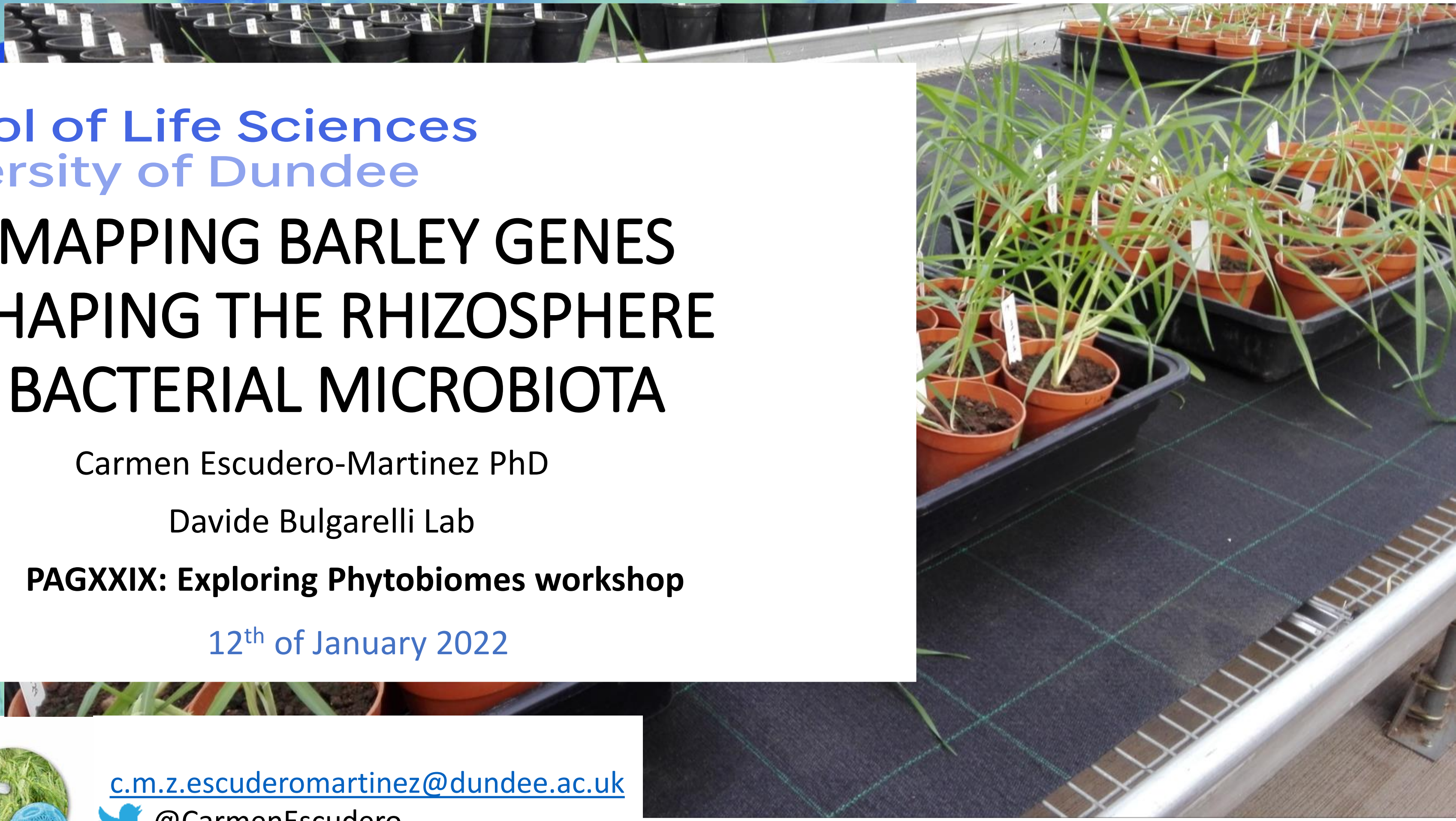
One of the UK's top 20 universities (*Guardian University Guide 2021*)



School of Life Sciences
University of Dundee

MAPPING BARLEY GENES SHAPING THE RHIZOSPHERE BACTERIAL MICROBIOTA

Carmen Escudero-Martinez PhD

Davide Bulgarelli Lab

PAGXXIX: Exploring Phytobiomes workshop

12th of January 2022

Plant Sciences

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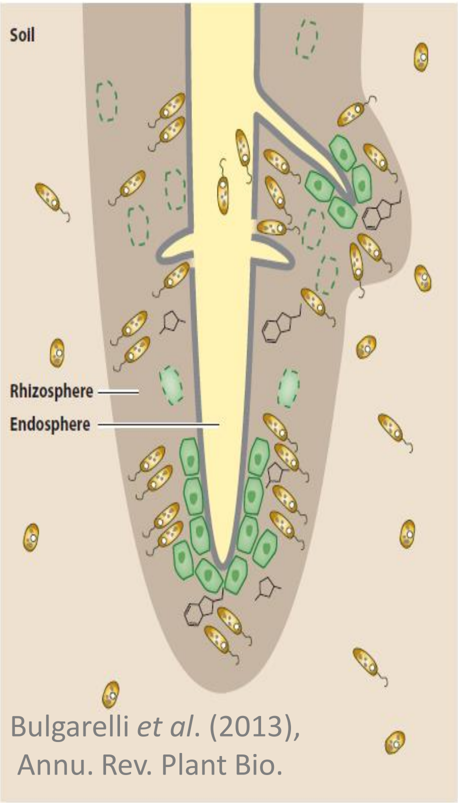
Soil microbes to improve sustainable agriculture and food security

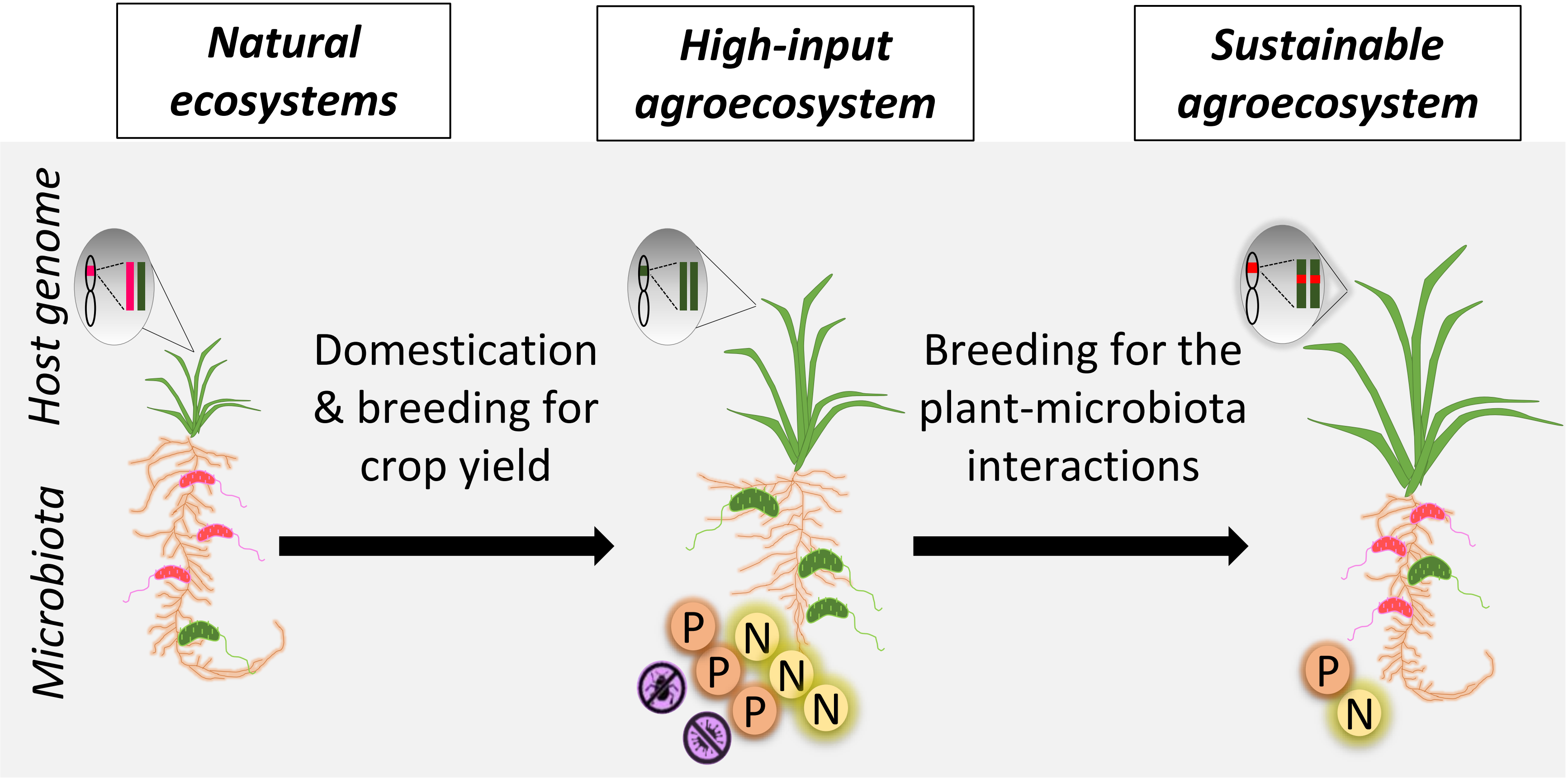


Bulgarelli *et al*. (2013), Annu. Rev. Plant Bio.

- **Access to soil-bound nutrients**
- **Pathogen protection**
- **Stress tolerance**
- **Enhancement of plant development and growth**

Berendsen *et al.*, 2018
Stringlis *et al.*, 2018
Zhang *et al.*, 2019
Yu *et al.*, 2021

Plant-microbiota interactions from natural ecosystems to future agricultural scenarios



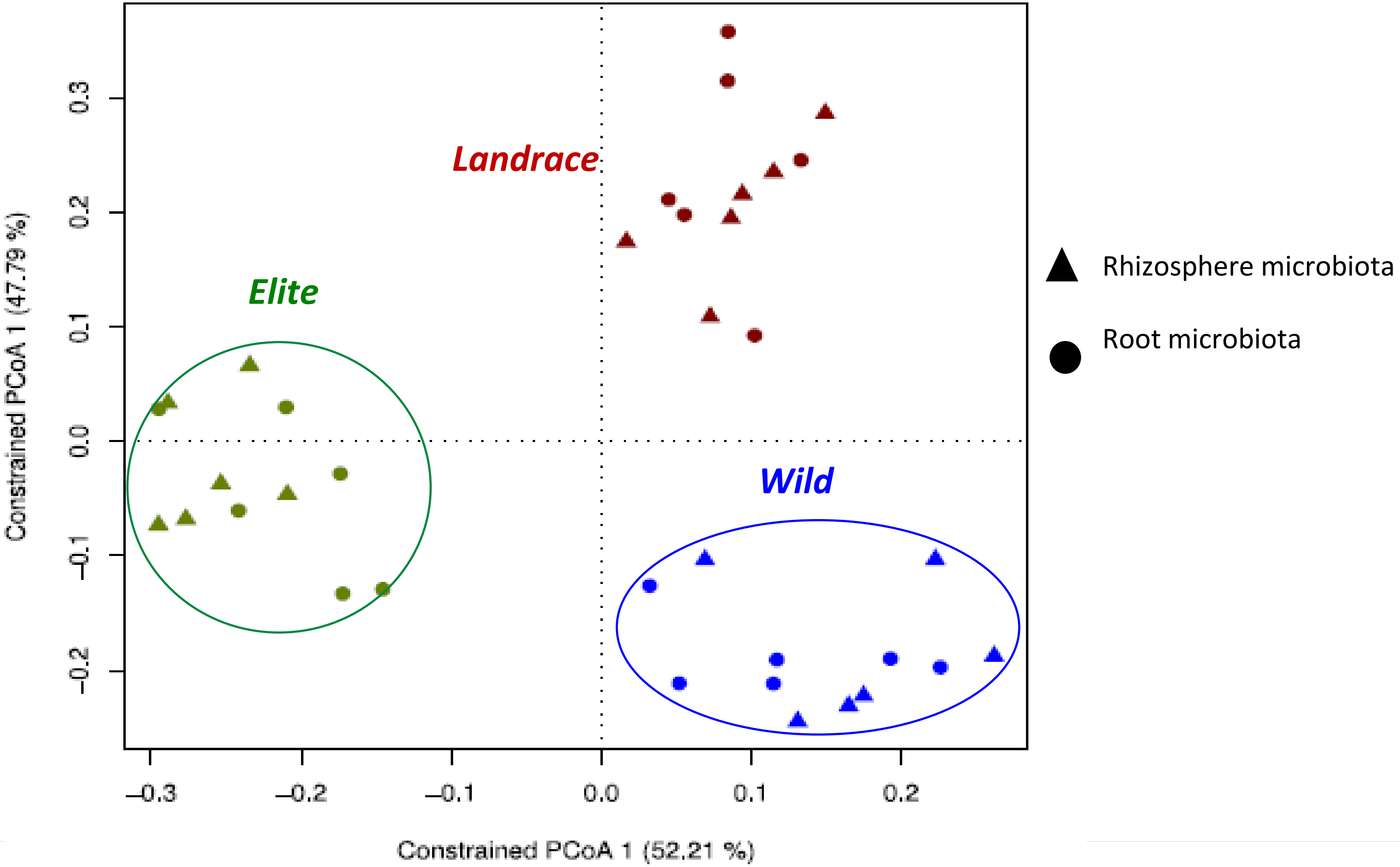
Escudero-Martinez & Bulgarelli (2019) Current Opinion in Microbiology

Barley as a model to study crop-microbiota interactions

- Global Crop: world 4th most cultivated cereal
- Large germplasm collections:
  Accessions, mutants, wild relatives
- Diploid genome
- Pan-genome
- Gene editing
- Speed breeding

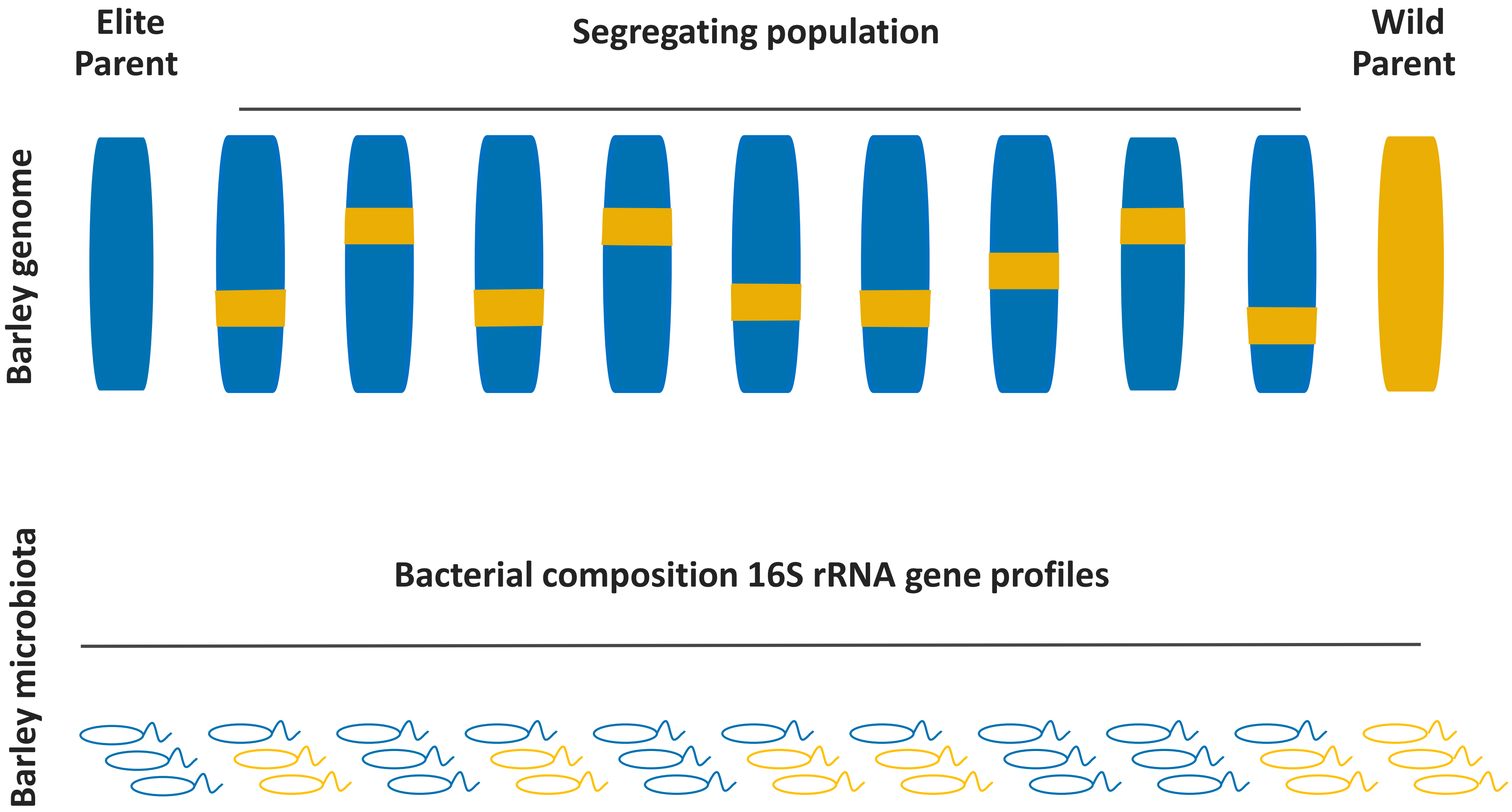
Rhizosphere microbiota diversifies between wild and elite barley

Constrained Principal Coordinate Analysis



Bulgarelli et al., (2015) Cell Host Microbe 17:392

Can we map barley genes associated with rhizosphere bacteria?


Elite Parent

Segregating population

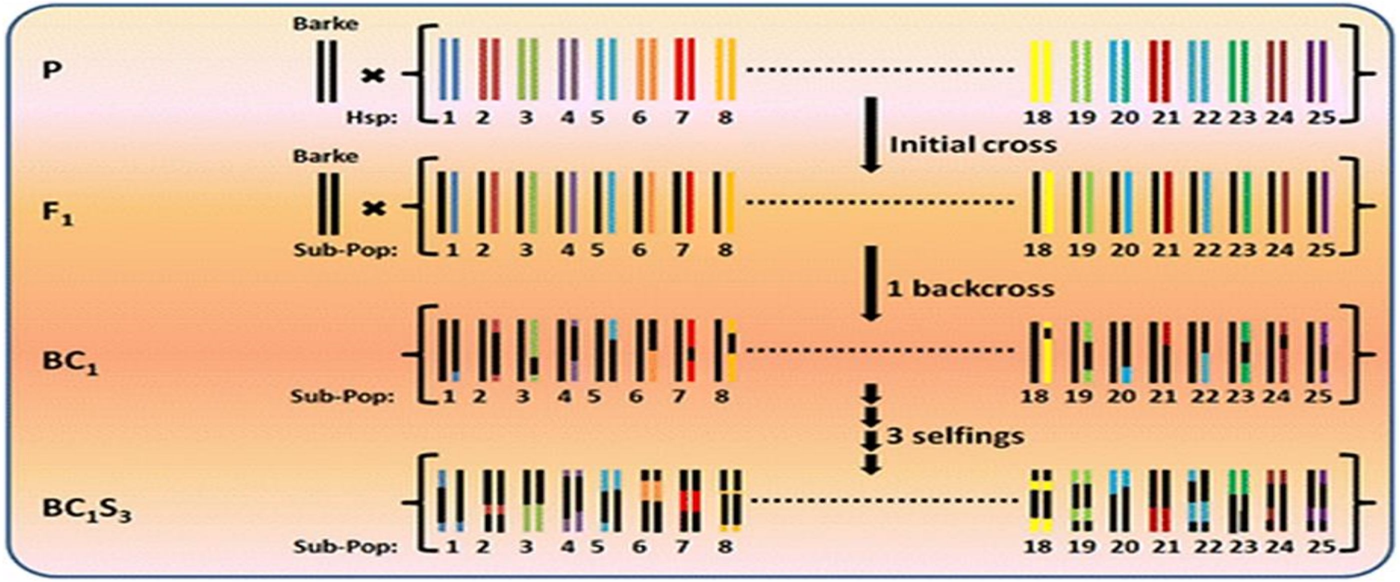
Wild Parent

Barley genome

Barley microbiota

Bacterial composition 16S rRNA gene profiles

NAM HEB-25: a useful resource in barley genetics

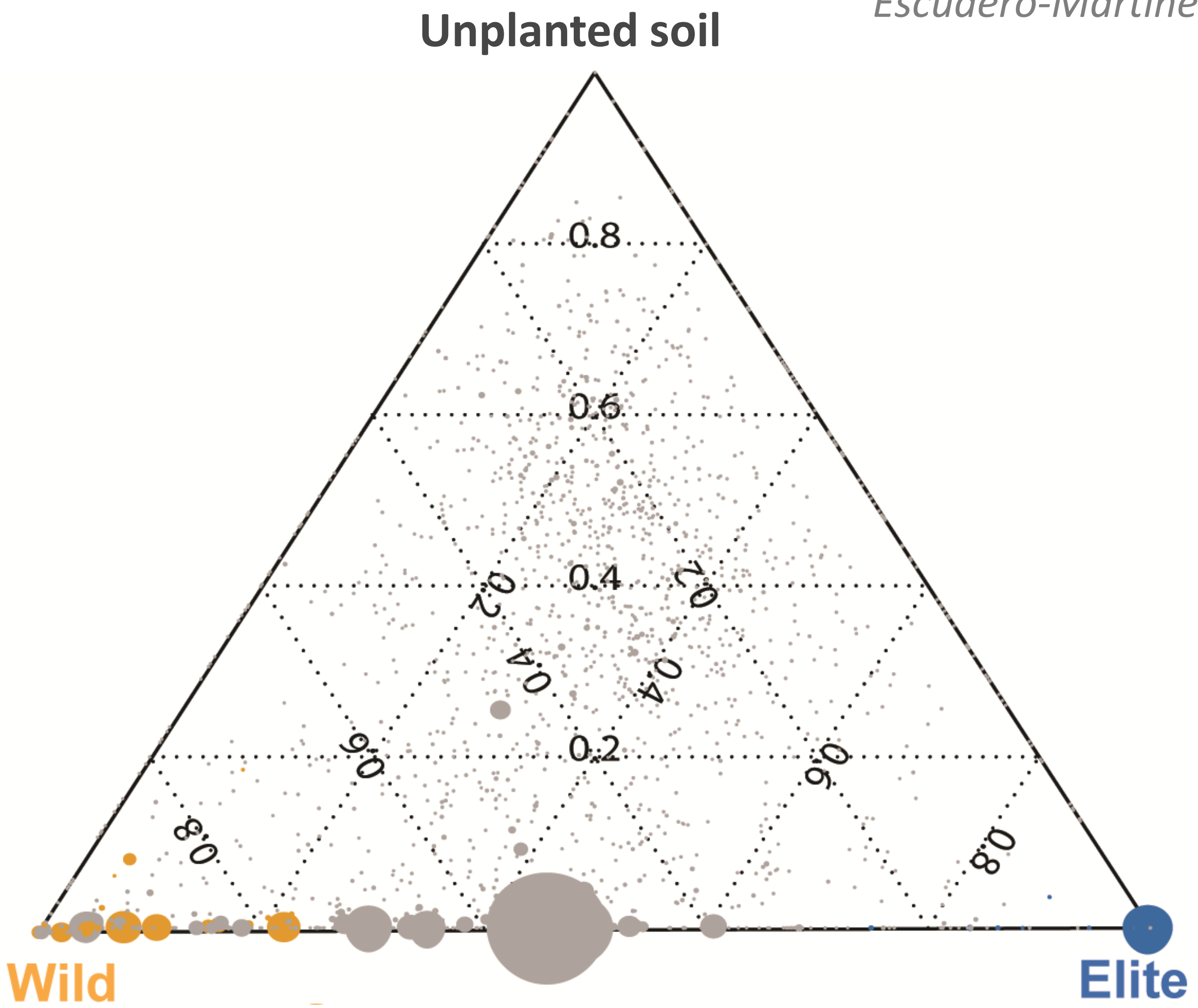


Maurer *et al.* (2015) BMC Genomics

- Multi-parent Nested Association Mapping (NAM)
- 25 **Wild barley** genotypes x Barke
- **Family 15**:HID144 wild parent crossed with the elite cultivar Barke
- Microbial composition/abundance as phenotype in mapping to identify the genetic region(s) responsible for the plant host microbiota recruitment

Is the NAM population suitable?

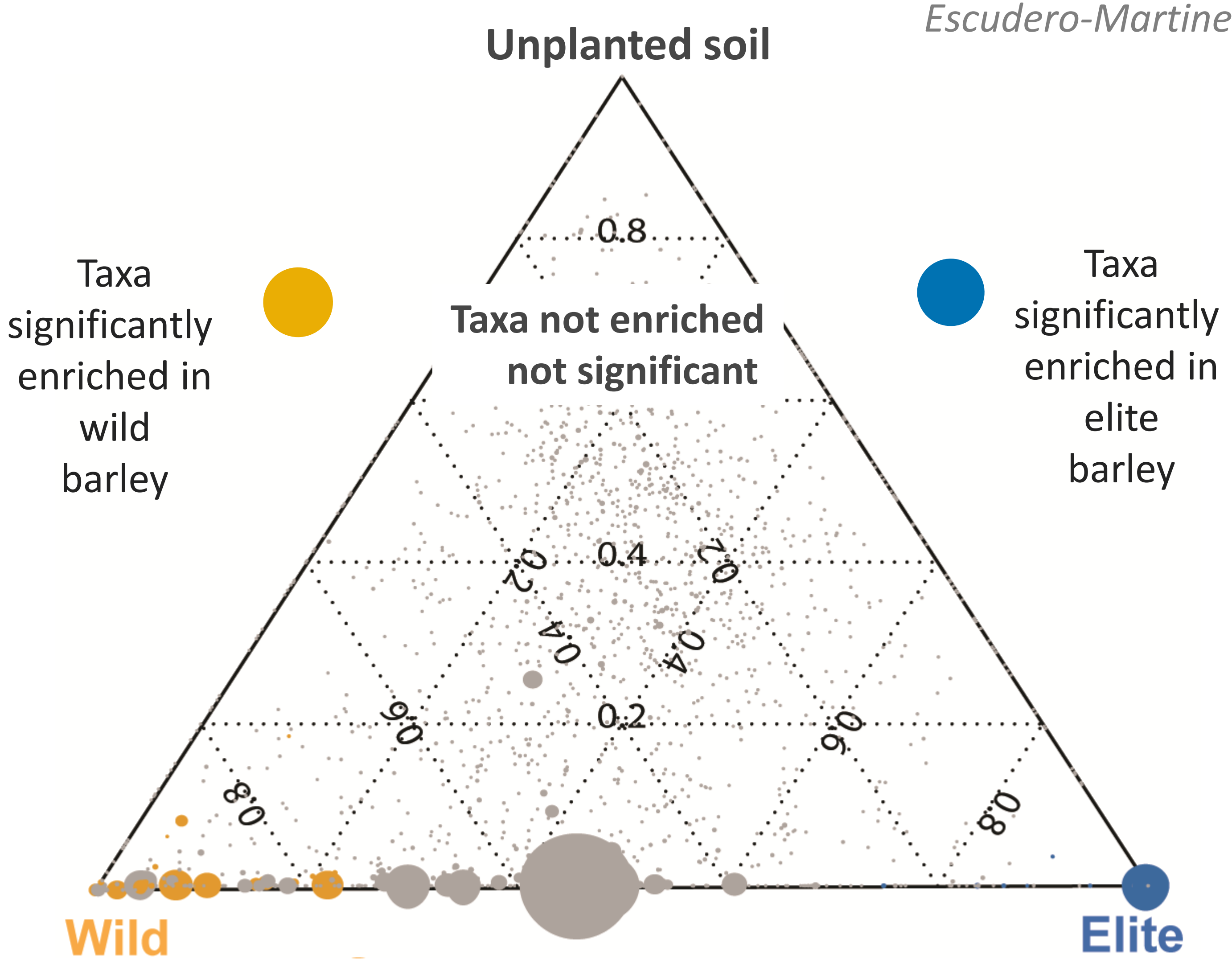
Escudero-Martinez, Coulter, et al. submitted



ASVs agglomerated at ASV level differentially enriched at P<0.05 (Wald test DESeq2, FDR corrected)

Wild and elite parents host contrasting bacterial microbiotas

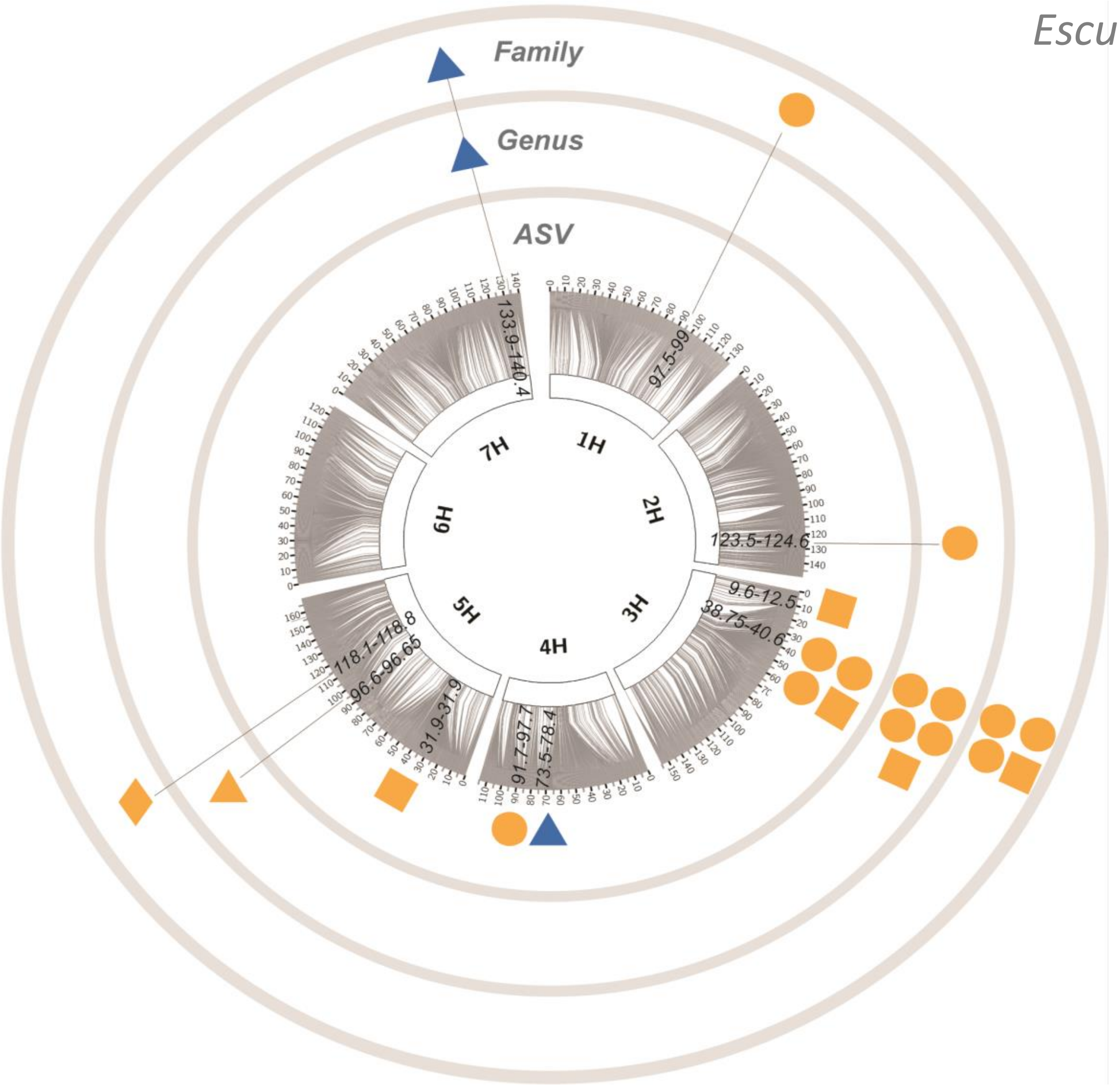
Escudero-Martinez, Coulter, et al. submitted



ASVs agglomerated at ASV level differentially enriched at P<0.05 (Wald test DESeq2, FDR corrected)

Genetic map of the barley determinants of the rhizosphere bacterial microbiota

Escudero-Martinez, Coulter, et al. submitted



Bacteria enriched in Wild

Bacteria enriched in Elite

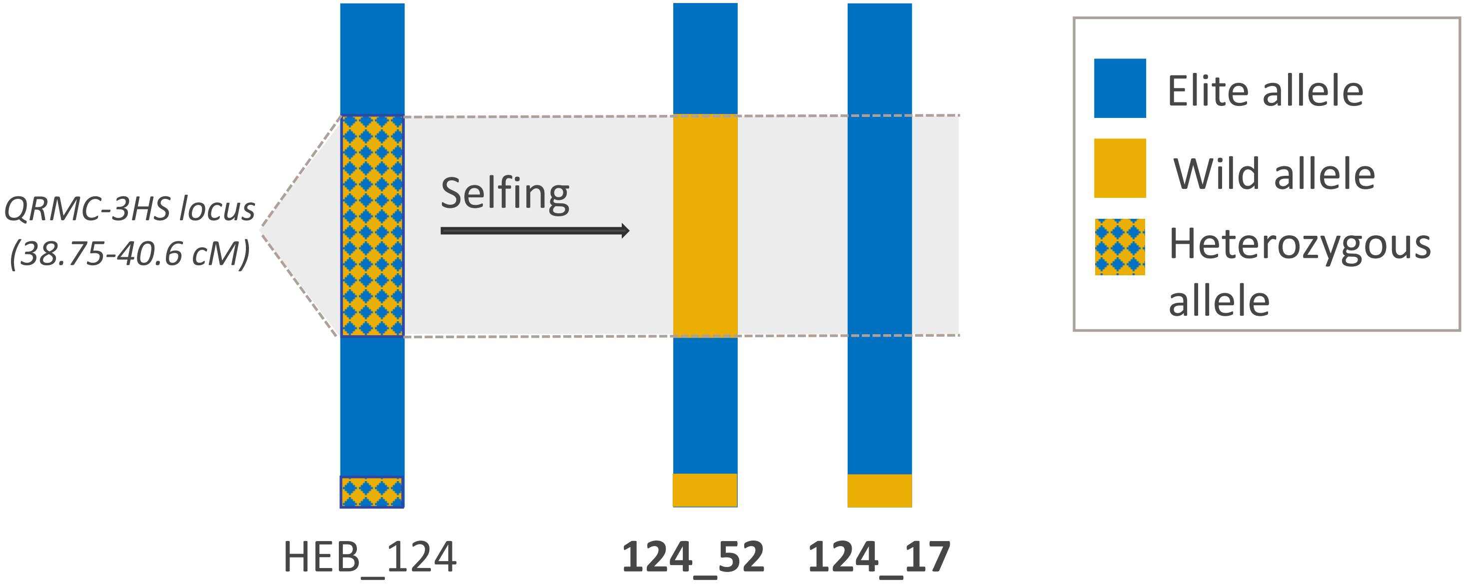
Taxonomy:

- ● Proteobacteria
- ▲ Actinobacteria
- ■ Acidobacteria
- ◆ Bacteroidota

The QRMC-3HS locus represents 5% of microbial reads

Map position defined with R/qtl using 'scanone' the mean abundance of the depicted bacterial as 'phenotypes' and the Illumina iSelect 9K markers, Expectation-maximization (EM) algorithm, LODs supported by marker regression or genome-wide significance threshold are presented

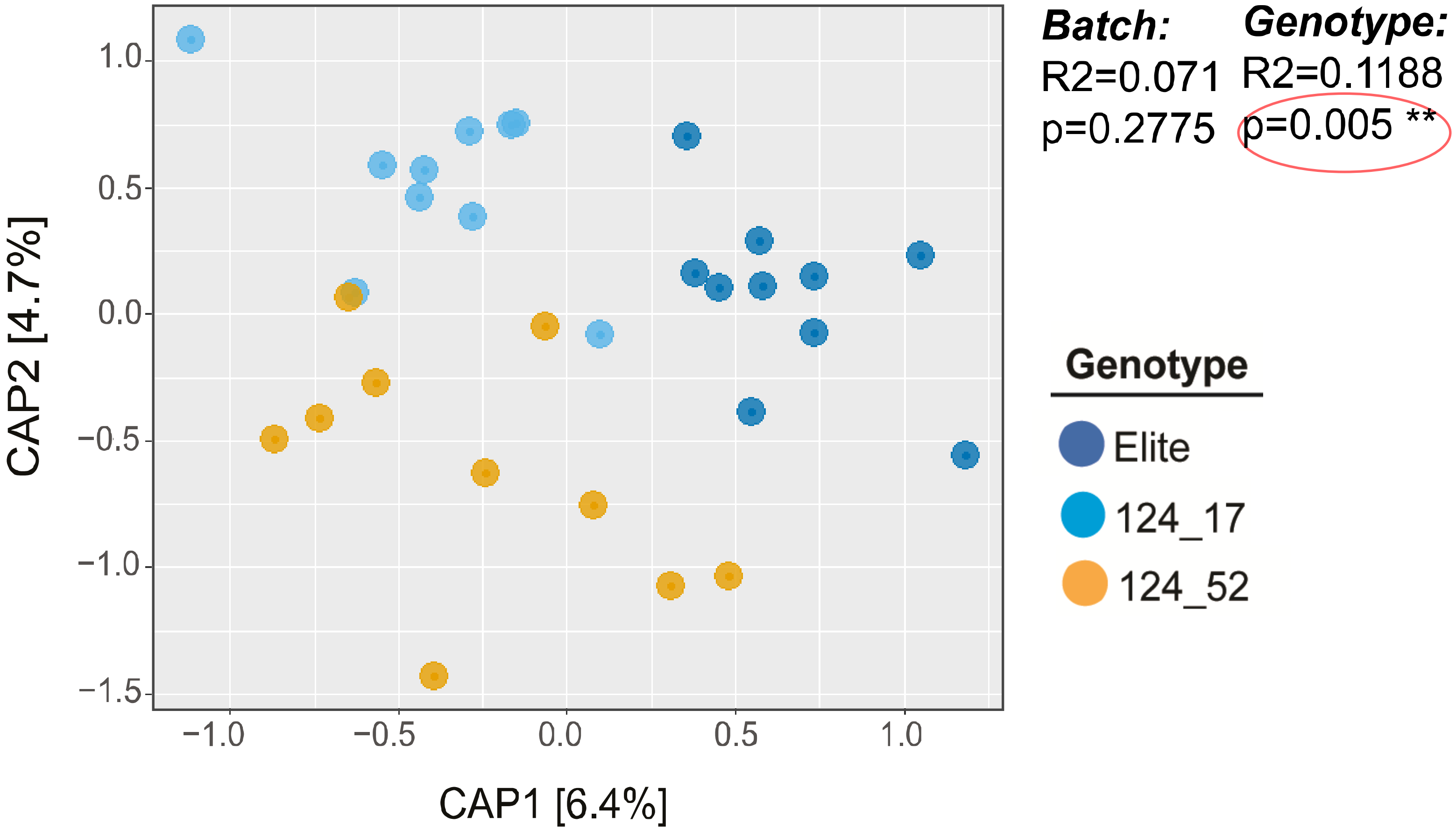
Generating sibling lines with “*wild*” and “elite” alleles at the QRMC-3HS locus



	Genotype	*3H locus type*	*Maker identity with Elite (%)*
●	**Elite parent**	Elite	100
●	**124_52**	Wild-like	93.3
●	**124_17**	Elite-like	95.5
	Wild parent	Wild	60.3

Marker genotyping: Data based on at least two independent lines per genotype processed with the 50k Illumina Infinium iSelect genotyping platform

Sibling lines with “wild” alleles at the QRMC-3HS locus shift the microbiota composition



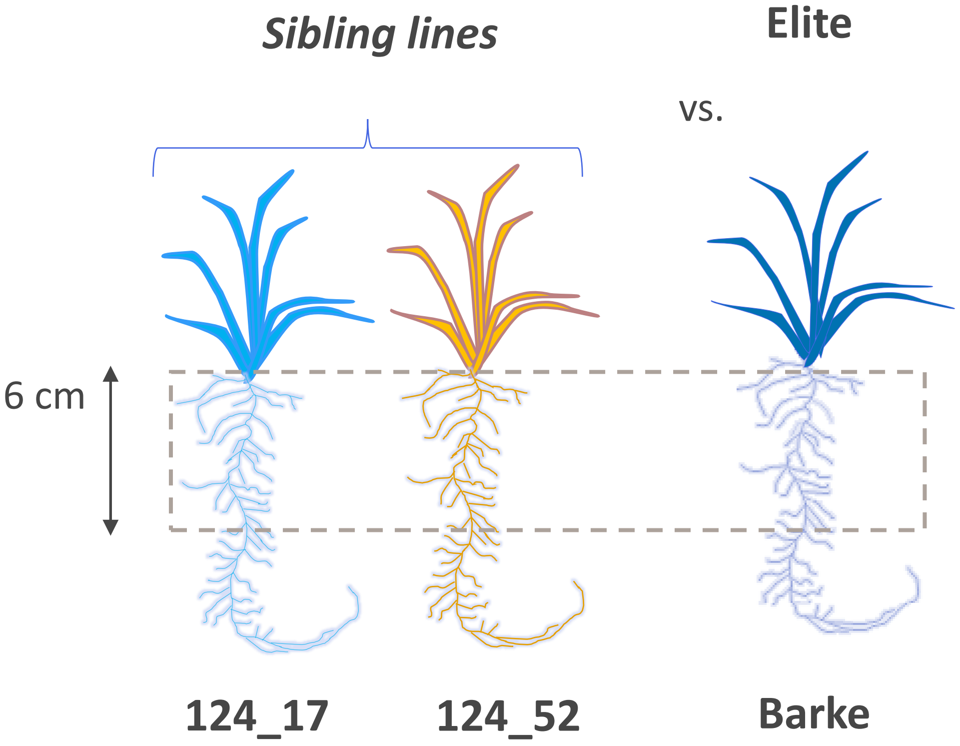
Batch:
R2=0.071
p=0.2775

Genotype:
R2=0.1188
p=0.005 **

Escudero-Martinez, Coulter, et al. submitted

Canonical Analysis of Principal coordinates (CAP) of Bray-Curtis. ANOVA p <0.01, 5.000 permutations

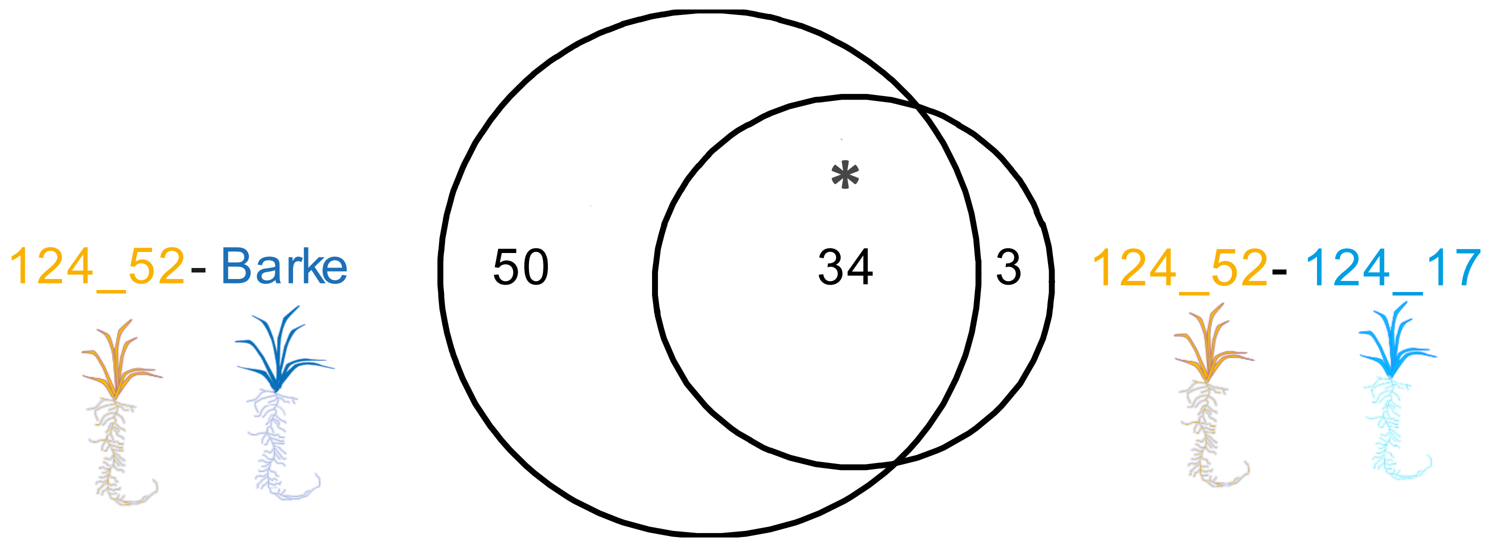
Can we use roots RNA-seq to identify candidate genes underpinning microbiota recruitment?



Raw RNA-Seq
(~100M, 2x150bp PE reads/rep)

RNA-seq confirms differentially expressed genes (DEGs) between the sibling lines and elite roots tissue

Escudero-Martinez, Coulter, et al. submitted

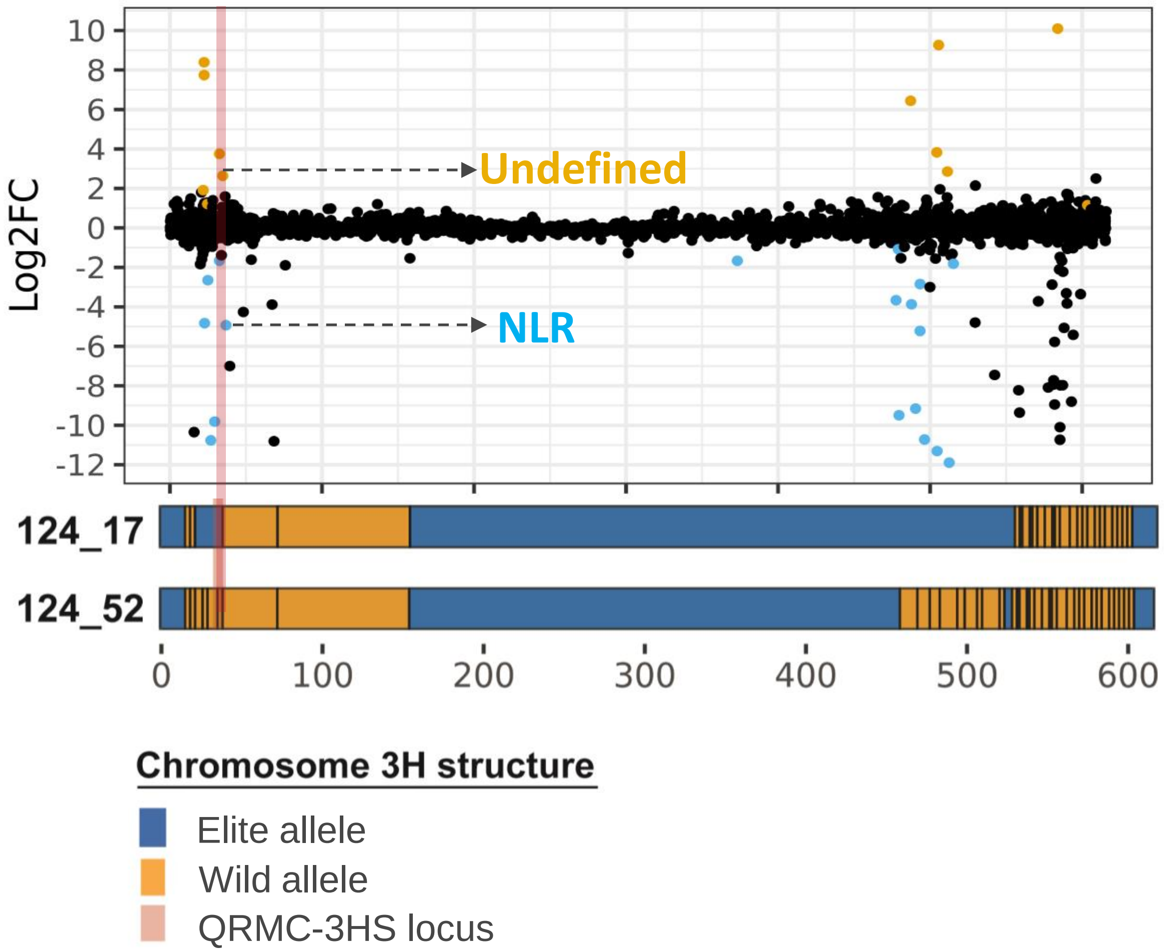


* 34 DEGs comparing line 124-52 (wild *QRMC-3HS* locus) to lines 124_17 and the elite

Differentially expressed genes displaying a P value < 0.01 (edgeR, FDR corrected)

Root DEGs mapping at the QRMC-3HS locus

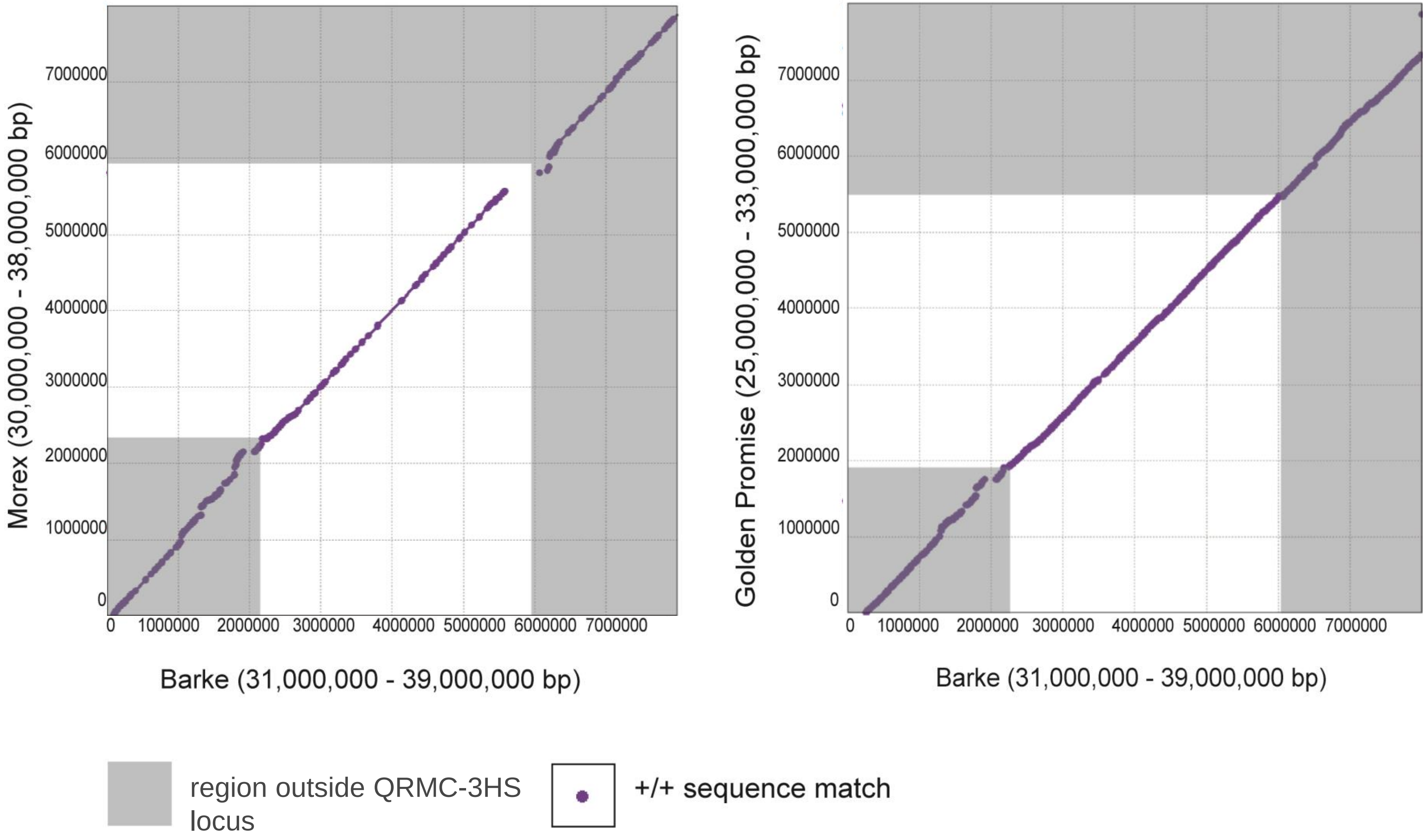
Escudero-Martinez, Coulter, et al. submitted



Differentially expressed genes displaying a P value < 0.01 (edgeR, FDR corrected)

A non-syntenic region at the QRMC-3HS locus

Escudero-Martinez, Coulter, et al. submitted



MUMmer was used to align QTL locus in Barke against multiple genomes

Conclusions

- Microbiota composition is a quantitative and genetically tractable trait in barley
- We have located and validated a major regulator of this trait on chromosome 3H
- RNA-seq on the root of the sibling lines identified candidate genes for the microbiota composition phenotype
- Only the resistant gene falls in a dissimilar region across the barley pangenome

Escudero-Martinez, Coulter, et al. 2021 BioRxiv

UKRI Biotechnology and Biological Sciences Research Council

BBSRC bioscience for the future

University of Dundee

The James Hutton Institute

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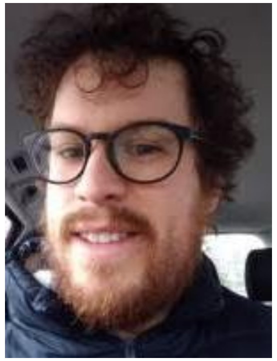
Davide


Max


Senga


Rumana


Aileen

Escudero-Martinez, Coulter, et al., (2021), BioRxiv

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Thank you for your attention!