Varietal differences in cotton – belowground

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Abstract

During work conducted between 2003 and 2006 on the potential for genetically modified cotton to affect soil microbiota it became apparent that variety type can influence microbiology associated with the rhizosphere. To further investigate this we commenced a project to determine the influence of current commercial cotton varieties on their associated rhizosphere microbial populations and functions. Over the duration of the project we have shown that different cotton varieties can significantly influence rhizosphere microbial populations and functions, and we have made some progress into understanding how these differences are brought about. The significance of these observations, with particular reference to reduced or more efficient input systems are discussed. Further research is required before field recommendations can be made.

Introduction

It has been accepted for many years that there is an interaction between plants and the microbiological life that exists in soil. This interaction is particularly important in the rhizosphere (Hiltner 1904), where plant exudates directly feed the microbial population, which in turn is responsible for nutrient cycling, production of growth promoters, and occasionally development of pathogenicity. These factors are important to plant health. However, the difficulties of studying such interactions in the soil and the inability to grow the majority of soil microorganisms in the laboratory have resulted in limited research in this area.

With the introduction of genetically modified (GM) crops into agricultural production systems public concern resulted in renewed interest and research into the possible environmental consequences from growing GM crops (Brookes and Barfoot 2005). This included the potential impact of GM crops on the soil microbiology. GM crops have the potential to influence soil microbiology through (i) the exudation of transgenic proteins from the root system, (ii) the release of the transgenic proteins from broken and dying roots, (iii) the incorporation of above ground plant material into the soil, and (iv) through differences in exudation chemistry (Gupta and Watson 2004; Knox *et al.* 2006; Saxena and Stotzky 2001).

Between 2003 and 2006 we assessed the potential for GM cotton, expressing either insecticidal Bt proteins, glyphosate tolerance or both traits, to influence the soil microbiota. The analysis of the rhizosphere microbiology showed some differences, but none that were specifically identified as being caused by the expression of the introduced transgenic material (Knox *et al.* 2004). The

results did, however, imply that cotton variety and family groupings were more likely to be associated with the observed differences in the rhizosphere microbiota.
In this paper we summarize the results of a project commenced in 2006 to investigate the impact and significance of variety driven alteration of rhizosphere microbiology for a number of Australian cotton varieties.

Materials and methods

Field establishment

Experimental plots for the 2006/07 and 2007/08 seasons were located at the NSW DPI, Myall Vale Research Station, Narrabri on fields B2 and A3, respectively. The 2006/07 study involved 15 varieties; Sicala 40, Sicala 40BR, Sicala 43, Siokra V16, Sicot 189, Auburn 623, DP 16, DP50, DP50B (Ingard), Coker 315, Sicala V2, Sicot 71B, Sicot 24B, Sicot 71 and Sicot 71BR. The experiment was planted over 16 rows of the field in 7 m length plots, each covering 4 rows and providing 4 replicated planting areas. In 2007/08 to enable increased measurements to be made, reflecting commercially available varieties, and to further interrogate the observations made in the previous year, varieties Sicot 189, Sicot 71, Sicot 71B, Sicot 71BR, Sicala 40, Sicala 40B, Sicala 40BR and Coker 315 were planted over 16 rows of the field in 20 m by 4 row plots in 4 replicate blocks. Planting was carried out in both years to a plan produced using DiGGER to reduce in-field variation.

Rhizosphere sampling

Sampling of rhizosphere soil was undertaken using methods developed in the previous project. Briefly, the process involved lifting cotton plants with a garden fork to expose the roots to a depth of about 20 cm. Soil adhering to the root surface was recovered by hand and carefully placed into labeled plastic bags, which were stored in an Esky for return to the laboratory. In the laboratory, soil was stored at 4°C, and most experimental procedures were initiated within 48 h of sampling. When longer delays were experienced, soil samples were frozen at -20°C and thawed overnight prior to experimentation. In the 2006/07 season, rhizosphere sampling was conducted at 55, 124 and 168 days after sowing, which corresponded to ‘first flower’, ‘cut out’ and ‘defoliation’ of the majority of the varieties planted in the experiment. In the 2007/08 season sampling was conducted at 63, 107, 141 and 176 days after sowing.

Microbial biomass and Soil Respiration

Soil microbial biomass was assessed from 1 g sub-samples of the rhizosphere soil collected from each experimental plot using an adaptation of the ninhydrin reactive N and chloroform fumigation method with extraction in 3 mL of 0.5M K_2SO_4 and soil removal with centrifugation at 3000 x g for 5 minutes (Sparling and Zhu 1993). For each sample a K_{ec} value of 29.3 was used to calculate the microbial biomass (Sparling *et al.* 1993). Soil respiration was not assessed using the recovered rhizosphere soils as this had been found to be too variable during the previous project. Instead intact soil cores were removed from field plots around the time of rhizosphere sampling, using a 2.5 cm diameter poly-pipe auger to a depth of 11 cm to provide a 50 cm^3 semi-intact core. All the cores were incubated for 7 days in the presence of a 0.5M NaOH trap at 25^{o}C in sealed Kilner jars

and CO_2 evolution was established using dual end point titration of the recovered NaOH trap with HCl (Gupta et al., 1994).

Composition of Bacterial population

The Denaturant Gradient Gel Electrophoresis (DGGE) technique was used to compare the community structure of the various rhizosphere bacterial communities based on 16SrDNA sequences. DNA was extracted from 0.2 g of each rhizosphere soil using the MoBio PowerSoil DNA extraction kit and 16SrDNA sequences were amplified using GC-clamped PCR primers as previously described (Duineveld et al., 1998). 16S rDNA PCR products were separated overnight using an IngenyPhor DGGE electrophoresis system, over a 45-70% urea-formamide gradient. DNA fragments were visualized by staining with SYBR gold. DNA fragment position and intensity was determined using the GelQuant software, followed by the use of then the Primer6 software package to assess differences between varieties.

Physiological profiling of microorganisms

The respiratory response of rhizosphere microbial communities to 23 carbon and nitrogen based substrates was determined using a modified MicroResp® technique as described by Campbell et al. (2003) modified with specific substrates selected for Australian cotton soils (Gupta, VVSR, CSIRO unpublished). Differences between varieties were analysed using canonical analysis (GenStat version 10.2.0.175, VSN International Ltd.)

Ammonium oxidizer populations

The populations of ammonium oxidizing (AO) bacteria were determined from a soil dilution (1 g in 10 mL of ¼ Ringer's solution) using a 96 well plate assay, which allowed the establishment of 8 replicates of each rhizosphere soil. The inoculated plates were incubated in the dark for 21 days at 21^{o}C before establishing the dilution point at which plated populations stopped oxidizing ammonium to nitrite (Weaver et al., 1994). Population numbers were estimated using a most probable number (MPN) assessment from each replicated dilution series.

Nitrification

The ability of 0.5 g sub-samples of the recovered rhizosphere soils to produce nitrite both with and without the addition of ammonium sulphate was assessed using 0.5 g sub-samples of the soil in an incubation study. Levels of nitrite produced after 0, 5 and 24 h were determined colourimetrically following the addition of a nitrite reagent, from absorbances read at 520 nm, against a sodium nitrite standard series. Rates of nitrification were assessed following removal of the background levels of soil nitrification.

Results

Microbial biomass and soil respiration

Data on microbial biomass showed a seasonal variation over the 2 years of this study. No significant differences were observed between the varieties at any sampling time (Figure 1). In general a high level of replicate variability was observed.

There were no significant and consistent differences in soil respiration between sampled varieties over the course of either season. Respiration differed between sampling times over a season and varieties. In general, the use of intact core method reduced variability compared to the use of soil samples from destructive sampling.

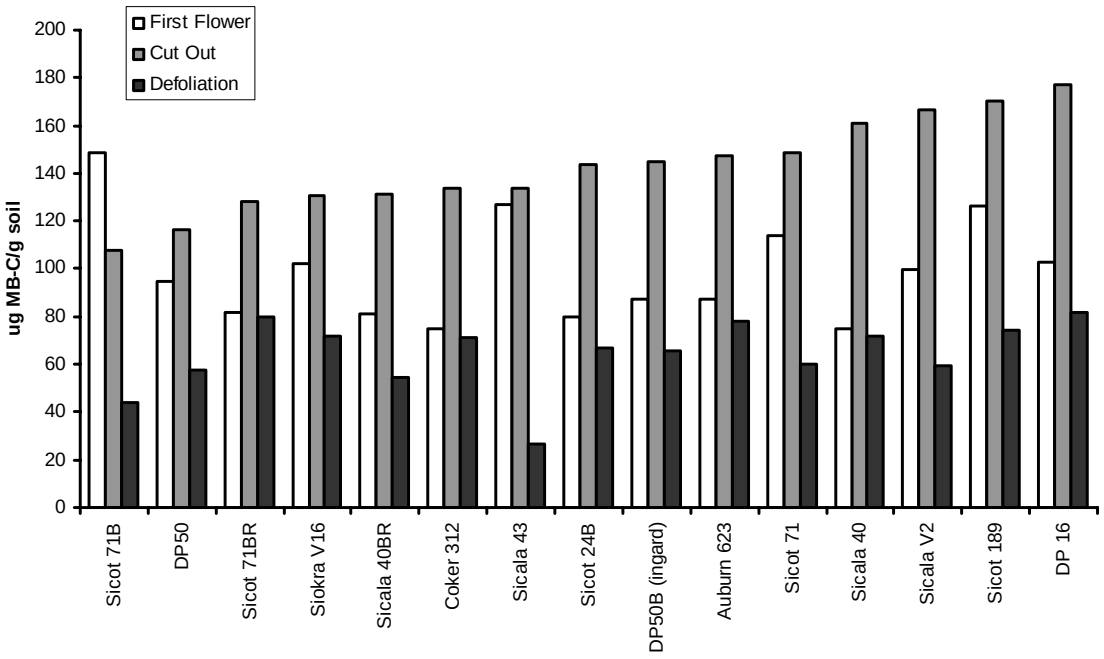

Figure 1 Ninhydrin reactive N assessed microbial biomass of rhizosphere soils sampled from various cotton varieties during the 2006/07 season.

Composition of bacterial population

Population analysis based on DGGE banding patterns of 16SrDNA was completed for the 2007/08 season. Analysis of the banding patterns indicated that there was quite a strong variety and family based association of specific rhizosphere microbial populations early in the season (Figure 2). This familial and variety based association was not observed later in the season.

Physiological profiling

MicroResp® results over the two years showed clear varietal influences on the capability of the rhizosphere microbiology to utilize specific compounds. Canonical analysis of induced respiration indicated that certain hexose sugars (fructose, glucose and sucrose) and amino acids (asparagines, valine and aspartic) were preferentially used by some rhizosphere communities over others (Figure 3).

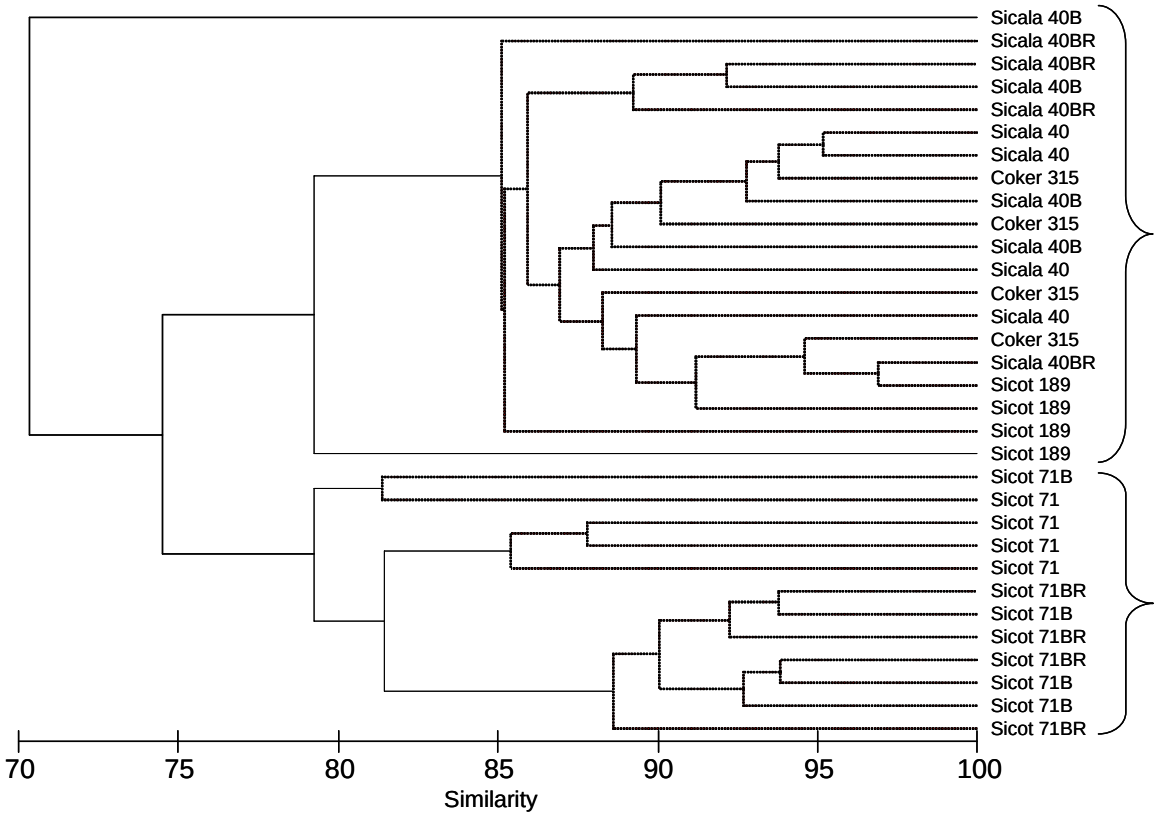

Figure 2 Cluster plot showing significant grouping of varieties by 16S DGGE analysis. Bracketed samples encompass groupings that are dissimilar (p<0.05); 2007/08 season.

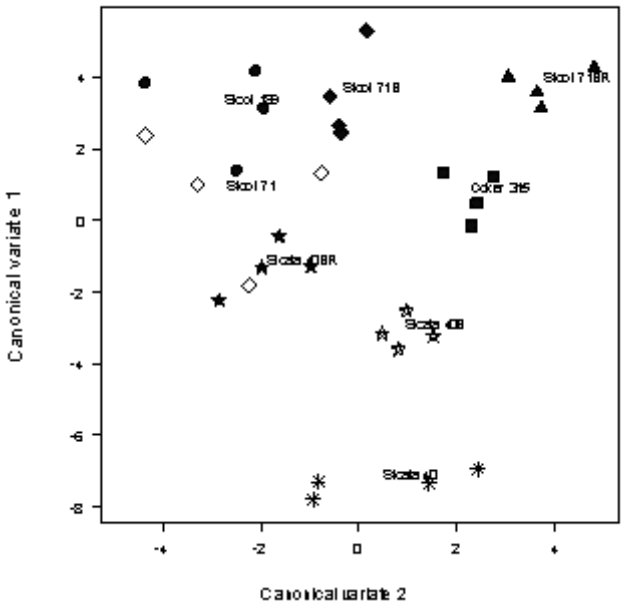

Figure 3 Canonical analysis of substrate induced respiration of rhizosphere microbiota (2006/07 season).

Ammonium oxidizer populations

As with the other microbial measurements, AO populations fluctuated over the season, but were often significantly different between varieties. Varietal trends observed in the 2006/07 season are shown in Figure 4. Although, significant differences were seen in the 2007/08 season trends did not remain the same for all varieties.

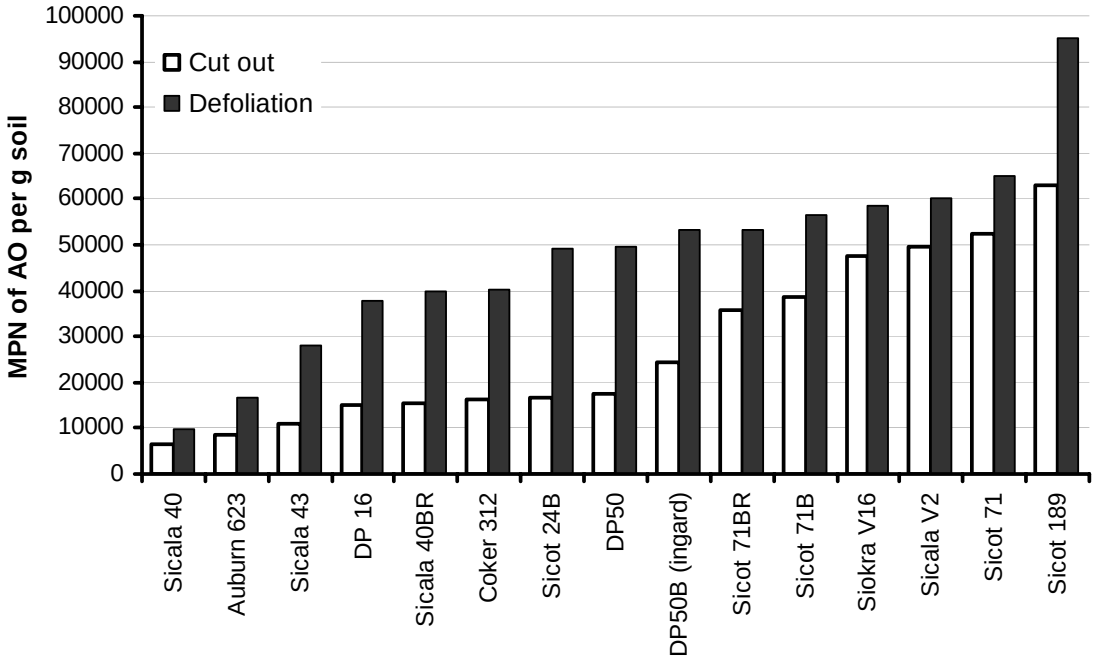

Figure 4 AO populations of rhizosphere soils from a number of difference cotton varieties sampled at cut out and defoliation in the 2006/07 season.

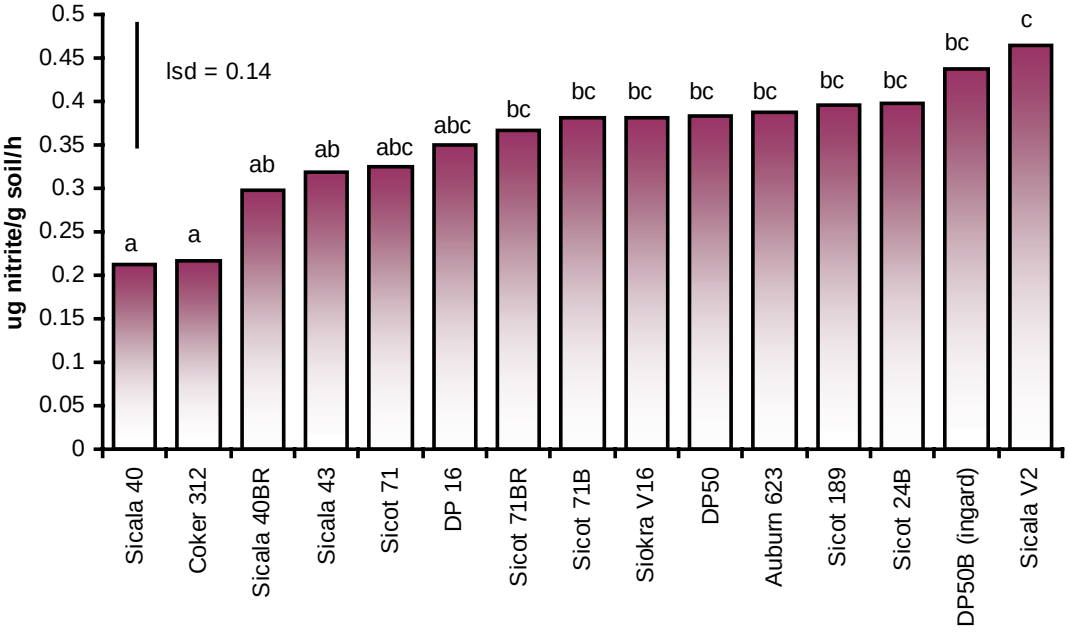

Figure 5 Mean rates of Nitrification for rhizosphere soils from different cotton varieties (2006/07 season).

Nitrification

Nitrification rates could not be determined early in the season due to the high levels of nitrate present in the soil from crop fertilization. Significant differences in the rates of nitrification were observed at some sampling time only, although in 2006/07 there was evidence of a trend existing between the varieties (Figure 5). This trend was not apparent in the 2007/08 data; rates of nitrification were similar in both years. There was no consistent and significant relationship between the levels of AO populations and the recorded rates of nitrification.

Discussion

The sampling strategy used in these experiments was adequate to determine the varietal based differences in rhizosphere populations but could be enhanced if required. The number of field assessments was limited by the time taken to process the samples with the resources available, and occasionally by the weather. This meant that investigation of issues such as rhizosphere changes with depth and root physiological differences between varieties were not pursued within this project.

The observed fluctuations in microbial biomass values throughout the season were taken as an indication of the influence of changes in exudation, as plants directed resources according to physiological requirements. Biomass results in the 2007/08 season were on average much higher than those obtained in 2006/07 (data not shown); this was assumed to be due to differences in the field used and environmental conditions, i.e. soil moisture and temperature.

Molecular interpretation of bacterial diversity in the rhizosphere clearly showed a variety and familial grouping occurring early in the 2007/08 season (Figure 2). Such differences in rhizosphere microbial diversity previously have been attributed to differences in the quality and quantity of rhizodeposition (Bowen and Rovira, 1999). Variety based differences in rhizosphere bacterial diversity has been reported for other crops (Gupta et al., 2004). However, in later samplings, e.g. in April 2008, this relationship was no longer evident. To address why this relationship was seen to collapse it is worth considering the following points; (i) as the season progressed the vertosols that supported our experiments would have undergone periods of shrinking and swelling that would have moved soil and its associated microbiota, (ii) work by Dr N. Hulugalle (NSW DPI) has demonstrated that root senescence can occur within the time frame in which we made measurements and at this stage the crop would have undergone significant physiological change. These factors are likely to have affected the quantity and quality of exudates, thus changing the associated bacterial community. (iii) Irrigation events throughout the season are likely to have had a significant effect on rhizosphere community structure as water would have carried bacteria through soil fissures and root channels between rows of varieties.

Altering our respiration assessment to a semi-intact core method reduced the variation we had encountered when analyzing loose rhizosphere soil, but did mean that we were likely to be analyzing soil that was not directly influenced by the plant. The use of the MicroResp® technique allowed us determine the composition of microbial community based on their ability to utilize different types of C and N substrates representing compounds that may be present in root exudates. Results showed a varietal based response of rhizoshere microbiota to specific hexose sugars and amino acids. We hypothesize from this that the rhizosphere microbial community of certain varieties had adapted to using specific compounds as a consequence of either increased quantity or quality of these compounds in root exudates. Had the work continued, studies would have been

undertaken to examine (i) differences in composition of exudates between varieties and (ii) the impact of these compounds on soil functional capabilities.
Analysis of AO populations and nitrification rates indicated that the availability of nitrate in the root environment varied between cotton varieties and so could determine physiological behaviour. We also observed varietal differences in the capability of rhizosphere soils to carry out non-symbiotic N_2 fixation. Much of the work on these microbial populations and functions associated with nitrogen cycling was often complicated by high levels of freely available nitrate present in the soil, which we monitored in by pass rain and irrigation water with 'Full Stop' devices. The results do, however, suggest that in the development of a reduced and more efficient input cotton system, varietal choice should be a key consideration. Our work in this area was preliminary and further work is needed before advice on variety selection can be given to farmers.

Conclusions

We observed that different cotton varieties can cause shifts in their associated rhizosphere microbial populations and functions. The potential to capitalize on this within the cotton system requires further investigation, but could be profitable, especially if a move to lower input systems becomes desirable. With the current increases in fuel and fertilizer costs such systems are likely to be considered in the near future, but more work on cotton variety and soil biological interactions is needed to capitalize on the preliminary work reported here.

Acknowledgements

This work was conducted with the support of the CSIRO Division of Entomology using funds provided by the Cotton Catchment Community CRC. Trial site management and support was provided by CSIRO Plant Industry and the NSW Department of Primary Industries. The preparation of this manuscript and continued involvement of Dr O Knox was made possible with the support of the Scottish Agricultural College.

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