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Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: CSE1401

Project Title: Management options enhancing beneficial microbial communities and functions in cotton soils

Project Commencement Date: 01/07/2013 **Project Completion Date:** 30/09/2016

CRDC Research Program: 1 Farmers

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Date Submitted: __Nov 30, 2016_____

Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report.)

Background

- **Outline the background to the project.**

Management practices in current Australian cotton farming systems, e.g. reduced tillage, crop rotation, residue retention, organic manure application and reduced insecticide use, can change the levels of key soil microbial functions. They need to be optimised to promote soil biological functions to sustain cotton production, improve nutrient use efficiency, reduce soilborne diseases and maintain environmental health. Preliminary results from research at ACRI suggest that management systems can be manipulated to optimise microbial functions to improve N and C cycling processes and improve soil biological health.

Diseases such as Fusarium wilt, Black root rot and Verticillium wilt have significant impact on cotton production. Crop rotation, stubble retention and tillage can either reduce the levels of pathogen inoculum or modify pathogen-soil microbe interactions thereby influencing disease. Currently the management of diseases is through the selection of genetically resistant cultivars (where available), agrochemical application and rotation with non-host crops. But even in our current high F-rank cultivars significant losses can occur from Fusarium disease under the right environmental conditions (Stiller W 2012 FUSCOM).

Soil fungal community has been shown to have capacity to affect pathogen inoculum levels and their disease causing potential. Examples of enhanced biological disease suppression have been suggested in cotton (suppression of black root rot). Biological mechanisms behind disease suppression in high-input cotton soils are not known and we are unable to extend individual observations to other sites or develop management options that promote biological disease suppression.

In the lower carbon cotton soils, composts can provide a source of organic carbon and nutrients for soil biota and increase soil fertility as well as provide other biological and structural benefits. But little is known about the effects of compost addition to cotton soils on soil biological health and fertility.

Long-term rotation trials at ACRI and in Qld provided a valuable resource to quantify management effects on key beneficial microbial communities and processes. In collaboration with these projects/experiments this project provided new knowledge on the underlying biological mechanisms that promote soil biological health.

Objectives

- **List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.**

Aim: To identify management options that promote biological functions through a better understanding of key beneficial microbial communities in cotton based farming systems.

Objectives:

1. To quantify the effect of management practices including rotation, stubble retention, tillage and organic manure application on microbial communities involved in nitrogen cycling, free-living N fixation and carbon turnover.
2. To characterize the genetic diversity of soil fungal communities as influenced by management practices and link it with disease incidence and suppression under different cotton farming systems.
3. To identify the 'best set' management practices that maximize the benefits from the key biological processes involved in biological disease suppression and free-living nitrogen fixation as part of myBMP for cotton based farming systems.

The polyphasic approach i.e. monitoring in field based experiments combined with glasshouse and controlled environment experiments, has provided new knowledge on key biological processes but more importantly helped to link them with management systems under field conditions.

1.1 Quantify soil type and cropping system based differences in free-living N fixing bacteria and amount of N fixation within cotton crop and during off-season including rotational crops.

1.2 Determine the seasonal and management effects on N and C cycling microbial communities in the long term cotton farming system trials - crop rotation and stubble retention effects over two seasons.

For this work, we used soils from selected treatments in the two long-term cropping system experiments located at ACRI conducted by the Late Dr. Ian Rochester and Dr. Nilantha Hulugalle.

I. Experiment # F6E – Nitrogen fertilizer x crop rotation experiment: Cotton-Wheat, Cotton-Vetch and Cotton-Fababean rotations sampled at the time of planting and in crop during 2013, 14 and 2015 seasons

II. Experiment # D1 – Rotation crops and stubble management on permanent beds in cotton farming systems: Cotton-Wheat-Vetch-Cotton; Cotton-Fallow-Cotton; Cotton-Wheat-Cotton; samples at the time of planting in 2013 season

All soils were analysed for microbial activity, catabolic diversity and potential, specific functional groups of microbial communities involved in N cycling (e.g. N fixation, nitrification, denitrification), microbial biomass and N mineralization potential and mineral N and dissolved organic carbon levels.
Results from this work were presented in annual progress reports and at the ACRC meetings in Toowoomba and Narrabri. Findings are being prepared for a journal manuscript.

Milestone 1.3 Conduct 1st controlled environment experiment (6-8 months duration) using soils (up to 2) representing field organic manure experiments to study the effect of quality of organic amendment on soil biology and nutrient levels.

Milestone 1.4 Conduct 2nd controlled environment experiment (up to one year) to investigate the effect of multiple applications of amendments on soil biological processes and their resilience.

As part of the above two milestones, two 6-month incubation experiments were conducted to test the effect of multiple organic compost amendments and the influence of added N fertilizer application on microbial, biochemical and chemical properties of soils using soil from the long-term organic amendment experiment in a field belonging to the farmer Jan Lefrenz in Qld. Additionally, surface 0-10cm soils collected from selected treatments collected during May 2015 (after 4 applications) were analysed for changes in microbial and biochemical properties.

Results from this work were presented at Australian Cotton Research Conference and the World Cotton Conference to disseminate the findings to researchers, cotton farmers and agronomists. A manuscript of this work is currently being prepared for publication in a scientific journal.

2.1 Describe the genetic composition of soil fungal communities and microbial activities as influenced by stubble retention (quantity and quality of stubble), crop rotation and fertilizer application (up to 2 samplings / season and two seasons).

2.2 Determine biological disease suppression potential in cotton soils from ongoing field experiments monitoring disease incidence – glasshouse experiment.

2.3 Identify links between diversity of soil fungal communities, disease incidence and suppression.

As part of the above milestones, soil samples collected from three regions/sites (ACRI (3 experiments), Goondiwindi and Cowan) during 2013, 15 and 16 cotton seasons were analysed for soil fungal communities, microbial activities to quantify the influence of rotation and stubble management practices. Data of disease incidence and crop performance was procured from the collaborators to identify potential links between fungal diversity and disease incidence/suppression. For the fungal diversity and community composition and population abundance we used next generation sequencing and qPCR methods. Soil samples were also analysed, with the help of collaborator assistance, for their disease suppression potential using the bioassay used by the pathogen inoculum measurements by Dr. Linda Smith's group.

Results from this work were presented at the annual FUSCOM meetings, ACRC meeting 2015 and World Cotton conference 2016. A manuscript of this work is currently being prepared for publication in a scientific journal.

3.1 Linkage between specific biological function and soil and management factors determined.

Soil samples from the experiments referred above were also analysed for overall microbial activity, N supply potential and catabolic diversity measures in order to determine the effect of management on biological functions. Based on findings during 2013 and 3014 seasons, e.g. higher levels of mineral N through cotton season and lower abundances of N fixing bacteria, additional analysis of samples from 2015-16 season are analysed using a comprehensive microarray method (Geochip) to determine the functional diversity of microbial community as influenced by N fertilizer addition. These analyses will provide information on the effect of N fertilizer addition on microbial functional capabilities for C, N, P, S turnover and other key functions. Data from this analysis is currently being analysed.

Methods

- **Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.**

Details of methodology used for various microbiological and chemical analyses are described under different sections in the Appendix document.

Results

- **Detail and discuss the results for each objective including the statistical analysis of results.**

See Appendix for detailed discussion about the results.

Outcomes

- **Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.**

Objective 1:

Expected science outcome: New knowledge on the diversity of FL-N fixing bacteria in cotton soils and management effects on N fixation. Biological fertility of cotton based farming systems established.

Expected industry/applied outcome: Quantification of microbial contribution to nutrient supply in cotton bases systems established which would allow development of sustainable management options for economic and environmental benefits to growers. For example, potential to moderate fertilizer use practices that support maximum FL-N fixation and achieve higher nutrient use efficiency through on-field management practices.

Biological processes are the key drivers of N cycling in soils and in cotton soils they are known to play significant roles in controlling N availability, loss and fertilizer use efficiency. There has been considerable research on the dynamics of plant available N levels and fertilizer use efficiency in cotton soils and management impacts but little was known about management impacts underlying these processes. Generally, a majority of the microbial populations involved in N cycling processes are considered to be concentrated in the surface 5 or 10 cm of the soil. We found that in cotton soils there are considerable populations of these organisms at 10-20 cm depth soil suggesting for the potential for greater biological contribution with appropriate management.

Vertisol, soils such as the ones at ACRI, can provide microsites for the proliferation and activity of non-symbiotic N fixing bacteria however their activity is modulated by soil mineral N and biologically available C levels. Findings from this project indicate that higher concentrations of mineral N through a large period of cotton growing season may have resulted in the lower abundances of these bacteria (*nifH* gene containing bacteria). Also, the amount of N fixed by these organisms is lower than that observed in similar soil types but with lower mineral N levels under cereal cropping. The effect of long-term crop management practices on these populations is evident from the differences between the two long-term cropping system experiments F6E and D1. These results suggest that with proper management, these populations could contribute greater amount of N fixed to supplement plant N demand.

Objective 2:
Expected science outcome: New knowledge on the diversity of soil fungal communities in relation to disease impacts in Australian cotton soils; no such information exists at present.
Expected industry/applied outcome: Improved management options to promote disease suppression capacity in cotton soils to complement integrated disease management in cotton farming systems.

Soil fungal community is an important part of soil biota playing key roles in plant nutrition, pathogen levels, disease incidence, plant health and soil structure related processes. Prior to this project little was known about the diversity of fungi in Australian cotton soils and management effects. Results from this project provide the first glimpse of the genetic composition and diversity of fungal community in cotton soils and the region based specificity of the fungal community. This provides a foundation for future research to better understand the factors that moderate pathogen populations and natural soilborne disease suppression. The finding that crop management practices such as rotation can significantly influence total fungal abundance and diversity suggest that an opportunity exists to design crop rotation practices that enhance the community diversity thereby either reduce pathogen multiplication and/or enhance biological disease suppression.
This project demonstrated that modern DNA-based tools could be successfully used in field experiments or farmer fields as part of an integrated investigation to derive critical information that assists in improving plant health and productivity.

Objective 3:
Expected science outcome: Quantitative information on the effects of organic amendment application on soil biological processes in Australian cotton soils.
Expected industry/applied outcome: Better management of organic amendment application for improved cotton production and overall soil health.

With many cotton growers looking at ways to improve soil health and soil microbial activity in an effort to increase productivity and ensure long term sustainability, interest in the use of compost application has grown because it is believed that compost provides several benefits to soils (cotton Info-soil health www.cottoninfo.com.au/soilhealth). This project provided detailed information, first of its kind for Australian cotton soils, using incubation experiments showed the short-term nature of the impact of compost application at recommended (commonly used) rates on microbial activities, key functional microbial groups and N mineralization. Compost addition effects were influenced by the addition of N fertilizer but the effect was short-lived. These observation provided the basis for the lack of change from multiple annual applications on microbial populations and biological processes under field conditions. However, repeated annual application of compost seem to modify the catabolic diversity of microbial communities suggesting that change in microbial composition may have an effect of specific biological functions? Additionally, it was found that composts vary significantly in their chemical composition of composts in terms of major nutrients and trace elements, biologically available carbon, in particular the gin trash compost. For industry, these findings suggest that long-term effects of composts may depend upon amount and frequency of application esp. for a change in microbial diversity and plant beneficial functions. Therefore, chemical analysis of the compost material before application is recommended to more fully consider its' potential benefits. It is important to note that these findings are from experiments in one soil type only and the effects need to be evaluated in different soil types and environments.

- **Please describe any:-**
  - **a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);**
  - **b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and**
  - **c) required changes to the Intellectual Property register.**

Information about the latest DNA based and biochemical techniques standardized within this project to be suitable for cotton soils is for public use (presented in publications and presentations) and thus no changes to the Intellectual property register are required.

Conclusion

- **Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?**

1. Cropping history and fertilizer management have a significant influence on the microbial community and biological processes involved in N cycling processes e.g. non-symbiotic N fixing bacteria and N_2-fixation and N mineralization in cotton soils. Higher levels of standing mineral N concentrations and lower C inputs in continuous cotton rotation resulted in lower non-symbiotic N fixing bacteria and N_2-fixation and overall microbial activities. Legumes in rotation have a significant positive effect on microbial catabolic diversity and activity and N mineralization potential.

Implication: Adoption of crop rotation and fertilizer application to increase and diversify plant types and C inputs and reduce standing concentrations of mineral N would benefit soil biological fertility and help with management of N nutrition and fertilizer efficiency in cotton soils.

2. Application of organic compost materials at 5 and 10 t/ha have only a short-term effect on microbial activity and biological processes but modify microbial composition. The magnitude of effect varied between compost types both in the laboratory and field experiments mainly due to the varied chemical composition, in terms of major nutrients and trace elements.

Implication: Chemical analysis of the compost material before application is recommended to more fully consider its' potential benefits. Long-term effects depend upon amount and frequency of compost additions esp. for a change in microbial genetic diversity with implications to plant beneficial functions. In addition, changes in the microbial catabolic diversity suggest that other properties related to disease suppression and pathogen abundance need to be considered. These effects need to be evaluated in different soil types and environments.

3. Soil type and environment (location) and cropping history have a significant influence on the genetic composition and diversity of soil fungal community in the surface 0-10 cm cotton soils. The total number of genera was generally lowest in the rotations including brassica crops and Continuous Cotton rotation compared to rotations that include other crops. These results indicate that, contrary to previous research which only concentrated on soil bacteria, diversity and abundance of soil fungal community varies significantly in the three cotton growing regions analysed and changes in soil fungal community would play a notable role in soilborne disease incidence in cotton.

Implication: Diversity and abundance of soil fungal community in cotton soils could be managed through crop rotation and stubble management which would have implications to disease impacts.

Extension Opportunities

- **Detail a plan for the activities or other steps that may be taken:**
  (a) to further develop or to exploit the project technology.
  - New project to characterize soil fungal communities in farmer fields and identify the links to management and disease incidence
  - Findings from the analysis of long-term experiments and compost effects lay the foundation and suggest the need to quantify the biological resilience of cotton soils and its impact on cotton nutrition and soil health.

(b) for the future presentation and dissemination of the project outcomes.

Two manuscripts based on the findings from this project are currently under preparation for publication in scientific journals – to be submitted during 2017.

Gupta, V.V.S.R., Linda Smith, Karen Kirkby, Linda Scheikowski, Ian Rochester, Nilantha Hulugalle, C.R. Penton (2016) Soil and environment has greater effect than management on fungal communities in Australian cotton soils. Soil Biology and Biochemistry. In preparation.

Gupta V.V.S.R., Kroker, S.K., Hicks, M. and Weir, D. (2016) Compost application effects on biological functions and microbial diversity are transitory in cotton soils. ISME Journal, in preparation.

Additionally, articles will be submitted during 2017 to Australian Cotton grower and Spotlight for the farmers and agronomists in cotton industry, e.g. article titled 'Not all composts are equal' draft prepared by Chrissy Brown for Spotlight.

(c) for future research.

Findings from this project formed the basis for the development of the new project during 2016-19 as part of an integrated project (collaboration with Dr. Linda Smith, DAF-Qld) with cotton disease survey based project 'Improving the management of cotton diseases in Australian cotton farming systems', to characterize the structure of fungi and beneficial microbial communities and assess management impacts and link them with disease incidence/suppression in fields in different cotton growing regions.

9. A. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

Gupta, V.V.S.R. (2016) Microbial diversity in surface soils in cotton fields compared to a nearby remnant vegetation: biomes of Australian Soil Environments (BASE) project. Report submitted to CRDC, p. 7

Gupta, V.V.S.R., Knox, O. and Bissett, A. (2016) How does cotton production system change the soil biology? The Australian Cotton Grower. Oct-Nov 2016. Pp. 46-49.

Gupta, V.V.S.R., Linda Smith, Karen Kirkby, Linda Scheikowski, Ian Rochester and Nilantha Hulugalle (2014) Crop rotation influences soil fungal communities in cotton soils. International FUSCOM 2014 Summaries, Ed. Stephen Allen, p. 7, ACRI, Narrabri, New South Wales, Australia

Presentations:

1. Gupta, V.V.S.R., Linda Smith, Karen Kirkby, Linda Scheikowski, Ian Rochester, Nilantha Hulugalle, C.R. Penton (2016) Effect of rotation and environment on fungal communities in Australian cotton soils. In: Abstracts of the World Cotton Research Conference-6 held during May 2-6 at Goiania, Brazil. p.82.
2. Gupta V.V.S.R., Kroker, S.K., Hicks, M., Nidumolu, B. and Weir, D. (2016) Does compost addition improve biological functions and microbial diversity in cotton soils. In: Abstracts of the World Cotton Research Conference-6 held during May 2-6 at Goiania, Brazil. p.92.
3. Gupta, V.V.S.R., Linda Smith, Kirkby, K., Scheikowski, L. and Rochester, I. (2015) What influences fungal communities in cotton soils? In: 2nd Australian Cotton Research Conference – Science securing cotton's future, pp 41, Sept 8-10, 2015, Toowoomba, Qld, The Association of Australian Cotton Scientists.
4. Gupta, V.V.S.R., Kroker, S.K., Hicks, M., Nidumolu, B. and Weir, D. (2015) Composts addition may improve biology in cotton soils. In: 2nd Australian Cotton Research Conference – Science securing cotton's future, pp 14, Sept 8-10, 2015, Toowoomba, Qld, The Association of Australian Cotton Scientists.
5. Gupta, V.V.S.R. and Rochester, I. (2013) Crop rotation influences microbial activity, diversity and nitrogen mineralization in cotton soils. Oral presentation. Abstracts of the Australian Cotton Research Conference, p.38, Sept 8-11th, Narrabri, NSW.
6. Gupta, V.V.S.R., Smith, L., Kirkby, K. (2013) Can sustainable biological disease suppression be achieved in cotton farming systems. Oral presentation. Abstract of the Australian Cotton Research Conference, p.37, Sept 8-11th, Narrabri, NSW.
7. Gupta, V.V.S.R. (2013) Beneficial soil microbial communities and functions in cotton soils. Talk given at the FUSCOM 2013 meeting held during July 17-19 in Toowoomba, Qld, Australia.
8. Gupta, V.V.S.R. (2013) Beneficial soil microbial communities and functions in cotton soils. FUSCOM 2013 meeting held during July 17-19 in Toowoomba, Qld, Australia

9. During 2013 and 2014, the Principal Researcher presented talks at the FUSCOM meetings in Toowoomba summarizing (i) current knowledge on the microbial diversity and biological functions in cotton soils and (ii) the research approach and results from this project.

B. Have you developed any online resources and what is the website address?

Not applicable

Part 4 – Final Report Executive Summary

For Sustainable cotton production, Australian cotton farming systems need to promote and optimize soil biological functions to by improving nutrient use efficiency, increasing biological nitrogen inputs, reducing disease impacts and maintaining environmental health. Currently cotton farming systems involve management practices such as reduced tillage, crop rotation, residue retention, compost and fertilizer addition and reduced insecticide use all of which can potentially alter key soil microbial functions. This project addressed three key areas of soil microbial community and biological processes with an aim to improve current knowledge and evaluate the impact of management, soil and environmental factors affecting them. They include:

1. Determined the effect of management practices including rotation, stubble retention and organic manure application on microbial communities involved in C and N cycling, free-living (FL) N fixation and carbon turnover.
2. Characterized the genetic diversity of soil fungal communities as influenced by management practices and linked it with disease incidence and suppression.
3. Quantified the effect of compost addition materials on soil biological fertility.

This project utilized on-going field experiments at ACRI and in Queensland which demonstrated significant effects of management practices on N cycling and uptake, disease incidence and crop yields. These studies were completed with targeted glass-house and laboratory incubation experiments. Therefore, new knowledge of key biological processes from long-term experiments can be effectively linked with management systems under field conditions.

Briefly, the advancement of knowledge provided in this project includes:

- In cotton soils, crop rotation and fertilizer management have a significant influence on the microbial community and biological processes involved in N and C cycling processes. Populations of FL N-fixing bacteria were significantly higher in the D1 experiment compared to that in the F6E experiment indicating the effect of stubble management type and timing of fertilizer application. In both the experiments, grain legume rotation crops (e.g. vetch and fababean) significantly improved microbial activity, catabolic diversity and N mineralization potential in surface soils, however the magnitude of effect varied significantly. Microbial activity and microbial biomass levels were lowest in the Continuous Cotton rotation suggesting rotation based management of N availability and fertilizer efficiency is possible.
- Soil type and environment (location) and cropping history have a significant influence on the diversity, genetic composition and fungal community in the surface 0-10 cm cotton soils. A total of 370 genera were found in the 5 cotton experimental sites, however, a few groups (20 families) were dominant. Fungal community composition varied significantly between the cotton growing regions and was also influenced by crop rotation. Lower diversity and abundance of total fungi were associated with higher disease incidence in intensively managed cotton systems e.g. rotations including brassica crops and Continuous Cotton rotation.
- Composts vary in their chemical composition significantly in terms of major nutrients and trace elements and biologically available carbon (BDOC). In a long-term field experiment, four years of compost addition on a Vertosol had no significant effect on microbial and nutrient properties. In controlled environment experiments, addition of composts increased microbial activity for two week only. The magnitude of the effect on biological functions and microbial diversity varied between different composts both in the laboratory and field experiments. Thus long-term effects of repeated compost application would depend upon amount and frequency of application *esp*. for a change in microbial diversity and plant beneficial functions. Therefore, chemical analysis of the compost material before application is recommended to more fully consider its' potential benefits. These effects need to be evaluated in different soil types and environments.

Overall, the new knowledge on the dynamics of microbial community and biological processes suggest that some of the key microbial groups and functions in cotton soils are regionally specific and can influenced by management. Thus a designer management may need to be applied to better harness

specific biological benefits and is the foundation for building and managing more resilient cotton production systems.

Appendix 1 – detailed report:

Part 1.1 Crop management and seasonal effects on carbon and nitrogen cycling microbial communities and biological processes (Milestones 1.1 and 1.2)
Milestone 1.1 Quantify soil type and cropping system based differences in free-living (FL) N fixing bacteria and amount of N fixation within cotton crop and during off-season including rotational crops
Milestone 1.2 Determine the seasonal and management effects on N and C cycling microbial communities in the long-term cotton farming trails – crop rotation and stubble retention effects over two season

Summary:
Soil microbial communities play an important role in a number functions related to carbon and nutrient availability, plant health and overall soil health. In cotton soils, optimum biological functions are necessary not only to improve the soil nutrient supply capacity, fertilizer use efficiency, reduce greenhouse gas emissions but also critical for carbon turnover and C-sequestration. Results from the analysis of soils from the two long-term cropping systems experiments at ACRI over 3 seasons indicated that cropping history, fertilizer and stubble management have a significant influence on the microbial activity, populations of microbial groups involved in N and carbon cycling processes. For example, abundance of free-living N fixing bacteria and N2-fixation were significantly lower in treatments that maintained higher levels of mineral N throughout the season and also in fallow rotations whereas legumes in rotation increased microbial catabolic diversity and N mineralization potential. There was a significant crop rotation and depth based differences in the composition and diversity of functional genes involved in N cycling processes such as nitrification, nitrogen fixation and denitrification. These results suggest that adoption of crop rotation and fertilizer application to increase and diversify plant types and C inputs and reduce standing concentrations of mineral N would benefit soil biological fertility and help with management of N nutrition and fertilizer efficiency in cotton soils.

Background:
Soil microbial populations, their diversity and the carbon, nutrient cycling and plant health related processes they mediate in the lower organic matter Australian cropping soils are generally significantly influenced by biologically available carbon, soil moisture and soil habitat properties (Gupta et al. 2011). Management practices in current Australian cotton farming systems, e.g. reduced tillage, crop rotation, residue retention, organic manure application and reduced insecticide use, can influence total and different groups of microorganisms both in terms of their populations and the levels of key functions they mediate. It is considered that biological functions in cotton soils need to be optimised to promote to sustain cotton production, improve nutrient use efficiency, reduce soilborne diseases and maintain environmental health. Preliminary results from research at ACRI suggest that management systems can be manipulated to optimise microbial functions to improve N and C cycling processes and improve soil biological health (Coleman et al 2010; Rochester…). Current knowledge about the effect of management practices e.g. stubble management, fertilizer application and rotation on microbial composition, abundance and biological processes related to C and N cycling is limited. Long-term rotation trials at ACRI and in Qld provided a valuable resource to quantify management effects on key beneficial microbial communities and processes.

Methods:
For this part of the research we used soil samples (0-10 and 10-20cm depths) from selected treatments in the long term experiments conducted by Drs. Ian Rochester and Nilantha Hulugalle.

- Experiment # F6E – Nitrogen fertilizer X crop rotation experiment: Cotton-Wheat, Cotton-Vetch and Cotton-Fababean rotations sampled at the time of planting.
- Experiment # D1 – Rotation crops and stubble management on permanent beds in cotton farming systems: Cotton-Wheat-Vetch-Cotton; Cotton-Fallow-Cotton; Cotton-Wheat-Cotton.

Samples were collected from the two experiments prior to planting in 2013 whereas in 2014-15 and 2015-16 seasons samples were only collected from the F6E experiment prior to planting and in-crop during Feb and March. These in-crop samples were also analysed 'in crop' (Feb 2014) to coincide with one of the key nutrient uptake phases for cotton) to quantify N supply potential of soil. All soils were

analysed for a number of microbial, biochemical and molecular properties such as microbial activity (Potential Mineralizable Carbon, PMC), microbial biomass (MB), N mineralization potential (PMN), catabolic diversity and populations of nitrifying (bacterial and archaeal amoA genes), FL N fixing (nifH gene) and denitrifying (nosZ gene) bacteria using qPCR methods (Knox et al. 2014). We used PMN and PMC as integrated measures of N and C cycling

Analysis of microbial communities involved in N cycling processes - Based on the results on the populations of free-living N-fixing bacteria measured as abundance of *nif*H gene copies which showed significant differences between the soils from the D1 experiment and the F6E experiment, the DNA samples from these experiments were analysed for diversity of functional genes involved in N cycling processes using a custom made (in CSIRO Hobart labs) functional microarray (Figure 1.1.8). Laboratory assays to quantify FL N fixation using the ^{15}N incubation assays for the samples from both experiments was conducted using a laboratory incubation assay (Gupta et al. 2014). All the results were subjected to statistical significance of treatment effects using ANOVA based analysis on Genstat v12-14.2 (www.vsni.co.uk). Data from catabolic diversity and microarray measurements were analysed with multivariate (e.g. canonical, PCA and nMDS analysis as required) and regression statistics using Genstat or Primer 6 software package (Primer-E Ltd, Plymouth, UK) (Gupta et al. 2014).

Results and Discussion:

Results showed that, in general, cropping history and fertilizer management have a significant influence on the microbial community and biological processes involved in N cycling processes e.g. non-symbiotic N fixing bacteria and N_2-fixation and N mineralization in cotton soils (Figure 1.1.1 – 1.1.7 and Table 1.1.1 and Table 1.1.2). Nitrogen fixation by free-living (or non-symbiotic N_2 fixing bacteria) have been reported to contribute agronomically significant amounts of N inputs in cropping soils in some of the agroecological regions of Australia (Roper and Gupta 2016). These bacteria are exposed to microaerophilic conditions that are one of the key requirement for the N fixation in soil. Vertosol soils with high clay content provide optimal conditions, within microaggregates for N_2 fixation to occur hence NS-N_2 fixation has been shown to be higher compared to sandy soils (Roper and Ladha 1995).

Abundance of nifH gene, one of the key genes involved in N_2 fixation has been used as an indicator genes representing the populations of N_2 fixing bacteria (diazotrophs). Results from the analysis of soils from the two long-term experiments at ACRI (F6E and D1) indicated that abundances of *nifH* genes ranged between 10^2 and 10^4 gene copies / ng of DNA (or 10^7 to 10^8 per gram soil) (Figure 1.1.1). The abundance of *nif*H gene copies were generally significantly higher in the soils from D1-experiment (ave. 16838 gene copies / ng DNA) compared to that in the F6E-experiment (ave. 3086 gene copies / ng DNA). Since there is only a small variation in soil texture between two experimental soils, this could be attributed to the lower concentrations of mineral N from the differences in crop management in the D1-experiment. Also, data for the nifH gene abundance over the three cotton seasons indicated that the lowest *nifH* gene abundance observed in 2014-15 season (Figure 1.1.1) coincided with highest mineral N levels (e.g. >50 kg N / ha) in the surface soil.

Crop residues in the F6E experiment are incorporated into the top 15 cm soil where as in the D1 experiment they are left on the surface thus causing significant variation in the rate of decomposition of residues, microbial community composition and associated nutrient transformation processes. Location of residue has been known to influence rate of availability of carbon and nutrients from the residue to microorganisms and thus can influence the composition of microbial communities including FL N fixing bacteria (Roper and Smith 1991; Gupta et al. 2011). Higher concentrations of mineral N and lower populations of nifH containing bacteria in the F6E experiment suggest lower N fixation by the FL N fixing bacteria in these soils, in particular in the wheat-cotton rotation. Results indicated that 0.5 to 1.1 mg N fixed per kg of soil per day from D1 experiment whereas the amount of N-fixed in the F6E soils was significantly lower (0.1. to 0.5 mg N fixed per kg soil per day) compared to the amounts reported for clay soils under grain cropping systems with stubble retention and practicing conservation tillage practices (Roper and Gupta 2016). Results from 2013-14 season indicated the presence of high levels of mineral N (e.g. >80 kg N/ha) in the surface soil during March 2014 which coincided with lower populations of free-living N fixing (*nifH* gene harbouring) bacteria. Research by Rochester et al. showed large amount of N inputs by the legume crops in rotation with cotton. Although, clay soils have been shown to harbour a

diverse FL N fixing bacterial community, higher mineral N levels (probably from the fertilizers applied) and differences in microbial carbon turnover may restrict N inputs by the FL N fixation. Results from the preliminary analysis of genetic diversity of FL N fixing bacterial community using nifH-amplicon sequencing method (in collaboration with a Grains RDC funded project; Mike Bell, DAFF Qld) indicated the presence of a diverse nifH-gene community and members of Proteobacteria group accounting for >54% of overall community in a clay soil from a crop rotation experiment at Colonsay, Qld (Gupta et al. 2014). These results suggest that adoption of crop rotation and fertilizer application practices that to increase C inputs and reduce standing concentrations of mineral N are required to promote more N inputs from non-symbiotic N_2 fixation. More importantly, the differences between the two long-term cropping system experiments at ACRI with different crop management practices and results from grain cropping soils from this region indicating soil-type based variation suggest for research to quantify the abundance and composition of FL-N_2 fixing bacteria and N_2 fixation in different cotton growing regions, in particular the effect of various fertilizer application and stubble management practices in farmer field conditions. Additionally, new crop rotations involving maize and sorghum need to be evaluated for their impact on N fixing bacteria.

Plant type can affect populations and functional capacity of different groups of biota in their rhizosphere soil and due to the difference in the quality of crop residues (Gupta and Knox 2008; Gupta et al 2011). Results from our analysis of soils from the two experiments indicated measurable and significant effect of rotation crops on a number of microbial and biochemical properties. The magnitude of the rotation effect varied between the F6E and D1 experiments. Microbial activity as represented by the PMC was higher in the two legume rotations (Vetch and Faba bean) compared to that in the wheat-cotton rotation (Figure 1). Also, in the D1-experiment soils from the Cotton-Fallow rotation exhibited lower microbial properties and C and N mineralization (Figure 1.1.6-1.1.7). In addition, in the D1 experiment there was a significant difference between the Top and Bottom half of the plots; for example most biological properties were higher in soils from the Bottom part of the plots compared to that from the Top part of the plots. This difference is likely to be due to the movement of residues and fine sediment and associated changes in soil chemical properties. In general, MB, microbial activity (Potential Mineralizable Carbon; PMC) and catabolic diversity index were higher in soils from Vetch rotation in both the experiments (Table 1.1.2; Figure 1.1.6). Results from the carbon substrate utilization profiling analysis showed significant differences in the catabolic diversity of microbial community (Figure 1.1.4. and 1.1.5) and confirming the rotation crop effects on microbial community composition in cotton soils. Significant differences were also seen with soil depth (Figure 1.1.5) and this observation concurs with that reported for soils under grain cropping systems (Gupta et al., unpublished). Fallow treatment significantly altered the catabolic diversity of microbial community in the D1 field experiment (Figure 1.1.4). In addition, the carbon substrate utilization ability and catabolic diversity index were also significantly ($P<0.05$) lower in soils from fallow-cotton rotation compared to the wheat-cotton and vetch-cotton rotation (Table 1.1.2).

Results for the in-crop soil samples from the F6E experiment indicated that the legume crop effects extended in to cotton growing season and mineral N levels in the top 20cm of soil were significantly higher (60 to 98 mg nitrate N per kg soil) in the cotton crop after legume compared to that in the cotton crop after wheat crop (Figure 1.1.7). Results for the 2013 pre-planting samples indicated significant effects of crop rotation on total microbial activity, microbial biomass and N cycling processes in both F6E and D1 crop rotation experiments. Surface soil samples were collected prior to planting in 2014 (October) to determine the seasonal variation in crop rotation effects. During 2014, crop rotation effects on total soil microbial activity in the surface 0-10 cm soils were smaller than those observed in 2013 season (Figure 4). Microbial biomass carbon and nitrogen were lower in Continuous cotton soils compared to that in the Vetch-Cotton and Wheat-Cotton soils (Table 1). Amount of dissolved organic C (DOC), an important source of energy for microorganisms was higher in CVC and WC rotations compared to CC (315 vs. 265 mg C/kg soil). Additionally, C:N ratio was wider in Wheat-Cotton rotation (52) compared to Continuous Cotton and Vetch-Cotton rotations (13.5-16). Results from 2013 season showed a lower DOC:DON ratio 4-6 indicating seasonal variation in soil properties.

Higher N content of the legume residues would have contributed to the higher levels of microbial activity and N mineralization compared to the low N containing wheat and cotton residues. In addition,

other differences in the chemical composition e.g. lignin content etc. of residues would have also contributed to the variation in microbial populations. These effects of legume crops on N mineralization and mineral N concentrations in cotton soils confirm the reports by Rochester et al in the long-term experiment and other crop rotation experiments. Legume crops in rotation with cereal crops have been reported to show similar changes in microbial biomass and N mineralization in grain cropping systems (Gupta et al. 2010; McBeath et al. 2014).

Microbial communities involved in N cycling processes:

The different biological processes that are part of the soil N cycle are conducted by a diverse microbial community with specialized physiological and biochemical requirements (Figure 1.1.8). The N-cycle array, developed by the CSIRO Marine labs, targets key functional genes of the microbial N cycle such as: *nifH* for nitrogen fixation, *amoA* for ammonia oxidation (part of N mineralization), *nosZ* for denitrification, *hzsA* for anaerobic ammonia oxidation, *nrfA* for assimilatory reduction of nitrite to ammonia, *nxrB* for the last stage of nitrification (nitrite to nitrate) (Figure 1.1.8). The array enables us to detect and characterise the functional gene communities harbouring these genes and carrying out these functions.

Overall, analysis of all the N-cycle array results indicated a significant crop rotation and depth based differences in all the functional gene categories related to N cycling. The depth based variation was observed in all the rotations analysed (Figure 1.1.9) i.e. the overall diversity of N cycle functional genes is generally higher in the surface 0-10 cm soil compared to that in the 10-20 cm soil, in particular in the Vetch-Cotton rotation (Figure 1.1.10) but there was limited depth based difference in the other crop rotation treatments. Depth based variation differed for the different functional genes. There was a significant difference in N-cycle communities in the two crop rotation experiments e.g. F64 vs. D1. For example, species richness index for Archaea *amo*A was higher for the 10-20 cm depth soils (d=5.26) compared to the surface 0-10 cm soils (d=4.81) but this was not the trend for the *nif*H gene communities. The trend was similar for the functional genes involved in greenhouse gas emissions (e.g. *nos*Z, *hzsA, nxrB*). Results from the abundance of archaea-*amo*A gene copies also indicated similar trend. Higher abundance of archaea-*amo*A genes at depth was previously reported in rainfed grain cropping regions in SA and WA. These results generally confirmed results from total *nif*H and *amo*A gene quantification, for example the total signal intensity of *nif*H gene categories was significantly higher in soils from the Wheat-Cotton rotation compared to the legume-cotton rotation and also higher in the surface 0-10 cm soils compared to that in the 10-20 cm soils. There was a significant seasonal variation in the abundance of *nosZ* gene harbouring (denitrifying) bacteria in surface 0-10 cm soils in all crop rotations in the F6E experiment and rotation based differences were only seen at some times (Figure 1.1.3). During 2016 season, the abundance of nosZ genes were higher in the surface soils and declined with depth but no effect of N fertilizer application was observed (Figure 1.1.3). Reports in the literature suggest significant effects of N fertilizer application on the denitrifying bacteria but such effect is dependent upon the mineral N levels in soil. At the time of sampling, smaller differences in mineral N levels between the two fertilizer plots may have contributed to the lack of differences in *nosZ* (denitrifying) populations.

In summary, results from the microarray analysis have provided an in-depth understanding of the changes in functional composition of the different members of N cycling microbial community. This information helped unravel the underlying mechanisms for the differences in rotation effects between F6E and D1 crop rotation experiments and would assist with identifying specific management factors that can be used to manipulate N availability in cotton soils. For example, rotation crops with wide C;N ratio plant residues would increase microbial N immobilization and could moderate standing mineral N levels. Additionally, adoption of fallow rotation option may has to be reconsidered depending upon soil organic matter levels in order to provide sufficient levels of biological organic carbon to maintain and improve microbial activity and diversity. Moreover, current N fertilizer application practices may need to be revisited to avoid accumulation of high concentrations of mineral N in cotton soils for longer periods?

Functional microbial community composition as influenced by N fertilizer application and depth:

Observations of in-season fluctuations and N fertilizer application in mineral N levels, microbial activity and catabolic diversity, N-cycling functional gene abundances in the F6E experiment in 2013-14 and 14-

15 seasons supported other observations on in-crop mineral N levels in cotton soils at different sites and seasons by Late Ian Rochester. Reports from other cropping systems within Australia and overseas suggest that N fertilizer management can influence the functional capacity of a wide variety of microbial groups e.g. microbial groups involved in C, N, P and S cycling and other biological functions. To test to test the effect of higher mineral N levels soil samples from 2015-16 season in the no-N fertilizer and 200kg N /ha treatments from depths 0-10, 10-30 and 30-50 cm were analysed for a comprehensive measurement of diverse functional groups of microbial communities using Geochip microarray system in collaboration with Dr. Zhou's lab at Oklahoma State University (Tu et al. 2014). Geochip microarray system analyses soil samples for functional diversity, composition, structure, metabolic potential and activity and dynamics of microbial communities. Data from this analysis is currently being processed. Detailed interrogation of this data will be done with observations by the Late Ian Rochester in order to evaluate the linkage between specific biological functions and management factors.

Conclusions:

- Cropping history and fertilizer management have a significant influence on the microbial community and biological processes involved in N cycling processes e.g. free-living N_2 fixing bacteria and N_2-fixation and N mineralization in cotton soils.
- A significant crop rotation and depth based differences was observed in all the functional gene categories related to N cycling e.g. nitrification (mineralization), denitrification and N_2 fixation. Abundance of free-living N_2 fixing bacteria and N fixation were lower in the F6E long-term crop rotation experiments compared to that in the D1 cropping system experiment. Higher levels of standing mineral N concentrations and lower C inputs in continuous cotton rotation resulted in lower Free-living N_2 fixing bacteria and N_2-fixation and overall microbial activities.
- The effect of depth was highest on nitrification genes, especially archaea-*amoA* whereas depth based variation in *nifH* gene abundance was smaller than that seen in grain cropping soils.
- Legumes in rotation have a significant positive effect on microbial catabolic diversity and activity and N mineralization potential.

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(A) In the Surface 0-10 cm soils (B) Depth and N fertilizer effects

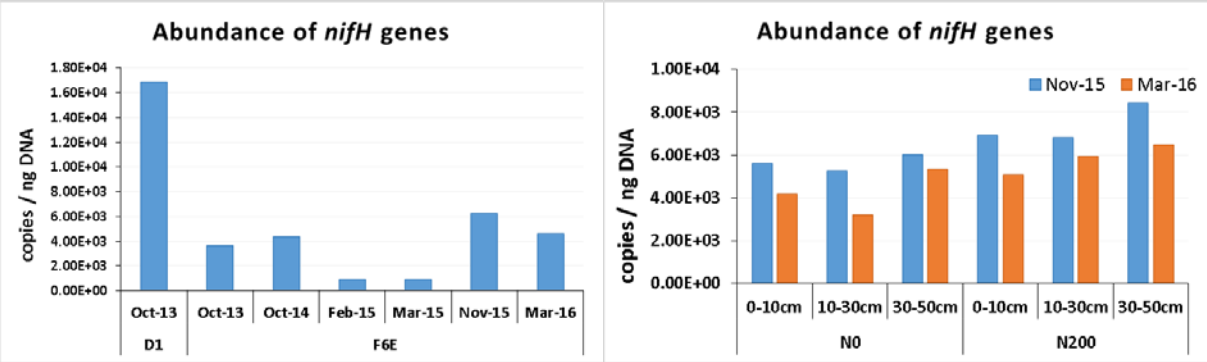


Figure 1.1.1. Changes in the abundance of nifH gene harbouring (diazotrophic/N_2 fixing) bacteria (A) over 3 cotton seasons in surface 0-10cm soils and (B) with depth and N fertilizer addition at the long-term field experiments at ACRI, Narrabri.

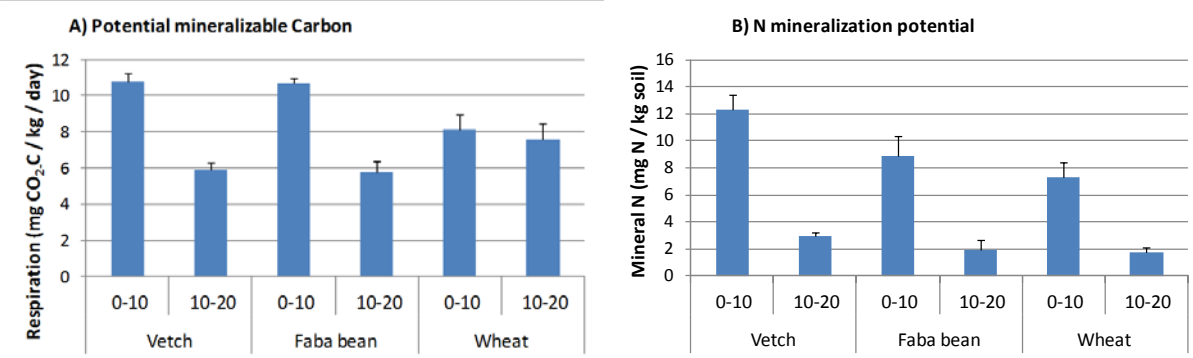


Figure 1.1.2. Effect of crop rotation on potential mineralizable carbon and N-mineralization potential in the surface soils collected prior at planting during 2013 from the cropping system experiment on F6E at ACRI, Narrabri.

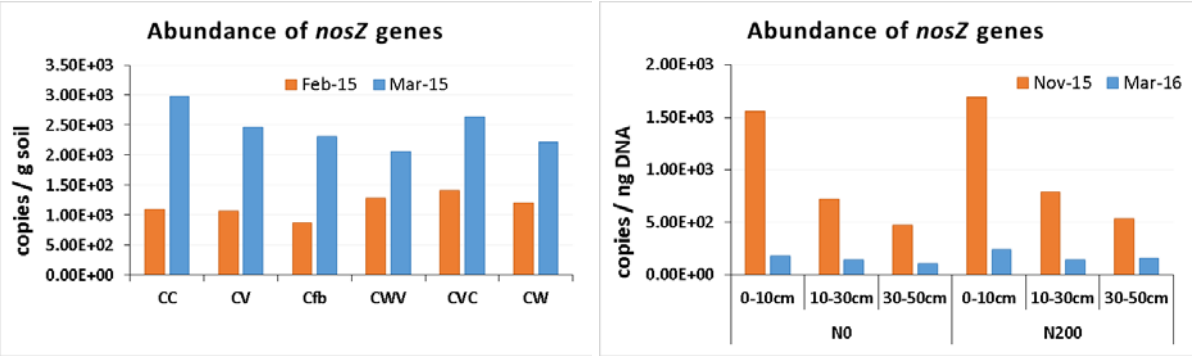


Figure 1.1.3. Changes in the abundance of *nosZ* gene harbouring (denitrifying) bacteria (A) over 3 cotton seasons in surface 0-10cm soils and (B) with depth and N fertilizer addition at the long-term field experiments at ACRI, Narrabri

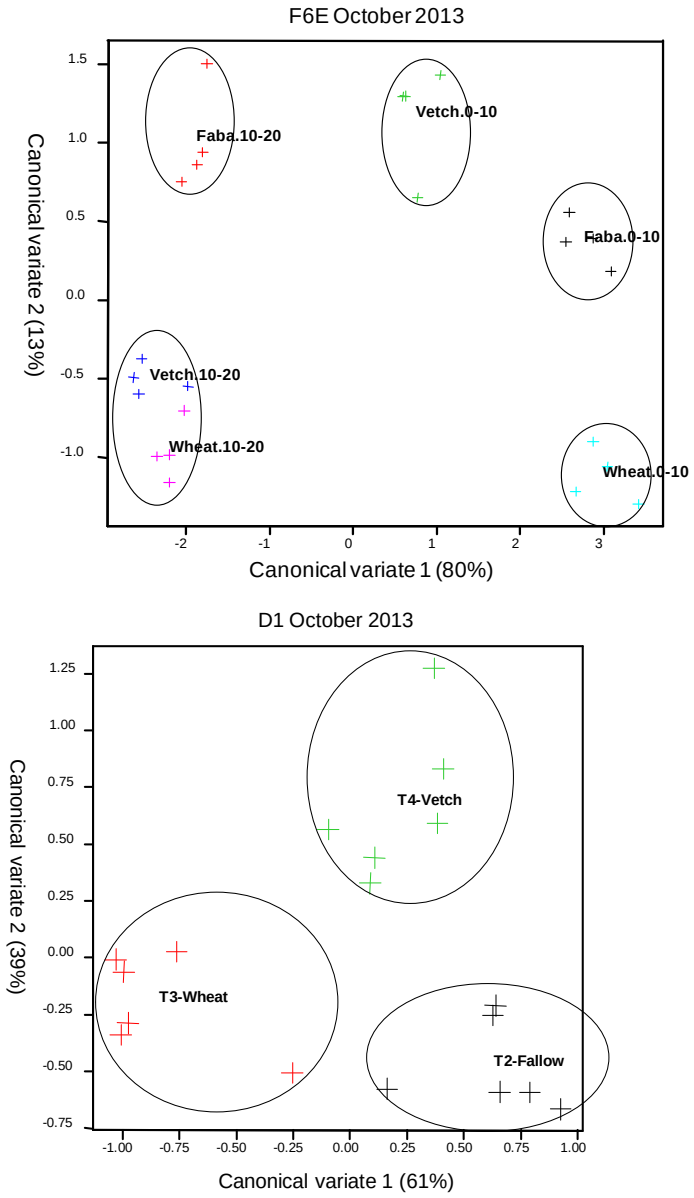


Figure 1.1.4. Effect of crop rotation and fallow on the catabolic diversity of soil microbial community in surface soils collected at planting during 2013 from the long-term experiments F6E and D1 at ACRI.

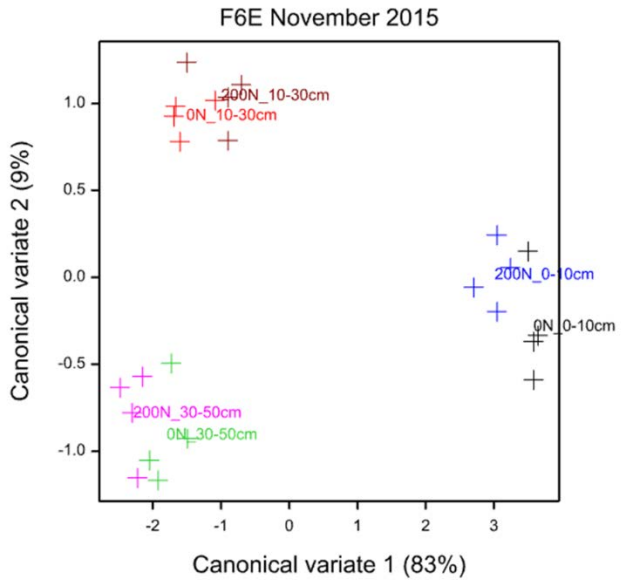


Figure 1.1.5. Effect of N fertilizer application on the catabolic diversity of soil microbial communities at different depths, during sowing 2015 from the long-term experiment (F6E) at ACRI, Narrabri.

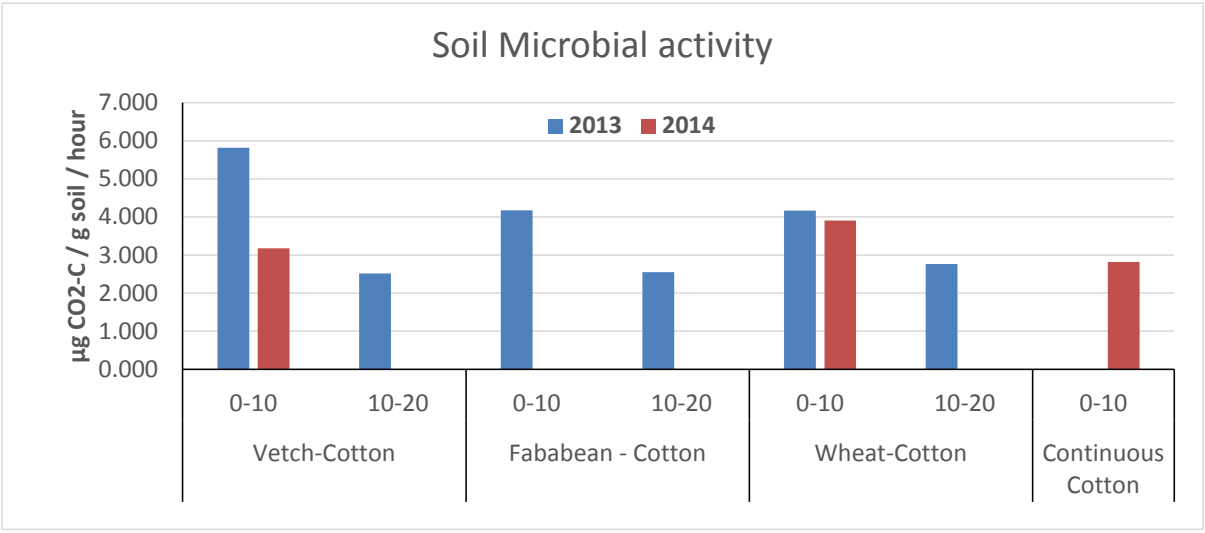


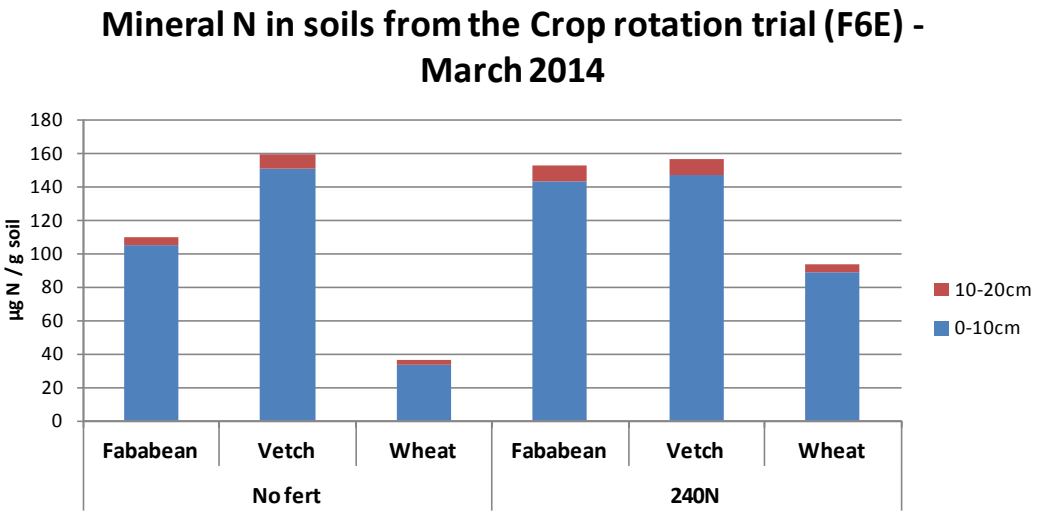
Figure 1.1.6. Substrate induced respiration (µg CO_2-C evolved per gram dry soil weight and per hour) as an indicator of soil microbial activity in surface soils collected prior to planting in 2013 and 2014. Treatment differences greater than 0.5 are significantly different at P<0.05).

Table 1.1.1. Effect of crop rotation on microbial biomass levels in surface soils in the F6E long-term rotation experiment at Narrabri.

Rotation	Depth	2013 October		2014 October	
	(cm)	MB-C	MB-N	MB-C	MB-N
		mg / kg soil			
Vetch-Cotton	**0-10**	849.3	121.3	1163.8	166.3
	10-20	456.9	65.3		
Fababean - Cotton	**0-10**	748.0	106.9		
	10-20	453.4	64.8		
Wheat-Cotton	**0-10**	686.0	98.0	1189.4	169.9
	10-20	337.9	48.3		
Continuous Cotton	**0-10**			976.9	139.5
LSD (P<0.05)		76.9	10.9	130.0	18.5

Table 1.1.2. Effect of crop rotation on microbial biomass, biologically available organic C and N and catabolic diversity at different depths in the F6E long-term rotation experiment at Narrabri.

Treat	Depth	Moisture %	MB-C µg MBC/ g	DOC µg NPOC/ g	TN	Min N µg N/ g soil	DON	CMD 0.25	CMD 0.1
0N	0-10cm	15.0	493.8	35.71	25.84	17.05	14.53	16.167	21.333
	10-30cm	27.6	181.8	14.44	7.29	5.34	6.67	4.083	9.417
	30-50cm	30.3	139.7	12.67	4.78	3.70	6.82	4.083	6.167
	Ave	**24.3**	**271.8**	**20.9**	**12.6**	**8.7**	**9.3**	**8.1**	**12.3**
200N	0-10cm	18.7	498.1	26.74	22.66	14.89	17.36	18.083	21.333
	10-30cm	29.0	184.8	12.89	12.28	9.62	2.13	4.167	9.833
	30-50cm	30.9	145.7	13.38	8.84	6.25	4.61	3.667	7.167
	Ave	**26.2**	**276.2**	**17.7**	**14.6**	**10.3**	**8.0**	**8.6**	**12.8**
Fprob	TRT	**0.039**	NS	**0.013**	**0.023**	NS	NS	NS	NS
	Depth	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
	TRT.Depth	NS	NS	**0.010**	NS	NS	NS	NS	NS
lsd	TRT	*1.788*		*2.462*	*3.351*				
	Depth	*2.190*	*84.5*	*3.016*	*4.104*	*4.720*	*4.939*	*1.177*	*2.439*
	TRT.Depth			*4.265*					



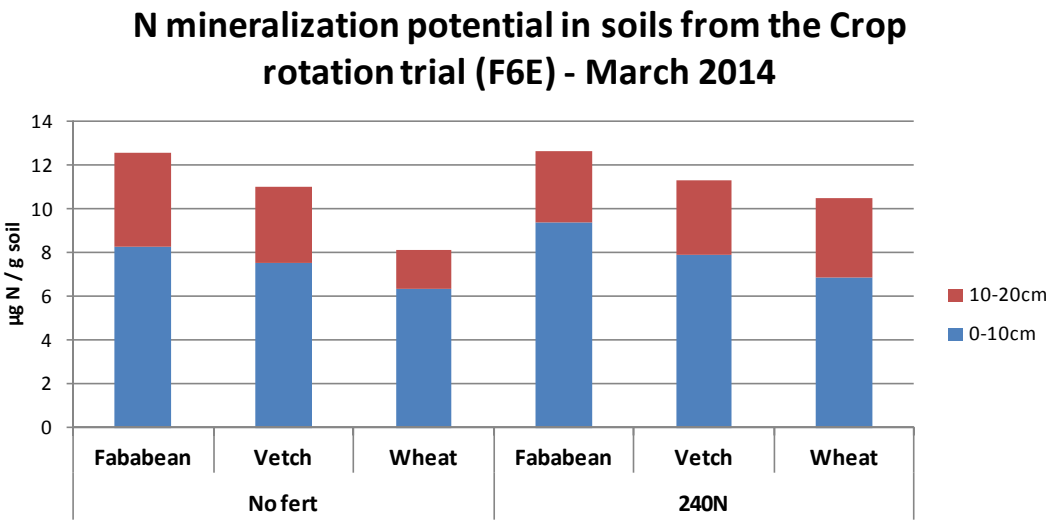


Figure 1.1.7. Mineral N concentrations (Top) and N mineralization potential (Bottom) in the soils collected in-crop (during the last week of February 2014) from the long-term crop rotation experiment (F6E) at ACRI, Narrabri.

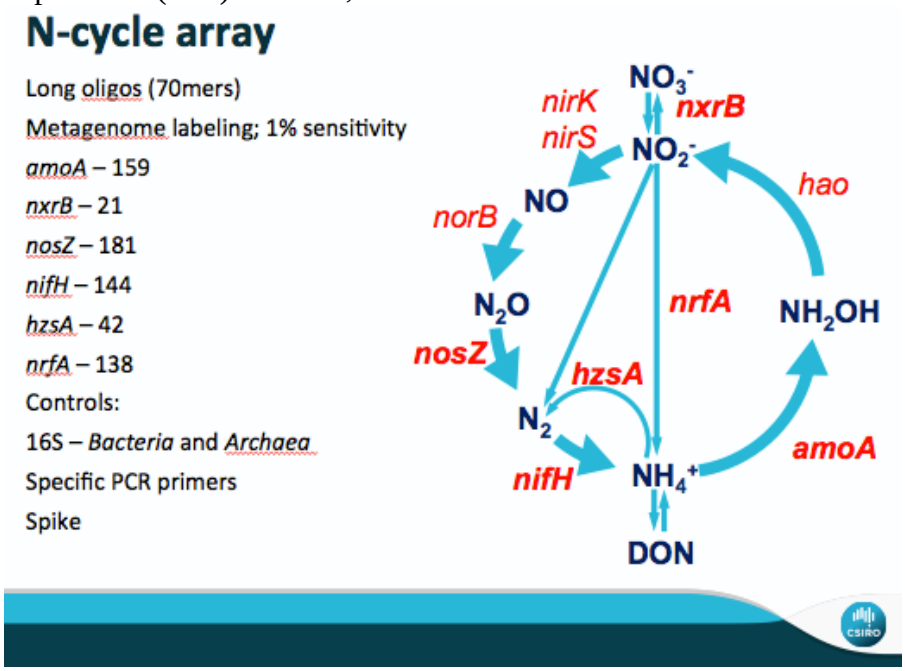


Figure 1.1.8. Details of the functional genes targeted in the N-cycle array (Bodrossy, L., CSIRO, unpublished).

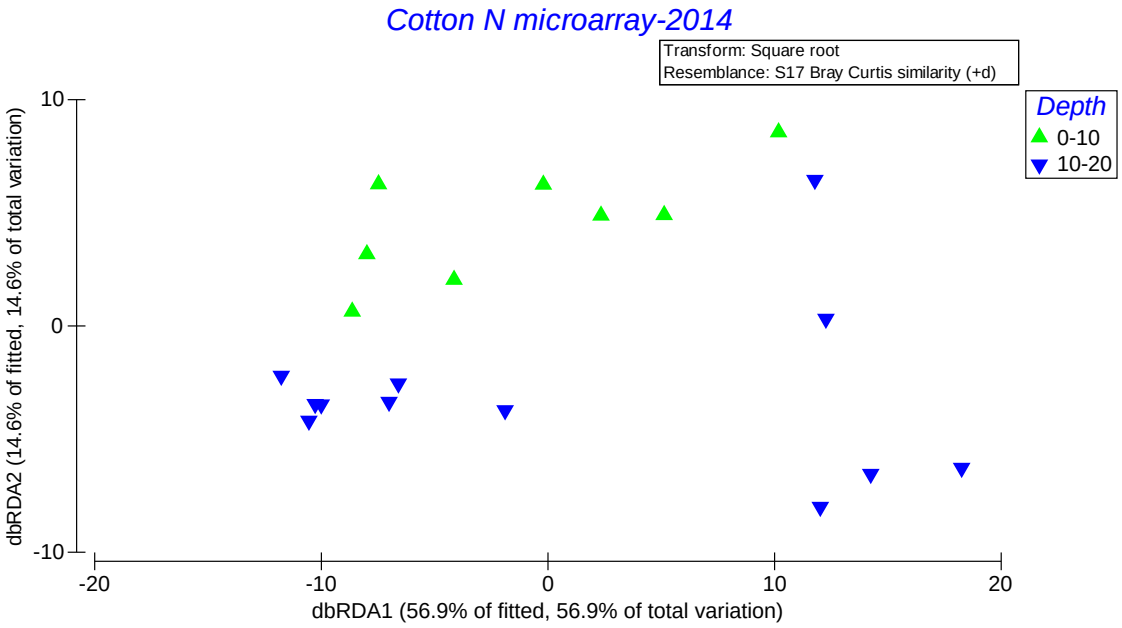


Figure 1.1.9. Distance-based redundancy analysis (dbRDA) of N cycling functional gene composition data shows a significant soil depth based difference in all the treatments from the F6E experiment at Narrabri. Each point in the graph represents summary of N cycle genes in one sample; data points closer are more similar than those further apart.

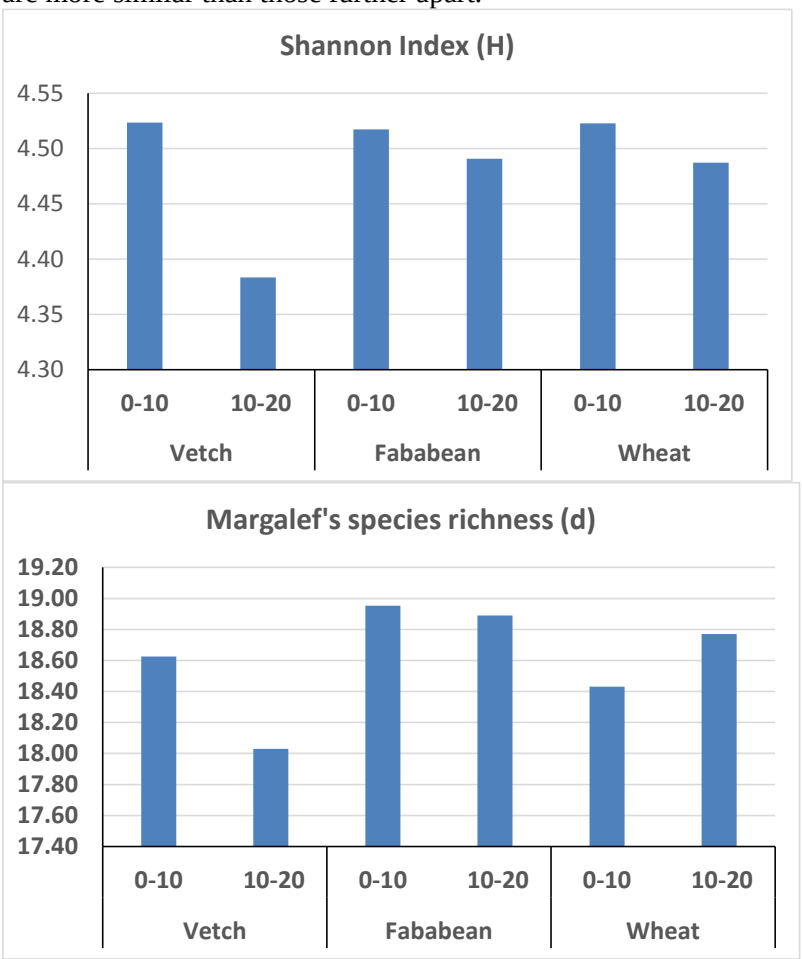


Figure 1.1.10. Diversity indices for the functional genes involved in N cycling processes as influenced by crop rotation and soil depth in the long-term experiment F6E at Narrabri (sowing 2013). Higher diversity indices value indicates greater diversity in terms of species richness (d) and overall diversity.

Part 1.2 Describe the genetic composition of fungal communities and microbial activities as influenced by stubble retention, crop rotation and fertilizer application **(Milestone 2.1)**

Summary:
Fungi are an important component of soil biota playing important roles in a number of plant essential functions. In general, soil type and crop rotation showed a significant effect on catabolic activity and diversity of soil microbial community. Results from the 28S LSU rRNA sequencing based analysis of 2013 samples indicated a total of 370 fungal genera in all the cotton soils and the top 25 genera in abundance accounted for the major portion of total fungal community. There were significant differences in the composition and genetic diversity of soil fungi between the different field sites from the three cotton growing regions, i.e. significantly affected by field location (soil type and environment) and cropping history. Results for diversity indices showed significantly greater diversity in the long-term crop rotation experiment at Narrabri (F6E) and experiments at Cowan and Goondiwindi compared to field sites with long-term disease history (e.g. Biofumigation experiments at ACRI, Narrabri). Diversity was lowest in the soils under brassica crop rotation in Biofumigation experiment. Surface soils from continuous cotton and cotton-fallow rotations showed lowest abundance of fungal populations and overall catabolic diversity of soil microbial communities. Overall, the diversity and abundance of soil fungal community varied significantly by cropping history suggesting that changes in soil fungal community may play a notable role in soilborne disease incidence in cotton. Biological based disease suppression can assist farmers in reducing the impact of diseases on cotton production.

1.2.1 Background:
Fungi are an important component of soil biota constitute >60% of microbial abundance in agricultural soils and play important roles in a number of plant essential functions. Soil fungal community has the capacity to affect pathogen inoculum levels and their disease causing potential. Soilborne diseases such as Fusarium wilt, Black root rot and Verticillium wilt have significant impact on cotton production. Currently the management of disease impacts is through the selection of genetically resistant cultivars (where available), agrochemical application and rotation with non-host crops. But even in our current high F-rank cultivars significant losses can occur from disease such as Fusarium under the right environmental conditions. Biological disease suppression mediated by soil microorganisms including soil fungi can assist farmers in reducing the impact of diseases on cotton production through crop management. Recent evidence from DNA sequencing based analysis has shown that there is a large diversity of uncultured fungi even in agricultural soils, but their role in plant essential functions including plant health is not known (Penton et al. 2014; Tedersoo et al. 2014). Soil type based variation in fungal populations in cropping soils has been documented (Cline and Zak 2014, Penton et al. 2014). Fungi can form hyphal networks that allow them access multiple microsites giving them competitive advantage with other microorganisms, however in the self-mulching property of Vertosol soils has a significant influence on fungal hyphal matrix in cotton soils. Therefore crop residues play a significant role in the dynamics of fungal community in cotton sols (Coleman et al. 2010). Currently our knowledge about management effects on soil fungi and links to pathogen inoculum levels and their disease causing potential is limited.

1.2.2 Methods:
We analysed surface soils from ongoing field experiments, in New South Wales (ACRI, Narrabri and Cowan) and Queensland (Goondiwindi), monitoring cotton performance and disease incidence in three cotton growing regions, collected prior to 2013 and 2015 planting, for the genetic diversity (28S LSU rRNA or ITS region sequencing) and abundance (qPCR) of fungi as influenced by soil type, environment and management practices and link it with disease incidence and suppression. Samples were also analysed for microbial catabolic diversity, microbial biomass and soil chemical properties.

This work was conducted in collaboration with the research teams led by Dr. Linda Smith and Dr. Karen Kirkby with the aim to link the observations on soil fungal community with disease incidence and yield. Selected treatments in 5 field experiments located at Narrabri (3), Cowan and Goondiwindi were used for these analyses, they include 'Cowan field rotation trial' and farmer fields at Goondiwindi, long-term

rotation experiments F6E (Late Dr. Ian Rochester), D1 (Dr. Nilantha Hulugalle) at ACRI and the plots in the Pathology block (Dr. Karen Kirkby) at Narrabri. Surface (0-10 cm) soils were collected prior to planting in 2013-14 and 2014-15 cotton seasons (October-November) using established sampling protocols for microbial community analysis from field experiments (Knox et al. 2014 and Gupta et al. 2012). Soils were transported to CSIRO labs in Adelaide in an eski, to reduce changes from transportation based disturbance, for microbial, chemical and biochemical properties. Field moist soils were stored -20 °C until used for DNA extraction. DNA was extracted from ~100 g of soil for each sample by the Root Disease Testing Service at SARDI (Adelaide) (Ophel-Keller et al. 2008).
Fungal community analysis was perfomed using ITS-TRFLP, ITS region and 28S LSU rRNA amplicon sequencing analysis using the methods described by xxx and Penton et al. (2014). For the 28S LSU rRNA PCR amplification including Tagged amplification was conducted in CSIRO labs at the Waite campus and amplicons for 454 pyrosequencing were sent to labs at the University of Utah, USA. The ITS region amplification was conducted on Illumina MiSeq platform (300bp paired end sequencing) using the primers ITS1F and 2R through AGRF (Adelaide, Australia, www.agrf.org.au).

For TRFLP analysis, soil DNA was amplified from 14 ng of template DNA using the ITS1F.FAM forward (5`-CTTGGTCATTTAGAGGAAGTAA-3`) and ITS4R.HEX reverse (5`-TCCTCCGCTTATTGATATGC-3`) primers [Garden and Bruns 1993; Penton et al. 2014). PCR was carried out in a 35 µL total volume using 0.4 µM of primers, 0.2 mM of dNTPs, 1x PCR buffer (Qiagen, Australia), and 4 units of HotStarTaq DNA Polymerase (Qiagen, Australia). The PCR conditions were 94 ºC for 1 min; 56 ºC for 1 min; 72 ºC for 1 min and 1.5 min for 35 cycles. The products were checked for size and specificity by agarose gel electrophoresis followed by purification using the MiniElute 96 UF PCR Purification Kit (Qiagen, Australia) and 100-150 ng of purified PCR product was digested for 3 h at 37 °C followed by 65 °C denaturation with the restriction enzymes *Alu*I and *Cfo*I. The digested DNA was purified using SigmaSpin® Post-Reaction Purification Columns (Sigma, Australia) and 10 µL of the purified T-RFs were analyzed for size by the AGRF (Adelaide, Australia) using capillary separation on an ABI 3730 DNA analyser with a LIZ500 size standard.

Data analysis – Paul Greenfield notes ITS
Bioinformatics analysis of the sequence data for 28S rRNA was conducted using the method described in Penton et al. (2014). Sequence data from ITS region analysis was processed using XXX. TRF size and intensity data were collected using the GeneMarker analysis software (version 1.85; SoftGenetics Inc.), with a minimum cut off of 100 intensity units. Relative abundances of TRFs were calculated and normalized against the total peak height of all TRFs in the profile. Relative abundances from the TRFLP and amplicon sequencing data were then analysed using the Primer6 software package (Primer-E Ltd, Plymouth, U.K.) by cluster analysis and non-metric multidimensional scaling (NMDS).

Bioassay experiments to determine the effect of soil biological activity in different soils, i.e. potential disease suppression capacity, on disease incidence potential was conducted using soils from ongoing field experiments (in collaboration with Dr. Linda Smith).

1.2.3 Findings and discussion:
There were significant differences in the composition and genetic diversity of soil fungi between the different field sites from the three cotton growing regions, i.e. significantly affected by field location (soil type and environment; New South Wales and Queensland) and within a site between experiments based on cropping history.
Results from the catabolic diversity analysis showed a clear treatment / rotation / field based dissimilarity at both sites (Figure 1.1). At Cowan, microbial communities in the continuous cotton (CCC) and cotton-fallow-cotton (CFC) treatments were distinctly different from that in the rotations involving maize (c-m-c), pigeon pea (c-pp-c) and sorghum (c-sgh-c) indicating that rotational crops cause a significant change in the catabolic capability of microbial communities. Additionally, overall catabolic diversity and activity was significantly lower in the soils from CCC and CFC treatments (ave. 8.6) compared to that in soils under other rotations (ave. 18.8). Crop rotation and management practices have been shown to influence microbial activity, catabolic diversity in agricultural soils both in the

rainfed and irrigated cropping systems (Gupta et al. 2007, Hartmann et al. 2015). Although seasonal variation in fungal community composition was observed it was found to be less evident than the site and management effects. Data for the abundance of total bacteria (16S rRNA gene abundance) and total fungi (ITS region abundance) also showed lower values for these treatments (data not shown here).

Data from the sequencing based analysis of fungal communities (28S LSU and ITS region) indicated a total of >350 genera in all the cotton soils analysed. There were significant differences in the composition (genetic diversity) of soil fungi between the different field sites from the three cotton growing regions and clear differences were seen at different hierarchical levels e.g. species (OTU), genus, family etc (Figures 1.2 and 1.3). Although fungal spores have the potential to travel long distances, Cline et al. (2014) indicated that phylogenetic differences in saprotrophic fungal communities increased with geographic distance and accounted for more variation in community composition than biogeochemical differences alone. Penton et al. (2014) found that location based variation (combined effect of edaphic and environmental factors) was greater than the soil biogeochemical and management based variation in fungal communities in the South Australian cropping soils. Results from both the 2013 and 2015 analysis in this study showing major location based variation support the reports from other environments suggesting the possibility that a region specific fungal community assembly may exist in the different cotton growing regions. This has implications to the development of management options to manipulate both beneficial and pathogenic fungi. However, this needs to be verified with a more extensive analysis of field samples from farmer fields.

In general, members Ascomycota were the most dominant group of fungi accounting for more than 80% of total fungi followed by Basidiomycota and Zygomycota in all soil analysed. Fungal genera belonging to the Classes Sordariomycetes and Dothideomycetes were the major groups and showed distinct differences between the locations, e.g. Dothideomycetes dominant in the Cowan soils whereas Sordariomycetes were dominant at other sites (Figure 1.2). This variation in relative proportion of various groups extended at family and genus levels with distinct differences in a number of groups between soils. Most striking difference is the difference between the soils under the long-term rotation experiment F6E and the adjoining block (KK-disease block) under a biofumigation experiment (Figure 1.2 and 1.3 and Table 1.1). The top 25 genera in abundance accounted for a large portion of total fungal community (Table 1.1). These results support the hypothesis that continuous cropping systems are generally dominated by a small and select group of soil microbial community which prefer the high inputs and regular disturbance. Soil samples from Cowan experiment in Queensland contained highest number of fungal genera (263) whereas the soils from Biofumigation experiment at ACRI showed lowest number of fungal genera (145). Results for Diversity indices showed significantly greater diversity in the long-term crop rotation experiment at Narrabri (F6E) and experiments at Cowan and Goondiwindi compared to the Biofumigation and D1 field experiments at ACRI, Narrabri (Table 1.2). It is known that different plant species and genotypes of same species growing in one soil can promote distinct microbial communities including fungal community (Knox et al. 2014, Bazghaleh et al. 2015). Plant produce specific bioactive substances e.g. C and N sources and signal molecules which either promote or inhibit specific microbial groups (Gupta and Knox 2010, xxx). Therefore cropping systems with greater plant diversity and diverse resource qualities, e.g. crop residue quality, have been shown to support higher phylogenetic diversity in microbial communities including fungi (Cline and Zak 2014) and thus cotton fallow system with lowest amount of crop residues and a more uniform residue quality seem to have resulted in lower fungal diversity.

Results from the analysis of cotton fields vs. nearby remnant vegetation field as part of BASE project showed a clear difference in the composition of soil bacterial, archaeal, fungal and eukaryotic communities between soils. For soil fungal communities, while the Ascomycetes dominated in cotton fields, in remnant vegetation soils they were only 50 and 30% in the 0-10 and 10-30 cm depths. In the remnant field soil Zygomycota and Basidiomycota were a significant part of the soil fungal community (See Appendix 2 for more details).

Although crop rotation effects were observed at all the sites the level of variation varied between the different experiments (Figure 1.4, 1.5, 1.6 & 1.7). For example, the effect of cropping history at the Goondiwindi site was highly significant compared to that observed in the 3 year field experiment at Cowan. At both sites continuous cotton rotation soils (e.g. Alcheringa furrow and C-C-C rotation)

showed lowest fungal diversity compared to rotations with Maize (e.g. Marella Windmill plots and C-M-C rotation). At Cowan site, both the abundance and species richness of fungi were lower in the C-C-C and Cotton-fallow-Cotton rotations compared to the rotations with sorghum, pigeon pea and maize crops (Figure 1.7). Previous observations by Linda Smith's group indicated that Maize as a rotational crop generally results in lower Fusarium disease on the following cotton crop. In addition, during 2008-09 Fusarium populations were significantly higher in the continuous cotton rotation than all other treatments (L. Smith personal communication). Thus, higher disease incidence in C-C-C rotations could be due to the combined effect of higher pathogen concentration and lower general fungal diversity in the surface soil. Previous results from the analysis of soils from other cotton experiments at ACRI indicated that Cotton-Fallow rotations generally exhibit lower microbial activities probably due to lower amount of total C inputs as crop residues and rhizodeposition. Research from grain cropping systems has indicated that soils with lower catabolic diversity could influence the disease suppression potential (Gupta et al. 2008). In particular, under the continuous cotton rotation which generally exhibits higher levels of pathogens and lower microbial activities may result in greater disease incidence as the incidence and severity of soilborne diseases is considered to be a product of pathogen inoculum-soil microbes-plant interactions modulated by environmental conditions (Gupta et al. 2011).

Soils from the Pathology block at Narrabri used by the pathology group showed a distinct soil fungal community composition, in particular when compared to that observed in the long-term crop rotation experiments on the neighbouring block (F6E) (Figure 1.5) with the presence of distinct fungal phylotypes in the brassica plant and cotton rotations and experimental plots. Plots in the pathology block in Field 4 at Narrabri are regularly exposed to long-fallow treatment which is known to adversely affect the populations of arbuscular mycorrhizal fungi (AMF). Brassica plants are known to significantly alter soil fungal community structure and even reduce the inoculum levels of soilborne fungal pathogens in grain cropping systems (Kirkegaard et al. 2008; Gupta et al. 2011). Observations by Dr. Karen Kirkby indicated significantly lower Black root rot disease and higher yields in the plots after the brassica crop compared to continuous cotton rotation.

It is known that seasonal environmental factors could have significant influence on the composition and abundances of soil bacterial and fungal communities both beneficial and pathogenic organisms (Gupta et al. 2008, Voriskova et al. 2013, Bevivino et al. 2014, Shen et al. 2015). It has been suggested that soil ecological and environmental factors and filtering processes related to substrate quality and availability, e.g. biologically available carbon, nitrogen, play a significant role in shaping microbial communities seasonally (Korando et al. 2013). Thus seasonal changes mainly reflect the fluctuations caused by temporal turnover of soil factors and microbial turnover due to management (Pereira e Silva et al. 2012). Penton et al. (2014) reported that location and soil type based differences in soil fungal communities were greater than the seasonal variation in total saprophytic communities. Comparison of results from 2013 and 2015 although show some seasonal variation in fungal community composition, location based effects were stronger (Figure 1.8), one again confirming the hypothesis that a region/location based fungal community exists in cotton soils. However, it is known that environmental factors such as rainfall, soil temperature etc would influence general microbial activity and pathogen populations thus can affect disease incidence and severity (Gupta et al. 2014).

Biological suppression of plant diseases in agricultural soils is linked to the development of communities that support microbial groups with antagonistic and competitive potential against plant pathogens along with those with plant growth promoting abilities (Gupta et al. 2011; Kinkel et al. 2011). The functional diversity of soil fungi and their capacity to colonize diverse microhabitats can influence pathogen levels and play a significant role in improving plant health, e.g. *Trichoderma* spp and mycorrhizal fungi. Soils with high disease suppressive potential have been found to exhibit high fungal diversity (Xu et al. 2012; Penton et al. 2014). Although we found low fungal diversity in continuous cotton fields/treatments, it needs to be evaluated if low diversity of soil fungi directly contributed to the low disease incidence. Data from the 5 sites is being interrogated to identify the presence of distinct members of fungal genera at sites / rotations varying in disease incidence. Additionally, the field sites we analysed not only had different cropping history but were also different in some soil physico-chemical properties. Currently we are analysing them for detailed soil chemical properties.

1.2.4 Conclusions:

Soil type and environment (location) and cropping history have a significant influence on the genetic composition and diversity of soil fungal community in the surface 0-10 cm cotton soils. The total number of genera was generally lowest in the rotations including brassica crops and Continuous Cotton rotation compared to rotations that include other crops. These results indicate that, contrary to previous research which only concentrated on soil bacteria, diversity and abundance of soil fungal community varies significantly in the three cotton growing regions analysed and changes in soil fungal community may play a notable role in soilborne disease incidence in cotton. This warrants detailed analysis of the community to identify the genera or groups that respond to cropping practices especially in farmer fields.

- Diversity and abundance of soil fungal community varied significantly in the cotton growing regions
- A total of 370 genera were found in the 5 cotton experimental sites, however, a few groups (20 families) were dominant.
- Cropping history had a significant influence on the composition and diversity but less than regional differences.
- There was a minor seasonal variation in community composition.
- Diversity of fungi was generally lowest in soils from brassica crop rotations (at ACRI), Continuous Cotton and Cotton-fallow compared to rotations that include other crops
- Lower diversity and abundance of total fungi were associated with higher disease incidence in the intensively managed cotton systems**
- These results suggest that the functional role of specific genera or groups in disease suppression and other functions esp. farmer fields needs to be clarified for the development of management practices that promote beneficial fungal communities

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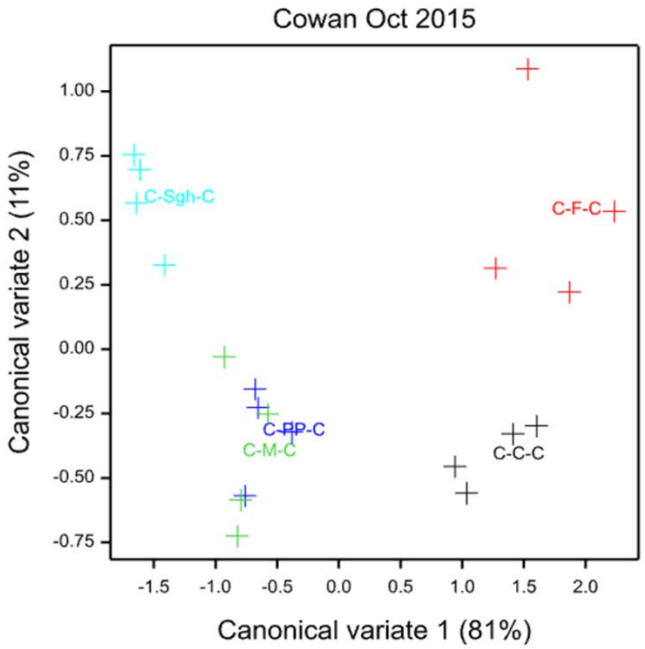
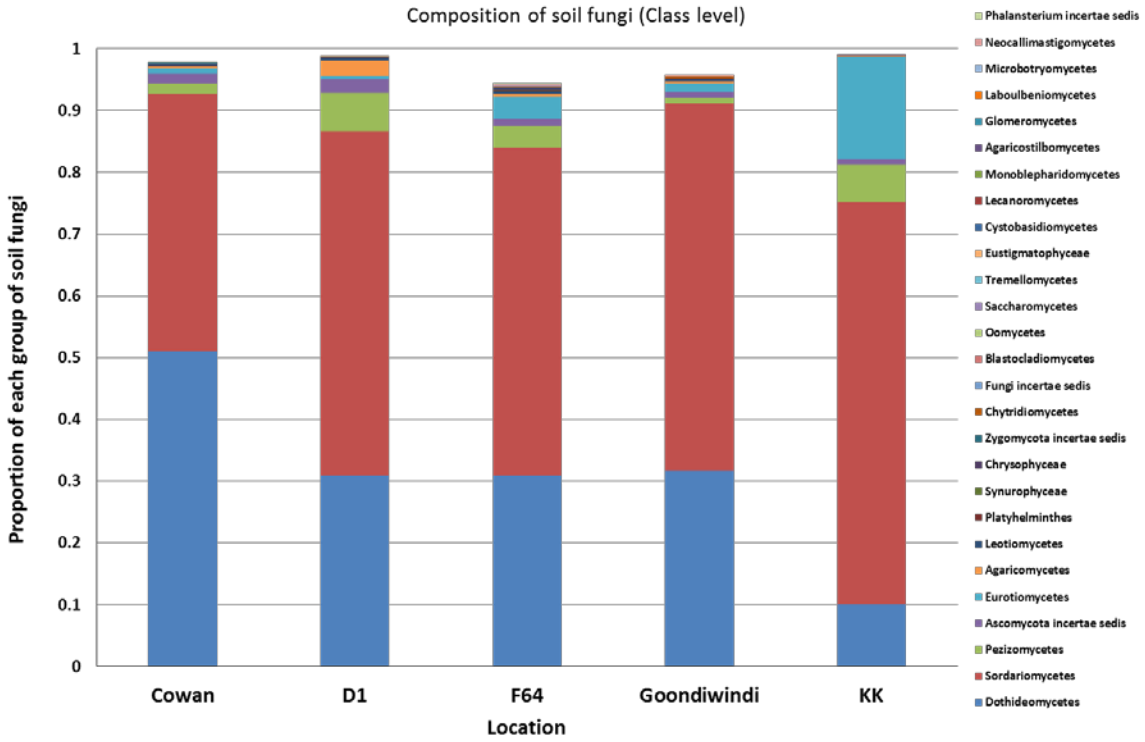


Figure 1.2.1. Canonical analysis results for the catabolic diversity profiles of microbial communities in soils from crop rotation treatments in field experiments at Cowan.

Table 1.2.1 Genera of soil fungal community most abundant in cotton soils from field experiments located at sites in different cotton growing regions:

Genus	Macquaire	McIntyre	Lower Namoi (Narrabri)		
	Cowan	Goondiwindi	D1	F64	KK
Alternaria	213	132	131	85	97
Cladosporium complex	152	116	24	133	17
Gibberella	104	266	118	246	180
Peyronellaea	94	29	42	50	16
Chaetomidium	62	51	72	52	397
Hydropisphaera	57	18	8	60	9
Anthostomella	46	5	29	6	4
Cercophora	45	90	94	63	37
Delitschia	44	7	7	9	2
Plectosphaerella	32	42	13	43	89
Cercospora	30	1	0	1	0
Phaeosphaeria	29	29	23	17	5
Pyrenochaeta	27	17	7	10	3
Nectria	24	19	6	10	12
Chlorophyta incertae sedis	23	46	11	3	10
Chaetomium	21	41	16	35	18
Phomopsis	18	8	2	15	1
Zygopleurage	17	28	73	9	2
Camarosporium	16	12	80	16	5
Peziza	15	2	33	4	5
Bartalinia	15	15	129	9	17
Leptosphaerulina	12	4	12	6	3
Parasarcopodium	12	30	5	25	1
Davidiella	11	8	1	8	0
Stachybotrys	11	17	101	24	16
Sphaeriothyrium	10	3	3	5	1
Massarina	10	5	6	10	2
Lewia	9	5	4	5	1
Zopfiella	9	7	3	5	11
Schizothecium	9	13	12	13	3
Sphaerulina	9	7	1	10	0
Westerdykella	8	6	13	8	3
Bionectria	8	5	4	4	3
Triangularia	7	36	32	12	5
Ecdysozoa incertae sedis	7	6	3	70	4
Corynascus	6	6	4	5	5
Melanospora	6	2	5	5	3
Podospora	6	2	3	5	5
Macrophomina	6	1	0	1	0
Pleospora	6	3	1	2	0
Chaetasbolisia	5	3	2	3	0
Neurospora	5	6	4	13	8
Immersiella	5	1	0	0	0
Pseudonectria	4	12	2	15	11
Cochliobolus	4	2	1	3	1
Corynespora	4	4	9	4	1
Neonectria	4	0	1	3	1
Preussia	4	4	15	35	5
Ascobolus	3	5	31	28	62
Melanopsamma	3	8	11	12	3

(A)



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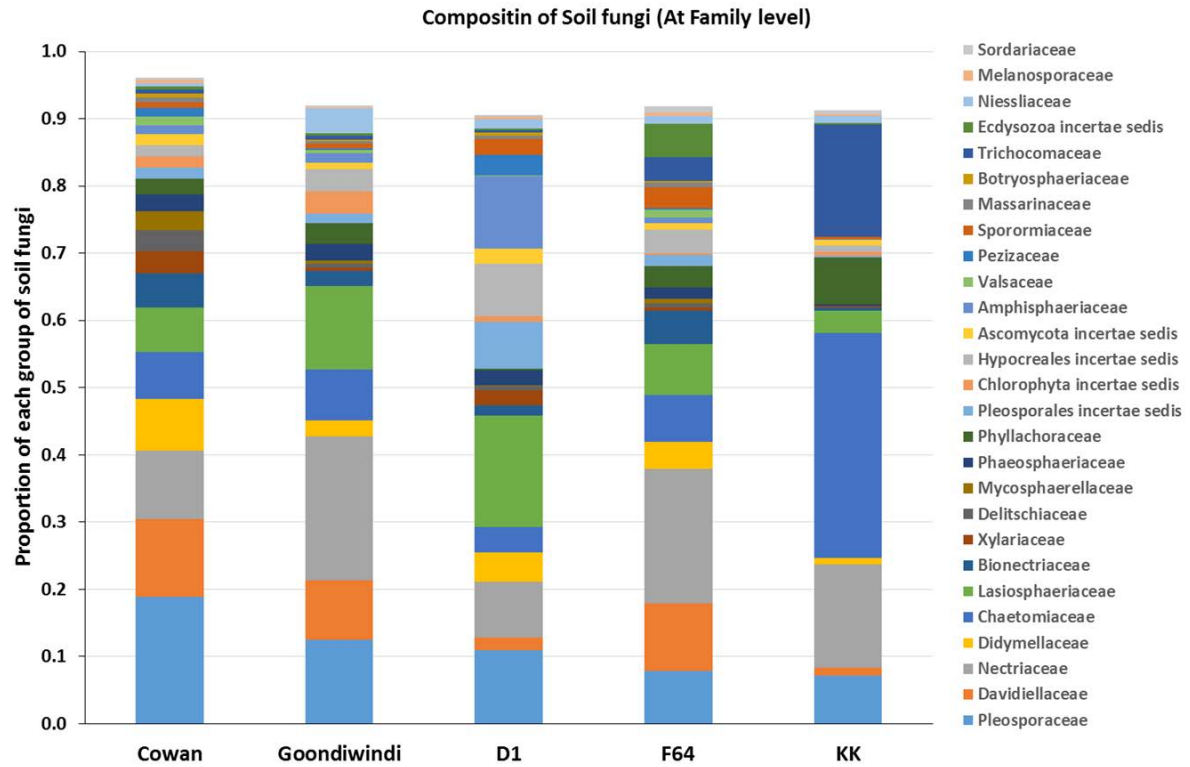

Figure 1.2.2. Relative abundance of various soil fungi (A) grouped at Class level and (B) family level in surface soils from field experiments located at sites in different cotton growing regions.

Table 1.2.2. Diversity indices of fungal community in cotton soils (0-10 cm) as influenced by soil type, location and management practice. At D1 experiment T2=fallow-cotton, T3=vetch-cotton and T4=wheat-cotton.

Diversity indices of soil fungal communities					
Site/Treatment	Species richness	Evenness	Shannon index	Simpson index	# OTUs
Cowan	**119.3**	**0.9566**	**5.443**	**1.0823**	**303.9**
c-c-c	107.5	0.9516	5.269	1.0951	254.0
c-f-c	116.9	0.9550	5.409	1.0845	292.3
c-w-c	146.6	0.9685	5.803	1.0655	401.5
f-m-c	110.2	0.9532	5.332	1.0867	272.5
f-pp-c	105.8	0.9505	5.262	1.0900	258.8
D1	**98.0**	**0.9495**	**5.187**	**1.0910**	**236.1**
T2	97.3	0.9508	5.190	1.0902	235.3
T3	100.9	0.9493	5.218	1.0901	244.0
T4	95.8	0.9484	5.153	1.0927	229.0
F64	**118.2**	**0.9570**	**5.448**	**1.0808**	**301.2**
fababean	132.2	0.9620	5.625	1.0727	349.3
vetch	106.4	0.9511	5.281	1.0888	260.0
wheat	111.4	0.9562	5.380	1.0836	278.3
Goondiwindi	**113.7**	**0.9562**	**5.400**	**1.0829**	**286.3**
Al C4	114.3	0.9589	5.443	1.0782	293.5
Furrow	120.5	0.9582	5.474	1.0811	306.0
Windmill East	104.0	0.9519	5.264	1.0891	254.0
Windmill West	110.5	0.9527	5.334	1.0874	272.0
KK	**69.0**	**0.9170**	**4.512**	**1.1389**	**138.9**
BF	69.8	0.9133	4.490	1.1438	136.5
Fallow	76.0	0.9303	4.738	1.1187	163.3
FR	61.5	0.9055	4.296	1.1566	115.8

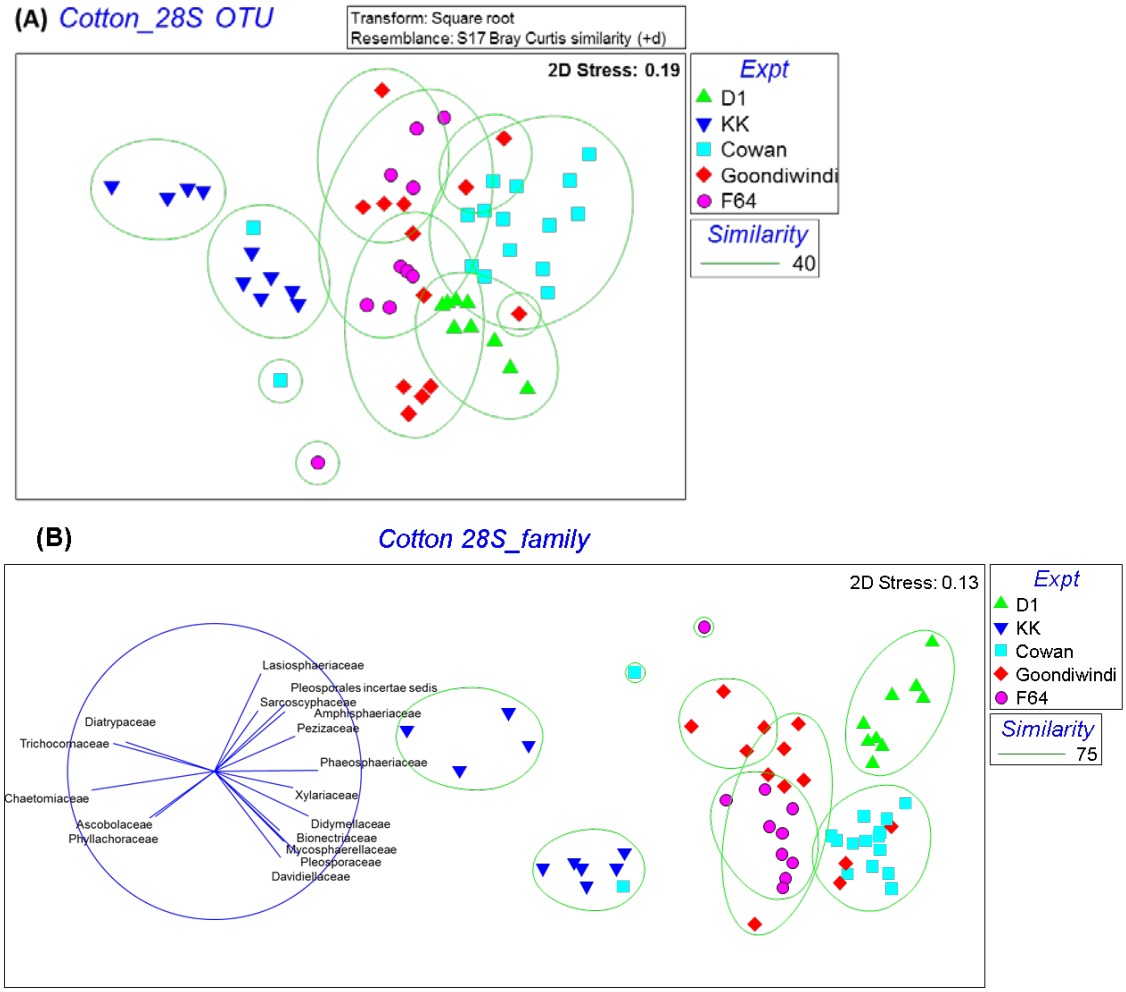

Figure 1.2.3. Genetic composition of fungal communities in surface soils from field experiments located at sites in different cotton growing regions: non-metric dimensional scaling (NMDS) based on Bray Curtis similarity plus a dummy variable (+d) with square root transformation of *28S LSU sequence data* for soils from field experiments at Goondiwindi (McIntyre), Cowan (Macquaire) and Narrabri (Lower Namoi). (A) Species level data - CV=24.6; P=0.001; (B) Family groups data (CV=16.4; P=0.01)

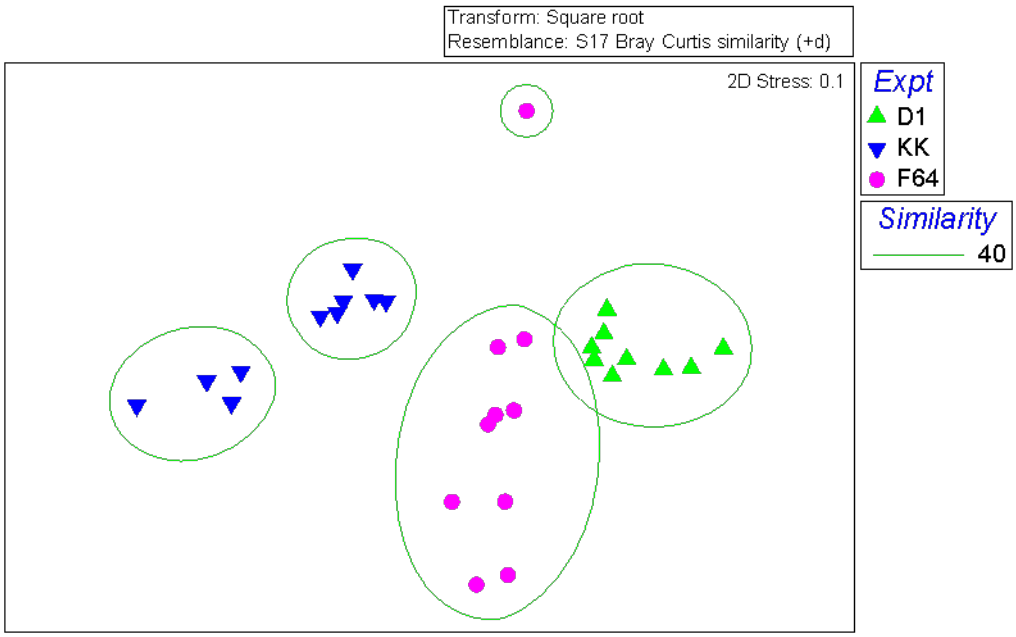

Figure 1.2.4. Differences in genetic composition of fungal communities in long-term experiments at ACRI varying in management history. NMDS graph based on Bray Curtis similarity plus a dummy variable (+d) with square root transformation of *28S LSU sequence data* for 2014 soil samples. D1- crop rotation experiment, F64 – crop rotation X fertilizer experiment, KK – Biofumigation experiment. ANOSIM global R=0.78, P=0.01; PERMANOVA CV=28%, P=0.01.

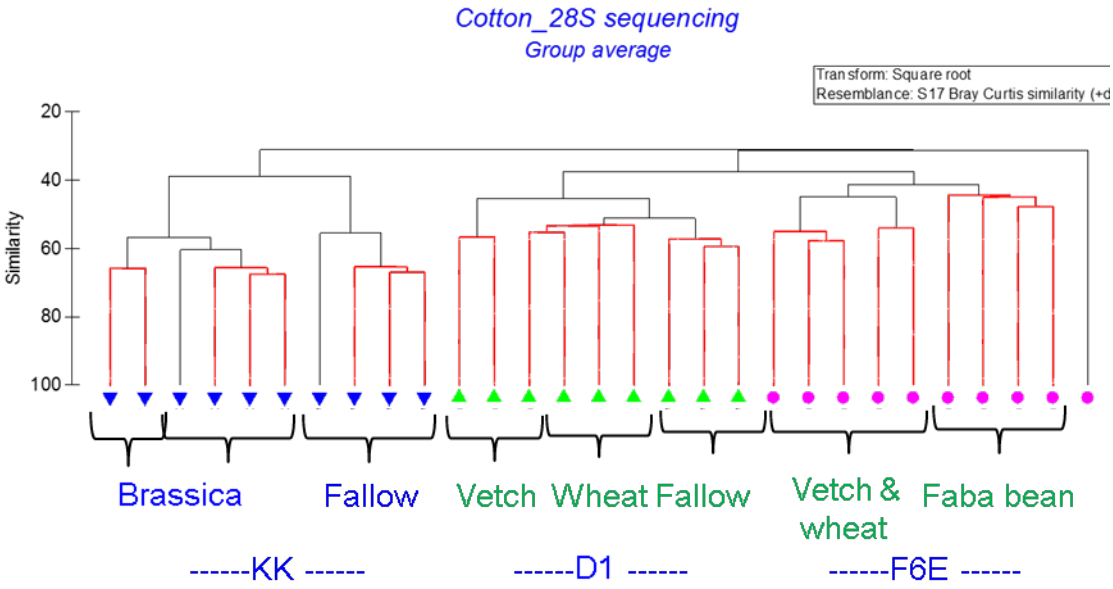

Figure 1.2.5. Genetic composition of soil fungal community (*28S LSU sequence data* for 2014) as influenced by crop rotation within a long-term experiment. D1- crop rotation experiment, F64 – crop rotation X fertilizer experiment, KK – Biofumigation experiment. CV=16.4; P<0.001, ANOSIM 0.631; P<0.01.

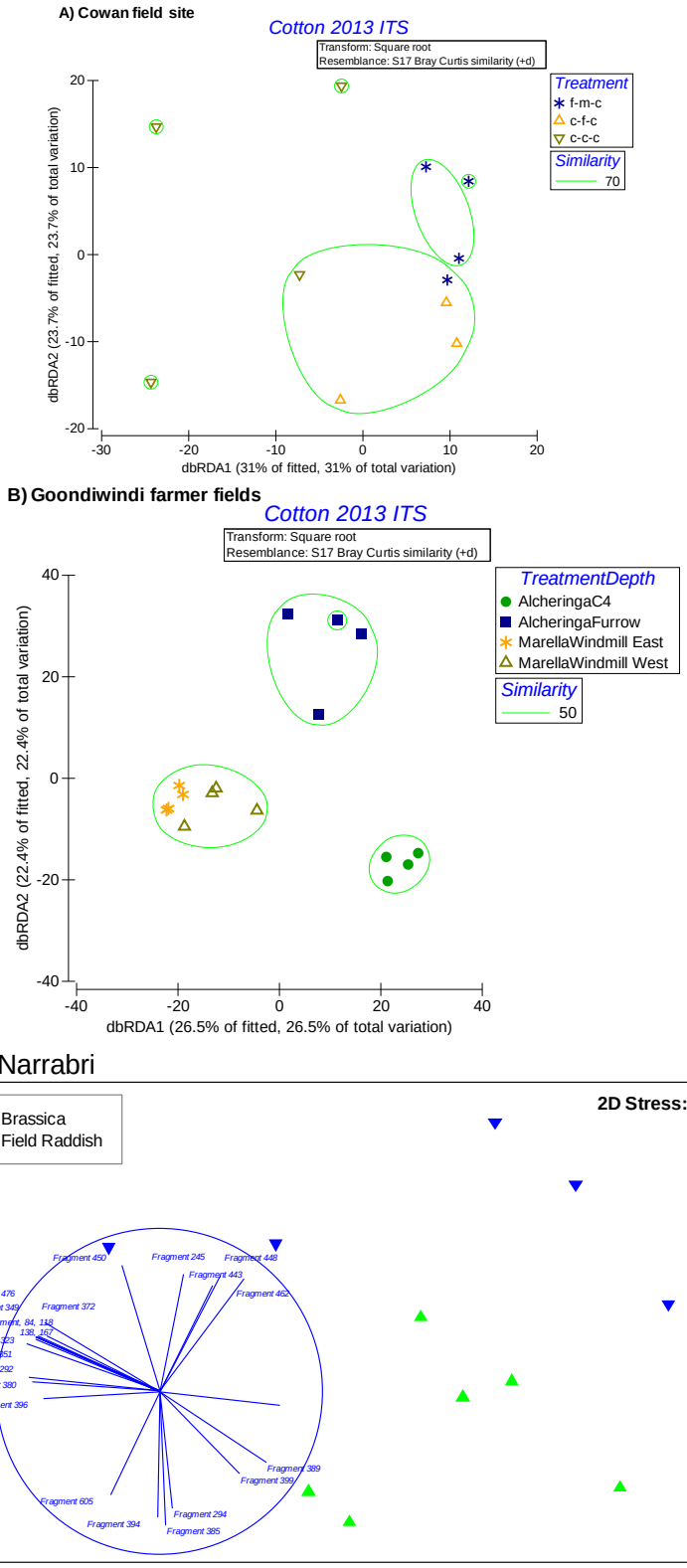

Figure 1.2.6. Differences in the composition of soil fungal communities as influenced by previous cropping history: (A) dbRDA plot based on Bray Curtis similarity plus a dummy variable (+d) with square root transformation of *ITS*-TRFs for soils from Crop rotation experiment at Cowan (CV=12.5%, P<0.004); (B) dbRDA plot for soils from Farmer fields at Goondiwindi (CV=21.7%, P<0.002); (C) non-metric dimensional scaling (NMDS) based on Bray Curtis similarity plus a dummy variable (+d) with square root transformation of *ITS*-region.

(A)

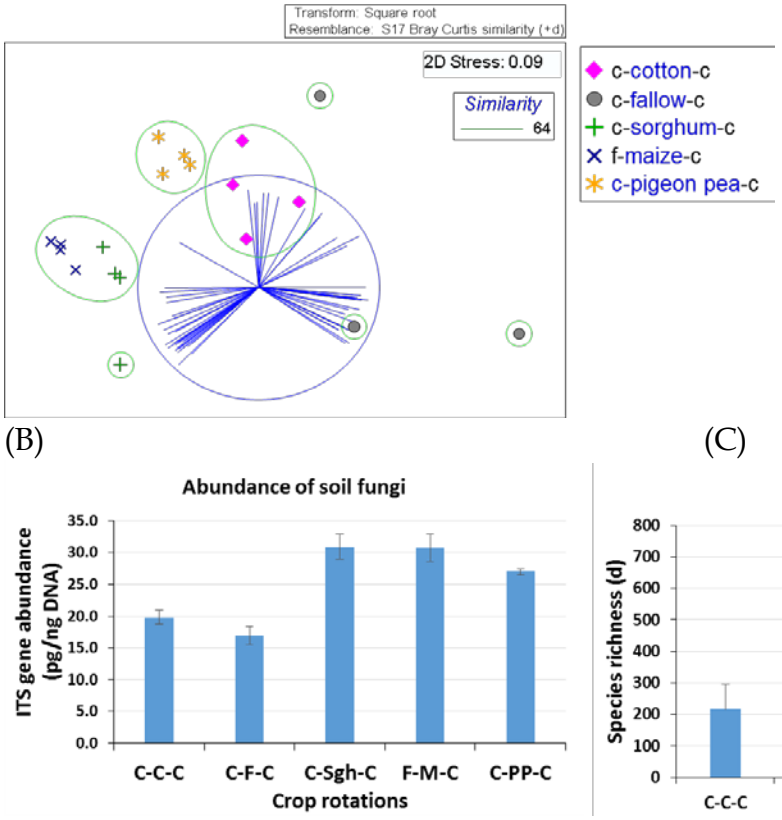

Figure 1.2.8. Effect of crop rotation history on the genetic composition, abundance and diversity of soil fungal communities (A) NMDS graph based on Bray Curtis similarity plus a dummy variable (+d) with square root transformation of *ITS region sequence data* for 2015 soil samples , PERMANOVA CV=19.6; P<0.001, ANOSIM 0.80; P<0.002; (B) Abundance of fungi based on ITS-qPCR data and (c) Margalef's Species richness (d).

(A) Between locations and seasons experiment

(B) Between seasons in Goondiwindi

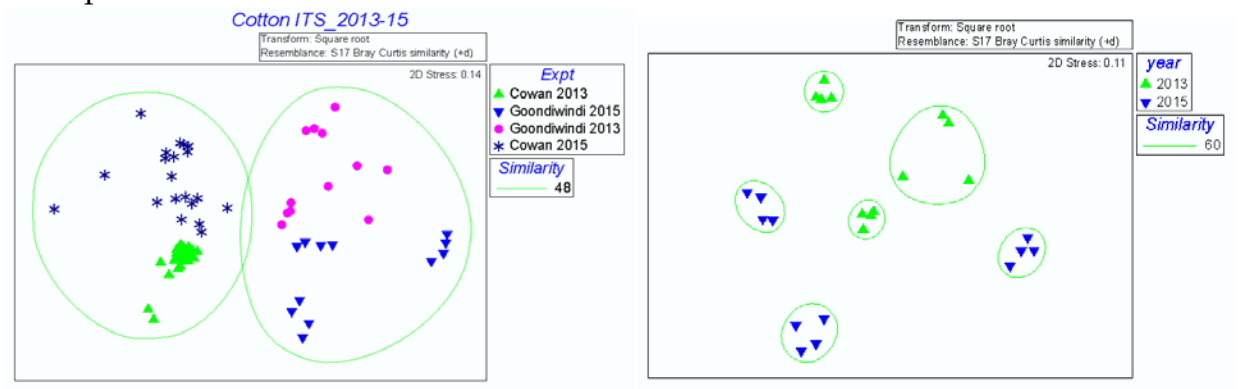

Figure 1.2.9. Seasonal variation in the composition of fungal communities in field experiments at Cowan and Goondiwindi. NMDS graph based on Bray Curtis similarity plus a dummy variable (+d) with square root transformation of *ITS region sequence data* for 2013 and 15 soil samples. (A) Between sites and seasons, ANOSIM global R=0.92, P=0.01; PERMANOVA CV=22%, P=0.01 and (B) Between seasons in the Goondiwindi experiment, ANOSIM global R=0.99, P=0.01; PERMANOVA CV=25%, P=0.01.

Part 2. Effect of compost addition on biological functions and microbial populations in cotton soils (This work is being conducted in collaboration with Duncan Weir, DAFF Qld) **(Milestone 1.3)**

Summary:
Composts vary in their chemical composition significantly in terms of major nutrients and trace elements, biologically available carbon, in particular the gin trash compost. In a long-term field experiment on a Vertosol soil, compost addition had no significant effect on microbial biomass, activity and N mineralization potential. In controlled environment experiments, addition of composts @ 5 and 10 t/ha generally increased microbial activity but the effect was only evident during the first two weeks of incubation. Compost effect on microbial biomass was only seen during the first 4 weeks after application after which MB levels generally decreased with or without added fertilizer N and no effect was seen at the end of the experiment. Similarly, application of nitrogen fertilizer after compost addition increased microbial activity for a very short period (~5 days) but altered microbial catabolic diversity, the magnitude of the effect varied between different composts. In general compost application had limited and no consistent effect on different microbial groups including those involved in N cycling processes. These results suggest that long-term effects of composts may depend upon amount and frequency of application *esp*. for a change in microbial diversity and plant beneficial functions. Therefore, chemical analysis of the compost material before application is recommended to more fully consider its' potential benefits. In addition, these effects need to be evaluated in different soil types and environments.

2.1 Background:

Australian cotton soils are often naturally low in biologically available organic carbon, which is the primary energy source required for soil microbial activity. Although management practices such as crop rotation, reduced tillage and fertilizer application can modify microbial activities and populations, the effects may require long period of adoption. Additionally seasonal factors such as soil moisture and temperature also influence biological functions.

With many cotton growers looking at ways to improve soil health and soil microbial activity in an effort to increase productivity and ensure long term sustainability, interest in the use of compost application has grown because it is believed that compost provides several benefits to soils (cotton Info-soil health www.cottoninfo.com.au/soilhealth). The rapid microbial turnover that is generally seen in the Vertosol soils and the climatic factors in cotton growing regions require large quantities of carbon inputs to sustain and improve microbial populations and biological functions. Composts can provide a source of organic carbon to 'feed' the soil biota and increases soil fertility as well as provide other biological activity and soil-structure benefits (Martinez-Blanco et al. 2003; Bastida et al. 2008; Roberts et al. 2015). Previous research on compost effects in cotton soils on soil fauna are variable (Knox et al. 2007). There is little information available on the influence of the addition of composts e.g. different types and rates, on microbial populations and biological functions in Australian cotton soils.

Aim: To identify the links between changes in soil microbial activity and biological functions to the chemical composition of composts – field & incubation experiments

2.2 Methods:

2.2.1 Field experiment

Surface soil (0-10 cm) samples were collected from the long term field experiment conducted by Duncan Weir in a field belonging to the farmer Jan Lefrenz during May 2015. This experiment was started in 2011 and the plots received different manure and compost materials @ 5 t/ha, annually. Multiple cores (6 / plot) collected from each plot were mixed to give one bulk sample and samples transported to Adelaide labs for analyses. Soils were analysed for microbial biomass, activity, nitrogen mineralization potential and populations of various microbial groups were measured similarly to that done in the laboratory incubation experiments.

2.2.2 Laboratory experiments 1 and 2

Two six month incubation experiments were completed evaluating the effects of addition of three different compost materials, e.g. rate of application, method of application and N fertilizer application, on microbial activity, catabolic and functional diversity and N mineralization in the surface soil from the control treatment plots in field trial at Jan Lefrenz. Surface (0-10 cm). Field soil was prepared to remove undecomposed plant material and stones and sieved to pass through 4 mm sieve. Three types of compost materials (Feedlot, Poultry manure and Gin trash compost) were added to the soil and the

samples incubated at moisture equivalent to 60% water-filled pore space and 25˚C. Known amounts of soil (100 g / core) or soil with or without compost materials and soil moisture adjusted as required was packed into 5.5 cm dia. cores at 1.1 bulk density. In experiment 1, compost materials were mixed at 5 t and 10 t/ha prior to preparing into cores. In experiment 2, compost materials @10t/ha were either mixed or placed on the surface of the soil. For the fertilizer N treatment, on day 26 urea (@100kg N / ha) was added in liquid form whereas distilled water was added for the other cores. Individual cores for each treatment were placed in 1 L glass jars and closed with lids during incubation. Soil moisture was maintained at the original level with weekly addition of distilled water as required. Multiple cores were prepared for each treatment to allow destructive sampling at regular intervals (Expt 1 - 2, 4, 8, 16 and 24 weeks and Expt 2 – 4, 8 and 22 weeks) for various biological measurements. They included: microbial biomass C and N, catabolic diversity (multiple-C utilization profiles using Microresp®), mineral N, dissolved organic C, populations of major microbial groups and functional groups involved in N cycling (gene abundances using qPCR assays). These include: total bacteria (*16S rRNA*), total fungi (*ITS* region), Pseudomonas species (*16S-pseudomonas* specific), nitrogen fixing bacteria (*nifH*), denitrifying microorganisms (*nosZ*), nitrifying bacteria and archaea (*amoA*) and chitinase degrading microorganisms (*chi*). Soil microbial respiration was measured regularly using a Gas Chromatograph (e.g. 2 times per week during the first three months and once a week afterwards). At the end of the experiments soil samples were analysed for organic C and total N levels.

2.3 Findings and Discussion:

2.3.1 Quality of compost materials: All the organic amendments will be analysed for their C and nutrient properties (Table 2.1). Different compost materials varied in their C and nutrient (major and trace element) concentrations significantly. The feedlot compost generally contained higher levels of dissolved organic carbon, total nitrogen and bicarbonate extractable phosphorus whereas the Gin trash compost had lower carbon and nutrient concentrations. Gin trash compost had lowest dissolved organic nitrogen but lower C:N ratio. Analysis of another set of Gin trash composts of different ages (1 month, 1 and 2 years old) showed lower levels of inorganic nitrogen. Overall, these results suggest compost materials vary widely in their quality.

2.3.2 Long-term field experiment: There was significantly higher concentration of dissolved organic carbon content in the compost amended soils although after four seasons there was not significant change in the total soil organic carbon (ave 1.31 $\pm$0.02; data from Duncan Weir) in the surface 10 cm of soil. Compost materials did cause a significant change in the soil phosphorus and some micronutrient concentrations (Duncan Weir, personal communication).Four years of addition of various compost materials @ 5 t/ha annually had little or no effect on the microbial biomass and activity measures including the nitrogen mineralization potential of soil (Table 2.7 and 2.8). But the differences in the microbial catabolic diversity profiles suggests that compost application may have modified the ability of soil microbial communities to utilize diverse carbon compounds (Figure 2.9). The different composts varied in their chemical composition and thus would have promoted different groups of microbes (Figure 2.9). Nevertheless, results for the abundances of various microbial groups (i.e. gene abundances) generally showed no significant differences in the majority of bacterial and actinobacterial populations and microbial groups involved in C and N cycling processes measured using soil enzyme analysis. This could be partly attributed to the smaller amounts of compost added coupled with the general high microbial turnover rates in Vertosol soils. However, soils receiving compost materials, especially poultry manure and gin trash compost materials showed higher abundance of soil fungi compared to standard fertilizer treatment (Figure 2.9). Coleman et al. (2010) found significant changes in soil fungal communities in response to addition of low quality wheat stubble. Additionally, Ye et al. (2016) suggested that bacteria present in the compost treatment had a high turnover facilitating quicker organic matter degradation. The self-mulching nature of Vertosol soils and the soil moisture and temperature regime during cotton season support high levels of microbial respiration and C and nutrient turnover which may not facilitate C sequestration supporting the reports by Hullegale et al. (2000 & 2013) on lack of significant C sequestration in irrigated Vertosols under cotton-based farming systems.

2.3.3 Laboratory incubation experiments: In both experiments, the addition of composts increased microbial activity for a short period only e.g. less than two weeks and the effect was highest with the Feedlot compost followed by Poultry manure compost whereas the Gin trash (Own) compost had the

lowest benefit in microbial activity. (Figure 2.1 and 2.2) Addition of fertilizer N as Urea solution @100kg N per ha caused a significant flush in microbial activity but disappeared within 5 days and the trends were similar with all the three compost types suggesting that N availability may play an important role in making the compost C bioavailable. In experiment 1, microbial biomass levels increased for feedlot and poultry composts only and the effect was greater at twice the field rate, whereas in experiment 2, MB levels at week 4 increased in all treatments but the increase was greater in the compost treatments compared to that in control (Table 2.2). This increase in MB from composts is mainly from the dissolved organic C and nutrients providing energy source to microorganisms. Gintrash compost contains less of bioavailable carbon and more recalcitrant carbon and thus it showed lowest effects. However, the short-term nature of this increase suggests other constraints for microorganisms to use the compost C for growth. In experiment 1, the effect of composts on microbial biomass reduced quickly, e.g. by week 4 sampling, whereas in experiment 2 there was a significant reduction after week 4 (Table 2.5). Overall, the reduction in MB continued until the end of the experiment and there was no difference between treatments (Table 2.2. and 2.5). Surprisingly, MB levels were significantly lower in the N fertilizer treatments compared to no-fertilizer treatments with (8.1-18.9%) or without (7.8%) compost addition (Table 2.5). There was a significant effect of compost addition on the catabolic diversity of microbial community in both experiments and the effect varied with compost type (Table 2.3 and Figures 2.4). Application of N fertilizer caused a significant change in the microbial catabolic profiles and the effect was greatest with the 'Own (Gin trash)' compost. The effect of composts was generally greater in the soil layer closer to compost addition, e.g. clear differences between composts in the 'Top' layer whereas no such differences were observed in the 'Bottom' layer (Figure 2.4). Implications from these changes in microbial catabolic diversity require further investigations. Gupta et al. (2007) found soils that were suppressive to rhizoctonia disease in cereal crops exhibited greater catabolic potential and diversity compared to disease conducive soils.

The addition of feedlot (beef) and poultry compost material significantly increased the levels of dissolved organic carbon (DOC) and nitrogen (DON) in soil compared to that in control soils while 'Gin trash' compost had no effect (Table 2.6 & Figure 2.5). These differences reflected in the catabolic diversity changes in the compost amended soils. In the Control treatment of experiment 1, a total of 24 µg of N / g of soil was mineralized during the 24 week incubation. Although the addition of composts increased the amount of mineral N in soil there was no additional benefit in the net N mineralization suggesting that the benefit is mainly through the N in the composts. The trend was similar in the experiment 2 i.e. 29-39 µg N/g soil mineralized during the 22 week incubation (Table 2.5 and Figure 2.5). Addition of N fertilizer had no additional benefit in N mineralization in the compost treatments but reduced the net N mineralized in the control treatment (Table 2.5). With the Poultry compost, N mineralization was lower in the soil layer close to the compost (Top layer) compared to the 2.5 cm away (Bottom layer) whereas no such difference was seen with the other composts (Table 2.5). These two observations suggest that application of composts with high levels of N and inorganic N fertilizer could reduce the mineralization of soil organic N.

The addition of all three compost materials had no consistent significant effect on the abundance of bacterial populations in experiment 1 and only increased with Poultry compost in experiment 2. However, there was a significant increase in the Pseudomonas species population in the soil receiving Feedlot (Beef) compost in experiment 1, whereas, the effect of Gintrash and Poultry composts varied in terms of time of change. Composts are known to contain complex carbohydrates and lignocellulotyic compounds and therefore have been shown to promote populations of Actinobacteria, Acidobacteria, Chloroflexi and some members of Gammaproteobacteria (Hartmann et al. 2015; Ye et al. 2016). The feedlot compost with its rich nutrient composition would have supported copiotrophic bacteria such as *Pseudomonas* species. We didn't measure in-depth bacterial composition analysis in these experiments. Unlike bacteria, populations of soil fungi significantly increased with the addition of Feedlot compost at both rates and Poultry manure but the effects varied between experiments (Figure 2.7 and 2.8). Similar to the changes in microbial biomass and catabolic diversity, the effect of compost addition was greater in the soil layer closer to the composts (Figure xx) suggesting that the observed changes in the treatments are mainly caused by the C and nutrient sources in the different composts.

The addition of compost material generally had limited effects on the populations of bacteria and actinobacterial groups involved in carbon (e.g. chitinase degrading-*chi*) and nitrogen cycling (e.g. nitrifying

bacteria – *AOB* and archaea - *AOA*, nitrogen fixing - *nifH*, denitrifying - *nosZ*) processes. Some of the observed changes include, a significant increase in chitinase degrading microbial populations in soils receiving Gintrash and Feedlot manure compost (Figure xx). This effect lasted until week 24 in the Gintrash compost treatment. Unlike the Feedlot and Poultry manure composts, Gintrash compost has lower nitrogen and dissolved organic carbon indicating a poor quality in terms of bioavailability or more recalcitrant material. Also, there were lower abundance of denitrifying bacteria in soils receiving Gintrash and Poultry manure composts compared to Control and Feedlot manure compost. Compost application has been reported to improve the suppression of plant diseases in grain crops and vegetable cropping systems either due to the increased microbial activity and/or due to the modification of microbial communities (Mazolla 2004; Campbell 2006; Ye et al. 2016). However many of these examples are based on application of large amounts and repeated application of compost material compared to that tested in these experiments. Such large applications may not be economical for the large scale cotton farming systems. Ye et al. (2016) reported that a combination of composts plus biochar-mineral complexes modified microbial community structure which can stimulate microbial processes in vegetable production systems.

Over all, the lack of significant change in the microbial activity measures reflects the quality and quantity of organic C (i.e. biological unavailable material) added. Based on the results from the laboratory incubation experiments it is clear that the compost addition, at the rates applied, has generally a short-term effect on microbial activities even after the addition of fertilizer N which could be attributed to not finding significant changes in the field experiment. **The finding of differences in the microbial community composition (e.g. catabolic diversity) requires further investigation into potential changes in the genetic composition of bacteria and fungi, in particular changes in key beneficial functional groups for plant health, nutrient availability** etc. Additionally, as soil type can significantly influence microbial composition, the effect of compost addition on soil biology and biological fertility needs to be evaluated on different soil types and environments (location, rainfall etc). The observation that **nature and magnitude of effect from compost addition varied between the three compost materials highlights the importance of conducting a chemical analysis of the compost material before application to more fully consider its' potential benefits to cotton soil biological fertility**.

Conclusions:

- Chemical composition of composts varies in terms of major nutrients and trace elements
- Gin trash generally has lower concentrations of biologically available carbon and nutrients compared to Feedlot and Poultry manure composts
- Addition of composts @ 5 and 10t/ha generally increased microbial activity but the effect was only evident during the first 2-3 weeks (short-term)
- Addition of N fertilizer after compost application increased microbial activity for a very short-period only. N fertilizer addition caused a small reduction in microbial biomass and resulted in changes in the catabolic diversity 4 and 18 weeks after fertilizer application.
- Addition of composts had small and variable changes in abundances of microbial groups and those involved in N cycling processes
- The magnitude of the effect on biological functions and microbial diversity varied between different composts both in the laboratory and field experiments
- Long-term effects depend upon amount and frequency of compost additions *esp*. for a change in microbial genetic diversity with implications to plant beneficial functions. In addition, changes in the microbial catabolic diversity suggest that

other properties related to disease suppression and pathogen abundance need to be considered.

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Table 2.1. Chemical composition of compost materials used in incubation experiments in cotton soils (supplied by Duncan Weir)

Type of compost	Total C	Total N	C:N	Dissolved Org C	Dissolved Nitrogen	DOC:DON	DOC as % total OC	Bicarbonate extractable P	Ca	K	Mg	Na	S
	%			%				mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Feedlot	23.4	2.5	9.42	1.36	0.40	3.37	5.84	4860	54600	14050	8660	4880	5520
Gin trash	9.5	1.3	7.31	0.16	0.15	1.11	1.70	849	22500	12800	5205	612.5	3120
poultry	15.3	1.8	8.36	0.51	0.18	2.87	3.33	4460	25750	11600	7370	5870	5585

Table 2.2. Microbial biomass carbon levels (µg C / g soil) as influenced by the addition of different compost materials.

Treatment	MB Carbon (ug C/g soil)				
	Start	wk 4	wk 8	wk 16	wk 24
Control	403.8	389.2	365.2	409.3	381.0
Feedlot L1	587.6	454.1	406.5	436.4	362.9
Feedlot L2	945.5	488.8	427.8	453.5	361.6
Own L1	423.1	399.5	366.3	374.2	341.3
Own L2	419.6	400.5	352.2	392.3	333.1
Poultry L1	500.7	438.6	386.4	399.4	342.2
Poultry L2	693.6	467.2	431.5	397.8	356.6
F test	**0.001**	**0.001**	**0.001**	**0.001**	**0.029**
LSD (P<0.05)	**30.4**	**19.8**	**19.1**	**18.6**	**27.6**

Table 2.3. Catabolic diversity of soil microbial communities measured by multiple-C substrate utilization responses as influenced by compost addition. – Experiment 1

Treatment	Ave Metabolic response (ug CO2/g/day)		Community Metabolic Diversity	
	Start	after 24 wk	Start	after 24 wk
Control	2.523	1.902	12.4	8.3
Feedlot 5t	2.532	1.597	15.5	7.5
Feedlot 10t	2.880	1.752	15.8	8.4
Gintrash 5t	2.665	1.645	14.1	7.8
Gintrash 10t	2.697	1.501	15.8	7.1
Poultry M 5t	2.729	1.549	15.8	8.1
Poultry M 10t	2.282	1.486	13.7	7.2
F value	**NS**	**0.002**	**0.01**	**NS**
LSD (0.05)	**-**	**0.232**	**1.66**	**-**

Table 2.4. Mineral nitrogen levels in soil (mg N / kg soil) as influenced by the addition of different compost materials – Experiment 1

Treatment	Mineral Nitrogen (mg N / kg soil)				Increase over Control			
	Start	wk 4	wk 8	wk 24	Start	wk 4	wk 8	wk 24
Control	14.2	24.2	28.3	37.2	0	0	0	0
Feedlot L1	25.2	34.1	41.9	50.0	11.1	9.8	13.7	12.8
Feedlot L2	37.6	46.0	52.2	59.2	23.5	21.8	23.9	22.0
Gin trash L1	18.1	27.6	34.3	42.8	3.9	3.4	6.0	5.6
Gin trash L2	23.5	33.1	37.0	47.0	9.4	8.9	8.8	9.8
Poultry L1	20.5	30.8	35.8	43.8	6.4	6.5	7.5	6.6
Poultry L2	28.8	37.8	45.4	51.5	14.7	13.6	17.1	14.3
F test	**0.001**	**0.001**	**0.001**	**0.001**				
LSD (P<0.05)	**1.23**	**2.1**	**2.94**	**2.52**				

Table 2.5. Microbial biomass and mineral N as influenced by compost type, method of application and fertilizer N addition (Experiment 2).

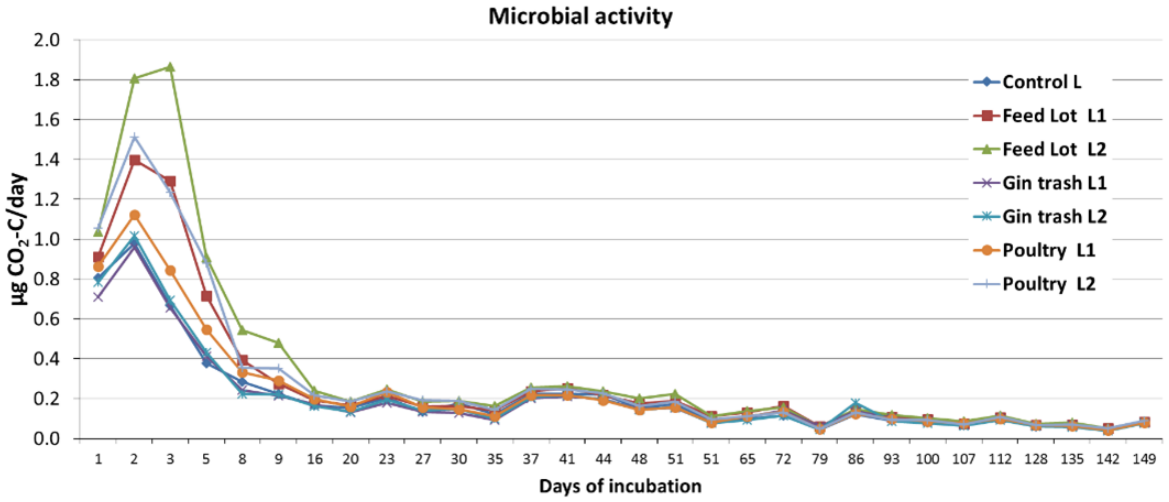
Treatments		Min N				MB-C			
		Wk1	Wk4	Wk9	W22	Wk1	Wk4	Wk9	W22
C	C	12.0	24.7	27.2	41.7	283.9	600.0	365.5	400.9
	CF			124.1	128.4			362.2	371.9
Beef	BM	21.7	32.4	28.6	54.2	311.8	667.7	397.4	401.4
	BS Top		27.6	35.0	51.7		725.9	452.4	414.2
	BS Bottom		30.8	31.8	53.1		625.8	381.1	359.5
	BF			128.1	154.0			389.9	352.4
Own	OM	24.6	36.7	38.6	54.8	330.0	686.9	395.6	405.4
	OS Top		36.9	38.0	53.4		716.3	386.9	408.2
	OS Bottom		34.5	37.9	55.1		709.3	391.3	401.0
	OF			125.4	153.1			389.4	375.0
Poultry	PM	38.4	57.2	50.7	78.1	642.8	788.4	425.2	419.9
	PS Top		64.4	59.2	74.1		837.5	418.9	446.5
	PS Bottom		46.6	56.5	76.2		766.3	404.9	366.0
	PF			152.8	181.4			388.8	353.1
	Fprob	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**0.016**	**<.001**
	lsd	**4.0**	**1.53**	**13.2**	**12.8**	**239.8**	**74.6**	**41.8**	**30.9**

Table 2.6. Dissolved organic C and N as influenced by compost type, method of application and fertilizer N addition (Experiment 2)

		Dissolved Org C		Dissolved N		DOC:DON	
		Wk1	Wk22	Wk1	Wk22	Wk1	Wk22
C	C	82.7	17.3	29.8	69.2	2.8	0.25
	CF		15.1		167.7		0.09
Beef	BM	105.5	28.9	39.9	86.3	2.6	0.33
	BS Top		36.6		84.3		0.43
	BS Bottom		15.6		85.0		0.18
	BF		23.6		193.6		0.12
Own	OM	88.6	25.5	44.3	90.3	2.0	0.28
	OS Top		32.1		86.4		0.37
	OS Bottom		17.9		89.4		0.20
	OF		21.8		195.0		0.11
Poultry	PM	138.3	31.4	42.1	125.1	3.3	0.25
	PS Top		42.5		118.7		0.36
	PS Bottom		16.3		123.1		0.13
	PF		26.0		227.5		0.11
	Fprob	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
	lsd	**10.1**	**4.4**	**1.5**	**14.1**	**0.3**	**0.04**

Figure 2.1. Effect of compost addition on microbial respiration rate in a vertosol soil in a laboratory incubation experiments using soils from field experiment.

(A) Experiment 1 – Compost type and rate of application



(B) Experiment 2 – Compost type and N amendment

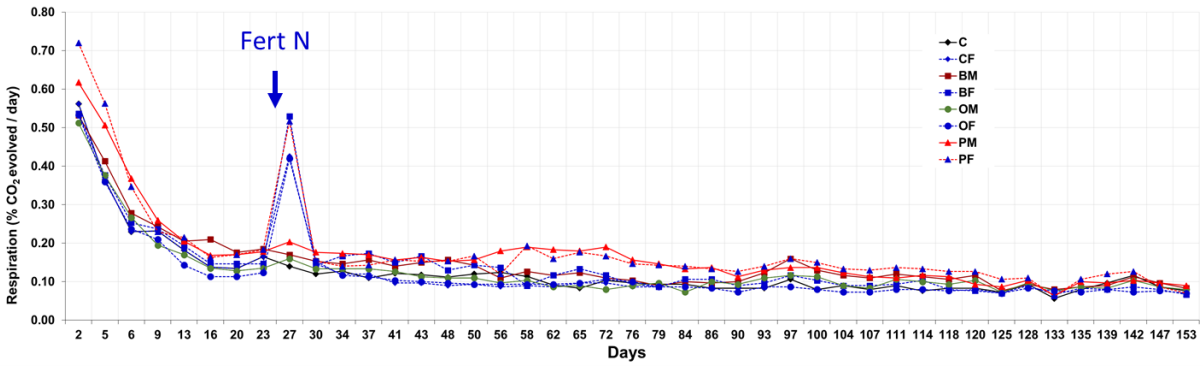


Figure 2.2. Effect of compost addition on cumulative microbial activity in a Vertosol soil in a laboratory incubation experiment (Experiment 1) using soils from field experiment.

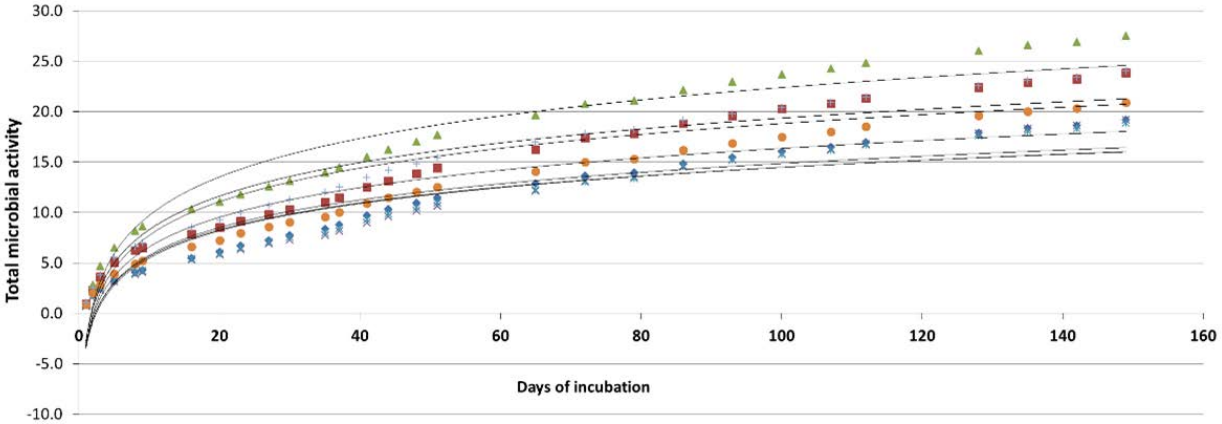
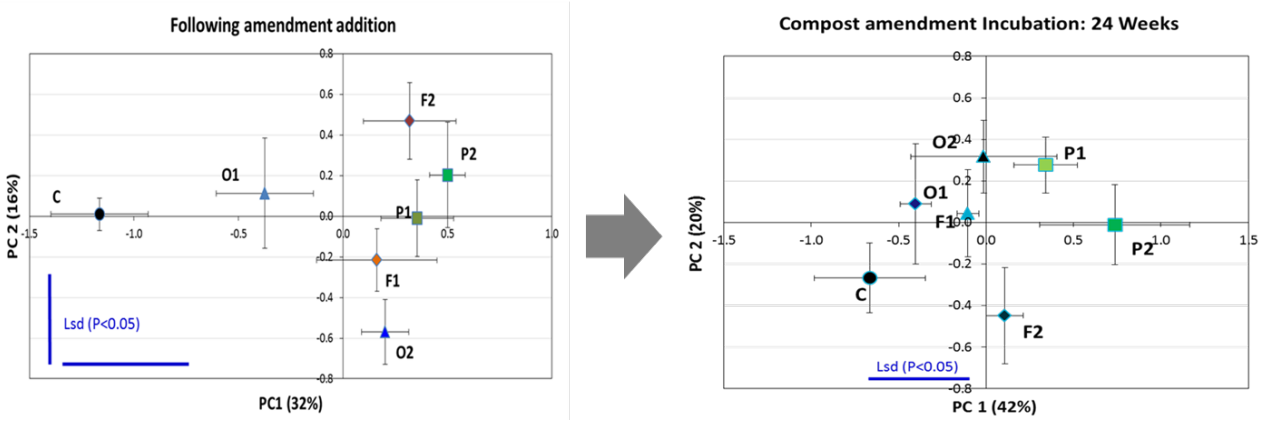
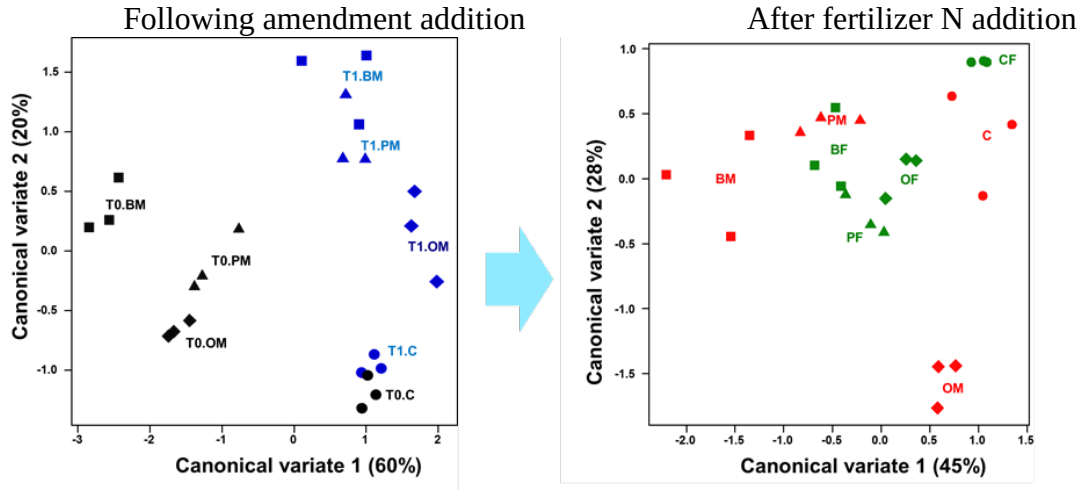


Figure 2.3. Effect of compost addition on microbial catabolic diversity in a Vertosol soil in laboratory incubation experiments using soils from field experiment. Data points closer to each other suggests greater similarity.

(A) Experiment 1 – compost type and rate of addition



(B) Experiment 2 – compost type and fertilizer addition



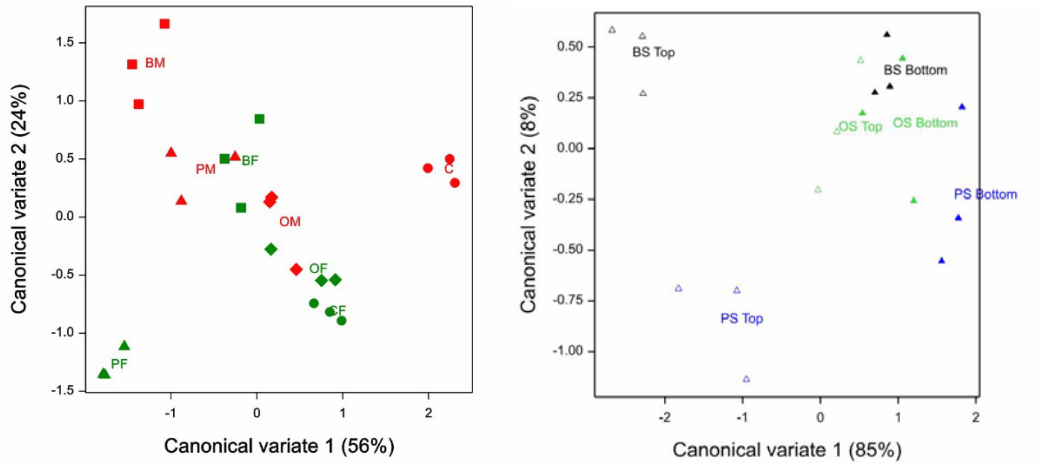
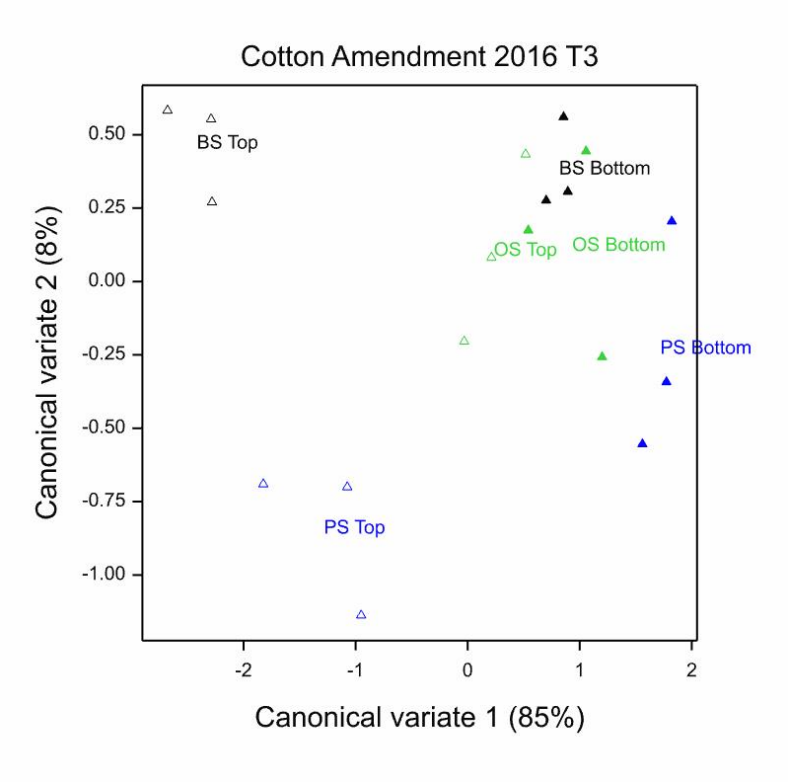


Figure 2.4. Effect of compost addition on microbial catabolic diversity in soil closer to compost and 2.5 cm away (expt 2). (A) Canonical analysis results for the Community catabolic profiles; Data points closer to each other suggests greater similarity and (B) Averages and standard error values for Community metabolic diversity.



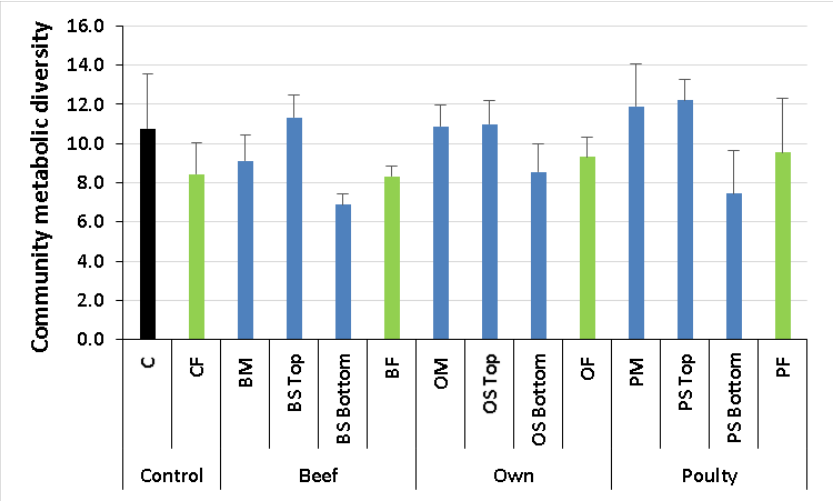


Figure 2.5. Dissolved organic C and mineral N levels in soils as influenced by compost addition in a vertosol soil in a laboratory incubation experiment (Experiment 1).

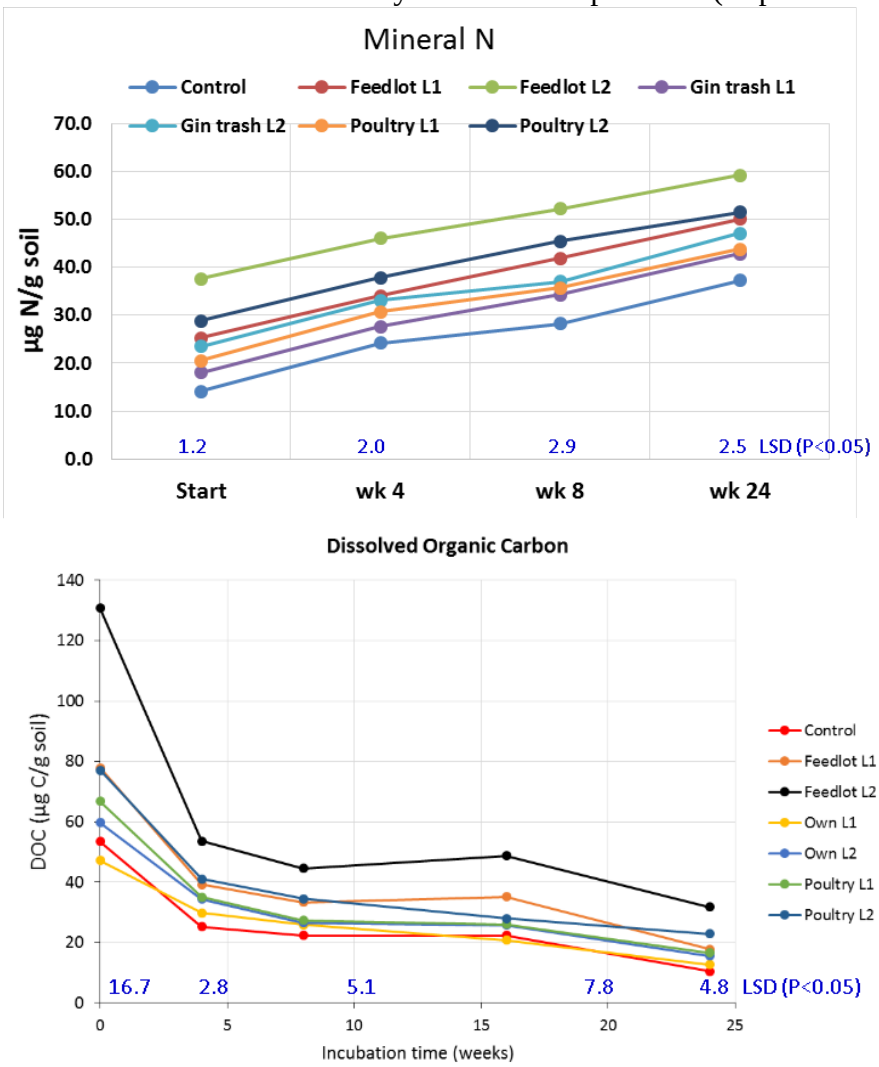


Figure 2.6. Effect of compost addition on microbial biomass carbon in soil.

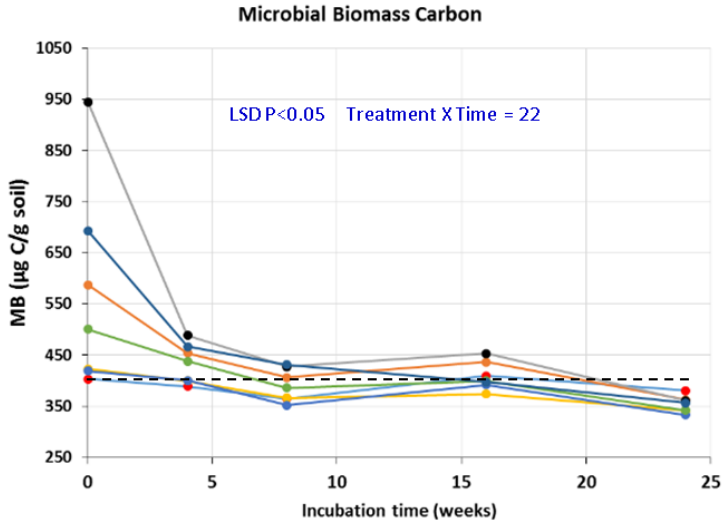
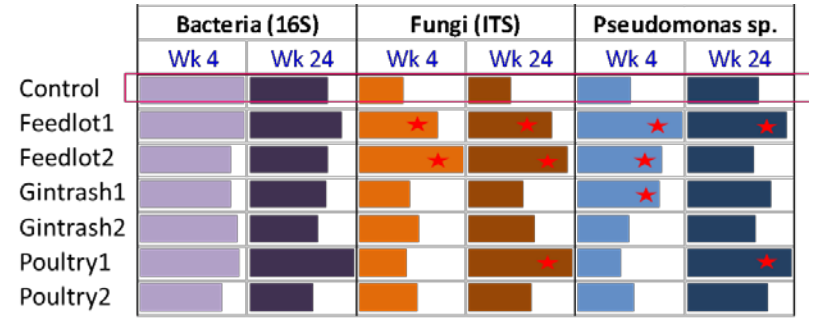


Figure 2.7. Effect of addition of different compost materials on the abundances of (A) bacteria, fungi, Pseudomonas group and (B) microbial groups involved in C and N cycling processes. Compost materials were added at two rates – 1=5 t/ha and 2=10t/ha). In each column bars with stars indicate significant differences with other treatments. Averages of gene abundances at the beginning (week 4) and end of the experiment (week 24).

(A)



(B)

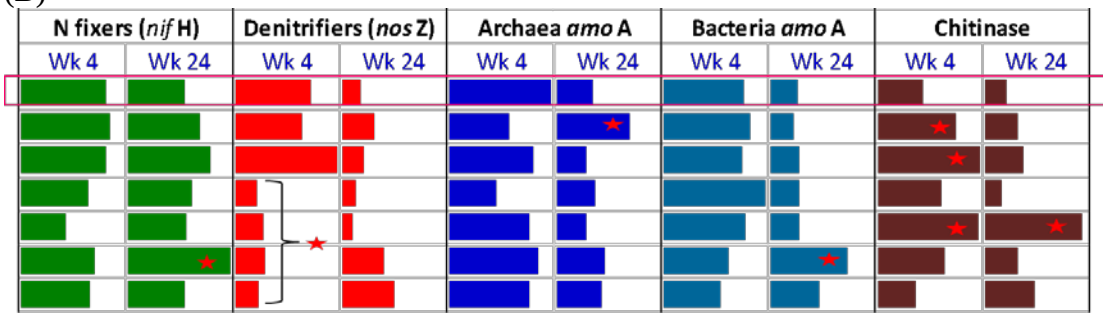
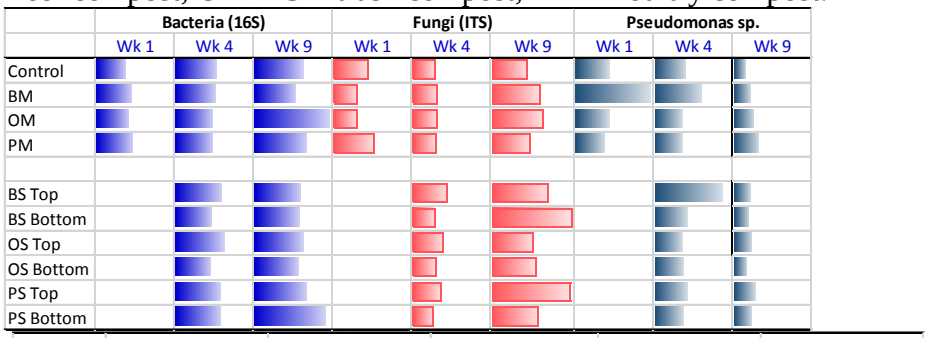


Figure 2.8. Effect of addition of different compost materials on the abundances of bacteria, fungi, and microbial groups involved in C and N cycling processes. Averages of gene abundances (copies / ng DNA) at the beginning (week 1) and week 4 of the experiment 2. BM – Beef compost, OM – Gin trash compost, PM – Poultry compost.



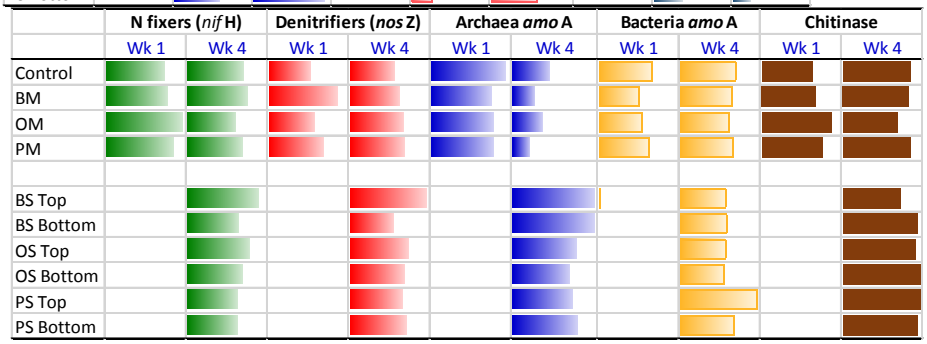


Table 2.7. Chemical properties of surface soils collected in May 2015 after 4 cotton seasons.

Soil chemical properties at May 2015

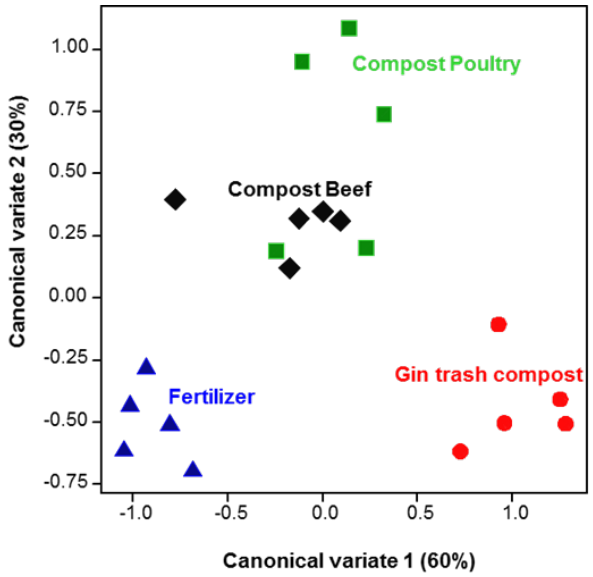
Treatment	Org C (%)		Nitrate N (mg N / kg)		BSES Phosphorus	
	0-10	10-30	0-10	10-30	0-10	10-30
Fertiliser	1.31	1.12	4.6	13.2	357	281
Gin trash Compost	1.38	1.06	6.4	18.0	352	241
Compost Poultry	1.30	1.05	6.0	17.8	490	231
Compost Beef	1.36	1.08	6.0	16.2	423	239
Raw Poultry	1.25	1.09	6.8	30.6	354	205
Raw Beef	1.26	1.04	4.8	10.8	317	226
Treatment	*NS*		*5.07*		*55*	
Depth	*0.04*		*2.93*		*32*	
Treat x Depth	*NS*		*7.17*		*78*	

Table 2.8. Microbial properties in the surface soils from the long-term organic amendment experiment.

Treatment	Microbial Biomass C kg C / ha	Microbial quotient %	N-mineralization potential kg N / ha	Dissolved Org C ug C/g soil
Fertilizer	308.9	2.1	3.25	26.8
Gin trash compost	306.4	2.0	4.21	32.1
Poultry compost	310.2	2.2	3.69	40.7
Beef compost	329.2	2.2	3.86	34.4
F-test	NS	NS	NS	0.02
LSD (P<0.05)	-	-	-	7.8

Figure 2.9. Effect of compost addition on the (A) C-substrate utilization profiles (ability to utilize different types of carbon substrates) of microbial communities and (B) microbial populations in cotton soils. (A) the two dimensional graph from the canonical analysis. Data points closer to each other suggests greater similarity; (B) Microbial populations are represented by gene abundances for 16S rRNA, ITS region and populations involved in N fixation (nifH), nitrification (amoA) and denitrification

(A) Catabolic diversity



(B) Phylogenetic and functional gene abundances (gene copies/ng DNA)

Treatment	Bacteria	Fungi	Actinomycetes	Nitrogenase	Nitrifiers		Denitrifiers
	16S	*ITS*	*16S*	*nifH*	Bacteria *amoA*	Archaea *amoA*	*nos Z*
Standard Fertilizer							
Gin trash @5t/ha							
Poultry @5t/ha							
Feed lot @5t/ha							

Appendix 2: Microbial diversity in surface soils in cotton fields compared to a nearby remnant vegetation: Biomes of Australian Soil Environments (BASE) project

Gupta V.V.S.R.
Database Reference: Bissett et al. *GigaScience* (2016) 5: 21 DOI 10.1186/s13742-016-0126-5

AP 2.1. Background:
The role of soil biota in providing ecosystem functions for sustainable productivity and soil and water resource us is well recognised in the intensive cotton cropping systems in Australia. Modern cotton cropping involves management practices such as reduced tillage, crop residue retention, crop rotation, reduced agrochemical use in order to improve soil biological diversity that supports economic productivity. Soil microorganisms along with fauna mediate carbon and nutrient cycles and play a critical role in disease suppression, degradation of agrochemicals and the maintenance of overall plant health and soil structure. Crop management practices such as crop rotation, tillage, crop residue retention, fertilizer and agrochemical application have been shown to influence the abundance and composition of soil biota communities with potential impact on biological functions (Coleman et al. 2011). Until recently the majority of attention has been given on a small percentage (<10%) of microorganisms because the majority of soil microorganisms are unculturable on generally used laboratory media. However, recent developments in high throughput DNA and RNA-sequencing methods allow the detailed analysis of the total microbial community composition, diversity and functionality of soil microbial communities. Currently, there is very little information available comparing the genetic composition of soil biota e.g. bacteria, archaea, fungi and eukaryotes, in cotton soils with that in remnant native bush soils.

The Biomes of Australian Soil Environments (BASE) project (http://www.Bioplatforms.Com/soil-biodiversity/) has generated a database of microbial diversity with associated metadata across extensive environmental gradients at the Australian continental scale. The main funding for the project was provided by Bioplatforms Australia through the Australian Government National Collaborative Research Infrastructure Strategy (NCRIS) and Education Investment Fund (EIF) Super Science Initiative coupled with support by a number of RDCs, State Government initiatives etc (Bissett et al. 2016). BASE now provides a database of amplicon and metagenomic data), complete with rich contextual information on edaphic, aboveground diversity and climate.

AP 2.2. Methods:
Taking advantage of the BASE project, soil samples collected from the long-term field experiment (F6E - 30°12'4.41"S; 149°35'44.43"E) at ACRI, Narrabri (Continuous Cotton and Cotton-Vetch-Cotton (CVC) roation treatments) and a nearby remnant bush (30°12'14.55"S; 149°35'39.91"E) were submitted for microbial (bacteria, fungi and archaea) and eukaryotic community analysis. The aim of this scoping study was to obtain detailed information on the genetic diversity of soil organisms in cotton fields compared to a remnant bush.
Soil samples were collected from 0-10 cm and 10-20 cm depths using standardized methods (see BASE data portal). Briefly, multiple soil cores collected from different replicates for each treatment (R=4) were pooled to give a bulk sample. Under the remnant bush soil cores were taken in multiple quadrats to give a representative sample. Samples were initially transported to CSIRO Adelaide labs for initial processing and submitted to the Adelaide node of the Australian Genome Research Facility (AGRF) laboratories for all the molecular analyses. Details of the analyses are given in the paper by Bissett et al. (2016). Subsamples of the soils were air dried and the chemical analyses were conducted by CSBP laboratories (Perth, WA), as per the BASE project requirement. Full details of DNA extraction, sequencing and bioinformatic analyses are described by Bissett et al. (2016). Output from the general bioinformatic analysis of the sequence data for cotton soils (Operational Taxonomic Unit – OTU) along with their taxonomic assignment information was used to develop the graphs showing taxonomic distributions and diversity estimates, using Excel and Primer6 software package (Primer-313 E Ltd, Plymouth, U.K.).

AP 2.3. Findings and Discussion:
The BASE database provides a unique resource on microbial diversity in the diverse set of environments at the Australian continental scale. As indicated by Bissett et al. (2016), it is an evolving platform that allows interrogation and integration of microbial diversity and function data across broad scale environmental or ecosystem level comparisons. For the cotton soils, the data provides a first-cut look at similarities/differences in the genetic composition of soil microbial and eukaryotic communities between cotton fields and a remnant bush. As the sampling procedure used to collect samples followed a rigorous protocol to incorporate (overcome) field based heterogeneity, this data provides a reliable summary of the genetic composition of soil biota communities in each of the three systems e.g. Continuous Cotton rotation, Cotton-Vetch-Cotton rotation and Remnant bush soils. However, a note of caution is that, the lack of replication at sequencing level doesn't allow statistical evaluation of the differences between fields.

Data in Figures AP1-AP3 show the genetic composition of bacteria, archaea, fungi and eukaryotic communities at a taxonomic level illustrating observable differences between fields and depths. In general there were clear and discernible differences in the composition of all soil biota groups between cotton fields and the remnant bush. However the field-based differences varied with depths for different communities. For example, a clear variation in soil fungal communities, both at phyla and class level, between fields was seen at both depths (Figure AP2 A&B). But with archaeal communities a variation was only seen at 10-20 cm depth (Figure AP1b).

Bacteria constitute a significant component of microbial diversity in soils and their activities play a critical role in a number of plant-health and nutrition related processes in cropping fields including cotton fields. Research overseas and in Australian cropping fields indicated that a number of abiotic and biotic factors and environmental factors can influence the community structure of soil bacteria and abundances of various functional groups (Coleman et al. 2011). For example, significant correlations have been found between soil pH, organic C and nitrogen level etc and bacterial diversity indices. Data in Figure 1A indicate a clear difference between cotton fields and remnant vegetation at both depths. For example, *Proteobacteria* accounted a larger component of bacterial community in Continuous Cotton fields compared to the CVC rotation and Remnant vegetation field. *Actinobacteria* and *Bacteriodetes* were higher in the surface 0-10 cm soils compared to that in 10-20 cm depth whereas *Chloroflexi* were higher in the Remnant vegetation soils. Bacterial diversity indices (e.g. Margaref species richness, Pilou's evenness index, Shannon diversity index) generally higher in the 0-10 cm soils compared to the 10-20 cm soils in all the fields, and the depth based variation was greater in the 10-20 cm depth soils. Bacterial diversity in soils has been shown to be influenced both by the soil and environmental factors (Fierer and Jackson, 2006). Therefore, the observed differences in the composition and diversity of soil bacteria between cotton fields and remnant vegetation could be partly due to the variation in soil chemical properties. For example, Organic C were generally higher in the Remnant vegetation soils compared to cotton field soils, whereas C:N ratio was narrower and soil pH was lower in the Remnant vegetation soils (Table 1). Surprisingly mineral N (ammonia N and nitrate N) and total N concentrations were considerably higher in the Remnant vegetation soils compared to cotton fields, although crop fields receive annual applications of fertilizers.

There is increasing evidence to show that soil archaea are playing a significant role in the cycling of carbon, nitrogen and plant-fungal interactions (Karlsson et al. 2012). With regards to the soil archaeal communities, members of *Nitrososphaeraceae* accounted for >99% of total archaeal communities, especially in the surface 0-10 cm soils from all fields and the 10-20 cm depth soils from Continuous cotton fields (Figure AP1B). Previous research using soils from the F6E long-term experiment indicated that crop rotation and addition of wheat stubble had a significant impact on the composition and abundance of archaeal communities (Gupta et al. 2014). However, this data only showed crop rotation based differences in the 10-20 cm depth soils. In the self-mulching Vertisol soils wetting and drying events causing the changes in physico-chemical characteristics could impact archaeal communities. Implications of differences in in archaeal community structure observed in this study to soil functions are yet to be fully understood

Members of soil fungi belonging to the phyla *Ascomycota* accounted for the major portion of fungal community (ave. 80%) in the cotton fields but in the remnant vegetation soils they were only 50 and 30% in the 0-10 and 10-30 cm depths. Differences were also seen between the two cotton rotation treatments in the surface 0-10cm depth. In the remnant field soil *Zygomycota* and *Basidiomycota* were a significant part of the soil fungal community. Mycorrhizal fungi are known to be more abundant and diverse in the native and undisturbed ecosystems which is supported by the data showing higher proportion of *Glomeromycota* fungi in the remnant bush soils (>2.2%) compared to the cotton field soils (<1.4%). This is mainly attributed to the P fertilization in cropping fields compared to no P application in the native vegetation. Similarly, *Dothideomycetes* were generally higher in cotton field soils compared to the remnant bush soils. Overall, the Margalef's species richness was higher in the 0-10 cm soil from Continuous cotton rotation compared to the remnant vegetation fields. Previous research by Coleman et al. (2012) and as part of the CRDC project CSE1401 showed cropping history and field location (soil type and environment) have a significant influence on the catabolic diversity of soil microbial communities and abundance of the soil bacterial and fungal populations.

Data in Figure 3 show distinct differences in the eukaryotic community in the cotton soils compared to the remnant vegetation fields, in particular the surface 0-10 cm soils. There was a limited depth based effect on eukaryotic community in the continuous cotton field compared to that seen in the Cotton-Vetch-Cotton rotation and remnant vegetation field. This could be partly attributed to the variation in the quality and quantity of above and below-ground crop residues and root growth patterns. It has been suggested that the intensive cotton cropping systems with higher disturbance may not provide ideal habitat for eukaryotic community in particular soil micro-, meso- and macro-fauna. However, our ability to make function related interpretation of the 18S-sequence based data is limited.

AP 1.4. Conclusions and Implications:

Data from this scoping study has provided a clear indication of differences in the composition of soil bacterial, archaeal, fungal and eukaryotic communities between soils from cotton field soils and remnant vegetation field. The general trends in the bacterial and fungal communities in cotton experiment soils are similar to those previously observed, e.g. Ascomycetes comprise the majority of soil fungal communities. Also, the presence of archaea from just one family raises the question about the true functional role of this group of organisms and their resilience. Soil archaea have been suggested to have a significant role in GHG gas (e.g. methane) emissions). Findings from grain cropping systems have indicated that Actinobacteria generally dominate soil bacterial community, whereas the Proteobacteria were the major group in all the soils in this study. The functional significance of this requires investigation, especially, a number of Proteobacteria have been shown to possess abilities related to plant growth promotion, disease suppression etc.

As a note of caution, the sequencing analysis conducted in this study is very limited and multi-site and well replicated analyses is warranted in order to confirm these preliminary observations. Additionally, all the work presented here is on the taxonomic / phenotypic composition of soil biota communities only. Further research that includes functional based analyses, e.g. metagenomic analysis, functional assays, and using soils from multiple sites (soils types, cotton growing regions) is required in order to derive new knowledge that aids in better harnessing of soil biological capability using the power of new analytical techniques.

Acknowledgement:

The author of this report extends his heartfelt appreciation to the Late Ian Rochester for his help with the collection of soils and general discussion with the selection of the Remnant vegetation field.

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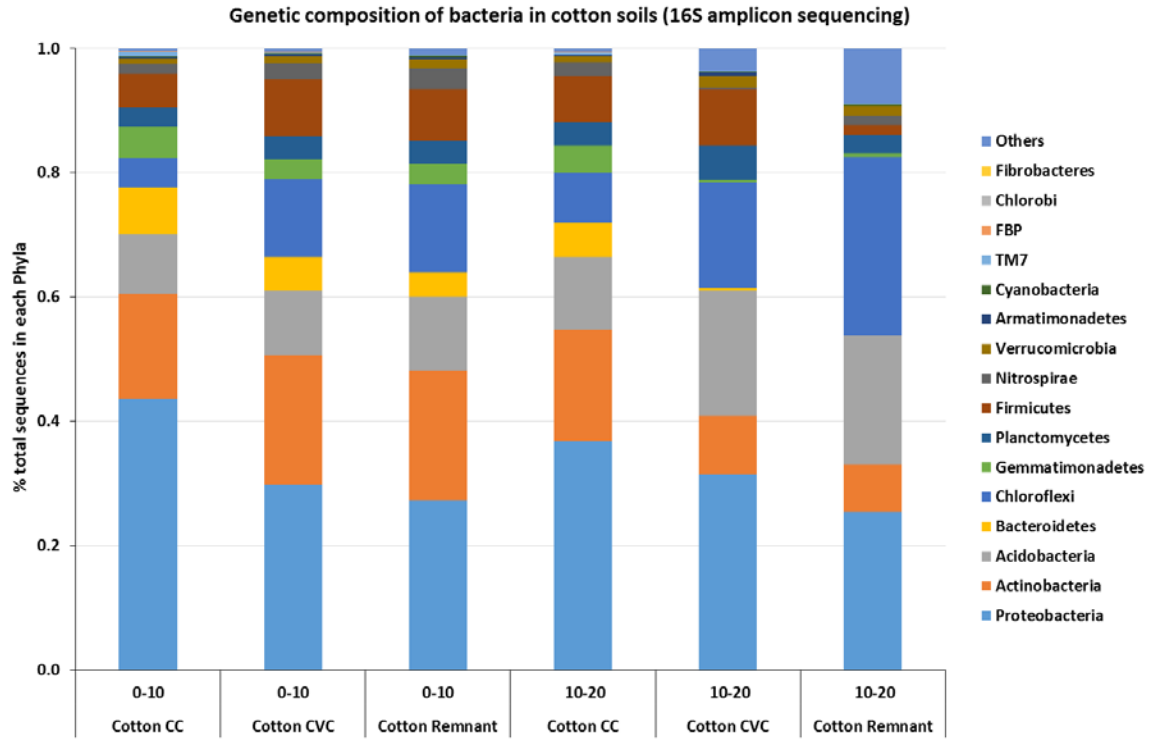
Table AP1. Soil chemical properties

Site	Depth	Ammonium N	Nitrate N	Colewell P	Colwell K	Sulphur	Organic Carbon	Conductivity	pH	Total N	C:N ratio
		mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	%	dS/m	(H2O)	%	
Continuous cotton	0-10	19	10	109	660	31.3	1.43	0.096	8.0	0.11	13.00
Cotton Vetch Cotton	0-10	32	20	128	745	60.8	1.52	0.200	7.1	0.09	16.89
Cotton Remnant	0-10	77	151	230	1512	34.4	4.90	0.516	6.4	0.72	6.81
Continuous cotton	10-20	15	2	91	543	13.1	1.14	0.075	7.9	0.06	19.00
Cotton Vetch Cotton	10-20	20	3	78	507	16.9	1.25	0.118	7.9	0.09	13.89
Cotton Remnant	10-20	46	52	137	1288	16.6	2.74	0.187	7.5	0.20	13.70

Site	Depth	DTPA Copper	DTPA Iron	DTPA Mn	DTPA Zinc	Exc. Al	Exc. Ca	Exc. Mg	Exc. K	Exc. Na	Boron Hot CaCl2
		mg/Kg	mg/Kg	mg/Kg	mg/Kg	meq/100g	meq/100g	meq/100g	meq/100g	meq/100g	mg/Kg
Continuous cotton	0-10	1.69	13.65	23.06	1.21	0.212	24.50	7.94	1.75	0.53	1.50
Cotton Vetch Cotton	0-10	1.66	12.06	47.40	1.56	0.118	20.64	6.99	1.91	0.32	1.49
Cotton Remnant	0-10	2.22	33.42	110.24	5.38	0.102	37.22	8.53	3.86	0.14	3.51
Continuous cotton	10-20	1.84	13.95	18.30	0.84	0.134	20.17	6.87	1.39	0.54	1.35
Cotton Vetch Cotton	10-20	2.49	10.94	17.59	0.87	0.187	20.91	7.25	1.30	0.37	1.25
Cotton Remnant	10-20	2.17	19.53	36.85	1.83	0.133	27.95	6.14	3.03	0.14	1.98

(A)



(B)

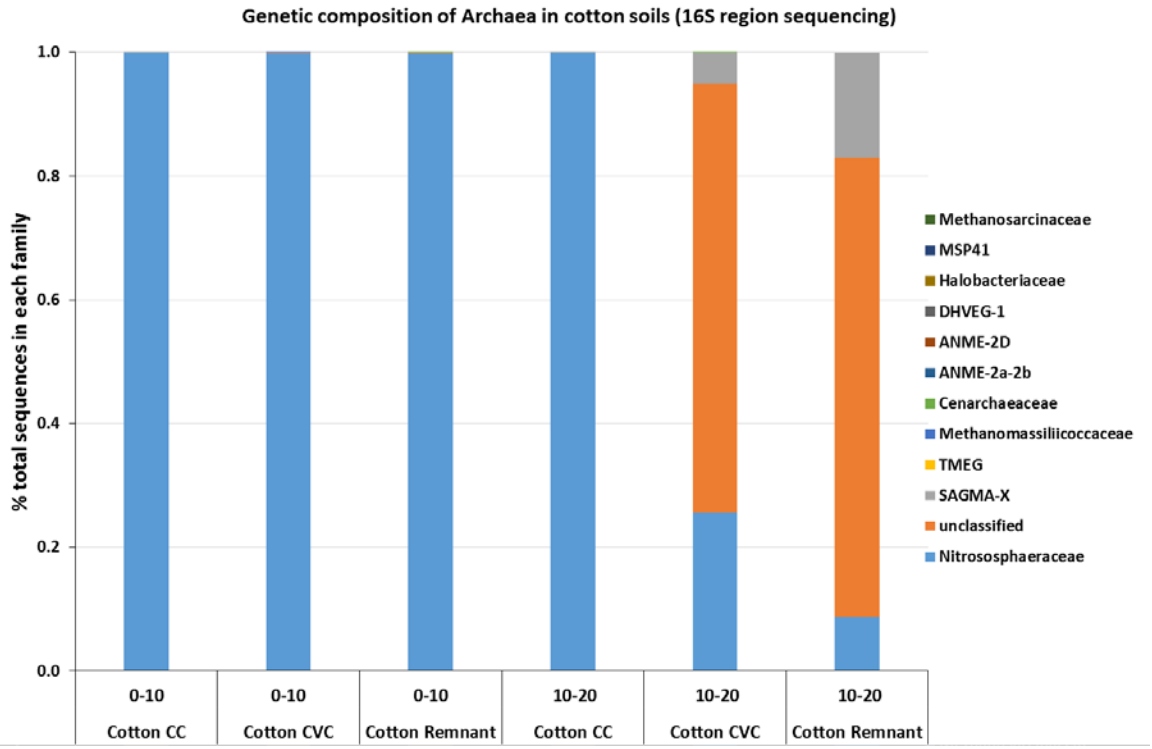
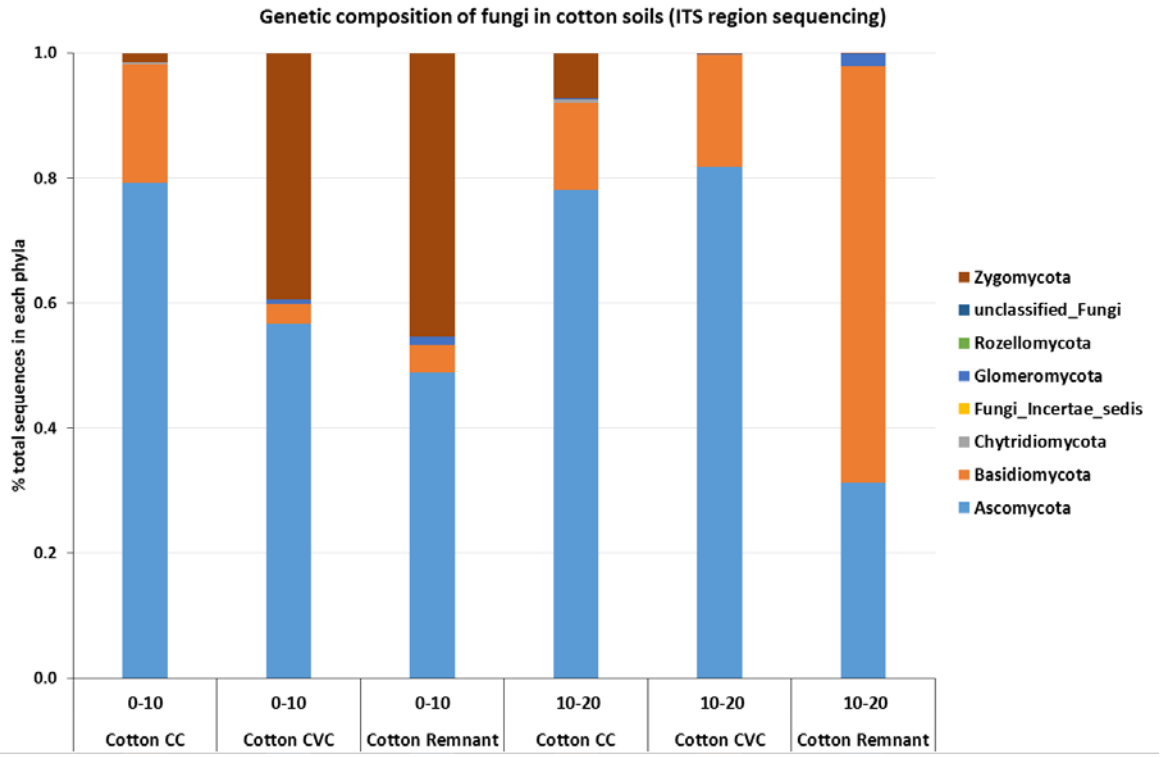


Figure AP1: The composition of soil bacterial and archael communities in soils from the long-term cropping system experiment (F6E) and the remnant vegetation at ACRI, Narrabri, NSW: (A) Bacterial phyla as % of total bacterial 16S rRNA gene amplicons-OTUs and (B) Archaeal families as % of total archaeal 16S rRNA gene amplicons (OTUs classified using Green Genes database (13-5)).

(A) At Phyla level



(B) At Class level

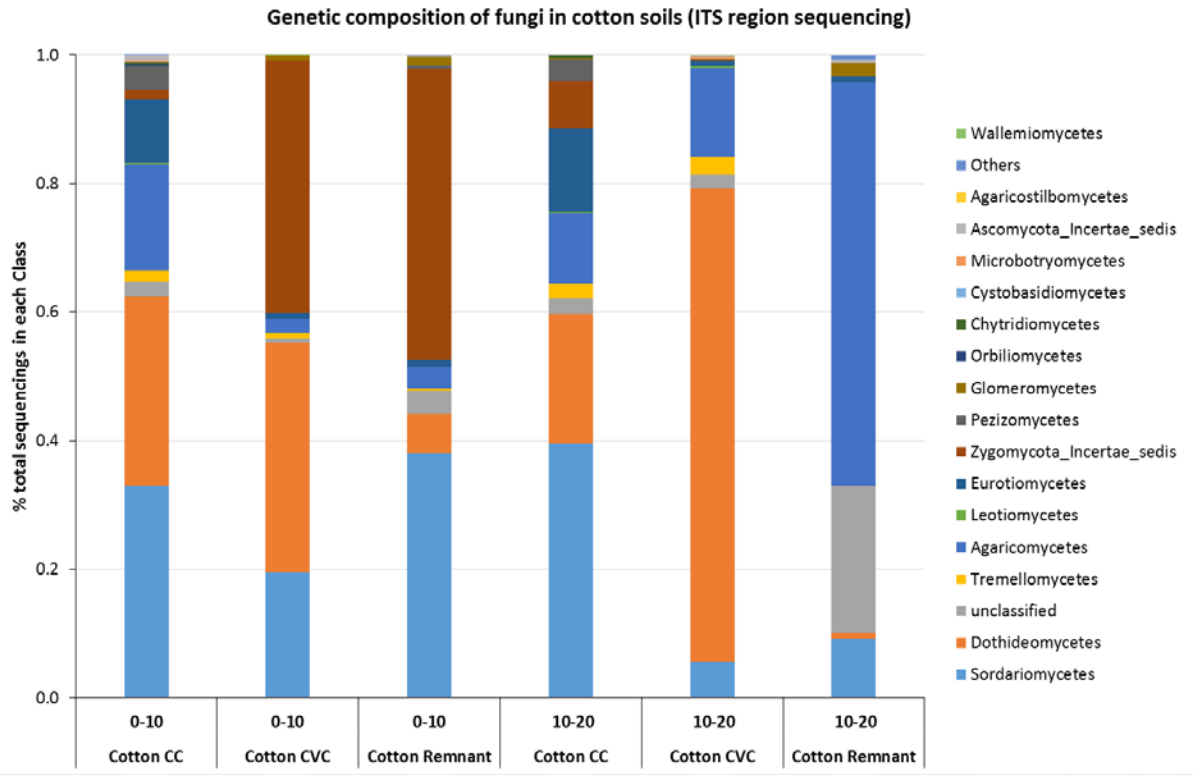


Figure AP2: The composition of soil fungal communities in soils from the long-term cropping system experiment (F6E) and the remnant vegetation at ACRI, Narrabri, NSW: Fungal phyla as % total fungal ITS1 region amplicons (OTUs classified using UNITE database (v7.0)).

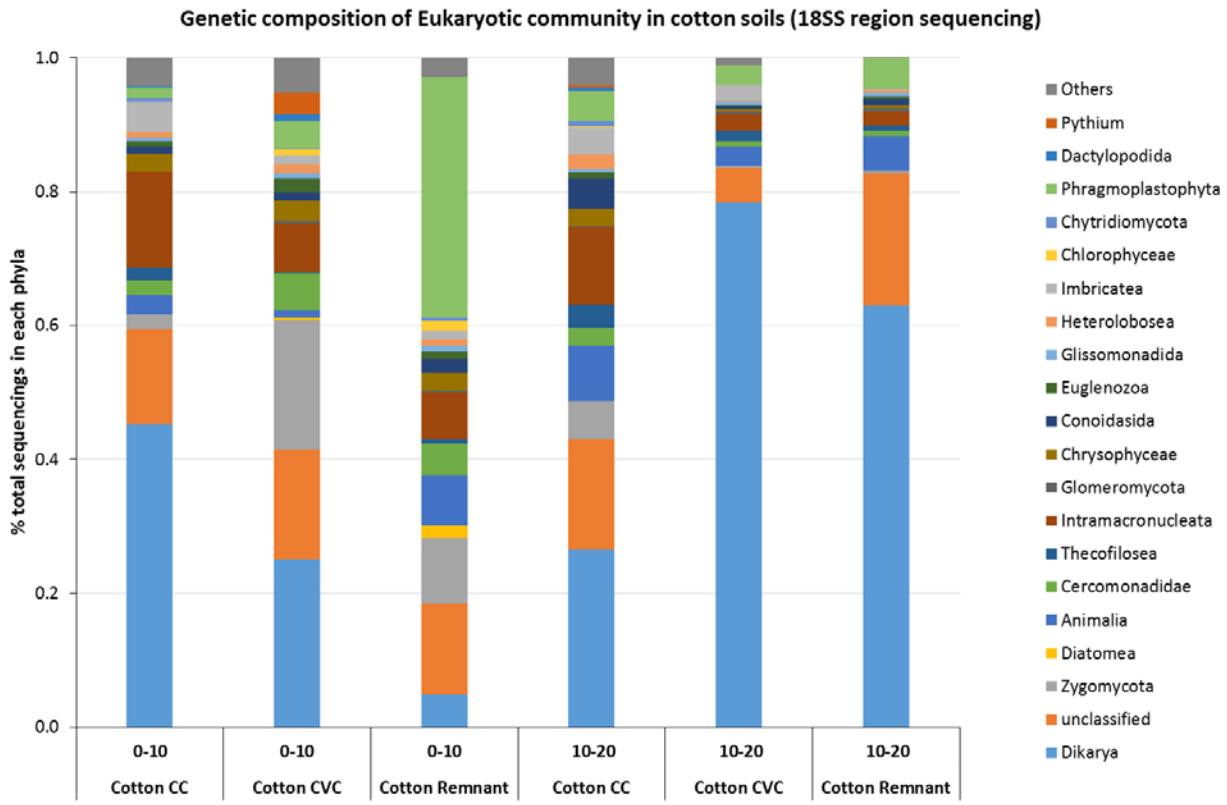


Figure AP3. The genetic composition of soil Eukaryotic communities in soils from the long-term cropping system experiment (F6E) and the remnant vegetation at ACRI, Narrabri, NSW: Eukaryote phyla (comprising >1% in at least one sample) as % total 18S amplicons (OTUs classified using SILVA (123)).

Figure AP4. Image of the field sites from where the soils were collected.