Rothamsted Research, Harpenden

Community outreach – September 2021

UK CROP MICROBIOME CRYOBANK

The purpose of today

- Promote the Cryobank and raise awareness to the user community
- To facilitates user engagement through access to resources and data
- Identify 'gaps' and help to improve approaches
- Ensure we adapt to meet the needs of users and identify opportunities for broader collaboration and the development of proposals.
- Use this valuable networking opportunity to discuss broader issues of relevance to the UK Crop, Soil and Agritech community including the UK Plant Microbiome Initiative and the KTN microbiome roadmap

11:00 Arrive and Networking

11:30 Project overview

12:30 Networking Lunch

13:30 Breakout sessions - Meeting user need and opportunities for collaborative research

Each participant will be assigned to one of four groups (A>D). Each group will then rotate to one of four led discussion sessions, each last half an hour on: 1) Crops/soil health, 2) Cryobanking, 3) Bioinformatics and Genomics & 4) Agritech / Industrial outreach

Coffee will be available at 14:30

15:30 Key points from breakout groups. Led by Paul Rogers with the Cryobank PI's.

15:45 Microbiome Infrastructure: KTN Microbiome and Biobanking (MJR) , the UK Plant Microbiome Initiative (TM) , funding opportunities – the role of CHAP and the Plant Microbiome Symposium (NH)

16:15 Close

The Project

- BBSRC – Bioinformatics Biological Resources Fund
- Worth £2 million at FEC (1.6M @ 80%) – 5 years
- Brings together biological resources, cryopreservation, crop health, microbial ecology and bioinformatics expertise from five leading UK agricultural research institutions
- Royal Botanic Gardens, Millennium Seedbank (sub-contract)

John Innes Centre
Unlocking Nature's Diversity

ROTHAMSTED RESEARCH

SRUC

CABI

The James Hutton Institute

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The Project Team (& sub-contract)

Institute / University	Team
CABI	**Dr Matthew Ryan (PI)** **VACANT (Named Researcher)** **Ms Helen Stewart (Curatorial / PGRA -0.5fte)** **Mr Miguel Bonnin (Curatorial / PGRA – 0.5fte)** **Mr Anthony Kermode (DSC work)**
Rothamsted Research	**Dr Tim Mauchline (PI)** **Dr Ian Clark (Named Researcher)** **Dr Rodrigo Taketani (Post Doc)**
John Innes Centre / UEA	**Dr Jake Malone (PI)** **Post Doc (TBA) FT**
Scotland's Rural College	**Prof Nicola Holden (PI)** **Payton Yau (Post Doc)**
James Hutton Institute	**Dr Pete Hedley** **Dr Sue Jones**
RBG Kew (Sub-Contract –DSC work)	**Prof Hugh Pritchard and Dr Dani Ballesteros**

Why we set up the project

Existing culture collections did not support microbiome research (in any field!)

Industry and stakeholder support e.g. through the UK Plant Microbiome Initiative

To respond to the need to link research to samples / metadata and tools for bioinformatic interpretation

A driver need to underpin crop microbiome research

To generate standards and methods / protocols

To provide the tools for future research and development – both for academia and industry

To improve our knowledge of soil and phytobiome for major UK crops

The Project Advisory Board

Institution	Organisation
Ruth Bastow	**CHAP**
Tom Bell	**Imperial College**
Cara Haney	**University of British Columbia**
Davide Bulgarelli	**James Hutton Institute**
Kellye Eversole	**Int'l. Phytobiomes Alliance**
Jim Prosser	**University of Aberdeen**
Susannah Bolton	**ADHB**
Rob Finn	**EBI**
Liz Shaw	**University of Reading**
Gina Swart	**Syngenta**

Additionally, David Smith (CABI) is available to advise on issues related to Nagoya, ABS & Biosecurity

Objective:

To establish a cryopreserved and characterised crop microbiome resource to underpin UK and international crop research, building on the UK Agritech capability provided through the Centre for Crop Health and Protection (CHAP).

The focus will be on the microbiomes of major UK crops: (barley, oats, oil seed rape, potato, sugar beet and wheat) from 3 different soil types obtained from across the UK.

***The UK-CMCB (Crop Microbiome CryoBank) will provide a comprehensive platform to facilitate research towards optimising plant yield in an integrated crop management framework*.**

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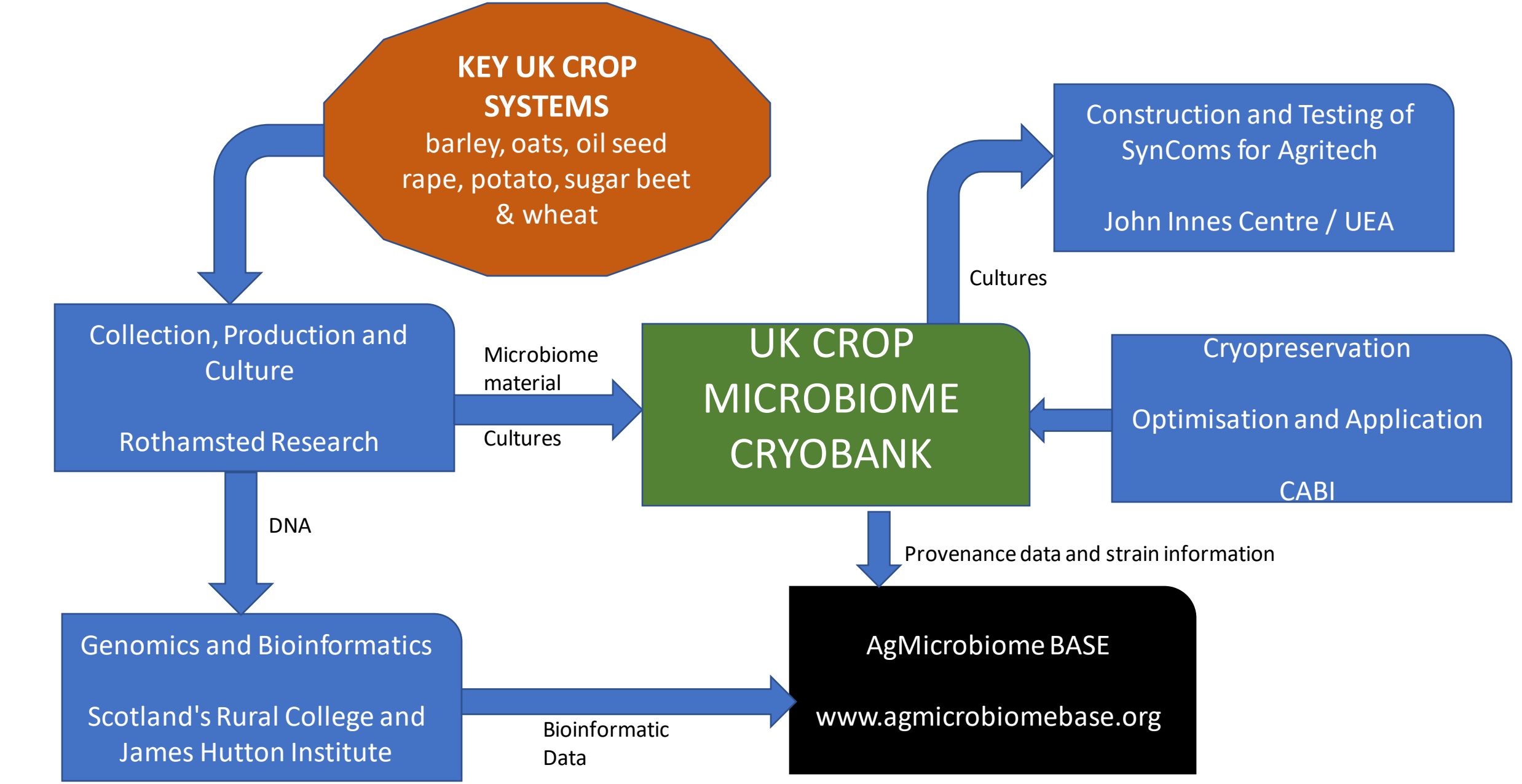
Key Aims:

- The validated resource will provide a facility for researchers to source data and samples for their work, including living microbial material from the rhizoplane and genomic sequences from different microbiome environments.

- This will provide a platform for soil scientists and plant researchers to assess and compare their work against validated datasets generated by the project



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Summary of the key projected outputs of UK Crop Microbiome CryoBank

● A cryopreserved resource of characterised material from crop microbiomes with a prioritised collection strategy. Frozen samples will be made publicly available to the user community through the CABI database and will be dynamically linked to genomic data.

● Robust methodologies for collection and storage of intact microbial communities in environmental samples and extracts of total DNA, which will be available to researchers.

● Enhanced capability to sustainably maintain the resource in a genotypically and phenotypically stable state.

● Genomic characterisation of the samples for assessing microbial diversity (including symbionts, endophytes, pathogens), from whole community taxonomies (bacteria, fungi, viruses) to individual isolate genomes.

● An added value demonstration of the utility of the UK-CMCB to the user community through PGPR isolation and synthetic community construction.

● A validated sequence resources database, 'AgMicrobiome Base' linked to EBI, available to Agritech sector and researchers, including model organisms and novel product outputs.

Types of studies the resource will facilitate – relevant to our discussion session

- Systematic identification & characterisation of plant growth-promoting & biocontrol microbes.
- Comparative analysis of soil communities from different crop systems and environments.
- Understanding population dynamics of microbial communities, individual species and their interaction eg. species interactions during microbial succession and changes in populations as a result of abiotic (environmental) and biotic stress factors.
- Identification of functional traits, e.g. in-depth metagenome and metatranscriptome profiling
- Studies over time, eg. ‘before and after’ studies for evolutionary comparisons or the effects of changing agricultural practices/other abiotic factors on crop production and plant health.
- The impact of gene mobility in the plant microbiome.

WORK PACKAGE DESCRIPTIONS

UK CROP MICROBIOME CRYOBANK

Task 1 Collection of soil, plant and microbial samples and DNA extraction

Leader: RR (Mauchline) ***Partner***: JHI (Holden)

Goal: Collection of samples for all downstream work (Tasks 2 to 4).

Task description:

200 kg of soil (including silt and sandy loams as well as a clay soil) sampled from 9 UK farm locations (3 of each soil type, with pH of around 6.5) available through the BBSRC ASSIST programme network

Soil sieved, homogenised and used to culture plants from surface sterilised seeds under controlled glasshouse conditions.

Wheat, barley, oats, oil seed rape, potato, faba beans, sugar beet and bulk soil

Five replicates from each of 7 crops (unplanted bulk soil will also be assessed), 11 soil sites (3 sand, 3 silt, 3 clay) each will be prepared, equating to 440 pot systems on which the core resource will be based. Plants will be harvested at the onset of flowering.

Samples collected will be split in two parts for:

A) Isolation and preservation of environmental microbial consortium

B) Extraction of total DNA from soil and plant material for amplicon and metagenomic sequencing.

Selected sites re

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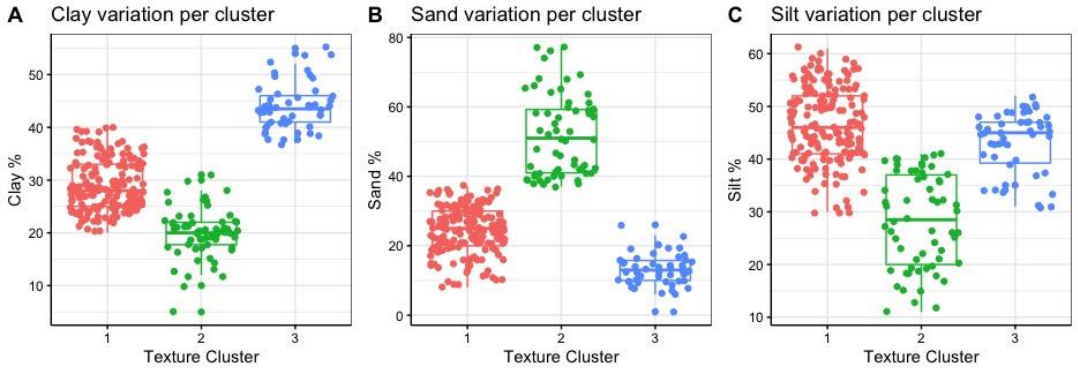

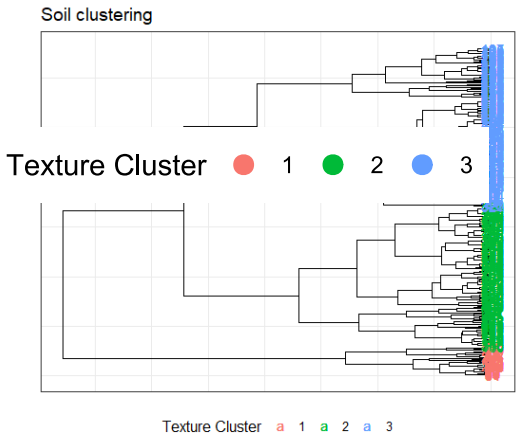

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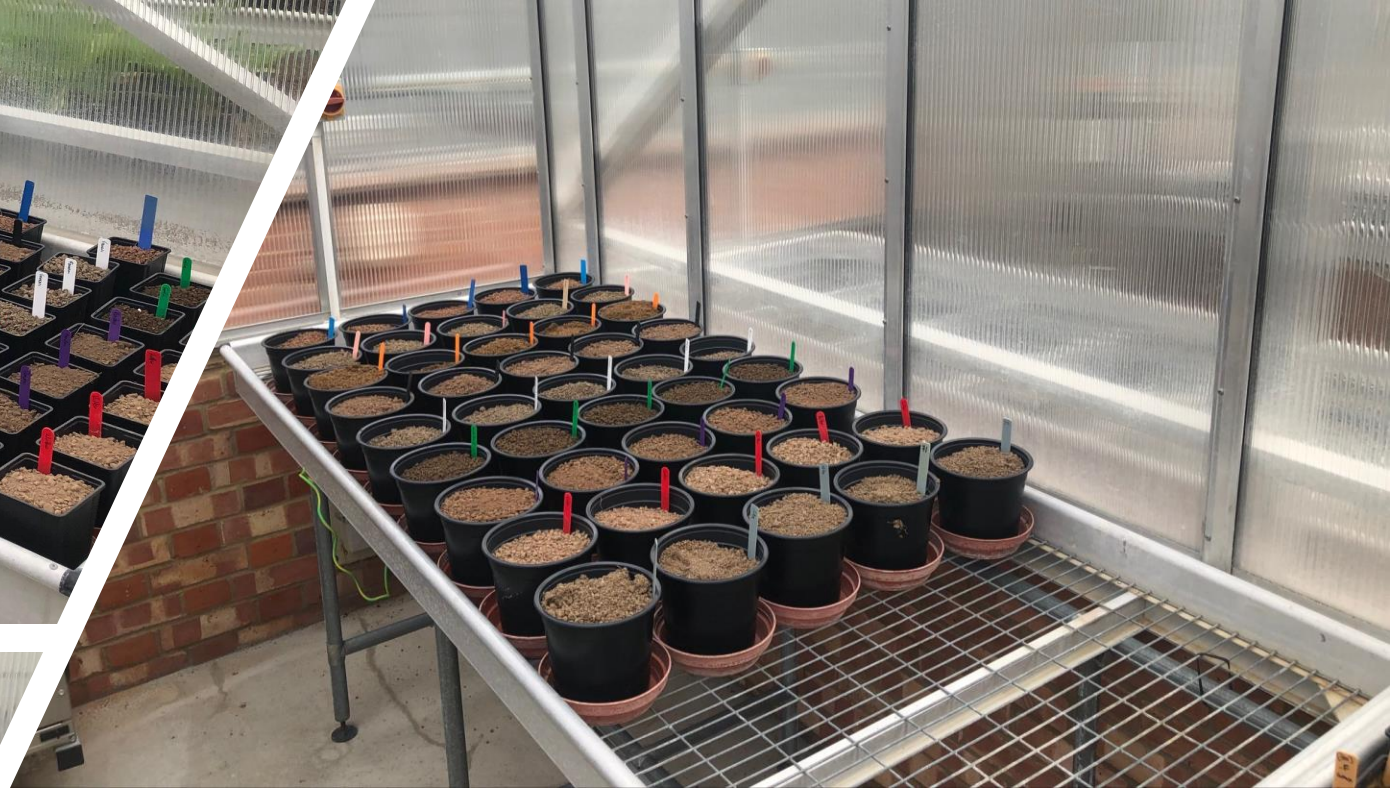
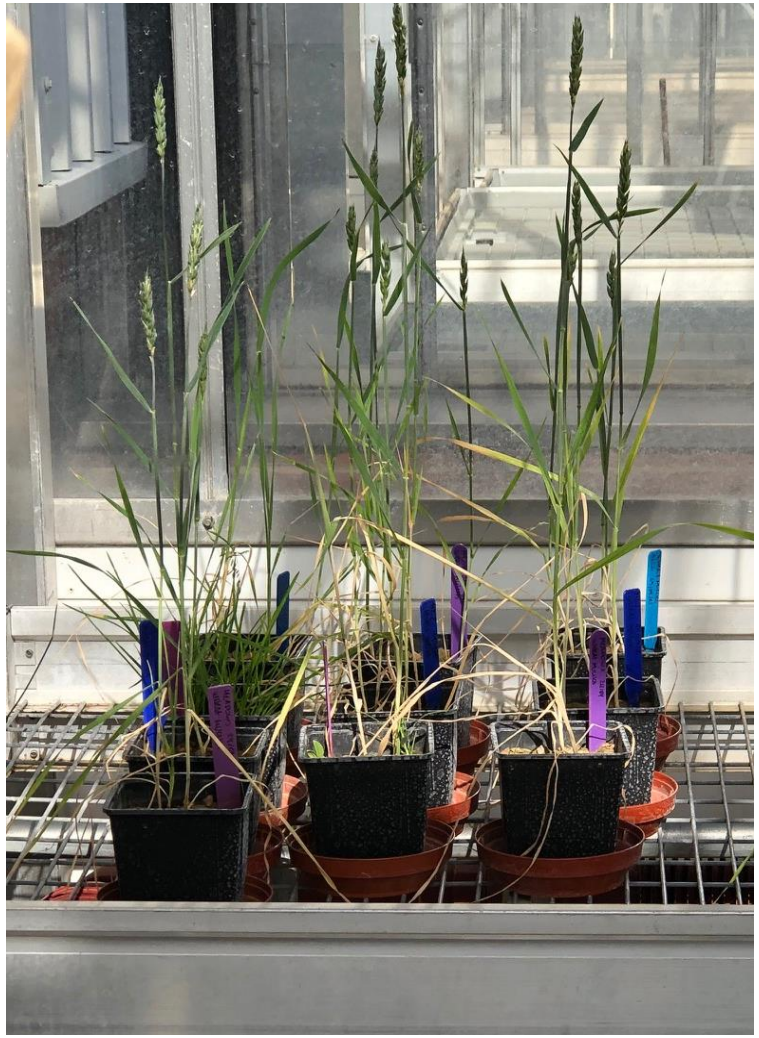
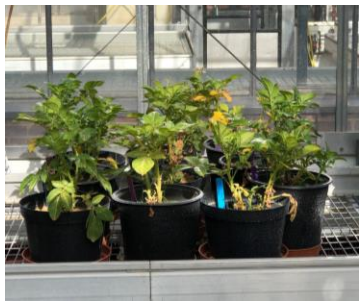
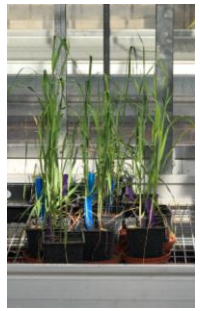


Spring Wheat	
Mulika	Senova
Spring Barley	
RGT-Planet	RAGT Seeds
Faba Beans	
Lynx	LS Plant Breeding
Spring Oats	
WPB Elyann	KWS
Spring OSR	
Campus	KWS
Potato	
Maris Piper	GB Seed Industry
Sugar Beet	
Degas	Strube

Trial experiment







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Culture-based approach

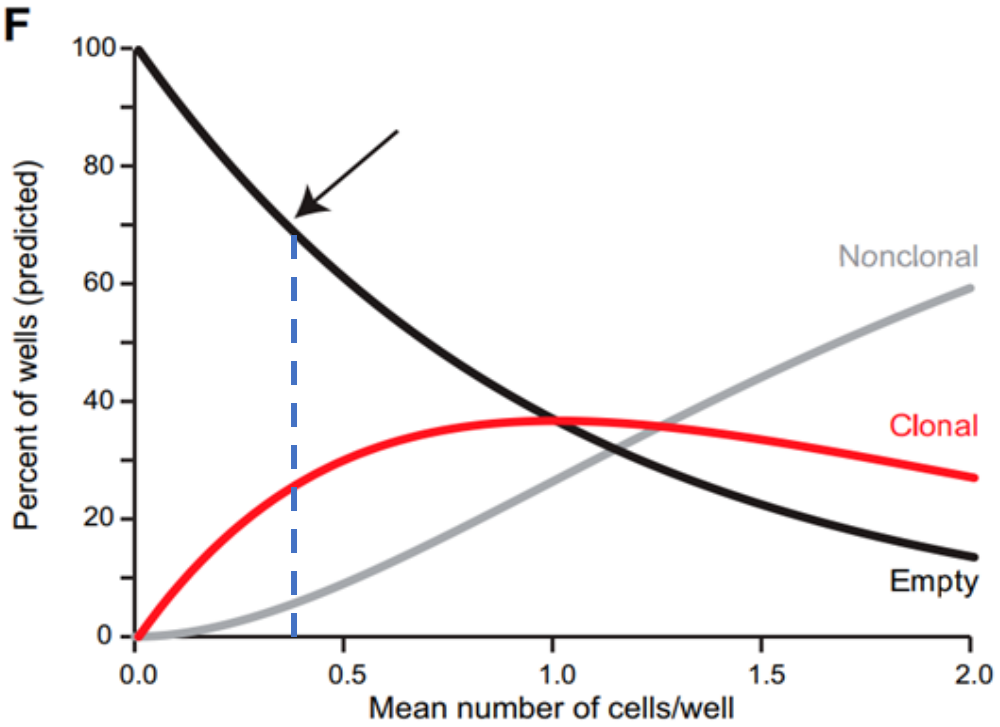
A combination of limiting dilution and traditional plating on a range of rich and minimal culture media is being used to maximise microbial recovery from the rhizoplane and bulk soil (Bai et al., 2015).

In total, we envisage collecting 96 microbial isolates per crop per pot system. This equates to 42,240 isolates when including unplanted bulk soil samples. Colonies will be sub-cultured in duplicate in 96-well plate format, to produce a total of 440 plates.

The original and duplicate 440-plate collections will be stored in separate -80 °C freezers at Rothamsted and CABI after cryopreservation to ensure robustness of the resource (Task 3).

All samples will undergo sequence barcoding and a sub-set of isolates genome sequenced (Task 2).

In addition, plant tissue samples (roots, shoots and seeds), rhizosphere and bulk soil samples will be collected and cryostored (Task 3).



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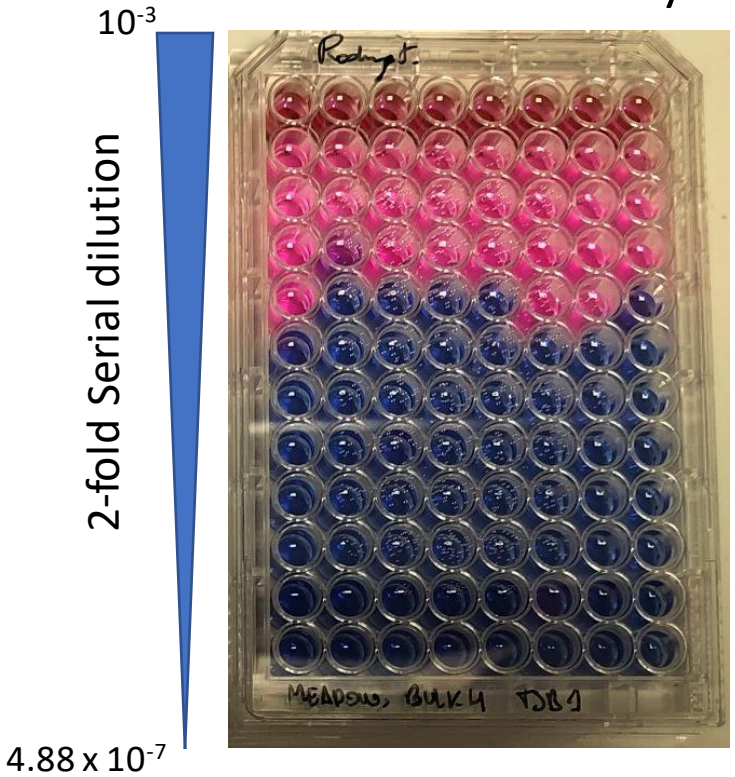
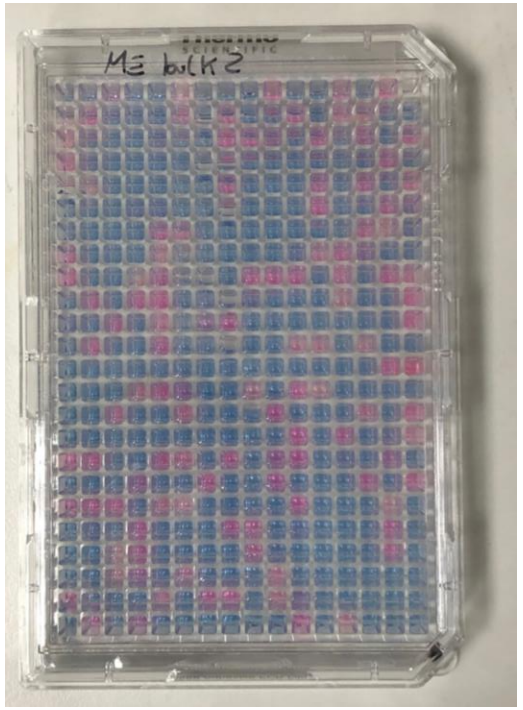
Limiting dilution isolation

Calculate CFU density



24hs

Plate 0.3 CFU/well



24hs

Transfer to 10% TSB

TSB 10% + 100 uM resazurin

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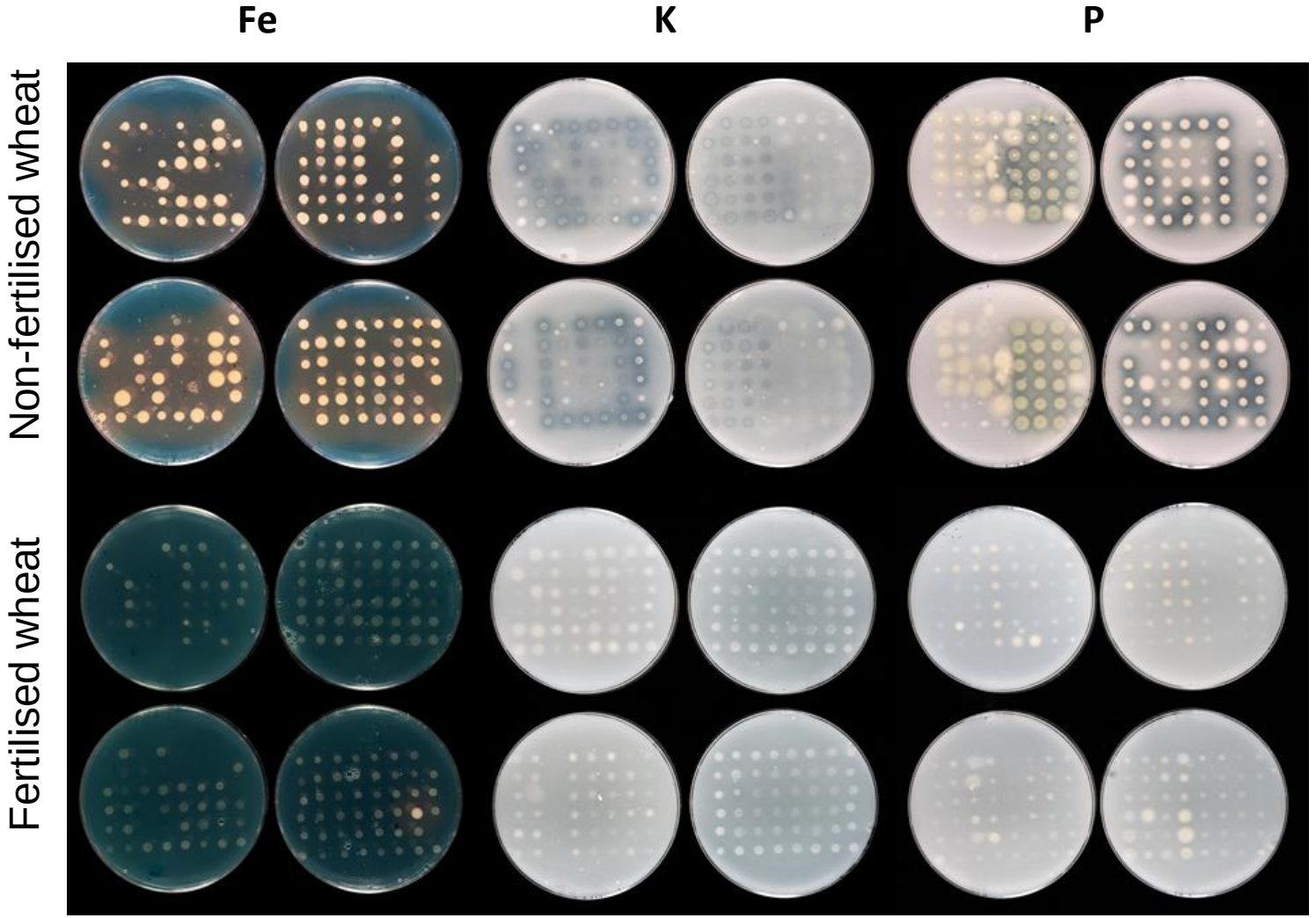
Functional screening of bacteria



Reid et al., 2021

Nutrient liberation
N (casein)
P (Phytate, AlPO4, Ca3PO4, FePO4)
K
Fe
Lipase

Abiotic
pH
ACC deaminase
Salt tolerance

Pathogen suppression
Septoria
Take-all
Fusarium

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Task 2: Unravelling the community structure and genomic composition of the crop microbiome

Leader: JHI (Holden), Rothamsted (Clark) ***Partners***: CABI (Ryan), JIC (Malone)

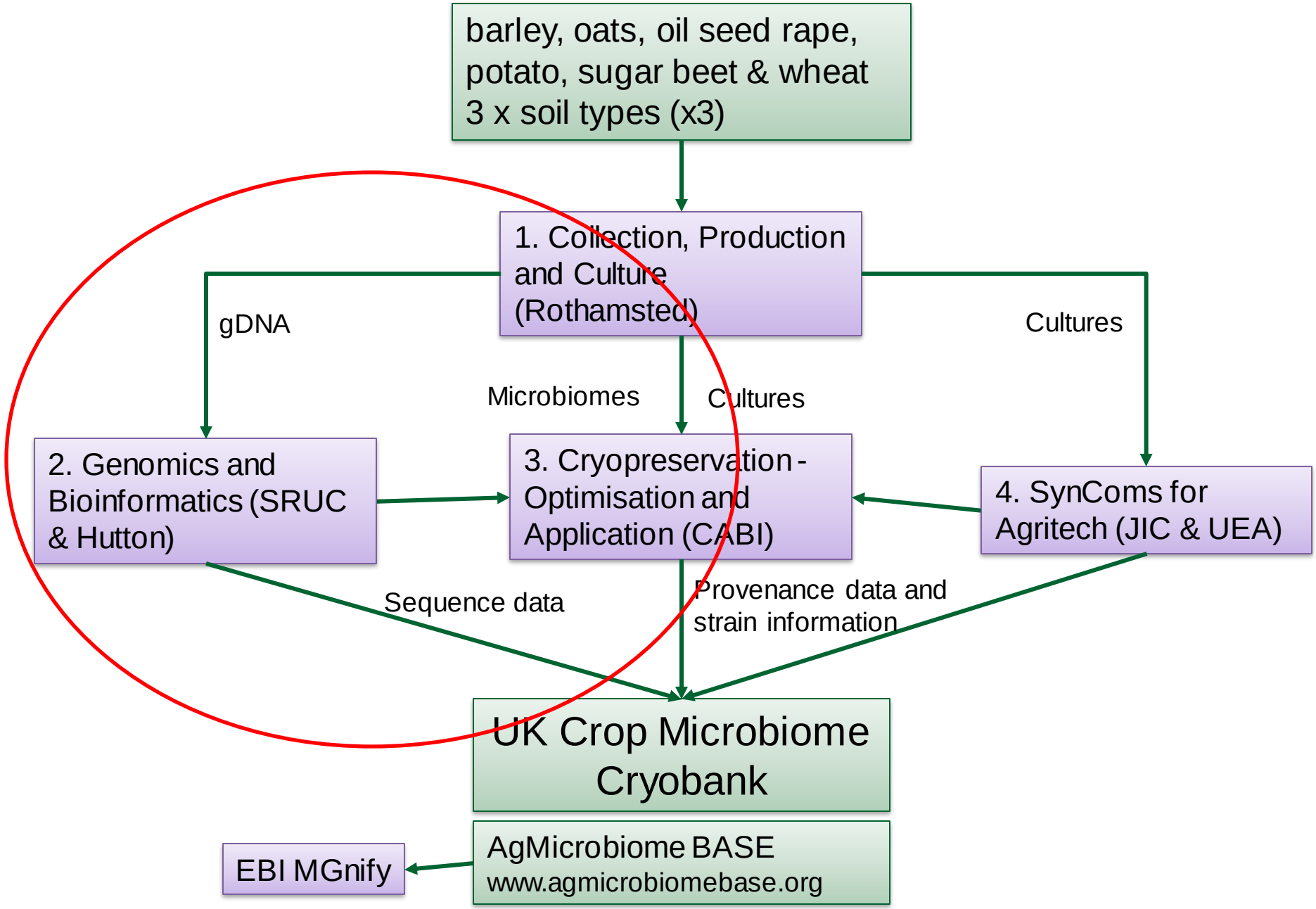
Goal: To identify the microbial community composition associated with each crop rhizosphere microbiome.

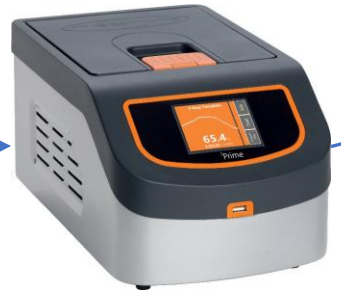
- Provides a comprehensive framework for downstream comparisons of microbiomes based on community structure and (for selected samples) implied function

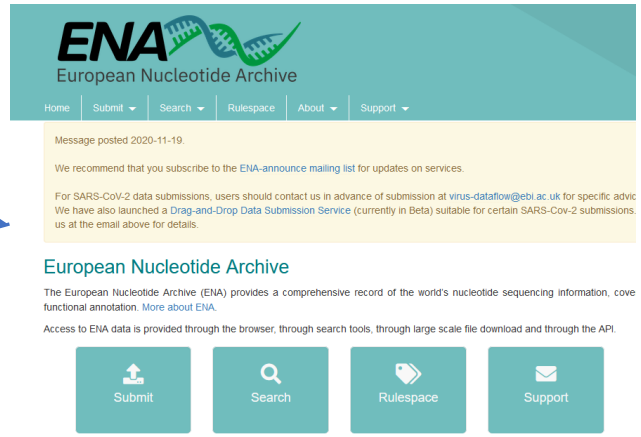
Task Description:

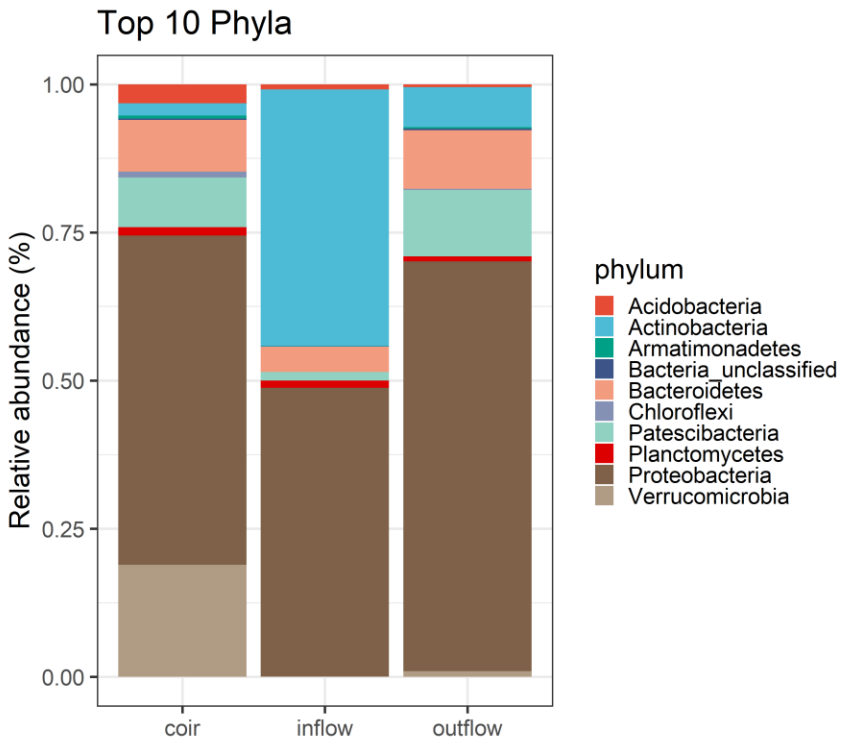
- rRNA amplicon sequencing (bacteria - 16S, [fungi - ITS2]) for community composition
- Selected samples for enhanced composition and functional analysis from shotgun metagenomic sequencing
- Selected culturable microbial isolates will be sequenced for a diversity to the whole genome level.
- Generation of cryo-preserved samples future-proofs the samples, enabling future sequencing and analysis approaches for direct comparisons.

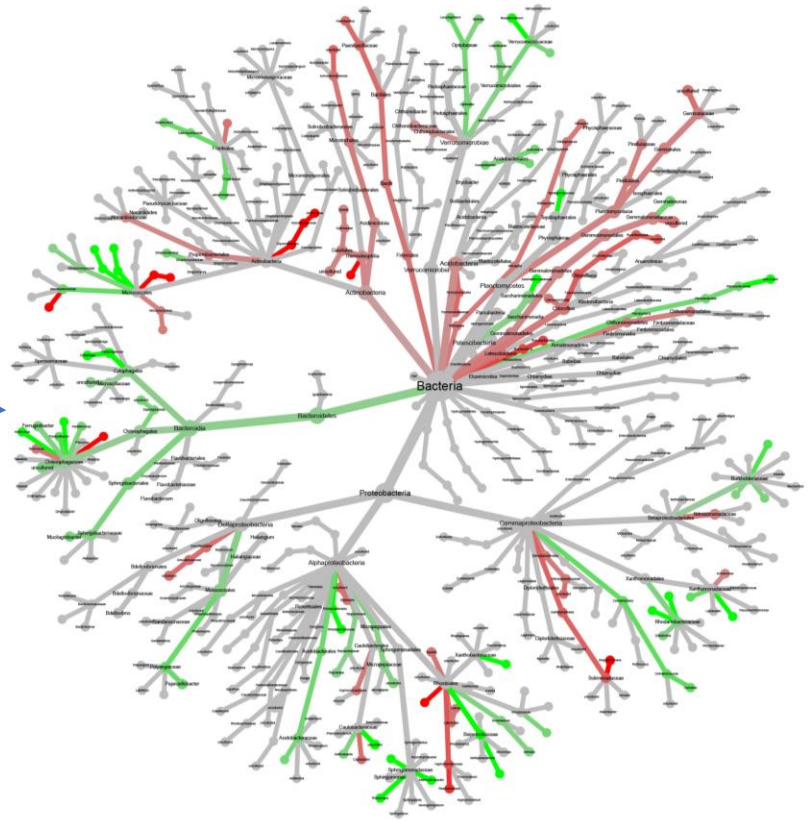
Approach: Amplicon libraries will be prepared at SRUC from gDNA supplied by WP1 (Rothamsted) and sequenced at the Hutton Institute. Isolate gDNA and metagenome libraries will be sequenced by 3rd parties. Sequence analysis & bioinformatics will be carried out at SRUC

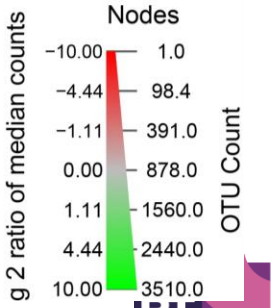

barley, oats, oil seed rape, potato, sugar beet & wheat
3 x soil types (x3)
1. Collection, Production and Culture (Rothamsted)
gDNA
Cultures
Microbiomes
Cultures
2. Genomics and Bioinformatics (SRUC & Hutton)
3. Cryopreservation - Optimisation and Application (CABI)
4. SynComs for Agritech (JIC & UEA)
Sequence data
Provenance data and strain information
UK Crop Microbiome Cryobank
AgMicrobiome BASE
www.agmicrobiomebase.org
EBI MGnify











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Task 3 – Long term storage of i) total crop microbiomes and ii) culturable components & SynCom panels by application of standard optimised cryopreservation approaches
Leader: CABI (M. Ryan) ***Partner:*** Rothamsted (Mauchline), John Innes Centre (Malone),

Goal: To conserve and store crop microbiome samples and develop "best practice" for preserving microbes in association with the plant microbiome.

Task description:
Samples and strains from WP1 and WP4 will be processed for long-term storage. This will include i) The 315 microbiome samples collected in WP1, which will be cryopreserved as bulk soil; ii) The 945 rhizosphere soil, root tissue and shoot/leaf tissue components generated from the WP1 pot experiments iii) The 315 plates containing the culturable microbes from WP1 & iv) The panels of synthetically constructed communities from WP4

Objectives:
To preserve the integrity of samples and the microbiome associated with them, samples will be transferred to CABI for long term cryostorage and will constitute a resource for future metagenomic and transcriptomic studies by the research community.

- We are applying sn AE (Alginate encapsulation-dehydration method) (Benson et al., 2018) and a controlled rate 'Stirling cycle' (Ryan et al., 2014) driven cryo-cooling approach will be applied. These procedures allow the optimal cryopreservation of samples, by reducing the prospects of freeze damage, thus retaining genomic integrity, and functional potential

- Culturable microbial isolates from task 1 will be cryopreserved in 96 deep well plates. These cultures will be frozen using a controlled rate cooling approach with the addition of a suitable cryoprotectant (-1oC min-1 / 10% Glycerol aq) and stored in duplicate at -80°C (at Rothamsted) and by ultra-low temperature storage at CABI. The resources generated through task 4 (Synthetic Community collection), will be transferred to CABI and cryopreserved

- Total DNA extracts (315) will also be stored for each crop microbiome and bulk soil as well as from each cultured microorganisms (30,240 in 96 well plates), providing a permanent reference and this (DNA) will be stored in a -135°C facility at CABI.

- At all stages, to assess the success of preservation and storage regime and as a quality control measure, molecular sequencing approaches will be utilised to assess the effectiveness of each cryopreservation regime, with functionality assessed using a total Phosphate enzyme assay for bulk microbiome samples.

- In order to ensure that the cooling process is consistent throughout samples, Differential Scanning Calorimetry (DSC) will be undertaken at Kew (Wakehurst Place). This will enable us to ensure that heat is evenly dispersed during cooling, safeguarding sample integrity and process reproducibility.

Initial Outputs

- 12 plant pot systems as well as bulk soil from all 11 sites have been cryopreserved to date. Consisting of 350 bulk soil and 350 encapsulated soil cryotubes.
- Paper on AgriRXIV Cafa G, Bonnin JM, Holden N, Malone, JG, Mauchline TH, Clark I, Taketani R & **Ryan, MJ** (2021) Cryopreservation of a soil microbiome using a Stirling Cycle approach – a genomic assessment https://doi.org/10.31220/agriRxiv.2021.00066
- Standards being developed
- Encapsulation / Dehydration method optimised
- AgMicrobiomeBase now 'Live'

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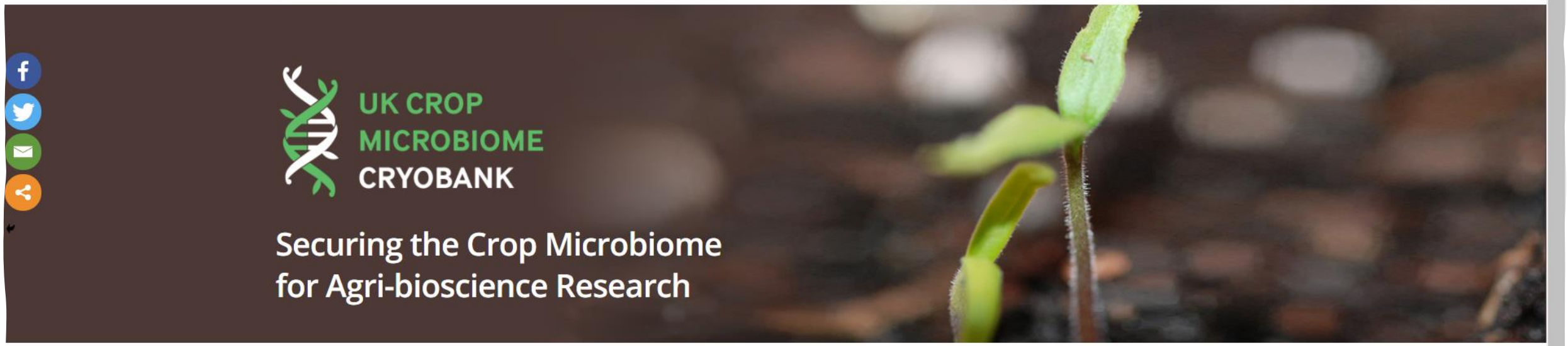
Introducing AgMicrobiome Base

- A curated and open access AgMicrobiome base of bioinformatic information, meta-data, annotated sequences, integrated with current sequence and microbiome databases such as MGnify - Linking data with sample provenance

- Full meta data sets including: Physical and chemical soil data, Microbial data sets, sample and isolation data, sequence data

www.AgMicrobiomeBase.org



Project Summary

The UK-CMCB will provide a facility for researchers to source data and samples for their work, including living microbial material as well as genomic and metagenomic sequences (DNA) from different microbiome environments, including rhizoplane.

Microbiomes are all the microbes present in any one ecosystem, in this case those associated with the crop plant, whether they are present in the leaves, seeds and stems or in the bulk soil around the roots. A beneficial microbiome results in a healthy plant and an improved crop yield and better quality food.

UK Research and Innovation

Task 4 – Analysis and validation of the microbial collection

- Characterise the microbial isolate collections from 5 microbiome samples from tasks 1-3 using high-throughput phenotypic assays.

- Assess the ability of a sub-set of sequenced plant-growth promoting microbes to promote host plant seedling growth in high-throughput gnotobiotic assays

- Interrogate datasets to identify relationships between phenotypes, and with genetic loci for sequenced isolates.

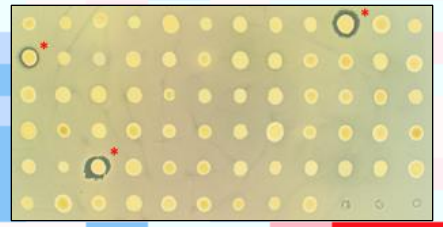
- Develop microbial synthetic communities (SynComs) containing combinations of the plant beneficial microbes identified in the previous step, and test:
  - **1**) the ability of individual, beneficial rhizosphere isolates to promote plant growth under microbial competition
  - **2**) the synergistic potential of representative microbial populations to promote host- and non-host plant growth.

Color Key

−1 −0.9 −0.7 −0.4 −0.2 0 0.2 0.4 0.7 0.9

Genotype vs Genotype correlation matrix

Phenotype/genotype/*in planta* screening



Tasks 1-3

Task 4

Pacheco-Moreno et al. *in revision*
Mauchline et al., Env Micro 2015
Mauchline and Malone, Curr Op Micro 2017

Type_VI_Sec
Massetolide
Pili_synthesi
Microcin_B1
LlpA
Phenazine_s
Peptide_synt
Pili_synthesi
Viscosin
NRPS
LapA_Type_
Pseudomoni
Cell_wall_1_
Tc_complexe
BapA1D
Pyocin_S1.2
pltBC
Enantio1pyo
NRPS_.gene
putative_prot
Chitinase
IAA_.Auxin._
Cell_wall_bio
Psl_operon
putative_met
Hydrogen_C
Pyoverdin_sy
Peptide_synt
Pili_synthesi
Pel_operon
Type_III_Sec
Pectate_lyas
Wss_cellulos
Curli_fimbrai
ACC_Deami
Hemophore

Breakout sessions

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1) Crops/soil health
2) Cryobanking,
3) Bioinformatics and Genomics
4) Agritech / Industrial outreach

Key questions:

What are the immediate research priorities in each area for Agritech? For Researchers?

What are the developing innovations in the area of relevance? From what you have heard so far, are there any critical areas that we should address immediately?

Where are the opportunities for collaboration / research and utilisation of the Cryobank?

From your research or interest area, are there gaps we should address things we should be doing or aren't.

The Cryobank is focusing on six crops (wheat, oats, OSR, Beet, corn, potato) what should be our next focus?

Coffee will be available at:

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Final Session

The UK Plant Microbiome initiative, the KTN & biobanking

Future Conference

Update: KTN Microbiome, the UKPMI and Biobanking

15th September

Matthew Ryan
Research Lead – Biological Resources @CAB International
Project Manager – UK Crop Microbiome Cryobank

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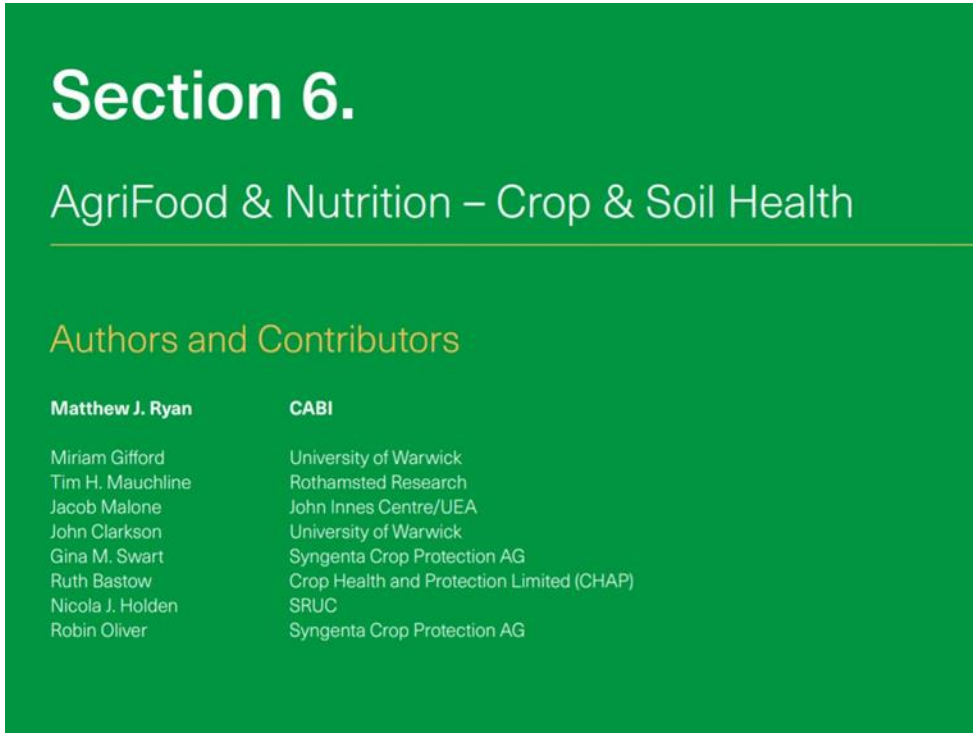
Priorities Identified in the Strategic Roadmap

1. Foster a “Microbiome Centres of Excellence” approach
2. Create Microbiome Research & Innovation Collaboration Networks
3. Encourage Microbiome entrepreneurship, seed funding, regulatory and intellectual property rights support
4. Ensure support for and access to emerging, enabling technology
5. Establish Microbiome research standards
6. Develop “Next Generation” bio-banking
7. Harness the potential for new and rapid diagnostics
8. Invest in Microbiome process development and pilot-scale manufacturing
9. Promote a supportive regulatory environment
10. Improve Microbiome education, skills, and talent pipeline
11. Prioritise support for specific opportunities where the UK has a distinct advantage
12. Increase strategic funding for Microbiome Research and Innovation



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Could the UK Plant Microbiome Initiative form the Crop Microbiome Network?

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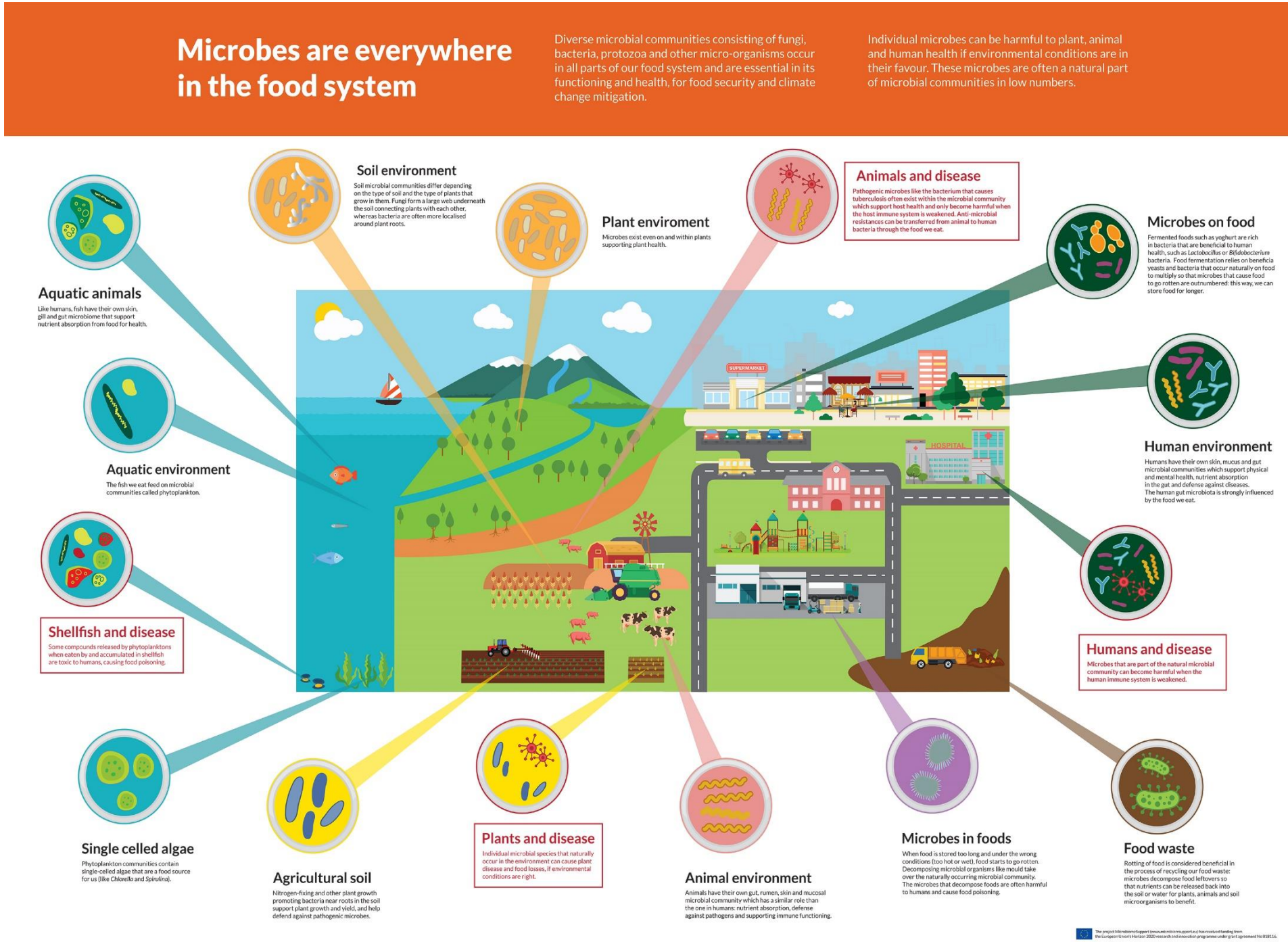


State of the 'ark':

Only axenic cultures are generally stored

Cultures are rarely stored as consortia or as 'microbiome'

Human and animal microbes are often prioritised over environmental and plant associated organisms

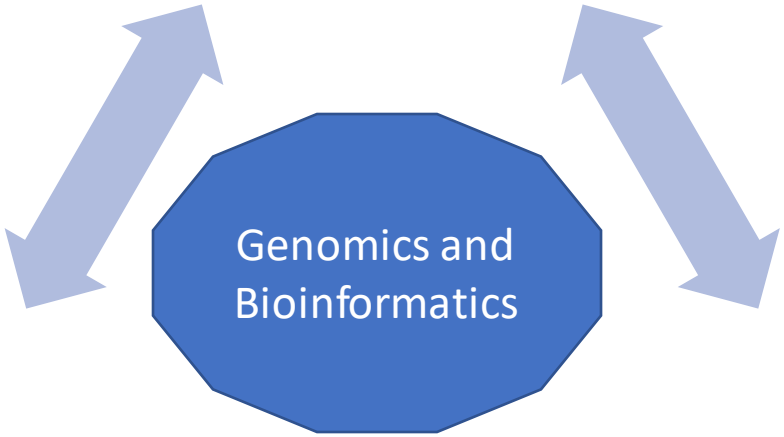
microbiome support

UKRI Biotechnology and Biological Sciences Research Council

There is currently only limited focus on Cryobanking to underpin Microbiome Research in the UK

Microbial Culture Collections e.g. CABI, NCIMB

Most centres are 'domain' focussed



Medical & Healthcare Biobanks

e.g. Biobank UK, ex vivo collections

Environmental Collections and Museums e.g. Kew, CryoArk, MSB

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Fragmentation of infrastructure is common in Europe and beyond

The UK ahead of the game?

UK BRCN
The United Kingdom Biological Resource Centre Network

But only for historical approaches?

UK CROP MICROBIOME CRYOBANK

Biotechnology and Biological Sciences Research Council

Trends in
Microbiology

CellPress

Scientific Life

Development of Microbiome Biobanks – Challenges and Opportunities

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The microbiome research field is The EU project MicrobiomeSupport assessed resource infrastructure needs in this important area of research (Figure 1). In this paper we consider why and what we need to preserve, and how it should underpin microbiome research.

Microbiomes in the Context of Biobanks and Culture Collections

Microbiomes are dynamic and complex systems consisting of bacteria, archaea, fungi, algae, protists, and viruses, and the principles of microbiome formation/functioning are the same regardless of host organism or environment. A recent revisit of microbiome definition proposes that it is the theatre of activities of microorganisms living in a given ecosystem [2].

Whilst every 'culture collection' has microorganisms isolated from microbiomes, these represent the culturable components preserved in an axenic state. The German DSMZ collection is one of the few collections with broader, collective deposits of culturable microbiome samples, including strains isolated from *Arabidopsis* [3], human intestinal microbiomes [4], and of the product available for subsequent use but will be translatable to scientists working in other domains such as food and agriculture. In the agricultural domain, the Rothamsted Sample Archive (UK) consists of wheat grain, straw, soil, and herbage together with fertilizers. Seed banks, for example, the Kew Millennium Seed Bank (UK), contain seeds and associated microbial endophytes. Whilst a culture collection will ensure that their microbes are preserved optimally [1] around a sustainability model of 'growth and supply', a biobank will generally store the sample not necessarily focusing on the viability or stability of all the constituent microbial components. This represents a clear demarcation of a living 'culture collection' and a 'biobank' archive repository, although there are occasional exceptions.

The Microbiota Vault (www.microbiotavault.org) represents the first major step towards a comprehensive microbiome resource. This initiative is a proposal for a vault for microbes important to humans and calls for an international microbiome preservation effort [6].

Why do we need biobanks?

- To aid the development of standards
- To allow deposits to ensure compliance with legislation including IP, Nagoya etc.
- As a source of new potential products for industry, medical and environmental applications
- To protect IP e.g. storage of SynComs, outputs from academia and industry
- For biodiversity conservation
- 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 meta data

Where is the science to underpin microbiome biobanking approaches?

- Cryobiology – The UK has a reasonable scientific base, but translation to microbial fields has not been optimal.
- The scientific infrastructure is quite fragmented
- The microbiome presents additional scientific challenges, including but not limited to: retaining functional potential of microbiome samples, maintaining viability of microbial consortia post storage and ensuring genomic integrity of preserved samples.
- Outside studies on human gut microbiota there is little science going on nationally or globally to address these issues. Our team at the UK Crop Microbiome Cryobank is starting to establish a baseline for crop and soil health.

What are we looking to do?

- Follow up the recommendations in the UK Microbiome Roadmap
- Pull together a group of experts from human, animal, plant & environmental domains representing collections, biobanks, bioinformatics and industry representatives
- Undertake a gap analysis, establishing what existing infrastructure and expertise and networks are are in the space e.g. UKBRCN, Cryobiology, CHAP etc. and what is missing
- Identify what the UK strategically requires to support research and industry
- Identify priority accession areas in association with Stakeholders e.g. avoid stamp collecting
- Optimise technology transfer between domains, without recreating wheels
- In association with KTN put together a report and proposal for discussion with UKRI and government, focussing on what the UK requires.

The 3rd International Plant Microbiome Symposium, Dundee (venue tbc)
24 – 26th May 2020

Day 1	**Provisionally 24th May 2022**
	Session 1: host genetic control of the plant microbiota
	Lunch & poster viewing
	Session 2: translational applications
	Reception
Day 2	**25th May**
	Session 3: 'Microbes to the rescue' the plant microbiota and pathogen protection
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	lunch break & poster session
	Session 4: metabolic dialogue between plants and the microbiota
	Social dinner
Day 3	**26th May**
	Session 5: What's next in in plant-microbiota interactions
	Final remarks and departure

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Phytobiomes Conference 2022

13-15 September 2022

Denver, CO, USA

www.phytobiomesconference.org

Main Scientific topics

- Climate/weather
- Environmental Data Set
- Plant fitness
- Microbial community assembly and function
- Network analyses within the phytobiome system
- Modeling
- Data – framework, tools and resources, big data
- Genetic linkages
- Carbon sequestration
- Interactions within phytobiomes for abiotic stress
- Engineering microbes and microbial communities
- Precision agriculture/digital Ag
- Fertilizer, nutrient, and chemical input efficiency
- Product development
- Regulatory requirements
- Greenhouse & Field trials
- Industry research needs

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Thank you for your participation!

- A summary of the outputs of today's meeting will be made available on agmicrobiomebase.org
- We hope to maintain and grow this network, please do spread the word to other stakeholders
- We hope you found attending the meeting to be worthwhile!

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