UK CROP MICROBIOME CRYOBANK

THE UK CROP MICROBIOME CRYOBANK, A UTILITY TO SUPPORT PHYTOBIOMES RESEARCH

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Summary

- The need to underpin Phytobiomes Research – Matthew Ryan
- The project, partners and ambition
- An approach to Cryobanking – why? and how?
- Resource generation and functional characterisation: Tim Mauchline
- The data paradigm – a genomic approach: Nicola Holden
- The utility of the resource: Jake Malone
- Looking to the future, compliance and opportunity

“State of the Ark”

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- Existing culture collections: Only axenic cultures are ‘generally’ stored
- Cultures are rarely stored as consortia or as ‘microbiomes’
- Human and animal microbes are often prioritised over environmental and plant associated organisms

- **Culture collections** (generally) preserve living organisms and their derivatives, often propagatable

- **Biobanks** are either reference collections of dead/fixed material such as tissue banks & museum collections or ‘viable’ seed banks or medical related e.g. blood banks, IVF clinics

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Examples of larger Culture Collections with global focus

NRRL Agricultural Research Service Culture Collection

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DSMZ Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH

The World Data Centre for Microorganisms provides a global view of microorganisms held, and there are almost 3.2 million strains available for reference and research, The data are extracted from 793 culture collections in 77 countries and regions (2019)

Example national / regional networks:
Latin American Federation for Culture Collections (FELACC)
European Confederation of Culture Collections (ECCO)
United States Culture Collection Network (USCCN)
UK Biological Resource Centre Network (UKBRCN)

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The Challenge – recognising the need

UNITED STATES CULTURE COLLECTION NETWORK

UK PLANT MICROBIOME INITIATIVE

microbiome support

2015, Fort Collins > Phytobiomes challenge

Innovate UK
Knowledge Transfer Network

Rothamsted Open Innovation Forum, 2017 and KTN Microbiome Roadmap

EU Microbiome Support 2018-2022

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Why do we need 'microbiome' biobanks?

- To aid the development of standards
- To allow deposits to ensure compliance with legislation including IP, Nagoya (ABS) etc.
- As a source of new potential products for industry, medical and environmental applications
- To protect IP e.g. stable storage of SynComs, LBP's, outputs from academia and industry
- For biodiversity conservation and to mitigate against future threats
- To provide resources to underpin research, furthering our scientific knowledge but also **to ensure the reproducibility and stringency of research**
- To ensure the link between provenance, sample and bioinformatic metadata

Fragmentation of infrastructure is common in Europe and beyond

Horizon2020
European Union Funding
for Research & Innovation

Ryan, M. J., et al. "**Development of Microbiome Biobanks–Challenges and Opportunities**." *Trends in Microbiology* (2020)

The aim of preserving the microbiome

For isolates: To store and recover culturable microbes, without change to their genomic or physiological integrity

For Nucleic acids: To store for future analysis where the host material may no longer be available

For environmental samples (e.g. soil) and biological material

To store samples, in such a format, that allows:

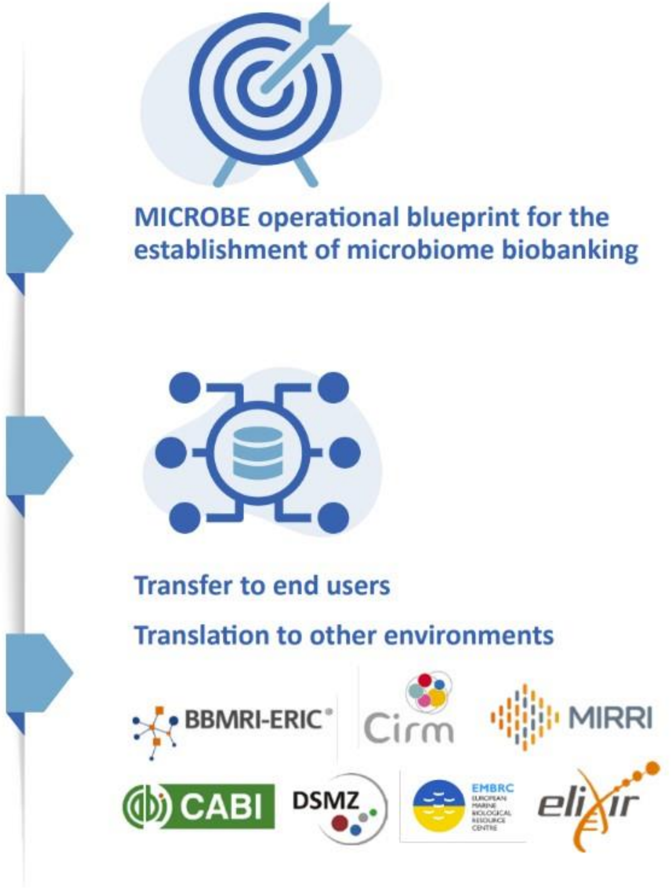
I) microbes to be recovered and utilized at a later date
ii) future culture of currently unculturable microbes where technologies may develop for future *in vitro* cultivation
iii) material to be utilised for future genomic analysis

MICROBE
the Microbiome Biobanking (RI) Enabler

The EU Microbiome (RI) Biobanking Enabler (EU Microbe)

Partners

AIT (Lead)
CABI
DSMZ
EMBL
HMGU
INRAe
MUG
RTD services
Sorbonne (Roscoff)



This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101094353

Project Outcomes:

- Validated protocols for preservation, isolation and co-cultivation of complex microbiome samples
- Novel isolates representative of key microbiome members from different domains
- Novel synthetic consortia reproducing the key functionalities of selected ecosystems
- Customised data infrastructure tools for microbiome biobanking
- Guidelines for implementation of standardised microbiome biobanking workflows in selected fields
- Guidelines for establishment of ethical and legal framework conditions that enable microbiome biobanking
- Business models for the implementation and exploitation of novel microbiome-based technologies and resources
- Portfolio of training and educational resources

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Translating to the Microbiome

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The UK Crop Microbiome Cryobank (UKCMCB) has established a cryopreserved and characterised crop microbiome resource (including baseline sample characterisation and metagenomic analysis thereof) to underpin UK and international crop research

***The UKCMCB provides a comprehensive platform to facilitate research towards optimising plant yield in an integrated crop management framework*. This provides an infrastructural baseline for other projects and science-based activities**

Focussing on key UK crops: wheat, OSR (Canola), barley, oats, sugar beet and beans

A collaboration between five institutes with expertise in crop health, bioinformatics and biological resource collection

UKRI Biotechnology and Biological Sciences Research Council

www.agmicrobiomebase.org

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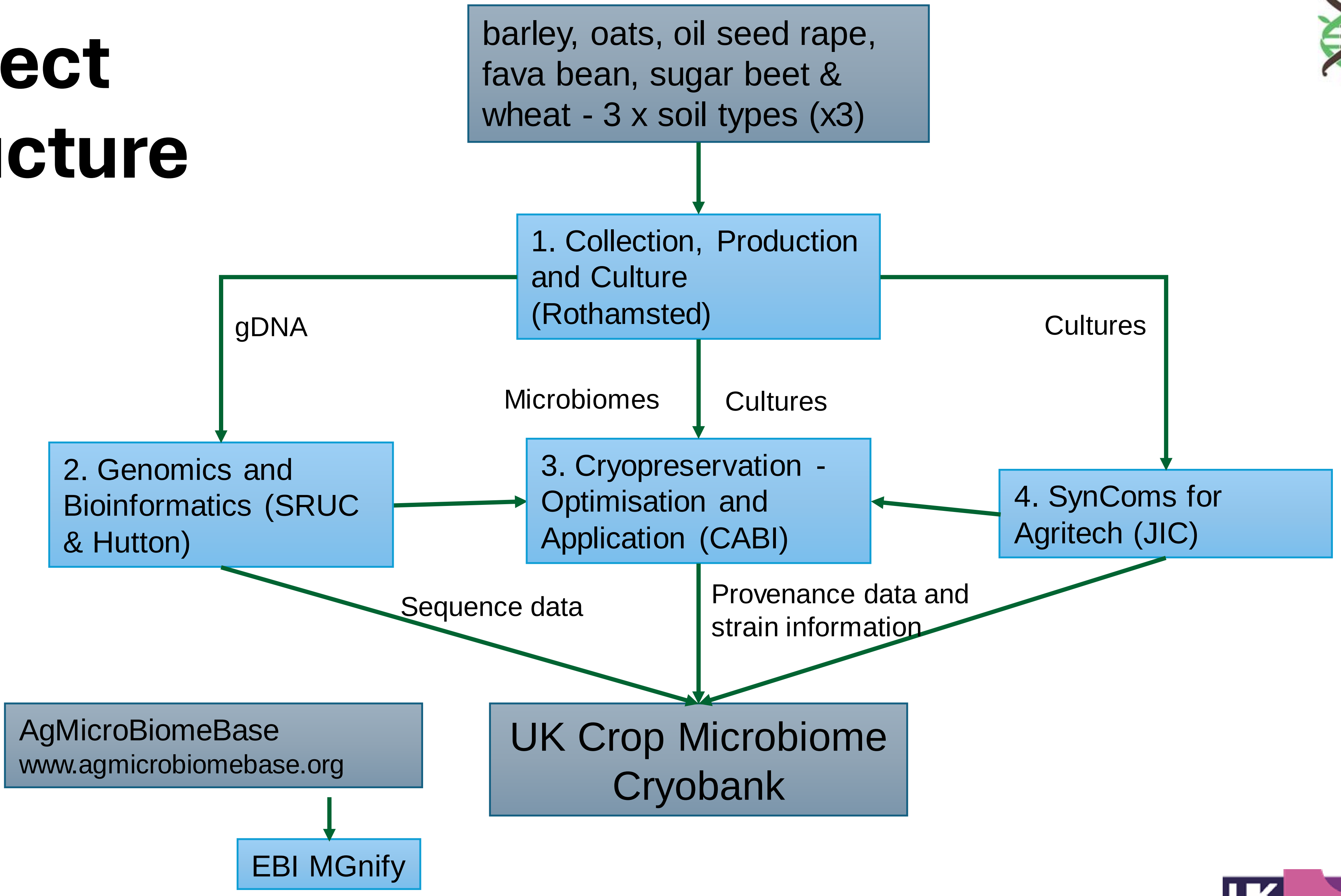
Project Structure



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Biotechnology and Biological Sciences Research Council

Cryopreservation - utilising two methods

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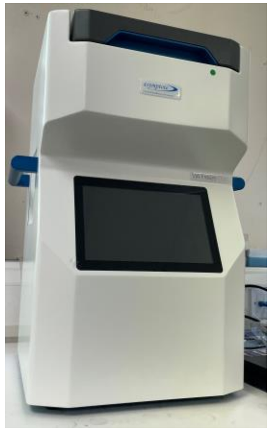
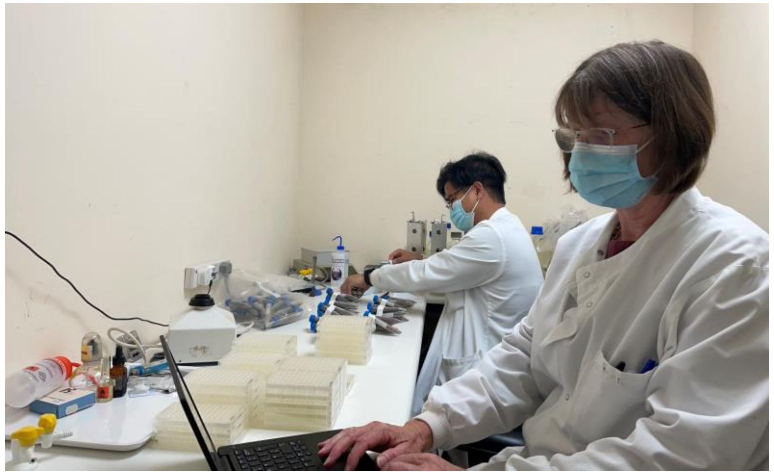


- **Controlled rate cooling** using a Stirling Cryocooler

Will capture most freeze tolerant organisms



- An **encapsulation vitrification** approach

Will capture more delicate and
freeze recalcitrant organisms

We chose not to select freeze drying (as proved later!) or sub-optimal storage at -80^{o}C

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Construction of the 'physical' resource is complete!

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We have preserved 4,800 individual cryovials consisting of:

- 2,400 preserved by Stirling Cycle Controlled Rate Cooling
- 2,400 preserved by encapsulation dehydration

Representing all bulk soil and rhizoplane samples from the Rothamsted pot experiments

- 36,000 isolates in microtitre plates @ -196 ^{o}C

Data is being uploaded to AgMicrobiomeBase & EBI platforms

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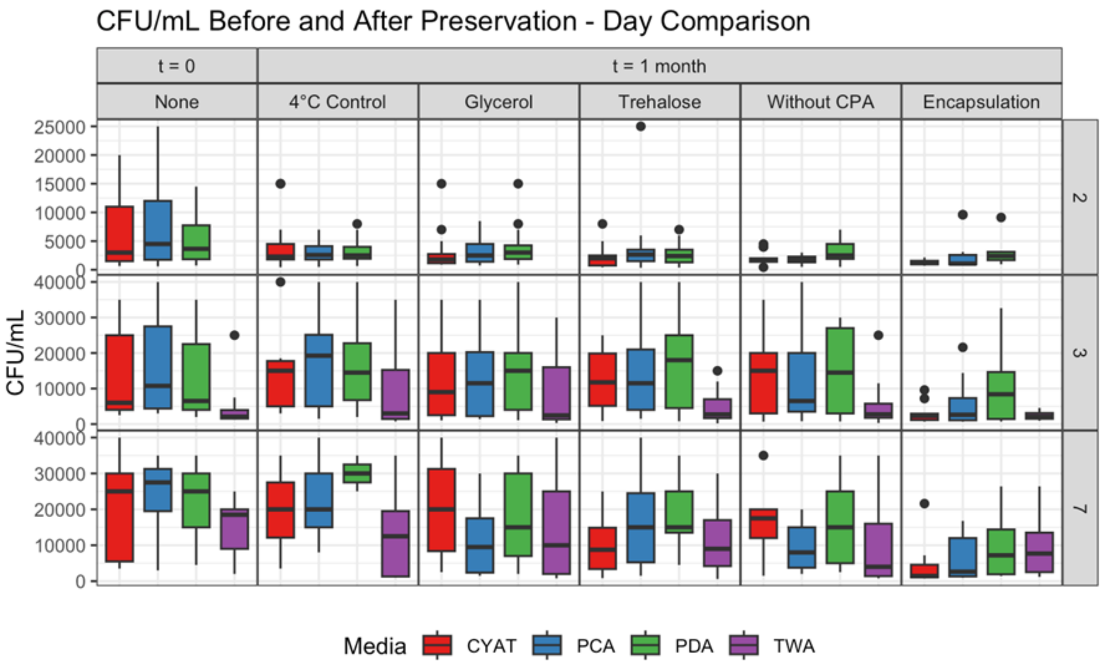
Assessing Success!

Cultures from the Rothamsted trials, preserved using Stirling Cycle controlled rate cooling, exhibit over 90% viability

Preservation methods used to preserve bulk soil and rhizosphere are successful

Awaiting data from shotgun sequencing

Functional activity is conserved after preservation but more research is required

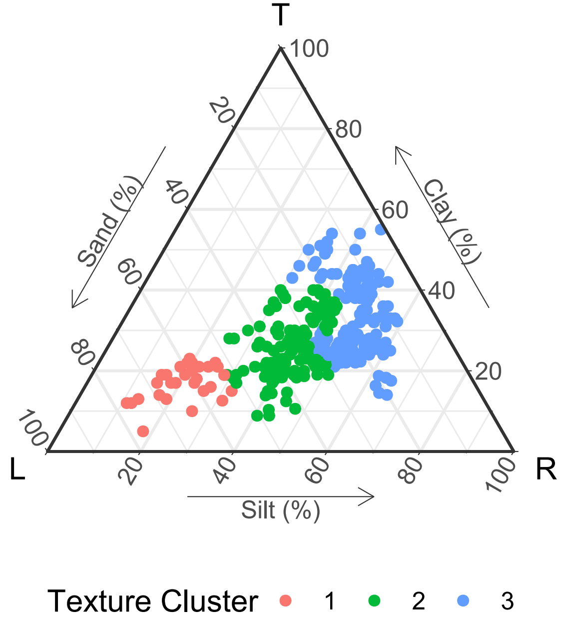


Example data, showing that fungal material is culturable post cryostorage (for illustration only)

Resource generation and functional characterisation: Tim Mauchline

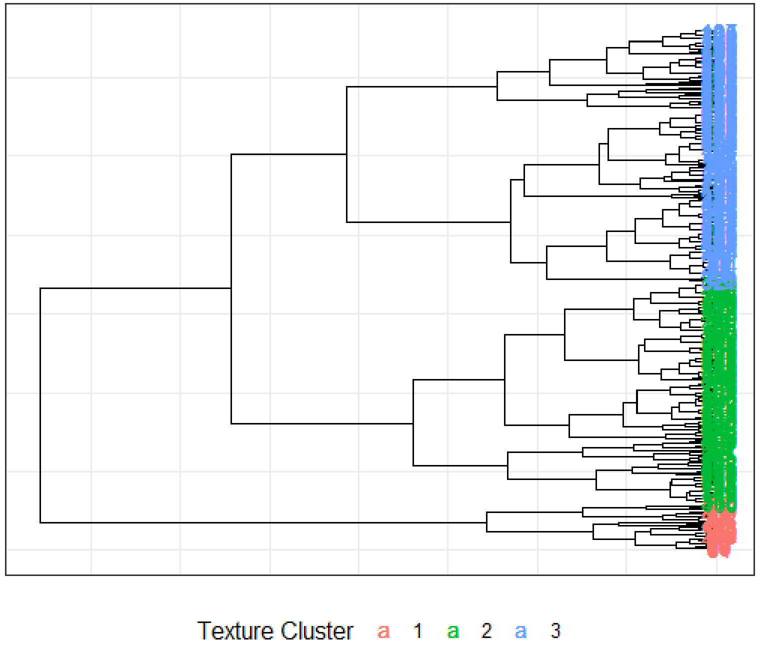
Collection of soil, plant and microbial samples and DNA extraction

Clustering Separation by texture

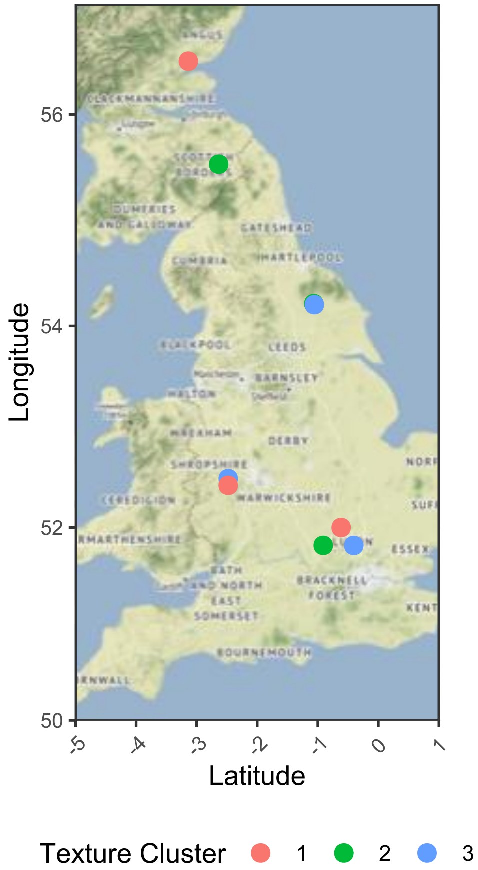


1 sand
2 clay
3 silt

Soil clustering



Selected sites



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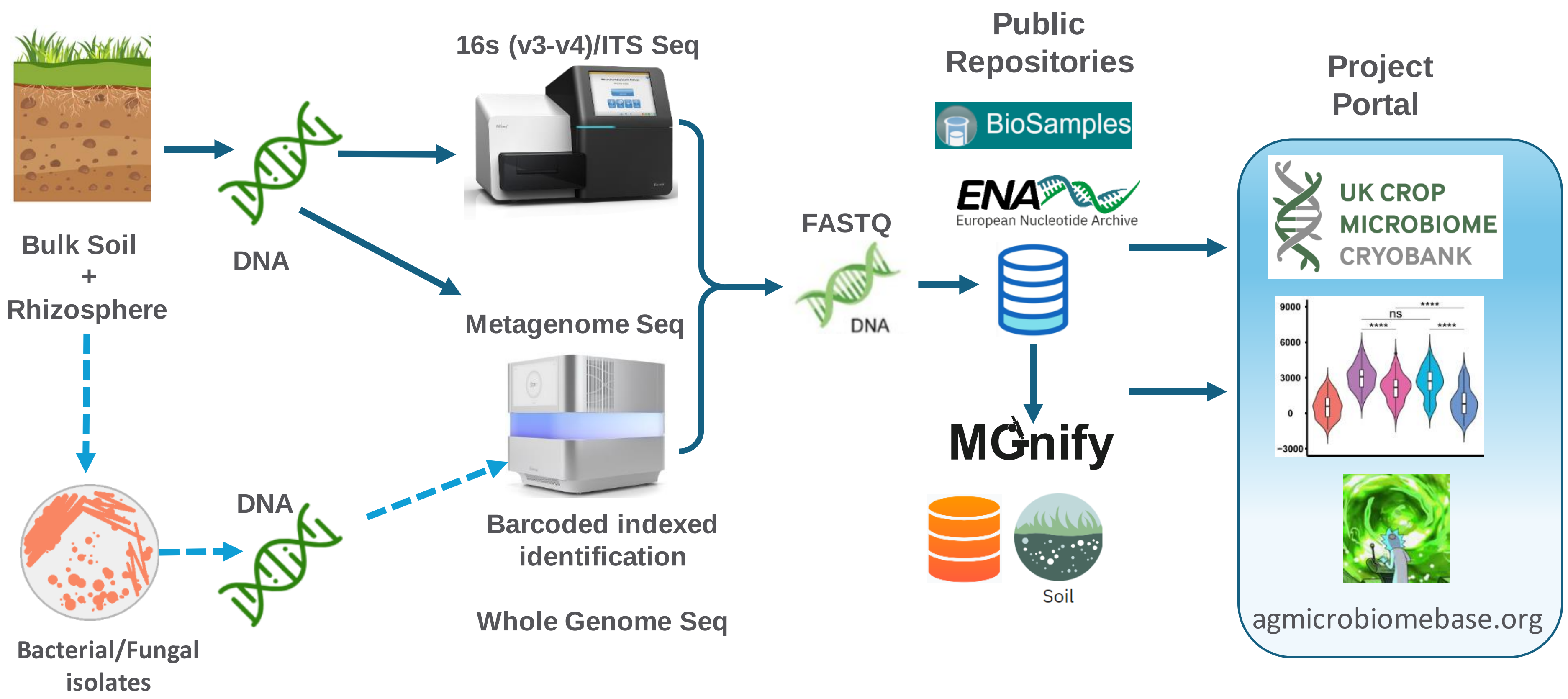
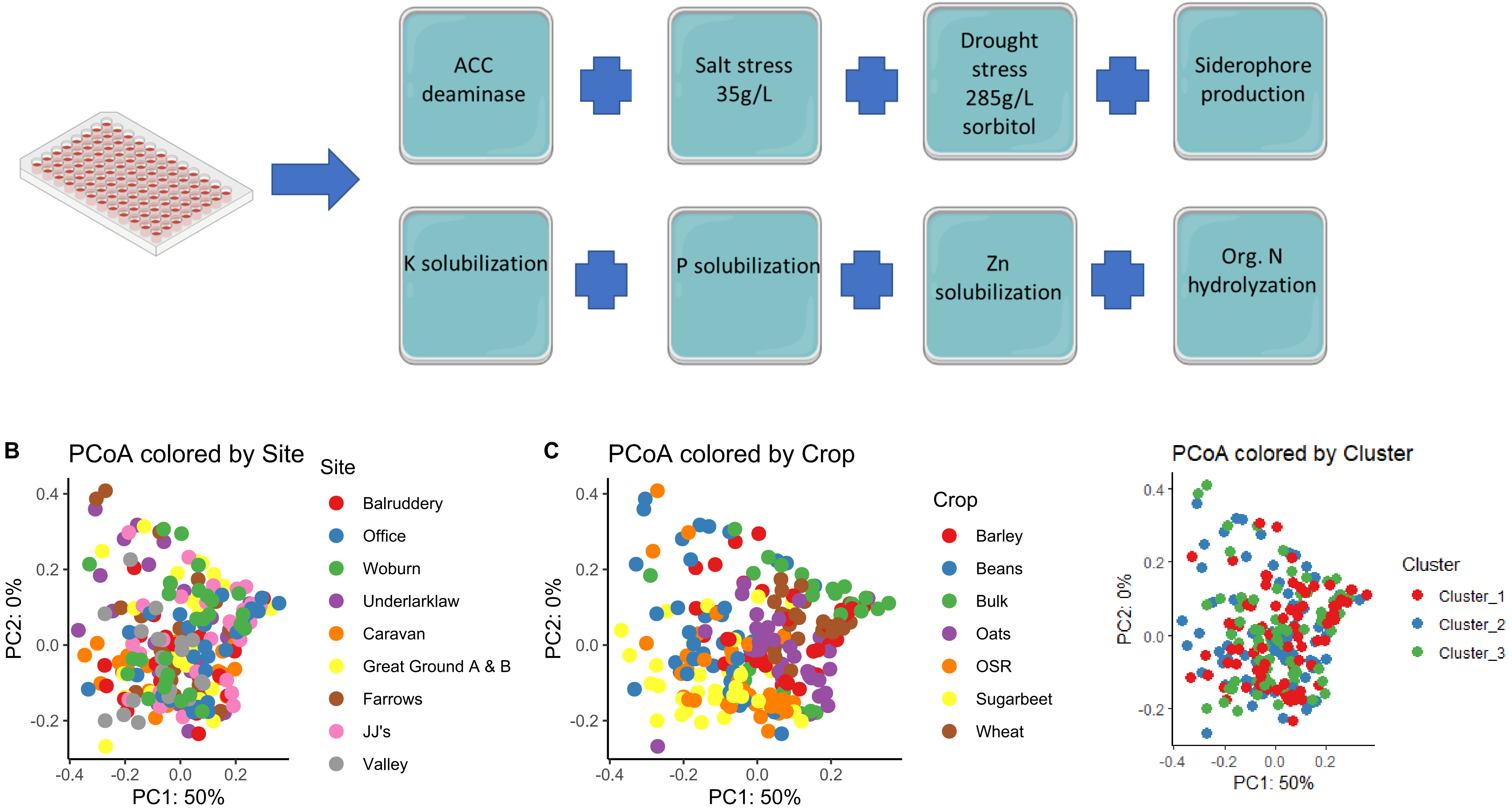
Focussing on key UK crops: wheat, OSR, barley, oats, sugar beet & beans

Crop Microbiome Cryobank Sequencing data





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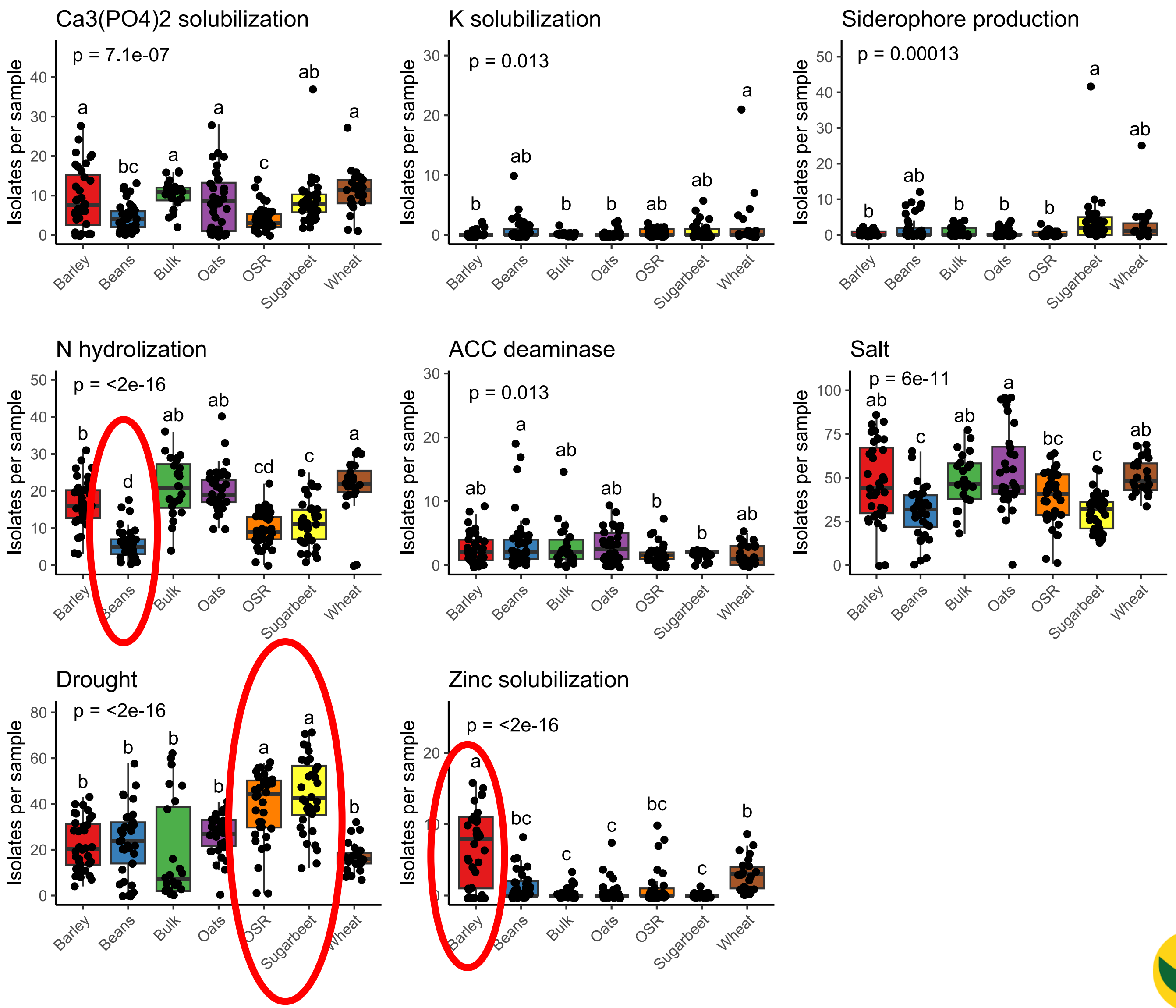
Frequency of functions per crop



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Resources for downstream analysis

- Resource based on 6 key crops + bulk soils (wheat, barley, oats, oil seed rape, faba beans and sugar beet)
- Well characterised soils (ASSIST network or from JHI and Rothamsted farms)
- Amplicon sequencing, metagenomics, soil metadata
- Cryopreserved isolates (36,000) and whole soils
- Isolate collection metabarcoding (in progress)
- Functions can be discriminated by crop type as opposed to soil cluster or location e.g. Barley with Zn
- Individual isolates typically have limited functional range

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The data paradigm – a genomic approach: Nicola Holden

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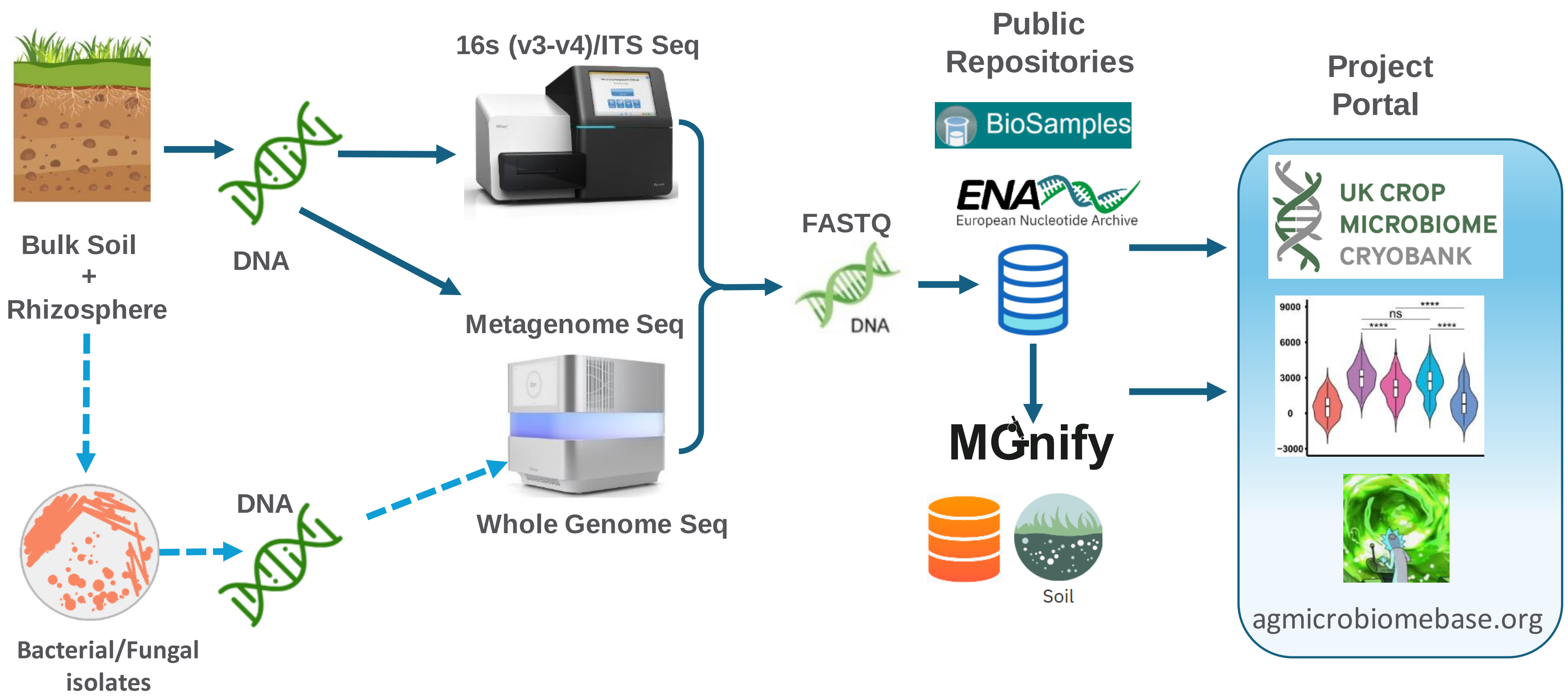
Crop Microbiome Cryobank Sequencing strategy



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Crop Microbiome Cryobank Data Organisation

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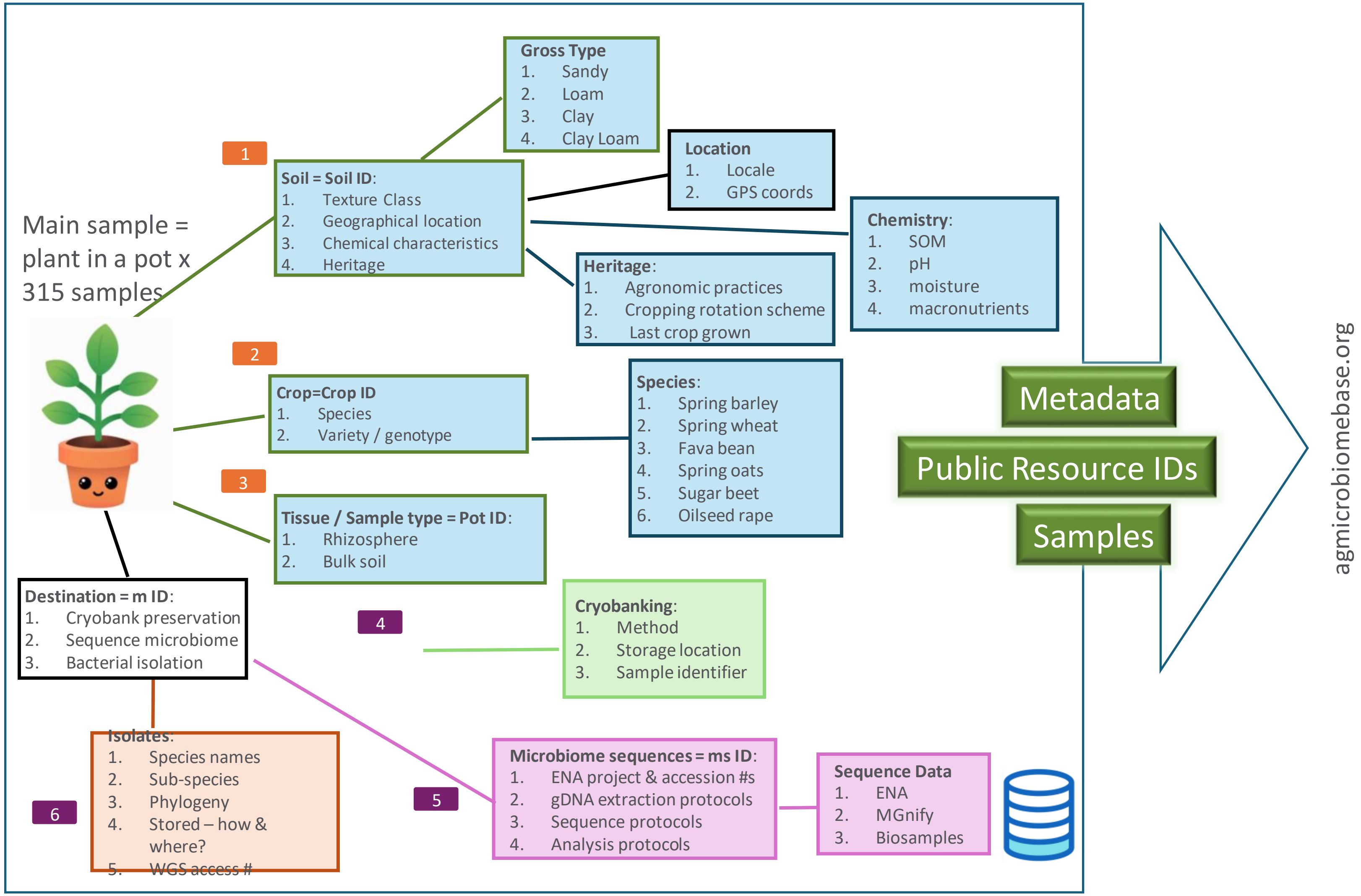

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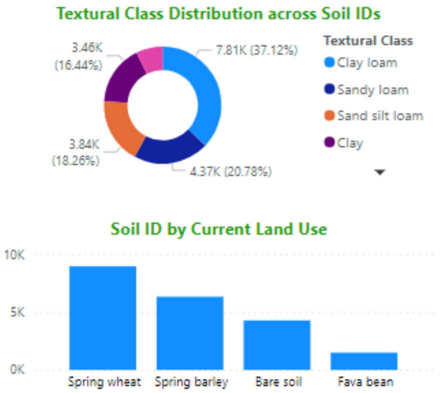
UK Crop Microbiome Cryobank Resource

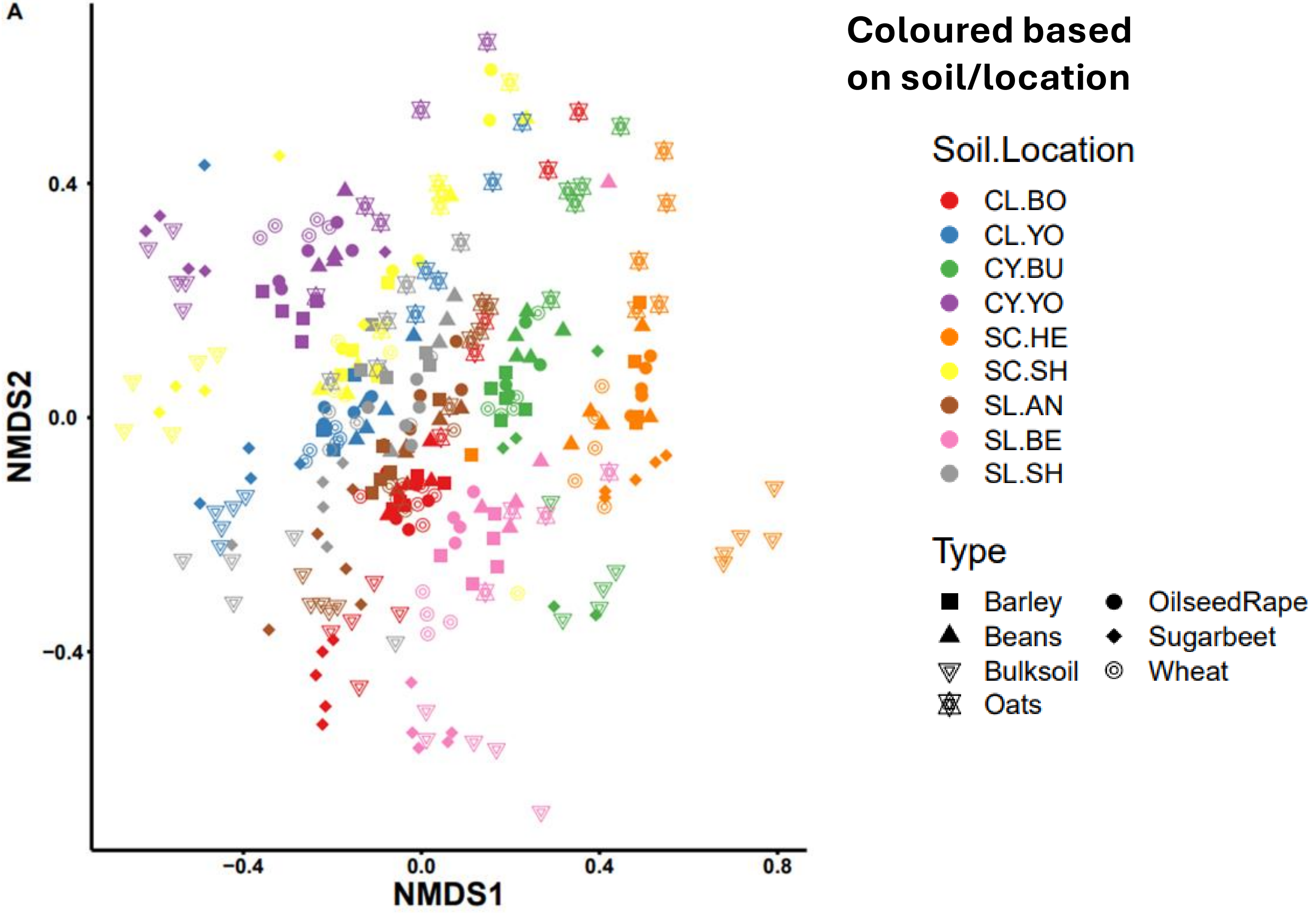
- agmicrobiomebase.org
- Open access data catalogue
- Interface to integrate, query and visualise sequences & metadata
- Direct link to sequence repositories, ENA & MGnify
- Development: 16S amplicon, Spring Wheat



https://agmicrobiomebase.org

Soil ID	Sample Name	Sample Volume	Storage Conditions	Total C	Total N	Olsen P	Total Organic C	Microbiome Run | AccessionLink
CL-BO	Underlarklaw	400 l	4° C	1.71	0.16	33.09		https://www.ebi.ac.uk/ena/browser/view/ERR12037116
CL-BO	Underlarklaw	400 l	4° C	1.71	0.16	33.09		https://www.ebi.ac.uk/ena/browser/view/ERR12037116
CL-BO	Underlarklaw	400 l	4° C	1.71	0.16	33.09		https://www.ebi.ac.uk/ena/browser/view/ERR12037116
CL-BO	Underlarklaw	400 l	4° C	1.71	0.16	33.09		https://www.ebi.ac.uk/ena/browser/view/ERR12037116

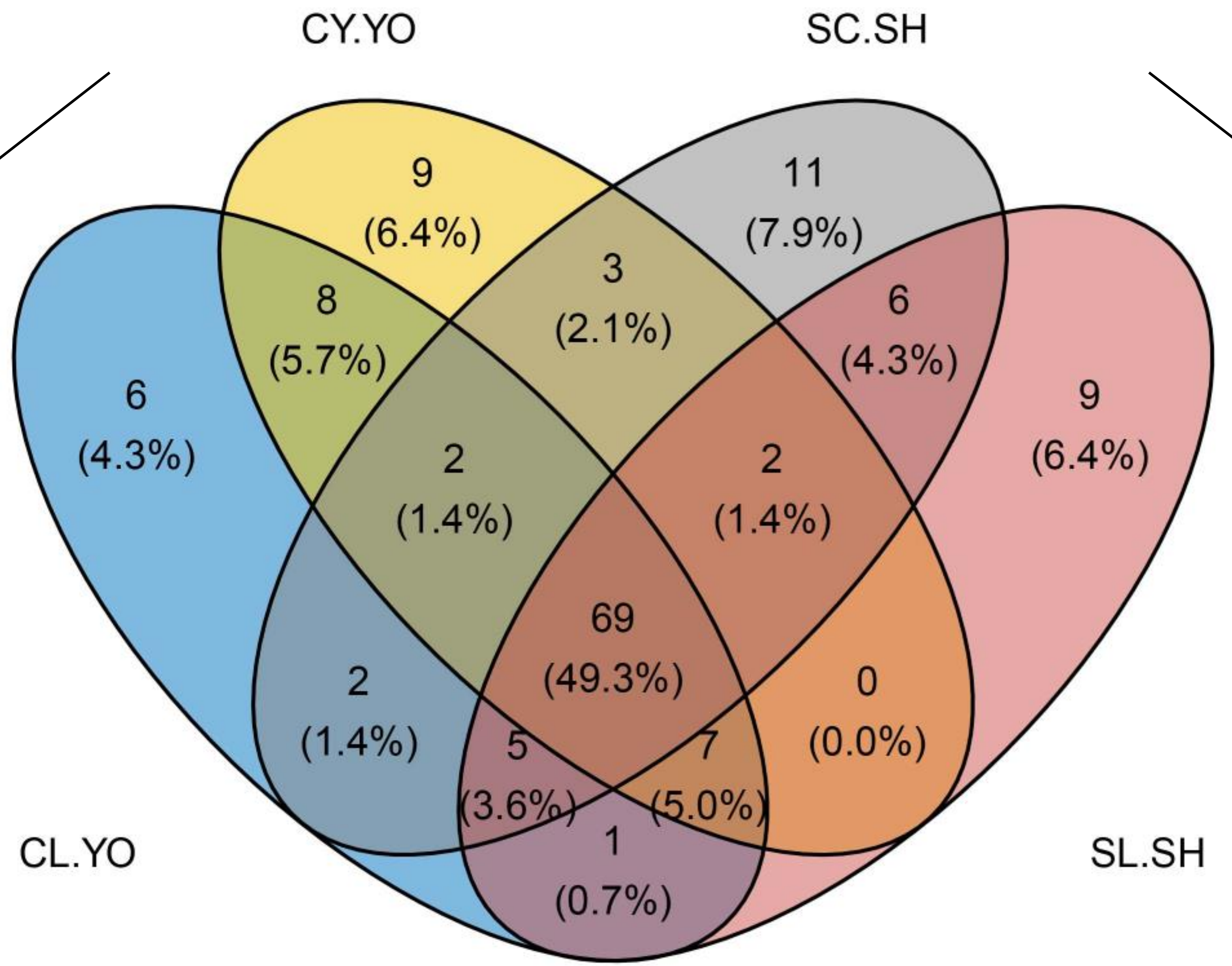
Amplicon sequence analysis:
Location Driven Microbe Recruitment



Coloured based on soil/location

Defining the core microbiome:
Understanding taxonomic commonalities

Same locale
Different clay
soils

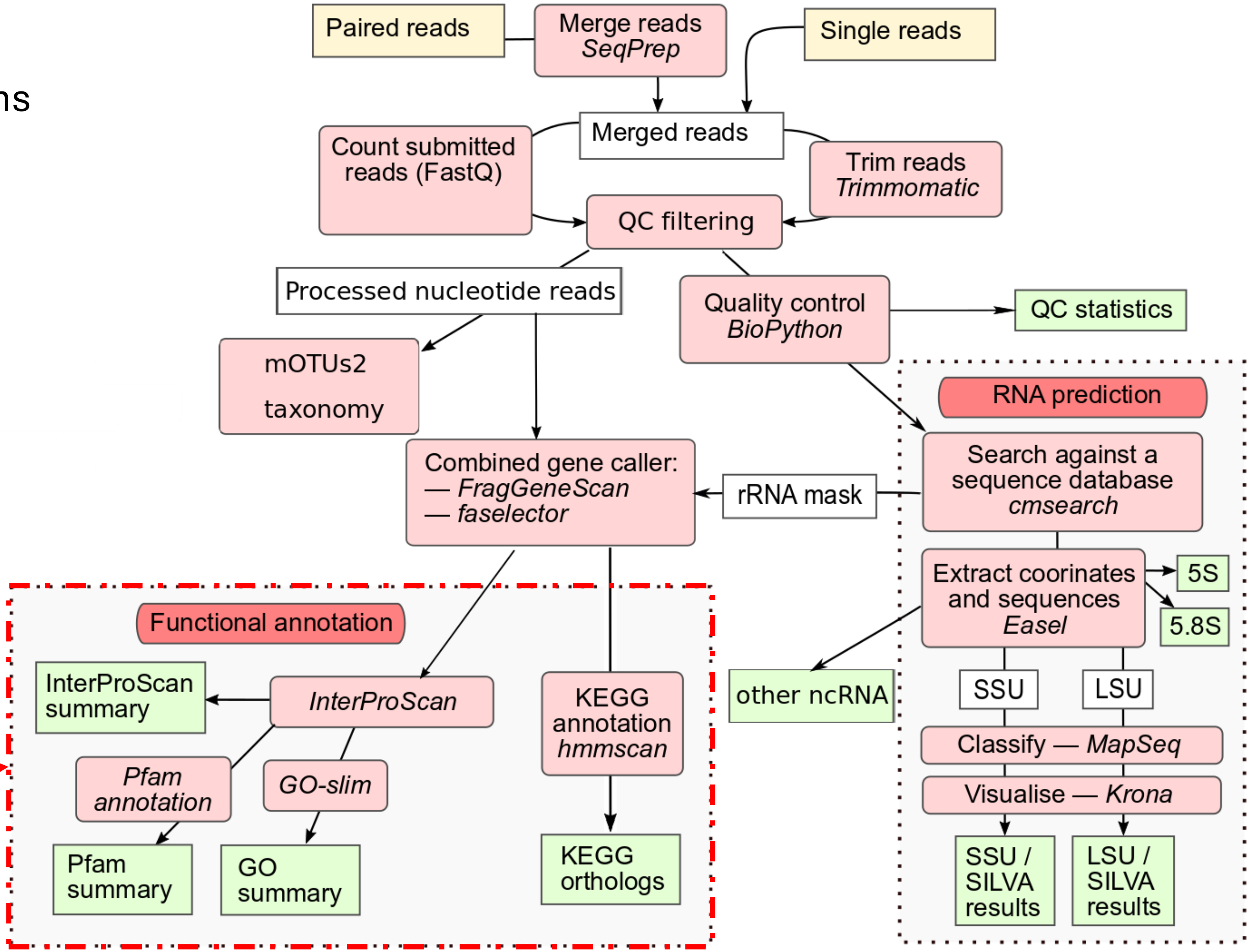


Same locale
Different silty
soils

Crop = sugarbeet

MGnify Metagenome pipeline

All soil/locale combinations
x
Spring wheat
Faba bean
No-plant control



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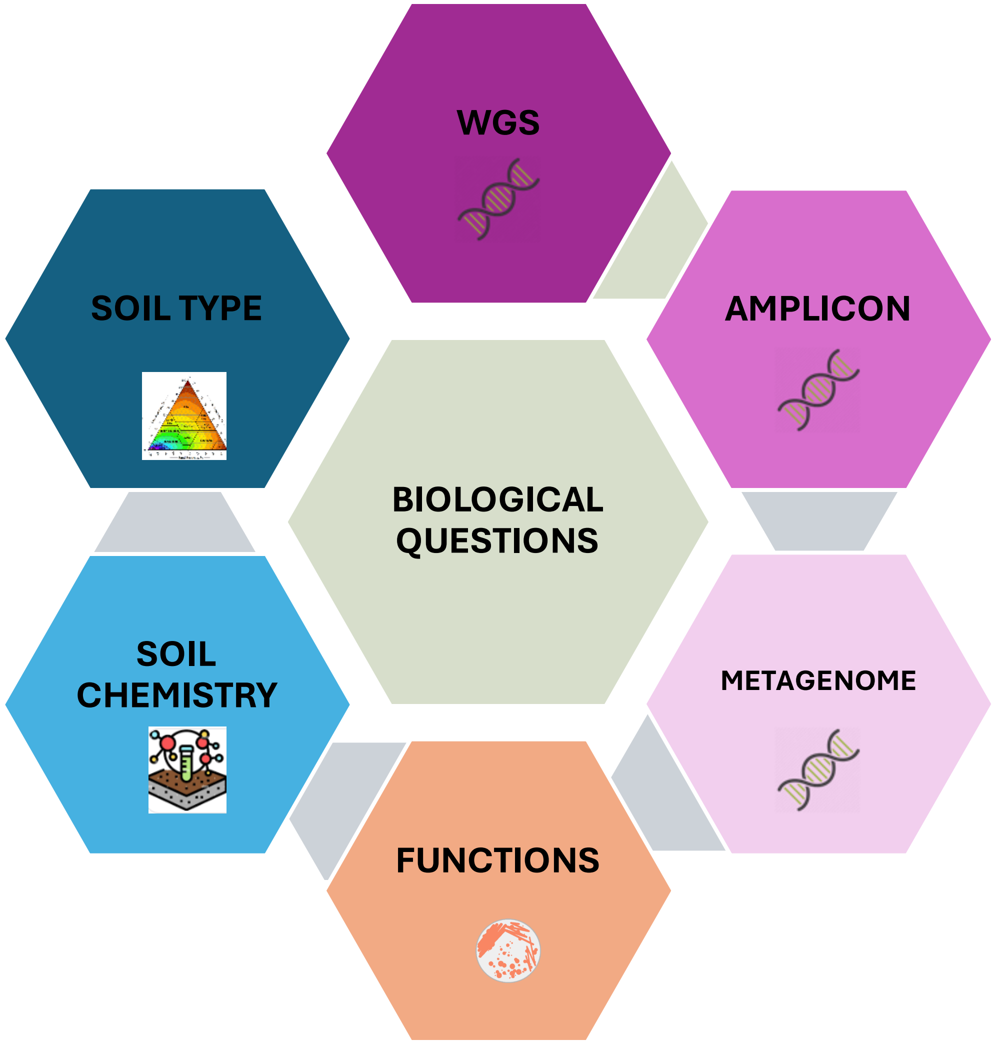
Data Integration



Soil Type
- Texture

Soil Chemistry
- pH
- Total C
- Total N

Community
- Metagenome sequence
- Biosynthetic gene clusters
- Diversity

Isolate
- Whole genome sequence
- Functional genes

Function
- Siderophores
- Pathogen inhibition
- Drought tolerance

The utility of the resource:
Jake Malone

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Demonstrating the utility of the Cryobank for PGPR isolation and SynCom construction

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Characterisation of culturable microbiota

Testing for positive plant growth-promoting traits

Creation of effective growth-promoting SynComs

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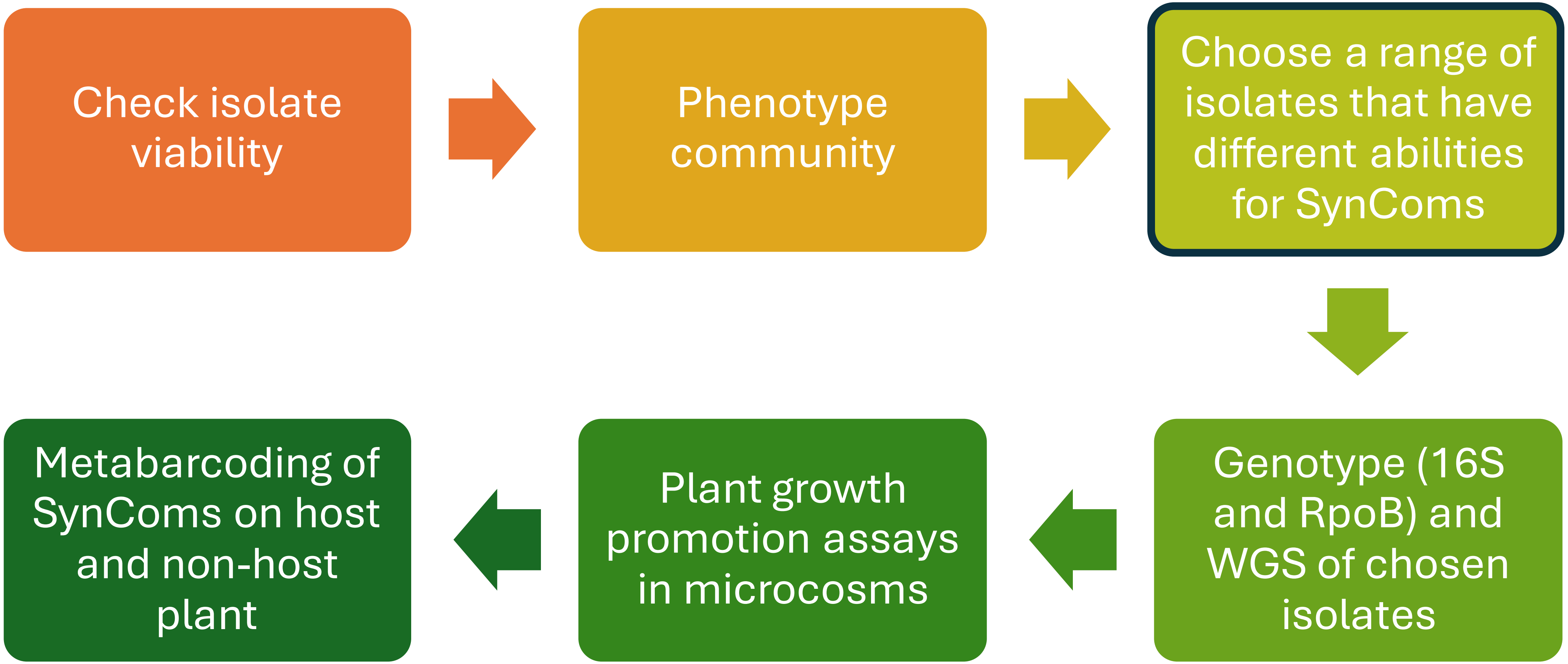
Workflow



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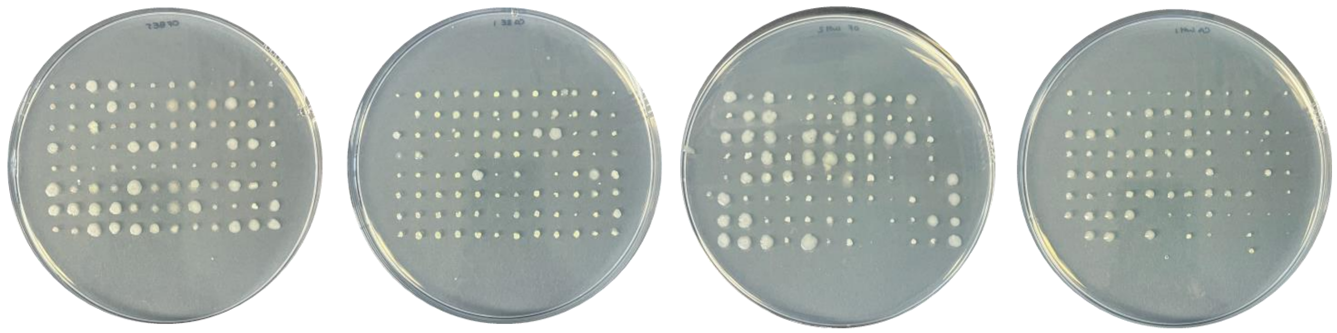
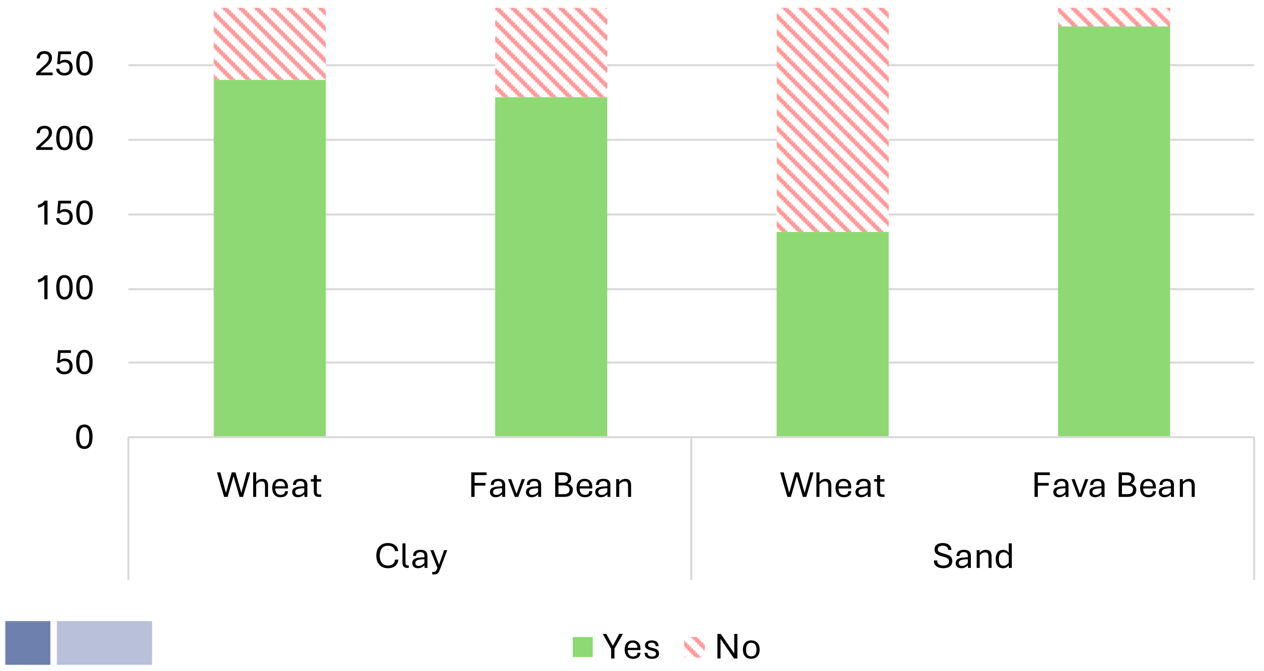
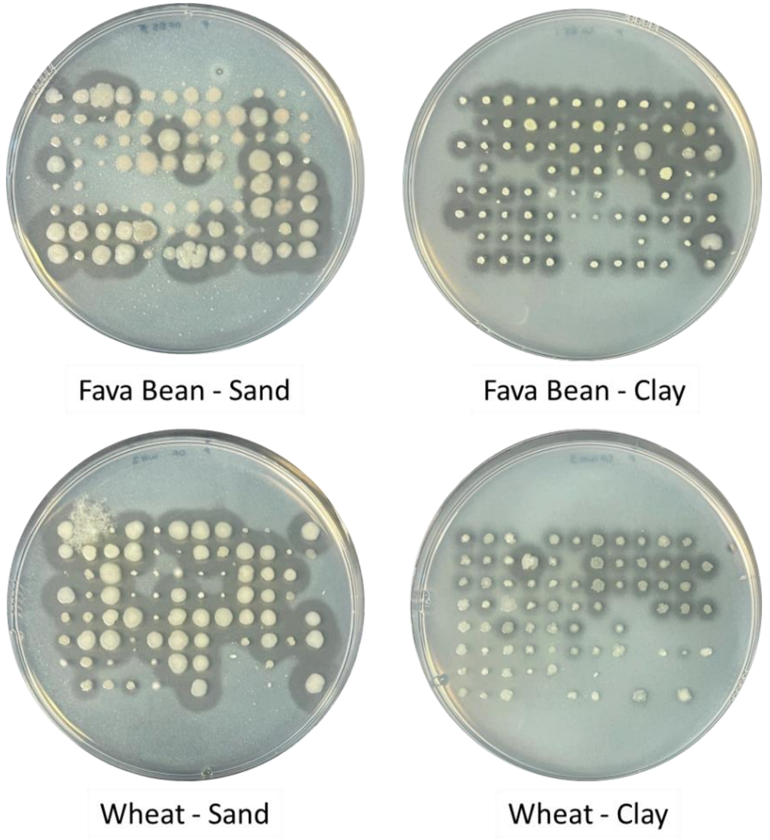
Viability of recovered isolates after 48 hours



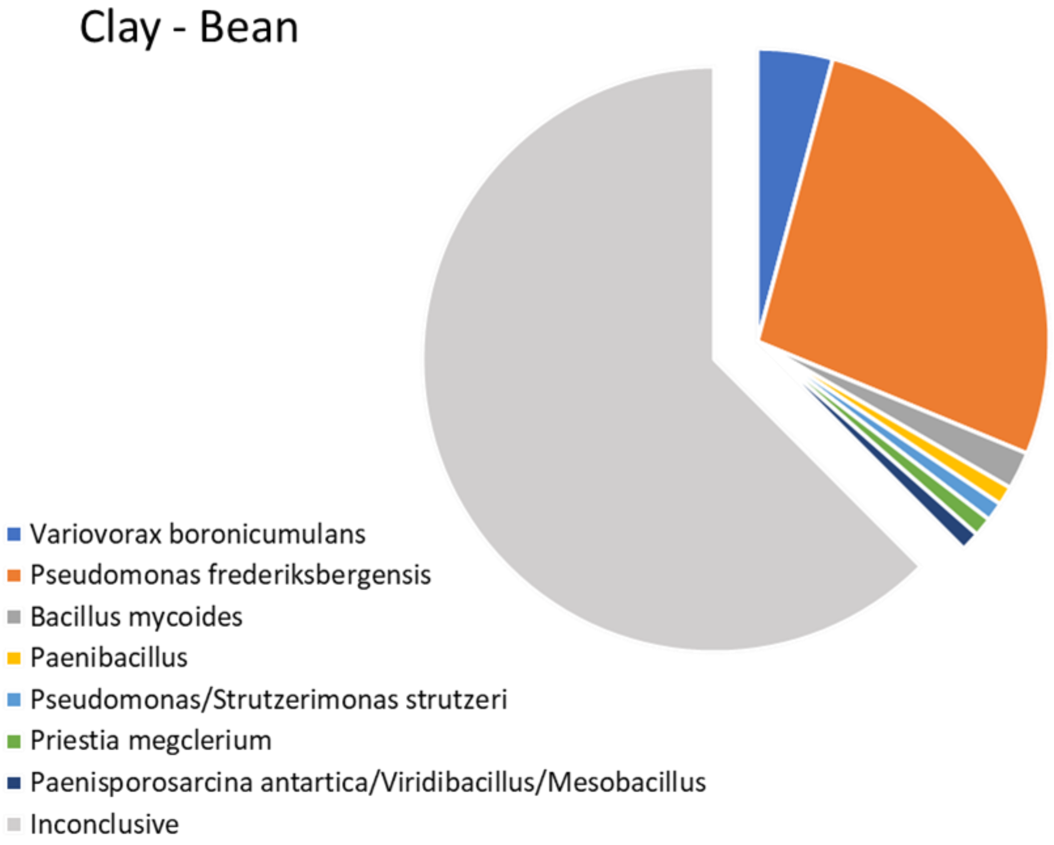
Fava Bean - Sand | Fava Bean - Clay | Wheat - Sand | Wheat - Clay

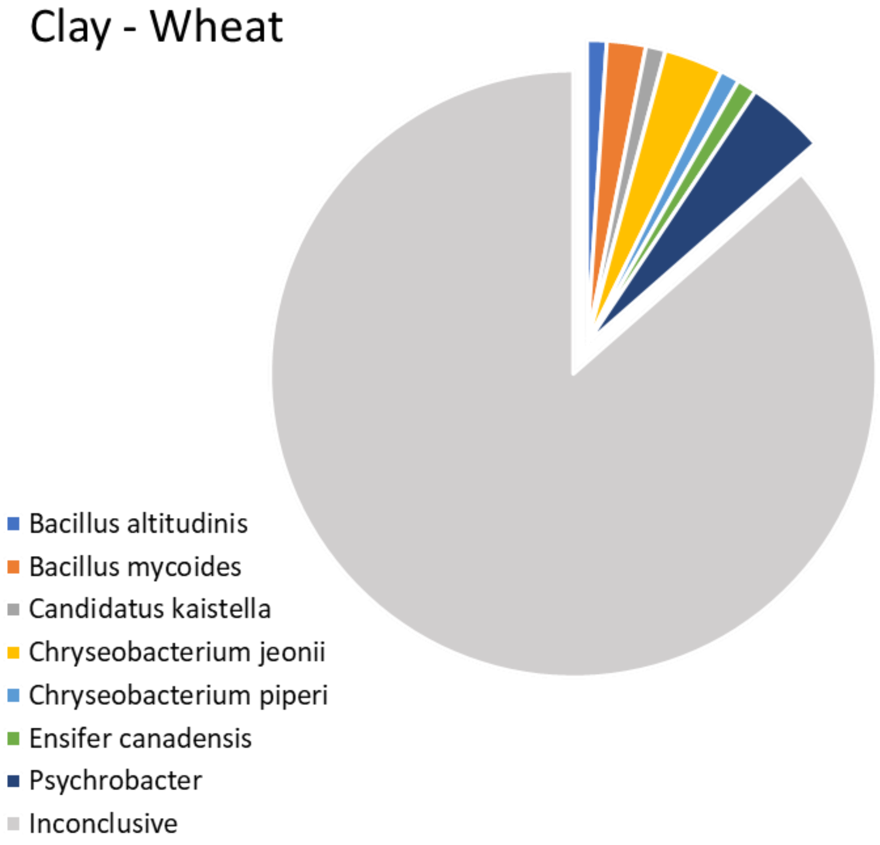


E.g. phenotyping - protease production



Genotyping the culture collection – initial steps





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Workflow

1. Check isolate viability
2. Phenotype community
3. Choose a range of isolates that have different abilities for SynComs
4. Genotype (16S and RpoB) and WGS of chosen isolates
5. Plant growth promotion assays in microcosms
6. Metabarcoding of SynComs on host and non-host plant

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Reproducible plant growth: EcoFab 2.0

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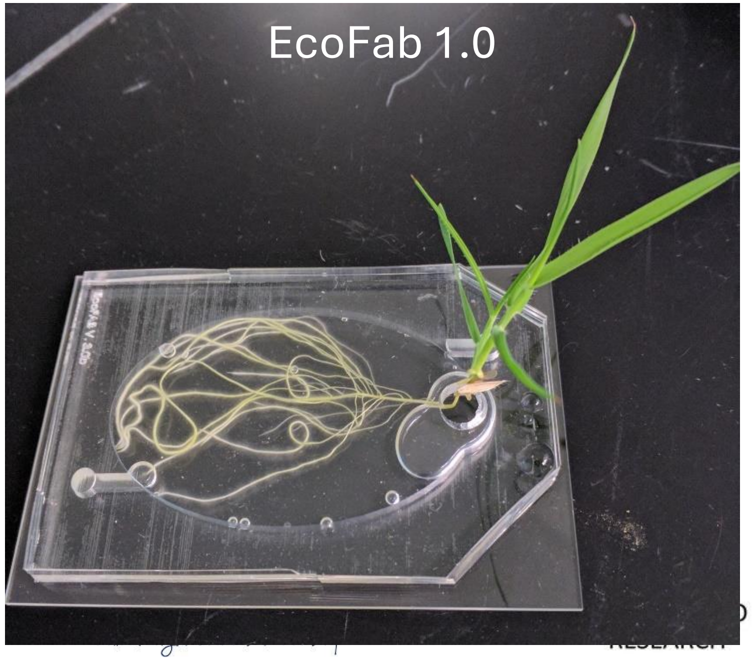
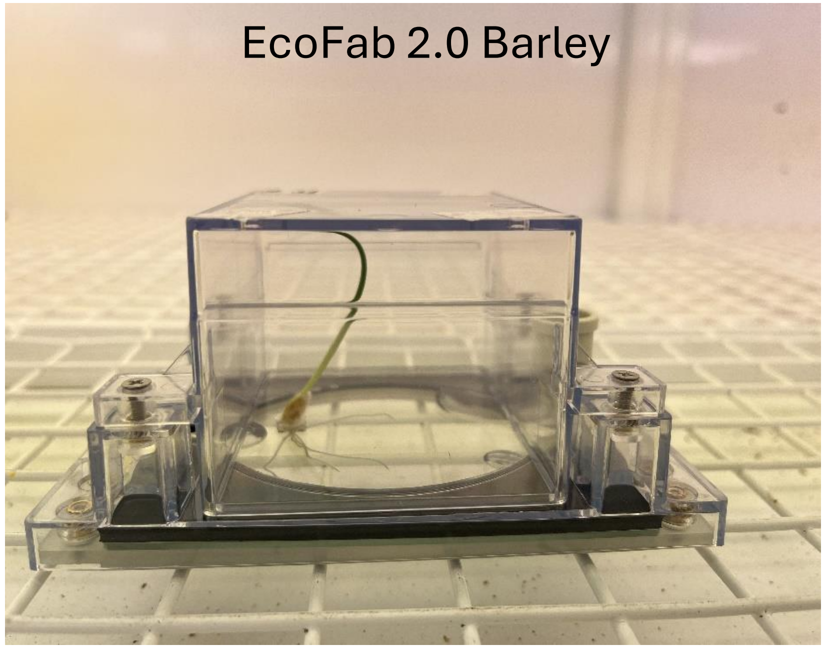
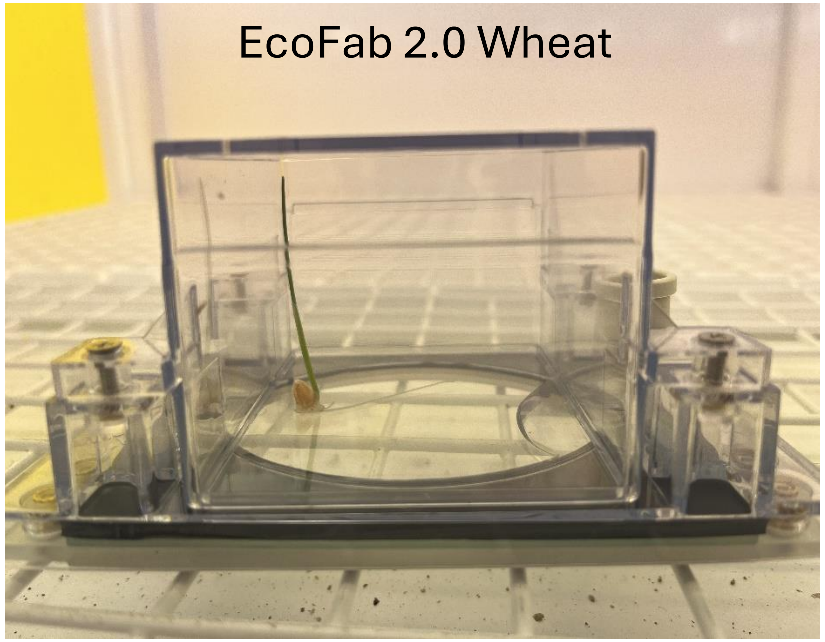
- Model lab ecosystem allowing for highly repeatable results world-wide
- Complements existing assays that will also be performed
- Has never been used for crop plants before

EcoFAB







SynCom strategy – next steps

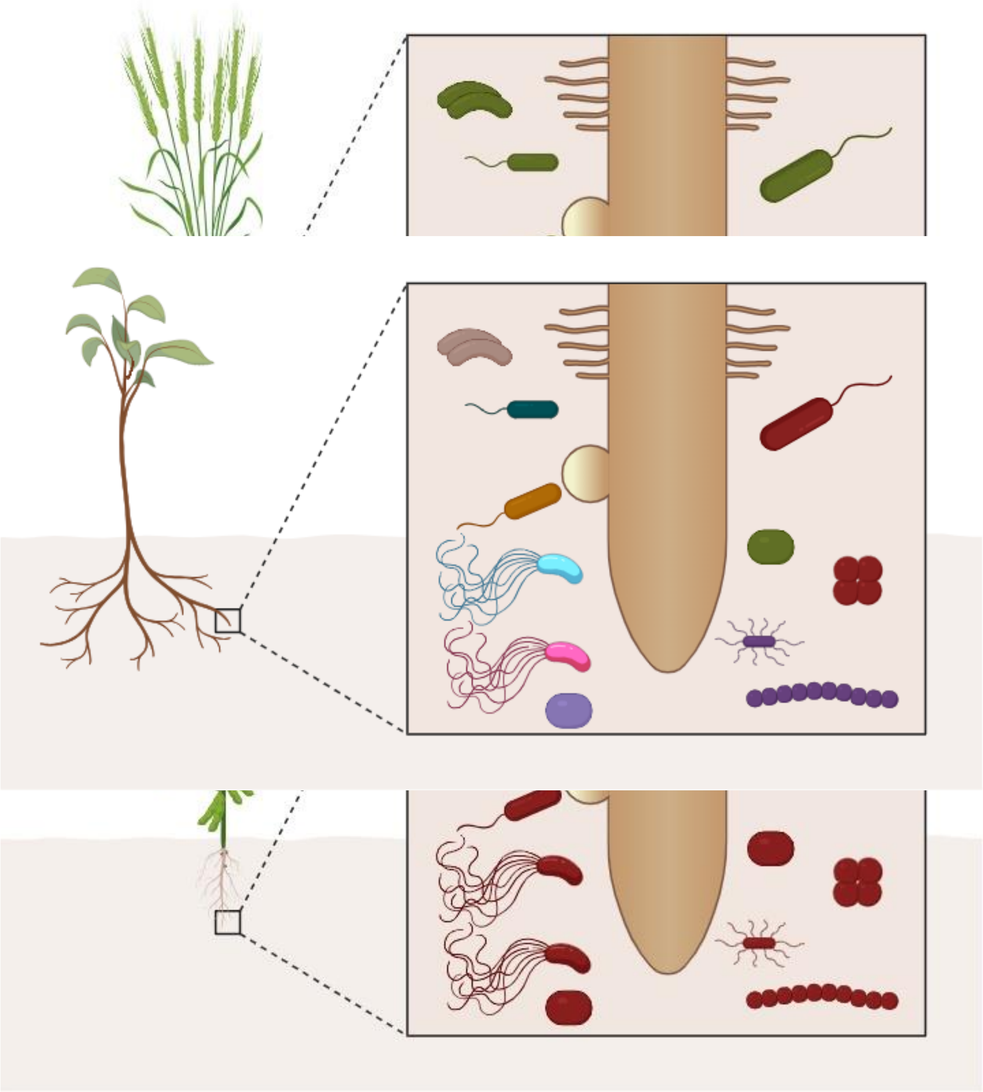
Genotype

↓

In depth phenotyping

↓

In planta assays



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Key Conclusions

UK CROP MICROBIOME CRYOBANK

- The UK Crop Microbiome Cryobank is a utility and model for supporting Phytobiomes research
- You can successfully preserve a soil sample to retain functional diversity
- The cryobank is a unique resource, linking physical sample with provenance data and genomic metadata linked to ENA and MGnify
- The resource can be interrogated to answer biological questions
- Drivers for community compositional variation are different to isolate function
- Preserved, characterised biological resources can provide promising leads for future development as Nature Based Solutions

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- **Imperial College** – Prof Tom Bell and Tom Lilley (functionality work)

- **EBI** – Rob Finn and team