‘A walk on the wild side’ the genetics of host-microbiota interactions in the barley rhizosphere

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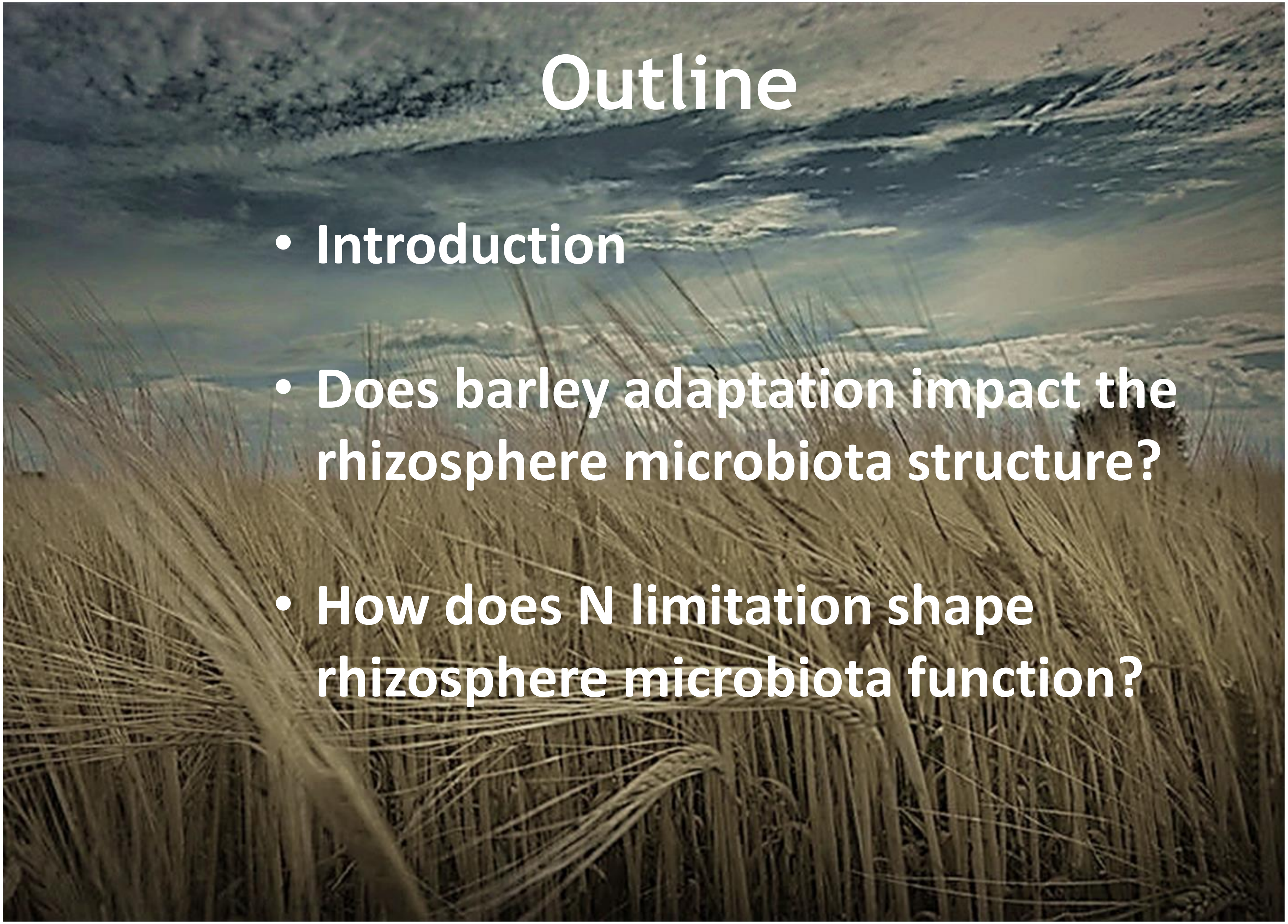
Phytobiomes Webminar March 2023

The James Hutton Institute

University of Dundee



Outline

- Introduction
- Does barley adaptation impact the rhizosphere microbiota structure?
- How does N limitation shape rhizosphere microbiota function?

Why is this important?

“Soil is where food begins! It is estimated that 95% of our food is directly or indirectly produced on our soils”.

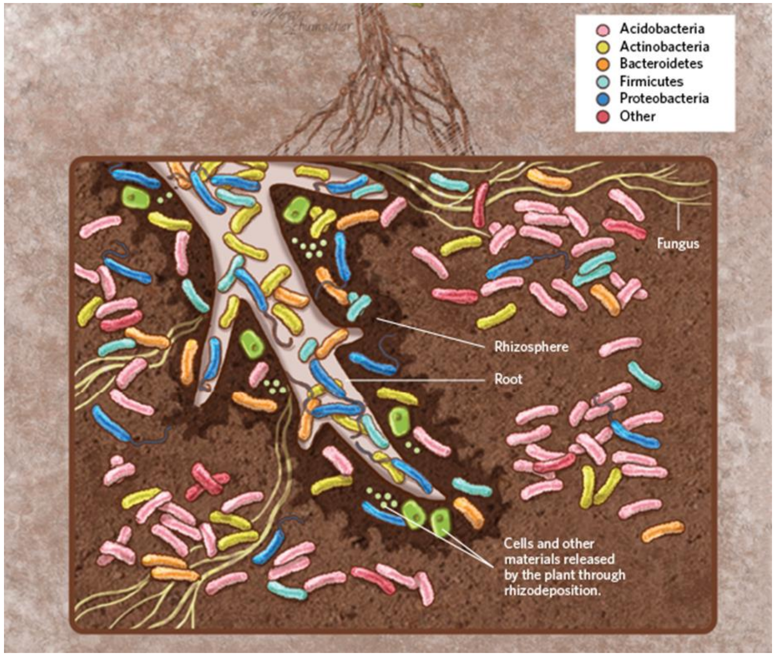
“A single gram of healthy soil contains Millions of organisms, **the microbiota**, capable of interacting with plant roots”

Novel insights into plant-microbiota interactions: unlocking the full potential of crop production

fao.org/soils-2015

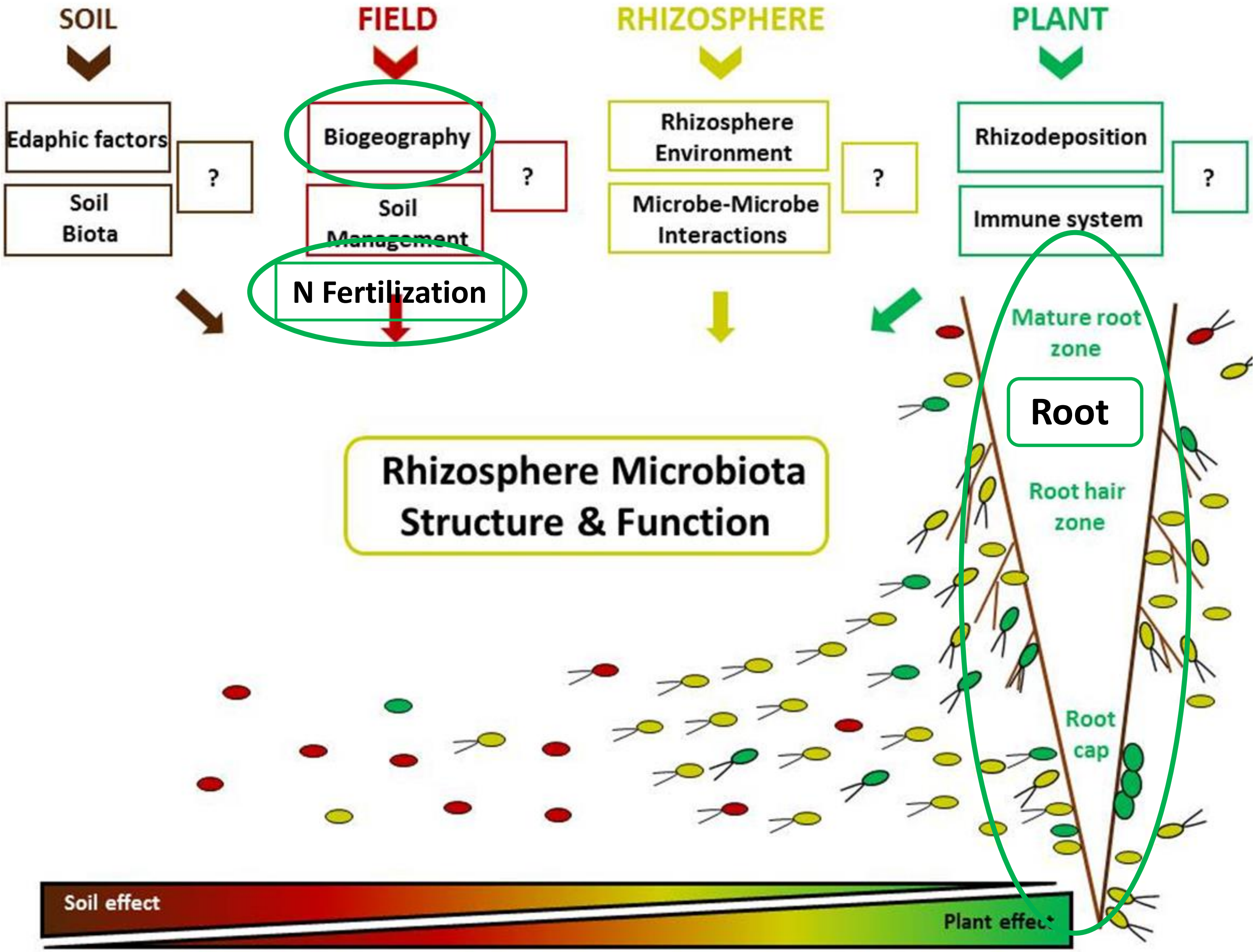
The rhizosphere microbiota: an “extended organ” of the barley plant

- Root-soil interface, plant nutrient uptake
- Rhizosphere effect
- Rhizosphere Microbiota
- Resource of plant probiotic functions
- Deployment in agriculture



Bulgarelli, 2018, The Scientist

Determinants of the microbiota at the root/soil interface



Alegria Terrazas et al., (2016) Adv Appl Microbiol. 95:1

Barley as a model crop to study host-microbiota interactions

Global Crop: world 4th most cultivated cereal

Large germplasm collections:
    Accessions, mutants, wild relatives

Diploid genome

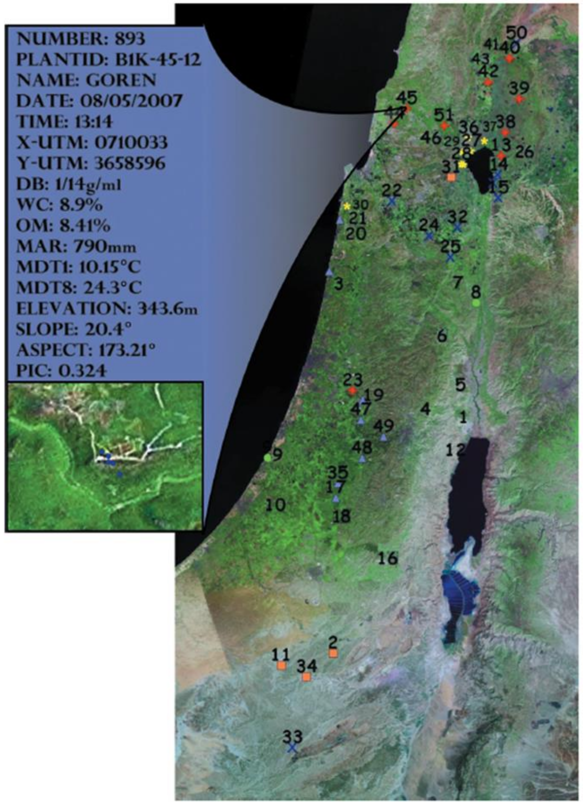
Pan-genome

Gene editing

Speed breeding

Wild barley as a genetic resource

- Wild barley adapted to marginal areas
- Adaptive niche of modern barley
- Genetically more diverse
- Interbreed with modern

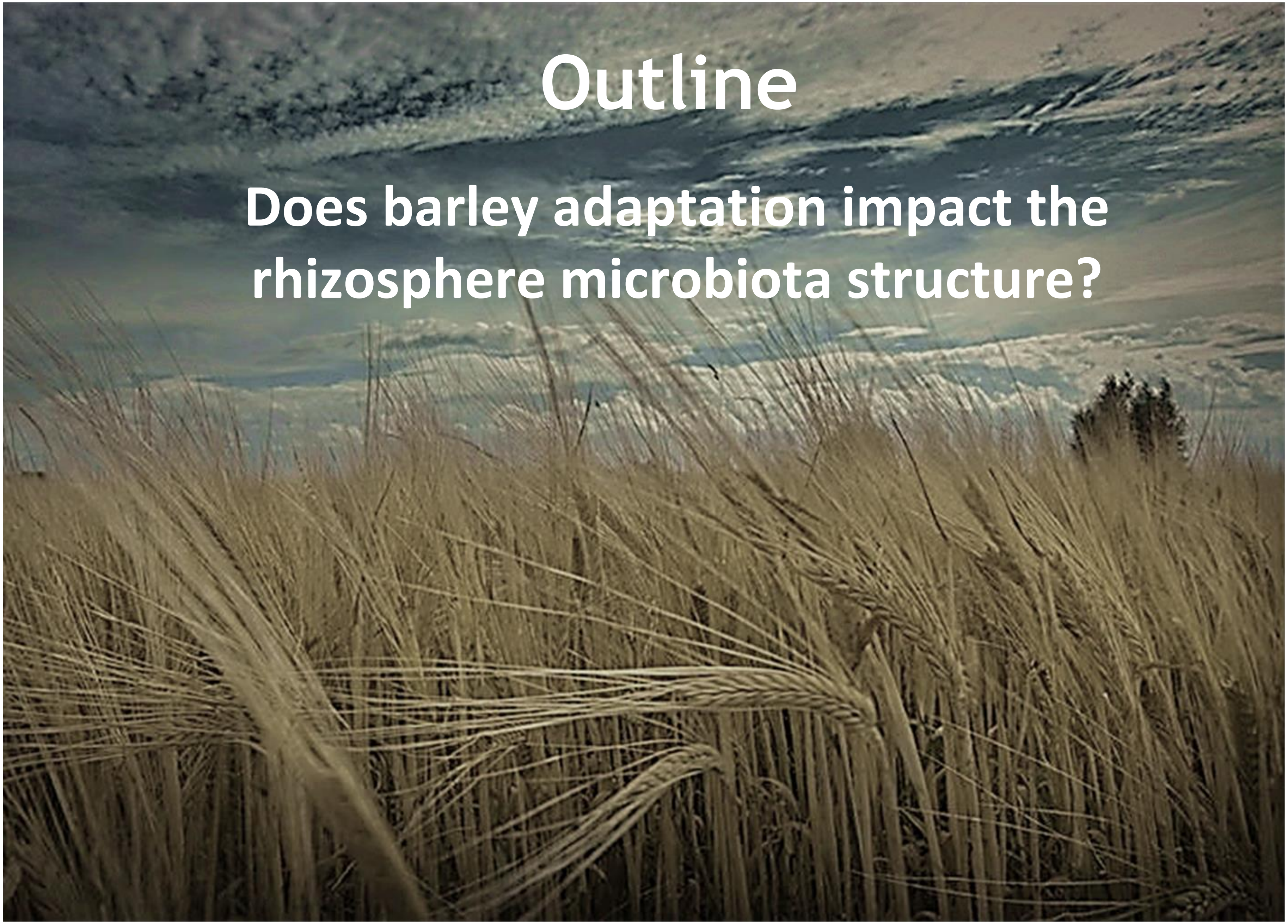


NUMBER: 893
PLANTID: B1K-45-12
NAME: GOREN
DATE: 08/05/2007
TIME: 13:14
X-UTM: 0710033
Y-UTM: 3658596
DB: 1/14g/ml
WC: 8.9%
OM: 8.41%
MAR: 790mm
MDT1: 10.15°C
MDT8: 24.3°C
ELEVATION: 343.6m
SLOPE: 20.4°
ASPECT: 173.21°
PIC: 0.324

Hordeum vulgare* ssp. *spontaneum

H. vulgare* ssp. *vulgare

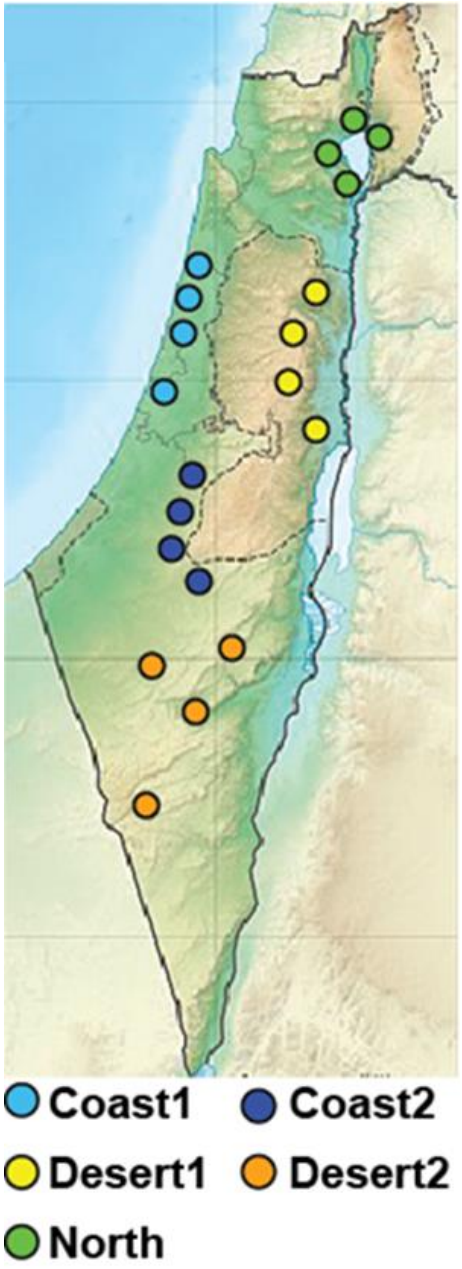
Hubner *et al.*, (2009) Mol Ecol: 18



Outline

Does barley adaptation impact the rhizosphere microbiota structure?

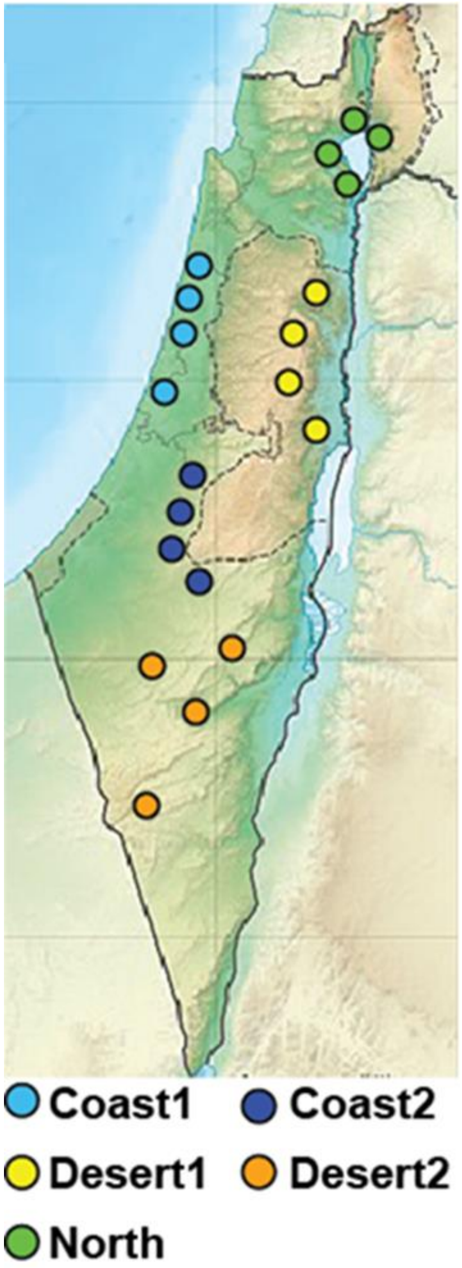
Barley Germplasm

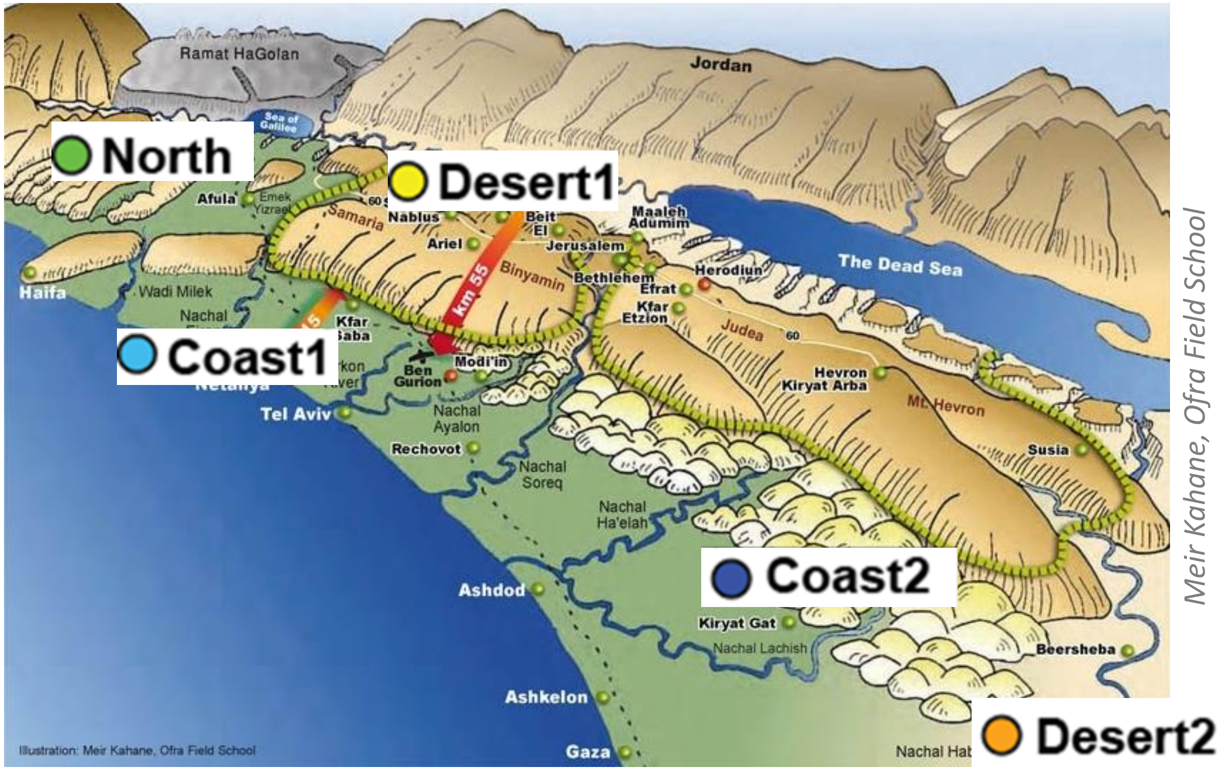


- Wild barley collection, B1K
- Seed sampled in natural location
- Three ecotypes: **North, Coast and Desert**
- Clustered according to genetic and phenotypic traits
- Elite cultivars: Barke, Bowman, Morex and Steptoe

Modified from Hübner et al. (2009) Mol Eco, 18

Barley Germplasm Eco-geography





Meir Kahane, Ofra Field School

Did eco-geographic factors leave a footprint in the barley genome?

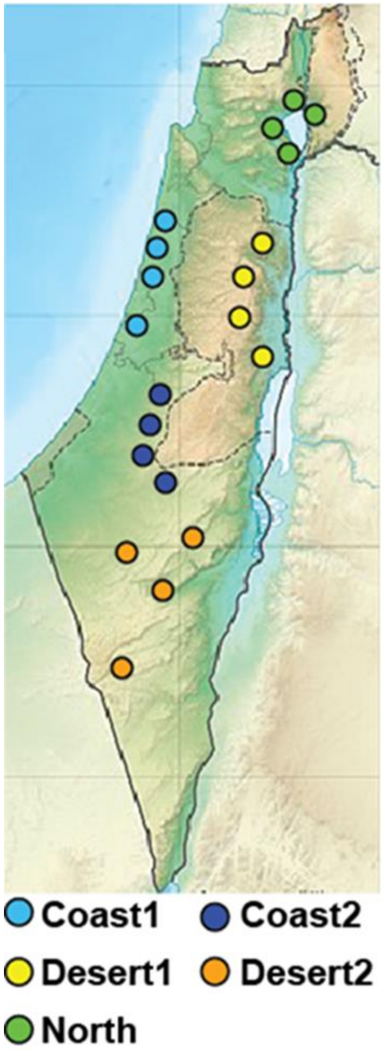
Is the barley genome related to the microbiota structure ?

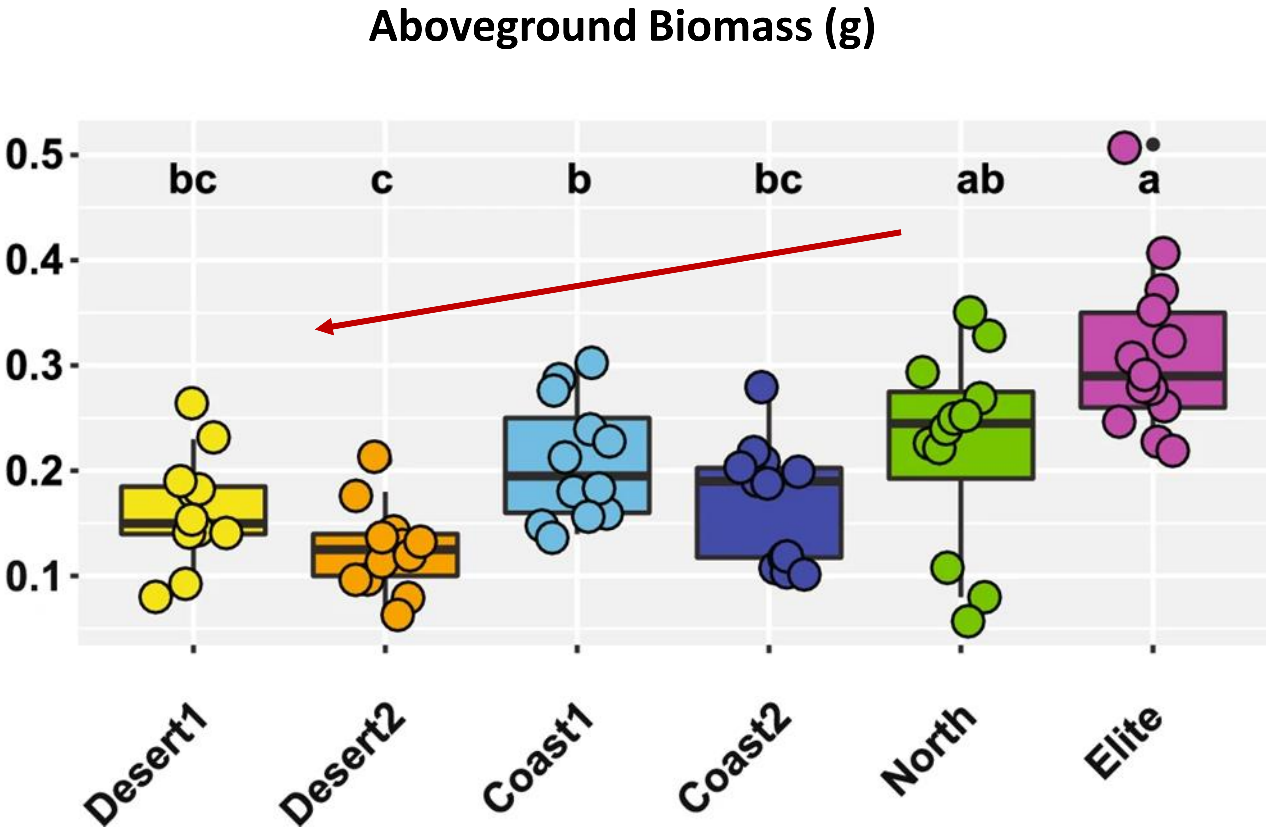
Adapted from Hubner et al., (2009) Mol Ecol: 18

Greenhouse Growth

- Scottish farm soils
- Early stem elongation
- Rhizosphere sampling
- Biomass

Elite varieties outperform wild ecotypes in aboveground biomass…

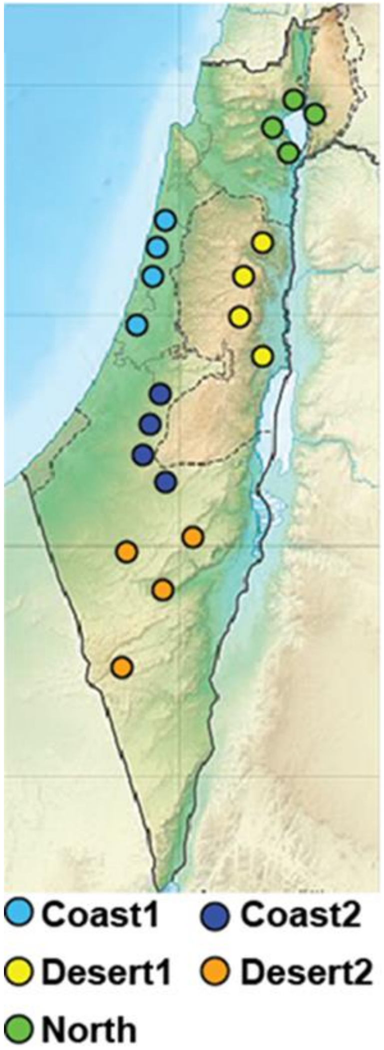




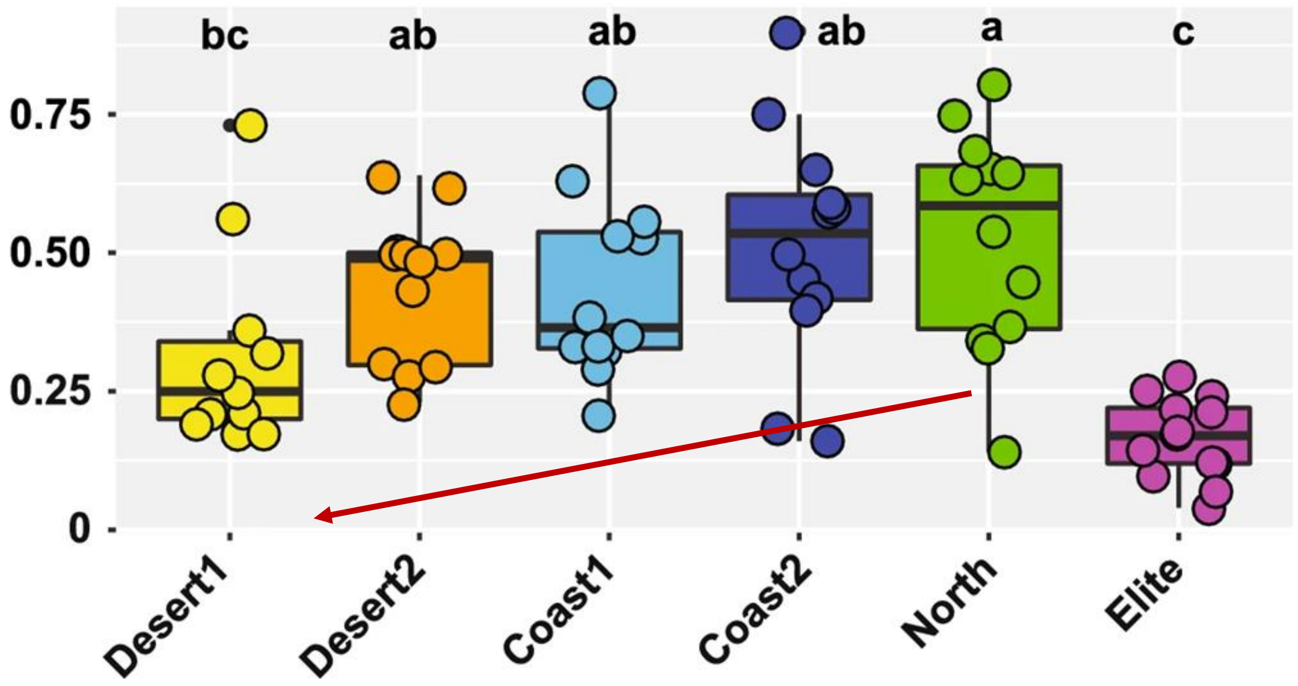
Kruskal–Wallis followed by Dunn's post hoc test.

Alegria Terrazas et al. Sci Rep 10, 12916 (2020)

…while wild ecotypes outperform elite varieties "belowground"



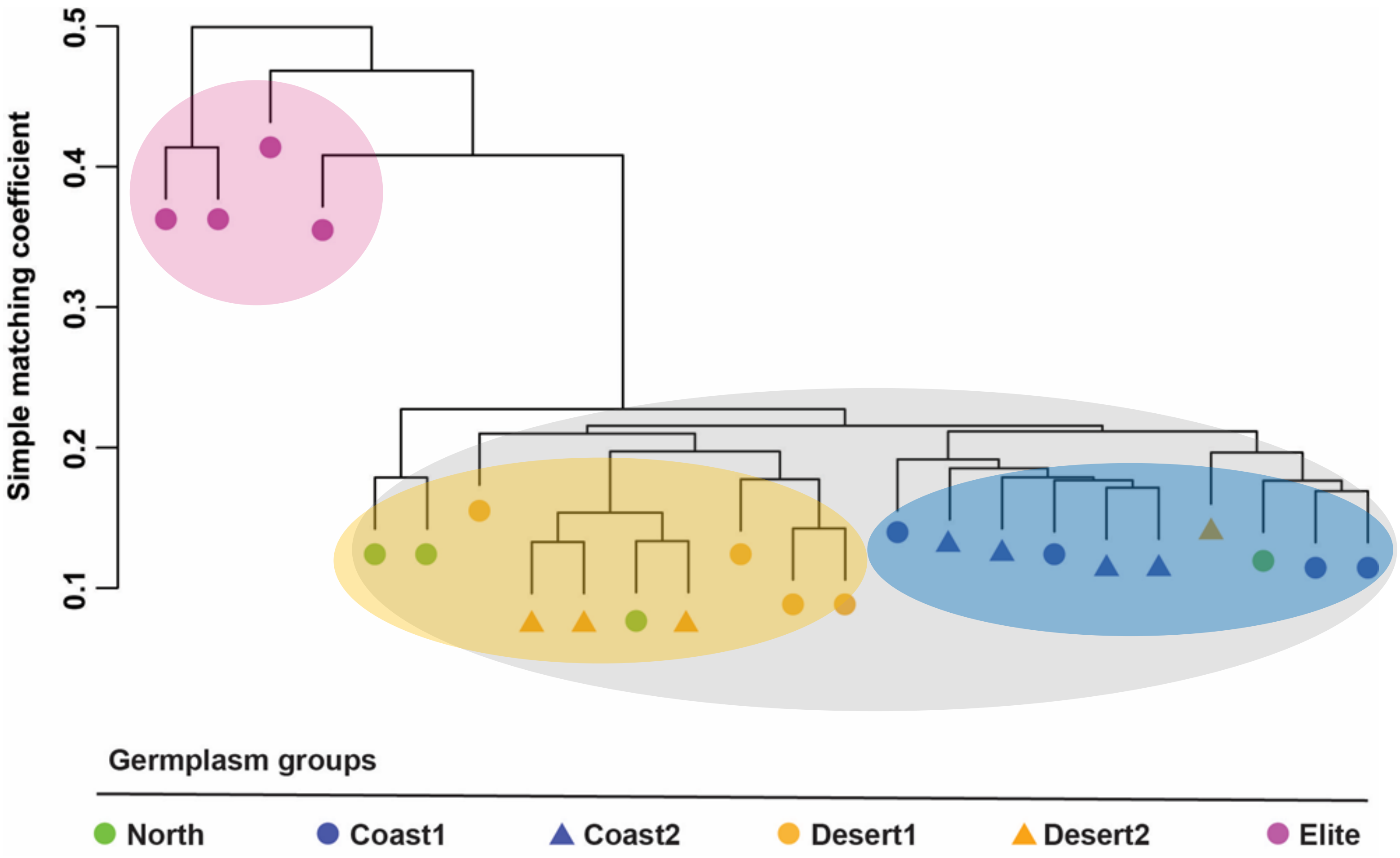
Root to Shoot Ratio (root DW/shoot DW)



Kruskal–Wallis followed by Dunn's post hoc test.

Alegria Terrazas et al. Sci Rep 10, 12916 (2020)

A footprint of eco-geographic adaptation on the barley genome



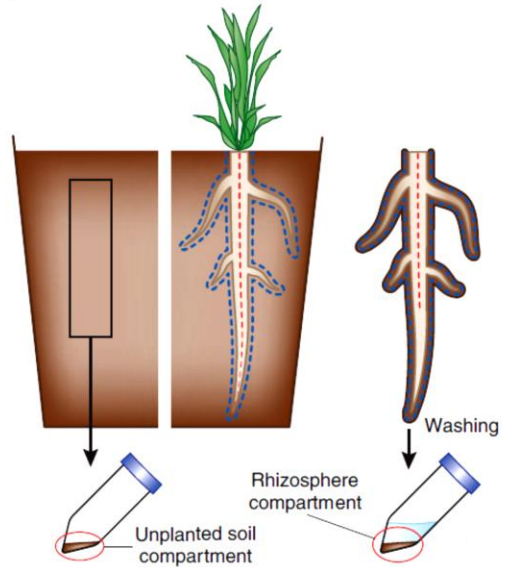
Hierarchical clustering of genetic distance by simple matching from SNPs of BOPA1 platform

Alegria Terrazas et al. Sci Rep 10, 12916 (2020)

16S rRNA Amplicon sequencing: who is there?

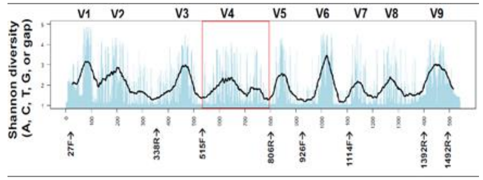
Hirsh & Mauchline (2012) Nat Biotech

Rhizosphere extraction



DNA extraction

16S rRNA gene libraries



Sequencing

illumina®

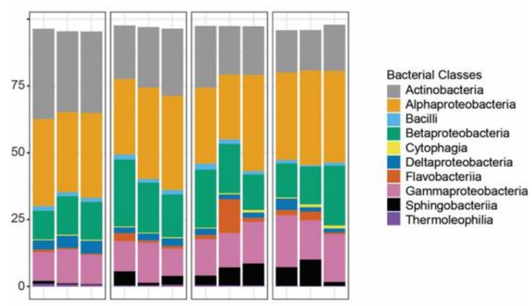
Data Analysis

Quantitative Insights

qiime

R

Taxonomic Composition



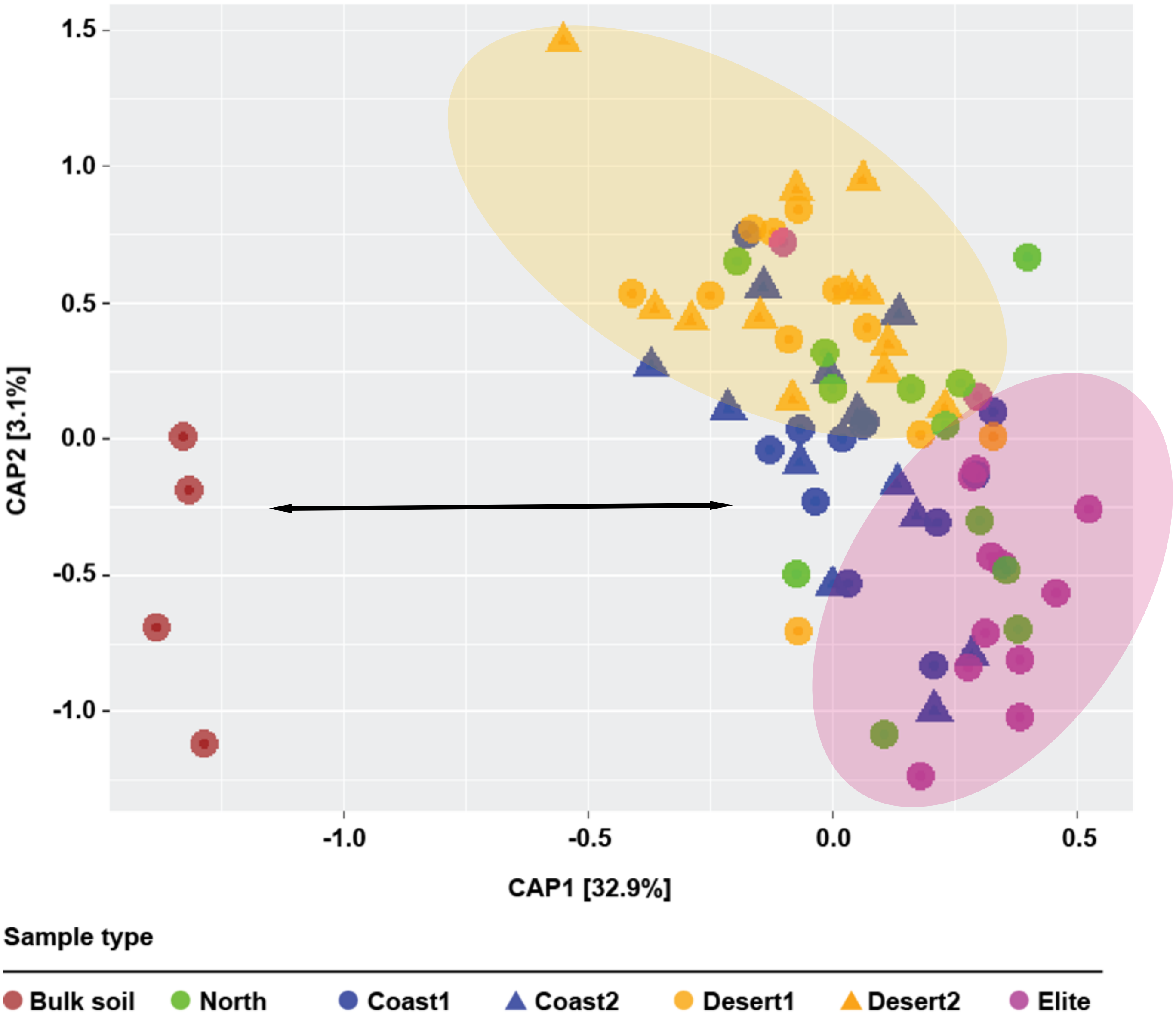
Lundberg *et al.* (2013) Nat Methods 10

Wild and elite barley genotypes fine-tune the composition of the rhizosphere bacterial microbiota

Variance explained:

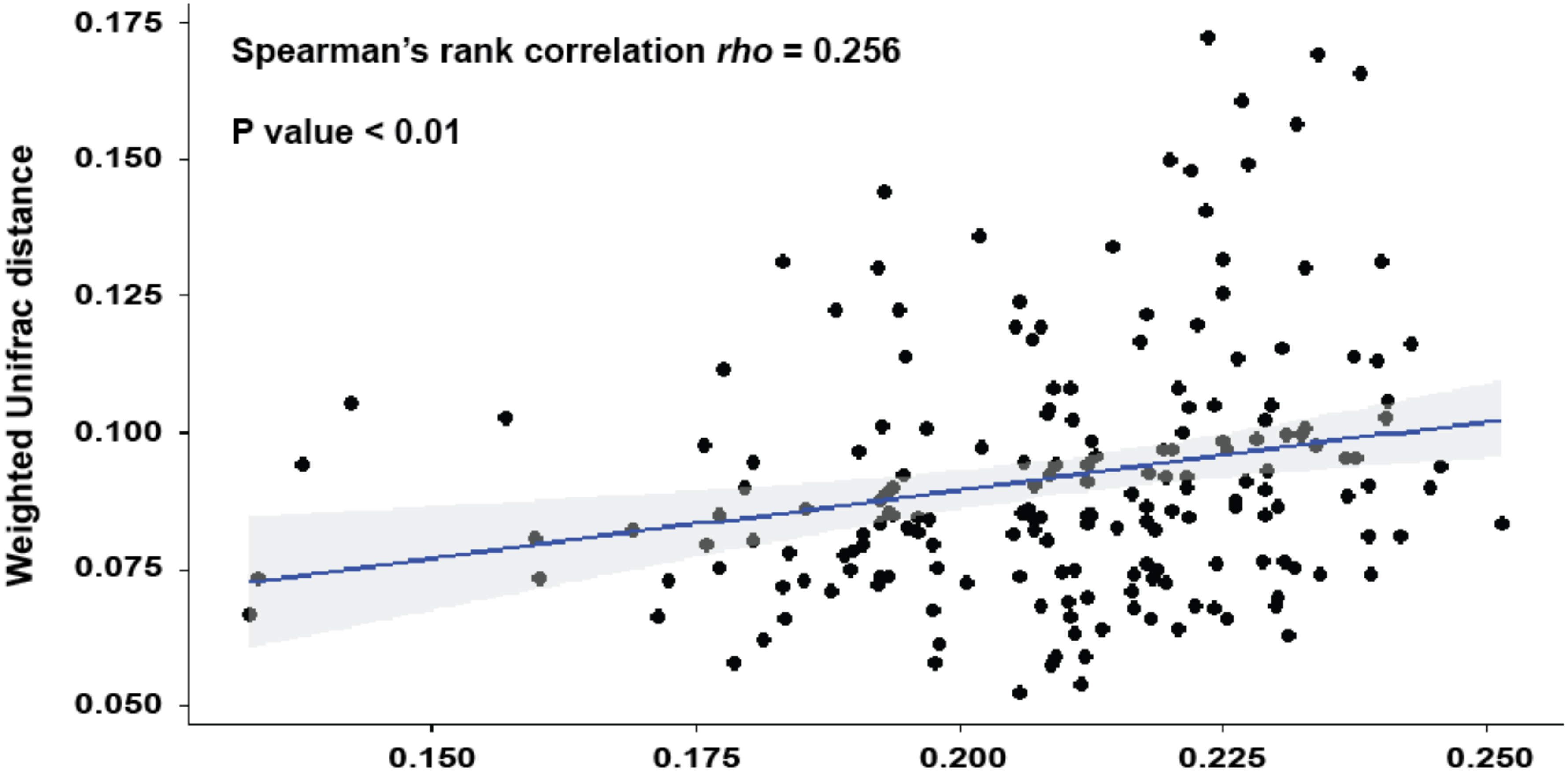
Microhabitat effect 30%

Ecotype effect 17%



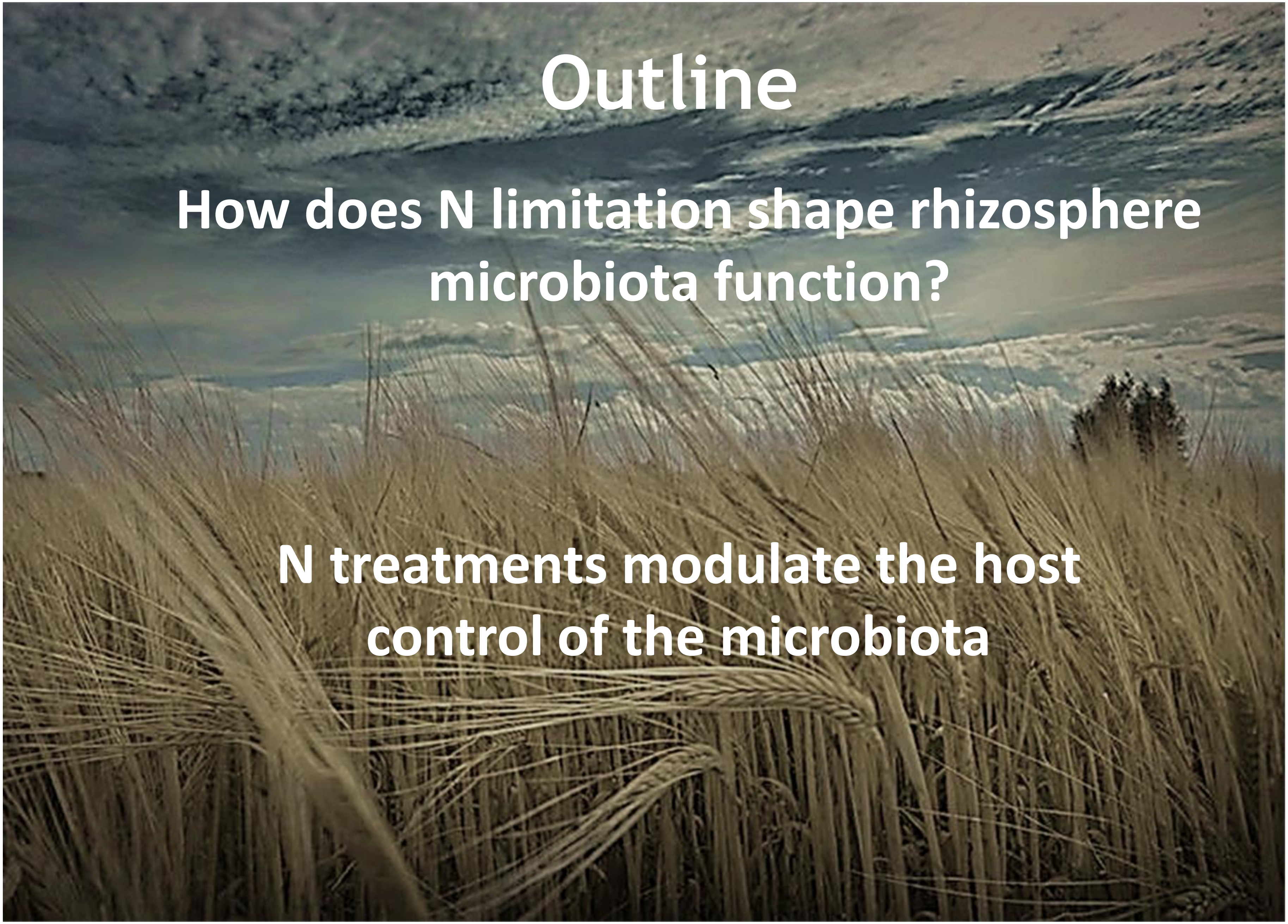
Alegria Terrazas et al. Sci Rep 10, 12916 (2020)
Canonical Analysis of Principal coordinates (CAP)
of the Weighted Unifrac ANOVA $p < 0.001$, 5,000 permutations

Genetic and Microbial diversity correlate within wild barleys



BOPA1 platform

Alegria Terrazas et al. Sci Rep 10, 12916 (2020)

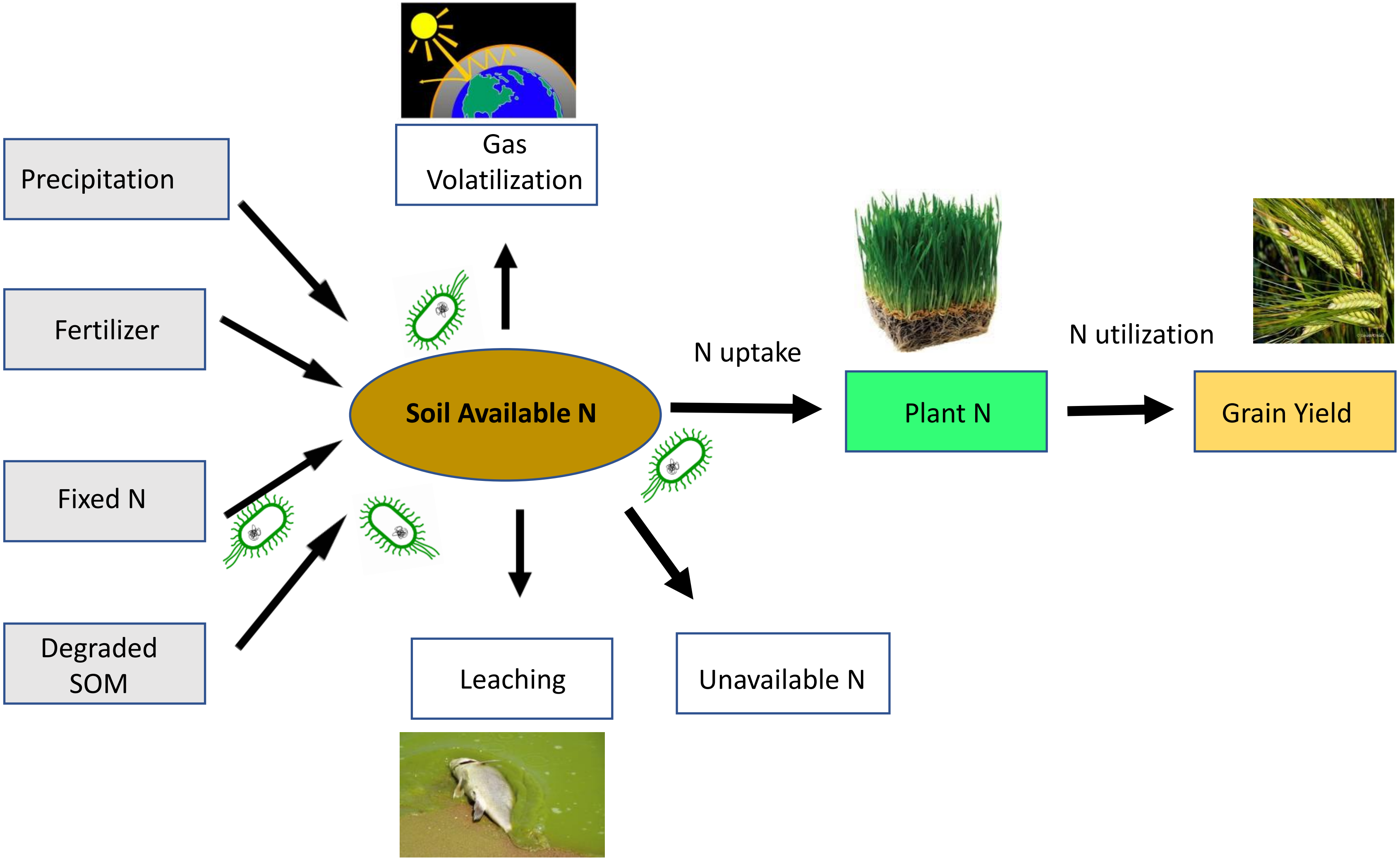


Outline

How does N limitation shape rhizosphere microbiota function?

N treatments modulate the host control of the microbiota

Nitrogen (N) cycle in the agroecosystem



Precipitation

Fertilizer

Fixed N

Degraded SOM

Gas Volatilization

Soil Available N

N uptake

Plant N

N utilization

Grain Yield

Leaching

Unavailable N

Modified from Good et al (2004) .*Trends Plant Sci 9*

Selected genotypes

Modern

Wild

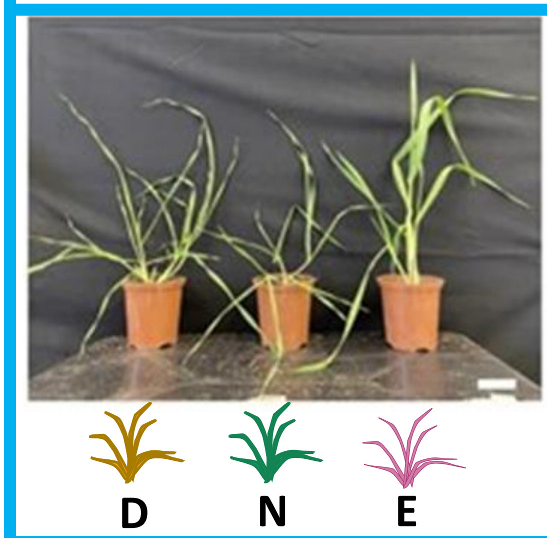
Morex = Elite

B1K - 31 = North

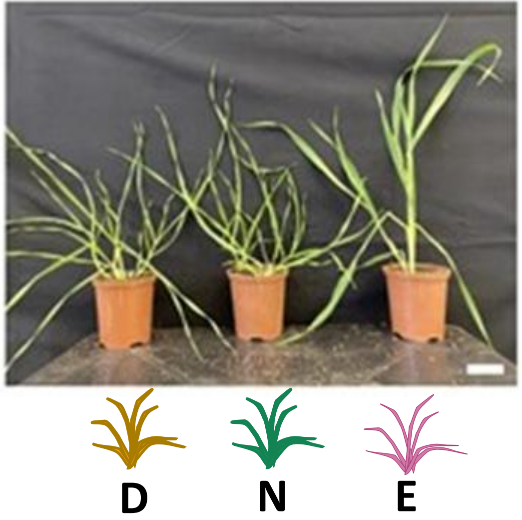
B1K - 12 = Desert

Nitrogen treatments

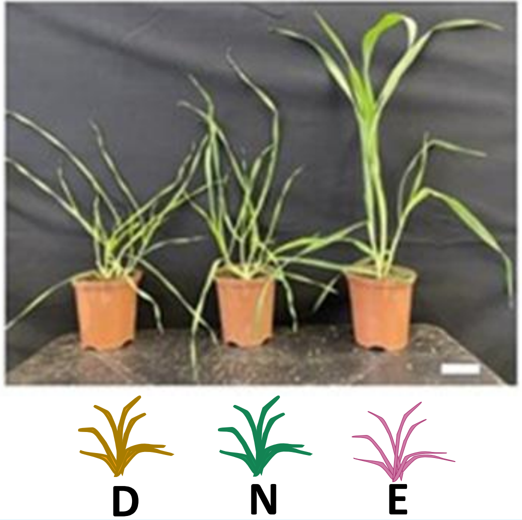
N 0%



N 25%

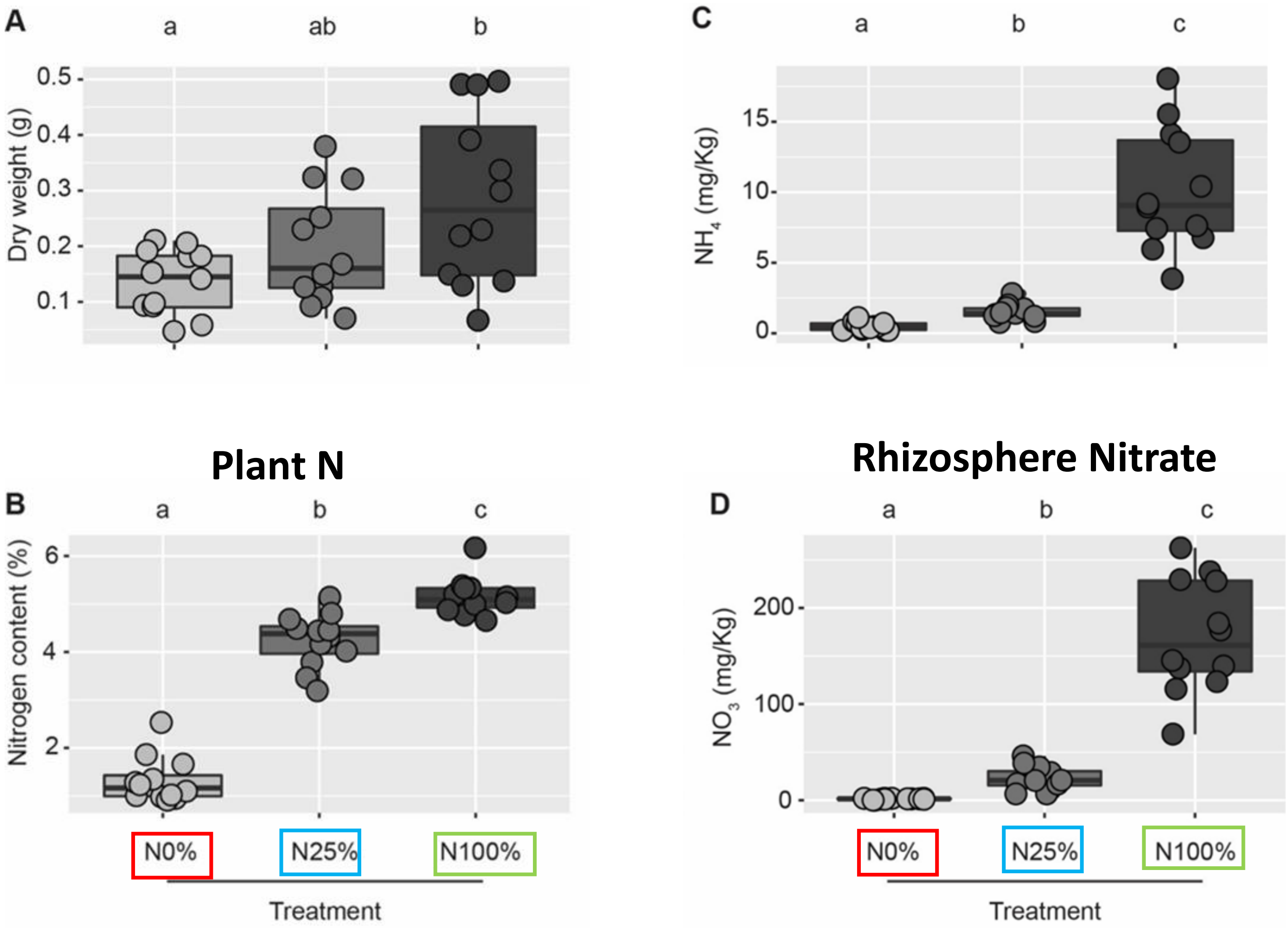


N 100%



Modified Hoagland solution with different N levels, all other plant nutrients at optimum level

Nitrogen availability impacts on plant-soil



Plant Biomass

Rhizosphere Ammonium

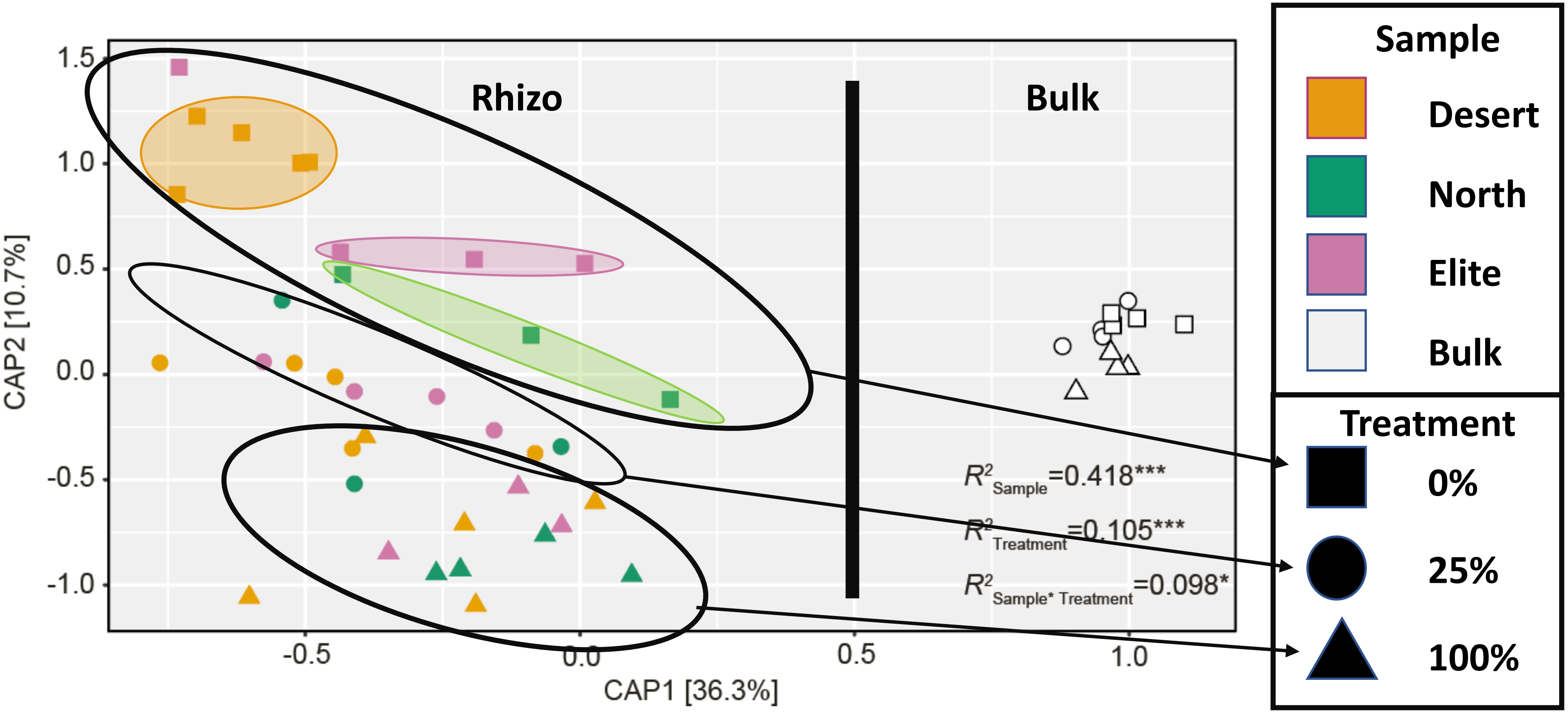
Plant N

Rhizosphere Nitrate

Kruskal–Wallis followed by Dunn's post hoc test.

Alegria Terrazas et al. mSystems 7, *00934 (2022)*

N treatment modulates the taxonomic composition



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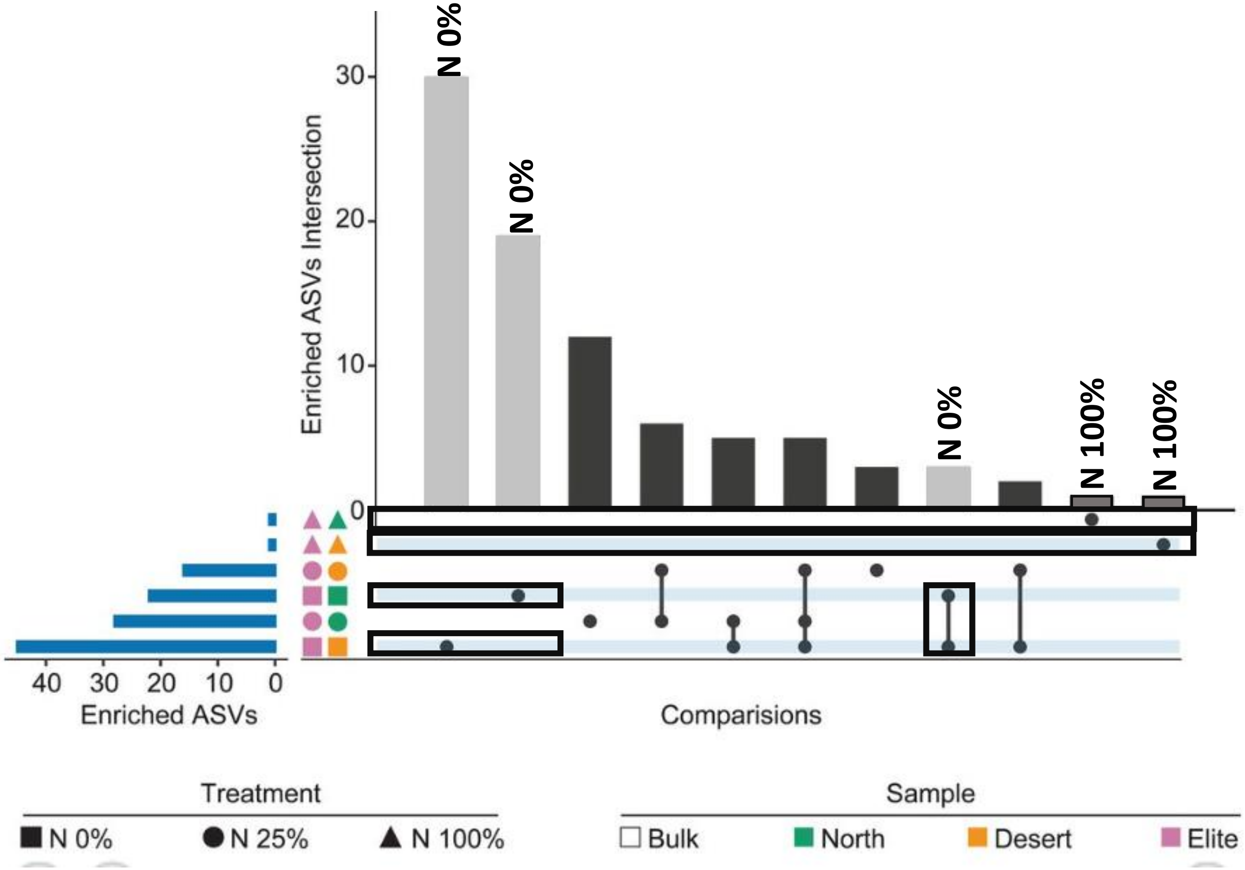
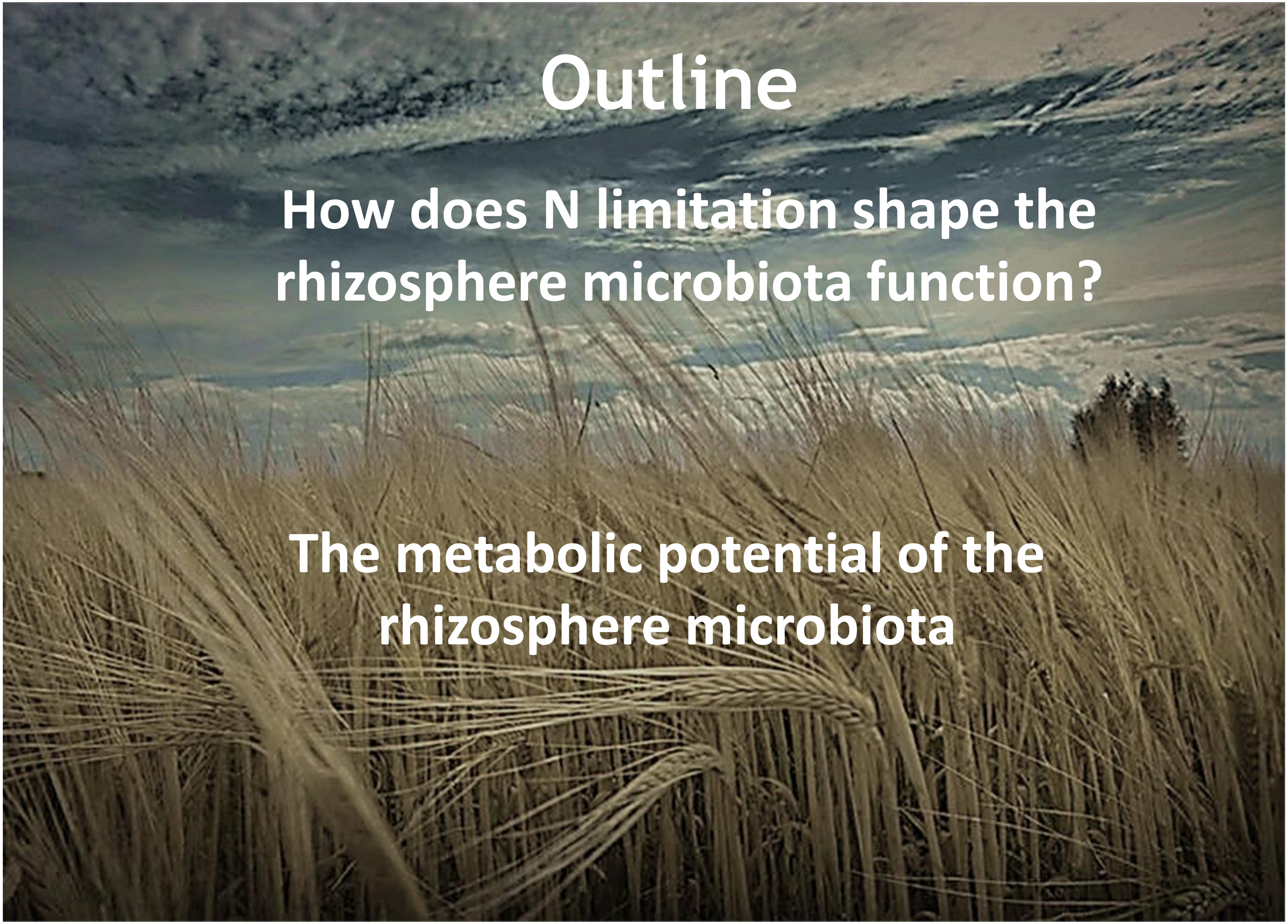
N limitation amplifies host selection on bacterial recruitment



Alegria Terrazas et al. mSystems 7, *00934 (2022)*

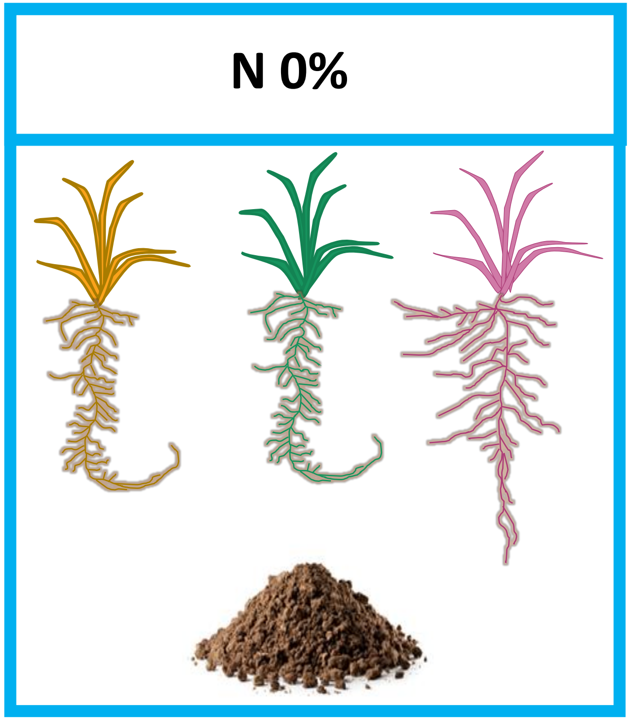


Outline

How does N limitation shape the rhizosphere microbiota function?

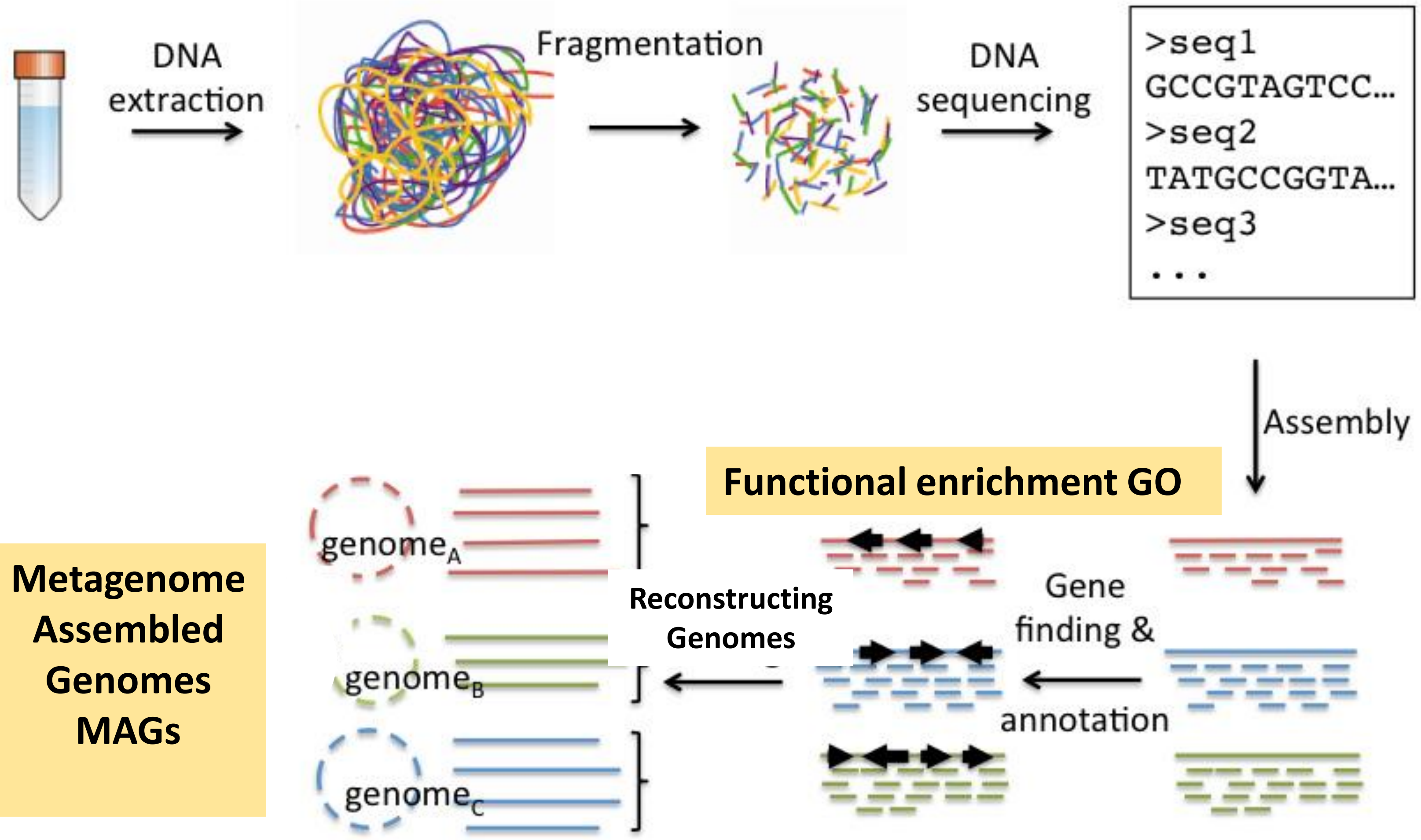
The metabolic potential of the rhizosphere microbiota

Experimental design



N 0% treatment for metagenomics

Metagenomics pipeline



Functional enrichment GO

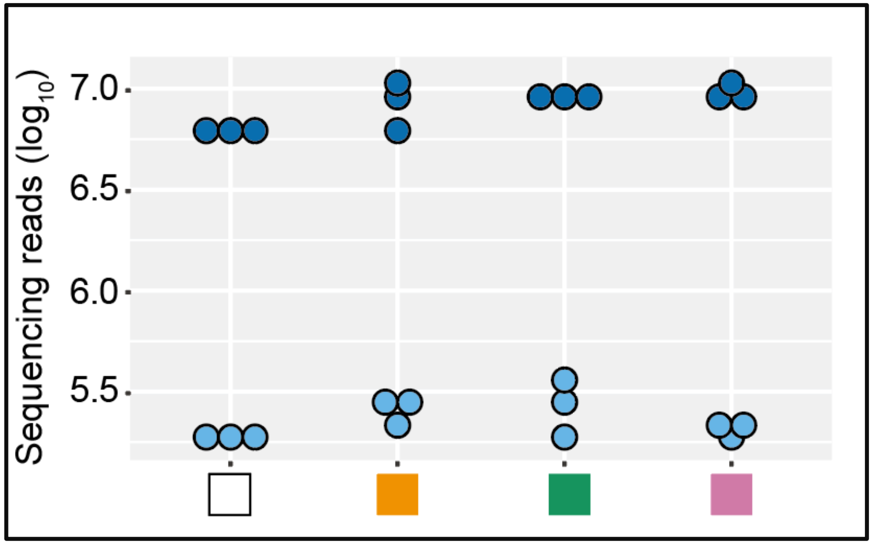
Reconstructing Genomes

Metagenome Assembled Genomes MAGs

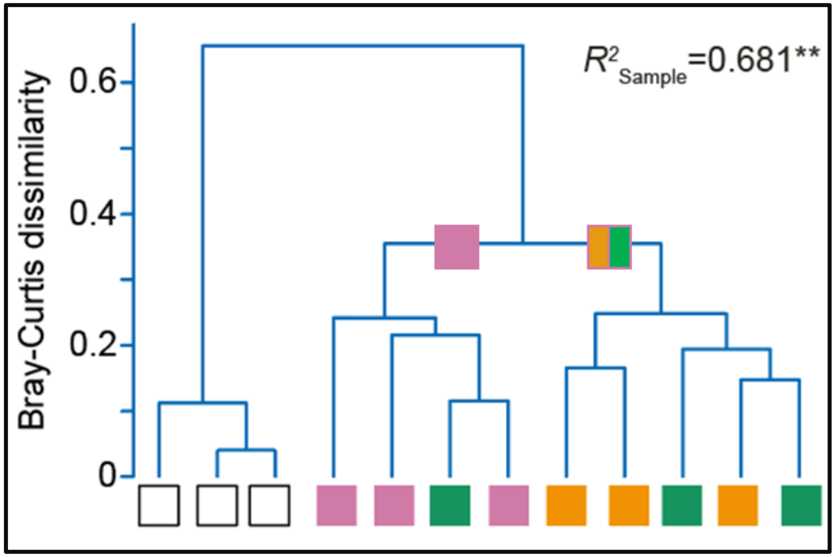
https://envgen.github.io/metagenomics.html#

Metagenomic profiles are dominated by bacteria and separate genotypes

Bacteria - Fungi



Bacteria



Sample

- 🟧 **Desert**
- 🟩 **North**
- 🟪 **Elite**
- ⬜ **Bulk**

● Bacteria ● Fungi

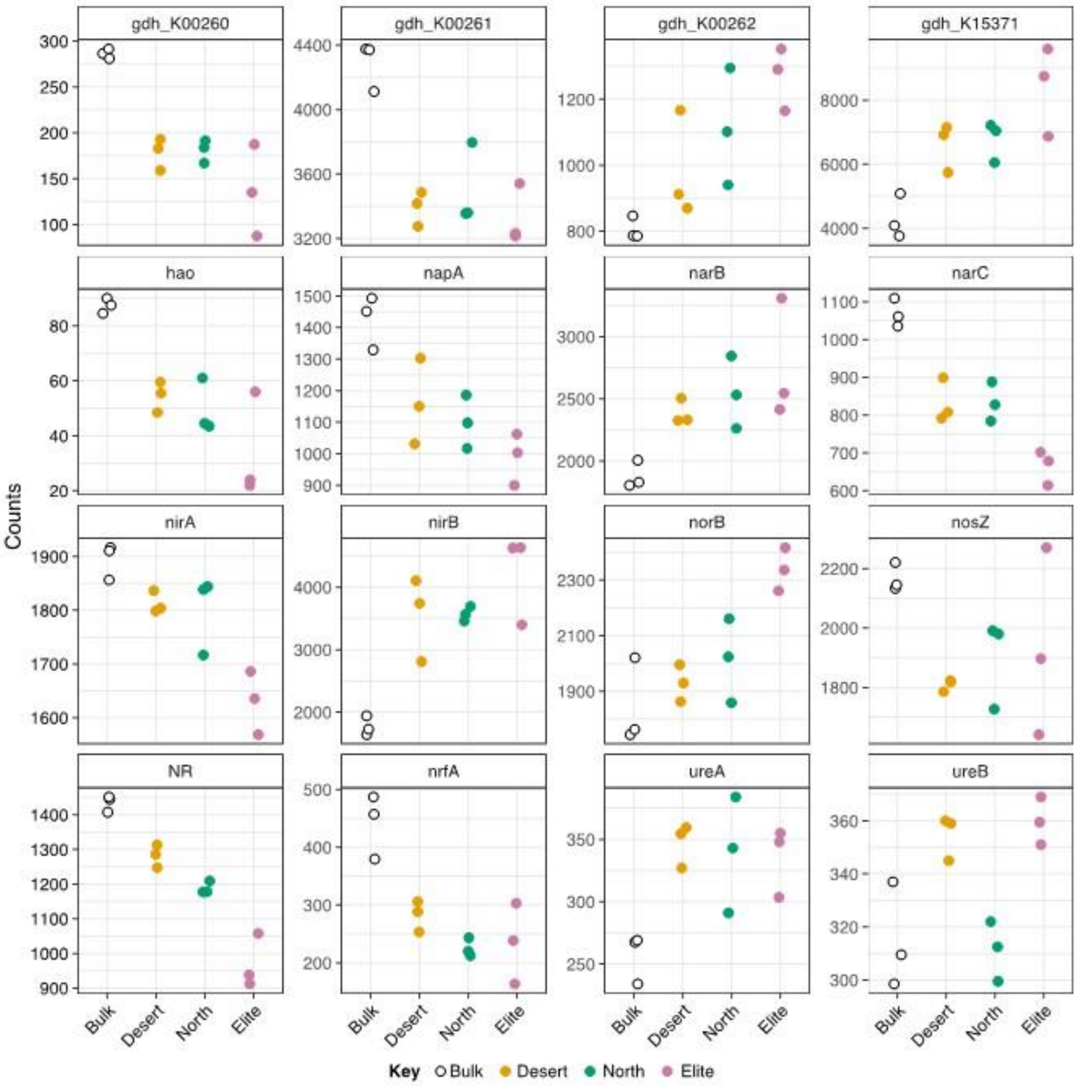
Numbers associated with the dendrogram depict the proportion of variance (R^2)

Alegria Terrazas et al. mSystems 7, *00934 (2022)*

Nitrogen biogeochemical cycle gene mining

- Nitrification
- Denitrification
- Nitrate reduction
- Synthesis and degradation of nitrogen containing organic compounds

Nitrogen biogeochemical cycle gene abundance didn't differentiate between genotypes



NCycProfiler/NCycDB/diamond method

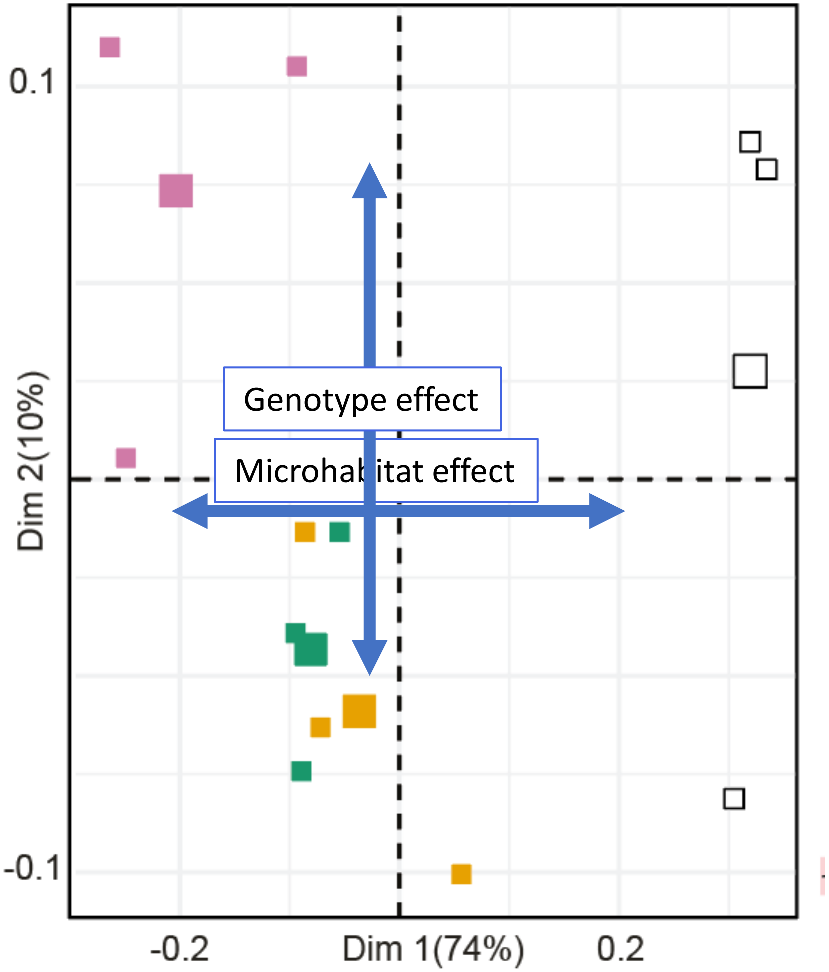
Alegria Terrazas et al. mSystems 7, *00934 (2022)*

Microhabitat and host genotype specialization define barley microbiota function

Sample

- 🟧 **Desert**
- 🟩 **North**
- 🟪 **Elite**
- ⬜ **Bulk**

What GO terms are enriched between these microhabitats?



PCA computed on annotated reads mapped to the terms of Gene Ontology Slim database
Alegria Terrazas et al. mSystems 7, *00934 (2022)*

Functional diversification of the microbiota

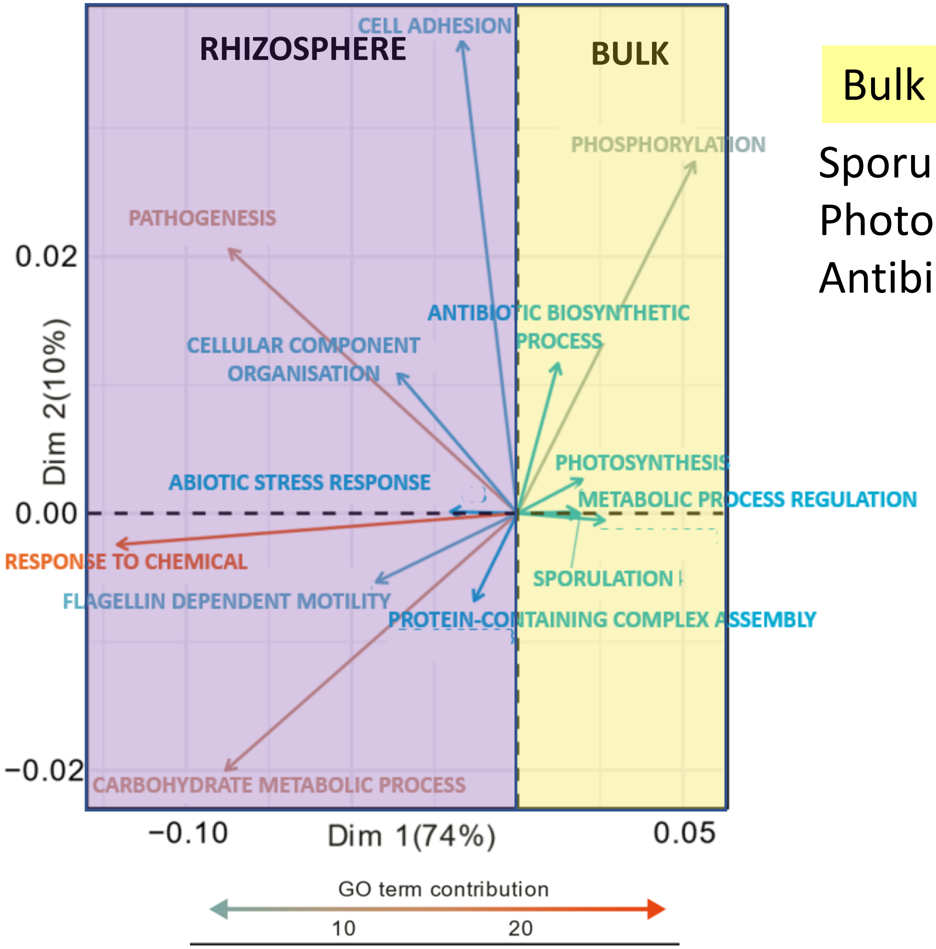
Nutrient stress in C poor

Elite

Cell adhesion
Pathogenesis
Response to abiotic stimulus

Wild

Carbohydrate metabolic processing
Responses to chemical
Protein containing complex assembly
Flagellum dependent motility



Bulk

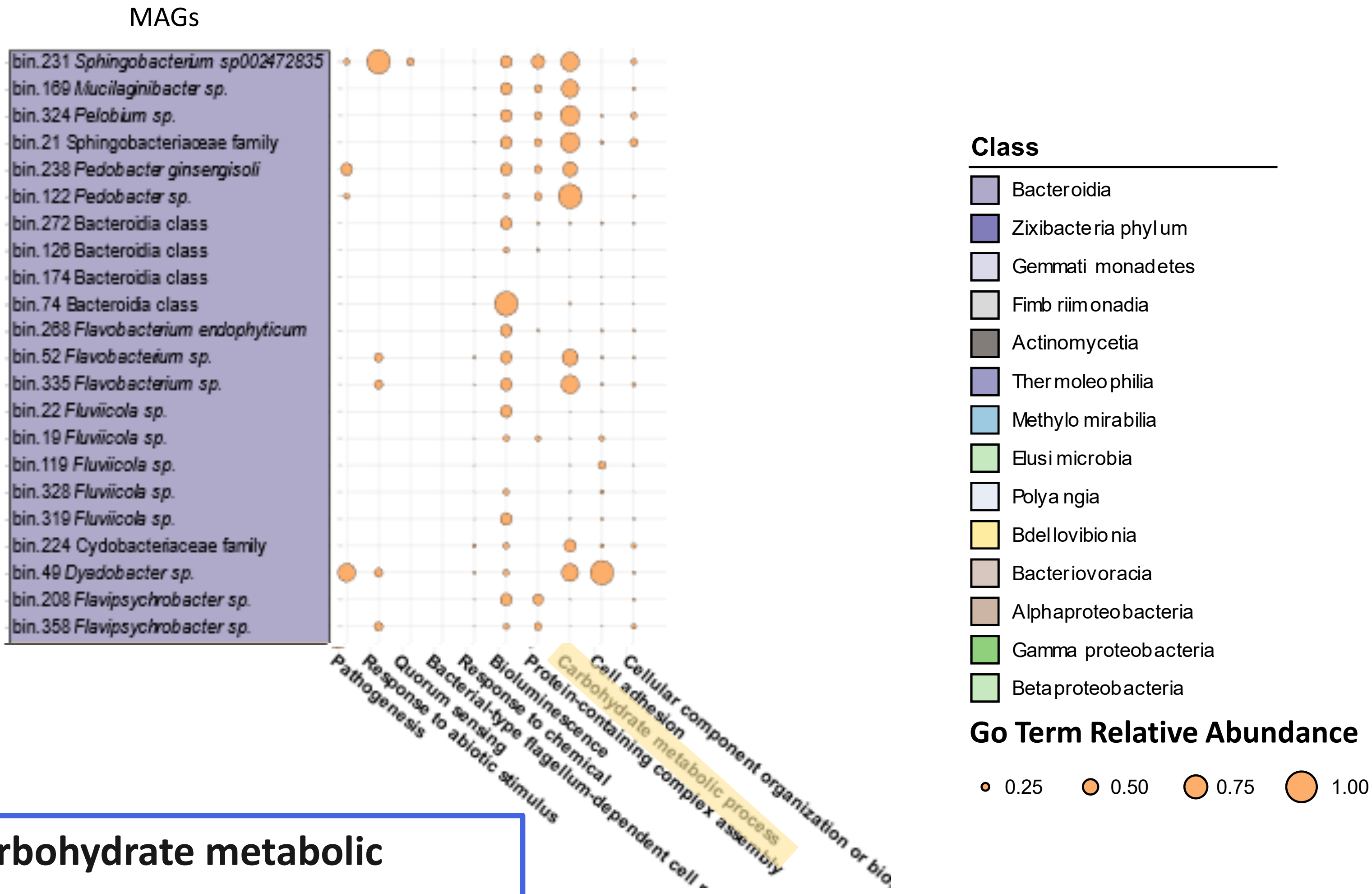
Sporulation
Photosynthesis
Antibiotic synthesis

Microbe-microbe/host interactio in C rich

PCA loadings representing the GO Slim terms sustaining the ordinatio

Alegria Terrazas et al. mSystems 7, *00934 (2022)*

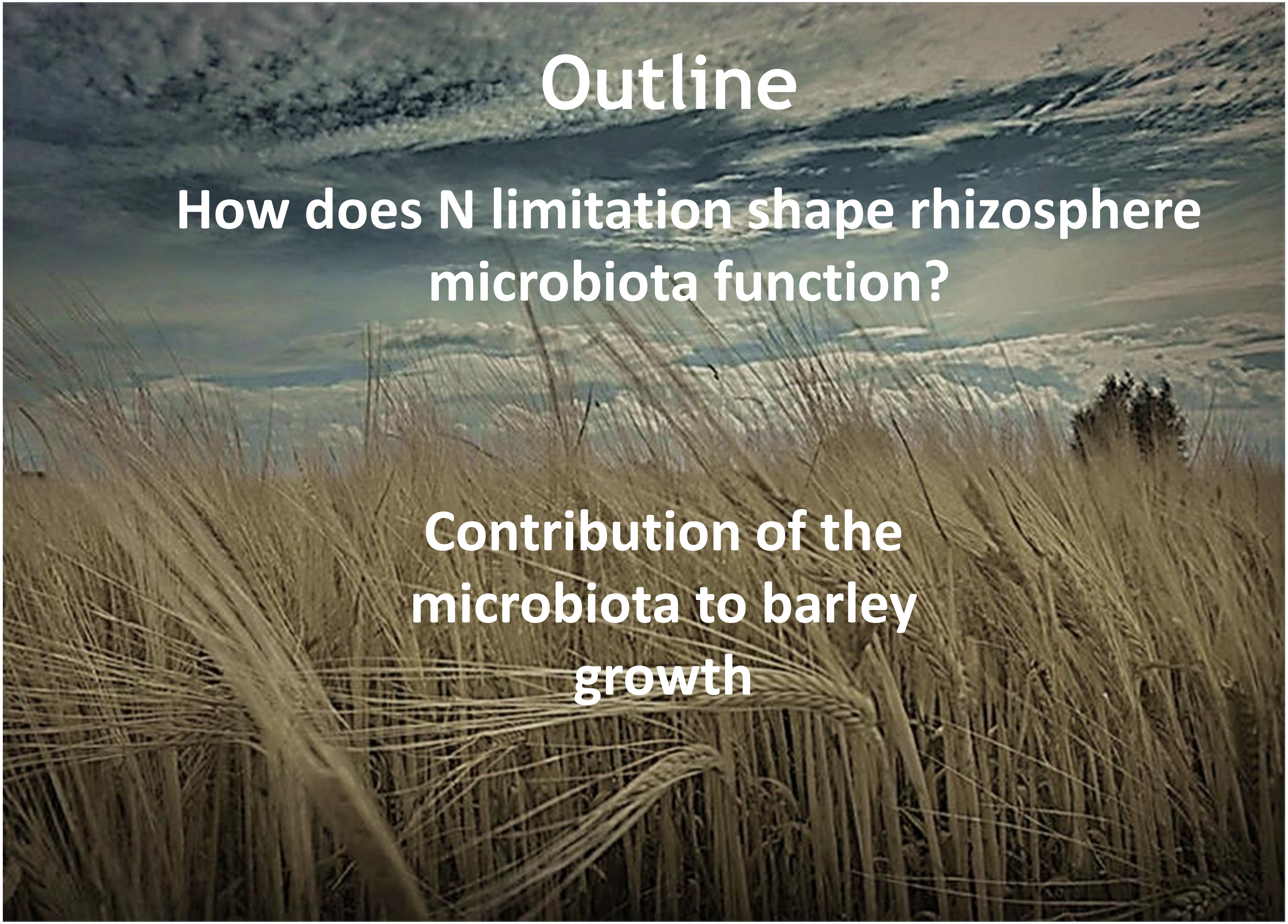
Genome reconstruction of bacteria (MAGs)
Who is doing what?



Carbohydrate metabolic process
Bacteroidetes
(11/15 elite)

67 metagenome-assembled genomes (MAGs) affiliated with 14 different bacterial classes

Alegria Terrazas et al. mSystems 7, *00934 (2022)*

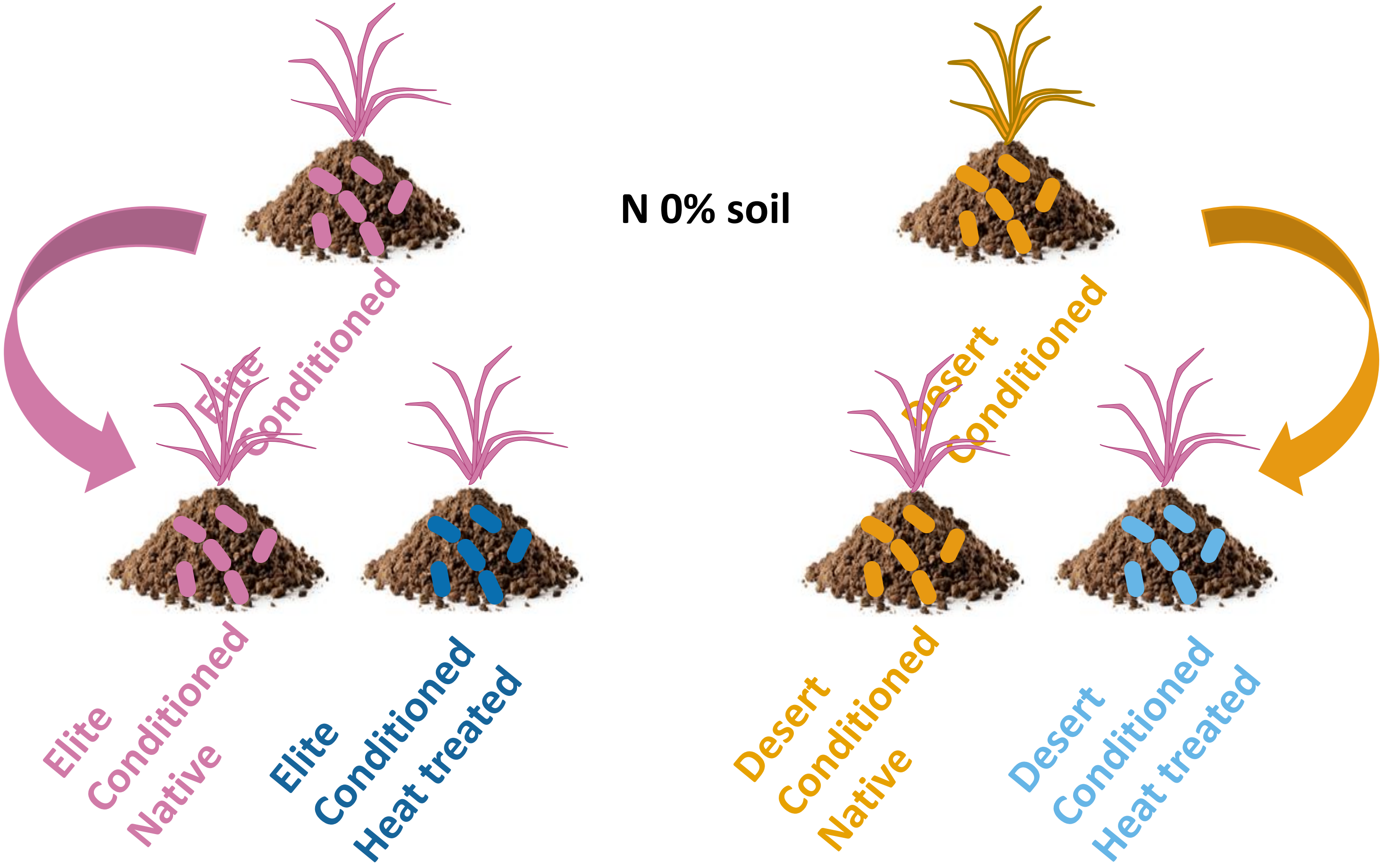


Outline

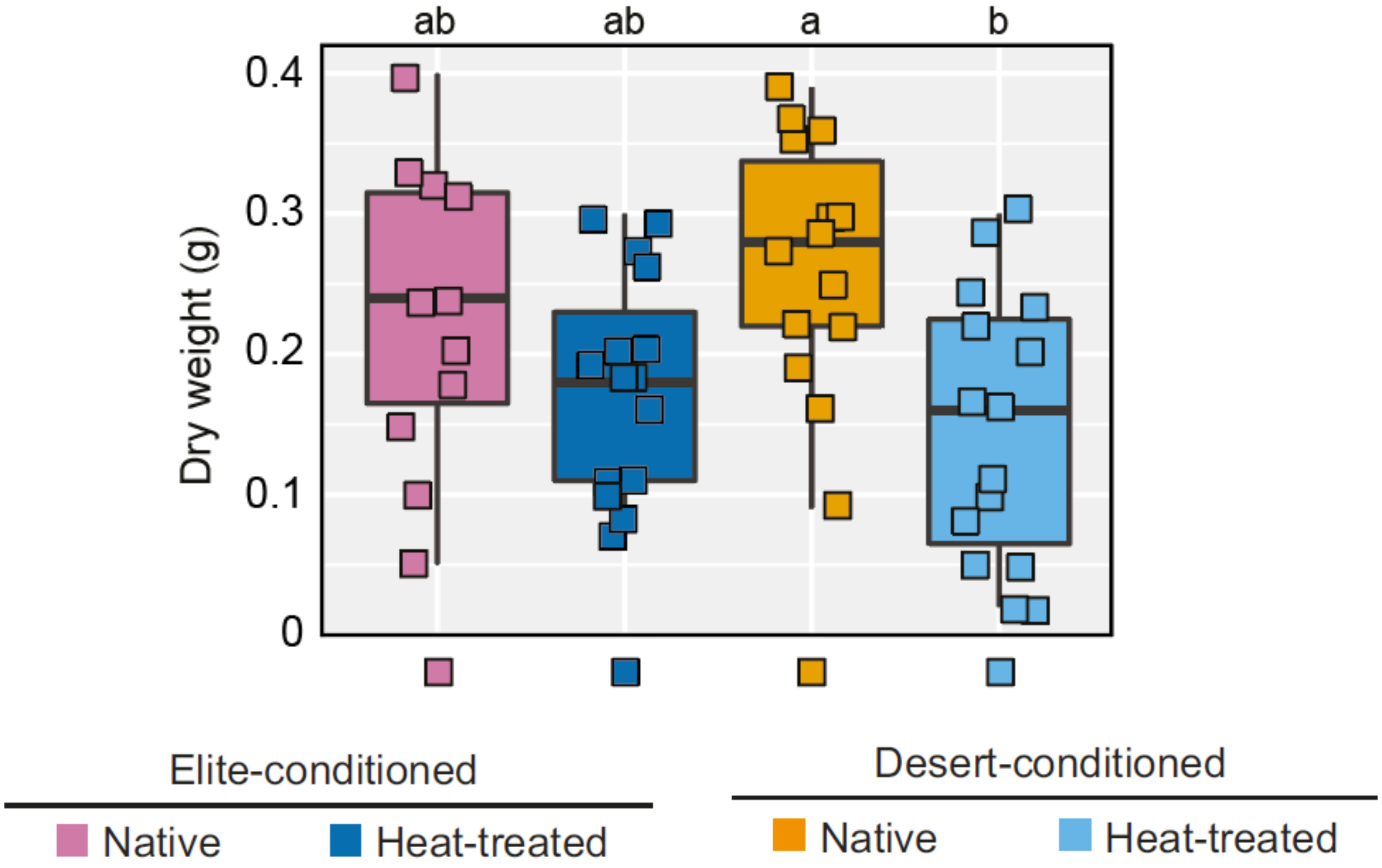
How does N limitation shape rhizosphere microbiota function?

Contribution of the microbiota to barley growth

Plant-soil feedback experiments



A functional microbiota is required for optimum barley growth



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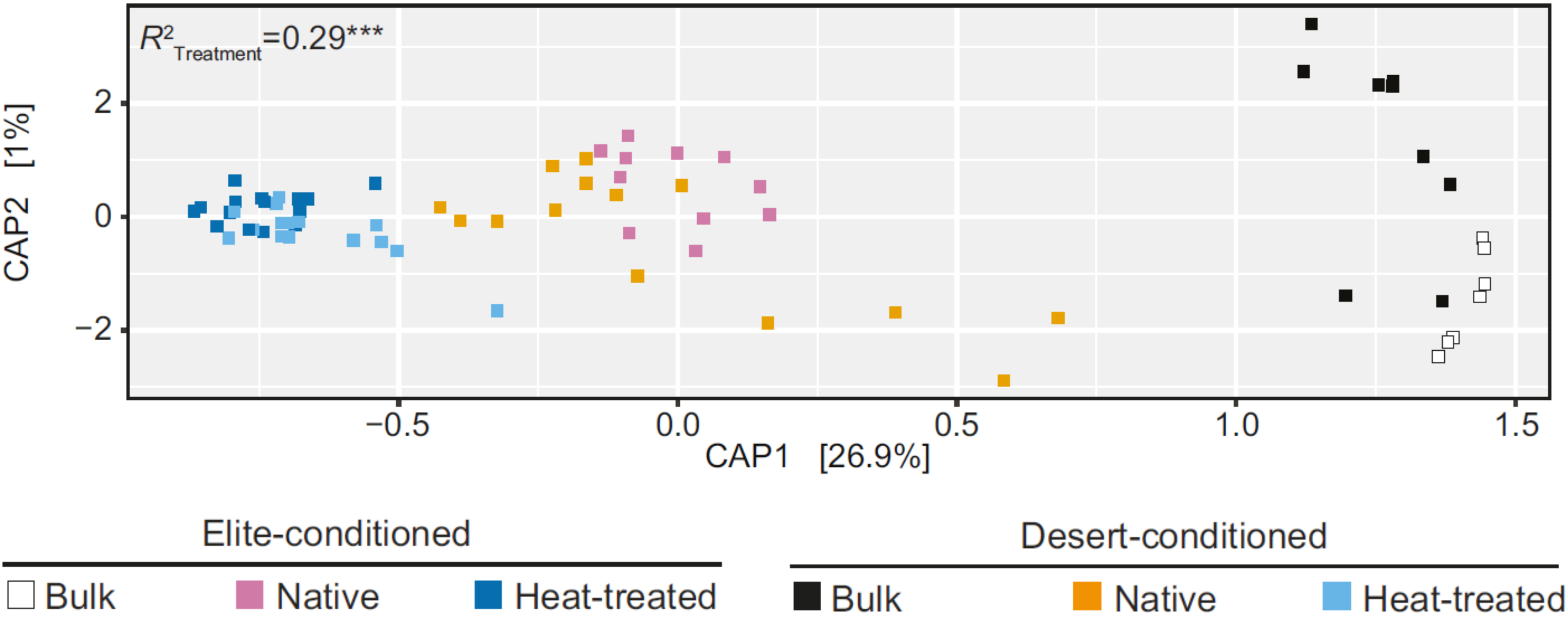
Distinct input microbiotas "converge" to a conserved composition



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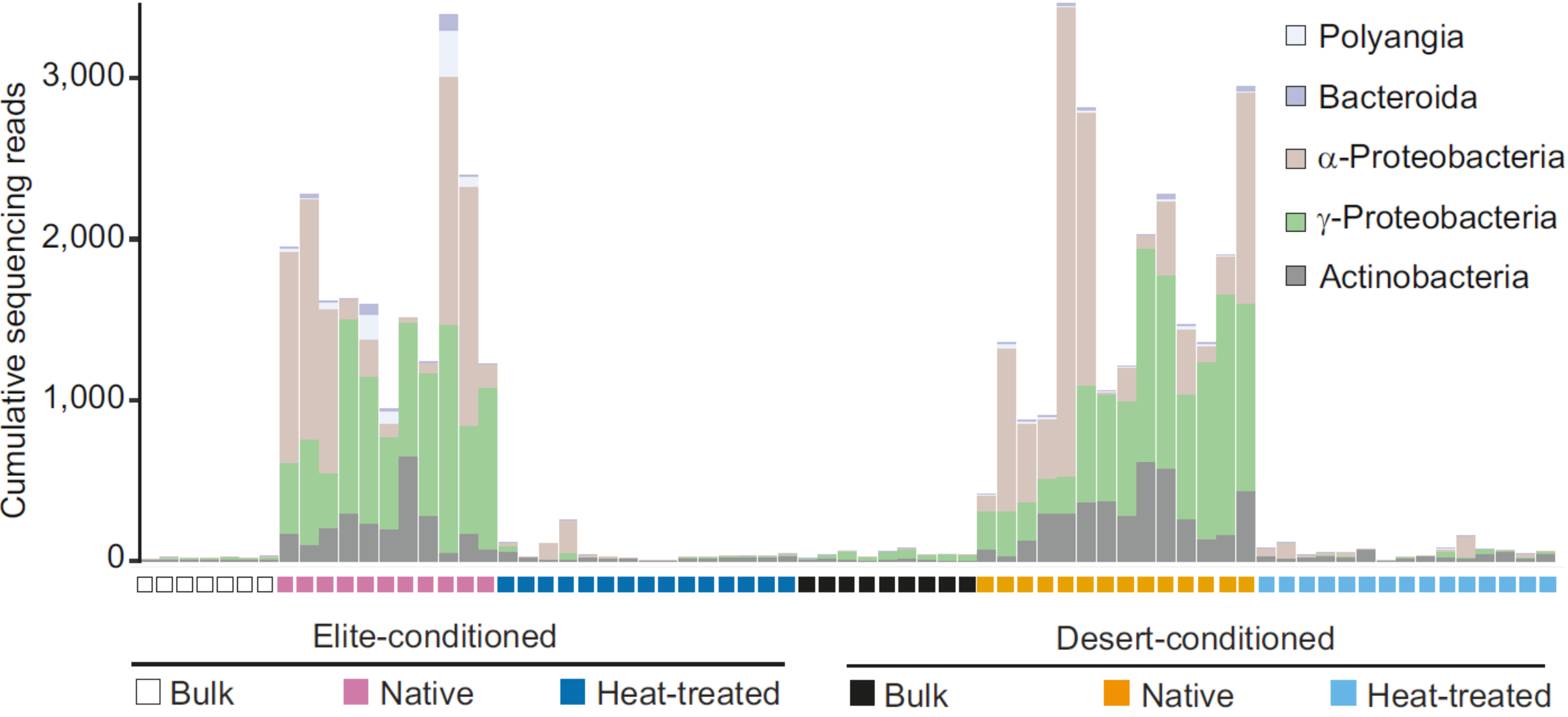
The plant genotype recruits it’s “own” microbiota



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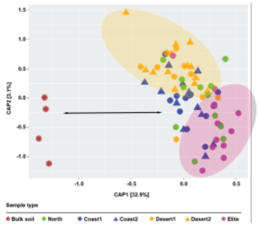
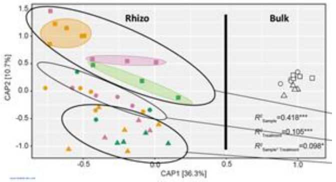
Take home messages

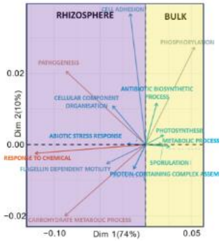
Plant adaptation not only shapes the phenotype and the genome but also the associated rhizosphere microbiota structure



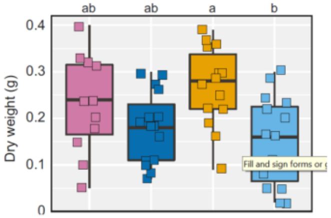
Nitrogen limitation amplifies the barley genetic control of the rhizosphere microbiota



Distinct barley genotypes recruit different functions in their rhizosphere microbiota



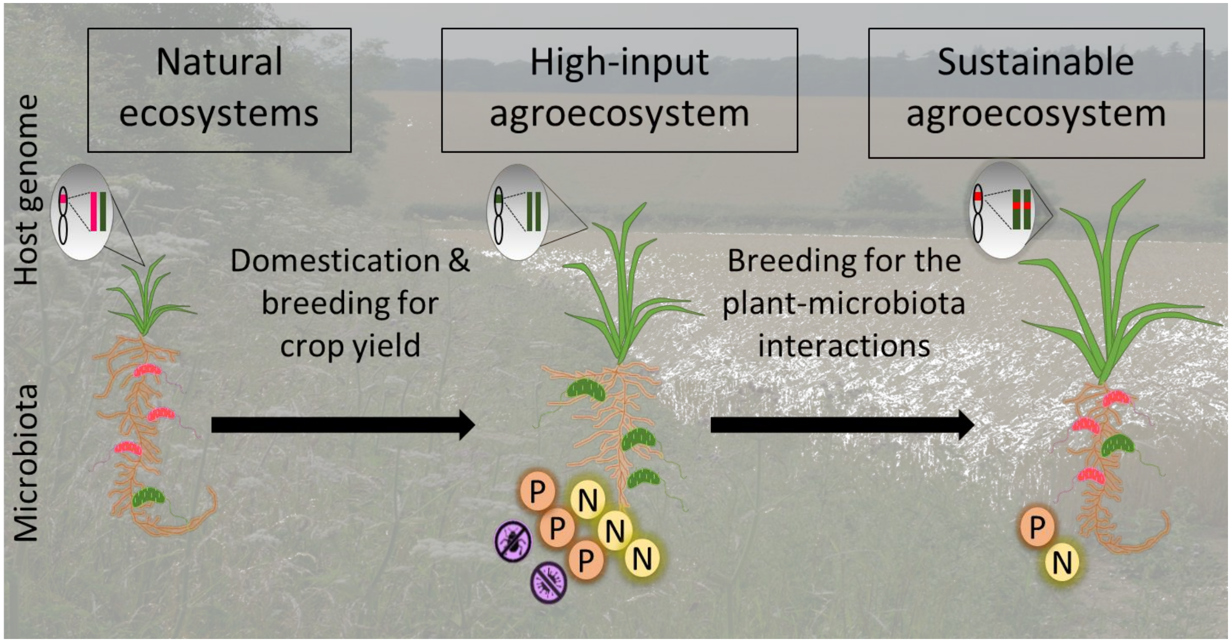
A host dependent microbiota is required for optimal barley growth under N-limiting conditions



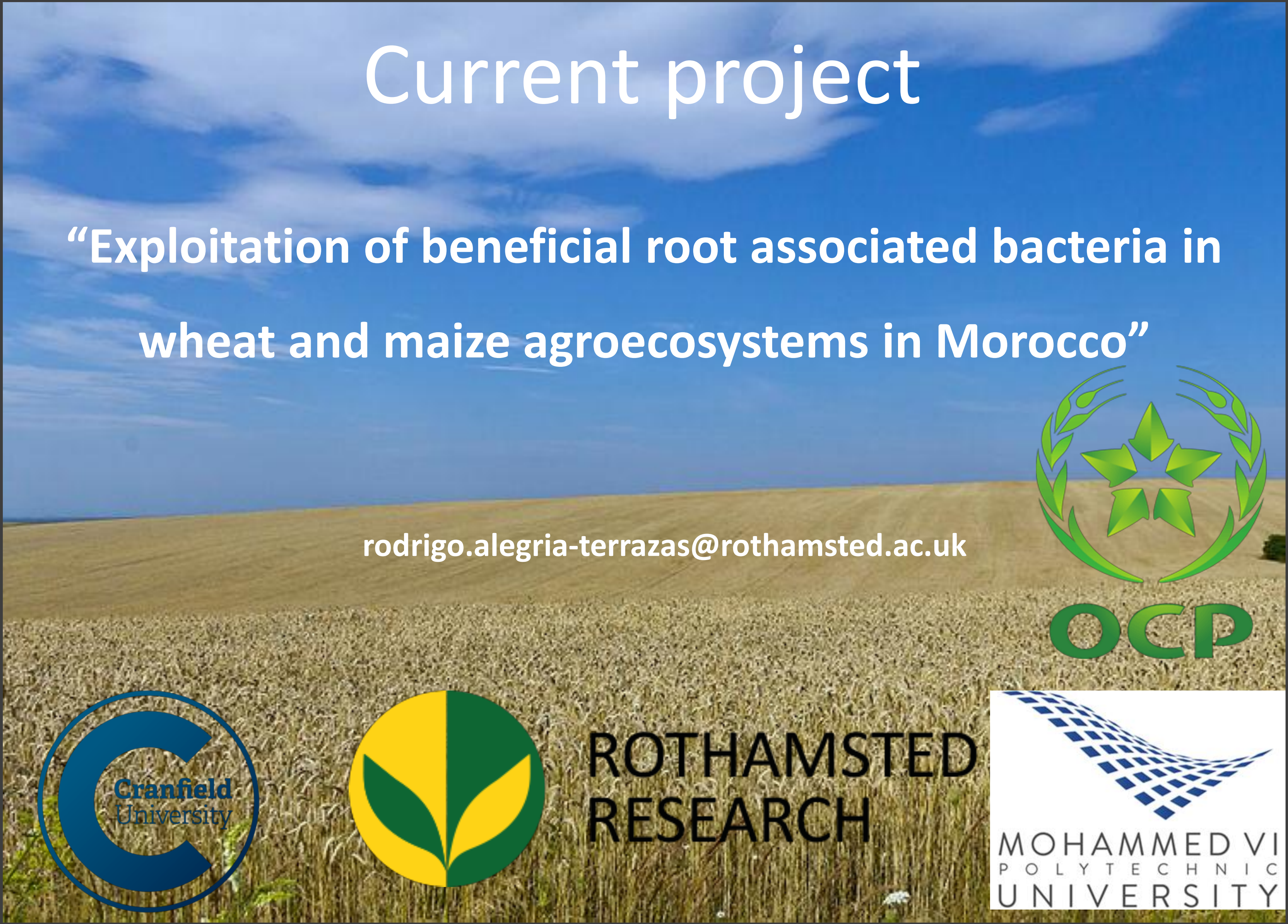
Perspectives

Plant adaptation shapes the plant genome which in turn modulates the taxonomic composition and function of the rhizosphere microbiota under low N conditions.

So potentially, we can manage the rhizosphere microbiota for crop beneficial functions through the plant genotype to make a more sustainable agriculture



Escudero-Martinez and Bulgarelli (2019) , Curr Opin Microbiol 49:34



Current project

“Exploitation of beneficial root associated bacteria in wheat and maize agroecosystems in Morocco”

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OCP

Cranfield University

ROTHAMSTED RESEARCH

MOHAMMED VI POLYTECHNIC UNIVERSITY

THANK YOU!

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