Innovations in Soil Science

– Soil Biology

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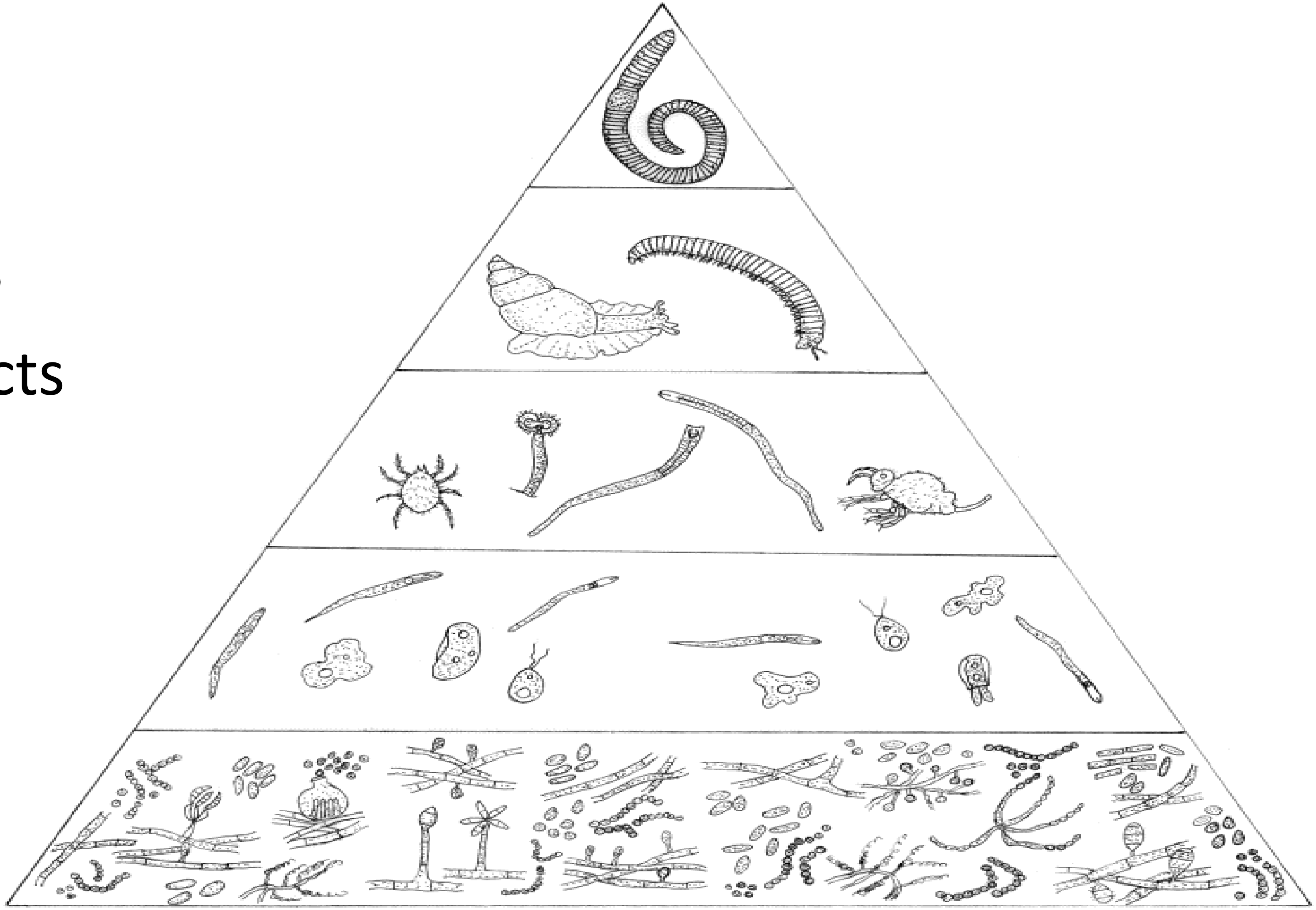
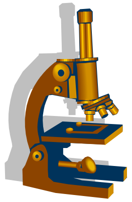
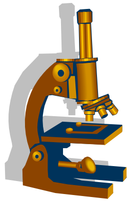
THE UNIVERSITY OF MELBOURNE

Outline

- Soil biology 101
- Soil biology tests
- Biological products
- Conclusions



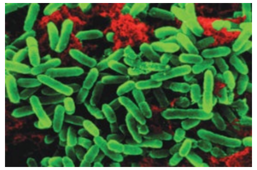
Who lives in our soils?

Group	Organisms	Size	Tools
Microbiota	Bacteria + Archaea Fungi	0.02–5 µm 1–4 µm	100 X magnification 
Microfauna	Protozoa Nematodes	5–200 µm 10 µm–2 mm	100 X magnification 
Mesofauna	Collembola Mites	250 µm–2 mm 100 µm–2 mm	> 40 X magnification 
Macrofauna	Earthworms Beetles Ants Termites	2 mm–1.5 m (visible)	10 X magnification 

Adapted from G. Vadakattu
‘Under the microscope’

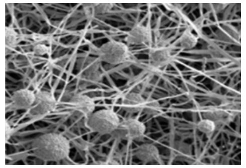
Soil as a physical habitat

Nematodes >30 µm

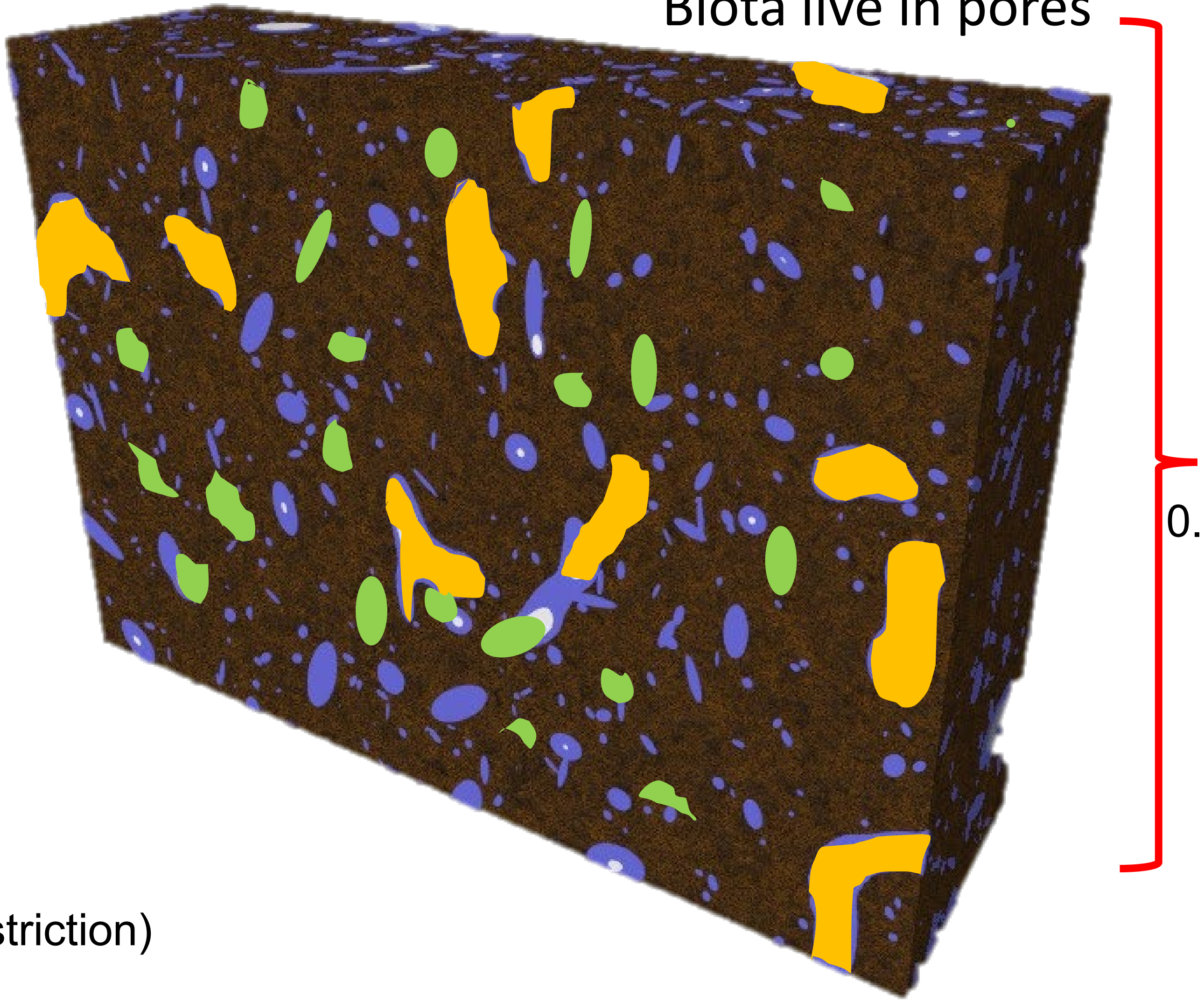


Bacteria <3 µm

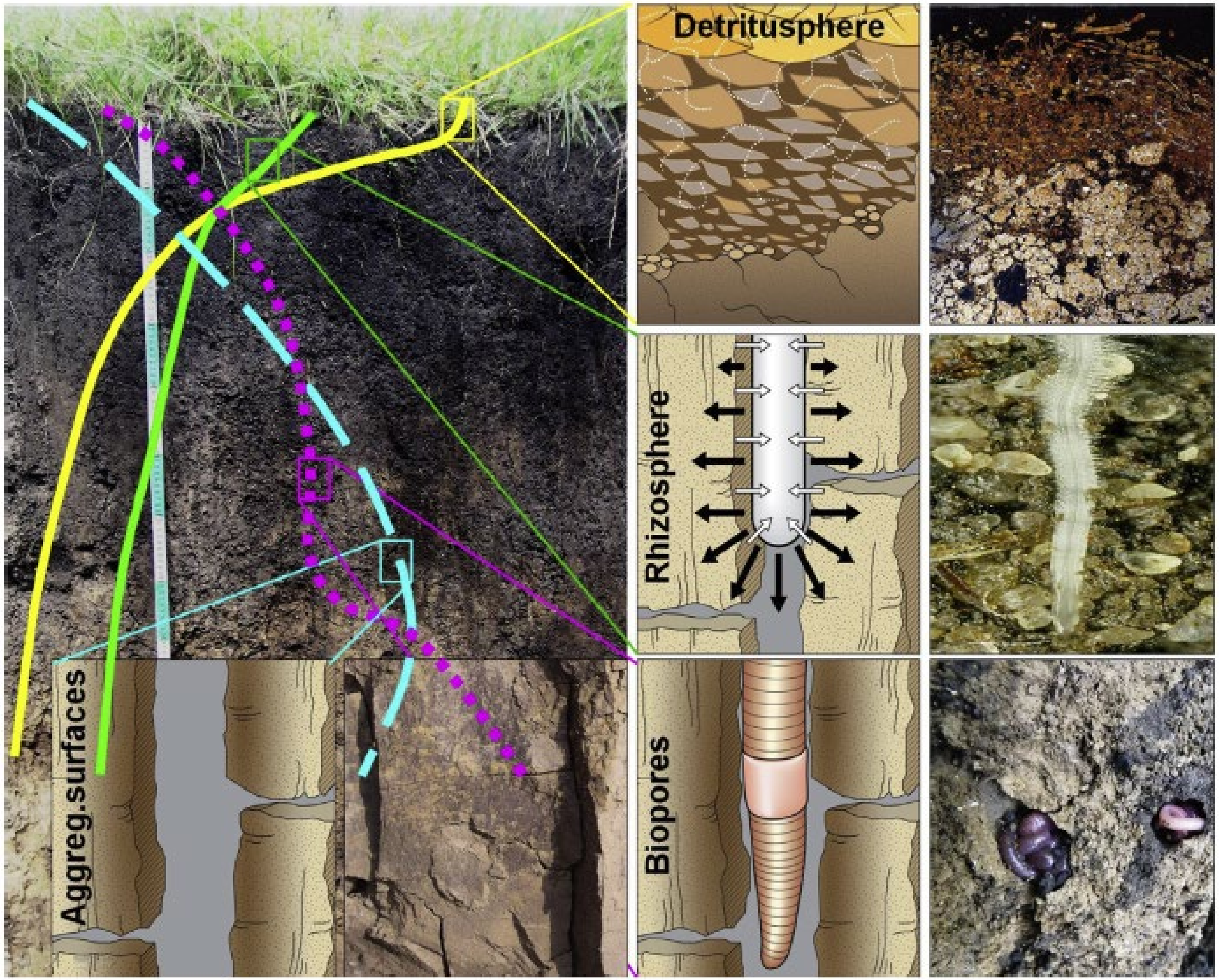
Protozoa 5-20 µm



Fungi (no pore size restriction)



Biological activity hotspots



Kuzyakov & Blagodatskaya 2015

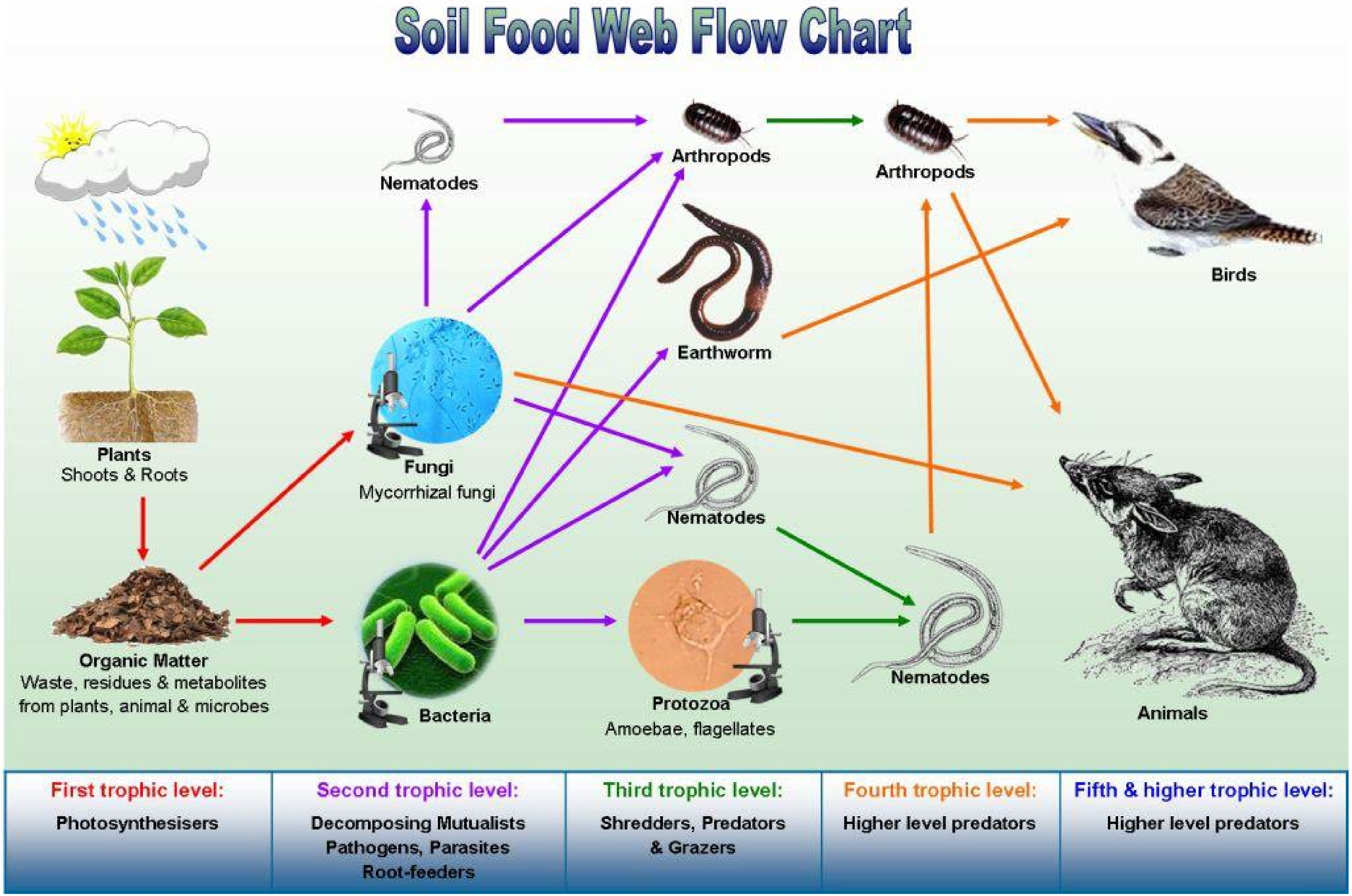
Soil biology ecosystem functions

Ecosystem function	Group	Biology active in function
Carbon transformation	Decomposers	Fungi, bacteria, microbivores, detritivores
Nutrient cycling	Nutrient transformers	N-fixers, mycorrhizae, decomposers, element transformers
Soil structure maintenance	Ecosystem engineers	Megafauna, macrofauna, fungi, bacteria
Biological population regulation	Biocontrollers	Predators, microbivores, hyperparasitism

Kibblewhite, Ritz and Swift 2008

Soil biology ecosystem functions

- Suppress soilborne diseases & pests
- Plant growth promotion
- Degrade pesticides and herbicides
- Regulate water quality e.g. filter nutrients
- Capture and release of greenhouse gases



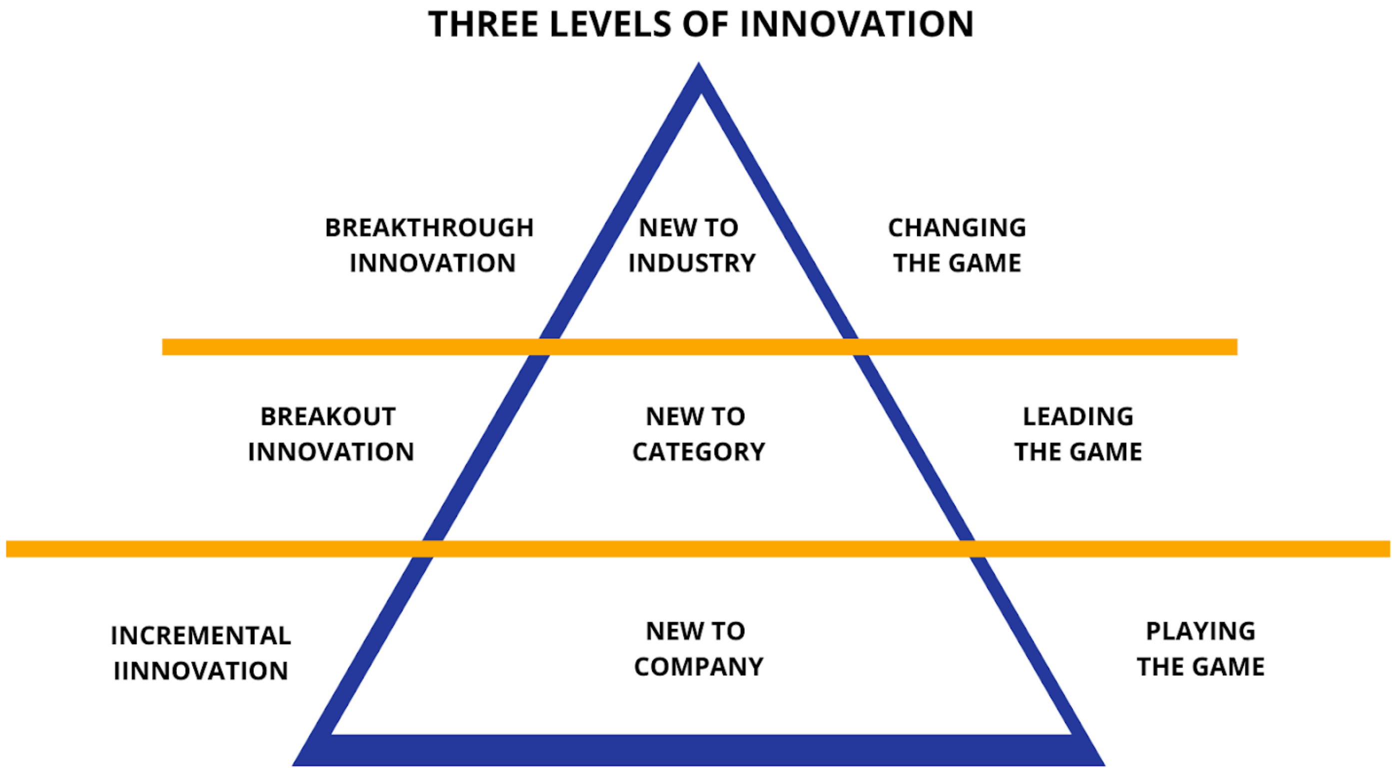
Source:agpath.com.au

Innovation

Definition

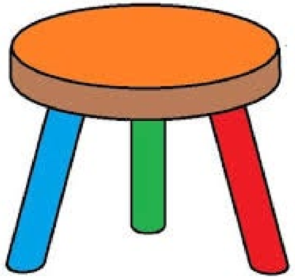
1) A new idea, method, device
2) Introduction of something new

- New or improved product, updated methods, new business model, new or improved services



Soil biology tests

Soil Health



Physical Properties

- Soil texture
- Bulk density
- Infiltration
- Aggregates

Photo: Richard Doyle

Chemical Properties

- Organic matter (colour/residues)
- pH
- CEC
- Salinity

Biological Properties

- Root material, ground cover
- Species abundance/richness (earthworms, dung beetles)
- Biomass
- Respiration
- Enzyme Activities

Considerations for soil biology

Will need to test >1 sample to gain useful information

Ask for an example of the interpretation provided with the test results

Target ranges for healthy & unhealthy soils are yet to be established for biology tests

How will the test help you make management decisions?

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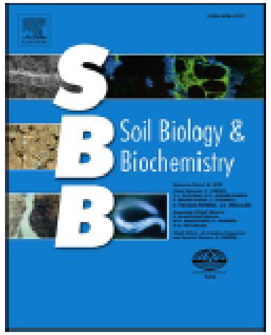

Perspectives Paper

How microbes can, and cannot, be used to assess soil health

Check for updates

Noah Fierer [a,*], Stephen A. Wood [b,c], Clifton P. Bueno de Mesquita [a]

[a] *Department of Ecology and Evolutionary Biology, Cooperative Institute for Research in Environmental Sciences, University of Colorado Boulder, Boulder, CO, 80309, USA*

[b] *The Nature Conservancy, Arlington, VA, 22203, USA*

[c] *Yale School of the Environment, New Haven, CT, 06511, USA*

- **Lists all the different tests and limitations**
- **Suggestions for where to start e.g things that can't be covered by cheap, easy tests**
- **Start by doing, but don't overpromise**

Soil testing rules of thumb

- Test every 3-5 years depending on intensity of system
- Test at the same time each year
- Use the same lab and tests for consistency
- Be aware of landscape, soil type (clay, loam, sandy) when collecting cores
- Use a grid, transect or zig zag to collect 20-30 cores for 1 sample (record pattern + GPS so you can repeat next time)
- Avoid stock camps, fence lines, headlands, dung pads
- Wait if lime or fertiliser applied (depends on soil moisture)

Soil biology test options + case study examples

Visual assessment of soil on farm	• Ground cover • Earthworms, mites, springtails, spiders etc • Depth of topsoil, healthy or damaged roots
Soil test at accredited commercial lab (ASPAC, NATA)	• Soil organic carbon, labile carbon • Potentially mineralizable N (PMN)
Specialised biology tests from commercial lab	• CO_2 respiration • Microbial Biomass C and N • Enzyme assays e.g. cellulose • Sensitive species/groups eg. mycorrhizae, nematodes • DNA tests - bacteria & fungi, functional genes, soil microbiome • Soilborne pathogens e.g. Predicta B

Accredited lab test – Soil chemistry

- Soil chemical measures e.g. soil organic carbon as an indirect measure of soil biology
- Benchmark for soil type and monitor over time

		pH	Nitrate Nitrogen	Ammonium Nitrogen	Phosphorus - Colwell	Organic Carbon	Active (labile) Carbon	Potentially Min. N	Silt	Clay	Sand	Texture
	Depth	water	mg/kg	mg/kg	mg/kg	%	mg/kg	mg/kg	%	%	%	
Ferrosol	0-10	6	46	6.8	26	5.83	1130	120	28.2	24.5	47.3	Silty Loam
	10-20	6	19	12	11	3.51	788	54	28.5	34.7	36.8	Silty Clay Loam
	20-30	5.9	12	4	8.3	2.21	658	49	23.4	44.3	32.3	Clay
Kurosol	0-10	6.4	43	6.7	23	5.62	1380	150	33.6	10	56.4	Silty Loam
	10-20	6.2	3.3	0.95	<5.0	1.61	469	26	35.9	21	43.1	Silty Loam
	20-30	5.5	<0.50	1.7	<5.0	0.94	332	19	33.7	37.4	28.9	Silty Clay Loam
Forest	0-10	4.5	<0.50	12	12	13.5	4050	220	22.4	3.7	73.9	Loamy Sand
	10-20	4.6	<0.50	1.6	<5.0	1.86	607	21	31	3.7	65.3	Silty Loam

PreDicta – soil monitoring for pathogens

- DNA-based soil testing service for growers to identify soilborne pathogens
  - Cereal cyst nematode
  - Root lesion nematode
  - Stem nematode
  - Crown rot
  - Take-all
  - Rhizoctonia barepatch
  - Blackspot of peas
  - Pythium
  - Sclerotinia
  - Aphanomyces

- Grower can gauge risk level use to inform management e.g crop rotation, resistant variety, non-host crop, improve crop nutrition, adjust tillage, improve drainage

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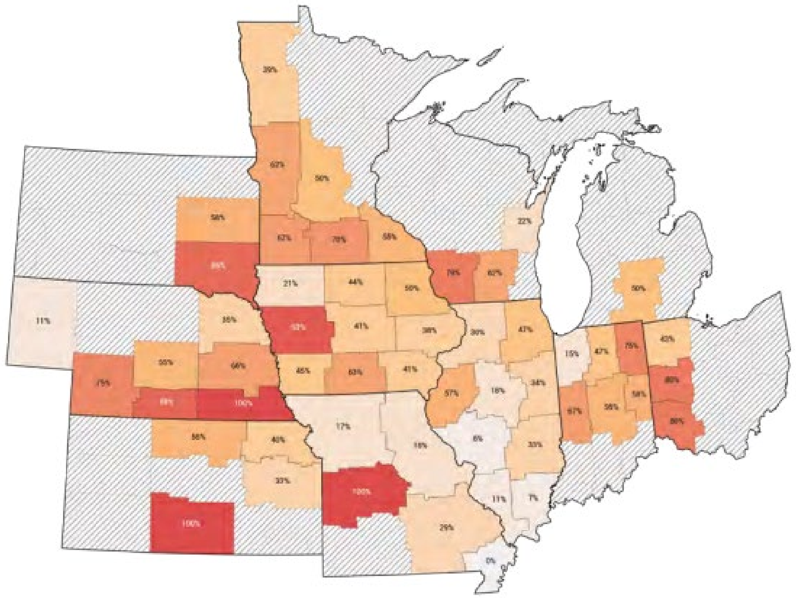
Soil microbiome for disease risk

Pattern Ag: soil microbiome analysis & recommendations for input optimization on farm.

- Focused on specific pest and disease pressures faced by **corn and soybean farmers** in USA
- Add-ons bio-fertility & chemistry nutrient analyses
- Recommends management options

Pattern Ag Analytic	Strongly linked to (with p<0.05; unbalanced ANOVA)
Gibberella Stalk Rot	Soil drainage class
Pythium Root Rot	Soil drainage class
White Mold	Soil drainage class
Sudden Death Syndrome	Soil drainage class
Mycorrhizal Fungi (AMF)	Tillage practice & soil drainage class
Plant Growth Promoters	Soil drainage class



Gibberella Stalk Rot Risk for 2023

So, let's do the math:

- *Treated Corn =* **$25-$30** per acre
- *On this grower's 400 acres, that would be somewhere between* **$10,000-$12,000**
- *Pattern Ag's Pressure Panel run on these acres totaled* **$1,400**

for a cost savings of approximately $10,000

Soil biology test – low resolution information

microbiology laboratories australia
agriculture • forestry • environmental

MICROBE ACTIVITY WISE PRO

V15-2020

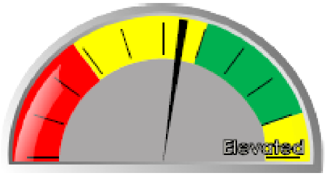
Name: Tahlia Kinrade   Sample: Baxter - soil pit area - ferrosol 0-10cm   Analysis no.: 3712-1-MAWP   Date: 14/03/2023

Customer name	NRM North	Date received	16/03/2023
Client name	Tahlia Kinrade	Agent	Microbiology Laboratories Australia
Sample name	Baxter - soil pit area - ferrosol 0-10cm	Advisor	
Crop	Pasture, permanent, mixed, improved	Authorised by	Dr Maria Manjarrez
Date sampled	14/03/2023 Ext. ID	Analysis number	3712-1-MAWP

Indicators

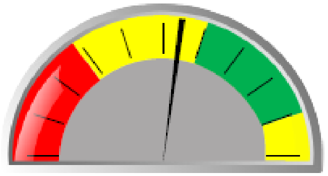
Data

Potential Microbial Activity



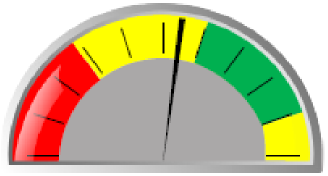
Microbial Potential		Yours	Guide
Microbial Activity Indicator		45.7	80.0
Soil Basal Respiration	mg CO_2/kg/day	407	668
Soil Microbial Biomass Carbon	mg/kg	2430	4000

Potentially Mineralisable Nitrogen



Nitrogen Potential		Yours	Guide
Potentially Mineralisable N (PMN)	mg/kg	791	1384
	kg/ha	949	1661

Potentially Releasable Phosphorus



Phosphorus Potential		Yours	Guide
Potentially Releasable P (PRP)	mg/kg	60	106
	kg/ha	72	127

Key

Low   Fair   Good

microbiology laboratories australia
agriculture • forestry • environmental

MICROBE ACTIVITY WISE PRO

V15-2020

Name: Tahlia Kinrade   Sample: Baxter - soil pit area - ferrosol 0-10cm   Analysis no.: 3712-1-MAWP   Date: 14/03/2023

Potential Microbial Activity

The microbial activity in your sample was fair. However, it could be increased by adopting management practices that encourage microbial activity. If your soil is low in carbon consider the addition of organic based soil conditioners. If your soil is low in nitrogen consider the addition of N fertiliser. It is very important to take the C:N ratio of your soil into account when adding any fertilisers high in C or N. In most farmed soils it is good practice to aim for a C:N ratio of less than 20:1 (12:1 is optimal for most soils, but may not be practicable for some production systems). Avoid the addition of large amounts of high C fertiliser to soils low in N, and the addition of large amounts of high N fertiliser to soils low in C, as these practices can further deplete Total C and Total N, and microbial activity.

Potentially Mineralisable N (PMN)

Potentially Mineralisable Nitrogen (PMN) was fair. However, crops grown in this soil will require some additional N inputs for optimal growth, particularly at germination. It may be possible to reduce the amount of N inputs required according to nutrient budgets by approximately 25% of the PMN amount. Soluble N is immobilised by soil microbiology into the soil microbial N pool. While this result indicates that N inputs can possibly be reduced, it is important not to underestimate the amount of PMN that will be immobilised by soil microbiology. Balance any soil carbon inputs with N applications. Aim for a soil C:N ratio of less than 20:1 (12:1 is optimal for most soils, but may not be practicable in some production systems).

Potentially Releasable P (PRP)

Potentially Releasable Phosphorus (PRP) was fair. Crops grown in this soil will require some additional P inputs for optimal growth, particularly at germination. However, it may be possible to reduce the amount of P inputs required by crop nutrient budgets by 5% of the PRP amount. Soluble P is easily immobilised by soil chemistry and microbiology. In many soils only 30% or less of soluble P additions to soil are available to plants and, while this result indicates that P can possibly be reduced, it is important not to underestimate the amount of P that will be immobilised by soil chemistry and microbiology.

Explanations

Microbe Activity Wise Pro measures the amount of carbon dioxide (CO_2) produced by microbes in your soil over 3 days to calculate potential microbial activity, soil basal respiration (SBR), soil microbial biomass carbon (SMBC), potentially mineralisable nitrogen (PMN) and potentially releasable phosphorus (PRP). These results allow you to assess your soil's capacity for total microbial activity and potential nutrient release under ideal conditions. In general, higher activity is linked to better soil health and nutrient availability because of the important roles microbes play in nutrient cycling and other important soil processes. For more information visit www.microbelabs.com.au

General soil biology tests – case study #1

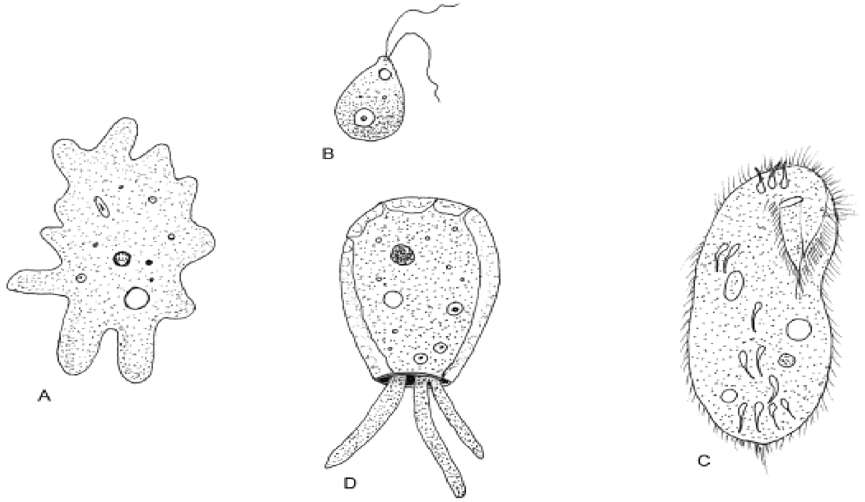
		Microbial Activity Indicator	Respiration	Microbial biomass carbon	Potentially mineralisable N	Phosphorus potential
	Depth		mg CO2/kg/day	mg/kg	mg/kg	mg/kg
Ferrosol	0-10	45.7	407	2430	791	60
Ferrosol	10-20	32.8	299	1838	567	43
Kurosol	0-10	45.5	405	2418	787	60
Kurosol	10-20	22.9	214	1387	397	30
Forest	0-10	54	474	2810	935	71
Forest	10-20	28.2	260	1626	488	37
Guide		80	668	4000	1384	106

- other soil data for same samples can support interpretation (pH, org C, CEC, bulk density)

Tests for specific organisms

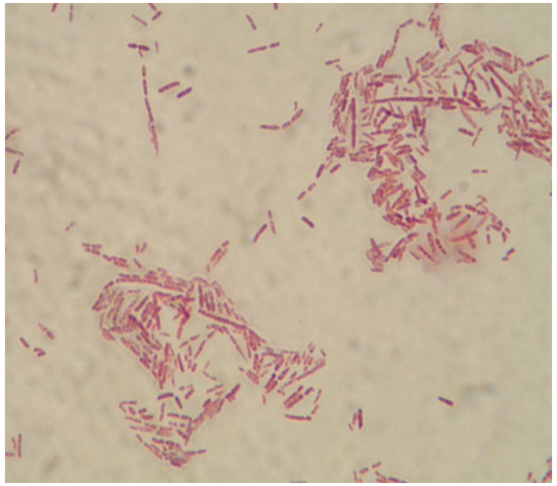
Microscopic inspection

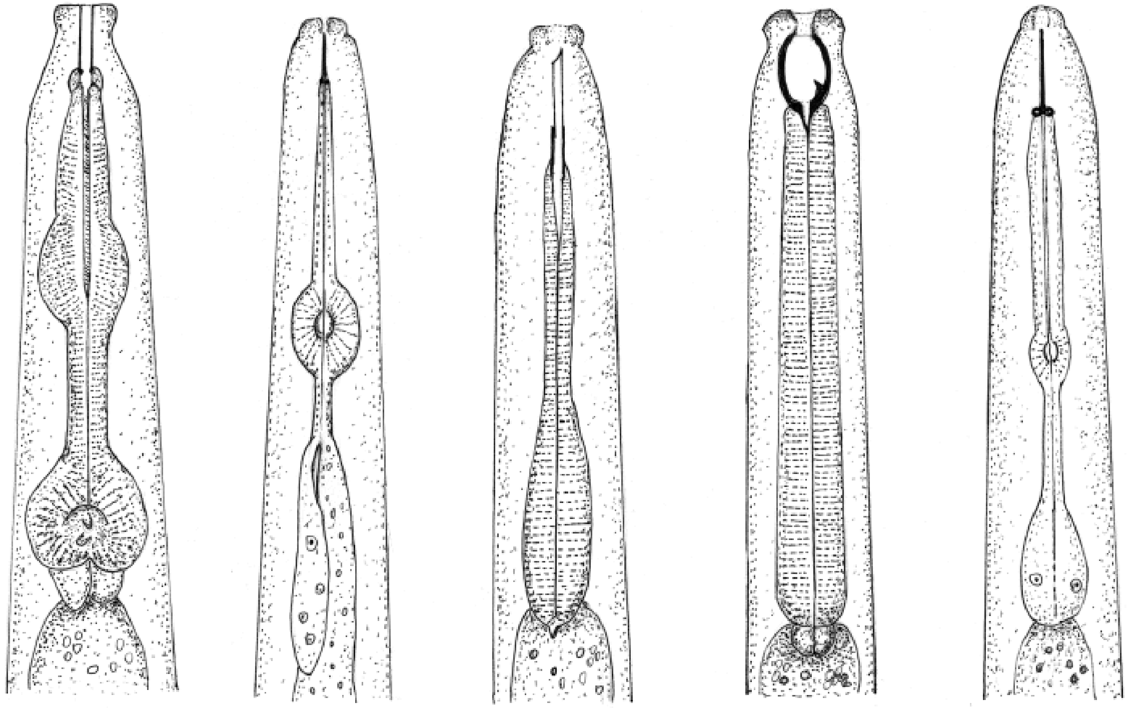
- identification of bacteria, fungi, protozoa
- mycorrhizal colonisation of roots
- nematodes diversity



Culturing on agar plates

- colony counts





Biology case study test #2

Microscope counting of nematode groups

– great expertise needed for test
– provides information about soil food web

Specialised organism test – soil nematodes

Sample details	Nematodes extracted per 200 g dry weight soil				
	Spiral (*Helicotylenchus* sp.)	Lesion (*Pratylenchus* sp.)	Stubby (*Paratrichodorus* sp.	Total plant parasites	Total free-living
Ferrosol	29	3	4	36	297
Kurosol	22	37	8	67	522
Native Veg	14	0	0	14	853

Interpretation:

The two agricultural soils had higher numbers of plant parasitic nematodes than the natural vegetation soil, but the total plant parasites were relatively low.

The main parasitic nematodes of concern in these samples are lesion and stubby root nematodes, however, the numbers observed would suggest that the damage potentials are currently quite low.

As expected, the natural vegetation soil had the highest number of free-living nematodes. While a detailed count was beyond the scope of this assessment, it was noted that the natural vegetation soil contained numerous omnivorous and predatory nematodes, while nematodes belonging to these functional groups were scarce in the agricultural soils.

Moderately pathogenic but not at these numbers

Specialised organism test – Free-living nematodes

Sample details	Nematodes extracted per 200 g dry weight soil				
	Spiral (*Helicotylenchus* sp.)	Lesion (*Pratylenchus* sp.)	Stubby (*Paratrichodorus* sp.)	Total plant parasites	Total free-living
Ferrosol	29	3			297
Kurosol	22	37			522
Native Veg	14	0			853

Native veg had lots of omnivores and predators = very healthy

New approaches to measuring soil biology with monitoring potential

- Soil fauna (beneficial nematodes)
    - ❑ Quantitative PCR (qPCR) DNA test replace microscopy
    - ❑ Nematode-based indices for soil health based on DNA (QPCR/sequencing)
- Microbial community composition (fungi, bacteria, archaea, protists)
    - ❑ 16S ribosomal RNA and Internal transcribed spacer (ITS) amplicon sequencing
    - ❑ Abundance measured by qPCR test or high throughput sequencing
- Microbial functional gene composition
    - ❑ Specific genes e.g. N cycle genes *amoA*, *nifH*
    - ❑ Abundance measured by qPCR or high-throughput shotgun metagenomic sequencing
- **<u>BUT….</u> Better interpretation services needed or specific frameworks for use**

Biology case study test #3

Soil DNA sequencing – Bacterial and Eukaryote microbiomes

– high resolution test, too much info?

Hey, what is a Eukaryote? Never heard of them!

All animals, plants, fungi, protists, and most algae are Eukaryotes. They can be single- celled or multicellular.

Soil microbiome – diversity interpretation

Soil·Health·Summary·Comparison¶

BLOCK·greenside·soil·pit·area·-·kurosol,·Greenside·spil·pit·area·-·ferrosol,·Native·vegetation·comparison·(Pasture)¶

SAMPLE·DETAILS·17-Mar-2023·Soil·-·DNA¶

¤	GREENSIDE·SOIL·PIT·AREA·-·KUROSOL¤	GREENSIDE·SPIL·PIT·AREA·-·FERROSOL¤	NATIVE·VEGETATION·COMPARISON¤
AMF·(VAM)¤	28.9¤	34.1¤	51.9¤
BACTERIA¤	77¤	43¤	6.7¤
FUNGI¤	52.6¤	74.1¤	18.5¤
MESOFAUNA¤	52.6¤	22.2¤	81.5¤
PROTISTS¤	94.1¤	94.8¤	91.1¤

Theory

Greater diversity = greater resilience of ecosystem functions

Soil microbiome – diversity interpretation

Bacterial Diversity Comparison

BLOCK greenside soil pit area – kurosol, Greenside spil pit area – ferrosol, Native vegetation comparison (Pasture)

SAMPLE DETAILS 17-Mar-2023 Soil – DNA

	GREENSIDE SOIL PIT AREA – KUROSOL	GREENSIDE SPIL PIT AREA – FERROSOL	NATIVE VEGETATION COMPARISON
ACIDOBACTERIA	57	32.6	14.8
ACTINOBACTERIA	88.2	75.6	5.2
BACTEROIDETES	80	70.4	48.1
CHLOROFLEXI	74.8	19.3	33.3
FIRMICUTES	45.9	80.7	48.1
GEMMATIMONADETES	40.7	78.5	7.4
NITROSPIRAE	8.9	11.8	13.3
PROTEOBACTERIA	57	64.4	3.7
VERRUCOMICROBIA	61.5	26.7	50.4

Soil microbiome – diversity interpretation

Nematode·Diversity·Comparison¶

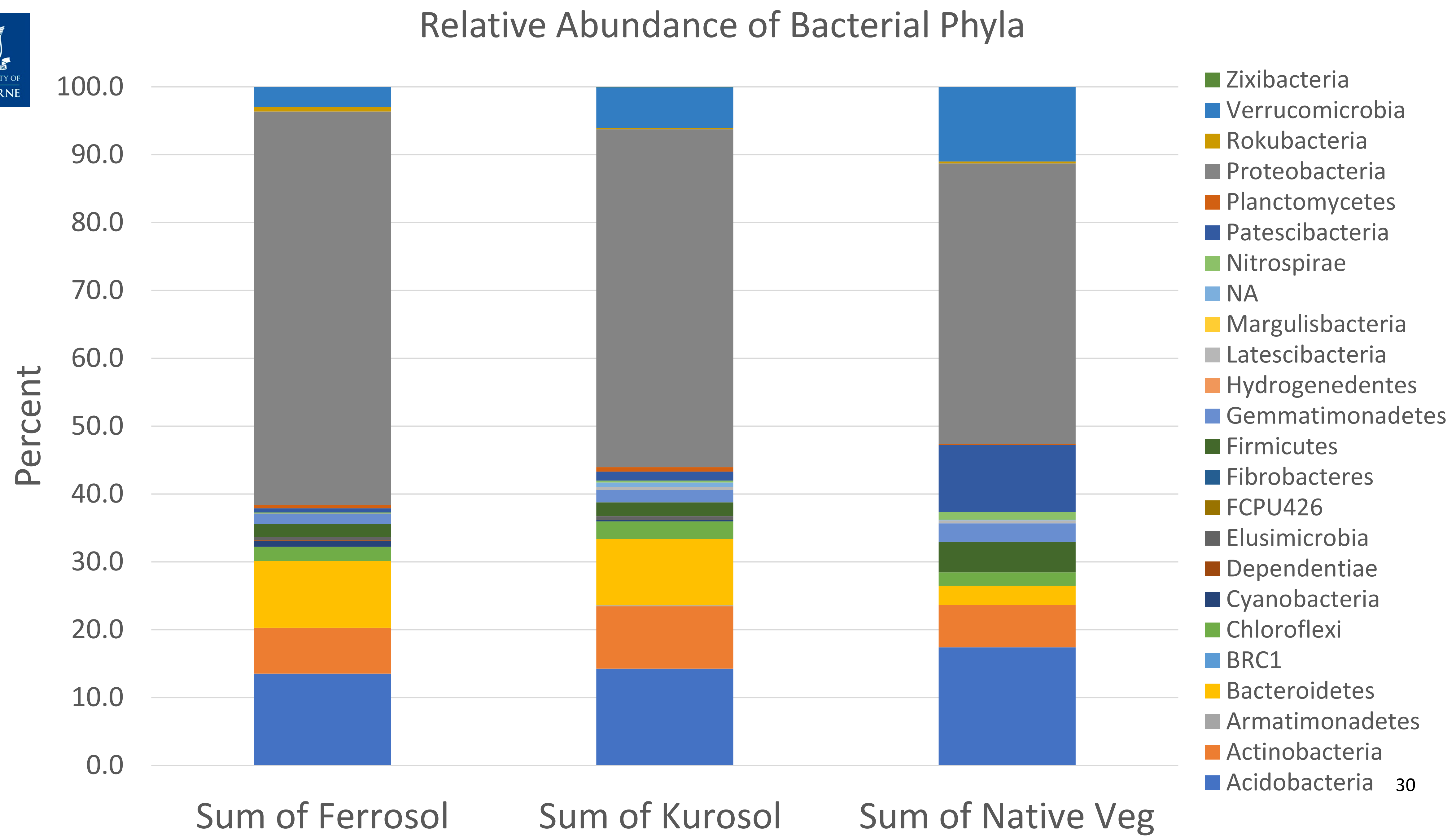
BLOCK·greenside·soil·pit·area·-·kurosol,·Greenside·spil·pit·area·-·ferrosol,·Native·vegetation·comparison·(Pasture)¶

SAMPLE·DETAILS·17-Mar-2023·Soil·-·DNA¶

¤	GREENSIDE·SOIL·PIT·AREA·-·KUROSOL¤	GREENSIDE·SPIL·PIT·AREA·-·FERROSOL¤	NATIVE·VEGETATION·COMPARISON¤
BACTERIVORE¤	22.2¤	17¤	17.8¤
EUKARYVORE¤	87.4¤	91.8¤	94.8¤
FUNGIVORE¤	76.3¤	80¤	83¤
OMNIVORE¤	42.2¤	50.4¤	68.2¤
PREDATOR¤	74.8¤	78.5¤	81.5¤

¶

Plant parasitic nematodes missing from this analysis, but trophic groups detected

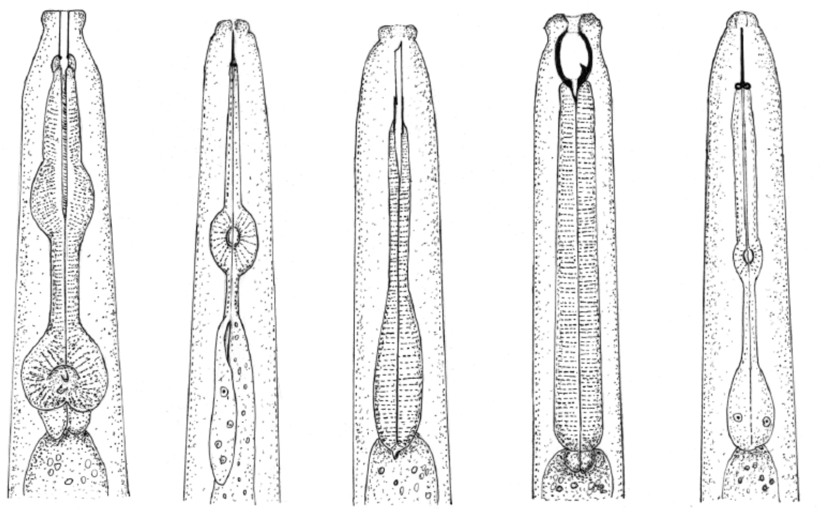

Relative Abundance of Bacterial Phyla
Percent
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Sum of Ferrosol
Sum of Kurosol
Sum of Native Veg
Zixibacteria
Verrucomicrobia
Rokubacteria
Proteobacteria
Planctomycetes
Patescibacteria
Nitrospirae
NA
Margulisbacteria
Latescibacteria
Hydrogenedentes
Gemmatimonadetes
Firmicutes
Fibrobacteres
FCPU426
Elusimicrobia
Dependentiae
Cyanobacteria
Chloroflexi
BRC1
Bacteroidetes
Armatimonadetes
Actinobacteria
Acidobacteria

More useful to target known beneficial groups? - strains of rhizobia

Ferrosol %	Kurosol %	Native Veg %	Order	Genus
0.49	0.00	0.00	Rhizobiales	Mesorhizobium
0.29	0.00	0.00	Rhizobiales	NA
0.24	0.00	0.00	Rhizobiales	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
0.12	0.00	0.03	Rhizobiales	NA
0.08	0.24	0.00	Rhizobiales	Mesorhizobium
0.05	0.00	0.00	Rhizobiales	NA
0.03	0.05	0.00	Rhizobiales	Mesorhizobium
0.00	0.20	0.00	Rhizobiales	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
0.00	0.00	0.30	Rhizobiales	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
0.00	0.00	0.28	Rhizobiales	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium

Case study #4 DNA-based nematode assessments for soil health

Microscope identification



Source: Stirling et al 2016

- Requires experienced and patient morphological specialist, not many labs can do this analysis

DNA tests by SARDI

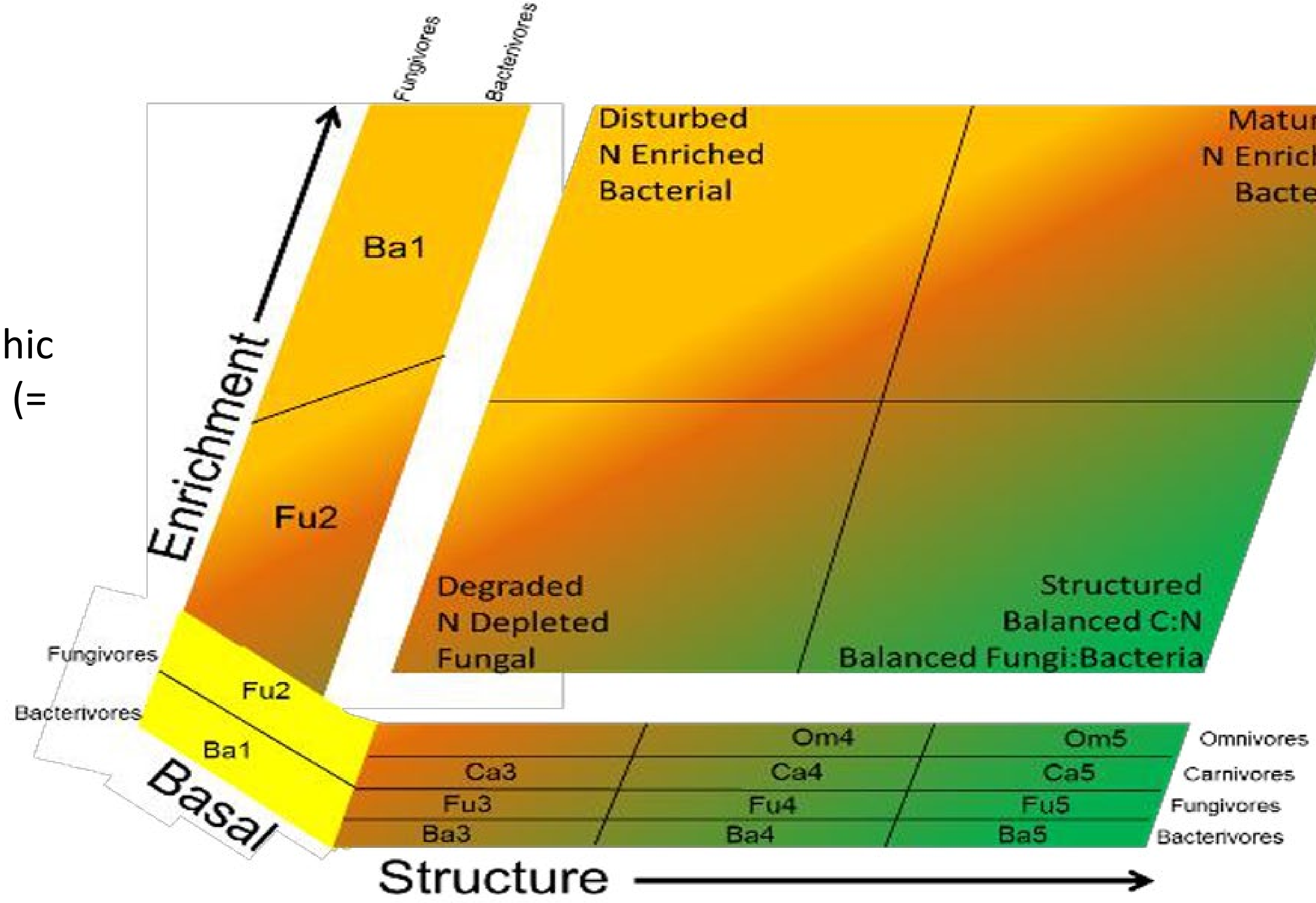
qPCR Assay	Feeding Group	Classification
Dorylaimida	Omnivore	Order
Mononchida	Predator	Order
Aphelenchidae	Fungivore	Family
Aphelenchoididae	Fungivore	Family
Cephalobidae	Bacterivore	Family
Mesorhabditinae	Bacterivore	Sub family
Rhabditinae 1 and 2	Bacterivore	Sub family
Panagrolaimidae	Bacterivore	Family
Tylenchinae 1 - 6	Plant associate	Sub family

Source: Katherine Linsell SARDI (DAS001111, BWD00245)

- Soil DNA extracted and groups identified with 15 qPCR assays
- Quicker way to count groups and calculate nematode-based indices for soil health

Nematode-based indices for soil health

Poor soil – 1 trophic group dominates (= enriched group)



Healthy soil – 4 trophic groups present (= structured soil food web)

- Indices based on
- A healthy soil requires a balance of the different nematode trophic groups to regulate organisms in the soil food web. Omnivores sensitive to disturbance, take longer to recover.

Biological products

Regenerative Agriculture

Table 1. Agronomic principles and practices considered to be part of Regenerative Agriculture and their potential impacts on restoration of soil health and reversal of biodiversity loss.

Principles	Practices	Restoration of soil health	Reversal of biodiversity loss
Minimize tillage	Zero-till, reduced tillage, conservation agriculture, controlled traffic	***	–
Maintain soil cover	Mulch, cover crops, permaculture	***	*
Build soil C	Biochar, compost, green manures, animal manures	***	–
Sequester carbon	Agroforestry, silvopasture, tree crops	***	**
Relying more on biological nutrient cycles	Animal manures, compost, compost tea, green manures and cover crops, maintain living roots in soil, inoculation of soils and composts, reduce reliance on mineral fertilizers, organic agriculture, permaculture	***	–
Foster plant diversity	Diverse crop rotations, multi-species cover crops, agroforestry	**	***
Integrate livestock	Rotational grazing, holistic [Savory] grazing, pasture cropping, silvopasture	**	?
Avoid pesticides	Diverse crop rotations, multi-species cover crops, agroforestry	*	***
Encouraging water percolation	Biochar, compost, green manures, animal manures, holistic [Savory] grazing	***	–

Based on McGuire (2018), Burgess et al. (2019) and Merfield (2019).

Giller et al 2021 Regenerative Agriculture: An agronomic perspective. Outlook on Agriculture

Biological amendments – Abbott et al 2018

- biostimulants (Chitosan, humic substances, seaweed extracts, amino acids)
- organic amendments (manures, composts, vermicompost, compost tea, biochar, biochar-enhanced products)
- microbial inocula
- pelletised formulations and extracts e.g. compost teas

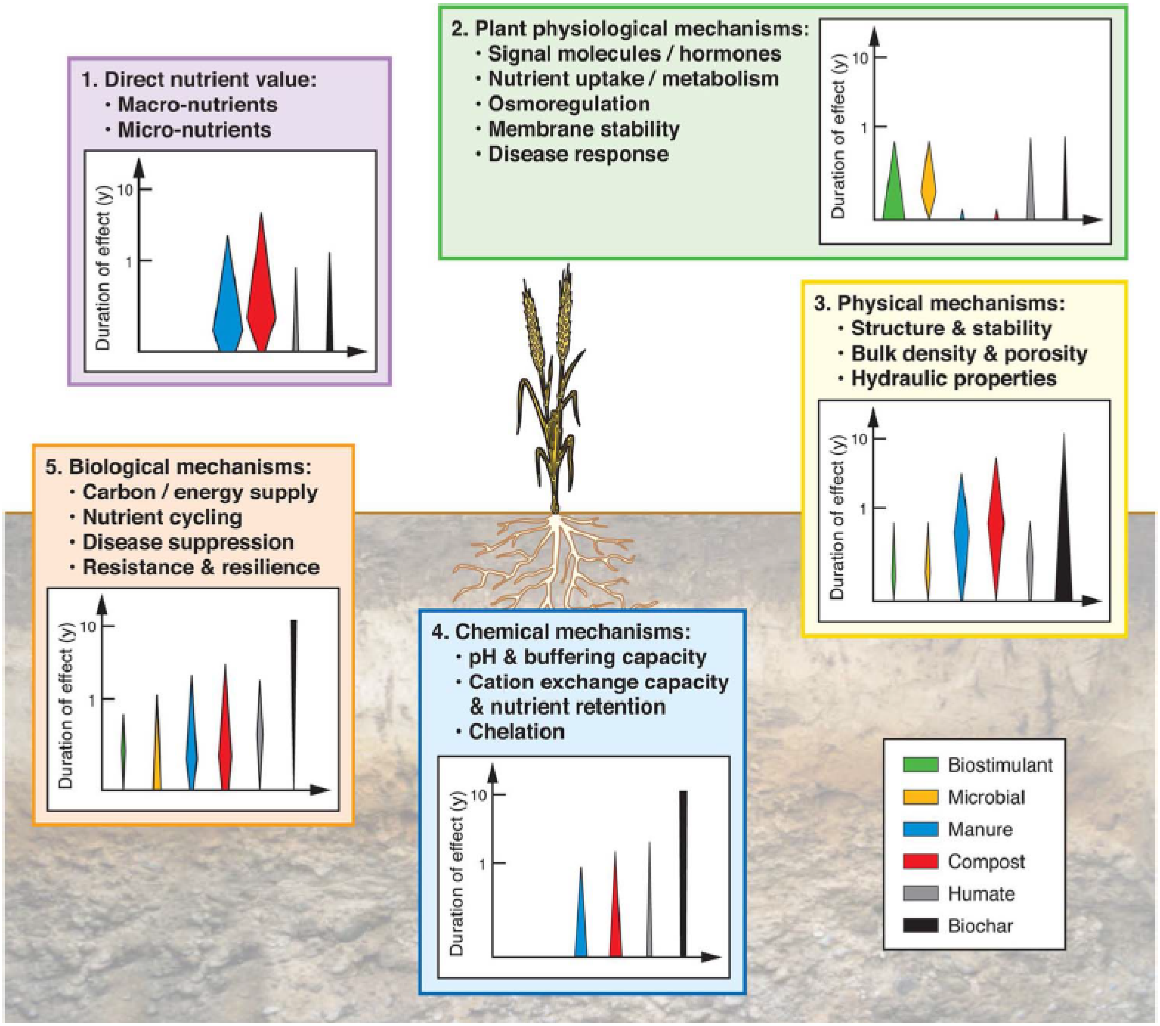
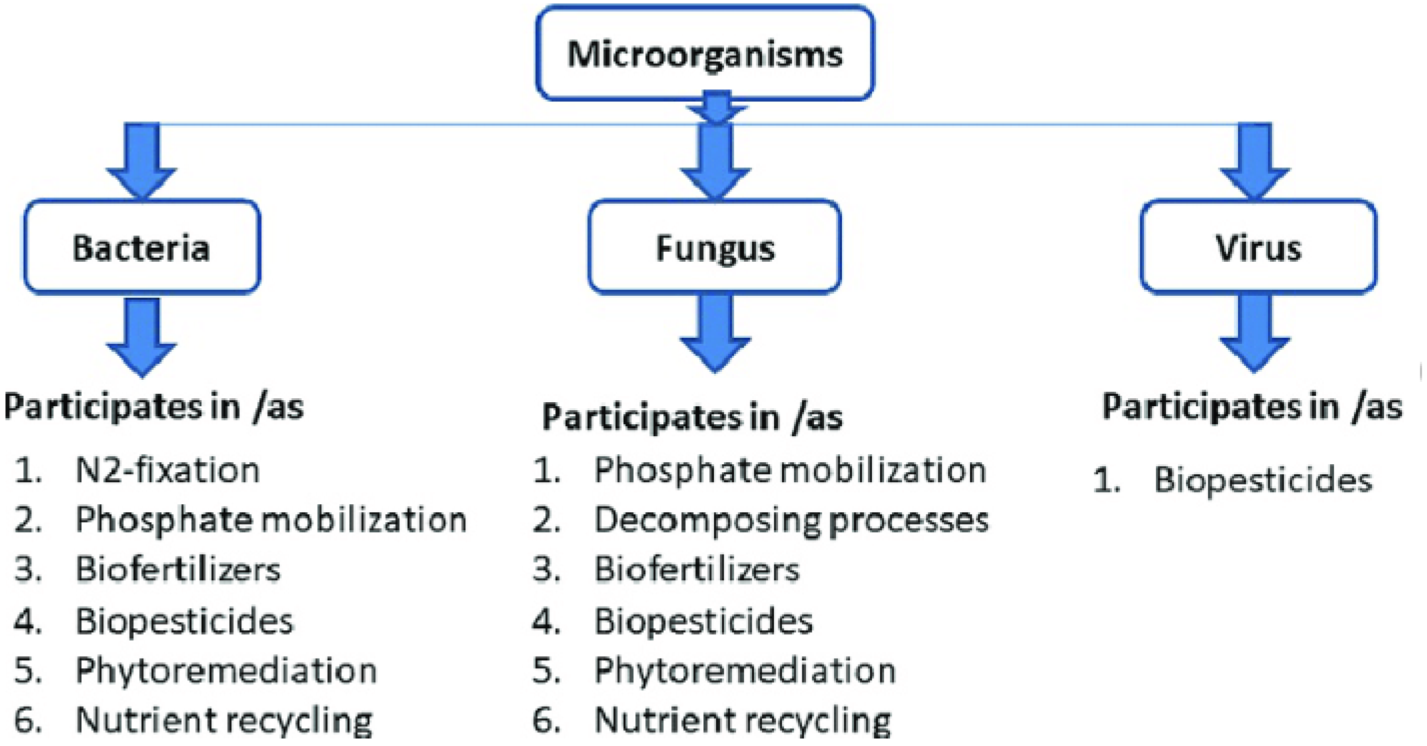


Fig. 1. Potential benefits from application of biological amendments in agriculture can be associated with direct nutrient contributions, plant physiological responses, and/or modifications in soil physical, chemical or biological components of soil health. The biological amendments are very varied but are categorised here as biostimulants (plant growth stimulants), microbial (including rhizobia for legumes and wider groups of microbial inoculants), manure and compost, humates (humic substances, some of which also fit the category of biostimulants), and biochar (includes biochars with a range of different properties). The width of bars indicates estimates of generalised intensity of response and the length of the bars indicates duration of response in years (y). Generalised effects include a range of methods of application and modes of action.

Biological amendment conclusions

1. Scientific evidence of field-scale benefits of most biological amendments with potential for use in rain-fed agriculture is not widely available, except in specific cases such as legume inoculant industry
2. Quantities of organic amendments required to make a significant contribution in rain-fed agriculture are often not available
3. Good evidence that long-term use of a number of these materials can be more beneficial than retention of stubble alone in cropping systems
4. Evidence of benefits of specific biostimulants in agriculture need to include field studies as well as glasshouse studies
5. Tools are needed for comparison of soil biological amendments based on their composition and likely impact of key soil biological, chemical, and physical functions, then develop a framework to guide decision-making by farmers, consultants and policy makers

Roles of microbes in sustainable agriculture

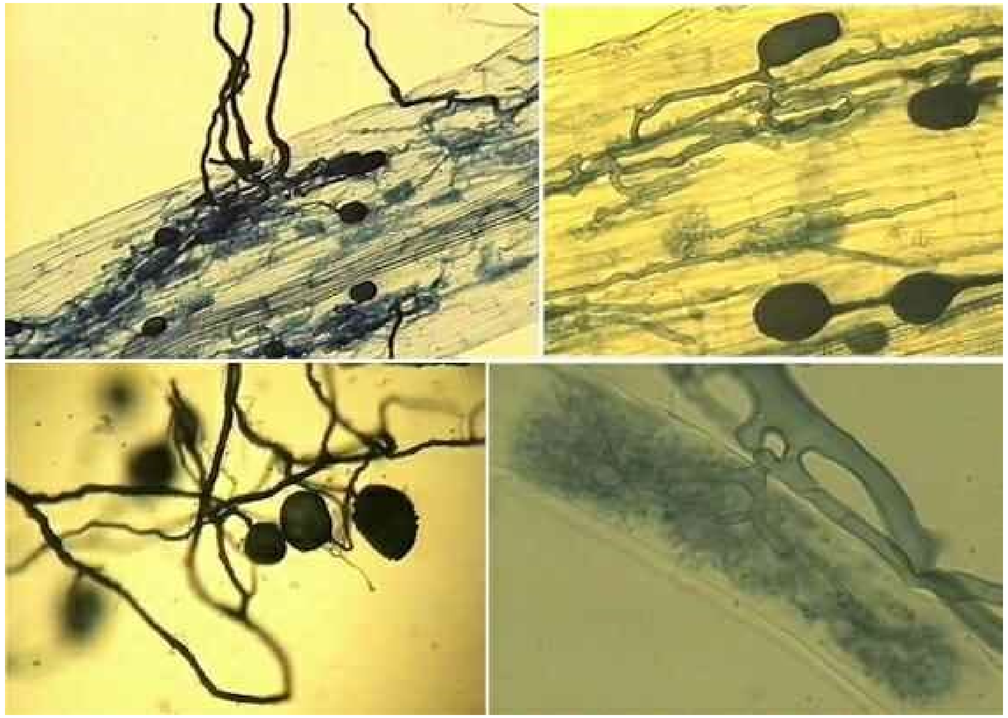


2 Various roles of microbes in development of sustainable agriculture.

Naturally occurring functions of soil microbes, how can we manipulate them for increased crop production, reduced disease or environmental protection/rehabilitation?

Microbial inoculants: enhanced nutrient assimilation

N-Fixation*
(rhizobia)

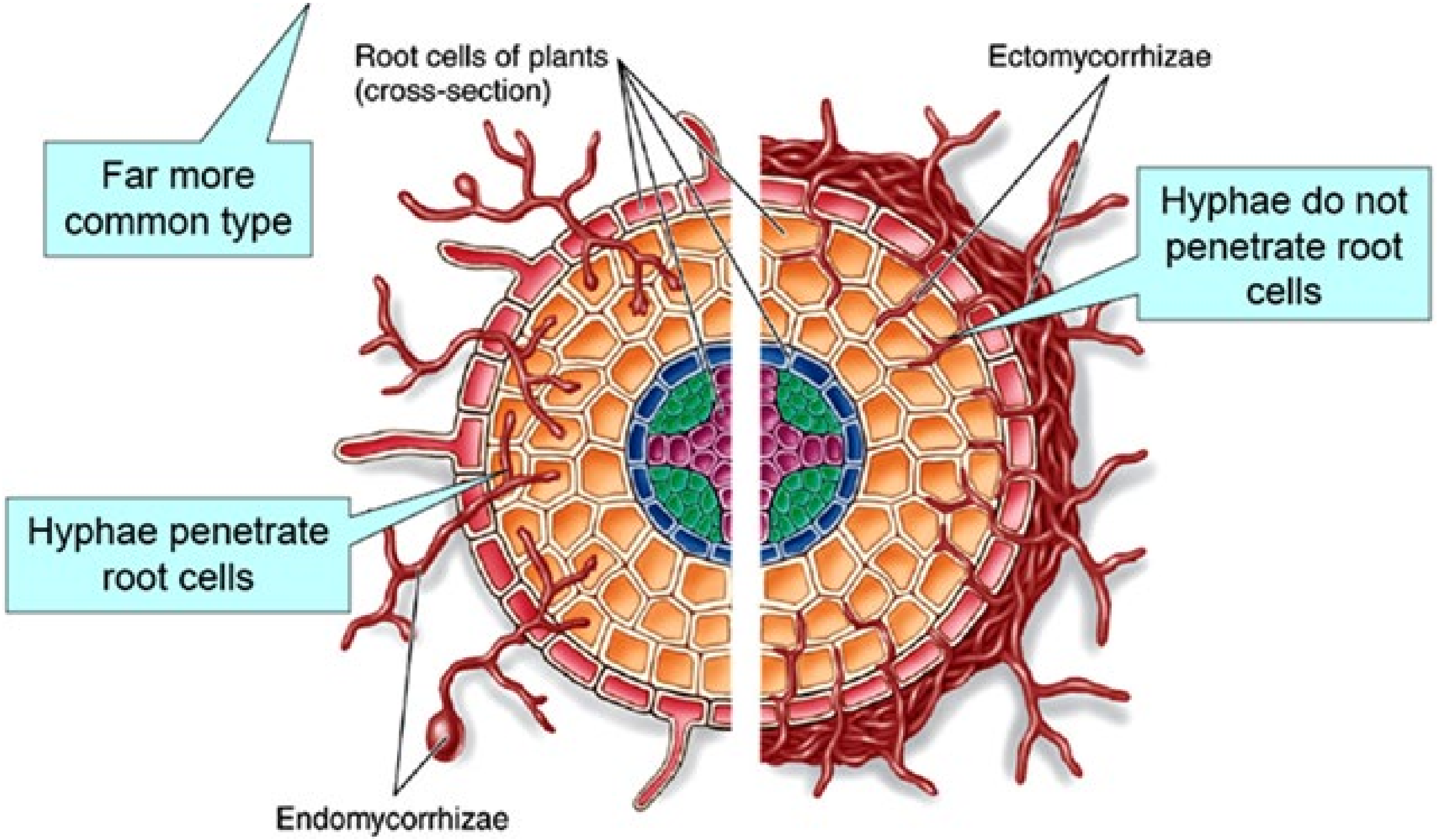


P-assimilation
Arbuscular mycorrhizal fungi

Source: INOCULATING LEGUMES: A PRACTICAL GUIDE

Mycorrhizal fungi – two types

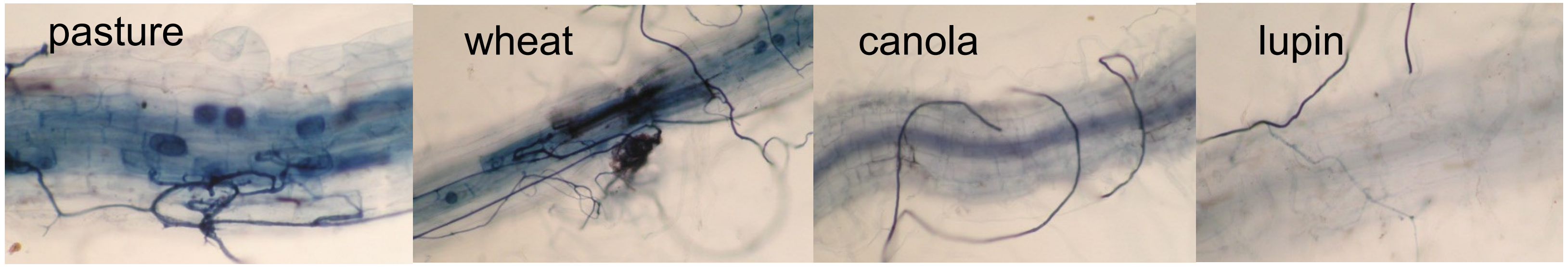
- These fungi live in a mutual symbiotic association with a plants
- Arbuscular Mycorrhizal fungi (AMF) are endomycorrhizae, always in phylum Glomeromycota



Ectomycorrhizae: More common in trees e.g. beech, fir, birch

Endomycorrhizae: Common in wheat, rice, grasses

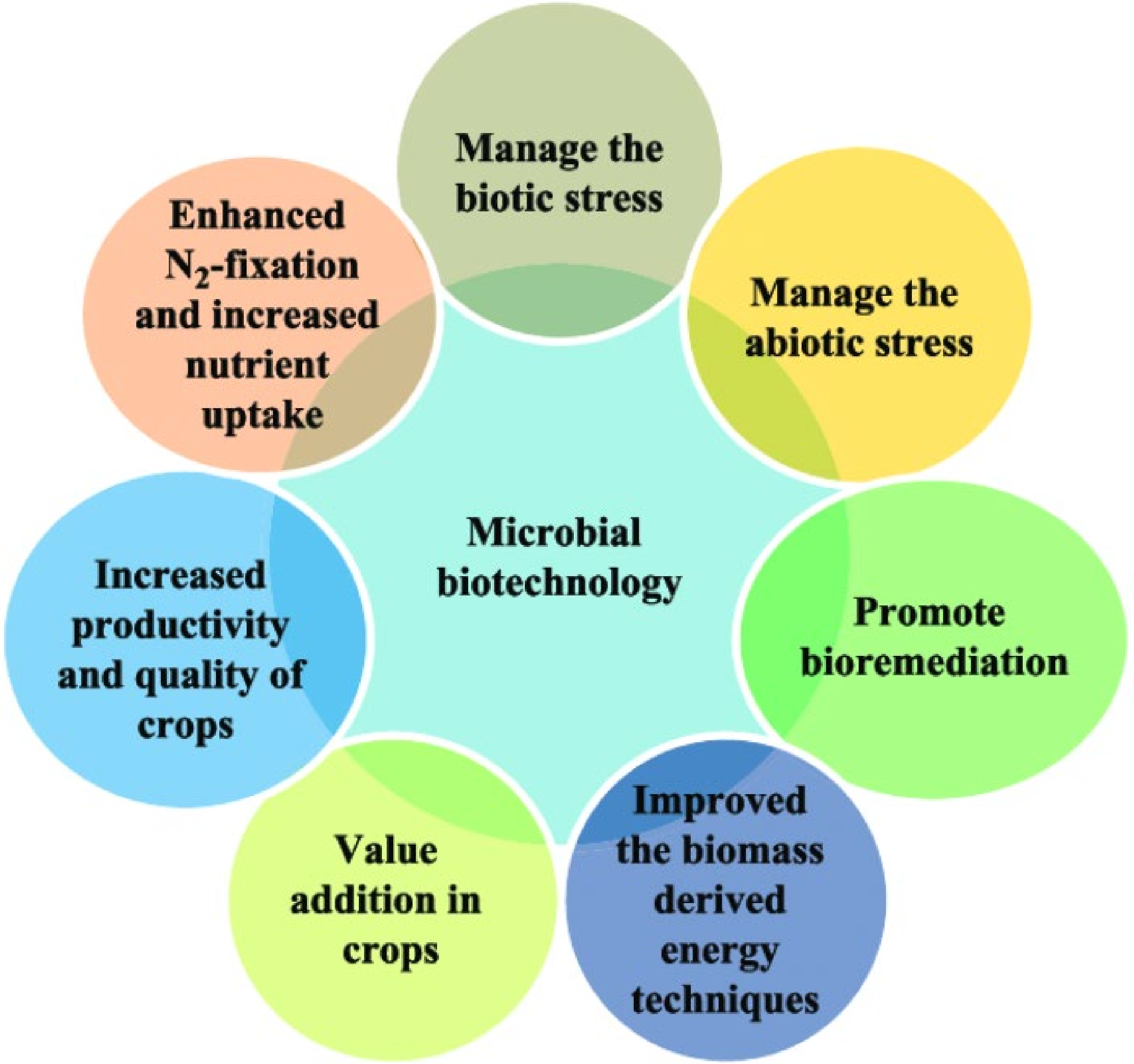
Phosphorus and microbes



1. Enhanced P uptake – AMF + plant
2. P-mineralisation
   - soil microbes transform and cycle organic P in soil organic matter (plant litter, dead microorganisms).
   - mineralised by phosphatase and phytase enzymes
3. P solubilisation- acid production
   - organic acid metabolites reduce soil pH & release inorganic forms of P (hydroxylapatite and calcium hydrogen phosphate dihydrate)

*Illmer, P., and Schinner, F. (1992) Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biology and Biochemistry* **24**: 389-395.

Biotech targets

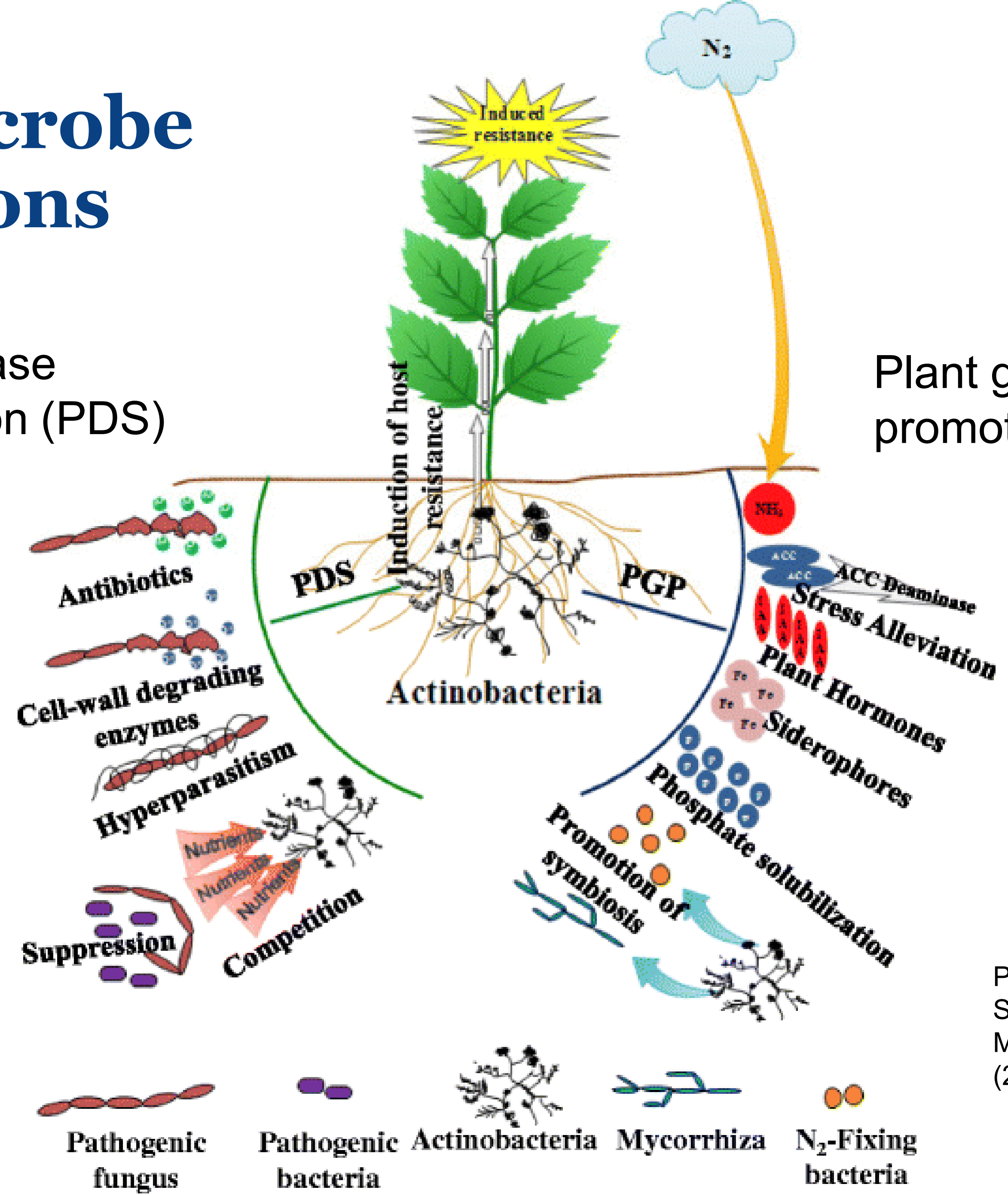


- Combinations of microbes with different functions “stacking”
- Indigenous microbes – local soil or centre of origin of crop
- Synthetic communities for product formulation

Plant-microbe interactions

Plant disease suppression (PDS)

Plant growth promotion (PGP)



Palaniyandi, S.A., Yang, S.H., Zhang, L. et al. Appl Microbiol Biotechnol (2013) 97: 9621.

Guideline for the regulation of biological agricultural products - APVMA

Australian Government
Australian Pesticides and Veterinary Medicines Authority

- Defn: A biological chemical product is an agricultural chemical product where the active constituent comprises or is derived from a living organism (plant, animal, micro-organism, etc), with or without modification.
- Group 3: microbial agents (bacteria, fungi, viruses, protozoa)
- Registered products are known as 'Biological Crop Protection' products i.e. used as a biopesticide, registered for use in the crop and situation it is applied in.
- Regulations skewed to microbial pesticides.
- Exempted products include soil ameliorants, fertilisers, …… legume inoculants based on bacteria or enzymes

Products register for Hort Industry

Biological Products Database - by Trade Name - April 2023

		Product type		Ingredient(s)		Use	
Trade Name	**Source**	**Registered Product Type (APVMA Registered)**	**Has been used as / for (not registered)**	**Active Ingredient(s) (APVMA Registered Products)**	**Main ingredient(s) of non registered products**	**Registered Use (APVMA Registered)**	**Has been used as / for (not registered)2**
Actinobact	microbial	not registered	growth stimulant/ regulator	not registered	*Streptomyces* sp. *Bacillus subtillus*	not registered	root system
Actinovate Biofungicide	microbial	fungicide	-	*Streptomyces lydicus* strain WYEC 108	-	Preventative control of foliar diseases (incl. **powdery mildew**) and soilborne diseases (incl. **Fusarium** wilt, **Rhizoctonia** root rot, **Pythium** damping off) + Biological soil amendment (to supplement nutrient availability, plant growth)	-
Actiwave	plant extract	not registered	growth stimulant/ regulator	not registered	plant extracts (no further information given)	not registered	crop health
Advance Promote	microbial	not registered	soil biology stimulant	not registered	VAM Fungi, Humates, Amino acids, micros, kelp, sugars	not registered	root system health
Agree WG biological insecticide	microbial	insecticide	-	*Bacillus thuringiensis* Berliner subsp *aizawai*	-	armyworm | cabbage moth | cabbage white butterfly | cotton budworm or bollworm | grapevine moth | light brown apple moth | looper moth | native budworm or bollworm | painted vine moth | pear looper | soybean looper	-
Agro-Vam	microbial	not registered	soil biology stimulant	not registered	one VAM (Vesicular Arbuscular Mycorrhizae) species: *Rhizophagus irregularis* (previously known as *Glomus intraradices*)	not registered	mycorrhizae

https://www.soilwealth.com.au/resources/global-scan-and-reviews/biological-products-database/

Establishing a quality management framework for commercial inoculants containing arbuscular mycorrhizal fungi

Salomon et al 2022

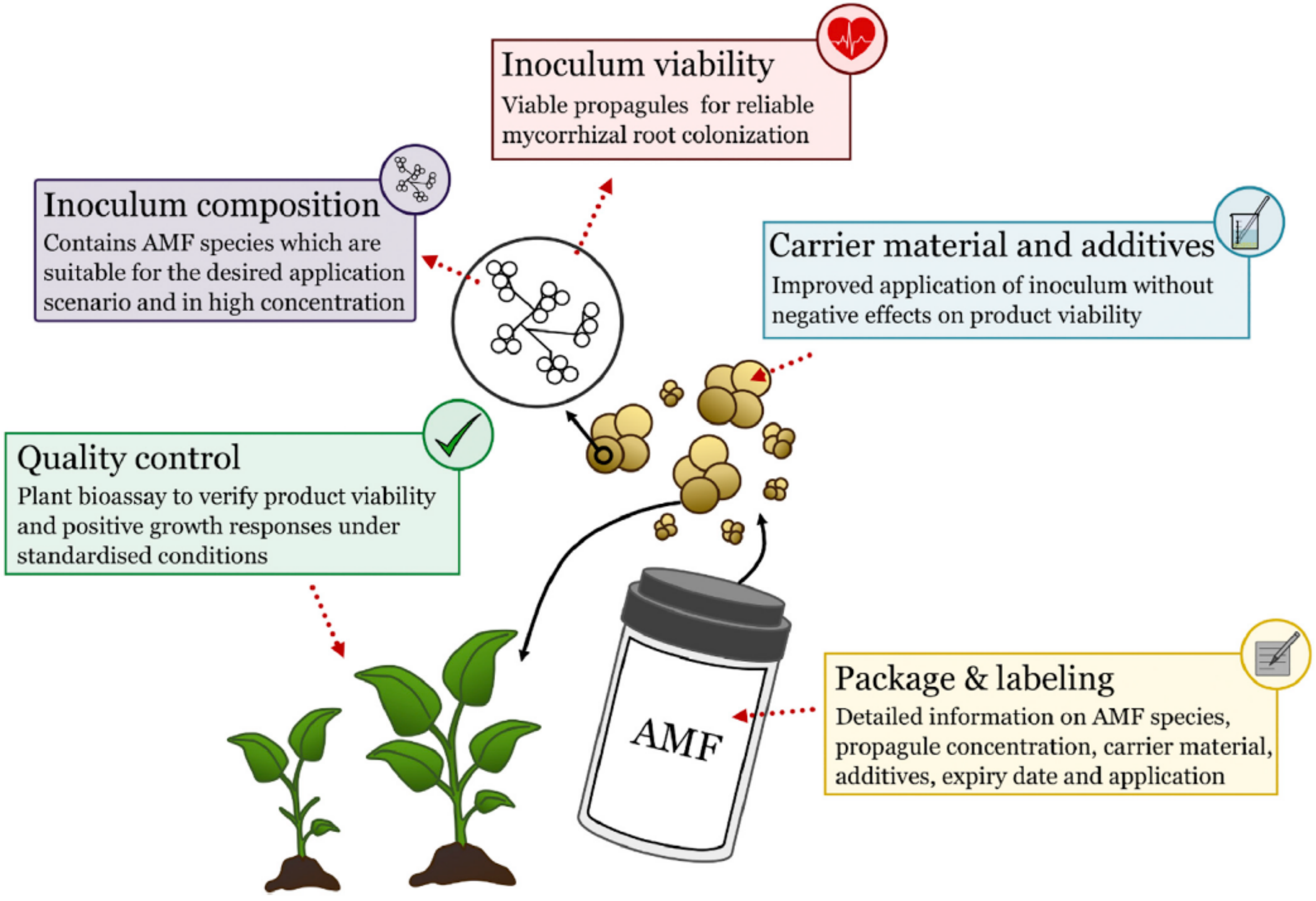


Figure 1. Quality criteria for microbial inoculants containing arbuscular mycorrhizal fungi.

Microbiome engineering for agriculture

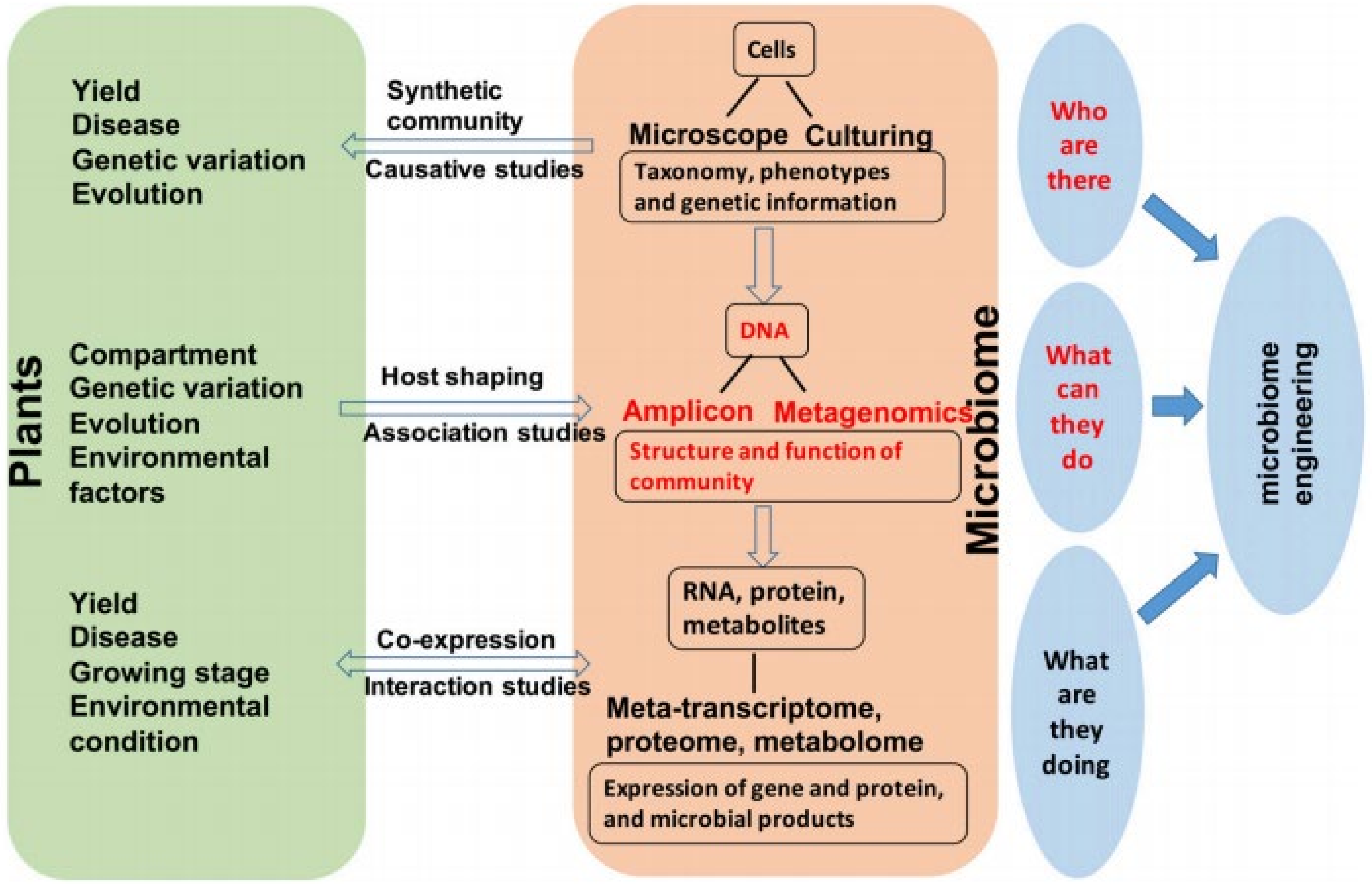


Fig. 6. Proposed methods and questions for citrus microbiome studies. Red color highlights the current progress of understanding the citrus microbiome.

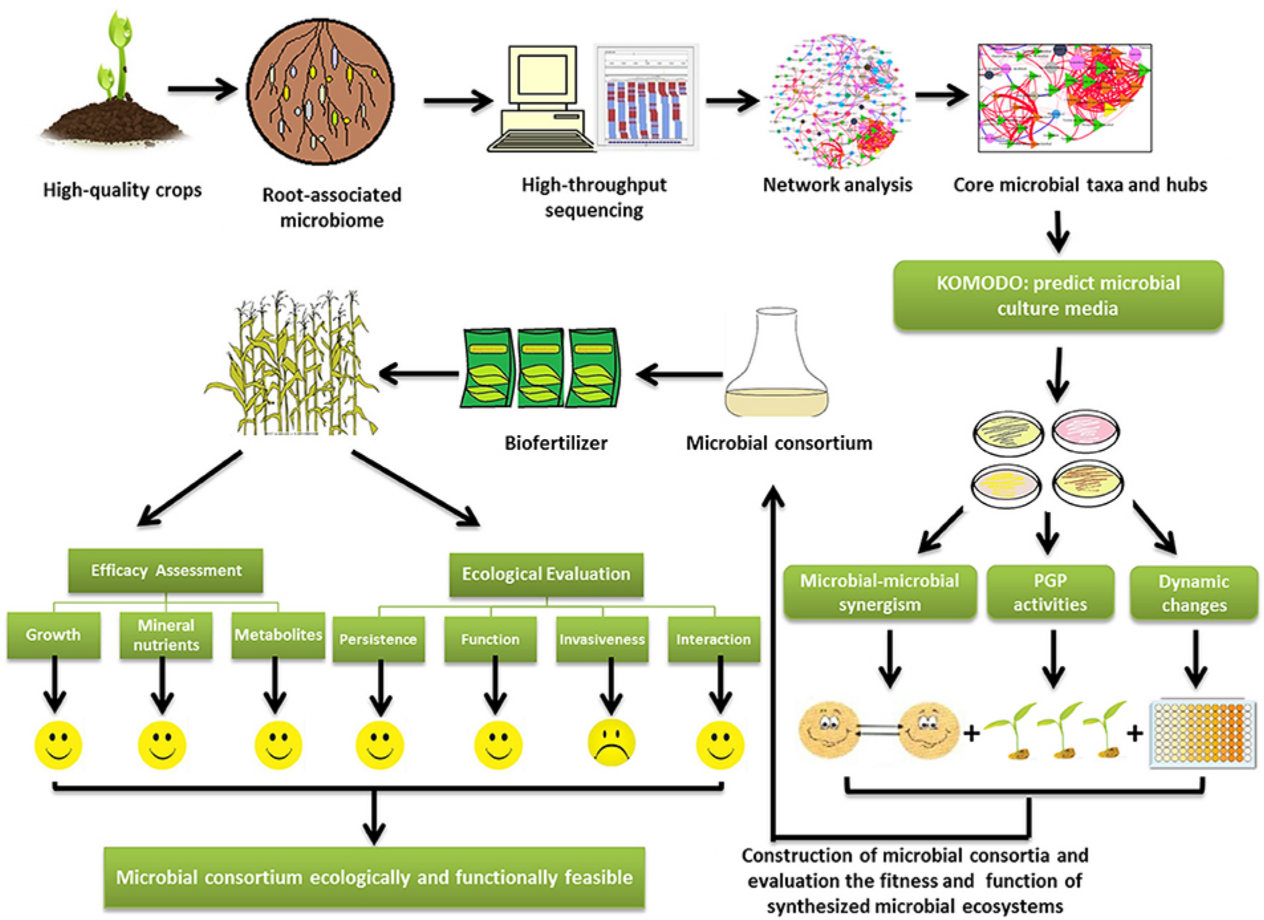
Zhang et al 2021 Phytobiomes Journal

Step 2: Move beyond DNA analysis and association studies (shown in red)

These are commonly done but need to move to more difficult experiments

Microbiome approach for bioproducts

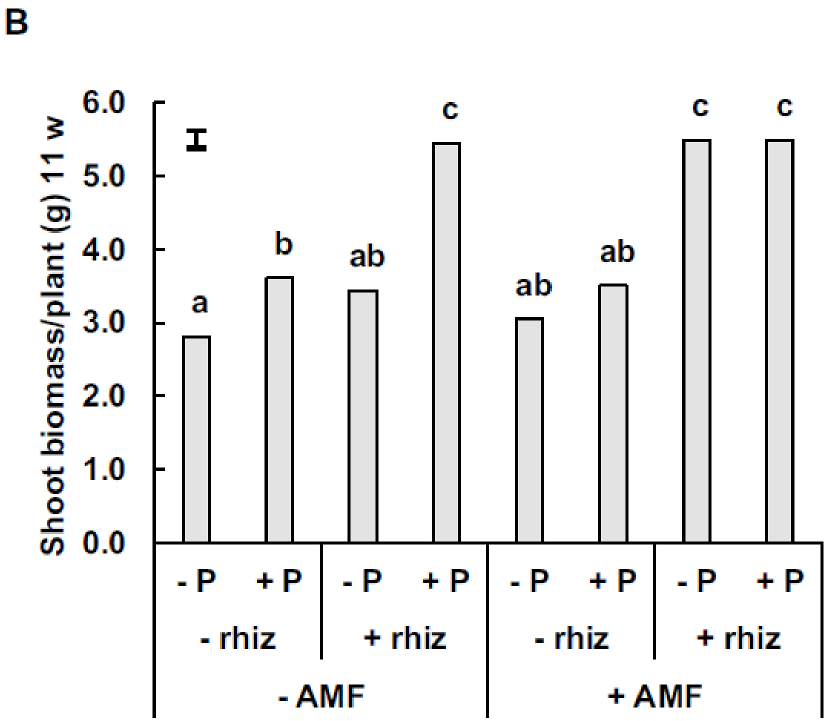
- Current biostimulants/biofertilisers contain a few microbes
- New idea: identify all microbes in root area, make synthetic microbial consortia (Syn-com) to potentially replace and/or reshape the structure and function of plant microbiome



Kong et al 2018
Front. Plant Sci 9: 1467

Applications: Rhizobium + AMF in mungbeans

Gough et al 2021

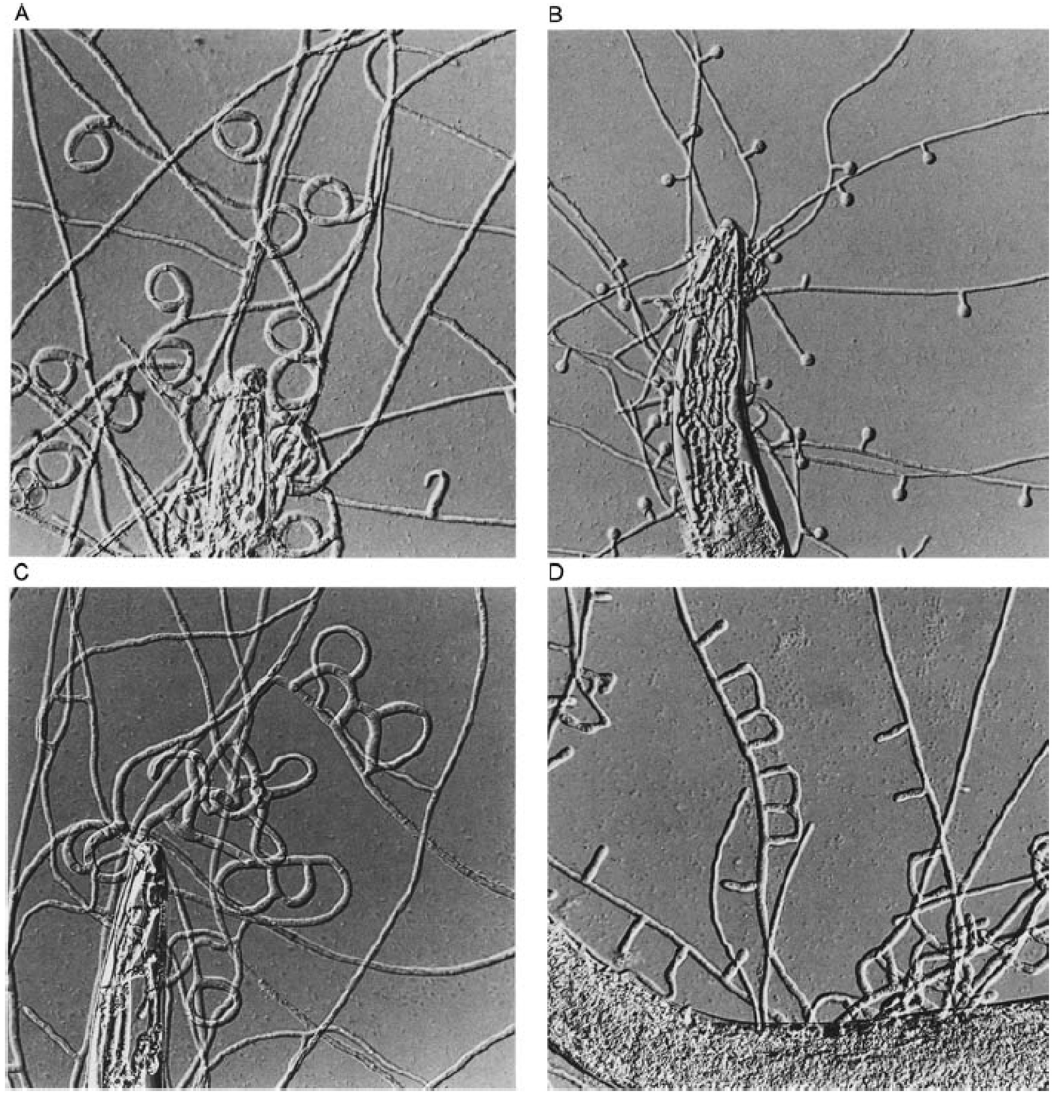


Conclusions

- inoculate legumes with optimal strain
- adoption of agricultural practices that encourage the proliferation of AMF in vertisols
- Improved biological N fixation, plant nutrient acquisition, biomass production, and grain yield, reduced fertilisers
- Monitor AMF+Rhizobium interaction with *Pratylenchus*

Fig. 8 The interactive effects of co-inoculation of (**A**) arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (Pt) x N on shoot biomass per plant of mung bean at 6 weeks (w) after sowing, (**B**) arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x P on shoot biomass of mung bean at 11 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d)

Nematode trapping fungi – Bioworma for livestock



Nematode-trapping fungi growing from parasitized nematodes in soil extract.
Jaffee et al., 1992

Duddingtonia flagrans (the active ingredient in BioWorma®) reduces the number of gastrointestinal nematodes larvae on pasture

Where to next for tests & products?

Sustainable soil management needs indicators

- United Nations' 2015 *Transforming our World: the 2030 Agenda for Sustainable Development*
  - 17 Sustainable Development Goals, 169 targets for action in critical areas for humanity and the planet until 2030
- Sustainable management of soils relevant to half goals

Table 4
Nature indicators scores after each round (R1 – R3), statistics and results for all participants.

	[a]Theme	Sub – Theme	[b]Indicator	[c]R1	R2	R3	[d]Results
NATURE	Atmosphere	Atmosphere	Net carbon sequestration in soil	4.49 (0.55)	4.57 (0.57)	4.48 (0.68)	Accepted
			Extreme weather events	3.60 (1.22)	3.64 (1.03)	3.81 (0.75)	Accepted
			Temperature daytime temperature during the growing season	N/A	3.29 (1.24)	3.48 (0.93)	Rejected
	Biodiversity	Biodiversity	Pedodiversity	3.95 (0.90)	3.82 (0.77)	4.00 (0.89)	Accepted
	Soil Properties	Physical	Aggregate diversity	4.38 (0.79)	4.29 (0.76)	4.25 (0.64)	Accepted
			Bulk density	4.16 (0.75)	4.21 (0.83)	4.24 (0.77)	Accepted
			Change in topsoil depth	4.33 (0.97)	4.37 (0.69)	4.10 (0.89)	Accepted
			Soil sealing	4.17 (1.03)	4.44 (0.70)	4.38 (0.74)	Accepted
			Strata composition and buffer capacity	N/A	4.07 (0.94)	3.76 (1.14)	Rejected
			Soil erosion	N/A	3.96 (1.00)	4.19 (0.93)	Accepted
		Chemical	Change in cation exchange capacity (CEC)	4.14 (0.86)	3.93 (0.73)	3.85 (0.75)	Accepted
			Soil contamination	4.44 (0.96)	4.46 (0.96)	4.38 (1.12)	Accepted
			Change in topsoil pH	4.14 (1.01)	4.36 (0.78)	4.33 (0.73)	Accepted
			Soil iron oxides content compared to reference value	N/A	3.61 (0.69)	3.24 (1.14)	Rejected
		Biological	Change in microbial biomass	4.17 (1.08)	4.39 (0.57)	4.24 (0.94)	Accepted
			Change in and absolute level of net N mineralization	4.16 (1.04)	4.21 (0.79)	4.24 (0.62)	Accepted
			Soil protective cover	4.44 (0.93)	4.50 (0.75)	4.24 (0.77)	Accepted
			Change in total soil organic matter (TSOM)	4.70 (0.56)	4.64 (0.49)	4.48 (0.68)	Accepted
			Change in flora diversity above ground	N/A	4.14 (0.71)	4.30 (0.57)	Accepted
			Change in fauna diversity above ground	N/A	4.04 (0.79)	4.14 (0.73)	Accepted

[a] Themes within one of the overarching dimensions of sustainable development.
[b] Proposed soil indicator for sustainable development.
[c] Rounds one to three with mean score, standard deviation in parenthesis.
[d] Results after round three considering score, standard deviation and comments from participants.

scientists, soil practitioners and policy people selected indicators

Jónsson et 2016

Product development oversight

https://phytobiomesalliance.org/

INTERNATIONAL PHYTOBIOMES CONFERENCE 2022

Working Groups

Working Groups are the implementation arm of the Alliance. They lead efforts and develop priorities on specific topic, disciplines, and technologies.

Microbiomes

The group focuses on identifying knowledge and resource gaps that need to be addressed to advance understanding of the role that microbes play within the broad phytobiome systems and how this can be used to improve agricultural sustainability.

Animal Microbiomes

The group works on identifying knowledge and resource gaps that need to be addressed to advance our understanding of the role that the phytobiome plays in influencing the nutrition, health, and net carbon emissions of livestock and poultry.

Regulatory

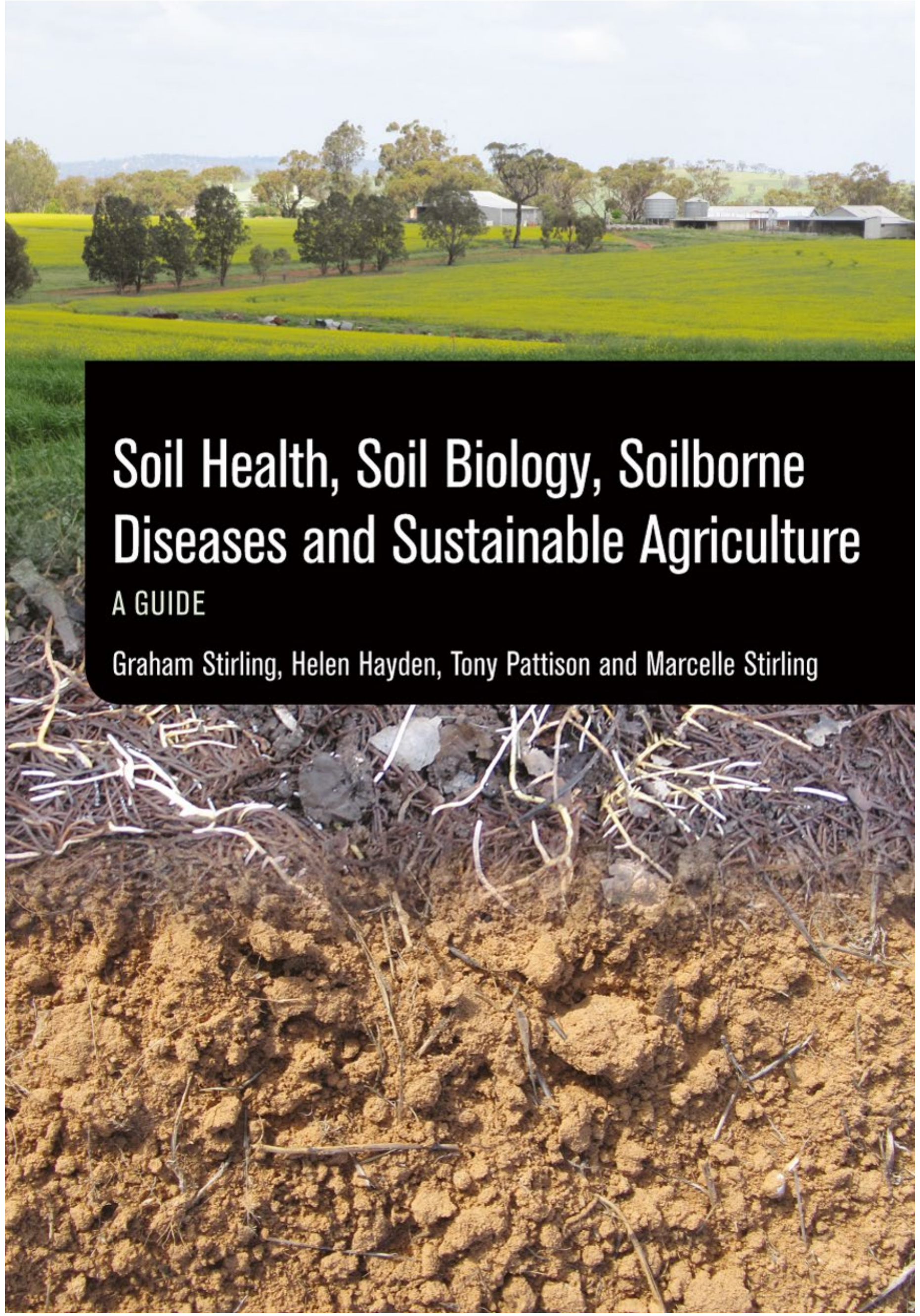
The group focuses on the development of a regulatory science roadmap to facilitate the commercialization of agricultural biologicals and microbial products.

Controlled Environment Agriculture

The aim of the group is to identify major controlled environment agriculture challenges that could be addressed by phytobiomes research.

Conclusions

- Soil biology are diverse, live throughout our soils and perform important ecosystem functions
- Improving soil health will lead to an active soil biological community
- Biology tests can provide a baseline for monitoring or be used to measure practice change effects on biology
- Biological products are a largely unregulated space so in-field trials recommended



https://www.publish.csiro.au/

Advertisement – seeking collaborators

- Precision agriculture
- Satellite/NVDI/drones
- Variable rate spreading
- EM38 soil surveys
- Yield data analyses

Work with us or host a student excursion!

Contact Dr Alexis Pang

alexisp@unimelb.edu.au

- Soil fauna research on farm and remnant vegetation
- Biology tests/indices for soil health/sustainability
- Rhizoctonia in broadacre crops

Contact Dr Helen Hayden

hhayden@unimelb.edu.au

Thank you