SOIL BIOLOGY SYMPOSIUM

From data to decisions... how far have we come?

Thursday, 15 May 2014
AgriBio-Bundoora, Melbourne
Foreword

The GRDC Soil Biology Initiative (SBI-II, 2009-2014) has partnered with research organisations throughout Australia to work towards the 10-year vision of increased profitability and sustainability of grain cropping as a result of harnessing the biological potential of soil.

Globally, research in soil biology is receiving renewed attention due not only to the increasingly urgent need to improve the long-term sustainability of food and fibre production but as a result of the rapid emergence of new technologies. These technologies, when locally applied, allow us to measure and monitor the specific biological mechanisms that underpin productive soils, and to consider their economic value and potential for strategic management. Biological mechanisms receiving particular attention in SBI-II include the capture and release of nutrients such as nitrogen, phosphorus and carbon and the protection of crops through disease suppression. Most importantly, SBI-II is up-skilling the next generation of soil researchers in the use of molecular and information technologies that until recently have been in the realm of the medical research community. This in turn is generating vast quantities of new information that landholders can begin to use to proactively and precisely manage the biology within their soils to achieve greater farm business efficiencies.

This symposium will showcase exciting new information that is emerging from three theme areas:

1) Soil quality monitoring
2) Nutrient management
3) Disease suppressive soils

It will become apparent that whilst significant progress has been made, there is an ongoing requirement for scientists to work closely with farmers and their advisers. By doing so, they will identify the on-ground issues from which to develop a solid, evidence-based foundation of knowledge that will lead to regionally specific on-ground action.

We encourage your lively participation and enjoyment in the symposium and in future RD&E efforts in soil biology.

Martin Blumenthal (GRDC Senior Manager Natural Resources)

Pauline Mele (SBI-II coordinator)
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Engaging with farmers

Geoff McLeod (Farmer, Finley, Southern NSW)
Geoff runs an irrigated cropping farm near Finley in southern NSW. The farm produces a range of winter cereal, oilseed and grain legume crops and soybeans using both overhead and surface irrigation systems.
Geoff has a degree in Agricultural Science and 30 years’ experience with irrigated and dryland farming systems in southern Australia. Geoff is a board member of Soy Australia and chairman of Southern Growers, a local grower group in the southern Riverina.
Geoff, a GRDC Southern Regional Panel member, also provides consultancy services to government, industry and catchment management authorities related to land and water management. He will provide a summary of the broad range of interests and expectations that land-holders have of soil biology RD&E in a talk titled ‘Satisfying diverse on-farm needs’.

Andrew Huffer (Consultant, Huffer & Associates, community engagement)
Andrew has a practical approach to facilitation and community engagement and is based in Perth, WA. Andrew, together with the regional cropping solutions network facilitator Sally Thomson, spent several days in February this year with GRDC Natural Resources Senior Manager, Martin Blumenthal, and GRDC soil biologists to gain greater insights into regional and local knowledge needs of growers and their advisers. Covering more than 2000km and speaking to more than 60 farmers both on farm and in lounge rooms and kitchens, this event uncovered some interesting insights and learnings.
Andrew will provide an overview of some of these learnings and articulate a way forward for improving the connections between soil biology R&D providers and farmers and advisers in order to fast track uptake. He will also assist in the facilitation of on-the-couch sessions during the day.

David Wolfenden (Farmer, Rand, Southern NSW)
David is a dryland sheep and cropping farmer from the eastern Riverina. David is interested in new technologies and how to introduce them into his business. He adopted minimum tillage in the early 1980s and has been retaining stubble since 1990.
David has been involved with many organisations that aim to aid the flow of information from the researcher to the grower. He is currently the Chair of the Victorian Grower Group Alliance. He sees the importance of farming systems groups and has been a member and on the committee of Riverine Plains. He will provide an insight into the benefits of soil biology in stubble retention systems based on in-paddock observations.

Allen Buckley (Farmer, Waikerie, SA)
Allen has a mixed farming enterprise in the SA Mallee at Waikerie. Allen is interested in new options for achieving higher forage value and at the same time providing surface cover. He has been a long-term supporter of no-till and stubble retention systems and has played an active role in research as part of the Mallee Sustainable Farming Systems group and as custodian of the MSF long-term site. Allen will provide observations he has made on the benefits of soil biology in low rainfall grain production systems.
Managing Expectations: The Microbiome in Agriculture

About the speaker:

Dr Jack A Gilbert is an Environmental Microbiologist at Argonne National Laboratory, Associate Professor in the Department of Ecology and Evolution at University of Chicago, and senior fellow of the Institute of Genomic and Systems Biology. Dr Gilbert has focused on analysing microbial function and diversity, community assembly processes, and dynamic interactions between taxa, with an aim of predicting the metabolic output from a community. He is currently working on microbial communities in natural, urban, built and human ecosystems. He is Principal Investigator for the Earth Microbiome Project (www.earthmicrobiome.org), Home Microbiome Project (www.homemicrobiome.com), Gulf Microbial Modelling Project (www.microbial-models.com), Hospital Microbiome Project (www.hospitalmicrobiome.com), and the Chicago River Microbiome Project.

Dr Gilbert became an advocate for GRDC’s investment in the Soil Biology Initiative, featuring on ABC’s award-winning Landline Program “Soil Secrets” (http://www.abc.net.au/landline/content/2012/s3630158.htm) in January 2012. Most recently Dr Gilbert contributed valuable insights and recommendations in a review of the science underpinning the Soil Biology Initiative (2009-2014). This will ensure that the technology applied and the new knowledge emerging from GRDC’s investment in soil biology will remain relevant for grain growers into the future.

The message:

Agriculture has always maintained an intricate association with microbiology, with a long history of innovation and exploration of the role bacteria, archaea, fungi and viruses play in nutrient cycling, plant productivity and disease suppression. This parallels the medical industry, in which understanding microbial pathogens has similarly led to significant changes in management practice over the last 150 years. Medicine is currently undergoing a revolution, with the bacterial communities associated with our bodies and our environments being implicated in influencing not just infections, but also allergic responses, neurological conditions and cancer. This realisation has resulted in the development of numerous large-scale research initiatives, costing $100s of millions, so that we can better understand and track down the individual bacteria or consortia of microbes that are associated with these conditions, and importantly prove causality and determine treatment strategies. Agriculture is arguably undergoing a similar revolution, both in animal husbandry, which maintains a similar focus to human medicine, and in cropping. With this renewed interest in the complex microbial interface between soil, crops and farming practice we are seeing a significant increase in investment in scientific research aimed at leveraging new technologies and ecological understanding, to identify key organisms and assemblages that influence crop productivity and disease resistance. In this brave new world, microbial ecologists are trying to illuminate the darkness surrounding plant-microbe interfaces, and this has resulted in a plethora of observational and exploratory investigations. These are vital for defining the road map that can help design the next generation of hypothesis driven experiments. We are now starting to see the fruits of these endeavours, with new understanding of how bacterial and fungal communities fight off infections, mediate nutrient concentrations resulting in loss or retention of expensive fertilisers, and alter plant hormone levels to influence productivity. Here we will examine these new findings, and highlight ongoing work within Australia to help generate scientific evidence that can guide management practice decisions to help improve crop productivity, disease resistance and economic viability of Australian farming practices in the 21st century.
Theme: Monitoring soil quality for better decision making


Project leader: Professor Daniel Murphy, The University of Western Australia
Email: daniel.murphy@uwa.edu.au

Objective:

Remove the barrier to adoption of management solutions that enhance soil biology and overall soil quality while maintaining economic viability.

Key findings:

Database of soil quality measurements (biological, chemical, physical) generated and populated into a National version of the soil quality web site for the Australian grains regions.

Figure 3.9: Example of the approaches used to develop a soil quality indicator package, highlighting the role of expert groups. These groups drew together a wide range of stakeholders with relevant information and experience including literature review and practical experience. Through facilitated discussion the groups derived the relationships between yield response and a range of soil properties to inform the interpretation of the indicators used within the soil quality monitoring framework (www.soilquality.org.au) which focuses on soil quality for crop production. Data presentation using the derived categories, descriptors and traffic lights shown for three soil indicators as examples.

Implications:

• The soil quality package can be used as a tool to identify potential biological, chemical and physical soil constraints to plant production. A primary outcome of the soil quality information is for a grower (i) not to do something (e.g. burn stubble) as opposed to changing practice or (ii) do something (e.g. lime) as a result of a poor soil quality score (e.g. low pH). This has been linked to other supporting programs such as the soil acidity work to provide tools and information to growers on managing constraints (e.g. lime calculator).

• Practice change is focused on education and knowledge regarding the loss of productive capacity associated with deterioration of the soil resource. This program demonstrates the value of strategic and regular soil testing and ties in with deeper testing for soil pH and nutrients. Expectation is that more growers are sampling, and to a greater depth.

Further reading: www.soilquality.org.au; Ground Cover TV Episode 6: January 2012, Soil Quality Supplement Part 1 and 2
http://www.grdc.com.au/Media-Centre/GroundCover-TV/2012/01/GCTV6-Jan2012/gCBozZUBVMw#sthash.BA1lifKG.dpuf
DNA Tests for nematode community analysis (DAS00111)

Project Leader: Dr Kathy Ophel-Keller  Key researcher: Katherine Linsell
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Objectives:

The objectives of this work are to characterise free-living nematode (FLN) communities in cropping soils and to develop DNA tests for monitoring soil health.

Background:

Nematodes are useful biological indicators of soil health because they occur widely and respond to physical and chemical changes. Nematode community analysis is typically performed by a trained nematologist able to classify free-living nematodes to a family level. This is a laborious task involving microscopic examination of individual nematodes extracted from soil samples and this places severe limitations on the number of samples that can be assessed. DNA based assays can potentially overcome this constraint.

This project has provided information on free-living nematode communities in key Australian cropping soils and developed DNA tests to quantify key indicator groups.

Results:

Nematode community analyses were performed on soils from 11 trial sites (SA, VIC, QLD & WA) across different environments (soil type, rainfall region) under different management practices (tillage, rotation, fertiliser, stubble) across multiple years. 40 FLN (identified to family or genus level) were counted, and 17 were found to be good indicators.

The two key drivers influencing changes within FLN communities were: 1) soil type which was correlated with rainfall, particularly 1-3 months prior to crop sowing and 2) application of nutrients, particularly N, P, S and Cu. The analysis also demonstrated that:

- Sampling times (pre and post sow) significantly influenced FLN communities suggesting there is an impact of rainfall and/or carbon exudates from roots.
- Significant shifts in nematode population structures were correlated to tillage regimes.
- Rotation history can influence FLN communities where canola, pastures and legumes were included in the cereal rotations.
- There was no evidence that stubble management (burnt, retained on surface or retained and worked into soil) influences FLN communities.

Nine DNA tests were developed incorporating 11 of the FLN indicators and are predicted to detect more than 80% of the free living nematode groups present in Australian cereal cropped soils.

Implications:

Free-living nematode communities have been characterised in grain-growing soils and the key environmental and management influences (seasonal rainfall, soil type, tillage and rotation,) determined to provide precise tools for assessing negative impacts of current management approaches and a means to manage to reduce these impacts.

DNA tests have been developed for key indicator groups. These require broader regional validation but will be accessible for growers to monitor free-living nematodes in soils as a measure of biological soil health.

Further reading: Fact Sheet (www.soilquality.org.au)
Molecular indicators of soil quality (UWA00142)

Project leader: Winthrop Professor Tony O’Donnell, UWA
Email: tony.odonnell@uwa.edu.au

Objectives:
(i) To identify a minimum set of molecular indicators for assessing soil quality.
(ii) Identify management options that sustain or enhance soil quality without affecting grain yields.

Key findings:
- We have shown that these molecular indicators can be routinely applied to a diverse range of soils and land uses from across Australia.
- That the methods are amenable to a centralised laboratory for DNA processing.
- Differences between soil can be related to known microbial taxa (i.e. groups) and that these differences can be related to environmental and management factors.

Historically soil organisms are considered as a single, undifferentiated unit (black box) termed the microbial biomass. This project has opened the black box through use of molecular tools to measure specific parts of the microbial community – thus providing greater insight into how management of soil impacts on soil biology and function.

Implications:
- Molecular probes could be upscaled for inclusion in a commercial soil quality monitoring platform.
- The microbial ‘black box’ has been successfully opened to provide greater information on the key groups present (e.g. Bacteria, Fungi, Archaea). Further validation of regional management impacts must be undertaken.

Further reading: The future version of www.soilquality.org.au that is being developed over the next 12 months will contain national molecular soil quality data from this project.
Monitoring soil biology with high resolution genomic technologies (DAV00102)

Project Leader: Dr Carolyn Bath/Dr Damian Bougoure  
Email: carolyn.bath@depi.vic.gov.au

Objective:
To trial three different molecular techniques to analyse the DNA of microorganisms in soil of different grain growing areas and compare the utility of these methods to monitor microbial and functional diversity relative to region and different management regimes. We plan to identify relationships between these measures and more stock standard measures of soil physics, chemistry and biology and yield data and to incorporate these into the soil quality website (www.soilquality.org.au).

Key findings:

- A total of 430 soil samples from cropping areas around Australia (a subset of the sites sampled in the broader soil quality monitoring effort) were analysed by a commonly used microbial community profiling technique known as Terminal Restriction Fragment Length Polymorphism (TRFLP)
  - Diversity measures for both bacteria and fungi were similar (~100 OTUs), which is not informative enough, as bacterial diversity is known to be much higher.
- A subset of 100 of these samples were used in DNA sequencing approaches that identify marker genes that describe to Genus level who is present in soil microbial communities
  - Initial sequencing of bacterial 16S rRNA tag libraries revealed 6,936 OTUs; much higher than the total of 424 OTUs found in TRFLP work.
  - Sequencing techniques look to be promising for providing diversity measures
    - 6,936 reads per sample available from 16S rRNA bacterial tag libraries (95 samples)
    - 760,000 reads per sample available from ITS fungal tag libraries (100 samples)
- The same subset of 100 samples were also used in a third DNA sequencing approach that measures all genes present in the sample (ie the ‘metagenome’) including those involved in significant functions
  - ~417 million reads of sequence in total is available from the 100 samples

Implications:

Identifying relationships between soil biology using genomic technologies and other soil quality measures will enable better monitoring of soil functions and validate the use of management regimes to ensure longer term soil sustainability. By selecting subsamples from key soil types and land use/management practices in each agro-ecological region we ensure relevance of the project findings over a wide geographical area (millions of ha). Identifying soil biology measures that can relate directly to other measures of soil health (and ideally yields) will serve to add value to monitoring programs that will validate current crop management regimes or provide evidence for the need for modification in the case of loss of functions. Further, these new measures could potentially provide great economic benefit in a time when farming systems are moving in a direction that will be more reliant on utilising biological components of the soil as substitutes or additives for currently used inputs, such as inorganic fertilisers.

Identification and characterisation of disease suppressive soils in the Western Region (DAW00201)

Project leader: Dr Daniel Hüberli, DAFWA
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Objectives:

1) Identify sites in Western Australia (WA) that are suppressive to one or more root diseases of wheat including take-all, rhizoctonia root rot, crown rot and root lesion nematodes (RLN);
2) Determine the components of suppressive sites; and
3) Identify microbial communities using molecular tools in collaboration with the other project in the Soil Biology Initiative.

Key findings:

- From 331 paddocks assessed during 2010 to 2012 in WA, 15 paddocks for rhizoctonia, six for take-all, 22 for crown rot and one for RLN were identified as “potentially” suppressive to disease.
- After confirmation with a pot bioassay, two paddocks were highly suppressive and five paddocks showed moderate suppression to rhizoctonia. Only two paddocks recorded were highly suppressive to crown rot. The bioassay for take-all failed to confirm any sites as being suppressive to this disease. The one RLN identified was not assessed.
- A selection of two suppressive sites for rhizoctonia were more similar to each other than the other farms for the bacterial microbial analysis than the two non-suppressive sites (collaborative research with Helen Hayden, DEPI).

Implications:

Suppressive sites, primarily for rhizoctonia, were found in WA. Sites changed to non-suppressive with change in crop type. It therefore is possible for grain growers to influence the capacity of WA soils to suppress a range of diseases though this may not endure. A range of management practices other than crop sequence effects need to be identified in order to promote a more robust and persistent disease suppressive state.

Further reading:

Biological Suppression of Root-lesion Nematodes in Grain-growing Soils (DAQ00164)

Project leader: Dr Nicole Seymour     Key researcher: Jady Li
Email: Nikki.seymour@daff.qld.gov.au

Objective: To better understand the suppressive nature of grain-growing soils and provide growers with management options to enhance suppressiveness of their soils to root-lesion nematodes (Pratylenchus spp)

Key findings: Repeated studies (in 2010-2013) of different soils in glasshouse and laboratory bioassays have consistently shown that general suppressiveness to root-lesion nematodes does exist in a variety of soils. We have also examined suppressiveness at different depths in the soil profile (since Pratylenchus nematode populations tend to be highest to depths of 30-60 cm) and to date have shown that soil from 0-15cm is much more suppressive than soil from 30-45cm. We are continuing to investigate mechanisms driving this change including food sources and moisture.

Over 130 soils from the northern region were surveyed for the presence of natural enemies (Pasturia bacteria, nematode-trapping fungi and predatory nematodes) to root-lesion nematodes. We are also compiling and analysing information on the soil characteristics and crop/management histories to understand which soils might better support these organisms. Glasshouse trials to increase populations of Pasturia on hosts of Pratylenchus thornei are being conducted but multiplication of the bacteria has been slow. Traditional methods of isolating fungi are being compared with new molecular methods being developed in collaboration with Dr Helen Hayden, Vic DEPI, thus new tools are now available to more easily determine where the fungi are present. Preliminary studies to examine the influence of wheat root endophytes on the multiplication of root-lesion nematodes have been conducted. In a wheat pot assay, the addition of a fungal endophyte (Fusarium nygamaei) previously isolated from wheat roots growing in a soil from a property near North Star, NSW, significantly reduced P. thornei multiplication. More studies are required to confirm this as a potential biocontrol organism.

Key farm practices that may enhance suppressiveness to root-lesion nematodes are being examined. A field trial at Hermitage Research Station, in southern Qld, was designed to study the impact of organic amendments (0, 5, 10 and 20 t organic matter/ha) and various cropping regimes (continuous fallow, sorghum with residue retained, sorghum with no residue retained) on P. thornei multiplication in wheat grown after these treatments. Two to three years after incorporating 20t/ha of organic matter, the earlier observed increases in suppression are not as evident and did not affect crop yield. However, improved soil biology especially in the surface soils was still present when soil was cropped continuously compared to fallow.

Implications:  This research has identified the soil bacterial species (Pasturia thornei) and the wheat root endophyte fungus, (Fusarium nygamaei) as having a key role in the suppression of root-lesion nematodes in northern grain-growing soils. On farms where Pasturia thornei is present, practices which maintain populations of root-lesion nematode such as the use of nematode-tolerant cultivars, minimisation of fallow periods, and continuity of cropping should increase populations of this host-specific parasite and eventually result in a reduction in nematode populations. The use of inoculants of P. thornei as an active intervention under these cropping regimes warrants further R&D. The general suppression of root-lesion nematodes by organic matter inputs and minimum tillage in the upper layers of northern grain-growing soils is more likely to provide effective control in shallower soils, and in situations where environmental conditions are dry rather than moist during summer (ie southern and western grain-growing regions).

Further reading:
Suppressive soils: Can we find a microbial finger-print using 'omics' technology?
(DAV00105)

Project leader: Dr Helen Hayden
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Objectives:
To identify soil microbial communities and functions associated with disease suppression using ‘omics’ technologies that will improve the management of disease especially options for early and rapid interventions.

What is a suppressive soil? Disease suppression refers to a lack of disease manifestation even in the presence of the pathogen, host plant and favourable environmental conditions. It is a biological property of the soil dependent on the microbial community present. Suppression may take different forms with general suppression referring to the inhibition of pathogens resulting from the total amount of microbial activity, while specific suppression refers to antagonistic activity against the pathogen by individual microorganisms or a more narrowly defined group of organisms. All soils have the ability to suppress soil-borne root diseases to some extent through the activity of soil microbes, though suppression may occur as a continuum from highly suppressive to poorly suppressive soils making it difficult to clearly identify the traits of a suppressive soil. Fungal pathogens suppressed in certain soils include Rhizoctonia solani, Gaeumannomyces graminis var. tritici (Ggt), Plasmodiophora brassicae, and Fusarium oxysporum.

Our study site at Avon, South Australia (refer to photo), has been previously characterised using pot trial assays and PreDicta B to be suppressive for Rhizoctonia solani AG-8, the cause of bare patch disease in grains crops. We sampled for two consecutive years from disease suppressive (background) and non-suppressive fields (foreground) using low and high resolution approaches to identify unique “fingerprints” or biomarkers for our suppressive soil.

Key findings:
- Suppressive and non-suppressive soils at Avon, SA, have similar physico-chemical properties.
- The microbial communities identified by their RNA and DNA were different in suppressive compared to non-suppressive soil providing candidate indicator species for suppressive soils.
- Proteins and metabolites, the products of microbial communities, were also found to be different in suppressive compared to non-suppressive soils. We are currently doing further characterisation of the metabolites that are unique to suppressive soils or more abundant in them to identity the exact molecular formulae of these potential biomarkers and biopesticide products.

Implications:
Microbial and functional biomarkers associated with R. solani AG-8 suppression have been identified and provide a novel strategy for early detection and control of bare patch disease in cereals. These biomarkers will require validation in other soils shown to be suppressive to R. solani AG-8.

Further reading:
GRDC Rhizoctonia Fact Sheet, Southern and Western regions “Management to minimise Rhizoctonia disease in cereals”, available at www.grdc.com.au
A molecular approach to unravel the dynamics of disease suppressive microbial communities (CSP00135)

Project leader: Dr Gupta Vadakattu, CSIRO
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Objectives:

1. To characterise disease suppressive microbial communities in detail, using high-throughput (HT) DNA techniques, with the aim to identify community signatures that represent highly disease suppressive communities.
2. To identify the crop-soil ecosystem features that can support disease suppression in cereal growing regions of southern and eastern Australia.

Background: An improved ability to identify and manage natural disease suppression (DS) capability would assist in the strategic management of soil-borne disease risks. Disease suppression is the ‘ability of a soil to suppress disease incidence or severity even in the presence of the pathogen, host plant and favourable environmental conditions’. There are a number of examples of biologically mediated disease suppression of soil-borne pathogens, such as rhizoctonia bare patch in cereals and take-all suppression, under Australian agricultural environments. It is believed that a diverse array of microbial communities can be involved in the continued effective expression of disease suppression in the field environment. At present we lack in knowledge of ‘what constitutes a suppressive community?’

Key findings:

The composition of soil biological communities in suppressive and non-suppressive soils at Avon and Minnipa in South Australia were measured using DNA techniques. The suppressive soils have been previously established as being highly suppressive against Rhizoctonia disease in cereals. We used a targeted polyphasic approach and the linkage to systems with known disease suppression potential would allow us to answer if a ‘disease suppressive index’ can be developed based on genetic structure of the microbial community. Some key findings include:

- Field observations of disease incidence (7 and 18 wks), plant growth (7 wks) and grain yield confirmed the differences in disease incidence, inoculum build-up and suppression levels.
- Comparative analysis of field soils with high and low suppression potential provides a realistic platform to apply state of the art molecular approaches to understand and solve biological constraints in field scenarios.
- Results from the genetic profiling of soil bacteria, nitrogen fixing bacteria, Pseudomonas species and soil fungal communities showing significant differences between soils based on suppression capacity supporting the hypothesis the observed disease suppression was biologically (microbial community) based.
- Successfully characterised soil fungal communities using high-throughput DNA sequencing and data processing which showed the presence of a diverse fungal community (917 distinguishable genera) in these agricultural soils and significant soil type/site based differences were observed. Detailed analysis of relative abundance data indicated significant suppression and soil type based differences in the diversity of fungal communities at different taxonomic groups. The majority of the differences associated with suppressive soils were attributed to less than 40 genera, in particular members of the Xylariaceae, Bionectraceae and Hypocreaceae families with known antifungal capabilities.
- Results from HT-DNA sequencing of bacteria indicated that specific bacterial taxa from alpha and delta proteobacteria, actinobacteria and acidobacteria GP6 groups contributed to the majority of differences in the diversity based on suppression. Soil type and time of sampling had a significant influence on the bacterial composition.
- Bioassay experiments conducted under controlled environmental conditions indicated the role of carbon
addition on disease suppression and its links to changes in specific soil bacterial and fungal communities.

Results from these experiments complement findings from field experiments and help identify the signatures of highly disease suppressive communities and identify factors that can enhance DSP in different soil types.

- A new glasshouse-based bioassay method to quantify the ‘Disease suppression potential’ (DSP) has been standardised for soils from northern NSW and southern Queensland making it possible to evaluate the potential for disease suppression to develop in these environments.
- In addition, the current DSP bioassay was successfully used to identify disease suppression in farmer fields in SA and WA.

Implications:

Soil-borne diseases are a major constraint to achieving maximum water and nutrient use efficiency in Australian rain-fed agriculture. An improved understanding of the genetic mechanisms and metabolic pathways involved with reduced disease incidence will greatly assist in the development of cropping practices with higher levels of natural disease suppression.

- This research is the first step in developing ‘microbial signature profiles’ for disease suppressive communities to identify and monitor their dynamics in field soils. The use of ongoing field experiments through links with farming system groups has improved the applicability of new information to field environments in Australia.
- Agronomically useful disease suppression capacity has been found in farmer fields under continuous cropping no-till systems in addition to long-term cropping system experiments in the rainfed cropping regions in southern Australia – this confirms that the improvement of disease suppression should be considered as one of the factors in the decision making about management practice or cropping system.
- Management practices that increase C inputs (e.g. crop residues) and turnover over a number of years (5-7 y) will improve disease suppression and reduce the impact of RBP, however carbon management alone wouldn’t improve disease suppression capacity in alkaline calcareous soil – other management practices (crop rotation to reduce pathogen inoculum levels, where available) need to be used to reduce soil-borne disease impacts.
- A diverse group of bacteria and fungi contribute to stable disease suppression in the field environment – which means enhancement of suppression through adoption of management practices should be a preferred option for reliable and stable method of controlling disease impacts.

Further reading:

Theme: Management systems for enhanced nutrient availability

Manipulating biological processes that improve nitrogen supply to cereal crops: Free Living Nitrogen (FLN) fixing bacteria (CSP00138)

Project leader: Dr Gupta Vadakattu, CSIRO
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Objectives:
1. Quantify N fixation by Free Living Nitrogen (FLN) fixing bacteria in cereal based cropping systems.
2. Determine the diversity of FLN bacteria in order to identify key players associated with soil and crop types in different agroecological zones.

Key findings:

- A laboratory based $^{15}$N method has been standardised for direct quantification of the amount of FLN fixation. It provides reproducible results and is suitable for a wide range of soil types (sands to clay soils) and for both bulk soil and rhizosphere samples. It allows the analysis of a large number of samples at each time and is cost effective. It is less influenced by the microscale variation compared to the ‘intact soil core’ assay.
- Estimates of FLN fixation ranged from <0.2 to 2.9 kg N / ha / day and the amount of N fixed is influenced by the soil type, time of sampling (in crop vs. non-crop period), type of previous crop (wheat vs. cereal rye), soil moisture content and mineral nitrogen levels.
- Carbon availability is a critical factor for FLN fixation and stubble removal either by burning or grazing has a negative impact on the amount of N fixed. FLN fixation was higher immediately after harvest and decreased as summer progressed.
- In the field FLN fixation is higher soon after rainfall events when the water content is adequate to carry the carbon to where FLN bacteria reside and also provide the required low oxygen concentration conditions.
- The amount of FLN fixation during summer significantly increased in the presence of summer active grasses e.g. Rhodes grass and Panicum species compared to cropping soils.
- Results from the genetic profiling of FLN bacteria (nifH gene based analysis) indicated a diverse community in Australian cropping soils. Soil and crop type and variety based differences in their diversity were observed. Different summer active grass species promoted the abundance of specific members of the FLN bacteria.

Implications:

- Our findings suggest that agronomically significant amounts of FLN fixation are produced in soils across Australia and these amounts can be manipulated.
- The quantity of cereal stubble influences the amount of FLN fixation during summer suggesting that how stubble is managed is important for maximising the amount of N fixed in cropping soils.
- Higher levels of mineral N in the surface (0-10 cm) soil (>25 kg N / ha) results in lower FLN fixation but the threshold value varies with soil type – this finding will assist in decisions for fertiliser N application.
- Summer active perennial grasses support higher levels of FLN fixation compared to winter-cereal crop only systems – suggests inclusion of perennial grasses in southern Australian cropping regions.

Further reading:

Harnessing the nitrogen cycle through novel solutions (UWA00139)

Project leader: Professor Daniel Murphy, UWA
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Objective:
To improve understanding of how farming systems can harness soil nitrogen supply by ensuring greater microbial/plant retention of nitrogen and lower loss to the environment.

Key findings:
Improved nitrogen use leads to lowered fertiliser costs and less off-site impact to the environment. Increasing soil inorganic N content decreased the microbial biomass yield suggesting a direct link between soil nitrogen management and microbial growth (see figure; Murphy, Banning, Crowley, Hoyle, Jones and Mele, unpublished data).

Implications:
- Increasing soil organic matter levels was not a management solution to decrease the Nitrification-to-Immobilisation ratio in soil (i.e. a ratio of potential loss versus microbial retention). However, root exudates were able to decrease this ratio thus highlighting the importance of considering rooting density/branching for nitrate retention in soils.
- Nitrification inhibitors were effective at 40°C indicating that new generation inhibitors are now a potential means of slowing nitrification rates in higher temperature soils that are susceptible to leaching.
- Plant root carbon has a significant influence on components of the soil microbial population. Manipulating plant-microbial signalling/interactions holds promise for rhizosphere engineering to benefit plant health and growth.
- PhD student Ms Louise Fisk trained in soil biology.

Further reading:
Managing soil biology to improve nitrogen supply in grain production systems (DAV00106)

Project leader: Dr Lori A. Phillips
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Objectives:

To determine whether agronomic practices can be used to manipulate nitrogen (N) cycling microbial communities using molecular tools to precisely identify key microbes involved.

What are the benefits of investigating nitrogen cycling microbes in soils?

Nitrogen fertilisers are the single largest variable input cost for grain growers, costing Australian producers over $3 billion per year. However, up to 60% of applied fertiliser N may be unavailable for crop uptake. Some N is lost from the soil by leaching and denitrification, while some is locked up in the soil in forms that plants cannot use. Soil microbial communities (bacteria, fungi, and archaea) control both the loss pathways, as well as the pathways that release crop-available nitrate. Understanding what microbial communities are active during each N-transformation step, and how agronomic practices influence that activity, is the first step towards understanding how to manipulate those communities to improve fertiliser N-use efficiency (NUE). A 10% increase in NUE would provide on-farm savings of over $0.3 billion per year.

Key findings:

- There are direct links between the microbes that release N from crop residues and soil sources, and the microbes that convert fertiliser N to crop-available N. These can be manipulated to improve NUE using standard agronomic practices:
  - strategic tillage can be used to manipulate the links between microbial N-cycling communities in Vertosols, optimising the timing of plant-available N release. Crop rotation combined with strategic tillage can increase the capacity of soil microbes to release N from organic sources in the next cropping season.
  - Fertilisation suppresses the release of organic N, by increasing the competition between the soil microbes that process organic N and those that convert fertiliser N.
  - There are limits to the rate at which microbes can process soil N into crop-available N, even in soils with high background total N (i.e. 0.5%).
  - Molecular tools that quantify specific microbial genes, such as those involved in organic N release, may be used as a substitute for chemical measures of soil mineralisation potential (eg PMN used in www.soilquality.org.au).

Implications:

- Treatment of crop residue: There is growing interest in the use of strategic tillage to manage weeds, disease, and improve stubble handling. Our results suggest that if such occasional tillage events occur after a legume crop, additional benefits may include:
  - Increasing the number of soil microbes able to process the N-rich organic material.
  - Reducing N loss by disrupting the microbial processes associated with leaching and denitrification.
- Timing of N fertiliser additions: Minimising or eliminating fertiliser applications at sowing will enhance microbial processing of soil organic N.
- Mineralisation measures: Molecular tools that measure the microbial genes associated with organic N release may be used to assess the mineralisation potential in a wide range of soils.

Further reading:

Management of microorganisms to unlock the phosphorus bank in soil (2012-2015) (UWA 00150)

Project Leader: Assoc Prof Deirdre Gleeson / Prof Daniel Murphy
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Objective:
To provide the agricultural sector with management options that harness soil microorganisms to unlock part of the $10 billion worth of fixed P in Australian arable soils and use P fertilisers more efficiently. We will achieve this by determining whether microbial release and plant uptake of fixed phosphorus (P) in soil can be increased by managing the carbon-to-nitrogen (C:N) ratio of organic matter inputs.

Key findings:
- Organic matter inputs with high C:N ratios decrease bacterial abundance yet increase bacterial diversity and change the structure of the bacterial community (Figure a and Figure b). Further analysis of our experimental data will show whether use of organic matter inputs with high C:N ratio will produce a diverse microbial community that also has more strategies to access fixed P in soil.
- We have obtained Arabidopsis mutants with altered C:N ratios in the plant residue that we are currently growing to obtain organic matter from the same species (but with different C:N ratios) which will be used to test whether organic matter with different C:N ratios alters microbial and plant uptake of P (Figure c).
- Computer aided tomography (CT) scanning can be used to visualise P fertiliser granules, microorganisms and roots in soil (Figure d).
- NanoSIMS can be used to visualise uptake of P by individual microbial and plant cells in the rhizosphere.

Implications:
- Organic matter inputs could be used to manipulate P cycling through the plant-soil-microbial continuum.
- Computer aided tomography (CT) scanning will allow us to re-build a 3-D image of a soil core showing the location of microbial populations relative to the position of P fertiliser granules.
- NanoSIMS will allow us to observe uptake of P by individual microbial and plant cells and competition between microbes and plants for P.
Project title: Assessing management options for enhanced soil phosphorus availability using rotations (UA00119)

Project leader: Associate Professor Annie McNeill    Key researcher: Ashlea Doolette
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Objective:

To identify if there are crop rotations that can provide an agronomic management solution for the release of fixed phosphorus (P) in a range of soils across the grain growing regions of southern Australia.

Background:

Sites were at Hopetoun and Longerenong in Victoria, Karoonda in SA and Junee in NSW and break crops were either green/brown manured or harvested at maturity. Crops were considered to mobilise P where P uptake plus available P in soil at peak growth exceeded the sum of applied fertiliser P plus available soil P at sowing. P mobilisation results from biological activity in the break crop root zone causing (a) solubilisation of fixed P and (b) mineralisation of organic matter.

Glasshouse work using $^{33}$P radio-isotope estimated P accumulation in intact root systems of lupin and directly traced that root residue P into following wheat.

Key findings:

- Break crops (lupins, peas, vetch, lentils, canola and cereal rye) at three of the sites appeared to mobilise P from the soil P bank; at one site wheat also mobilised P. The amount of P estimated as mobilised was greatest for rye (30 kg/ha) and ranged from 8-23 kg/ha for the other crops. Soil microbial P only increased at one site during the break crop season.

- Large differences in P inputs by break crops as mature shoot residues (0.97–6.7kgP/ha) or green/brown manures (12.3–17.0 kgP/ha) did not translate to different amounts of available P or microbial P prior to sowing wheat.

- At all sites wheat following break crops accumulated more P than wheat after wheat (8.3–19.1 kg/ha P compared with 7–12.6kgP/ha) – reflected as greater dry matter, yield and P uptake – but was not related to the estimated break crop P mobilisation.

- Neither the pre-season soil P test nor wheat P uptake were strongly correlated with P input from break crop residues; but at three of the sites wheat P uptake was correlated, to varying extents, to available N at sowing.

- Estimated P content of intact lupin root systems was almost double the P amount in recovered roots alone. In an unfertilised soil 19% of the lupin root system P accumulated in wheat plants five weeks after sowing. This represented 12% of P uptake by the young wheat plant.

Implications:

Break crop management increases P uptake and yield in wheat, although any link to break crop P mobilisation was not evident from measures of microbial P or available P. Further work is required to explore how the P mobilisation potential of break crops is influenced by soil type and P fertiliser interactions, and to quantify the contribution of mobilised P in residue inputs including break crop roots to wheat under field conditions.
Can arbuscular mycorrhizal (AM) fungi be harnessed to enhance P nutrition and grain yield in rotations? (UA00128)

Project leader: Prof Andrew Smith, UA
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Objectives:
The project aimed to determine if populations of AM fungi (AMF) in soil are influenced by growth of different break crops, so influencing AM colonisation in the following wheat crop and delivery of soil phosphate (P) to the plants via the AMF. We use a novel method of estimating this delivery separately from direct P uptake by the roots, using radioactive P ($^{33}$P). The project involves bioassays of field soil with wheat in the glasshouse to test AM-infectivity, and measurement of P uptake via AMF. This is followed in the field with the same methodology; shown as follows:

Experiments were carried out at the Mallee Sustainable Farming site at Karoonda, South Australia, in 2012 & 2013. Tubes with $^{33}$P were buried in large PVC cylinders, open at the base, and containing most of the roots. Sets of cylinders were located in ‘cages’, as shown:

There were four cages in each experiment. In 2012 a cage was placed in each of four strips in which wheat was grown after pea, lupin, canola, or wheat in 2011. The experiment was terminated after c.10 weeks, determined by ‘decay’ of $^{33}$P. Plant analysis was at the Waite laboratory, Adelaide. $^{33}$P uptake was negligible due to negligible AMF colonisation.

In 2013 the aim was changed to assessing variability in a single crop treatment, with the four cages along a single strip with wheat grown after wheat in 2012. The experiment was successful.

Key findings:
- $^{33}$P appeared in the wheat, but with high variability (0-24% of total shoot P uptake, mean 12%).
- The range was similar to percent of plant root length colonised by AMF (0-29%, mean 7%).
- Surprisingly, application of commercial AMF inoculum did not improve colonisation or $^{33}$P transfer. More soil tests of the inoculum are being conducted.

Implications:
- Management of AMF activity using break crops may not be appropriate at sites we have tested.
- Much more research is needed to understand factors resulting in low AM infectivity that contrasts with sites on Eyre Peninsula where plants are highly colonised and AMF can deliver at least 50% of total P in wheat, at least in glasshouse experiments (results of previous SAGIT project).
- $^{33}$P tracking can be used with other approaches to understand and improve soil biology management.

See also the poster (this Symposium): Tracking phosphate uptake via arbuscular mycorrhizal fungi into field-grown wheat. Authors: Andrew Smith, Maria Manjarrez, Annie McNeill & Sally Smith.
Carbon Storage: Identifying drivers and key modulators in grain cropping systems (UWS00008)

Project leader: Prof Brajesh K Singh
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Objective:

The overarching aim of this project is to identify the microbial drivers and key modulators of soil C storage in different soil types and under different management practices in grain-based ecosystems. This will be achieved by the following four complementary and inter-linked objectives:

(1) To identify the interaction between soil aggregate size and soil microbial communities and determine the consequences for soil C storage under contrasting soil types and management practices (aggregate size, fertiliser application and other soil characteristics).

(2) To identify how changes in soil organic matter (SOM) turnover are related to the structure and functioning of soil microbial communities and to identify the key microbial groups which influence soil C storage.

(3) To distinguish the role of substrate quality, nutrient availability and microbial communities in soil C storage and therefore determining their relative importance.

(4) Bring together the new data generated from this project with that of existing, closely related, SBI projects to improve understanding of the biological constraints of soil C storage and to add value of existing SBI research projects.

Key findings:

Our preliminary analysis of available data suggests that:

- Management practices have differential effect on the ability of soil to store C.
- The amount of C decreases with depth irrespective of soil type or treatments.
- The ability of soils to accumulate C correlates well with the substrate metabolising abilities of microbial communities.
- Soil type and management practices allow build-up of distinct taxonomic (analysed by advanced molecular methods including next generation sequencing) and functional groups (analysed by high throughput microarray ‘GeoChip’) of microbial communities which can influence various soil processes including C turnover.
- There is a significant partitioning of C among different soil aggregates wherein the smallest sized aggregates (<50 um) store significantly higher amounts of C.
- The amount of C in different aggregate co-related with differences in substrate utilisation pattern and soil enzymatic activities of microbial communities.

Implications:

- Improved knowledge of the role of various soil microbial groups in C storage in different soil aggregate sizes in major grain producing systems in Australia. Rigorous statistical analysis of our dataset is underway to provide guideline for adoption
- These findings highlight and identify management practices and soil type which improve soil aggregate C storage in grain cropping systems.

Further reading:
