The Big Soil Community Community Level Report

Original thinking... applied

Report version 1.0

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1. Introduction

Thank you for participating in the Big Soil Community in 2018 - the inaugural year for this ambitious programme which aims to collaboratively unlock the biological potential of soil health.

Soil biology is widely recognised as a key component of soil health but the measures available to assess these communities and our understanding of the link between biology and agriculture remain limited. In the Big Soil Community we have used DNA sequencing and data science to address this complexity and reveal the breadth of the bacterial and fungal communities within a sample. Your participation in the Big Soil Community not only reduces and shares the cost of analyses but also generates a unique dataset which will accelerate research into how soil communities influence our farming systems.

In this first year we have analysed 228 samples. These samples come from across the UK; from a range of soil types, from heavy clays to peat soils; and a range of farming systems, from intensive to extensive, organic to conventional. This variety mirrors the diversity of the biological communities with thousands of different bacterial and fungal taxa successfully matched to extant reference libraries.

In this report we provide an overview of the results for the Big Soil Community as a whole. Reporting on key statistics, findings and relationships that were identified through our meta-analysis of the data from this inaugural year. This report can be used in parallel with your individual sample report to explore how your soil compares to the wider sample pool.

We have worked to ensure that we simplify the complexity inherent within these analyses and provide background information and guidance to support with the interpretation and understanding of biological populations. For further support we have put additional reference material and Frequently Asked Questions on the Big Soil Community website (www.fera.co.uk/bigsoilcommunity) and will also be hosting webinars and workshops where we can discuss specific components of the results. We are happy to answer any questions which you may have about your results so please feel free to contact us via the website.

Thanks again for your participation and for your continued support for the Big Soil Community.

Guy Thallon, on behalf of the Big Soil Community Team at Fera Science.

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1.1. Overview

The Big Soil Community was launched in 2018 as a coordinated effort to accelerate the understanding of soil biological communities in the context of agricultural production systems. The programme is based upon the commercial provision of Next Generation Sequencing (NGS) analyses which are used to provide insight into the makeup and structure of biological communities within a given sample using an approached called metabarcoding. In this first year these approaches have been used to identify communities of soil bacteria and fungi. Farmers and growers from across the UK were invited to sign up to the community by purchasing a sample via the fera.co.uk website. Sampling was then undertaken by participants during a set window from Mid-October to Mid-November using a Standard Operating Procedure (SOP) which defined the sampling protocol and minimised the risk of sample contamination. Samples were then returned to Fera via post for storage, sample preparation, DNA extraction and analysis. The DNA sequences were then aggregated and analysed using various computation techniques (described in section 4.2) to prepare this community level report and the individual sample report.

1.2. Community description

In total, 228 samples were received and sequenced. These samples arrived from locations across the country, included various pH ranges and diverse percentages of soil organic matter.

Some Big Soil Community participants submitted more than one sample from the same field (Table 1). This information was valuable as it highlighted how similar samples can be when taken from the same location. Large variations in intra-field population diversity were not observed in these instances and there is work ongoing in this area.

Table 1: Sampling densities (number of samples submitted per location by number of participants)

Number of sample submitted per location	Number of participants	Total number of samples
1	60	60
2	2	4
3	1	3
4	4	16
5+	5	121
	Total	204

For this community level report, representative samples from each of the fields were used. This was a conscious effort to not bias the analysis by having multiple samples from the same location, and thus a similar diversity and metadata. This finally resulted in 86 samples being selected for this community report analysis.

Visualisations of selected metadata can be seen in the 'Metadata Visualisations' section of this report (1.4).

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1.3. Sample metadata

204 of the 228 samples received came with metadata. 18 of the metadata categories were deemed complete enough to be used for further analysis. These categories included:

- How would you score the health of your soil? (5 = very good; 0 = very poor)
- How would you score the performance of your field? (5 = very good; 0 = very poor)
- How would you describe your cropping system?
- 2018 Harvest Year Crop
- Yield(t/ha)
- Is the field organic or conventional?
- Was the previous crop irrigated?
- What is your soil texture?*
- Please score your earthworm activity for this soil (5 = Very Good; 0 = Very Poor)
- What is the level of Soil Organic Matter (%, w/w) in the soil?
- Does the area sampled have any known nutrient deficiencies?
- Cultivation Description**
- Have you applied any of the following fungicides in the previous crop?
- Have you applied any of the following herbicides in the previous crop?
- Have you applied any of the following insecticides in the previous crop?
- Mean pH in water (value determined by lab analysis at Fera)
- Mean pH in CaCl2 (value determined by lab analysis at Fera)
- Mean Moisture Content % (value determined by lab analysis at Fera)

* Data originally collected from this question was reclassified into 3 distinct categories - Heavy soils, Medium soils and Light soils.

**Data originally collected from cultivation methods was reclassified and grouped into 5 distinct categories - No soil disturbance, Minimal soil disturbance, Mild soil disturbance, Moderate disturbance and Heavy disturbance

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1.4. Metadata visualisations

Summary of metadata for those samples locations with complete records (86).

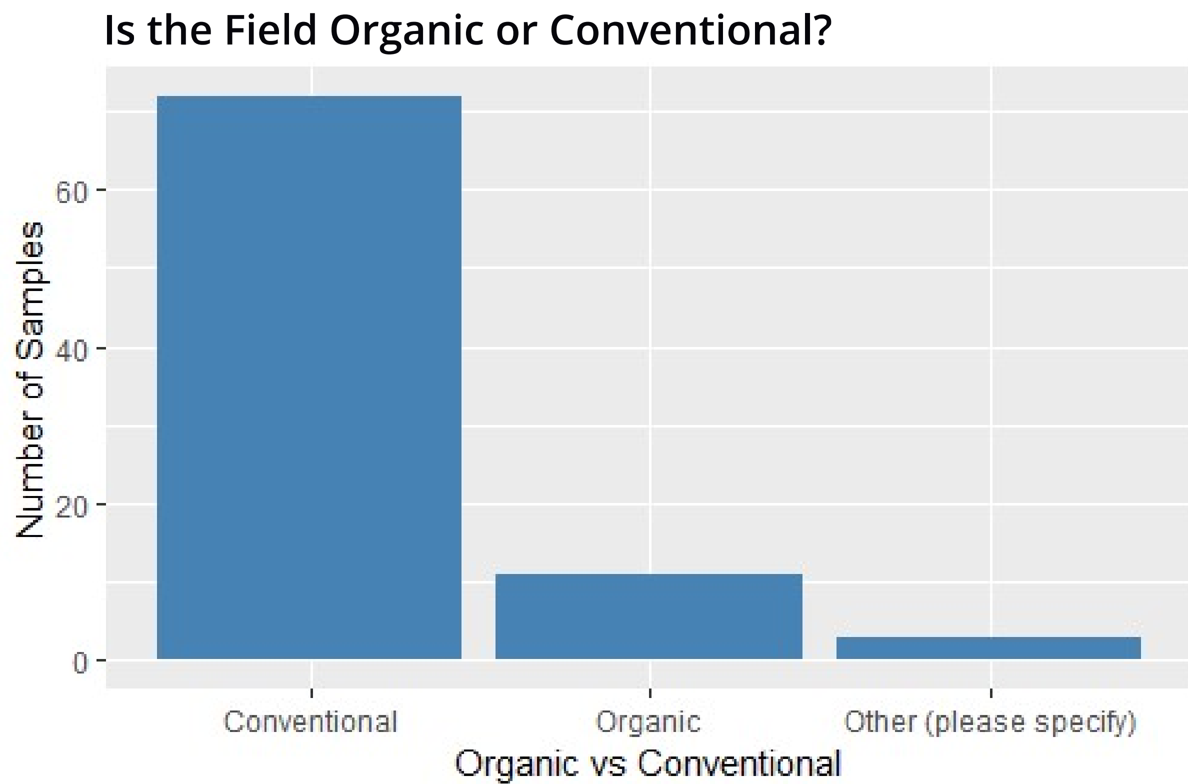

Figure 1. Total number of samples received from conventional (78), organic (11) or other (3) systems.

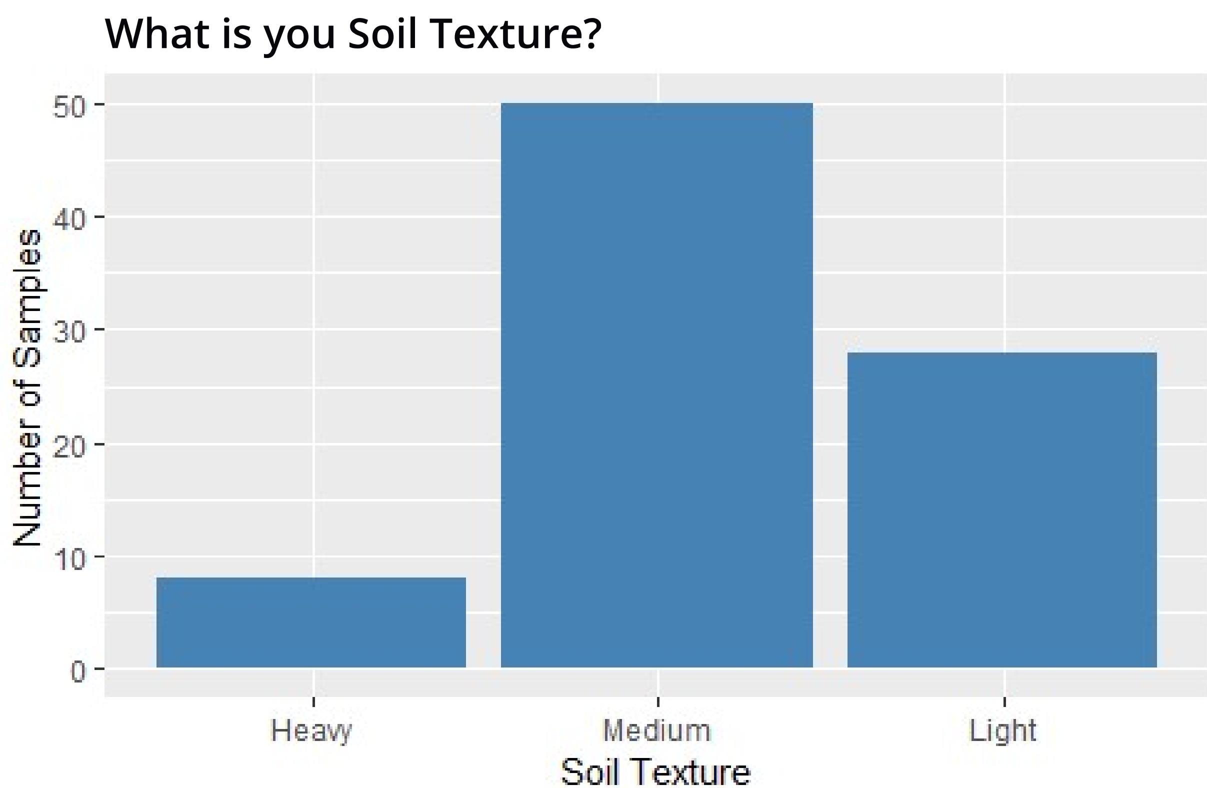

Figure 2. Total number of samples received that were either heavy (8), medium (50) or light (28) soil textures.

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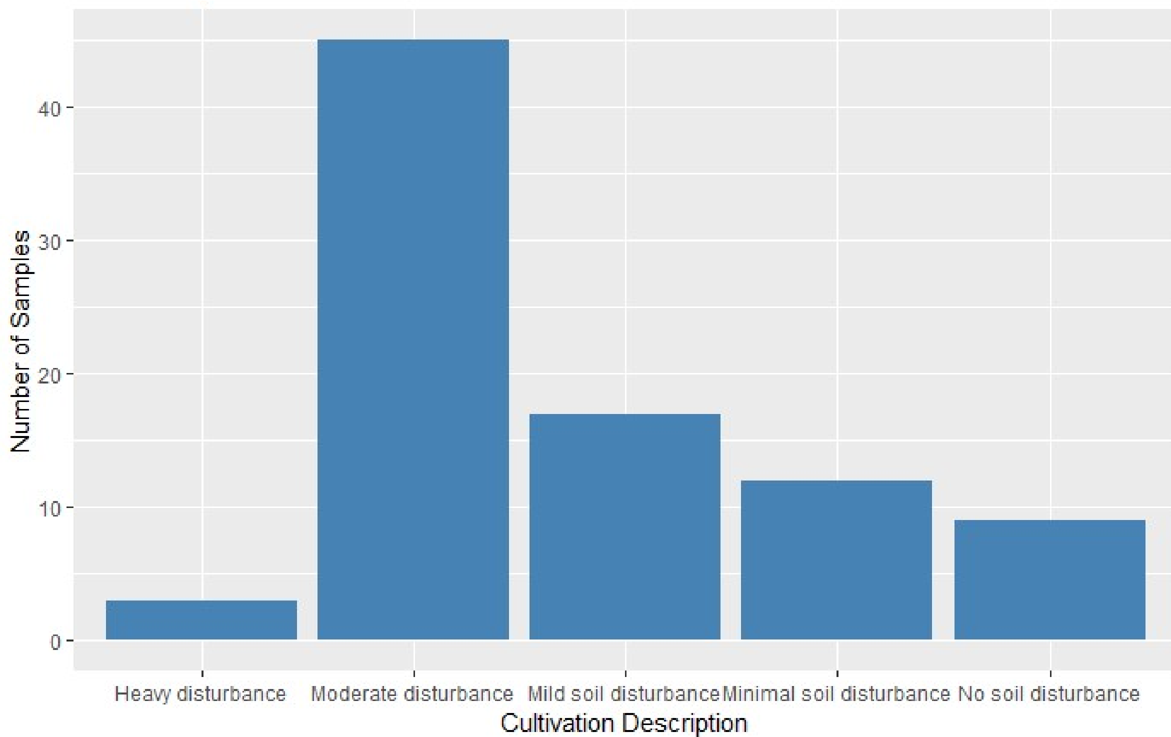

Figure 3. Total number of samples received and a description of the cultivation of those samples. Heavy disturbance (3), mild disturbance (17), minimal disturbance (12), moderate disturbance (45) and no disturbance (9).

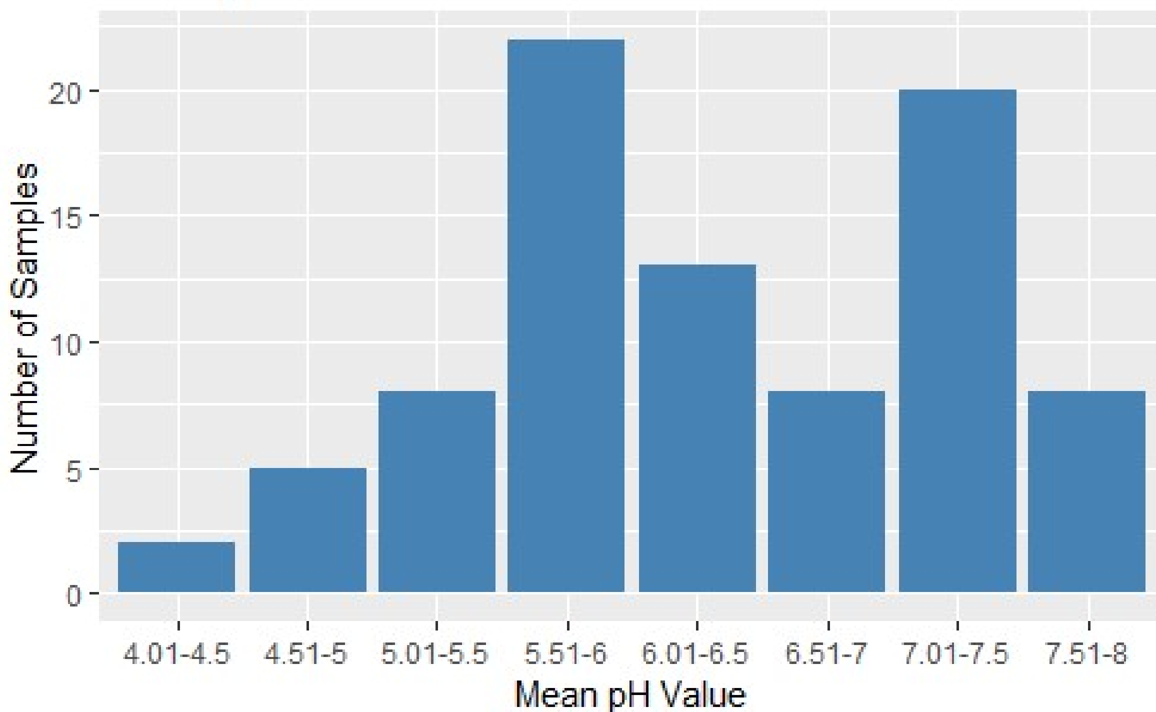

Figure 4. Total number of samples received and the pH range that these samples fall into. 4.01-4.5 (2), 4.51-5 (5), 5.01-5.5 (8), 5.51-6 (22), 6.01-6.5 (13), 6.51-7 (8), 7.01-7.5 (20), 7.51-8 (8).

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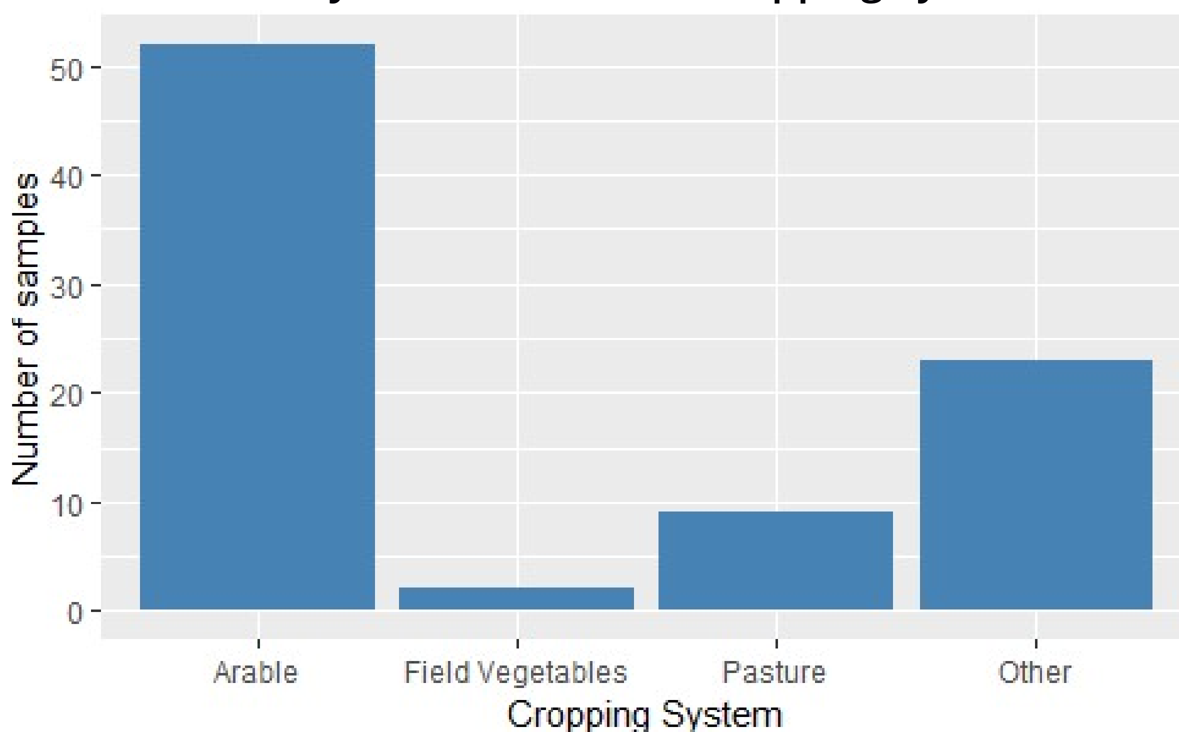

Figure 5. Total number of samples received and the main cropping systems these samples are taken from. Arable (52), Field vegetables (2), Pasture (9) and Other (23).

1.5. Analytical approach

Each soil sample received from the Big Soil Community was tested at Fera to determine water content and pH (in water and in CaCl2). DNA was extracted from 10 g sub-samples and purified to remove humic acids and other soil materials that can inhibit analysis of the DNA. We then used a technique called DNA metabarcoding to measure the underlying microbial biodiversity found in each soil sample. This first involves a method known as PCR to amplify DNA that is specific to the different taxonomic groups of organisms – their 'barcode'. We then identified the barcodes of bacteria and fungi in each soil sample using high-throughput DNA sequencing technology. This generated thousands of DNA sequences that could be grouped together according to their sequence similarity.

With metabarcoding were able to measure microbial diversity at various taxonomic levels (see 2.1). We first quality-controlled the DNA sequences obtained and then used established computational methods to compare them with DNA sequences in specialised bacterial and fungal databases curated by 3rd parties. Those sequences have been annotated with taxonomic information by the curators; this enables us to identify the organisms from which our sequences originated. Many of the computational tools which we used are accessible via two software systems named "QIIME2" and "R". We used the SILVA database as a reference for bacterial metabarcode sequences, and the UNITE database for fungi. We modified the latter by renaming taxa in accordance with the taxonomic classifications used by the National Centre for Biotechnology Information (NCBI), and also by removing some entries which are annotated to low resolution.

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2. Ecological approaches and soil biology

2.1. Why is soil biology important?

Soils are the most complex ecosystem on the planet, harbouring enormous biological diversity that is hugely influential on several processes that sustain plant and animal productivity and health. Healthy soils contain a rich diversity of beneficial bacteria and fungi that:

- Form beneficial relationships with plant roots, such as nitrogen fixation through the symbiosis between rhizobia and legumes and increased nutrient uptake by mycorrhizal fungi associated with the roots of many crop plants;
- Recycle nutrients by decomposing organic matter from plant roots, crop debris, manures and other organic amendments and releasing carbon, nitrogen, phosphorus and sulphur in plant available forms;
- Stabilise soil structure by secreting polymers that bind soil particles, creating aggregates with an open pore network that traps water and allows free movement of air and nutrients;
- Help control plant diseases by suppressing soil-borne plant pathogens through competition for nutrients and habitats or production of antimicrobial substances;
- Ultimately improve crop production and quality by sustaining overall soil fertility.

As an important reservoir of biodiversity, our soils contain up to a third of all living organisms on the planet. Soil microorganisms are hugely diverse and play a range of critical roles in most soil processes. For some of these microorganisms (see 2.2), their functions have been well defined. However, a large proportion of bacteria and fungi found in soil are unculturable and have yet to be named and their functions and role in soil health have yet to be identified.

Measurements of biodiversity can be used to compare the general biological health of different soils. Farm management practices can both help and hinder the biological processes happening in the soil. Soil biodiversity can be lost by reductions in the abundance of beneficial micro-organisms. This can occur through:

- Negative effects of land management (e.g. over-intensive production, loss of organic matter, overuse of inorganic fertilizers and pesticides or compaction due to heavy machinery);
- Environmental issues (e.g. flooding, drought or erosion).

The government's 25-year environment plan has given high importance to soil health. The government's agricultural bill will likely set targets to maintain high soil health across the country by 2030, with possible incentives to improve soil management practices. This report represents a first step in trying to assess the range of biodiversity across UK agricultural and horticultural soils and how this can be affected by common soil management practices. By combining farming knowledge and scientific expertise through the Big Soil Community, we are creating an inaugural step towards being able to manage soil conditions to provide the best food and living conditions for soil organisms so that they can function in harmony and maintain fertile and productive soils into the future.

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2.2. Taxonomic diversity explained

All living cellular organisms have evolved into three large clusters of related organisms, called domains:

- Archaea
- Bacteria
- Eukaryota

Archaea and Bacteria are also known as prokaryotes. They are small, relatively simple cells surrounded by a membrane and a cell wall, with a circular strand of DNA containing their genes. All other life— including fungi, insects, plants and animals — belongs to the third domain, Eukaryota. Eukaryotic cells are more complex than prokaryotes, and the DNA is linear and found within a nucleus.

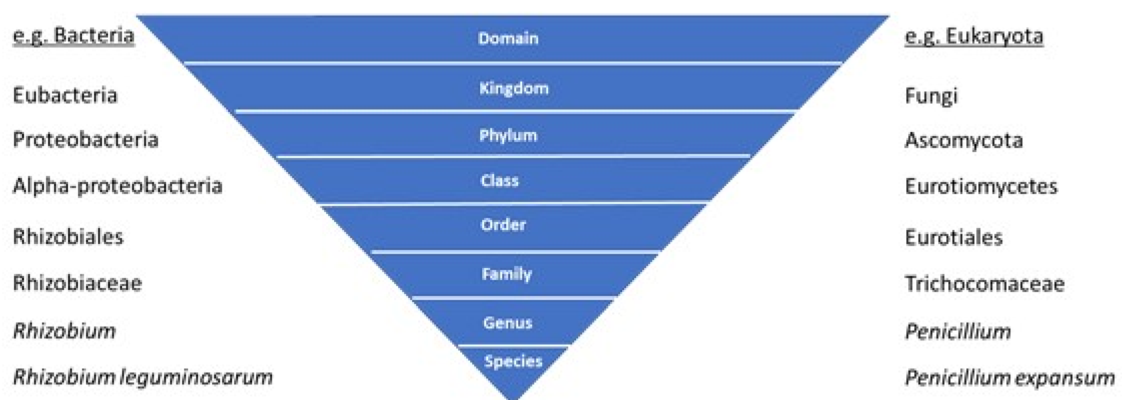

Figure 6: Additional taxonomic ranks further differentiate organisms within each domain:

By analysing DNA sequences of taxonomic marker genes that are unique to bacteria or fungi, the diversity of organisms present within each domain can be determined. In the following analyses, organisms with taxonomically distinct DNA sequences have been grouped into operational taxonomic units (OTUs). These can then be assigned taxonomic rank where the sequences match those of known organisms held in sequence databases. The relative abundance of each organism in the sample can then be estimated. It is then useful to identify those that make up most of the overall community. Many soil microorganisms have yet to be fully classified, so it is not always possible to assign all the taxonomic ranks from a unique OTU.

For the bacteria and fungi, the most abundant organisms in each soil sample have been identified at the taxonomic ranks of phylum, class, order, family and genus. An overall figure has also been calculated that represents the population profile of the community of organisms within each soil sample. This characteristic, known as 'α-diversity', depends on both the number of different organisms and their relative abundances. The α-diversity is expected to differ from sample to sample depending on factors such as geographical location, soil type and texture, cropping pattern, cultivation practices and inputs. Comparing changes in α-diversity across different soils is a major step towards understanding the most important factors that contribute to biodiversity and how this information can be used to encourage sustainable biological soil health into the future.

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2.3. Influential soil-borne microorganisms

It is possible to screen the DNA extracted from each soil sample to determine possible presence of specific organisms that are known to be beneficial or detrimental to crop health and production. Such organisms are thought to be useful markers of good or bad soil health. This report indicates the potential presence of any of these organisms (at genus level) in the sample submitted.

Table 2: Beneficial and detrimental soil-borne microorganisms that can influence soil health

Type of microorganism and their biological function (Beneficial/Detrimental)	Key taxa (phylum or division and examples of the main genera involved)
Nitrogen-fixing bacteria that assimilate atmospheric nitrogen into fixed nitrogen (inorganic compounds usable by plants).	Free-living bacteria **Cyanobacteria** (blue green algae) e.g. *Anabaena* & *Nostoc* spp. **Proteobacteria** e.g. *Azospirillum*, *Azotobacter*, Beijerinckia, *Gluconobacter*, *Herbaspirillum*, **Firmicutes** e.g. *Clostridium* **Bacteroidetes e.g.** *Flavobacterium* Mutualistic bacteria associated with certain plants ***Proteobacteria*** e.g. species in the genera *Allorhizobium, Neorhizobium, Pararhizobium, Rhizobium, Bradyrhizobium, Mesorhizobium* and *Sinorhizobium* (on legumes) & *Azospirillum* (on grasses & cereals) ***Actinobacteria*** e.g. *Frankia* (on certain dicots)
Nitrifying bacteria that convert soil ammonia to nitrates, compounds usable by plants.	• **Proteobacteria** e.g. *Nitrobacter*, *Nitrospina*, and *Nitrococcus* (convert ammonium to nitrite and nitrate); *Nitrosomonas*, *Nitrosolobus*, *Nitrosococcus*, & *Nitrosolobus* (convert nitrites to nitrates)
Denitrifying bacteria that convert nitrates in soil to nitrous oxide (a greenhouse gas) or free atmospheric nitrogen, thus depleting soil fertility and reducing agricultural productivity.	**Proteobacteria** e.g. some species of *Thiobacillus*, *Paracoccus*, *Serratia*, *Pseudomonas*, and *Achromobacter.*
Decomposer bacteria that break down organic materials, especially in the early stages of decomposition when moisture levels are high.	Many phyla of soil bacteria including: **Proteobacteria** e.g. *Pseudomonas,* **Firmicutes** e.g. *Bacillus* **Actinobacteria** e.g. *Streptomyces* (usually active at non-acid soil pH >5)
Saprobic (*saprotrophic and saprophytic*) decomposer fungi that break down organic materials, usually in the later stages of decomposition and also at low pH.	Many phyla of soil fungi including: **Ascomycota** e.g. *Aspergillus and Penicillium* (decompose plant litter) **Basidiomycota** (decay wood as well as decompose plant litter) **Zygomycota** (moulds such as *Mucor* & *Rhizopus*) **Chitridiomycota** **Blastocladiomycota**

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Sulphur oxidising bacteria that covert sulfides into sulfates, a form of sulfur which plants can use.	**Proteobacteria** e.g. *Thiobacillus*
Arbuscular mycorrhizal fungi that penetrate the roots of many vascular plants (but not brassicas) and help to capture nutrients such as phosphorus, sulphur, nitrogen and micronutrients from the soil.	**Glomeromycota** e.g. *Diversispora*, *Redeckera*, *Pacispora*, *Acaulospora*, *Scutellospora*, *Funneliformis, Glomus, Rhizophagus, Sclerocystis, Septoglomus*, *Claroideoglomus*, *Paraglomus, Innospora, Archaeospora.*
Plant growth promoting bacteria that occupy the rhizosphere of many plant species and have beneficial effects by enhancing plant growth and stimulating their defence mechanisms to better protect from disease and abiotic stresses.	**Proteobacteria** e.g. *Acinetobacter*, *Azomonas*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Kluyvera*, *Proteus*, *Pseudomonas*, *Psychrobacter*, *Rahnella*, *Serratia*, *Sphingomonas*, *Stenotrophomonas*, *Variovorax*, *Xanthomonas* **Firmicutes** e.g. *Bacillus*, *Brevibacillus, Paenibacillus* **Actinobacterium** e.g. *Bravibacterium* **Bacteroidetes** e.g. *Flavobacterium*
Plant pathogenic soilborne fungi that can reside in soil for long periods and cause disease by infecting through crop roots under favourable conditions.	**Ascomycota** e.g. *Fusarium* (head blight, root rot, wilt), *Colletotrichum* (black dot), *Gaeumannomyces* (take-all), *Helminthosporium* (silver scurf), *Phomopsis* (black root rot), *Polyscytalum* (skin spot), *Sclerotinia* (white mould), *Sclerotium* (white rot), *Verticillium* (wilt) **Basidiomycota** e.g. *Rhizoctonia* (damping off, root rot, black scurf) **Chytridiomycota** e.g. *Synchytrium* (wart)
Soilborne fungi with potential biocontrol activity against plant pathogens and pests.	**Ascomycota** e.g. some species of *Trichoderma, Plectosphaerella* and *Beauveria,* **Entomophthoromycota** e.g. *Entomophaga*

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