

DETAILS

Project number	M106/12
Project title	Evaluation of soil microbial dynamics under conventional and conservation maize-production systems
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Final abstract

Modern conventional agriculture dictates the use of practices such as ploughing and monoculture cropping that lead to the degradation of soils. Evidence of these practices can be seen in the decrease of soil carbon, especially on the Highveld and poses a risk for sustainable farming. One way to combat the deterioration of production soils is through conservation agriculture (CA). CA promotes the use of multiple cropping systems, little or no disturbance of the soil and a soil cover comprising of crop residues. Although CA has been widely studied, very little is known with regards to its effects on soil microbiology in South African agriculture. Changes in agricultural practices such as no till, residue management, and crop rotation may induce major shifts in the population and composition of soil microbial fauna and flora. With this in mind, the study aimed to evaluate the impact that CA systems have on soil microbial activity in local maize production. CA trials were first established at two localities i.e., Buffelsvallei (comprised of a loamy sand soil) and Erfdeel (comprised of a sandy soil) in the Free State and North West Province during the 2008/09 maize growing season. During the five growing seasons, soil sampling from both conservation agriculture (CA) field trials, viz. Buffelsvallei and Erfdeel were done. Soil samples collected from the maize crop treatments were subjected to various chemical and microbiological analyses. Microbiological data collected included enumeration of selected important soil microbes viz., actinomycete and bacterial as well as filamentous fungal counts. Soil microbial enzyme activities from the collected soil samples were also determined using β -glucosidase and urease enzymes that are involved in carbon and nitrogen cycles in the soil, as well as levels of glomalin activity in the soil. Glomalin is a carbon-containing compound that can provide an indication of mychorrizal status and of carbon in the soil. Soil samples from the rhizosphere were also subjected to denaturing gradient gel electrophoresis (DGGE) profiling for possible microbial community shifts. Results obtained showed that no clear trends with regards to soil microbial

parameters could be established. However, on the loamy sand soil shifts in the microbial community are beginning to occur. After five years of CA practices filamentous fungi are starting to be more prevalent compared to the conventional systems with a predominant bacterial component. The CA based systems have in all likelihood not yet reached biological stability.

Keywords: conservation agriculture, soil enzymes, soil microbial activity, DGGE fingerprinting

Introduction

In order to farm sustainably, soil management and efficient use of natural resources are becoming more important. The persistent use of conventional agricultural practices such as ploughing and monoculture cropping often leads to the degradation of soils (Montgomery, 2007). This is evident in the decrease of carbon content in our arable soils of the Highveld, which poses an inherent risk for farmers to grow crops sustainably. Conservation Agriculture (CA) is recognised as a way to combat soil deterioration brought about by conventional cultivation practices. CA is governed by principles that include minimal soil disturbance, multiple cropping systems and a soil cover of plant material (Bockus and Shroyer, 1998, Verhulst *et al.*, 2010).

Soil microbial activity under conventional tillage systems has been grossly neglected because soil has always been seen as a lifeless substrate acting as a food and water reservoir for plant growth. Lately, with the popularisation of CA principles and other biological farming approaches the living component of soil has become a reality. By applying CA practices soil properties such as the soil microbial population and composition can be altered to improve soil quality (Andersen, 1999). However, due to the ages of neglect very little is known about it and little is understood about its effects on and contributions to crop production under different cultivation systems.

The importance of soil microbial activity, especially in CA is presently unknown in local maize-producing soils on the Highveld of SA. Soil factors such as organic matter content, soil structure, nutrient cycling, nutrient availability and water-holding capacity are all dependent on soil microorganisms. By studying soil microbe-plant interactions the necessary measures could lead to adaptation of existing production practices, which might improve efficiency and economy of maize production at farm level.

Also, questions regarding quality of crop yield may be answered by addressing soil microbial interactions. Thus, the importance of microbial diversity in promoting plant productivity could not be emphasised more. Results from this project will provide maize farmers from commercial as well as resource-poor systems with alternatives as under scribed by CA in order to make sound decisions with regard to management practices that suit their activities best. All the intended trial procedures are well known and do not pose any risk. Field trials could be damaged by adverse weather conditions, which may cause delay in reporting. At present it is difficult to quantify the cost and amount of influence that soil microbes have on the maize industry but as an environmentally friendly approach there is no doubt that the proliferation of

soil microbes will enhance food security. Information created in this project will provide facts and figures for determining the role of microorganisms in CA.

Effect of soil management practices on soil biological properties

Soil functioning may decline through management practices that reduce soil quality, while proper management systems can be expected to restore ecosystem functions (Reeves, 1997). It is clear that soil microorganisms play an essential role in soil quality and plant productivity through various key processes. Therefore, it is important to know the effect of agricultural management practices on the soil microbial community for a broader understanding of soil quality and to establish sustainable management practices (Hill *et al.*, 2000). However, our knowledge of soil ecosystem function is limited in part by the complexity of measuring soil microorganisms.

Measuring soil microbial communities

Key approaches that are used to measure soil microbial communities include microscopy, biochemical methods, physiological assays, and molecular analyses such as DNA-fingerprinting (Torsvik *et al.*, 1996). Classical or traditional techniques commonly rely on phenotypic characteristics and are restricted to organisms that can be isolated or cultured. Since <1% of soil microorganisms can be cultured, these techniques can underestimate population size and diversity (Amann *et al.*, 1995). These techniques may however be useful in discerning relative differences between soil microbial communities, without determining the abundance or identity of specific microorganisms in the population (Mazzola, 2004).

Process-level studies can also be used, where microbes themselves are not isolated or identified but their activities measured (Dick, 1994; Kirk *et al.*, 2004). However, the most promising advances have been made in the use of molecular methods (Thies, 2006), with soil-extracted nucleic acids, which do not rely on the capacity to culture organisms.

Soil microflora are most frequently assessed in terms of their abundance, activity or function, and diversity or community composition. The total microbial community or specific members of the community can be assessed. Alternatively, indicators that reflect the capacity of the soil to function can also be measured (Doran and Parkin, 1994).

Abundance

The use of traditional methods in measuring abundance include culturing organisms on artificial media, direct microscopy and extraction of specific cell components or molecules through measuring their concentration (Thies, 2006). One of the most common biochemical

methods used to assess abundance are fumigation-extraction (Vance *et al.*, 1987) that measure microbial biomass carbon and/or nitrogen. Analysis based on phospholipid fatty acids (PLFA) or fatty acid methyl esters (FAME) are useful due to their presence in all living cells. Specific groups of organisms can be distinguished through their unique fatty acids but cannot be characterized to species level (Zelles, 1999). Other methods include detection of specific molecules (e.g. glomalin, ergo sterol) associated with the soil (Jenkinson and Ladd, 1981).

Microbial activity and function

In general, the rate of a specific biochemical processes can be measured, for example the ability to transform one compound to another (carbon or nitrogen mineralisation) (Thies, 2006), or the ability to metabolise specific compounds. Studies of microbial activity have been commonly conducted at a broad scale level, through measuring microbial respiration (Hill *et al.*, 2000). Enzymatic activities have been used as an indicator of the overall microbial activity in soils while also producing useful functional information on the capacity of a soil to carry out specific activities important in maintaining soil fertility (Dick, 1994).

Diversity and microbial population composition

The heterogeneity of DNA recovered from soil can also be used as a reflection of community diversity (Torsvik *et al.*, 1990). Extracted DNA can be used either with DNA-DNA hybridization to detect specific genes in the soil (Torsvik *et al.*, 1990), or with primers to amplify portions/regions of the DNA, generating electrophoretic patterns of DNA fragments separated in different ways, resulting in profiles used as genetic fingerprints (Thies, 2006). These methods include terminal restriction fragment length polymorphisms (T-RFLP) (Thies, 2007), denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al.*, 1993) and automated ribosomal intergenic spacer analysis (ARISA) (Ranjard *et al.*, 2001). Some DNA approaches compare individual rDNA sequences to a database of previously encountered sequences in order to assess diversity (Olsen and Woese, 1993). These DNA-based methods in a large part make use of PCR for amplification of the small subunit rRNA gene found universally in all life forms and composed of highly conserved regions as well as regions with considerable sequence variation (Ward *et al.*, 1992). Another method used is diversity indices, for example the Shannon-Weaver index which includes parameters such as species richness and evenness (Pankhurst *et al.*, 1997).

With the above as background the aim of the project was to quantify the effects of conservation agriculture practices on soil microbial parameters. Moreover, in determining how minimal soil disturbance, residue management and crop rotation affect soil microbial activities and how

these treatments alter the microbial community to obtain a perspective on soil microbial dynamics.

Materials and methods

Field trial

Two field trials at Buffelsvallei in the North West province and Erfdeel in the Free State province were planted in the 2008 maize growing season. Treatments consisted of (1) conventionally cultivated, mono-cropped maize (T1), (2) mono-cropped maize with minimal soil disturbance (T2), (3) a two-year system with maize in rotation with cowpea or sunflower with minimal soil disturbance (T3.1) and (4) a three-year system with maize in rotation with cowpea or sunflower and pearl millet with minimal soil disturbance (T4.1) with systems 2-4 as variants of CA. Soil samples were taken from the field trial at the Buffelsvallei farm comprising of a loamy sandy soil in a cool semi-arid climate with 600mm summer rainfall in the North West Province. Samples were also collected from the Erfdeel trial comprised of a sandy soil in the Free State Province. The soil samples were taken on only the maize crop during the active growing phase at 0-5, 5-15, 15-30 cm depths as well as 70 and 100 days and kept refrigerated until further use. Day 70 and 100 were root soil samples, whilst all other samples were taken on the row of the maize crop.

Laboratory analyses

Conventional microbial counts

A serial dilution series (10^{-1} to 10^{-6}) of soil samples was prepared for every sample using distilled water. Viable cell counts were obtained using the aseptic spread plate technique. Different microbial growth media designed to be selective for heterotrophic bacteria; actinomycetes and filamentous fungi were used in the microbial analyses. These microbial populations were subjected to the physiological ability of microbes to grow on each of the selective media. General heterotrophic plate counts were done on nutrient agar (NA), (Biolab, Midrand, South Africa).

Actinomycetes were enumerated on Actinomycete isolation agar (Sigma-Aldrich, South Africa). To obtain filamentous fungal counts, malt extract agar (MEA), (Biolab, Midrand, South Africa) was used supplemented with 30ppm chloramphenicol and 50 ppm streptomycin.

Enzyme assays

The microbial activities of β -glucosidase, and acid phosphatase were determined using 1g of air-dried soil and incubated for 1h (37 °C) with the appropriate substrate for each enzyme at their respective optimal pH values (Tabatabai, 1994). In the case of urease, 5g of air-dried soil was used. Methods used are summarised in Table 1. These selected enzymes have been

implicated in the carbon, nitrogen and phosphorous soil cycles, respectively.

Table 1 The methods used to determine enzyme activity in soils.

EC number ^a	Recommended name ^b	Assay conditions ^c [Substrate]	Optimum pH
3.1.3.2	Acid phosphatase	<i>p</i> -Nitrophenyl phosphate [25mM]	6.5
3.2.1.21	β -glucosidase	<i>p</i> -Nitrophenyl- β -glucopyranoside [25mM]	6.0
3.5.1.5	Urease	Urea [80mM]	Non-buffered

^a EC number denotes enzyme class

^b Methods according to Tabatabai (1994 and 1982)

^c Values in parentheses are substrate concentrations under the respective assay conditions. The product of reactions for glucosidase and phosphatase is *p*-Nitrophenol=PN

Another soil biological parameter, glomalin activity that is implicated in carbon sequestration and contributing towards the soil organic carbon pool was also performed according to Wright and Upadhyaya (1996).

Statistical analysis

Analysis of variance (ANOVA) was used to detect significant differences between the effects of cropping system (treatments) on microbial parameters employing Statgraphics plus (version 5) software. A 5% probability level was used for accepting or rejecting the Null hypotheses. The comparison of means with $P < 0.05$ was used for detecting significant differences.

Molecular fingerprinting

Denaturing gradient gel electrophoresis (DGGE) profiling

Community genomic DNA from soil samples were extracted using the Powersoil DNA isolation kit (Mobio, USA) and subjected to conventional PCR using universal bacterial and fungal specific primers (Table 2). Soil samples were also subjected to PCR-denaturing gradient gel electrophoresis (DGGE) as described by Muyzer *et al.* (1993) to study microbial community shifts. Both bacterial and fungal communities were studied. Acrylamide gels (Sigma-Aldrich, USA), 8 and 7% were prepared with 40 to 60% and 20 to 50% urea-formamide denaturing

gradients used for the two microbial groups. Equal volumes of PCR products were loaded on these gels and subjected to electrophoresis in 0.5x TAE buffer (20mM Tris, 10mM acetate, 0.5mM Na₂EDTA, pH 7.8) at 60°C and 60V for 16h using the Dcode™ Universal Mutation system (BioRad Laboratories, Ltd, South Africa). The DGGE banding patterns (products) were visualised by GelRed (Biotium, USA) staining and UV transillumination.

Table 2 PCR primers and cycling conditions for amplification of 16S and 18S rRNA genes of Bacteria and Fungi

Primer set	Expected size (bp)	Initial denaturin g temp. (°C), time (min)	No. cycles	Denaturin g temp. (°C), time (s)	Annealing temp. (°C), time (s)	Extension temp. (°C), time (s)
16S: ^a ‡PRBA338F/PR UN518R	220	95, 5	35	95, 30	63, 45	72, 60
18S: ^b ‡NS1/GCfung	350	95, 5	35	95, 30	50, 45	72, 60

‡GC clamp added to the 5'end of primers (PRBA338F and NS1), 5'CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G 3'.

^a Lane *et al.* 1991 and Muyzer *et al.* 1993

^b May *et al.* 2001

Results

Season 2008/09

During the first season microbial plate counts and the glucosidase assay was performed on the soil samples of the two localities (Buffelsvallei and Erfdeel). This was to establish a baseline for microbial activities. Results showed no significant differences among the various treatments.

Season 2009/10

For the second season glucosidase activity as well as heterotrophic bacterial, actinomycete and filamentous fungal counts were done. During the second season (2009/10) β -glucosidase activity in soil of the trial at Erfdeel differed between various soil depths, the highest levels in the 0-5cm layer (Fig 1) with the lowest levels in the 5-15 and 15-30cm depths (not indicated). However, no significant trends in β -glucosidase activity between maize treatments were observed at both Erfdeel and Buffelsvallei trials at 0-5cm (Fig 1. and Fig 2). This also applied for total heterotrophic bacterial and fungi counts. However, at Erfdeel β -glucosidase activity showed a correlation with soil pH. In the case of the mychorrhizal protein glomalin, its levels also showed no significant difference between maize treatments.

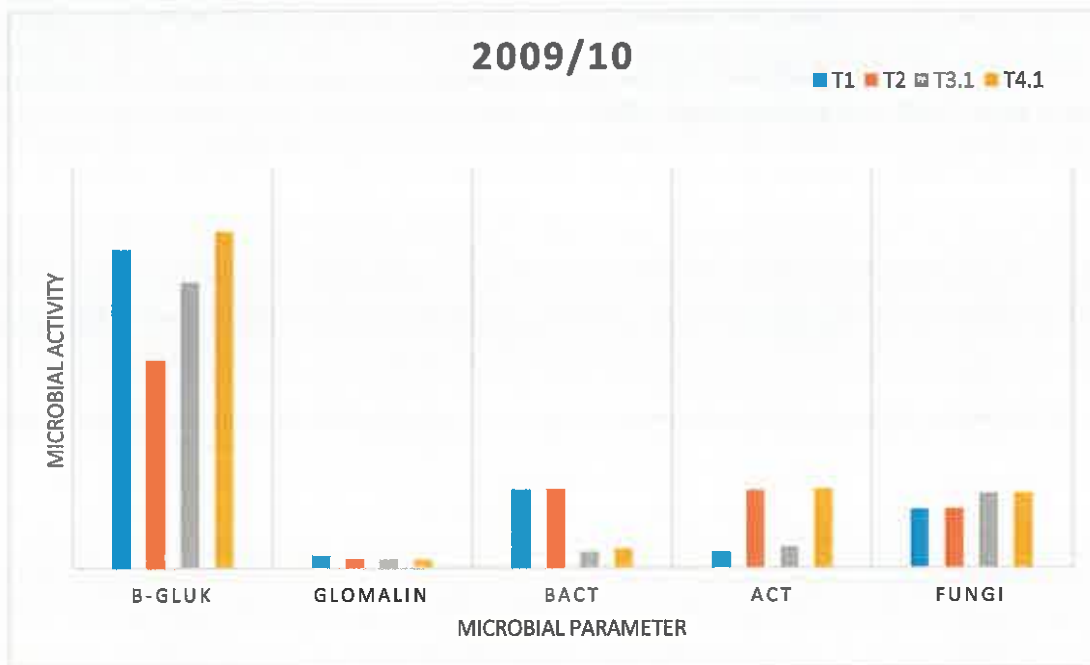


Figure 1 Effect of treatments on various microbial activities during active growing phase of maize crop at Erfdeel. Measurements for β -glucosidase activity (mg PNP released.kg⁻¹ soil.h⁻¹), glomalin activity, heterotrophic bacteria counts (BACT) (Log cfu.g⁻¹), actinomycetes counts (ACT) (Log cfu.g⁻¹), and filamentous fungi counts (FUNGI) (Log cfu.g⁻¹), were determined from soil samples at 0-5 cm depth.

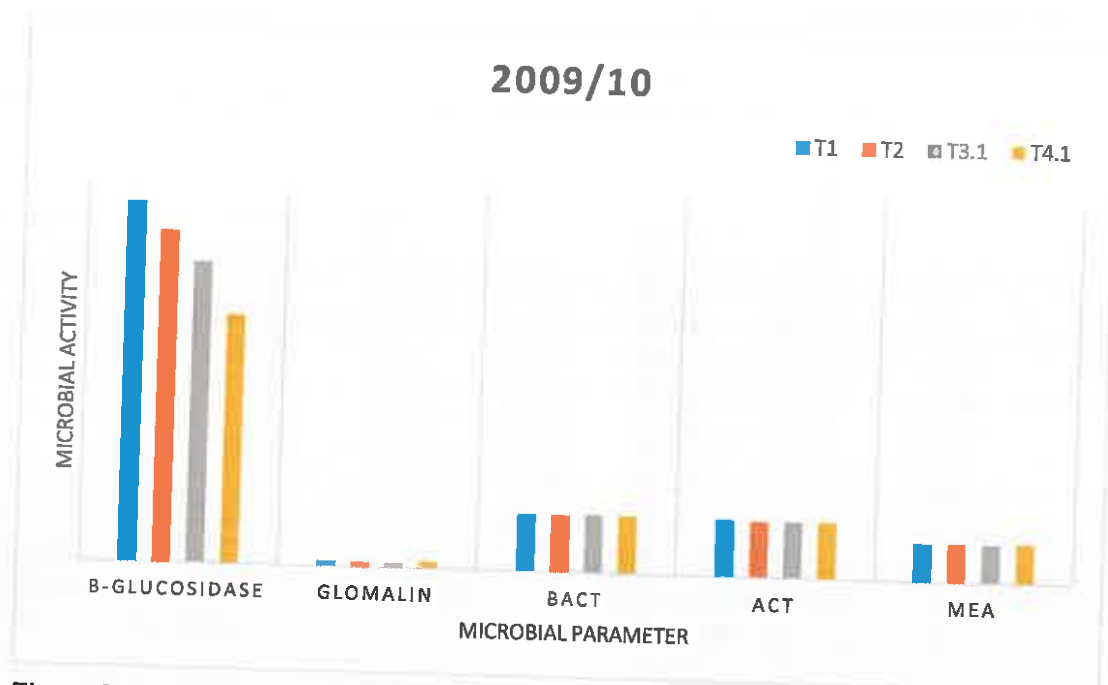


Figure 2 Effect of treatments on various microbial activities during active growing phase of maize crop at Buffelsvalleil. Measurements for β -glucosidase activity ($\text{mg PNP released.kg}^{-1} \text{ soil.h}^{-1}$), glomalin activity, heterotrophic bacteria counts (BACT) (Log cfu.g^{-1}), actinomycetes counts (ACT) (Log cfu.g^{-1}), and filamentous fungi counts (FUNGI) (Log cfu.g^{-1}), were determined from soil samples at 0-5 cm depth.

Season 2010/11

Similarly to the second season (2009/10) β -glucosidase activity in soil of the trial at Buffelsvallei differed between various soil depths, with the highest levels occurring in the 0-5cm layer and the lowest levels observed in the 5-15 and 15-30cm depths (Figs 3-5). β -glucosidase activity levels differed significantly between maize treatments in the trial at Buffelsvallei. The trial also showed that fungal activities differ significantly amongst maize under monoculture and maize under rotation. Total heterotrophic bacterial and fungal counts were not significantly different between various treatments and depths (Figs 6-8 & 12-14). Levels of actinomycetes differed significantly between maize treatments at lower depths (Figs 9-11). Glomalin activities at lower depths at Buffelsvallei correlated with yield data. In the case of the mycorrhizal protein glomalin, its levels also showed a significant difference between maize treatments and depths for the Buffelsvallei trial (Figs 15-17). Due to liming correction of the soil at Erfdeel no trends could be established between treatments. Denaturing Gradient Gel Electrophoresis (DGGE) analysis showed similar DNA banding profiles at various depths between treatments in both trials. The results for the third season are promising especially in the Buffelsvallei trial. However, conservation agricultural practices may take up to seven years

to show any significant effects.

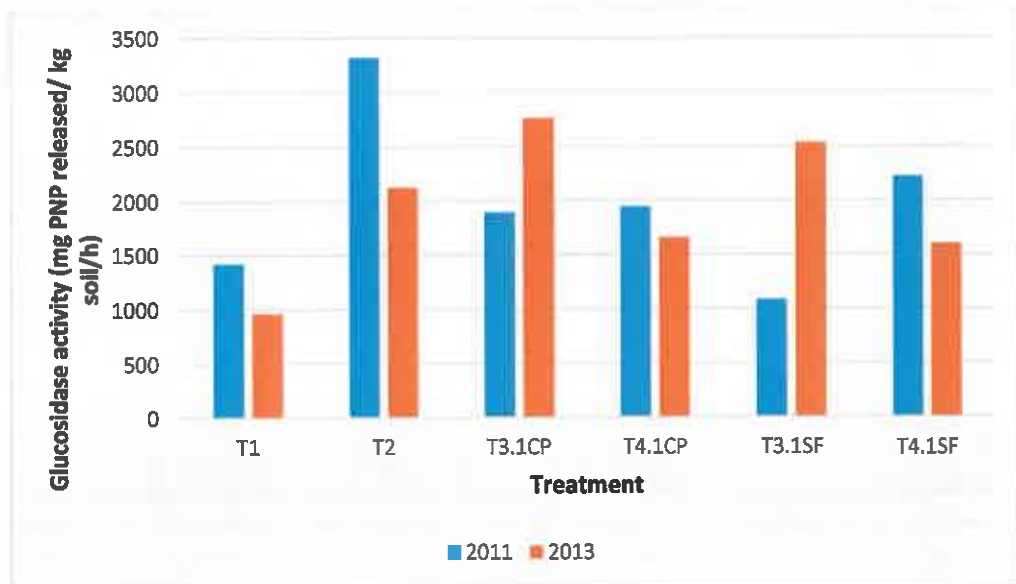


Figure 3 Effect of treatments on glucosidase activity at 0-5 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

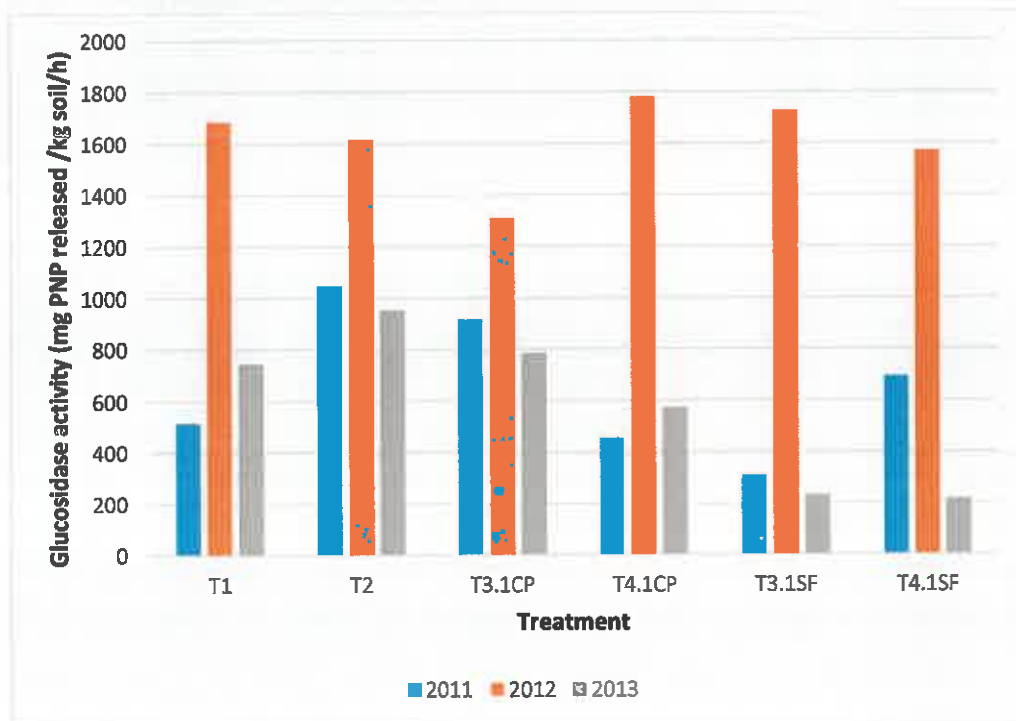


Figure 4 Effect of treatments on glucosidase activity at 5-15 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

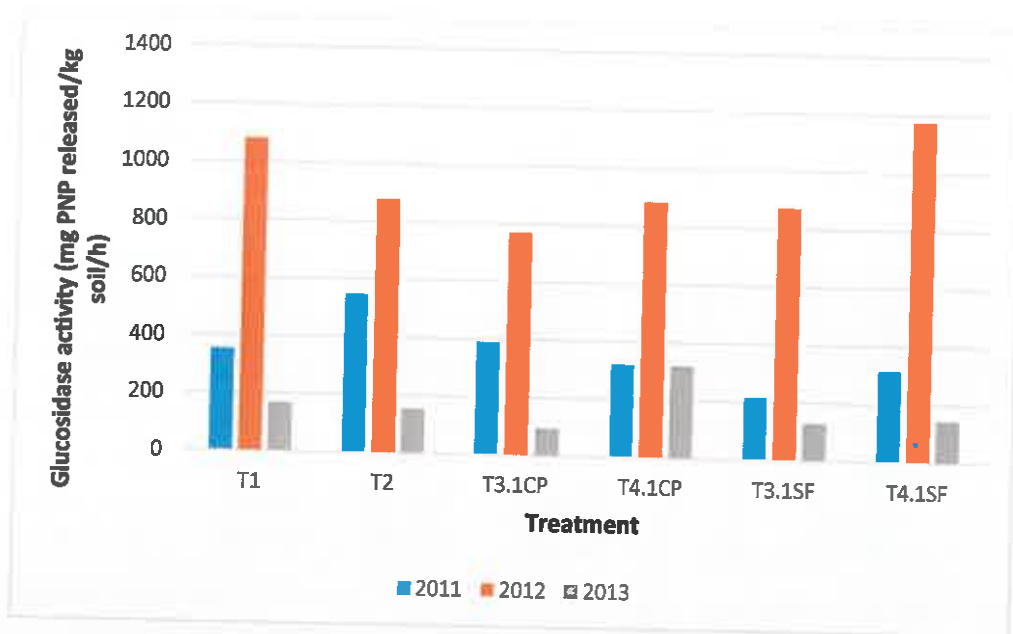


Figure 5 Effect of treatments on glucosidase activity at 15-30 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.



Figure 6 Effect of treatments on heterotrophic bacterial counts at 0-5 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

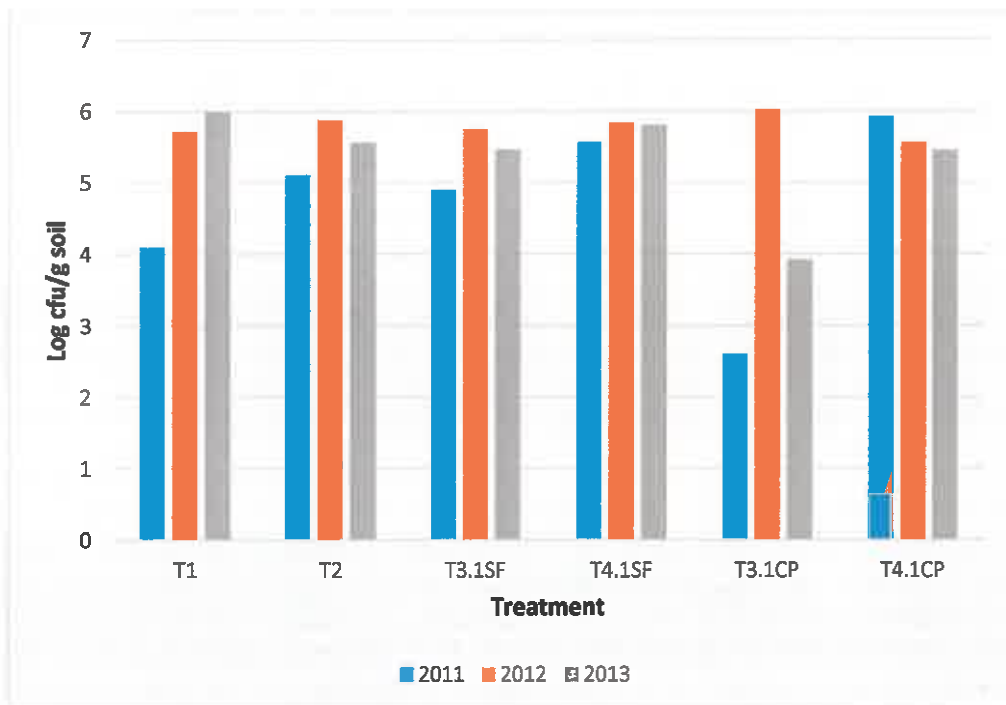


Figure 7 Effect of treatments on heterotrophic bacterial counts at 5-15 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

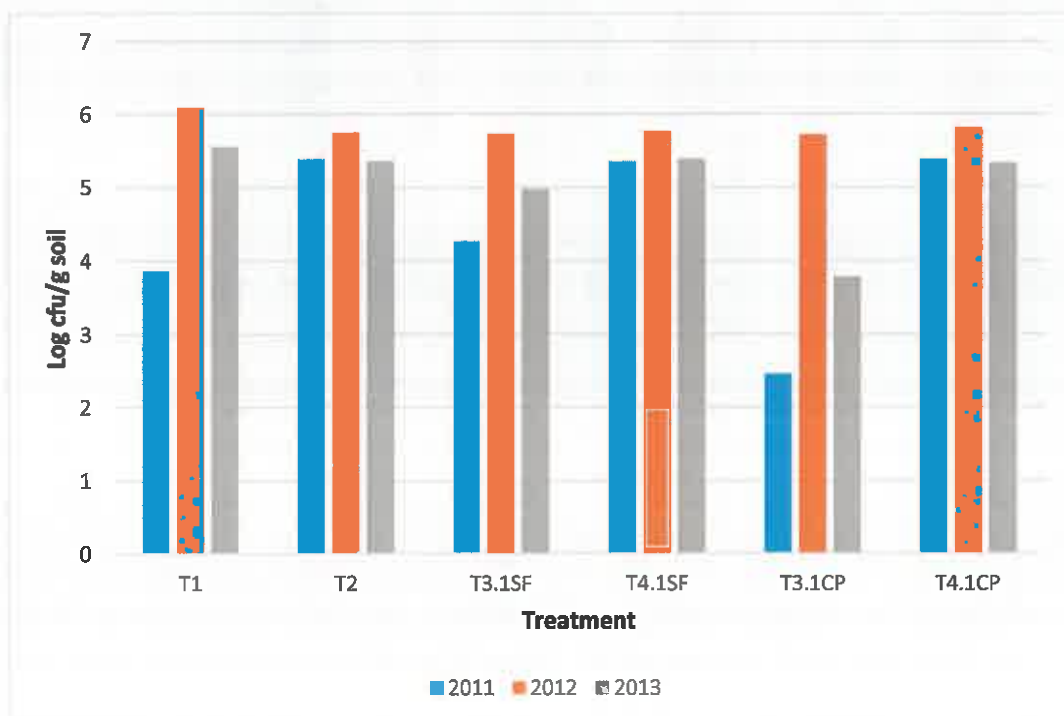


Figure 8 Effect of treatments on heterotrophic bacterial counts at 15-30 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

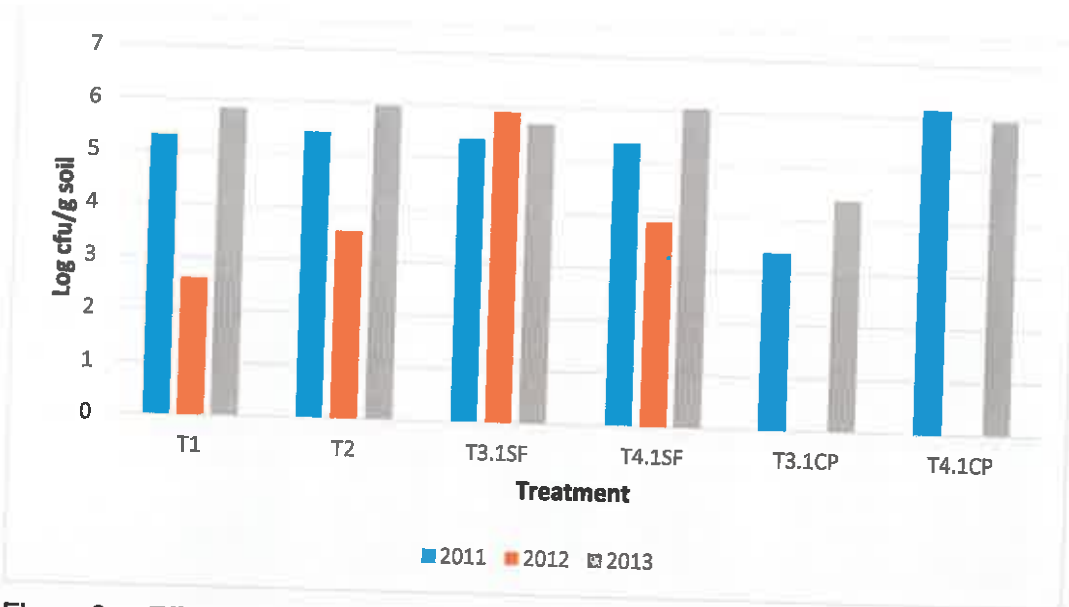


Figure 9 Effect of treatments on actinomycete counts at 0-5 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

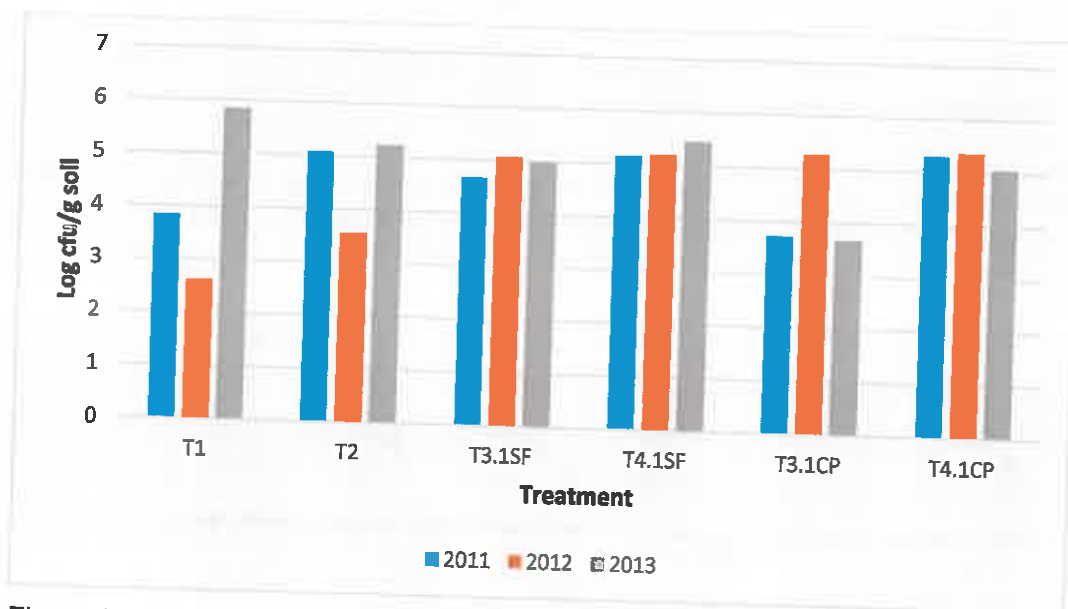


Figure 10 Effect of treatments on actinomycete counts at 5-15 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

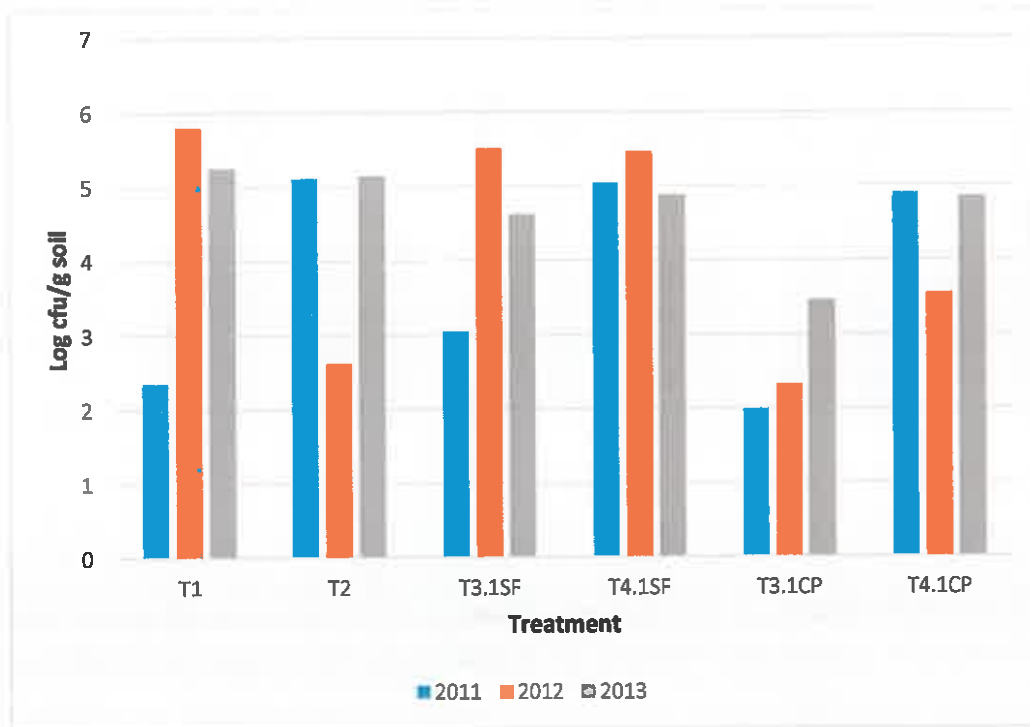


Figure 11 Effect of treatments on actinomycete counts at 15-30 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

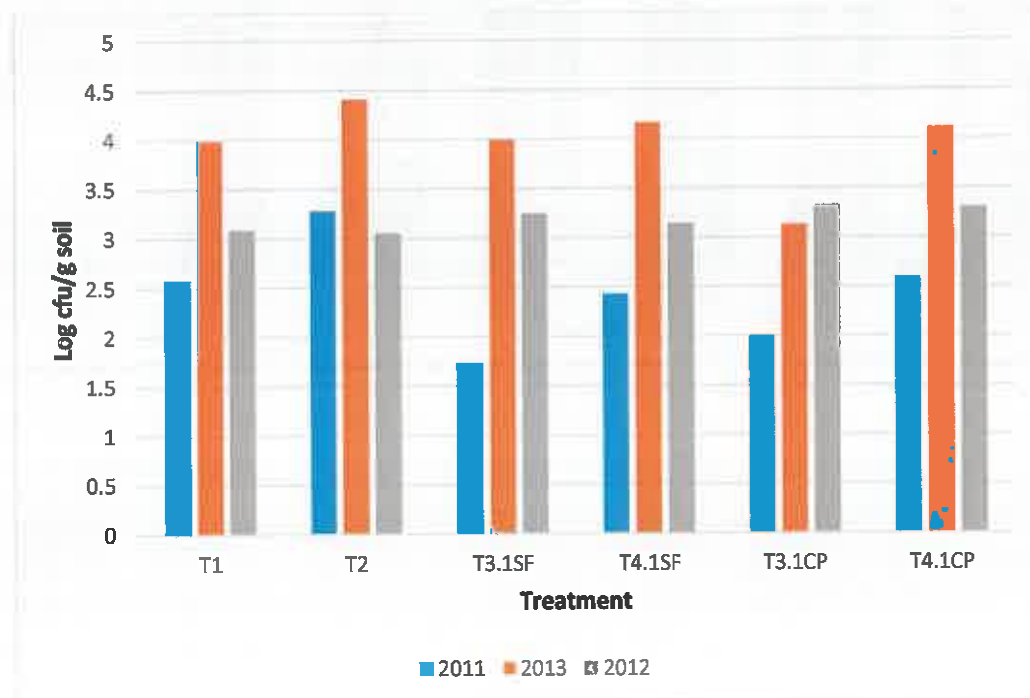


Figure 12 Effect of treatments on filamentous fungal counts at 0-5 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

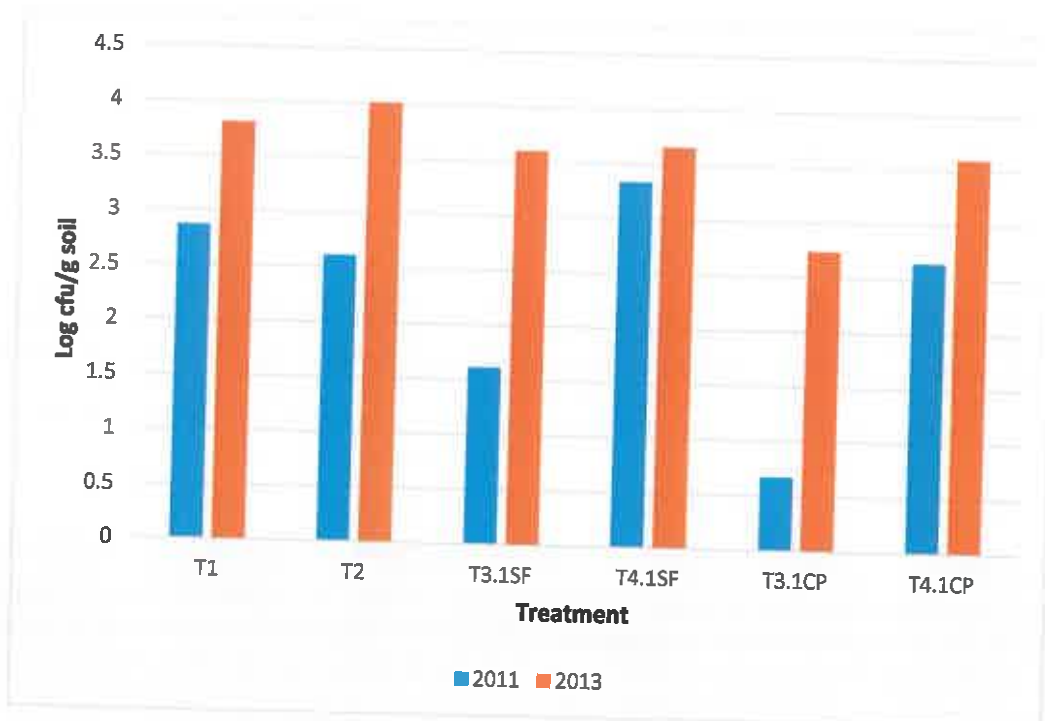


Figure 13 Effect of treatments on filamentous fungal counts at 5-15 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.



Figure 14 Effect of treatments on filamentous fungal counts at 15-30 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

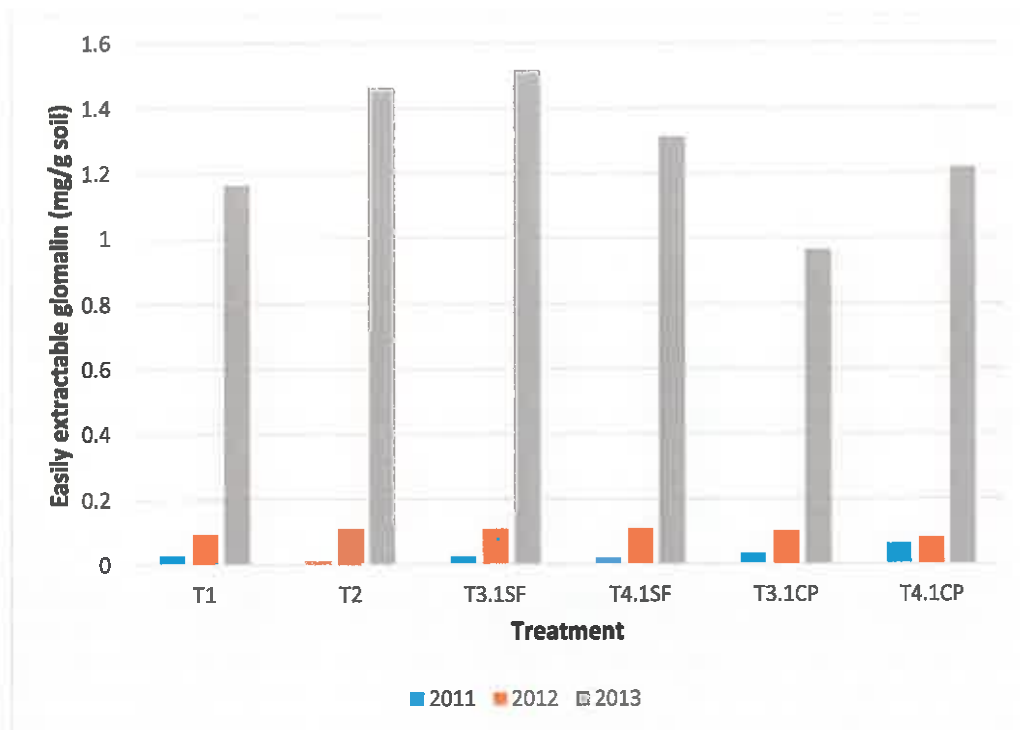


Figure 15 Effect of treatments on glomalin activity at 0-5 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

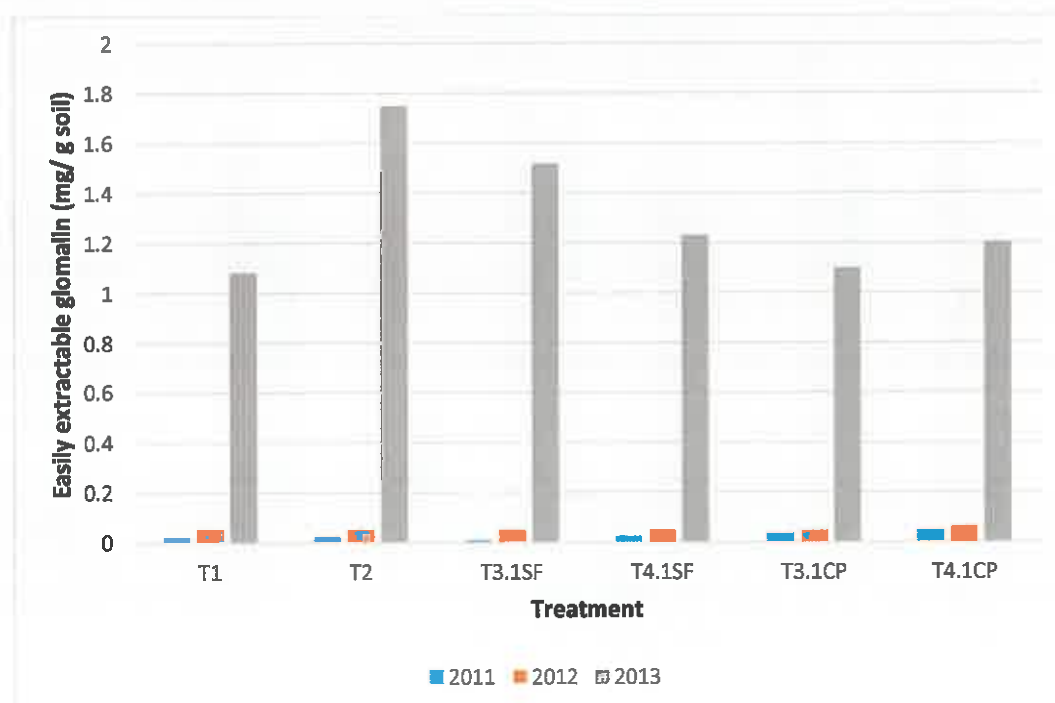


Figure 16 Effect of treatments on glomalin activity at 5-15 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

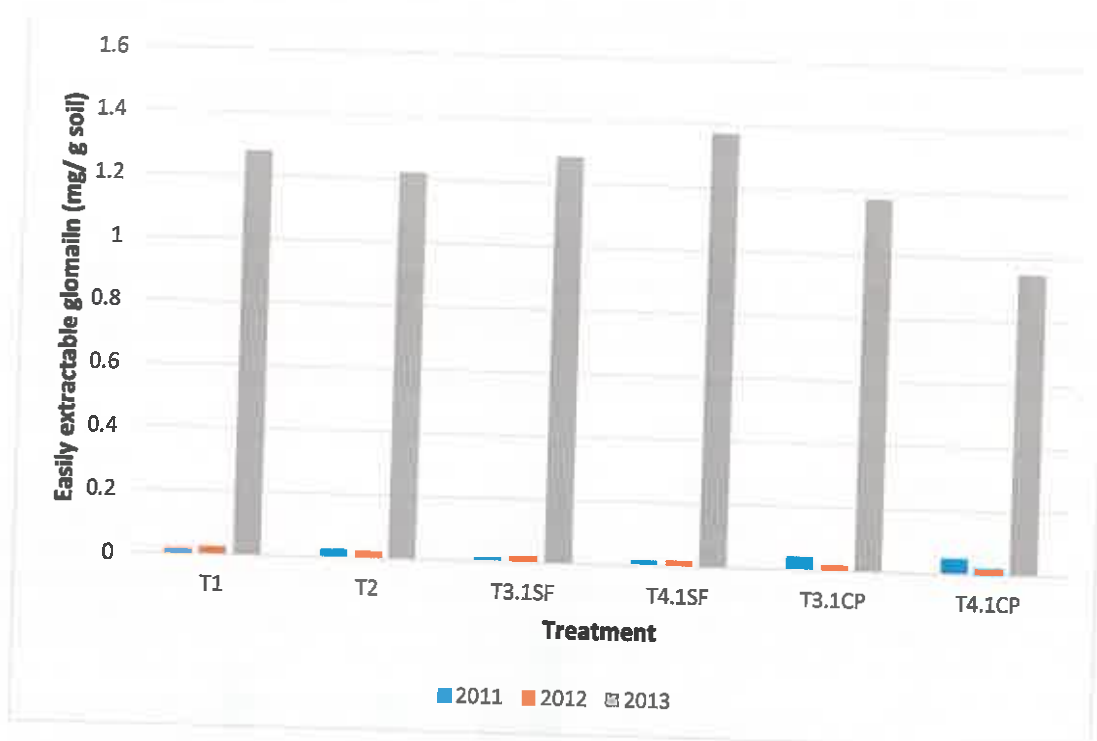


Figure 17 Effect of treatments on glomalin activity at 15-30 cm for seasons 2010/11 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower respectively.

Season 2011/12

For the 2011/12 season soil samples were taken at three different depths (0 - 5, 5 - 15 and 15 - 30 cm) from all maize plots that included treatments under monoculture, and varying degrees of CA practices i.e., minimum soil disturbance and crop rotation. Bacterial levels in the soil at the Buffelsvallei trial differed between treatments in the 0 - 5 cm layer compared to the previous season (2010/11) (Fig 6). In this layer bacterial activities were higher in the two-year system maize in rotation with cowpea and sunflower with minimum soil disturbance (Figs 6). However, bacterial activities showed no difference between treatments in the 5-15 and 15-30 cm soil depths (Figs 7 - 8). Actinomycete levels did not differ significantly between maize treatments at lower soil depths (15 - 30 cm) (Fig 11). Total fungal counts were also not significantly different between treatments and depths (Figs 12 - 14). In general, β -glucosidase and urease activities at Buffelsvallei were not significantly different between treatments. As compared to the third season a similar trend was observed between β -glucosidase levels at various soil depths. The levels of β -glucosidase were highest in the top soil (0 - 5 cm) whilst the lowest were detected in the lower depths (5 - 15, and 15 - 30 cm) (Figs 3 - 5). However, urease activity levels in the topsoil (0 - 5 cm) were significantly higher between the three-year system with maize in rotation with cowpea/sunflower and pearl millet with minimal soil

disturbance (Fig 18). In the case of glomalin levels no difference between treatments and depths were detected. The trial at Erfdeel showed no significant differences between treatments in the 0 - 5 cm soil layer for actinomycetes, bacterial and fungal counts as well as for enzyme activities (not shown). A similar finding was observed in the 5 - 15 cm and 15 - 30 cm soil layer between treatments. Although not significant, fungal counts were higher in the 15-30cm layer between the CA and maize monoculture treatments. In the case of DGGE analysis for Erfdeel similar DNA profiles were detected between treatments and depths. At Buffelsvallei DGGE profiles showed microbial community shifts between the cropping systems with a higher bacterial diversity in CA treatments. Early indications are that changes occurred at microbial community level with different structural diversity when switching from conventional agricultural to CA practices.

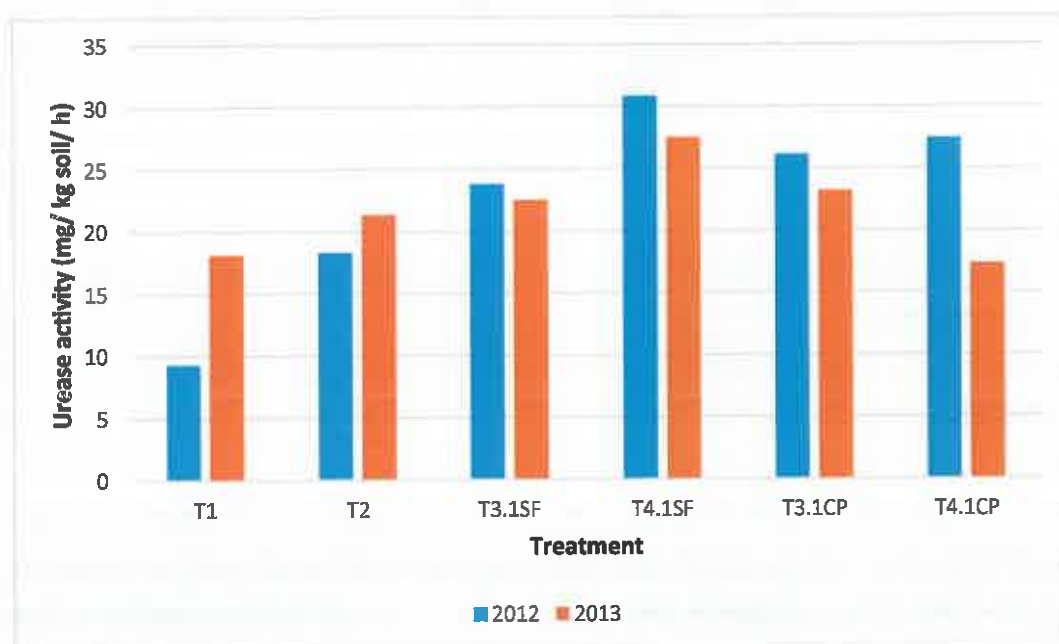


Figure 18 Effect of treatments on urease activity at 0-5 cm for seasons 2011/12 to 2012/13 at Buffelsvallei. CP and SF denote preceding cowpea and sunflower rotation crop respectively.

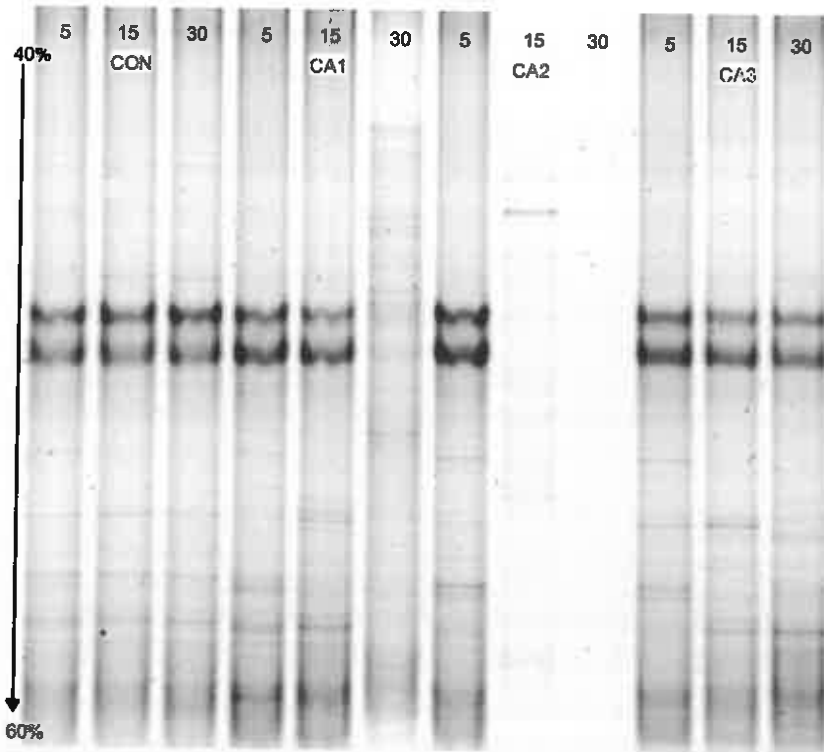


Figure 19 DGGE profiles of dominant bacterial communities present in loamy sand soil under conventional agricultural and CA practices for season 2011/12. 16S rDNA PCR amplicons were run on an 8% PAGE gel with 40-60% denaturing gradient when PCR-DGGE analysis was performed. Numbers 5, 15 and 30 depict soil samples at 0-5, 5-15 and 15-30 cm depths, respectively.

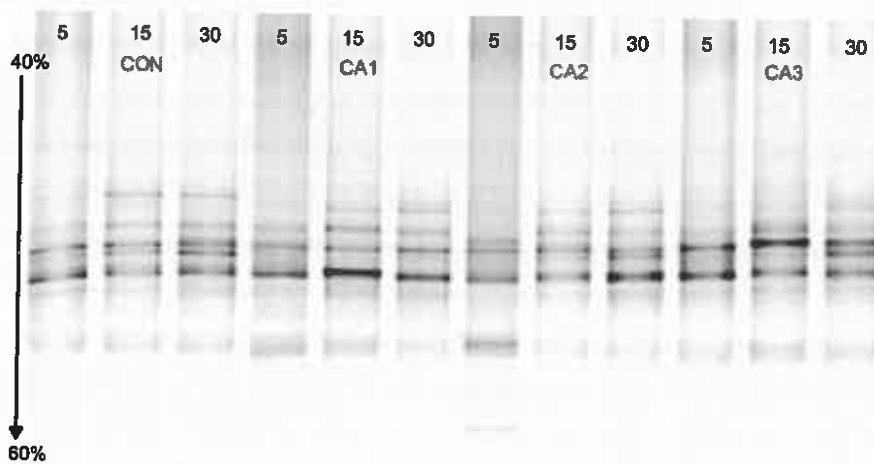


Figure 20 DGGE profiles of dominant fungal communities present in loam sandy soil under conventional agricultural and CA practices for season 2011/12. 18S rDNA PCR amplicons were run on a 7% PAGE gel with 20-50% denaturing gradient when PCR-DGGE analysis was performed. Numbers 5, 15 and 30 depict soil samples at 0-5, 5-15 and 15-30 cm depths, respectively.

Season 2012/13

During the 2012/13 growing season soil sampling from both conservation agriculture (CA) field trials, viz. Buffelsvallei and Erfdeel were done. Only soil samples collected from the maize crop treatments were subjected to various chemical and microbiological analyses. Contrasting to the fourth season (2011/12) bacterial counts in the soil at the Buffelsvallei trial did not differ significantly between treatments in the 0-5cm layer (Figs 6 - 14). However, bacterial activities were significantly higher in conventional monoculture practices compared to the two-year system of maize in rotation with cowpea with minimum soil disturbance in the 15 - 30 cm depths (Fig 8). Contrary to the fourth season Actinomycete levels showed significant differences between treatments in the 5 - 15 and 15 - 30 cm soil depths (Fig 10 - 11). Similarly, total fungal counts were significantly different between treatments at deeper depths (15 - 30 cm) (Fig 14). Similarly to the fourth season, β -glucosidase and urease activities at Buffelsvallei were not significantly different between treatments (Figs 3 - 5 & 18). As compared to the third season a similar trend was observed between β -glucosidase levels and soil depths. β -glucosidase levels were highest in the top soil (0 - 5 cm) whilst the lowest levels were detected in the lower depths (5 - 15 and 15 - 30 cm). However, urease activity levels in the topsoil (0 - 5 cm) were significantly higher between the three-year system with maize in rotation with cowpea/sunflower and pearl millet with minimal soil disturbance. In the case of glomalin levels no difference between treatments and depths were detected. The trial at Erfdeel showed no significant differences between treatments in the 0 - 5 cm soil layer for actinomycetes, bacterial and fungal counts as well as for enzyme activities. A similar situation applied in the 5 - 15 cm and 15 - 30 cm soil layer between treatments. Although not significant, fungal counts were higher in the 15 - 30 cm layer between the CA and maize monoculture treatments. Denaturing Gradient Gel Electrophoresis (DGGE) analysis showed similar DNA banding profiles between treatments and depths at Erfdeel. On the other hand, DGGE profiles showed microbial community shifts between the cropping systems with a higher bacterial diversity in CA treatments at the Buffelsvallei trial. While system stability has not been reached, results are already showing that microbial diversity is changing when converting from conventional to CA practices when 70 day soil samples were studied. Shannon diversity indices showed that CA cropping systems are starting to switch from a predominant bacterial system to a fungal system (Table 3).

Table 3 Shannon-Weaver indices based on dominant DGGE banding profiles for bacteria and fungi respectively in selected cropping system for day 70 samples (2012/13 season).

	Cropping system			
Bacteria	T1	T2	T3.1	T4.1
Shannon-Weaver index	23.24	30.57	26.63	15.63
Fungi				
Shannon-Weaver index	8.36	8.75	13.56	17.33
fungi: bacteria ratio	0.36	0.29	0.51	1.11

Discussion

The study showed that soil type could play a significant factor determining possible trends. No clear trends could be established in the effects of various cropping systems on microbial parameters at Erfdeel consisting of a sandy soil. The soil pH also had to be corrected with lime and gypsum more than once, which could have led to an ambiguous result.

In the case of the loamy sand soil with higher clay content (Buffelsvlei) effects of cropping system were more pronounced. For one season (2011) mono-cropped maize with minimal soil disturbance (T2), β -glucosidase enzyme activities were higher than conventionally cultivated, mono-cropped maize (T1) systems. β -glucosidase (E.C. 3.2.1.21), an enzyme involved in cellulose degradation, plays an important role in the soil organic carbon cycle. Cellulose is the most abundant organic compound in the biosphere. So a product of its enzymatic hydrolysis is important as an energy source for soil microorganisms. Since the soil samples were collected within the growing season, crop residues are ploughed into the soil of the conventionally cultivated, mono-cropped maize system (T1) before planting. This could have led to higher glucosidase activity immediately after ploughing hence reflecting a decrease level of bacterial glucosidases later during the season. This is supported in a study by Roldan *et al.* (2004), whereby tillage had negative effects on the hydrolase activities (urease, protease-BAA, phosphatase and β -glucosidase), at all soil depths, mainly with the adoption of mouldboard ploughing.

During the 2012/13 season glomalin levels in the loamy sand soil increased and were significantly higher at the lower depths in the mono-cropped maize with minimal soil disturbance system (T2) compared to conventionally cultivated, monocropped maize (T1), suggesting that mycorrhiza are colonizing the root system of the maize plant. Without any soil disturbance it creates the ability through its extensive hyphal network to deposit the glomalin into the deeper depths. The preceding season 2011/12 showed actinomycete levels also increased significantly in the two-year system with maize in rotation with sunflower with minimal soil disturbance (T3.1) and three-year system with maize in rotation with a legume or sunflower and pearl millet with minimal soil disturbance (T4.1) compared to conventionally cultivated, mono-cropped maize (T1). This occurred up to 15 cm depth signifying an increase in active degrading aerobic filamentous bacteria. In turn this will help with the breakdown of organic material in the top soil. At the 0 - 5 cm depth urease activity levels increase among the CA maized based system compared to conventionally cultivated, mono-cropped maize (T1).

The 2010/11 season showed that filamentous fungi were significantly higher in the mono-cropped maize with minimal soil disturbance (T2) and at 15 - 30 cm depth in the loamy sand soil, a three-year system with maize in rotation with sunflower and pearl millet with minimal soil disturbance (T4.1) compared to the conventionally cultivated, mono-cropped maize (T1) system. This is indicative of no or little soil disturbance allowing fungi to proliferate within this zone. No tillage significantly increased crop residue accumulation on the soil surface, which enriched this soil in labile organic matter. No tillage may promote fungal growth and the proliferation of fungal hyphae that contribute to macro-aggregate formation (Roldán *et al.*, 1994). This result is in agreement with Doran *et al.* (1980), which indicated that populations of fungi were significantly higher in the surface (0 - 7.5 cm) of no tillage soils than in the surface of tilled soil.

Based on the DNA fingerprinting profiles a pattern is starting to develop whereby the soil microbial community is changing in its composition. This is observed in the Shannon-Weaver diversity H' index in that the microbial community is shifting from a mainly bacterial dominant conventionally cultivated, mono-cropped maize to a more fungal based CA system. This situation is desired in terms of what is already described to create an environment that foster soil aggregation, improve water holding capacity, suppressing diseases and building a diverse and robust microbial population.

Conclusions

The switch from conventional to CA farming can create complex interactions within the soil ecosystem. These interactions cannot be studied over a short period of time but requires more measuring data points over an extended time period to provide a genuine representation of the soil biological interactions occurring in transitioning to a CA based system. Measuring these parameters at several intervals throughout the crop-growing season allows one to determine how, and at what magnitude, seasonal fluctuations affect soil microbiology. Climatic conditions such as drought and rainfall may also contribute in providing a masking effect in detecting differences among the various farming practices. For future studies redundancy discriminant analysis (RDA) might provide a better understanding to the relevant role players or factors that are active in the soil ecosystem.

This study highlighted that microbial activities are affected by various farming or management systems such as CA. However, since soil is a dynamic environment it creates a challenge in accurately monitoring changes. The study also revealed that switching from a conventionally monocropped system to a CA based system takes time to observe significant changes in soil microbial life and activities. System stability in terms of observing these biological changes will probably only be reached within a number of years of practicing CA.

References

- AMANN, R.L., LUDWIG, W. & SCHLEIFER, K., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59: 143 - 169.
- ANDERSEN, A., 1999. Plant protection in spring cereal production with reduced tillage. II. Pests and beneficial insects. *Crop Prot.* 18: 651 - 657.
- BOCKUS, W.W. & SHROYER, J.P., 1998. The impact of reduced tillage on soilborne plant pathogens. *Annu.Rev. Phytopathol.* 36: 485 - 500. York.
- LANE, D.J., 1991. 16S/23S rRNA sequencing. p. 115–175. In E. Stackebrandt and M. Goodfellow (ed.) *Nucleic acid techniques in bacterial systematics*. John Wiley & Sons, New York.
- DICK, R.P., 1994. SOIL ENZYME ACTIVITIES AS INDICATORS OF SOIL QUALITY. IN: DORAN, J.W., COLEMAN, D.C., BEZDICEK D.F. & STUART, B.A. (Eds.), *Defining soil quality for a sustainable environment. SSSA Special publication No.35*, Madison, Wisconsin, pp. 107 - 124.
- DORAN, J.W. & PARKIN, T.B., 1994. Defining and assessing soil quality. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A. (Eds.), *Defining soil quality for a sustainable environment. SSSA Special Publication 35*, Madison, WI, pp. 1 - 45.
- DORAN, J.W., 1980. Soil microbial and biochemical changes associated with reduced tillage. *Soil Science Society of America Journal.* 44: 765 - 771.
- SPEEDING, T.A., HAMELA, C., MEHUYSA, G.R. & MADRAMOOTOO, C.A., 2014. Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. 2004. *Soil Biol. Biochem.* 36: 499 - 512.
- HILL, G.T., MITKOWSKI, N.A., ALDRICH-WOLFE, L., EMELE, L.R., JURKONIE, D.D., FICKE, A., MALDONADO-RAMIREZ, S., LYNCH, S.T. & NELSON, E.B., 2000. Methods for assessing the composition and diversity of soil microbial communities. *Appl. Soil Ecol.* 15: 25 - 36.
- JENKINSON, D.S. & LADD, J.N., 1981. Microbial biomass in soil: measurement and turnover. In: Paul, E.A., Ladd, J.N. (Eds.), *Soil Biochemistry*, Vol. 5, Marcel Dekker, New York, pp. 415

- 471.

KIRK, J.L., BEAUDETTE, L.A., HART, M., MOUTOGLIS, P., KLIPRONOMOS, J.N., LEE, H. & TREVORS, J.T., 2004. Methods of studying soil microbial diversity. *J. Microbiol. Meth.* 58: 169 - 188.

LIU, W.T., MARSSH, T.L., CHENG, H. & FORNEY, L.J., 1997. Characterization of microbial diversity by determining terminal restriction fragment polymorphisms of genes encoding 16S rRNA. *Appl. Env. Microbiol.* 63: 4 516 - 4 522.

MAY, L.A., SMILEY, B. & SCHMIDT, M.G., 2001. Comparative denaturing gradient gel electrophoresis analysis of fungal communities associated with whole plant silage. *Can J. Microbiol.* 47: 829 - 841

MAZZOLA, M., 2004. Assessment and management soil microbial community structure for disease suppression. *Annu. Rev. Phytopathol.* 42, 35 - 59.

MONTGOMERY, D.R., 2007. Soil erosion and agricultural sustainability. *P. Natl. Acad. Sci. USA* 104: 13 268 - 13 272.

MUYZER, G., DE WAAL, E.C. & UITTERLINDEN, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59: 695 - 700.

FUENTES J.P., BEZDICEK D.F., FLURY M., ALBRECHT S. & SMITH. J.L., 2006. Microbial activity affected by lime in a long-term no-till soil. *Soil & Tillage Res.* 88: 123 - 131.

OLSEN, G.J. & WOESE, C.R., 1993. Ribosomal RNA: a key to phylogeny. *The FASEB Journal*, 7: 113 - 123.

PANKHURST, C.E., DOUBE, B.M. & GUPTA, V.V.S.R., 1997. Biological Indicators of Soil Health. CAB International, Wallingford.

PANKHURST, C.E., 1994. Biological indicators of soil health and sustainable productivity. In: Greenland, D.J., Szabolcs, I. (Eds.), Soil Resilience and Sustainable Land Use, CAB International, Wallingford, UK, pp. 331 - 351.

- RANJARD, L., POLY, F., LATA, J.C., MOUGEL, C., THIOULOUSE, J. & NAZARET, S., 2001. Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. *Appl. Environ. Microbiol.* 67: 4 479 - 4 487.
- REEVES, D.W., 1997. The role of soil organic matter in maintaining soil quality in continuous cropping systems. *Soil Till. Res.* 43: 131 - 167.
- ROLDÁN A., SALINAS-GARCÍA J.R., ALGUACIL, M.M., G. DÍAZC, G. & CARAVACA, F., 2004. Changes in soil microbial activity following conservation tillage practices in a sorghum field under subtropical conditions. ISCO 2004 - 13th International Soil Conservation Organisation Conference - Brisbane, July 2004. *Conserving Soil and Water for Society: Sharing Solutions.*
- ROLDÁN, A., GARCÍA-ORENES, F. & LAX, A., 1994. An incubation experiment to determine factors involving aggregation changes in an arid soil receiving urban refuse. *Soil Biology and Biochemistry*, 26: 1 699 - 1 707.
- TABATABAI, M.A., 1994. Soil enzymes. In: Weaver RW, Angle JS, Bottomley PS (Eds.) *Methods of soil analysis, part 2. Microbiological and biochemical properties.* SSSA Book Series No. 5. Soil Sci. Soc. Am. Madison, Wis., pp. 775-833.
- TABATABAI, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, R.H. and Keeney, D.R., Editors, 1982. *Methods of Soil Analyses, Part 2, Chemical and microbiological properties.* (2nd Ed.). *Agronomy*, 9: 903 - 943.
- THIES, J.E., 2007. Soil Microbial Community Analysis using Terminal Restriction Fragment Length Polymorphisms. *Soil Sci Soc Am J.*, 71: 579 - 591.
- THIES, J.E., 2006. Measuring and assessing soil biological properties. In: *Biological approaches to sustainable soil systems.* In: Uphoff, N., Ball, A.S., Fernandes, E., Herren, H., Husson, O., Laing, M., Palm, C.A., Pretty, J., Sanchez, P.A., Sanginga, N., Thies, J. (Eds.), *Biological approaches to sustainable soil systems.* CRC Taylor and Francis, Boca Raton, pp. 655 - 670.
- TORSVIK, V., SORHEIM, R. & GOKSOYR, J., 1996. Total bacterial diversity in soil and sediment communities- a review. *J. Indus. Microbiol.* 17, 170-178.

TORSVIK, V., GOKSOYR, J. & DAAE, F.L., 1990. High diversity in DNA of soil bacteria. *Appl. Environ. Microbiol.* 56: 782 - 787.

VANCE, E.D., BROOKES, P.C. & JENKINSON, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19: 703 - 707.

VERHULST, N., GOVAERTS, B., VERACHTERT, E., CASTELLANOS-NAVARRETE, A., MEZZALAMA, M., WALL, P., DECKERS, J. & SAYRE, K.D., 2010. Conservation Agriculture, Improving Soil Quality for Sustainable Production Systems? In: Lal, R., Stewart, B.A. (Eds.), *Advances in Soil Science: Food Security and Soil Quality*. CRC Press, Boca Raton, FL, USA, pp. 137 - 208.

WRIGHT, S.F. & UPADHYAYA, A., 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science*, 161: 575 - 586.

WARD D.M., BATESON M.M. & WELLER R., 1992. Ribosomal RNA analysis of microorganisms as they occur in nature. *Adv Microb Ecol*, 12: 219 - 286

ZELLES, L., PALOJÄRVI, A., KANDELER, E., VON LUTZOW, M., WINTER, K. & BAI, O.Y., 1997. Changes in soil microbial properties and phospholipids fatty acid fractions after chloroform fumigation. *Soil Biol. Biochem.* 29: 1 325 - 1 336.

Publications

RHODE, O.H.J., 2013. Grondkwaliteit die basis van volhoubare landbou. *Graan SA/ Grain SA*, September 2013, 15(9): 52.

RHODE, O.H.J., 2012. Bewaringsboerdery: Bestuur die biologie jou grond. *Graan SA/ Grain SA*, September 2012. 14(9): 40.

VAN COLLER C., 2012. Erdwurms: Die produsent se belangrike vennoot in bewaringsboerdery. *Graan SA/Grain SA*, December 2012 Vol 12 No.12.

RHODE, O.H.J., 2010. Bewaringsboerdery: Grondbiologie is deurslaggewend. *Graan SA/ Grain SA*, 1/12/2010, 12(12): 38.

RHODE, O.H.J., 2010. Grondmikrobes - 'n belangrike vennoot. *Landbouweekblad*, 1641: 24-26. 19 February 2010.

Poster presentations

RHODE, O.H.J., NEL, A.A. & BEZUIDENHOUT, C.C., 2013. Soil microbial community response to conservation agricultural practices in local maize production. SASM2013. Bela Bela. 24 - 27 November 2013.

RHODE, O.H.J., NEL, A.A. & BEZUIDENHOUT, C.C., 2013. Changes in soil microbial activities under conservation agricultural practices in local maize production. Combinedcongress, Durban, 21 - 24 January 2013.

RHODE, O.H.J., NEL, A.A. & BEZUIDENHOUT, C.C., 2012. Early soil microbial activities affected by conservation agricultural maize production systems. Combinedcongress 2012. North West University, Potchefstroom. January, 2012.

RHODE, O.H.J., NEL, A.A. & BEZUIDENHOUT, C.C., 2011. Preliminary evaluation of soil microbial activities as affected by conventional and conservation agricultural (ca) practices in maize based systems in South Africa. FEMS, Geneva Switzerland 26 - 30 June 2011.

RHODE, O.H.J., BEZUIDENHOUT, C.C. & VAN WYK, D.A.B., 2010. Preliminary investigation of yeast diversity in maize producing soils. Combinedcongress 2010, University of the Free State, Bloemfontein, 18 - 21 January 2010.

Radio talks

RHODE, O.H.J., 2013. Grondgesondheid, die basis van volhoubare landbou. RSG. 7 August 2013

RHODE, O.H.J., 2012. Grondmikrobiologie in bewaringsboerdery. RSG 1 August 2012

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