Office Use Only	
Project Code	
Project Type	

FINAL REPORT 2021

Applicants must read the *SAGIT Project Funding Guidelines 2021* prior to completing this form. These guidelines can be downloaded from www.sagit.com.au

Final reports must be emailed to admin@sagit.com.au as a Microsoft Word document in the format shown ***within 2 months*** after the completion of the Project Term.

PROJECT CODE	:	**CS1118**

PROJECT TITLE	Improved capture of native soil nitrogen and urea fertiliser in wheat

PROJECT DURATION

*These dates **must** be the same as those stated in the Funding Agreement*

Project Start date	**July 1, 2018**					
Project End date	Dec 30, 2020					
SAGIT Funding Request	18-19		19-20			

PROJECT SUPERVISOR CONTACT DETAILS

The project supervisor is the person responsible for the overall project

Title:	**First Name:**	**Surname:**
	Gupta	Vadakattu
Organisation:		
CSIRO Agriculture and Food		
Mailing address:		

Telephone:	**Facsimile:**	**Mobile:**	**Email:**

PROJECT REPORT

Provide clear description of the following:

Executive Summary (200 words maximum)

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

This project investigated the links between wheat N uptake and microbial gene regulators of N cycling in the root and rhizosphere of wheat varieties varying in root growth and efficiency of N uptake using established and new varieties agronomically adapted to SA conditions. Field and controlled environment experiments were conducted to quantify variations in microbial biomass, activity and populations of different N-cycling microbes and their response to N-fertilizer application. Rhizosphere soils in all the experiments indicated differences both in the total microbial activity and microbial catabolic diversity, suggesting that belowground C inputs (quantity and quality) varied with variety. Findings in this project provide the first report of significant varietal based variation in N-cycling microbes, involved in the different N cycling processes such as mineralization of organic N, conversion of urea N into ammonia N, nitrification and non-symbiotic N_2-fixation, in wheat crops under realistic field conditions. But, the magnitude of the variation between varieties was soil dependant. significant relationship between increase in wheat yields from older to newer varieties and variations in microbial groups involved in nitrification and organic N mineralization was observed. These findings raise the potential for breeding this trait into modern, elite cultivars of wheat. Successful exploitation of this approach requires selection of germplasm that more readily associate with beneficial microbiomes.

This project also demonstrates the potential for using sophisticated DNA based methods to quantify changes in microbial groups in field-based experimentation.

Project Objectives

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

To identify traits in varieties that enhance the efficiency of nitrogen utilization in South Australia adapted wheat varieties by comparing wheat varieties agronomically adapted to SA, featuring an established difference in nitrogen uptake per unit root length. Specifically to:

i) Screen varieties for rhizosphere traits including microbial activity, N mineralization and nitrogen uptake in field experiments conducted in SA.
ii) Quantify of differences in the abundance of key microbial functional genes involved in N cycling and availability and relate them to variation in N uptake by plants.

Overall Performance

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

Project Team:

Vadakattu Gupta, CSIRO – Project Supervisor and Researcher

Marcus Hicks (CSIRO) Research officer

Stasia Kroker (CSIRO) Research officer

Victor Sadras (SARDI) Researcher
Mariano Cossani (SARDI) Research officer

As per the original project plans, 3 field experiments and 2 controlled environment experiments were successfully completed, and the proposed project objectives achieved. The unexpected COVID19 related workplace restrictions resulted in completing the 2nd controlled environment experiment in 2020 and some of the laboratory analysis of field samples within the original timeline. An extension of the final end date to Dec 2020 allowed the proper completion of all of the experiments and analyses.

Key Performance Indicators (KPI)

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

KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
Main Contract with SAGIT and subcontract with SARDI signed	Yes	
Establish, maintain, monitor and harvest plots of core trial (60 plots). Sample collection from field experiment completed (234 samples). Initial processing of soil samples for DNA extraction microbial gene abundance analysis completed.	Yes	
Laboratory analysis of all soil samples and analysis of plant biomass, grain yield and N uptake completed. Annual report submitted. Experimental protocols for 2nd year experiments finalized.	Yes	
Second year field sampling completed and processing of soil samples for DNA extraction microbial gene abundance analysis, plant biomass and N uptake completed.	Yes	
All soil and plant sample analysis and data processing completed.	Yes	
Industry paper communicating 2019 results through AgCommunicators.	No	Presented in talks at GRDC updates, proposed an article for industry paper
Final report submitted to SAGIT	Yes	

Technical Information (Not to exceed **three** pages)

Provide sufficient data and short clear statements of outcomes.

1. Methods and Materials:

A total of three field experiments (Roseworthy 2018 & 2019, Riverton 2019) were conducted evaluating 15 wheat varieties and all experiments received agronomic operations that are considered normal for this region (more details in the Table A1). In addition, two controlled environment (CT) experiments were conducted using (i) a red-brown earth soil from 2018 field site and (ii) a Mallee sandy soil from Loxton for in-depth analysis of varietal based differences and the influence of fertilizer N application on N cycling microbes associated with roots and rhizosphere. Rhizosphere soil (soil

surrounding roots) and root samples collected at 10 (field experiments) to 12 weeks (Controlled environment expts) after sowing were analysed for microbial biomass (Sparling et al. 199X), microbial catabolic diversity (Gupta et al. 2019) and abundances of total bacteria (16S rRNA), fungi (ITS region) and microbial groups involved in different N cycling processes (functional gene qPCR). Total plant biomass, grain yield and N uptake were measured at harvest (field expts) or 12 weeks after sowing (CT expts). Soils from pot experiments All the data analysed using standard statistical methods (Genstat v19.1, VSN Intl Ltd; Primer-e, v7.10.7, www.primer-e.com).

Both the controlled environment pot experiments plants were grown on a 10h day (25 C)-14 hnight (20 C) cycle with soil moisture maintained at 70-80% field capacity. The CT experiment testing variety x N level interactions was conducted using a red-brown earth soil (Roseworthy: Org C-2.4%, Total N-0.195%)) and a Mallee sand (Loxton: Org C – 0.5%, Total N-0.39%). Pots for +N treatments received Urea @50kgN/ha. At harvest (~12 weeks after sowing) rhizosphere and root samples were analysed for microbial and biochemical properties and plant biomass and N uptake were also measured. Field experiments at Roseworthy were on a Red Brown Earth soil (org C - 1.9 to 2.1%, pH - 8.2) and at Riverton on a Sandy loam soil (org C - 2.15%, pH - 5.35). At sowing, mineral N levels in 1M soil profile were 95 to 106 kg N/ha. Microbial biomass (MB)-N levels were 80 to 116 kg N/ha at Roseworthy experiments (2018 & 19, respectively) and 50 kg N/ha in the Riverton (2019) experiment. Both crop seasons were generally dry (~decile 5) with average grain yields of 2.21±0.07 t/ha (2018) and 2.61 ±0.08 t/ha (2019) at Roseworthy and 2.33 ±0.09 t/ha (2019) at Riverton experiments.

Table 1. Details of field and controlled environment experiments conducted.

Experiment	Year	Site/Soil	# cultivars
Field #1	2018	Roseworthy	13 Wheat
CT expt #1	2019	Roseworthy	14 - Wheat & 2 - Durum Wheat
Field #2	2019	Roseworthy	14 - Wheat
Field #3	2019	Riverton	15 - Wheat
CT expt #2	2020	Roseworthy	5 Wheat & 1 Durum x 2 Nitrogen rates
		Loxton	5 Wheat & 1 Durum x 2 Nitrogen rates

1. Significant and systematic variation in crop performance in terms of grain yield and N uptake

In general, there was a significant and systematic variation in grain yields between the 14 varieties, i.e. 0.60 to 0.76 t/ha yield increase in newer varieties (e.g. Scepter, Mace, Gladius) compared to that for older varieties (Heron, Gamenya, Halberd) in all three experiments. The varieties also varied significantly in a number of yield attributes such as harvest index and thousand grain weight. Differences in total plant biomass didn't always follow the same trend as grain yield in all experiments. These results for crop performance generally confirmed the previous reports that selection of cultivars/varieties since 1950s has steadily increased wheat grain yield due to changes in some key yield contributing factors (Aziz et al., 2017; Sadras et al., 2016; Sadras and Lawson, 2013). The trend of higher crop yields with recent varieties was also reflected in total N uptake.

Wheat varieties developed during 50's – 60s with greater root biomass compared to recent varieties (Aziz et al. 2017; Corneo et al. 2016). It has been suggested that breeder's selection of cultivars mainly for yield and grain quality has led to significant changes in wheat plant phenotype including changes to root system size, e.g. root length, root biomass etc. However, previous research by Aziz et al. (2017) with these varieties

has shown an overall reduction in root system size but an increase in N uptake per unit root length. Detailed analysis of root tip dynamics and distribution of roots through soil profile didn't provide consistent trends, as soil profile characteristics can significantly influence local root distribution. It was there hypothesized that rhizosphere microbial properties may have a role in the differences in N uptake between older and newer varieties.

2. Significant variation in total microbial biomass, catabolic diversity in rhizosphere soils

Results for the amount of microbial biomass C and N in the rhizosphere soils of the various varieties showed significant differences in the 2018 field trial only but differences were not significant in the 2019 field experiments. Microbial biomass levels were generally higher in the Roseworthy soils (550 to 1250 ug MB-C / g soil) compared to that in the Riverton field soil (<350 MB-C/g soil). In general, there was no systematic trend in the amount of MB, similar to that seen in grain yield, from older to newer varieties. Microbial biomass in the rhizosphere soil is mainly dependant on the C released through root exudation and root turnover. Analysis of results from microbial community metabolic profiling (CLPP) assays indicated significant differences in the CLPP and catabolic diversity of different varieties in all the three field experiments. The finding of significant variation in rhizosphere microbial catabolic diversity suggests that different varieties may vary in terms of the quantity and quality of root exudation. However, the lack of systematic trend in microbial activity and catabolic diversity from older to newer varieties suggests that the reduction in root biomass may not have directly translated into increased root exudation and root turnover. These results suggest that soil type based variation in microbial biomass and turnover is a dominant factor in determining the N availability in wheat crop and the varietal base differences may be secondary in terms of microbial turnover and its direct contribution to N mineralization.

3. Abundances of N-cycling microbial groups in rhizosphere soils were significantly different between different varieties

In general, there were significant varietal based variations between rhizosphere soils of different wheat varieties in terms of the abundance of various microbial groups involved in the different N cycling processes [mineralization of organic N (npr, apr), conversion of urea N into ammonia N (*ure*C), nitrification (B-*amo*A, A-*amo*A) and non-symbiotic N_2-fixation (*nif*H)]. Significant varietal based variation in the abundances of N-cycling functional genes in the field experiments were further confirmed from the observations in the two pot experiments including with fertilizer N application. The varietal based differences in gene abundances varied for different N-cycling genes and the trends were not the same across all the functional genes (e.g. R^2=0.51, P<0.05, Roseworthy 2019). Additionally, absolute abundances and magnitude of variation between varieties for different functional genes varied between the field experiments suggesting a soil type based variation. In general, there was a systematic increase in B-amoA gene abundance from older to newer varieties similar to that observed in grain yields. Application of Urea N fertilizer caused a significant increase in nitrifying bacteria (B-amoA) both in the Roseworthy red brown earth and Loxton Mallee sand, although the N fertilizer effects on other groups varied between the two soil types. Fertilizer application generally didn't mask the varietal based variation. Soil type, in terms of physico-chemical properties (e.g. biologically available organic C, soil texture), can have a significant influence on the composition and abundances of total microbial communities which seems to have

reflected in N-cycling microbes. Also, varietal performance in terms of root growth can differ based on soil structure and other properties influencing populations of N-cycling microbes. Overall, results for the abundances of various N-cycling microbial groups indicate significant varietal based variation that was not masked by differences in soil properties and seasonal effects.

Table 1. Summary of results for the statistical significance of varietal based variation in the various microbial properties in the rhizosphere soils from the field and controlled environment experiments.

Biological property	Field trial	Field trial	Field trial	Pot Trial	2020 Pot trial					
	2018	2019		2018	Roseworthy			Loxton		
	Roseworthy	Roseworthy	Riverton	Roseworthy	Variety	Fertilizer	VxF	Variety	Fertilizer	VxF
Microbial Biomass C	Not sig	Not sig	Not sig	0.014	0.03	0.011	Not sig	Not sig	<.001	Not sig
Average Metabolic Response (AMR)	0.004	0.001	<0.001	Not sig	0.006	<0.001	0.006	<0.001	Not sig	Not sig
Community Metabolic Diversity (CMD)	0.003	0.007	<0.001	Not sig	0.007	Not sig	Not sig	Not sig	Not sig	0.02
Respiration	Not sig	Not sig	Not sig	Not sig	Not sig	Not sig	Not sig	Not sig	<.001	Not sig
npr	<0.001	Not sig	<0.001	0.001	Not sig	Not sig	0.03	Not sig	Not sig	Not sig
apr	<0.001	Not sig	<0.001	0.001	Not sig	Not sig	Not sig	0.004	Not sig	Not sig
B-ureC	0.023	0.045	0.017	Not sig	0.05	Not sig	Not sig	<0.001	Not sig	Not sig
F-ureC	-	0.072	<0.001	-	0.05	Not sig	0.004	0.51	Not sig	Not sig
B amoA	<0.001	0.05	0.003	0.001	<0.001	0.04	Not sig	0.01	Not sig	Not sig
A amoA	0.030	Not sig	Not sig	0.001	0.013	Not sig	0.026	0.008	0.002	Not sig
nifH	0.002	0.03	0.005	0.001	0.047	0.009	Not sig	<0.001	<.001	0.003
Total amoA	0.030	0.05	0.027	0.001	0.028	0.007	Not sig	<0.001	<.001	0.003
A/B ratio	0.023	0.05	0.05	<0.001	0.011	Not sig	Not sig	0.001	0.002	0.004
Rate of Nitrification	0.045	Not sig	Not sig	<0.001	Not sig	<0.001	Not sig	<0.001	<0.001	<0.001

4. Rhizosphere microbial properties showed significant relationship with grain yield and N uptake

It is known that rhizosphere and root associated microorganisms are vital for the cycling of nutrients and mediate the availability of N for plant uptake. Results in this project are the first to show a significant varietal based variation in N cycling microbes between wheat varieties in realistic field conditions. Although, there were significant varietal based differences in the amount of MB and microbial activity, there was no significant relationship between variations in grain yield and N uptake. In the field experiments on red-brown earth soils at Roseworthy during 2018 and 2019, a significant positive relationship was observed between nitrifying bacterial populations (indicated by B-amoA gene abundance) and grain yield and N uptake. For example, in the 2018 experiment variation in wheat grain yield was significantly correlated to the abundance of nitrifying (B-amoA and A-amoA) and ammonifying (ureC) microorganisms (multiple $R=0.786$; $P<0.02$). Whereas, in the field experiment at Riverton, grain yield and N uptake differences were significantly related to organic N mineralizing microbes, i.e. alkaline peptidase (apr gene) harbouring microbes. This could be partly attributed to the low in soil organic C (0.5%) and general N mineralization potential in this soil, thereby microorganisms involved in organic N mineralization were found to have significant influence on crop performance.

Conclusions Reached &/or Discoveries Made (Not to exceed <u>one</u> page)

Please provide concise statement of any conclusions reached &/or discoveries made.

Findings from the field and CT experiments, using a set of 13 wheat varieties developed over 50 years and agronomically adapted to SA, provide the first report of significant varietal based variation in N-cycling microbes in the rhizospheres of wheat crops under realistic field conditions. The project also identified significant positive relationship between the increase in wheat grain yields for wheat varieties released between 1958 and 2015 and the N-cycling microbes involved in nitrification and organic N mineralization.

Previous research by Aziz et al. (2017) has suggested that the increasing yields and N uptake (total and per unit root length) in the recently released wheat varieties was due to the progressively increasing the efficiency of their root system in capturing N. This project provides evidence suggesting that the increased N uptake by the newer varieties may partly linked to the changes in the specific N-cycling microbes involved in N mineralization. Additionally, it was suggested that the reduction in root biomass in the newer varieties may have resulted in less carbon being released. Carbon released by roots through exudation and root turnover provides a key source of energy for rhizosphere microbial communities. Results observed in this study for the amount of microbial biomass carbon, microbial catabolic diversity and potential suggest no reduction in root released C in the newer varieties.

This project also demonstrates the potential for using sophisticated DNA based methods to quantify changes in key microbial groups involved in N cycling and availability to cereal crops in field-based experimentation. The methodology used in this project could form a framework for the use of DNA-based methods for other microbial groups.

Intellectual Property

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

Findings from this study are presented in grain industry grower meetings and scientific conferences and published in scientific journals hence available to other researchers and end users. Also, methods used are reported in publications hence there is no potential for commercialization.

Application / Communication of Results

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

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

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

This project provided foundation knowledge on the varietal based differences in microbial communities and its implication to N availability and NUE in wheat.

- Wheat varieties vary in terms of microbial communities that regulate different N-cycling processes and N availability to plants thereby influencing grain yield and N uptake.
- The amount of microbial biomass and catabolic diversity levels in the rhizosphere soils are significant different between older and newer varieties developed the last 50 years.
- Higher N uptake per unit length by the newer wheat varieties is linked to the increased populations of specific microbial groups involved in nitrification and organic N mineralization.
- Application of N fertilizer didn't mask the effects of varietal based variation in N-cycling microbes, but the magnitude of effect was higher in the low organic matter Mallee sand compared to a red brown earth soil.

Findings from this project indicate the potential to manage N availability to wheat crops through manipulation of N-cycling microbial communities. Also, the presence of

significant varietal based variation suggests that breeders may consider incorporating host genetics for specific microbial communities as part of future varietal development.

Publications and presentations:
Gupta VVSR, Hicks M, Kroker S, Cossani M and Sadras V (2021) Rhizosphere functional microbiomes drive N availability to wheat. Oral presentation accepted for presentation at the International Nitrogen Initiative scheduled for May 30th-June4th 2021, Berlin, Germany.
Gupta V.V.S.R. (2019) Plant-Microbiome interactions: Opening the biota black box. Invited key note talk at the Rhizosphere 5 Conference, Saskatoon, Canada during July 7-11.
Gupta VVSR, Sadras V and McBeath T (2019) Factors that drive N availability in wheat; including soil microbiology; Presentation at the GRDC Research update on Feb 12th 2019, Adelaide.

POSSIBLE FUTURE WORK

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

New findings from this pilot study, showing significant variation in the abundances of key microbial functional groups involved in N cycling and availability (gene x variety interaction) for high nitrogen use efficiency in the newer varieties of wheat could be extended to (i) NVT wheat trials and other crop variety trials to investigate the extent of such differences in different agro-climatic regions and soil types and (ii) detailed investigations using mapping lines to identify host genetics factors (e.g. QTLs) for root/rhizosphere N cycling microbes. Once established this information could be adapted through varietal development programs by the Plant Breeding companies with an aim to develop high NUE varieties through the selection for specific N-cycling microbes.

APPENDIX:

Plant's microbiome, above- and below-ground microbiomes, is an integral part of the wider plant genome and is now considered as the extended phenotype of all plants. Microorganisms associated with rhizosphere and roots regulate the cycling of nutrients in soil and microbial processes mediate the availability of key macro (N, P and S) and micronutrients to plants. Nitrogen inputs account for a large part of production costs for Australian wheat crops and involve the risk of unrealised profit in dry seasons. We investigated links between growth, yield and N uptake and microbial gene regulators of N cycling in the rhizosphere and roots of wheat varieties varying in root growth and N uptake efficiency using established and new varieties agronomically adapted to SA conditions (Aziz et al., 2017).

Plant genotype shapes the root microbiome composition hence microbial diversity in the rhizosphere is generally lower than in the bulk soil due to selective recruitment, enrichment or inhibition of specific members. This plant based variation in microbial diversity, composition and functions has been shown to occur between different crop types and to some extent their cultivars (Gupta and Sharma 2020). The degree with which crop/cultivar based differences in rhizosphere and root microbes has also been shown to vary with soil habitat e.g. soil type and management. Plant genotype effects on root microbiome composition has been reported in total microbial community (i.e. bacteria and fungi) composition and with some of the specific functional groups involved in nutrient cycling. Therefore, successful exploitation of soil microbial capabilities for improved nutrient availability and uptake could happen from the development of designer plant-microbiome combinations through selection of germplasm (e.g. new varieties) that more readily associated with specific beneficial microbiomes.
The objectives of this project were:

i) Screen varieties for rhizosphere traits including microbial activity, N mineralization and nitrogen uptake in field experiments conducted in South Australia.
ii) Quantify of differences between varieties in the abundance of key microbial functional genes involved in N cycling and availability and relate them to variation in crop N uptake.

To address this, we measured differences in microbial biomass, activity and populations of various functional groups of microorganisms involved in N cycling processes. The populations of N-cycling microbial groups were estimated based on the abundances of relevant functional genes using quantitative-PCR techniques.

2. Methods and Materials:

A total of three field experiments (Roseworthy 2018 & 2019, Riverton 2019) were conducted evaluating 15 wheat varieties and all experiments received agronomic operations that are considered normal for this region (more details in the Table A1). In addition, two controlled environment (CT) experiments were conducted using (i) a red-brown earth soil from 2018 field site and (ii) a Mallee sandy soil from Loxton for in-depth analysis of varietal based differences and the influence of fertilizer N application on N cycling microbes associated with roots and rhizosphere. Rhizosphere soil (soil surrounding roots) and root samples collected at 10 (field experiments) to 12 weeks

(Controlled environment expts) after sowing were analysed for microbial biomass (Sparling et al. 199X), microbial catabolic diversity (Gupta et al. 2019) and abundances of total bacteria (16S rRNA), fungi (ITS region) and microbial groups involved in different N cycling processes (functional gene qPCR). Total plant biomass, grain yield and N uptake were measured at harvest (field expts) or 12 weeks after sowing (CT expts). Soils from pot experiments All the data analysed using standard statistical methods (Genstat v19.1, VSN Intl Ltd; Primer-e, v7.10.7, www.primer-e.com).

Both the controlled environment pot experiments plants were grown on a 10-14 day (25 C)-night (20 C) cycle with soil moisture maintained at 70-80% field capacity. The CT experiment testing variety x N level interactions was conducted using a red-brown earth soil (Roseworthy: Org C-2.4%, Total N-0.195%)) and a Mallee sand (Loxton: Org C – 0.5%, Total N-0.39%). Pots for +N treatments received Urea @50kgN/ha. At harvest (~12 weeks after sowing) rhizosphere and root samples were analysed for microbial and biochemical properties and plant biomass and N uptake were also measured.

Table 1. Details of field and controlled environment experiments conducted.

Experiment	Year	Site/Soil	# cultivars
Field #1	2018	Roseworthy	13 Wheat
CT expt #1	2019	Roseworthy	14 - Wheat & 2 - Durum Wheat
Field #2	2019	Roseworthy	14 - Wheat
Field #3	2019	Riverton	15 - Wheat
CT expt #2	2020	Roseworthy	5 Wheat & 1 Durum x 2 Nitrogen rates
		Loxton	5 Wheat & 1 Durum x 2 Nitrogen rates

3. Results and Discussion:

Field experiments at Roseworthy were on a Red Brown Earth soil (org C - 1.9 to 2.1%, pH - 8.2) and at Riverton on a Sandy loam soil (org C - 2.15%, pH - 5.35). At sowing, mineral N levels in 1M soil profile were 95 to 106 kg N/ha. Microbial biomass (MB)-N levels were 80 to 116 kg N/ha at Roseworthy experiments (2018 & 19, respectively) and 50 kg N/ha in the Riverton (2019) experiment. Both crop seasons were generally dry (~decile 5) with average grain yields of 2.21±0.07 t/ha (2018) and 2.61 ±0.08 t/ha (2019) at Roseworthy and 2.33 ±0.09 t/ha (2019) at Riverton experiments.

3.1 Crop performance and yield

In general, there was a significant and systematic variation in grain yields between the 14 varieties, i.e. 0.60 to 0.76 t/ha yield increase in newer varieties (e.g. Scepter, Mace, Gladius) compared to that for older varieties (Heron, Gamenya, Halberd) in all three experiments (Figure S1). The varieties also varied significantly in a number of yield attributes such as harvest index and thousand grain weight (Table A2). There was a significant ($P<0.001$) increase in grain number from older varieties (5620 to 6639 per M sq) to newer varieties (7146 to 8457 per sq M). Differences in total plant biomass didn't always follow the same trend as grain yield and there was no significant difference in total biomass at Roseworthy during 2019. Although trends in grain yield and harvest index at the two sites were not the same (R^2=0.51 and 0.56 between the two sites in 2019, respectively), their performance in terms of Thousand grain weight was highly correlated between the two sites in 2019 (between the two sites R^2=0.81). These results for crop performance generally confirmed the previous reports that selection of cultivars/varieties since 1950s has steadily increased wheat grain yield due to changes in some key yield contributing factors (Aziz et al., 2017; Sadras et al., 2016;

Sadras and Lawson, 2013). The trend of higher crop yields with recent varieties was also reflected in total N uptake (Table A3).

Wheat varieties developed during 50's – 60s with greater root biomass compared to recent varieties (Aziz et al. 2017; Corneo et al. 2016). It has been suggested that breeder's selection of cultivars mainly for yield and grain quality has led to significant changes in wheat plant phenotype including changes to root system size, e.g. root length, root biomass etc. However, previous research by Aziz et al. (2017) with these varieties has shown an overall reduction in root system size but an increase in N uptake per unit root length. Detailed analysis of root tip dynamics and distribution of roots through soil profile didn't provide consistent trends, as soil profile characteristics can significantly influence local root distribution. Another point of importance is the suggestion that more recent varieties have thinner roots than the early varieties and thinner roots may favour greater N and water uptake. Furthermore, it was suggested that lower root biomass could result in less amount of C released from roots potentially influencing rhizosphere microbial populations.

3.2 Microbial biomass, catabolic diversity and activity

Results for the amount of microbial biomass C and N in the rhizosphere soils of the various varieties showed significant differences in the 2018 field trial only but differences were not significant in the 2019 field experiments (Figure A4). Microbial biomass levels were generally higher in the Roseworthy soils (550 to 1250 ug MB-C / g soil) compared to that in the Riverton field soil (<350 MB-C/g soil). In general, there was no systematic trend in the amount of MB, similar to that seen in grain yield, from older to newer varieties. Microbial biomass in the rhizosphere soil is mainly dependant on the C released through root exudation and root turnover. Rhizosphere microbial communities are known to respond to the quantity and quality of C released by the growing crop and root turnover. Root exudates are known to comprise a variety of C and nutrient substrates including carbohydrates, amino acids and carboxylic acids thereby supporting a diverse microbial community in the rhizosphere environment. The ability of microbial communities in the rhizosphere soils of different wheat varieties from field experiments to utilize 31 different C and N substrates ('C substrate utilization profile -CLPP) was measured using a high-throughput lab assay. Analysis of results from these assays indicated significant differences in the CLPPs of different varieties in all the three field experiments (Figures A5). For example, rhizosphere microbial catabolic diversity was more similar between Scepter and Halberd compared to that between Condor and Gladius (Figure AX). Although there were a systematic trend in the CLPP profiles from older to newer varieties; microbial activity responses were generally lower with older varieties such as Heron, Gamenya and Halberd compared to that with Wyalkatchem and Gladius (Figure A6 and A7). Also, microbial activity responses to added C substrates were generally higher for rhizosphere samples of varieties such as Machete, Condor, Janz compared to that for Warigal, Spear, Yitpi. Similar to the CLPP profiles, there significant differences were observed in the microbial community metabolic diversity and average metabolic response values (Table A4). The finding of significant variation in rhizosphere microbial catabolic diversity suggests that different varieties may vary in terms of the quantity and quality of root exudation. However, the lack of systematic trend in microbial activity and catabolic diversity from older to newer varieties suggests that the reduction in root biomass may not have directly translated into increased root exudation and root turnover. Since microbial biomass and activity

(turnover) are key factors affecting N mineralization and tie-up this would impact on mineral N available for plant uptake. Results also indicated that rhizosphere soil in this red brown earth at Roseworthy 2018 experiment contained 563 to 691 mg C / kg soil which is equivalent to 80 to 100 kg N in microbial populations suggesting a significant potential for immobilization (tie-up) of N particularly during the early crop growth. Whereas, the soil at the Riverton 2019 field experiment only contained 39 kg N in the MB suggesting a lower mineralization and immobilization potential (Figure A4). Additionally, differences between varieties in terms of MB levels were smaller and not significant in the red earth soil at Riverton compared to that in the red brown earth soils at Roseworthy in both 2018 and 2019 seasons. These results suggest that soil type based variation in microbial biomass and turnover is a dominant factor in determining the N availability in wheat crop and the varietal base differences may be secondary in terms of microbial turnover and its direct contribution to N mineralization. Future research should address quantitative relationships between microbial turnover and N release in the rhizosphere of wheat varieties.

3.3 Abundances of N-cycling functional groups of microorganisms

Results from the analysis of rhizosphere and root samples indicated measurable levels of all the functional genes involved in N-cycling. Also, the observation of reproducible data from multiple field samples and in both seasons suggests that DNA based methods could be used to monitor populations of different N-cycling microorganisms for effects of crop, variety, and management practices.

In general, there were significant varietal based variations between rhizosphere soils of different wheat varieties in terms of the abundance of various microbial groups involved in the different N cycling processes [mineralization of organic N (npr, apr), conversion of urea N into ammonia N (*ure*C), nitrification (B-*amo*A, A-*amo*A) and non-symbiotic N_2-fixation (*nif*H)] (Figure A8-A10). Significant varietal based variation in the abundances of N-cycling functional genes in the field experiments were further confirmed from the observations in the pot experiment using soil from the Roseworthy 2018 field experiment. The varietal based differences in functional gene abundances varied for different N-cycling genes and the trends were not the same across all the functional genes. Additionally, absolute abundances of different functional genes varied between the field experiments suggesting the existence of soil type based variation in N-cycling microbial groups.

The functional genes npr and apr are involved in the mineralization organic N (neutral (npr) and alkaline (apr) metallo peptidases) i.e. they mediate the enzymatic proteolysis or breakdown of proteins into amino acids and smaller peptides. This is the one of the early processes in the mineralization of organic N into mineral form. The significant differences in the abundances of npr and apr reflects differences in abundance and composition of relevant microbial groups which may contribute to the overall N mineralization process. The enzyme urease represented by the functional genes B-ureC (bacterial urease) and F-ureC (fungal urease) catalyzes the hydrolysis of urea (derived from plant residues, SOM or fertilizer) into ammonia. Results from the 2019 field experiments indicated that the abundance of B-ureC gene were generally higher than that of F-ureC gene. Significant varietal based differences were observed for both the urease related genes (Figure A9 and A10). However, there were no significant differences in B-ureC abundance between the varieties in the pot experiment in

Roseworthy soil (Figure A11), probably because no fertilizer was added and also undecomposed crop residues were removed as part of soil preparation prior to using in the experiment.

Ammonia N oxidation, the conversion of ammonia N (from fertilizer or SOM) into nitrate form of N, is the first step in the nitrification process represented by B-amoA (bacterial) and A-amoA (archaeal) genes. The abundance of B-amoA gene was generally higher than that of A-amoA in all experiments and soils (Figure A8-A11). These results match with other reports showing a general greater abundance of bacterial ammonia oxidizers in surface soils and archaeal ammonia oxidizers may be more abundant in constrained soils e.g. extreme pH, high salinity, deeper profile soils. Significant differences in the abundances of both bacterial and archael amoA genes in all experiments. Additionally, a systematic trend of increasing abundance of B-amoA gene from older varieties to newer varieties was observed (Figure A12). Whereas, for A-amoA gene such trend was only observed in the pot experiment with Roseworthy soil (Figure A11). The differences in abundances of ammonia oxidizers was also reflected in the rate of nitrification measured for the rhizosphere soils (data not presented). These results indicate that the potential for the production of nitrate N is generally higher for the newer wheat varieties compared to that for the older varieties rhizosphere soils of newer varieties suggesting greater N mineralization potential.

Diazotrophs or non-symbiotic (NS) N-fixing microorganisms (represented by nifH-gene abundance) associated with cereal crops such as have been suggested to contribute N inputs for crop use (Roper and Gupta 2016). Results for the abundance of nifH gene showed significant varietal based variation in the rhizosphere of wheat varieties in both the field and pot experiments (Figures A8-A11). But, a systematic increasing trend in nifH gene abundance from older to newer varieties was only seen in the 2019 Roseworthy field and 2018 Roseworthy pot experiments. The population of NS-N fixing microbes is generally known to be influenced by the concentrations of nitrate N hence higher nitrate N levels may mask the varietal based variation.

Results from these experiments also indicated that differences in the actual magnitude of variation between varieties in the different soils. Soil type, in terms of physico-chemical properties (e.g. biologically available organic C, soil texture), can have a significant influence on the composition and populations of general microbial communities (Gupta et al. 2019). Also, varietal performance in terms of root growth can differ based on soil structure and other properties.

Overall, results for the abundances of various N-cycling microbial groups indicate significant varietal based variation that may not be masked by differences in soil type and seasonal effects. This suggests that rhizosphere and root associated microbial communities have the potential to influence N supply to wheat crops.

3.4 Effect of N fertilizer application

Using the soils from the 2019 Roseworthy field experiment and a sandy soil from the Mallee (Loxton) a pot experiment was conducted under controlled environment conditions to determine the response of N cycling microbes to N fertilizer application with different wheat varieties. In addition to 5 selected varieties the Durum variety DBA-Aurora (sourced from Dr. Jason Able, Univ Adelaide) will also be included.

Treatments included 6 varieties and two N levels (no fertilizer and urea @25 kgN/ha) and all treatments had four replicates. The experiment was harvested 9 weeks after germination and rhizosphere soil, root and plant samples were analysed.
There was a significant improvement in plant growth performance to N fertilizer application in terms of shoot weight and chlorophyl content (measured using SPAD meter) in both soils, although the effect was greater in the Loxton Mallee sand compared to that in the Roseworthy soil (Figure A13). This could be attributed to the lower mineral N levels at the time of sowing were lower in the Loxton sand (ave 15 ug N / g soil) compared to that in the Roseworthy soil (ave. 54 ugN/g soil). However, significant increase in the N uptake by the plants was only seen in the Loxton soil and the effect was seen with all the varieties (Figure A14). Similar to the observations in the field experiment, newer variety Scepter generally showed higher total N uptake than the older variety Gladius whereas the performance of Condor was different i.e. showed highest amount of total N uptake esp. in the Roseworthy soil. The Durum variety Aurora generally had lowest plant biomass and N uptake in both soils.

Application of N fertilizer generally reduced the amount of MB-C in the rhizosphere soil and the effect was greater and seen with all the varieties in the Loxton sand (Figure A15). Application of fertilizer also altered the microbial catabolic diversity and potential in both soils, but the effect varied with different varieties (Table AX). The reduction in the MB level and catabolic activity could be partly attributed to the reduction in soil pH (0.1 to 0.35 units) in the N-fertilized samples.

Significant variety based variation in the abundances of N-cycling functional genes in both the soils but the effect of fertilizer application was only seen with B-amoA, A-amoA, nifH genes in the Loxton sand and B-amoA in the Roseworthy soil (Tables A6 and A7). In the Loxton sand, application of fertilizer reduced A-amoA abundance whereas a significant increase in the B-amoA abundance was observed with all the varieties. Application of urea fertilizer significantly increased B-amoA abundance in the Roseworthy soil with all the varieties except for the Condor samples (Figure A6). The increase in the ammonia oxidizing bacterial populations (represented by B-amoA) with fertilizer application reflects the response of these microorganisms to the addition of the N substrate. The lack of such effect on the urease producing enzyme related genes (ureC) may be due to the smaller amount of fertilizer applied not requiring additional urease production. Overall, results from this pot experiment confirmed the previous observation for varietal based variation in N-cycling microbial communities in the Loxton sand. Additionally, the effect of fertilizer application seems to vary with soil type probably due the differences in the initial mineral N levels.

4. Relationship between microbial properties, yield and N uptake

It is known that rhizosphere and root associated microorganisms are vital for the cycling of nutrients and mediate the availability of N for plant uptake. Results in this project are the first to show a significant varietal based variation in N cycling microbes between wheat varieties in realistic field conditions. Although, there were significant varietal based differences in the amount of MB and microbial activity, there was no significant relationship between variations in grain yield and N uptake. There was a systematic increase in the abundance of some groups of N-cycling microbes from older varieties to newer varieties, e.g. bacterial-ammonia oxidizing organisms, a group of nitrifying organisms. In the field experiments on red-brown earth soils at Roseworthy

during 2018 and 2019, a significant positive relationship was observed between B-amoA gene abundance and grain yield and N uptake (Figure A7 and A8). For example, in the 2018 experiment variation in wheat grain yield was significantly correlated to the abundance of nitrifying (B-amoA and A-amoA) and ammonifying (ureC) microorganisms (multiple $R=0.786$; $P<0.02$). Whereas, the abundance of the B-amoA gene was the major factor for the variation in the grain yield and N uptake in the 2019 Roseworthy experiment. Similarly, in the pot experiment in Roseworthy 2019 and Loxton sand soils, plant biomass and total N uptake were significantly positively correlated to nitrifying microorganisms (B-amoA gene abundance). It has been suggested that the process of nitrification, the final step in the production of nitrate N is a key process in the production of nitrate N, both in the organic N mineralization and conversion of ammonia and urea fertilizers. Thus the observation of significant link between the abundance of nitrifying microorganisms and grain yield and N uptake suggests that newer varieties of wheat may be benefiting from the activity of this group of microbes. Whereas, in the field experiment at Riverton, grain yield and N uptake differences were significantly related to organic N mineralizing microbes, i.e. alkaline peptidase (apr gene) harbouring microbes (Figure A10). This could be partly attributed to the low in soil organic C (0.5%) and general N mineralization potential in this soil, thereby microorganisms involved in organic N mineralization were found to have significant influence on crop performance. In this experiment, significant positive relationship ($P<0.01$) was also observed between nitrifying microbes (amoA gene abundance) and bacterial urease harbouring community (B-ureC) suggests the role of different N-cycling groups in the overall N mineralization in this low organic matter soil.

Overall, results from the field and CT experiments present the first report of significant varietal based variation in N-cycling microbes in the wheat crops under realistic field conditions i.e. 13 wheat varieties developed over 50 years, agronomically adapted to SA and featuring an established difference in N uptake per unit root length. Also, the project identified significant relationship between the increase in wheat grain yields and the N-cycling microbes involved in nitrification and organic N mineralization. This project also demonstrates the potential for using sophisticated DNA based methods to quantify changes in key microbial groups involved in N cycling and availability to cereal crops in field based experimentation. The methodology used in this project could form a framework for the use of DNA-based methods for other microbial groups.

List of Tables and Figures with results from field and controlled environment experiments

Table 1. Details of field and controlled environment experiments conducted.

Experiment	Year	Site/Soil	# cultivars
Field #1	2018	Roseworthy	13 Wheat
CT expt #1	2019	Roseworthy	14 - Wheat & 2- Durum
Field #2	2019	Roseworthy	14 - Wheat
Field #3	2019	Riverton	15 - Wheat
CT expt #2	2020	Roseworthy	5 Wheat & 1 Durum x 2 Nitrogen rates
		Loxton	5 Wheat & 1 Durum x 2 Nitrogen rates

Table A1. Details of field experiments.

Sowing Operations	Details
Sowing date	May 27th, 2018
Conditions at sowing	Cloudy, calm, warm. Soil a bit moisture than seeding 1 but still very dry on top.
Pre-seeding herbicides	Seeder on identical settings to seeding 1. 2 L/ha of Triflur X, 2 L/ha of Glyphosate, 35 g/ha of Triasulfuron and 118 g/ha of Sakura immediately prior to seeding
Seeding depth	4 cm below press wheel trench
Fertiliser at seeding	230 kg/ha of single super, banded 3-4 cm below seed. 25 kg N /ha as Urea
Soil conditions	Dry on top, some moisture below (slightly more than seeding 1), seeded a little deeper to get into moisture. Lots of volunteer barley established (2-3 leaves).
	Used crop rows in front of the trials to set AB lines for each trial, then A+ for plots at right angles. AB lines @ 15 m intervals used for trip lines and A+ @ 1.8 m for plot runs.
Sowing equipment	Autosteer used TopCon local network (UHF 36)

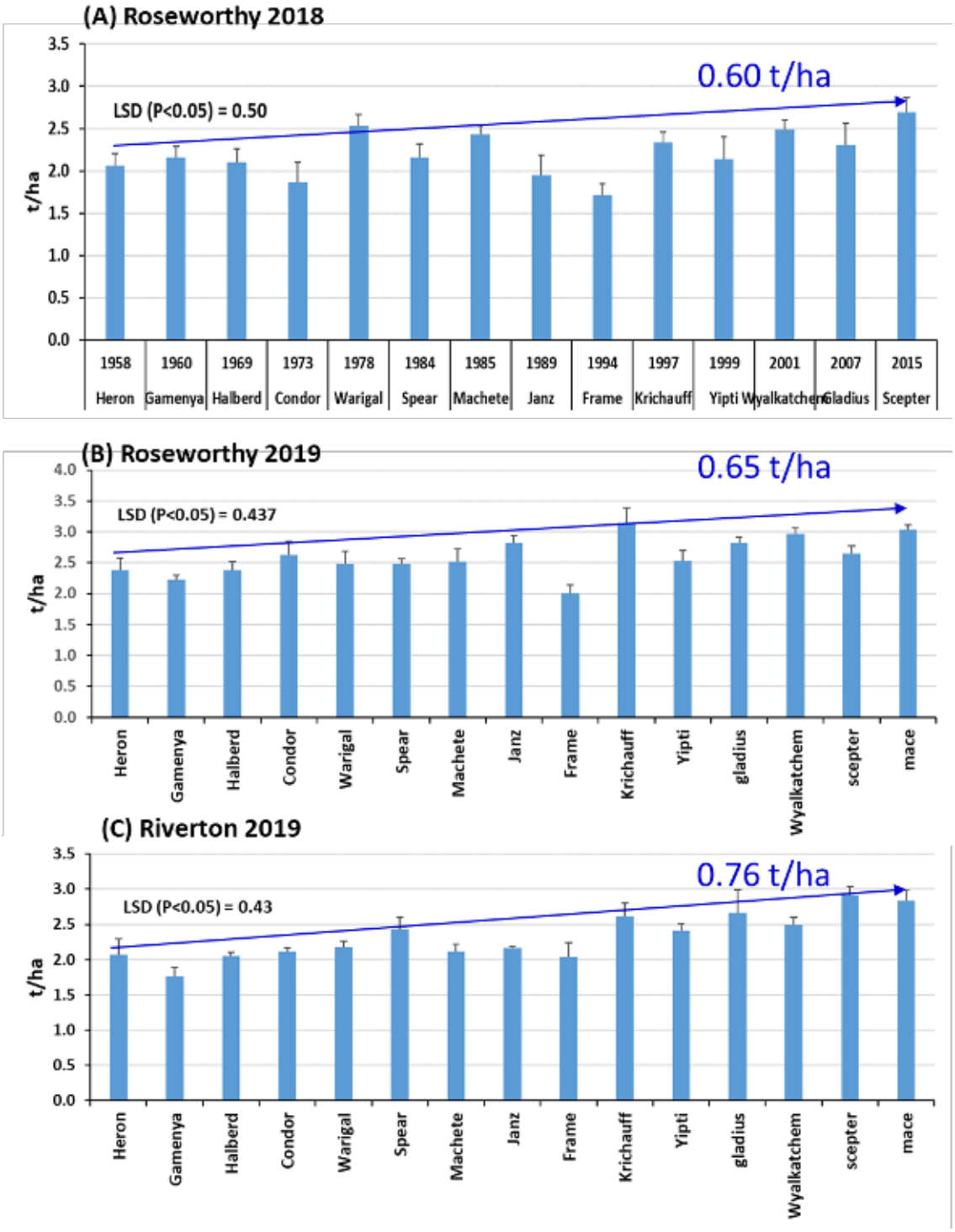


Figure S1. Grain yield (t/ha) of wheat varieties in the field experiments at Roseworthy and Riverton during 2018 and 2019 crop seasons. All varieties had 4 replicate plots.

Table A2. Grain yield components for different varieties in the field experiments at Roseworthy and Riverton during 2018&19 seasons.

Variety	Year of release	Aboveground Biomass (t/ha)			Harvest Index			Thousand grain weight (g)			Grain number (per sq M)		
		Roseworthy 18	Roseworthy 19	Riverton19	Roseworthy 18	Roseworthy 19	Riverton19	Roseworthy 18	Roseworthy 19	Riverton19	Roseworthy 18	Roseworthy 19	Riverton19
Heron	1958	4.259	6.118	5.792	0.49	0.392	0.353	36.57	37.83	32.41	5620	6315	6326
Gamenya	1960	4.358	5.733	5.204	0.49	0.389	0.345	31.28	32.73	29.98	6899	6818	5926
Halberd	1969	4.636	5.859	6.128	0.45	0.407	0.335	33.17	34.28	31.22	6333	6952	6639
Condor	1973	4.409	6.480	5.713	0.42	0.404	0.371	29.41	31.02	27.43	6337	8450	7729
Warigal	1978	5.695	6.600	5.904	0.44	0.376	0.369	32.19	30.38	29.23	7849	8146	7470
Spear	1984	5.021	6.091	6.502	0.42	0.408	0.373	36.00	36.32	32.08	5942	6835	7564
Machete	1985	4.817	6.050	5.297	0.50	0.414	0.402	33.87	35.34	33.58	7219	7110	6329
Janz	1989	4.292	6.453	5.676	0.45	0.436	0.383	30.24	33.95	26.70	6449	8298	8230
Frame	1994	4.400	5.861	6.025	0.39	0.344	0.339	37.91	40.03	36.46	4542	4995	5636
Krichauff	1997	5.184	7.684	6.995	0.45	0.409	0.373	27.61	32.04	29.35	8398	9844	8915
Yipti	1999	5.026	6.381	6.580	0.42	0.398	0.368	39.77	40.84	35.78	5407	6215	6776
Wyalkatchem	2001	5.328	6.834	6.447	0.47	0.413	0.411	33.23	38.69	36.31	7445	7288	7321
Gladius	2007	4.683	6.825	5.928	0.49	0.435	0.421	31.76	37.29	35.03	7217	7987	7117
Mace	2007	-	6.776	6.675	-	0.449	0.425	-	42.67	37.50	-	7188	7582
Scepter	2015	5.299	6.336	7.262	0.51	0.419	0.403	37.57	39.68	34.63	7146	6695	8457
F-test		***Not sig***	***0.059***	***0.012***	***<0.001***	***<001***	***<0.001***	***<0.001***	***<001***	***<0.001***	***<0.001***	***<001***	***<0.001***
LSD (P<0.05)			***1.050***	***1.080***	***0.0376***	***0.033***	***0.02954***	***4.07***	***2.636***	***2.73***	***1138***	***1299***	***1340***

2019 grain yield R2=0.51; biomass R2=0.26 HI R2=0.56; TGW R=0.81, Grain #R2=0.54 Roseworthy 19

Table A3. Differences in N uptake by wheat varieties in field experiments at Roseworthy and Riverton during 2018&19 seasons.

Variety	Year of release	Protein (%)			Grain N uptake (kg N / ha)			Total N uptake (kg N / ha)		
		Roseworthy 18	Roseworthy 19	Riverton19	Roseworthy 18	Roseworthy 19	Riverton19	Roseworthy 18	Roseworthy 19	Riverton19
Heron	1958	4.259	8.1475	5.792	0.49	33.840	0.353	36.57	48.81	32.41
Gamenya	1960	4.358	9.0825	5.204	0.49	35.256	0.345	31.28	50.52	29.98
Halberd	1969	4.636	8.325	6.128	0.45	34.565	0.335	33.17	48.86	31.22
Condor	1973	4.409	8.9525	5.713	0.42	40.558	0.371	29.41	56.30	27.43
Warigal	1978	5.695	9.725	5.904	0.44	41.770	0.369	32.19	56.96	29.23
Spear	1984	5.021	8.4025	6.502	0.42	36.120	0.373	36.00	51.21	32.08
Machete	1985	4.817	9.605	5.297	0.50	41.837	0.402	33.87	56.35	33.58
Janz	1989	4.292	8.4	5.676	0.45	41.062	0.383	30.24	56.53	26.70
Frame	1994	4.400	9.4075	6.025	0.39	32.723	0.339	37.91	48.98	36.46
Krichauff	1997	5.184	8.6875	6.995	0.45	47.797	0.373	27.61	64.50	29.35
Yipti	1999	5.026	8.0525	6.580	0.42	35.680	0.368	39.77	51.27	35.78
Wyalkatchem	2001	5.328	9.145	6.447	0.47	47.217	0.411	33.23	63.52	36.31
Gladius	2007	4.683	9.17	5.928	0.49	44.993	0.421	31.76	62.57	35.03
Mace	2007		8.6125	6.675		45.597	0.425		61.10	37.50
Scepter	2015	5.299	8.5375	7.262	0.51	39.464	0.403	37.57	54.40	34.63
F-test		***Not sig***	0.01	***0.012***	***<0.001***	***0.002***	***<0.001***	***<0.001***	***0.065***	***<0.001***
LSD (P<0.05)			0.9392	***1.080***	***0.0376***	***8.040***	***0.02954***	***4.07***	***11.62***	***2.73***

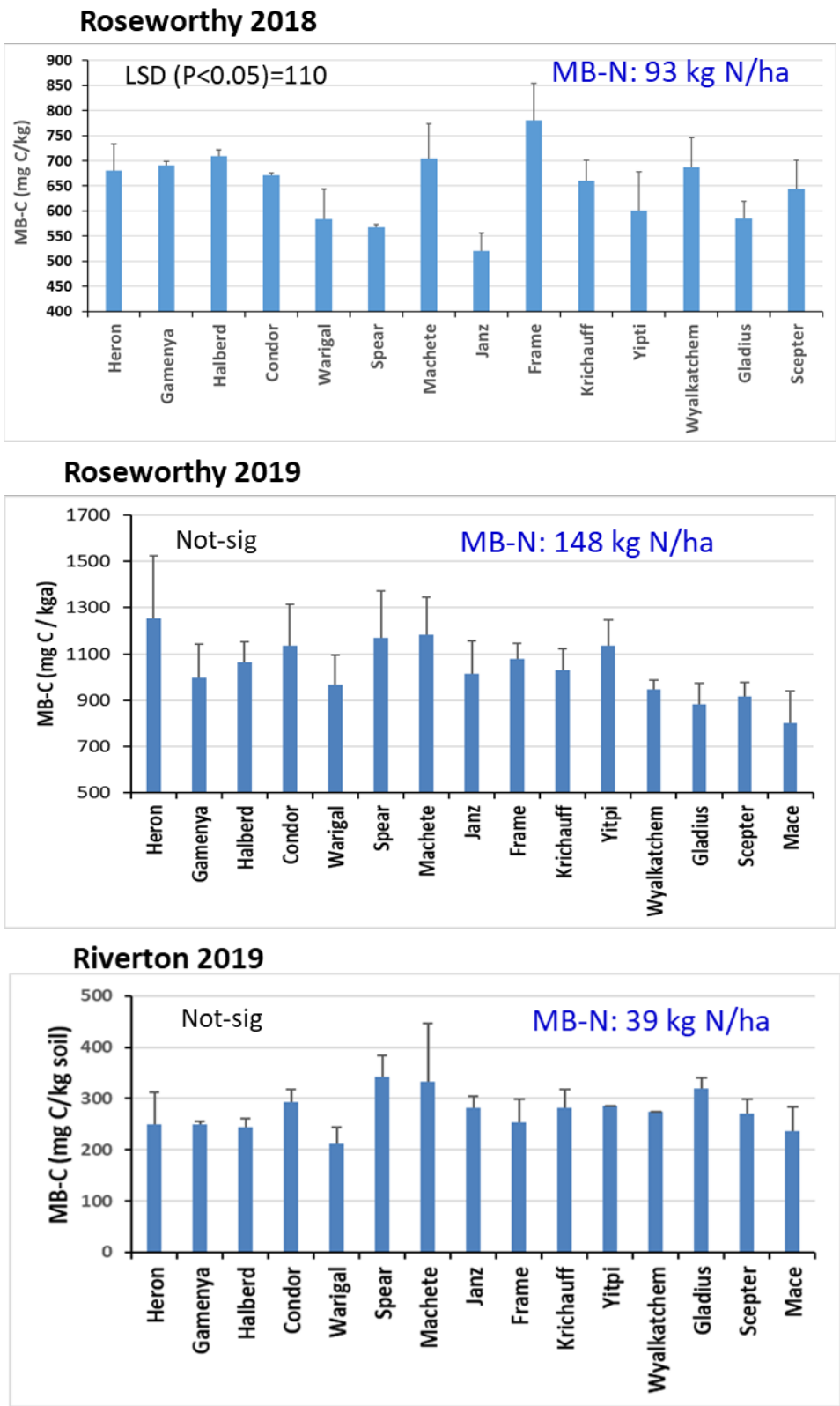


Figure A4. The amount of microbial biomass (MB) C in the rhizosphere soils of different wheat varieties in field experiments during 2018 and 2019 crop seasons. Values for MB-N represent the average amounts observed in the surface soils at the time of sowing.

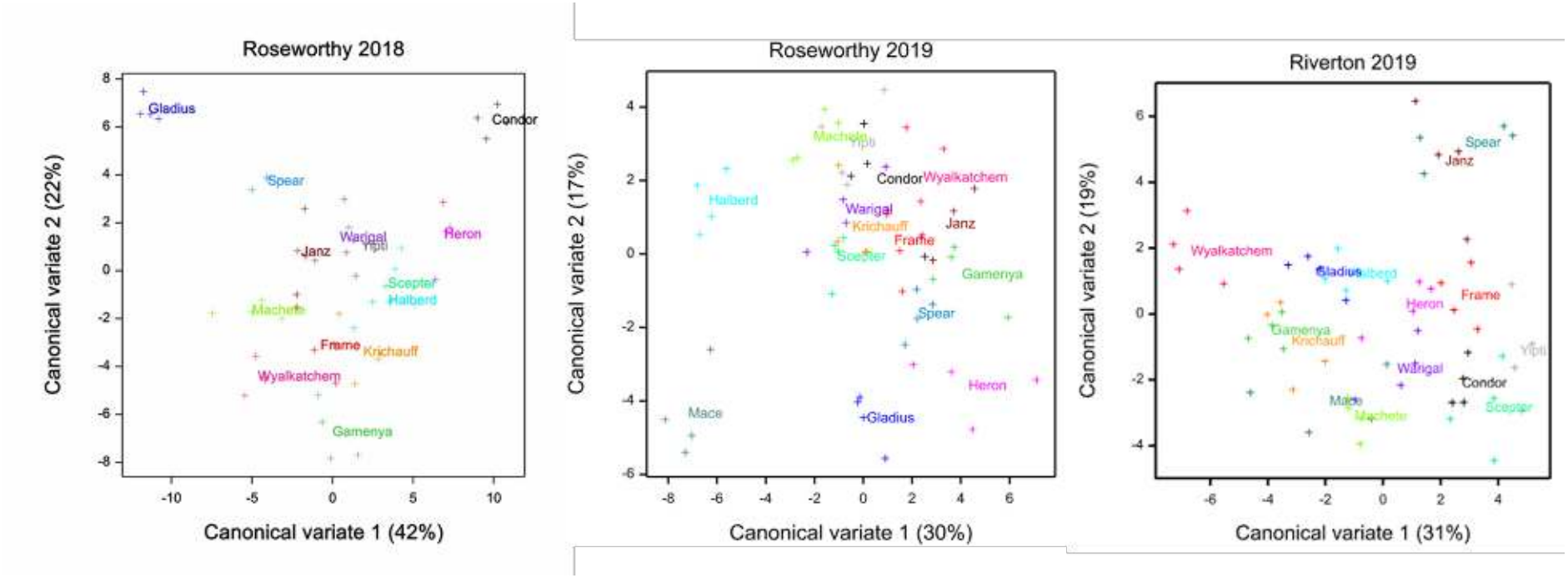


Figure A5. Results from the canonical variate analysis of microbial catabolic activity profiles (ability of microbes to utilize 31 different C and N substrates). Distance between the data points for different varieties represents the similarity between them i.e. points that are closer are more similar. For example, rhizosphere microbial catabolic diversity is more similar between Scepter and Halberd compared to that between Condor and Gladius. The first canonical variates accounted for the majority of the variation (64% of total variation) between treatments.

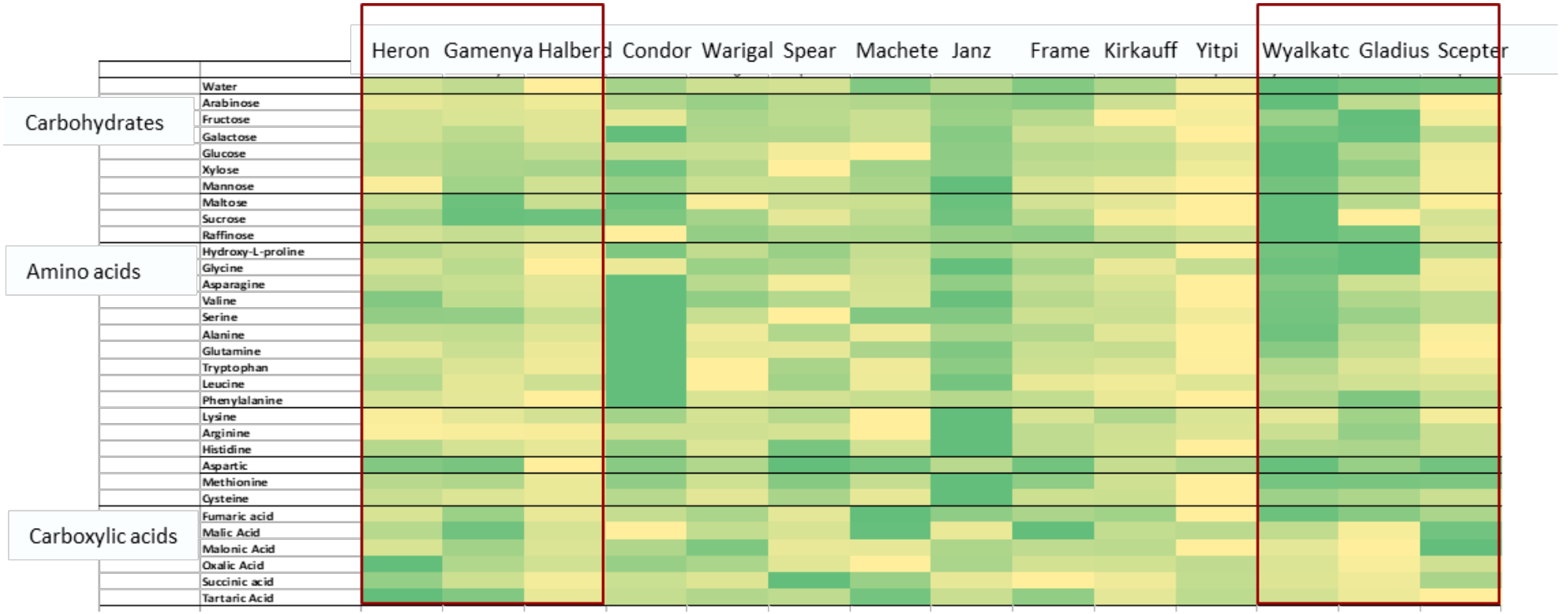


Figure A6. Carbon substrate utilization profiles for rhizosphere soils from the Roseworthy field experiment 2018 measured as microbial activity responses to 31 different carbon substrates. The level of microbial response is represented by colours yellow (low activity) to green (high microbial activity).

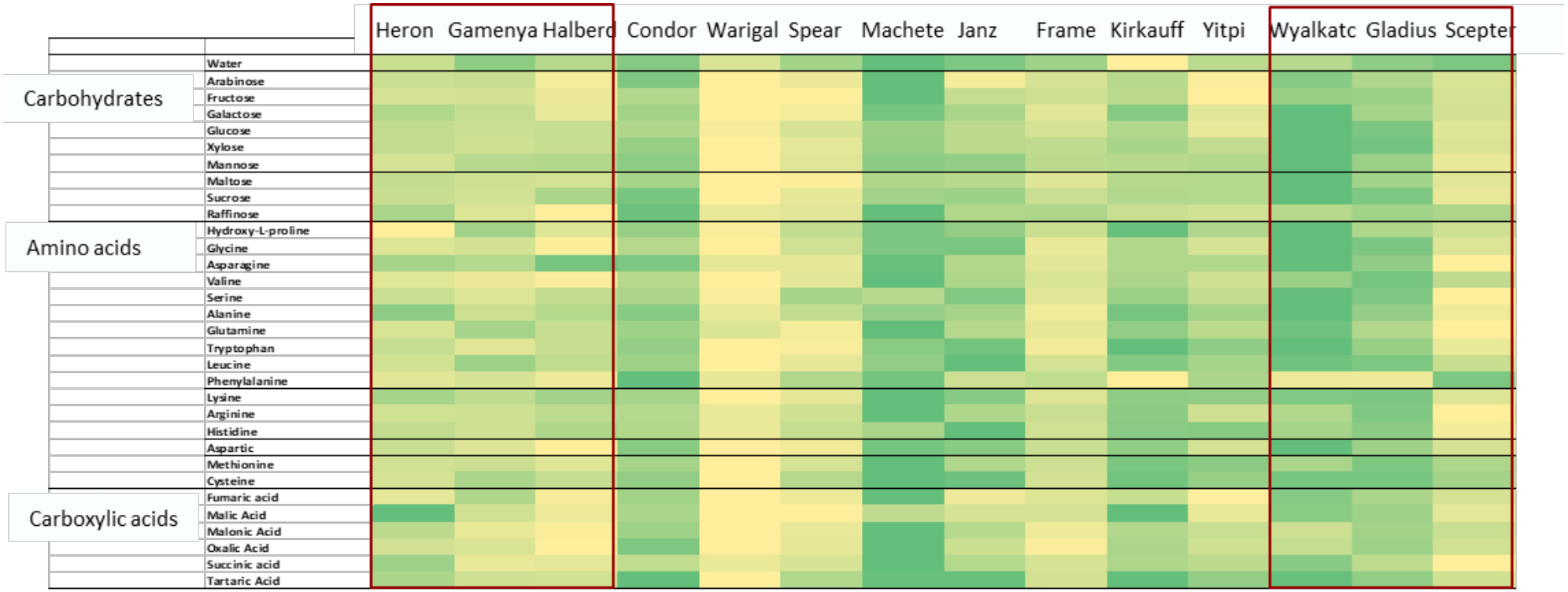
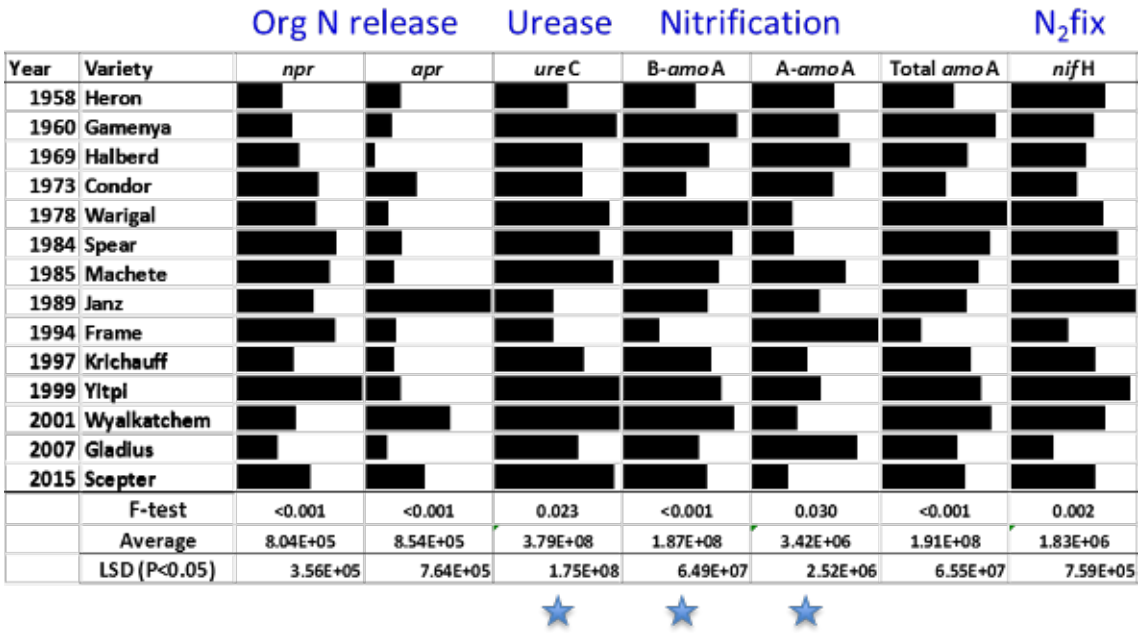


Figure A7. Carbon substrate utilization profiles for rhizosphere soils from the Riverton field experiment measured as microbial activity responses to 31 different carbon substrates. The level of microbial response is represented by colours yellow (low activity) to green (high microbial activity).

Table A4. Rhizosphere microbial catabolic activity (AMR, respiration) and metabolic diversity as influence by wheat variety, nitrogen application and soil type in a controlled environment experiment.

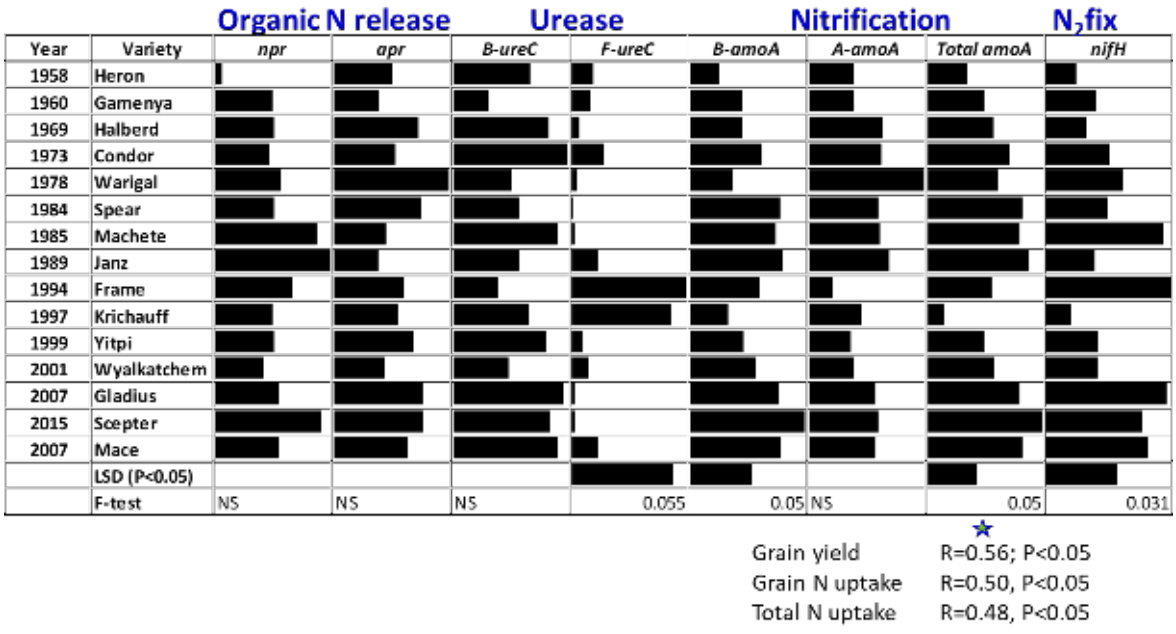
Variety	Year of release	Average Metabolic Response (AMR)			Community Metabolic Diversity (CMD)			Respiration		
		Roseworthy 18	Roseworthy 19	Riverton19	Roseworthy 18	Roseworthy 19	Riverton19	Roseworthy 18	Roseworthy 19	Riverton19
Heron	1958	0.323	6.119	2.524	16.75	18.000	6.167	1.24	1.49	0.65
Gamenya	1960	0.354	5.013	2.211	17.58	15.417	3.750	1.27	0.96	0.78
Halberd	1969	0.270	4.727	2.181	13.67	13.917	4.833	1.14	1.11	0.70
Condor	1973	0.343	4.634	3.032	15.58	13.500	9.250	1.32	0.95	0.79
Warigal	1978	0.321	3.836	1.613	16.25	12.167	1.500	1.24	0.89	0.62
Spear	1984	0.327	5.633	1.859	14.75	15.333	2.667	1.24	1.24	0.73
Machete	1985	0.341	5.465	3.368	16.17	16.583	10.417	1.40	1.00	0.86
Janz	1989	0.371	5.421	2.654	18.25	15.917	7.250	1.30	0.97	0.81
Frame	1994	0.332	4.839	2.078	16.25	15.583	4.250	1.39	0.94	0.74
Krichauff	1997	0.277	4.120	3.069	14.58	13.500	8.833	1.31	0.96	0.55
Yipti	1999	0.250	5.248	2.338	12.00	15.667	4.917	1.17	1.12	0.69
Wyalkatchem	2001	0.396	4.135	3.644	17.75	13.417	11.500	1.46	0.79	0.69
Gladius	2007	0.318	3.504	3.101	15.25	10.917	9.250	1.43	0.91	0.77
Mace	2007	-	3.952	1.612	-	12.583	2.417	-	1.03	0.74
Scepter	2015	0.287	3.547	1.844	12.00	10.833	2.500	1.42	1.16	0.81
F-test		***0.004***	***0.001***	***<0.001***	***0.003***	***0.007***	***<0.001***	***Not sig***	***Not sig***	***Not sig***
LSD (P<0.05)		***0.0668***	***1.273***	***0.805***	***3.179***	***3.629***	***4.358***			



Year	Variety	*npr*	*apr*	*ure*C	B-*amo*A	A-*amo*A	Total *amo*A	*nif*H
1958	Heron							
1960	Gamenya							
1969	Halberd							
1973	Condor							
1978	Warigal							
1984	Spear							
1985	Machete							
1989	Janz							
1994	Frame							
1997	Krichauff							
1999	Yitpi							
2001	Wyalkatchem							
2007	Gladius							
2015	Scepter							
	F-test	<0.001	<0.001	0.023	<0.001	0.030	<0.001	0.002
	Average	8.04E+05	8.54E+05	3.79E+08	1.87E+08	3.42E+06	1.91E+08	1.83E+06
	LSD (P<0.05)	3.56E+05	7.64E+05	1.75E+08	6.49E+07	2.52E+06	6.55E+07	7.59E+05

Grain yield (t/ha) = -5.06+(0.207*B*amo*A)-(0.553*A*amo*A)+(1.069**ure*C); R=0.79; p<0.018

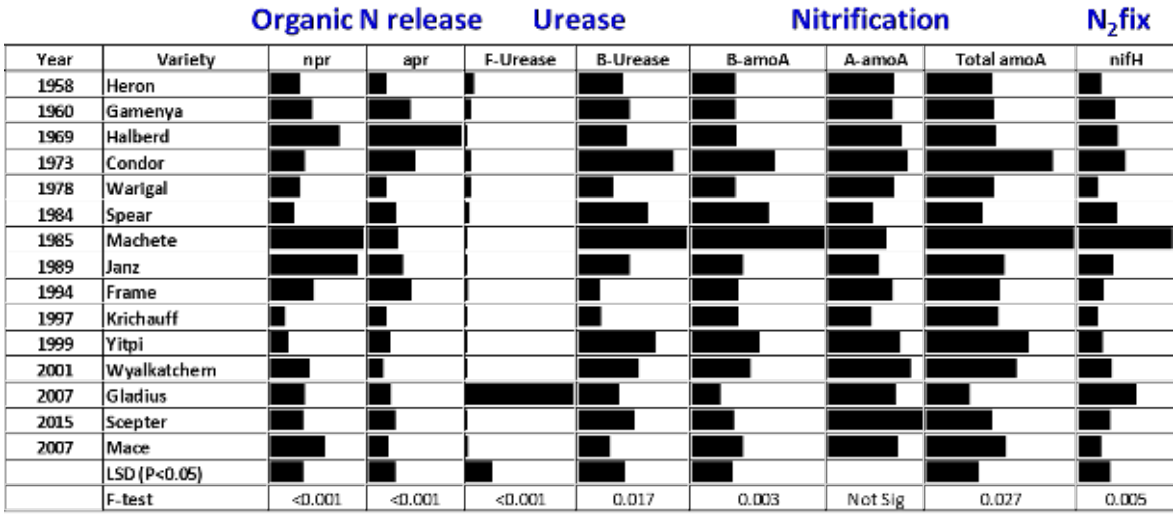
Figure A8. Abundances of microbial functional groups (gene copy number/gram soil) that regulate different N cycling processes in the rhizosphere of wheat varieties in a field experiment on a red brown earth soil at Roseworthy, SA during 2018 crop season.



Year	Variety	*npr*	*apr*	*B-ureC*	*F-ureC*	*B-amoA*	*A-amoA*	*Total amoA*	*nifH*
1958	Heron								
1960	Gamenya								
1969	Halberd								
1973	Condor								
1978	Warigal								
1984	Spear								
1985	Machete								
1989	Janz								
1994	Frame								
1997	Krichauff								
1999	Yitpi								
2001	Wyalkatchem								
2007	Gladius								
2015	Scepter								
2007	Mace								
	LSD (P<0.05)								
	F-test	NS	NS	NS	0.055	0.05	NS	0.05	0.031

Grain yield	R=0.56; P<0.05
Grain N uptake	R=0.50, P<0.05
Total N uptake	R=0.48, P<0.05

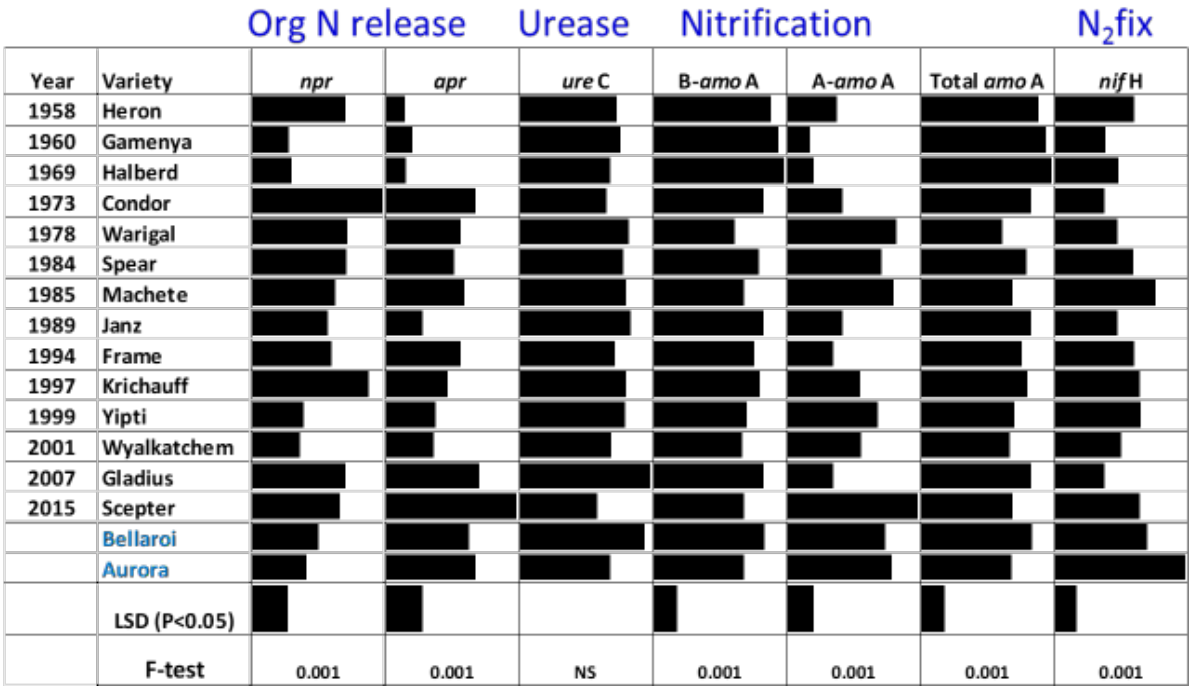
Figure A9. Abundances of microbial functional groups (gene copy number/gram soil) that regulate different N cycling processes in the rhizosphere of wheat varieties in a field experiment on a red brown earth soil at Roseworthy, SA during 2019 crop season.



Year	Variety	npr	apr	F-Urease	B-Urease	B-amoA	A-amoA	Total amoA	nifH
1958	Heron								
1960	Gamenya								
1969	Halberd								
1973	Condor								
1978	Warigal								
1984	Spear								
1985	Machete								
1989	Janz								
1994	Frame								
1997	Krichauff								
1999	Yitpi								
2001	Wyalkatchem								
2007	Gladius								
2015	Scepter								
2007	Mace								
	LSD (P<0.05)								
	F-test	<0.001	<0.001	<0.001	0.017	0.003	Not Sig	0.027	0.005

Grain yield vs. apr – R^2 =0.496, P<0.05
Grain N uptake vs. apr – R^2=0.570, P<0.01

Figure A10. Abundances of microbial functional groups (gene copy number/gram soil) that regulate different N cycling processes in the rhizosphere of wheat varieties in a field experiment on a red brown earth soil at Riverton, SA during 2019 crop season.



Year	Variety	*npr*	*apr*	*ure* C	B-*amo* A	A-*amo* A	Total *amo* A	*nif* H
1958	Heron							
1960	Gamenya							
1969	Halberd							
1973	Condor							
1978	Warigal							
1984	Spear							
1985	Machete							
1989	Janz							
1994	Frame							
1997	Krichauff							
1999	Yipti							
2001	Wyalkatchem							
2007	Gladius							
2015	Scepter							
	Bellaroi							
	Aurora							
	LSD (P<0.05)							
	F-test	0.001	0.001	NS	0.001	0.001	0.001	0.001

Figure A11. Abundances of microbial functional groups (gene copy number/gram soil) that regulate different N cycling processes in the rhizosphere of wheat varieties in the controlled environment pot experiment on a red brown earth soil from Roseworthy, SA.

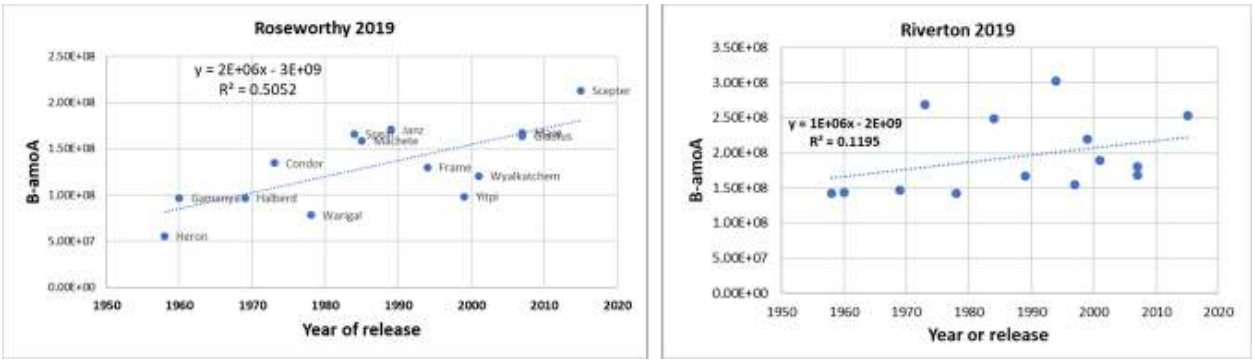


Figure A12. The abundance of bacterial ammonia oxidizers (B-amoA gene abundance) in the rhizosphere samples for the different wheat varieties grown in field experiments during 2019 season.

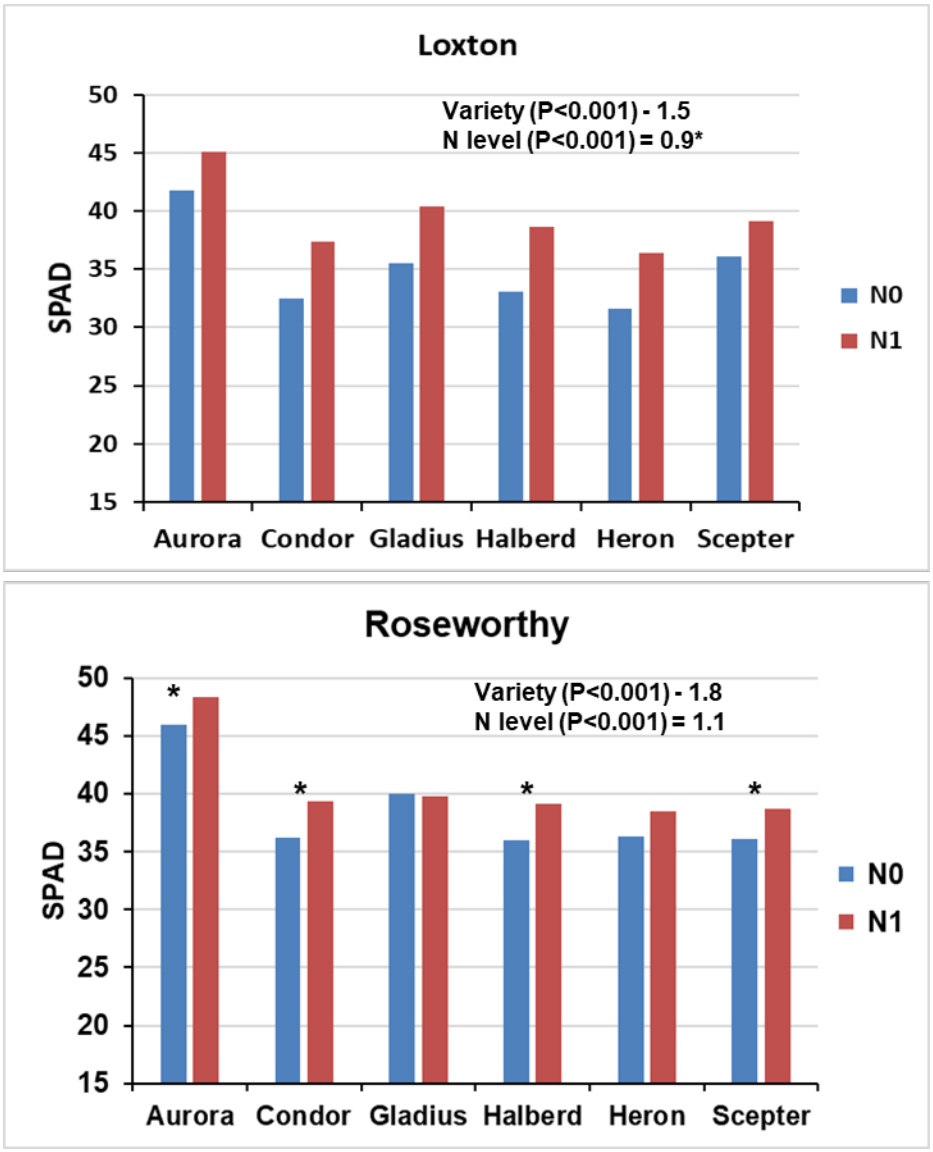
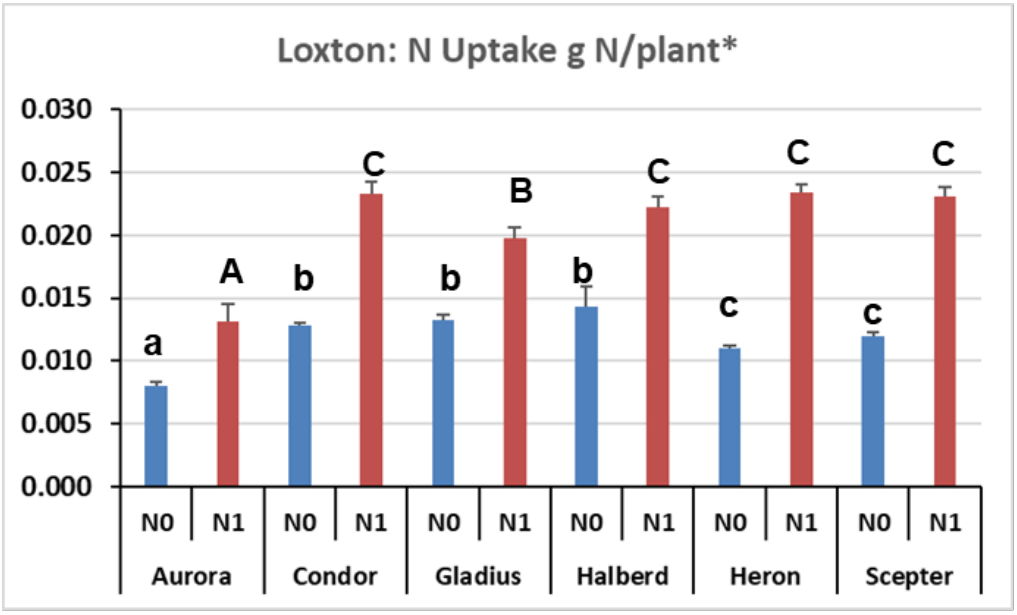


Figure A13. Plant growth performance of wheat varieties as influenced by N application and soil type in the CT environment experiment.



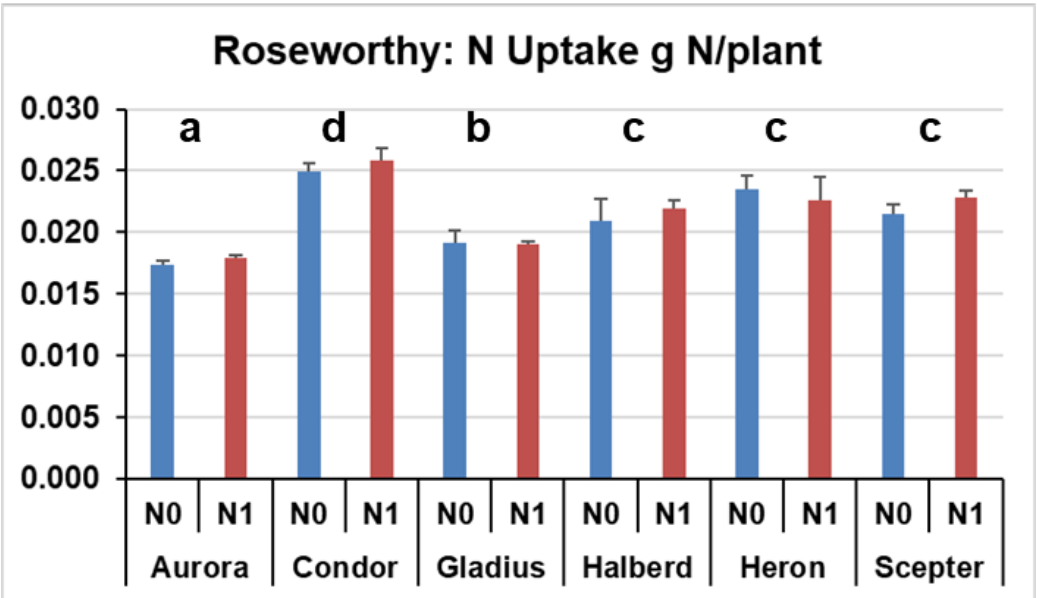


Figure A14. Effect of N fertilizer application on plant N uptake by different wheat varieties in Loxton and Roseworthy soils.

Variety	Aurora	Condor	Gladius	Halberd	Heron	Scepter
	17.66	25.4	19.09	21.43	23.03	22.15
	a	d	b	c	c	c

N_level	N0	N1
	21.22	21.7

Variety	N_level	N0	N1
Aurora		17.37	17.95
Condor		24.95	25.85
Gladius		19.09	19.09
Halberd		20.92	21.95
Heron		23.46	22.6
Scepter		21.52	22.77

Variety	Aurora	Condor	Gladius	Halberd	Heron	Scepter
	10.53	18.03	16.49	18.28	17.18	17.5

N_level	N0	N1
	11.87	20.81

Variety	N_level	N0	N1
Aurora		7.97	13.1
Condor		12.77	23.29
Gladius		13.27	19.72
Halberd		14.34	22.22
Heron		10.96	23.41
Scepter		11.92	23.08

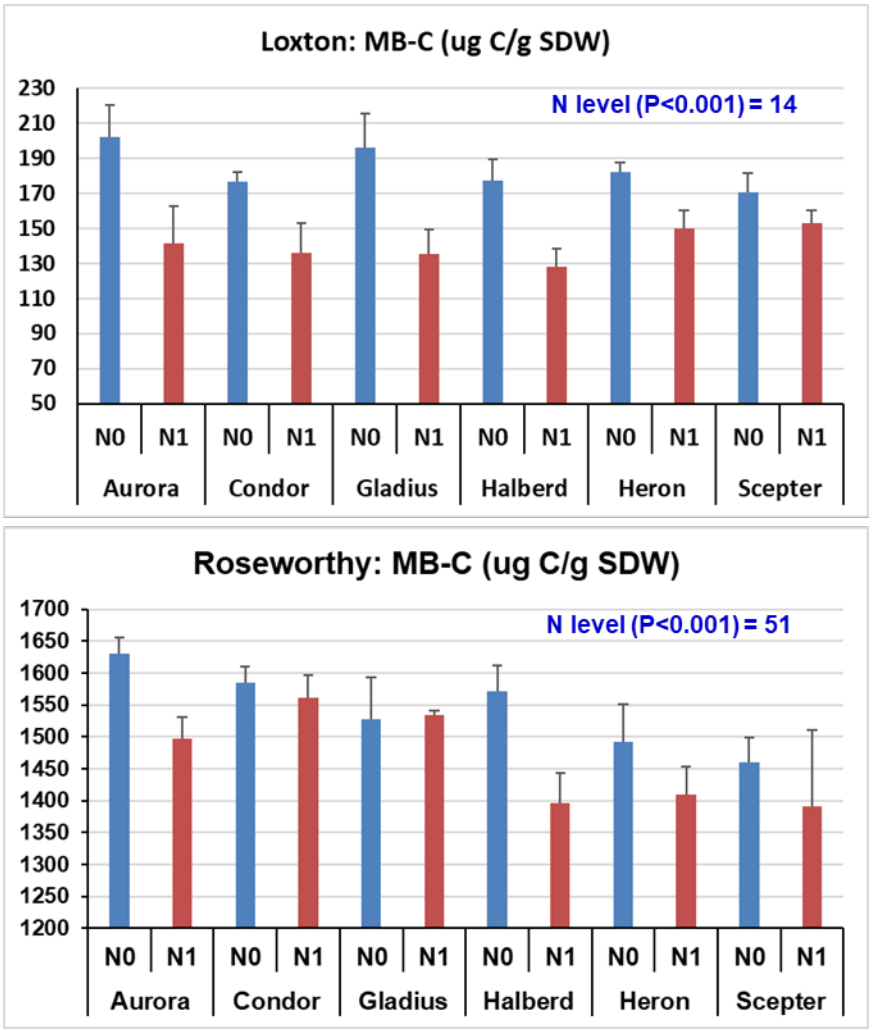


Figure x. Rhizosphere microbial biomass levels as influenced by N application, variety and soil type in the CT environment experiment.

Table A5. Rhizosphere microbial catabolic activity (AMR, respiration) and metabolic diversity as influence by wheat variety, nitrogen application and soil type in a controlled environment experiment.

Variety	Year of release	Roseworthy Pot Trial		
		AMR	CMD	Respiration
Condor 0N	1973	6.687	17.667	1.092
Condor 1N		7.034	17.444	1.007
Gladius 0N	2007	6.192	17.556	0.906
Gladius 1N		7.762	18.222	1.014
Halberd 0N	1969	8.239	19.333	0.999
Halberd 1N		7.192	18.778	1.057
Heron 0N	1958	6.739	19.556	1.133
Heron 1N		8.293	19.000	1.037
Scepter 0N	2015	6.614	17.556	1.114
Scepter 1N		7.042	17.444	1.258
Aurora 0N		6.885	20.222	1.078
Aurora 1N		9.316	19.444	0.987
Soil 0N		7.710	20.111	1.098
Soil 1N		8.539	18.778	1.028
F-test	***Var***	***0.006***	***0.007***	***Not sig***
	Fert	***<.001***	***Not sig***	***Not sig***
	Var.Fert	***0.006***	***Not sig***	***Not sig***
LSD (P<0.05)	***Var***	***0.82***	***1.446***	
	Fert	***0.438***		
	Var.Fert	***1.159***		

Variety	Year of release	Loxton Pot Trial		
		AMR	CMD	Respiration
Condor 0N	1973	0.646	0.333	0.586
Condor 1N		0.686	0.333	0.479
Gladius 0N	2007	0.560	0.000	0.525
Gladius 1N		0.693	1.000	0.437
Halberd 0N	1969	0.868	1.667	0.550
Halberd 1N		0.729	0.111	0.471
Heron 0N	1958	0.630	0.778	0.526
Heron 1N		0.699	0.556	0.471
Scepter 0N	2015	0.608	0.333	0.487
Scepter 1N		0.760	0.556	0.457
Aurora 0N		0.540	0.000	0.482
Aurora 1N		0.460	0.222	0.437
Soil 0N		0.342	0.000	0.480
Soil 1N		0.481	0.000	0.467
F-test	***Var***	***<.001***	***Not sig***	***Not sig***
	Fert	***Not sig***	***Not sig***	***<.001***
	Var.Fert	***Not sig***	***0.02***	***Not sig***
LSD (P<0.05)	***Var***	***0.1337***		
	Fert			***0.03243***
	Var.Fert		***0.8971***	

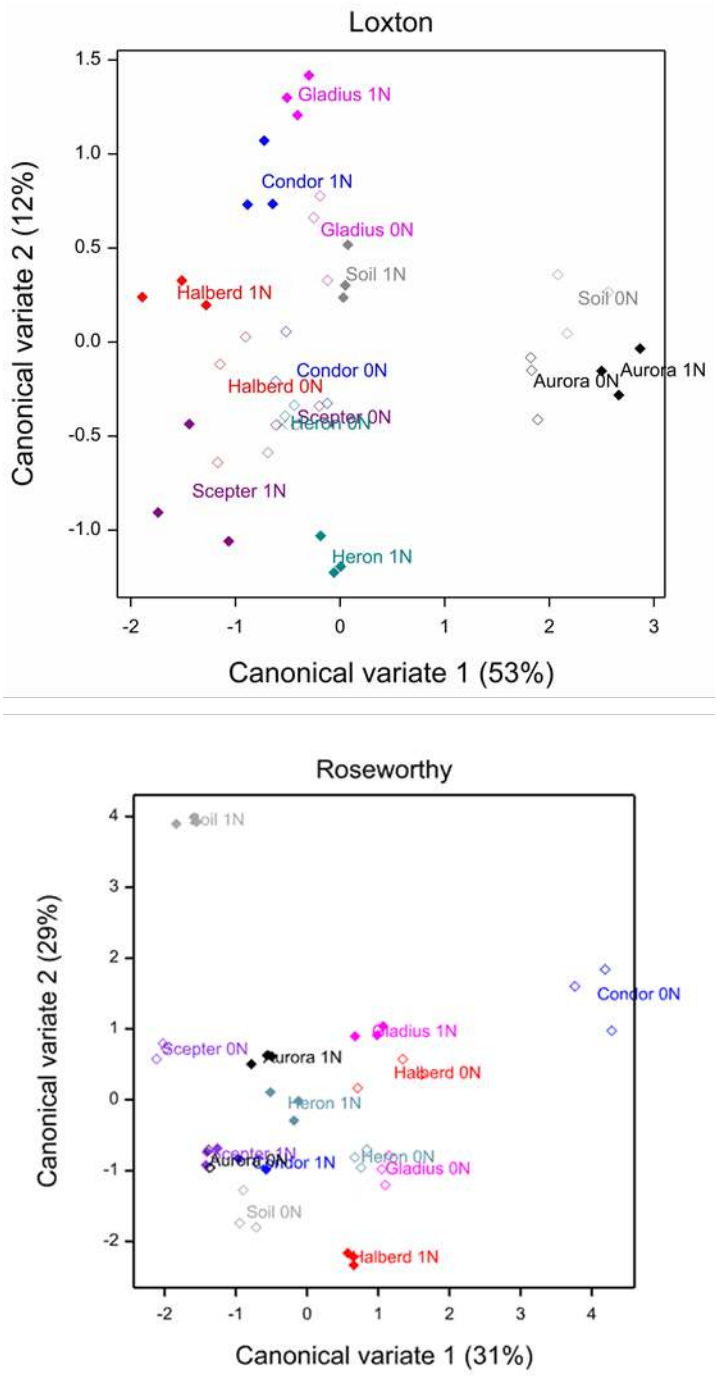


Figure A16. Results from the canonical variate analysis of microbial catabolic activity profiles for rhizosphere microbial communities as influenced by N fertilizer application, variety and soil type (ability of microbes to utilize 31 different C and N substrates). Distance between the data points for different varieties represents the similarity between them i.e. points that are closer are more similar. The first canonical variates accounted for the majority of the variation (64% of total variation) between treatments

Table A6. Abundances of microbial functional groups (gene copy number/gram soil) that regulate different N cycling processes and total bacterial (16S) and fungal (ITS) populations in the rhizosphere of wheat varieties in the controlled environment pot experiment on a red brown earth soil from Roseworthy, SA.

Variety	N Level	*S16*	*ITS*	*Npr*	*APR*	*BUrease*	*FUrease*	*AAmo*	*BAmo*	*Total amoA*	*NifH*	*npr/16S*	*BamoA/16S*	*AamoA/16S*	*APR/16S*	*nifH/16S*	*Bure/16S*	*A/B amoA*
Heron	**0N**	8.367E+10	3.692E+04	1.052E+06	4.765E+06	1.714E+09	3.401E+06	2.930E+07	5.665E+08	5.958E+08	4.409E+07							
	1N	6.476E+10	3.501E+04	1.621E+06	3.956E+06	1.636E+09	5.325E+06	5.973E+07	7.703E+08	8.300E+08	3.711E+07							
Halberd	**0N**	6.783E+10	5.622E+04	1.561E+06	4.533E+06	2.174E+09	3.701E+06	8.271E+07	1.145E+09	1.228E+09	7.354E+07							
	1N	6.494E+10	6.949E+04	1.823E+06	8.043E+06	1.994E+09	3.494E+06	5.638E+07	1.077E+09	1.133E+09	6.618E+07							
Condor	**0N**	4.753E+10	7.753E+04	1.231E+06	4.740E+06	2.413E+09	2.045E+06	3.871E+07	8.650E+08	9.037E+08	5.552E+07							
	1N	3.929E+10	2.293E+04	1.260E+06	5.049E+06	1.129E+09	2.290E+06	7.860E+07	9.597E+08	1.038E+09	5.032E+07							
Gladius	**0N**	5.288E+10	2.271E+04	2.507E+06	1.273E+07	1.993E+09	2.903E+06	1.340E+08	1.082E+09	1.216E+09	7.058E+07							
	1N	7.644E+10	9.141E+04	1.242E+06	6.296E+06	3.763E+09	4.277E+06	4.148E+07	1.410E+09	1.451E+09	7.717E+07							
Scepter	**0N**	5.557E+10	5.588E+04	8.604E+05	5.312E+06	2.718E+09	3.368E+06	4.814E+07	6.845E+08	7.326E+08	5.694E+07							
	1N	9.434E+10	5.070E+04	1.316E+06	3.932E+06	2.873E+09	4.945E+06	1.148E+08	1.068E+09	1.182E+09	5.211E+07							
Aurora	**0N**	4.566E+10	5.826E+04	1.502E+06	8.139E+06	1.363E+09	2.900E+06	1.064E+08	8.022E+08	9.086E+08	6.473E+07							
	1N	9.694E+10	8.142E+04	2.452E+06	7.195E+06	2.742E+09	4.281E+06	1.396E+08	1.201E+09	1.341E+09	9.228E+07							
Soil	**0N**	5.112E+10	7.014E+04	8.351E+05	2.665E+06	2.728E+09	6.116E+05	8.887E+07	9.064E+08	9.953E+08	5.553E+07							
	1N	4.767E+10	4.389E+04	1.293E+06	5.522E+06	1.185E+09	6.386E+05	2.018E+08	1.422E+09	1.624E+09	4.961E+07							
Average	**0N**	5.78E+10	5.40E+04	1.36E+06	6.13E+06	2.158E+09	2.70E+06	7.54E+07	8.65E+08	9.40E+08	6.013E+07	2.59E-05	1.64E-02	1.45E-03	1.18E-04	1.13E-03	0.041	0.084
	1N	6.92E+10	5.64E+04	1.57E+06	5.71E+06	2.189E+09	3.61E+06	9.89E+07	1.13E+09	1.23E+09	6.068E+07	2.60E-05	1.83E-02	1.74E-03	9.68E-05	9.59E-04	0.033	0.089
	All Samples	6.35E+10	5.52E+04	1.47E+06	5.92E+06	2.173E+09	3.16E+06	8.72E+07	9.97E+08	1.08E+09	6.041E+07	2.60E-05	1.74E-02	1.60E-03	1.08E-04	1.05E-03	0.037	0.087
F-test	**NVariety**	0.038	NS	NS	0.046	0.051	<.001	0.013	0.047	0.028	0.011	NS	0.005	0.02	NS	0.026	NS	NS
	Level	0.056	NS	NS	NS	NS	0.041	NS	0.009	0.007	NS	NS	NS	NS	NS	NS	NS	NS
	NVariety.Level	0.021	0.025	NS	NS	0.004	NS	0.026	NS	NS	NS	NS	NS	0.019	0.035	NS	NS	NS
LSD (P<0.05)	**NVariety**	2.21E+10			3.70E+06	8.78E+08	1.61E+06	5.63E+07	3.61E+08	3.78E+08	2.14E+07		7.39E-03	1.31E-03		4.68E-04		
	Level	1.18E+10					8.62E+05		1.93E+08	2.02E+08								
	NVariety.Level	3.12E+10	4.67E+04			1.25E+09		7.96E+07						1.85E-03	1.12E-04			

Table A7. Abundances of microbial functional groups (gene copy number/gram soil) that regulate different N cycling processes and total bacterial (16S) and fungal (ITS) populations in the rhizosphere of wheat varieties in the controlled environment pot experiment in a Mallee sand soil from Loxton, SA.

Variety	N Level	*S16*	*ITS*	*Npr*	*APR*	*BUrease*	*FUrease*	*AAmo*	*BAmo*	*Total amoA*	*NifH*	*npr/16S*	*BamoA/16S*	*AamoA/16S*	*APR/16S*	*nifH/16S*	*Bure/16S*	*A/B amoA*
Heron	**0N**	1.462E+09	3.491E+04	5.808E+06	1.266E+06	5.884E+07	1.100E+05	1.128E+05	8.974E+06	9.086E+06	4.533E+06							
	1N	9.631E+08	2.043E+04	4.251E+06	1.162E+06	9.724E+07	8.347E+04	9.719E+04	6.268E+07	6.278E+07	1.763E+06							
Halberd	**0N**	8.277E+08	2.583E+04	1.000E+06	7.983E+05	1.334E+08	1.769E+05	2.482E+05	4.586E+06	4.834E+06	1.289E+06							
	1N	1.201E+09	3.773E+04	3.676E+06	1.445E+06	1.139E+08	7.898E+04	1.139E+05	6.199E+07	6.211E+07	1.841E+06							
Condor	**0N**	1.866E+09	3.329E+04	5.746E+06	2.565E+06	8.069E+07	9.034E+04	3.573E+05	2.509E+07	2.545E+07	4.897E+06							
	1N	6.598E+08	1.611E+04	3.705E+06	1.784E+06	1.043E+08	1.050E+05	1.018E+05	6.640E+07	6.650E+07	1.729E+06							
Gladius	**0N**	1.019E+09	3.524E+04	3.889E+06	2.074E+06	9.265E+07	2.580E+05	1.411E+05	6.825E+06	6.966E+06	2.100E+06							
	1N	1.346E+09	4.338E+04	4.644E+06	1.219E+06	1.162E+08	1.287E+05	8.955E+04	4.646E+07	4.655E+07	2.322E+06							
Scepter	**0N**	1.423E+09	4.485E+04	4.108E+06	1.093E+06	5.949E+07	1.133E+05	1.467E+05	2.229E+07	2.243E+07	1.862E+06							
	1N	7.966E+08	4.979E+04	4.101E+06	9.841E+05	4.306E+07	1.838E+05	9.068E+04	5.518E+07	5.527E+07	2.124E+06							
Aurora	**0N**	1.104E+09	4.230E+04	6.944E+06	2.020E+06	8.061E+07	1.291E+05	1.681E+05	1.525E+07	1.542E+07	2.915E+06							
	1N	1.096E+09	3.382E+04	5.186E+06	9.687E+05	4.704E+07	1.091E+05	1.555E+05	1.356E+08	1.358E+08	2.334E+06							
Soil	**0N**	3.245E+08	3.300E+04	2.270E+06	2.779E+05	6.993E+07	4.455E+04	6.365E+04	5.441E+06	5.505E+06	1.479E+06							
	1N	1.361E+08	2.012E+04	1.681E+06	1.531E+05	7.067E+07	2.458E+04	5.211E+04	1.916E+07	1.921E+07	4.351E+05							
Average	**0N**	1.15E+09	3.56E+04	4.25E+06	1.44E+06	8.22E+07	1.32E+05	1.77E+05	1.26E+07	1.28E+07	2.73E+06	4.52E-03	1.38E-02	1.65E-04	1.30E-03	2.89E-03	1.22E-01	2.39E-02
	1N	8.85E+08	3.16E+04	3.89E+06	1.10E+06	8.46E+07	1.02E+05	1.00E+05	6.39E+07	6.40E+07	1.79E+06	5.95E-03	9.40E-02	1.70E-04	1.37E-03	2.39E-03	1.45E-01	1.87E-03
	All Samples	1.02E+09	3.36E+04	4.07E+06	1.27E+06	8.34E+07	1.17E+05	1.38E+05	3.83E+07	3.84E+07	2.26E+06	5.23E-03	5.39E-02	1.68E-04	1.33E-03	2.64E-03	1.34E-01	1.29E-02
F-test	**NVariety**	NS	NS	0.004	<.001	0.51	0.01	0.008	<.001	<.001	0.001			<.001				
	Level	NS	NS	NS	NS	NS	NS	0.002	<.001	<.001	0.002			NS				
	NVariety.Level	NS	NS	NS	NS	NS	NS	NS	0.003	0.003	0.004			NS				
LSD (P<0.05)	**NVariety**	NS	NS	2.05E+06	7.10E+05	7.64E+07	7.57E+04	8.52E+04	2.33E+07	2.34E+07	1.06E+06			9.36E-05				
	Level	NS	NS	NS	NS	NS	NS	4.55E+04	1.25E+07	1.25E+07	5.69E+05			NS				
	NVariety.Level	NS	NS	NS	NS	NS	NS	NS	3.30E+07	3.30E+07	1.51E+06			NS				

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