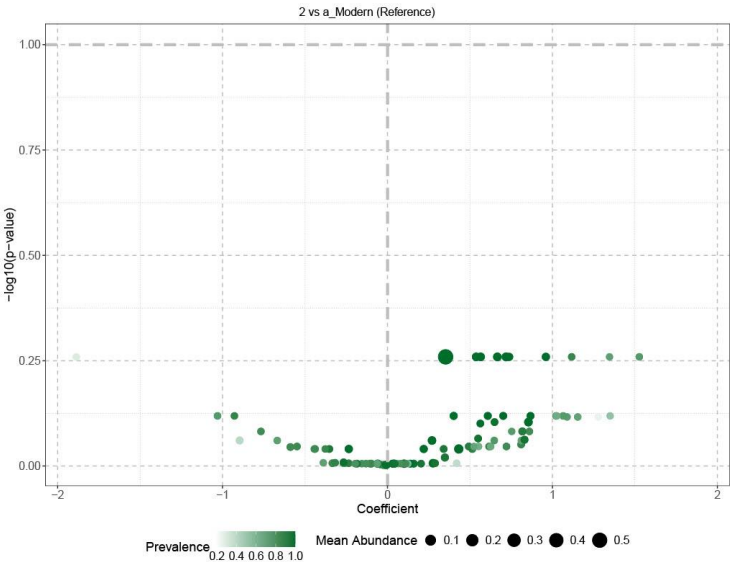
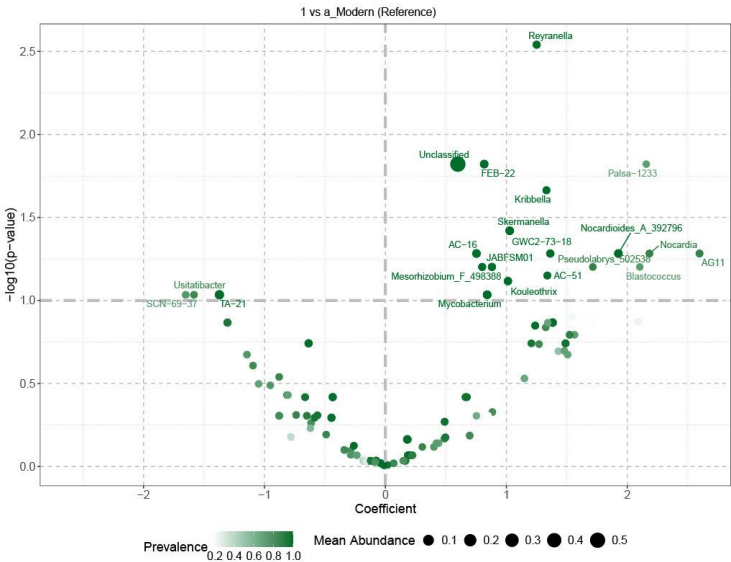


## N-cycling



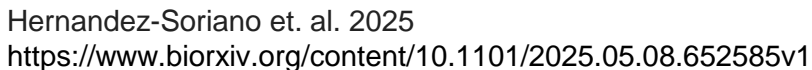
# Microbiome recruited by bread wheat landraces and modern cultivars:

## Differential abundance (LinDA)





## Dissimilarity network

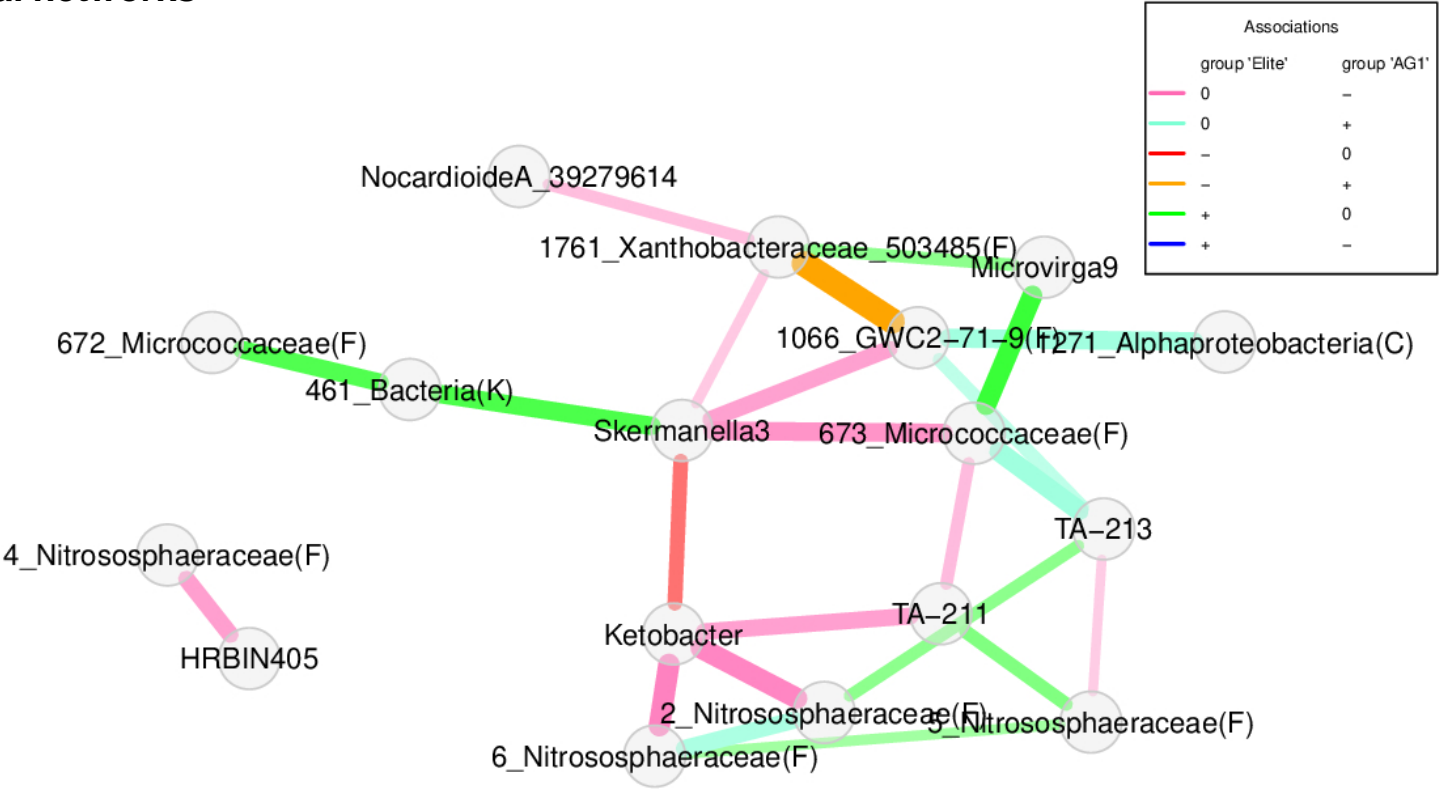






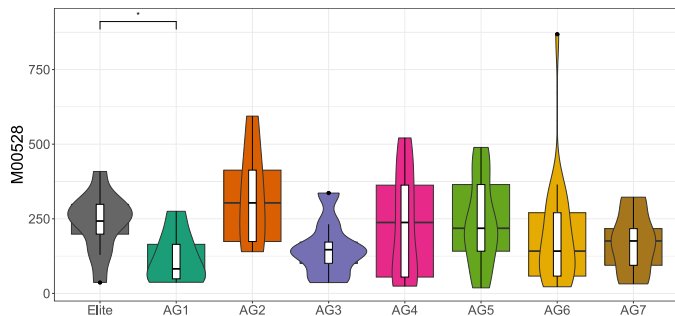
# Microbiome recruited by bread wheat landraces and modern cultivars:

## Differential networks

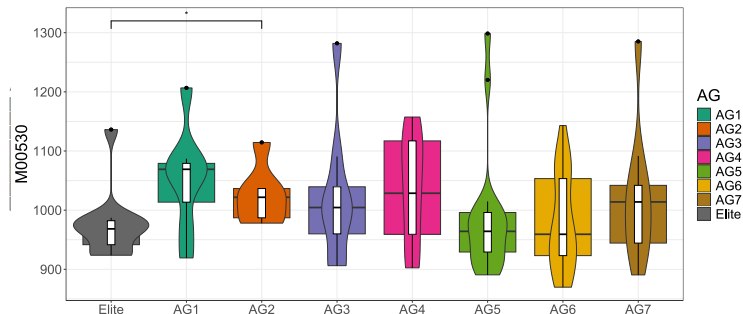


# Microbiome recruited by bread wheat landraces and modern cultivars:

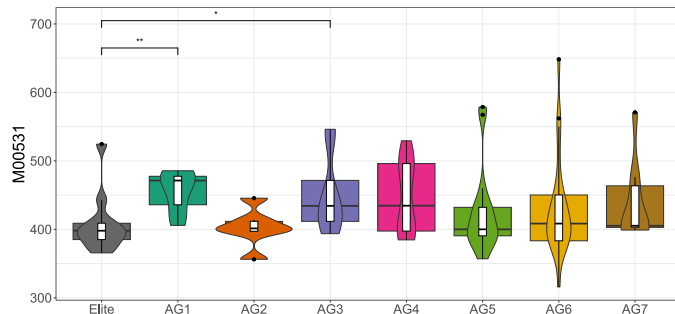
## Predicted functions



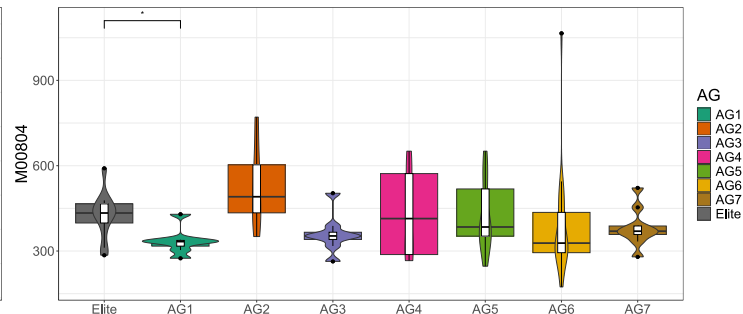
M00528 Nitrification, ammonia => nitrite



M00530 Dissimilatory nitrate reduction, nitrate => ammonia



M00531 Assimilatory nitrate reduction, nitrate => ammonia



M00804 Complete nitrification, comammox,  
ammonia => nitrite => nitrate




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## **Unearthing the rhizosphere microbiome recruited by ancestral bread wheat landraces**

 Maria C HERNANDEZ SORIANO,  Frederick James Warren,  Falk Hildebrand,  Luzie U Wingen,  
 Anthony J Miller,  Simon Griffiths

doi: <https://doi.org/10.1101/2025.05.08.652585>

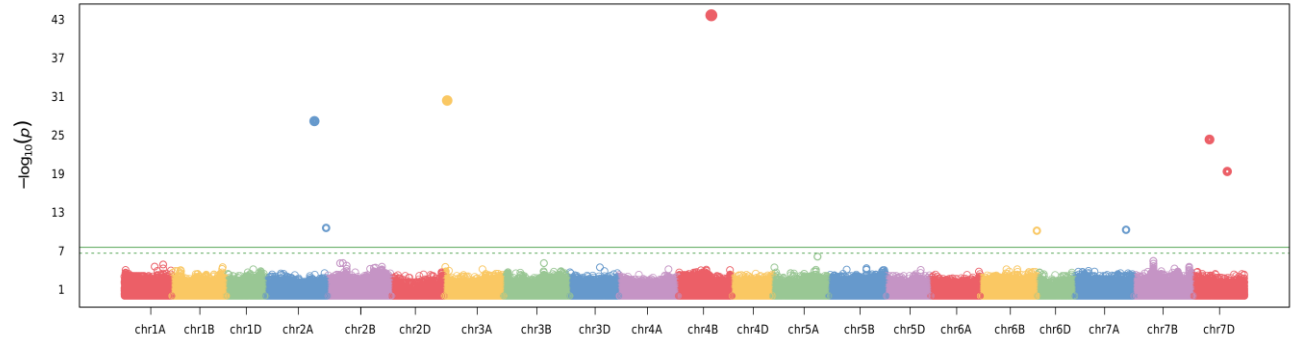
**GWAS** (Genome-wide association)

Identify genomic variants that are statistically associated with a particular trait.

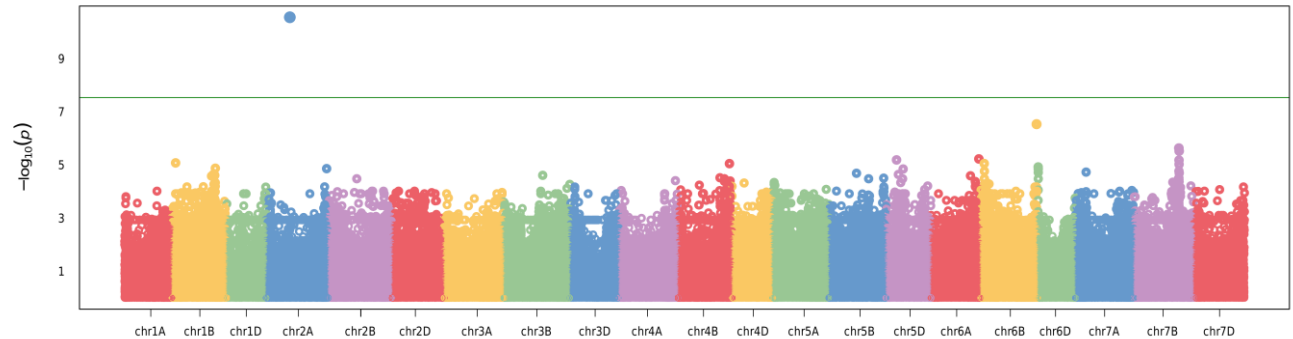


**Dr. Samer Amer**

### MLMM.Kribbella



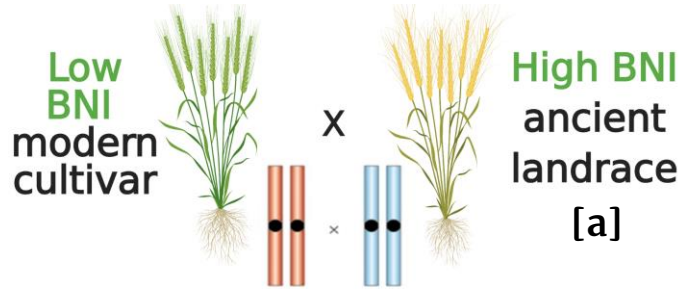
### MLMM.TA.21





## 2. Identification of QTL for BNI capacity to breed this trait into modern wheat varieties

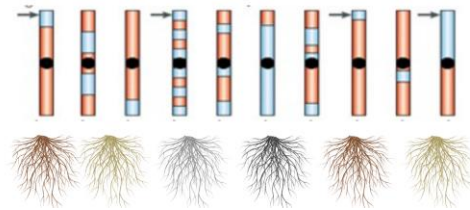
Parental lines differ genetically for the trait



QTL analysis links:

- phenotype for each lines
- genotype of markers that vary between parental lines
- Statistical techniques: probability that a marker/ interval between two markers is associated with a QTL affecting the trait

Quantitative trait loci (QTL) mapping:  
linking BNI activity - wheat genes [b]



Recombinant inbred lines (RIL) [b] of bread wheat:  
individuals contain different fractions of the genome of each  
parental line

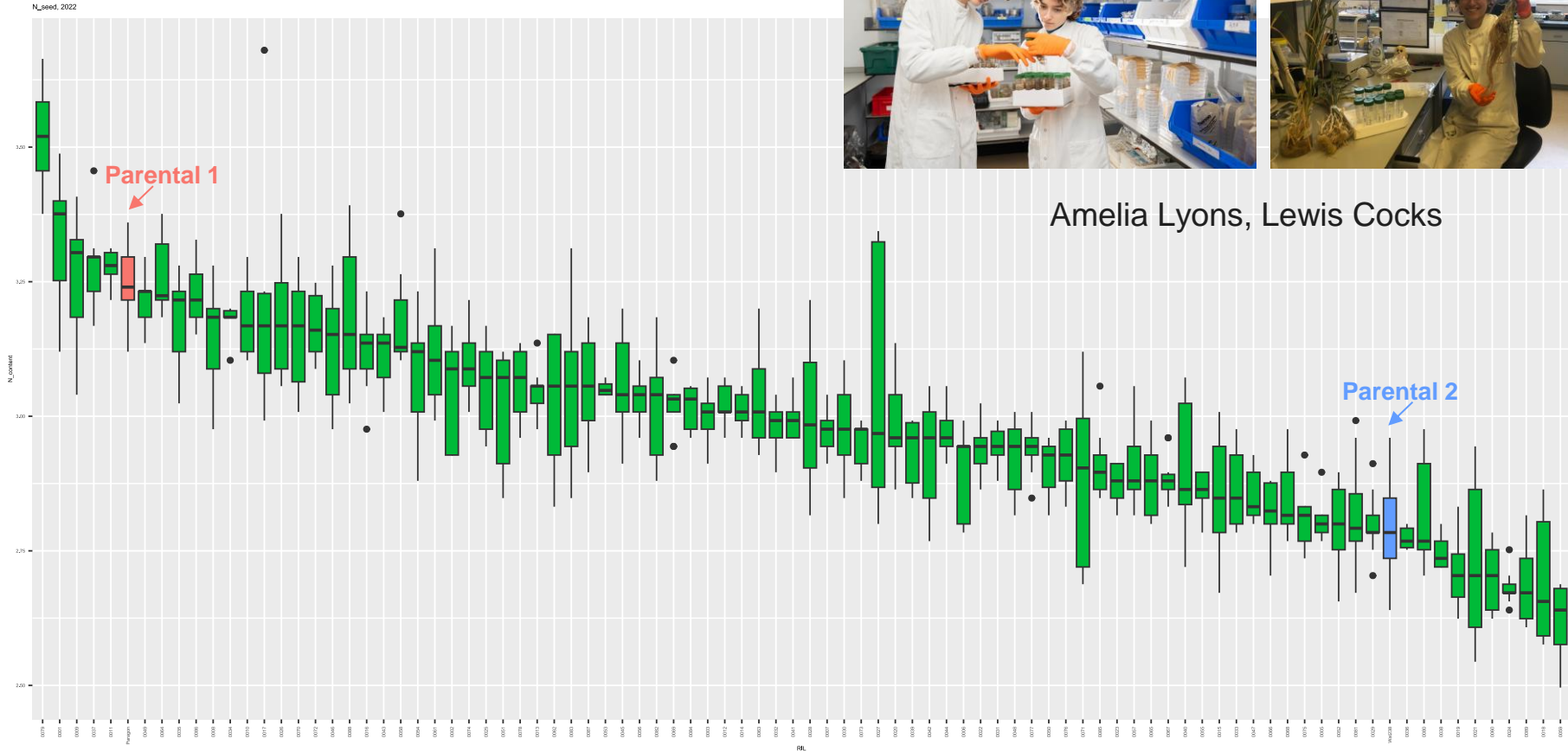


Luzie U. Wingen, JIC

[a] O'Sullivan et al. 2016 **Plant Soil** 404:61-74

[b] Wingen et al. 2017 **Genetics** 205(4):1657-1676

# Nitrogen content in seed across a RIL population



Amelia Lyons, Lewis Cocks

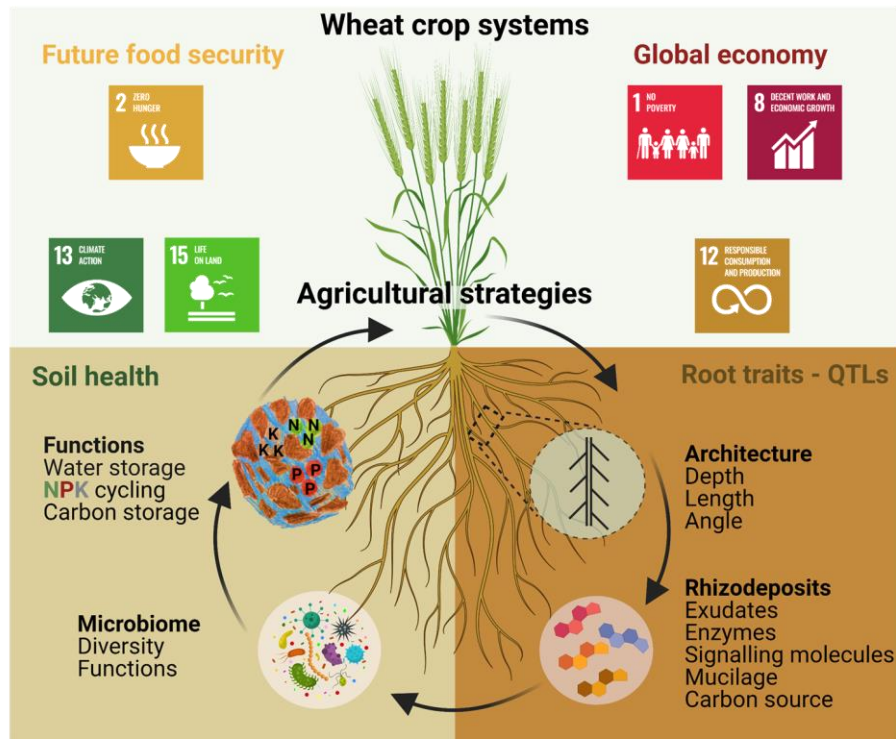
# WISH-ROOTS: **W**heat **I**mproving **S**oil **H**ealth through **ROOTS** traits

## Our objective:

To enhance the potential beneficial effects of wheat cultivation on soil health through the identification of **root traits** that can optimize **nitrogen (N) cycling** and improve **soil structure**.

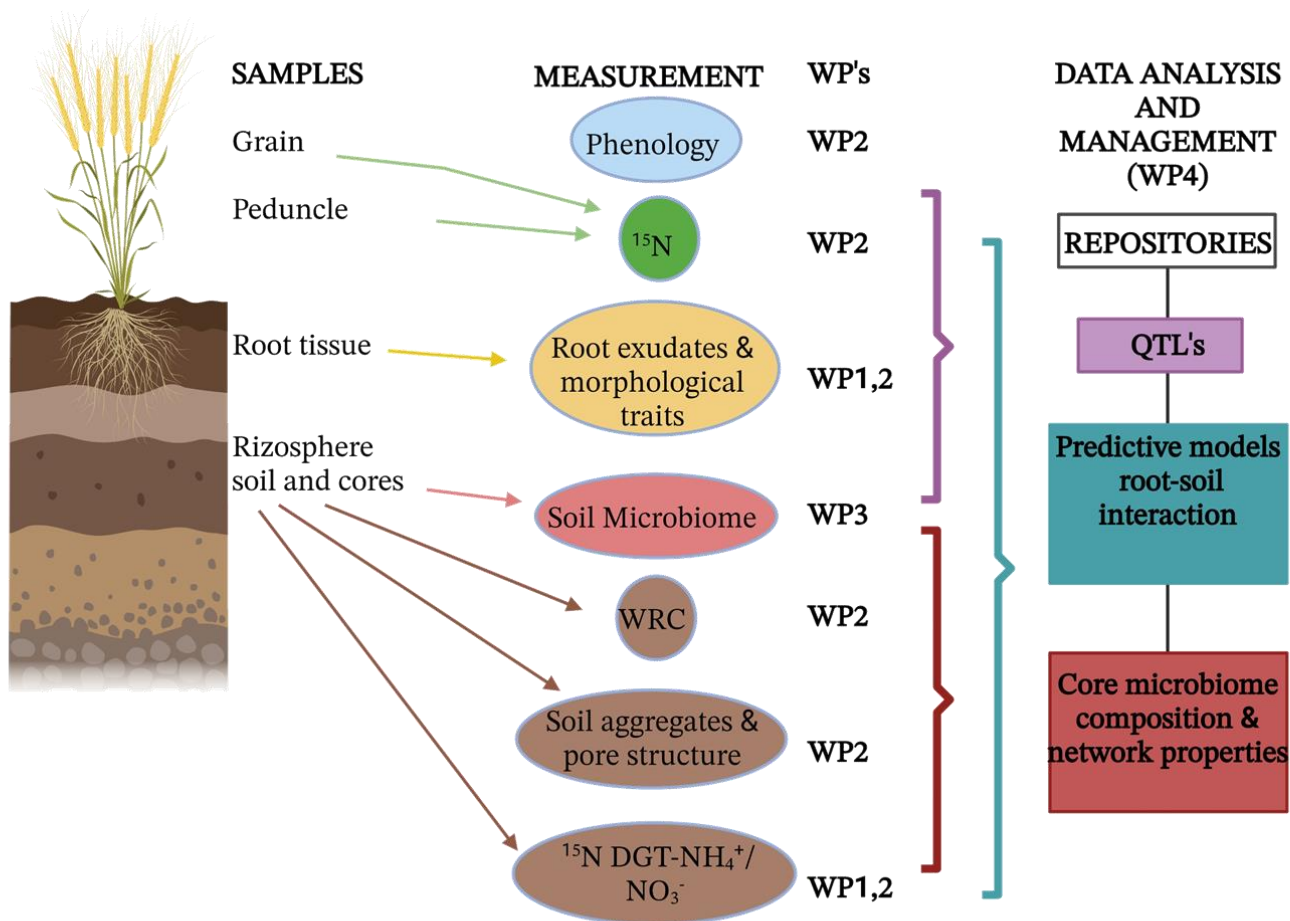


WISH-ROOTS consortium: plant genetics, soil science, microbial ecology



Making soil health a wheat breeding target.

EJP Soil WISH-ROOTS Consortium. Review in preparation.



# Linking genes for Root System Architecture (RSA) with soil physical properties

wide root angle, shallow roots



narrow root angle, deep roots



RSA

- root growth angle
- total root length
- total root surface
- average root length
- primary root surface
- primary root length
- primary root volume



- Carbon storage - removal of CO<sub>2</sub> from atmosphere
- Soil structure - decrease erosion, improve water retention
- Feeding/attracting beneficial organisms

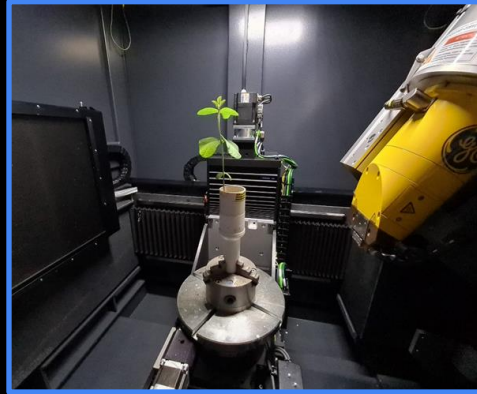






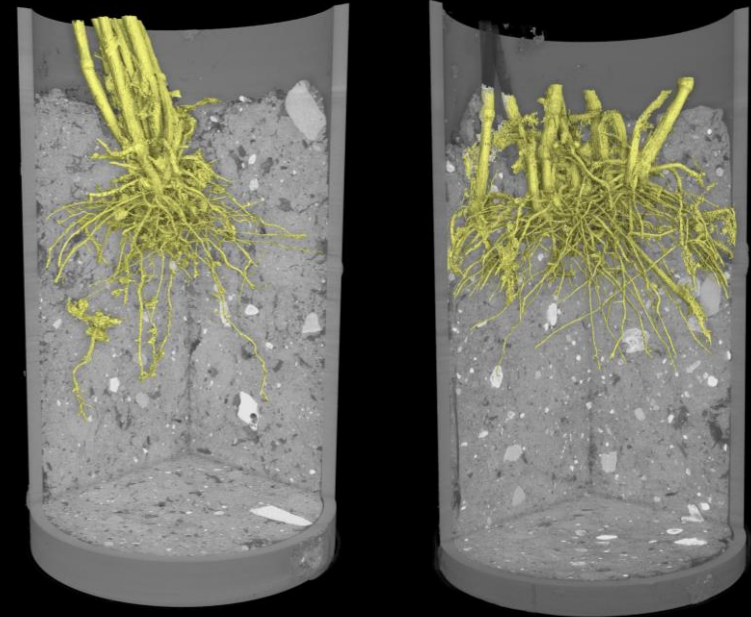
# X-ray Computed Tomography

**Hounsfield Facility**  
3D X-ray imaging

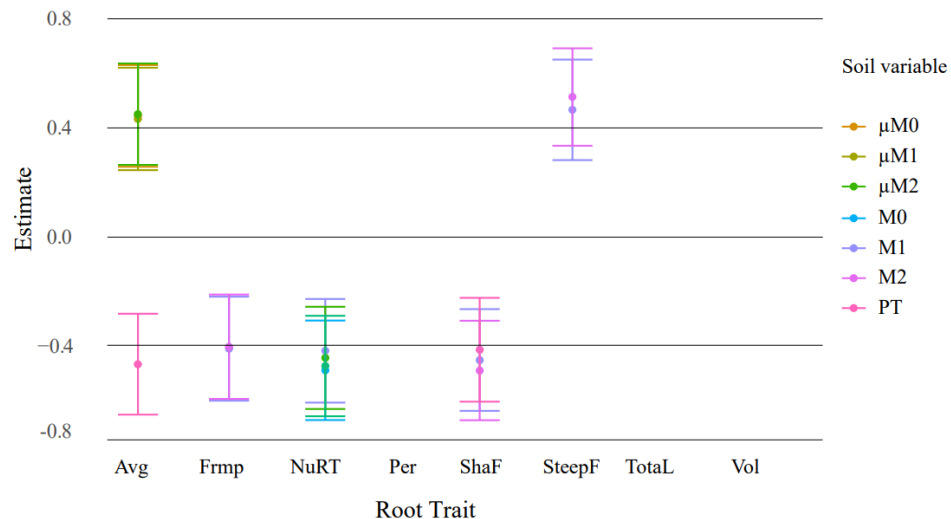
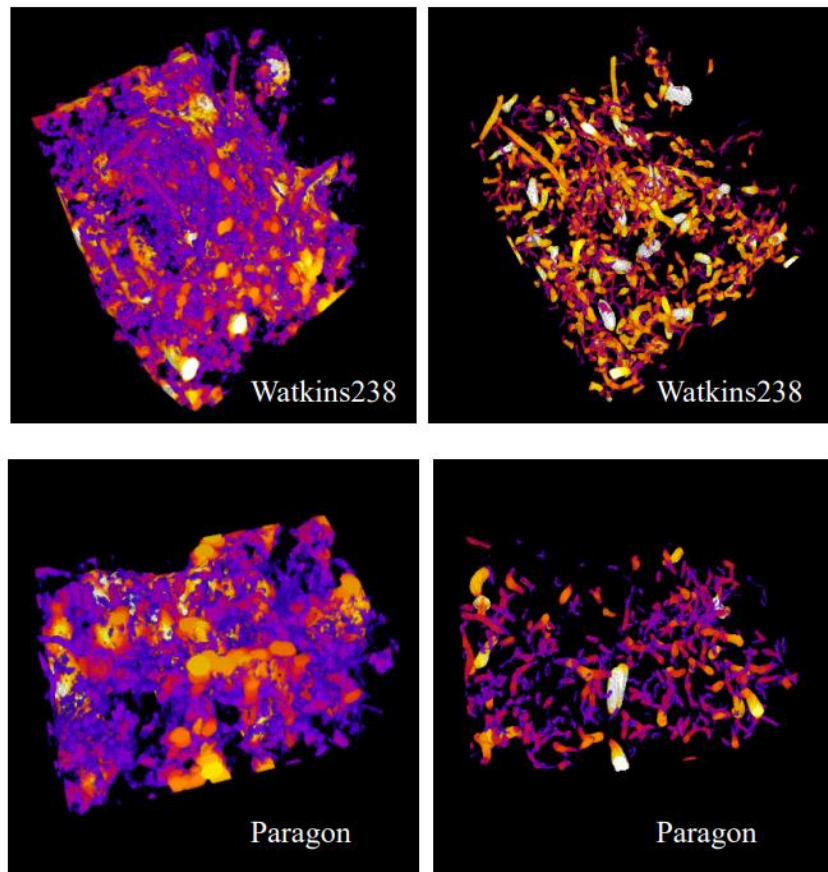


A non-invasive technique for visualizing & quantifying the interior of a solid object in 3D.

- **Soil porosity**
- **Total volume of soil pores**
- **Pore thickness**
- **Connectivity & complexity of the pore network**

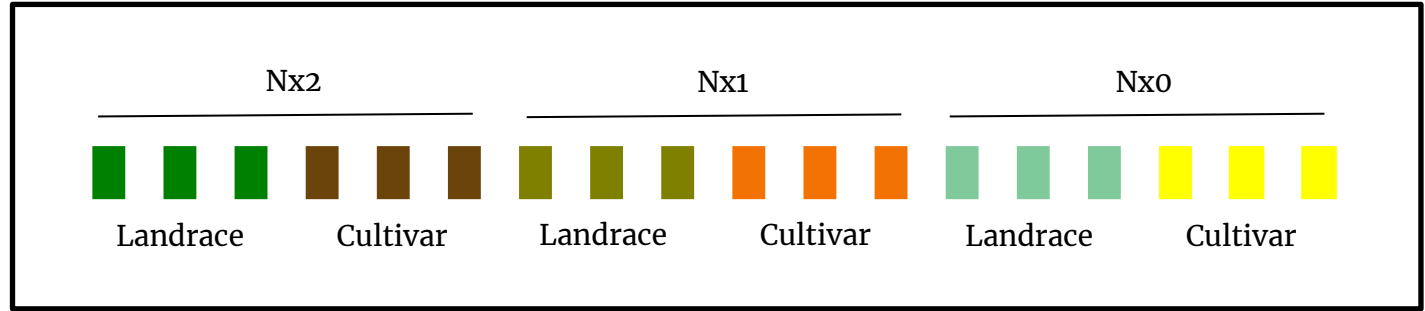


# Root traits of different wheat cultivars influence soil structure: an X-ray computed tomography and root morphology study

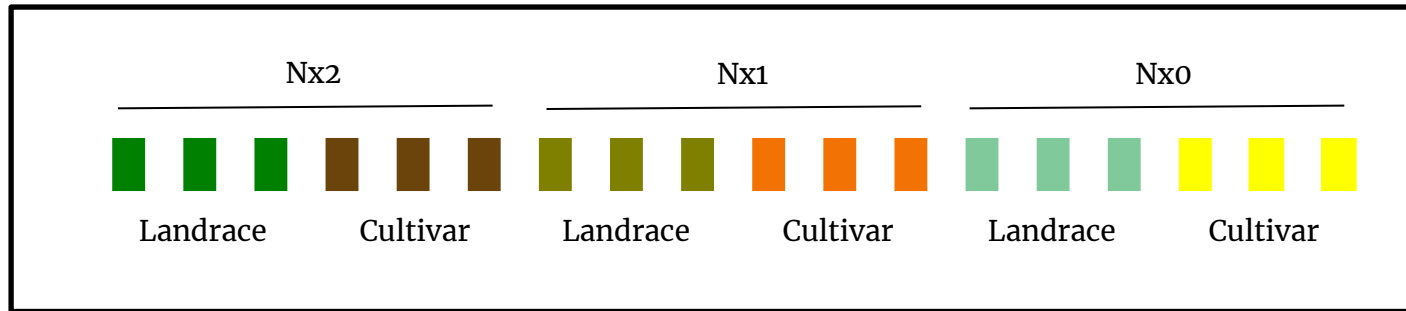


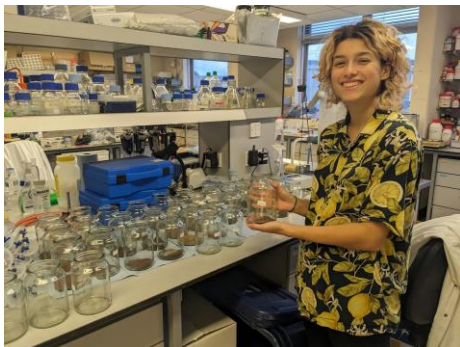


## Ongoing field trails



- 3 regimes of N-fertilizer application: 0/48/96 kg N/ha
- 2 wheat cultivars – contrasting BNI capacity

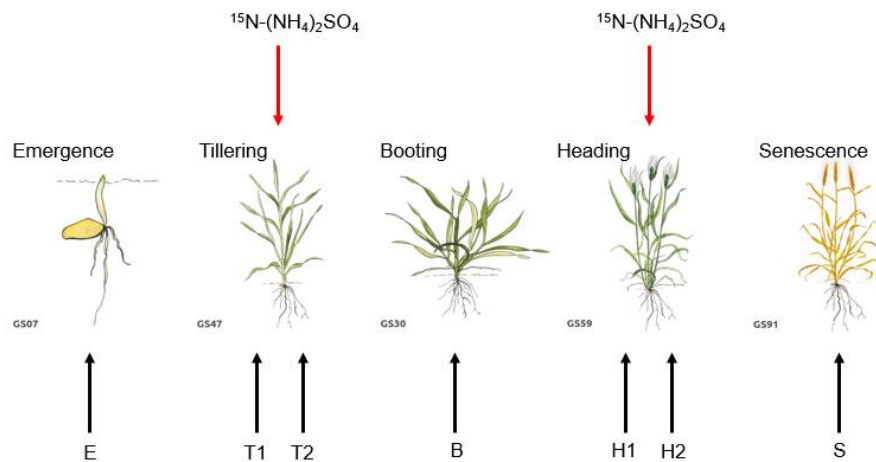




**Amelia Lyons**

### Field trial:

- 8 wheat genotypes – contrasting BNI capacity
- Sampling at key developmental stages



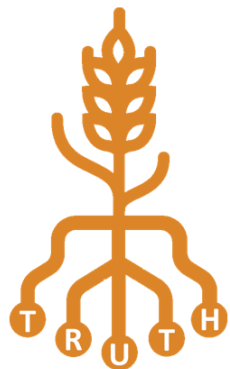
## Learning objectives:

- 🎯 Root and rhizosphere traits - plant-microbe interactions ✓
- 🎯 Root traits influence microbial communities, nitrogen cycling, and overall soil health ✓
- 🎯 **WISH-ROOTS: Wheat Improving Soil Health - ROOT traits.** ✓
- 🎯 Potential applications in sustainable agriculture.



Farming Innovation Programme

<https://bofin.org.uk/truthproject/>



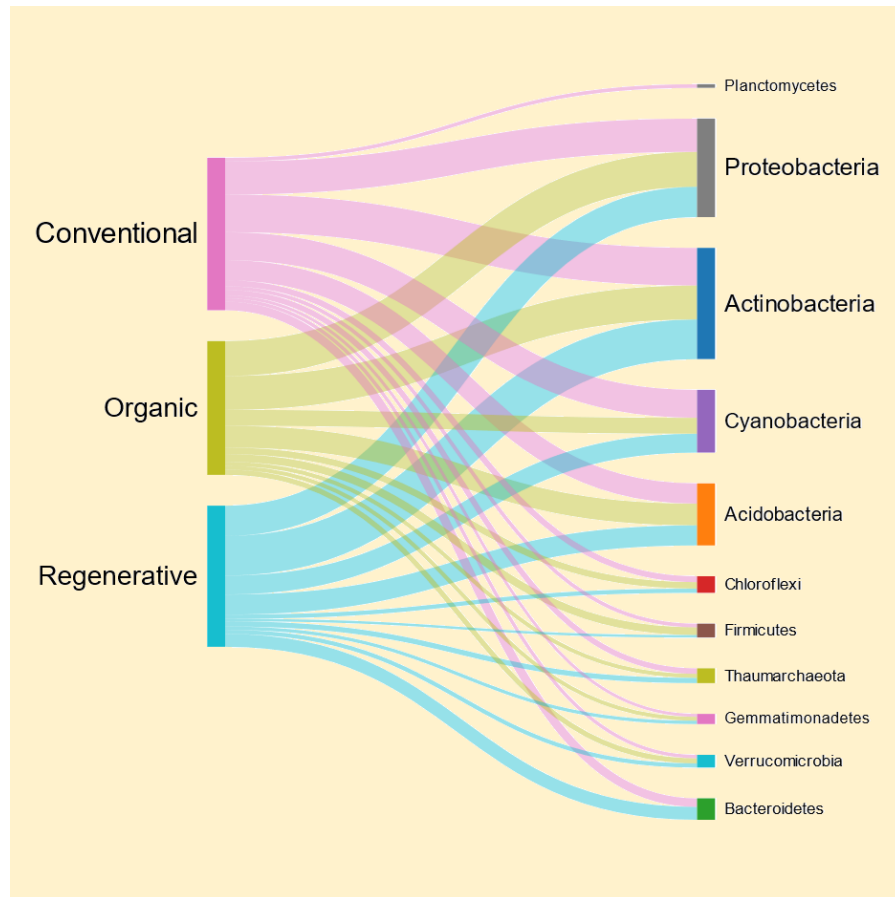
# **TRUTH: Thriving Roots underpinning Total Soil Health**



# Microbiome recruitment and land management

Fingerprint of the microbiome composition at phyla resolution across contrasting land management.

Soils under conventional farming:  
Planctomycetes were more prevalent, while  
Bacteroidetes were significantly reduced.



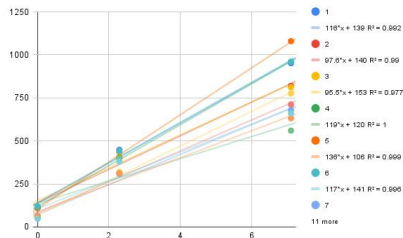
# Potential? nitrification rates



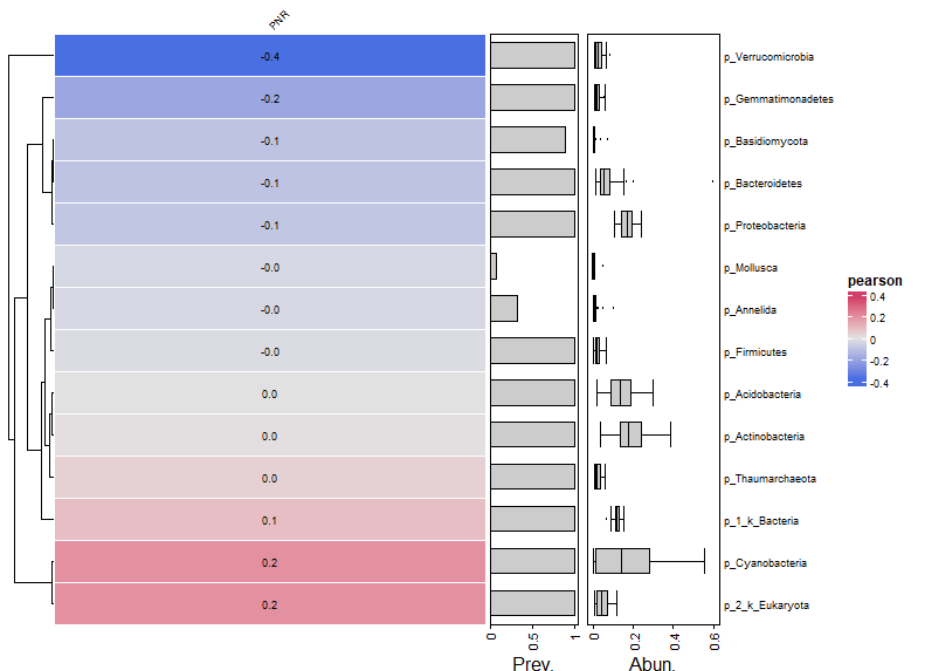
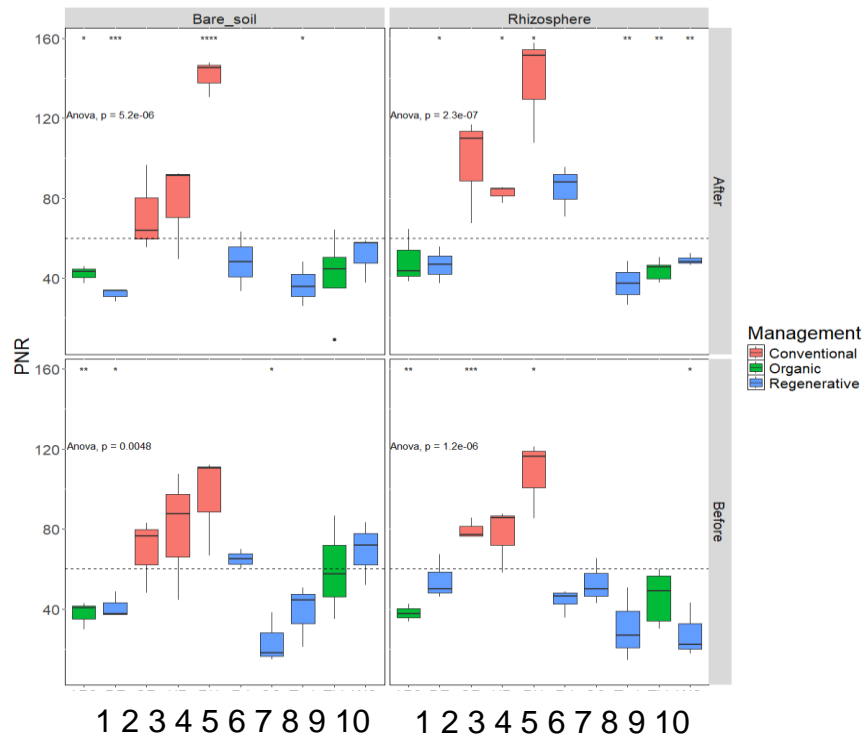
Soil from wheat rhizosphere added with  $(\text{NH}_4)_2\text{SO}_4$



$\text{NO}_3^-$  concentration measured at 0, 3 and 7 days using a nitrate-selective electrode



Higher slopes indicate faster conversion of ammonia into nitrate by soil microorganisms



*Characterization of a novel biostimulant  
targeting crop root system and soil microbiome  
through WISH-ROOTS methodological approach  
and field trials.*

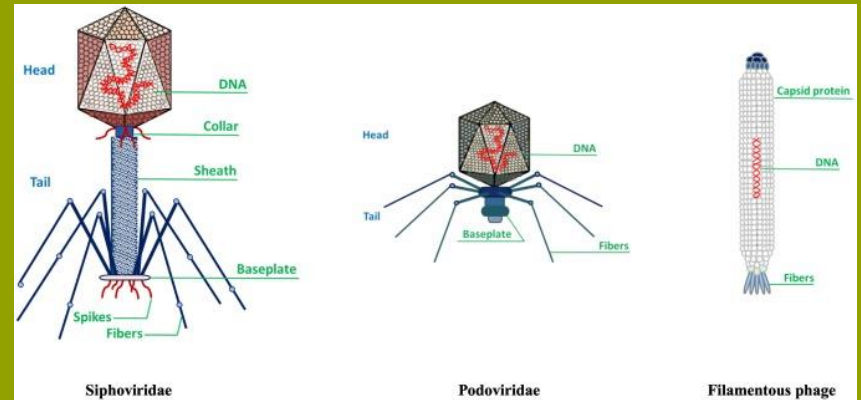


# BIOSTImax

- Biostimulants currently available in the market are either based on natural chemical compounds (such as humic acids) or single strains of microorganisms.
- *BIOSTImax* contains a mixture of humic acids and bacteriophages (phages) that can thrive within the soil microbiome networks and become established, with reported long-term capacity to modulate a wide range of soil microbiome functions.



Phages are viruses that infect and replicate within host bacteria and archaea



Mao Y. et al. *Environment international* 129 (2019): 488-496.



## Take home messages:

- **Root exudates** released in the rhizosphere are a **complex mixture** of metabolites
- The release of root exudates is a trait **linked to a wide range of plant functions** through enrichment and depletion of bacterial (also archaea, fungi) communities
  - plant defence against abiotic and biotic stresses
  - nutrient acquisition
- Release of root exudates **pairs with other root traits** to support plant and soil functions
- **Analysis of rhizobiome composition and functionality** is an emerging approach to **phenotype** traits linked to root exudates and microbiome recruitment.



## Take home messages:

- Wheat **roots can control nitrification** through the release of biological nitrification inhibitors in the rhizosphere
- The **trait** has only been found for a group of landraces - **lost in elite varieties**
- Analysis of the **rhizobiome composition** and **functionality** has allowed **phenotyping** the BNI trait in wheat
- Analysis of the **rhizobiome composition combined with functional assays** is an approach to phenotype traits linked to root exudates
- **Root architectural traits** can be identified with **imaging** techniques
- **Selection of breeding methods** needs to consider the **potential loss** of beneficial traits.



# Acknowledgements

## WISH-ROOTS consortium John Innes Centre

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- Simon Griffiths
- David Laurie
- Charlie Philp
- James Laurie
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- Acacia Simpson
- Amelia Lyons
- Lewis Cocks

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- Falk Hildebrand

## The James Hutton Institute

- Tim George

## The University of Manchester

- Henry Birt
- David Johnson

## Biome Makers Inc.

- Alberto Acedo
- Irene Baena



Fields4 Ever Action -Horizon 2020  
Grant agreement No 947084



Daphne Jackson Trust



Unlocking Nature's Diversity

Biotechnology and Biological Sciences Research Council



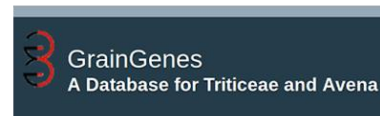
oloBion

EJP SOIL - Horizon 2020 Grant No. 862695  
BBSRC Grant No. BB/X003000/1

BIOME MAKERS



<https://www.seedstor.ac.uk>  
<https://designingfuturewheat.org.uk/>

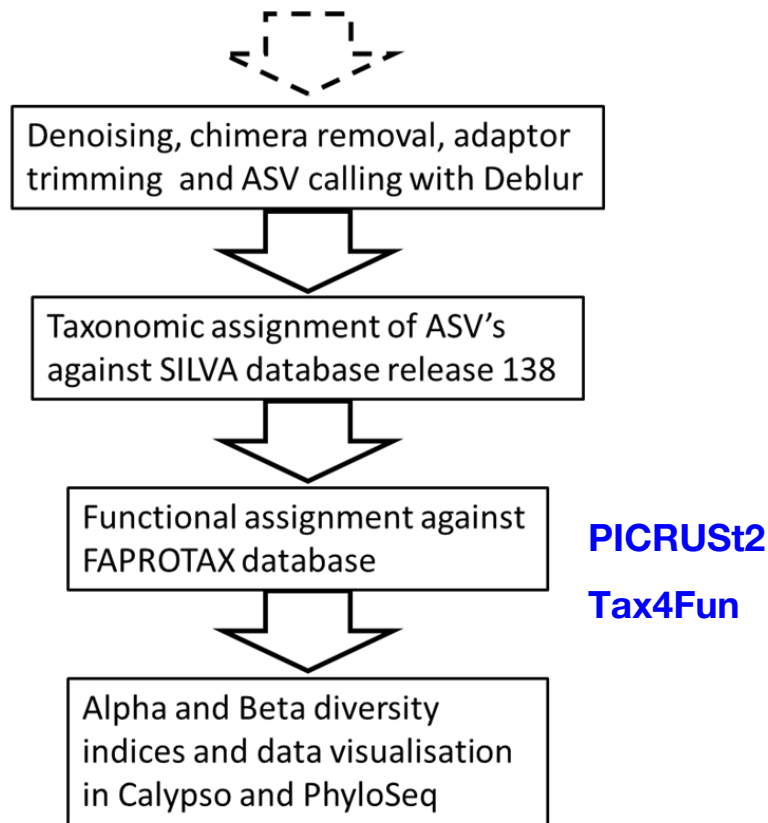
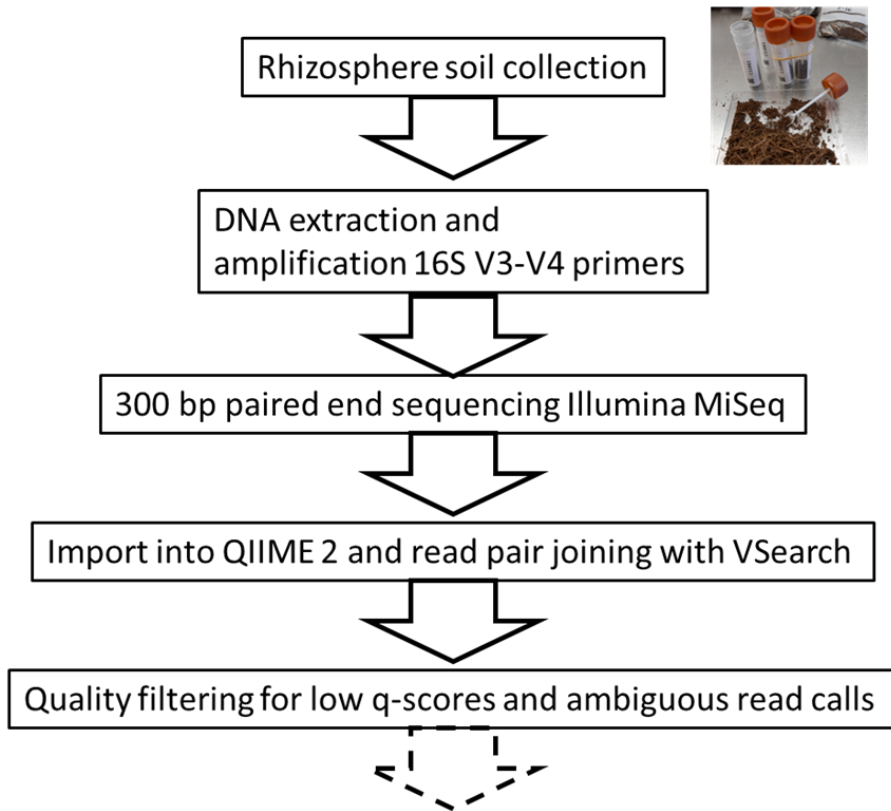


[https://wheat.pw.usda.gov/GG3/global\\_durum\\_genomic\\_resources](https://wheat.pw.usda.gov/GG3/global_durum_genomic_resources)



# How to identify rhizosphere microorganisms: bacteria and archaea

## 16S sequencing and bioinformatic work flow



## Soil microbiome - metagenomics.

16S rRNA sequencing focuses on a specific gene (16S rRNA) for taxonomic profiling, while shotgun metagenomics sequences all DNA in a sample.



Soil from wheat rhizosphere, margins and bare soil collected by Root Rangers



DNA extraction (JIC), library preparation (QIB) and metagenome sequencing (Novogene)

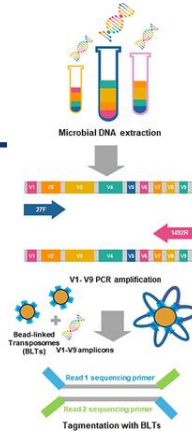


Dave Baker



**Novogene**

Advancing Genomics, Improving Life



Microbiome taxonomy and functions annotation



Falk Hildebrand

<https://github.com/hildebra/MG-TK>



Data analysis

- Taxonomic and functional profiles
- Microbiome composition, diversity and similarity of functional profiles
- Variation and differential abundance



**John Innes Centre**  
Unlocking Nature's Diversity

Maria Hernandez-Soriano

## Library Preparation - purpose

- Preparing DNA/RNA for Sequencing:**

DNA or RNA needs to be converted into a form compatible with the sequencing platform.

- Creating a Library of Fragments:**

Breaking down the DNA or RNA into smaller, manageable fragments of a specific size.

- Attaching Adapters and Indexes:**

Adapters, which are short DNA sequences, are attached to the ends of the fragments. These adapters contain sequences that allow the fragments to be amplified and sequenced on the chosen platform. Indexes (also called barcodes) are used to identify different samples during sequencing.

## Key Steps

- Nucleic Acid Isolation:** isolation of the DNA or RNA from the sample.

- Fragmentation:** The DNA or RNA is then fragmented into smaller pieces, often using enzymatic or mechanical methods.

- End Repair:** The ends of the fragments are repaired to ensure that they are suitable for adapter ligation.

- Adapter Ligation:** Adapters are attached to the ends of the DNA or RNA fragments.

- Amplification (Optional):** e.g. PCR amplification step to increase the amount of DNA fragments.

- Library Quantification:** to ensure that there is enough DNA for sequencing.

- Library Quality Control:** to ensure that the library is of high quality and suitable for sequencing.

## From raw reads to taxonomic and functional abundance matrices

### **MATAFILER** (now MG-TK)

Pipeline to process metagenomic data from raw reads to taxonomic and functional abundance matrices.

MG-TK offers assembly-dependent (*gene catalog*) and assembly-independent (*direct mapping to appropriate databases*) workflows.

- Assemble metagenomes, profile miTags, profile functions, profile taxonomy using a variety of approaches (MATAFILER.pl)
- Build a gene catalog based on these assemblies and predicted genes, build abundance matrices from these and annotate the genes functionally (geneCat.pl)

<https://github.com/hildebra/mg-tk/>  
<https://zenodo.org/records/5831723>

## Exploring your data:

Tools for microbiome analysis  
R packages

```
library(readxl)  
library(tidyverse)  
library(ggpubr)  
library(tibble)  
library(dplyr)  
library(ggplot2)  
library(patchwork)
```

```
library(phyloseq)  
library(phylosmith)  
library(vegan)  
library(microViz)  
library(micro4all)  
library(Maaslin2)  
library(NetCoMi)  
library(microbiome)  
library(Microbiomest)
```

