CSIRO Land and Water

Management of herbicide effects on soil biological processes essential for plant health and nutrition

V.V.S.R. Gupta
CSIRO Land and Water, Glen Osmond, SA 5064

Collaborator:
Grant Roberts, CSIRO Plant Industry, CRC Cotton Research Unit
Narrabri, NSW 2390

Cotton
RESEARCH & DEVELOPMENT

Cotton
RESEARCH & DEVELOPMENT

Final Report

Part 1 - Summary Details

REPORTS

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

CRDC Project Number: **SLM2C**

Annual Report: ☐ Due 30-September

Progress Report: ☐ Due 31-January

Final Report: ☐ Due 30-September

Project Title: Management of herbicide effects on soil biological processes essential for plant health and nutrition

Project Commencement Date: 01/06/2000 **Project Completion Date:** 31/12/2003

Research Program: Soils

Part 2 – Contact Details

Administrator: Ms. Rebecca Dunn

Organisation: CSIRO Land and Water

Postal Address: PMB No 2, Glen Osmond, SA 5064

Ph: 08-83038507 **Fax:** 08-83038550 **E-mail:** Rebecca.Dunn@csiro.au

Principal Researcher: Gupta Vadakattu V.S.R.

Organisation: same as above

Postal Address: Same as above

Ph: 08-83038579 **Fax:** 08-83038550 **E-mail:** Gupta.Vadakattu@csiro.au

Supervisor: Same as above

Organisation:

Postal Address:

Ph: **Fax:** **E-mail:**

Researcher 2 Dr. Grant Roberts

Organisation: CSIRO Plant Industry, CRC Cotton Research Unit

Postal Address: Myall wale, Wee Waa road, Narrabri, NSW 2390

Ph: 02-67991500 **Fax:** 02-67931186 **E-mail:** Grant.Roberts@csiro.au

Signature of Research Provider Representative:

Part 3.3 – Final Reports

(The points below are to be used as a guideline when completing your final report. Postgraduates please note the instructions outlined at the end of this Section.)

1. Outline the background to the project.

Microorganisms regulate a majority of processes in soil that are essential for plant growth (nutrient availability and disease incidence), soil health (soil structure and agrochemical degradation) and sustained productivity (Gupta, 1998). A large, diverse and active soil biota could help provide soil conditions for sustainable cotton production through (i) crop residue decomposition and improvement of nutrient supplying potential of soils, (ii) preventing aggressive plant pathogens taking hold and improve plants ability to withstand disease effects, (iii) reducing the loss of inorganic fertilizers through erosion and leaching by short-term immobilization (iv) stabilizing soil structure and (v) reducing the reliance for agrochemicals and reduced persistence of pesticides in soil and thus less off-site impacts. In a high-input cropping system such as cotton, it is essential to maintain activities of key microbial groups to maximise input efficiency and to reduce non-target environmental negative effects.

Herbicide use is a vital component of modern agriculture; in particular under reduced till systems. With increased adoption of stubble retention and reduced till practices and the introduction of new herbicides, herbicide use will remain as an essential practice in the near future. More than one million kg active ingredients of herbicides are applied every year in order to obtain weed-free cotton fields in Australia (Charles, 1991). Herbicide use is an essential management practice in cotton growing and multiple applications of a wide array of herbicides in a single season are a common practice. Application of herbicides may not affect the overall size of the microorganism pool but selectively affect specific groups of biota which may result in altering the balance of biological activity (soil biological health) and consequently nutrient availability, disease incidence and plant growth.

Non-target effects of herbicides could be either positive or negative. Figure 1 illustrates two basic types of inhibitions caused by herbicide application i.e. irreversible and reversible against the base line of a given population / activity remains constant (untreated control). If a specific herbicide has been shown to cause irreversible inhibition of key groups of microorganisms then avoiding its use is the simple and best option. The two reversible inhibitions shown in Figure 1 differ in the magnitude of maximum depression (the greatest difference between treated and untreated/control soil samples) and the duration of the herbicide effect (recovery period for the microbial function in treated samples to reach that in control soil samples). This information is essential for developing management options that either reduce the negative effects or compensate for the loss of a biological function. Management of the use of herbicides that cause reversible inhibitions is difficult, as reaching a balance between high herbicide efficiency and minimum non-target effects requires a better understanding of herbicide-microorganism-environment interactions.

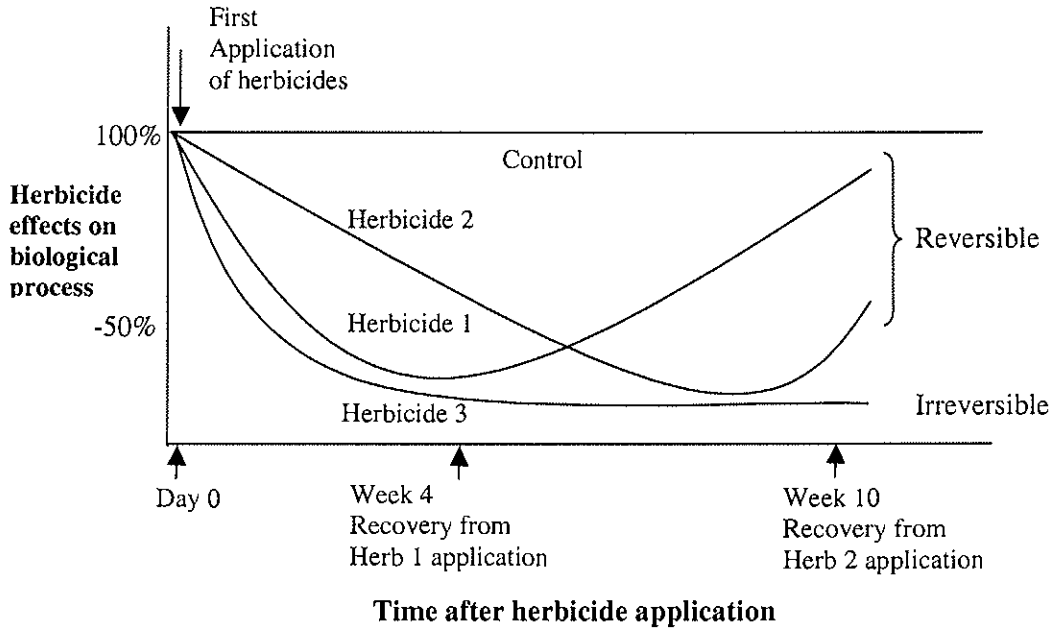


Figure 1. A conceptual diagram showing different types of herbicide effects on soil biological processes in cotton soils.

Using the above conceptual frame work the effects of single application of herbicides on different soil microbial populations and their activities were determined in a previous project (SLM1C). Results from these investigations on the effects of a single application of selected herbicides in cotton soils indicated that:

1) A number of herbicides currently used in cotton soils have a negative impact on key groups of microorganisms;
2) Most of the negative effects were reversible partly or fully within 10-weeks after herbicide application;
3) Some herbicides caused a significant shift in bacteria : fungi ratio, reduced decomposition rate of cotton stubble and has the potential to influence disease incidence;
4) Some herbicides applied in cotton had negative impact on symbiotic N2-fixation by legumes in rotation and
5) Application of a post-emergent herbicide Staple caused significant changes in the rhizosphere microbial activity and the composition of microbial community. In a collaborative study (funded by the CRC for S&LM) we found that herbicide effects on rhizosphere microbial community were different for conventional and Ingard® cotton.

Even though the effects from a single application were reversible, the impact of additional herbicides applied prior to the full recovery may result in an overall adverse effect on key microbial activities. For example, a continued herbicide induced stress on general microbial activity in rhizosphere and near crop residues may encourage the dominance of pathogenic microorganisms. No information is available on the effects of multiple herbicide applications on soil biological processes in cotton soils in Australia. Similarly no information is available on the effect of herbicides on the survival and growth of pathogenic fungi on cotton residues.

Transgenic cotton varieties (GM-cotton) will play a major role in the development of sustainable cotton industry in the future and more than 60,000 ha was sown to Ingard cotton during 1998-99 season. Herbicide tolerant cotton varieties may soon be available for farmer use in Australia. No information is available on herbicide effects in the rhizosphere and near decomposing crop residues of transgenic cotton varieties. Information on the effects of herbicide application (especially multiple herbicides in one season) on soil biota and biological processes is necessary for the successful incorporation of GM-cotton into Australian cotton industry, particularly herbicide-tolerant cotton.

2. List the project objectives and the extent to which these have been achieved.

1. To quantify the effects from multi-herbicide use on key soil biological processes in soils

2. To quantify the magnitude and duration of the effects from multi-herbicide applications (pre- and post-emergence) during the cotton season on populations and activities of key microbial groups essential for nutrient availability and uptake, crop residue decomposition and plant growth

– Experiments conducted both under field conditions (2000/01, 2001/02 and 2002/03) at ACRI and glasshouse conditions at CSIRO L&W in Adelaide.

3. To evaluate the effects of multi-herbicide application on microbial processes in the rhizosphere and near crop residues of genetically modified cotton varieties and determine their impact on cotton growth and the maintenance of soil health.

Since the microbiology of the GM cotton residues was found to be significantly different to that of conventional GM cotton residues, experiments were conducted to determine the extent of differences between GM and conventional cotton varieties (as part of collaboration with CLW1C) as a foundation to any future experimentation to investigate the herbicide x GM cotton interaction.

4. To provide recommendations to changes in herbicide usage patterns based on potential impacts on key soil processes.

- In order to provide practical recommendations, additional field experiment was conducted during 2002/03 season combined with glasshouse based intact core experiments.

3. Detail the methodology and justify the methodology used.

Non-target effects of herbicides could be either positive or negative. Figure 1 illustrates two basic types of inhibitions caused by herbicide application i.e. irreversible and reversible against the base line of a given population / activity remains constant (untreated control). If a specific herbicide has been shown to cause irreversible inhibition of key groups of microorganisms then avoiding its use is the simple and best option. The two reversible inhibitions shown in Figure 1 differ in the magnitude of maximum depression (the greatest difference between treated and untreated/control soil samples) and the duration of the herbicide effect (recovery period for the microbial function in treated samples to reach that in control soil samples). This information is essential for developing management options that either reduce the negative effects or compensate for the loss of a biological function. Management of the use of herbicides that cause reversible inhibitions is difficult, as

reaching a balance between high herbicide efficiency and minimum non-target effects requires a better understanding of herbicide-microorganism-environment interactions.

In this project, the impact of multi-herbicide application on soil biological processes were investigated using both field and glasshouse experiments. Field experiments were conducted on soils with known herbicide history. The sites of field experiments were at Myall Vale, NSW. A combination of pre- and post-emergence herbicides were selected {e.g. Diuron, Prometryn (Gesagard®), Trifluralin, Fluometuron (Cotoran®) and Pyrithiobac (Staple®)} based on their effects observed in the previous project and in consultation with experts in the industry. For each soil the comparisons were made between plots with no test herbicide application and plots with two or more herbicide applications. Glasshouse experiments were conducted using intact soil cores collected from control plots in field experiments, in order to obtain detailed information on specific biological processes and for herbicide combinations that cause significant changes in soil biota in field experiments. Details of various herbicides tested in this project are given below:

Common name	Active Ingredient	Rate	Mode of action	Group	Time of application
Treflan	Trifluralin	2.8L/ha	Absorbed through roots and shoots, disrupts tubulin protein invloved in cell division	D	Pre emergence
Stomp	Pendimethalin	4 L/ha	Absorbed through roots and shoots, disrupts tubulin protein invloved in cell division	D	Pre emergence
Diuron	Diuron	2.5 kg/ha	Root absorption, inhibits photosynthesis	C	Pre emergence and Post (usually Pre)
Gesagard	Prometryn	3-5 L/ha	Root absorbption mailny some foliar, inhibits photosynthesis	C	Pre-emergence (Can be both)
Staple	Pyrithiobac-sodium	120 g/ha	Absorbed through foliage - ALS inhibitor	B	Post-emergence
Cotoran	Fluometuron	3-5 L/ha	Root absorbption mailny some foliar, inhibits photosynthesis	C	Pre emergence

Field experiments:

1. Field experiments were conducted at the ACRI farm during 2001 and 2002 cotton seasons to determine the effect of multiple herbicide application on soil biological processes in cotton soils. All treatments were replicated four times.

Table 1. Herbicide treatment details for the field experiments in 2001 and 2002 cotton seasons.

Treatment	Pre plant + incorporated	Rate	Post plant – Pre emergence	Rate	Lay by	Rate
1 Best	Trifluralin	2.8	Cotoran	4 L/ha		

		L/ha				
2 Best	Trifluralin	2.8 L/ha	Cotoran	4 L/ha	Staple	120 g/ha
3 Worst	Stomp	4 L/ha	Diuron	2.5 L/ha		
4 Worst	Stomp	4 L/ha	Diuron	2.5 L/ha	Gesagard	4 L/ha
5 Control	No herbicide application					

2. During the 2002-2003 season, in a factorial experiment, two herbicide treatments (given below) along with a no herbicide treatment were tested in soils with a rotational history (vetch or fallow) and irrigated or dryland cotton.

Table 2. Herbicide treatment details for the field experiment during 2002-03 cotton season.

Treat	**Pre plant + incorporated**	**Rate**	**Lay by**	**Rate**
1	Trifluralin + Diuron	2.8 + 2.5 L/ha	Staple	120 g/ha
2	Stomp + Cotoran	4 + 3 L/ha	Gesagard	4 L/ha
3	Control (no herbicide)			

3. Residual effects of cotton herbicides on rotations Vetch crop - In order to determine the effect of residual herbicides, originally applied during cotton season, on nitrogen fixation, Vetch crop was grown following the harvest of the cotton crop in each of the three seasons. There was no additional herbicide application in the Vetch crop it self to avoid any interactions or masking that may occur.

4. Effect of herbicide application on the decomposition of cotton residues – Litter bag experiments (12-15 weeks) were conducted in the field plots at Narrabri in all three years, to test the effects of various herbicides on residue decomposition and associated microbial properties.

Briefly, litterbags with known amount of cotton stubble (stubble cut ~ 4cm length) treated with appropriate herbicides (or no herbicide) were buried in the experimental plots after the sowing of cotton. Selected litterbags were retrieved at regular intervals (3, 6, 9, 12, 15 weeks after incubation) and transported to Adelaide laboratories for dry weights, chemical (C and N concentrations) and biological analysis (microbial activity).

Glasshouse experiments:

1a. Effect of single or multiple herbicide application on microbial activity and selected microbial functions - A glasshouse experiment was conducted using 'Intact' soil cores (PVC cores with 10 cm dia and 10 cm deep) collected from the control plots in field experiments during 2001 and 2002. A combination of one, two or three types of herbicide application was tested for their impacts on microbial activity, microbial biomass and microbial functions such as nitrification in a two-month incubation experiment (at 25°C). Following an initial 3 days of pre-incubation with adequate soil moisture condition, the first set of herbicides were applied and the second ('lay by') herbicide application was done ~35 days after the start of the experiment.

where microbiological, biochemical and chemical analysis were conducted. Care was taken during collection and transport (in Eski with ice) of soil samples in order to reduce moisture loss and alterations to microbiological properties. Only methods standardised in our laboratory during previous years experimentation were used for collection, transport and preparation before laboratory analyses of soil and residue samples. The biological parameters measured include: microbial activity, populations of cellulolytic bacteria and fungi, nitrifying bacteria, functional groups of decomposing microorganisms and associated processes such as rate of nitrification, mineralization of nitrogen, mineralization of carbon (and activities of enzymes involved in cellulose decomposition) and the size and metabolic status of microbial biomass.

Populations of cellulolytic bacteria and fungi were determined using most-probable-number methods (Gupta and Roper, 1994). Chloroform fumigation-direct extraction methods were used to measure the amounts of microbial biomass. Short term incubation methods (laboratory based) were used to measure the level of microbial activity, nitrification rates and mineralization potentials of nitrogen. The metabolic status of microorganisms was determined using the substrate induced respiration method. The activities of functional groups of carbon mineralizers were analysed using BIOLOG plates with specific carbon substrates selected for cotton rhizosphere (Gupta et al., 1998). General information on soil physical (texture and structure) and chemical properties (organic C and total N, P and S) was also obtained.

In summary, the significance of the changes in populations and associated processes was assessed in relation to management practices that may be adopted to minimise their impact on crop productivity and soil health. For any specific herbicide, the damage assessment was made against a nil herbicide control. We used a two-tier system to evaluate the herbicide effects e.g. populations of ammonia oxidizing microorganisms coupled with the rate of nitrification as the two types of measurements on the microbial functional group involved in N mineralization. This two-tier approach using information on key functional groups would be better suited, compared to single biological property studies, to evaluate the herbicide effects in different soil types and cropping systems.

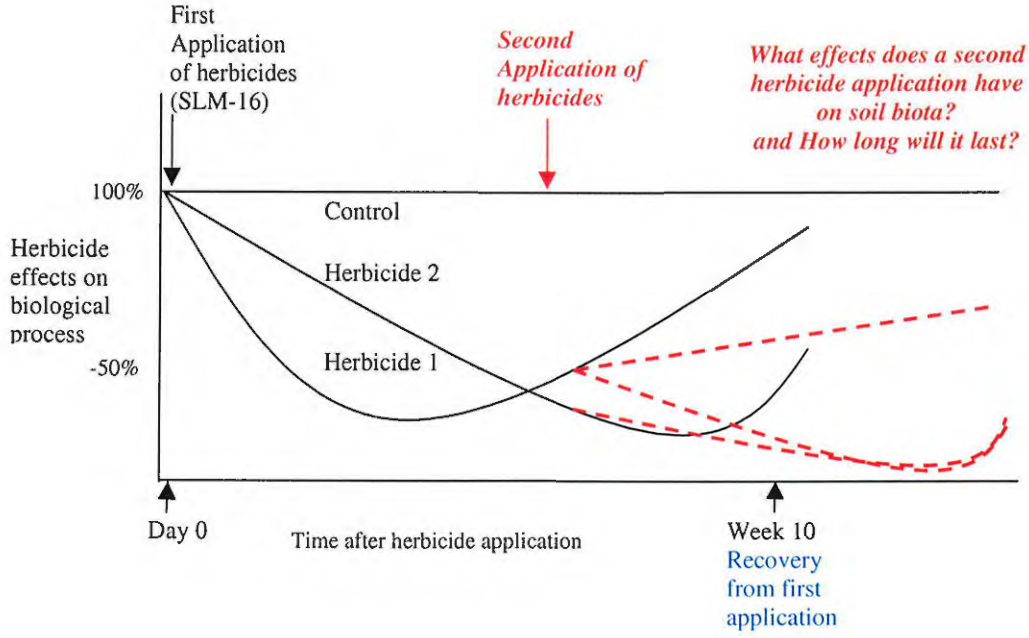


Figure 2. The role of this project (italics) in determining the impact of multi-herbicide use on soil biological processes in cotton soils

4. Detail and discuss the results including the statistical analysis of results.

1. Field experiments:

A) Effects of multiple herbicide application on soil biological properties (2000-01 and 01-02 seasons):

Surface soil samples collected from field experimental plots, at 4 times to coincide herbicide application events, were analysed for various microbial, chemical and biochemical properties. The first sampling was always done prior to herbicide application to know the status of microbial community prior to any herbicide treatment and the later samplings were done 7-10 days after herbicide application (first set of herbicides and 'lay by' herbicide application) and the final samples were collected after a minimum of 4 weeks from the 'lay by' herbicide application. Microbial biomass levels in the control plots ranged from 200 to 520 mg C/kg soil and these levels accounted for 2 to 5% of soil organic carbon. During 2000-01 cotton season MB levels were highest during the first sampling, i.e. prior to herbicide application (435 mg MB-C / kg soil). MB levels were generally lower in all plots and at all the three later samplings (<250 mg C / kg soil). During the 2000-01 season experimental plots at ACRI experienced repeated flooding and soil was in a saturated condition for periods of more than a week. During this season our first experiment was abandoned due to the flooding immediately after the application of first herbicides. A majority of soil microorganisms require aerobic soil conditions and the prolonged water logging could be one of the reasons for the lower levels of MB in the later samples. During 2001-02 season MB levels increased as cotton season progressed, an indication of cotton rhizosphere induced increase in microbial biomass.

The application of different combination of herbicides had varying effect on the level of microbial biomass in the two seasons. During 2000-01 season application of herbicides caused a decline in MB at the 7 day sampling after herbicide application. But the levels of MB recovered from then onwards and the rate of recovery was different in the different herbicide treatments. Application of 'lay by' herbicides slowed the recovery in MB levels compared to the treatments with initial herbicide application only (Figure shown below). At the final sampling, in both years of field experiments, MB levels in the treatments with 'lay by' herbicide application were lower than those in the single application of herbicides (10-15%). In general MB levels in the Trifluralin+Cotoran treatment were higher than Stomp+Diuron treatment. During 2001-02 season, MB levels in different herbicide treated plots were similar or higher than that in the 'no herbicide application.

Application of herbicides had a significant impact on the level of microbial activity and metabolic status of microorganisms. The effect of herbicides was highest at the second sampling i.e. second week after initial herbicide application when the decline in the microbial activity in herbicide treated plots compared to the control plots ranged from 10-20%. A recovery in microbial activity was observed in most herbicide treated plots except in the treatment receiving the lay by herbicide 'Gesagard'. Our results from the previous work indicated that application of herbicide Gesagard caused significant reductions in microbial activity.

The microbial quotient values (qCO_2, microbial activity per unit microbial biomass) for the samples collected during the second week after initial herbicide application, during 2000-01 season, were higher than that for control soils. A reduction in MB levels coupled with higher qCO2 values generally indicates a stress on the growth of microbial community. As the microbial community recovered from herbicide impacts by the final sampling the qCO_2 values lowered.

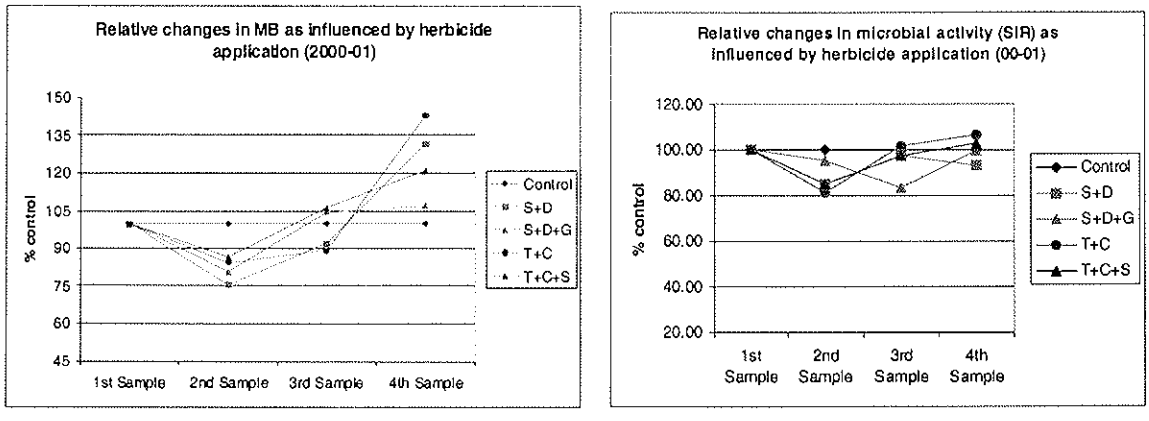


Figure 3. Microbial biomass levels

Surface soil samples from the field experiments were also analysed for any changes due to the application of various herbicides, in the populations of microbial communities involved in carbon turnover and nitrogen mineralization. Most probable numbers (MPN) for the populations of cellulolytic bacteria (CB) and

cellulolytic fungi (CF) ranged between 10^5 to 10^6 per gram soil and 10^4-10^5 per gram soil, respectively.

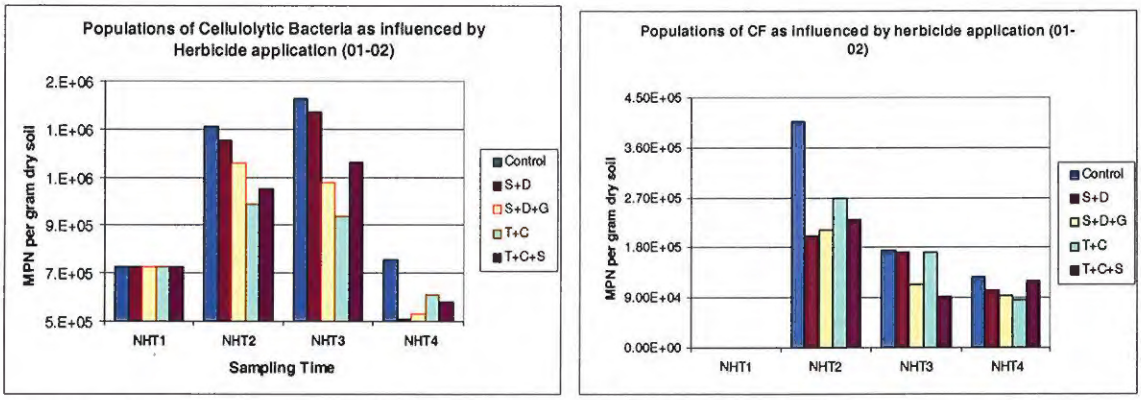


Figure 4. Populations of cellulolytic bacteria (CB) and cellulolytic fungi (CF)

Populations of CF generally remained 10^5 per gram soil through out the season during 2001-02, where as during 2000-01 season there was a 10-fold decline in CF populations at the final sampling. Seasonal changes in the populations of CB and CF reflect the soil and environmental conditions present, e.g. availability of crop residues, soil moisture and temperature are some of the factors influencing the populations of CB and CF. The effect of different management practices including application of herbicides on microbial populations is both due to the direct effect of herbicides on microorganisms and indirect effects through changes in soil and environmental conditions. For example, if an application of a herbicide results in the addition of large quantities of dead weed plant biomass then it could cause an increase in microbial populations involved in decomposition.

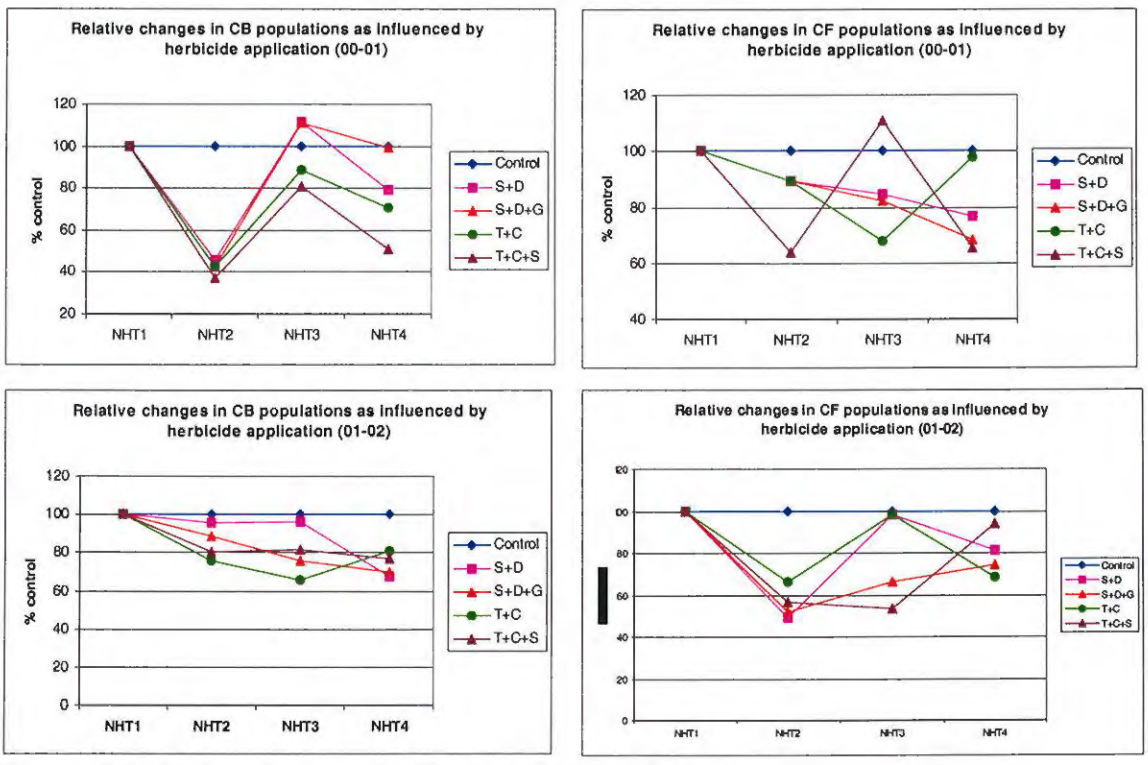
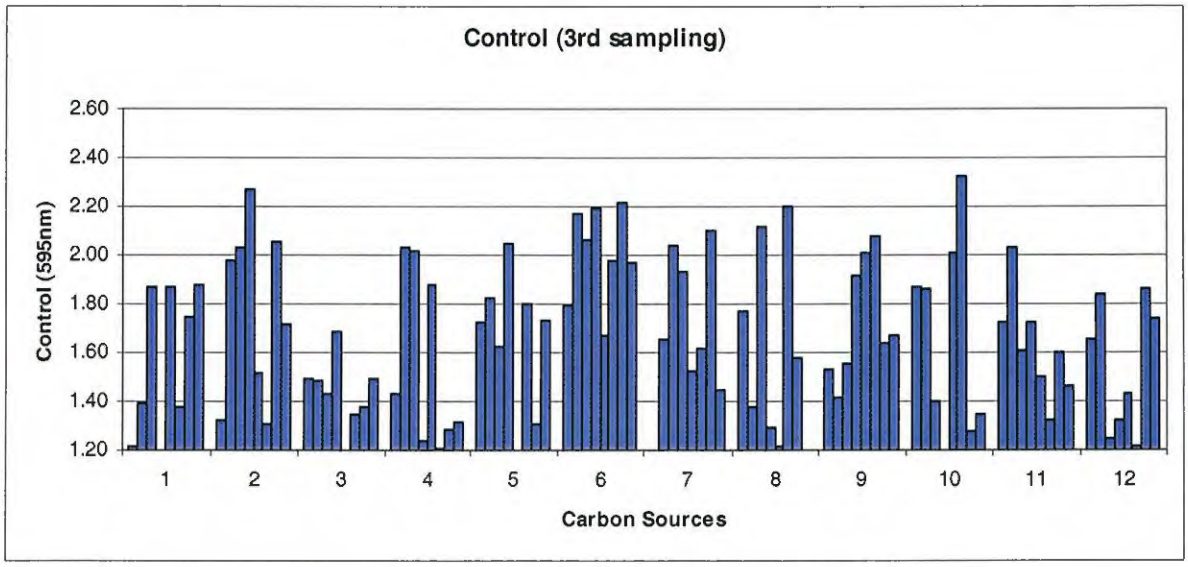


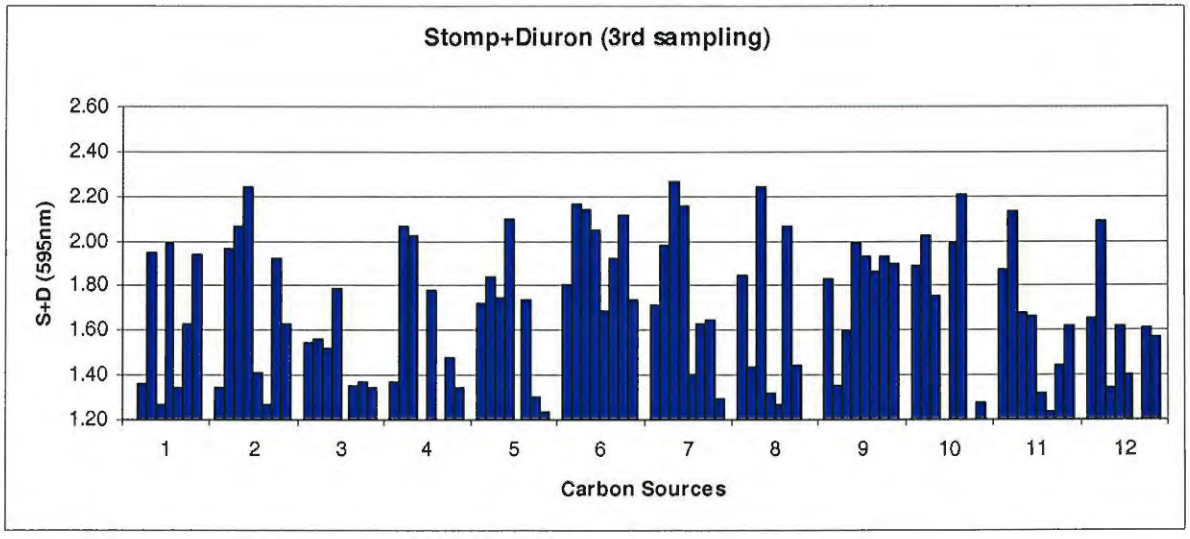
Figure 5. Relative changes in CB and CF populations.

Data on the populations of CB and CF indicated significant changes in populations of these microorganisms in herbicide treated plots compared to no-herbicide treated control plots (Figures 5). The changes in CB and CF populations were different in different herbicide treatments and the two seasons except that the effect was negative in the samples collected during the second week after initial herbicide application. Any recovery in population levels varied between herbicide treatments and seasons.

Results from the litterbag experiments indicated a proliferation of fungi near decomposing cotton stubble treated with herbicides (more details later in this section).

In addition to the CB and CF populations we measured the changes in populations of soil bacteria based on their ability to use 95 different carbon substrates (using BIOLOG-GN plates). Results from these analysis indicated that herbicide application caused significant changes in soil bacterial community composition (based on BIOLOG substrate utilization profiles) and the effects were different for different herbicide combinations. Application of 'lay by' herbicides also caused changes in substrate utilization profiles compared to the initial herbicide application only (Figure 6). At the final sampling the BIOLOG-GN profile for soils from Stomp+Diuron+Gesagard were remained different to that of control soils where as the BIOLOG-GN profile for Trifluralin+Cotoran+Staple was more similar to the control soils. These results suggest that the composition of bacterial community was modified with the application of herbicides either due to the direct effects of herbicides on specific bacterial community or because of the changes in available carbon sources. It has been suggested in the literature that soil faunal activity could modify the composition of microflora. We have no measured the population or activity of soil fauna i.e. mesofauna and macrofauna such as earthworms.





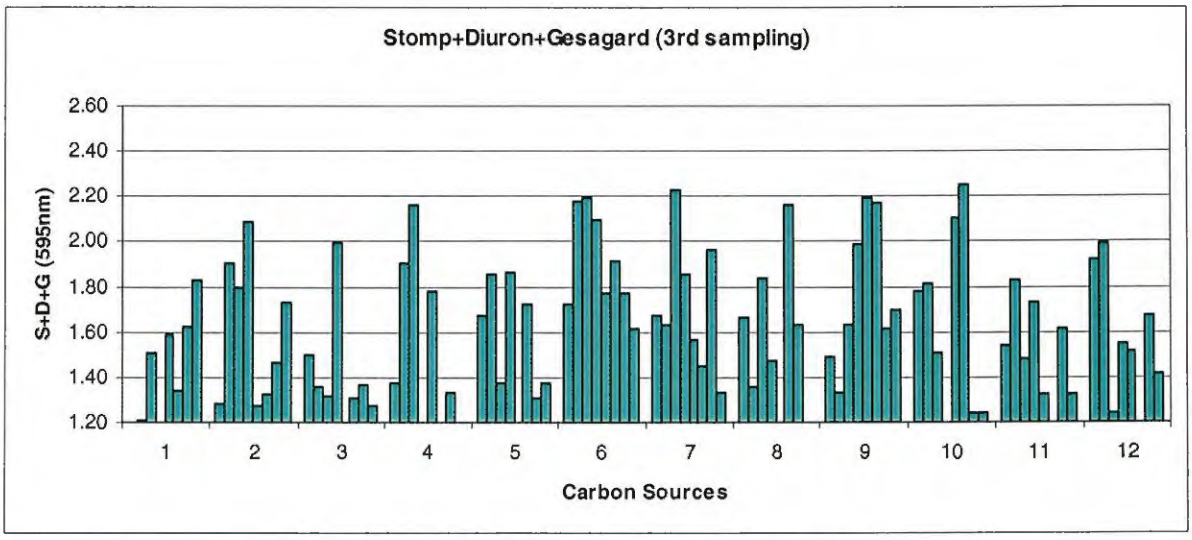


Figure 6: Bacterial composition as indicated by the substrate utilization profiles measured using the BIOLOG-GN plates. Results indicate that herbicide application influenced both the spectrum and level of use of various carbon substrates by bacteria in the soil samples.

Nitrification is one of the important biological process both in the mineralization of nitrogen from soil organic matter and transformation of fertilizer nitrogen in to plant available nitrate nitrogen. The two key groups of microorganisms involved in nitrification are 'ammonia oxidizing microorganisms' (AO) and 'nitrite oxidizing microorganisms' (NO). Populations of AO and NO in the soils from field experiments ranges from 10^4 to 10^6 and 10^4 per gram soil, respectively. Populations of AO were generally higher than NO except that at the first sampling, in both seasons. Application of herbicides had significant negative impact on both the AO and NO populations in both seasons, e.g. 20-40% reduction in AO populations and 20-80% reduction in NO populations at the 7 days after initial herbicide application. A recovery in AO and NO populations was observed by the third sampling, however the recovery was different depending upon the herbicide used and the application of 'lay by' herbicides (Figures 7). For example, during 2000-01 season, the application of 'lay by' herbicides had greater impact on NO populations.

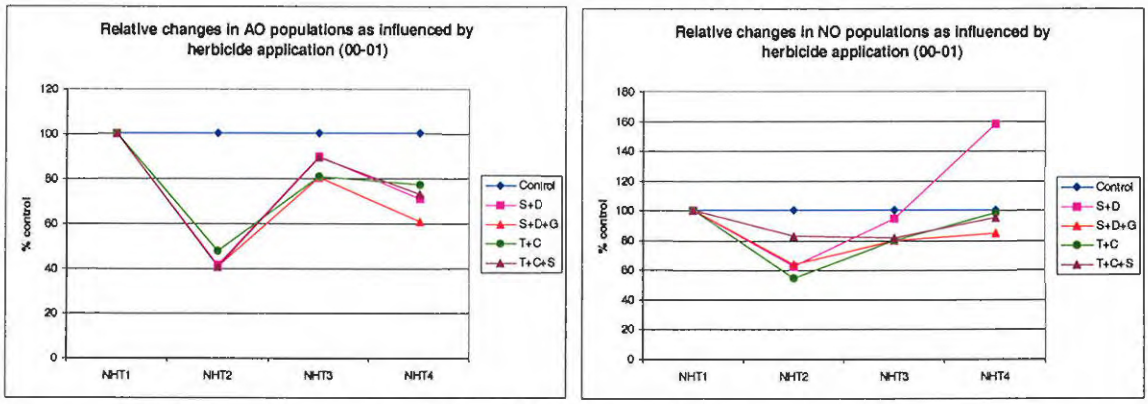


Figure 7: Relation changes in AO and NO populations.

The negative impact of herbicide application on AO and NO populations was also reflected in the rate of nitrification it self. Data given in the Table below shows the effect of different herbicide combinations on the rate of nitrification following the application of 'lay by' herbicides.

Table 4. Rate of nitrification, estimated using 24 h incubation assay, as influenced by herbicide application (2001-02 season, third sampling)

Treatment	**µg Nitrite N per gram soil per hour***	
	0-24 h	**5-24 h**
Control	1.2466a#	1.1689a
Stomp+Diuron	1.0059b	0.6662c
Stomp+Diuron+Gesagard	0.825c	0.6024c
Trifluralin+Cotoran	0.9489b	0.809c
Trifluralin+Cotoran+Staple	0.9332b	0.7173bc

*derived from the slope of the regression line

#values followed by the same letter are not significantly different from the control value at $P<0.05$

Our estimation of net N mineralization using a laboratory incubation assay indicated that herbicide application caused a reduction in net N mineralization in all herbicide

treatments at the 7day sampling (NHT2) in both seasons. Net N mineralization in the surface soil from field experimental plots ranged from 4.0 to 9.0 mg N mineralized per kg soil per day and the values were generally higher for samples from 2001-02 season compared to the 2000-01 season. The effect of 'lay by' herbicides was only observed during 2001-02 season; e.g. 15 to 25% reduction in net N mineralization in the 'lay by' treatments compared to control. The observed effect of herbicides on net N mineralization is a combination of their effects on nitrification of fertilizer N and mineralization of soil organic N. Since the cotton crop is generally grown with a large amount of applied N fertilizer, the actual significance of herbicides on N mineralization to plant N requirement is less significant but it may be more relevant to the efficiency of fertilizer inputs or its losses.

B) Impact of crop management practices on herbicide effects on soil biota:
It is known that cropping history i.e. previous crop type has the potential to impact on the populations and activities of different soil microorganisms. During the third year of experimentation the effects of two sets of multiple herbicide application were determined in soils with two cropping histories i.e. Fallow-Cotton and Vetch-Cotton. All these treatments were tested in both under Irrigated cotton and Dryland cotton systems.

Analysis of soils collected at the time of cotton planting indicated that the populations and activities of microbial populations were higher in soils after Vetch crop compared to that after fallow. For example, populations of cellulolytic bacteria in soils after vetch crop were double compared to that after fallow soils. Populations of Cellulolytic fungi averaged 10^5 MPN per gram soil and they were higher after vetch than in Fallow soils. Similarly microbial biomass levels were higher in soils after vetch (320 mg MB-C / kg soil) compared to that after Fallow treatment (249 mg MB-C / kg soil).

Due to unsuitable soil/environmental conditions we were unable to collect samples 7 days after initial herbicide sprays, however samples were collected prior to the application of 'lay by' herbicides. Application of herbicides caused significant changes in the CB and CF populations under all the management systems i.e. vetch-cotton or fallow-cotton and irrigated or dryland systems. Results from the samples collected prior to application of 'lay by' herbicides showed that application of the two herbicide treatments (Stomp+Cotoran and Trifluralin+Diuron) caused an increase in CB populations (2-fold or greater) and a reduction in CF populations compared to control soils in the fallow-cotton systems. However in the vetch-irrigated cotton systems application of herbicides caused significant reductions in both the CB and CF populations; Stomp+Cotoran causing higher reductions in CB populations. Populations of CB were generally higher in the irrigated cotton ($6x10^5$-$1x10^6$ MPN per gram soil) compared to dryland cotton ($2x10^5$-$6x10^5$ MPN / g soil) systems. Also CB populations were higher in vetch-cotton systems compared to fallow-cotton systems. There was no difference in CB and CF populations in the soil samples from different treatments collected after 'lay by' herbicide application except for the vetch-dryland cotton system in which herbicide application caused an increase in CF populations and reduction in CB populations. Overall, the results on

CB and CF populations suggest significant changes in CB and CF populations due to the two types of herbicides tested.

Application of herbicides caused reductions in the populations of nitrifying microorganisms (AO populations) and the effect of the herbicide combination of 'Stomp+Cotoran' was higher than that of 'Trifluralin+Diuron' application (Figure 8). The effects of herbicides on AO populations also reflected in the rate of nitrification measured using a laboratory incubation assay. For example, in the fallow-dryland cotton system where herbicides caused more than 2-fold reduction in AO populations was also reflected in a decline in the rate of nitrification (Table 4). Herbicide effects on rate of rate of nitrification in soils from irrigated cotton systems did not directly reflect changes in AO populations, in particular in the Fallow-Irrigated cotton systems.

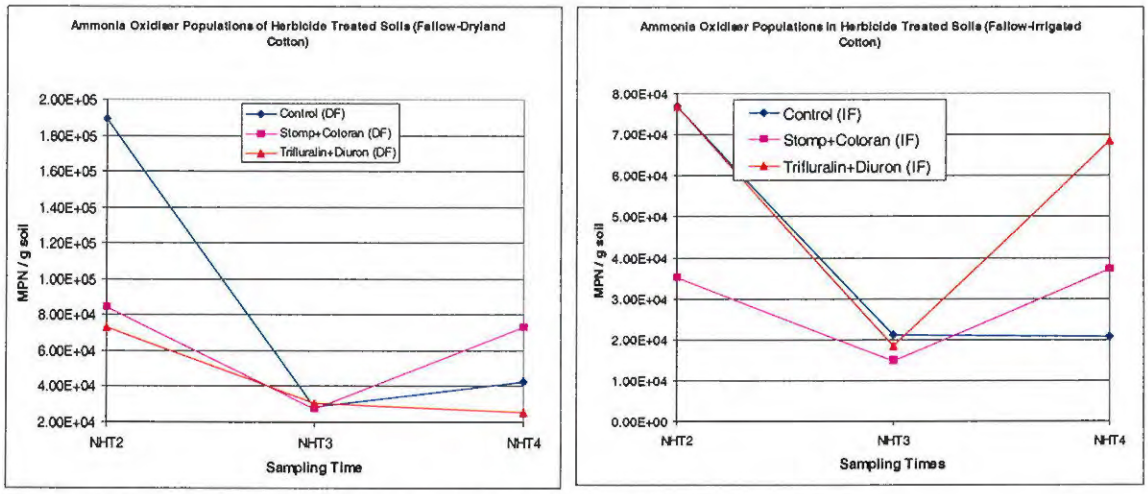


Figure 8: Populations of Ammonia oxidizing microorganisms

Microbial biomass carbon values in the dryland cotton systems were an average 25% lower than Irrigated cotton systems. MB levels in the samples collected prior to the application of 'lay by' herbicide application indicated little differences between herbicide treated and control samples, except for the Stomp+Cotoran treatment under Vetch-Dryland cotton system (15% reduction in herbicide treated soils compared to control). This lack of difference in MB values suggests a recovery in MB levels following the effects of herbicides applied at planting.

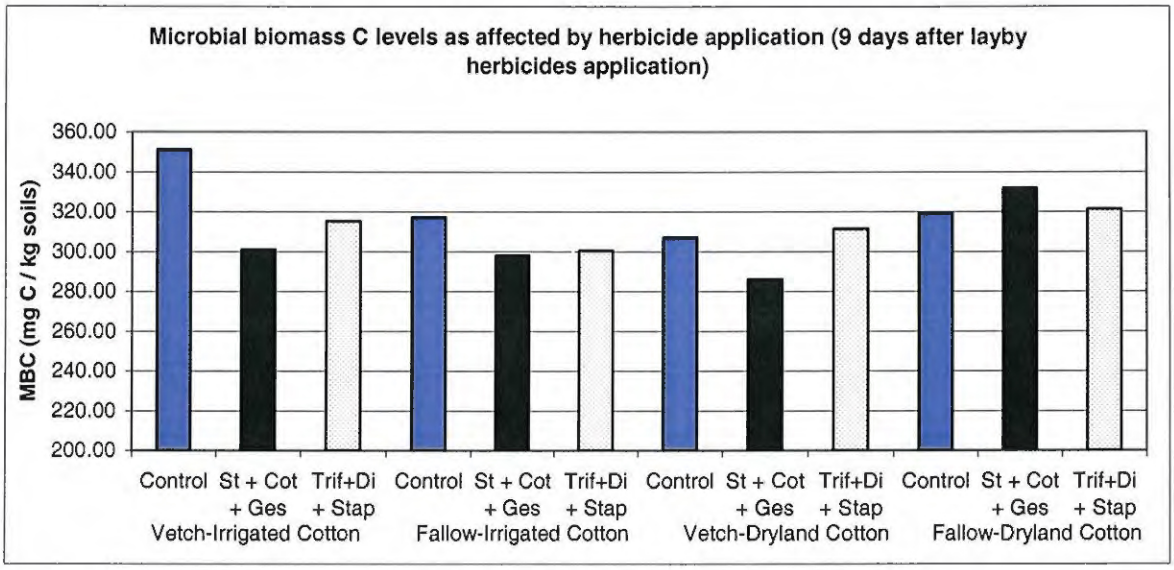


Figure 9: Microbial biomass levels in surface soils.

The effect of 'lay by' herbicide application was most seen in the Vetch-Irrigated cotton system (15% reduction in the 'Stomp+Cotoran+Gesagard' plots compared to control plots) followed by the Vetch-Dry land cotton system. Herbicide application had no significant effect on MB levels in the Fallow-Dryland cotton systems (Figure 9). The negative effect of 'Stomp+Cotoran+Gesagard' herbicides on MB levels was evident even at the final sampling (e.g. 18% reduction in MB levels in the Vetch-Irrigated cotton system). These results on the reduction of MB in herbicide treated plots; in particular in the Vetch-Cotton rotations are also seen in our 'intact core' experiments (see the section below).

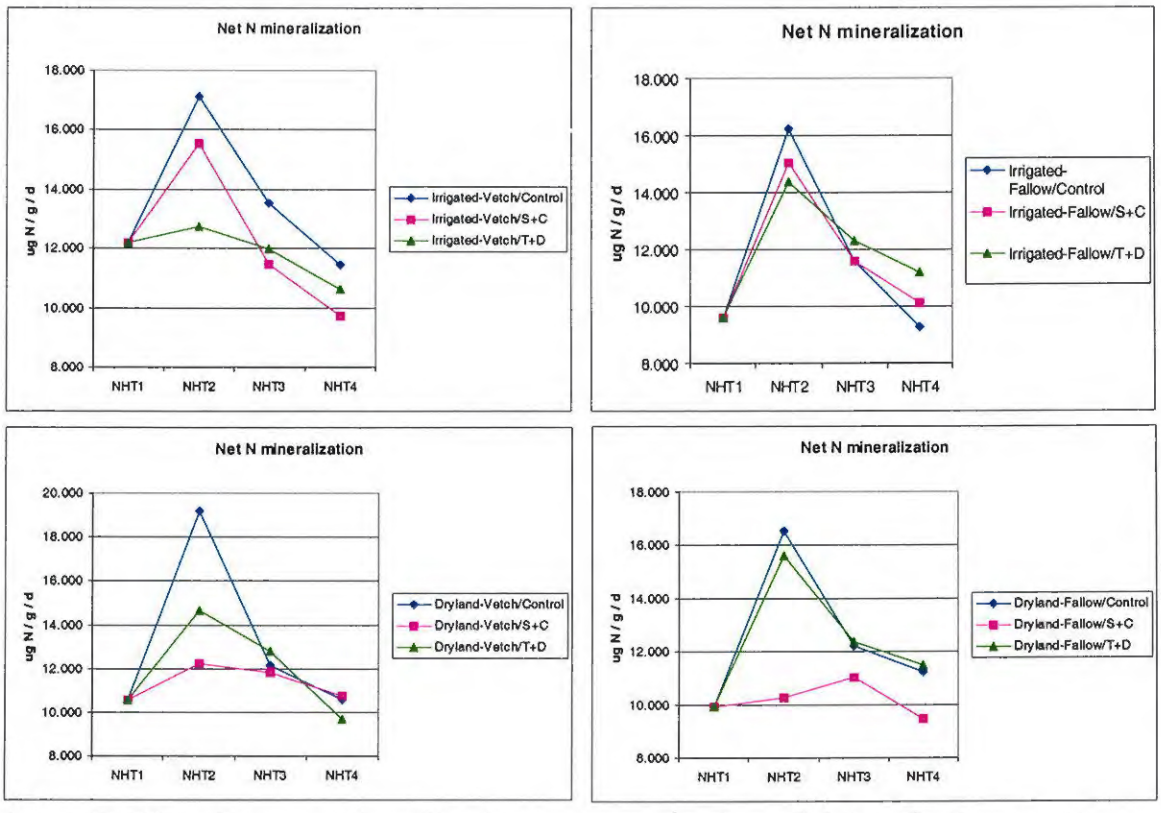


Figure 10: Net nitrogen mineralization measured using a lab incubation assay.

Net N mineralization measured using a laboratory incubation assay showed higher levels of N mineralization (8-12%) in soils from Vetch-Cotton systems compared to the Fallow-Cotton systems during the first three samplings. It is known that growing a legume off-season crop not only increases the soil N levels as a result of N fixation by the legume-Rhizobium symbiosis but also the addition of N-rich legume plant material forms a source of organic N for microbial N mineralization. Net N mineralization reduced as the season progressed probably an indication of disappearing organic N rich legume residues. The effect of herbicides on net N mineralization was strongest at the second sampling and in the Dryland system, the Stomp+Cotoran herbicide combination had greater impact than the Trifluralin+Diuron herbicide combination (Figure 10).

The net N Mineralization values from this laboratory incubation assay are only an indicator of N mineralization potential of the soils. Overall the Stomp+Cotoran+Gesagard herbicide combination seems to have a significant (greater) impact on various microbial populations and processes involved in N cycling and availability (e.g. AO populations, rate of nitrification, net N mineralization potential and microbial biomass values) compared to the Trifluralin+Diuron+Staple herbicide combination.

C) Cotton residue decomposition and associated microbial activity:

Litterbag studies have been recommended by a number of studies to investigate crop decomposition and associated microbial activities and the type of litterbags vary depending up on the nature of study. For example, the pore size of the material determines the type of soil fauna that can get access to the residue material inside. In our studies we used litterbags made using polyester or nylon cloth material with 2-3mm pores and litterbags were 15 x 15 cm in size. Observations of litterbags from field experiments in all the seasons indicated that the litterbags withstood the harsh conditions of wetting and drying in cotton fields and retain their integrity through out the 15week experimental period. We routinely observed varying soil faunal types (microfauna and mesofauna) inside the litterbags. Due to the pore size of litterbag material it was unlikely that the residues were accessible to soil macrofauna. Each litterbag contained cotton stubble of varying thickness and ~7-8 g dry wt.

In general, during the 15week litterbag trial, the decomposition of cotton residues ranged between 41 to 62% (ash free dry wt basis) of the residue material. The cotton residue we used in the litterbag studies contained an average 0.5% N with a carbon to nitrogen ratio of 86 to 88. Application of some herbicides caused significant changes to the decomposition of cotton residues. For example, decomposition was highest with the herbicide treatment Stomp+Diuron (60%) and lowest with Trifluralin+Cotoran treatment (41%). Application of the herbicide Gesagard to the Stomp+Diuron mix reduced the decomposition. Results from our single herbicide application experiments indicated that herbicides such as Gesagard, Stomp and Cotoran negatively affected residue decomposition. Application of herbicide Diuron along with Stomp seems to have increased the level of Cotton residue decomposition. There was a significant decline in the %C levels of decomposing residues in all treatments whereas the %N levels increased as the experiment

progressed with greatest increase occurring between 6 to 12 weeks. The percent C values for decomposing residues indicated that the residues treated with herbicides, in particular Trifluralin+Cotoran combination, were higher than the no-herbicide treated residue. Microbial activity associated with residues was lower with residues treated with Triflularin+Cotoran or T+C+G herbicides compared to the control residues whereas a higher level of microbial activity was associated with residues treated with S+D or S+D+G. Observations on the growth of fungi on the surface of decomposing residues indicated higher levels of fungal growth with residues treated with various residues, especially during the first 3-6 weeks of incubation (Figure 11).

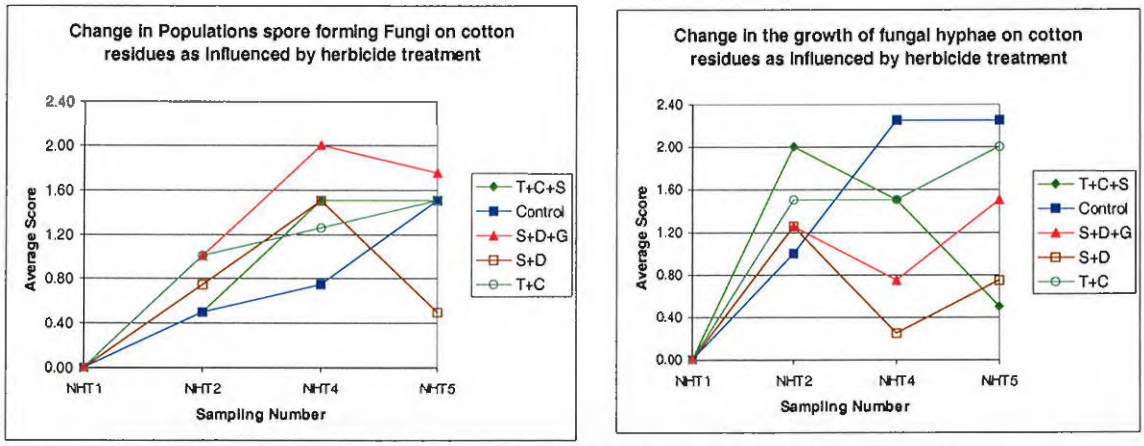


Figure 11: Populations of fungi associated with cotton stubble samples

Decomposition of cotton residues during the 2002-2003 season ranged between 41 to 58% (ash free DW basis) and 52-53% decomposition was observed with control residues both under Vetch-Cotton and Fallow-Cotton treatments. Application of Stomp+Cotoran caused significantly reduced cotton residue decomposition (41-43%) compared to control whereas the application of Trifluralin+Diruron had no effect or increased the decomposition. Cotton residue decomposition in terms of amount of carbon also indicated similar trends, e.g. after 12 weeks of incubation application of S+C herbicides caused 15 to 23% decline in C decomposition compared to the control.

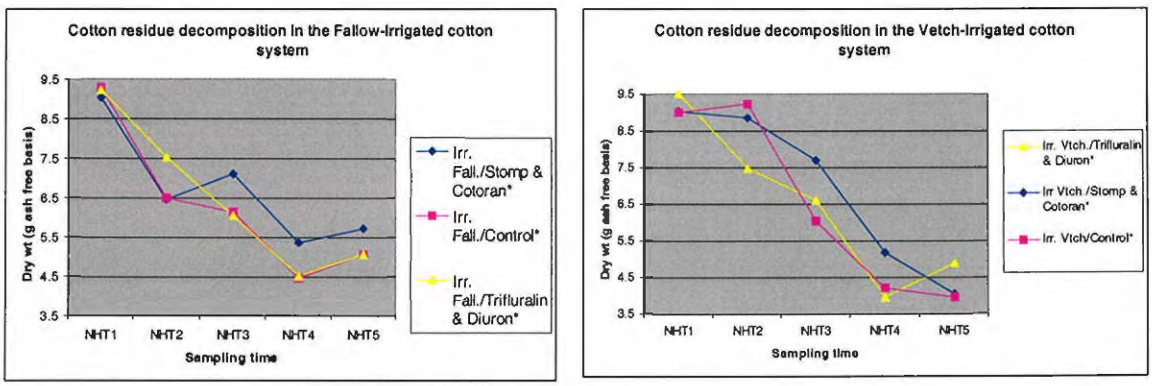
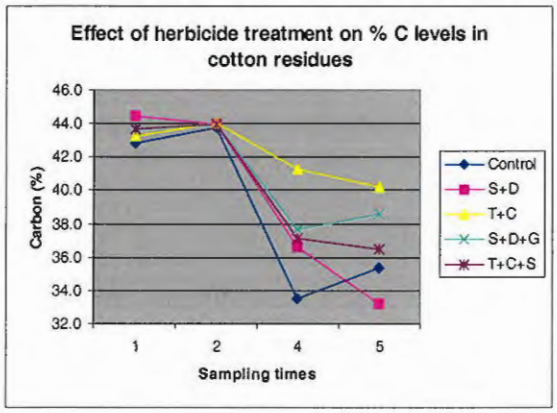


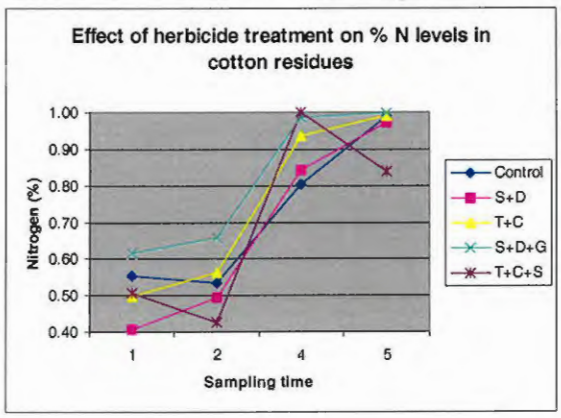
Figure 12: Decomposition of cotton stubble in litterbag experiments.

Similar to the previous years experiments %N level of residues increased as the experiment progressed i.e. week 1 to week 15 and the trend was seen in all

treatments. At week 6, residues treated with T+D had higher levels of N (mg/g ash free wt basis) compared to control residues where as the residues treated with S+C had lower levels of N.

Figure 13: Concentrations of C and N in cotton residues from litter bags.





D) Nitrogen fixation by Vetch-*Rhizobium* symbiosis as influenced by Cotton herbicide residues:

Traditionally cotton crop is grown with a fallow during off-season, however during the last 5-10 years legume crops such as vetch, fababean have been recommended as rotational crops mainly to gain benefits from the nitrogen fixed and the associated benefits to soil fertility. Our objective was to determine whether the residual concentrations of herbicides applied cotton season have any impact on the nitrogen fixation by the Vetch-*Rhizobium* symbiosis.

Results of the percent nitrogen derived from atmosphere (%ndfa), a measure of N_2 fixation, from the control plots ranged from 62 to 74% from the three years of experimentation. A %ndfa value of more than 65% is generally considered sufficient to gain maximum benefits from legume-*Rhizobium* symbiosis. The % ndfa values for Vetch crop in the dryland cotton system were generally lower (53-61%), based on our results from the 2003 experiments. The %ndfa values for the Vetch grown in herbicide treated cotton plots were 7-16% lower than those for no herbicide control plots. This herbicide induced reduction in %ndfa equated to 4 to 18% reduction in nitrogen fixed by the Vetch crop. Even though, the effects of three herbicides applied in cotton were generally greater than two-herbicide application the differences were not significant. Since the persistence of each herbicide is different and the effects of individual herbicide on legume-*Rhizobium* symbiosis depend up on their mode of action it may be difficult to clearly separate herbicide mixes for their effects.

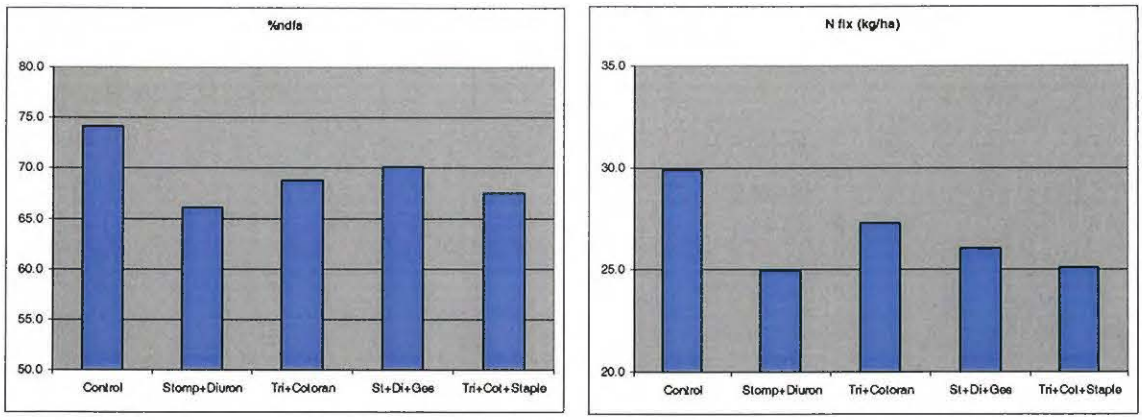


Figure 14: Parameters related to nitrogen fixation by legume crops.

In contrast to their effects on N_2 fixation, residual cotton herbicides had an impact on the N uptake by the Vetch from the soil, i.e. during the 2002 season uptake of soil N by the Vetch crop in the herbicide treated plots increased by 15 to 42%.

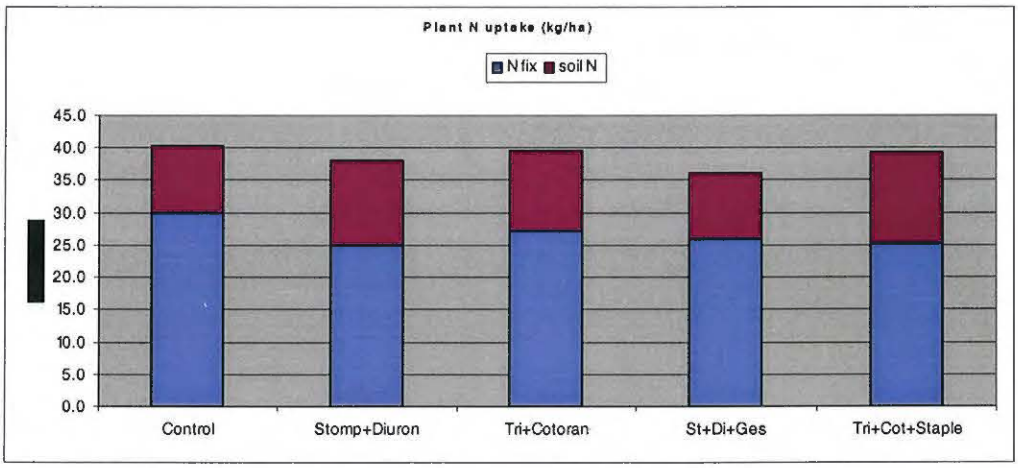


Figure 15: Sources of N taken up by legume crop in rotation with cotton.

This suggests that the low levels of residual herbicides, which had a negative impact on N_2 fixation, may not affect the overall plant N uptake.

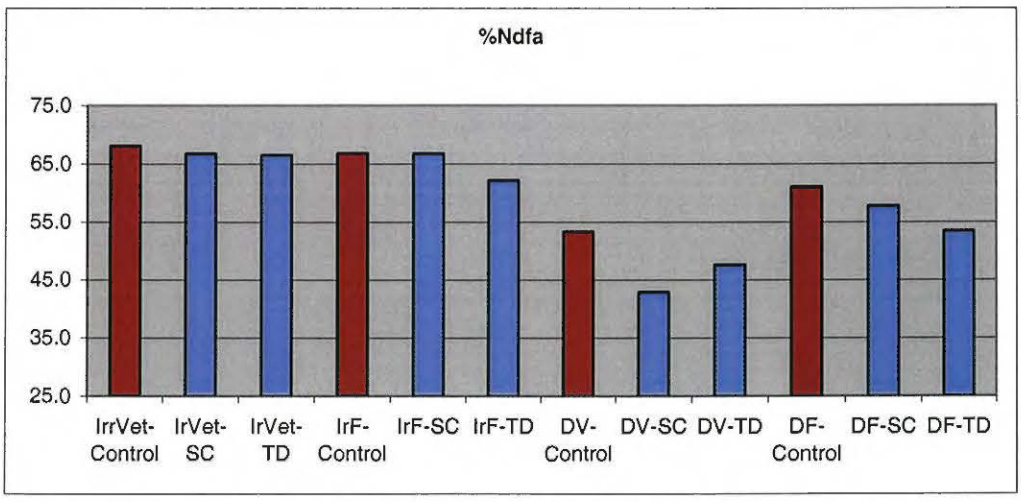


Figure 16: Percent nitrogen derived from fixation (%Ndfa) by the legume crop.

Vetch grown in the dryland cotton system had lower %ndfa values (31-63%) compared to that in the irrigated-cotton system (54-75%). The effects of residual cotton herbicides were only negative in the dryland system whereas in the irrigated system N_2 fixation by the Vetch grown in the herbicide plots was similar or greater than that in the control plots. The highest negative effect was observed in the vetch grown in dryland cotton-fallow system with Stomp+Cotoran herbicide application and the residual effects of Trifluralin+Diuron were either mildly negative or similar.

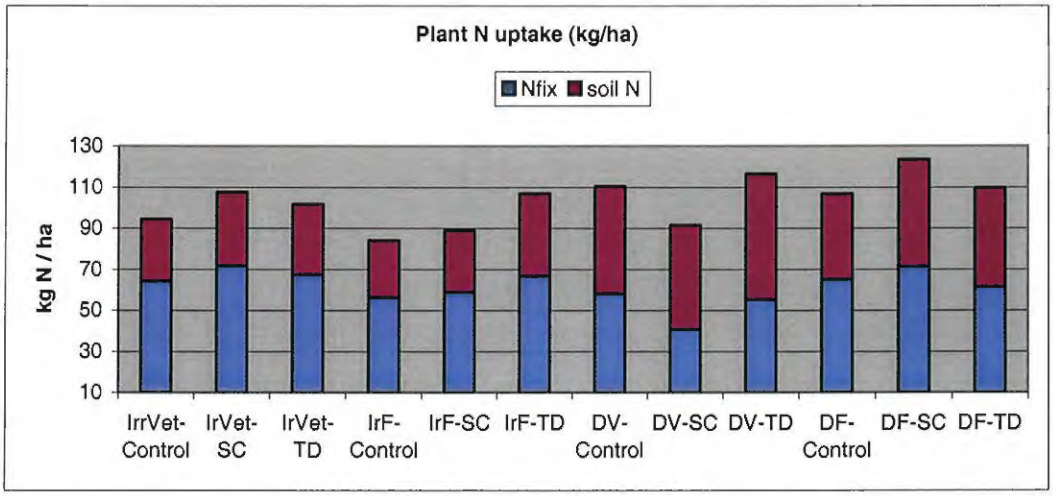


Figure 17: Sources of N taken up by the vetch crop as influenced by cotton herbicides.

2. Intact core experiments:

A) Experiment 1

- Effect of multi-herbicide (two or three herbicides) application was generally greater than single herbicide application although not all multi-herbicide applications had greater impact on biological properties (e.g. Trifluralin vs Trifluralin+Cotoran+Staple). However the single application some herbicides that are persistent (e.g. Stomp, Gesagard) had greater impact on microbial activity (e.g. substrate induced respiration, SIR) compared to the application of herbicides that are degraded faster (e.g. Diuron, Trifluralin).

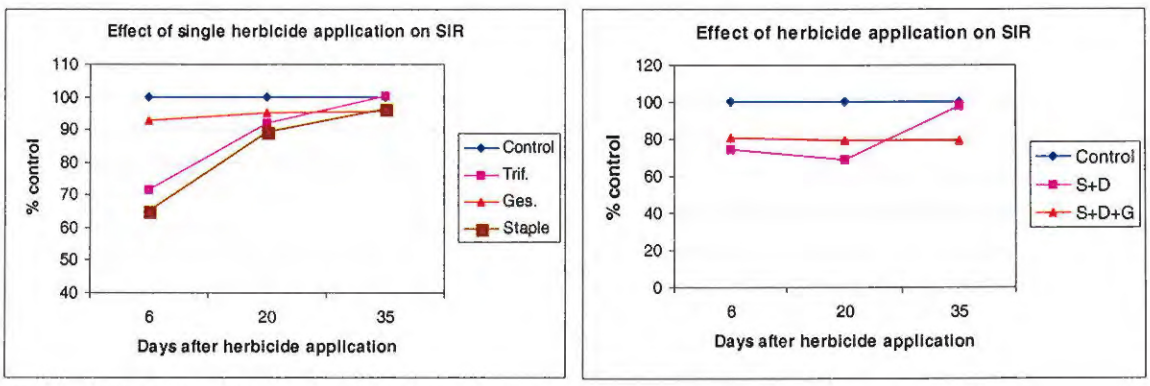


Figure 18: Microbial activity levels in soils from intact core experiments

- The negative effects of persistent herbicides were seen even after a 3 month dry period similar to the fallow conditions in the field, e.g. negative impact of herbicides such as Stomp+Diuron on potential carbon mineralization and microbial biomass level.

- Application of herbicides such as Stomp caused a reduction in the rate of nitrification however other herbicides had a positive influence on the rate of nitrification (e.g. Staple). These effects of herbicides on nitrification also persisted the dry fallow period suggesting that the residual herbicides from previous crop/season have the potential to impact on important microbial functions such as nitrification.

B) Experiment 2

- Effects of herbicides on microbial activity were not only negative but also greater in soils from irrigated cotton system than in the dryland cotton system. Herbicide effects in soils from dryland system were variable.

- Application of Stomp+Cotoran caused greater impact than the herbicide combination of Trifluralin+Diuron. Herbicide effects on microbial biomass levels were negative in all systems except in soils from the dryland cotton-fallow system.

C) Experiment 3

- Application of inorganic nitrogen fertilizer at the time of herbicide application caused an increase in microbial activity initially in soils from both cotton-vetch and cotton-fallow rotations. However the total microbial activity during the initial 20 days of incubation from the herbicide + N-fertilizer treatment was lower in the cotton-fallow cores compared to the control soil cores. In addition the increases in microbial activity in the fertilizer added cores reduced 2 months after the herbicide application and this was more evident in the cotton-fallow cores.

- Application of inorganic nitrogen fertilizer at the time of herbicide application caused a significant decline in the levels of microbial biomass in soil cores from both the cotton systems even though the application of herbicides alone caused little or no effect on MB-C levels. It is considered that conditions that result in negative or no change in MB levels but cause an increase in microbial activity (i.e. CO_2 production) indicate conditions of metabolic stress on microbial community. Results from experiments 2 and 3 suggest that under conditions of higher levels of nitrogen availability either from fertilizer N or crop residue derived N (similar to that seen in vetch-cotton systems), herbicide application resulted in greater stress on microbial community.

3. Methodology to determine the impacts of herbicides on soil microbial populations and biological processes:

As a part of the project we developed / standardized a number of laboratory methods to measure herbicide effects on soil microbial communities. Some of examples include, bacterial community profile based on carbon substrate utilization abilities, populations AO microorganisms etc. In this project soil samples collected from field experiments were handled in ways to reduce significant changes in microbial properties prior to laboratory analysis in order to correctly determine the biological properties relevant to field conditions. For example, following the collection of soil samples from field experiments they were stored / transported in

cold containers (eski with freezer bags) to Adelaide laboratories where they were stored in cold room until analysis. Prior to the actual laboratory analysis soils were subjected to a pre incubation period (up to 3 days) at temperatures relevant to field conditions. Following the evaluation of various sample handling and preparation conditions we finalized the conditions that best reflected field situations. For most soil samplings we collected samples from field experiments after an irrigation or rainfall event i.e. moist soil conditions. Since soil moisture is one of the key regulating factors controlling microbial activity we only determined microbial properties in moist samples. Prior to pre-incubation, when required, minor adjustments to soil moisture were made to bring soil moisture close to ~65% water holding capacity and similar in all soil samples.

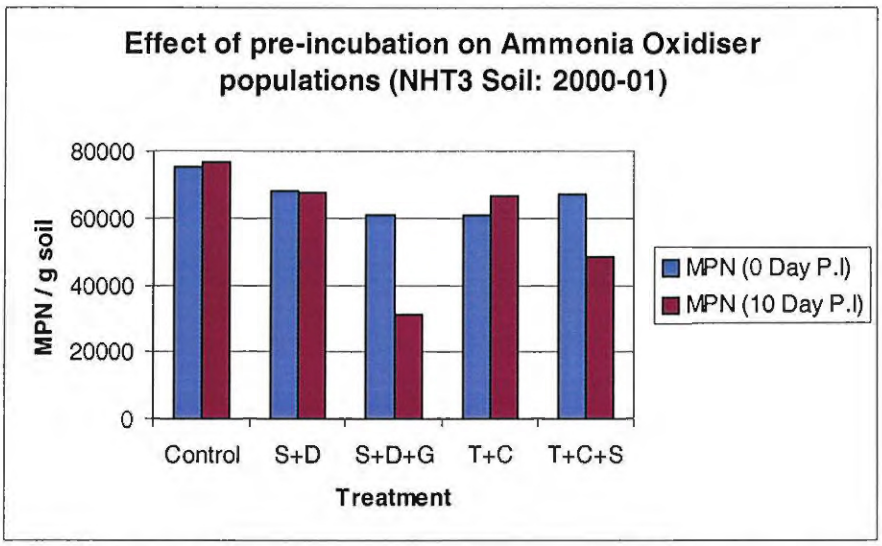


Figure 19: Evaluation of a lab incubation method to test the effect of herbicides.

For example, the populations of AO are influenced by the exposure to changes in soil temperature and moisture, and therefore the measurement of AO populations in such disturbed soils may not provide a true picture of herbicides on their populations. Data given in the above graph indicates that the herbicide impacts could be better differentiated after the pre-incubation of soil samples, i.e. effect of 'lay by' herbicides, compared to direct analysis of field samples. Since our aim was to compare the effects of herbicides on microbial properties compared to that in control (no herbicide soils), we tried to remove any variability due to environmental and sample collection and handling variables. These results also suggest that an incubation assay which incorporates these principles could be used as an indicator for testing the effects of agrochemicals on microbial functions in cotton soils.

As indicated before, during the first two seasons all the treatments in field experiments had four replicates in an RBD experiment however the treatments in the 2002-03 experiment had only three replicates (in order to keep the total number of samples at a manageable level). All the treatments in the litterbag experiments and 'intact core' experiments also had four replicates. Laboratory analysis for most of the biological and chemical properties for each field sample was done in duplicate. Statistical analysis of the data and calculation of averages, standard error of means, ANOVA and regression relationships was done using statistical packages such as Statistix® and Genstat® and Excel® packages.

5. Provide a conclusion as to research outcomes compared with objectives. What are the "take home messages"?

Based on results from field based and glasshouse experiments, presented in the previous section, we demonstrated that application of multiple herbicides (applied in 2 or more applications) had greater and longer impact on microbial populations or activities compared to no herbicide control or single application of herbicides (objectives 1 & 2). Using the litter bag technique we were able to quantify the effects of herbicides on crop residue decomposition (stubble disappearance, C and N turnover). Our experiments with rotational legume crop provided evidence for effects of residual herbicides on symbiotic nitrogen fixation. In addition, by selecting different herbicide combinations, based on information from single herbicide impacts, we were able to demonstrate that it is possible to reduce herbicide use impacts on key soil biological process and microbial populations even under a multiple herbicide use regime. In addition, by testing herbicide impacts under different rotation and other management systems coupled with glass house experiments we have demonstrated the possibilities to manage herbicide impacts through farming system practices. All these results clearly demonstrate that by changing herbicide usage pattern (in terms of type of herbicide and time of application) cotton farmers not only could manage the weeds but also reduce any long-term impacts from herbicide use. Some key 'Take home messages' are given below.

Take home messages based on results from the current project:

1) Application of 'lay by' herbicides slowed the recovery in MB levels compared to the treatments with initial herbicide application only. At the final sampling microbial biomass levels in the treatments with 'lay by' herbicide application were lower than those in the single application of herbicides (10-15%). The presence of herbicides such as Prometryn, Fluometuron and Pendimethalin in the herbicide regime significantly reduced the decomposition of cotton residues and modified associated microfloral composition.
2) Some herbicides applied in cotton had negative impact on symbiotic N_2-fixation by legumes in rotation (e.g. Pendimethalin, Prometryn > Trifluralin, Diuron). Results also suggest that it is possible to reduce any residual effects of cotton herbicides on N_2-fixation by legumes in rotation through using less persistent herbicides.
3) An appropriate recovery period for soil biota should be allowed between herbicide applications and modification of herbicide regimes may be possible to avoid the application of herbicides that either reduces microbial activity or cause microbial stress in sequence before the soil has recovered from the effects of previous application of herbicides.
4) In low-fertility Australian soils application of N fertilizer generally causes an increase in microbial activity and biomass levels. However our results suggest that herbicide induced effects are greater under conditions of higher N availability.
5) Using a standardized laboratory incubation assay it is possible to evaluate the long-term impacts of herbicides in cotton soils.

Take home messages based on research conducted in collaboration with a previous project and CLW 1C:

1) It is essential to use an integrated approach of testing key groups of biota and associated activities in order to evaluate and predict unforeseen non-target effects from herbicide use. Effects of each herbicide need to be considered separately as the herbicide-microbe-soil interactions vary for different herbicides.

2) Short-term impacts of most of the herbicides we tested are reversible partly or fully within 10-weeks after herbicide application, hence it may be possible to develop management options to reduce non-target negative impacts.
3) A number of herbicides currently used in cotton soils have a negative impact on key groups of microorganisms, however not all herbicides caused negative impacts.
4) Some herbicides caused a significant shift in bacteria:fungi ratio, reduced decomposition rate of cotton stubble and populations and activities of nitrifying microorganisms, the magnitude of herbicide impacts varied with season
5) Response of rhizosphere microbial populations to 'in-crop' herbicide application is different for conventional and GM cotton varieties.

6. Detail how your research has addressed the Corporation's three Outputs - Economic, Environmental and Social?

As soil biota are the key participants in the maintenance of long-term soil quality and health, results from this project directly contribute to the CRDC-Output 1 (Sustainability of natural resources) especially to the output for the 'Soils' program. Herbicide use is an essential practice for economically efficient cotton production.

Inefficient use of added resources and the persistence of added agrochemicals may contribute to the environmental problems in high input cropping systems. A balanced and properly functioning soil biota is necessary for the efficient use of added resources and reduced persistence of added chemicals. Any efforts by the cotton farmer to maintain or improve biological functioning require better understanding of biota response to added herbicides. Since the research conducted in this project helped in the identification of potential non-target environmental effects of herbicides they would assist in better management of herbicide use and would contribute to the economic benefits through increased efficiency of added resources as a result of improved biological functions. Public knowledge about the adoption of environmentally friendly management practices would also improve the 'clean and green image' that the Australian cotton industry pursue.

7. Provide a summary of the project ensuring the following areas are addressed:

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.)

No

b) other information developed from research (eg discoveries in methodology, equipment design, etc.)

During the course of the project we developed/standardized a number of laboratory methods involved in the measurement of populations of specific functional groups of microorganisms and specific biological functions in cotton soils. Examples include:

1. Method to determine the metabolic status of microbial community
2. Method to estimate the bacterial community diversity using substrate utilization profiles
3. An incubation method to determine the long-term impacts of residual herbicides on soil biological processes
4. Method to prepare crop residue and root samples for scanning electron microscopy studies.

c) are changes to the Intellectual Property register required?

No

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

Following the completion of this project there is no current funding for future research on this topic. Based on our results from this project it is clear that even though not all herbicides used in cotton farming have negative impacts, a number of herbicides have significant negative impacts on key microbial properties and activities. It may not be possible to keep testing every new herbicide for potential impacts if full scale field experiments but preliminary screening of herbicides in 'intact soil cores' may be done and needed to acquire information for growers to use and avoid unexpected negative consequences.

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

A leaflet / information sheet to add to the CRDC's 'Weedpak' with results and recommendations from this project is currently being prepared.

Paper to be presented at the 12th Australian Cotton conference to be held during August 2004 – Impact of herbicide application on soil biological properties in cotton soils?

A series of talks by the principle investigator at CRC Cotton sponsored

(c) for future research.

Results from this and previous project indicate that the use of a number of herbicides in cotton farming modifies microfloral composition e.g. cellulolytic bacteria and fungi and the growth of fungi on cotton residues. Since fungal growth and composition has the potential to significantly influence cotton plant health any future research on herbicide impacts should concentrate on determining the effects of herbicide use on economically important fungi. In addition research from our project CLW1C shows that cultivation of GM cotton varieties significantly increases fungal growth and modifies species composition.

As the results from this project clearly indicate the negative effects of a majority of herbicides on nitrifying microorganisms, future research to screen all the herbicides that are available for use in cotton farming for their effects on the populations and activities of the microorganisms involved in N cycling may be very useful, especially the new herbicides.

Results from our glasshouse experiments indicated that 'in-crop' application of herbicides have the potential to impact on rhizosphere microbial functions and cotton plant-microbe interactions (Gupta, V.V.S.R., Crisp, P. and Neate, S.M. (1998) Herbicide effects on the microbial diversity in the rhizosphere of genetically modified and conventional cotton. Proceedings of the 14th Australasian Biotechnology Conference held in Adelaide during April, 1998, p120)

.Little or no information available on the herbicide induced changes in the new cotton farming systems with herbicide tolerant GM cotton varieties. Future

research in this area could benefit successful long-term incorporation of new varieties and farming systems.

9. List the publications arising from the research project and/or a publication plan.

Gupta, V.V.S.R. and Roberts, G. (2000) Impact of herbicide application on essential microbiological processes in cotton soils. Paper presented at the 2000 Joint Scientific meeting of the Australian and New Zealand Societies of Microbiology held at Cairns, Qld. Australia Microbiology. 21(3): A148.

Gupta, V.V.S.R. and Roberts, G.N. (2001) Biological components of soil health – A cotton farming system perspective. Paper presented at the 'Soil health' workshop organized by the CRDC during December 6-7 at Narrabri, NSW, Australia.

Gupta, V.V.S.R. (2004) Impact of herbicide application on soil biological processes in cotton soils. Paper presented at the 12th ACGRA conference held at Gold Coast, Qld.

Gupta, V.V.S.R. and Roberts, G.N. (2005) Effects of single or multiple herbicide application on soil biological functions in cotton soils in Australia. Oral presentation submitted for Beltwide conference to be held during Jan 2005.

Results from this work would form the basis for the following journal publications in preparation:

Gupta V.V.S.R. and Roberts, G. Impact of herbicides used in cotton farming systems on soil biota and biological processes: (1) Effects on microbial activity, composition and nitrogen transformations. For Soil Biology and Biochemistry (information on the effects of single herbicide application).

Gupta, V.V.S.R. and Roberts, G. Impact of herbicides used in cotton farming systems on soil biota and biological processes: (2) Effects of multi-herbicide application. For Soil Biology and Biochemistry

Gupta, V.V.S.R. and Roberts, G. Impact of herbicides used in cotton farming systems on soil biota and biological processes: (3) Effects on the decomposition of cotton stubble. For Soil Biology and Biochemistry

Gupta, V.V.S.R. and Roberts, G. Impact of herbicides used in cotton farming systems on soil biota and biological processes: (4) Effects on nitrogen fixation by the legume crop in rotation. For Plant and Soil

10. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry or the Australian community.

A detailed cost : benefit analysis was not part of this project hence it is difficult to make specific recommendations. However, results in this project show that not all herbicides cause negative impacts on microbial populations and activities and it is possible to choose herbicide(s) to minimize any potential negative impacts. In addition, with appropriate management options e.g. adequate recovery period between herbicide applications and selection of appropriate herbicide combinations cotton farmers could still benefit (through better weed management) from the herbicide use.

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Soil biota communities are one of the most diverse groups of earth's biota. Soil organisms regulate a number of processes in soils that are not only critical for productivity but are also essential for maintenance of ecosystem health. Herbicide use is a vital component of modern agriculture, in particular under reduced till systems. With increased adoption of stubble retention and reduced till practices and the introduction of new herbicides, herbicide use will remain an essential practice in the near future. Non-target effects of herbicides on soil biological activities may (i) cause undesirable effects on essential transformation processes (e.g. reduced nitrification and nitrogen mineralisation) or (ii) result in unexpected damage to crops (e.g. increased diseased incidence). Non-target effects of herbicides could be either positive or negative. If herbicide application is to remain a viable practice in sustainable farming systems, evaluation of herbicide effects especially from repeated and / or multiple herbicide use is essential to ensure optimum nutrient availability and plant growth. We measured the impact of multiple herbicide application on the populations of selected functional groups of soil microorganisms and the biological processes they mediate, using surface soil samples from field experiments were conducted at ACRI farm sites.

A brief summary of the results from our herbicide related research is as follows:

1) It is essential to use an integrated approach of testing key groups of biota and associated activities in order to evaluate and predict unforeseen non-target effects from herbicide use. Effects of each herbicide need to be considered separately as the herbicide-microbe-soil interactions vary for different herbicides.
2) Short-term impacts of most of the herbicides we tested are reversible partly or fully within 10-weeks after herbicide application, hence it may be possible to develop management options to reduce non-target negative impacts.
3) A number of herbicides currently used in cotton soils have a negative impact on key groups of microorganisms, however not all herbicides caused negative impacts.
4) Some herbicides caused a significant shift in bacteria : fungi ratio, reduced decomposition rate of cotton stubble and populations and activities of nitrifying microorganisms, the magnitude of herbicide impacts varied with season.
5) Application of 'lay by' herbicides slowed the recovery in MB levels compared to the treatments with initial herbicide application only. At the final sampling microbial biomass levels in the treatments with 'lay by' herbicide application were lower than those in the single application of herbicides (10-15%).
6) An appropriate recovery period for soil biota should be allowed between herbicide applications and modification of herbicide regimes may be possible to avoid the application of herbicides that either reduce microbial activity or cause microbial stress in sequence before the soil has recovered from the effects of previous application of herbicides.
7) Some herbicides applied in cotton had negative impact on symbiotic N_2-fixation by legumes in rotation (e.g. Pendimethalin, Prometryn > Trifluralin, Diuron). Results also suggest that it is possible to reduce any residual effects of cotton herbicides on N_2-fixation by legumes in rotation through using less persistent herbicides.

Finally these results from cotton soils and other work from dryland cropping systems suggest that appropriate use of herbicides could be less destructive to soil biota and biological processes and by changing herbicide usage pattern (in terms of type of herbicide and time of application) cotton farmers not only could manage the weeds but also reduce any long-term impacts from herbicide use

Acknowledgements:
This work was funded by the Cotton Research and Development Corporation and CSIRO Land and Water. The Principle investigator expresses his sincere appreciation to Dr. Grant Roberts, main collaborator, for all his efforts in conducting the field experiments and valuable discussions related to experimental set up and the field relevance of the research. The Principle investigator also wishes to thank Ms. Clare Fenton-Taylor for her assistance with field related work and Mr. Marcus Hicks for his efforts in the laboratory.

Copyright and Disclaimer

Important Disclaimer:

Cover Photograph:

A photo of cotton herbicide field trial conducted at the ACRI farm in Narrabri, NSW