

Figure 5.8-2: (a) The average soil microbial species richness under high and low fertilizer levels, and (b) different cropping systems and tillage practices.

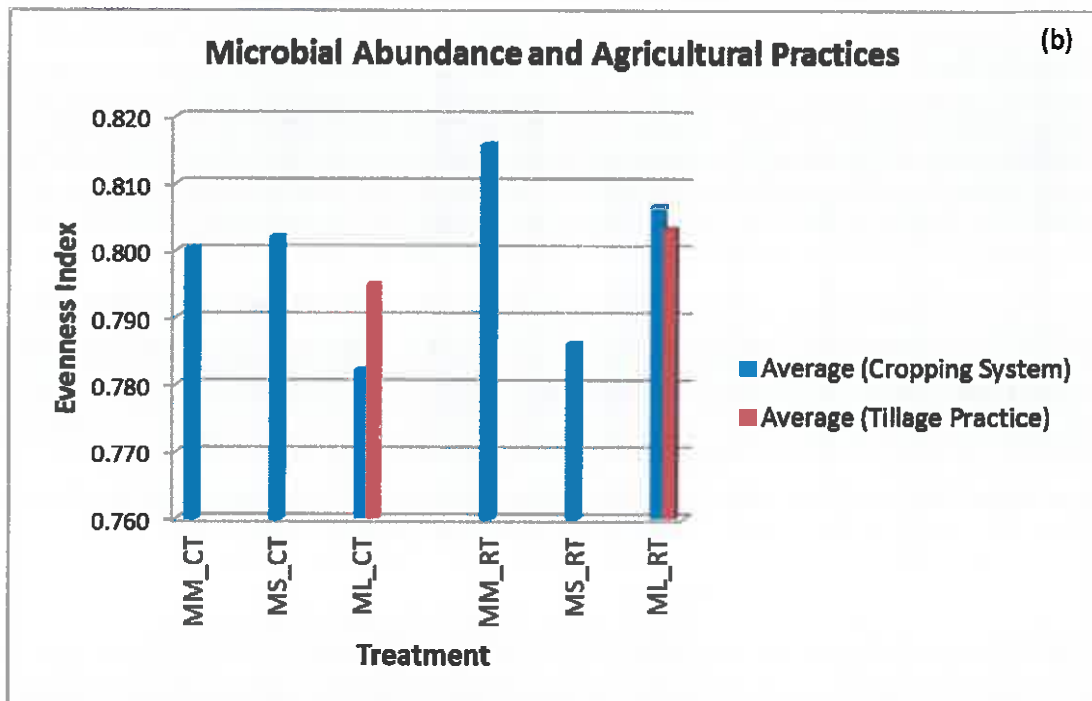
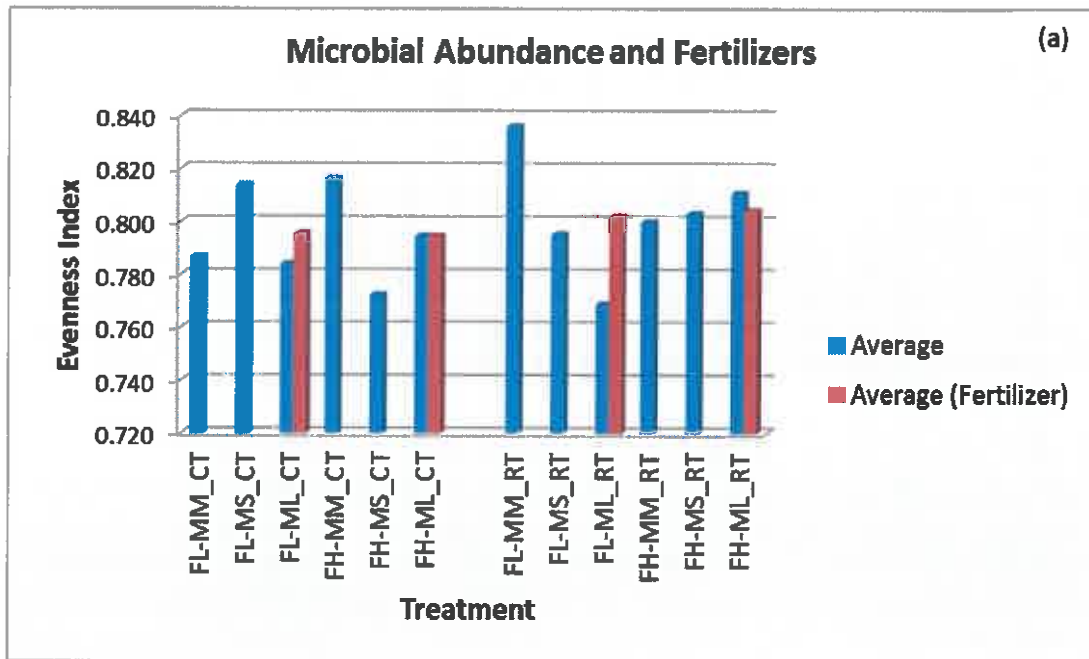


Figure 5.8-3: (a) The average soil microbial abundance under high and low fertilizer levels and (b) different cropping systems and tillage practices.

Soil microbial enzymatic activities, viz. Microbial activity

Enzyme assays are process-level indicators and culture-independent biochemical methods. The activity of any enzyme assayed in a soil sample is the sum of active and potentially active enzymes from all the different sources. Results are presented as a means of determining the potential of soil microbial communities to degrade or convert substrates. Enzymatic activities, i.e. microbial activity, in relation to the cycling of carbon, nitrogen (ammonification, nitrification, denitrification) and release of inorganic phosphorus in soil have been used to evaluate the fertility of the soil and the functioning of the ecosystem. The influence of the various conservation agriculture management practices on the activities of four enzymes, i.e. β -glucosidase (C-cycling), alkaline phosphatase and acid phosphatase (P-cycling), and urease (N-cycling), were analysed over a five-year period as a measure of determining the soil's potential to degrade or convert substrates (see Appendix 3.2).

The impact of agricultural practices on nutrient cycling processes within soil ecosystems is clearly demonstrated in Table 5.8-2. An increase in overall microbial activity can be observed from the 2009/10 to 2012/13 seasons, with the highest overall activity observed during the 2012/13 season under RT. Significant increases in soil microbial enzyme activity could be observed over time under the various agricultural management systems. The FH-MM and FL-MM treatments presented the most considerable and slightest increase, respectively, in overall microbial activity under CT from 2009/10 to 2012/13, whereas the contrary was valid for the same treatments under RT. From Table 5.8-2 it is evident that, by the 2012/13 season, FH-MM under CT and FL-MM under RT demonstrated the highest carbon and nitrogen conversion rates (i.e. β -glucosidase and urease activity, respectively), whereas FH-ML under CT and FL-MS under RT demonstrated the highest phosphorus conversion rate by microbial populations (i.e. alkaline phosphatase activity). On the other hand, FL-MM, FL-MS, and FH-MS demonstrated the lowest carbon, phosphorus and nitrogen conversion rates, respectively, under CT, while FH-MS under RT demonstrated the lowest overall nutrient conversion rate. It is interesting to note that the maize monoculture at low fertilizer levels (FL-MM) under CT resulted in a significant decline in phosphorus conversion rates. The maize/soybean rotation system at high fertilizer levels (FH-MS) under RT resulted in a decline in phosphorus and carbon conversion rate, with the latter being significant. Alternatively, the same treatment under RT at low fertilizer levels (FL-MS) resulted in a significant increase in carbon and phosphorus conversion rates.

Table 5.8-2: Comparison of soil microbial enzymatic activities under various agricultural practices during January 2010 and 2013

	β -glucosidase Activity	Alkaline Phosphatase Activity	Acid Phosphatase Activity	Urease Activity
FH-ML_CT10	1091.047 ^{cdefgh}	822.115 ^{abc}	1555.200 ^a	39.465 ^{defg}
FH-ML_CT13	1062.626 ^{cddefg}	941.265 ^{cddef}	1875.914 ^c	45.876 ^{gh}
FH-ML_RT10	1098.339 ^{cdefgh}	844.238 ^{abcd}	1564.571 ^a	35.853 ^{bode}
FH-ML_RT13	1202.671 ^{efhi}	975.379 ^{def}	1871.372 ^{bc}	50.569 ^{jk}
FH-MM_CT10	923.512 ^{bc}	913.975 ^{bcdef}	1562.270 ^a	31.618 ^{bc}
FH-MM_CT13	1383.756 ^{ijk}	931.455 ^{cddef}	1871.400 ^{bc}	57.163 ^k
FH-MM_RT10	1207.951 ^{gh}	950.593 ^{cdef}	1539.529 ^a	32.324 ^{bc}
FH-MM_RT13	1267.992 ^{hij}	949.728 ^{cddef}	1883.363 ^c	53.752 ^{jk}
FH-MS_CT10	705.743 ^a	816.005 ^{abc}	1517.071 ^a	33.711 ^{bcd}
FH-MS_CT13	1077.997 ^{cddefg}	888.417 ^{abcde}	1866.851 ^{bc}	41.861 ^{efgh}
FH-MS_RT10	1196.938 ^{efgh}	1024.875 ^{efg}	1563.680 ^a	44.967 ^{efhi}
FH-MS_RT13	972.756 ^{bcd}	911.948 ^{bcde}	1874.509 ^{bc}	46.876 ^{hij}
FL-ML_CT10	934.129 ^{bc}	805.563 ^{abc}	1556.679 ^a	38.250 ^{cdef}
FL-ML_CT13	1169.210 ^{efgh}	929.429 ^{cddef}	1881.179 ^c	51.926 ^{jk}
FL-ML_RT10	1073.400 ^{cdefg}	869.431 ^{abcd}	1581.809 ^a	10.255 ^a
FL-ML_RT13	1023.073 ^{cddef}	1066.055 ^{fg}	1875.391 ^c	47.618 ^{gh}
FL-MM_CT10	943.277 ^{bc}	1227.900 ^h	1539.227 ^a	32.506 ^{bcd}
FL-MM_CT13	1007.872 ^{cdde}	885.655 ^{abcde}	1872.811 ^{bc}	45.740 ^{gh}
FL-MM_RT10	992.939 ^{bode}	751.112 ^a	1603.766 ^{abc}	32.518 ^{bcd}
FL-MM_RT13	1481.330 ^k	981.286 ^{defg}	1882.143 ^c	64.652 ^l
FL-MS_CT10	818.253 ^{ab}	766.533 ^{ab}	1537.120 ^a	34.952 ^{bode}
FL-MS_CT13	1143.142 ^{defgh}	839.681 ^{abcde}	1877.470 ^c	50.637 ^{jk}
FL-MS_RT10	1172.307 ^{efgh}	869.840 ^{abcd}	1591.444 ^{ab}	30.130 ^b
FL-MS_RT13	1405.486 ^{kl}	1150.778 ^{gh}	1870.727 ^{bc}	54.823 ^k

*Means followed by the same letters do not differ significantly ($p > 0.05$)

According to results obtained, the soil's ecosystem function is greatly influenced by cropping systems and tillage practices, and to a lesser extent by fertilizer levels (Figs. 5.8-5, 6 & 7). Of the cropping systems, ML treatments exhibited the highest β -glucosidase and acid phosphatase activities, with MS treatments the lowest (Fig. 5.8-4b, 6b – blue bars). ML treatments also exhibited the highest alkaline phosphatase activity (Fig. 5.8-5b – blue bars), but with the highest urease activity under CT (Fig. 5.8-7b – blue bars). The lowest alkaline phosphatase and urease activity was presented in MS treatments under CT, as well as in MM treatments under RT (Fig. 5.8-5b, 7b – blue bars).

Soil disturbance also exerted a strong influence on microbial activity, with the highest β -glucosidase, alkaline phosphatase, and acid phosphatase activity demonstrated under RT treatments, compared to CT (Fig. 5.8-4, 5 & 6). The highest urease activity, however, was observed under CT (Fig. 5.8-7b – red bars).

The influence of cropping systems and/or tillage practices on soil microbial activity can be better illustrated with the use of dendograms (Fig. 5.8-8) assigning treatments into groups, so that treatments in the same cluster are more similar to each other than to treatments in other clusters.

An unambiguous transformation in soil microbial activity could be observed from the 2009/10 to the 2012/13 planting season, with all the treatments in the 2009/10 season grouping to the right of the dendogram (Fig. 5.8-8 – red block), and the treatments in the 2012/13 season to the left (Fig. 5.8-8 – blue block). Closer inspection of treatments in the 2009/10 season illustrated a visible clustering of treatments according to tillage practice, with CT treatments clustering to the right and RT treatments to the left.

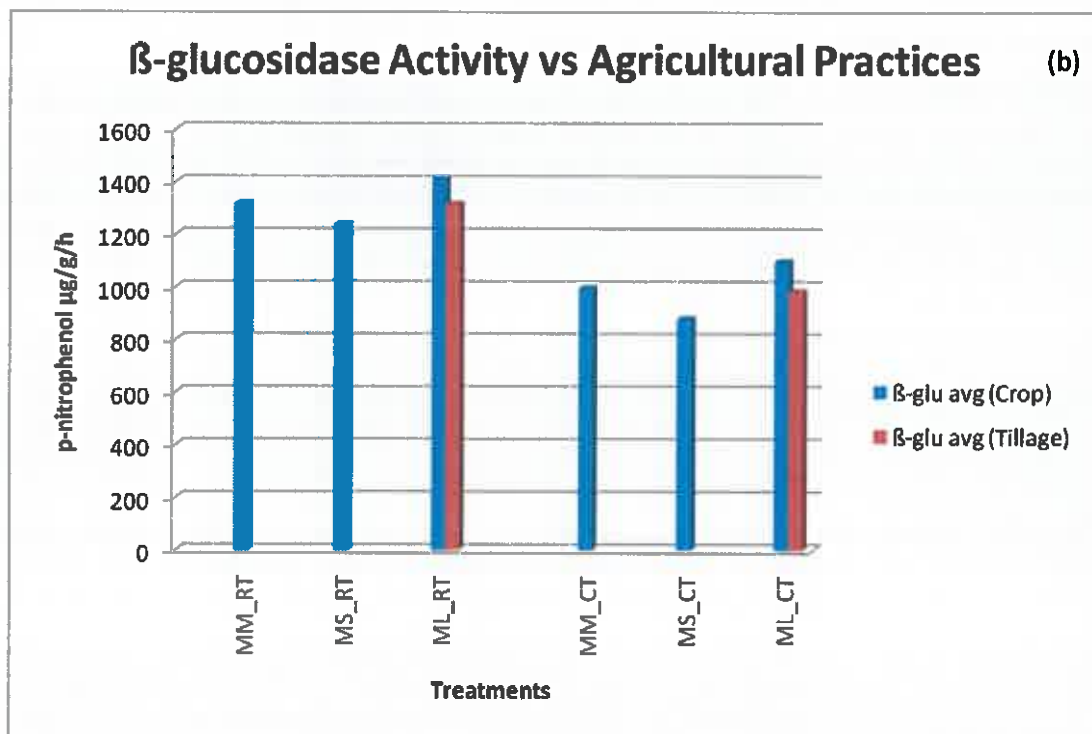
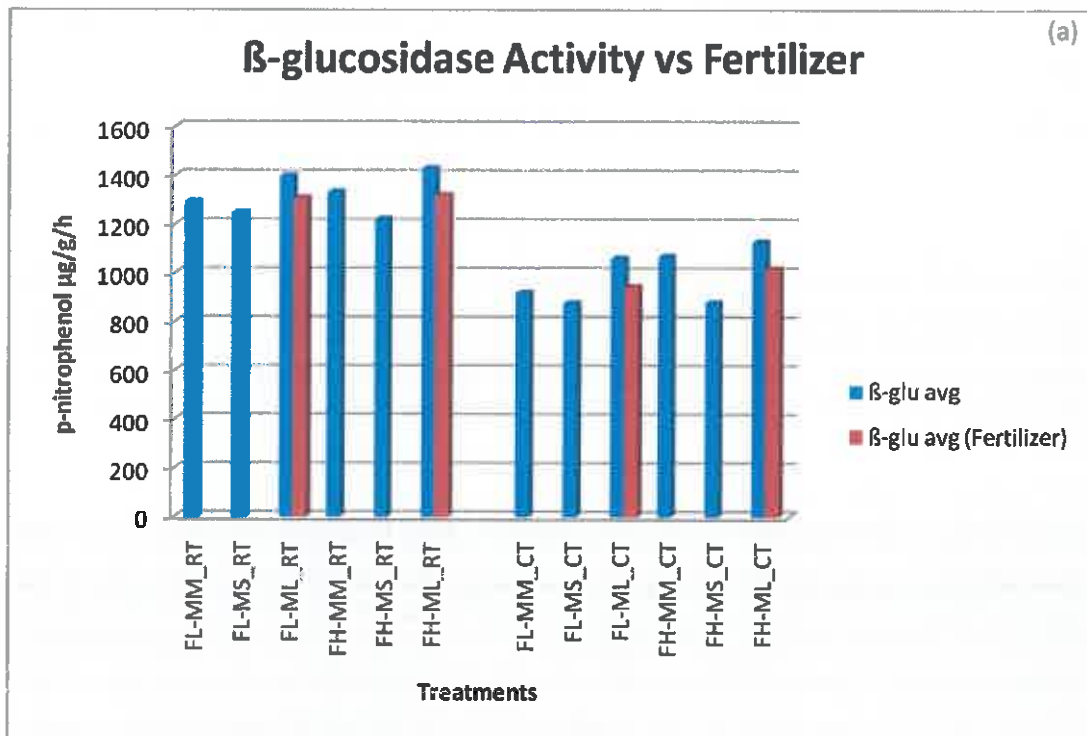


Figure 5.8-4: The average β-glucosidase activity under (a) fertilizer levels (FL, FH), and (b) different cropping systems (MM, MS, ML) and tillage practices (CT, RT).

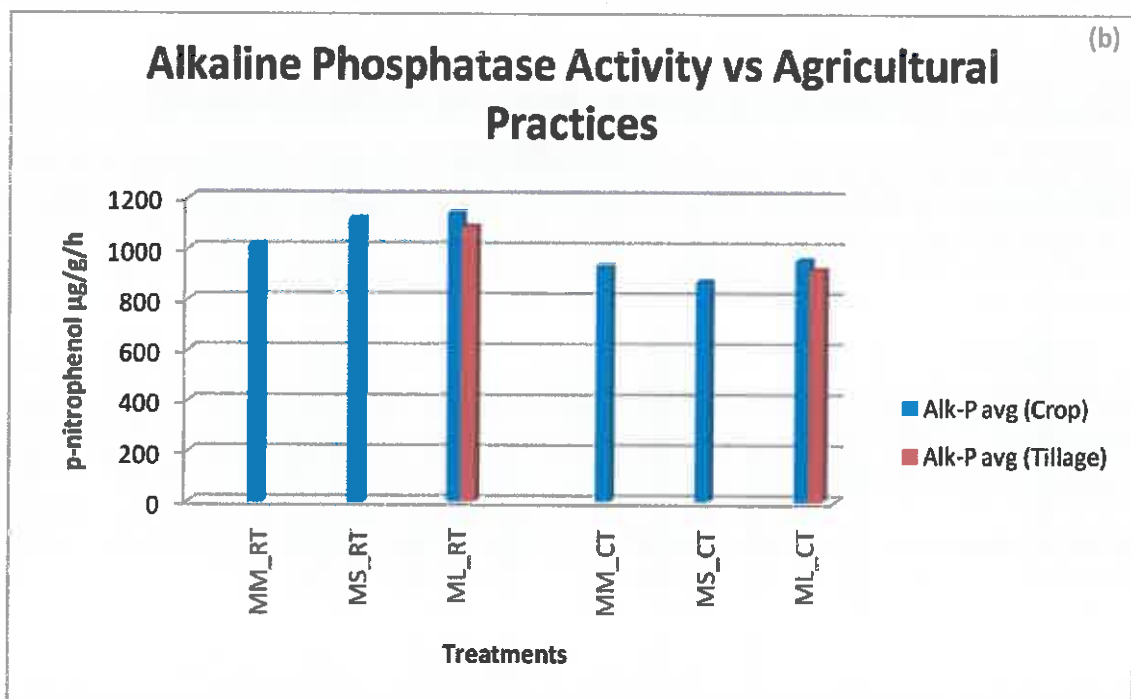
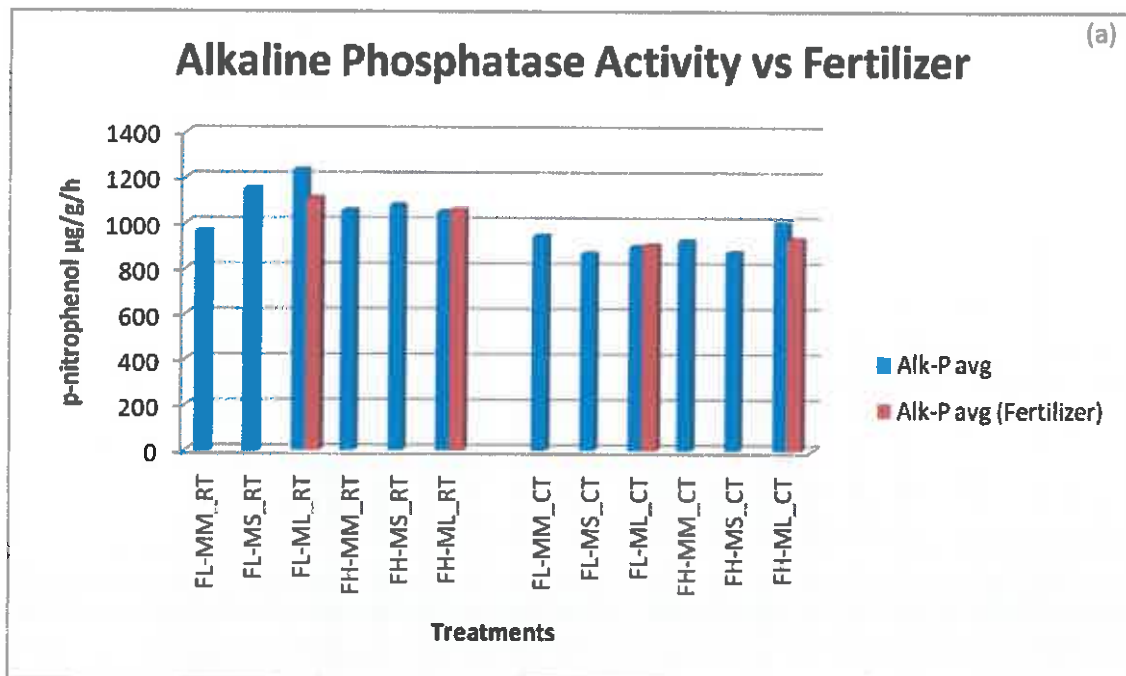


Figure 5.8-5: The average alkaline phosphatase activity under (a) fertilizer levels (FL, FH), and (b) cropping systems (MM, MS, ML) and tillage practices (CT, RT).

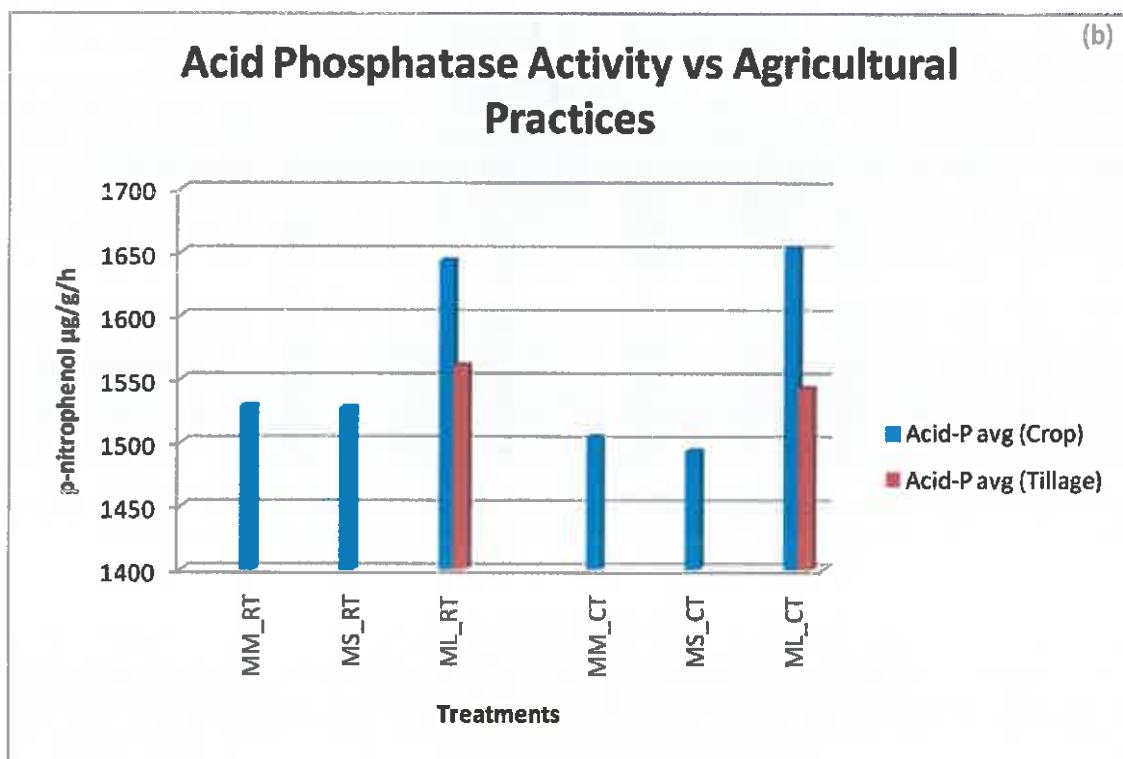
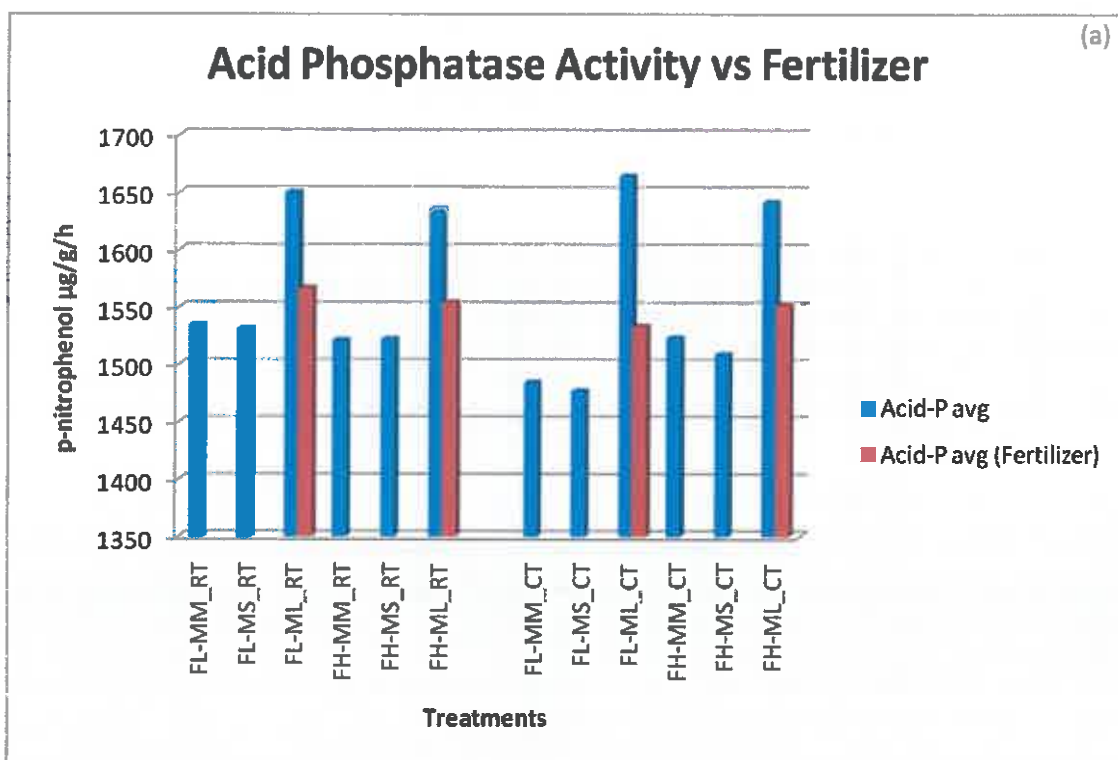


Figure 5.8-6: The average acid phosphatase activity under (a) fertilizer levels (FL, FH), and (b) cropping systems (MM, MS, ML) and tillage practices (CT, RT).

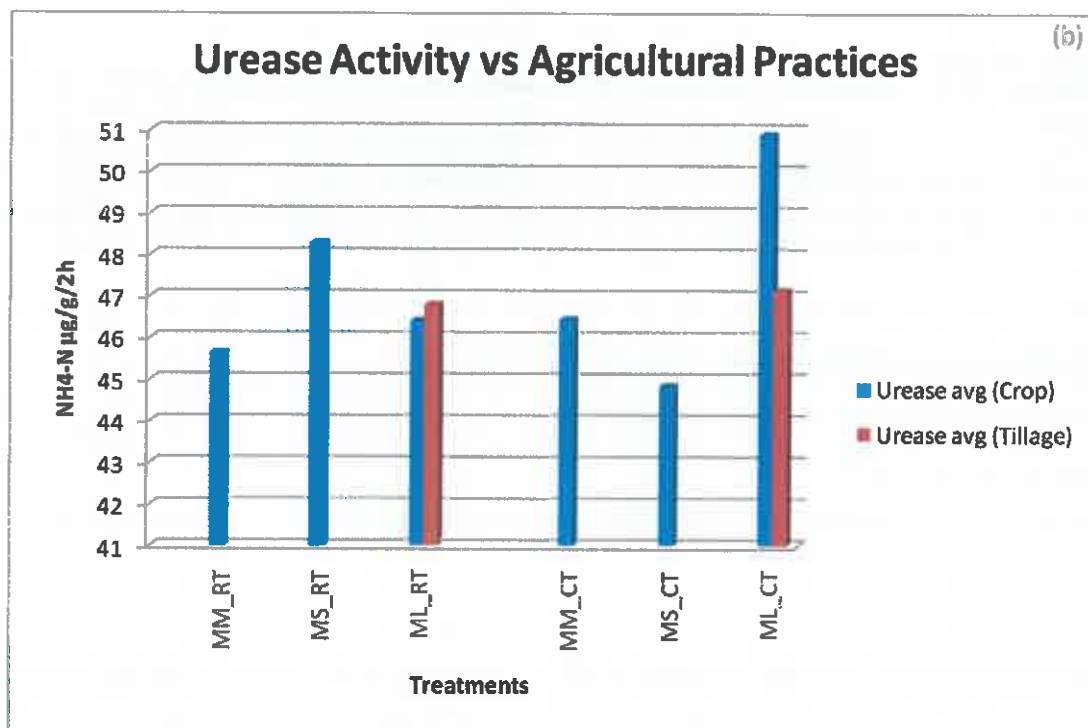
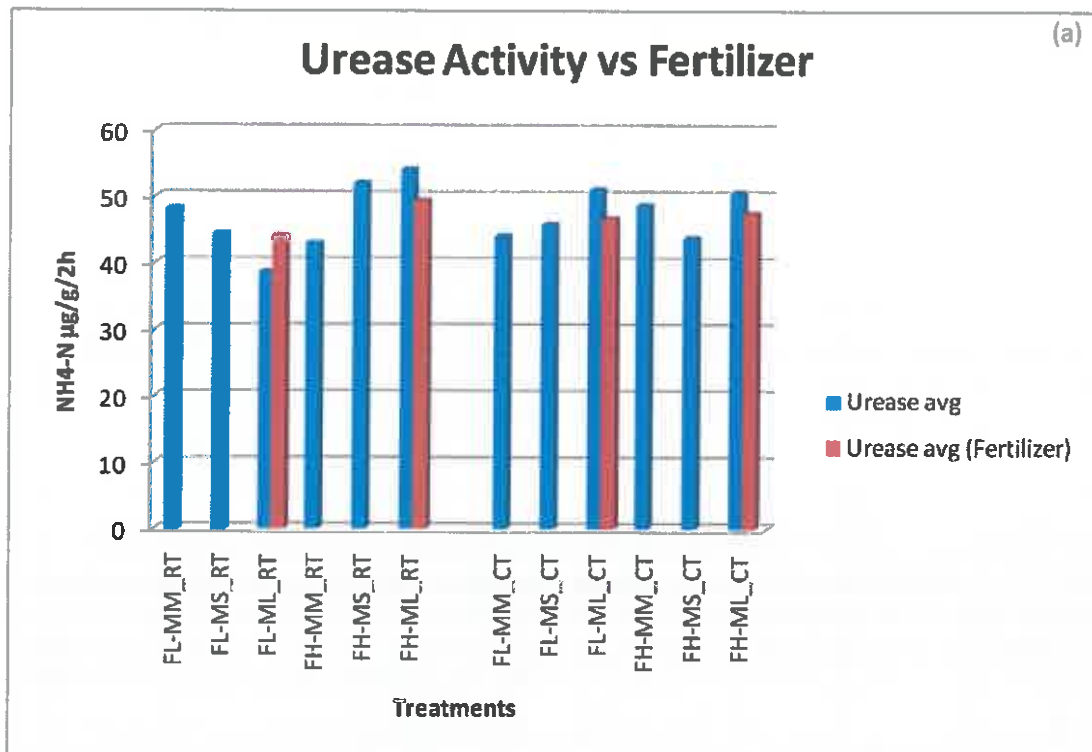


Figure 5.8-7: The average urease activity under (a) fertilizer levels (FL, FH), and (b) cropping systems (MM, MS, ML) and tillage practices (CT, RT).

Treatments in the 2012/13 season seemingly clustered according to cropping systems and tillage practices, with MM and ML clustering in opposite sides (Fig. 5.8-8 – blue block) and MS in the middle, together with the majority of CT practices.

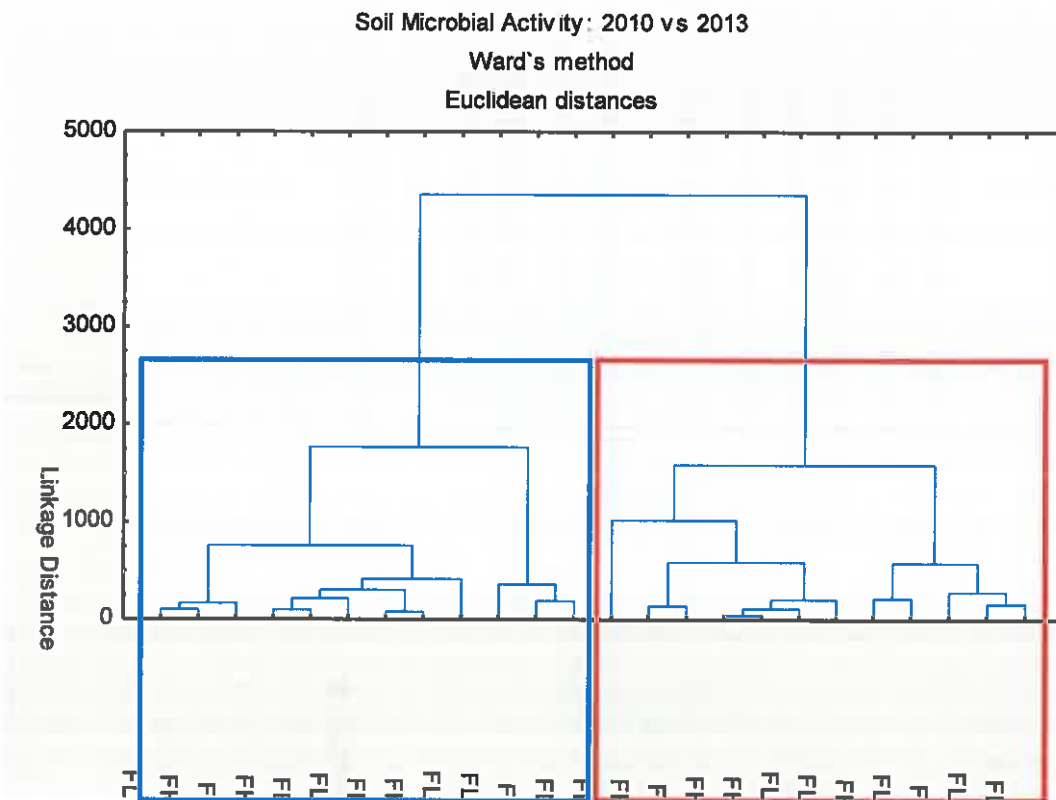


Figure 5.8-8: Dendrogram comparing the influence of different CA practices on overall soil microbial activity during the 2009/10 and 2012/13 seasons.

Correlation Analysis

Table 5.8-3 indicates correlation coefficients and correlation sums among soil microbial activity and diversity indices. The highest positive correlations (coefficient > 0.44; $p < 0.05$) were observed between β -glucosidase, acid phosphatase activity, urease activity, microbial richness (Shannon-Weaver Index), and microbial abundance (Evenness Index). The positive correlation of microbial richness with acid phosphatase and β -glucosidase activities suggests that the number of microbial species will increase as more inorganic phosphates are solubilised and more glucose is released as an energy source through increased cellulose degradation. Acid phosphatase and urease activities strongly correlated with β -glucosidase, indicating the importance of C:N:P ratios in soil fertility. The correlation sum gives an indication of the most

representative indicators, with acid phosphatase, urease, and β -glucosidase activities presenting the highest correlation sums.

Table 5.8-3: Correlation coefficients and correlation sums among soil microbial activity and diversity indices

	β -glucosidase Activity	Alkaline Phosphatase Activity	Acid Phosphatase Activity	Urease Activity	Shannon-Weaver Index	Evenness Index
β -glucosidase Activity	1.00	0.38	0.51*	0.59*	0.44*	0.22
Alkaline Phosphatase Activity	0.38	1.00	0.27	0.31	0.14	-0.31
Acid Phosphatase Activity	0.51*	0.27	1.00	0.77*	0.54*	0.31
Urease Activity	0.59*	0.31	0.77*	1.00	0.32	0.25
Shannon-Weaver Index	0.44*	0.14	0.54*	0.32	1.00	0.60*
Evenness Index	0.22	-0.31	0.31	0.25	0.60*	1.00
Correlation sum	3.14	2.41	3.40	3.25	3.03	2.69

* Marked coefficients differed significantly ($p < 0.05$)

Conclusions

CA practices, including crop rotation and RT, can maintain or enhance soil quality by improving physical, chemical and biological soil properties (Carter, 1998). Different levels of disturbances have different effects on soil microbial populations. In order to enhance biodiversity in agricultural soils, we need to understand how diversity is impacted by different agricultural management strategies. The sensitivity of soil microbial diversity and enzymatic activity could be observed in the influence that tillage practices, cropping sequence, and fertilizer inputs exerted on these indicators of soil health and soil fertility over a period of five seasons (2008/09 to 2012/13).

Conventional tillage practices resulted in very little change in the amount of soil microbial species (richness) between treatments, but distinct differences could be observed between species richness in treatments over time under RT practices. Slightly higher species abundance could also be observed under RT, when compared to CT. Regardless of tillage practice, continuous high level fertilizer (FH) application presented slightly higher microbial species richness and abundance, compared to low level fertilizer (FL) application. This observation might be attributed to the influence of fertilizer application management on soil organic matter (SOM)

and cropping systems (Fisk & Fahey, 2001), resulting in increased SOM due to FH, giving rise to increased numbers of microbial species to convert the available substrates.

Cropping systems, fertilizer application levels, and tillage practices seem to exert a stronger influence on soil microbial activity than on soil microbial diversity. FH-MM under CT demonstrated the highest carbon and nitrogen conversion rate, whereas FL-MS under RT demonstrated the highest phosphorus conversion rate. FH-MS under RT demonstrated the overall lowest microbial enzyme activity. Overall, RT seemingly had a more favourable effect on soil microbial activity, compared to CT. According to results obtained, there appears to be a correlation between increased microbial activity in treatments with FL under RT, and FH under CT, whereas FL under CT and FH under RT, corresponded to decreased microbial activity.

Cropping systems, fertilizer applications, and tillage practices promoting the selection/stimulation of soil microbial communities beneficial to soil health could be incorporated or selected through specific agricultural practices to increase soil microbial diversity and activity, and therefore increased soil health/quality and crop yields. This implies that soil microbial communities could be characterised under various cropping systems with the aid of molecular techniques.

The importance of monitoring soil microbial diversity and enzymatic activity over time cannot be over-emphasised. Long-term microbiological evaluation of different combinations of agricultural practices can promote the elimination of seasonal fluctuations and inherent differences in activity. It is consequently recommended that these trends be monitored over time in order to attain a more complete reflection on the impact that the different treatment groupings would have on microbial diversity and activity as indicators of soil fertility and health. As shown in the Results and Discussion, enzymatic activities (thus, soil microbial activity) obtained as trends over time from January 2009 to January 2013, already indicated very encouraging increases in soil microbial activity. Not only is it recommended that microbial activity and diversity be monitored as trends over time, but the incorporation of additional "whole soil" techniques that do not require the extraction and culturing of soil microorganisms is also suggested. Owing to the influence of various external factors, trends-over-time measurements will enable the attainment of a better wide-ranging indication of the impact that tillage practices, fertilizer inputs, and cropping systems would have on soil microbial diversity and activity as potential indicators of soil health and quality. Based on results obtained, it is difficult to determine which agricultural practices, or combinations thereof, are most beneficial

or detrimental to soil microbial populations due to the presence of a large number of variables. It is believed that integration/correlation of existing soil microbial data with soil physical-chemical properties, crop yield, disease index and molecular data, will bring researchers one step closer to a better understanding of the role soil microbial communities play in soil health and quality.

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5.9 Soil Flora: Mycorrhizae

*Arbuscular mycorrhizal fungi as an indicator of soil health under conservation and conventional agricultural practices*⁶

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Introduction

Arbuscular mycorrhizal fungi (AMF) that form symbiotic associations with plants such as maize, soybean, cowpea and vetch impact on plant growth, soil fertility and productivity. Therefore they are essential in adding to ecosystem services delivered by microorganisms (Gianinazzi *et al.*, 2010). From host roots, AM fungal hyphae spread to the surrounding soil and develop an extensive network of mycelia. Plants benefit from this extensive network which aid in the uptake of nutrients, especially phosphorus (P), nitrogen (N), copper (Cu) and zinc (Zn) (Giovannetti & Avio, 2002; Smith & Read, 2008; Blanke *et al.*, 2011). AMF symbiosis, in many cases, increases plant tolerance to root pathogens, pests and abiotic stresses, such as drought and salinity (Augé, 2001; Evelin *et al.*, 2009; Sikes *et al.*, 2009). The increased production of beneficial secondary plant metabolites results in the availability of safer and higher quality food on the market (Ceccarelli *et al.*, 2010; Giovannetti *et al.*, 2012). Above all AMF contribute to soil carbon (C) sequestration and organic matter conservation as the extensive network of mycelia produce significant quantities of a sticky protein based hydrophobic substance, commonly known as glomalin. This glyco-protein accumulates in soil as a glomalin-related soil protein (GRSP) (Rillig & Mummey, 2006; Bedini *et al.*, 2009). Chitin and chitosan production by AMF, which are recalcitrant polymers, further contribute to the build-up of C and organic matter (Zhu & Miller, 2003).

Cropping management systems are believed to impact on the survival and beneficial effects that AMF have in cropping ecosystems. Studies by Douds *et al.* (1995), Jansa *et al.* (2002; 2003), Oehl *et al.* (2004), Castillo *et al.* (2006) and Brito *et al.* (2012) have demonstrated that different crop management systems involving high intensity of mechanisation or high inputs of chemicals may affect AMF species composition, AMF spore abundance and mycorrhizal colonisation. Thereby

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the potential benefits from AMF/crop symbiosis are often not realised and soil quality is not improved. To date no such related study has been conducted on field cropping management systems under South African conditions in summer rainfall areas.

The aim of the present study was to evaluate under field conditions the impact of conventional and reduced tillage practices, in combination with high and low fertilizer applications, as well as cropping systems on the spore production, root colonisation and GRSP production of AMF.

Materials and Methods

Study site and experimental design

See section 3.

Plant and soil sampling

In all four growth seasons (2008/09 to 2011/12) soil and plant samples were collected in mid-February. During the first two growth seasons five random samples in each plot were collected. Then three samples/plot for the last two growth season in order to reduce soil disturbance within the plots. The samples were collected by removing the whole root system of plant (maize or legume) together with its surrounding soil respectively into brown paper bags (10 kg) and immediately transported to a storage room at the ARC-PPRI, Roodeplaat campus. Roots were removed and soil samples were allowed to air dry for two weeks at 25 °C. Sub-samples of 250 g of soil were collected from the main sample for spore isolation and glomalin quantification and were stored in plastic bags at 4 °C in a cold room until processing.

Immediately after collection of samples, plant samples were washed with tap water for 2-3 days. After washing the roots were placed in plastic bags and stored in a 4 °C cold room. Sub-samples of feeder roots for root colonisation assessment were collected and cut into approximately 1-2 cm root pieces. The sub-samples were placed in 20 ml McCartney bottles with 50% ethanol solution and stored at 25 °C.

Assessment of AM fungi in the soil

AM fungal spores extraction and enumeration

A 100 g quantity of air-dried soil was measured from the stored 250 g sub-sample and placed in a 2000 ml beaker containing 1900 ml of tap water. The suspension was agitated and then allowed to settle for 1-5 min. The suspension was decanted through a nest of sieves with mesh

sizes of 425 µm, 250 µm, 106 µm and 53 µm. The contents of the 425 µm sieve were discarded and the residue on the remaining sieves was washed into 15 ml conical centrifuge tubes. The spore suspension was centrifuged at 3000 rpm using a bench-top centrifuge for 5 min and the supernatants were discarded. The pellet was re-suspended in 60% w/v sucrose solution and centrifuged for 5 min at 3000 rpm. The supernatant was decanted into a 45 µm sieve and washed to remove sucrose from the spores (Brundrett *et al.*, 1996; Sylvia, 1994).

The spore suspension was washed onto a 90 mm diameter filter paper disc which was fitted into a Buchner funnel connected to a vacuum pump. The filter paper was divided into 1 cm² grids. After drying the filter papers were transferred into a clean Petri dish to ease spore counting under a dissecting microscopy. The enumeration included dead and viable spores that were distinguished from one another by their appearance and light reflection under dissecting microscope (Brundrett *et al.*, 1996; Sylvia, 1994). Spore numbers per sub-sample were recorded and the total number of spores per treatment was calculated to be representative of the AM fungal spore population in 100 g of soil.

Identification of spore morphotypes

Spores were either directly mounted in polyvinyl alcohol (16.6 g), lactic acid (100 ml), glycerol (100 ml), water (100 ml) mixture (PVGL) or were first stained with Melzer's reagent which is an aqueous solution of chloral hydrate (100 g), potassium iodide (15 g) and iodine (0.5 g) suspended in water (22 ml) before being mounted on microscope slides. The spores were identified by morphological descriptive characteristics such as colour, size, attachment, spore walls and germinating structures to determine genus (Koske & Walker, 1986; Trappe, 1982). Descriptions were compared to those given on the INVAM website (INVAM, 2014).

Glomalin extraction and quantification

Easily extractable glomalin (EEG) was extracted from soil using a modified method of Wright & Upadhyaya (1996; 1999) as described by Janos *et al.* (2008). Soil (1 g) from sub-samples was weighed and placed in 15 ml conical centrifuge tubes and then suspended by adding 20 mM sodium tri-citrate (pH 7.0) solution. The EEG was then extracted by autoclaving the suspension at 121 °C for 45 min. After autoclaving the tubes were immediately centrifuged at 5000 rpm for 15 min. The supernatants were transferred into 2 ml Eppendorf tubes and stored at 4 °C (Janos *et al.*, 2008).

The EEG concentration (mg EEG/g soil) was determined according to the Bradford assay and with bovine serum albumin (BSA) as standards. The concentration of the standard solutions ranged from 1.25-5.00 mg BSA/ml water. Water was used as a negative control (blank). Duplicates of 5 μ l of BSA standards, water and test samples were pipetted into 96 well micro-titre plates. A volume of 250 μ l of Bradford Coomassie Brilliant Blue as dye was added to each well and left at 25 °C for 10-15 min to allow for colour development (Bradford, 1976). Absorbance was measured at 595 nm using a spectrophotometer, and was corrected for the blank. The values for the standard readings were calculated and plotted against their concentration using a scatter graph with linear regression values and equation. The regression equation of $y = ax + b$ from a standard curve was used to determine samples concentration (x) which is the unknown concentration also corrected for amount of soil and extractant.

Root analyses for AM fungal structures

Root staining for mycorrhizal colonisation

Fixed roots, as previously described, were washed with distilled water and further treated according to the methods described by Brundrett *et al.* (1996). After washing, the roots were covered with 5% w/v potassium hydroxide (KOH)/water solution and incubated in a 90 °C water bath for 45 min. Potassium hydroxide was discarded and the roots were washed with dH₂O. Subsequently the roots were bleached with freshly prepared alkaline hydrogen peroxide (H₂O₂) solution (3 ml NH₄OH, 30 ml 10% v/v H₂O₂, 567 ml dH₂O) for 30 min or until the roots turned white or clear. The solution was discarded and the roots were again washed with dH₂O. Furthermore, the roots were acidified overnight in a 0.1 M HCl solution (22.79 ml HCl, 2 L dH₂O). The HCl solution was discarded and the roots were covered with lacto-glycerol solution (520 ml lactic acid, 480 ml glycerol, 640 ml dH₂O) containing 0.82 g trypan blue as a stain (Koske & Gemma, 1989). Subsequently it was incubated for 45 min in a 90 °C water bath. The staining solution was decanted and the roots were destained in clear lacto-glycerol and after 2 days, 10 root pieces of 1-2 cm length were mounted on slides in PVLG. After placing the cover slip, the root pieces were squashed to ease observation of fungal infection during microscopic examination (Brundrett, 2009).

Percentage root colonisation assessment

Stained root pieces were further analysed for percentage root colonisation using a modified slide intercept method (Daniels & Skipper, 1982). The roots were analysed for AM fungal structures (arbuscules, vesicles and intercellular hyphae) using of a compound microscope at

40x magnification. A minimum of 100 fields of view were examined. The presence of AM fungal structures inside the root pieces was recorded and total number of root pieces colonised was calculated (Kormanik & McGraw, 1982) and expressed as percentage root colonised. The following formula was used (McGonigle *et al.*, 1990):

$$\text{Percentage AM fungal colonization (\%)} \\ = \frac{\text{Root pieces colonized with AM fungi}}{\text{Total number of pieces observed}} \times 100$$

Statistical procedures

Data was analysed using the SAS/STAT statistical software (SAS Institute Inc., 1999). The data was subjected to an appropriate ANOVA. The standardised residuals were tested for deviations from normality using the Shapiro-Wilk's test (Shapiro & Wilk, 1965). In cases where deviations from normality were evident because of skewness, outliers were removed until residuals were normally or symmetrically distributed. The Student's unprotected t-LSDs were calculated at the 5% level of significance to compare means (Snedecor & Cochran, 1967).

Results

Spores

The ANOVA and the analysed results showing the unprotected LSDs according to the student t-test for spore numbers in 100 g soil are respectively presented in Tables 5.9-1 & 2. In the first two growth seasons the number of resident spores per 100 g soil was very low and the mean number of spores ranged between 19 and 33 for the respective years. Due to these low inoculum levels, at planting all the plots were inoculated with a commercial mycorrhizal inoculant supplied by MycoRoot, Grahamstown. The inoculum contained isolates of *Glomus clarum*, *G. mosseae*, *G. etunicatum*, *Paraglomus occulum* and *Gigaspora gigantea* identified with morphological and molecular methods. The inoculum had a minimum of 10 spores/g carrier and infectivity (most probable number) of between 80-100 propagules/g carrier. Subsequently the number of spores recovered 8 weeks after planting increased 3.5 times. Only a slight increase was recorded in the last growth season. Notwithstanding the low numbers of spores recovered in the first season, significant differences ($P = 0.05$) between treatments were recorded. The best treatments were R-FH-MC, R-FH-Mv, C-FL-Mv, R-FH-MS and R-FL-Mo. Reduced tillage and high fertilizer application were the main drivers in the higher number of spores recovered.

Table 5.9-1: ANOVA for the number of AM spores/100 g soil over four growth seasons

Spore counts	Season 1			Season 2			Season 3			Season 4		
	2008/ 2009			2009/2010			2010/2011*			2011/2012		
Source of Variation	df	Mean Square	P value*	df	Mean Square	P value*	df	Mean Square	P value*	df	Mean Square	P value*
Block stratum	2	70.33	0.83	2	3052.22	0.10	2	210433.95	0.05	2	36235.06	0.60
Tillage	1	279.67	0.46	1	1972.97	0.14	1	58.76	0.95	1	45317.22	0.46
Error (a)	2	339.51		2	337.82		2	11470.75		2	53759.71	
Fertilizer application	1	30.10	0.59	1	1352.87	0.04	1	1161.25	0.57	1	6353.15	0.37
Tillage X Fertilizer application	1	688.45	0.01	1	118.84	0.54	1	5118.31	0.23	1	13.35	0.97
Cropping System	5	113.09	0.38	5	379.55	0.33	5	8634.34	0.05	5	13759.78	0.14
Tillage X Cropping system	5	126.98	0.32	5	478.38	0.21	5	3539.40	0.42	5	3409.43	0.82
Fertilizer application X Cropping system	5	118.64	0.36	5	748.12	0.06	5	3160.71	0.48	5	11102.20	0.23
Tillage x Fertilizer application X Cropping system	5	382.44	0.01	5	422.96	0.27	5	4058.50	0.34	5	954.56	0.99
Error (b)	41	104.09		44	316.74		43	3454.12		44	7773.70	
Total	68			71			70			71		

2010/11 was inoculated with mycorrhiza

In the second season, which was a rotational cropping season, the outstanding treatments were all conventional tillage treatments. High fertilizer application and cropping systems were the main drivers in the number of spores recovered. The best performing treatments were the following: C-FH-Mc, C-FH-Ms and C-FL-Mv.

After application of AM inoculum at planting in the third season, which was a non-rotational season, the MC rotational treatments had a significant ($P = 0.05$) effect on the number of spores recovered. The best treatment for that season as determined through the unprotected Student t LSD test was R-FH-MC where 277 spores/100 g soil was recovered. The treatment with the worst effect on the number of spores/100 g soil was C-FL-MM.

Table 5.9-2: Mean number of AM spores/100 g of dry soil over four growth seasons

Tillage	Fertilizer	Cropping system	Season 1	Season 2	Season 3	Season 4
			2008/2009	2009/2010	2010/2011 [#]	2011/2012
Grand Mean			19	33	118	133
Tillage						
Conventional Tillage			17 a	38 a	119 a	159 a
Reduced Tillage			21 a	28 a	118 a	108 a
<i>P value</i>			0.46	0.14	0.95	0.46
Fertilizer						
High Fertilizer			20 a	37 a	123 a	143 a
Low Fertilizer			18 a	29 b	114 a	124 a
<i>P value</i>			0.59	0.04	0.57	0.37
Cropping system						
MM			16 b	28 a	82 b	163 ab
MS			18 ab	37 a	121 ab	93 b
MC			19 ab	30 a	164 a	121 ab
Mc			18 ab	40 a	96 b	126 ab
Mo			20 ab	26 a	121 ab	113 ab
Mv			24 a	36 a	130 ab	185 a
<i>Unprotected LSD (P = 0.05)</i>			9	15	49	73
Tillage X Fertilizer						
Conventional Tillage						
High Fertilizer			15 b	41 a	115 a	168 a
Low Fertilizer			19 ab	35 a	123 a	149 ab
Reduced Tillage						
High Fertilizer			25 a	33 ab	133 a	117 ab
Low Fertilizer			17 b	22 b	104 a	99 b
<i>Unprotected LSD (P = 0.05)</i>			7	12	40	59
Tillage X Cropping System						
Conventional Tillage						
MM			13 b	28 bc	67 d	206 ab
MS			16 ab	45 ab	103 bcd	116 bc
MC			13 b	34 ab	157 ab	145 abc
Mc			22 ab	53 a	106 abcd	145 abc
Mo			16 ab	22 c	119 abcd	12 bc
Mv			22 ab	46 ab	159 ab	228 a
Reduced Tillage						
MM			18 ab	28 bc	96 bcd	119 bc
MS			19 ab	29 bc	140 abc	70 c
MC			27 a	26 bc	173 a	97 c
Mc			14 b	27 bc	87 cd	108 bc
Mo			24 a	29 bc	119 abcd	115 bc
Mv			28 a	26 bc	102 bcd	141 abc
<i>Unprotected LSD (P = 0.05)</i>			12	21	69	103

2011/12 was inoculated with mycorrhiza

R = reduced tillage; C = conventional tillage; FL = fertilizer low input; FH = fertilizer high input; MM = maize monoculture; MS = maize/soybean rotation; MC = maize/cowpea rotation; Mc = maize/cowpea intercropping; Mo = maize/oats intercropping; Mv = maize/vetch intercropping

LSD = Unprotected least significant difference with P value = 0.05

Table 5.9-2 (continued)

Tillage	Fertilizer	Cropping system	Season 1 2008/2009	Season 2 2009/2010	Season 3 2010/2011 [#]	Season 4 2011/2012
Fertilizer X Cropping System						
High Fertilizer						
	MM		16 ab	29 bc	81 b	180 ab
	MS		23 ab	46 ab	115 b	92 b
	MC		22 ab	27 bc	209 a	118 b
	Mc		19 ab	58 a	101 b	88 b
	Mo		17 ab	26 bc	112 b	148 ab
	Mv		22 ab	38 abc	136 b	231 a
Low Fertilizer						
	MM		15 ab	27 bc	82 b	145 ab
	MS		13 b	28 bc	127 b	94 b
	MC		15 ab	34 bc	127 b	124 b
	Mc		17 ab	22 c	92 b	165 ab
	Mo		24 ab	26 bc	130 b	79 b
	Mv		26 a	35 bc	124 b	138 ab
	<i>Unprotected LSD (P = 0.05)</i>		12	21	69	103
Tillage X Fertilizer X Cropping system						
Conventional Tillage						
High Fertilizer						
	MM		13 cde	28 bcde	70 bc	223 ab
	MS		14 cde	57 ab	105 bc	107 bc
	MC		9 e	23 de	164 b	146 abc
	Mc		27 abcd	78 a	104 bc	108 bc
	Mo		14 cde	21 de	80 bc	136 bc
	Mv		11 cde	40 bcde	165 b	289 a
Low Fertilizer						
	MM		14 cde	28 bcde	64 c	188 abc
	MS		18 bcde	33 bcde	101 bc	124 bc
	MC		17 bcde	46 bcd	150 bc	143 bc
	Mc		17 bcde	28 cde	107 bc	182 abc
	Mo		19 bcde	23 de	166 b	88 bc
	Mv		32 ab	53 abc	152 bc	167 bc
Reduced Tillage						
High Fertilizer						
	MM		19 bcde	29 bcde	93 bc	136 bc
	MS		31 ab	35 bcde	126 bc	76 c
	MC		42 a	31 bcde	277 a	90 bc
	Mc		11 de	38 bcde	97 bc	68 c
	Mo		20 bcde	31 bcde	143 bc	160 abc
	Mv		39 a	36 bcde	107 bc	173 abc
Low Fertilizer						
	MM		17 bcde	26 cde	100 bc	103 bc
	MS		7 e	23 de	154 bc	64 c
	MC		11 cde	22 de	104 bc	104 bc
	Mc		17 bcde	16 e	78 bc	147 abc
	Mo		29 abc	29 bcde	94 bc	70 c
	Mv		20 bcde	17 de	96 bc	109 bc
	<i>Unprotected LSD (P = 0.05)</i>		17	29	98	145

Results for the last season were inconclusive according to the ANOVA (Table 5.9-1), but when the unprotected Student t LSD test was applied, it showed that the best treatment was C-FH-Mv. In Table 5.9-3 the best treatments and interactions over the four growth seasons were ranked according to the highest number of spores/100 g soil. The highest number of spores was recovered from the conventional tillage treatments. High fertilizer application had a prominent effect on the number of spores recovered. In general the cropping systems that included vetch ranked the highest. Overall the C-FH-Mv treatment had the best effect on the number of spores produced in the soil surrounding the root system of the maize plants. In the last season it was also this treatment that outperformed the other treatments.

Table 5.9-3: Ranking of best treatment for spore formation

Treatment	Ranking	Spores			
		Season 1 2008/2009	Season 2 2009/2010	Season 3 2010/2011	Season 4 2011/2012
Tillage	1	R	C	C	C
Fertilizer	1	FH	FH	FH	FH
Cropping System	1	Mv	Mc2	MC	Mv
	2	Mo	MS	Mv	MM
Tillage x Fertilizer	1	RxFH	CxFH	RxFH	CxFH
	2	CxFL	CxFL	CxFL	CxFL
Tillage x Cropping System	1	RxMv	CxMc2	RxMC	CxMv
	2	RxMC	CxMv	CxMv	CxMM
	3	RxMo	CxMs	CxMC	CxMc2
Fertilizer x Cropping System	1	FLxMv	FHxMc2	FHxMC	FHxMv
	2	FLxMo	FHxMs	FHxMv	FHxMM
	3	FHxMS	FHxMv	FLxMo	FLxMc2
Tillage x Fertilizer x Cropping System	1	RxFHxMC	CxFHxMc2	RxFHxMC	CxFHxMv
	2	RxFHxMv	CxFHxMS	CxFLxMo	CxFHxMM
	3	CxFLxMv	CxFLxMv	CxFHxMv	CxFLxMM
	4	RxFHxMS	CxFLxMC	CxFHxMC	CxFLxMc2
	5	RxFLxMo	CxFHxV	RxFLxMS	RxFHxMv

Identification of AM fungal spores

Identification of resident, indigenous background spores to species level was not possible due to the low number of spores extracted from the trial site soils. Using only morphological features from the limited number of spores tentative identifications to genus level were made. Similar spore morphologies were observed for both low and high fertilizer application, cropping systems and both reduced and conventional tillage. The spores were identified to be from the genera *Glomus*, *Gigaspora* and *Scutellospora*. Spores were examined microscopically and described and identified as follows:

The genus *Scutellospora*

Spores belonging to the *Scutellospora* genus were identified as white to yellow in colour, globose to subglobose with an average diameter of 100-200 μm (Plate 5.9-1 A). The spores had bulbous subtending hyphae also referred as a sporogenous cell (Plate 5.9-1 B, C – sc). The walls of the spores had a plate-like "germination shield" on the surface of the innermost flexible inner wall (Plate 5.9-1 A, D – gs). The germination shield was yellow to brownish in colour and 172 μm in size with small compartments, including 10 μm multiple germ tubes and a 10 μm germinal whole (Plate 5.9-1 A, D – gs). Spore wall consisted of outer and inner, flexible layers that could not be properly distinguished (Plate 5.9-1 A-D – L1, L2).

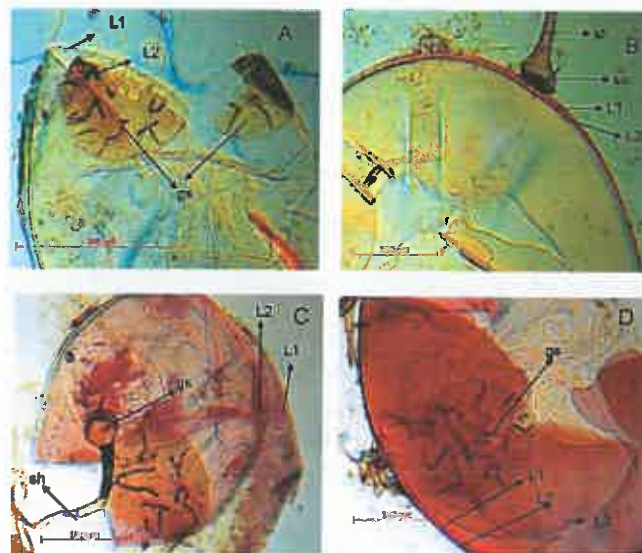


Plate 5.9-1: Spores of *Scutellospora* from Zeekoegat soil samples. A)-Brown yellow spore crushed in PVLG showing yellow brown germinating shield (gs), outer wall layer (L1), inner wall layer (L2). B and C)-Yellowish brown spore in PVLG with defined outer layer and a bulbous sporogenous cell (sc). Spore patchily stained with melzers reagent, subtending hyphae (sh), germinating shield (gs), wall layer (L1 and L2). D)-A purple-pink stained spore in melzers reagent and PVLG with germinating shield (gs), wall layer (L1, L2).

The genus *Gigaspora*

The genus *Gigaspora* was identified from spores that were globose to sub-globose in shape, varying in size from 250-448 μm with a mean of 349 μm in diameter (Plate 5.9-2 A). Spores had pale yellow subtending hyphae (Plate 5.9-2 E – sh) that was 20 μm in diameter at it widest point (Plate 5.9-2 E – sc). Spores had 2 distinct spore walls layers (Plate 5.9-2 D, F – L1, L2) that were pale yellow in colour.

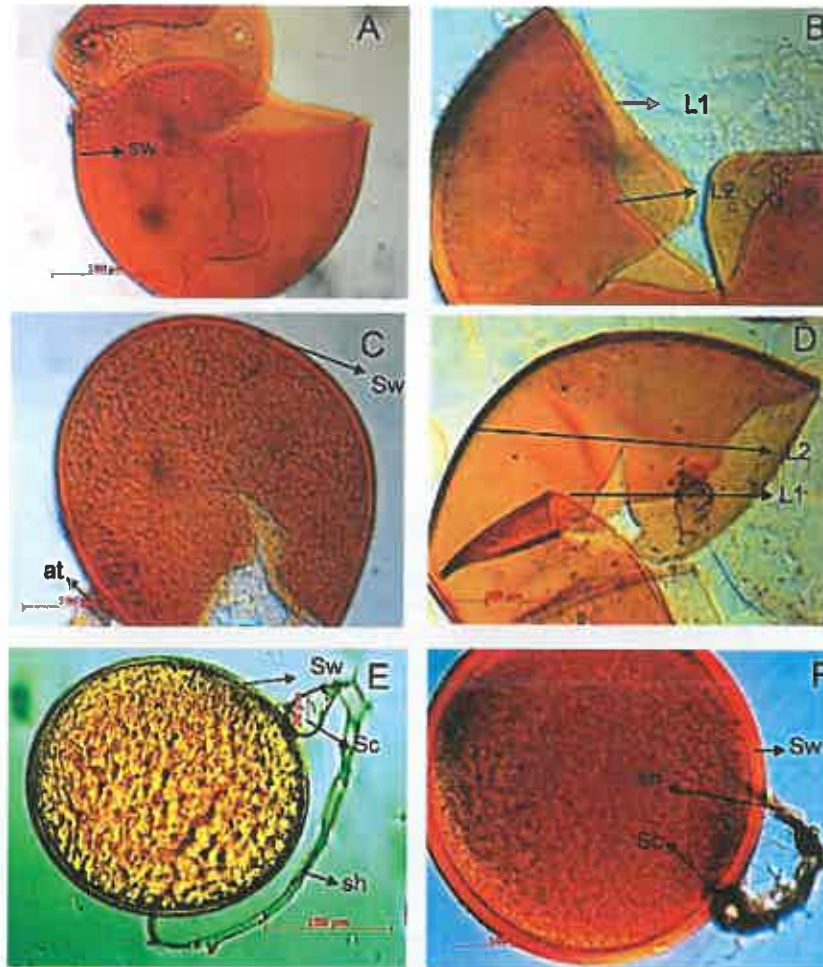


Plate 5.9-2: Spores of *Gigaspora* from Zeekoegat soil samples. A)-Brownish yellow spore stained in melzers reagent and PVLG showing spore wall (sw) after crushing. B) Spore in PVLG showing spore wall layers (L1 and L2). C) Spore showing spore wall (sw) and attachment (at). D) Brown spore crushed in PVLG with layer one and two (L1 and L2). E) Yellow spore with sw-spore wall, bulbous sporogenous cell (sc) with funnels shaped connectible and subtending hyphae (sh). F) Spore red in melzers stain with spore wall (sw), bulbous attachment (sc) and subtending hyphae (sh).

The genus *Glomus*

A compact cluster of spores joined together by sporogenous hypha were identified as belonging to the genus *Glomus*. Spores were globose in shape and pale yellow (Plate 5.9-3 A). Hyphal attachments were hyaline to pale yellow (Plate 5.9-3 A, B). Spores had a single wall layer possibly surrounded by an outer covering. The subtending hyphae were cylindrical in shape (Plate 5.9-3 B – h).

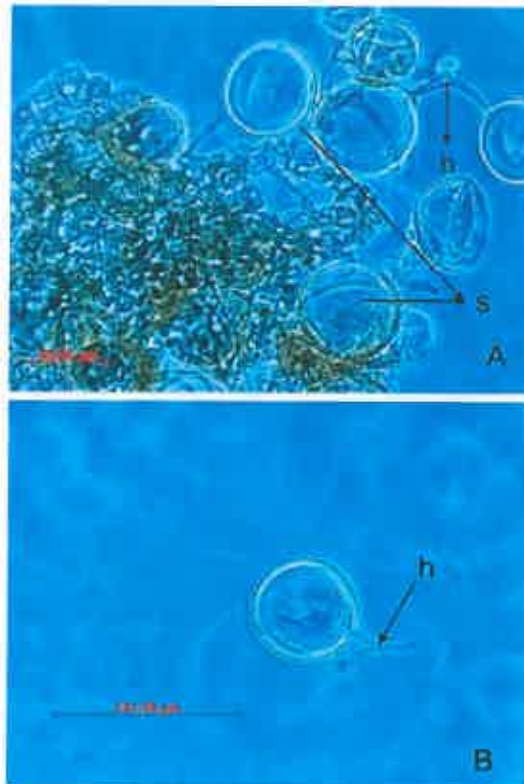


Plate 5.9-3: *Glomus* spores from Zeekoegat soil samples. A) Spore with hyphal attachment (h) of aggregate spores (s). B) Spores showing spore wall (sw) and subtending hyphae of a single spore.

Easily extractable glomalin

The ANOVA (Table 5.9-4) showed that in the second season there was a significant interaction between the fertilizer/cropping system treatments in the amount of easily extractable glomalin (EEG) resident in the soil surrounding the maize root system (Table 5.9-5). The most EEG was extracted from the soils in the FH-MS and the FH-Mc treatments. After application of mycorrhiza inoculum to the plots at planting in the third growth season, the overall concentration of EEG increased from 1.72 to 2.53 mg EEG/1 g soil and this correspond with the overall increase in the number of spores recovered. When ranked (Table 5.9-6), the R-FH-Mc treatment was the best in supporting EEG production over the monitoring period.

Table 5.9-4: ANOVA for the concentration of glomalin (mg EEG/g soil) for all growth seasons

Glomalin	Season 1			Season 2			Season 3			Season 4		
	2008/2009			2009/2010			2010/2011 [#]			2011/2012		
Source of Variation	df	Mean Square	P value*	df	Mean Square	P value*	df	Mean Square	P value*	df	Mean Square	P value*
Block stratum	2	0.859	0.08	2	0.170	0.39	2	0.052	0.06	2	1.697	0.09
Tillage	1	0.114	0.35	1	0.075	0.50	1	0.019	0.13	1	0.021	0.76
Error (a)	2	0.080		2	0.110		2	0.003		2	0.170	
Fertilizer application	1	0.001	0.81	1	0.001	0.64	1	0.001	0.44	1	0.003	0.9
Tillage X Fertilizer application	1	0.004	0.59	1	0.000	0.95	1	0.001	0.52	1	0.190	0.23
Cropping System	5	0.010	0.64	5	0.008	0.20	5	0.000	0.93	5	0.073	0.78
Tillage X Cropping system	5	0.031	0.09	5	0.006	0.35	5	0.001	0.84	5	0.076	0.76
Fertilizer application X Cropping system	5	0.012	0.58	5	0.018	0.01	5	0.001	0.46	5	0.122	0.54
Tillage x Fertilizer application X Cropping system	5	0.026	0.14	5	0.007	0.25	5	0.001	0.67	5	0.139	0.47
Error (b)	44	0.015		44	0.005		42	0.010		44	0.148	
Total	71			71			69			71		

2010/11 was inoculated with mycorrhiza

Table 5.9-5: Mean concentration of glomalin (EEG) from 1 g dry soil over four growth seasons

Tillage	Fertilizer	Cropping system	Season 1	Season 2	Season 3	Season 4
			2008/2009	2009/2010	2010/2011 [#]	2011/2012
Grand Mean			1.85	1.72	2.53	1.92
Tillage						
Conventional Tillage			1.89 a	1.69 a	2.51 a	1.94 a
Reduced Tillage			1.81 a	1.75 a	2.55 a	1.91 a
<i>P value</i>			0.35	0.50	0.13	0.76
Fertilizer						
High Fertilizer			1.86 a	1.73 a	2.53 a	1.93 a
Low Fertilizer			1.84 a	1.72 a	2.52 a	1.92 a
<i>P value</i>			0.81	0.64	0.44	0.9
Cropping system						
MM			1.83 a	1.7 b	2.53 a	2.07 a
MS			1.86 a	1.76 a	2.53 a	1.89 a
MC			1.89 a	1.7 b	2.53 a	1.89 a
Mc			1.88 a	1.71 ab	2.54 a	1.91 a
Mo			1.81 a	1.74 ab	2.54 a	1.84 a
Mv			1.85 a	1.71 ab	2.53 a	
<i>Unprotected LSD (P = 0.05)</i>			0.10	0.06	0.03	0.32
Tillage X Fertilizer						
Conventional Tillage						
High Fertilizer			1.89 a	1.69 b	2.52 b	1.89 a
Low Fertilizer			1.89 a	1.68 b	2.52 b	1.99 a
Reduced Tillage						
High Fertilizer			1.82 ab	1.76 a	2.55 a	1.96 a
Low Fertilizer			1.80 b	1.75 a	2.54 a	1.85 a
<i>Unprotected LSD (P = 0.05)</i>			0.08	0.05	0.02	0.26
Tillage X Cropping System						
Conventional Tillage						
MM			1.89 ab	1.71 c	2.51 bc	2.01 a
MS			1.94 a	1.72 bc	2.52 abc	1.85 a
MC			1.94 a	1.68 c	2.51 bc	1.85 a
Mc			1.87 ab	1.68 c	2.52 abc	1.94 a
Mo			1.91 ab	1.69 c	2.53 abc	1.92 a
Mv			1.81 abc	1.67 c	2.49 c	2.06 a
Reduced Tillage						
MM			1.77 bc	1.69 c	2.54 ab	2.12 a
MS			1.79 bc	1.81 a	2.54 ab	1.94 a
MC			1.84 abc	1.71 bc	2.54 ab	1.94 a
Mc			1.89 ab	1.75 abc	2.55 a	1.87 a
Mo			1.72 c	1.80 ab	2.54 ab	1.79 a
Mv			1.88 ab	1.74 abc	2.55 a	1.76 a
<i>Unprotected LSD (P = 0.05)</i>			0.14	0.09	0.04	0.45

2011/12 was inoculated with mycorrhiza

R = reduced tillage; C = conventional tillage; FL = fertilizer low input; FH = fertilizer high input; MM = maize monoculture; MS = maize/soybean rotation; MC = maize/cowpea rotation; Mc = maize/cowpea intercropping; Mo = maize/oats intercropping; Mv = maize/vetch intercropping

LSD = Unprotected least significant difference with P value = 0.05

Table 5.9-5 (continued)

Tillage	Fertilizer	Cropping system	Season 1	Season 2	Season 3	Season 4
			2008/2009	2009/2010	2010/2011 ^a	2011/2012
Fertilizer X Cropping System						
High Fertilizer						
	MM		1.85 a	1.68 bc	2.51 a	2.06 a
	MS		1.91 a	1.78 a	2.54 a	1.83 a
	MC		1.86 a	1.74 abc	2.54 a	2.00 a
	Mc		1.89 a	1.77 a	2.55 a	1.98 a
	Mo		1.79 a	1.73 abc	2.54 a	1.68 a
	Mv		1.84 a	1.66 c	2.53 a	2.01 a
Low Fertilizer						
	MM		1.81 a	1.72 abc	2.54 a	2.08 a
	MS		1.81 a	1.76 ab	2.53 a	1.96 a
	MC		1.92 a	1.67 c	2.52 a	1.79 a
	Mc		1.86 a	1.66 c	2.53 a	1.83 a
	Mo		1.84 a	1.75 ab	2.54 a	1.99 a
	Mv		1.86 a	1.76 ab	2.52 a	1.84 a
	<i>Unprotected LSD (P = 0.05)</i>		0.14	0.09	0.04	0.45
Tillage X Fertilizer X Cropping system						
Conventional Tillage						
High Fertilizer						
	MM		1.84 abc	1.71 bcde	2.49 abc	1.93 a
	MS		1.92 a	1.71 bcde	2.51 abc	1.88 a
	MC		1.94 a	1.70 bcde	2.52 abc	1.82 a
	Mc		1.91 a	1.70 bcde	2.53 abc	1.81 a
	Mo		1.91 a	1.69 cde	2.53 abc	1.76 a
	Mv		1.80 abc	1.64 e	2.51 abc	2.16 a
Low Fertilizer						
	MM		1.93 a	1.71 bcde	2.52 abc	2.09 a
	MS		1.95 a	1.73 abcde	2.53 ab	1.84 a
	MC		1.94 a	1.66 cde	2.49 abc	1.88 a
	Mc		1.83 abc	1.65 e	2.51 abc	2.08 a
	Mo		1.91 a	1.68 cde	2.53 ab	2.09 a
	Mv		1.82 abc	1.69 cde	2.47 c	1.96 a
Reduced Tillage						
High Fertilizer						
	MM		1.86 abc	1.65 de	2.52 abc	2.19 a
	MS		1.90 a	1.84 a	2.57 a	1.79 a
	MC		1.77 abc	1.76 abcd	2.57 a	2.18 a
	Mc		1.87 ab	1.84 a	2.56 a	2.16 a
	Mo		1.66 c	1.77 abcd	2.54 ab	1.86 a
	Mv		1.86 ab	1.67 cde	2.56 ab	1.86 a
Low Fertilizer						
	MM		1.68 abc	1.74 abcde	2.56 a	2.07 a
	MS		1.67 abc	1.78 abc	2.56 abc	2.09 a
	MC		1.90 a	1.67 cde	2.54 ab	1.71 a
	Mc		1.89 a	1.66 de	2.54 ab	1.59 a
	Mo		1.77 abc	1.83 a	2.55 ab	1.90 a
	Mv		1.89 a	1.82 ab	2.55 ab	1.73 a
	<i>Unprotected LSD (P = 0.05)</i>		0.20	0.12	0.06	0.63

Table 5.9-6: Ranking of best treatment for glomalin production

Treatment	Ranking	Glomalin			
		Season 1 2008/2009	Season 2 2009/2010	Season 3 2010/2011	Season 4 2011/2012
Tillage	1	C	R	R	C
Fertilizer	1	FH	FH	FH	FH
Cropping System	1	MC	MS	Mc2	MM
	2	Mc2	Mo	Mo	Mv
Tillage x Fertilizer	1	CxFL	RxFH	RxFH	CXFL
	2	CxFH	RXFL	RxFL	RxFH
Tillage x Cropping System	1	CxMC	RxMS	RxMc2	RxMM
	2	CXMS	RxMo	RxMv	CxMv
	3	CxMo	RxMc2	RxMC	CxMM
Fertilizer x Cropping System	1	FLxMC	FHxMS	FHxMc2	FLxMM
	2	FHxMS	FHxMc2	FHxMS	FHxMM
	3	FHxMc2	FLxMv	FLxMo	FHxMv
Tillage x Fertilizer x Cropping System	1	CxFLxMS	RxFHxMc2	RxFHxMS	RxFHxMM
	2	CxFHxMC	RxFHxMS	RxFHxMc2	RxFHxMC
	3	CxFLxMC	RxFLxMo	RxFHxMC	CxFHxMv
	4	CxFLxMM	RxFLxMv	RxFLxMM	RxFHxMc2
	5	CxFHxMS	RxFLxMS	RxFHxMv	CxFLxMo

Each growth season, when the EEG and spore concentrations between treatments were compared, there was a slightly negative correlation between spore and EEG production. (This is a possible indication that some treatments that are advantageous to spore production are not supportive of EEG production.)

Root colonisation

The ANOVA and the results for the colonisation of maize roots by mycorrhizal fungi are presented in Tables 5.9-7 & 8 respectively. Prior to inoculation of the trial with mycorrhizal fungi in the non-rotational maize growth season, no colonisation by AM fungi were observed in the roots. Eight weeks after inoculation an overall root colonisation rate of 63% by AMF was recorded. Although there was no significant difference between treatments, the best supportive treatments towards root colonisation were the R-FL-Mo and the R-FL-Mc treatments (Table 5.9-9). In the last growth season the colonisation of the roots by AMF declined to an insignificant level of 2%.

Table 5.9-7: ANOVA for percentage root colonisation by AM fungi for 2010/11 and 2011/12 growth seasons

	Season 1			Season 2			Season 3			Season 4		
Root colonization	2008/2009			2009/2010			2010/2011			2011/2012		
Source of Variation	df	Mean Square	P value*	df	Mean Square	P value*	df	Mean Square	P value*	df	Mean Square	P value*
Block stratum							2	2334.71	0.22	2	10.15	0.53
Tillage							1	793.80	0.39	1	28.76	0.25
Error (a)							2	675.34		2	11.48	
Fertilizer application							1	579.07	0.08	1	0.39	0.84
Tillage X Fertilizer application							1	22.82	0.73	1	0.65	0.79
Cropping System							5	148.49	0.55	5	12.23	0.28
Tillage X Cropping system							5	43.26	0.95	5	4.63	0.55
Fertilizer application X Cropping system							5	68.67	0.87	5	15.20	0.18
Tillage x Fertilizer application X Cropping system							5	66.21	0.87	5	7.37	0.57
Error (b)							43	184.50		41	9.40	
Total							70			68		

Table 5.9-8: Mean values of AM fungal percentage root colonisation

Tillage	Fertilizer	Cropping system	Season 1	Season 2	Season 3	Season 4
			2008/2009	2009/2010	2010/2011 [#]	2011/2012
Grand Mean			0	0	63	2
Tillage						
Conventional Tillage			0	0	67 a	1 a
Reduced Tillage			0	0	60 a	2 a
<i>P value</i>					0.39	0.25
Fertilizer						
High Fertilizer			0	0	66 a	2 a
Low Fertilizer			0	0	60 a	2 a
<i>P value</i>					0.08	0.84
Cropping system						
MM			0	0	21 a	2 a
MS			0	0	26 a	1 a
MC			0	0	18 a	2 a
Mc			0	0	21 a	1 a
Mo			0	0	27 a	3 a
Mv			0	0	20 a	1 a
<i>Unprotected LSD (P = 0.05)</i>					11	3
Tillage X Fertilizer						
Conventional Tillage						
High Fertilizer			0	0	57 ab	2 a
Low Fertilizer			0	0	61 b	1 a
Reduced Tillage						
High Fertilizer			0	0	63 ab	2 a
Low Fertilizer			0	0	70 ab	2 a
<i>Unprotected LSD (P = 0.05)</i>					9	2
Tillage X Cropping System						
Conventional Tillage						
MM			0	0	65 ab	2 ab
MS			0	0	53 b	0 b
MC			0	0	55 ab	1 ab
Mc			0	0	61 ab	1 ab
Mo			0	0	61 ab	1 ab
Mv			0	0	62 ab	0 b
Reduced Tillage						
MM			0	0	66 ab	3 ab
MS			0	0	64 ab	1 ab
MC			0	0	63 ab	3 ab
Mc			0	0	70 a	0 b
Mo			0	0	71 a	5 a
Mv			0	0	67 ab	2 ab
<i>Unprotected LSD (P = 0.05)</i>					16	4

2011/12 was inoculated with mycorrhiza

R = reduced tillage; C = conventional tillage; FL = fertilizer low input; FH = fertilizer high input; MM = maize monoculture; MS = maize/soybean rotation; MC = maize/cowpea rotation; Mc = maize/cowpea intercropping; Mo = maize/oats intercropping; Mv = maize/vetch intercropping

LSD = Unprotected least significant difference with P value = 0.05

Table 5.9-8 (continued)

Tillage	Fertilizer	Cropping system	Season 1 2008/2009	Season 2 2009/2010	Season 3 2010/2011 [#]	Season 4 2011/2012
Fertilizer X Cropping System						
High Fertilizer						
	MM		0	0	62 a	3 ab
	MS		0	0	56 a	0 b
	MC		0	0	56 a	0 b
	Mc		0	0	60 a	1 ab
	Mo		0	0	62 a	5 a
	Mv		0	0	66 a	0 b
Low Fertilizer						
	MM		0	0	69 a	2 ab
	MS		0	0	61 a	1 b
	MC		0	0	62 a	4 ab
	Mc		0	0	71 a	0 b
	Mo		0	0	70 a	1 ab
	Mv		0	0	63 a	2 ab
<i>Unprotected LSD (P = 0.05)</i>					16	4
Tillage X Fertilizer X Cropping system						
Conventional Tillage						
High Fertilizer						
	MM		0	0	64 ab	2 b
	MS		0	0	55 ab	0 b
	MC		0	0	50 b	0 b
	MC2		0	0	54 ab	2 ab
	Mo		0	0	57 ab	2 ab
	Mv		0	0	64 ab	0 b
Low Fertilizer						
	MM		0	0	67 ab	3 ab
	MS		0	0	51 b	0 b
	MC		0	0	61 ab	2 b
	MC2		0	0	67 ab	0 b
	Mo		0	0	65 ab	0 b
	Mv		0	0	60 ab	0 b
Reduced Tillage						
High Fertilizer						
	MM		0	0	60 ab	4 ab
	MS		0	0	57 ab	1 b
	MC		0	0	64 ab	1 b
	MC2		0	0	65 ab	0 b
	Mo		0	0	67 ab	7 a
	Mv		0	0	68 ab	0 b
Low Fertilizer						
	MM		0	0	71 ab	1 b
	MS		0	0	71 ab	2 b
	MC		0	0	62 ab	5 ab
	MC2		0	0	74 a	0 b
	Mo		0	0	74 a	2 ab
	Mv		0	0	65 ab	4 ab
<i>Unprotected LSD (P = 0.05)</i>					23	5

Table 5.9-9: Ranking of best treatment for root colonisation

Treatment	Ranking	Root Colonization			
		Season 1 2008/2009	Season 2 2009/2010	Season 3 2010/2011	Season 4 2011/2012
Tillage	1			R	R
Fertilizer	1			FL	FH
Cropping System	1			Mo	Mo
	2			MM	MM
Tillage x Fertilizer	1			RxFL	RxFL
	2			RxFH	RxFH
Tillage x Cropping System	1			RxMo	RxMo
	2			RxMc2	RxMC
	3			RxMv	RxMM
Fertilizer x Cropping System	1			FLxMc2	FHxMo
	2			FLxMo	FLxMC
	3			FLxMM	FHxMM
Tillage x Fertilizer x Cropping System	1			RxFLxMo	RxFHxMo
	2			RxFLxMc2	RxFLxMC
	3			RxFLxMM	RxFHxMM
	4			RxFLxMv	RxFLxMv
	5			RxFHxMv	CxFLx MM

Discussion

AM fungal spore extraction and enumeration

In the soil arbuscular mycorrhizal fungi produce propagules such as spores, colonised root fragments, soil borne auxillary vesicles (some genera) and hyphae as a source of inoculum (Jasper *et al.*, 1989). In this study the presence of these propagules was used as a measure of the activity and infectivity of these fungi in the soil (Bedini *et al.*, 2007; Castillo *et al.*, 2006). Any changes in the propagule numbers can be a useful marker to indicate the effect of agricultural practices on soil health, with spore enumerations being the most widely used measurement (Avio *et al.*, 2013; Aggarwal *et al.*, 2010; Boddington & Dodd, 2000; Castillo *et al.*, 2006; Mathimaran *et al.*, 2007; Utobo *et al.*, 2011).

Spore numbers extracted and enumerated in this study were low, and from literature low numbers of AM fungal spores have been directly related to soil disturbance (Jasper *et al.*, 1989). These findings are similar to several others studies (Avio *et al.*, 2013; Bedini *et al.*, 2007; Castillo *et al.*, 2006). The low number of spores recorded in this study are similar to another pilot study conducted in the Bophirima District in the Ganyesa sub-region of the North West Province where low number of spores in cultivated soil were also found as compared to natural, grassland and pastures soil (Ingleby & Dirk, 2006). In contrast, Castillo *et al.* (2006) recorded counts ranging from 182 to 238 spores/100 g of dry soil under conventional tillage, and recorded counts of 280 to 641 spores/100 g of dry soil under no-till. These findings confirm that different tillage

practices have varying effect on the number of spores of AM fungi because they create varying forms of disturbance in the soil chemical, physical and biological properties of soil (Aggarwal *et al.*, 2010; Douds *et al.*, 1995; Kabir *et al.*, 1998; Schalamuk & Cabello, 2010). They also shows that their effect is interlink with soil type and AM fungal species.

These results show that regardless of the low number of indigenous spores extracted, there were no significant differences between tillage practices at a 95% confidence level after four growth seasons (2008/09 to 2011/12) (Table 5.9-2). However, there was a slight non-significant increase in spore numbers under conventional tillage for growth seasons 2009/10, 2010/11 and 2011/12 (Table 5.9-1). The slight increase in the number of spores found under conventional tillage may be due to intensive preparation of the soil at the beginning of the trial that could have shifted propagule distribution by mixing of top and below ground soil diluting the concentration of these propagules, and that could have reduced their effectiveness (Douds *et al.*, 1995; Franke-Snyder *et al.*, 2001; Galvez *et al.*, 2001; Honermeier, 2007). Soil preparation can result in destruction of hyphal networks that can alter the life cycle (Bethlenfalvay, 1992) and functionality of these fungi (Castillo *et al.*, 2006; Douds *et al.*, 1995). The increase in spore numbers for growth season 2010/11 was solely due to the application of inoculum which was applied in a less invasive manner. These results do confirm that different types of agricultural management practices affect AM fungal spore numbers in different ways (Curaqueo *et al.*, 2011).

These results show that reduced tillage, high fertilizer application and cropping systems positively influenced the number of AM fungal spores in the soil, even though very low spore numbers were recorded (0 to 33 spores/100 g of dry soil) in the first two growth seasons (Table 5.9-1). AM fungal spores were also increased under conventional tillage (mouldboard plough) and low fertilizer application interaction especially during maize rotation (Table 5.9-1). Due to the low spore numbers abundance or dominance of AM fungal genera was established. These results indicate that AM fungal spores studied here may be adapted to intensive management practices as it is well documented that tillage treatments can select and favour certain spores of AM fungal species over others (Jasper *et al.*, 1993; Jansa *et al.*, 2003). In a study by Galvez *et al.* (2001) they found that *Glomus etunicatum* spores were abundant under chisel disk tillage while *G. occultum* spores were more abundant under no-till, and *G. geosporum* spore were not affected by tillage. Douds *et al.* (1995) reported similar results. Tilling of the soil redistributes spores by mixing of top and below ground soil explaining the abundance of spores at 20 cm

depth under conventional tillage plots. However, the soil sampling in this current study was at 20 cm depth. These findings contradict with studies that have recorded a high number of spores at this depth especially under conventional tillage. The disturbance of soil at different levels can determine and estimate AM fungal propagules. It is important to include soil depth during sampling of these fungi. Studies that looked at soil depth as a contributing factor to AMF distribution in the soil found that spores vary in their distribution at different soil depth. Abbott & Robson (1991) conducted a study on the distribution of AMF at different depth under tilled soil and they found that at number of spores were high at 8 cm depth as compared to 8-15 cm depth. Therefore soil depth does play a role in the distribution and enumeration of AM fungal spores under tilled soil.

Historical soil management practices may also be responsible for the low AM spore numbers. The trial site was fallow for five years before the trial was established. Initially this effect was not taken into consideration but lack of suitable host plant material would have resulted in reduced spore numbers in the soil. Kabir *et al.* (1999) found decreasing number of AM fungal spores with increasing length of fallow period from 86, 74 and 54 spores/100 g of soil respectively. Duponnois *et al.* (2001) found a higher number of spores in 4 year old fallow soil, than in an 11 years old fallow soil and intermediate numbers in the 19 year old fallow with spore counts ranging from 116 to 419 spores/100 g of soil. It is recommended that future studies should consider historical fallow periods as a contributing factor negatively affecting soil health.

This study indicated that crop rotation and intercropping with legumes positively affected the number of AM fungal spores in the soil. AMF are intimately linked to their host plant and spore numbers can be affected by the host plant's mycorrhizal status (Aggarwal *et al.*, 2010; Bedini *et al.*, 2007; Troeh & Loynachan, 2009). This emphasises the need to consider the mycorrhizal status of crops used for crop rotation and intercropping in order to support and increase AM fungal diversity (Kahiluoto *et al.*, 2009; Mozafar *et al.*, 2000). AM fungal spore distribution is closely related to above-ground plant species. The development and changes in plant species can either increase or decrease AM fungal activity. It is important to note that sporulation is a survival mechanism for AMF, allowing them to overcome unfavourable conditions (Smith & Read, 1997). Agricultural management practices combined with other factors like host plant and soil environmental conditions are determinators of the occurrence and spore numbers of AM fungal species in this site and this could account for the variations observed in this study. Kramadibrata *et al.* (1995) in Indonesia found 383 to 12 266 spores/100 g of soil in soybean

rhizosphere. Medina *et al.* (1988) in Florida found 5 to 679 spores/100 g of dry soil under four forage legumes. In this study it is clear that mycorrhizal status of agricultural crops play an important role in the activity of these fungi in the soil.

Tillage combined with one or two cropping sequences has been shown in this study to support a higher number of AM fungal spores. These findings are similar to several other studies that have reported differences in spore numbers (Aggarwal *et al.*, 2010; Borie *et al.*, 2006; Castillo *et al.*, 2006; Mathimaran *et al.*, 2007; Oehl *et al.*, 2003). However, the effect of these cropping sequences can be highly dependent on the host plant's mycorrhizal status. Crops can be highly mycorrhizal, moderate to non-mycorrhizal (Troeh & Loynachan, 2009). Cropping systems with high mycorrhizal crops can support a high AM fungal spore population (Kahiluoto *et al.*, 2009; Mozafar *et al.*, 2000). The effect of cropping practices on AM fungi is also dependent on several factors such as growth season, soil depth and stage of crop development (Aggarwal *et al.*, 2010; Douds *et al.*, 1995; Galvez *et al.*, 2001), but the effects of these factors were not considered in this study.

It is important to consider fallow period as a contributing factor in this study as it could have played a role in the decrease in spore numbers. Thompson (1987) found poor growth and less root colonisation by AM fungi of various crop species such as wheat (*Triticum aestivum*), chickpea (*Cicer arietinum*), grain sorghum (*Sorghum bicolor*), sudan grass (*S. sudanense*), sunflower (*Helianthus annuus*), soybean and maize after long fallow periods in cracking clay soils of the northern areas of the Australian grain belt. This was termed long fallow disorder for periods in excess of 12 months.

Inoculation of this trial increased number of spores for growth season 2010/11 (Table 5.9-2 and Fig. 5.9-1 A-G). The increase of spores shows that inoculation was successful in re-establishing AMF in this trial, after two growth seasons without activity (Table 5.9-6). The inoculation has proved to be a good practice for soils that are degraded and have low numbers of AMF. Number of AM fungal spores in the soil can be directly linked to soil aggregates stability and soil structure (Sylvia, 1992; Wright *et al.*, 1999).

In the fourth growth season the number of spores decreased compared to previous growth season. The decrease of spores indicates that in degraded soils where spores are low, inoculation of the soil may be required for several seasons in order to re-establish the

mycorrhizal population. It further emphasises the importance of inoculating agricultural soils. Predictions are that changes will start to show in the next two to five growth seasons especially under reduced tillage (Pellegrino *et al.*, 2011; Salami & Osonubi, 2002). Salami & Osonubi (2002) found an increase or maintenance of crop yields after two years of field inoculation in a case study of farmers in Nigeria.

Spore morphotype identification

In this study three resident AM fungal spore morphotypes were tentatively identified to genus level (see Plates 5.9-1, 2 & 3). The spores were separated into three genera *Glomus*, *Scutellospora* and *Gigaspora*. The recovery of few morphotypes is directly associated with low AMF species and community composition. So the species and community composition may be low due to the recovery of only three morphotypes. These findings are similar to others studies (Bedini *et al.*, 2007; Castillo *et al.*, 2006). In a Cerreto farm in Sovicille (Seina, Tuscany, Italy (Bedini *et al.*, 2007) three morphotypes of AM fungal spores were found in three different land use types that was used to cultivate maize for more than 50 years, confirming that agricultural management practices especially monoculture has a negative effect on these fungi (Castillo *et al.*, 2006; Douds *et al.*, 1995; Galvez *et al.*, 2001; Jansa *et al.*, 2003). Survey of background population of AMF in South African under intensively managed vineyards shows the prevalence of the five genera *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora* (Meyer *et al.*, 2005). However, more studies on mycorrhizal populations under different land use types in South Africa are required to establish baseline populations.

In agricultural soils the number of AMF species that can be identified ranges from one to 58. Jansa *et al.* (2002) found 17 species of the five genera *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora* and *Entrophospora*. Dodd (1994) found 22 AMF species, six *Glomus* spp., six *Acaulospora* spp., four *Scutellospora* spp., one *Archaeospora* sp. and one *Pacispora* sp. Morphotypes in this study are typical of agricultural soils. However, the presence of *Scutellospora* spores contradicts previous studies where this genus was not recovered after many years of cultivation (Jasper *et al.*, 1993; Jansa *et al.*, 2003).

The presence of *Gigaspora* spores in this study confirms that these fungi are not easily affected by soil disturbance because they depend on spores for their life cycle than on hyphae. Spores survive longer in soil than other propagules (Jansa *et al.*, 2003). *Glomus* spores are well known to dominate agricultural soils (Jansa *et al.*, 2002; 2003; 2008; Oehl *et al.*, 2004; Schalamuk &

Cabello, 2010; Schalamuk *et al.*, 2006). The present of *Gigaspora* spores is similar to two studies by Hamel (1996) and Johnson & Pflieger (1992) who found soybean monoculture favouring *Gigaspora* spp., compared to rotational plots that favoured *Glomus* spp. Medina *et al.* (1988) found six species of AM fungi, namely *Gi. heterogana*, *G. margarita*, *G. etunicatum*, *G. intraradices*, *Glomus* sp. and *A. spinosa*, in Florida soil cultivating four tropical forage legumes. No-till or reduced tillage support Glomeraceae species better than the other families with few exceptions (Oehl *et al.*, 2003; Jansa *et al.*, 2003; Galvez *et al.*, 2001). Galvez *et al.* (2001) found that species of *Acaulospora*, *G. occultum*-like and *Scutellospora* sp. survived well in reduced tillage (Castillo *et al.*, 2006; Jansa *et al.*, 2003; Oehl *et al.*, 2004) whilst *S. calospora* are generalists (Oehl *et al.*, 2003).

Species like those of the *G. mosseae* group, *G. geosporum*, *G. albidum* of the *G. occultum* group, *G. diaphanum*, *G. constrictum*, *G. fasciculatum* group, *G. geosporum* and *G. etunicatum*, *G. intraradices*, *G. occultum*, and *G. etunicatum*-like spores only survive under intensive tillage practices (Douds *et al.*, 1995; Galvez *et al.*, 2001; Jansa *et al.*, 2003; Schalamuk & Cabello, 2010). AM fungal spp. are also known to favour some host crops over others, for example species of *G. albidum* and *G. etunicatum* were found to be dominant under maize and *G. constrictum* under soybean (Troeh & Loynachan, 2009). Schenck & Kinloch (1980) found spores of *Gi. margarita*, *Gi. gregaria* and *Gi. gigantea* numerous from soil around soybeans. Some species of AMF require longer periods of germination than others (Tommerup, 1984). Therefore, the type of spore morphotype that can be recovered may differ from one growth season to another. In future studies based on the recovery of this morphotypes under different crops, tillage, fertilizer and growth seasons should consider such factors (Castillo *et al.*, 2006; Douds *et al.*, 1995; Galvez *et al.*, 2001; Troeh & Loynachan, 2009). For example, in the central area of South Korea, Lee & Eom (2009) found the species *G. clarum*, *G. etunicatum*, *G. mosseae*, *A. longula*, *A. spinosa*, *Gi. margarita* and *Paraglomus occultum* by morphological and molecular characteristics. Bedini *et al.* (2007) found species of *Glomus* such as *G. rubiforme*, *G. sinuosum*, *G. constrictum*, *G. mossea* and two unidentified *Glomus* sp. morphotypes dominating three different vegetation sites namely poplar, maize monoculture and grassland. On the contrary, a study in the semi-arid region of Namibia by Uhlmann *et al.* (2004) showed that the AM fungal community was affected more by vegetation cover and rainfall regimes than land use management systems. So absence or presence of certain AMF species in the soil can be indicative of the effect of agricultural practices on soil health or can indicate changes in environmental and climatic conditions.

Glomalin quantification

The concentration of EEG over the four growth seasons was low with readings ranging from 1-2.9 mg/g of soil (Table 5.9-5). These concentration ranges are taken as the minimum levels of glomalin that can be detected in a sample in other studies, because the detectable amounts by others researchers can be up to 105 mg/g of dry soil in forested Chilean Andisol soils (Borie *et al.*, 2000). The low levels of EEG concentration could be the result of the low number of AM fungal spores recorded in this study. In several studies EEG concentration has been directly linked to the production, decomposition and standing stock of this protein that is released into the soil from dead hyphae and spores walls (Driver *et al.*, 2005; Rillig *et al.*, 2003; Wright *et al.*, 2006).

The increase of EEG concentration in the third growth season (2010/11) ranged from 2.09-2.86 mg/g of soil under reduced and conventional tillage (Table 5.9-5). The values recorded are similar to other studies conducted under different soil types and somewhat similar conditions (Bedini *et al.*, 2007; Wright *et al.*, 2007). Wright & Upadhyaya (1999) found mean values from 1.25-5 mg of protein in the volume of extract of strips of horticultural film (16-32 cm²) of Sudan grass colonised by *Gi. rosea* FL 224-1, *G. intraradices* EY 113/114, and *G. caledonium* UK 301-1. In a tropical rain forest landscape of the La Selva soil, Lovelock *et al.* (2004) found concentration of EEG ranging between 1-8 mg/g of soil. Wright *et al.* (2007) recorded EEG concentrations within the ranges of 2.5-15 mg/g of 1-2 mm aggregates for 11 undisturbed Ultisols soils in the Mid-Atlantic state geographic area, with mean values of 2.86, 2.27, 2.09 mg/g of soil for whole soil GRSP under no-till, conventional tillage and organic farming respectively. Knorr *et al.* (2003) found average concentrations of 0.67-1.28 mg/g soil in 1994 and 0.81-1.28 mg/g soil in 2000, with no significant changes in glomalin even after 6 years of sampling. It is predicted that for glomalin levels to increase it may require several years to start to show differences as it is dependent on an active population of AMF which produce and deposit the glomalin in soil on degradation.

There was a significant difference in EEG concentration for growth season 2009/10 under the interaction of fertilizer application and cropping systems (Table 5.9-5). The interaction of between and within tillage with fertilizer application and cropping systems had no significant difference in EEG concentration. The relationship between glomalin with tillage and treatment has been reported in other studies where tillage strongly influenced glomalin concentration (Borie *et al.*, 2006; Bedini *et al.*, 2007; Lee & Eom, 2009; Preger *et al.*, 2007; Rillig *et al.*, 2003;

Wright & Anderson, 2000; Wright *et al.*, 1999; 2007). Hence, Bedini *et al.* (2007) found GRSP mean values of 3, 1.6, 5.7 mg/g of soil in grass/poplar grove and grass/maize of Entisol, fluvent soil respectively. Low EEG level is a good indication that no production of glomalin has taken place for a long time (Lovelock *et al.*, 2004). This is similar to others studies in agricultural soils as already discussed (Wright & Anderson, 2000). In contrast, the concentration of EEG was not significantly affected by tillage or other treatments. These findings contradict other studies in agricultural soils where differences in EEG were observed under different practices (Bedini *et al.*, 2007; Borie *et al.*, 2006; Lee & Eom, 2009; Preger *et al.*, 2007; Wright & Anderson, 2000; Wright *et al.*, 2007).

Root infectivity by AM fungi

Lack of AM fungal structures such as hyphae, vesicles and arbuscules from stained roots for the first two growth seasons indicates that AM fungal infectivity of field crops was non-existent. The observation of soil borne fungus hyphal networks inside root pieces indicates that there was no activity or root colonisation by background AM fungal propagules for the first two growth seasons. No interaction was recorded between agricultural management practices with AM fungal root colonisation percentage in this study as a result of the application of an inoculum (Mycoroot) which was applied to the entire trial site in row to stimulate mycorrhizal activity in the third growth season. Other studies have shown that root colonisation may be highly affected by agricultural practices, hence Jansa *et al.* (2003) found higher root colonisation under no-till than ploughed and chiselled soil. *Scutellospora* sp. was present under no-till, *G. intraradices* under intensive tillage, and *G. mosseae*, *G. caledonuim* and *Gigaspora* sp. were found under all tillage practices. It is suggested that reduced infectivity of propagules could be the result of the reduction in propagules number through intensive dilution by tilling, or by changes in the soil environment (Habte *et al.*, 1988).

Increased percentage root colonisation by the AM fungi from Mycoroot inoculum indicates that inoculation encourages activity and infectivity of these fungi especially in degraded soil. The absence of the suspected soil borne root pathogen confirmed that the colonisation of host roots by AMF effectively discourages pathogens. Several studies have shown that mycorrhizal root colonisation of host plants by AM fungal species control the development and severity of root borne diseases (Behn, 2008; Gosling *et al.*, 2006; Mummey *et al.*, 2009; Upadhyaya *et al.*, 2010). This emphasises the importance of AMF in protecting their host against pathogens attack (Garcia-Garrido *et al.*, 2000; Ozgonen *et al.*, 2010; Vos *et al.*, 2012). Inoculation with AMF also

provides protection for crops against pathogens and parasites especially in no-till or reduced tillage treatments that do not have other means of pathogenic control (Vos *et al.*, 2012). Colonisation of roots by AMF not only gives protection against pathogens, it also enhances host fitness due to early symbiotic benefits to the host (Karasawa *et al.*, 2002; Pellegrino *et al.*, 2011).

Similarly root colonisation levels of between 70 and 90% were found in vineyards that were previously inoculated with commercial AM fungal inoculum at two farms in the Stellenbosch region of South Africa (Meyer *et al.*, 2005). Jansa *et al.* (2008) found 50% of root length of medic and leek colonised within 4 weeks by *G. mosseae* and *G. intraradices*, he also found that *G. mosseae* colonised root faster than *G. intraradices* although root colonisation levels were similar at 8 weeks. It is also important to note that different AM fungal species will exhibit differences in infectivity with some species such as those belonging to the Glomeraceae colonising roots within 4 weeks while members of the Acaulosporaceae and Gigasporaceae are slow colonizers requiring about 8 weeks (Hart & Reader, 2002). Lack of colonisation in the first two growth seasons may have resulted from other factors not considered in this study such as soil type, crops, environmental conditions and host plant type (Oehl *et al.*, 2010). From previous studies it is reported that root colonisation can be influenced by AM fungal species type, soil type, agricultural practices (crop types) and environmental factors such as pH, temperature and soil moisture (Aggarwal *et al.*, 2010; Oehl *et al.*, 2010). As a result certain species are adapted to performing well under certain soil conditions and if any changes occur to this soil conditions, this fungal species will no longer be active (Aggarwal *et al.*, 2010). Therefore, the decrease in root colonisation can also be influenced by the preference or adaptation of some AM species to certain soil type, environmental conditions and crop types.

Summary and Conclusion

Over the four growth seasons (2008/09 to 2011/12) since the establishment of this trial, regardless of the low number of spores, EEG concentrations and no evidence of root colonisation from background population, statistical differences between reduced and conventional tillage and the sub-treatments (FL = fertilizer low input; FH = fertilizer high input; MM = maize monoculture; MS = maize soybean rotation; MC = maize cowpea rotation; Mc = maize cowpea intercropping; Mo = maize oats intercropping; Mv = maize vetch intercropping) at a 95% confidence level were detected, but it is too early to make conclusive deductions. The results obtained from this study provide a baseline dataset for this key fungal group and will serve as future reference. Inoculation of this field trial successfully re-introduced AMF after 8 weeks and

increased spore numbers and root colonisation was recorded. This emphasises the importance of inoculation with AMF for improving soil health, particularly when background populations have been significantly reduced. Therefore, in order to maintain mycorrhizal populations after inoculation one could combine reduce tillage and intercropping of perennial host plants like annual rye grass (*Lolium multiflorum*), and perennial ryegrass (*Lolium perenne*). This will provide sufficient root material for the mycorrhizal fungi to continue to propagate in the soil.

Crop rotation and intercropping with highly mycorrhizal crops are good practices for maintaining high AM fungal activity in the soil (Kahiluoto *et al.*, 2009; Mozafar *et al.*, 2000), but this was not evident in this study, emphasizing the need to assess the mycorrhizal status of the soil to determine whether inoculation is required. Inoculating with AMF increased spore number and colonisation of crops in the field, which can encourage high mycorrhizal activity that will build up good soil aggregate binding and stabilize soil structure through hyphal networks (Hamel, 1996; Jeffries *et al.*, 2003; Wright *et al.*, 1999) and production of a glue-like glycoprotein glomalin in the soil (Bedini *et al.*, 2007; Gosling *et al.*, 2006; Harrier, 2001; Lutgen *et al.*, 2003; Schloter *et al.*, 2003; Wright & Anderson, 2000). The longer-term effect of inoculation on establishing populations was not within the scope of this study but subsequent inoculations would greatly improve re-establishment of fungal populations.

The enumeration of AM fungal spores from soil samples show that background population is low, with little or no activity in the soil. Determination of AM fungal species indicates that species diversity and composition is low. Estimation of infectivity of AMF propagules indicates that the background population in the soil was unable to form symbiotic associations with the crops that were planted. Quantification of easily extractable glomalin that is freshly deposited indicates that there is little production of glomalin in this soil. Estimation of spores and their infectivity, determination of species type and composition, and quantification of EEG correlate, and all these parameters indicate that AM fungi are not actively operating in this soil.

The low number of spores, with no root infectivity of background population, resulted in the inability to establish pot cultures. To improve on these results, determination of AM fungal activity with molecular identification of both field and pot cultures with new and more sensitive techniques such as PCR-based methods with taxon and species specific primers (Johansson *et al.*, 2004; Ma *et al.*, 2005) is recommended. Studies should further include estimation and enumeration of others propagules such as hyphae. Determination of root infection must include

measurements of other contributing factors like environmental factors such as temperature, soil moisture and pH. Quantification of AMF must include determination of glomalin with more sensitive methods like specific monoclonal antibody MAb32B11. Changes to the populations and further diversity studies should also involve more sensitive techniques such as 454-pyrosequencing and identification of operational taxonomic units (Krüger *et al.*, 2012). Therefore, AMF are indicative of soil health under different agricultural practices and confirm that these fungi are very sensitive to any changes to soil properties (Schalamuk & Cabello, 2010). So, it is important to manage soil with these fungi in mind in order to support an active population that will positively influence the soil physical, chemical and biological properties, including plant health.

In conclusion, agricultural studies in disturbed soil need to be restored, maintained, sustained and monitored by high mycorrhizal activity that is interlinked with soil chemical, physical and biological components. It is recommended that future agricultural management of soil must include the monitoring of AM fungal spore number, activity, infectivity, species composition, community structure, diversity and glomalin production, deposition and standing stocks in the soil as they positively influence soil physical, chemical and biological properties. Similar studies to the present one should be used to further investigate the role of AMF as bio-indicators under agricultural soils in South Africa. Sustainable agriculture management practices cannot be properly implemented without AMF because they are a good bio-indicator of soil health. Therefore, measurements of AM fungal activity, spore numbers, abundance, and production of glomalin are essential in indicating changes in soil chemical, physical and biological properties of agricultural soils (Alguacil *et al.*, 2008; Castillo *et al.*, 2006) and are well established indicators of soil health under changing conditions (Ibijbijen *et al.*, 1996).

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5.10 Greenhouse Gas Emissions: CO₂

Preliminary results on greenhouse gas emissions from selected treatments⁷

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Introduction

Agricultural soil is interestingly at the centre of many seemingly unrelated issues, such as food security, climate change and degradation of natural resources. Doran & Safley (1997) indicate that maintaining soil quality is essential for ensuring sustainable agriculture and environmental quality. While agricultural soils are integral in challenges like food provision and food security, intensive cultivation often degrades natural resources and reduces the production potential of soil over the long term. Agricultural production is furthermore directly affected by climate change while it simultaneously also contributes to climate change by emitting greenhouse gases (GHGs). For example, the agricultural sector contributes the major GHGs (carbon dioxide [CO₂], methane [CH₄] and nitrous oxide [N₂O]) while cultivated agricultural soils are specifically associated with CO₂ and N₂O emissions (Desjardins *et al.*, 1993).

The dynamics of C and N in agricultural soils are affected by management and cultivation actions. The induced differences in C and N dynamics can affect the C sequestration and GHG emissions from agricultural soils. As such, agricultural management interventions can greatly influence GHG emissions from agricultural soils. For example, management actions like conservation agriculture (CA) not only increase soil organic matter (SOM) and the associated soil quality, but also increase C sequestration (Beukes *et al.*, 2011), which in turn will result in reduced GHGs in the atmosphere. Similarly, better N management will lead to less N fertilizer applied, which in turn could reduce pollutant exports (such as NO₃ leaching) and GHG emissions. Collecting empirical data on GHG emissions and C and N sequestration on such a large scale is impractical and costly, therefore a modelling approach is more appropriate. A modelling tool which yields confidence in estimates will improve the ability to compile GHG inventories for cultivated land. This information will be valuable to government departments which are currently lacking data

⁷ Data in this section will contribute to a PhD thesis chapter and peer-reviewed article (Assessment of C and N dynamics in cultivated South African soils and associated greenhouse gas emissions) to be submitted later in 2014.

for GHG inventories, as well as mitigation and adaptation planning. As part of a PhD project on GHG emissions from cultivated South African soils, soil water and CO₂ data from the Zeekoegat trial will be used to populate a mechanistic Soil Water Balance (SWB-Sci) crop model (Van der Laan *et al.*, 2010; Annandale *et al.*, 1999).

Materials and Methods

In situ CO₂ measurement: Closed chamber method

A custom-made Perspex chamber of 290 mm inner diameter and 230 mm height, with a fitting for a Vaisala probe, was used to measure CO₂ *in situ*. Aluminium rings were deployed in 24 plots, including four different treatments in both tillage systems, replicated three times, one set each for Blocks A, B and C. At the day of sampling, the chamber was secured on the ring, and made airtight with masking tape. Vaisala CO₂ measuring probes were used to record CO₂ concentrations and temperature inside the chamber. Each sampling unit was measured for up to 10 minutes with an interval of 1 min. Before each measurement, the probe was allowed to return to the background reading. The surrounding air was continuously monitored. CO₂ data was downloaded, and emission rates were determined by fitting a regression line to the data. The slope of the regression line gave the rate of soil respiration (CO₂ concentration in ppm per minute interval).

The CO₂ emissions were taken at various intervals throughout the trial, from 2008 to 2013. Initially the CO₂ concentrations were measured by Vaisala measuring device as well as gas samples analysed on a gas chromatograph (GC) by Pelindaba Analytical Laboratories. Gas samples were later discontinued (Swanepoel *et al.*, 2013). Making use of different sampling methods complicated data analysis. For example, gas sampled for GC analysis was collected after an hour closure time in the chamber, while the chamber with the Vaisala measuring device was only closed for up to 10 or 15 min. Where possible, corrections were made for different methods; alternatively the data was discarded.

Rainfall simulation

During the winter 2013, a final attempt was made to measure CO₂ emissions under various conditions. CO₂ emissions in dry soil were low. A rainfall simulation was initiated to measure CO₂ emissions from a dry to a wet soil, under cool atmospheric conditions. Each sampling site was prepared in advance by securing a tyre with a cross-section of 270 mm around each aluminium ring. The soil was saturated with 40 L of water and left for 24 hours to reach field capacity. Six

additional tyres were installed (two tyres per block: one for RT and one for CT) and saturated with water, in order to take destructive soil samples for gravimetric water content. On days 1, 2, 3, 4, 7 and 8 after the rainfall simulation, CO₂ emissions were measured with the Vaisala CO₂ measuring device and soil samples were taken from the dedicated points to determine gravimetric soil water content.

Soil Water Balance model

SWB-Sci is a mechanistic, real-time, generic crop, soil water balance, irrigation scheduling model, and can give a detailed description of the soil-plant-atmosphere continuum by using weather, soil and crop management data. SWB-Sci can simulate changes in soil water content, soil temperature as well as mineralisation of organic matter. Since CO₂ and N₂O emissions are largely dependent on soil water, temperature and availability of organic N and C, this model can be applied to estimate potential CO₂ and N₂O emissions from different cultivation practices. The dataset for the rainfall simulations was used to validate soil water simulations for that period.

Results and Discussion

CO₂ emissions for Zeekoegat

CO₂ emission rates for Zeekoegat from 2009 to 2013 are shown in Fig. 5.10-1. Emissions during summer were higher as a result of more microbial activity under warmer and wetter soils. Emissions during colder winter months, or drier periods, were lower, as expected. Tillage had an effect on CO₂ emissions with RT emitting lower CO₂ than CT, while cropping systems did not have a measurable effect on CO₂ emissions (data not shown). Reduced emissions under RT are a positive result for CA, and could indicate that practising CA reduces GHG emissions in the agricultural sector. This data is still preliminary and more work will be done with other trials, and combined with modelling, to determine the potential for RT as a mitigation option in the long term.

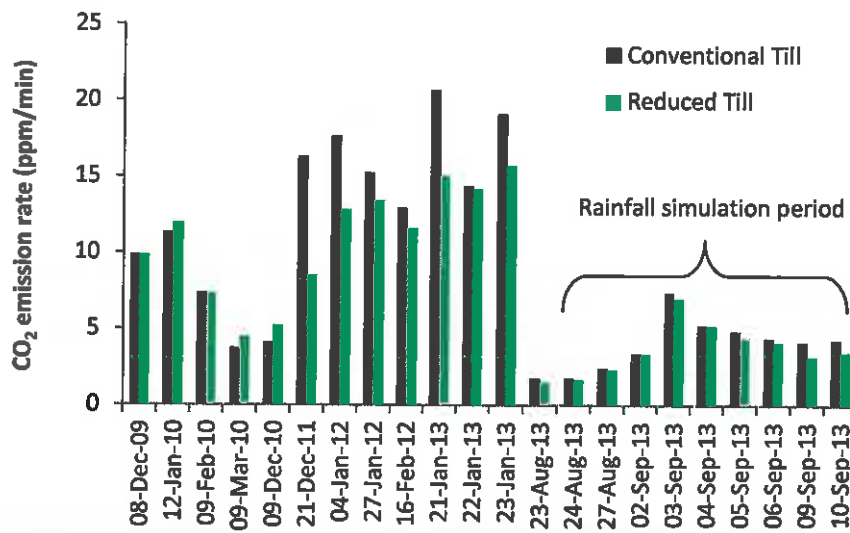
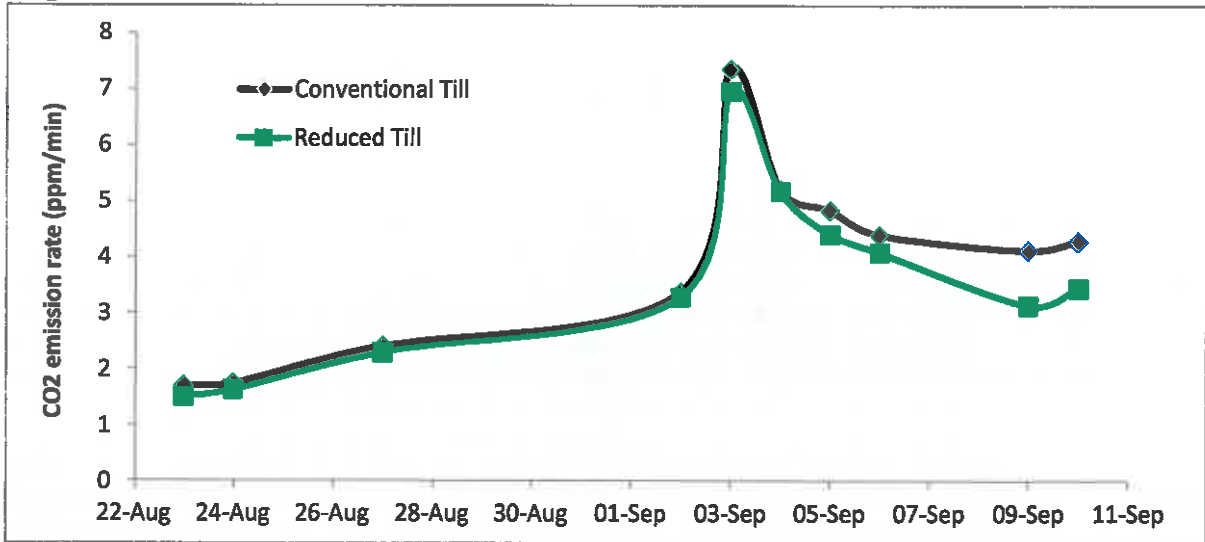


Figure 5.10-1: Comparison of CO₂ emission rates under RT and CT (2009 - 2013).

Rainfall simulation

CO₂ emissions are mostly affected by soil water, soil temperature, availability and quality of organic matter and microbial activity (Singer & Munns, 1992). During winter periods, when the soil water and soil temperature are relatively low, microbial activity is reduced, and subsequently the CO₂ emissions are also expected to be low. The results from the Zeekoegat trial confirm this, as the CO₂ emissions during August were relatively low with an average emission rate of 1.71 ppm/min for CT and 1.51 ppm/min for RT. This increased slightly when temperatures increased to an average of 3.38 (CT) and 3.29 ppm/min (RT) (Fig. 5.10-2). A sharp increase was observed 1 day after the rainfall simulation, with an average of 7.36 (CT) and 6.96 ppm/min (RT). The emission rate decreased as the soil water decreased, and after 8 days, levels of CO₂ emissions were similar to those prior to the rainfall simulation. It is interesting to note that the CO₂ emissions under CT were consistently higher than those of RT, even though the soil water content under RT was higher compared to CT.

Figure 5.10-2: CO₂ emissions from conventional tilled and reduced tilled soils after a rainfall



simulation in 2013.

A relatively good correlation ($R^2=0.64$) was measured for CO₂ emissions and soil water content (Fig. 5.10-3). The SWC was determined during the rainfall simulation trial and a range of water contents from dry to field capacity were obtained. This data was collected within a 20-day period, when external variables such as temperature and soil organic matter were relatively similar, and the major variable was the soil water.

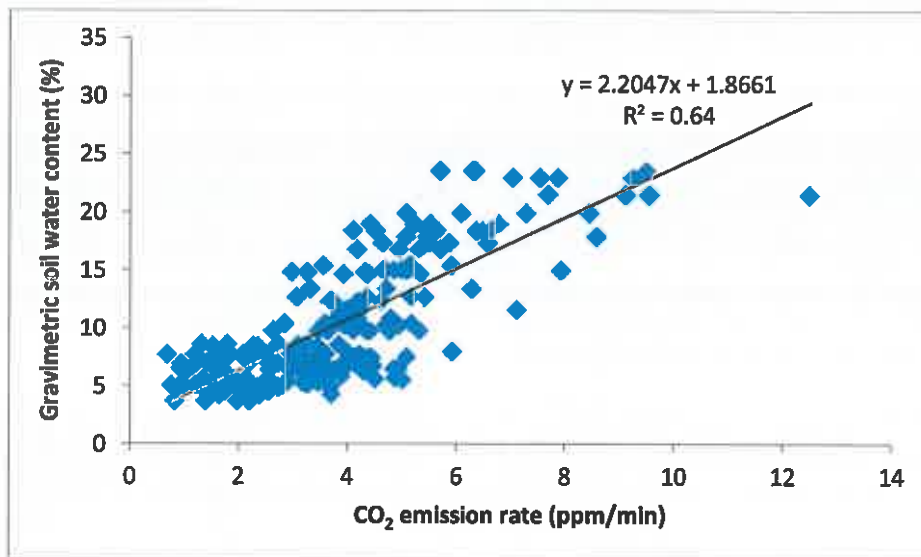


Figure 5.10-3: Correlation between CO₂ emissions and gravimetric soil water content.

A weak correlation ($R^2=0.198$) exists between CO_2 and ambient temperature (Fig. 5.10-4). It is expected that a better correlation of CO_2 emission and temperature could be obtained if soil temperature is used instead of ambient temperature, and if CO_2 emissions are measured under a wider range of temperature.

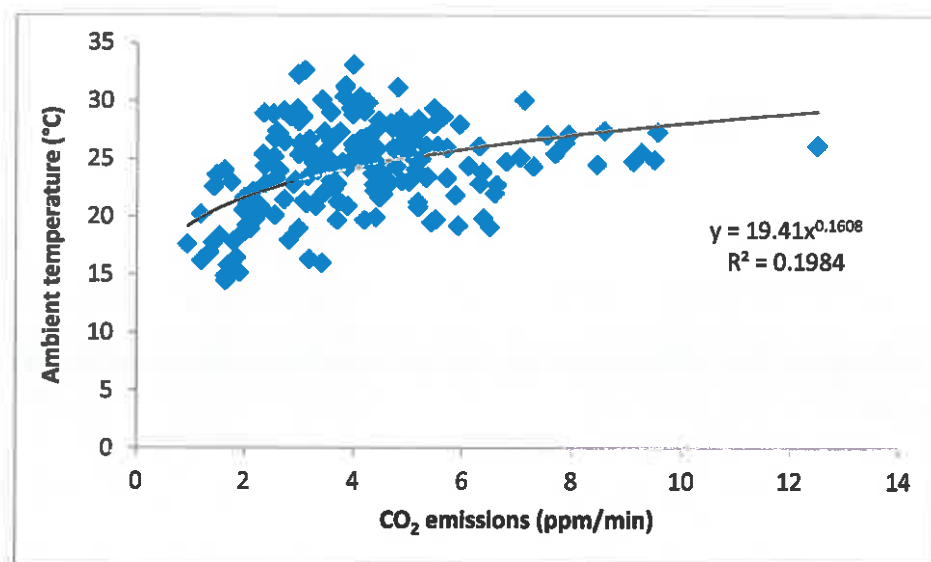


Figure 5.10-4: Correlation of CO_2 emissions with ambient temperature.

SWB-Sci

The SWB-Sci model predicts soil water particularly well, and for this exercise a simulation was run to determine how accurately it could predict results obtained from Zeekoegat. First the gravimetric SWC values were converted to volumetric data, by taking into account the bulk density of the soil (1.3 g/cm^3), as the model output is in terms of volumetric water content. Fig. 5.10-5 compares model simulation with data collected. The close fit in the simulated and measured soil water is a very positive result and can be used to estimate subsequent CO_2 and N_2O emissions, as these emissions are also closely related to SWC.

Currently, SWB-Sci does not simulate different soil water contents for different tillage systems. This will be adjusted to take the increased soil water-holding capacity under RT into account.

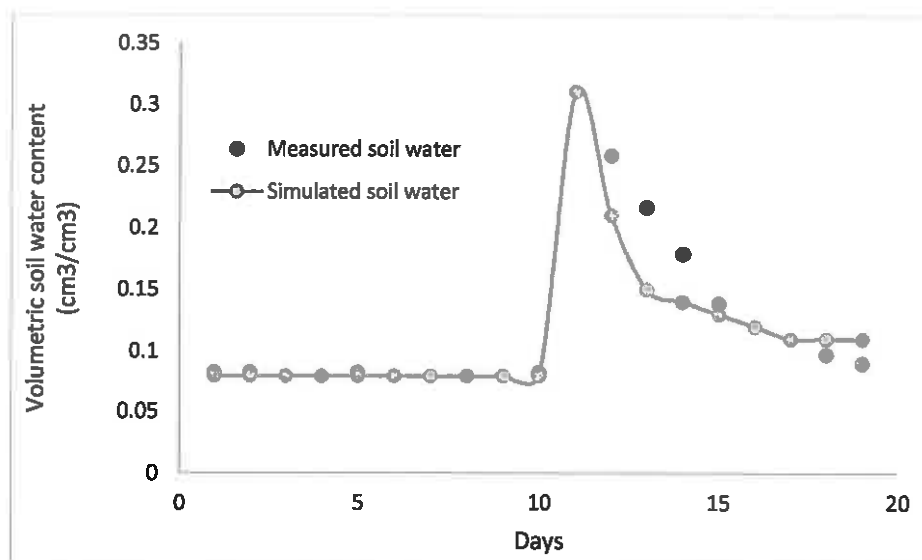


Figure 5.10-5: Comparison of SWB-Sci simulated data with soil water content measured in field collected soil samples.

Conclusions

Preliminary results show that practising RT could possibly reduce CO₂ emissions from soil. Conventional tillage consistently had a higher CO₂ emission rate. A relatively poor correlation between ambient temperature and CO₂ emissions was determined, while CO₂ responded well to soil water content. The SWB-Sci model accurately predicts soil water content and can be used to determine long-term emission rates of CO₂ and N₂O.

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5.11 Economic Analysis

*Economic Viability vs Sustainability: An evaluation of two farming systems*⁸

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Background

Conservation agriculture (CA) aims to achieve sustainable and profitable agriculture (FAO, 2007) and is based on optimising yields and profits, rather than maximising yield as with conventional agriculture. In the process, CA aims to achieve a balance between agricultural production, economic and environmental benefits, while improving the livelihoods of farmers and protecting the environment at the same time (Dumanski *et al.*, 2006). The economic benefits associated with CA include a reduction in labour requirements and lower input costs such as fuel, fertilizer and machinery (Hobbs, 2007). Agriculture benefits from CA mainly by the increase of soil organic matter (SOM) and all the subsequent advantages such as increased infiltration, aggregate stability, better water-holding capacity etc., that are associated with a higher SOM content (FAO, 2007; Hobbs, 2007). Environmental benefits include the reduction of erosion, improved water quality, biodiversity increase, carbon sequestration and conserving of natural ecosystems (IIRR and ACT, 2005). It is believed that by combining production and protecting the environment, the economy and the local community can benefit more than from increasing production alone. With CA, farming communities become providers of more healthy living environments through reduced use of fossil fuels, pesticides and other pollutants, and through conservation of environmental integrity and services (Dumanski *et al.*, 2006).

Trial layout

In 2007 the Agricultural Research Council (ARC) initiated an on-station field trial at Zeekoegat, north of Pretoria. The trial included tillage practices (reduced tillage [RT] and conventional tillage [CT]), in combination with various cropping systems. The latter included maize monoculture, two rotation systems with maize/cowpea and maize/soybean, and three intercropping systems with maize/cowpea, maize/oats and maize/vetch. The treatments per year are indicated in Table 5.11-1.

⁸ Prepared for submission to *SA Grain*.

Table 5.11-1: Crops planted in each year for various cropping systems

Treatment	2007/08	2008/09	2009/10	2010/11	2011/12	2012/13
Maize monoculture	Maize	Maize	Maize	Maize	Maize	Maize
Maize/cowpea rotation	Cowpea	Maize	Cowpea	Maize	Cowpea	Maize
Maize/soybean rotation	Soybean	Maize	Soybean	Maize	Soybean	Maize
Maize/cowpea intercropping	Maize & Cowpea	Maize & Cowpea	Maize & Cowpea	Maize & Cowpea	Maize & Cowpea	Maize & Cowpea
Maize/Oats intercropping	Maize & Oats	Maize & Oats	Maize & Oats	Maize & Oats	Maize & Oats	Maize & Oats
Maize/Vetch intercropping	Maize & Vetch	Maize & Vetch	Maize & Vetch	Maize & Vetch	Maize & Vetch	Maize & Vetch

In the maize monoculture cropping system, maize was planted each year at a density of 37 000 plants/ha, with row width of 0.9 m. Cowpeas and soybeans were planted in rows of 0.45 m width, while oats and vetch were broadcast at densities of 50 kg/ha and 30 kg/ha, respectively. For the first two years all the maize stands were planted at this density, with the intercrops planted in the 0.9 m space between maize rows. However, it was decided to switch to tramlines in the third season (2009/10), as the intercrops were highly disadvantaged competing with maize. Due to the small size of the experimental plots (7 x 8 m) only one row of maize was planted, and one row skipped, resulting in a single row tramline (Fig. 5.11-1). This resulted in a reduction of 50% of density in the maize plant stand. For larger areas a better alternative would be tramlines where two maize rows are used instead of one. This would result in a maize plant stand two-thirds that of a full plant stand.

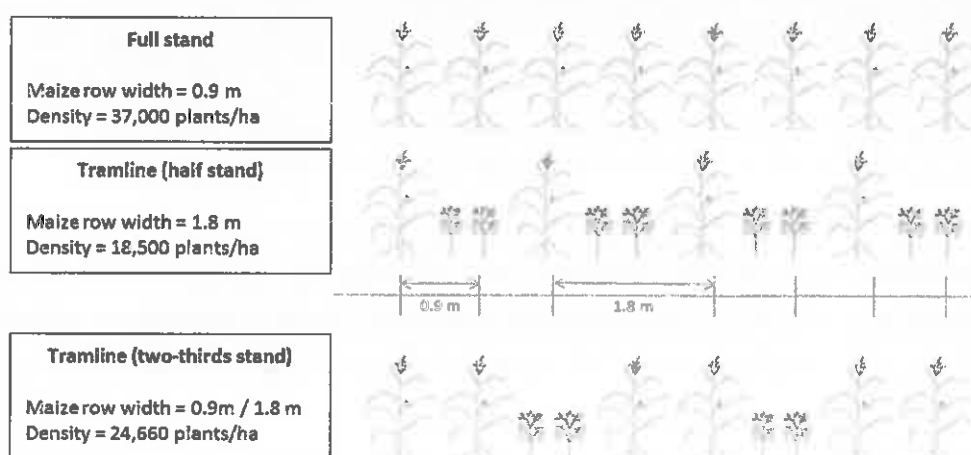


Figure 5.11-1: Illustration of plant densities when comparing full maize plant stands with one-row tramlines and two-row tramlines.

Comparison of farming systems

After six trial seasons, all the data has been compiled to quantify the effects of CA and conventional farming systems on soil and plant properties (see Appendix 4). However, comparing two farming systems proves to be very difficult. There are different ways to evaluate successful farming systems: soil health could be quantified by comparing soil organic carbon, or microbial activity; soil physical properties could be evaluated by comparing aggregate stability or water-holding capacity; agronomic success could be determined by comparing yields or biomass production. But the nature of the treatments makes certain comparisons complicated. For example, comparing maize grain yield across different treatments is not effective, as every second year the maize/cowpea and maize/soybean rotation treatments do not have any maize crops. Additionally the maize in the intercropping treatments was planted in tramlines (18 600 plants/ha) compared to monocropping where it was planted at a density of 37 000 plants/ha. When comparing biomass, the biomass generated from maize residue is much more than that generated from leguminous crops. One method is to compare the farming systems in terms of economics. However, expressing systems in terms of economic value alone has many drawbacks. For instance, in a CA system, the build-up of soil organic matter results in all the desired advantages, such as improved water-holding capacity, nutrient cycling, better soil structure and stable yields. But if all the biomass is removed and sold (as is assumed in an economic analysis), none will be available to build up the organic matter to result in the improvements associated with CA. Nonetheless, in this report we aim to economically evaluate the different farming practices. We stress that such an economic comparison is limited since CA aims to produce long-term sustainable yields by improving soil health. In comparison, conventional farming aims to maximise yields and will always outperform CA in the short term.

Economic considerations

Economic analysis can be quite complex and some comprehensive models include all the possible expenditure. These models go into much detail, including long-term loans, insurance, infrastructure repairs, diminishing values on capital equipment, etc. For the purpose of this study we kept it simple, focussing on the more direct expenses and income generated as a result of different farming systems. The economic analysis of this trial is not a reflection of true gross

profit. Since many farming expenses are not included here, the analysis is rather an effort to compare completely different systems with each other.

In our analysis we took into account direct income which includes i) grain yield from maize, cowpea and soybean, and ii) fodder from maize residue, cowpea, soybean, oats and grazing vetch. Expenses included costs incur for seeds, fertilizer, and land preparation. Expenses such as labour, herbicide and pesticide, harvesting costs, tractors, and insurance, infrastructure and property rates were not included as these expenses would be similar for both systems. For our purposes it is thus sufficient to compare conventional farming systems and various conservation agriculture systems at a basic financial level.

The data used in the calculations was obtained from various sources. The grain yields and biomass data was obtained from the trial itself. Direct costs such as cultivation and labour expenses were more difficult to determine. Since the plots were small and one implement was used to do all the cultivation in one day, it is difficult to calculate exact costs for each system. Instead we used production models developed by Grain SA to estimate litres of fuel per hectare for reduced and conventionally tilled soils. The diesel price for each year was used to calculate the costs for the different tillage systems. General retail prices for seeds were obtained and the ideal planting density was used to calculate seed cost. When seed prices for a specific year were not available they were estimated from current data. Potential income from biomass was determined by consulting small advertisements in the *Landbouweekblad* dating back to 2008. The average selling prices for various fodder, such as maize rests, soybean hay, cowpea hay and oats hay were obtained. In the absence of vetch hay prices we used data for lucerne. All values are expressed per hectare. The total income and total expenses were calculated, and the gross profit was calculated by subtracting the expenses from the income.

When comparing soil properties or plant properties between the different farming systems, in most instances some form of CA proved to be the most beneficial. Reduced tillage improved soil water-holding capacity and aggregate stability. Multi-cropping, especially the leguminous intercrops such as cowpeas and grazing vetch, significantly improved soil nutrient status and contributed to improved maize grain yields. But the picture changes when economic analyses are taken into account. The highest incomes were closely linked to maize grain yields. In systems where maize was planted, the income was higher than in systems without maize. This can clearly be seen in the rotation plots. In the years where soybeans or cowpeas were planted, the income

was drastically reduced, while during the 'maize' year of the rotation (Table 5.11-1), the income become comparable with other maize-based treatments. Regardless of the tillage practice, wherever the maize grain yield was highest, so was the income (Fig. 5.11-2). The reduction in cost of land preparation under reduced tillage was not enough to compensate for the loss of income from lower grain yields.

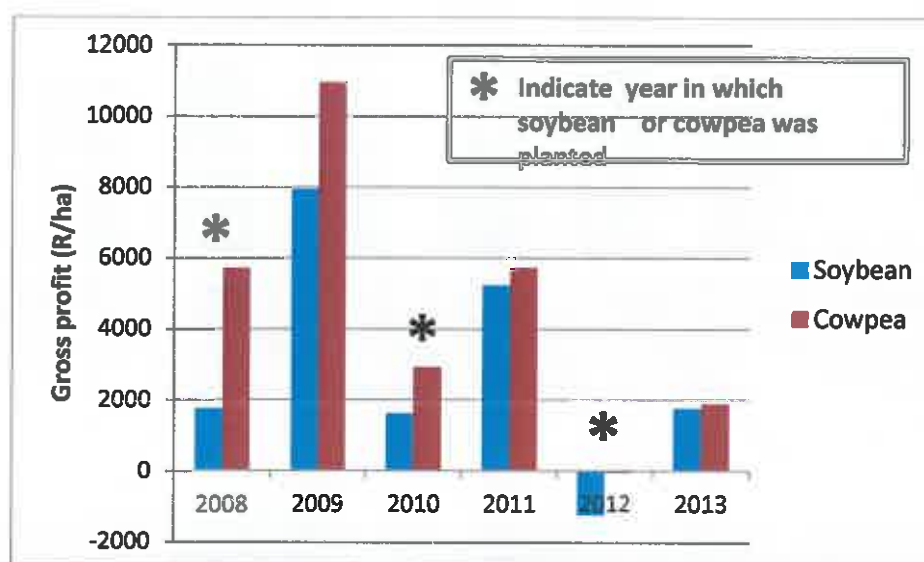


Figure 5.11-2: Comparison of gross profit for maize/soybean and maize/cowpea rotation treatments. The years 2009, 2011 and 2013 were 'maize' years.

However, there are certain constraints when extrapolating small plots to large areas. Although maize monoculture under CT seems to perform just as well as maize/vetch under RT, this is not an accurate reflection of the various farming systems. It must be kept in mind that maize in the maize/vetch treatment was planted in tramlines, at a density of 18 500 plants/ha, while maize in the maize monoculture treatments was planted at a density of 37 000 plants/ha. If maize grain yield per plant is compared, the picture changes, since CA treatments such as leguminous intercrops do improve maize grain yield. Fig. 5.11-3 illustrates that the average grain yield from a maize plant under monoculture treatment actually resulted in the poorest yields, compared with those from rotational and intercropping systems. Grain yield for maize monoculture was only 58.2 g/plant, while grain from the maize plants that were planted with vetch intercropping produced an average of 89.6 g/plant. This is a 54% improvement in grain yield per plant compared to monoculture treatments. In our trial the maize/oats treatment did not perform statistically better than that of the maize monoculture, mainly due to the area not being suited for oats production. In other areas, such as winter rainfall areas, or sites with more available soil

water during the winter, oats are proven to be potentially a very good intercrop that can generate copious amounts of biomass, which would in turn result in the positive changes associated with CA.

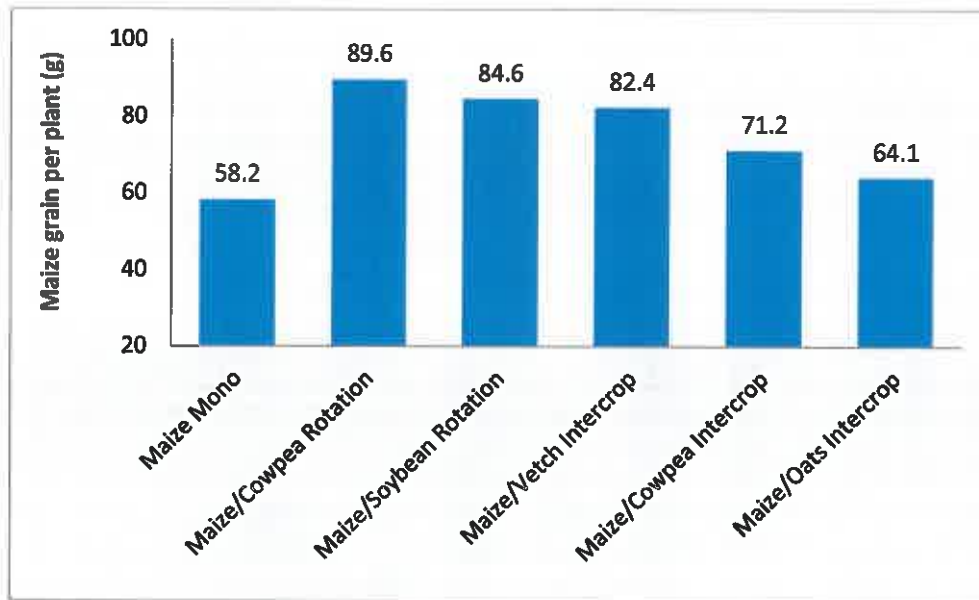


Figure 5.11-3: Comparing maize grain yield per plant under various cropping systems.

Even though the maize grain yield per plant was significantly better under CA systems compared to monoculture systems, the income generated from maize monoculture was the second highest after that of maize/vetch intercropping (Fig. 5.11-4). The reason for this high income was because the maize monoculture plots were planted at maximum planting density, while intercrops were planted at 50% maize density (row width of 1.8 m).

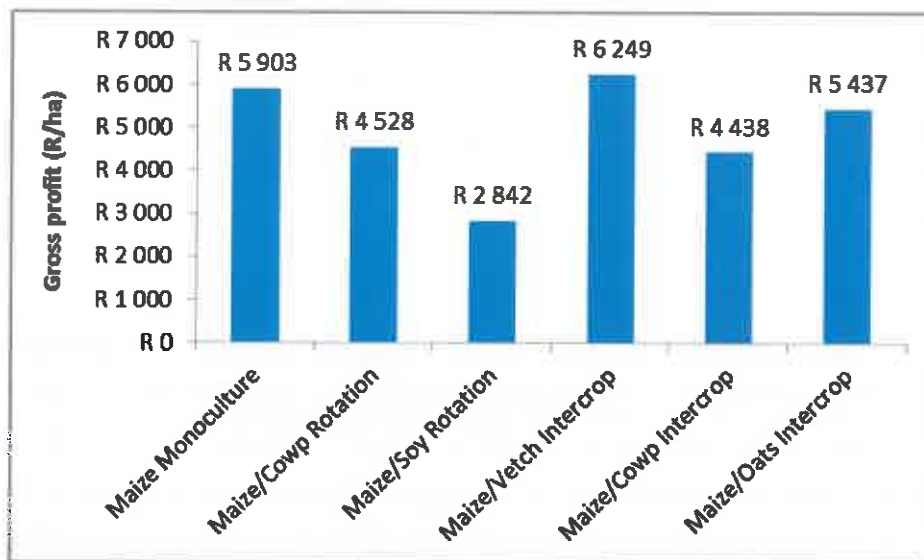


Figure 5.11-4: Gross profit (R/ha) for various treatments, expressed in terms of cropping systems, using the single row maize tramline system.

A better system would have been a tramline with a double maize row, followed by a legume intercrop (see Fig. 5.11-1). This would give a total of 24 660 plants/ha, thereby changing the economic analysis considerably. Additionally, if this trial was planted in an area with a better rainfall distribution or period (like the first two trial seasons), or even in a system where irrigation is an option, the inputs for economic analysis would be different. With adequate rainfall during the critical growing periods, the yields and biomass production would be better, which would positively affect not only the direct income, but also improve soil health and in return also benefit crop growth and yields.

Conclusion

According to the economic analysis, the conventional farming systems were only outperformed by one CA treatment, namely the maize/vetch intercropping system. Regardless of the improved soil C content, or the increased water-holding capacity, the higher yield per plant, or better soil structure that was measured under CA systems, the conventional farming practices outperformed most CA treatments when compared economically. Economic analysis thus failed to adequately compare these various farming systems. The economic analysis mostly responded to maize grain yield and therefore favoured all the maize-based cropping systems, especially maize monoculture which was planted in full stands. It did not take into account the poor quality of the grain from maize monoculture or the poor yield per plant, nor was the dwindling soil quality picked up in the evaluation. CA treatments, on the other hand, supported better grain

yield per plant, improved soil nutrient status, and better soil and plant health, which make this farming system more sustainable in the long term. Evaluating farming systems that have an influence on the environment and on resources cannot be effectively evaluated by putting prices on everything. The conditions necessary to achieve economic efficiency rely on unrealistic and inappropriate assumptions (Gowdy *et al.*, 2010).

Economic growth and conservation is often viewed as mutually exclusive. If a decision must be made between conserving pristine grassland and developing a new mine, the grassland stands little chance against the onslaught of economic growth. Decision making in agriculture is also mostly based on economic principles. However, pure economic analysis is inherently flawed as it fails to take into account the complexities of human behaviour and the constraints imposed by the environment (Wilson, 1998). Economic analysis only focusses on direct income and expenses for the farmer, but will not take into account sustainability, soil health or long-term use of resources such as soil; nor does it include ecosystem services, or the cost when degrading or losing these services.

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6 CONCLUSIONS AND RECOMMENDATIONS

The Zeekoegat CA trial was designed to compare several conservation agriculture practices such as tillage (reduced and conventional), in combination with various cropping systems (maize monoculture, maize/cowpea rotation, maize/soybean rotation, maize/cowpea intercropping, maize/oats intercropping and maize/vetch intercropping), and two fertilizer levels (high fertilizer and low fertilizer). Data from the trial was either collected on the entire trial (all 72 plots) (such as yield, soil analyses, mycorrhizae), or on key plots (36 plots) (such as weeds, soil water). Statistical analyses of the data distinguished between tillage alone, cropping systems alone, fertilizer level alone, as well as a combination thereof. One of the initial objectives of this study was to identify the contribution of various CA components to soil health. By separating the various components (tillage, cropping systems and fertilizer level), the contribution of each of these components can be identified.

Tillage systems

Tillage systems were responsible for some differences in results, including the following:

Maize grain yield: Initially maize grain yield was higher under reduced tillage (2008, 2009 and 2010), but in the last 3 years it was higher under conventional tillage (2011, 2012 and 2013). The lower yield under RT is attributed to topsoil compaction in the absence of tillage. The 6-year average yields between the tillage systems were similar with 2.9 ton/ha for RT and 3.1 ton/ha for CT.

Biomass: Similar to grain yield, the biomass initially performed better under RT, but in the last 3 years CT supported the highest biomass production. As with grain yield, the topsoil compaction under RT reduced root development and disadvantaged the entire crop.

Soil nutrient dynamics: A gradual build-up of organic C was observed under RT. Tillage also had an effect on K, Mg, NO₃ and total C, but the trend was not clear over time.

Aggregate stability: A positive correlation was drawn between aggregate volumes and aggregate stability and RT.

Soil water: Soil water content was consistently higher under RT compared to CT. This trend has been established since the first season.

Soil temperature: The soil was cooler under RT, possibly due to a combination of higher water content and surface cover.

Penetration resistance: Increased soil penetration resistance was measured in the topsoil of the RT treatments. It is hypothesised that a compaction layer developed in the absence of

ploughing due to the use of implements such as tractors. This increased soil resistance had a carry-over effect, reducing root development, which in turn compromised crop growth and grain yield. Poorer crop growth resulted in less biomass which compromised the entire CA system.

Weeds: RT initially resulted in lower weed biomass, but over time this changed, and weed biomass increased under RT, while it stayed similar under CT. A shift in weed species composition was also measured, with CT supporting mainly two dominant pioneer weeds, while weed diversity increased under RT over time.

Microbial activity: RT had a favourable effect on microbial activity. Clear trends over time were observed in specie richness under RT treatments.

Mycorrhizae: Initially RT had a positive influence on AM fungal spores in the soil. However, in subsequent years, CT outperformed RT.

CO₂: Higher CO₂ emissions were measured under CT compared to RT.

Tillage had no effect on nutrients in maize leaves, germination rate, nematodes or glomalin levels.

Cropping systems

Maize grain yield: Cropping systems had a significant effect on yield. The CA systems, specifically maize/vetch and maize/cowpea intercropping systems, outperformed conventional practices (monoculture).

Biomass: The trend is that cropping systems had some effect on biomass, although not always significant. Maize monoculture and maize/vetch produced the highest biomass. Maize monoculture produced high biomass because maize was planted in a full stand, and this is compared to maize planted in tramlines. Maize/vetch intercropping produced the most effective soil cover.

Soil nutrient dynamics: Cropping systems had a clearly measurable effect on soil nutrients and affected Ca, K, Mg, NH₄, NO₃, total C and total N. The maize/vetch cropping system was mostly responsible for increased nutrient status in the soil, while maize monoculture or maize/oats intercropping were associated with low nutrient status.

Leaf analyses: Initially maize/cowpea was the most beneficial in terms of nutrient uptake, but was outperformed by maize/vetch intercropping towards the end of the trial. The nutrient uptake in leaves also corresponded with crop growth of biomass and yield.

Aggregate stability: Significant correlations were found between cropping systems and organic C, which in turn significantly influenced aggregate stability and volume.

Mycorrhizae: Crop rotation and intercropping with legumes positively affected the number of AM fungal spores in the soil.

Cropping systems had a limited effect on nematodes, AM fungi and glomalin levels.

Fertilizer level

Overall the effect of fertilizer level was negligible, however, fertilizer application did have an effect on the following:

Soil nutrient dynamics: Highly significant changes were measured for soil P under the topsoil (0-5 and 5-10 cm), as a result of high fertilizer application.

Leaf analyses: Fertilizer application resulted in significant differences in nutrient uptake for Mg, Ca and K.

Mycorrhizae: Higher fertilizer application resulted in higher AM fungal spore counts.

Fertilizer had little or no effect on maize grain yield, aggregate stability, nematodes, AM fungi or glomalin levels.

Economic comparison between the CA and conventional farming systems indicated that maize monoculture and maize/vetch intercropping were the most beneficial. However, the economic analysis failed to take into account sustainable and long-term use of resources.

In summary it can be said that the results obtained from the Zeekoegat CA trial support conservation agriculture practices. Reduced tillage was responsible for many positive results and outperformed conventional tillage in most variables measured. The maize/vetch and maize/cowpea intercropping treatments were the most successful cropping systems, while maize monoculture and maize/oats intercrop often performed poorest. Fertilizer application had a very limited effect on the variables measured. A combination of reduced tillage and CA cropping systems would therefore be the most effective. Although a general positive linkage was observed for CA, there were some negative results as well. After three seasons the biomass and yield under RT were lower than under CT. This is most likely due to the increased penetration resistance that was measured in RT plots. The increased penetration resistance could indicate

topsoil compaction in the absence of tillage. Weed diversity and biomass also increased over time under RT, which can complicate weed management. The negative results do not necessarily indicate that CA is a less desirable farming system than conventional systems but rather give us more insight in how to apply CA to get optimal results.

7 THE WAY FORWARD

Some of the original objectives that were key in initiating the Zeekoegat CA trial, such as capacity building, inter-institute collaborations, and contribution to basic knowledge, were successful achieved. In the process, the following lessons were learned:

- Very few medium to long-term CA field trials exist in South Africa, and the importance thereof has been highlighted in this study.
- The importance of multi-disciplinary collaboration has been highlighted in complex systems. The research team valued the experience and access to data from other disciplines. This added tremendous value to the overall project.
- It is valuable to have an on-station field trial, as opposed to an on-farm trial. In an on-station trial the responsibility lies with the research institution, while for on-farm trials, the farmer is ultimately responsible. Experience with on-farm trials has shown that treatments can be changed without prior notice to the researcher, or undesired routine activities can occur due to a lack of understanding by farm workers.
- Accessibility to the trial is important. Zeekoegat was ideally situated at Roodeplaat, only 30 km from the ARC-ISCW and less than 5 km from the ARC-PPRI. This allowed for frequent visits by researchers and ensured good quality control. Data collection is also much easier, especially for frequently measured data such as soil water. It also reduced costs (no long distance travelling, flights, overnight accommodation, etc.).
- The trial layout needs to be as simple as possible, with as few treatments as possible. Our experience with the Zeekoegat trial was that 72 plots were difficult to sample, and frequent analyses on all plots were time consuming and expensive. As a result only 36 key plots were selected on which all the data was collected.
- Plots sizes need to be large enough to ensure intensive data collection over the medium term. The 7 x 8 m plots of Zeekoegat were too small, and posed some challenges in terms of data collection.
- The research team feels that the Zeekoegat CA trial was a good learning experience. We would like to continue this collaboration and implement the lessons learned.
- It would be ideal if several medium- to long-term CA trials in different agro-ecological zones could be initiated.

8 INCOME AND EXPENSES

The project was jointly funded by the ARC-ISCW and the Maize Trust (MT). Income received from both funders is presented in Table 5.12-1 and expenditure is summarised in Table 8-1 and 8.2.

Table 8-1: Income received for Zeekoegat project

Year	ARC-ISCW	MT	Total
2007/08	R555 560		R555 560
2008/09	R500 000		R500 000
2009/10	R650 000	R429 910	R1 079 910
2010/11	R734 100	R472 900	R1 207 000
2011/12	R775 500	R494 000	R1 269 500
2012/13	R119 500	R544 000	R663 500
2013/14		R460 000	R460 000
Total	R3 334 660	R2 400 810	R5 735 470

Table 80-2: Expenditure for Zeekoegat project

	2007/08	2008/09	2009/2010	2010/11	2011/12	2012/13	2013/14
ARC-ISCW Core funding: GW 59/010							
Administration	R 21 281	R 39 632	-R 2 470	R 0	R 770	R 0	
Field trial: direct expenses	R 28 750	R 0	R 0	R 0	R 8 195	R 0	
Inter-Institute	R 741	R 2 020	-R 2 599	R 7 831	R 0	R 0	
Knowledge dissemination	R 26 940	R 62 280	R 77 040	R 156 744	R 101 690	R 0	
Lab analyses	R 78 801	R 219 274	R 411 575	R 591 906	R 541 603	R 74 829	
Manpower & overheads	R 399 047	R 176 794	R 166 454	R -22 381	R 123 242	R 44 671	
TOTAL ARC-ISCW	R 555 560	R 500 000	R 650 000	R 734 100	R 775 500	R 119 500	
Maize Trust funding: GW 59/010/02							
Administration			R 3 821	R 1 866	R 3 441	R 3 251	R 3 015
Field trial: direct expenses			R 63 472	R 29 969	R 75 505	R 33 767	R 33 151
Inter-Institute			R 233 878	R 302 729	R 207 748	R 75 845	R 3 250
Knowledge dissemination			R 22 472	R 9 332	R 20 625	R 15 001	R 14 801
Lab analyses			R 36 600	R 36 240	R 47 574	R 185 638	R 25 662
Manpower and overheads			R 69 667	R 92 764	R 139 107	R 230 498	R 380 121
TOTAL MT			R 429 910	R 472 900	R 494 000	R 544 000	R 460 000

APPENDICES

Appendix 1: Publication List

To date, the following publications and presentations have resulted from the Zeekoegat Conservation Agriculture project:

Technical Reports

1. Smith HJ, Swanepoel CM, Kidson MV, Trytsman G, Bloem JF, Habig J, Stronkhorst LD, Fyfield TP, Beukes DJ. 2008. Quantifying the effects of conservation agriculture (CA) practices on soil and plant properties, social acceptability and economic viability. First annual progress report. ARC-ISCW Report No. GW/A/2008/21. ARC-Institute for Soil, Climate and Water, Pretoria.
2. Swanepoel CM, Smith HJ, Marais M, Swart A, Habig J, Koch S, Sekgota WM, Kidson MV, Fyfield TP, Trytsman G, Beukes DJ. 2009. Quantifying the effects of conservation agriculture (CA) practices on soil and plant properties, social acceptability and economic viability. Second annual progress report. ARC-ISCW Report No. GW/A/2009/24. ARC-Institute for Soil, Climate and Water, Pretoria.
3. Swanepoel CM, Beukes DJ, Marais M, Swart A, Habig J, Koch S, Sekgota WM, Kidson MV, Trytsman G., Fyfield TP, Smith HJ. 2010. Quantifying the effects of conservation agriculture (CA) practices on soil and plant properties, social acceptability and economic viability. Third annual progress report. ARC-ISCW Report No. GW/A/2010/25. ARC-Institute for Soil, Climate and Water, Pretoria.
4. Smith HJ, Barnard RO, De Villiers MC, Beukes DJ, Lange D, Putter T. 2010. Final Report to FAO Representative in South Africa on The Establishment and Management of Demonstration Sites and Synthesis of Conservation Agriculture (CA) in South Africa. Southern Africa Project OSRO/RAF/904/USA. ARC-ISCW Report No. GW/A/2010/37. ARC-Institute for Soil, Climate and Water, Pretoria.
5. Swanepoel CM, Marais M, Swart A, Habig J, Koch S, Sekgota WM, Kidson MV, Trytsman G, Beukes DJ. 2011. Quantifying the effects of conservation agriculture (CA) practices on soil and plant properties, social acceptability and economic viability. Fourth annual progress report. ARC-ISCW Report No. GW/A/2011/36. ARC-Institute for Soil, Climate and Water, Pretoria.
6. Swanepoel CM, Marais M, Swart A, Habig J, Koch S, Sekgota WM, Mampana R, Trytsman G, Beukes DJ. 2012. Quantifying the effects of conservation agriculture (CA) practices on soil and plant properties. Fifth annual progress report. ARC-ISCW Report No. GW/A/2012/33. ARC-Institute for Soil, Climate and Water, Pretoria.
7. Swanepoel CM, Marais M, Swart A, Habig J, Koch S, Sekgota WM, Mampana R, Trytsman G, Beukes DJ. 2013. Quantifying the effects of conservation agriculture (CA) practices on soil and plant properties. Sixth annual progress report. ARC-ISCW Report No. GW/A/2013/15. ARC-Institute for Soil, Climate and Water, Pretoria.

Presentations

1. Beukes DJ. 2010. CA Research and demonstration site at Zeekoegat Experimental Farm. (*Oral presentation at FAO Workshop: Synthesis of CA Information in South Africa, Pretoria, 30 March 2010.*)

2. Habig J. 2010. Approaches to measuring the abundance, activity and diversity of soil microbial populations & Micro-organisms in No-Till Systems. (*Oral Presentation at OMNIA Agriculturalist Conference, 26/05/2010.*)
3. Habig J. 2010. The role of bio-fertilizers in crop production and conservation agriculture. (*Oral Presentation at Bio-Fertilizer Workshop hosted by ARC-GCI, 25/08/2010.*)
4. Habig J. 2010. Soil microbial community dynamics in the Zeekoegat Conservation Agriculture Trial. (*Oral Presentation at Plant Pathology & Microbiology Science Meeting at ARC-PPRI, 08/09/2010.*)
5. Habig J. 2010. Grondmikrobiese gemeenskapsdinamika in die Zeekoegat Bewaringsboerdery-proef, Roodeplaat. (*Oral Presentation at the Suid-Afrikaanse Akademie vir Wetenskap en Kuns Jaarkongres hosted by ARC-PPRI, 01/10/2010.*)
6. Habig J. 2010. Soil microbial population dynamics in the Zeekoegat Conservation Agriculture Trial, Roodeplaat. (*Oral Presentation at Combined Congress hosted by University of Pretoria, 20/01/2011.*)
7. Kidson MV, Swanepoel CM, Beukes DJ. 2011. Chamber design for in-field soil CO₂ flux measurement. (*Poster presentation at Combined Congress, University of Pretoria, 20/01/2011.*)
8. Sekgota WM. 2011. Arbuscular mycorrhizal (AM) fungi as an indicator of soil health. (*Oral presentation at ARC-PPRI Science day, Seminar Room at Roodeplaat-East, 23/02/2011.*)
9. Sekgota WM, Koch SH, Dames JF. 2011. Arbuscular mycorrhizal (AM) fungi as indicators of soil health. (*Poster presentation at Combined Congress hosted by the University of Pretoria, 20/01/2011.*)
10. Swanepoel CM, Beukes DJ. 2011. Effects of Conservation Agriculture on selected soil properties and yields under dry-land. (*Oral Presentation at Combined Congress, UP, 20/01/2011.*)
11. Beukes DJ. 2011. Theory and practice of neutron scattering to measure soil water content. (*ARC-ISCW Seminar.*)
12. Swanepoel CM. 2012. Evaluating a low-technology closed-chamber method for measuring greenhouse gas emissions from agricultural soil. (*Combined Congress, North-West University, Potchefstroom, 16-17 January 2012.*)
13. Beukes DJ. 2012 Soil aggregate stability. (*Zeekoegat Information Day, February 2012.*)
14. Mampana R. 2012. Cropping system effects on soil water, soil temperature and dry-land maize productivity. (*Oral Presentation at University of Pretoria Post Graduate Seminar.*)
15. Swanepoel CM, Beukes DJ. 2014. Interpretation of soil penetration values under various cultivation practices: A case study. (*Combined Congress, Grahamstown, 20-23 January 2014.*)
16. Beukes DJ, Swanepoel CM. 2014. The effect of conventional and conservation agriculture practices on soil aggregate stability and related properties. (*Combined Congress, Grahamstown, 20-23 January 2014.*)
17. Habig J. 2014. Effect of Conservation Agriculture on soil microbial community dynamics. (*Oral Presentation at 1st Africa Congress on Conservation Agriculture, Lusaka, Zambia, 18-21 March 2014.*)
18. Marais M, Swart A, Habig J. 2014. The effect of conservation agriculture on nematode populations and soil microbial community dynamics. (*International Congress of Nematology, Cape Town, April 2014.*)

Popular Articles

1. Habig J. 2010. Soil Microbiology: Linking Conservation Agriculture with the Underground. *Plant Protection News* No. 83: 16.
2. Hittersay P. 2009. ARC starts researching CA. *Farmer's Weekly* 19 June 2009.
3. Marais M. 2009. Plant-parasitic nematodes of the Zeekoegat Conservation Agriculture trial. *Plant Protection News* No. 80, April-June 2009, ARC-PPRI.

Peer-Reviewed Articles

1. Main R, Cho MA, Mathieu R, O'Kennedy M, Abel Ramoelo A, Koch S. Comparing published spectral indices for the estimation of leaf chlorophyll. *ISPRS Journal of Photogrammetry and Remote Sensing* (accepted).
2. Van den Berg E, Tiedt LR, Coyne DL, Ploeg AT, Navas-Cortes JA, Roberts PA, Yeats GW, Subbotin SA. 2013 Morphological and molecular characterisation and diagnostics of some species of *Scutellonema* Andrassy, 1958 (Tylenchida: Hoplolaimidae) with a molecular phylogeny of the genus. *Nematology* 15. Open source, online January 2013.
3. Habig J. 2013. Grondmikrobiese gemeenskapsdinamika in die Zeekoegat Bewaringsboerdery-proef, Roodeplaat. *Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie* 32(1), Art. #796, 2 pp.
4. Habig J. 2014. Effects of Conservation Agriculture on soil microbial community dynamics. *Book of Condensed Papers: 1st Africa Congress on Conservation Agriculture, 18-21 March 2014, Lusaka, Zambia* (accepted).
5. Mampana RM, Beukes DJ, Swanepoel CM. 2014 Soil elements other than hydrogen that can affect the field calibration of a neutron water meter. *South African Journal of Plant and Soil* (submitted).
6. Swanepoel CM, Habig J, Swanepoel LH. 2014. Temporal variation in weed occurrence and biomass under conservation agriculture and conventional tillage practices at Zeekoegat, Gauteng, South Africa. *South African Journal of Plant and Soil* (submitted).

Theses

1. Mampana RM. 2014. Cropping effects on soil water, soil temperature and dry-land maize productivity. MSc (Agric) thesis, Department of Plant Production and Soil Science, University of Pretoria (completed and passed).
2. Sekgota WM. 2014. Arbuscular mycorrhizal fungi as a bio-indicator of soil health under agricultural management practices in South Africa. MSc thesis, Department of Biochemistry and Microbiology, Rhodes University (submitted).
3. Baker C. Weed dynamics under conservation agriculture compared to conventional farming systems. M.Inst Agrar thesis, Department of Plant Production and Soil Science, University of Pretoria (to be completed in 2015).
4. Swanepoel CM. Assessment of carbon and nitrogen dynamics in cultivated South Africa soils and associated greenhouse gas emissions. Chapter in PhD thesis. Department of Plant Production and Soil Science, University of Pretoria (to be completed in 2017).

Information Days

1. Zeekoegat CA Information Day. 11 March 2009.

2. Soil Ecology and Conservation Agriculture Workshop. 5 October 2009. Presenter: Dr Jill Clapperton, University of Lethbridge, Canada.
3. Zeekoegat CA Information Day. 24 February 2010.
4. Zeekoegat CA Information Day. 29 February 2012.

Awareness Material

1. **Pamphlet:** Conservation Agriculture: A Research Trial at Roodeplaat. *ARC-ISCW, EcoPort & FAO*.
2. **Poster:** Practices Conservation Agriculture: Increase Profits and Combat Climate Change. *Ecoport, FAO & ARC-ISCW (Poster Serial Number 78415)*.
3. Agricultural Research Council. 2011. Quantifying effects of conservation agriculture on soil health, as well as on crop growth and yield: Section summary in ARC Annual Report, 2010-2011.

TV/Radio Broadcasts

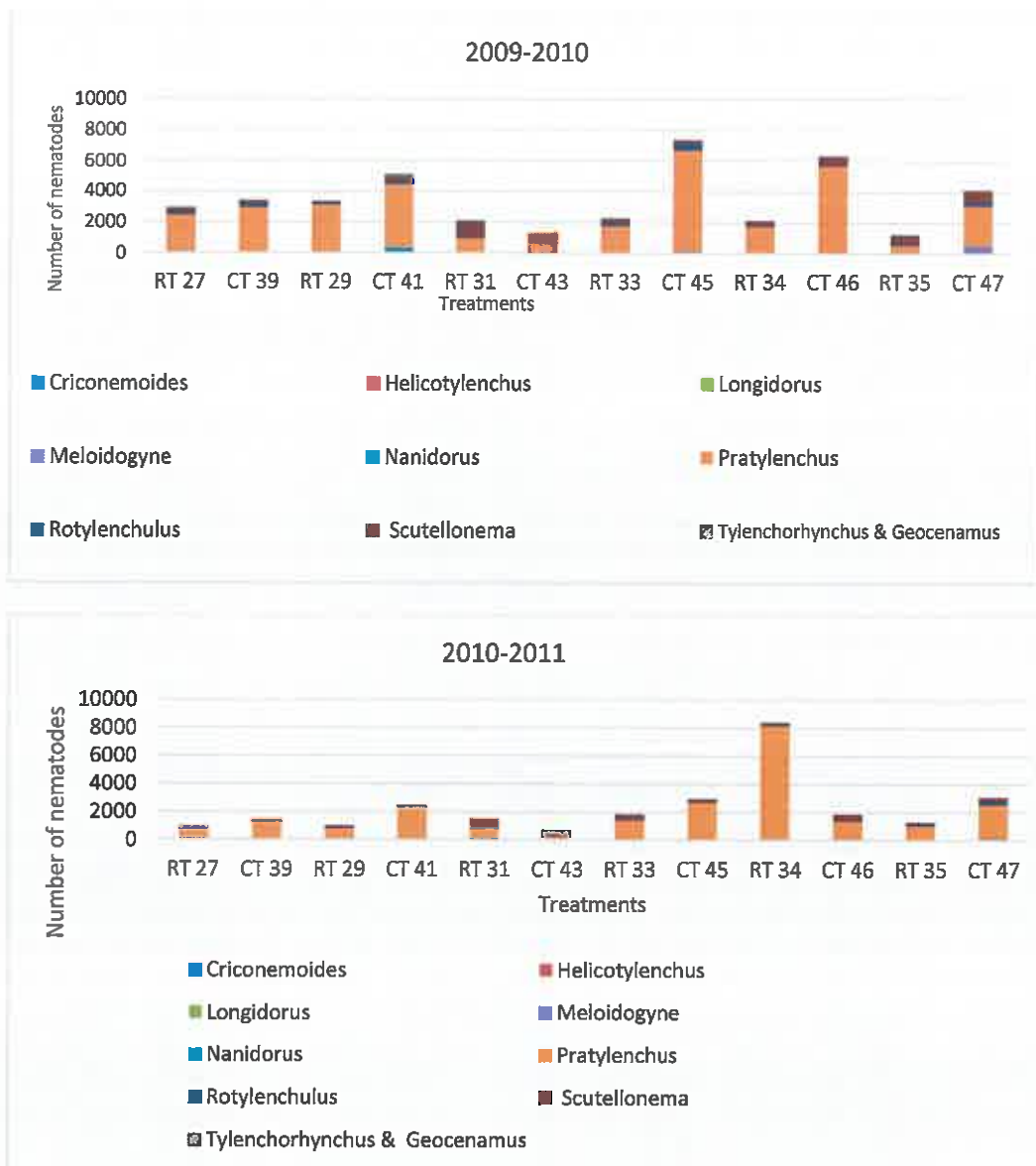
1. Information Day – ARC. Broadcast on 29 April 2009, AgriTV.
2. ARC Soil Ecology and Conservation Agriculture Workshop. Broadcast on 19 October 2010, AgriTV.
3. Information Day – ARC. Broadcast on 12 March 2012, AgriTV & 20 March 2012, Landbousake, KykNet.
4. Conservation Agriculture Field Visit (20/03/2014) as part of 1st African Congress on CA - Lusaka, Zambia. Interviewed by China Central Television (CCTV), Ethiopia – Broadcast date to be confirmed.
5. Conservation Agriculture Field Visit (20/03/2014) as part of 1st African Congress on CA - Lusaka, Zambia. Interviewed by Newmark Group Ltd, Kenya – Broadcast date to be confirmed.

Appendix 2: Nematology

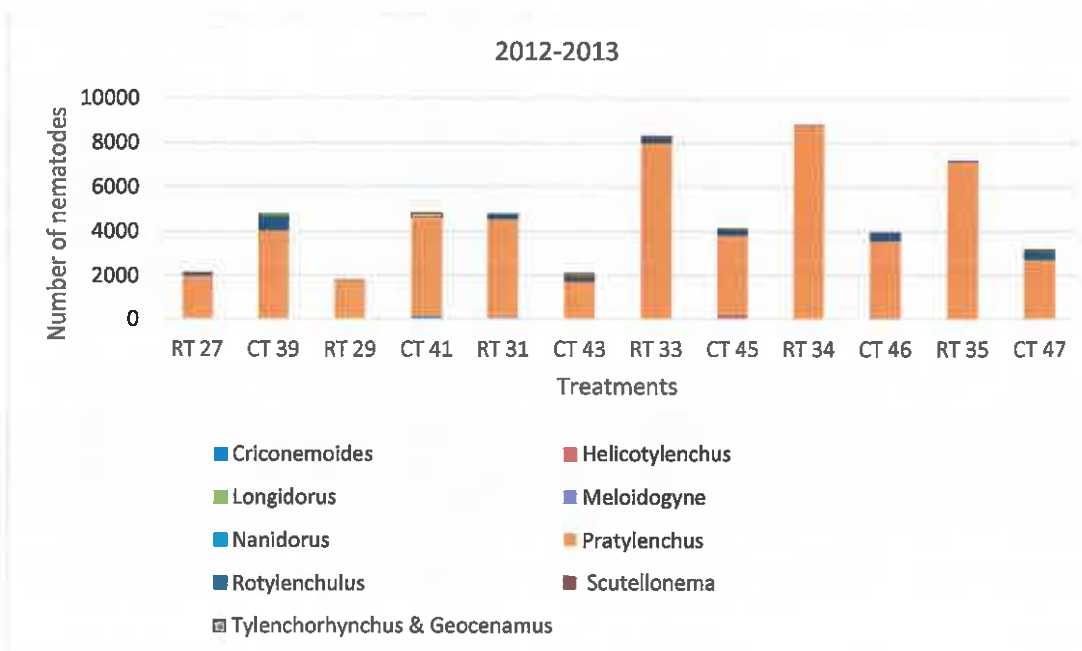
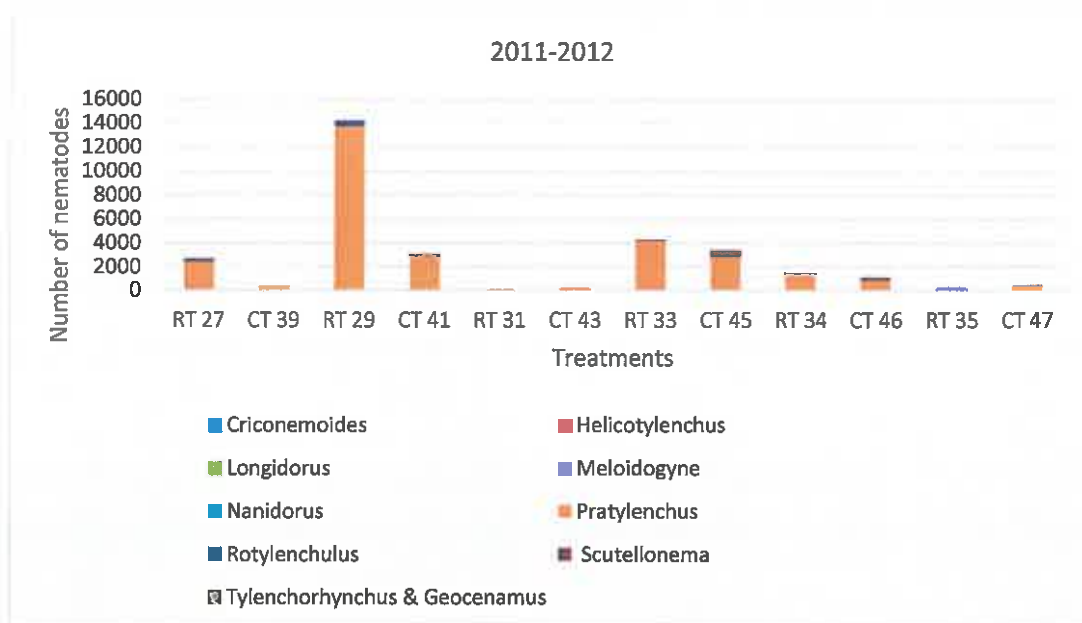
Supplement Table N1: Nematodes found in the soil and roots of key plots of Block B

Plot number	Tillage	Bacterivore	Fungivore	Omnivore	Predator	<i>Criconemoides sphaerocephalus</i>	<i>Criconemoides xenoplax</i>	<i>Helicotylenchus sp.</i>	<i>Helicotylenchus digonicus, H. dihystra</i>	<i>Helicotylenchus dihystra</i>	<i>Hemicyclophora typica</i>	<i>Longidorus pisi</i>	<i>Meloidogyne sp. (I)</i>	<i>Meloidogyne javanica</i>	<i>Nanidorus minor</i>	<i>Pratylenchus brachyurus, P. penetrans, P. zeae</i>	<i>Rotylenchulus pervus</i>	<i>Scutellonema brachyurus</i>	<i>Tylenchorhynchus sp., T. brevilineatus, Geocenamus brevidens</i>	Total free-living	Total herbivore	Total nematodes	% Herbivore	% Free-living	% Bacterivore	% Fungivore	% Omnivore			
37	C 200 T 9	-	-	-	-	0	0	0	185	0	0	0	0	0	14	1930	110	0	0	0	3355	-	-	-	-	-	-	-	-	-
38	C 200 T 9	-	-	-	-	0	0	0	165	0	15	0	0	0	70	960	390	0	0	0	1600	-	-	-	-	-	-	-	-	
39	C 201 T 0	53	180	0	5	0	0	0	0	0	0	0	0	0	35	2885	280	19	0	715	3395	4110	83	17	13	4	0	0	0	
39	C 201 T 1	85	455	20	15	0	0	0	0	10	0	0	0	0	40	1215	60	80	0	134	1405	2750	51	48	9	31	5	7	0	
39	C 201 T 2	12	150	0	10	0	0	0	0	0	0	0	0	0	0	280	0	5	0	280	285	565	50	49	6	21	5	0	0	
39	C 201 T 3	27	440	0	0	5	0	0	0	0	0	5	0	0	35	3995	550	20	180	710	4790	5500	87	12	9	5	8.0	0	0	
40	C 200 T 9	-	-	-	-	0	0	0	117	0	0	0	0	0	13	455	70	0	0	1970	-	-	-	-	-	-	-	-	-	
41	C 200 T 9	-	-	-	-	0	5	0	250	0	0	0	0	0	55	225	135	0	0	670	-	-	-	-	-	-	-	-	-	
41	C 201 T 0	27	265	0	5	5	0	0	0	0	0	0	0	0	30	4170	125	29	230	545	5120	5665	90	9.6	5	4.7	0	0	0	
41	C 201 T 1	28	130	20	0	10	0	0	0	5	0	0	0	0	30	2225	85	75	60	430	2490	2920	85	14	7	10	4.5	7	0	
41	C 201 T 2	65	190	0	10	0	0	0	0	0	0	0	0	0	80	2795	40	16	0	265	3075	3340	92	7.9	2	5.7	0	0	0	
41	C 201 T 3	50	315	0	15	0	0	0	0	0	0	5	0	0	13	4500	35	80	70	380	4820	5200	93	7.3	1	6.1	0	0	0	
42	C 200 T 9	-	-	-	-	0	0	0	220	0	10	0	0	0	10	625	95	0	300	-	1260	-	-	-	-	-	-	-	-	
43	C 200 T 9	-	-	-	-	0	0	0	280	0	0	0	0	0	45	45	70	0	740	-	1190	-	-	-	-	-	-	-	-	
43	C 201 T 0	18	158	10	5	20	0	0	0	0	0	0	0	77	0	508	45	62	60	356	1332	1688	79	21	11	9.4	0	6	0	

Supplement Figure N1: Population numbers of herbivore genera



Supplement Figure N1 (continued)



Appendix 3.1: Microbiology (1)

Soil microbial diversity under various agricultural practices over a period of five seasons, i.e. January 2009 to January 2013.

	Shannon-Weaver		Evenness
FH-MS_CT-10	2.127 ^a	FL-ML_RT-12	0.686 ^a
FL-MS_CT-10	2.143 ^{ab}	FL-ML_RT-13	0.698 ^{ab}
FL-MS_RT-10	2.173 ^{abc}	FH-MS_CT-12	0.699 ^{abc}
FL-ML_RT-12	2.187 ^{abc}	FL-MM_CT-10	0.724 ^{abcd}
FL-ML_CT-12	2.212 ^{abcd}	FL-ML_CT-12	0.724 ^{abcde}
FL-ML_CT-10	2.217 ^{abcd}	FH-MM_RT-11	0.737 ^{abcdef}
FH-MS_CT-12	2.222 ^{abcd}	FH-ML_CT-10	0.741 ^{abcdefg}
FL-MS_RT-12	2.231 ^{abcde}	FH-MS_RT-12	0.747 ^{abcdefgh}
FL-MM_CT-10	2.279 ^{abcdef}	FL-ML_CT-10	0.748 ^{abcdefgh}
FL-MS_CT-12	2.290 ^{abcdefg}	FH-MM_RT-10	0.748 ^{abcdefgh}
FH-MS_RT-10	2.308 ^{abcdefg}	FH-ML_CT-13	0.755 ^{abcdefgh}
FL-ML_RT-13	2.309 ^{abcdefg}	FL-MS_RT-10	0.768 ^{abcdefghi}
FH-ML_CT-10	2.314 ^{abcdefgh}	FH-MS_RT-10	0.768 ^{abcdefghi}
FL-ML_CT-11	2.326 ^{abcdefgh}	FH-MS_CT-13	0.770 ^{abcdefghij}
FL-MM_RT-10	2.336 ^{abcdefgh}	FL-MS_CT-12	0.778 ^{bcdefghijk}
FH-MS_CT-09	2.346 ^{abcdefghi}	FH-MS_CT-09	0.783 ^{bcdefghijkl}
FH-MS_RT-12	2.351 ^{abcdefghi}	FH-MS_RT-09	0.784 ^{bcdefghijkl}
FH-ML_CT-13	2.356 ^{abcdefghi}	FH-MS_CT-11	0.786 ^{cdefghijkl}
FL-MS_RT-11	2.371 ^{abcdefghi}	FL-MM_CT-09	0.788 ^{defghijkl}
FH-MM_RT-11	2.372 ^{abcdefghi}	FH-ML_RT-12	0.788 ^{defghijkl}
FL-MS_CT-11	2.380 ^{abcdefghi}	FL-MS_RT-09	0.789 ^{defghijkl}
FL-MM_RT-11	2.394 ^{abcdefghi}	FL-MM_CT-12	0.789 ^{defghijkl}
FH-MS_CT-11	2.399 ^{abcdefghi}	FH-MM_CT-09	0.790 ^{defghijkl}
FH-MS_CT-13	2.404 ^{abcdefghi}	FL-MS_CT-09	0.791 ^{defghijkl}
FL-ML_CT-13	2.428 ^{abcdefghij}	FL-MS_RT-11	0.795 ^{defghijkl}
FH-MM_CT-09	2.429 ^{abcdefghij}	FH-MM_RT-09	0.795 ^{defghijkl}
FL-MS_RT-13	2.432 ^{abcdefghij}	FL-MM_RT-11	0.804 ^{defghijkl}
FL-MS_RT-09	2.433 ^{abcdefghij}	FH-MM_CT-10	0.807 ^{defghijkl}
FH-MM_RT-10	2.436 ^{abcdefghij}	FL-MS_RT-12	0.807 ^{defghijkl}
FH-ML_CT-12	2.437 ^{abcdefghijk}	FH-ML_RT-11	0.811 ^{efghijkl}
FH-ML_RT-10	2.462 ^{abcdefghijkl}	FH-MM_CT-13	0.811 ^{fghijkl}
FL-MS_CT-09	2.475 ^{abcdefghijkl}	FL-MM_CT-13	0.813 ^{fghijkl}
FH-MS_RT-09	2.476 ^{abcdefghijkl}	FH-ML_RT-10	0.817 ^{fghijkl}
FL-MM_CT-09	2.477 ^{abcdefghijkl}	FL-MS_RT-13	0.818 ^{fghijkl}
FH-ML_RT-12	2.483 ^{abcdefghijkl}	FL-MM_RT-09	0.818 ^{fghijkl}
FH-MM_CT-13	2.496 ^{abcdefghijkl}	FL-ML_CT-13	0.819 ^{fghijkl}
FL-MS_CT-13	2.515 ^{bcdefghijkl}	FL-MM_CT-11	0.819 ^{fghijkl}
FH-MM_RT-09	2.524 ^{cdefghijklm}	FH-ML_CT-12	0.821 ^{fghijkl}
FL-MM_CT-12	2.536 ^{cdefghijklm}	FL-MS_CT-11	0.824 ^{fghijkl}
FL-MM_RT-09	2.569 ^{defghijklm}	FH-MS_CT-10	0.825 ^{ghijkl}
FH-MM_CT-12	2.584 ^{defghijklm}	FL-MS_CT-10	0.825 ^{ghijkl}
FH-ML_RT-13	2.606 ^{efghijklm}	FL-MM_RT-10	0.825 ^{ghijkl}
FL-MM_CT-11	2.608 ^{fghijklm}	FH-ML_RT-13	0.828 ^{ghijkl}
FH-MM_CT-11	2.616 ^{fghijklm}	FH-MM_CT-12	0.831 ^{hijkl}

FL-ML_RT-11	2.624 ^{fghijklm}		FL-ML_RT-10	0.833 ^{hijkl}
FH-ML_RT-11	2.644 ^{fghijklm}		FH-MM_CT-11	0.843 ^{ijkl}
FL-MM_CT-13	2.652 ^{fghijklm}		FL-ML_CT-11	0.846 ^{ijkl}
FH-MS_RT-11	2.653 ^{fghijklm}		FL-MS_CT-13	0.850 ^{ijkl}
FH-MM_CT-10	2.655 ^{fghijklm}		FH-MM_RT-12	0.858 ^{jkl}
FL-ML_RT-10	2.658 ^{ghijklm}		FH-MS_RT-13	0.858 ^{kl}
FH-MM_RT-13	2.692 ^{hijklm}		FL-ML_RT-11	0.859 ^{kl}
FH-ML_CT-11	2.719 ^{ijklm}		FH-MS_RT-11	0.859 ^{kl}
FL-MM_RT-13	2.786 ^{jklm}		FH-ML_CT-11	0.860 ^{kl}
FH-MS_RT-13	2.814 ^{klm}		FH-MM_RT-13	0.861 ^{kl}
FH-MM_RT-12	2.836 ^{lm}		FL-MM_RT-12	0.864 ^{kl}
FL-MM_RT-12	2.901 ^m		FL-MM_RT-13	0.868 ^l

* Means within a row followed by the same letter, do not differ significantly ($p > 0.05$).

Appendix 3.2: Microbiology (2)

The activities of four soil microbial enzymes under various agricultural practices over a period of five seasons, i.e. January 2009 to January 2013.

	2009	2010	2011	2012	2013
Sample name	β-glucosidase Activity (<i>p</i>-nitrophenol $\mu\text{g/g/h}$)				
FL-MM_RT	559.133	992.939	2641.955	810.443	1481.330
FL-MS_RT	628.022	1172.307	2199.695	854.772	1405.486
FL-ML_RT		1073.400	2538.257	966.651	1023.073
FH-MM_RT	623.576	1207.951	2652.648	920.797	1267.992
FH-MS_RT	555.705	1196.938	2419.701	969.851	972.756
FH-ML_RT		1098.339	2327.177	1102.909	1202.671
FL-MM_CT	524.067	943.277	1429.032	705.168	1007.872
FL-MS_CT	574.026	818.253	1199.910	660.473	1143.142
FL-ML_CT		934.129	1371.703	779.520	1169.210
FH-MM_CT	565.503	923.512	1475.376	1006.952	1383.756
FH-MS_CT	633.297	705.743	1349.166	639.761	1077.997
FH-ML_CT		1091.047	1455.599	914.058	1062.626
Sample name	Alkaline Phosphatase Activity (<i>p</i>-nitrophenol $\mu\text{g/g/h}$)				
FL-MM_RT	451.819	751.112	2268.824	377.071	981.286
FL-MS_RT	486.969	869.840	2395.611	886.617	1130.778
FL-ML_RT		869.431	2094.445	905.815	1066.055
FH-MM_RT	537.332	950.593	2139.818	700.465	949.228
FH-MS_RT	440.101	1024.875	2121.436	894.729	911.948
FH-ML_RT		844.238	1613.168	767.470	975.379
FL-MM_CT	411.894	1227.900	1432.703	757.460	885.655
FL-MS_CT	553.343	766.533	1275.809	901.159	839.681
FL-ML_CT		805.563	1267.206	587.446	929.429
FH-MM_CT	434.583	913.975	1498.618	836.451	931.455
FH-MS_CT	445.864	816.005	1510.010	698.793	888.417
FH-ML_CT		822.115	1628.901	654.432	941.265
Sample name	Acid Phosphatase Activity (<i>p</i>-nitrophenol $\mu\text{g/g/h}$)				
FL-MM_RT	997.822	1603.766	2051.199	1140.498	1882.143
FL-MS_RT	993.644	1591.444	2031.542	1171.200	1870.727
FL-ML_RT		1581.809	2011.796	1133.837	1875.391
FH-MM_RT	1002.209	1539.529	2005.894	1176.239	1883.363
FH-MS_RT	982.193	1563.680	2043.680	1147.267	1874.509
FH-ML_RT		1564.571	2007.287	1100.071	1871.372
FL-MM_CT	994.709	1539.227	1995.350	1021.451	1872.811
FL-MS_CT	992.823	1537.120	1983.142	995.316	1877.470
FL-ML_CT		1556.679	1973.930	1246.532	1881.179
FH-MM_CT	989.067	1562.270	2028.915	1166.390	1871.400
FH-MS_CT	986.405	1517.071	2033.215	1141.686	1866.851
FH-ML_CT		1555.200	2042.476	1093.546	1875.914

Sample name	Urease Activity (NH ₄ -N µg/g/2h)				
FL-MM_RT	36.286	32.518	57.562	49.878	64.652
FL-MS_RT	20.911	30.130	60.135	56.436	54.823
FL-ML_RT		10.255	49.770	47.125	47.618
FH-MM_RT	37.498	32.324	52.245	39.709	53.752
FH-MS_RT	37.462	44.967	66.361	64.584	46.876
FH-ML_RT		35.853	60.679	69.407	50.568
FL-MM_CT	36.672	32.505	49.793	55.945	45.740
FL-MS_CT	41.494	34.952	48.161	54.164	50.637
FL-ML_CT		38.250	56.606	57.579	51.926
FH-MM_CT	37.763	31.618	52.855	64.423	57.163
FH-MS_CT	41.762	33.711	50.371	51.532	41.661
FH-ML_CT		39.465	50.075	67.339	45.876

Appendix 4: Economic Analysis

2008

Crop: main	Crop: inter	Till	Treat	Grain yield ton/ha			Grain income R/ha	Fodder / biomass ton/ha			Fodder income R/ha	Fert cost R/ha	Seed cost R/ha		Land prep R/ha	2008 Gross profit R/ha
				Maize ton/ha	R/ton	Soybean/Cowpea ton/ha		R/ton	R/ton	R/ton			Maize	Cowpea/Soybean/Oats/Vetch		
Maize		R	1	7.04	1652		11629	7.04	550		3872	444	360	201	14496	
Cowpea		R	2			0	0			3	2700	116		201	2183	
Soybean		R	3			0.7	1834			1.5	900	116		201	2209	
Maize	Cowpea	R	4	7.39	1652		12208	7.39	550	0.5	4515	560	360	201	15453	
Maize	Oats	R	5	7.21	1652		11904	7.21	550	2.486	8936	560	360	201	19370	
Maize	Vetch	R	6	7.75	1652		12804	7.75	550	1.740	6873	560	360	201	17557	
Maize		R	7	6.80	1652		11224	6.80	550		3737	887	360	201	13514	
Cowpea		R	8			0	0			3	2700	232		201	2067	
Soybean		R	9			0.6	1572			1.5	900	232		201	1831	
Maize	Cowpea	R	10	5.93	1652		9793	5.93	550	0.5	3711	1119	360	201	11674	
Maize	Oats	R	11	6.55	1652		10819	6.55	550	3.072	9747	1119	360	201	18535	
Maize	Vetch	R	12	7.64	1652		12619	7.64	550	1.854	6982	1119	360	201	16922	
Maize		C	1	6.15	1652		10161	6.15	550		3383	444	360	402	12339	
Cowpea		C	2			0	0			3	2700	116		402	1982	
Soybean		C	3			0.57	1493			1.5	900	116		402	1667	
Maize	Cowpea	C	4	6.61	1652		10918	6.61	550	0.5	4085	560	360	402	13532	
Maize	Oats	C	5	6.52	1652		10764	6.52	550	2.674	8932	560	360	402	18025	
Maize	Vetch	C	6	6.28	1652		10381	6.28	550	1.291	5394	560	360	402	13453	
Maize		C	7	7.26	1652		11998	7.26	550		3995	887	360	402	14345	
Cowpea		C	8			0	0			3	2700	232		402	1866	
Soybean		C	9			0.48	1258			1.5	900	232		402	1316	
Maize	Cowpea	C	10	4.07	1652		6729	4.07	550	0.5	2691	1119	360	402	7388	
Maize	Oats	C	11	6.16	1652		10174	6.16	550	3.627	10642	1119	360	402	18585	
Maize	Vetch	C	12	7.30	1652		12059	7.30	550	2.112	7183	1119	360	402	16261	

2009

Crop: main	Crop: inter	Till	Treat	Grain yield ton/ha			Grain income	Fodder / biomass ton/ha			Fodder income	Fert cost	Seed cost R/ha		Land prep	2009
				Maize ton/ha	R/ton	Soybean/Cowpea ton/ha		R/ton	Maize	R/ton			Cowpen/Soybean/Oats/Vetch ton/ha	R/ton		
Maize		R	1	3.92	1208		4734	3.92	550		2155	1089	500	270	5031	
Maize		R	2	8.52	1208		10293	8.52	550		4686	1089	500	270	13120	
Maize		R	3	4.59	1208		5544	4.59	550		2524	1089	500	270	6209	
Maize	Cowpea	R	4	3.42	1208		4131	3.42	550	0.5	2431	1386	500	200	4206	
Maize	Oats	R	5	3.93	1208		4745	3.93	550	0.852	3566	1386	500	370	5785	
Maize	Vetch	R	6	4.77	1208		5762	4.77	550	0.64	3584	1386	500	1200	5990	
Maize		R	7	3.78	1208		4572	3.78	550		2082	2177	500	270	3706	
Maize		R	8	9.52	1208		11502	9.52	550		5237	2177	500	270	13792	
Maize		R	9	5.55	1208		6700	5.55	550		3051	2177	500	270	6804	
Maize	Cowpea	R	10	5.45	1208		6586	5.45	550	0.5	3549	2773	500	200	6392	
Maize	Oats	R	11	3.43	1208		4142	3.43	550	1.718	4721	2773	500	370	4951	
Maize	Vetch	R	12	5.52	1208		6664	5.52	550	1.515	5307	2773	500	1200	7229	
Maize		C	1	5.55	1208		6705	5.55	550		3053	1089	500	540	7629	
Maize		C	2	5.92	1208		7149	5.92	550		3255	1089	500	540	8276	
Maize		C	3	6.39	1208		7721	6.39	550		3515	1089	500	540	9107	
Maize	Cowpea	C	4	3.87	1208		4681	3.87	550	0.5	2681	1386	500	200	4735	
Maize	Oats	C	5	3.17	1208		3827	3.17	550	1.015	3417	1386	500	370	4447	
Maize	Vetch	C	6	5.28	1208		6378	5.28	550	0.783	4079	1386	500	1200	6831	
Maize		C	7	4.31	1208		5207	4.31	550		2371	2177	500	540	4361	
Maize		C	8	6.72	1208		8121	6.72	550		3697	2177	500	540	8601	
Maize		C	9	7.32	1208		8843	7.32	550		4026	2177	500	540	9652	
Maize	Cowpea	C	10	4.02	1208		4861	4.02	550	0.5	2763	2773	500	200	3611	
Maize	Oats	C	11	3.31	1208		4001	3.31	550	0.497	2641	2773	500	370	2459	
Maize	Vetch	C	12	4.89	1208		5912	4.89	550	1.222	4525	2773	500	1200	5425	

2010

Crop: main	Crop: inter	Till	Treat	Grain yield ton/ha			Grain income	Fodder / biomass ton/ha			Fodder income	FERT cost	Seed cost R/ha		Land prep	2010
				Maize ton/ha	R/ton	Soybean/Cowpea ton/ha		R/ton	Maize R/ton	Cowpea/Soybean/Oats/Vetch ton/ha			R/ton	Maize R/ha		
Maize		R	1	1.756	1102	0.2815	2800	5.83	600		3500	613	450	204	4957	
Cowpea		R	2							2.87	3731	157		204	2921	
Soybean		R	3			0.68783	2800			1.93	1062	157		204	2127	
Maize	Cowpea	R	4	0.661	1102	0.0415	2800	4.19	600	0.67	3384	424	225	204	3025	
Maize	Oats	R	5	0.723	1102			4.15	600	1.92	4987	424	225	204	4531	
Maize	Vetch	R	6	0.658	1102			7.19	600	4.53	11111	424	225	204	9584	
Maize		R	7	2.070	1102			9.85	600		5910	1227	450	204	6311	
Cowpea		R	8			0.201	2800			2.89	3757	313		204	3353	
Soybean		R	9			0.72720	2800			1.14	627	313		204	1646	
Maize	Cowpea	R	10	0.682	1102	0.108	2800	4.25	600	0.46	3149	848	225	204	2576	
Maize	Oats	R	11	0.782	1102			4.05	600	1.57	4469	848	225	204	3653	
Maize	Vetch	R	12	0.935	1102			4.19	600	4.21	8829	848	225	204	7182	
Maize		C	1	1.532	1102			5.68	600		3411	613	450	408	3629	
Cowpea		C	2			0.0415	2800			2.16	2808	157		408	1910	
Soybean		C	3			0.54775	2800			2.36	1298	157		408	1767	
Maize	Cowpea	C	4	0.623	1102	0.0225	2800	5.07	600	0.61	3834	424	225	408	3177	
Maize	Oats	C	5	0.541	1102			2.68	600	3.14	5688	424	225	408	4827	
Maize	Vetch	C	6	0.840	1102			4.03	600	2.8	6621	424	225	408	5090	
Maize		C	7	1.388	1102			6.69	600		4015	1227	450	408	3460	
Cowpea		C	8			0.2075	2800			2.56	3328	313		408	2738	
Soybean		C	9			0.58695	2800			0.91	501	313		408	923	
Maize	Cowpea	C	10	0.528	1102	0.093	2800	3.29	600	0.35	2429	848	225	408	1440	
Maize	Oats	C	11	0.543	1102			2.35	600	2.22	4296	848	225	408	3013	
Maize	Vetch	C	12	0.616	1102			3.45	600	3.56	7409	848	225	408	5206	

2011

Crop: main	Crop: inter	Till	Treat	Grain yield ton/ha			Grain income	Fodder / biomass ton/ha			Fodder income	Fert cost	Seed cost R/ha		Land prep	2011
				Maize ton/ha	R/ton	Soybean/Cowpea ton/ha		R/ton	Maize R/ton	Cowpea/Soybean/Oats/Vetch ton/ha			R/ton	Maize		
Maize		R	1	0.95	1931		1834	5.996	500		2998	736	620	225	3251	
Maize		R	2	1.81	1931		3501	9.058	500		4529	736	620	225	6449	
Maize		R	3	1.15	1931		2226	5.734	500		2867	736	620	225	3512	
Maize	Cowpea	R	4	0.67	1931		1299	4.720	500	0.4441	2804	513	310	225	2705	
Maize	Oats	R	5	0.41	1931		785	3.540	500	0.89	3016	513	310	225	2322	
Maize	Vetch	R	6	0.74	1931		1426	6.534	500	2.5383	7074	513	310	225	5852	
Maize		R	7	0.99	1931		1904	8.318	500		4159	1473	620	225	3746	
Maize		R	8	1.53	1931		2954	9.149	500		4575	1473	620	225	5211	
Maize		R	9	1.67	1931		3223	6.634	500		3317	1473	620	225	4222	
Maize	Cowpea	R	10	0.46	1931		882	3.977	500	0.9383	2927	1027	310	225	1896	
Maize	Oats	R	11	0.40	1931		764	5.570	500	0.7766	3872	1027	310	225	2644	
Maize	Vetch	R	12	0.68	1931		1312	4.112	500	3.8083	7768	1027	310	225	5918	
Maize		C	1	1.71	1931		3293	6.230	500		3115	736	620	450	4602	
Maize		C	2	2.05	1931		3953	6.485	500		3243	736	620	450	5389	
Maize		C	3	2.13	1931		4117	7.700	500		3850	736	620	450	6160	
Maize	Cowpea	C	4	1.42	1931		2750	5.364	500	0.4033	3085	513	310	450	4212	
Maize	Oats	C	5	1.18	1931		2283	3.533	500	1.8166	4310	513	310	450	4890	
Maize	Vetch	C	6	2.09	1931		4026	5.190	500	2.1083	5758	513	310	450	6910	
Maize		C	7	1.87	1931		3615	8.098	500		4049	1473	620	450	5122	
Maize		C	8	2.08	1931		4020	8.800	500		4400	1473	620	450	5877	
Maize		C	9	2.67	1931		5155	8.858	500		4429	1473	620	450	7041	
Maize	Cowpea	C	10	1.05	1931		2029	3.541	500	0.415	2186	1027	310	450	2078	
Maize	Oats	C	11	0.91	1931		1765	3.821	500	1.518	4036	1027	310	450	3584	
Maize	Vetch	C	12	2.49	1931		4802	5.510	500	2.325	6242	1027	310	450	7659	

2012

Crop: main	Crop: inter	Till	Treat	Grain yield ton/ha			Grain income	Fodder / biomass ton/ha				Fodder income	Fert cost	Seed cost R/ha		Land prep	2012
				Maize ton/ha	R/ton	Soybean/Cowpea ton/ha		R/ton	Maize	R/ton	Cowpea/Soybean/Oats/Vetch ton/ha			R/ton	R/ha		
Maize		R	1	1.433	2069		2964	3.301	675		2228	732	650	300	3510		
Cowpea		R	2				0			1.05	1000	177		300	48		
Soybean		R	3		0.00000		0			0.1166	630	177		300	-1003		
Maize	Cowpea	R	4	1.281	2069		2649	1.938	675	0.6433	1000	1952	499	300	3077		
Maize	Oats	R	5	0.628	2069		1300	1.209	675	0.063	1400	904	499	300	630		
Maize	Vetch	R	6	0.886	2069		1834	3.344	675	0.2816	1500	2680	499	300	1590		
Maize		R	7	2.082	2069		4308	5.097	675		4115	1466	650	300	6008		
Cowpea		R	8				0			1.123	1000	1123	354	300	-56		
Soybean		R	9		0.00000		0			0.1366	630	86	354	300	-1167		
Maize	Cowpea	R	10	1.081	2069		2236	2.598	675	0.68	1000	2434	998	300	2646		
Maize	Oats	R	11	0.682	2069		1411	1.221	675	0.0083	1400	896	998	300	174		
Maize	Vetch	R	12	0.991	2069		2049	1.777	675	0.0466	1500	1269	998	300	-104		
Maize		C	1	1.893	2069		3916	4.691	675		3166	732	650	600	5100		
Cowpea		C	2				0			1.49	1000	1490	177	600	188		
Soybean		C	3		0.00000		0			0.0633	630	40	177	600	-1337		
Maize	Cowpea	C	4	1.986	2069		4109	2.960	675	1.3166	1000	3315	499	600	5600		
Maize	Oats	C	5	1.337	2069		2767	2.364	675	0.255	1400	1953	499	600	2846		
Maize	Vetch	C	6	2.110	2069		4366	4.224	675	0.455	1500	3534	499	600	4677		
Maize		C	7	2.462	2069		5094	7.190	675		4853	1466	650	600	7232		
Cowpea		C	8				0			1.0767	1000	1077	354	600	-402		
Soybean		C	9		0.00000		0			0.0266	630	17	354	600	-1537		
Maize	Cowpea	C	10	1.307	2069		2705	3.622	675	0.4366	1000	2881	998	600	3263		
Maize	Oats	C	11	1.182	2069		2446	3.858	675	0.0666	1400	2698	998	600	2770		
Maize	Vetch	C	12	1.624	2069		3360	3.525	675	0.2416	1500	2742	998	600	2379		

2013

Crop: main	Crop: inter	Till	Treat	Grain yield ton/ha			Grain income	Fodder / biomass ton/ha			Fodder income	Fert cost	Seed cost R/ha		Land prep	2013
				Maize ton/ha	R/ton	Soybean/Cowpea ton/ha		R/ton	Maize	Cowpea/Soybean/Oats/Vetch ton/ha			R/ton	R/ha		
Maize		R	1	0.584	1958		1143	2.708	750		2031	910	940	345	980	
Maize		R	2	1.047	1958		2050	4.413	750		3310	910	940	345	3166	
Maize		R	3	0.580	1958		1137	2.548	750		1911	910	940	345	853	
Maize	Cowpea	R	4	0.242	1958		474	1.193	750	0.2681	1000	600	470	400	-178	
Maize	Oats	R	5	0.557	1958		1091	2.758	750	0.088	1400	600	470	500	1368	
Maize	Vetch	R	6	0.425	1958		833	1.597	750	0.1583	1500	600	470	2000	-1148	
Maize		R	7	1.263	1958		2473	4.550	750		3413	1825	940	345	2776	
Maize		R	8	0.878	1958		1719	3.317	750		2488	1825	940	345	1096	
Maize		R	9	0.770	1958		1508	3.172	750		2379	1825	940	345	777	
Maize	Cowpea	R	10	0.435	1958		851	2.038	750	0.5668	1000	1204	470	400	527	
Maize	Oats	R	11	0.513	1958		1005	1.532	750	0.06	1400	1204	470	500	-281	
Maize	Vetch	R	12	0.578	1958		1132	2.501	750	0.23	1500	1204	470	2000	-666	
Maize		C	1	1.292	1958		2529	3.488	750		2616	910	940	690	2606	
Maize		C	2	1.296	1958		2538	3.192	750		2394	910	940	690	2392	
Maize		C	3	0.910	1958		1781	4.643	750		3483	910	940	690	2724	
Maize	Cowpea	C	4	0.962	1958		1883	3.131	750	0.376	1000	600	470	400	2447	
Maize	Oats	C	5	0.688	1958		1348	2.342	750	0.265	1400	600	470	500	1215	
Maize	Vetch	C	6	0.963	1958		1886	3.257	750	0.0533	1500	600	470	2000	648	
Maize		C	7	1.620	1958		3171	5.363	750		4023	1825	940	690	3739	
Maize		C	8	0.947	1958		1854	3.403	750		2553	1825	940	690	951	
Maize		C	9	1.489	1958		2915	4.312	750		3234	1825	940	690	2694	
Maize	Cowpea	C	10	0.868	1958		1699	2.444	750	0.253	1000	1204	470	400	1021	
Maize	Oats	C	11	0.602	1958		1179	2.152	750	0.15	1400	1204	470	500	139	
Maize	Vetch	C	12	0.663	1958		1298	3.189	750	0.0783	1500	1204	470	2000	-556	

Appendix 5.1: Zeekoegat CA Trial Treatments

1. Tillage (whole plots – 2 treatments)

- a) Reduced tillage (RT)
- b) Conventional (CT)

2. Fertilizer (2 treatments)

a) *Low-input (F1):*

Maize

Type – with plant: 2:3:2 (22)

Amount: 2 bags (100 kg); 6.3kg N : 9.6 kg P : 6.3 kg K

Type / amount – top-dress: 0

Legume

Type – with plant: Mixture LAN and KCl (ratio of 2:1)

Amount: 1 bags Supers (100 kg) and 0.5 bag KCl (50 kg)

Type / amount – top-dress: 0

b) *High input (F2): (Potential of 4 ton/ha)*

Maize

Type – with plant: 2:3:2 (22)

Amount: 4 bags (200 kg); 12.6 kg N : 19.2 kg P : 12.6 kg K

Type / amount – top-dress: 4 bags LAN (200 kg) (54 kg N)

Legume

Type – with plant: Mixture LAN and KCl (ratio of 2:1)

Amount: 2 bags Supers (100 kg) and 1 bag KCl (50 kg)

Type / amount – top-dress: 0

3. Cropping systems (7 treatments)

- a) 1 x Maize monoculture – C1
- b) 2 x Maize / Legume rotation
 - a. Maize + Cowpea – C2
 - b. Maize + Soybean – C3
- c) 1 x Maize / Legume intercropping (Legume: Cowpea) – C4
- d) 2 x Maize delayed intercropping with temperate crops

- a. Maize + oats – C5
- b. Maize + vetch – C6

Total number of treatments (plots):

3 repetitions (blocks) x 2 tillage x 2 (fert) x 6 (crop) = 72 plots

Experimental design:

Split-plot Randomised Complete Block Design (RCBD), replicated 3 times, with each replicate split into 2 tillage systems (whole plots) and then each whole plot (conventional & reduced tillage) subdivided into 12 treatments (6 Crop x 2 Fert).

Treatment Number (T)	Treatment Combination (Crop + Fert)
1	F1C1
2	F1C2
3	F1C3
4	F1C4
5	F1C5
6	F1C6
7	F2C1
8	F2C2
9	F2C3
10	F2C4
11	F2C5
12	F2C6

Appendix 5.2: Zeekoegat CA Trial Layout and Key Plots

BLOCK A	Reduced Tillage	PIT 1	1 / 7 / 1	13 / 7 / 2	Conventional Tillage
			2 / 2	14 / 2	
			3 / 9 / 4	15 / 9 / 3	
			4 / 3 / 5 / AC	16 / 3 / 6 / AC	
			5 / 1 / 8 / AC	17 / 1 / 7 / AC	
			6 / 11	18 / 11	
			7 / 4 / 9 / AC	19 / 4 / 10 / AC	
			8 / 12	20 / 12	
			9 / 6	21 / 6	
			10 / 8	22 / 8	
			11 / 10 / 12	23 / 10 / 11	
			12 / 5	24 / 5	
BLOCK B	Reduced Tillage	PIT 2	25 / 5	37 / 5	Conventional Tillage
			26 / 11	38 / 11	
			27 / 10 / 13	39 / 10 / 14	
			28 / 8	40 / 8	
			29 / 1 / 16 / AC	41 / 1 / 15 / AC	
			30 / 2	42 / 2	
			31 / 9 / 17	43 / 9 / 18	
			32 / 6	44 / 6	
			33 / 7 / 20	45 / 7 / 19	
			34 / 4 / 21 / AC	46 / 4 / 22 / AC	
35 / 3 / 24 / AC	47 / 3 / 23 / AC				
36 / 12	48 / 12				
BLOCK C	Conventional Tillage	PIT 3	49 / 1 / 25 / AC	61 / 1 / 26 / AC	Reduced Tillage
			50 / 11	62 / 11	
			51 / 2	63 / 2	
			52 / 5	64 / 5	
			53 / 10 / 28	65 / 10 / 27	
			54 / 8	66 / 8	
			55 / 9 / 29	67 / 9 / 30	
			56 / 3 / 32 / AC	68 / 3 / 31 / AC	
			57 / 12	69 / 12	
			58 / 7 / 33	70 / 7 / 34	
59 / 6	71 / 6				
60 / 4 / 36 / AC	72 / 4 / 35 / AC				

1-72 / 1-12 / 1-36 = Plot / Treatment / NWM access tube / AquaCheck probe (AC)



Key plots