Office Use Only	
Project Code	
Project Type	

FINAL REPORT 2023

Final reports must be submitted using the online application form at www.sagit.com.au with this Word document attached ***within two months*** after the completion of the Project Term.

PROJECT CODE	USA118
PROJECT TITLE	
Comparative effects of agricultural pesticides on SA soil microbial functions	

PROJECT DURATION *These dates **must** be the same as those stated in the Funding Agreement.*						
Project start date	**11/02/2019**					
Project end date	**31/05/2023**					
SAGIT Funding	2018/19		2019/20		2020/21	

PRINCIPAL INVESTIGATOR *(responsible for the overall project and reporting)*			
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ADMINISTRATION CONTACT DETAILS *(responsible for all administrative matters relating to project)*			
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Mobile:			

PROJECT REPORT: *Please provide a clear description for each of the following:*

Executive Summary (200 words maximum)

A few paragraphs covering what was discovered, written in a manner that is easily understood and relevant to SA growers. A number of key dot points should be included which can be used in SAGIT communications.

This project tested 20 commercial pesticides, commonly used in SA, for their short- and long-term effects on the microbial activities in three contrasting soils and demonstrated the following:

- The short-term effects of pesticides varied across soil type and pesticide category. Pesticides showed most effects in the alkaline loam soil from the Clare Valley. The fungicides azoxystrobin and flutriafol, herbicide chlorsulfuron and insecticide fipronil inhibited nitrogen cycling associated microbial functions in this particular soil.

- For the short-term effects on the carbon cycle, some pesticides tested, especially insecticides, have stimulated the cellulolytic and chitinolytic microbial activities in the acidic sandy loam soil from Wolseley. The pesticides did not exert any significant effects on phosphorus cycle in any of the three soils.

- The lab-to field validation focused on the effects of insecticide fipronil, herbicide propyzamide and fungicide flutriafol and used the Hart field site. In particular, the longevity and cumulative effects of multiple pesticide applications were investigated. The herbicide propyzamide and fungicide flutriafol inhibited all tested enzymatic activities (microbial functions) associated with nitrogen, carbon and phosphorus cycling following the second application at the end of the two-year field study. In addition, the herbicide propyzamide altered the microbial community structure at the end of the field study. Our results suggest that repeat pesticide applications, especially of the herbicide propyzamide, may influence soil health and microbial functionalities and that more information should be collected to inform risk-based policy development.

Project objectives

A concise statement of the aims of the project in outcome terms should be provided.

This project aimed:

- To compare the effect of 20 pesticides on the microbial composition and function in soil types of key importance to SA farmers.
- To validate laboratory results using laboratory-to-field assessment.
- To determine the cumulative effects and persistence of negative impacts on selected soil-pesticide combinations.

Overall Performance

A concise statement indicating the extent to which the project objectives were achieved, a list of personnel who participated in the Research Project including co-operators, and any difficulties encountered and the reasons for these difficulties.

This project made use of high-throughput methods for molecular microbial ecology and the assessment of key soil enzymatic activities to compare the effects of 20 pesticides on the microbial composition and function in three SA soils. Lab-to-field validation of the laboratory results and the cumulative tests (repeated pesticide application over a long-term incubation) were also performed with selected pesticides in a selected soil. This was in line with the project proposal and as such the objectives of the project were achieved.

Personnel involved during the project:

- Dr Allan Mayfield provided expert advice on the selection of commonly used pesticides for this project.
- Dr Casey Doolette communicated with six suppliers; Adama, Bayer, BASF, FMC, Nurfam and Syngenta to acquire the 20 pesticides and provided assistance throughout the project.

- Dr Sarah Noack and Rebekah Allen from HART, Amanda Cook and Brian Dzoma from SARDI and Dr Melissa Fraser from PIRSA assisted with the soil collections and the field trial.
- PhD student, Jowenna Sim performed all the laboratory incubations and analyses (chemical and molecular), supervised by Dr Casey Doolette, Dr Barbara Drigo and Prof Enzo Lombi.
- Dr Sotirios Vasileiadis and Dimitrios Karpouzas (University of Thessaly, Greece) provided their guidance on bioinformatic analysis and manuscript reviewing and editing.
- DNA Sequencing Center (Brigham Young University), University of Technology Science (UTS) and Ramaciotti Centre for Genomics sequenced samples for metagenomic analysis.

Difficulties encountered

One of the main difficulties encountered throughout the project was the major delays on the project progress due to the COVID-19 pandemic. For instance, (i) both metagenomic analyses were delayed for at least 4 months each, due to mandatory closures in the US (DNA Sequencing Center; Brigham Young University) and Sydney (University of Technology Science; UTS). Delays in sample shipment and receipt of data had flow on effects due to the reliance on these result for subsequent experiments ie. the field trial (where the selection of pesticides was dependent on the results from earlier experiments).

KEY PERFORMANCE INDICATORS (KPI)

*Please indicate whether KPIs were achieved. The KPIs **must** be the same as those stated in the Application for Funding and a brief explanation provided as to how they were achieved or why they were not achieved.*

KPI	Achieved	If not achieved, please state reason.
First soil collected and characterized for microcosms (Hart soil)	Yes ☒ No ☐	
Pesticides acquired	Yes ☒ No ☐	
1st set of microcosm experiments initiated (Hart soil)	Yes ☒ No ☐	
Residual effect trial started	Yes ☒ No ☐	
Enzyme activity work for 1st set of microcosms completed	Yes ☒ No ☐	
2nd soil collected and set of microcosm experiments initiated (SE soil)	Yes ☒ No ☐	
3rd soil collected and set of microcosm experiments initiated (EP soil)	Yes ☒ No ☐	
Molecular analyses of all sets of microcosms completed	Yes ☒ No ☐	
Enzyme activities work for 2nd set of microcosms completed	Yes ☒ No ☐	
Field trial initiated	Yes ☒ No ☐	
Enzyme activities work for 3rd set of microcosms completed	Yes ☒ No ☐	
First sampling and analysis of field trial completed	Yes ☒ No ☐	
Bioinformatics and statistical data of the microcosm experiment completed	Yes ☒ No ☐	
Degradation measurement completed	Yes ☒ No ☐	
Second field trial sampling and analysis completed	Yes ☒ No ☐	
Development of factsheets completed	Yes ☒ No ☐	

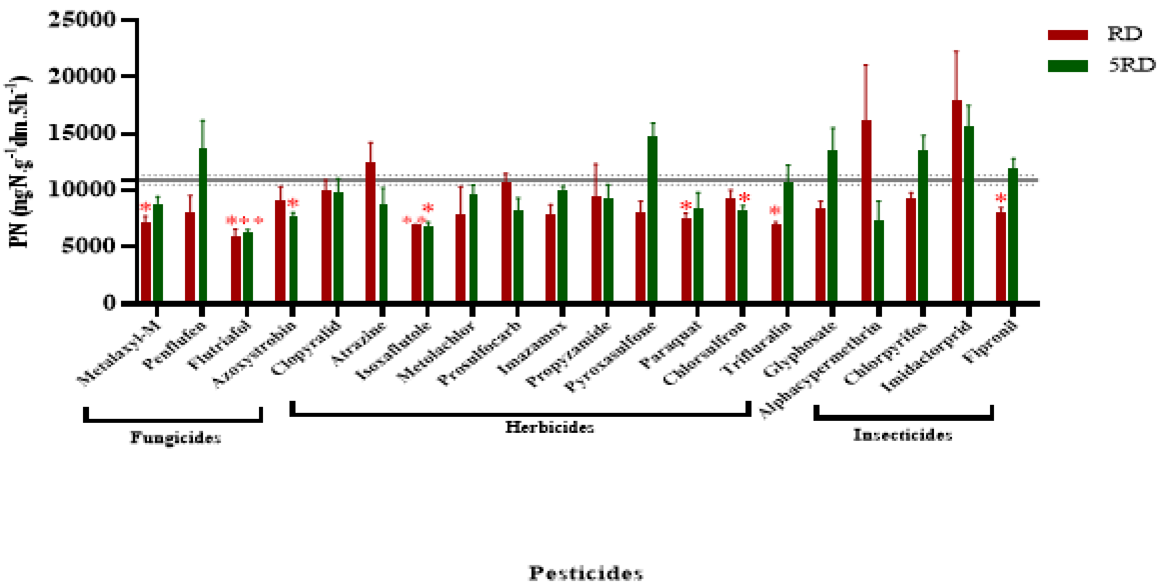
TECHNICAL INFORMATION (Not to exceed **three** pages)

Provide sufficient data and short clear statements of outcomes.

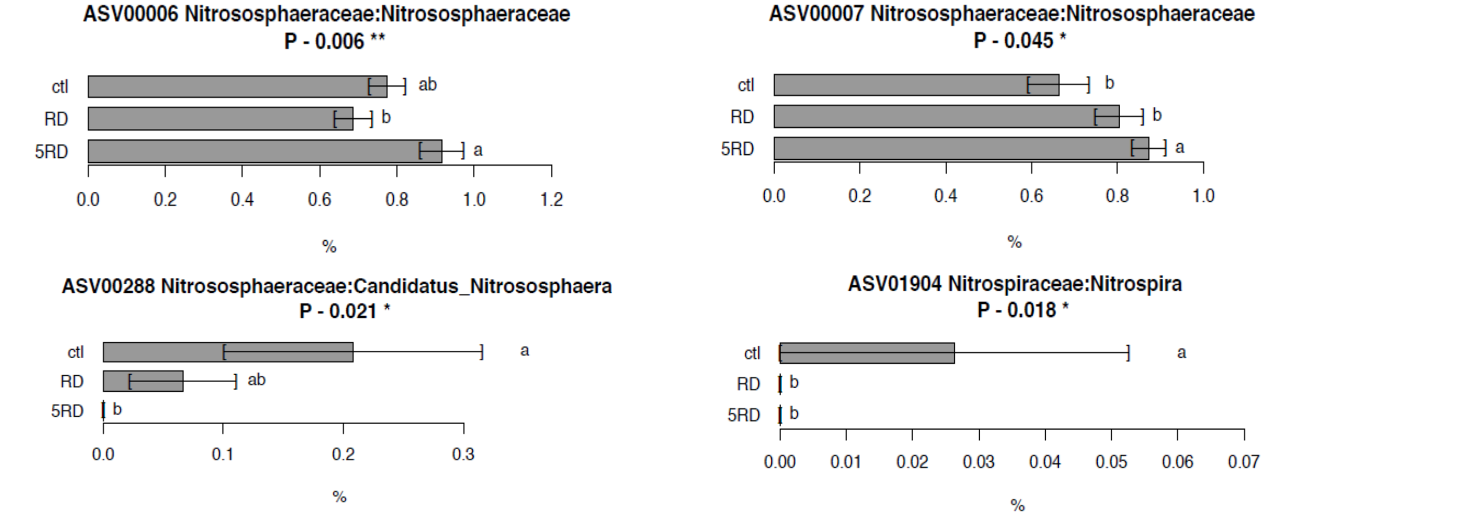
Findings of this project are broken down into three parts:

a) Short term effect of pesticides on nitrogen (N) cycling associated microbial activities

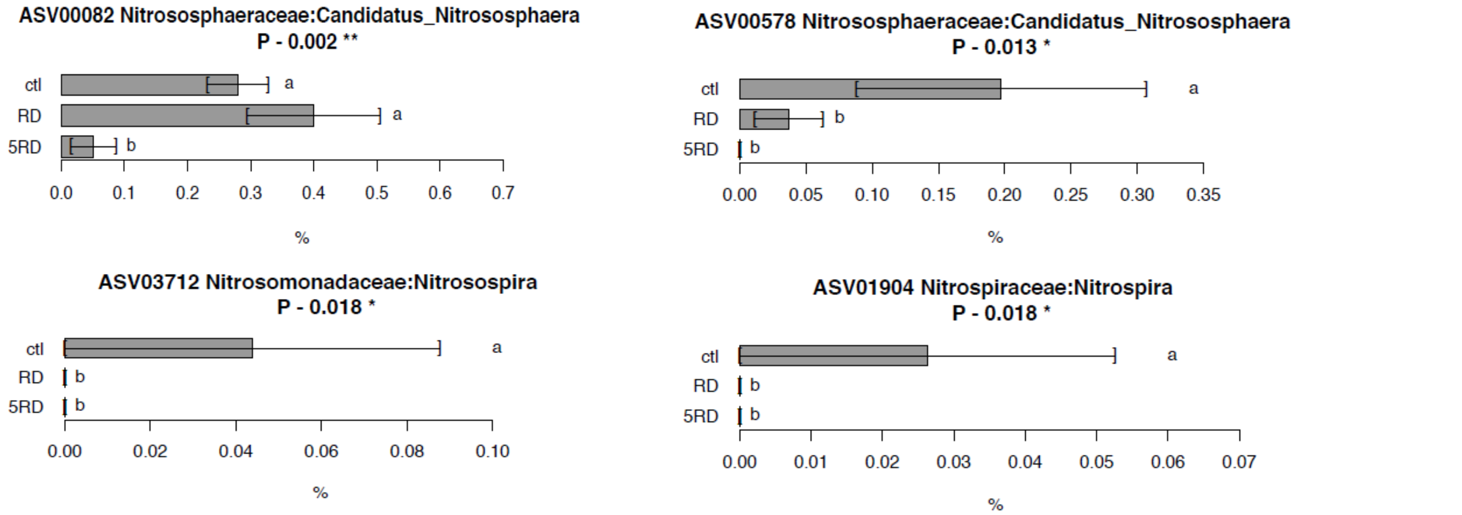
- Pesticide effects varied among soil type and pesticide category. The N cycle in the Hart soil was significantly affected by the application of pesticides.
- β-1,4-N-acetyliglucosaminidase (NAG) and Potential Nitrification (PN) were the most responsive parameters to pesticide application. In fact, while the effect of all 20 pesticides was mostly insignificant for all parameters investigated, nine and eight pesticides caused inhibitory effects on NAG and PN, respectively in the alkaline low organic matter soil (Hart). Below is an example of the effect of pesticides on PN with red asterisks showing significant pesticide effects when applied at the recommended dose (RD) and five times the RD (5RD).



- Because of the significant pesticide effects observed in the alkaline loam soil, we focused our amplicon sequencing on this particular soil. In our findings, we identified 135 amplicon sequence variants (ASVs) in our dataset. The most dominant ASV were members from the family *Nitrososphaeraceae* which are ammonia oxidizing archaea comprising 61 ASVs (i.e. almost half of the ASVs identified).
- Out of the 135 ASVs, 22 ASVs were significantly affected by pesticide exposure. The abundant members of *Nitrososphaeraceae* tolerated or even benefited from herbicide and insecticide exposure. In contrast, less abundant members such as *Nitrosospira* ammonia-oxidizing bacteria and *Nitrospira* nitirite-oxidising bacteria were negatively affected. Some examples are shown in the bar plots below (x-axis: abundances of ASV in percentage; y-axis: pesticide application rate (ctl = control/untreated soil sample; RD = recommended dose; 5RD = five times recommended dose); P-value indicates significant differences between control soil and soil with pesticide application within each ASV).

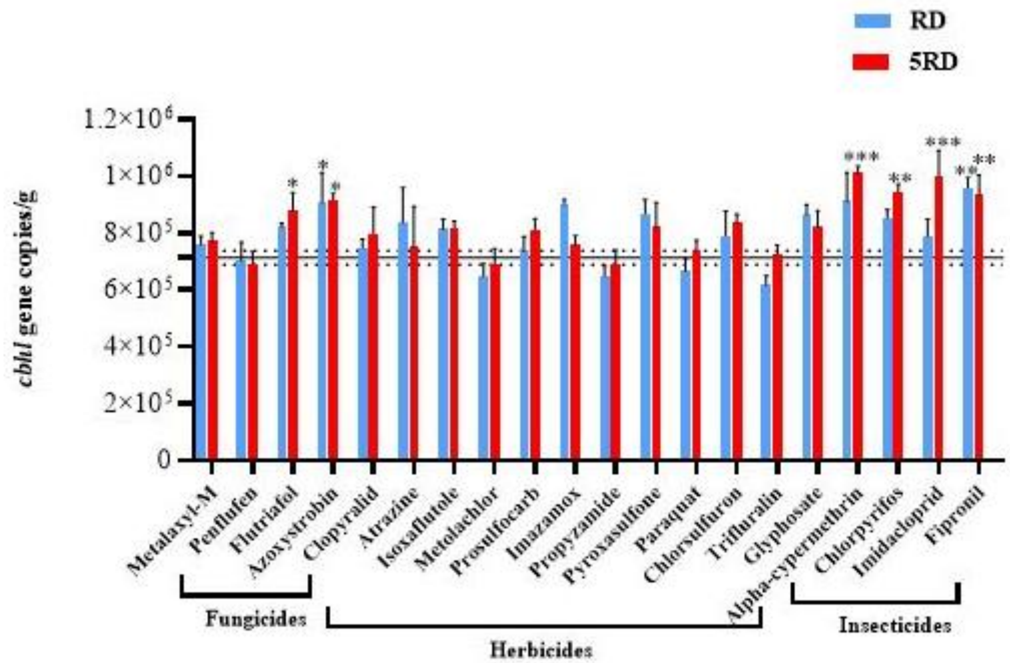


- These contrasting results indicate that the effect on microbial functions linked to the N cycle may not be significant. However, the diversity of the microorganisms responsible for these functions decreased and this may have implications for the overall resilience of the system.
- Fungicide application resulted in significant inhibiting effects on the microbial communities. Eleven nitrifiers were found to be significantly affected by fungicide exposure with their abundances significantly reduced compared to the control, with some example bar plots of these observations shown below

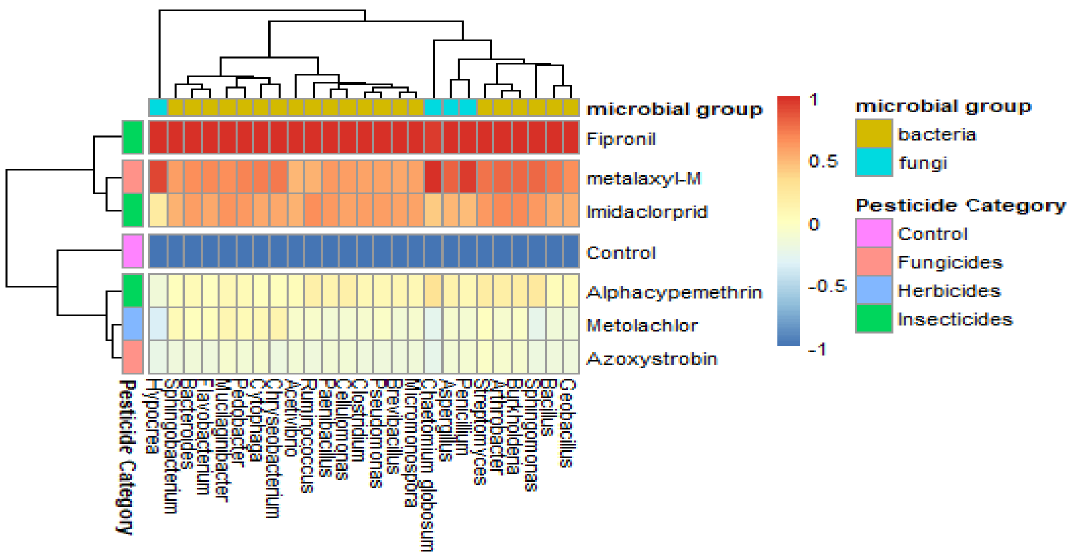


b) Short term effects of pesticides on carbon cycling associated microbial activities

- In relation to the carbon cycle, pesticide effects, especially those caused by insecticides, were most prominent in the acidic sandy loam soil. The abundance of *chiA* and *cbhl* genes were the most responsive parameters (see example below for the effect of the pesticides on *cbhl* absolute gene abundances in the acidic loam soil with asterisks showing significant pesticide effects when applied at the recommended dose (RD) and five times the RD (5RD)).



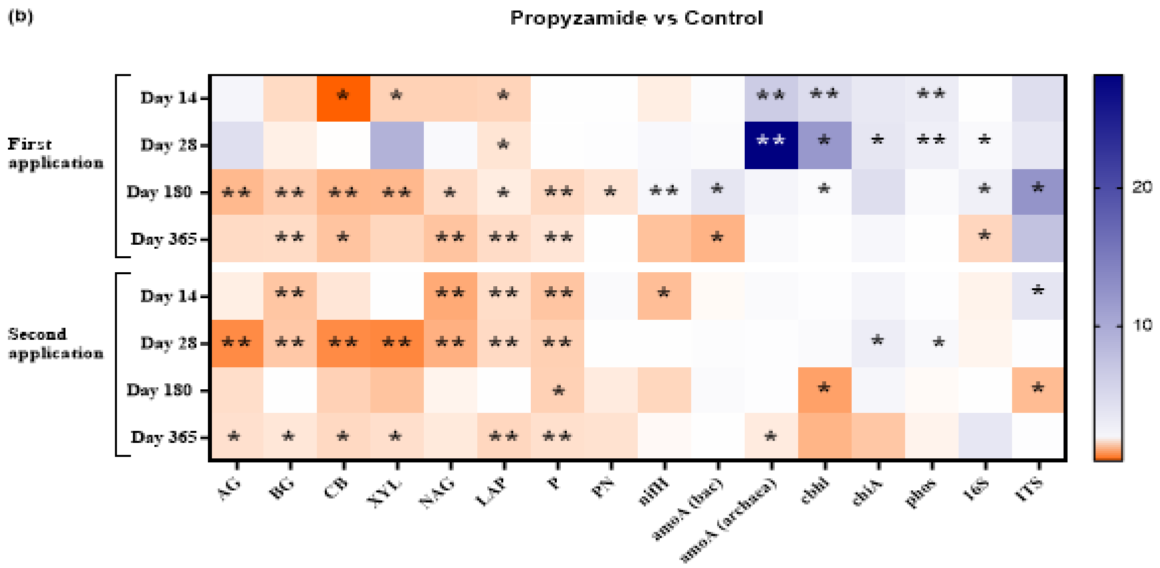
- With the significant insecticide effects observed, we selected three insecticides, two fungicides and one herbicide for shotgun metagenomic analysis to validate the significant effect of insecticides. Overall an increase in the abundance of carbon cycle associated microorganisms were determined.
- Metalaxyl-M however, increased the abundance of the fungal community more compared to the bacterial community. This may affect the carbon cycle and disrupt the formation of soil organic matter and structure stabilization. Below is an example heat map showing the abundance of carbon cycle associated microorganisms as affected by the six tested pesticides.



c) Long term effect of selected pesticides on the alkaline loam soil (Hart)

- Based on the short-term pesticide effects observed, we selected the insecticide fipronil, the herbicide propyzamide and the fungicide flutriafol to investigate the long-term effect on both laboratory and field settings.
- Surprisingly, the pesticide effect was found to be more detrimental in the field than the laboratory.
- The field effect of pesticides on the abundance of microbial community was transient, up to 6 months after the second application.

- However, the herbicide propyzamide and the fungicide flutriafol inhibited the microbial functions and altered the microbial community structure at the end of the 2-year field study (i.e. two pesticide applications). Below is a heatmap showing the significant field effect of propyzamide on the microbial parameters tested (scales are adjusted based on the activities/abundances in relative to the control; orange color indicates inhibiting effect while purple color indicates stimulating effect, compared to the control; * indicates *P < 0.05*; ** indicates *P < 0.01*).



- More information can be found in our three published manuscripts (please see the details given below under ‘Application/communication of results’)

CONCLUSIONS REACHED &/OR DISCOVERIES MADE (Not to exceed **one** page)
Please provide concise statement of any conclusions reached &/or discoveries made.

- Overall, pesticide effects differed among soil type and pesticide categories.
- In the alkaline loam soil, fungicides had more detrimental effects on N cycling microbial functions compared to insecticides and herbicides.
- Insecticides stimulated C cycling associated microbial functions and modified the community composition.
- Fungicide metalaxyl-M may affect soil organic matter formation and soil structure stabilization.
- All 20 pesticides had no significant effect on phosphorus cycling related microbial activities.
- Repeat applications of the herbicide propyzamide and fungicide flutriafol showed cumulative effects on microbial functions, where all enzymatic activities involved in carbon, nitrogen and phosphorus cycle were significantly inhibited. The herbicide propyzamide also altered the microbial structure at the end of the two-year field trial despite the transient effect on the abundance of microbial community.
- Our findings provide novel insights into the effects of 20 commonly used pesticides, (with a particular focus on the selected pesticides fipronil, propyzamide and flutriafol) on the soil ecosystem by providing comprehensive analyses at both functional and taxonomical levels, and laboratory and field scales.

INTELLECTUAL PROPERTY
Please provide concise statement of any intellectual property generated and potential for commercialisation.

Not applicable.

APPLICATION / COMMUNICATION OF RESULTS

A concise statement describing activities undertaken to communicate the results of the project to the grains industry. This should include:

- *Main findings of the project in a dot point form suitable for use in communications to farmers;*
- *A statement of potential industry impact*
- *Publications and extension articles delivered as part of the project; and,*
- *Suggested path to market for the results including barriers to adoption.*

Note that SAGIT may directly extend information from Final reports to growers. If applicable, attach a list of published material.

We have delivered the significance of this project via several platforms including conferences, article publications, newsletter articles, UNISA research day and SAGIT annual update presentations.

- An article outlining this study and explaining its relevance to the farming community was published in the **EP Farming Systems Summary 2019**, mainly to deliver the project objectives to SA farmers.

- Oral presentations have been given by Jowenna domestically and internationally during **conferences** to share the findings to the agriculture industries and researchers:
    - '*Do pesticides affect soil nitrogen cycling?*' on the 2021 Joint Soil Science Australia, NZ Society of Soil Science and ASPAC Conference.
    - *'Pesticides driving impact on soil microbial interactions across nitrogen, carbon, and phosphorus sources'* on the 16th Congress of the Federation of Asian and Oceanian Biochemists and Molecular Biologists
    - *'Impact of 20 pesticides on soil carbon and nitrogen cycles related microbial functions and community composition'* on the 18th International Symposium on Microbial Ecology

- A presentation on the *'Effect of pesticides on soil nitrogen and carbon microbial functions and community composition'* was given by Enzo and Jowenna at the **SAGIT Annual Update 2022** to share the latest project findings to the farming community and other researchers.

- Two manuscripts have been published as **journal articles** with one more submitted for publication:
    - *'Pesticide effects on nitrogen cycle related microbial functions and community composition'* published in Science of Total Environment.
    - *'Impact of twenty pesticides on soil carbon microbial functions and community composition'* published in Chemosphere.
    - *'Repeated applications of fipronil, propyzamide and flutriafol affect soil microbial functions and community composition: a laboratory-to-field assessment'* published in Chemosphere.

POSSIBLE FUTURE WORK

Provide possible future directions for the research arising from the project including potential for further work and partnerships.

Our results showed that pesticide effects differed among soil type and the physicochemical properties of both soils and pesticides play a key role in the interactions of pesticides with soil microbiota. Therefore, to provide a comprehensive understanding of the impact of pesticides on SA soil ecosystems, a large number of soil-pesticide combinations require evaluation.

We will commence another SAGIT project (USA 03323) on the 1st of July 2023 to focus on increasing the number of SA soil types and testing only those pesticides which have previously been found to have significant effects.

Other possible further work includes: (i) testing multiple pesticides from the same class of action to determine if the pesticide's mode of action is a key determinant of the effects on soil microorganisms (ii) testing the effect of active ingredients and the adjuvants separately (instead of the commercial pesticide formulation) (iii) using different field trial sites to further investigate the effect of soil type in realistic agricultural settings.