FINAL REPORT 2020/21

DETAILS

Project number		P05000100 (MT)
Project title		On-farm monitoring of selected soil fauna and beneficial microbes as bio-indicators in local soils under conservation agriculture regimes
Project manager		OHJ Rhode
Co-worker(s)	Internal	P du Toit, C Myburgh, CCM Abrams
	External	North-West University, Stellenbosch University, ARC-Nietvoorbij/Infruitec
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Final abstract report

The three conservation agriculture (CA) principles are advocated to improve soil quality, optimize crop yields and reduce input costs when the three CA principles are applied effectively. Due to the diversity of crops and their different root systems, organic matter is deposited in various soil strata (Verhulst, et al., 2010). Increased soil microbial diversity and activities are encouraged by crop diversification. Cropping systems that return crop residues to the field significantly increase the activity of a wide range of soil enzymes, compared to unamended soils, due to the stimulation of microbial activity. With this in mind, the study aims to monitor on-farm selected soil fauna and beneficial microbes as bio-indicators in local soils under conservation agriculture. Subsequently, this study made use of trials established by the Maize Trust project, P05000011. Trials promoting some of the principles of CA such as crop rotation were first established at two localities i.e., Brooksby and Lareystryd in the Mahikeng and Lichtenburg areas of North West Province during the 2019/20 and 2020/21 maize growing season. During these growing seasons, soil sampling from both field trials was done. Soil samples collected from the maize crop treatments were subjected to various microbiological analyses. Soil microbial enzyme activities from the collected soil samples were also determined using β-glucosidase, alkaline phosphatase and urease enzymes that are involved in carbon and nitrogen cycles in the soil, as well mycorrhiza colonization of crop roots and enumeration of mites and springtails. Using the soil alteration index three (AI3) the technology is based on a calculated balances between three microbial-secreted enzymes, potentially enables differences between soils due to contrasting management practices to be quantified in relative terms (Meyer et al., 2014). For all localities maize under conventional mono-

cropping displayed higher enzyme activities compared to CA treatments. A more negative AI3 index score for the maize based soils supported this. This was also the case for mycorrhiza and springtail counts for both seasons at Lareystryd and Brooksby. These findings suggest that maize cropping systems for this particular region provide the highest activities concerning microbial activities and soil micro fauna. However, this trend must be corroborated with on-going monitoring.

Keywords: conservation agriculture, soil enzyme activities, soil fauna, mycorrhiza

INTRODUCTION

In South Africa 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 and address soil deterioration brought about by conventional cultivation practices.

This conversion process achieved through CA, is governed by three main principles namely crop diversification, minimum soil disturbance, and permanent soil cover. Ensuing advantages such as increased infiltration, aggregate stability, increased water holding capacity, etc., are associated with a higher soil organic matter (SOM) content. Since SOM is responsible for the energy supply in a soil ecosystem, the application of these three main CA principles has a considerable impact on soil biology. Soil organisms can be classified into three main groups, which describe the principal function they perform in the soil: chemical engineers representing bacteria and fungi, biological regulators consisting of protozoa, springtails, mites and ecosystem engineers namely earthworms. The latter two are classified under soil fauna. With regard to soil fauna, microbial mass diversity and biological activity are higher in undisturbed soil or a soil system that is managed using CA techniques compared to those receiving deep cultivation (Nsabimana et al., 2004; Spedding et al., 2004). Currently we have limited information on the microbial community in soil and its role in CA. What is lacking are the impacts and contribution that biological regulators and ecosystem engineers are making in local CA systems and if the scenario is similar to that of e.g. Argentina. On-farm monitoring of these soil fauna could prove to be helpful to improve overall soil quality and grain crop performance, specifically for developing farmers. The three conservation agriculture (CA) principles are advocated to improve soil quality, optimize crop yields and reduce input costs when the three CA principles are applied effectively. Due to the diversity of crops and their different root systems, organic matter is deposited in various soil strata (Verhulst, et al., 2010). Increased soil microbial diversity and activities are encouraged by crop diversification. Cropping systems that return crop residues to the field significantly increase the activity of a wide range of soil enzymes, compared to unamended soils, due to the stimulation of microbial activity.

Soil enzymes are important mediators and catalysts of important soil functions that regulate decomposition, transformation and release of inorganic nutrients for plants growth, help in nitrogen fixation etc. The cropping systems, crops and fertiliser doses affect not only soil biota but also influence soil enzyme activities. Bacteria, actinobacteria, fungi, yeast, algae and protozoa (Pelczar et al. 2010). All facilitate metabolic processes in soils mainly through enzyme synthesis (Dick 1994). The activities of soil enzymes may be affected by agricultural management practices (Dick 1994). β-

glucosidase, urease and phosphatase, which are active in the cycling of, respectively, carbon (C), nitrogen (N) and phosphorus (P), have been shown to be sensitive to management-induced changes in the soil environment by Caravaca et al. (2002). Typically, enzyme activities correlate with soil organic matter (OM) content, with soil OM being the site of enzyme synthesis and enzyme stabilisation (Tabatabai 1994). Activities of these enzymes are indicative of short-term (Bandick and Dick 1999) and medium- to long-term (Jin et al. 2009) changes in their respective pools of organic nutrients. Potentially, enzyme activity may be a useful indicator of the effects of different soil management practices on soil conditions, which in turn may affect crop performance. However, insofar as monitoring microorganisms are concerned, it is not possible to evaluate the ecological significance of a microorganism simply by determining their numbers. It is of greater importance to obtain information on their activity (Alexander, 1977). This view was reiterated by Naseby and Lynch (1997), who considered enzymatic determinations more useful than microbial measures, since they can be made with higher precision.

The majority of reports are thus, in favour of using enzyme activity as valid indicators of soil health (Dick 1994, Dick et al. 1996, Marx et al. 2001; Paz-Ferreiro et al. 2009). However, some studies on individual enzyme activities suggest strong temporal and spatial variability, dependent on the function of the enzyme and the type of land use under consideration thus, often leading to conflicting results (Aon et al. 2001; Trasar-Cepeda et al. 2008; Garcı´a-Ruiz et al. 2009). It is this complexity of the behaviour of the soil enzymes that raises doubts, among some researchers, about the suitability of enzyme activities as a measure of general soil health. However, the activities of individual enzymes are rather difficult to interpret and a number of reports proposed using simultaneous estimation of multiple enzyme activities, equations or single numerical values (indices), as indicators of soil health or quality (Pankhurst et al. 1997; Trasar-Cepeda et al. 1998; Saviozzi et al. 2001; Killham and Staddon 2002). One such index, alteration index three (AI3), quantifies the balance between the three. Latest research regarding the use of enzymes as indicators of soil health involves an innovative approach using a soil alteration index three (AI3). This technology is based on a calculated balances between three microbial-secreted enzymes, potentially enables differences between soils due to contrasting management practices to be quantified in relative terms (Meyer et al., 2014). The ability of AI3 to distinguish between apple orchard soils under conventional and organic production protocols, and to reflect tree performance, were tested in a maturing apple orchard. AI3 was proofed to be a useful indicator of relative apple tree performance under organic and conventional soil surface management practices. This inspire confidence that the AI3 index could also be used as soil management tool in CA systems.

Stimulation of microorganisms in the rhizosphere and the improved physical condition of soils in crop rotations have been observed, particularly when the cropping systems have contained legume species. Synergistic associations between soil biota and plant roots (rhizosphere) are facilitated through the release of root exudates, leading to improved nutrient cycling, plant growth stimulation, and disease resistance, resulting in increased soil quality, crop health and yield. This could possibly be indicative of better mycorrhiza fungi root colonisation. As mycorrhiza fungi cover and extend the crop roots, there may be less room for pathogenic fungi to infect.

Conventional tillage does not only alter the soil's physical and chemical properties, but also the spatial integrity of the soil as a support matrix for the functioning of the soil microbial communities. Conventional tillage breaks crop residues into smaller pieces and redistributes them throughout the ploughed layer. Contrary to conventional tillage, no-till does not disrupt existing soil food webs, leading to increasing soil microbial diversity and activity. It has been argued that increased soil microbial diversity will increase the potential of an ecosystem to function more efficiently under a variety of environmental conditions suggesting greater resilience. It is therefore important to study communities rather than single species. Concerning micro-fauna, Cochran *et al*. (1994) suggest that management practices that favour bacteria would also be expected to favour protozoa, since bacteria are their main food source. In addition, the abundance of meso-fauna (in particular, pot worm) was greater where CA was practiced in comparison to compacted soil (Röhrig *et al*., 1998). The negative effects on micro arthropod populations are caused, in part, by the physical disturbance of the soil from plough-based tillage. Soils managed according to CA principles show significantly decreased bulk density at the surface. This results from the exiting mulch layer on top of non-tilled soils (Beisecker, 1994) that provides organic matter and food for soil fauna, which loosens surface soil because of burrowing activities.

Thus the aim of the study is to determine the effect of on-farm conservation agricultural promoted practices, tillage practices, and cropping systems, on soil fauna and selected microbes that can serve as bio-indicators over a three-year monitoring period in the North West under communal farming. To generate information that would add to the existing knowledge base as to which soil fauna are prevalent under established CA systems under communal farming.

MATERIALS AND METHODS

This study is underpinned by the agronomic practices of Maize Trust project P05000011. Soil samples were collected during grain filling stage of maize crops at the trial sites at Brooksby (2019/2020), and Lareystryd (for 2019/20 and 2020/21) in the Lichtenburg and Mafikeng areas.

Rhizosphere soil samples from various treatments comprising of strip plots of selected crops namely maize, sunflower, soybean and cowpeas were taken for further analyses. Subsequently, selected laboratory analyses were performed on these samples.

Lareystryd trial

Due to the removal of the crop residue of the previous season (2019/20) by livestock accessing the plot, no crop cover remained. As a result of this event, no soil cover was left. In view of this, the focus was on the effect of crop rotation.

Brooksby trial

Due to theft of maize at the Brooksby plot during the growth period in 2020, the trail could not be harvested. However, soil samples could still be sampled. As indicated by the report of Mr P du Toit, the trial plot at Brooksby was planned to be planted on 20 January 2021 following several delays but due to unforeseen circumstances resulted in a failure of the trial at Brooksby.

Laboratory analyses

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	*p*-Nitrophenyl phosphate [25mM]	6.5
3.2.1.21	β-glucosidase	*p*-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

AI3 values were calculated with the equation: AI3 = (7.87 x β-glucosidase) – (8.22 x phosphatase) – (0.49 x urease) where enzyme activities were expressed in micromoles of, respectively, p-nitrophenyl-β-D-glucoside and p-nitrophenylphosphate per gram of soil per hour, and micrograms of urea per gram of soil per hour.

Soil fauna assessment

The On-Farm Soil Monitoring Handbook was used as guide how to sample soil and how to extract and count the dominant soil meso-fauna (mites and springtails). Data was recorded and subjected to statistical analyses using Statgraphics software package.

Mycorrhiza colonization

Mycorrhiza colonization was determined by using the method described by Vierheilig et al 1998.

RESULTS

Lareystryd trial

Results were obtained and are presented as follows: Selected soil enzyme assays (β-glucosidase, alkaline phosphatase and urease) revealed no significant differences between the various treatments at Lareystryd (Table 2). However, the soil alteration index three (AI3) showed a more negative value in the maize plots compared to other treatments at Lareystryd. The AI3 index quantifies the balance between the three enzymes into a singular numerical value. A more negative value indicates an improvement in soil quality. This means the soil quality could be better in the maize plots compared to the rest of the treatments. Furthermore, this is also supported by the higher colonization (34%) percentage of maize treatments compared to cowpeas (12%) and soybean (13%) (Table 5). We also detected that in the maize and sunflower treatments the mites: springtails ratio is lower than in soybean treatments. In the second season at Lareystryd enzyme activities were all higher in maize plots preceded by maize or a legume in the previous season than maize preceded by sunflower (Table 4). The AI3 index also supported this trend. Springtail numbers were also significant higher in the maize rotated with maize treatments as well as mychorrhizal colonization (Table 7). However, the highest ratio of mites to springtails were detected in the maize preceded by soybean. The lowest ratio of mites to springtails were recorded in the maize preceded by sunflower.

Brooksby trial

At Brooksby the selected soil enzymes showed no significant differences among treatments (Table 3). A more negative value for the AI3 index was also obtained in the maize plots. A similar finding for the mites: springtails ratio occurred in the soybean and cowpea treatments that was higher compared to the sunflower and maize treatments (Table 6). Mycorrhiza colonization was also higher in the maize and sunflower treatments compared to the legume treatments.

Table 2. Soil microbial enzyme activity as affected by treatment at Lareystryd during 2019/20 season. Glucosidase - and phosphatase activities were measured in $mg.kg^{-1}$ $soil.h^{-1}$. Urease activity was measured in $mg.kg^{-1}$ $soil.2h^{-1}$

Treatment	df	Glucosidase	Urease	Phosphatase	AI3
Maize		1509a	5.15a	1213a	-9888
Soybean		1445a	4.36ab	1134a	-9241
Cowpea		1409ab	4.42ab	1128a	-9194
Sunflower		1379ab	3.98ab	1052ab	-8571
ANOVA					
Source of variation					
Treatment	12	NS	NS	NS	

NS = Not significant at the 0.05 probability level

Table 3. Soil microbial enzyme activity as affected by treatment at Brooksby during 2019/20 season. Glucosidase - and phosphatase activities were measured in $mg.kg^{-1}$ $soil.h^{-1}$. Urease activity was measured in $mg.kg^{-1}$ $soil.2h^{-1}$

Treatment	df	Glucosidase	Urease	Phosphatase	AI3
Maize		943a	4.15a	1008a	-8234
Soybean		936a	4.16a	978a	-7988
Cowpea		879a	4.28a	983a	-8032
Sunflower		885a	3.64ab	964a	-7876
ANOVA					
Source of variation					
Treatment	12	NS	NS	NS	

NS = Not significant at the 0.05 probability level

Table 4. Soil microbial enzyme activity as affected by treatment at Lareystryd during 2020/21 season. Glucosidase - and phosphatase activities were measured in $mg.kg^{-1}$ $soil.h^{-1}$. Urease activity was measured in $mg.kg^{-1}$ $soil.2h^{-1}$

Treatment	df	Glucosidase	Urease	Phosphatase	Al3
Maize-maize		1704a	5.02a	1521a	-12408
Soybean-maize		1690a	5.23a	1529a	-12475
Cowpea-maize		1586ab	5.12ab	1455a	-11872
Sunflower-maize		1488c	4.74b	1367b	-11154
ANOVA					
Source of variation					
Treatment	12	NS	NS	NS	

Effect of crop rotation on legumes and sunflower

No significant crop rotation effect was observed on legumes and sunflower for enzyme activities (data not shown).

Table 5: Soil flora and fauna results for Lareystryd for 2019/20 season

Treatment	Mites (per m^2)	Springtails (per m^2)	Mites: Springtails ratio	Mycorrhizal colonization (%)
1- Maize	600a	1800b	1:3.0	44
2- Soybean	300c	2300a	1:3.2	13
3- Cowpeas	620a	1950b	1:3.14	12
4-Sunflower	490b	1500c	1:3.3	25

Means followed by different letters within a column are significantly different at $P < 0.05$

Table 6: Soil flora and fauna results for Brooksby for 2019/20 season

Treatment	Mites (per m^2)	Springtails (per m^2)	Mites: Springtails ratio	Mycorrhizal colonization (%)
1- Maize	300a	900a	1:3.0	31
2- Soybean	130c	850b	1:6.5	21
3- Cowpea	210ab	820b	1:3.9	15
4-Sunflower	450a	750ab	1:1.7	28

Means followed by different letters within a column are significantly different at $P < 0.05$

Table 7: Soil flora and fauna results for Lareystryd for 2020/21 season for maize crop rotation

Treatment	Mites (per m^2)	Springtails (per m^2)	Mites: Springtails ratio	Mycorrhizal colonization (%)
1- Maize-Maize	554a	1923a	1:3.5	51
2- Soybean-Maize	426b	1834b	1:4.3	18
3- Cowpea-Maize	517a	1789b	1:3.5	16
4-Sunflower-Maize	478ab	1528b	1:3.2	29

Means followed by different letters within a column are significantly different at $P < 0.05$

DISCUSSION

It is evident from the results obtained that maize treatments under monocropping showed higher enzyme activities in both localities *viz.* Lareystryd and Brooksby. Soil enzymes regulate ecosystem functioning and in particular play a key role in nutrient cycling. These enzyme activities are the direct expression of the soil community to metabolic requirements and available nutrients (Spedding et al., 2004). The glucosidase is an important enzyme in terrestrial carbon cycle in producing glucose, which contributes important energy source for microbial biomass (Tabatabai, 1994). The glucosidase activity has been suggested as a good indicator of soil quality (Bishnu et al., 2008) The AI3 index also showed a more negative value in maize treatments indicative of a possible better soil quality (Meyer et al., 2014). Furthermore, mycorrhiza colonization in maize and sunflower is higher than in legume treatments. This is an expected result since it is known that arbuscular mycorrhiza is strongly dependent on maize and sunflower to lesser extent to form strong mutualistic associations (Arihara and Karasawa, 2000).

Concerning springtails, they are often sampled as bio-indicators of healthy soils. They are considered beneficial insects that eat decaying plant material, fungi or bacteria, breaking down material and improving soil structure. The results suggest that maize treatments are a good source of food for springtails.

CONCLUSIONS

This study highlighted that microbial activities are affected by various farming or management practices 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 maize based system takes time to observe significant changes in soil microbial life and activities. The stability of the system in terms of observing these biological changes will probably only be reached within a number of years of practicing CA. Despite these slow changes, there are already trends that serve as a baseline for further studies and tracking any changes that may occur in the soil microbiome. With continued monitoring the soils, mites and types of springtails as well as mycorrhiza fungi will become more familiar. Relationships between these factors and the environment will also be clearer and provide guidance to the farming communities especially our local developing (communal) farmers transitioning to commercial CA farming.

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