The University of Sydney

Root Turnover and Microbial Activity in Cotton Farming Systems

Nicholas Luelf

Year IV Undergraduate Research Project

Faculty of Agriculture, Food & Natural Resources

Supervisor: Dr Daniel Tan

2005

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N.W.Luelf

Faculty of Agriculture, Food and Natural Resources, University of Sydney, NSW, 2006 Australia

Abstract

Soil organic carbon levels have been declining in the cotton producing regions of Australia ever since the introduction of cultivation. In response, cotton growers must modify cotton farming systems to slow this decline, or ideally reverse it. A study was carried out at Myall Vale near Narrabri, NSW, Australia to compare cotton-based rotations in relation to root production and turnover, soil microbial biomass, and soil microbial activity. The long-term rotations started in 2002 and were; (CV) cotton-vetch, (CC) continuous cotton, (CW) cotton- wheat (with tillage), (CWV) cotton- wheat- vetch (minimum-tillage). Cotton root dynamics and below ground carbon production were measured using the minirhizotron, core break and root washing methods during the 2004/2005 growing season. The fumigation-extraction (FE) method and Ninhydrin reactive N were used to measure microbial biomass. Microbial activity was measured by soil respiration (CO_2) using the NaOH trap method. Root growth rates, root numbers and root length were all highest at 72 days after sowing (DAS) in the CW rotation. Microbial biomass at this time was also highest in the CW rotation (10-20cm) indicating that high cotton root growth and possibly root exudations, and incorporation of wheat residues was most favourable to microbial populations. Both cotton-based rotations including a wheat phase (CW and CWV) produced the highest root mass throughout the season and hence, the largest amounts of carbon (27% w/w carbon in roots) in their root systems. There were no significant differences in microbial activity between rotations throughout the season, suggesting that soil carbon losses through CO_2 respiration could be similar for all treatments. Therefore, the two cotton-based rotations incorporating a wheat phase (CW and CWV) may return the largest amount of carbon into the soil through their

roots. Lint yields were also highest in rotations CWV (2.58 t/ha) and CW (2.37 t/ha) suggesting that the inclusion of a wheat phase in the rotation may also improve cotton yield.

1. Introduction

Traditionally in eastern Australia, irrigated cotton (*Gossypium hirsutum*) was produced as a monoculture using conventional tillage operations and the burning of crop residues. Despite continuous cotton production generally being the most economically productive, sowing rotation crops benefits soil nitrogen and alleviates soil compaction (Constable and Forrester 1995; Hulugalle *et al.* 1997). Rotations in cotton production have been widely accepted by farmers over recent years. The introduction of rotations involving wheat and legumes such as lucerne, vetch and chickpeas (Cooper 1999), as well as a trend towards permanent beds and minimum tillage, has led to a complete change in soil structural, chemical and biological properties (Hulugalle *et al.* 1997). The impact of these rotation systems on cotton root production is relatively unknown other than the beneficial effects of structural and chemical improvements.

In Australia, the major cotton growing regions are dominated by Vertosol soils or “cracking clays”. This cracking nature results in the formation of macropores that is ideal for the proliferation of roots attempting to establish at depth. These soils have clay particle contents in the range of 50-80% and exhibit shrink-swell characteristics as moisture levels vary. These soils characteristically possess a very high water holding capacity, high natural fertility, initially high infiltration and high structural stability. The soil pH ranges from slightly acidic to acid in the topsoil, to alkaline in the subsoil (McKenzie *et al.* 1995). Other soil types used for cotton production include the duplex Chromosols in the Macquarie Valley (minor production), and Sodosols in Queensland and parts of Northern NSW. The sodic Sodosols incur production difficulties due to the hardsetting or “crusting” nature of the soils which causes poor germination rates and slower water infiltration. Tillage operations and soil organic matter decline tend to exacerbate the problems associated with these soils. The main soil stress factors that influence the root production of the cotton plant are; water stress, soil mechanical resistance, bulk density (aeration) and temperature. Soil temperatures higher than 35^{o}C

significantly reduce metabolic activity and elongation rates of cotton roots. In addition, root branching is also influenced by changes in soil temperature (Nielson 1974). Compaction is a major problem within the cotton industry due to the fragile nature of the Vertosol soils when they are wet. Compacted soil layers typically form immediately below the plough layers (approximately 20 cm). This leads to the formation of tillage pans that have high bulk densities, few macropores, and mechanical impedance which is detrimental to root proliferation in subsoils.

Maintaining and where possible, increasing soil organic matter is commonly viewed as a key to conserving healthy soils (Hulugalle and Entwistle 1997; Nortcliff 2002). Soil organic matter (SOM) helps stabilise soil aggregates, thus decreasing erosion. It also improves soil structure, enhances aeration and water penetration, increases water holding capacity, and stores and supplies nutrients for growth of both plants and soil microorganisms (Miller 2001).

Since the introduction of cultivation into Australian agriculture, soil organic carbon (OC) levels have decreased dramatically. The main reasons for this decline are the use of conventional tillage practises, the use of pesticides, burning stubble residues, and removal of produce. This continuous system led to large carbon losses through multiple tillage operations and burning stubble residues. Burning causes the carbon within the cotton plant to be lost as atmospheric CO_2, as well as losing considerable amounts of nitrogen and sulphur. Minimum tillage and crop rotation have become a feature of many cotton farming systems (Constable and Forrester 1995). Reasons for the adoption of minimum tillage operations include, soil erosion control, moisture conservation, maintenance of soil structure, improved nutrition and fuel savings (Constable *et al.* 1991). Minimum tillage has the potential to enhance soil carbon sequestration (Wright *et al.* 2004). However, the impacts of tillage on soil organic matter vary due to soil type, cropping system, residue management, and climate (Paustian *et al.* 1997).

The retention of stubble residues instead of burning them has led to a decrease in atmospheric CO_2 losses. The use of leguminous winter manure crops has also helped to increase soil organic matter. The roots of the cotton plant also play a significant role in the cycling of carbon. When the roots turnover (die and decompose) they provide organic carbon to the soil.

Plants acquire carbon from the atmosphere through photosynthesis. Using carbon dioxide (CO_2) from the atmosphere and energy from sunlight, plants convert CO_2 to carbon as they produce stems, leaves and roots (McVay and Rice 2002). Plants and soil can lose carbon through inefficiencies and it is released as atmospheric CO_2. The cycle of life and death of plants results in the accumulation of decomposing plant tissue both above and below the ground, and produces a significant amount of soil organic carbon.

Root production is an extremely important aspect of plant productivity, particularly during the early stages of germination and establishment (Knox *et al.* 2001). The root system is responsible for the extraction of water and minerals from the soil as well as for anchorage (Kramer and Boyer 1995). Roots are therefore vital to fruit production in cotton and play an important role in the final yield of the plant.

Minirhizotrons are an effective tool to observe and quantify root system dynamics, providing a unique method by which individual root segments can be repeatedly measured over multiple time intervals (Crocker *et al.* 2003; Firth *et al.* 2003; Liedgens and Richner 2001). Unlike other root investigation methods, the minirhizotron has the ability to separate the processes of root production and mortality (Phillips *et al.* 2000; Tingey *et al.* 2005). By quantifying root mortality, minirhizotrons can be used effectively to develop ecosystem carbon budgets (Johnson *et al.* 2001). They provide a non-destructive, in situ, method for the direct and continuous observation of fine roots (Crocker *et al.* 2003).

The core-break method of measuring plant root system development consists of retrieving a vertical core of soil, breaking it to reveal a cross section at the depth of interest, and counting the number of roots visible (Bland 1989). Soil coring techniques have the primary advantage of reduced time and labour inputs over excavation methods (Bohm *et al.* 1977). A large number of cores can be collected in a relatively short period of time (Taylor *et al.* 1991), however results have a large variance creating the need for large sample numbers (Firth *et al.* 2003). This method is destructive and does not lend itself to repeated measurements in a research plot (Benjamin and Nielson 2004).

Many cotton practices and management techniques disturb the soil habitat and imbalances occur. This can lead to the dominance or suppression of individual microbial species. These microbial species may be beneficial or detrimental to cotton production and farmers must vary their management strategies in response to this. Cotton producers disrupt the equilibrium of microbial species through tillage, crop rotations, and use of fertilisers and agrochemicals (Gupta *et al.* 2004). Similarly, there are relationships formed by the cotton plant with soil organisms that can either promote or restrict growth. The most effective management techniques that can be adopted by producers to enhance microbial activity are; water management to control moisture and soil oxygen levels, and nutrient management to control the pH (Martin 1991).

When roots grow they produce exudates that support soil microbial populations. Similarly when cotton roots turnover or decompose, microbial populations use this carbon source as a form of energy. The quantitative analysis of microbial activity is important for the assessment of biological soil health and quality. Common techniques include physiological methods (soil respiration, glucose-induced respiration) and enzyme activity analysis [2,3,5,-triphenoltetrazolium (TTC) and 2,3,5-phenyltetrazolium (INT) dehydrogenase activity, dimethylsulfoxide (DMSO) reduction] (Bauer *et al.* 1991).

Soil microbial biomass is the living component of soil organic matter and excludes soil animals and roots (Dalal 1998). Many techniques have been used to determine the total amount of soil microbial biomass including plate counting, direct microscopic counting, fumigation-extraction, and substrate-induced respiration (Lin and Brookes 1996). Fumigation/extraction techniques involve the fumigation of a soil sample of which the extractable C, N or P can be measured and compared to the sample which has not been fumigated (Jenkinson *et al.* 2004). Soil organisms die after their cell membranes are lysed by chloroform (Joegensen 1995). Microwave irradiation has also been proposed as a rapid and non-toxic alternative to chloroform fumigation (Islam and Weil 1998). In the last 20 years, relatively rapid assessment of soil microbial biomass has been possible based on the development of new physiological, biochemical and chemical techniques (Horwath and Paul 1994).

Although the use of minirhizotrons has been extensive to date, there is very little information on the use of minirhizotrons in cotton root investigations or its relationship to the core break and root washing methods. Similarly, there have been few attempts to quantify the contribution of decomposed root carbon in reversing the decline of soil organic carbon. The effect that different cotton farming systems have on root production/turnover and on microbial biomass and microbial activity is also an area which has largely been ignored.

To investigate these gaps in the literature, cotton was grown near Narrabri, NSW, Australia, under four cotton-based rotations. The root production dynamics were quantified throughout the growing season using the core break, root washing and minirhizotron techniques. Soil microbial attributes were measured using the NaOH trap method for microbial activity, and the Ninhydrin reactive N method for microbial biomass. The objective of this study was to determine whether different cotton-based rotations change the cotton root production and turnover dynamics, and increase microbial biomass and activity compared to a cotton monoculture.

2. Materials and Methods

2.1. Site Location and Experimental Design

The study area was field D1 at the Australian Cotton Research Institute (ACRI). ACRI is located at Myall Vale approximately 40 km west of Narrabri, NSW, Australia (150^{o}E, 30^{o}S, Figure 2.1). There has been a long term crop rotation experiment running in D1 since 2002 which forms the basis for this study, and has the following treatments:

- CV = cotton – vetch (green-manured) – cotton (1)
- CC = continuous cotton (2)
- CW = wheat – long fallow (stubble incorporated) – cotton (3a)
- CWV = wheat – summer fallow (standing stubble) – vetch – cotton (4a)

The experimental design was a randomised, complete block design with 3 replicates (i.e. 12 plots in total). The plot dimensions were 20m x 165m. Cotton was grown in all four treatments and was sown on the 27/10/2004.

2.2. Data Collection and Measurements

During the months from December 2004 to February 2005 numerous measurements were made to collect data to quantify cotton root production and turnover characteristics. This included the use of the core break method, root washing method, and the minirhizotron. Microbial activity was measured on four occasions using the NaOH trap method and microbial biomass was assessed twice during this period using the chloroform fumigation- extraction (CFE) prior to Ninhydrin-reactive N analysis.

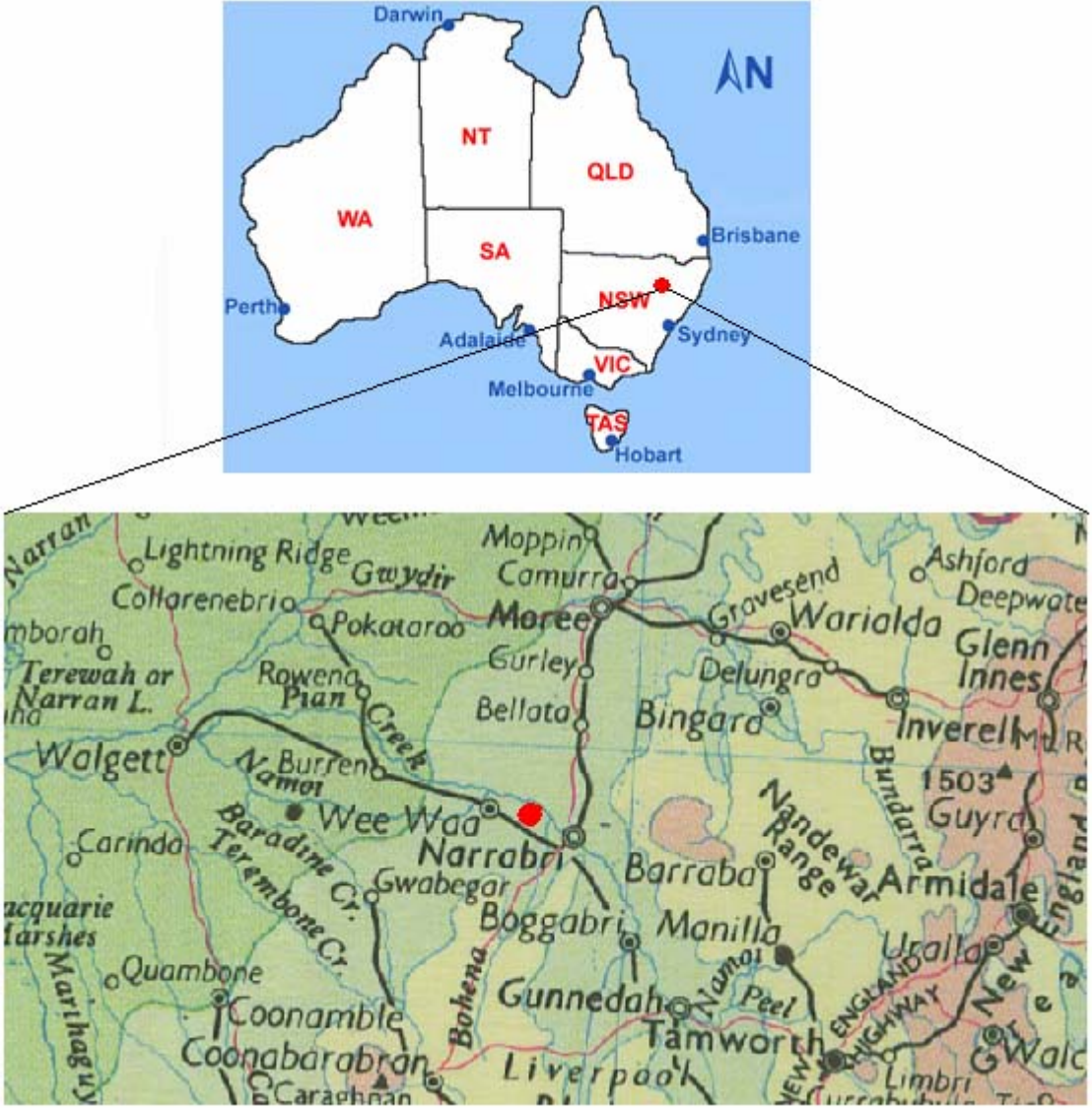

Fig. 2.1. Location of experimental site. (a) Map of Australia with star on Myall Vale NSW, (b) Map of region surrounding Myall Vale NSW (red dot).

2.2.1. *NaOH trap for determining microbial respiration*

A fresh solution of 0.5M NaOH (20g NaOH in 1L of distilled water) was prepared. Twelve plastic vials were marked with an identification code. An additional plastic vial was used as a blank standard. 25mL of the NaOH solution was dispensed into each plastic vial, sealed instantly and placed into an air tight container (desiccator).

In the field, traps were set so that there is one trap per plot, or 3 traps per treatment. When setting up a trap, the plastic vials were unsealed and placed upright on the soil bed, between two cotton

plants. These vials were covered with a cup of known volume and diameter, and the starting time was recorded (Figure 2.2). Fiberglass poles were used to identify where the trap was located.

After 8 h of incubation, the traps were retrieved. The cup was removed, the vials sealed, and the finish time recorded. Traps were stored in a dessicator with an open vial of NaOH to trap any excess CO_2. The traps were then stored in a cool location for 24 h prior to titration (Tiessen *et al.* 1981).

Fig. 2.2. Photo illustrating the procedure for initializing NaOH trap method.

The samples were analysed by the method of Tiessen *et al* (1983). Using a titrator/autoburette, 2 ml of each trap solution and the blank were titrated in the following manner: 2ml of trap solution was added to 100 ml of sterile distilled water. The solution was placed onto the titration platform (with stirrer, acid titration head and pH electrode). Then, 3 N HCl was added until the pH dropped below pH 11. Five drops of 1mg/ml carbonic anhydrase was added using a Pasteur pipette to provide a catalyst

for the reaction (Underwood 1961). The pH was lowered to 9.2-9.8 by adding 1 N HCl.. Using the titrator, 0.05 N HCl was added to lower the pH to 8.3. The titrator/autoburette was then activated to lower the pH to 3.7. The amount of titrant added was recorded.

The amount of titrant added to the blank sample was subtracted from the amount of titrant added to the test samples. The equation; mg C = ml 1 N HCl added x 12 (molecular weight of carbon in CO_2 is 12 as opposed to 1 for hydrogen), was used to calculate the amount of CO_2-C produced. The trap duration was used to convert this value to a daily value (see Appendix 1a). Finally, the trap circumference and diameter were used to convert this value to a per m basis (see Appendix 1b). Higher amounts of CO_2-C being produced indicate a higher level of microbial activity.

2.2.2. *Ninhydrin Reactive N (NHD-N) for quantifying microbial biomass*

Preparation of soil extracts:

The NHD-N was assessed by the Chloroform Fumigation Extraction (CFE) method (Jenkinson 1988). Soil samples were collected in a container from the root zone of the cotton plants and sealed. In the laboratory, two soil samples (~10g) were removed and used to estimate the gravimetric water content (GWC) by weighing pre and post oven dried (105^{o}C) soil. Then, 30 ml of 0.5M K_2SO_4 (pH 6.8) was added to one sample, and shaken at 180 rpm for 60 min. The other sample was fumigated prior to extraction. The soil extract was then filtered through a Whatman #42 filter paper and the filtrate collected. Extracts were either stored at -20^{o}C long term or below 4^{o}C for no more than 2 days (to prevent fungal growth in extracts).

The end of a 40 cm glass column was blocked with a glass-wool plug, and the column was filled to a height of 30 cm with aluminium oxide, tapping the side with a rubber bung to slightly compact the column. Prior to use, the column was dried at 100^{o}C overnight or for 3 days at 80^{o}C. The

column was mounted onto a retort stand in the fume hood. A funnel was sealed with Parafilm to the top of the column and a conical flask was placed at the bottom. Approximately 60 ml of chloroform was passed through the column and 40 ml was collected in the conical flask. The filtered chloroform was then transferred to a beaker containing bumping beads and deposited at the bottom of the desiccator.

Preparation of desiccator and soil samples:

Two wet paper towels were placed in the bottom of the desiccator. This prevented the soil from drying out during fumigation. Several glass jars were labeled using pencil to correspond to the samples. Approximately 10 g of soil was weighed out and placed into the glass jars. The chloroform beaker was placed into the desiccator and the soil samples were positioned around it. The dessicator was maintained under vacuum applied via a water pump until the chloroform boiled. If the chloroform did not boil after 1 h or the vacuum was lost during incubation, the vacuum procedure would be repeated the following day.

After the vacuum procedure, the desiccator was then incubated in the dark for 7 days at 25^{o}C. After incubation, the desiccator was flushed in the fume hood. Chloroform was removed and the air was exchanged in the dessicator twice using the vacuum before the samples were removed. Then, 30ml of 0.5M K_2SO_4 was added to the samples and shaken at 180 rpm for 60 min, storing samples at -20^{o}C prior to NHD-N analysis (Martens 1995).

NHD-N Analysis:

The samples were removed from the freezer and allowed to thaw for 12-24 h in the laboratory prior to the analysis. Gentle heating in a microwave ensured that the samples had dissolved after thawing. The 20 ml boiling tubes were numbered and labeled. 1ml of unfumigated soil extract was

added to one set of tubes. 0.5ml of fumigated soil extract and 0.5ml of K_2SO_4 was added to another set of tubes. For standards (leucine and ammonium sulphate), 0.1, 0.25, 0.5, 0.75 and 1 ml of the standard was made up to 1 ml with K_2SO_4. This gives 0.35, 0.875, 1.75, 2.625 and 3.5 μg N per standard. At least two distilled water blanks were prepared with about 4 ml of water.

2.5 ml of citric acid buffer was added to the 1 ml of test samples and mixed by vortex. 1.75 ml of Ninhydrin reagent was added, and mixed by vortex. The tubes were then placed in the boiling bath for 25 min. The rack of tubes was then removed from the bath and placed in ice cooled water. The lids were removed and 4 ml of ethanol (50%) diluent was added. The spectrophotometer was blanked on the distilled water blanks. The test tubes were mixed by vortex, and the OD_{570} (absorbance at 570nm) was read and recorded using 1 ml of each reaction in the spectrophotometer. Samples were then diluted in a 1 in five ratio and sent to Adelaide for C and N analysis.

A standard curve was prepared from the readings for the standards (Sparling *et al.* 1993). The absorbance was plotted against μg N/ml. The line through the points for each standard was plotted and the equation for the best trend line ($R>0.96$ usually) was taken and used to calculate the μg N/ml in each sample. Then, the μg N/g soil, flush of fumigation (FOF) and MB-C (see Appendix 2 for calculations) were calculated.

2.2.3. *Core break method*

Cores of soil, 100 mm diam. and 1 m in length were extracted from three random locations in each plot using a tractor-mounted hydraulic soil-coring machine (Bohm *et al.* 1977). Using a knife and ruler, the core was separated into depth intervals of 10 cm. Each 10cm core was broken in half to reveal a cross section (Figure 2.3). The faces of each half were rinsed with a wash bottle containing

distilled water to enhance visibility and clean roots. The numbers of live roots (shiny and white) visible on both faces were counted and an average was calculated (Bland 1989). This process is then repeated for each depth interval for each plot. After counting, one core from each plot was separated into depth intervals and placed into labeled plastic bags to be processed by the root washing method. These samples were stored at below 4^{o}C prior to washing.

Fig 2.3. Photo illustrating the core break method after a core sample has been broken in half.

2.2.4. *Root Washing Method*

The root samples were removed from the 4^{o}C cold room and soaked in warm water containing a 2:1 solution of 10% sodium hexametaphosphate: 1 M sodium hydroxide for a period of 4-12 hours. The soaking period depends on the dispersability of the soil. Once dispersed, the suspension was washed through a 0.2 mm sieve. The remaining silt and sand material was separated from the root and other organic material by flotation and decantation.

The remaining organic material (including roots) was then stained with a 0.1% Congo red solution for a period 2-4 hours, followed by washing in ethanol (95 %). The Congo red stains the live roots in the sample to a bright red colour, whereas the dead organic material remains black. The live roots were separated from the dead material using forceps under a bright light (Jose *et al.* 2001). Root separation was done by spreading out the sample in a shallow white, plastic tray. The trays were filled with 2 to 3 mm of water. In this way, roots may be easily separated, but are prevented from floating around in the tray. Once the live roots had been separated from the dead material, they were stored in a 25% ethanol solution until the length is measured using a line interception method with a calibrated 1cm x 1 cm grid (Newman 1966) (Figure 2.4). Root samples were then dried, ground and sent to Adelaide for C and N analysis.

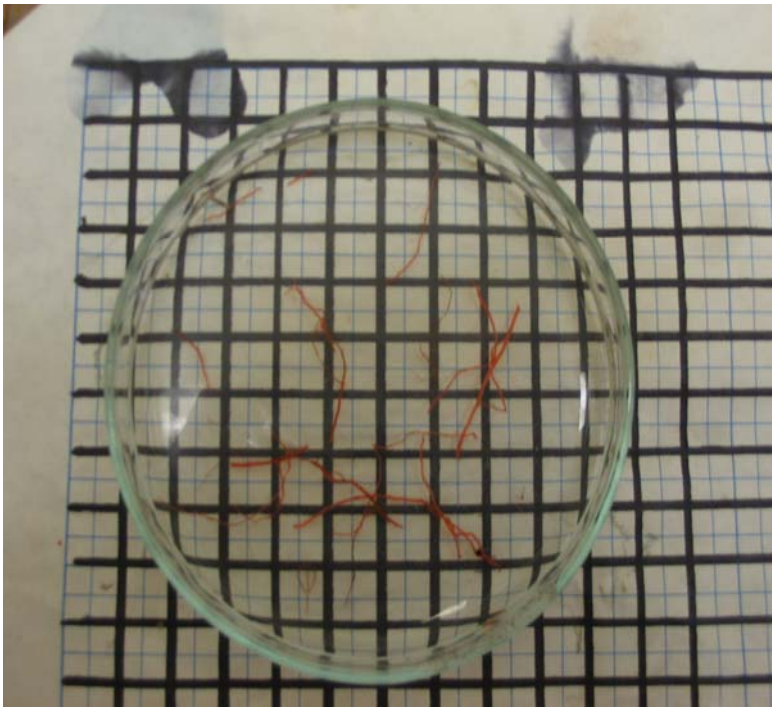

Fig 2.4. Sub-samples of live roots overlaying a 1cm grid. No. of intersections will be counted as part of the Newman line intersection method.

2.2.5. *Minirhizotron method*

Between the months of December 04 and February 05, cotton root growth was measured at approximately 3-week intervals using the minirhizotron. The observation sites were located in Block 2 with 6 transparent PVC access tubes being installed in each treatment. Images were collected from all tubes on 21/12/2004, 07/01/2005, 02/02/2005 and 22/02/2005 (equivalent to 55, 72, 98 and 119 days after sowing).

Minirhizotron image recording and processing:

Clear plexiglass minirhizotron access tubes with sealed bottoms (1.2m long and 5.1cm inner diameter) were installed into the root zone of the cotton plants at a 45^{o} angle using a tractor-mounted hydraulic coring device (Johnson *et al.* 2001). This was performed with minimal soil disturbance and optimal contact between the tube and the soil (Taylor *et al.* 1991). The 20 cm section of the clear access tube left above the ground was covered with PVC pipe to prevent water and sunlight entering the tube. A fibreglass pole was also used to indicate the location of the tubes for machinery operators.

The colour micro-video minirhizotron camera was lowered through the access tubes with a calibrated handle provided by the manufacturer to a depth of 90 cm. Images were taken by the digital camera at 10 cm intervals from a depth of 10 to 90 cm. There were two images taken at each depth interval, 180^{o} apart. Using the BTC I-CAP Image Capturing System, images were captured and stored on a computer in the field. The computer software labels each image according to location and depth and saves the images in a format that can be exported to Rootracker® software for further analysis.

Before analysing images in Rootracker, a calibration was performed. By default, Rootracker will record all root measurements in pixels. **Calibration > New calibration** will allow for a calibration based on the minirhizotron setup so that measurements can be done in units (mm) that are more

relevant. Once calibrated, images were traced and measured using the software program. Changes in root dynamics over time were also measured and results exported to Excel for further analysis. Root growth/turnover, root volume/area/length, and root carbon content were all assessed using the minirhizotron system (see Appendix 4). Root carbon content was calculated based on carbon and nitrogen analysis on the roots. Root dimensions were calculated using a combination of the core break and root washing results, and the area dimensions determined in Rootracker.

2.3. Statistical Analysis

The statistical analysis was performed using Genstat release 8.1. Probability plots and residual analyses were conducted on data to ensure normality. Transformations were made if necessary using either the square root or natural log function, and back-transformed means were presented in graphs and tables. For all experiments, data was subject to an analysis of variance (ANOVA) to test for any significant differences between rotational systems (Appendix 6). The data from the minirhizotron was analysed using ANOVA using “repeated measurements”. The analysis also incorporated possible interactions between blocks, depths and time.

3. Results

3.1. General Soil Characteristics

Many of the general soil properties such as pH, EC, soil organic carbon, sodicity and clay % were similar for all experimental plots (See Appendix 5). Soil depth and origin was also relatively uniform throughout the experimental site.

3.2. Minirhizotron Readings

At the first sampling date (55 DAS [days after sowing]), all treatments had similar mean root growth rates (Figure 3.2). On the second sampling date (72 DAS), the root growth rates of CW and CWV were significantly higher than CV and CC.

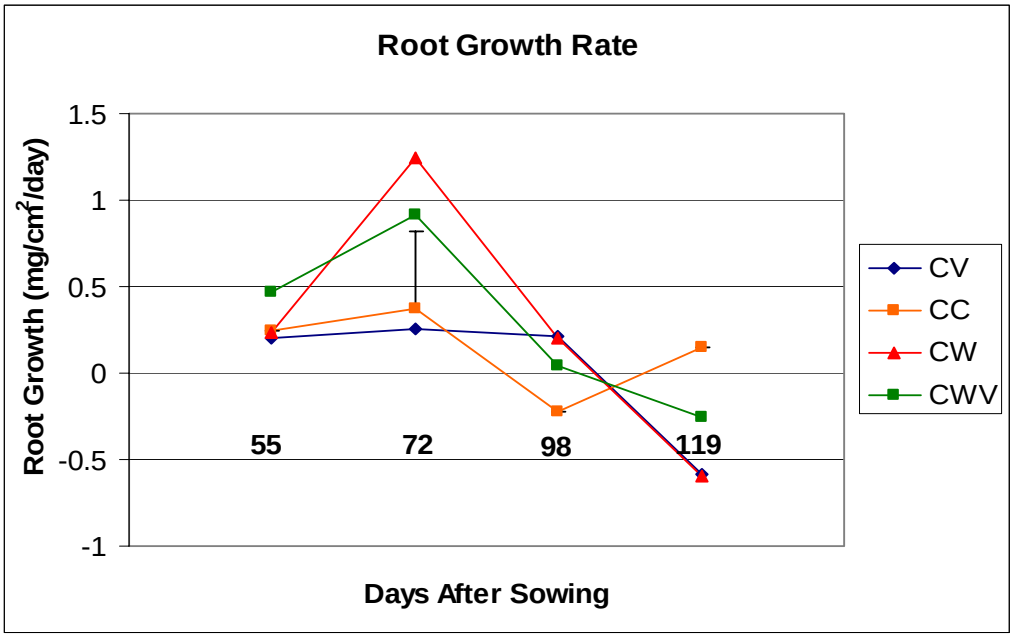

Fig. 3.2. Root growth rates for cotton-vetch (CV), continuous cotton (CC), cotton-wheat (CW) and cotton-wheat-vetch (CWV) rotations over the 2005 growing season using the minirhizotron. Vertical bar represents the l.s.d at P=0.05 at 72 days after sowing (DAS)

On the third sampling date (98 DAS), all rotations experienced a steep decline in root growth rates except for CV, with rotation CC having a net loss of roots. The root growth rate of CV remained constant in comparison to other sampling dates.

On the final sampling date (119 DAS), all rotations had a decrease in root growth rates except for CC. Overall, the average root growth/turnover was higher ($P<0.05$) in rotations including wheat, CW (3.267 mg/cm^2/day) and CWV (3.255 mg/cm^2/day) compared to CC (3.233 mg/cm^2/day) and CV (3.229 mg/cm^2/day) over the 64-day period they were measured.

3.3. Core Break Analysis

The core break method was used twice during this study with sampling dates approximately two months apart. CW rotation had higher ($P<0.01$) cotton root numbers (10, 20, 50, 60, 70, and 80cm depths) than all other treatments on the first sampling date (07/01/2005) (Fig. 3.3). Both CC and CWV rotations have higher ($P<0.01$) live root numbers than CV.

In the second core break sample on 25/02/05, the average root number for CC and CW were very similar with both rotations having a significantly higher root numbers than CV and CWV. The data from the two core break samples were used to ground truth the minirhizotron results.

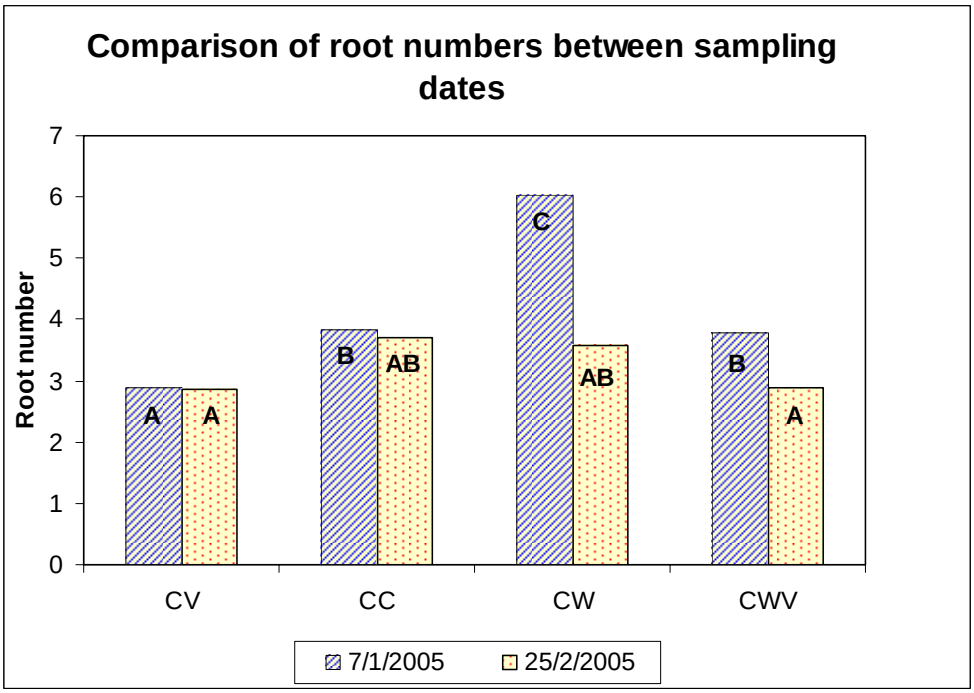

Fig. 3.3. Backtransformed mean numbers of live roots for the cotton-vetch (CV), continuous cotton (CC), cotton-wheat (CW) and cotton-wheat-vetch (CWV) rotations using core break measurements. Backtranformed means with the same letters are not significantly different at P = 0.05.

3.4. Root Washing Analysis

The data from the root washing analysis were used in conjunction with the first core break measurements to develop calibrations to ground truth the minirhizotron readings. The CW rotation has the highest ($P<0.01$) average root length, and CC and CWV had higher ($P<0.01$) mean root lengths than CV (Fig. 3.4.1).

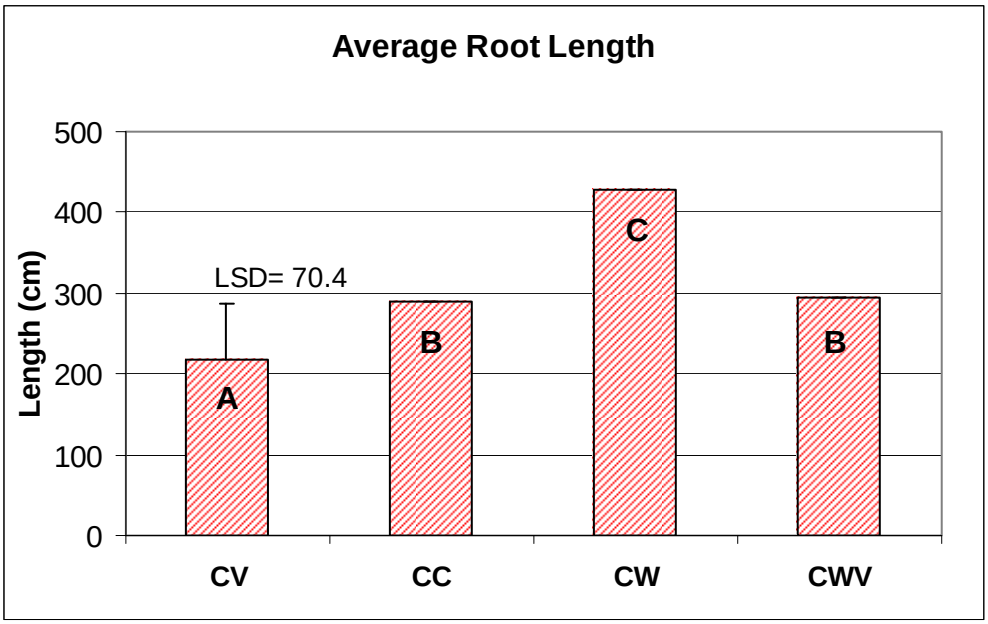

Fig. 3.4.1. Mean root length (cm) of the cotton-vetch (CV), continuous cotton (CC), cotton-wheat (CW) and cotton-wheat-vetch (CWV) rotations using the root washing method. Means with the same letters are not significantly different at P = 0.05.

There was no significance difference in the root mass between treatments. Root length/mass ratio (0-20 cm) for CW was higher ($P<0.05$) than the CC rotation in the 0-20 cm depth interval (Fig. 3.4.2a) and higher ($P<0.05$) than CV in the 20-80 cm depth interval (Fig. 3.4.2b). The CW rotation also had the highest mean root length: soil volume ratio for the 0-20cm depth interval (Fig. 3.4.2c). This shows that cotton roots were longer per unit mass in the CW rotation which could provide better extraction of water and nutrients. Both CW and CWV had higher ($P<0.05$) root length: soil volume ratios than CV in the 20-80 cm depth interval suggesting that longer roots were present deeper in the soil profile possibly due to roots following macropores and channels left by the previous wheat crop (Fig. 3.4.2d).

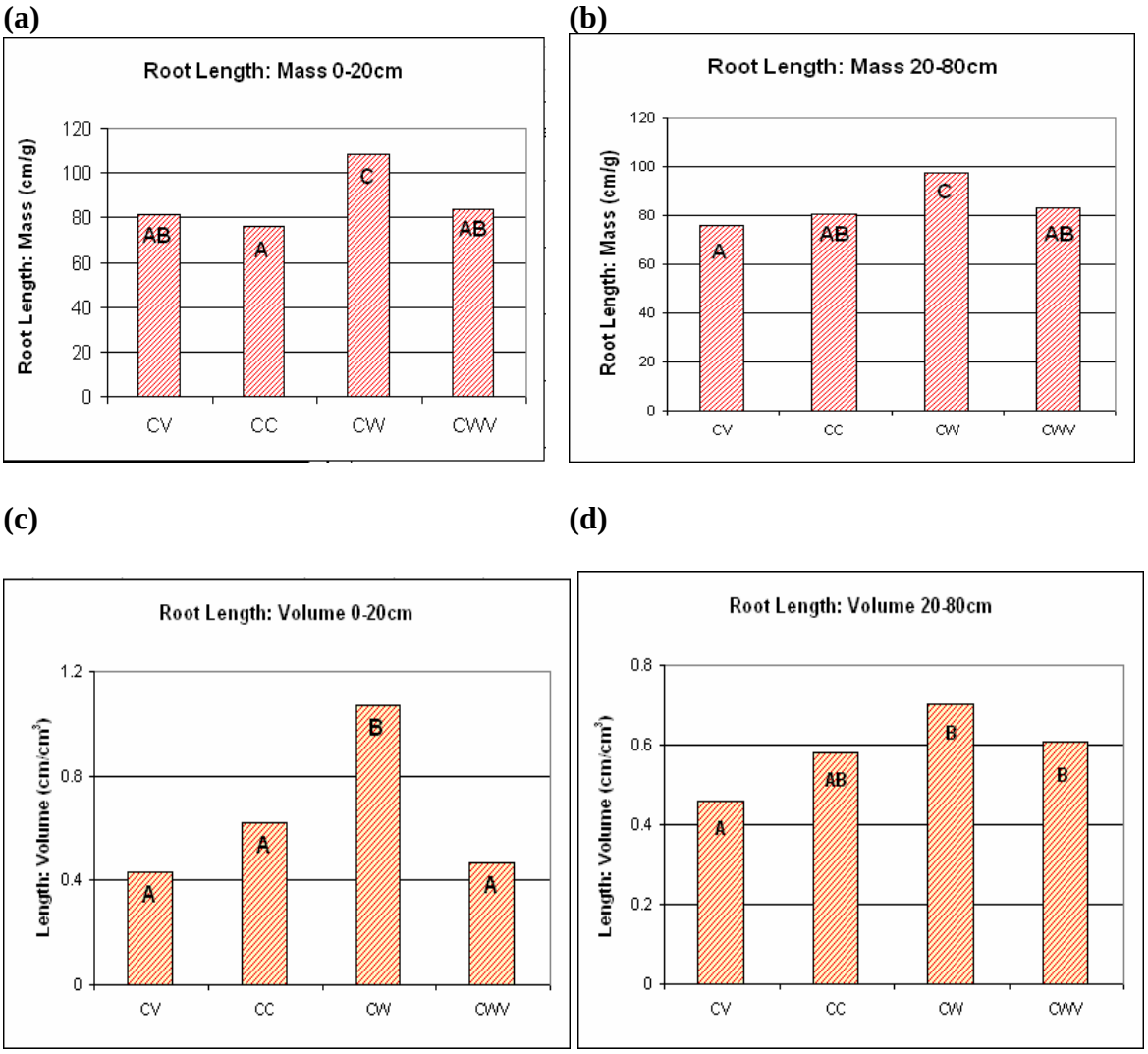

Fig. 3.4.2. Backtransformed root length/root mass (cm/g) ratios in (a) 0-20 cm and (b) 20-80 cm and root length/volume of soil (cm/cm^3) ratios in (c) 0-20 cm and (d) 20-80 cm for the cotton-vetch (CV), continuous cotton (CC), cotton-wheat (CW) and cotton-wheat-vetch (CWV) rotations using the root washing method. Backtransformed means with the same letters are not significantly different at P = 0.05.

3.5. Root Mass and Root Carbon

Root length from the minirhizotron readings were converted to net root mass using calibrations of the root length and root dry weight measurements from the root washing method (0.0389mg/mm root). Similarly, net root carbon was calculated using the conversion of 27.09% from the laboratory analysis of these roots. The CWV rotation had the highest net root mass of 330.31g/m^2 and was

significantly higher (P<0.05) than both CV and CC (Fig. 3.5). Net root mass of CW and CC were higher (P<0.05) than CV. The net root carbon values showed the same trends due to the relationship between root carbon and root mass.

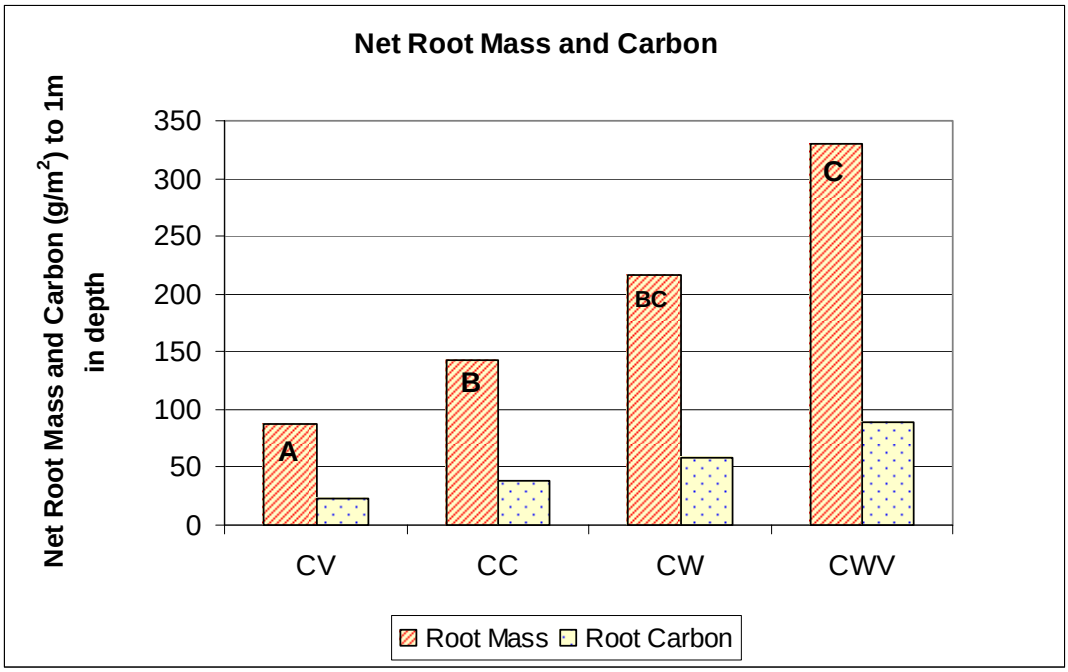

Fig. 3.5. Backtransformed net root mass (g/m^2) and net root carbon (g/m^2) of the cotton-vetch (CV), continuous cotton (CC), cotton-wheat (CW) and cotton-wheat-vetch (CWV) rotations for the cotton growing season (Dec 04 - Feb 05) based on minirhizotron readings. Backtransformed means with the same letters are not significantly different at P = 0.05.

3.6. Microbial Biomass

Microbial biomass was measured twice during the growing season on 04/01/2005 and 15/02/2005. The first microbial measurement used soil cores from the first core break sampling. Rotation CW in the 10-20cm depth interval had a significantly higher soil microbial biomass than all other treatments (Fig. 3.6). Treatments CV, CC and CWV had similar soil microbial biomass levels.

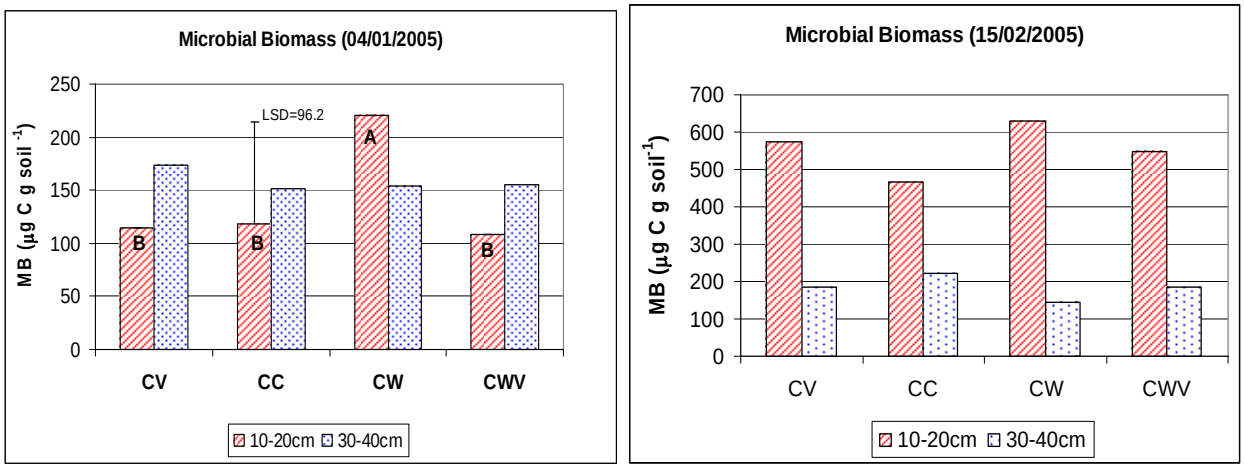

Fig. 3.6. Microbial biomass of the cotton-vetch (CV), continuous cotton (CC), cotton-wheat (CW) and cotton-wheat-vetch (CWV) rotations measured using the Ninhydrin reactive N technique on (a) 04/01/2005 and (b) 15/02/2005 at the 10-20 cm and 30-40 cm depth intervals. Means with the same letters are not significantly different at P = 0.05.

In the 30-40cm depth interval, there were no significant differences in microbial biomass. For all treatments, microbial biomass for CW in the 10-20cm depth interval was higher ($P<0.05$) than the other treatments (Fig. 3.6a). The microbial biomass in the 30-40 cm depth interval in the second sampling was lower than at the 10-20cm depth interval (Fig. 3.6b).

3.7. Microbial Activity

Microbial activity was measured 4 times throughout the experiment. The data from each the measurements were quite inconsistent and in most cases not significantly different. Due to the vulnerability of microbial functions to environmental conditions such as soil moisture and temperature, it is difficult to use this sampling technique in the field. For future studies however, analyses could be made with soil samples in the laboratory.

3.8. Yield Analysis

At the end of the growing season the experimental site was harvested and the yield of each treatment were analysed in relation to seed cotton bales/ha, lint (t/ha), seed (t/ha) and trash content

(t/ha). Overall yield was higher (P<0.05) in CWV (11.39 bales /ha) than rotations CV (8.24/ha) and CC (8.45/ha) (Fig. 3.8a). Lint yield followed very similar trends to the seed cotton bales/ha (Fig. 3.8b). Rotation CWV had higher (P<0.05) lint yields than both CV and CC, and CW was higher (P<0.05) than CV.

Seed content was higher (P<0.05) in CWV and CW than CV and CC (Fig. 3.8c). There were no statistical differences in trash content between treatments. Overall, the cotton rotations (CW and CWV) including a wheat phase had the highest yields.

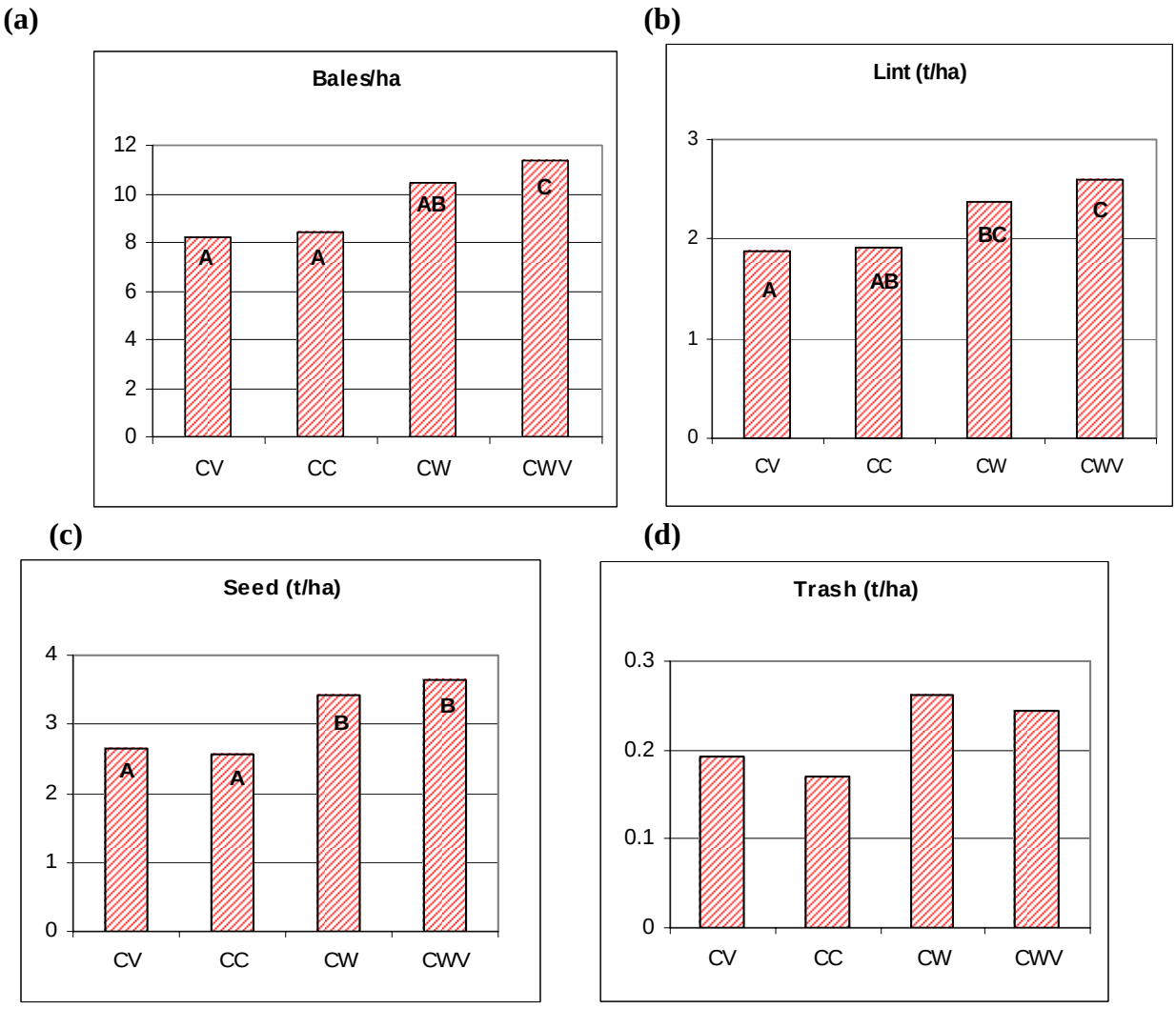

Fig. 3.8. Backtransformed yield (a) seed cotton 227 kg bales/ha, (b) lint (t/ha), (c) cotton seed (t/ha) and (d) trash (t/ha) of the cotton-vetch (CV), continuous cotton (CC), cotton-wheat (CW) and cotton-wheat-vetch (CWV) rotations for growing season 2004/05. Backtransformed means with the same letters are not significantly different at P = 0.05.

4. Discussion

Cotton rotations that include wheat phases increased cotton root production, root carbon levels and yields in this study. Microbial populations can also be higher in cotton rotations including wheat compared to continuous cotton and cotton-vetch rotations. In this study, implementation of wheat-based rotations has resulted in higher cotton root lengths, root mass which led to an increase in soil organic carbon, microbial biomass and yields.

Root length and root mass data determined by the minirhizotron was well correlated with the core break (R^2= 0.84 [0-20cm], R^2=0.45 [20-100cm]) and root washing data. Similar relationships have been observed in other agronomic crops. For example, Box and Ramseur (1993), reported significant correlation between minirhizotron root areas and root biomass in winter wheat (*Triticum aestivum* L.). These authors observed a much weaker correlation (R^2= 0.48) than that found in the 0-20cm depth (R^2= 0.84) in our study.

Although the minirhizotron technique offers an attractive alternative to destructive root sampling, it has been shown to underestimate rooting density in comparison to soil core sampling in the soil surface layers (Jose *et al.* 2001). Hence, a calibration using the core break and root washing methods has been used to calculate root dynamics in the 0-10cm depth.

Rotations CW and CWV had the highest root growth rates 72 days after sowing (DAS) and there were no significant differences between rotations at 119 DAS. The measurements on these two minirhizotron sampling dates are consistent with core break data sampled at the same time. In the first core break sampling (72 DAS), root numbers were highest in CW rotation and there were no significant results in the second sampling (122 DAS). Both CW and CWV had a marked decrease in root numbers during this time (119 DAS) in the growing season. This corresponds to the cotton plants' natural tendency to turnover roots during the fruiting stage of growth to direct more of its energy into

boll formation for survival. Minirhizotron readings supported this turnover of roots with CW and CWV having a net loss of live roots between 07/01/2005 (72 DAS) and 02/02/2005 (122 DAS). Surprisingly, the CC rotation only had a small decrease in root numbers and CV actually showed evidence of root production throughout this phase.

Root washing data were also consistent with that of the core break and minirhizotron measurements showing that rotation CW had the longest average root length. Rotation CW produced the longest length of roots per unit volume of soil and CWV produced longer roots per unit volume of soil than CV. This suggests that the cotton roots in wheat-based rotations are better distributed throughout the soil profile than the other rotations.

Cotton rotations that include wheat have the highest cotton root mass. Net root mass produced by CWV was higher ($P<0.05$) than treatments CV and CC, and rotation CW had a higher ($P<0.05$) root mass than CV. This increased root growth could be due to macropores that have been created or left behind by previous wheat crops. Macropores are tubular pores left by plant roots after they decay or burrowed by soil animals (eg. earthworms), which provide channels for deep rooting and improve crop access to water and nutrients (Nakamoto 2000). Deep-rooting is particularly important in the case of the less mobile nutrients that move by diffusion (Nye and Tinker 1977). This observation is consistent with the data of Hodgson and Chan (1984), who found that the extensive root system of wheat plants was ideal for drying out heavy clay profiles to a depth of up to 95cm prior to the sowing of cotton. In doing so, the wheat plants created an extensive network of macropores in the soil profile for the following cotton crop. Similarly, fractal analysis of the distribution of cotton roots has shown that macropores contributed greatly to root elongation in soils with high clay content (Hatano and Sakuma 1990).

As well as producing macropores, wheat roots have been shown to maintain and extend existing macropores. In a study of wheat roots growing in a heavy clay soil of South Australia, 80% of

wheat roots were located in or within 1 mm of macropores (Pierret *et al.* 1999). This preferential location of roots also reduces mechanical impedance of root growth which is extremely important in heavy clay soils.

In addition to increasing root production, macropores also improve the soil system's ability to distribute valuable water and nutrients. Increased soil nutrient supply can improve plant growth while the plant can increase the absorbing surface by root growth and extension, thereby also increasing nutrient availability. Hatano and Sakuma (1990) found that macropore systems allowed unevenly distributed roots to take up water easily and also provided pathways for the rapid movement of water and solutes. By distributing water and solutes to the entire root system, plant stress is minimised and plant productivity is increased.

Production of root mass was highest in rotations CWV and CW. Throughout the season this carbon was released into the soil through root turnover (decomposition). Similarly, at the end of the growing season the remaining roots decomposed releasing carbon into the soil. The efficiency of this process in still highly debated. There was only a weak correlation between soil carbon change and the net amount of live fine root mass produced by native grasses in one study (Guo *et al.* 2005). However, the soil carbon changes were positively correlated with live fine root length density. It was suggested that the increase in soil carbon was caused more by the exudation of live fine roots than by decomposition of fine root mass to humus. Root exudation played a major role in release of carbon into the soil (Rees *et al.* 2005). However, quantifying inputs from decomposing roots and release of carbon-based exudates was difficult.

Due to the higher root growth in the wheat-based rotations, carbon turnover from decomposing roots and root exudates could possibly be higher than in other rotations in this study. However, the rotations including vetch should not be overlooked. Namoi Woolly Pod Vetch (*Vicia villosa*) can fix up to 265 kg N/ha (root and shoot) and by incorporating the legume into the soil, it can add 1.6 tons

organic carbon per ha (Rochester 2005). Similarly, by using vetch as a green manure crop in cotton rotations over a number of years, the nitrogen and carbon levels can increase in the soil eliminating or reducing the need for fertilisers (Rochester *et al.* 1998).

However, the benefits of green manure crops can vary depending on other crops in the rotation and the tillage practices used. The amount of nitrogen and carbon that vetch supplies when green manured is significantly higher in minimum tillage practices compared to conventional tillage (Jantalia *et al.* 2003). Therefore, the amount of vetch-fixated nitrogen and carbon could be higher in the CWV (minimum till) rotation in the long term compared to CV.

Decomposition of organic matter is regulated by microorganisms, and results in the mineralisation of nitrogen and other nutrients. In this study, the incorporation of wheat stubble and higher root growth led to a high microbial biomass in the CW rotation in the 10-20cm depth interval at 72 DAS (07/01/2005). This observation is consistent with previous reports that wheat stubble incorporation increases availability of plant nutrients and microbial biomass (Lal and Mishra 2002). Similarly, another study incorporating wheat residues increased microbial biomass which led to a yield increase in the following rice crop (Singh and Singh 1995). Carbon is the core element of organic matter (OM) and is a vital energy source for soil biota (Gupta *et al.* 2004). Soil microbial biomass is strongly influenced by the presence of readily available carbon-based substrates (Hoyle and Murphy 2004).

As well as the incorporation of wheat stubble residues, the high root growth in the CW rotation could have resulted in the increased microbial biomass. This notion is supported by a study that showed that microbial biomass was positively correlated to the dissolved organic carbon (DOC) concentration and root biomass (Lu *et al.* 2002). In this study, root exudates were strongly correlated to root length and mass. Similarly, plant root exudates have been shown to accelerate the processes of carbon and nitrogen transformation in the soil. The activity of extracellular enzymes in the rhizosphere

depends on both the composition of root exudates and the addition of organic carbon to the soil (Mergel *et al.* 1998). Data from the second microbial biomass sampling on 15 Feb 05 (112 DAS) were not significantly different. This could be related to the decreased root length and mass which suggests that root exudation may play a major role in the stimulation of microbial biomass. For future studies, root exudates should be measured to determine the role of carbon-based root substrates on microbial dynamics.

There were no significant differences in microbial respiration in this study. Numerous measurements were made but there were large variations in results possibly due to the vulnerable nature of soil microbes. Lee and Jose (2003) experienced similar difficulties comparing the differences in soil respiration between forest and pasture soils. In their study, respiration values fluctuated between 177 to 776 mg $CO_2/m^2/h$ depending on soil temperature, moisture and time of day. Likewise, it has been reported that soil respiration has a very weak correlation with chloroform-extracted microbial biomass which is consistent with our results (Wang *et al.* 2003).

Since there were no significant differences between the microbial respiration rates of our rotations, it may be possible to speculate on the fate of the root-derived carbon. If soil respiration rates were similar, it is reasonable to assume that similar amounts of root-derived carbon are lost from each treatment through microbial respiration (CO_2). Hence, rotations that produce the highest root mass may replace the largest amount of carbon into the soil, which can benefit the following crops.

Rotations including a wheat phase had higher lint yield than continuous cotton and cotton-vetch rotations. These results support other findings showing that cotton lint yield and quality, and gross margins/ha and gross margins/ML, were always higher in cotton rotations including wheat (Hulugalle *et al.* 2001). Lint yield and fibre quality also decreased when leguminous rotation crops were sown. In contrast, cotton lint yield was unaffected by rotation crops in another study assessing wheat and legumes in cotton rotations. However, the legume used was field peas and wheat rotations still

remained the most economical in terms of profitability and water use efficiency (Hulugalle *et al.* 1999). The higher lint yields and quality in the wheat-based rotations can also be contributed to the higher root growth which increased the absorptive surface area of the roots. This increases the plant's ability to absorb soluble nutrients and water from the soil.

For future experiments, it would have been useful to have a "control" for microbial data using samples from areas outside the influence of the root zone. This would separate the effect of previous crop residues from current root exudates.

5. Conclusion

Root growth rates, root production, and root numbers were highest in cotton-based rotations including wheat. Hence, net carbon produced in roots was also highest for these rotations. Since carbon losses through soil microbial respiration did not differ between rotations, the cotton-based rotations including a wheat phase (CW and CWV) rotations could potentially replace the largest amounts of carbon into the soil when cotton roots turnover.

Microbial biomass at 72 days after sowing in the 10-20cm depth interval was greatest in the Cotton-Wheat (CW) rotation which coincided with this rotation having the highest root numbers and growth rates. This indicates that the incorporation of wheat residues, and possibly elevated root exudation due to high root production could increase microbial biomass and potentially improve soil health. Lint and cotton seed yields were also highest in the cotton-based rotations including a wheat phase (CW and CWV).

6. Acknowledgements

The biggest thanks must go to my supervisor, Dr Daniel Tan for his constant support, enthusiasm and wealth of knowledge. I am very grateful that I was able to work with such an excellent supervisor who made this such a great learning experience.

To Dr Oliver Knox, CSIRO Land and Water, and Dr Nilantha Hulugalle, NSW Department of Primary Industries, who were my supervisors throughout my summer scholarship at the Australian Cotton Research Institute, I am extremely grateful for their generosity and support during my time in Narrabri. Also, their constant assistance throughout my final year and thesis writing is greatly appreciated.

To Tim Weaver and Lloyd Finlay for their assistance in the collection of data for this experiment and their constant help while I was in Narrabri. They both contributed greatly to this research and for that I am grateful.

Finally, Dr Damien Field for his efforts in assisting in the writing of this thesis.

7. References

Bauer E, Pennerstorfer C, PHolubar P, Braun R (1991) Microbial activity measurement in soil- a comparison of methods. *Journal of Microbiological Methods* **14**, 109-117.

Benjamin JG, Nielson DC (2004) A method to seperate plant roots from soil and analyse root surface area. *Plant and Soil* **267**, 225-234.

Bland WL (1989) Estimating root length density by the core-break method. *Soil Science Society of America Journal* **53**.

Bohm W, Maduakor H, Taylor HM (1977) Comparison of five methods for characterising soybean rooting density and development. *Agronomic Journal* **69**, 415-419.

Box JE, Ramseur EL (1993) Minirhizotron wheat root data- comparison to soil core data. *Agronomic Journal* **85**, 1058-1060.

Constable GA, Forrester NW (Eds) (1995) 'Challenging the future.' (CSIRO: Melbourne)

Constable GA, Rochester IJ, Daniells IG (1991) Cotton yield and nitrogen requirement is modified by crop rotation and tillage method. *Soil and Tillage Research* **23**, 41-59.

Cooper JL (1999) A grower survey of rotations used in New South Wales cotton industry. *Journal of Experimental Agriculture* **39**, 743-755.

Crocker LT, Hendrick RL, Ruess RW, K.S. P, Burton AJ, Allen MF, Shan J, Morris LA (2003) Substituting root numbers for length: improving the use of minirhizotrons to study fine root dynamics. *Applied Soil Ecology* **23**, 127-135.

Dalal RC (1998) Soil microbial biomass- what do the numbers really mean? *Australian Journal of Experimental Agriculture* **38**, 649-665.

Firth DJ, Whalley RDB, Johns GG (2003) Distribution and density of the root system of macadamia on krasnozem soil and some effects of legume groundcovers on fibrous root density. *Australian Journal of Experimental Agriculture* **43**, 503-514.

Guo LB, Halliday MJ, Siakimotu SJM, Gifford RM (2005) Fine root production and litter input: its effects on soil carbon. *Plant and Soil* **272**, 1-10.

Gupta VVSR, Neate SM, Leonard E (2004) 'Life in the Soil.' CRC for Soil and Land Management, Adelaide.

Hatano R, Sakuma T (1990) The role of macropores on rooting pattern and movement of water and solutes in various field soils. In '14th International Congress of Soil Science'. Kyoto, Japan pp. 130-135

Hodgson AS, Chan KY (1984) Deep moisture extraction and crack formation by wheat and safflower in a vertisol following irrigated cotton rotations. *Reviews in Rural Science* **5**, 299-304.

Horwath WR, Paul EA (1994) Microbial Biomass. In 'Methods of Soil Analysis'. (Ed. RW Weaver) pp. 754-773. (Soil Science Society of America: Madison, Wisconsin)

Hoyle F, Murphy D (2004) Can we manipulate resource availability to drive changes in microbial carbon assimilation and nitrogen cycling. In 'Soil Biology in Agriculture'. Tamworth, NSW. (Ed. R Lines-Kelly)

Hulugalle NR, Entwistle P (1997) Soil properties, nutrient uptake and crop growth of an irrigated vertisol after nine years of minimum tillage. *Soil and Tillage Research* **42**, 15-32.

Hulugalle NR, Entwistle P, Lobry de Bruyn LA (1997) Residual effects of tillage and crop rotation on soil properties, soil invertebrate numbers and nutrient uptake in an irrigated vertisol sown to cotton. *Applied Soil Ecology* **7**, 11-30.

Hulugalle NR, Entwistle P, Scott F, Kahl J (2001) Rotation crops for irrigated cotton in a medium-fine, self-mulching, grey Vertosol. *Australian Journal of Soil Research* **39**, 317-328.

Hulugalle NR, Scott F, Entwistle P, Nehl DB, Allen SJ, Cooper JL, Finlay LA (1999) Sowing wheat or field pea as rotation crops after irrigated cotton in a grey vertosol. *Australian Journal of Soil Research* **37**, 867-890.

Islam KR, Weil RR (1998) Microwave irradiation of soil for routine measurement of microbial biomass carbon. *Biological Fertility and Soils* **27**, 408-416.

Jantalia CP, Santos HP, dos Denardin JE, Kochhann R, Alves BJR, Urquiaga S, Boddey RM (2003) Influence of crop rotations in soil carbon and nitrogen stocks under no tillage and conventional tillage. *Agronomia* **37**, 91-97.

Jenkinson D (1988) Determination of microbial biomass carbon and nitrogen in soil. In 'Advances in nitrogen cycling in agricultural ecosystems.' (Ed. J Wilson) pp. 368-386. (CAB International: Wallingford, UK)

Jenkinson DS, Brookes PC, Powlson DS (2004) Measuring soil microbial biomass. *Soil Biology and Biochemistry* **36**, 5-7.

Joegensen RG (1995) Quantification of the microbial biomass by determining Ninhydrin-reactive N. *Soil Biology and Biochemistry* **28**, 301-306.

Johnson MG, Tingey DT, Phillips DL, Storm MJ (2001) Advancing fine root research with minirhizotrons. *Environmental and Experimental Botany* **45**, 263-289.

Jose S, Gillespie AR, Seifert JR, Pope PE (2001) Comparison of minirhizotron and soil core methods for quantifying root biomass in a temperate alley cropping system. *Agroforestry Systems* **52**, 161-168.

Knox, Ladiges, Evans, Saint (Eds) (2001) 'Biology (2nd edn).' (McGraw-Hill Book Company: Sydney)

Kramer PJ, Boyer JS (1995) 'Water Relations of Plants and Soils.' (Academic Press, INC.: San Diego)

Lal JK, Mishra B (2002) Effect of recycling of crop residues in situ on soil fertility and crop yield. *Journal of Research* **14**, 181-185.

Lee KH, Jose S (2003) Soil respiration and microbial biomass in a pecan - cotton alley cropping system in Southern USA. *Agroforestry Systems* **58**, 45-54.

Liedgens M, Richner W (2001) Relation between maize (*Zea mays* L.) leaf area and root density observed with minirhizotrons. *European Journal of Agriculture* **15**.

Lin Q, Brookes PC (1996) Comparison of methods to measure microbial biomass in unamended, ryegrass-amended and fumigated soils. *Soil Biology and Biochemistry* **28**, 933-939.

Lu YaHai Watanabe A, Kimura M (2002) Contribution of plant-derived carbon to soil microbial biomass dynamics in a paddy rice microcosm. *Biology and Fertility of Soils* **36**, 136-142.

Martens R (1995) Current methods for measuring microbial biomass C in soil: potentials and limitations. *Biological Fertility and Soils* **19**, 87-99.

Martin A (1991) 'Introduction to Soil Microbiology.' (Krieger Publishing Co.: New York)

McKenzie DC, Hall DIM, Daniels IG, Abbott TS, Kay AM, Sykes JD (1995) 'Soil Management for Irrigated Cotton.' (NSW Agriculture: Agfact P5: 3.6)

McVay KA, Rice CW (2002) 'Soil Organic Carbon and the Global Carbon Cycle.' (US Department of Agriculture: Kansas State University)

Mergel A, Timchenko A, Kudeyarov V (1998) Role of plant root exudates in soil carbon and nitrogen transformation. In '5th Symposium of the International Society of Root Research'. Clemson University, South Carolina, USA pp. 43-54. (Dordrecht, Netherlands)

Miller ML (2001) 'Soil Organic Matter.' Alberta Agriculture Food and Rural Development.

Nakamoto T (2000) The distribution of wheat and maize roots as influenced by biopores in a subsoil of the Kanto loam type. *Plant Production Science* **3**, 140-144.

Newman IE (1966) A method for estimating the total length of root in a sample. *Journal of Applied Ecology* **3**, 139-145.

Nielson KF (1974) Roots and root temperature. In 'The plant root and its environment.' (Ed. EW Carson) pp. 293-335. (University Press of Virginia: Charlottesville)

Nortcliff S (2002) Standardisation of soil quality attributes. *Agriculture, ecosystems and environment.* **88**, 161-168.

Nye PH, Tinker PT (1977) Solute movement in the soil - root system. Blackwell Scientific Publishers Oxford.

Paustian K, Collins HP, Paul EA (Eds) (1997) 'Soil organic matter in temperate ecosystems: Long term experiments in North America.' Management controls in soil carbon. (CRC Press: Boca Rotan, FL)

Phillips DL, Johnson MG, Tingey DT, Biggart C, Nowak RS, Newsom JC (2000) Minirhizotron installation in sandy , rocky soils with minimal soil disturbance. *Soil Science Society of America Journal* **64**, 761-764.

Pierret A, Moran CJ, Pankhurst CE (1999) Differentiation of soil properties related to the spatial association of wheat roots and soil macropores. *Plant and Soil.* **211**, 51-58.

Rees RM, Bingham IJ, Baddeley JA, Watson CA (2005) The role of plants and land management in sequestering soil carbon in temperate arable and grassland ecosystems. *Geoderma* **128**, 130-154.

Rochester IJ, Peoples MB, Constable GA, Gault RR (1998) Faba beans and other legumes add nitrogen to irrigated cotton cropping systems. *Australian Journal of Experimental Agriculture* **38**, 253-260.

Rochester IP, M. (2005) Growing vetches (Vicia villosa Roth) in irrigated cotton systems: inputs of fixed N, N fertiliser savings and cotton productivity. *Plant and Soil* **271**, 251-274.

Singh H, Singh KP (1995) Effect of plant residue and fertilizer on grain yield of dryland rice under reduced tillage cultivation. *Soil and Tillage Research* **34**, 115-125.

Sparling G, Gupta VVSR, Zhu C (1993) Determination of microbial biomass C. *Soil Biology and Biochemistry* **25**, 1803-1805.

Taylor HM, Upchurch DR, Brown JM, Rogers HH (1991) Some methods of root investigation. In 'Plant roots and their environment'. (Eds BL McMichael and H Persson). (Elsevier Science Publishers, INC.: New York)

Tiessen H, Bettany J, Stewart J (1981) An improved method for the determination of carbon in soils and soil extracts by dry combustion. *Communications in Soil Science and Plant Analysis* **12**, 211-218.

Tiessen H, Roberts T, Stewart J (1983) Carbonate analysis in soils and minerals by acid digestion and two-endpoint titration. *Communications in Soil Science and Plant Analysis* **14**, 161-166.

Tingey DT, Phillips DL, Johnson MG, Rygiewicz PT, Beedlow PA, Hogsett WE (2005) Estimates of Douglas-fir fine root production and mortality from minirhizotrons. *Forest Ecology and Management* **204**.

Underwood A (1961) Carbonic Anhydrase in the titration of carbon dioxide solutions. *Analytical Chemistry* **33**, 955-956.

Wang WJ, Dalal RC, Moody PW, Smith CJ (2003) Relationships of soil respiration to microbial biomass, substrate availability and clay content. *Soil Biology and Biochemistry* **88**, 933-939.

Wright AL, Hons FM, Matocha Jr JE (2004) Tillage impacts on microbial biomass and soil carbon and nitrogen dynamics of corn and cotton rotations. *Applied Soil Ecology* **29**, 85-92.

Appendices

1a) μg of CO_2-C/day = (A_1-A_2)* [1000(B) * 12(C) * 0.05(D) *(25/2)(E) /fraction of day]

A_1 = titrant added to test sample (ml)

A_2 = titrant added to blank (ml)

B = to convert mg to μg

C = molecular weight of carbon in CO_2 is 12 opposed to 1 for hydrogen

D = molarity of titre acid

E = concentration of NaOH

b) μg of CO2-C/m^2/day = (result of formula 1a / [10000 / area trapped (cm^2)]

2a) μg N g^{-1} soil = (μg N ml^{-1} x total volume of extract) / dry weight of extracted soil

b) FOF (μg N g^{-1} soil) = (NHD-N fumigated) – (NHD-N unfumigated)

c) MB-C (μg C g^{-1} soil) = FOF x k_{EC}

NB: The k_{EC} value changes depending on soil type and length of fumigation. For Narrabri clay soils the k_{EC} is 29.3 for 7-10 day fumigation (Sparling *et al.* 1993).

3) Root length = 0.8168x – 1.2543, where x = number of intersections on grid

4a) Since there were no images taken in the 0-10cm depth interval, the number of live roots from the core break method for that depth had to be converted to root length to analyse with the minirhizotron. The following equation was derived using a regression analysis (see Appendix 4a, Figure 1).

$L = -5.146 + 34.9372\ N, \quad R^2 = 0.84, \quad (0\text{-}20cm)$

Where,

L = length and,

N = number of roots in core break method

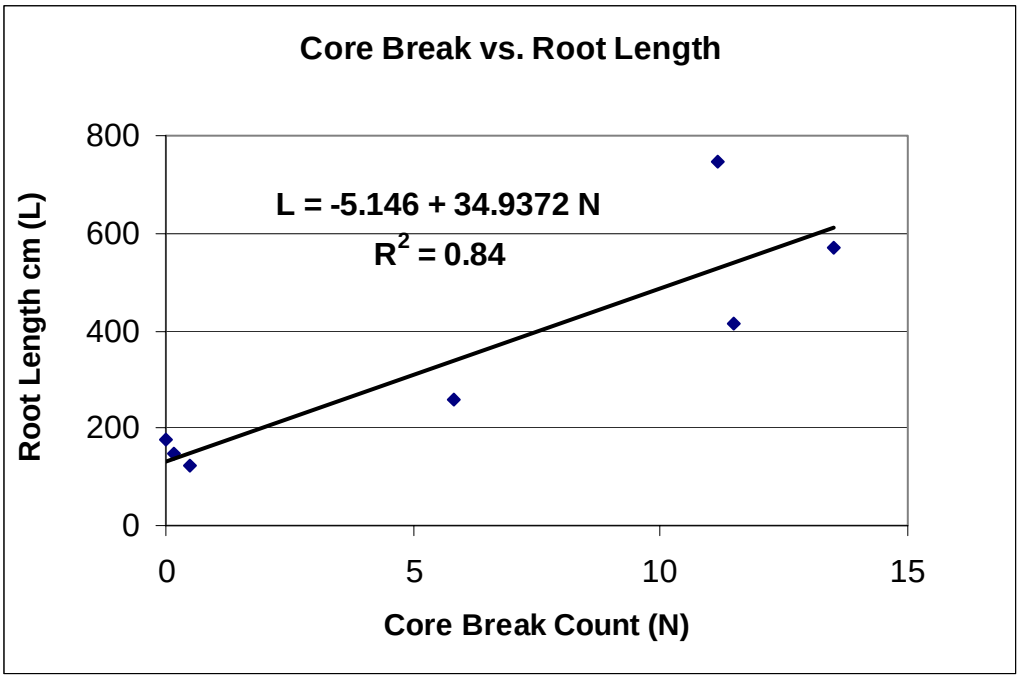

Fig 1. Results of regression analysis for calibration of minirhizotron.

b) Root length could be converted to root mass using a calibration based on the results of the root washing method. The average root mass/root length was calculated.

Root mass/Root length = 0.0389 mg/mm of root

c) The amount of carbon returned to the soil when roots were turned over was calculated based on the results of the C and N analysis of the root samples in Adelaide.

Average root carbon = 27.09%

5) Soil physical and chemical analysis of all plots in the study.

Replication	Treatment	Depth	pH	$EC_{1:5}$	SOC	Ca	Mg	Na	K
1	CV	0-10 cm	6.8	0.22	1.69	23.18	13.69	0.74	1.79
2	CV	0-10 cm	6.6	0.18	0.92	23.21	13.71	0.63	1.58
3	CV	0-10 cm	7	0.19	0.89	25.39	14.81	0.85	2.01
1	CV	10-30 cm	6.9	0.18	0.49	23.11	13.66	1.58	1.16
2	CV	10-30 cm	6.9	0.17	0.55	24.29	14.79	1.69	1.27
3	CV	10-30 cm	7.1	0.19	0.35	24.38	15.90	1.70	1.38
1	CV	30-60 cm	7.1	0.22	0.75	22.08	15.77	2.73	0.84
2	CV	30-60 cm	7.2	0.28	0.72	20.09	16.91	3.38	0.74
3	CV	30-60 cm	7.2	0.3	0.61	20.18	16.99	3.29	0.96
1	CV	60-120 cm	7.1	0.27	0.53	13.70	14.76	4.64	0.74
2	CV	60-120 cm	7.2	0.29	0.58	14.80	17.97	6.24	0.95
3	CV	60-120 cm	7.3	0.36	0.47	13.77	18.01	6.04	1.17
1	CC	0-10 cm	6.7	0.51	1.26	24.23	11.59	0.42	1.79
2	CC	0-10 cm	6.8	0.32	0.79	24.24	14.75	0.74	1.79
3	CC	0-10 cm	6.8	0.47	0.80	25.33	14.77	0.53	2.22
1	CC	10-30 cm	7.1	0.21	0.69	24.15	11.55	0.95	1.16
2	CC	10-30 cm	7	0.19	0.45	25.29	15.81	1.69	1.16
3	CC	10-30 cm	6.9	0.21	0.37	25.43	15.89	1.59	1.48
1	CC	30-60 cm	7.1	0.22	0.93	24.25	12.65	1.37	0.74
2	CC	30-60 cm	7.1	0.26	0.66	20.10	16.93	3.17	0.74
3	CC	30-60 cm	7.2	0.3	0.62	20.15	16.97	3.18	0.95
1	CC	60-120 cm	7.1	0.19	0.79	17.92	13.71	1.79	0.74
2	CC	60-120 cm	7.3	0.33	0.67	13.75	17.98	5.92	0.95
3	CC	60-120 cm	7.4	0.33	0.51	15.87	17.98	5.61	1.06
1	CW	0-10 cm	6.9	0.26	1.26	24.12	10.49	0.42	1.68
2	CW	0-10 cm	6.9	0.15	0.75	25.35	14.79	0.74	1.90
3	CW	0-10 cm	7	0.21	0.85	26.46	15.88	0.74	2.12
1	CW	10-30 cm	7.1	0.25	0.77	24.12	11.54	0.63	1.26
2	CW	10-30 cm	6.9	0.19	0.40	25.44	15.90	1.59	1.38
3	CW	10-30 cm	6.9	0.29	0.71	25.34	15.84	1.37	1.58
1	CW	30-60 cm	7.1	0.21	0.80	25.20	12.60	1.16	0.84
2	CW	30-60 cm	7.2	0.27	0.59	20.11	16.93	2.96	0.85
3	CW	30-60 cm	7.2	0.26	0.56	20.10	17.99	2.75	0.95
1	CW	60-120 cm	7.1	0.23	0.66	17.89	12.62	1.58	0.74
2	CW	60-120 cm	7.2	0.33	0.58	15.93	19.12	5.42	0.96
3	CW	60-120 cm	7.4	0.33	0.52	14.85	19.09	5.30	1.17
1	CWV	0-10 cm	6.9	0.23	1.37	24.27	11.61	0.42	1.48
2	CWV	0-10 cm	7	0.21	0.86	25.42	14.83	0.85	1.80
3	CWV	0-10 cm	7	0.25	0.82	26.47	15.88	0.74	2.01
1	CWV	10-30 cm	7	0.15	0.74	24.24	11.59	0.95	1.26
2	CWV	10-30 cm	7.1	0.21	0.35	26.47	15.88	1.80	1.38
3	CWV	10-30 cm	7.1	0.21	0.72	25.38	16.92	1.69	1.37
1	CWV	30-60 cm	7.1	0.19	0.89	25.34	13.73	1.58	0.84
2	CWV	30-60 cm	7.1	0.25	0.60	21.17	16.94	3.07	0.95
3	CWV	30-60 cm	7.2	0.28	0.62	20.10	16.93	3.07	0.95
1	CWV	60-120 cm	7.1	0.24	0.77	17.94	13.72	2.22	0.84
2	CWV	60-120 cm	7.2	0.3	0.60	15.95	18.08	5.32	1.06
3	CWV	60-120 cm	7.4	0.32	0.50	14.83	18.01	5.19	1.17

Replication	Treatment	Depth	ratio(Ca:Mg)	ESP	sodicity	Clay, %	Nitrate-N, mg/kg	DI,
1	CV	0-10 cm	1.69	1.87	0.30	60.0	2.92	10.8
2	CV	0-10 cm	1.69	1.62	0.28	62.2	3.24	11.5
3	CV	0-10 cm	1.71	1.97	0.22	63.1	3.10	10.8
1	CV	10-30 cm	1.69	3.99	0.11	61.4	1.91	8.4
2	CV	10-30 cm	1.64	4.02	0.10	64.9	2.28	8.3
3	CV	10-30 cm	1.53	3.91	0.11	65.9	2.34	12.6
1	CV	30-60 cm	1.40	6.60	0.08	62.5	2.18	8.2
2	CV	30-60 cm	1.19	8.23	0.08	67.7	2.31	11.2
3	CV	30-60 cm	1.19	7.95	0.09	67.8	1.69	8.9
1	CV	60-120 cm	0.93	13.71	0.06	62.8	1.40	9.4
2	CV	60-120 cm	0.82	15.61	0.05	67.2	2.64	16.1
3	CV	60-120 cm	0.76	15.49	0.06	66.0	1.83	7.2
1	CC	0-10 cm	2.09	1.11	1.21	61.0	261.09	9.5
2	CC	0-10 cm	1.64	1.78	0.43	62.5	55.76	12.9
3	CC	0-10 cm	1.71	1.23	0.89	64.0	149.97	9.6
1	CC	10-30 cm	2.09	2.50	0.22	61.0	17.99	6.8
2	CC	10-30 cm	1.60	3.84	0.11	64.5	5.35	9.4
3	CC	10-30 cm	1.60	3.58	0.13	66.1	18.22	16.0
1	CC	30-60 cm	1.92	3.51	0.16	62.5	2.87	9.6
2	CC	30-60 cm	1.19	7.75	0.08	65.1	2.61	9.9
3	CC	30-60 cm	1.19	7.71	0.09	65.4	2.73	16.5
1	CC	60-120 cm	1.31	5.25	0.11	59.0	1.70	11.0
2	CC	60-120 cm	0.76	15.34	0.06	66.3	1.64	9.5
3	CC	60-120 cm	0.88	13.84	0.06	65.4	1.46	9.8
1	CW	0-10 cm	2.30	1.14	0.62	58.2	71.10	10.8
2	CW	0-10 cm	1.71	1.73	0.20	62.3	6.87	12.0
3	CW	0-10 cm	1.67	1.64	0.28	62.6	19.72	13.6
1	CW	10-30 cm	2.09	1.68	0.40	61.9	52.27	8.2
2	CW	10-30 cm	1.60	3.59	0.12	66.5	6.24	15.7
3	CW	10-30 cm	1.60	3.11	0.21	66.4	40.90	12.0
1	CW	30-60 cm	2.00	2.90	0.18	60.0	12.05	8.5
2	CW	30-60 cm	1.19	7.25	0.09	67.0	7.05	15.0
3	CW	30-60 cm	1.12	6.58	0.09	66.5	7.98	16.5
1	CW	60-120 cm	1.42	4.81	0.15	60.9	4.07	8.3
2	CW	60-120 cm	0.83	13.08	0.06	68.0	2.47	9.5
3	CW	60-120 cm	0.78	13.12	0.06	66.0	2.95	16.5
1	CWV	0-10 cm	2.09	1.12	0.54	59.1	4.25	9.6
2	CWV	0-10 cm	1.71	1.98	0.25	64.4	2.21	11.0
3	CWV	0-10 cm	1.67	1.64	0.34	64.9	18.57	12.3
1	CWV	10-30 cm	2.09	2.49	0.16	62.2	2.23	11.1
2	CWV	10-30 cm	1.67	3.95	0.12	66.4	2.46	14.6
3	CWV	10-30 cm	1.50	3.73	0.12	66.4	3.51	10.3
1	CWV	30-60 cm	1.85	3.82	0.12	62.5	2.73	11.0
2	CWV	30-60 cm	1.25	7.29	0.08	68.5	2.31	9.8
3	CWV	30-60 cm	1.19	7.47	0.09	68.0	2.19	14.8
1	CWV	60-120 cm	1.31	6.38	0.11	61.7	1.86	7.9
2	CWV	60-120 cm	0.88	13.16	0.06	67.6	1.84	7.1
3	CWV	60-120 cm	0.82	13.24	0.06	68.6	1.89	11.3

6) The following documents are from the analysis of data in Genstat.

a) Minirhizotron growth rates (mg/cm^2/day)

Analysis of variance

Variate: Minirhizotron growth rates

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Treatment	3	0.0050200	0.0016733	3.96	0.014
Residual	44	0.0185768	0.0004222	0.77	
Subject.Time stratum					
d.f. correction factor 0.6713					
Time	3	0.4339750	0.1446583	262.21	<.001
Time.Treatment	9	0.0094714	0.0010524	1.91	0.088
Residual	132	0.0728241	0.0005517		
Total	191	0.5398673			

(d.f. are multiplied by the correction factors before calculating F probabilities)

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

Subject 40	0.0267	s.e.	0.0098
Subject 42	0.0274	s.e.	0.0098
Subject 8 Time 2	-0.0563	s.e.	0.0195
Subject 26 Time 3	-0.0956	s.e.	0.0195
Subject 27 Time 3	0.0683	s.e.	0.0195
Subject 42 Time 1	0.0635	s.e.	0.0195

Tables of means

Variate: Minirhizotron growth rates

Grand mean 3.2592

Time	1	2	3	4
	3.2300	3.2460	3.2208	3.3401

Treatment	1	2	3	4
	3.2557	3.2528	3.2654	3.2629

Time	Treatment	1	2	3	4
1		3.2269	3.2282	3.2281	3.2369
2		3.2290	3.2334	3.2671	3.2545
3		3.2267	3.2100	3.2260	3.2203
4		3.3403	3.3397	3.3402	3.3400

Standard errors of differences of means

Table	Time	Treatment	Time Treatment
rep.	48	48	12
s.e.d.	0.00479	0.00419	0.00930
d.f.	88.62	44	123.42
Except when comparing means with the same level(s) of Treatment			0.00959
d.f.			88.62

Correction factors have been applied to residual d.f.(see analysis-of-variance table for details)

Least significant differences of means (5% level)

Table	Time	Treatment	Time Treatment
rep.	48	48	12
l.s.d.	0.01019	0.00845	0.01971
d.f.	88.62	44	123.42
Except when comparing means with the same level(s) of Treatment			0.02038
d.f.			88.62

Correction factors have been applied to residual d.f.(see analysis-of-variance table for details)

b) Core break root numbers for both sampling dates, 07/01/2005 and 25/02/2005.

Analysis of variance

Variate: SqrtRoot

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	2		17.3999	8.6999	19.12	
Block.*Units* stratum						
Rotation	3		22.6459	7.5486	16.59	<.001
Depth	9		291.6725	32.4081	71.21	<.001
Time	1		0.2985	0.2985	0.66	0.418
Rotation.Depth	27		22.6165	0.8376	1.84	0.006
Rotation.Time	3		11.2507	3.7502	8.24	<.001
Depth.Time	9		103.3337	11.4815	25.23	<.001
Rotation.Depth.Time	27		21.3972	0.7925	1.74	0.012
Residual	568	(70)	258.5090	0.4551		
Total	649	(70)	652.3709			

Message: the following units have large residuals.

Block 1 *units* 97	-1.932	s.e.	0.599
Block 2 *units* 120	3.020	s.e.	0.599
Block 2 *units* 144	2.814	s.e.	0.599
Block 3 *units* 119	2.104	s.e.	0.599

Tables of means

Variate: SqrtRoot

Grand mean 1.743

Rotation	1	2	3	4
	1.551	1.743	2.027	1.651

Depth	1	2	3	4	5	6	7
	2.404	2.409	2.457	2.129	2.074	1.806	1.493

Depth	8	9	10
	1.141	0.888	0.629

Time	1	2
	1.723	1.763

Rotation	Depth	1	2	3	4	5	6
1		2.308	1.949	2.040	2.045	1.976	1.552
2		2.120	2.406	2.848	2.272	1.995	1.620
3		2.687	2.739	2.550	2.130	2.585	2.351
4		2.501	2.543	2.391	2.069	1.739	1.699

Rotation	Depth	7	8	9	10
1		1.279	0.886	0.903	0.569
2		1.427	1.243	0.615	0.885
3		1.921	1.573	1.134	0.595
4		1.343	0.861	0.899	0.467

Rotation	Time	1	2
1		1.450	1.652
2		1.578	1.909
3		2.188	1.865
4		1.675	1.628

Depth	Time	1	2
1		2.640	2.167
2		2.620	2.198
3		2.939	1.975
4		2.481	1.777
5		2.374	1.774
6		1.824	1.787
7		1.275	1.710
8		0.671	1.610
9		0.363	1.413
10		0.037	1.221

Rotation	Depth	Time	1	2
1	1		2.092	2.523
	2		2.102	1.796
	3		2.500	1.579
	4		2.463	1.626
	5		2.072	1.880
	6		1.493	1.612
	7		1.000	1.557
	8		0.347	1.426
	9		0.236	1.571
	10		0.191	0.947
2	1		2.266	1.974
	2		2.793	2.020
	3		3.486	2.209
	4		2.495	2.048
	5		2.089	1.902
	6		1.190	2.051
	7		0.927	1.926
	8		0.550	1.936
	9		-0.023	1.252
	10		0.003	1.767
3	1		3.263	2.110
	2		3.232	2.246
	3		2.949	2.150
	4		2.542	1.719
	5		3.217	1.954
	6		2.853	1.848
	7		1.902	1.940
	8		1.266	1.880
	9		0.622	1.646
	10		0.033	1.156
4	1		2.941	2.061

2	2.354	2.732
3	2.819	1.964
4	2.422	1.716
5	2.117	1.361
6	1.761	1.638
7	1.272	1.415
8	0.522	1.199
9	0.618	1.181
10	-0.078	1.012

Standard errors of differences of means

Table	Rotation	Depth	Time	Rotation Depth
rep.	180	72	360	18
d.f.	568	568	568	568
s.e.d.	0.0711	0.1124	0.0503	0.2249

Table	Rotation Time	Depth Time	Rotation Depth Time
rep.	90	36	9
d.f.	568	568	568
s.e.d.	0.1006	0.1590	0.3180

Least significant differences of means (5% level)

Table	Rotation	Depth	Time	Rotation Depth
rep.	180	72	360	18
d.f.	568	568	568	568
l.s.d.	0.1397	0.2208	0.0988	0.4417

Table	Rotation Time	Depth Time	Rotation Depth Time
rep.	90	36	9
d.f.	568	568	568
l.s.d.	0.1975	0.3123	0.6246

c) Root length (cm) from root washing analysis.

Analysis of variance

Variate: sqrt_length

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	2		281.48	140.74	6.61	
Block.*Units* stratum						
Rotation	3		406.01	135.34	6.35	<.001
Depth	9		2143.71	238.19	11.18	<.001
Rotation.Depth	26	(1)	925.59	35.60	1.67	0.047
Residual	70	(8)	1491.18	21.30		
Total	110	(9)	4821.01			

Message: the following units have large residuals.

Block 1 *units* 20	-12.94	s.e.	3.53
Block 2 *units* 20	9.27	s.e.	3.53
Block 2 *units* 31	-9.07	s.e.	3.53

Tables of means

Variate: sqrt_length

Grand mean 16.66

Rotation	1	2	3	4
	14.09	16.41	19.28	16.84

Depth	1	2	3	4	5	6	7
	19.84	23.86	21.96	19.44	16.41	16.10	13.46

Depth	8	9	10
	12.00	11.85	11.63

Rotation	Depth	1	2	3	4	5	6
1		15.44	20.50	21.14	16.43	9.70	9.37
2		18.98	24.71	22.23	18.06	17.43	14.85
3		29.12	28.73	23.11	24.90	20.09	21.00
4		15.81	21.53	21.37	18.37	18.43	19.17

Rotation	Depth	7	8	9	10
1		8.21	12.55	12.42	15.18
2		13.55	11.77	11.12	11.39
3		16.72	12.28	10.47	6.37
4		15.35	11.39	13.40	13.58

Standard errors of differences of means

Table	Rotation	Depth	Rotation Depth
rep.	30	12	3
d.f.	70	70	70
s.e.d.	1.192	1.884	3.769

Least significant differences of means (5% level)

Table	Rotation	Depth	Rotation Depth
rep.	30	12	3
d.f.	70	70	70
l.s.d.	2.377	3.758	7.516

d) Root length: mass relationship (cm/g) for the 0-20cm depth interval based on root washing results.

Analysis of variance

Variate: sqrt_LM

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	103.1	51.6	0.09	
Block.*Units* stratum					
Rotation	3	3651.0	1217.0	2.15	0.130
Residual	18	10204.8	566.9		
Total	23	13958.9			

Message: the following units have large residuals.

Block 2 *units* 6 59.0 s.e. 20.6

Tables of means

Variate: sqrt_LM

Grand mean 87.3

Rotation	1	2	3	4
	81.1	76.2	108.1	83.8

Standard errors of differences of means

Table	Rotation
rep.	6
d.f.	18
s.e.d.	13.75

Least significant differences of means (5% level)

Table	Rotation
rep.	6
d.f.	18
l.s.d.	28.88

e) Root length: volume relationship (cm/cm^3) for the 0-20cm depth interval based on root washing results.

Analysis of variance

Variate: length_volume

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.02019	0.01009	0.23	
Block.*Units* stratum					
Rotation	3	1.54870	0.51623	11.85	<.001
Residual	18	0.78433	0.04357		
Total	23	2.35322			

Tables of means

Variate: length_volume

Grand mean 0.648

Rotation	1	2	3	4
	0.432	0.623	1.070	0.466

Standard errors of differences of means

Table	Rotation
rep.	6
d.f.	18
s.e.d.	0.1205

Least significant differences of means (5% level)

Table	Rotation
rep.	6
d.f.	18
l.s.d.	0.2532

f) Root length: mass relationship (cm/g) for the 20-80cm depth interval based on root washing results.

Analysis of variance

Variate: sqrt_L_M

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	5330.8	2665.4	2.81	
Block.*Units* stratum					
Rotation	3	4736.6	1578.9	1.67	0.183
Residual	66	62520.9	947.3		
Total	71	72588.3			

Message: the following units have large residuals.

Block 2 *units* 12	77.8	s.e.	29.5
Block 2 *units* 16	-72.5	s.e.	29.5
Block 2 *units* 20	-76.2	s.e.	29.5
Block 3 *units* 19	-95.1	s.e.	29.5

Tables of means

Variate: sqrt_L_M

Grand mean 84.2

Rotation	1	2	3	4
	75.9	80.4	97.6	83.0

Standard errors of differences of means

Table	Rotation
rep.	18
d.f.	66
s.e.d.	10.26

Least significant differences of means (5% level)

Table	Rotation
rep.	18
d.f.	66
l.s.d.	20.48

g) Root length: volume relationship (cm/cm^3) for the 20-80cm depth interval based on root washing results.

Analysis of variance

Variate: sqrt_L_V

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	2		0.30907	0.15454	3.45	
Block.*Units* stratum						
Rotation	3		0.53685	0.17895	3.99	0.011
Residual	64	(2)	2.87050	0.04485		
Total	69	(2)	3.70549			

Message: the following units have large residuals.

Block 2 *units* 18 -0.579 s.e. 0.200

Tables of means

Variate: sqrt_L_V

Grand mean 0.588

Rotation	1	2	3	4
	0.460	0.582	0.702	0.608

Standard errors of differences of means

Table	Rotation
rep.	18
d.f.	64
s.e.d.	0.0706

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	Rotation
rep.	18
d.f.	64
l.s.d.	0.1410

h) Results of first microbial biomass sampling (MB - C).

Analysis of variance

Variate: MB_C

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Block stratum	2		7864.	3932.	1.32	
Block.*Units* stratum						
treatment	3		11797.	3932.	1.32	0.309
depth	1		2063.	2063.	0.69	0.420
treatment.depth	3		14767.	4922.	1.66	0.225
Residual	13	(1)	38635.	2972.		
Total	22	(1)	75125.			

Message: the following units have large residuals.

Block 2 *units* 6 115. s.e. 40.

Tables of means

Variate: MB_C

Grand mean 150.

treatment	1	2	3	4
	145.	135.	187.	132.

depth	1	2
	140.	159.

treatment	depth	1	2
1		115.	174.
2		118.	152.
3		220.	154.
4		108.	155.

Standard errors of differences of means

Table	treatment	depth	treatment depth
rep.	6	12	3
d.f.	13	13	13
s.e.d.	31.5	22.3	44.5

(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	treatment	depth	treatment depth
rep.	6	12	3
d.f.	13	13	13
l.s.d.	68.0	48.1	96.2

(Not adjusted for missing values)

i) Results of second microbial biomass sampling (MB - C).

Analysis of variance

Variate: MB_C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	267.	133.	0.01	
Block.*Units* stratum					
Treatment	3	6287.	2096.	0.13	0.940
Depth	1	819591.	819591.	51.33	<.001
Treatment.Depth	3	44837.	14946.	0.94	0.449
Residual	14	223518.	15966.		
Total	23	1094500.			

Message: the following units have large residuals.

Block 3 *units* 1	223.	s.e.	97.
Block 3 *units* 5	-228.	s.e.	97.

Tables of means

Variate: MB_C

Grand mean 369.

Treatment	1	2	3	4
	380.	344.	387.	367.

Depth	1	2
	554.	185.

Treatment	Depth	1	2
1		575.	185.
2		465.	223.
3		629.	145.
4		548.	185.

Standard errors of differences of means

Table	Treatment	Depth	Treatment Depth
rep.	6	12	3
d.f.	14	14	14
s.e.d.	73.0	51.6	103.2

Least significant differences of means (5% level)

Table	Treatment	Depth	Treatment Depth
rep.	6	12	3
d.f.	14	14	14
l.s.d.	156.5	110.6	221.3

j) Results of first microbial activity sampling (mg/CO^2-C/m^2/day).

Analysis of variance

Variate: mg_CO2_C_m2_day

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1040.9	520.5	3.30	
Block.*Units* stratum					
Treatment	3	395.8	131.9	0.84	0.521
Residual	6	946.9	157.8		
Total	11	2383.6			

Tables of means

Variate: mg_CO2_C_m2_day

Grand mean 48.2

Treatment	1	2	3	4
	49.2	57.2	42.3	44.2

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	6
s.e.d.	10.26

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	6
l.s.d.	25.10

k) Results of second microbial activity sampling (mg/CO^2-C/m^2/day).

Analysis of variance

Variate: log_CO2_C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.05728	0.02864	0.33	
Block.*Units* stratum					
Treatment	3	0.09892	0.03297	0.38	0.772
Residual	12	1.05430	0.08786		
Total	17	1.21050			

Message: the following units have large residuals.

Block 1 *units* 6 0.506 s.e. 0.242

Tables of means

Variate: log_CO2_C

Grand mean 3.819

Treatment	1	2	3	4
	3.912	3.745	3.791	3.928
rep.	3	6	6	3

Standard errors of differences of means

Table	Treatment	
rep.	unequal	
d.f.	12	
s.e.d.	0.2420	min.rep
	0.2096	max-min
	0.1711	max.rep

Least significant differences of means (5% level)

Table	Treatment	
rep.	unequal	
d.f.	12	
l.s.d.	0.5273	min.rep
	0.4567	max-min
	0.3729	max.rep

l) Results of third microbial activity sampling (mg/CO^2-C/m^2/day).

Analysis of variance

Variate: CO2_C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block.*Units* stratum					
Treatment	3	209.35	69.78	0.85	0.484
Residual	16	1306.45	81.65		
Total	19	1515.80			

Information summary

Aliased model terms
Block stratum

Message: the following units have large residuals.

Block 1 *units* 3 17.5 s.e. 8.1

Tables of means

Variate: CO2_C

Grand mean 41.8

Treatment	1	2	3	4
	39.7	43.2	37.9	46.3

Standard errors of differences of means

Table	Treatment
rep.	5
d.f.	16
s.e.d.	5.72

Least significant differences of means (5% level)

Table	Treatment
rep.	5
d.f.	16
l.s.d.	12.12

m) Results of fourth microbial activity sampling (mg/CO^2-C/m^2/day).

Analysis of variance

Variate: mg_CO2_C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	82.0	41.0	0.39	
Block.*Units* stratum					
Treatment	3	643.5	214.5	2.02	0.213
Residual	6	638.3	106.4		
Total	11	1363.8			

Tables of means

Variate: mg_CO2_C

Grand mean 32.2

Treatment	1	2	3	4
	30.8	28.6	25.1	44.4

Standard errors of differences of means

Table	Treatment
rep.	3
d.f.	6
s.e.d.	8.42

Least significant differences of means (5% level)

Table	Treatment
rep.	3
d.f.	6
l.s.d.	20.61

n) Yield analysis in t/ha.

Analysis of variance

Variate: lint_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.02660	0.01330	0.20	
Block.*Units* stratum					
Rotation	3	1.09670	0.36557	5.43	0.038
Residual	6	0.40420	0.06737		
Total	11	1.52750			

Tables of means

Variate: lint_t_ha

Grand mean 2.185

Rotation	T1	T2	T3a	T4a
	1.870	1.917	2.367	2.587

Standard errors of differences of means

Table	Rotation
rep.	3
d.f.	6
s.e.d.	0.2119

Least significant differences of means (5% level)

Table	Rotation
rep.	3
d.f.	6
l.s.d.	0.5186

o) Yield analysis in bales/hectare.

Analysis of variance

Variate: bales_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.518	0.259	0.20	
Block.*Units* stratum					
Rotation	3	21.185	7.062	5.39	0.039
Residual	6	7.864	1.311		
Total	11	29.567			

Tables of means

Variate: bales_ha

Grand mean 9.62

Rotation	T1	T2	T3a	T4a
	8.24	8.45	10.43	11.39

Standard errors of differences of means

Table	Rotation
rep.	3
d.f.	6
s.e.d.	0.935

Least significant differences of means (5% level)

Table	Rotation
rep.	3
d.f.	6
l.s.d.	2.287

p) Analysis of seed content (t/ha)

Analysis of variance

Variate: seed_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.0654	0.0327	0.25	
Block.*Units* stratum					
Rotation	3	2.6369	0.8790	6.73	0.024
Residual	6	0.7834	0.1306		
Total	11	3.4857			

Tables of means

Variate: seed_t_ha

Grand mean 3.07

Rotation	T1	T2	T3a	T4a
	2.64	2.57	3.42	3.64

Standard errors of differences of means

Table	Rotation
rep.	3
d.f.	6
s.e.d.	0.295

Least significant differences of means (5% level)

Table	Rotation
rep.	3
d.f.	6
l.s.d.	0.722

q) Analysis of trash content (t/ha)

Analysis of variance

Variate: trash_t_ha

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.003150	0.001575	0.55	
Block.*Units* stratum					
Rotation	3	0.016825	0.005608	1.95	0.223
Residual	6	0.017250	0.002875		
Total	11	0.037225			

Tables of means

Variate: trash_t_ha

Grand mean -0.218

Rotation	T1	T2	T3a	T4a
	-0.193	-0.170	-0.263	-0.243

Standard errors of differences of means

Table	Rotation
rep.	3
d.f.	6
s.e.d.	0.0438

Least significant differences of means (5% level)

Table	Rotation
rep.	3
d.f.	6
l.s.d.	0.1071