## Project title:

Using microbiome profiles as an indicator of soil health to investigate the impacts from compaction and potato cropping.

## Applicant(s):

Ashleigh Holmes, Sue Jones, Lauren Watts and Sonia Humphris

## Background to the project (200 words max): As in original application

Soil compaction is a growing issue in soil health due to the intensification of farming and increased dependence on heavy machinery. The effects of compaction on the soil have been well documented, such as increasing mechanical resistance, but less is understood about the impact to the soil microbes (referred to herein as soil microbiome). The soil microbiome can be an indicator of soil health, where microbial richness and metabolically diverse species demonstrate a healthy soil. Soil compaction causes reduction in pore space and oxygen availability which can reduce the diversity and function of the soil microbiome. The reduction in oxygen favours certain microorganisms that then outgrow the rest of the population. This can alter the metabolic activity of the soil, e.g denitrification of the soil, but can also lead to an increase in pathogen populations that favour low oxygen conditions, eg *Pectobacterium* (blackleg). These metabolic changes can impact the chemical composition of the soil, e.g. sulphur and calcium concentrations. Making targeted soil amendments to correct these changes, therefore, may help to reduce the negative effects of soil compaction.

## Aims and objectives (100 words max): As in original application

This study aims to determine how treatments to overcome soil compaction (or anaerobicity) can improve microbial diversity and overall soil health, helping to reduce the presence of harmful pathogens.

The treatments tested:

- 1. Fulvic+humic acid (20L/ha)
- 2. Mineral application (CalMax Ultra 2L/ha)
- 3. Acid + mineral combination
- 4. No treatment control (standard practice).

**Research results** (Please include figures, images, diagrams etc as appendixes – 1 page):

Where our RESAS supported research is to investigate the effects of amendments on blackleg disease progression, this project allowed us to use the existing field trial to broaden the scope to the effects on the soil communities (microbiome). In May 2022, we planted two replicate field trials (one in West Loan and the other in Loan) with Fontane PB tubers. The soil resistance, measured by a shear vane, in West Loan was greater and consistent across the plots (Appendix Figure 1) so all further samples were taken from this field trial for the study. We also measured the starting moisture content and pH of the plots to track how it changes during the growing season (Appendix Figure 2). The average moisture content was significantly different between the drills and the tramlines within the plot in May, but this difference was lost by the time of sampling in August before burndown of the foliage. The pH was an average of 5.3 across the trial in May, and this significantly increased in the drills only to ~5.43 by August. This is likely due to the potatoes root structure and exudates secreted in the drill soil.

To investigate if the amendments influenced the plant nutrient content, petioles were sampled from 50 plants across the replicate 4 plots for each treatment, in August, and sent for micronutrient analysis (YARA). There was no significant effect of the treatment regime on the amount of nutrients in the plants, except for Nitrate where the levels from plants with the Acid treatment were approximately 10-fold greater than the other samples but it was still considered a low concentration of nitrate present (Appendix Figure 3). The field was routinely assessed for the presence of blackleg symptoms (May – August 2022) but no diseased plants were found, even with increased irrigation to promote onset of disease, which could be due to the unseasonably warm temperatures in the summer of 2022. At harvest, tubers from the central drill were collected for average yield and blemish disease assessment, in collaboration with Jennie Brierley. The treatment regime resulted in a slightly higher average yield of tubers from acid treated plots, but no difference in the incidence of blemish diseases (Appendix Figure 4).

Total DNA was extracted from soil samples recovered from the drills, tramlines and potato root surface (rhizosphere) for 16S rDNA amplicon sequencing. The alpha diversity of a sample measures the abundance (Chao1) and diversity (Shannon) of a bacterial population within a sample (Appendix Figure 5A). Before planting, the overall abundance and diversity of sequences present in the drills and tramlines across treatments is similar. Before burndown in August, the addition of the rhizosphere group of samples shows a reduced alpha diversity compared to the bulk soil (drills and tramlines; Figure 5B). The beta diversity measures the abundance and diversity of a bacterial population between samples. From these graphs (Appendix Figure 6), although treatment (A:blue/green) has no effect on the bacterial populations recovered from the drills (circle), tramlines (square) or rhizosphere (triangle), there is a change in diversity between the populations from bulk soil and the rhizosphere (B). This difference in population diversity is described as the rhizosphere effect where the sugars, amino acids and other components of the potato root exudate change the chemistries in the soil surrounding the root and drives the recruitment and selection of bacterial species.

Although beyond the scope of this project, it would be possible to analyse the data further to determine the relative abundance of different species of bacteria and predict their potential functions in the system. The bacteria present in higher relative abundance were from the *Proteobacteria* family. This included plant growth promoting *Pseudomonas fluorescens* and disease causing *Pectobacterium spp*. In further work it could be possible to achieve species level assignments for a restricted set of bacterial sequences observed in our initial amplicon sequence variant data. In this way we could further investigate which species are adapted for growth with potatoes and what benefits, or detriment, they may offer the plant.

*Outcomes* (- *Comment on the proposed outcomes and describe who has been helped by this work and how - 200 words max):* 

Two main outcomes were anticipated from this project:

- 1. To provide evidence about the effects of soil compaction on the microbial communities and inform on how increased microbial diversity can improve soil health within a potato cropping rotation.
- 2. To provide a dataset that can be included to inform on the current BBSRC funded project developing a decision support tool for blackleg management (Des-BL).

There were no marked effects of treatment on the bacterial communities of the field soil or potato rhizosphere and the lack of diseased plants in the trial meant it was not possible to assess the impact of blackleg on the bacterial communities. However, this project has provided useful dataset for further research as the microbiome from the soil around the potato roots is of interest to compare to those described from other studies using different potato varieties to determine how the communities are influenced by variety and field location. From helpful feedback from the SSCR Potato Winter meeting, we are reducing the fertiliser rate and introducing *Pectobacterium* infected tubers to our next trial. This project also provided a valuable training opportunity for Lauren Watts, who prepared the next generation sequencing libraries for the project.

*Next steps* (e.g. Information on further funding etc – 100 words max):

The results and experience from undertaking this project is contributing to not only scientific research proposals and the RESAS supported projects but also for future knowledge exchange. The project partners are applying to SEFARI Innovation Knowledge Exchange fund to drive discussions on maximising the impact from microbiome datasets such as the one from this project. Ashleigh Holmes presented the work at Scottish Government ENRA Policy event in Our Dynamic Earth, Thursday 18<sup>th</sup> May and will present at EAPR Pathology and Pests section meeting in Arras, France in September 2023.

Appendix



Figure 1: Shear strength of field soil (Loan or West Loan) in May



Figure 2: Moisture content (MC; % dry weight) and pH of West Loan field soil, from drills and tramlines, in May and August.



Figure 3: Nutrient analysis of potato petioles from West Loan comparing standard practices (purple) to amendments humic+fulvic acid (blue), mineral supplement (red) and combination acid+mineral (green). Elements measured as percentage (%) or parts per million (ppm)



Figure 4: Tuber statistics: average yield (left) and blemish disease assessment (right).

May



August



Figure 5: Alpha diversity of bacterial communities (16S rDNA sequencing) from bulk soils in May and August and root-associated (rhizosphere) soil in August. Alpha diversity describes the species diversity within a sample. Legend: A = acid amendment; M = mineral amendment; C =combination acid+mineral; S = standard practice.



Figure 6: Beta diversity of bacterial communities (16S rDNA sequencing) from bulk (drill or tramline) and root-associated (rhizosphere) soils in August. Beta diversity compares the species diversity between samples. The amendment treatment does not influence the bacterial community diversity (A) but the communities associated with the area surrounding the root differs from the bulk soil (B).