Evaluating soil health at rotational scale – testing molecular-based indicators.
The effects of long-term crop rotation and cultivation treatments in the STAR project (Sustainability Trial in Arable Rotations) on soil health.

Soil biology is widely recognised as a key component of soil health but measures to assess the below-ground communities are only just being developed and our understanding of the link between soil biology and agriculture remains limited. Soils are an important reservoir of biodiversity, and 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. The functions of some microorganisms have been well defined. However, a large proportion of bacteria and fungi found in soil are unculturable and have yet to be named; consequently their functions and role in soil health have yet to be identified. While currently used indicators such as pH and worm counts are already providing valuable information on soil health, new measures are being evaluated and added, such as total nitrogen, microbial biomass carbon, potential mineralisation nitrogen. For the future, DNA-based measures of the soil community including pathogens, nematodes and other soil fauna are likely to become a key component of regular soil analysis.

Changes to crop rotations, for example using cover cropping, and reduced tillage practices have been adopted to increase the sustainability of cropping systems. However, in the field, the impacts on productivity and environmental resilience can only be investigated over the medium/long-term. NIAB have been working with a number of charity partners (including the Felix Cobbold Trust, the JC Mann Trust, the Chadacre Trust and the Morley Agricultural Foundation) to run multi-factorial trials of farming systems for the last 10-15 years. The STAR project (Sustainability Trial in Arable Rotations) is a long-term rotational and cultivation trial established in 2006 on a (heavy) clay loam soil at Stanway Farm, Otley, Suffolk, with a fully replicated comparison of four rotations and four cultivation systems (48 plots). In the 2017-2018 cropping season, the trial was uniformly cropped with winter wheat (2nd wheat, cv Shabras, drilled 13th October 2017) thus allowing the long-term impacts of the treatments on soil health to be assessed separately to any short-term impacts resulting from the presence of different crops.

The aim of the study (funded by TMAF and the JC Mann Trust) was to evaluate biological indicators of soil health using real-time PCR approaches (alongside established chemical and physical indicators) at rotational scale. NIAB measured a range of physical, chemical and biological indicators of soil health and worked with partners at Fera to implement DNA extraction, real-time PCR and preliminary biostatistical analysis. Soil health indicators were measured in all plots (48) in spring (11th April 2018). Crop development and yield were monitored in the wheat crop; harvested on 26th July 2018. In the autumn, soil health was assessed again for all cultivation treatments but in the continuous wheat plots only (12) when the soil wetted up (5th November 2018). The soil health sampling protocol used in the AHDB/BBRO Soil Biology and Soil Health Partnership was used. In-field approaches were used to assess soil structure (VESS) and count earthworms. Soil samples were collected and sent for commercial analysis of chemical and biological measures that have been proposed as indicators of soil health. Samples were also sent to Fera to measure the molecular-based inidcators.

The analytical process for molecular-based indicators
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. With the spring samples, a Fera-developed DNA extraction method was also used on larger 250 g sub-samples for comparison. A technique called DNA metabarcoding was used 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'. The barcodes of bacteria and fungi in each soil

sample were then identified using high-throughput DNA sequencing technology. 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. Organisms with taxonomically distinct DNA sequences are grouped into operational taxonomic units (OTUs). These can then be assigned taxonomic rank where the sequences match those of known organisms held in specialised bacterial and fungal databases curated by third parties. The SILVA database was used as a reference for bacterial metabarcode sequences, and the UNITE database for fungi together with the taxonomic classifications used by the National Centre for Biotechnology Information (NCBI). The relative abundance of each organism in the sample can then be estimated. 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. Some OTUs remain unknown even at family level (Figure 1). The DNA sequencing approach enables us to quickly assess the presence and relative amounts of many hundreds or even thousands of types of organism, irrespective of whether they form a relatively large or small part of the community - but it does not tell us the absolute amounts of each type of bacterium or fungus present.

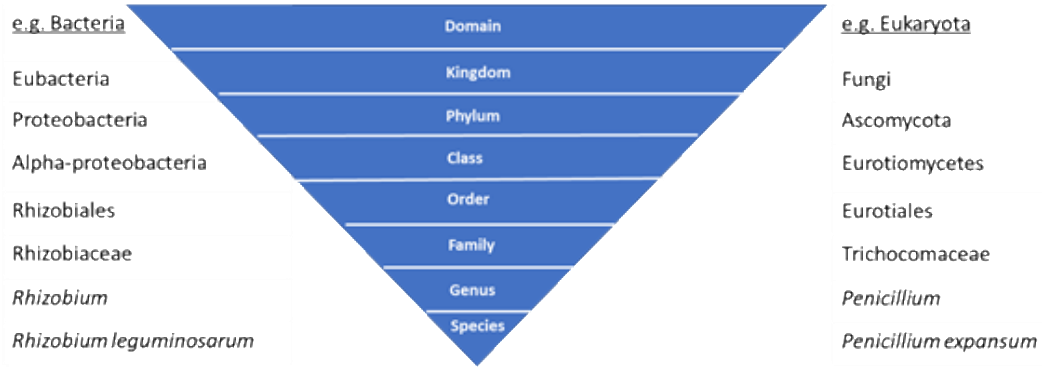

Figure 1: Organisms are described according to a number of hierarchical taxonomic ranks; these are shown together with an example for a known bacterial and fungal species.

Populations of known plant pathogenic micro-organisms, *Gaeumannomyces graminis* var. *tritici*, *Fusarium culmorum*, *Fusarium graminearum*, *Rhizoctonia solani* AG2-1, and *Rhizoctonia solani* AG8. were detected and quantified using quantitative polymerase chain reaction (qPCR) technology. This involves amplification and quantification of DNA markers specifically designed to target the known pathogen.

Soil health indicators

The data for soil indicators was compiled into soil health scorecards using the approach developed in the AHDB/BBRO Soil Biology and Soil Health Partnership (Figure 2). The site overall has a low/moderate soil K reserve and crops are likely to respond to fresh K additions. For a medium/heavy soil, the soil organic matter is at the lower end of the typical range; and microbial activity measures and earthworm numbers are also lower than typical. The low/moderate biological activity scores are not unexpected as the soils are in continuous arable cropping and organic manures have not been applied recently. Biological indicators (earthworms, organic matter and microbial activity) showed no clear differences as a result of rotation or cultivation treatments. Overall, the soil health scorecards showed no marked differences between rotation and cultivation treatments. The clay loam topsoil had good to moderate structure when assessed in spring and autumn with some evidence of low porosity blocky aggregates, which reflects both the soil texture and moderate levels of SOM. Higher nutrient offtakes in the winter cropping rotation mean that this has slightly lower soil P and K reserves on average. The topsoil showed noticeable visual differences

in residue locations as a result of the cultivation treatments following the incorporation of winter wheat straw in autumn 2017 (Figure 3).

a) Averages for STAR rotation treatments (spring 2018 sampling)

Soil health parameter	Winter cropping	Spring cropping	Wheat / fallow	Continuous wheat
pH	6.9	7.2	7.2	7.0
Extractable P (mg/l)	13.1	17.4	20.4	17.3
Extractable K (mg/l)	101.7	111.5	117.5	101.5
Extractable Mg (mg/l)	62.6	60.7	61.2	58.8
Organic matter (%LOI)	3.8	3.8	3.8	3.8
Structure; VESS score	2.9	2.7	2.7	2.6
CO_2 Burst	131	125	124	120
Earthworm count (total number in a 20 x 20 x 20 cm block)	6.5	5.3	6.6	6.3

b) Averages for STAR cultivation treatments (spring 2018 sampling)

Soil health parameter	Plough	Shallow non-inversion	Deeper non-inversion	Managed
pH	7.2	7.1	7.1	6.9
Extractable P (mg/l)	17.1	17.9	17.6	15.6
Extractable K (mg/l)	106.2	110.0	112.3	103.8
Extractable Mg (mg/l)	57.7	63.8	61.1	60.6
Organic matter (%LOI)	3.7	4.0	3.9	3.7
Structure, VESS score	2.4	2.8	3.0	2.7
CO_2 burst	112	130	136	121
Earthworm count (total number in a 20 x 20 x 20 cm block)	7.7	5.9	4.9	6.2

Figure 2 Data presented as Soil Health Scorecards (using the benchmarks under development in SBSH)

Plough Shallow non-inversion Deeper non-inversion

Figure 3. Topsoil profiles (VESS scoring blocks) from the three main cultivation systems, taken in wet conditions in spring 2018.

Molecular-based indicators

There are many different definitions of biological diversity, with different units and different calculations. The simplest definition is simply the number of different types of organism in the sample (this is often referred to as "richness"). However, this ignores the possibility that two communities with the same number of organisms could have very different abundance profiles. For example, in one community the four types of organism could be present in equal amounts; in another, a one organism could dominate, with the others present in very low abundance. Most measures of diversity take such distributions into account, and the data is often presented using a measure called Shannon Diversity. A low Shannon Diversity (less than 1) would mean that the community is practically concentrated in one type, and the other types are very rare (even if there are many of them). A large Shannon Diversity means that there are both a large number of species and also that all species are equally abundant within the community.

In the samples collected from STAR, a total of 11,266 bacterial and 301 fungal OTUs were identified. These soil biological communities show high diversity typical of UK agricultural soils. The Shannon Diversity for the bacterial community is typically in the range 6 - 7.5 and is much higher than that for the fungal community which is typically 3 - 4.5. The fungal community is more dominated by a few common species, whereas the bacterial community is more diverse (Table 1). Many of these different bacterial and fungal OTUs were matched successfully to the reference libraries at genus level.

Metabarcoding approaches also showed no clear pattern of differences in α-diversities of bacterial or fungal richness (numbers of species) with rotation or cultivation treatments. However, there were differences in fungal community composition, β-diversity, as a result of different rotational and cultivation treatments. This was also seen in the individual pathogen assays with higher populations of Takeall fungus (*Gaeumannomyces graminis* var. *tritici*) detected in rotations with continuous wheat or alternate fallow compared with rotations containing spring or winter break crops. In contrast, a higher average population of *Fusarium culmorum* was detected in plots following deep or shallow non-inversion tillage compared with annual ploughing.

Relationships to wheat yield

There were no differences in the 2018 grain yield with rotation or cultivation treatments; overall average 9.4 t ha^{-1}. None of the soil health measures were well correlated with wheat yield; all show relatively little variation between the long-term treatments at this site. Despite the 13 year differences in cultivation and rotational management, there has been little impact on either crop yield on soil health on this (heavy) clay loam soil in continuous arable cropping with no organic manure applications.

Table 1: The most abundant bacterial and fungal phyla in 48 soil samples across all treatments in the long-term STAR trial experiment determined by (a) 16S rRNA metabarcoding (1,160437 sequence reads) and (b) ITS rRNA metabarcoding (764,783 sequence reads) respectively.

a)

Bacterial phyla	Relative abundance %	
Proteobacteria	20.5	Enormous range of metabolic diversity, including opportunistic pathogens, plant growth promoters, symbiotic and free-living N fixers, nitrifiers and de-nitrifiers, sulphur oxidisers; diversity within this phylum may be changed by agricultural management
Bacteroidetes	18.5	Wide range of metabolic diversity, some free living N fixers and plant growth promoters; often found to increase in relative abundance under agricultural management
Acidobacteria	17.7	Wide range of metabolic diversity; only recently described; common in soils, but relative abundance often declines under agricultural management
Verrucomicrobia	10.3	Recently described, common in soils; relative abundance may decline under agricultural management
Thaumarchaeota	9.3	Ammonium oxidisers, relative abundance may increase under agricultural management
Planctomycetes	9.2	Dominantly aquatic bacteria
Chloroflexi	6.5	Phylum includes free-living photosynthetic bacteria; relative abundance may decline under agricultural management
Actinobacteria	3.5	Decomposers with wide enzymatic capacity, form mycelial colonies similar to fungi, source of many antibiotics, some plant symbiotes, relative abundance may decline under agricultural management
Gemmatimonades	3.2	Recently described, relative abundance may decline under agricultural management

b)

Fungal phyla	Relative abundance %	
Ascomycota	43.6	Active decomposers commonly hyphal but also a wide range of parasites and symbionts (including some ectomycorrhizal fungi); often with a high degree of specialisation within the phylum. Include some fungi with biocontrol activity such as *Trichoderma* and *Beauveria* spp. and a range of plant pathogens (e.g. *Fusarium*, *Verticillium*, *Gaeumannomyces*)
Mortierellomycota	36.0	Recently proposed phylum containing decomposers in the order Mortierellales. Saprotrophs on decaying leaves, roots and other organic material.
Basidiomycota	20.4	Mushroom, rust and smut fungi; includes a range of symbionts including ectomycorrhizae of trees and some pathogens such as *Rhizoctonia*.

What next?

The project showed differences between spring and autumn sampling for biological indicators and DNA extraction methods. It is therefore important to standardise methodology and sampling time for comparative studies of soil biological indicators. Molecular-based analysis of the soil microbial community (and soil fauna too) is a new developing tool that will revolutionise the understanding of soil biological function and underpin an increased focus on the management of soil biology, alongside soil chemistry and physical structure. This project has generated a unique dataset which will accelerate research into how soil microbial communities influence our farming systems. More information is still likely to come from this dataset itself as a result of on-going analysis. Much more science is needed before molecular-based analysis of the soil microbial community is of practical value to farmers. However, these approaches are likely to generate new soil health indicators over the next few decades.

But don't wait for those indicators before you begin to look more closely at your own soil health. It's already possible to link together soil health indicators measured in the field, such as earthworms and visual evaluation of soil structure (VESS) and the data from soil samples sent for lab. analysis. Looking at a range of soil indicators together adds value to any single measure and will help enhance our understanding of the impacts of current soil management - even if, as on the STAR trial, the impact is low. In the future it may also be possible for farmers to also pool these wider sets of soil health data to create a farm citizen science resource allowing benchmarking for farm data and to support selection of soil-improving practices. Then when the molecular indicators become available they will slot straight in.