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Effects of oats and vetch cover crops on light organic matter fractions and activities of selected enzymes in an irrigated maize based conservation agriculture system on Alice Jozini Ecotope in the Eastern Cape, South Africa

By

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DECLARATION

I, Caroline Mukumbareza, declare that the dissertation hereby submitted for the degree of Master of Science in Agriculture (Crop Science) at the University of Fort Hare is entirely my work and that all reference materials contained in this dissertation have been duly acknowledged. This dissertation has not been previously submitted to another university for any other degree.

Signed at Alice this.....day of……2014

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Caroline Mukumbareza

PREFACE

This dissertation consists of seven chapters. Chapter 1 introduces the study area giving the background and justification as well as stating the broad objective of the study. Chapter 2 is the literature review relevant to the study. Chapters 3-5 are on three specific experiments presented in paper format, complete with the introduction, specific objectives, hypotheses, materials and methods, results and a brief discussion. Chapter 6 discusses the main findings of the work, as well as the general conclusion and recommendations for future studies. All the literature cited in the study is listed in the reference list. Appendices containing outputs of the statistical analyses of the results are at the end of the dissertation.

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ABSTRACT

Low soil fertility in the Eastern Cape Province (EC) is attributed to poor agricultural management, which reduces organic matter, among other factors. Conservation agriculture (CA), consisting of no-till, soil cover and rotations, is being promoted to improve soil quality and productivity. Soil biological parameters respond to the overall effects of management on the physical and chemical components of soil.

This study was conducted to evaluate the effects of oat (*Avena sativa*) and grazing vetch (*Vicia dasycarpa*) cover crops on soil biological activity after five years under CA and evaluate light organic matter fractions, MBC and activities of selected enzymes as early indicators of organic matter build up under in no-till maize based CA system. The study was based on soil samples collected from a five year trial with rotations of maize and sole cover-crops and two younger trials (4 and 28 months) with oat and grazing vetch bicultures.

In the five year old trial, oat and grazing vetch were planted at four fertiliser regimes (i) Fertilizer applied to cover crops and maize (F1), (ii) fertilizer applied to cover crops only (F2), (iii) fertilizer applied to maize only (F3) and (iv) no fertilizer applied (F4)) to give a 2 × 4 factorial plus control laid out in a randomized complete block design (RCBD) with three replications. Fertilizer was applied at 10 kg P ha^{-1}, as a compound (6.7% N; 10% P; 13.3% K) at planting and grazing vetch was inoculated with *Rhizobium legunominosarium* biovar *viciae*. Oat was top dressed using limestone ammonium nitrate (LAN – 28% N) at 7 weeks after planting (WAP) to make a total of 45 kg N ha^{-1}. Three weeks after termination of the cover crops, all plots were split in half and maize was planted and fertilizer applied at 0 and 60 N kg ha^{-1}. A third of the N was applied at planting (6.7% N; 10% P; 13.3% K).

In the biculture trials, the treatments were 90% oat + 10% vetch, 70% oat + 30% vetch, 50% oat+ 50% vetch, 100% vetch and 100% oat and laid out in a RCBD with three replicates. Only basal fertilizer was applied to the cover crop at planting at 13.34 kg N ha^{-1}, 20 kg P ha^{-1} and 26.66 kg K ha^{-1} using 2:3:4 (30 + 0.5% Zn) compound fertiliser. After cover crop termination, SC701 maize variety was planted, and fertilised at 60 kg N ha^{-1} with a third of the N applied as a basal.

In the five year old trial, oat and vetch gave significantly ($P < 0.05$) higher MBC and activities of all soil enzymes measured than the weedy fallow at 0-5 and 5-20 cm depths. Fertilization of cover crop (F2) and maize (F3) gave similar MBC. The F4 in cover crops

gave similar dehydrogenase activity with F3 under weedy fallow and that for vetch rotation was the same for F3 in oat. The F4 for grazing vetch had similar β-glucosidase activity as F1 and similar urease activity as F1, F2 and F3 of oat rotations. Acid and alkaline activity in F1 had similar results to F2.

In the younger trials, biculture treatments did not improve total C, and N when compared with the weedy fallow. Dehydrogenase, β-glucosidase, arylsulphatase and alkaline phosphatase activities were higher in the 28 month trial compared to the 4 month one while urease was higher in the 4-month old trial. Treatments with more than 50% oat content had higher acid phosphatase activity in the 4- than 28 month old trial. Effects of cover crop, as the main factor, was significant in all enzymes ($P < 0.05$), with 70% oat + 30% vetch treatment having the highest dehydrogenase and arylsulphatase while for alkaline phosphatase it had similar results to 90% oat + 10% vetch. For β-glucosidase and urease, effects of cover crop as the main factor were also significant with sole vetch giving the highest activity for both enzymes. Acid phosphatase activity was highest in sole oat.

Particulate OM was highest in the 100% oat treatment and declined with decrease in the proportion of oat in the biculture. The 50% oat + 50% vetch treatment had similar POM to the 70% oat +30% vetch in the 4 month old trial and to the 90% oat + 10% vetch in the 28 months old trial in the 0-5 cm depth ($P < 0.05$). In the 5-20 cm depth, the 70% oat +30% vetch treatment had similar POM to the 90% oat + 10% vetch in the 28 month old trial. The 70% oat+30% vetch had greatest WSC and MBC followed by the 90% oat +10% vetch, with the 50% oat + 50% vetch being similar to 100% vetch and 100% oat (MBC only). The 28 months old trial had greater POM and WSC than the 4 months trial.

The findings of this study imply that the use of grazing vetch and fertilization of cover crops only improves soil biological activity, represented by MBC and enzyme activities, after 5 years of maize-cover crop rotations in low input conservation agriculture system. Bicultures, particularly the 70% oat + 30% vetch, work better than their sole crops in improving organic matter fractions, MBC and enzyme activities and that POM, WSC, MBC, enzyme activities are useful early indicators of soil organic matter build-up of CA systems.

Keywords: Soil enzymes, conservation agriculture, microbial biomass carbon, particulate organic matter, water soluble carbon.

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LIST OF ACRONYMS

SA	-South Africa
EC	-Eastern Cape Province
UFH	-University of Fort Hare
FAO	-Food and Agriculture Organization
GMRDC	-Govan Mbeki Research and Development Centre
NRF	-National Research Foundation
ANOVA	-Analysis of variance
CV	-Coefficient of variation
RCBD	-Randomized Complete Block Design
$LSD_{0.05}$	-Least significant difference at 5% probability level
ns	-Non significant
*	-Significant at 5 % probability level
**	-Significant at 1 % probability level
***	-Significant at 0.1 % probability level
MBC	- Microbial biomass carbon
POM	-Particulate organic matter
WSC	-Water soluble carbon
C	-Carbon
S	-Sulphur
P	-Phosphorus
N	-Nitrogen
CA	-Conservation Agriculture
C: N	-Carbon to nitrogen ratio
g	-Gram
µg	-Microgram
mg	-Milligram
%	-Percentage
kg	-Kilogram
mm	-Millimeter
L	-Litre
H_2SO_4	-Sulphuric acid
HCl	-Hydrochloric acid

KCl	-Potassium chloride
$CaCO_3$	-Calcium carbonate
TTC	- 2,3,5 triphenyl tetrazolium chloride
K_2SO_4	-Potassium sulphate
$K_2Cr_2O_7$	-Potassium dichromate
H_3PO_4	-Phosphoric acid
$Fe(NH_4)_2(SO_4)$	-Ferrous ammonium sulphate
$CaCl_2$	-Calcium chloride
TPF	-Triphenyl formazan
MUB	-Modified universal buffer
THAM	-Tris-hydroxymethyl aminomethane
KCl-PMA	-Potassium chloride-phenylmercuric acetate
NaOH	-Sodium hydroxide
LAN	-Lime Ammonium Nitrate
m	-Meter
SOM	-Soil Organic matter
WAP	-Weeks after planting

1. GENERAL INTRODUCTION

1.1 Introduction

Most soils of the Eastern Cape (EC) Province of South Africa (SA) have agricultural sustainability problems due to soil erosion and fertility decline. The majority of these soils are derived from shales and mudstones, and are high in silt and fine sand content which makes them unstable and highly erodible (Laker, 1999). In addition to soil texture, these soils have high levels of sodium (Na) and/or magnesium (Mg), which cause dispersion and erosion (Van der Merwe & De Villiers, 1998). Soil organic matter (SOM), which improves soil aggregate stability, is low in most soils of the EC province, further enhancing soil erosion. Soil erosion is conventionally perceived as the main reason for declining crop yields in South Africa (Laker, 2004). Soil OM has been used as an indicator of soil quality (Gregorich & Ellert, 1993), as it is related to other important soil parameters.

According to Doran *et al.* (1994), soil quality is "the capacity of a soil to function within an ecosystem and sustain biological productivity, maintain environmental quality and promote plant and animal health". The decline in soil fertility is a threat to crop production hence food security in the smallholder farming systems. Soil productivity deterioration affects soil physical, chemical and biological properties and processes. Biological degradation includes low SOM, and declining microbial and mesofaunal activity and population (Eswaran *et al*., 2001). Monoculture cereal production, coupled with insufficient or no fertility replenishment, intensive tillage, overgrazing, short to no fallow periods and the virtual absence of crop rotation systems, are among the major causes of soil quality decline (Barnard & Newby, 1999). The three principles of conservation agriculture (CA) consisting of minimal soil disturbance, permanent soil cover and crop rotations have the potential to arrest and reverse the effects of soil erosion.

Research on CA in the EC Province has been focusing on ways to improve soil fertility, curb soil erosion as well as reduce weed infestations in arable lands. A number of cover crops including oats (*Avena sativa*) and grazing vetch (*Vicia dasycarpa*) have been tested in this regard and maize grown after these winter cover crops has shown an increase in yields (Murungu, 2010). Growing of high biomass yielding winter cover crops saw improvement in soil cover resulting in decreased weed infestation in irrigated maize based systems (Murungu, 2010). The killing of the cover crops could result in increased levels of OM in surface soil resulting in better structural stability, nutrient cycling and water dynamics and increased microbial population and activities (Derpsch, 2003).

Under no-till, the continued practice of cover cropping could be an investment in building healthy soil over the long term by providing OM (Lal, 2000). Work at the University of Fort Hare (UFH) has shown increased maize yields due to cover crops. This has been attributed to weed control and nutrient cycling. Changes in soil organic carbon (SOC) may not be detectable in the short term because of low magnitude of change, high background carbon levels and high natural variability of soils (Friedel *et al.*, 1996). Dube *et al*. (2012) showed improvements in light organic matter fractions in maize-cover crop (oat and vetch) rotations carried out over a five year period at the University of Fort Hare Research Farm. Dube (2012) also found improvements in phosphorus (P) cycling in these systems. While the physical and chemical soil parameters can improve in CA systems, it is the biological parameters that indicate the overall productivity of the soil. It is therefore essential to understand the changes in soil biological properties in CA systems.

Soil management practices (such as crop rotation, mulching, tillage and application of fertilisers and pesticides) have diverse effects on soil microbial activities and on various

enzymes (Tabatabai, 1994). There is evidence that suggests that the incorporation of cover crops plays a vital role in ensuring that the soil supports biological activity (Derpsch, 2007a, b). The long term on-going CA trials for continuous irrigated maize at UFH farm provide an opportunity to investigate the role of CA in improving soil health.

Grass and legume cover crops have been shown to differ in terms of their contributions in CA systems. Grass cover crops tend to provide greater mulch, contributing to water conservation while legumes, due to their lower C:N ratios contribute more to nutrient cycling. Both these benefits could make significant contributions in small holder production systems. Bicultures of grass and legume cover crops could make this combined benefit possible, either in an additive or synergistic way. Soil biological properties could be the most sensitive approach to determine whether or not soil productivity is improved through the use of cover crops, either as sole crops or bicultures in the short-medium term.

Although SOM has been related to soil quality, particulate organic matter (POM) is a short term or early indicator of long term changes in soil quality. It has been shown to be much more sensitive than SOC to changes in agricultural management and as such has been proposed as an early indicator of soil quality (Gregorich & Ellert, 1993). It would be important to investigate the effects of the CA systems on POM.

The living and active part of SOM, microbial biomass carbon (MBC), and water soluble carbon (WSC) have been suggested as sensitive measures of changes in SOM (Friedel *et al.,* 1996). With a comparatively rapid rate of turnover of 1-2 years, it is possible to detect changes in the microbial biomass fraction long before they are detectable in the total OM. Water soluble carbon (WSC) is the most mobile and reactive soil carbon source and it is a major substrate (C-source) for microorganisms, and as such, can easily reflect the effects of land use on soil quality (Nelson *et al.,* 1994). Water soluble carbon reflects the equilibrium

between dissolved and solid phases and is closely related to microbial activity thereby being a sensitive indicator of total SOM and changes in soil management (Haynes & Francis, 1993). It is therefore essential to investigate the responses of MBC and WSC to the CA systems including those being tested in the Eastern Cape.

Enzymes (dehydrogenase, β-glucosidase, urease, acid and alkaline phosphatases and arylsulphatase) that are involved in the cycling of important nutrients (C, N, P and S) can also be used as possible early indicators of changes in soil quality. Enzymes are protein catalysts synthesised by soil microorganisms, plant roots and soil fauna to catalyse important soil processes. Enzyme activities and microbial biomass are closely related because transformations of the important organic elements occur through microorganisms (Wang *et al.,* 2013). Enzymes produced by soil microbes are sensitive to stress on the ecosystem, and can provide robust measures of the ecosystem health and sustainability (Boerner & Brinkman, 2003; Dick, 1994). Many studies have reported significant correlations among soil enzyme activities, microbial biomass and various soil properties. Dick (1997) found a strong correlation between dehydrogenase activity and MBC.

Enzymes exist as intracellular or extracellular enzymes. Extracellular enzymes exist both in viable cells and in soil solution and are complexed to inorganic and organic soil colloids. The activity of extracellular enzymes does not necessarily reflect microbial activity at the time of assay but reflect the cumulative effect of previous microbial activity (Boerner *et al.* 2005). However, certain enzymes like dehydrogenase can only exist in viable cells and their activities provide an index of overall microbial activity. The measurement of soil enzymes can be used as indicative of the biological activity or biochemical processes (Dick *et al.,* 1988). Soil enzyme activities have the potential to provide a unique integrative biological assessment of soils because of their relationship to soil biology, easy of measurement and rapid response to changes in management (Kirchner *et al.,* 1993). The investigation of POM,

MBC, WSC and soil enzymes could go a long way in understanding the contributions of the long term trials at the UFH farm, and any other new trials in the Eastern Cape, to soil OM and ecosystem health.

1.2 Main objective and main hypothesis

The overall objective of this study was to determine the effects of rotations of maize with oat and grazing vetch cover crops, in long-term sole crops and short-term bicultures, on soil microbial biomass, enzyme activities and light organic matter fractions in no-till maize based system. The null hypothesis was that the rotation of maize with oat and grazing vetch cover crops either as sole crops or bicultures did not improve the soil microbial biomass, enzyme activities and light organic matter fractions in no-till maize based system.

Specific objectives and hypotheses of specific experiments are outlined in the respective chapters 3-5.

2. LITERATURE REVIEW

2.1 Soil degradation and soil quality improvements

Soil degradation has been defined by different authors differently; Lal (1993) defined it as the decrease in soil's actual or potential productivity owing to land misuse while Bai *et al.* (2008) defined it as the long term loss of ecosystem function and productivity caused by disturbances from which the land cannot recover unaided. Decline in soil quality caused by its improper use, usually for agricultural, pastural, industrial or urban purpose is a serious global problem. Soil quality is faced by a number of threats which include (i) soil erosion, (ii) soil contamination, (iii) decline in organic matter and biodiversity, (iv) soil compaction, (v) salinization, (vi) floods and landslides, and (vii) soil sealing. These processes reduce soil quality by changing the soil attributes, such as nutrient status, organic and labile carbon, texture, water-holding capacity, structure, maximum rooting depth, and pH. Soil erosion is the most severe consequence of soil degradation and most of the soil degradation processes are interlinked, and are often linked by similar causative factors (De la Rosa, 2005).

Soil erosion being the most widespread form of soil degradation, land area globally affected by erosion is 1094 million ha (Mha) by water erosion, of which 751 Mha is severely affected and 549 Mha by wind erosion, of which 296 Mha is severely affected (Oldeman, 1994). South Africa (SA) has not been spared from this land degradation. Implementing agricultural practices that reduce soil degradation has the potential to increase agricultural sustainability and soil conservation. The main ways to reverse soil degradation, increase soil organic matter content and improve soil health seem to be the three principles of conservation agriculture (CA) which are: i) minimal to no soil disturbance, ii) use of crop rotations and iii)

permanent soil cover. Implementation of these practices can help restore a degraded agro-ecosystem into a sustainable and productive state (FAO, 2008).

According to a number of estimates, in 2009, CA was practiced on roughly 110 million ha (Mha) worldwide that is approximately 6-7% of the world's total cropland. Derpsch *et al.* (2010) estimated that no-till agriculture (which they equate with CA) was practiced on nearly 116 Mha in 2009, and is increasing at a rate of 6 Mha per year. Kassam *et al.* (2009) distinguished no-till agriculture from CA (which they define as involving no-till or minimal tillage plus permanent soil cover and diversified crop rotations), and estimate that CA was used on 106 million ha world-wide in 2009. Africa has the lowest portion of area under CA or no-till farming, accounting for only about 0.3% of the world total and its adoption rates vary widely by country in Africa.

Although the practice of CA on a large scale emerged from Brazil and Argentina, same developments are now happening in the rest of the world. There has been an increase in no-till systems in Brazil with >11 Mha planted in 1998/1999 (Derpsch, 2001). Zero tillage is now practices on more than 95 Mha worldwide with highest adoption in North and South America (Derpsch, 2005) followed by South America with 47%, USA and Canada with 39%, Australia with 9% and about 3.9% in the rest of the world including Africa, Asia and Europe. In South Africa the extent of CA adoption is quite limited and restricted to a small number of summer grain producers in the Free State, winter grain in the Western Cape and grain and sugarcane farmers in the KwaZulu-Natal (Fowler, 2000). There is also a general lack of information and statistics concerning CA in SA and for this reason estimates made by the Crop Estimates Committee were resorted to and these estimates refer to conservation tillage. It is estimated that reduced tillage is practiced on approximately 34.6% of total cultivable area and 8.6% is under no tillage. South Africa has a total land area of approximately 122

Mha and only 1.2 Mha is under irrigation and account for almost 30% of crop production (Scotney & van der Merwe, 1992; Goodland, 1995). The rest, about 60% have low organic matter content which makes them prone to soil degradation and only 3% of the possible 14% arable land is classified as being of high agricultural potential (van der Merwe & de Villiers, 1998). Management of type of tillage and their frequency can stop soil degradation and improve soil quality (Franzluebbers *et al.*, 1999b).

2.2 Approaches used to improve soil quality

A number of approaches in the past have been used to improve soil quality mainly by reducing soil erosion**.** These approaches included use of contour ridges and ridge drains, windbreaks and shelterbelts, strip cropping and terracing, planting of cover crops to cover the soil during high risk periods, reduced tillage, leaving residues on soil surface and regular addition of organic matter to improve soil structure enhance water and nutrient holding capacity. Three of these approaches have now been widely adopted as the principles of conservation agriculture which aims at maintaining and/or improve soil quality.

Soil quality maintenance is an integral part of agriculture sustainability hence, in order to carry out sustainable farming system, it is necessary to apply soil management systems which improve or maintain soil quality. Conservation agriculture (CA) has been proposed as a widely adapted set of management principles that can assure more sustainable production. Conservation agriculture is an approach that fosters natural ecological processes to increase agricultural yields and sustainability by:

- Minimising soil disturbance by tillage and thus seeding directly into soil, eliminating tillage altogether once the soil has been brought to good condition, and keeping soil disturbance from cultural operations to the minimum possible,

- Maintaining permanent or year round OM cover over the soil including specially introduced cover crop and intercrops and or mulch provided by retained residues from previous crop,
- Diversifying crop rotation sequences and associations adapted to local environmental conditions, and including appropriate nitrogen fixing legumes. Such rotations contribute to maintaining biodiversity above and below the soil, contribute N to the soil or plant system, and help avoid build-up of pest populations (FAO, 2008; Derpsch, 2005).

The major objective of CA is to achieve sustainable and profitable agriculture for both mechanized and non-mechanized agriculture.

2.3 Successes and challenges of CA in the Eastern Cape Province

Majority of people in Africa depend on agriculture for their sustenance and livelihoods. However, Africa is witnessing severe degradation of its farmlands and EC province of South Africa has not been spared from this. Destructive management practices particularly cereal monocropping, burning of crop residues and intensive tillage contribute to this degradation, as they reduce the OM of the soil and destroy the soil structure (Mills & Fey, 2003; Mandiringana *et al.*, 2005). This leads to a fragile ecosystem hence a downward trend into poverty for farmers. Conservation agriculture comprise of a number of technologies that when used together can limit, arrest, or reverse the effects of unsustainable agricultural practices, especially soil erosion, SOM decline, and physical degradation of the soil, while at the same time reducing excessive fertiliser, pesticide and fuel use (FAO, 2008; Pretty, 2008). Conservation agriculture is seen as an encompassing solution to soil erosion, poor soil fertility and high weed infestation facing the resource poor smallholder farmers in the EC.

In SA, conservation agriculture has always been used mostly by commercial farmers with different approaches being taken (Fowler, 1999). According to Bollinger *et al*. (2006) SA has of late been actively involved in CA. The Agricultural Research Council's Institute for Soil, Climate and Water Land Care projects in EC tried to solve the issue of soil acidity and low fertility using agrochemicals and cultural methods. Legumes such as cowpea, lablab, and soyabean were incorporated as rotations, intercrops with maize, and mixtures of cover crops under-sown into maize as relay crops were used to fight against late summer weeds and improve soil health. The Massive Food Production Programme, a brainchild of the EC Department of Agriculture, is another project with a mandatory embracement of CA principles.

Conservation Agriculture Thrust (CAT) was established as a joint initiative between the EC Department of Agriculture and University of Fort Hare (UFH) from experiences of the Brazilian Cerrado, to promote CA in EC (FAO, 2011). However, all these programmes from such organisations as CAT have not made significant impact besides the benefits of CA. The adoption of full CA system has been poor, especially by smallholder farmers due to socio economic and biophysical limitations (Bollinger *et al.*, 2006). Among the various challenges, one major limitation has been low or lack of cover achieved in the CA systems as currently practiced, often resulting in weed problems. In mixed farming systems, competition for crop residues with livestock is another reason putting pressure on crop residues for soil cover (Twomlow *et al.*, 2008; Derpsch & Friedrich, 2009).

Studies carried out at UFH had a number of successes that were recorded and these include selection of winter cover crop species for improved biomass yields, soil cover, weed control and nitrogen fixation (Ganyani, 2010; Murungu *et al*., 2011; Musunda, 2010).

Grazing vetch, a legume cover crop was noted to improve maize yields mainly due to fertility service. However, their residues do not persist longer than grass cover crop residues. The grass cover crops were more effective in moisture conservation and weed control hence white oat was selected as the best in this regard (Murungu, 2010).

2.4 Selection of cover crops

The loss of soil fertility, destruction of OM, increased acidity and weed competition are some of the reasons why the use of cover crops in smallholder farmers is becoming pertinent. Various cover crop alternatives can be used as vegetative cover, such as grains, legumes, root crops and oil crops. All of these are beneficial to the soil, however, a right combination of suitable cover crops must be identified for each climatic region and this involves field trials over years before appropriate cover crops can be identified for recommendation to farmers (Anon, 2004). Cover crops that will meet the farmers' needs should possess some of these characteristics: easy to establish, fast growing to cover the soil within a short period, ability to grow in mixed stands, presence of extensive or deep rooting system to utilise nutrient and water at lower depths, ability to suppress weeds and pests (Varhallen *et al.*, 2003). However, performance of cover crops depends on a number of factors. It varies by species, growth environment and management (Cherr, 2004). While a farmer might want as many attributes and goals from a cover crop, it is important to decide on the few most important ones to ease the selection of cover crops. For majority of the EC smallholder farmers, soil fertility and weed control are the major goals besides soil protection.

In EC, several screening trial have been conducted at UFH for winter cover crops (Murungu *et al.*, 2010; Musunda, 2010). White oat and grazing vetch were the best candidates for the intended soil preservation against erosion, nutrient supply and weed

suppression characteristics. Although these two cover crops were found to be the best in these attributes, their input to what is happening below ground is not known hence this study looking at their effects on the biological activities in the soil. To be effective when adopted cover crops should produce high biomass, be easily killed by mechanical methods, suppress weed seed germination and weed growth and provide nutrients to the soil for the follow-on crop. Other parameters such as residue quality, decomposition and mineralisation rate are also a basis for cover crop selection and this is guided ultimately by the major goals to be achieved and the ability to fulfil those goals (Fosu *et al.*, 2004).

2.5 Approaches of using cover crops

Cover crops are grown during fallow periods, between harvest and planting of commercial crops, utilizing the rest of the moisture in the soil. They are killed either before the next crop is sown, or after sowing the next crop, but before competition between the two crops start. There is growing interest in the use of short-season summer annual legumes or grasses as cover crops in the crop production systems. Cover crops can provide a significant source of nitrogen (N) for subsequent crops; reduce erosion, runoff, and potential pollution of surface waters; capture soil N that might otherwise be lost to leaching; add organic matter to the soil; improve soil physical properties; impact insect and disease life cycles; and suppress nematode populations and weed growth. There can be potential drawbacks, such as cooler soils in the spring, and the additional cost of seeding the cover crop. These factors must be considered depending on the particular cash crops and cover crops being grown.

Cover crops can positively or negatively affect the follow-on crop due to their influence on N and water dynamics (Thorup-Kristensen *et al.*, 2003). The positive effects like those on yield have been attributed to an increase in soil N mineralization during decomposition of

cover crop residues (Kumar & Goh, 1999). Although decomposition of crop residues can lead to N mineralization, on the other hand it can lead to N immobilization hence adversely affecting the growth and yield of the following crop (Wagger & Mengel, 1993). Legumes and grasses have been used extensively as winter cover crops (Clark, 2007; Wagger & Mengel, 1993) and in this study white oat and grazing vetch were selected as the best candidates for the soil type and climatic conditions of the area and also for their attributes to increase soil fertility and crop yields.

Oats take up excess N and small amounts of phosphorus (P) and potassium (K) when planted early enough. It can be used as a catch crop after summer legume plough down to hold some N over winter where it winterkills. Some of the N in the winterkilled oats may be lost by spring either through denitrification or by leaching from the soil profile (Delgado & Lemunyon, 2006). Oats germinate quickly hence a great smother crop that outcompetes weeds and provides allelopathic residue that can hinder weed seed germination. Mixing oats with a legume helps slow establishing legumes such as vetches, clovers and winter peas while increasing biomass. It also helps in reducing autumn weeds. The oats will winterkill while improving the legume's winter survival.

Legumes vary widely in their ability to prevent erosion, suppress weeds and add OM to the soil. In general, legumes are lower in carbon and higher in nitrogen than grasses. This lower C:N ratio results in faster breakdown of legume residues (Jung *et al.,* 2004). Therefore, the N and other nutrients in legume residues are usually released faster than from grasses. Weed control may not last as long as for an equivalent amount of grass residue and they do not increase SOM as much as grasses (Fisk *et al.,* 2001). If there is a need for a cover crop to take up excess nutrients, a grass or a mixture is usually a better choice.

Mixing two cover crops species, a grass and a legume in a single stand is known as biculturing. This technology combines the benefits of both legumes and grasses, including biomass production, N scavenging and additions to the system, as well as weed and erosion control (Sainju *et al.,* 2005). Mixtures of two or more cover crops are more effective than planting a single species. Biculturing offers the best of both worlds combining the benefits of grasses and legumes, or using the different growth characteristics to fit the farmer's need (Delgado *et al.,* 2006). Mixing fast growing grasses and slow developing legumes, usually provides better erosion control because more of the ground is covered, as well as an increased root mass to stabilize the topsoil. The vegetation intercepts more raindrops before they can dislodge soil particles. Sunlight is used more efficiently because light that passes through the tall crops is captured by the low growing crops (Clack *et al.,* 1994). A grass-legume biculture adjusts to the amount of available soil N, if there is a lot of N the grass dominates if there is not much available N the legume will dominate. Vetch, a vinyl crop will climb oat that has a straight growth habit so it can get more sunlight and fix more N (Ranells & Wagger, 1996). In either case the farmer gets combined benefits of N scavenging by the grass and N addition from the legumes.

In summary, cover crops mixtures are used to improve: winter survival, use of solar radiation, ground cover, biomass and N production, duration of active growing period, weed control, tolerance to adverse conditions, range of beneficial pests and response to variable soil traits. Some disadvantages of cover crop mixing may include high cost of seed, more complicated management and too much residue although the advantages outweigh the disadvantages.

2.6 Effects of cover crops on organic matter and nutrient cycling

Organic matter includes thousands of different substances derived from decayed leaves, roots, microorganisms, manure and even soil fauna that died in their burrows. These substances function in different ways to build healthy soil. Different plants leave behind different kinds of organic matter as they decompose, so the choice of a cover crop will largely determine which soil benefits will be received (Varhallem *et al.,* 2003). Oats as a grass cover crop provides little N for the follow-on crop and they are less economical as they need large amounts of fertiliser N to reach acceptable biomass compared to legumes.

The use of appropriate cover crops can reduce the use of chemical fertilisers, and yet produce yields equivalent to those produced with conventional fertiliser rates (Corak *et al.,* 1991). Cover crop influence on soil fertility depends on a number of factors, including: i. Soil and weather conditions present during development and decomposition of the cover crop. ii. Length of time that the cover crop is present and actively growing. iii. Quantity of biomass eventually produced by the cover crop. iv. Cover crop species. Climate and location influence the first three factors, for example in the Northern hemisphere, cover crop establishment may not occur until near or after harvest in September or October. The cover crop has a limited time to develop and cause effects before the beginning of the freezing cold spell and maize planting may occur again before the cover crop makes significant spring growth (Rannells & Wagger, 1997). In such conditions, potential effects of cover crop are small. In the Southern hemisphere, however, maize may not utilize as much of the available growing season and winters may be quite mild, allowing more opportunity for cover crop development and effect. Farmers often plant winter cover crops in an attempt to increase SOM concentrations. While the cover crop will certainly add biomass to the soil when it is killed or incorporated, the long-term changes in organic carbon are often small or negligible.

Grazing vetch was observed to increase soil mineral N and as reported by Murungu (2010) unfertilised maize grown on grazing vetch residues had higher yields compared to fertilized maize plots with either oats or lupin residues. The conclusion was that decomposing grazing vetch residues were able to compensate for the lack of maize fertilisation by releasing mineral N and modest extractable P amounts. Muzangwa (2012) reported that in oat-vetch bicultures, an improvement in soil N and P was observed and that N and P uptake ranged from 188-220 kg ha^{-1} and 20-28 kg ha^{-1}, respectively. Cover crops help to bring nutrients back into the upper soil profile from deep soil layers. Calcium (Ca) and K are the two macronutrients that are easily leached and they can be brought up from deeper soil layers by a deep rooted cover crop like oats and released back into the active OM when the cover crop dies and decomposes. Cover crops may also play a role in P availability although it is generally not leached as it is slightly water soluble (CTIC, 2005).

In a 4 year trial under CA, SOM levels increased from as low as 10 g kg^{-1} to ranges above 20 g kg^{-1} in 0-20 cm soil depth as reported by Dube (2012). Also observed was that lot of SOM was in the particulate form hence high particulate organic matter carbon (POM-C) and this was because greater biomass was produced by winter cover crops which are a greater source of POM than the weedy fallow. Hot water soluble carbon, which is a potentially mineralisable active fraction, was also found to increase in soil under cover crops than weedy fallow.

2.7 Roles and importance of soil microbial biomass and soil enzymes on nutrient cycling and soil quality.

- **Microbial biomass**

Soil microbial biomass, which can be either a source or sink of available nutrients, plays a critical role in nutrient transformation in the soil (Singh *et al.,* 1989). It only comprises 1-4% of organic carbon but due to its fast turnover time, it plays a key role in controlling nutrient cycling and energy flow. Despite its small size, the soil microbial biomass pool is known to play a fundamental role in SOM dynamics and facilitates fundamental processes such as OM decomposition, organic C, N and P mineralisation, maintenance of soil structure and plant growth and development (Acosta-Martinez *et al.*,2004). Soil microbial biomass is greatly influenced by many factors such as plant community composition, seasonal variations of temperature and rainfall, plant development, and organic matter accumulation (Wardle, 1992; Devi & Yadava, 2006). Fluctuations or any changes in the size of the microbial biomass during crop growing season may affect soil organic matter turnover. Thus, the soil microbial activity has a direct influence on ecosystem stability and fertility (Smith *et al.,* 1993). Increases in the soil microbial biomass have been associated with positive changes in soil quality under systems that are managed to support an extensive rooting system, protection of soil surface by crop residue application or green manures and reduced tillage operations (Acosta-Martinez *et al.,* 2010). In general, microbial biomass has a short turnover time and is highly sensitive to soil environmental conditions and disturbances hence it is a useful index to assess soil quality and diagnose early changes in soil C stabilisation and nutrient dynamics.

- **Enzymes**

Enzymes are proteins that act as biological catalysts which speed up rates of reactions without undergoing permanent change (Rowell, 1994). The extracellular enzymes in soils

play a major role in the degradation of cellulose, chitin and proteins. The specific location of extracellular enzymes within the soil matrix has attracted much attention, especially in relation to SOM quality and turnover (Kandeler *et al.,* 1999). Soil enzymes in particular play a key role in nutrient cycling and they also play a key biochemical function in the overall process of OM decomposition in the soil system (Dick, 1997; Dick *et al.,* 1994). Soil enzymes are also important for the life processes of the micro-organisms in soils and the stabilisation of soil structure, the decomposition of organic wastes, OM formation and nutrient cycling (Dick *et al.,* 1994). These enzymes are constantly being synthesised, accumulated, inactivated and/or decomposed in the soil, hence playing an important role in agriculture and particularly in nutrient cycling (Dick 1997; Tabatabai, 1994).

The enzyme levels in the soil vary primarily due to the different OM content, composition and activity of its living organisms and intensity of the biological processes (Narasimha *et al.,* 2011). In practice, the biochemical reactions are bought about largely through the catalytic contribution of enzymes and variable substrates that serve as energy sources for microorganisms. It is generally assumed that soil enzymes are largely of microbial origin, but it is also possible that animals and plants may contribute enzymes to soils (Dick & Tabatabai, 1993).

A better understanding of the role of soil enzymes activity in the ecosystem will provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement and their rapid response to changes in the soil management practices (Bandick & Dick, 1999; Dick, 1997; Dick, 1994).

Dehydrogenase

Dehydrogenases are enzymes which catalyse the removal of hydrogen atom from different metabolites (Nelson & Cox, 2000). The dehydrogenase enzyme activity is

commonly used as an indicator of biological activity in soil (Quilchano & Maranon, 2002). This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellularly in the soil. Dehydrogenase enzyme is known to oxidise SOM by transferring protons and electrons from substrates to acceptors. These processes are part of respiration pathways of soil microorganisms and are closely related to the type of soil. Studies on the activities of dehydrogenase enzymes in the soil is very important as it may give indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility. They conduct a number of oxidative processes that are responsible for SOM degradation and its activities can reflect changes in the respiratory activity of a given population size in response to changes in the soil management (Margesin *et al*., 2000). Dehydrogenases are often used as a measure of disruption to the soil caused by type of soil management, trace elements and pesticides (Frank & Malkomes, 1993; Wilke, 1991) and can indicate the type and significance of pollution of soils as well as direct measure of soil microbial activity (Garcia & Hernandez, 1997).

β-glucosidase

β-glucosidase is a common and predominant enzyme in soil. It is named according to the type of bond that it hydrolyses. This enzyme plays an important role in soils because it is involved in catalysing the hydrolysis and biodegradation of various β-glucosides present in plant debris decomposing in the ecosystem (Ajwa & Tabatabai, 1994). Its final product is glucose, an important C energy source of life to microbes in the soil (Esen, 1993). It plays a major role in the initial phases of the decomposition of OC compounds. β-glucosidase is useful as a soil quality indicator and may give a reflection of past biological activity, the capacity of soil to stabilise the SOM and can be used to detect management effects on soils (Ndiaye et al., 2000; Bandick & Dick, 1999). This has greatly facilitated its adoption for soil quality testing. Generally β-glucosidase can provide advanced evidence of changes in OC

long before it can be measured by other methods. Acosta-Martinez & Tabatabai (2000) reported that β-glucosidase is sensitive to pH changes. This property can be used as a good biochemical indicator for measuring ecological changes resulting from soil acidification. Understanding the activity of this enzymes and factors that influence it may contribute significantly to soil fertility and soil health.

Urease

The presence of urease enzymes in the soil is of paramount importance to the utilization of urea inn agriculture. Urease is an enzyme that belongs to the amidohydrolase, it catalyses the hydrolysis of urea into NH_3 and CO_2. Urea has been considered as a slow release fertiliser since it undergoes two transformations in the soil before it becomes available to most crops. The first transformation of urea is its hydrolysis to carbonate and ammonium ions catalysed by urease enzyme. The second one is when ammonium ion is oxidized in the soil through microbial processes to nitrite and subsequently nitrate, a process called nitrification (Killham, 1994). Urease activity tends to increase the pH of the environment in which it is as it produces ammonia, this in turn results in a rapid N loss to the atmosphere through NH_3 volatilisation (Zimmer, 2000).

Urea is often the main source of N in many crops including maize in many parts of Africa. Its activity tends to be high in non-calcareous soils under dense vegetation and low in saline and soils lacking OM (Bremner & Mulvaney, 1978). Despite the importance of urea fertiliser, its efficiency has been reported as low due to substantial N lost to the atmosphere through volatilisation, a process mediated by urease enzyme (Klose & Tabatabai 2000). The rate of urea hydrolysis increases with an increase in urea concentration until the urease becomes saturated with the substrate (Ping *et al*., 2000). The activity and stability of this enzyme in the system is affected by several factors and these include cropping history, OM

content of the soil, soil depth, soil amendments, heavy metal and environmental factors such as temperature (Watson, 2000). Generally urease activity increases with increasing temperature.

Arylsulphatase

Plants take up sulphur in the form of inorganic sulphate (SO_4^{-2}) and its availability depends on its mineralisation or mobilisation from aromatic sulphate esters ($R\text{-}O\text{-}SO_3^-$) (Deng & Tabatabai, 1997). This is due to the fact that certain proportions of sulphur in different soil profiles are bound into organic compounds and are indirectly available to plants. In this regard, its availability will depend on the extracellular hydrolysis of these aromatic sulphate esters or intracellular oxidation of soluble OM absorbed by the microorganisms to yield energy and carbon skeletons for biosynthesis by which some sulphate-sulphur are released as a by-product. All these processes are dependent on arylsulphatases enzymes.

Arylsulphatases are responsible for the hydrolysis of sulphate esters in the soil and are secreted by bacteria into the external environment as a response to sulphur limitation (Kertesz & Mirleau, 2004). Its occurrence is often correlated with microbial biomass and rate of sulphur immobilisation (Klose *et al.*, 1999; Klose & Tabatabai, 1999). Its role in the hydrolysis of aromatic sulphate esters ($R\text{-}O\text{-}SO_3^-$) to phenols (R-OH) and sulphate or sulphate sulphur (SO_4^{-2} or SO_4-S) is shown in the following simple chemical equation (Tabatabai, 1994).

$$R\text{-}O\text{-}SO_3^- \xleftrightarrow{\text{arylsulphatases/hydrolysis}} R\text{-}OH + SO_4^{-2}$$

The release of sulphate from soluble and insoluble sulphate esters in the soil is affected by various environmental factors such as OM content and type, concentration of organic

sulphate esters, the extent to which organic sulphate esters are protected against enzymatic hydrolysis, pH changes, heavy metal pollution, and the activity persistence of extracellular arylsulphatases in the soil (Acosta-Martinez & Tabatabai, 2000). A better understanding of the roles of arylsulphatases in S mobilisation in agricultural soils is important as S is to plant nutrition.

Phosphatases (Acid and Alkaline)

Phosphatases particularly catalyse the hydrolysis of P-ester bonds binding P to C (C-O-P ester bonds) and anhydrides of phosphoric acid in OM (Tabatabai, 1994) with release of inorganic P without concominant release of C. These enzymes are believed to play critical roles in the P-cycles as evidence shows that they are correlated to P stress and plant growth. Phosphatases are concentrated in the surface layer and rhizosphere where most of the fresh and less humified OM is prevailing. The ability to solubilise mineral elements by these phosphomonoesterases is expected to be higher in biologically managed systems because of a higher quantity of OC found in those systems. Plants have evolved many morphological and enzymatic adaptations to tolerate low phosphate availability. This includes transcription activity of acid phosphatase which tends to increase with high P stress (Miller *et al.,* 2001; Li *et al.,* 2002). For example, when there is a signal indicating P deficiency in the soil, acid phosphatase secretion from plant roots is increased to enhance the solubilisation and remobilisation of phosphate, thus influencing the ability of the plant to cope with P-stressed conditions (Mudge *et al.,* 2002; Li *et al.,* 1997).

Acid phosphatase is mainly produced by plants and also released by soil microorganisms; it has also been detected in the rhizodermal and root cap cells, soil fungi and bacteria, in mucilage covering roots and in microbial membranes in soil (Dick *et al.,* 2000).

The amount of acid phosphatase exuded by plant roots has been shown to differ between crop species and varieties as well as crop management practices (Ndakidemi, 2006). Research has shown that legumes secrete more phosphatase enzymes than cereal. This may probably be due to a higher requirement of P by legumes in the symbiotic N-fixation processes as compared to cereals (Yadav & Tarafdar, 2001). The activity of acid and alkaline phosphatases was found to correlate with OM and soil pH is a factor that influences their rate of synthesis, release and stability (Acosta-Martinez & Tabatabai, 2000; Tabatabai, 1994). Apart from being good indicators of soil fertility, phosphatase enzymes play key roles in the soil system.

3. EFFECTS OF OAT (*Avena sativa L.*) AND GRAZING VETCH (*Vicia dasycarpa L.*) COVER CROPS ON MICROBIAL BIOMASS AND ACTIVITES OF SELECTED ENZYMES AFTER FIVE YEARS OF ROTATIONS WITH MAIZE ON A SANDY LOAM SOIL IN SOUTH AFRICA

Abstract

The study was carried out to determine the effects of rotation of maize with oat and grazing vetch cover crops with or without fertilization on soil microbial biomass and activities of selected enzymes after 5 years of conservation agriculture. Treatments consisted of oat (*Avena sativa*) and grazing vetch (*Vicia dasycarpa*) winter cover crops and a weedy winter fallow control, in rotation with maize. Four fertilisation regimes were used: (i) fertiliser applied to cover crop and maize (F1), (ii) fertiliser applied to cover crops only (F2), (iii) fertiliser applied to maize only (F3) and (iv) no fertiliser applied (F4). This gave a 2 × 4 factorial plus control plots laid out in a randomized complete block design with three replications. After 5 years of rotations, soil samples were collected from the 0-5 cm and 5-20 cm depths and analysed for microbial biomass C, and activities of dehydrogenase, β-glucosidase, urease and acid and alkaline phosphatase enzymes. Oat and vetch gave significantly ($P < 0.05$) higher MBC and activities of all soil enzymes measured than the weedy fallow at 0-5 and 5-20 cm depths. Fertilization of cover crop (F2) and maize (F3) gave similar MBC. The F4 in cover crops gave similar dehydrogenase activity with F3 under weedy fallow and that for vetch rotation was the same for F3 in oat. The F4 for grazing vetch had similar β-glucosidase activity as F1 and similar urease activity as F1, F2 and F3 of oat rotations. Acid and alkaline activity in F1 had similar results to F2. The findings of this study imply that the use of grazing vetch and fertilization of cover crops only improves soil biological activity, represented by MBC and enzyme activities, after 5 years of maize-cover crop rotations in low input conservation agriculture system.

Keywords: Fertiliser management, soil enzymes, winter cover crops

3.1 Introduction

Most soils in smallholder farmlands of South Africa (SA) are susceptible to land degradation as a result of low organic matter (OM) content (Van der Merwe & de Villiers, 1998), among other things. Monoculture cereal production, intensive tillage, short or no fallow and inadequate biomass input result in decline in soil organic matter (SOM) (Banard & Newby, 1999), and also leave the soil bare, which exposes it to agents of soil erosion. Conservation agriculture (CA) has the potential to increase SOM, improve soil cover and reverse the effects of soil degradation and yield declines in South Africa (FAO, 2008; Laker, 2004; Derpsch, 2003).

Integrating cover crops into existing cropping systems could increase biomass input, provide permanent soil cover and overcome soil degradation problems (Rueben & Lee, 2000). Where cover crop biomass is limited because of poor soil fertility, small doses of fertilizer can be invested on cover crops to enhance biomass yield. Studies have been carried out to evaluate cover crop species and fertilizer effects on biomass input and maize grain yield response in maize-based CA systems of the Eastern Cape (Murungu, 2010; Musunda, 2010). Oat (*Avena sativa L.*) and grazing vetch (*Vicia dasycarpa L.*) were identified as the most promising cover crop species in this regard because of their ability to produce high biomass yields and also control weeds as actively growing crops or their residues in summer grown maize. Winter cover crops may increase SOM content in the topsoil, which in turn leads to higher microbial biomass and activity (McGill *et al.*, 1996). Dube *et al*. (2012) reported that rotations of maize with white oat and grazing vetch, over a five-year period, improved total soil C, particulate organic matter and water soluble C which are labile pools of total C, known to provide readily available substrates to soil microbes and are sources of important nutrient elements in the short term. Improvement of OM could be related to soil

function. While OC has improved, its effects on nutrient cycling will depend on the soil biological function.

Microbial biomass C and enzyme activities are closely related because transformations of the important organic elements occur through microorganisms (Wang *et al.,* 2013). Soil microbial biomass is a living fraction and part of active pool of SOM which responds much more rapidly to changes in soil management than total SOM (Li & Chen, 2004), and can be influenced by several factors, such as plant community composition, SOM level, soil moisture, and temperature (Wardle, 1992). Determination of soil microbial biomass carbon (MBC) provides better insights of soil organic C turnover (Wang et al., 2004; Omay *et al*., 1997).

Soil enzyme activities are used as indicators of soil quality because they play an important role in decomposition and nutrient cycling, and their rapid responses to changes in soil management (Dilly *et al.,* 2003; Bandick & Dick, 1999; Dick, 1994). They can provide information on important biochemical processes that affect soil functions, including the cycling of main biologically important nutrients (C, N and P). Dehydrogenase enzymes are used as indicators of overall microbial activities because they are intracellular enzymes, and they play a critical role in the biological oxidation of SOM. β-glucosidase is involved in the last limiting step of cellulose degradation (C-cycle) (Deng & Tabatabai, 1996b), while urease catalyses the hydrolysis of urea in the N-cycle. The phosphatases are involved in the hydrolysis of P-ester bonds thereby releasing P into the soil for plant uptake. They are significantly affected by soil pH, which controls phosphorus availability despite OM content and levels of disturbance. Therefore, there is need to understand both acid and alkaline phosphatase activities in the soil.

Many researchers have studied the effects of tillage (Curci et al., 1997; Deng &

Tabatabai, 1996a, b), crop rotation (Dick, 1984), and fertilizer amendment (Melero et al., 2009; Goyal *et al.,* 1999) on enzyme activities. In a long-term study, Kandeler *et al.* (1999a, b) found that xylanase, protease and phosphatases (acid and alkaline) enzyme activities were significantly increased in the top 10 cm of the profile after only 2 years of minimum and reduced tillage compared to conventional tillage. No-till soils become stratified with high OM, N mineralization potential, microbial biomass and enzyme activities near the soil surface and often lower measures of these properties in the shallow subsurface. Dick (1984) reported significant effects of crop rotations on soil enzymes (acid and alkaline phosphatases, arylsulphatase, invertase, amidase and urease) in the 0-7.5 cm depth. High enzyme activities were observed more in maize-oat-alfalfa rotation than maize-soya-beans rotation. Goyal *et al.* (1999) observed an increase in SOM and soil enzyme when farmyard manure, wheat straw, green manure was applied along inorganic fertilizers. This indicates that SOM level and soil microbial activities vital for nutrient turnover are enhanced by use of organic and inorganic fertilizers. The objective of this study was to determine the biological status of the soil after 5 years of maize-cover crop (oat and grazing vetch) rotations under different fertiliser regimes. The hypothesis was that cover cropping with fertiliser use increase soil biological activity.

3.2 Materials and methods

3.2.1 Experimental site

The study was carried out at the University of Fort Hare Research Farm within the Alice Jozini Ecotope. The ecotope is characterised by soils formed from weak physical and chemical weathering and it has a single parent material of alluvium form that accumulated through river deposits. It has a slope of 0.5% and vegetation is mainly cultivated land with wheat as a previous crop. The farm is located at 32^{o} 47′ 51″ S and 27^{o} 50′ 55″ E at an altitude of about 535 m. The area has a warm temperate climate with an annual mean temperature of

18.1^{o}C and an average winter and summer rainfall of 167 mm and 420 mm respectively (Austin, 1989). The soils are of alluvial origin, deep and are classified as the Oakleaf form (Soil Classification Working Group, 1991), Haplic Cambisol (IUSS Working Group, 2006) and Typic Haplustalf (USDA Soil Taxonomy, 1999). These are among the most commonly cropped soils in smallholder farms of the Eastern Cape.

3.2.2 Treatments and experimental design

The study was based on a trial that was established on the 1st of June 2007 as described by Murungu (2010). White oat (*Avena sativa* cv. Sederbrg) and grazing vetch (*Vicia dasycarpa* cv. Max) winter cover crops were planted with and without fertilizer. Control plots with no winter cover crops and no fertilizer were included. In summer, all plots were split in half and maize (cv. PAN 6479) was planted. Fertilizer in maize was applied at two levels (0 and 60 N kg ha-1), half of the plot was not fertilised and the other half was fertilized (Table 3.1). The splitting of the winter plots and application of two fertilizer levels (0 and 60 kg N) to maize in the first cycle gave rise to four fertilizer regimes namely F1, F2, F3 and F4. The F1 treatments were fertilized in both winter and summer seasons. Fertilizer was only applied in winter cover crops with no fertilization in the subsequent maize for the F2 treatments. For the F3 treatments, there was no fertilization of winter cover crops while the summer maize was fertilized. No fertilizer was applied in the F4 treatments both in winter and summer. There were thus two factors in the study; type of cover crop and fertilizer regimes giving a 2 × 4 factorial plus control plots laid out in a randomized complete block design with three replications.

Table 3.1: Treatment layout in winter and summer

Cover crop	Winter	Summer	Fertiliser regime
Oat	Fertilised oat	Fertilized maize	F1
	Fertilized oat	Non-fertilized maize	F2
	Non-fertilised oat	Fertilised maize	F3
	Non-fertlised oat	Non-fertilised maize	F4
Vetch	Fertilised vetch	Fertilized maize	F1
	Fertilized vetch	Non-fertilized maize	F2
	Non-fertilised vetch	Fertilised maize	F3
	Non-fertlised vetch	Non-fertilised maize	F4
Weedy fallow	Weedy fallow	Fertilized maize	F3
	Weedy fallow	Non-fertilised maize	F4

3.2.3 Agronomic practices

The cover crops were planted at recommended seed rates of 90 kg ha^{-1} for white oat and 35 kg ha^{-1} for grazing vetch (Clark *et al.*, 1994) into small furrows opened using hoes. The fertilizer was applied at 10 kg P ha^{-1}, as a compound (6.7% N; 10% P; 13.3% K) at planting. Grazing vetch was inoculated with Rhizobium leguminosarium biovar viciae at planting. Oat had 45 kg N ha^{-1} applied of which a third was applied as a compound (2:3:4 (30)) at planting and the remainder was top dressed as limestone ammonium nitrate (LAN – 28% N) at 7 weeks after planting (WAP). Normal rains were supplemented with overhead irrigation based on Class A evaporation pan readings as summarised in Table 3.2. Vetch had reached the flowering stage and oat was just starting grain filling when they were killed (their growth was terminated) by rolling with a tractor mounted roller and glyphosate ((N-phosphonomethyl) glycine) (360 g L^{-1}) applied at a rate of 5 L ha^{-1}. Three weeks later, all plots were split in half and maize (cv. PAN 6479) was planted using hand operated ‘matraca’ planters with no-till. The maize rows were spaced at a distance of 90 cm and the plants spaced at 30 cm to give a planting density of 37 000 plants ha^{-1}. The planters were calibrated to drop the required

fertilizer about 4 cm from the maize seeds in fertilized plots. Fertilizer was applied at two levels (0 and 60 N kg ha-1) mimicking smallholder irrigation farmer practice in the EC (Fanadzo *et al.*, 2010). A third of the maize N was applied at planting as a compound (6.7% N; 10% P; 13.3% K) and the rest as LAN at 6 WAP by banding. Early weeds during maize growth were controlled using Basagran (a.i. thiadiazine 480 g L^{-1}) applied to all plots at 5 L ha^{-1}. Subsequent to maize harvesting, maize stalks were rolled, glyphosate applied at 5 L ha^{-1} and oat and grazing vetch cover crops were planted and managed as in the previous seasons. The trial was continued into the second, third, fourth and fifth years under the same experimental design, trial management and rotations. Soil samples for this study were collected in October 2011, after cover crop termination.

Table 3.2: Mean monthly rainfall and irrigation received at the University of Fort Hare Research Farm during cover crop growth from May to September in 2011 season

	Rainfall (mm)			Irrigation (mm)
Month	2011	30-year mean	CV (%)	2011
May	136.6	24.8	112	18
June	8.1	23.7	94	27
July	74.4	20.6	103	50
August	31	31.8	107	48
September	5.5	32.6	92	10
Total	255.9			153

3.2.4 Soil sampling, storage and analyses

Three soil samples from each plot were taken at intervals along an imaginary zig-zag line, avoiding one metre boundaries on all sides. Samples were collected at depths of 0-5 cm (using a small trowel) and 5-20 cm (using a 7 cm diameter precision auger). Samples from

the same depth in each plot were mixed together to make a composite soil sample. The soil was then taken to a shade house where it was sieved (2 mm) and immediately stored in the cold room at 4^{o}C until analyses. Moisture content of the samples was determined by oven-drying a subsample at 105^{o}C for 24 h.

Moisture of the samples was adjusted to 80% of field capacity and the samples were incubated at 37^{o}C for seven days to activate the soil microorganisms before analysis of soil MBC and enzyme activities as follows:

3.2.5 Soil microbial biomass carbon

Microbial biomass carbon (MBC) was determined by the modified chloroform fumigation–extraction followed by the dichromate oxidation of the C (Witt *et al*., 2000). A portion of each sample was fumigated using chloroform while the other portion was not fumigated. Both the fumigated and unfumigated samples were then kept in dark for 24 h at 25^{o}C. Organic carbon in both the fumigated and unfumigated samples was extracted with 0.5M K_2SO_4 solution, and determined after digestion at 150^{o}C for 30 min with 0.07N $K_2Cr_2O_7$, 98% H_2SO_4 and 88% H_3PO_4 and titrated with 0.01N $Fe(NH_4)_2(SO_4)_2$ in 0.4M H_2SO_4. Soil MBC was calculated using the following equation:

$$MBC = (Ecf - Ecu)/Kc$$

where: Ecf is organic carbon extracted from fumigated soil and

Ecu is organic carbon extracted from unfumigated soil.

Kc is a constant. The value of *Kc* used was 0.38 (Vance *et al*., 1987).

3.2.6 Soil enzyme assays

Dehydrogenase enzyme

Twenty grams of soil (<2 mm) was mixed with 0.2 g of $CaCO_3$ and 6 g of this mixture placed in each of three test tubes, to which 2.5 mL distilled water and 1 mL of 3% aqueous solution of 2,3,5-Triphenyltetrazolium chloride (TTC) was added. The contents of each tube were mixed with a glass rod and stoppered before incubation at 37^{o}C for 24 h. After incubation, 10 mL of methanol was added, the tube shaken for one minute, and filtered through a funnel plugged with absorbent cotton into a 100 mL volumetric flask. The tube was washed with methanol and soil transferred to the funnel, and additional methanol (in 10 mL portions) was added until the reddish colour disappeared from the cotton plug. The filtrate was diluted to 100 mL volume with methanol. The intensity of the reddish colour was measured using a spectrophotometer at a wavelength of 480 nm with methanol as a blank. The amount of Triphenyl formazan (TPF) produced (related to dehydrogenase activity) was calculated by reference to the calibration graph prepared from TPF standards (Casida, 1977).

β-Glucosidase

Activity of β-Glucosidase was determined using the method of Dick *et al.* (1996). One gram of soil (<2 mm) was placed in a 50 mL Elernmeyer flask and 0.25 mL of toluene, 4 mL of modified universal buffer (MUB) pH 6.0, 1 mL of ρ-Nitrophenyl-β-D-glucoside (PNG) solution added. The flask was swirled for ten seconds to mix the contents, stoppered and incubated at 37^{o}C for 1h. After incubation 1 mL of 0.5M $CaCl_2$ and 4 mL of 0.1M tris-hydroxymethyl aminomethane (THAM) buffer pH 12 were added, swirled for ten seconds and filtered through a Whatman no. 2 filter paper. The intensity of the yellow colour produced was measured at wavelength of 420 nm. The amount of ρ-nitrophenol released

(related to β-glucosidase activity) was measured by reference to a calibration graph plotted from the results obtained with p-nitrophenol standards.

Urease

Urease activity was based on determination of urea remaining after incubation with added urea (Tabatabai & Bremner, 1972a). Five grams of soil (oven-dry equivalent) was placed in a 65 mL glass bottle and the soil treated with 5 mL of urea solution (10 mg of urea), stoppered and incubated at 37^{o}C for 5 h. After incubation, 50 mL of 2M potassium chloride-phenylmercuric acetate (KCl-PMA) solution was added, stoppered and shaken for 1 h. The suspension was filtered under suction through a Whatman no.42 filter paper. To determine the urea remaining, an aliquot (1-2 mL) of the extract containing up to 200 μg of urea was pipetted into a 50 mL volumetric flask and made to 10 mL with 2M KCl-PMA solution. Colour reagent (30 mL) was added and the flask swirled for six seconds to mix the contents. The flask was placed in boiling water bath for 30 minutes, and then cooled immediately in running water for about 15 minutes, made to 50 mL with distilled water and mixed thoroughly. The intensity of the red colour produced was measured at a wavelength of 527 nm. The urea content of the extract analysed was calculated by reference to the calibration graph plotted from the results obtained with the standards.

Acid and alkaline phosphatase

Activities of acid and alkaline phosphatase were based on the method of Tabatabai (1994). One gram of soil was placed in a 50 mL Elernmeyer flask and 0.2 mL of toluene, 4 mL of MUB (pH 6.5 for assay of acid phosphatase or pH 11 for assay of alkaline phosphatase), and 1 mL of ρ-nitrophenyl phosphate solution added and the mixture swirled for ten seconds, stoppered and incubated at 37^{o}C for 1 h. After incubation, 1 mL of 0.5M $CaCl_2$ and 4 mL of 0.5M NaOH was added and the contents swirled for ten seconds before

filtration through a Whatman no. 42 filter paper. The intensity of the yellow colour was measured at wavelength of 420 nm.

3.2.7 Data analysis

Data of soil MBC and enzyme activities, at each depth, were subjected to an analysis of variance (ANOVA) as an RCBD with 3 replications to test the effects of cover crop mulch and fertiliser regime using Genstat Statistical Package 12th edition. An extra factor (cover cropping) was included while cover crop type × fertiliser regime was nested within cover cropping to include analysis of controls in the ANOVA (Gomez & Gomez, 1984). Where significant differences occurred, separation of means was done using the least significant difference (LSD) at 5% level of significance.

3.3 Results

Microbial biomass carbon

The interaction effects of cover cropping, fertiliser regime and cover crop type on MBC at 0-5 and 5-20 cm soil depths were not significant ($P > 0.05$). There was a significant interaction effect of cover cropping and cover crop type on MBC at 0-5cm ($P < 0.05$) and 5-20 cm depth ($P < 0.05$). Oat-maize rotations had higher MBC (1525 µg g^{-1}soil) than vetch-maize (1370 µg g^{-1}soil) and weedy fallow-maize (1117 µg g^{-1}soil) rotations at 0-5 cm soil depth (Table 3.3a). Interaction effects of cover cropping × fertiliser regime were significant at both soil depths ($P < 0.05$) (Table 3.3b). Winter cover cropping gave higher MBC as compared to the weedy fallow. In the weedy fallow-maize rotations, fertilisation of maize (F3) and no fertiliser treatment (F4) had comparable results. Comparisons between treatments showed that the fertilised cover crop only (F2) and fertilised maize only (F3) had comparable

MBC. Fertilisation of both winter cover crop and summer maize (F1) increased MBC while a lack of fertilisation (F4) had the least MBC at both soil depths.

Dehydrogenase

The interaction effect of cover cropping, fertiliser regime and cover crop type on dehydrogenase activity was significant ($P < 0.05$) in 5-20 cm depth (Figure 3.1) while in 0-5 cm depth it was not ($P > 0.05$). The interaction effect of cover cropping and cover crop type on dehydrogenase activity at 0-5 cm depth was significant ($P < 0.05$) (Table 3.3a). Dehydrogenase activity was significantly higher in vetch-maize with 851 µg TPF g^{-1}soil h^{-1} than oat-maize and weedy fallow-maize rotations with 739 and 577 µg TPF g^{-1}soil h^{-1} respectively at 0-5 cm depth, with the weedy fallow having the least activity of the enzyme (Table 3.3a). Interaction effects of cover cropping × fertiliser regime were significant ($P < 0.001$) at 0-5 cm depth (Table 3.3b). In the weedy fallow at 0-5 cm depth, F3 and F4 gave comparable results of dehydrogenase activity while at 5-20 cm F3 had significantly higher enzyme activity than F4 (Table 3.3b). Comparison between treatments and the control showed that F4 under winter cover cropping having comparable results to F3. Fertilisation of both cover crops and maize (F1) gave higher enzyme activity while fertilisation of cover crop only (F2) did not differ from fertilisation of maize only (F3).

At 5-20 cm depth, all fertiliser regimes were significantly different from each other with F1 having the highest dehydrogenase enzyme activity of 962 µg TPF g^{-1}soil h^{-1}. Enzyme activity was in the order of F1 >F2 >F3 >F4 with lack of cover cropping having less dehydrogenase activity than under cover crops (Figure 3.1). Vetch-maize rotations gave higher dehydrogenase activity compared to the oat-maize rotations. In oat-maize rotations, F3 had comparable results to F3 and F4 under vetch-maize rotation although the two were not

similar. Fertilisation of maize only (F3) under the weedy fallow had similar results to F4 under oat-maize rotations.

Table 3.3a: Cover cropping × cover crop type effects on MBC (μg g^{-1}soil) and dehydrogenase activity (μg TPF g^{-1}soil h^{-1}) at 0-5 cm depth.

	Cover cropping		Weedy fallow	Significance	LSD_b	LSD_c
MBC	Oat	Vetch				
	1525	1370	1117	**	116.3	94.9
Dehydrogenase	739	851	577	**	78.5	64.1

LSD_b: for comparisons of controls with other treatments, minimum replication and maximum replications; LSD_c: for treatment comparisons only, with controls excluded, maximum replications.

Table 3.3b: Cover cropping × fertiliser regime effects on MBC (μg g^{-1}soil) and dehydrogenase activity (μg TPF g^{-1}soil h^{-1}) at 0-5 and 5-20 cm depths.

	Soil depth	Winter cover crops				Weedy fallow		LSD_a	LSD_b	LSD_c
		F1	F2	F3	F4	F3	F4			
MBC	0-5 cm	1721	1476	1379	1215	1134	1100	189.9	164.4	134.2
	5-20 cm	1622	1411	1331	1150	709	680	200.2	173.4	141.6
Dehydrogenase	0-5 cm	1016	797	752	614	599	555	128.2	111.0	90.6
	5-20 cm	962	786	723	644	573	537	31.1	26.9	22.0

LSD_a: for control to control comparisons only, minimum replications; LSD_b: for comparisons of controls with other treatments, minimum replication and maximum replications; LSD_c: for treatment comparisons only, with controls excluded, maximum replications.

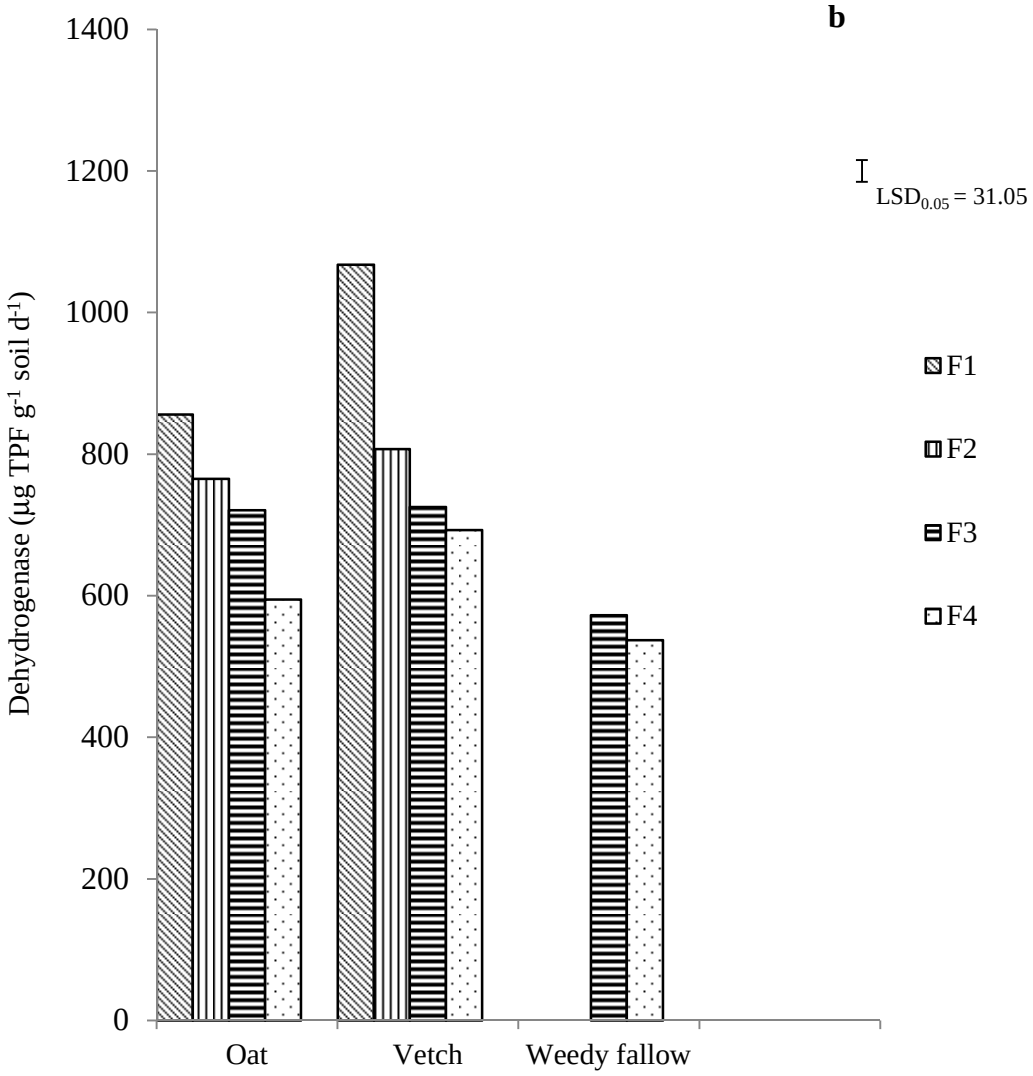

Figure 3.1: Cover cropping × cover crop type × fertiliser regime effects on dehydrogenase activity at 5-20 cm depth following five years of continuous practice. Error bar represents LSD.

β-glucosidase

The cover cropping × cover crop type × fertiliser regime interaction effect was significant with respect to β-glucosidase at 0-5 and 5-20 cm depth ($P < 0.05$) (Figure 3.2). The type of cover crop significantly affected the activity of β-glucosidase enzyme at both soil depths. Rotations with vetch had higher enzyme activity than those with oat while absence of a cover crop had the least activity of β-glucosidase. Fertilisation of cover crop and/or maize

significantly affected the activity of the enzyme, in 0-5 cm depth. Fertilisation of maize only and lack of fertilisation (F3 and F4) in oat plots and the weedy fallow had comparable β-glucosidase activity. Fertilisation of both cover crop and maize (F1) in oat-maize rotations had comparable results to F3 and F4 of vetch-maize rotations. In 5-20 cm depth, there were significant differences among fertiliser regimes with β-glucosidase activity high in the order F1> F2> F3> F4 (Figure 3.2). Fertilisation of maize only (F3) under the weedy fallow-maize rotation had comparable results to unfertilised oat-maize rotations (F4). Lack of fertilisation reduced β-glucosidase activity both in cover crop rotations and weedy fallow rotations.

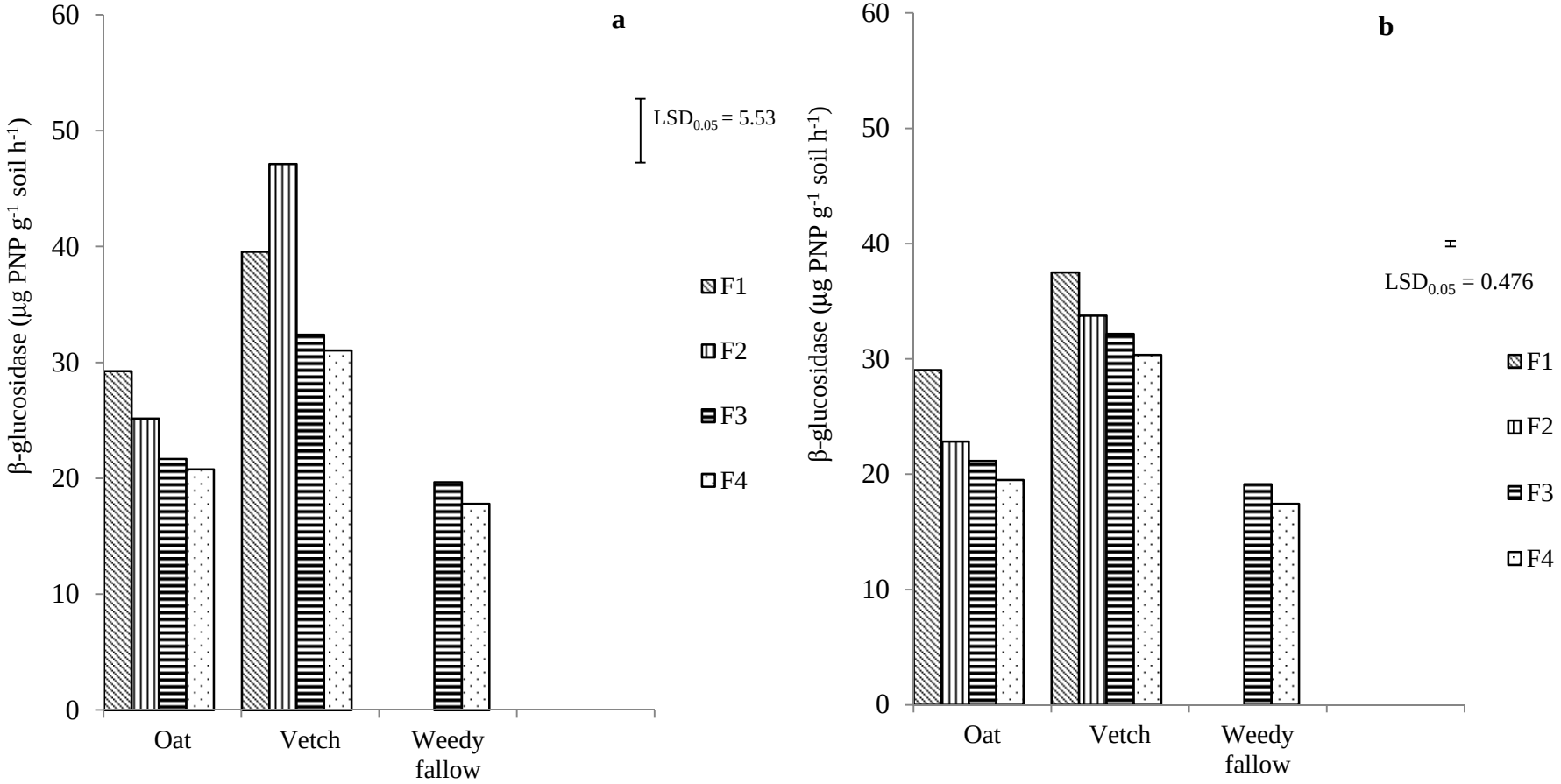

Figure 3.2: Cover cropping × cover crop type × fertiliser regime effects on β-glucosidase activity at 0-5 cm (a) and 5-20 cm (b) depth following five years of continuous practice. Error bars represents LSD.

Urease

Cover cropping × cover crop type × fertiliser regime interaction effect was significant ($P < 0.05$) (Figure 3.3a) with respect to urease activity at 0-5 cm depth. Vetch-maize rotations gave higher urease activity than oat and weedy fallow-maize rotations (Figure 3.3a). In the weedy fallow-maize rotations, fertilizer regimes were significantly different from each other while in oat-maize rotations, similar results were observed where maize only was fertilised and where no fertiliser was applied (F3 and F4). In the same oat-maize rotation, all fertiliser regimes except for F4 had similar results to the F4 under vetch-maize rotations. Fertilisation of maize only and cover crop only (F2 and F3) in vetch-maize rotations also had similar results.

The interaction effect of cover cropping × cover crop type × fertiliser regime on urease activity was not significant at 5-20 cm depth ($P > 0.05$). However, cover cropping × fertiliser regime and cover cropping × cover crop type interaction effects were both significant ($P < 0.05$). The use of cover crops compared to the weedy fallow increased urease activity significantly with vetch (1971.49 μg urea g^{-1}soil $5h^{-1}$) performing better than oat (1968.40 μg urea g^{-1}soil $5h^{-1}$) (Table 3.4 a). Fertilisation of maize only (F3) resulted in higher urease activity than where fertiliser was not applied (F4) in both the weedy fallow and winter cover crop treatments. In the weedy fallow-maize rotation, F3 and F4 regimes gave similar results. Fertiliser regime F4 within winter cover crops was significantly different from other regimes which had similar results (Table 3.4b).

Acid phosphatase

The interaction effect of cover cropping × cover crop type × fertiliser regime was significant ($P < 0.05$) with respect to acid phosphatase activity at 0-5 cm depth (Figure 3.3b).

Oat-maize rotations gave higher acid phosphatase activity than vetch-maize and weedy fallow-maize rotations.

The interaction effect of cover cropping × cover crop type × fertiliser regime was not significant ($P > 0.05$) with respect to acid phosphatase activity at 5-20 cm depth. However, interaction effect of cover cropping × cover crop type on acid phosphatase was significant ($P < 0.05$) (Table 3.4a). Oat gave the highest enzyme activity of 42.80 µg PNP g^{-1}soil h^{-1} and there were no comparable results between the two winter cover crops neither was it between the winter cover crops and the weedy fallow. Cover cropping × fertiliser regime interaction effect on acid phosphatase was significant ($P < 0.05$) (Table 3.4b). Fertilisation of both maize and cover crop (F1) gave the highest results (43. 40 µg PNP g^{-1}soil h^{-1}) although it was comparable to fertilisation of cover crop only (F2) (42.83 µg PNP g^{-1}soil h^{-1}). The F2 regime gave similar results of acid phosphatase activity as the F3 regime (41.17 µg PNP g^{-1}soil h^{-1}). Although lack of fertilisation gave the least acid phosphatase activity (39.66 µg PNP g^{-1}soil h^{-1}), it had similar results to the F3 regime.

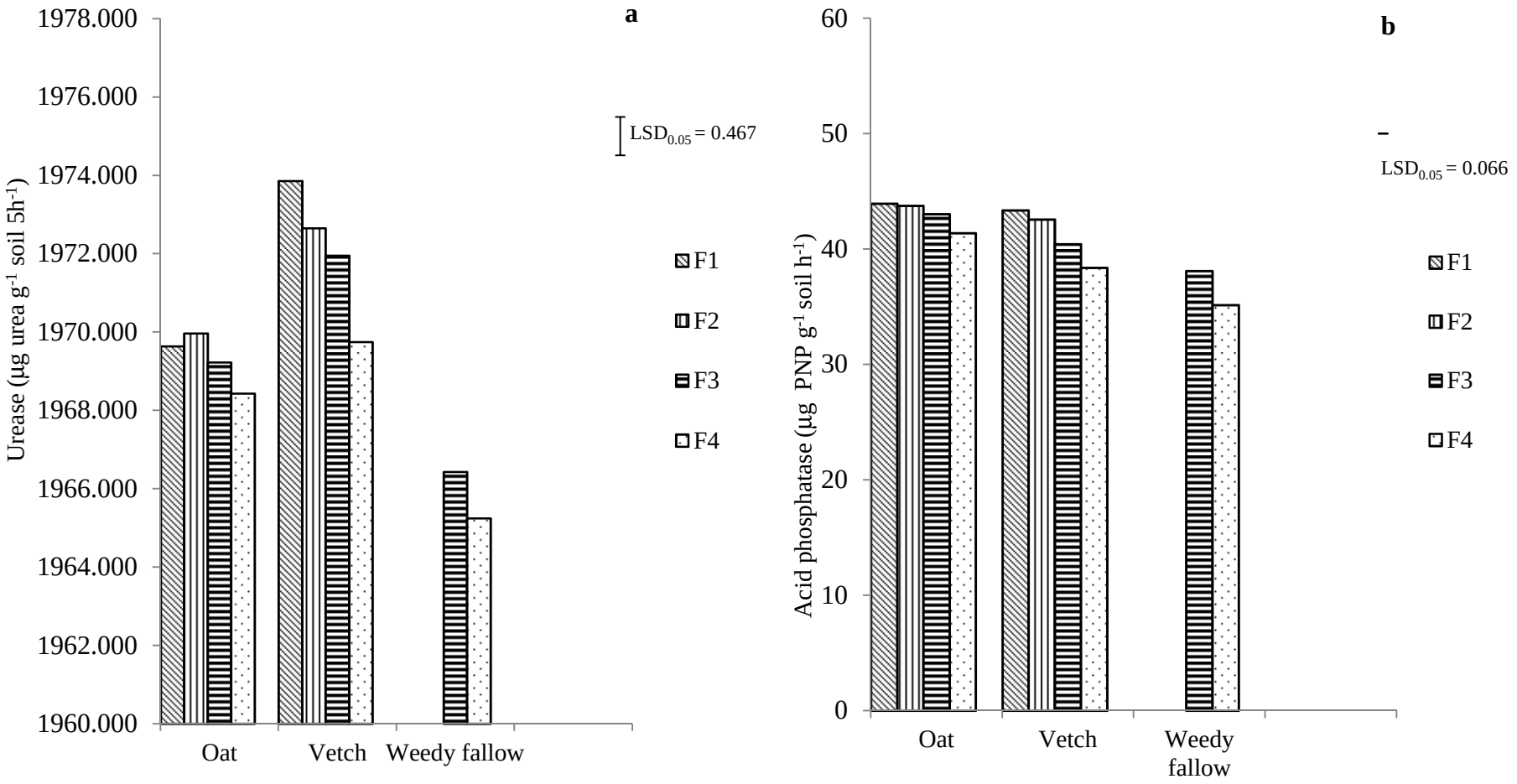

Figure 3.3: Cover cropping × cover crop type × fertiliser regime effects on urease (a) and acid phosphatase (b) activities at 0-5 cm depth following five years of continuous practice. Error bars represents LSD.

Table 3.4a: Cover cropping × cover crop type effects on urease (µg urea g^{-1}soil $5h^{-1}$) and acid phosphatase (µg PNP g^{-1} soil h^{-1}) activities at 5-20 cm depth.

	Cover cropping		Weedy fallow	Significance	LSD_b	LSD_c
	Oat	Vetch				
Urease	1968.40	1971.49	1965.29	***	1.298	1.060
Acid phosphatase	42.80	40.73	34.31	**	1.550	1.266

LSD_b: for comparisons of controls with other treatments, minimum replication and maximum replications; LSD_c: for treatment comparisons only, with controls excluded, maximum replications.

Table 3.4b: Cover cropping × fertiliser regime effects on urease (µg urea g^{-1}soil $5h^{-1}$) and acid phosphatase (µg PNP g^{-1} soil h^{-1}) activities at 5-20 cm depth.

	Winter cover crops				Weedy fallow		LSD_a	LSD_b	LSD_c
	F1	F2	F3	F4	F3	F4			
Urease	1970.50	1970.72	1970.23	1968.32	1965.89	1964.69	2.120	1.836	1.499
Acid phosphatase	43.40	42.83	41.17	39.66	36.77	31.85	2.531	2.192	1.790

LSD_a: for control to control comparisons only, minimum replications; LSD_b: for comparisons of controls with other treatments, minimum replication and maximum replications; LSD_c: for treatment comparisons only, with controls excluded, maximum replications.

Alkaline phosphatase

The cover cropping × cover crop type × fertiliser regime interaction effect was significant ($P < 0.05$) with respect to alkaline phosphatase at 0-5 and 5-20 cm depth (Figure 3.4). The type of cover crop significantly affected the activity of alkaline phosphatase. Oat-maize and vetch-maize rotations gave higher alkaline phosphatase activity than the weedy fallow with vetch-maize rotations performing better than oat-maize rotations. The absence of a soil cover crop reduced the enzyme activity when compared to the soils under winter cover crops.

In 0-5 cm depth, fertilisation of maize in the weedy fallow rotation had comparable results to unfertilised soils in oat rotations (Figure 3.4a). Alkaline phosphatase activity in F1 under oat-maize rotation had comparable results to F2 under vetch-maize rotation. In 5-20 cm depth, fertiliser regimes were significantly different from each other ($P < 0.001$). Alkaline phosphatase activity was high in the order F1> F2> F3> F4 (Figure 3.4b).

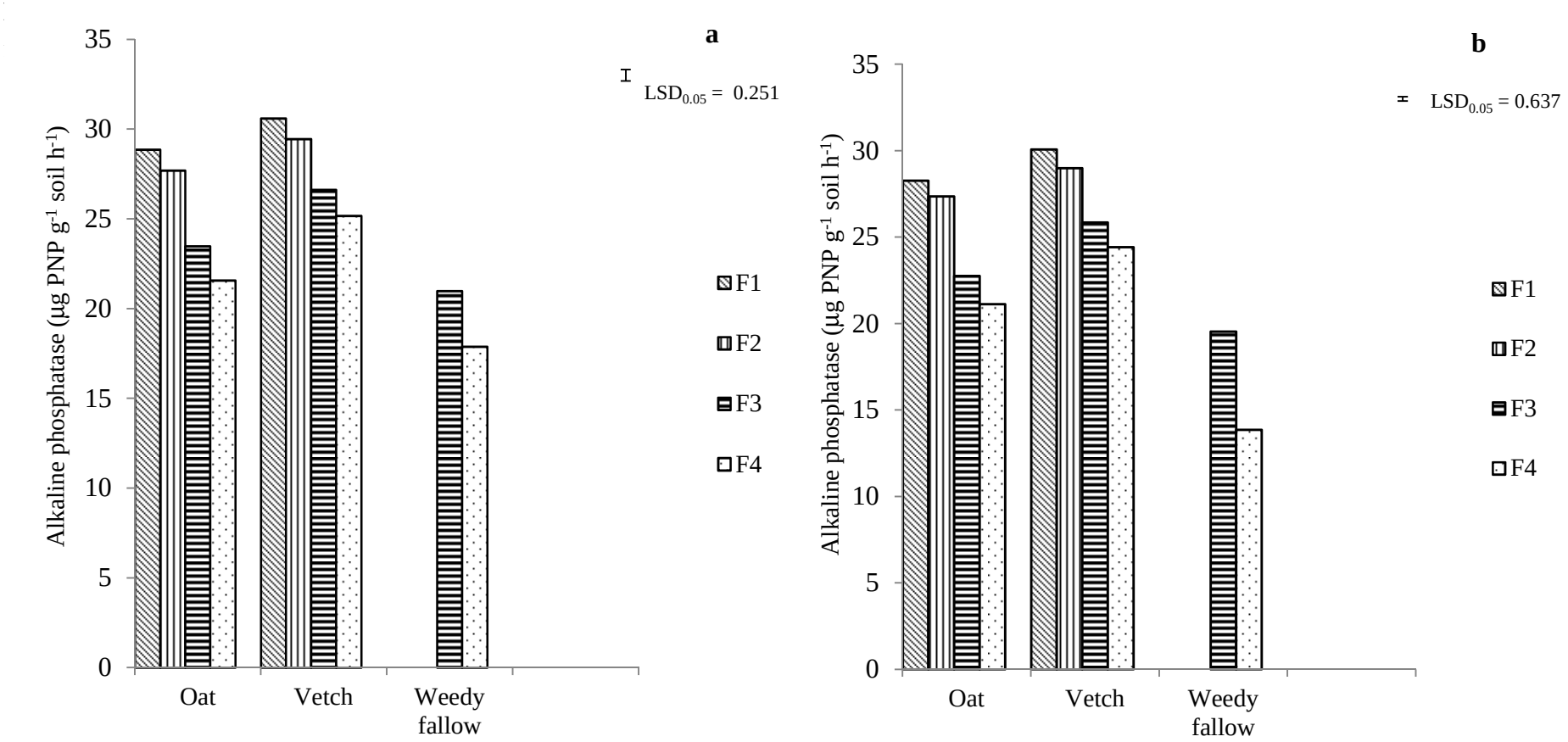

Figure 3.4: Cover cropping × cover crop type × fertiliser regime effects on alkaline phosphatase activity at 0-5 cm (a) and 5-20 cm (b) depths following five years of continuous practice. Error bars represents LSD.

3.4 Discussion

The introduction of CA in local environments of the Eastern Cape could improve soil quality and productivity, including in smallholder systems that are highly degraded. While CA has been found to improve the chemical and physical quality of the soil, it is the biological component that gives an overall indication of soil productivity. Dube *et al*. (2012) reported increased particulate organic matter and water soluble C in five year maize-vetch and maize-oat rotations in the Eastern Cape. The POM and WSC organic matter pools provide ready substrate for microorganisms and could therefore have an effect on microbial biomass and enzyme activities.

The higher MBC in soils under cover crops than weedy fallow, observed in this study indicated that the presence of a cover crop enhances labile C pools and this was in agreement with observations in annual cropping systems by Jackson (2000) and Jackson *et al.* (2004; 2003). The rate of organic C input from plant biomass is generally considered the dominant factor controlling the amount of microbial biomass in soil (Campbell *et al.*, 1997) and this could explain the higher MBC in the maize-oat than the maize-vetch rotation, which was also higher than the weedy fallow. Spedding *et al.* (2004) also found that residue management had more influence than tillage system on microbial characteristics. The decrease in MBC with depth could also be a result of accumulation of organic inputs (substrates) at the surface as a result of minimum disturbance of the soil. Spedding *et al.* (2004) also observed higher MBC levels in plots with residue retention than with residue removal only in the 0-10 cm layer. High enzyme and microbial activities in oat and vetch rotations may be due to the moisture retention and favourable temperature because of their residues.

The increase in MBC in the fertilised plots, particularly where both the cover crop and the maize are fertilised, could be due to increased organic matter inputs due to greater plant

biomass production. Paustian *et al.* (1992) reported that fertiliser N addition increased SOC contents by 15-19% by increasing net primary productivity and residue carbon input, while Franzluebbers *et al.* (1994) observed that SOC of 0-5cm depth was 62% higher in wheat cultivation with fertiliser than without fertiliser, implying synergy in organic and inorganic resources inputs.

Many studies proposed soil enzyme activities as early indicators of changes in soil properties as a consequence of land management (Bandick & Dick, 1999; Dick *et al.,* 1996; Dick, 1994). Enzymes control nutrient release for plant and microbial growth (Gregorich *et al.,* 1994), and therefore, high levels of microbial activity are crucial to maintain soil fertility. Management practices such as tillage, crop rotation and residue management may have diverse effects on various soil enzymes (Tabatabai, 1994) and in this way may alter the availability of plant nutrients.

The higher dehydrogenase, β-glucosidase, urease and alkaline phosphatase enzyme activities in soils under cover crops than weedy fallow, observed in this study indicated that cover crops enhance labile C pools, which was in agreement with observations in annual cropping systems by Jackson (2000) and Jackson *et al.* (2004; 2003). Dehydrogenase, an oxidoreductase, present only in viable cells, has a significant role in the initial organic matter oxidation as it is related to microbial respiratory processes (Dick *et al.,* 1998). β-glucosidase is an important enzyme involved in the last step of cellulose degradation, related to the release of low-molecular weight sugars (Jimenez *et al.,* 2002). It has been demonstrated to be the most consistent in showing separation of treatment effects among other C-cycle enzymes (Lagomarsino *et al.,* 2009; Bandick & Dick, 1999). Phosphatases are extracellular enzymes that catalyse the hydrolysis of organic phosphates to inorganic orthophosphates thus are an

important link between biologically unavailable phosphorus and available P. Higher enzyme activities in maize-vetch than maize-oat rotations may be better explained by the contribution of vetch to soil's nutrient status as reported by Murungu *et al.* (2011). The higher activities of these enzymes in the maize-vetch than in maize-oat rotations suggest that the quality of the organic matter additions could be more important for these enzymes. In a study done by Dick (1994), an observed significant increase in enzymatic activity in 2 years after initiation of cover crop treatments was attributed to an increase in OM inputs from cover crops.

While the rate of organic C input from plant biomass is generally considered the dominant factor controlling the amount of microbial biomass in soil (Campbell *et al.*, 1997), the quality of the organic input could be more important for the most enzymes involved in nutrient cycling. Although dehydrogenase activity reflects a measure of the total viable microbial cells, the MBC was higher in maize-oat while dehydrogenase activity was higher in the maize vetch. The reason for such a contrast is not clear.

The higher activities of β-glucosidase, which catalyses hydrolytic processes in the organic matter breakdown (Deng & Tabatabai, 1996b), arylsulphatase, urease and alkaline phosphatases, which are important in the mineralisation of S, N and P compounds, could contribute to improved soil fertility particularly in the maize-vetch rotation (Deng & Tabatabai, 1997) compared to maize-oat treatment. This could be explained by the C:N ratio of vetch, which make the residues to break down more rapidly. The increased activities of the enzymes in the cover crop treatments particularly those with vetch suggest that such CA systems will result in the decomposition of the residues accompanied by cycling of N, P and S, making these nutrients available for the subsequent crop.

High activity of acid phosphatase compared to that of alkaline phosphatase in all treatments may suggest that acid phosphatase is much more efficient in the hydrolysis of its

substrate than alkaline phosphatase (Klose & Tabatabai, 2000; 1999). The similar trends in the results of acid phosphatase activity and those of MBC (both higher in maize-oat than maize-vetch rotations), suggests that this enzyme is more dependent on the quantity of organic matter addition than the quality. The differences in the trends of activities of acid and alkaline phosphatase activities suggest that P cycling in maize-oat is driven by acid phosphatase while in maize-vetch it is the alkaline phosphatase that could be more important. Different responses of acid and alkaline phosphatases to oat and vetch cover crops and fertilisation supports findings that phosphatases are inducible enzymes and the intensity of their release by microorganisms and plants is determined by their requirement for orthophosphatase, which is strongly affected by soil pH (Amador *et al.*, 1997).

Even though variation in type of cover crop residue accounts for most of the variation in enzyme activities, other factors like soil moisture were obviously operational in the soils examined. Vetch biomass decomposes quickly than oat biomass due to the low C/N ratios and N is mineralised and nitrified at a rapid rate. This rapid mineralisation can make vetch inefficient as a cover crop in terms of synchronisation on N availability with summer maize crop as it exhibits its peak N demand several weeks after nitrate peaked leaving nitrate vulnerable to leaching (Stute & Posner., 1995)

The increase in activities of all enzymes in the fertilised plots, particularly where both the cover crop and the maize are fertilised, could be due to increased organic matter inputs due to greater plant biomass production (Franzluebbers *et al.,* 1994; Paustian *et al.*, 1992). The similarity of F3, F2 and at times F1 in the maize-oat rotation with F4 in the maize-vetch rotation could be explained by the benefits of biological nitrogen fixation of vetch, which could have resulted in higher N in the system.

The decrease in soil enzyme activities, like MBC, with soil depth could be explained by lower levels of OM with depth in no-till systems. This was in agreement with Green *et al.* (2007) and Curci *et al.* (1997), who observed a general decrease of enzyme activities with soil depth as this follows their substrate availability within the soil profile. Deng & Tabatabai (1997) and Tabatabai (1994) showed that the activities of enzymes decreased markedly with increasing soil depth and they attributed it to differences in soil pH and organic carbon content resulting in varied microbial population and diversity. The top 0-5 cm receives both aboveground biomass and roots whereas only roots contribute to the inputs in deeper layers.

3.5 Conclusions and recommendations

Oat-maize rotations gave higher MBC and acid phosphatase activity than vetch-maize rotations which had higher dehydrogenase, β-glucosidase, urease and alkaline phosphatase activities after five years under conditions of the Alice Jozini Ecotopes. The use of grazing vetch and fertilization of cover crops only improved soil biological activity, represented by MBC and enzyme activities, after 5 years of maize-cover crop rotations in low input conservation agriculture system. Considering the differences in persistence of the residues of oat and vetch coupled with the need to benefit from mulch and nutrient cycling effects of the two cover crops, biculture could be used particularly in smallholder systems. This work was based on a CA system that was 5 years old and it is essential to determine the sensitive parameters that could be useful as early indicators of changes in soil quality in younger trials, which will be of value in smallholder systems where CA is being tested.

4. EFFECTS OF OAT (*Avena sativa* L.) AND GRAZING VETCH (*Vicia dasycarpa* L.) COVER CROP BICULTURES ON ACTIVITIES OF SELECTED ENZYMES IN SHORT-TERM ROTATIONS WITH MAIZE ON A SANDY LOAM SOIL IN A WARM TEMPERATE REGION OF SOUTH AFRICA

Abstract

The study evaluated the effects of winter planted white oat (*Avena sativa*) and grazing vetch (*Vicia dasycarpa*) cover crop bicultures grown in rotation with maize (*Zea mays*) on soil enzymes, as early indicators of soil quality, in an irrigated maize-based conservation agriculture (CA) system. Activities of a range of enzymes related to the cycling of the main biologically important nutrients C, N, P and S were investigated. The treatments used were sole oat (100% oat), sole vetch (100% vetch), 90% oat + 10% vetch, 70% oat + 30% vetch, 50% oat + 50% vetch and a weedy fallow as the control. These were laid out in a randomised complete block design with three replications. Maize was planted in summer in same plots after termination of cover crops. Soil samples were collected from the 4 month (after first cover crop season) and 28 month (after three cover crops and two maize crops) trials from the 0-5 cm and 5-20 cm depths and were analysed for activities of dehydrogenase, β-glucosidase, urease, arylsulphatase and acid and alkaline phosphatase enzymes. All enzymes except for urease were more concentrated in the 0-5 cm depth than 5-20cm depth. Treatment × trial age interaction effects were also significant ($P < 0.05$) at both soil depths. Dehydrogenase, β-glucosidase, arylsulphatase and alkaline phosphatase activities were higher in the 28 month trial compared to the 4 month one while urease was higher in the 4-month old trial. Treatments with more than 50% oat content had higher acid phosphatase activity in the 4- than 28 month old trial. Effects of cover crop, as the main factor, was significant in all enzymes ($P < 0.05$), with 70% oat + 30% vetch treatment having the highest dehydrogenase and arylsulphatase while for alkaline phosphatase it had similar results to 90% oat + 10% vetch. For β-glucosidase and urease, effects of cover crop as the main factor were also significant with sole vetch giving the highest activity for both enzymes. Acid phosphatase activity was highest in sole oat. The overall results suggest that sole vetch and the biculture 70% oat + 30% vetch promotes activities of the majority of enzymes, except acid phosphatase, as early as after the first season of cover crops and that these enzymes can be used as biological indicators to detect early changes in soil quality in CA systems.

Keywords: conservation agriculture; soil enzymes; cover crops

4.1 Introduction

Most soils used for crop production in South Africa (SA) have low soil fertility (Mandiringana *et al.,* 2005; Mills & Fey, 2003), causing low maize yields (< 3t ha^{-1}) in irrigated smallholder practises in the Eastern Cape (EC) province of SA (Fanadzo *et al.*, 2010). Improvements in SOM can result in several benefits, including improved soil nutrient storage capacity, nutrient availability, biological activity, soil structure and resistance to erosion (Brady & Weil, 2008).

Tillage has been observed to dilute soil SOM through mixing with soil within the plough layer and to expose aggregate protected organic matter to microbial decomposition, exacerbating the vulnerability of soils to erosion (Burke *et al.* 1995). The promotion of conservation Agriculture (CA) in the low input smallholder farming systems of the EC is meant to improve and restore soil organic matter and fertility. Use of winter cover crops has been identified as an avenue of introducing CA on smallholder farms. Oat *(Avena sativa)* a grass and vetch *(Vicia dasycarpa)* a legume, have been tested and identified as the best species to address soil fertility challenges in the smallholder irrigated practices (Murungu *et al.,* 2010).

The cycling of nutrients in soils is dependent on the energy supply to and through the soil biota. The soil microbial biomass is an important labile pool of C, N, P and S and fluctuations in its size and activity can significantly influence crop productivity. Analyses of the activity of soil enzymes provide information on the biochemical processes occurring in the soil and this study concentrated on the following enzymes based on their roles in nutrient cycling: dehydrogenase (estimates microbial activity), β-glucosidase (C), urease (N), arylsulfatase (S), acid and alkaline phosphatase (P).

The use of maize-oat and maize-vetch rotations has shown improvements in light organic matter fractions (Dube *et al*., 2012) and microbial biomass and enzyme activities (Chapter 3). Maize-oat rotations have been found to produce residues that degrade slower, and are more persistent to provide mulch with moisture conservation and weed suppression benefits. On the other hand, residues from maize-vetch rotations breakdown faster, releasing nutrients for the benefit of the subsequent crop (Murungu *et al*., 2011). In low input smallholder systems, combined benefits from these two rotations could make a significant contribution in crop and soil productivity. Bicultures of these two winter cover crops could be important to realise these benefits. The effectiveness of such biculture could depend on the proportions of the cover crops in the bicultures. In this regards, sensitive indicators of changes in soil quality could be required to evaluate these benefits in the short-term. Microbial biomass C and enzyme activities, shown to be improved after 5 years in CA system could provide such sensitive measures. The objective of this study was to determine effects of oat-vetch bicultures on activities of selected enzymes as early indicators of changes in soil quality. The hypothesis was that rotations of maize and cover crop bicultures, with different ratios of oat and grazing vetch, would improve activities of selected soil enzymes in maize based CA systems in the short-term.

4.2 Materials and methods

4.2.1 Experimental site

This experiment was carried out at the same site with the winter cover crop study described in Chapter 3. Details of location and climatic conditions of the study site are as described in section 3.2.1.

4.2.2 Treatments and experimental design

The biculture experiment evaluated five species ratios of grazing vetch (*Vicia dasycarpa* var Max) and white oat (*Avena sativa* var Pallinup) and a weedy fallow control laid in a randomised complete block design replicated three times. The treatments were 90% oat + 10% vetch, 70% oat + 30% vetch, 50% oat + 50% vetch, 100% oat, 100% vetch and weedy fallow. The ratios were based on the percentage of the recommended seed rate used in the sole crop (100 kg/ha for oat and 50 kg/ha for vetch). A maize experiment was conducted in the summer to evaluate the residual effect of the biculture treatments following their termination at the end of winter. The maize trial, superimposed on the biculture treatments, was laid as a randomised complete block design replicated three times, with the treatments as in the biculture experiment.

The cover crop trial was first established on 1 June 2009 (winter season) and was repeated yearly for three cover crop and two maize cropping seasons. The weedy fallow control was left with maize residues in cycle two and three of the cover crop-maize rotations and was only under weeds in the first cycle. Measurements in these trials captured effects of cover crops after three cycles of cover crop-maize rotation. In June 2011, the same cover crop trial was planted on separate plots on the Fort Hare Research farm to evaluate the first year effects of cover crops on light organic matter fractions.

4.2.3 Agronomic practices

At initiation of the biculture experiment, the field was ploughed and disked. No ploughing was done in subsequent seasons in line with the principles of CA. Weeds were controlled by spraying glyphosate (360 g L^{-1}) at a rate of 3 L ha^{-1} before planting of cover crop. Seeds of cover crops were drilled into small furrows spaced 30 cm apart in plot sizes of

5 m × 4 m. Grazing vetch was inoculated with *Rhizobium leguminosarium* bio var *viciae* inoculants having 5 x 10^8 rhizobial cells g^{-1} (Stimuplant CC, Zwavelpoort 0036, SA) at planting. Only basal fertilizer was applied at a rate of 200 kg ha^{-1} compound fertilizer to the cover crop at planting. The fertiliser had N: P: K ratio of 2:3:4 (30 + 0.5% Zn) and supplied 13.3 kg N ha^{-1}, 20 kg P ha^{-1} and 26.7 kg K ha^{-1}.

Neither weed nor pest control was done during the growth of the cover crop. Cover crop growth was terminated at early flowering stage by tractor-drawn roller and application of glyphosate (N-phosphonomethyl glycine) herbicide (360g L^{-1}) at a rate of 5L ha^{-1}. In summer, maize (variety SC 701) was planted in plots previously grown to the cover crop and weedy fallow treatments. The maize was spaced 30 cm in row and 90 cm inter-row targeting a population of 37 000 plants ha^{-1}. Jab planters were used to drop 2-3 seeds which were thinned to one plant per station at 3 weeks after planting (WAP). Fertilizer application in the maize was at a rate of 60 kg N ha^{-1} (smallholder farmer practice in the EC) with a third of the N applied as a basal compound fertilizer with an N: P: K ratio of 2:3:4 (30+Zn) at planting. The remainder was applied as a top dressing of lime ammonium nitrate (LAN) (28% N) at 6 weeks after planting (WAP). Weed control was done using Basagran (a.i: thiadiazine 480 g L^{-1}) applied at 5 L ha^{-1}.

To supplement the normal winter rains, overhead irrigation water was applied to all treatments based on Class A evaporation pan readings as summarised in Table 3.2, with all plots receiving the same amounts of water. To ensure all plots received the same amount of water, irrigation pipes were laid at a same distance from each other, this included at the boundaries so that all crops including boarder crops received same amount of water. Irrigation time was also the same across all plots. Soil samples were collected two weeks after cover crop termination before maize planting in each plot. Soil sampling from 0-5 cm and 5-20 cm depth was done in October 2011, both from the trial that was established in June

2011 (first cycle - 4 month trial) and from the older trial that was established in June 2009 (third cycle - 28 month trial). The samples were stored in the cold room at < 4^{o}C.

4.2.4 Soil enzymes analyses

Samples were adjusted to 80% of field capacity moisture content and incubated at 37^{o}C for seven days to activate the soil microorganisms.

4.2.4.1 Soil enzyme assays

The samples were analysed for dehydrogenase, β-glucosidase, urease, acid and alkaline phosphatase enzymes activities as described in section 3.2.5. Arylsulphatase activity was also analysed.

Arylsulphatase

Arylsulphatase was based on the method of Tabatabai (1994). One gram of soil (<2 mm) was placed in a 50 mL Elernmeyer flask and 0.25 mL of toluene, 4 mL of acetate buffer, and 1 mL of ρ-Nitrophenyl sulphate solution added. The flask was swirled for ten seconds to mix the contents, stoppered and incubated at 37^{o}C for 1h. After incubation, 1 mL of 0.5 M $CaCl_2$ and 4 mL of sodium hydroxide (NaOH) were added, swirled for ten seconds and filtered through a Whatman no. 2 filter paper. The intensity of the yellow colour produced was measured at wavelength of 420 nm. The amount of ρ-nitrophenol released (related to arylsulphatase activity) was measured by reference to a calibration graph plotted from the results obtained with standards.

4.2.5 Data analysis

Data of soil enzyme activities, at each depth, were subjected to two way analysis of variance (ANOVA) using Genstat Statistical Package 12th edition. Where significant

differences occurred, separation of means was done using the least significant difference (LSD) at 5% level of significance.

4.3 Results

Dehydrogenase

There was a significant interaction effect of cover crop treatment × trial age on dehydrogenase activity at both the 0-5 cm ($P < 0.001$) and 5-20 cm depths ($P < 0.001$) (Table 4.1). Treatments and age of trial as main effects were highly significant both at 0-5 and 5-20 cm ($P < 0.001$) depths. Dehydrogenase enzymes were highest in the 70% oat + 30% vetch treatment in the two depths and trial ages. All treatments were significantly different from each other except for 100% oat and 100% vetch treatments that had similar dehydrogenase activities in the 4 month old trial at 5-20 cm depth. Soil dehydrogenase activity in the 28 months trial was greater than in the four month trial and that in the 0-5 cm depth was also greater than in the 5-20 cm depth ($P < 0.001$).

β-glucosidase

The interaction effect of cover crop treatment and trial age on β-glucosidase at 0-5 and 5-20 cm depths were significant ($P < 0.001$) (Table 4.1). Treatments and trial age as main effects were both significant ($P < 0.001$). β-glucosidase activity increased with increase in the proportion of vetch in the biculture and was highest in 100% vetch and least in the weedy fallow. The 100% vetch for the 4 month old trial had similar results to the weedy fallow in the 28 month old trial under 0-5 cm depth. In the 5-20 cm depths, oat and weedy fallow had similar results both in the 4 and 28 month old trials. Treatments 90% oat + 10% vetch and

70% oat + 30% vetch had comparable results in both 4 and 28 month old trials. Generally β-glucosidase activity increased in the treatments as the ratio of vetch in the biculture increased.

Table 4.1: Cover crop treatment × trial age interaction effects on β-glucosidase and dehydrogenase activities at 0-5cm and 5-20 cm soil depth.

Cover crop treatment	**Dehydrogenase** (μg TPF g^{-1} soil 24 h^{-1})						**β-glucosidase** (μg PNP g^{-1} soil h^{-1})					
	0-5 cm depth		Main effect	5-20 cm depth		Main effect	0-5 cm depth		Main effect	5-20 cm depth		Main effect
	4 months	28 months		4 months	28 months		4 months	28 months		4 months	28 months	
100% oat	122.3^{c}	1238.9^{i}	680.6^{c}	67.7^{b}	696.2^{h}	381.9^{c}	21.48^{b}	33.28^{g}	27.38^{b}	17.83^{ab}	21.80^{de}	19.81^{a}
90% oat +10% vetch	291.6^{e}	1599.1^{k}	945.3^{e}	112.9^{d}	883.5^{j}	498.2^{e}	22.54^{c}	37.05^{h}	29.79^{c}	19.07^{bc}	25.32^{ghi}	22.19^{b}
70% oat + 30% vetch	348.0^{f}	1636.2^{l}	992.1^{f}	184.3^{e}	922.3^{k}	553.3^{f}	23.45^{d}	41.36^{j}	32.41^{d}	20.54^{cd}	25.66^{hi}	23.10^{bc}
50% oat + 50% vetch	199.4^{d}	1358.4^{j}	778.9^{d}	84.6^{c}	731.7^{i}	408.2^{d}	27.06^{e}	40.08^{i}	33.57^{e}	23.30^{ef}	23.97^{fg}	23.63^{c}
100% vetch	75.2^{b}	1205.0^{h}	640.1^{b}	64.0^{b}	660.6^{g}	362.3^{b}	28.86^{f}	43.69^{k}	36.27^{f}	24.54^{fgh}	26.07^{i}	25.31^{d}
Weedy fallow	50.8^{a}	1137.1^{g}	594.0^{a}	43.3^{a}	539.5^{f}	291.4^{a}	20.36^{a}	29.41^{f}	24.89^{a}	17.42^{a}	21.12^{d}	19.27^{a}
Significance	***		***	***		***	***		***	***		***
$LSD_{0.05}$	11.79		8.34	13.99		9.89	0.576		0.407	1.509		1.067
CV (%)	0.9			2.0			1.1			4.0		

Means followed by different letters differ significantly at 5% level of significance. ***P < 0.001

Urease

There was a significant interaction effect of cover crop treatment × trial age on urease activity at both 0-5 cm ($P < 0.001$) and 5-20 cm depths ($P < 0.001$) (Table 4.2). Treatments and age of trial as main effects were highly significant both at 0-5 and 5-20 cm ($P < 0.001$). Urease enzyme activity was highest in 100% vetch in the 4 month old trial of both 0-5 and 5-20 cm depths. In the 28 month old trial at 0-5 cm, 100% vetch had similar results to the three bicultures while at 5-20 cm depth it was similar to the all treatments except the weedy fallow. As main effects, 70% oat + 30% vetch and 90% oat + 10% vetch treatments had similar results at both soil depths. Soil urease activity in the 4 month trial was greater than in the 28 month trial and that in the 0-5 cm depth was also greater than in the 5-20 cm depth ($P < 0.001$) except for the weedy fallow that had greater urease activity in 28 than 4 month old trial at both soil depths and 100% oat at 5-20 cm depth (Table 4.2).

Arylsulphatase

There was a significant interaction effect of cover crop treatment × trial age on arylsulphatase activity at both the 0-5 cm ($P < 0.001$) and 5-20 cm depths ($P < 0.001$) (Table 4.2). Treatments and trial age as main effects were highly significant both at 0-5 and 5-20 cm ($P < 0.001$). Arylsulphatase activity was high in 70% oat + 30% vetch treatment while the weedy fallow had the least enzyme activity at both soil depths. In the 0-5 cm depth, treatments 70% oat + 30% vetch and 90% oat + 10% vetch in the 4 month trial had similar results while in the 28 month trial 50% oat + 50% vetch and 100% oat also had similar results. In the 5-20 cm depth, all treatments were significantly different from each other. The bicultures had higher enzyme activity than their sole crops (100% oat and 100% vetch) and the 28 months old trials in both depths had higher arylsulphatase activity than the 4 month old trial.

Table 4.2: Cover crop treatment × trial age interaction effects on urease and arylsulphatase activities at 0-5cm and 5-20 cm soil depth.

Cover crop treatment	**Urease** (mg urea g^{-1} soil $5h^{-1}$)						**Arylsulphatase** (µg PNP g^{-1} soil h^{-1})					
	0-5 cm depth		Main effect	5-20 cm depth		Main effect	0-5 cm depth		Main effect	5-20 cm depth		Main effect
	4 months	28 months		4 months	28 months		4 months	28 months		4 months	28 months	
100% oat	1.972^{d}	1.968^{bc}	1.970^{b}	1.966^{bc}	1.968^{cd}	1.967^{b}	18.36^{b}	35.69^{h}	27.02^{b}	15.85^{b}	29.51^{i}	20.52^{a}
90% oat +10% vetch	1.976^{e}	1.970^{bcd}	1.973^{c}	1.974^{e}	1.968^{cd}	1.971^{c}	19.22^{c}	37.98^{i}	28.60^{c}	16.56^{c}	33.61^{k}	25.08^{d}
70% oat + 30% vetch	1.980^{f}	1.971^{cd}	1.975^{cd}	1.976^{e}	1.969^{d}	1.972^{c}	19.78^{c}	38.94^{j}	29.36^{d}	17.45^{d}	34.59^{l}	26.02^{e}
50% oat + 50% vetch	1.982^{f}	1.972^{d}	1.977^{de}	1.979^{f}	1.969^{d}	1.974^{d}	20.71^{d}	35.55^{h}	28.13^{c}	18.54^{e}	30.75^{j}	24.64^{c}
100% vetch	1.986^{g}	1.972^{d}	1.979^{e}	1.982^{g}	1.970^{d}	1.976^{e}	22.19^{e}	34.21^{g}	28.20^{c}	19.55^{f}	27.54^{h}	23.54^{b}
Weedy fallow	1.960^{a}	1.967^{b}	1.964^{a}	1.958^{a}	1.965^{b}	1.961^{a}	17.09^{a}	31.74^{f}	24.41^{a}	14.81^{a}	26.23^{g}	20.52^{a}
Significance	***		***	***		***	***		***	***		***
$LSD_{0.05}$	0.0037		0.0026	0.0025		0.0018	0.821		0.58	0.605		0.428
CV (%)	0.1			0.1			1.8			1.5		

Means followed by different letters differ significantly at 5% level of significance. ***P < 0.001

Acid phosphatase

The interaction effect of cover crop treatment and trial age on acid phosphatase activity at both the 0-5 cm and 5-20 cm depths was significant ($P < 0.001$) (Table 4.3). Treatments and trial age as main effects were highly significant both at 0-5 and 5-20 cm ($P < 0.001$). Acid phosphatase activity was highest in sole oat (100% oat) and decreased in the bicultures with an increase in vetch content. Weedy fallow had the lowest acid phosphatase activity in both depths and trial ages. In 0-5 cm depth, 50% oat + 50% vetch in the 4 month old trial had similar results to all the treatments in the 28 month old trial except for the 70% oat + 30% vetch. Acid phosphatase activity in treatments 100% oat, 90% oat +10% vetch and 70% oat + 30% vetch was higher in 4 month old trial than the 28 month old trial while in 50% oat + 50% vetch, 100% vetch and weedy fallow it was the opposite. In 5-20 cm depth, 50% oat + 50% vetch in 4 month old trial had similar results to the weedy fallow in 28 month old trial. The 70% oat + 30% vetch treatment in 4 month old trial had similar results to all treatments in 28 month old trial except the weedy fallow. Enzyme activity in treatments 100% oat and 90% oat +10% vetch was higher in 4 month old trial than the 28 month old trial while in 70% oat + 30% vetch, 50% oat + 50% vetch, 100% vetch and weedy fallow it was the opposite.

Alkaline phosphatase

The interaction effect of cover crop treatment and trial age on alkaline phosphatase activity at both 0-5 cm and 5-20 cm depths was significant ($P < 0.001$) (Table 4.3). Treatments and trial age as main effects were highly significant both at 0-5 and 5-20 cm ($P < 0.001$). Alkaline phosphatase activity was highest in 70% oat + 30% vetch and lowest in the weedy fallow in all depths. In 0-5 cm depth, 70% oat + 30% vetch in the 4 month old trial had similar results to 50% oat + 50% vetch and 100% oat in the 28 month old trial. Also 90%

oat + 10% vetch (4 month old trial) had similar results to 100% vetch (28 month old trial). The 28 month old trial had higher alkaline phosphatase activity than the 4 month old trial except for the weedy fallow. In 5-20 cm depth, 100% vetch in 4 month old trial had similar results to the weedy fallow in 28 month old trial. Treatment 50% oat + 50% vetch and 70% oat + 30% vetch both in 4 month old trial had similar results. Alkaline phosphatase activity was higher in 28 month old trial than the 4 month old trial.

Table 4.3: Cover crop treatment × trial age interaction effect on acid and alkaline phosphatases activities at 0-5 and 5-20 cm soil depth.

Cover crop treatment	**Acid phosphatase** (μg PNP g^{-1} soil h^{-1})						**Alkaline phosphatase** (μg PNP g^{-1} soil h^{-1})					
	0-5 cm depth		Main effect	5-20 cm depth		Main effect	0-5 cm depth		Main effect	5-20 cm depth		Main effect
	4 months	28 months		4 months	28 months		4 months	28 months		4 months	28 months	
100% oat	46.63^{g}	34.38^{cd}	40.51^{f}	40.27^{g}	33.70^{de}	36.98^{f}	28.02^{d}	35.26^{f}	31.64^{b}	19.75^{c}	28.41^{g}	24.08^{bc}
90% oat +10% vetch	44.40^{f}	34.32^{cd}	39.36^{e}	36.01^{f}	33.94^{e}	34.97^{e}	37.78^{g}	45.76^{h}	41.77^{c}	23.45^{e}	32.22^{i}	27.84^{d}
70% oat + 30% vetch	36.51^{e}	35.42^{de}	35.96^{d}	33.37^{de}	34.15^{e}	33.76^{d}	34.10^{f}	48.91^{i}	41.51^{c}	21.86^{d}	34.03^{j}	27.94^{d}
50% oat + 50% vetch	33.01^{c}	33.49^{c}	33.25^{c}	31.04^{c}	32.46^{d}	31.75^{c}	30.07^{e}	34.22^{f}	32.15^{b}	21.25^{d}	26.60^{f}	23.92^{b}
100% vetch	29.69^{b}	34.14^{cd}	31.92^{b}	26.52^{b}	33.35^{de}	29.93^{b}	26.19^{c}	37.12^{g}	31.66^{b}	18.23^{b}	30.91^{h}	24.57^{c}
Weedy fallow	25.20^{a}	33.20^{c}	29.20^{a}	22.85^{a}	30.77^{c}	26.81^{a}	24.24^{b}	19.45^{a}	21.85^{a}	16.79^{a}	17.59^{b}	17.19^{a}
Significance	***		***	***		***	***		***	***		***
$LSD_{0.05}$	1.616		1.143	1.348		0.953	1.392		0.984	0.747		0.528
CV (%)	2.7			2.5			2.5			1.8		

Means followed by different letters differ significantly at 5% level of significance. ***P < 0.001

4.4 Discussion

Sensitive soil indicators are required to determine the response of soil quality to changes in management, like using cover crops, especially in smallholder CA systems where cover crop and maize residue input is likely to be lower as a result of poor management and possible grazing by animals. Microbial biomass carbon and enzyme activities have been found to be relatively more sensitive to changes of management like tillage compared to physical and chemical properties (Bandick & Dick, 1999; Dick, 1994; Gregorich *et al.*, 1994;). Soil enzyme activities are not only affected by the type and chemical nature of the organic matter. Other factors like temperature, soil moisture and soil pH strongly affect the biochemical processes. Moisture availability strongly affects soil microbial activity, community composition and consequently soil enzymatic activities. As soils dry, the water potential increases, and as well microbial activity as intracellular enzyme activity slows down (Geisseler *et al.*, 2011). In the case of moist soils, increased moisture could bring into soil solution soluble OM that might be responsible for an increase of microbial population number (Subhani *et al.*, 2001).

Among all enzymes in the soil environment, dehydrogenase is one of the most important, and is used as an indicator of overall soil microbial activity, because they occur intracellularly in all living microbial cells (Yuan & Yue, 2012; Zhao *et al.*, 2010). They may give a measure of the total viable microbial cells. Dehydrogenases play a significant role in the biological oxidation of SOM by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al.*, 2010). The difference in dehydrogenase activity under different cover crop treatments shows that they also affect the microbial population and their activities.

Mulching generally increases enzymes activities in soils, and the supply of readily available substrate for microorganisms (Monreal & Bergstrom, 2000). Cellulose is the most important organic compound in plant residues and its mineralisation and degradation in soil is a major process within the C-cycle. β-glucosidase belongs to the group of enzymes that catalyse the hydrolytic conversion of cellulose to glucose (Deng & Tabatabai, 1996b). The increase in activity of β-glucosidase with increase in proportion of vetch in the bicultures may be due to the low C:N ratio of vetch, as according to Tian *et al.* (1992) plant residue mineralisation is controlled by its C:N ratio, lignin and polyphenols content. The high concentration of residue and roots of previous crops in the 28 month trial may have increased the enzyme activity and as Balota *et al.* (2004) have reported, that glycosidases increase in no till systems as compared to conventional tillage system due to an increase in mulch content.

The effects of cover crop residue on the level of urease (the enzyme that catalyzes the hydrolysis of urea) activity in the upper soil depth (0-5 cm) could be a reflection of the amount of residue retained and rate of decomposition which in turn would influence the size and composition of the microbial population. High urease activity probably resulted from an increase in SOM content and microbial population which promoted the secretion of urease enzyme although no urea fertilizer was applied in the field. Similar results have been reported by Garcia *et al*. (1994); Pascual *et al*. (1999). The higher urease activity in the 4 month old trial than 28 month old trial could be because of more rapid N mineralisation.

Higher arylsulphatase enzyme activity in both soil depths under cover crops than weedy fallow is in agreement with Deng & Tabatabai (1997) who observed that mulching and no till increases arylsulphatase activity significantly. Arylsulfatase is the enzyme that is involved in mineralization of ester sulphate in soils (Tabatabai, 1994), and its activity has

varied widely in relation to soil properties and management (Bandick & Dick, 1999; Gupta & Germida, 1988). The greater arylsulfatase activity under 70% oat + 30% vetch treatments and in 28 month than 4 month old trial may reflect an increase of fungal biomass because arylsulfatase has strong correlation with ergosterol, which is almost exclusively found in fungi (Newell *et al.*, 1987). The 70% oat + 30% vetch ratio may have favoured the microbial proliferation and microbial activity hence the higher production of the enzyme arylsulfatase than other bicultures. The lack of soil tilling in this study may have increased fungal growth as in consistent with Frey *et al.* (1999) who found greater fungal than bacterial growth under reduced tillage. They observed that fungal hyphal length was 1.9 to 2.5 times higher in no till than conventional tillage. One of the reasons is that no till facilitates establishment and maintenance of hyphae compared to tillage that disrupts fungal networks. This further supports our findings of high arylsulphatase activities in the older trial than the younger one. Furthermore, fungi have up to 42% of its S as ester sulphate, which is the substrate for arylsulphatase, while bacteria have around 10% ester sulphate-S (Saggar *et al.,* 1981).

Phosphorus is one of the major soil nutrient limiting crop yields, and South African soils are generally low in this nutrient (Barnard & du Preez, 2004). Large proportions of the phosphorus in many soils are organically bound and the mineralisation of these portions is of agricultural and economic importance. Organic phosphorus compounds in soil can constitute 5-50% of total P and the assimilation of this P by plants and microorganisms is preceded by soil enzymes. Acid and alkaline phosphatases particularly catalyse the hydrolysis of P-ester bonds binding P to C in OM. Acid and alkaline phosphatases were high in 0-5 cm than 5-20 cm soil layer and this is confirmed by literature which states that phosphatases are more concentrated in the surface layer and rhizosphere where most of the fresh and less humified OM is prevailing (Deng & Tabatabai, 1997; Tabatabai, 1994). The different activities of phosphatases in soil at different soil depth can be attributed to difference in soil pH and

organic C content resulting in varied microbial population and diversity. It is an interesting element in that relatively small amounts are actually required by plants but its efficiency in soil is relatively low.

4.5 Conclusion and recommendation

Rotations of maize and bicultures resulted in elevated dehydrogenase, arylsulphatase and alkaline phosphatase particularly in the 70% oat + 30% vetch treatments, while β-glucosidase and urease increased with proportion of vetch and acid phosphatase with increase in proportion of oat, as early as in the first season of cover crops. Enzyme activities increase with the age of the trial. The activities of enzymes studied can be used as early indicators of changes in soil quality in CA systems that make use of bicultures of oat and grazing vetch. Similar work on light organic matter fractions is essential.

5. EFFECTS OF OAT (*Avena sativa* L.) AND GRAZING VETCH (*Vicia dasycarpa* L.) COVER CROP BICULTURES ON LIGHT ORGANIC MATTER FRACTIONS IN SHORT-TERM ROTATIONS WITH MAIZE ON A SANDY LOAM SOIL IN A WARM TEMPERATE REGION OF SOUTH AFRICA

Abstract

This study evaluated the effects of winter planted white oat (*Avena sativa*) and grazing vetch (*Vicia dasycarpa*) cover crop bicultures grown in rotation with maize (*Zea mays*) on light organic matter fractions in an irrigated maize-based conservation agriculture (CA) system. The treatments used were sole oat (100% oat), sole vetch (100% vetch), 90% oat + 10% vetch, 70% oat + 30% vetch, 50% oat + 50% vetch and a weedy fallow as the control. These treatments were laid out in a randomised complete block design with three replications. Maize was planted in summer in same plots after termination of cover crops. Soil samples were collected from the 4 month (after first cover crop season) and 28 month (after three cover crops and two maize crops) trials from the 0-5 cm and 5-20 cm depths and were analysed for total carbon, total N, microbial biomass carbon (MBC), particulate organic matter (POM) and water soluble carbon (WSC). Total C, N, MBC, POM and WSC were more concentrated in the 0-5cm depth than 5-20cm depth. Total C, N, C:N and MBC, at both soil depths, and WSC at the 0-5 cm depth, were not affected by treatment × trial age interaction effects ($P > 0.05$), the interaction effects were significant for POM. The biculture treatments did not improve total C, and N when compared with the weedy fallow. Particulate OM was highest in the 100% oat treatment and declined with decrease in the proportion of oat in the biculture, except in the 0-5 cm depth of the 28 months old trial where the 70% oat + 30% vetch treatment had higher concentrations than the 90% oat + 10% vetch. The 50% oat + 50% vetch treatment had similar POM to the 70% oat +30% vetch in the 4 month old trial and to the 90% oat + 10% vetch in the 28 months old trial in the 0-5 cm depth ($P < 0.05$). In the 5-20 cm depth, the 70% oat +30% vetch treatment had similar POM to the 90% oat + 10% vetch in the 28 month old trial. The soil POM content in the 28 months trial was greater than in the four month trial ($P < 0.001$). The 70% oat + 30% vetch had greatest WSC followed by the 90% oat + 10% vetch, with the 100% vetch being similar to 50% oat + 50% vetch. The 28 months old trial had greater POM and WSC than the 4 months trial. The 70% oat + 30% vetch treatment had the highest MBC followed by 90% oat + 10% vetch, with the sole crops and 50% oat + 50% vetch having similar levels. The findings suggest that POM,

MBC and WSC are useful early indicators of soil organic matter build-up of CA systems and bicultures seem to work better than their sole crops.

Keywords: Conservation agriculture; particulate organic matter; water soluble carbon; microbial biomass carbon.

5.1 Introduction

The majority of smallholder farmers in the Eastern Cape (EC) Province of South Africa (SA) practice maize monoculture and usually use conventional mouldboard tillage with little or no fertility replenishment (Mandiringana *et al.*, 2005). Consequently, maize yields realised by these farmers are low, averaging less than 1 t ha^{-1} under dry land systems and 3 t ha^{-1} under irrigation (Fanadzo, 2007). Poor crop productivity in the EC has been attributed to soil degradation. The majority of soils have low soil organic matter (SOM) due to practices such as intensive tillage, short to no fallow periods, virtual absence of crop rotations, overgrazing and removal of crop residues (Mandiringana *et al.,* 2005; Laker, 2004; Fox & Rowntree, 2001). The land degradation observed over many years has prompted promotion of alternative tillage methods such as no-till and the practice of conservation agriculture (CA) to restore the productivity of agricultural land in the EC. Emphasis has been placed on provision of permanent soil cover through the practice of winter cover cropping.

Previous work by Murungu *et al.* (2010) and Musunda (2010) identified grazing vetch *(Vicia dasycarpa),* a legume, and white oat *(Avena sativa),* a grass, as candidate cover crop species capable of addressing soil fertility and weed challenges experienced by smallholder farmers in the EC. However, Murungu *et al*. (2010) showed that neither grazing vetch nor white oat could singly address both objectives of improving soil fertility and reducing weed competition and suggested the use of grass/legume bicultures as a possible solution. Bicultures have been reported to moderate C:N ratios resulting in intermediate decomposition

and mineralization, compared to their sole crops (Muzangwa, 2012; Sainju *et al*., 2005). In low external input cropping systems like the EC smallholding farms, the mineralisation of OM contributes to soil fertility which is generally measured by the amount of soil C.

Soil organic carbon (SOC) is proposed as a primary indicator of soil quality (Conteh *et al.,* 1997; Reeves, 1997), especially in the surface soil (Franzlubbers, 2002), the vital horizon that receives much of the seed, fertilizer and pesticides. Returning more residues to the soil is associated with an increase in SOC concentration (Dolan *et al.,* 2006; Wilhelm *et al.,* 2004) and bicultures of cover crops could have a significant effect in this regard. In order to establish whether the CA system based on oat and grazing vetch bicultures works, effects on soil organic matter build up in the early stages need to be established. While improvements in soil quality are often linked to SOM levels, this parameter is less sensitive to changes in management in the short term, and long periods (up to six years) may be required to observe significant responses (Carter et al., 2002). However, more sensitive parameters that are related to SOM improvements are available and they include particulate organic matter (POM), water soluble carbon (WSC) and microbial biomass carbon (MBC).

Dube *et al.* (2012) reported high levels of light organic matter fractions (particulate OM and water soluble C) in CA systems involving rotations of maize with either oat or grazing vetch. In the same trials, microbial biomass C and activities of selected enzymes were also elevated when compared to the controls (Chapter 3). Bicultures of oat and vetch, in rotation with maize, elevated activities of enzymes involved in important nutrient cycles were observed as early as after the first cover crop termination. The improved enzyme activity could be related to organic matter build up especially the light fractions that are sensitive to changes in management.

Particulate organic matter is the most labile fraction of SOM that is readily formed and decomposed and is an important substrate for soil mineralisation processes (Cambardela & Elliot, 1992). Water soluble carbon is the most dynamic C pool in soils and forms a small proportion of the total SOM and is present in soil solution (Herbert & Bertsch, 1995). Microbial biomass is the living component of SOM and generally comprises 1-5% of total OM content (Nsabimana *et al.,* 2004). Microbial biomass C is the most active part of SOC that has been suggested as a useful and more sensitive measure of a change in SOC status (Friedel *et al.*, 1996). It is possible to detect the changes in microbial fraction long before they are detectable in the total organic matter because of its rapid rate of turn-over of 1-2 years (Sparling, 1992). The three parameters (POM, WSC and MBC) could be used to detect short term responses of the soil to increased biomass input through cover crop bicultures. The objective of this study was to determine effects of oat-vetch bicultures on labile organic matter pools as early indicators of changes in soil quality in no-till irrigated maize based CA system. The hypothesis was that rotations of maize and cover crop bicultures, with different ratios of oat and grazing vetch, would improve concentrations of POM, WSC and MBC in maize based CA systems in the short-term.

5.2 Materials and methods

5.2.1 Experimental site

The study was carried out at the University of Fort Hare Research Farm. Details of location and climatic conditions of the study site are as described in section 3.2.1. This study was based on the experimental set up described in Chapter 4.

Details of treatments and experimental design are as described in section 4.2.2, while those of agronomic practices are described in section 4.2.3.

5.2.2 Light organic matter fractions

Soil samples were collected at depths of 0-5 cm (using a small trowel) and 5-20 cm (using a 7 cm diameter precision auger) in October 2011, from the experimental plots described in Chapter 4. Three soil sub-samples were taken for each depth per plot along an imaginary zig-zag line, avoiding one metre boundaries on all sides. Samples from the same depth in each plot were mixed together to make a composite soil sample. The soil was then taken to a shade, where it was sieved (2 mm). Portions of the samples were air-dried before analysis of total C, N, C/N, POM and WSC.

5.2.2.1 Total C and N was analysed by dry combustion using LECO CNS analyser (LECO Corporation, 2003) and C:N ratios were calculated from observed C and N results.

5.2.2.2 Particulate organic matter was determined using a method by Cambardella & Elliot (1992). Soil samples (50 g) were dispersed in 100 mL of 10% Calgon solution (sodium hexametaphosphate). The dispersed soil suspensions were sieved through a nest of sieves, with pore diameter of 250 μm and 50 μm, mounted on an electromagnetic wet sieving shaker (Filtra Vibración S.L model FTLVH-0150) and shaken at 50 rpm, with several water rinses. Particulate OM from the 250 μm and 50 μm mesh size was back-washed and collected in a beaker. Since POM floats in water, it was separated from silt and fine sand by repeated decantation. The collected POM samples were oven dried at 65^{o}C for 24 h and weighed, and the weight was expressed as a percentage of the initial soil (oven-dry equivalent).

5.2.2.3 Water soluble carbon was determined using a method by Haynes & Francis (1993). Soluble organic carbon was extracted by suspending soil samples (3 g) in 30 mL cold distilled water in 50 mL centrifuge tubes followed by shaking at 200 rpm for 30 minutes on a rotary shaker. The suspension was centrifuged (Eppendorf 5810, Eppendorf Hamburg, Germany) at 822 × g for 20 minutes. The supernatant was filtered through Whatman no. 42 filter paper and WSOC in the solution analysed by the dichromate oxidation method (Witt *et al*., 2000). Two blanks that had only the reagents without the supernatant were included, one of them was digested together with the treatments and the other was not digested. These were the heated and the unheated blanks respectively. Water soluble organic carbon in the soil was calculated using the following equation:

$$Water\ soluble\ organic\ carbon\ (\%) = \left(\frac{A \times M \times 0.003}{m}\right) \times \left(\frac{E}{S}\right) \times 100$$

Where $A = (mL_{HB}\text{-}mL_{sample}) \times (mL_{UB}\text{-}mL_{HB}/mL_{UB}) + (mL_{HB}\text{-}mL_{sample})$

HB = heated blank

UB = unheated blank

M = molarity of ferrous ammonium sulphate (≈ 0.033 M)

m = dry soil mass (g)

E = extraction volume (mL)

S = digest sample volume (mL)

5.2.2.4 The soil sub-samples for the analysis of MBC had their moisture adjusted to 80% of field capacity and the samples incubated at 37^{o}C for seven days to activate the soil microorganisms. Microbial biomass carbon (MBC) was determined by the modified chloroform fumigation–extraction method as described in section 3.2.4.

5.2.3 Data analysis

Data of total C, N, C:N, POM, WSC and MBC, were analysed as in section 4.2.5. Regression analyses were conducted to test the relationship of MBC versus POM and MBC versus WSC.

5.3 Results

5.3.1 Total C, N and C:N

Cover crop treatment x trial age interaction effects on total C, total N and C:N in 0-5cm and 5-20 cm soil depth were not significant ($P > 0.05$). Both main effects of cover crop treatment and age of trial were not significant with respect to total C, N and C:N ratio in both 0-5cm (Table 5.1) and 5-20 cm depths ($P > 0.05$).

5.3.2 Particulate organic matter

There was a significant interaction effect of cover crop treatment and trial age on POM at both the 0-5 cm ($P < 0.05$) and 5-20 cm depths ($P < 0.001$). Treatments and age of trial as main effects were highly significant both at 0-5 and 5-20 cm ($P < 0.001$). Particulate OM was highest in the 100% oat treatment and declined with decrease in the proportion of oat in the biculture, except in the 0-5 cm depth of the 28 months trial where the 70% oat +30% vetch treatment had higher POM than the 90% oat + 10% vetch. The 50% oat + 50% vetch treatment had similar POM to the 70% oat +30% vetch in the 4 month old trial and to the 90% oat + 10% vetch in the 28 month old trial in the 0-5 cm depth ($P < 0.05$) (Table 5.2). In the 5-20 cm depth, the 70% oat +30% vetch treatment had similar POM to the 90% oat + 10% vetch in the 28 month old trial (Table 5.2). The soil POM content in the 28 month old trial was greater than in the four month old trial ($P < 0.001$).

Table 5.1: Carbon, Nitrogen and C:N ratios under different cover crop treatments and different trial ages.

Cover crop treatment	Carbon						Nitrogen			C:N ratio		
	0-5 cm depth		Main effect	5-20 cm depth		Main effect	0-5 cm depth		Main effect	0-5 cm depth		Main effect
	4 months	28 months		4 months	28 months		4 months	28 months		4 months	28 months	
100% oat	1.06	1.07	1.07	0.91	0.92	0.91	0.11	0.10	0.11	9.69	10.45	10.07
90% oat +10% vetch	1.22	1.19	1.21	0.95	0.92	0.93	0.12	0.12	0.12	10.22	9.65	9.93
70% oat + 30% vetch	1.35	1.23	1.29	0.93	0.95	0.94	0.14	0.13	0.13	9.88	9.72	9.80
50% oat + 50% vetch	1.27	1.19	1.23	0.93	0.99	0.96	0.13	0.12	0.13	10.02	9.77	9.90
100% vetch	1.05	1.16	1.10	0.96	0.99	0.97	0.11	0.12	0.11	9.55	9.90	9.72
Weedy fallow	1.15	1.21	1.18	0.93	0.85	0.89	0.16	0.12	0.14	8.41	10.07	9.24
Significance	ns		ns	ns		ns	ns		ns	ns		ns
$LSD_{0.05}$	-		-	-		0.115	-		0.030	-		1.560
CV (%)	11.2			10.3			20.5			13.3		

ns- not significant at 5% level of significance

Table 5.2: Cover crop treatment × trial age effect on POM (%) at 0-5 and 5-20 cm soil depth.

Treatments	0-5 cm			5-20 cm		
	4 months	28 months	Main effect	4 months	28 months	Main effect
100% oat	1.88^{f}	2.23^{g}	2.05^{f}	1.52^{i}	1.90^{j}	1.71^{f}
90% oat +10% vetch	1.38^{d}	1.59^{e}	1.67^{e}	1.01^{f}	1.39^{h}	1.20^{e}
70% oat + 30% vetch	1.14^{c}	1.81^{f}	1.47^{d}	0.91^{e}	1.35^{h}	1.13^{d}
50% oat + 50% vetch	1.02^{c}	1.59^{e}	1.31^{c}	0.77^{d}	1.24^{g}	1.01^{c}
100% vetch	0.86^{b}	1.36^{d}	1.11^{b}	0.58^{b}	0.96^{ef}	0.77^{b}
Weedy fallow	0.56^{a}	0.86^{b}	0.71^{a}	0.38^{a}	0.64^{c}	0.51^{a}
Significance	*		***	***		***
$LSD_{0.05}$	0.150		0.106	0.055		0.039
CV (%)	6.4			3.1		

Means followed by different letters differ significantly at 5% level of significance, * P < 0.05, *** P < 0.001

5.3.3 Water soluble C

The interaction effect of cover crop treatment and trial age on WSC at 0-5 cm depth was not significant ($P > 0.05$) (Table 5.3). Treatments and trial age as main effects were both significant ($P < 0.001$). Water soluble C was highest in the 70% oat + 30% vetch and least in the weedy fallow and the 100% oat treatment. The 70% oat + 30% vetch had greater WSC than the 90% oat + 10% vetch which was also greater than the 100% vetch (similar to 50% oat + 50% vetch). The 28 month old trial had more WSC than the 4 month old trial (Table 5.3).

In the 5-20 cm depth cover crop treatment and trial age interaction effect was significant ($P < 0.01$), while the main effects were also significant ($P < 0.001$) (Table 5.3). Biculture treatments had greater WSC than the two monoculture treatments (100% oat and 100% vetch) which were comparable and had greater WSC than the weedy fallow, for both trial ages (Table 5.3). The highest WSC in the 4 months trial was in the 90% oat + 10% vetch and was similar to the 70% oat + 30% vetch and 50% oat + 50% vetch treatments, whereas in the 28 months trial 70% oat + 30% vetch treatment had the greatest and was higher than in the 90% oat + 10% vetch.

5.3.4 Microbial biomass C

The interaction effect of cover crop treatment and trial age on MBC was not significant at both depths ($P > 0.05$) but treatments and age of trial as main effects were significant in both soil depths ($P < 0.001$) (Table 5.4). For both trial ages, MBC was highest in the 70% oat + 30% vetch followed by the 90% oat + 10% vetch, while the weedy fallow had the least. The rest of the treatments had comparable levels except that the 100% oat had lower MBC than 100% vetch and 50% oat and 50% vetch in the 5-20 cm depth. The 28 month trial had greater MBC than the 4 month trial.

Table 5.3: Cover crop treatment × trial age effect on WSC (mg g^{-1} soil) at 0-5 and 5-20 cm soil depth.

Treatments	0-5 cm			5-20 cm		
	4 months	28 months	Main effect	4 months	28 months	Main effect
100% oat	1.45	1.77	1.61^{ab}	1.29^{a}	1.45^{b}	1.37^{b}
90% oat +10% vetch	1.64	1.94	1.79^{c}	1.52^{b}	1.78^{d}	1.65^{d}
70% oat + 30% vetch	1.82	2.21	2.02^{d}	1.51^{b}	1.95^{e}	1.73^{e}
50% oat + 50% vetch	1.51	1.89	1.70^{bc}	1.45^{b}	1.63^{c}	1.54^{c}
100% vetch	1.60	1.87	1.73^{c}	1.32^{a}	1.51^{b}	1.42^{b}
Weedy fallow	1.49	1.63	1.56^{a}	1.05^{a}	1.28^{a}	1.17^{a}
Significance	ns		***	**		***
$LSD_{0.05}$	-		0.091	0.103		0.073
CV (%)	4.4			4.1		

Means followed by different letters differ significantly at 5% level of significance, ns- not significant, ** P < 0.01, *** P < 0.001

Table 5.4: Cover crop treatment × trial age effect on MBC at 0-5 and 5-20 cm soil depth.

Treatments	0-5 cm			5-20 cm		
	4 months	28 months	Main effect	4 months	28 months	Main effect
100% oat	963.6	2155.4	1559.5[b]	658.5	1959.4	1309.0[b]
90% oat +10% vetch	1306.0	2645.2	1975.6[c]	1112.4	2416.6	1764.5[c]
70% oat + 30% vetch	1518.4	2775.8	2147.1[d]	1372.0	2677.9	2024.9[d]
50% oat + 50% vetch	883.5	2188.0	1535.7[b]	691.9	1992.1	1342.0[b]
100% vetch	914.5	2122.7	1518.6[b]	720.5	2024.7	1372.6[b]
Weedy fallow	638.5	1959.4	1299.0[a]	490.2	1796.1	1143.2[a]
Significance	ns		***	ns		***
$LSD_{0.05}$	-		101.96	-		69.55
CV (%)	5.1			3.9		

Means followed by different letters differ significantly at 5% level of significance, ns- not significant, *** P < 0.001

A regression analysis of MBC and POM was highly significant ($P < 0.001$), although it was significant, the relationship was very low. At 0-5 cm it was 37.89% while at 5-20 cm depth it was 26.92%.

A regression analysis of MBC and WSC showed a significant ($P < 0.001$) relationship. High WSC resulted in a corresponding higher MBC in both 0-5 and 5-20 cm soil depths (Figure 5.1).

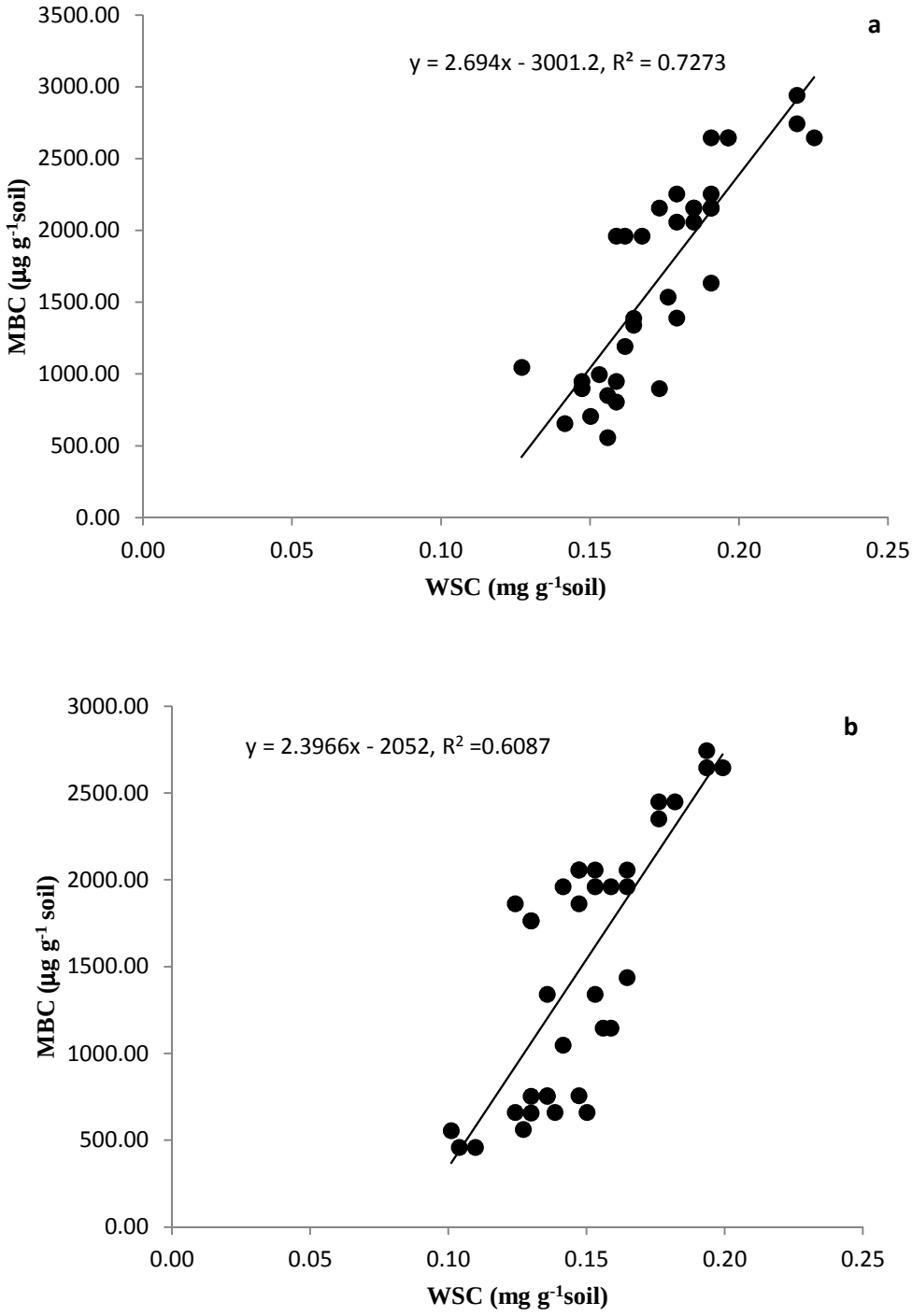

Figure 5.1: MBC response to increase in WSC at 0-5 (a) and 5-20 cm (b) soil depths.

5.4 Discussion

Soil OM has been considered as an indicator of soil quality because of its character of nutrient sink and source that can enhance soil physical and chemical properties, and also promote biological activity (Salazar *et al.*, 2011).

The lack of differences, in total C and N among treatments and between trial ages could be explained by the poor sensitivity of these parameters in the soil. Due to the large turnover of total C, measurement of this parameter alone was not adequate to reflect changes in soil quality and nutrient status (Bezdicek, 1996; Franzluebbers *et al.,* 1994) in this study. Although Gale & Cambardella (2000) reported that roots are a great contributor to SOM than the aboveground plant biomass, such effects were not evident in this study.

Labile C fractions were more sensitive or indicative of changes in C contents than total C. Although POM concentrations were lower in the 4 month trial, the trends of the treatment effects at both depths were similar to those of the 28 months trial. This suggests that POM is highly sensitive and can be used to detect early changes in soil quality in CA systems that use bicultures of oat and grazing vetch. The increase in POM with increase in proportion of oat in the bicultures suggests that oat contributes more towards POM build-up than grazing vetch. The finding could be explained by the C:N ratio of oat (18.2) and grazing vetch (10.0) determined from the same trial by Muzangwa (2012). The higher proportion of the grass with greater C:N ratio (more recalcitrant compounds) (Quemada & Cabrera, 1995a, b) could have resulted in the higher POM observed in oat and bicultures with higher proportion of oat. The lower C:N ratio in vetch makes it more prone to rapid decomposition and the persistence of POM from vetch in soil could be low. The high biomass produced by oat and grazing vetch compared to the weedy fallow, as reported by Muzangwa (2012), and retention of residues on the soil surface without soil disturbance could explain the POM

build-up in the 0-5 cm depth (Franzluebbers & Arshad, 1997) and this was also in agreement with findings from a long term cover crop study by Sasal *et al*. (2010).

Water soluble C is among the most labile pools of SOM and it serves as an important reservoir of plant nutrients since it acts as a readily-decomposable substrate for soil microorganisms (Gregorich *et al.,* 1994) especially in agricultural ecosystems. The decrease in WSC with depth could have been due to the close relationship between WSC and total SOM, which is more concentrated in the surface layer (Carter, 2002; Gregorich *et al.,* 1994). Although there was an increase in WSC from the 4 months- to the 28 months trial, the trend among the treatments were similar, which suggested that WSC is as sensitive as POM as an indicator of soil quality in early stages of CA systems.

The similar WSC in the oat treatment compared to the weedy fallow suggests oat contributes more to POM accumulation and minimally to WSC. The greater POM in oat than in vetch, and the greater WSC in vetch than oat, suggested that the two species in the bicultures have a synergistic effect of improving both POM and WSC particularly at 70% oat and 30% vetch. The 70% oat +30% vetch treatment appears the optimum biculture in terms of WSC although this could be a result of a moderated C:N ratio together with increased biomass addition. Muzangwa (2012), using the same trial, found that the 70% oat + 30% vetch and the 90% oat + 10% vetch had greater dry matter than the other treatments. Dry matter addition through the follow on maize was also highest in the 70% oat + 30% vetch (Muzangwa, 2012). A great proportion of WSC observed in this study could have been readily biodegradable, promoting the growth of soil microorganisms and contributing to ready nutrient release.

The similarity of trends of MBC and WSC (highest in the 70% oat +30% vetch and the 90% oat +10% vetch) suggests that microbial biomass was utilising water soluble organic

matter as a substrate. This was supported by the strong relationship between MBC and WSC where 67% of the MBC variation could be explained by WSC. Whereas 3.8% to 39.9% of water soluble C, in forest soil leachates, is readily degradable (Boissier & Fontvieille, 1993), 85% was found to be biodegradable in agricultural soils (Zsolnay & Steindl, 1991). The decline of MBC with depth was also similar to that of WSC further supporting the view that microorganisms were utilising WSC as a substrate.

Management has been reported to have a major impact on microbial biomass in agricultural soils, due largely to its ability to impact the amount of OC entering the soil. The amount of labile carbon present in soil is of particular importance for the microbial biomass. In many agricultural soils where concentrations of labile carbon are low, the microbial biomass is often starved because it does not have enough organic C (Murphy *et al.,* 1998).

5.5 Conclusions and recommendations

Particulate organic matter was more responsive in bicultures that had a greater component of oat, while WSC and MBC were higher with increasing proportion of vetch in the biculture. The two cover crops are synergistic, with the 70% oat + 30% vetch working better than sole crops in building up POM, WSC and MBC, all of which can be used as highly sensitive indicators of SOM build up in early stages of CA systems. This work was based on a CA system that was less than 3 years old, and extending the trial for a longer period would be necessary to check the accuracy of the predictions based on POM, WSC, and MBC on long term build-up of organic matter. Similar work could also be done on a variety of soils to determine the effects of soil types.

6. GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1. General discussion

Soil degradation has resulted in decline in productivity in South Africa, particularly in the Eastern Cape. Conservation agriculture (CA) has proved to be successful in improving productivity of poor soils, particularly in South America, and has been introduced and is being promoted for the same purpose in South Africa. Preliminary studies in the EC on cover cropping under CA showed improvements on weed suppression, high biomass production, soil fertility improvements and other secondary benefits (Murungu, 2010, 2011; Musunda 2010; Muzangwa, 2012). Although, soil fertility has been shown to improve, soil biological properties are better indicators of overall soil productivity as they respond to both the physical and chemical environment in the soil. Soil microbial biomass, enzyme activities and light organic matter fractions could be used as measures of soil productivity in the short-to-medium term. The broad objective of this study was to determine the effects of rotations of maize with oat and grazing vetch cover crops, as sole crops or bicultures, on soil microbial biomass, enzyme activities and light organic matter fractions in no-till maize based system.

Grazing vetch, grown as a sole cover crop, improves the activities of dehydrogenase, β-glucosidase, alkaline phosphatase and urease enzymes, while oat supported acid phosphatase and MBC (Chapter 3). Oat continuously gave higher acid phosphatase and vetch β-glucosidase and urease both in sole crop cover crops systems, while in bicultures, activities of the other enzymes were high in the 70% oat + 30 % vetch treatment (Chapter 4). Chapter 5 showed that oat produced higher POM than vetch and their bicultures while WSC and MBC were high in 70% oat + 30 % vetch treatment.

The increased enzyme activities could be explained by increases in organic matter inputs and the different fractions of organic matter in the cover crop treatments. This can be confirmed by the results from the 5-year trial where rotations of maize and cover crops resulted in increased total C and N (Dube *et al.*, 2012), and from all the trials (4 and 28 months and 5-years) where rotations with cover crops, either as sole crops or as bicultures, resulted in elevated POM and WSC. These effects were in response to higher inputs of biomass both above ground (Murungu, 2011; Muzangwa, 2012) and from roots. The accumulation of soil enzymes, their activities and distribution in the 5 year monoculture trial was high in the 0-5 cm layer and this is supported by Dube *et al.* (2012) findings which gave large amounts of SOM mostly in the particulate form in the 0-5 cm layer. The low activity of enzymes under the weedy fallow may be due to low reserves of microbial energy source as the weeds gave lower biomass than the cover crops since soil enzymes follow the concentration of their food source. The synergistic effect of the bicultures could pronounce the build-up of organic matter, productivity and ecosystem health of poor soils cultivated with low inputs.

The activity of microorganisms usually follows where readily available C energy sources are accumulating. In this study POM and WSC were the two labile energy sources investigated and in all treatments both POM and WSC accumulated more in the 0-5 cm layer than 5-20 cm layer. The observed POM and WSC as the main source of microbial biomass energy in deep soil may have been mainly the left over root biomass. It is suggested by Puget & Drinkwater (2001), that greater biochemical recalcitrance of root litter also increase MBC contents in soil depending upon the root biomass produced. Chan (1997) also found out that straw application increased particulate organic carbon in surface soil but not at lower depths. The relationship between MBC and POM in the bicultures was generally low with 38% and 27% in the 0-5 cm and 5-20 cm depths respectively while that of MBC with WSC was 73%

and 61% in 0-5 cm and 5-20 cm depths respectively. It is not surprising that the three measures of labile organic matter were closely correlated since they are closely interrelated properties. This result confirms the value of these fractions as sensitive indicators for detecting changes in SOM in the short term, before they are readily measurable in total C. The increased total C, POM and WSC act as substrates for microorganisms, as supported by results of MBC in all the trials, from 4 months to 5 years, higher biomass was observed in cover crop treatments, which was a similar trend to the OM fractions

Water soluble C, believed to be derived from plant roots, litter and soil humus and is a labile substrate for microbial activity, increased in surface soil (0-5 cm) and was highest in the 70% oat + 30 % vetch. Although the WSC concentration decreased with soil depth, the observed WSC in the 5-20 cm depth could be explained by decomposition of crop residues or translocation from surface soil (Kalbitz *et al.,* 2000). The POM fraction is a labile SOC pool mainly consisting of plant residues partially decomposed and not associated with soil minerals. In the present study, the treatments with sole oat contained significantly higher POM in both soil depths analysed than that in vetch or biculture of oat and vetch treatments. Oat as a high biomass yielding cover crop could have enhanced the POM accumulation. In no till systems POM declines with increase in soil depth.

The quality and quantity of organic residues entering the soil have an influence on the soil enzymatic activities as the material may contain intra- or extracellular enzymes and also stimulate microbial activity (Pascual *et al.,* 1998). The distribution of soil enzymes in the soil profile follow the distribution of SOM as they are mainly produced by soil microorganisms to degrade available substrates (Deng & Tabatabai, 1996a, b; Dick, 1984). While two years of rotations of maize with cover crop bicultures had no effect on the soil total C and N content, soil enzymes were more sensitive to effects of cover cropping, fertilization and cover crop biculturing.

Acid phosphatase activity was higher in cover crop treatments with oat than vetch and this was similar to the response of POM, while WSC and all the other enzymes appeared to respond more to vetch, with bicultures having a synergistic effect. Dehydrogenase activity has been proposed as a measure of overall microbial activity since it is an intracellular enzyme involved in the oxidative phosphorylation processes. The high dehydrogenase activity observed in vetch treatments could be because of the more easily decomposable components of vetch residues, hence an improvement in microbial activity. The increase in activity of hydrolases (urease, β-glucosidase, arylsulphatase, acid and alkaline phosphatase) due to cover cropping showed that cover crop residues significantly increased microbial and enzyme activity. This is in agreement with Tejada *et al.* (2007), who observed an increase in enzyme activity after green manure addition to soil. Whereas alkaline phosphatase and arylsulphatases were high in 70% oat + 30 % vetch treatment, urease and β-glucosidase had their highest activity under vetch treatments, while acid phosphatase activity was high under oats. These differences could be due to the differences in the origin, state, persistence of different groups of enzymes and also the quality of crop residues. For example alkaline phosphatase is believed to be found in microorganisms and not in higher plants (Tabatabai, 1994). The different activities of phosphatases are also believed to be attributed to soil pH and organic C content resulting in varied microbial population and diversity (Deng & Tabatabai, 1996a, b). Enzyme results reported in this study suggests that no till coupled with cover crop rotations increased activities of β-glucosidase, urease, phosphatases and arylsulphatase thereby increasing C, N, P and S cycling in soils. This is supported by Beare *et al.* (1993) results which showed that microbial growth is favoured in soil environments with crop residue retention and cover crop rotation which resulted in greater enzyme activities.

Soil management systems that lead to accumulation of greater quantities of labile C pools contribute greater energy and C fluxes through the activity of soil microorganisms which

improve nutrient mineralisation. The introduction of CA in low input smallholder systems could benefit from the use of oat and grazing vetch cover crops, whether as sole crops or as bicultures, with bicultures (particularly the 70% oat and 30% vetch) showing greater potential in terms of biomass input, POM, WSC and activities of enzymes involved in C, N, P and S cycles. In order to better predict improvements in soil function in low input CA systems, highly sensitive parameters are required. The rapid response of POM, WSC, MBC and the activities of the selected enzymes suggests that these parameters could be used to indicate such changes as early as the first season after cover crops. From the results of the different trials, POM and acid phosphatase activity would be more useful with oat as a cover crop, whereas WSC, MBC and all the other enzymes are better predictors where vetch is used.

The results in this study also showed that biculturing oat and vetch increased MBC as compared to growing them as monocultures, and high levels of MBC were more in the younger trials than the older 5 year trial. The observed high MBC in the cover crops than the weedy fallow was in agreement with Lynch & Panting (1980), who reported that eight months after application of straw manure to a loamy arable soil the microbial biomass was almost twice as high as compared to a control. Ocio *et al.* (1991) also observed a significant increase in microbial biomass after straw inputs in field conditions. The higher MBC in monoculture oat-maize rotations and in the 70% oat + 30 % vetch biculture could be due to the observed high POM and WSC from the same treatments that act as carbon source activators of microorganisms. Increases in SOM are usually associated with similar increases in microbial biomass because the SOM provides principal substrates for the microorganisms (Melero *et al.*, 2009).

The fertilisation of both cover crop and maize has been found to result in greater biomass inputs (Murungu, 2011) with greater effects on total organic C and N, POM and WSC (Dube *et al.*, 2012) and MBC and activities of the different enzymes (Chapter 3). In low input

cropping system, this approach may not be as attractive to the farmers. Fertilisation of the cover crop alone (particularly oat) or maize alone resulted in significant increases in total C and N, POM, WSC (Dube *et al.,* 2012), MBC and enzyme activities (Chapter 3). The low MBC content in the treatments where cover crop and/or maize was not fertilised (F2 or F3 and F4) may be due to the imbalanced use of fertilisers which decreased MBC due to limitation imposed by major nutrients like P and K, which are essential for higher crop production as well as for microbial cell synthesis. While fertilisation of the cover crop alone could be beneficial, farmers may prioritize the main crop. In low input systems where maize is fertilised and cover crop is not, grazing vetch could make better contributions in terms of improving soil productivity. In bicultures, less fertilizer could be required than in oat to achieve these benefits. It is essential to understand the effects of fertilization of bicultures on soil OM build up and productivity, particularly under the 70% oat + 30% vetch biculture.

Smallholder agricultural systems crop residues are often used as animal feed. Where CA systems with winter cover crops are used without protection from animals, the above ground biomass of cover crops and the main crop could be grazed. The contribution of larger root biomass contributions from the cover crop treatments and the main crop could be substantial. This view is supported by increased POM, WSC, enzyme activities in the 5-20 cm depth, although the above ground biomass could also contribute. In the realities of these systems, there is need to understand the effects of rotations of maize and cover crops, either as sole crops or in bicultures on soil organic matter and function when the system includes grazing of the above ground biomass. The sensitive parameters determined in this study, which include POM, WSC, MBC and enzyme activities could be useful in this regard.

6.2. Conclusions

- Rotation of maize with oat and grazing vetch cover crop, as sole crops or bicultures, improve organic matter and soil biological activity.
- Fertilisation of both cover crop and maize or maize only or cover crop alone, improves the contribution of the CA system on soil biological activity.
- Particulate OM, WSC, MBC and enzyme activities are sensitive indicators that can be used to determine the effectiveness of CA systems in the short-term.

6.3. Recommendations

- Similar work could also be done on a variety of soils to determine the effects of soil types on organic matter pools and activity of soil enzymes.
- More work needs to be done to establish the effect of fertilisation of cover crop bicultures on organic matter build up and biological activity.
- Extending the biculture trial for a longer period would also be necessary to check the accuracy of the predictions based on POM, WSC, and MBC on long term build-up of organic matter.

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APPENDICES

APPENDIX 1: Chapter three analysis of variance (ANOVA) tables

Variate: MBC at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	667.	333.	0.03	
Replicate.*Units* stratum					
Cover_cropping	1	525184.	525184.	42.87	<.001
Cover_cropping.Cover_crop_type					
	1	144530.	144530.	11.80	0.003
Cover_cropping.Fertilizer_regime					
	4	807901.	201975.	16.49	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime					
	3	38664.	12888.	1.05	0.394
Residual	18	220490.	12249.		
Total	29	1737435.			

Variate: MBC at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	2612.	1306.	0.10	
Replicate.*Units* stratum					
Cover_cropping	1	2244605.	2244605.	164.74	<.001
Cover_cropping.Cover_crop_type					
	1	160463.	160463.	11.78	0.003
Cover_cropping.Fertilizer_regime					
	4	690839.	172710.	12.68	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime					
	3	48630.	16210.	1.19	0.342
Residual	18	245249.	13625.		
Total	29	3392398.			

Variate: Dehydrogenase at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	9308.	4654.	0.83	
Replicate.*Units* stratum					
Cover_cropping	1	227197.	227197.	40.70	<.001
Cover_cropping.Cover_crop_type					
	1	74922.	74922.	13.42	0.002
Cover_cropping.Fertilizer_regime					
	4	502022.	125506.	22.48	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime					
	3	44224.	14741.	2.64	0.081
Residual	18	100478.	5582.		
Total	29	958152.			

Variate: Dehydrogenase at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	509.1	254.6	0.78	
Replicate.*Units* stratum					
Cover_cropping	1	240262.4	240262.4	733.36	<.001
Cover_cropping.Cover_crop_type					
	1	47567.3	47567.3	145.19	<.001
Cover_cropping.Fertilizer_regime					
	4	331058.3	82764.6	252.62	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime					
	3	36601.6	12200.5	37.24	<.001

Residual	18	5897.2	327.6		
Total	29	661896.0			

Variate: β-glucosidase at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	20.85	10.43	1.00	
Replicate.*Units* stratum					
Cover_cropping	1	707.40	707.40	68.08	<.001
Cover_cropping.Cover_crop_type					
	1	1063.68	1063.68	102.36	<.001
Cover_cropping.Fertilizer_regime					
	4	482.93	120.73	11.62	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime					
	3	149.98	49.99	4.81	0.012
Residual	18	187.04	10.39		
Total	29	2611.88			

Variate: β-glucosidase at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	0.16579	0.08290	1.08	
Replicate.*Units* stratum					
Cover_cropping	1	479.99985	479.99985	6229.32	<.001
Cover_cropping.Cover_crop_type					
	1	637.60933	637.60933	8274.73	<.001
Cover_cropping.Fertilizer_regime					
	4	236.20620	59.05155	766.36	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime					
	3	6.92162	2.30721	29.94	<.001
Residual	18	1.38699	0.07705		
Total	29	1362.28979			

Variate: Urease at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	0.8688	0.4344	1.33	
Replicate.*Units* stratum					
Cover_cropping	1	112.5367	112.5367	344.05	<.001
Cover_cropping.Cover_crop_type					
	1	44.8656	44.8656	137.16	<.001
Cover_cropping.Fertilizer_regime					
	4	26.5300	6.6325	20.28	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime					
	3	6.3515	2.1172	6.47	0.004
Residual	18	5.8877	0.3271		
Total	29	197.0404			

Variate: Urease at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	3.612	1.806	1.18	
Replicate.*Units* stratum					
Cover_cropping	1	104.002	104.002	68.09	<.001
Cover_cropping.Cover_crop_type					
	1	57.347	57.347	37.55	<.001
Cover_cropping.Fertilizer_regime					
	4	23.985	5.996	3.93	0.018
Cover_cropping.Cover_crop_type.Fertilizer_regime					
	3	9.856	3.285	2.15	0.129
Residual	18	27.493	1.527		
Total	29	226.294			

Variate: Acid phosphatase at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	0.012276	0.006138	4.14	
Replicate.*Units* stratum					
Cover_cropping	1	143.906138	143.906138	97162.36	<.001
Cover_cropping.Cover_crop_type	1	20.476665	20.476665	13825.41	<.001
Cover_cropping.Fertilizer_regime	4	64.366091	16.091523	10864.65	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime	3	5.881474	1.960491	1323.68	<.001
Residual	18	0.026660	0.001481		
Total	29	234.669304			

Variate: Acid phosphatase at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	4.930	2.465	1.13	
Replicate.*Units* stratum					
Cover_cropping	1	266.806	266.806	122.53	<.001
Cover_cropping.Cover_crop_type	1	25.807	25.807	11.85	0.003
Cover_cropping.Fertilizer_regime	4	87.774	21.943	10.08	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime	3	6.768	2.256	1.04	0.400
Residual	18	39.193	2.177		
Total	29	431.278			

Variate: Alkaline phosphatase at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	2.4270	1.2135	8.80	
Replicate.*Units* stratum					
Cover_cropping	1	252.4071	252.4071	1831.43	<.001
Cover_cropping.Cover_crop_type	1	39.2792	39.2792	285.00	<.001
Cover_cropping.Fertilizer_regime	4	173.5160	43.3790	314.75	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime	3	4.1553	1.3851	10.05	<.001
Residual	18	2.4808	0.1378		
Total	29	474.2653			

Variate: Alkaline phosphatase at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	2	1.20748	0.60374	28.16	
Replicate.*Units* stratum					
Cover_cropping	1	425.02873	425.02873	19823.87	<.001
Cover_cropping.Cover_crop_type	1	36.29563	36.29563	1692.87	<.001
Cover_cropping.Fertilizer_regime	4	216.88961	54.22240	2529.00	<.001
Cover_cropping.Cover_crop_type.Fertilizer_regime	3	3.33330	1.11110	51.82	<.001
Residual	18	0.38592	0.02144		
Total	29	683.14067			

APPENDIX 2: Chapter four analysis of variance (ANOVA) tables

Variate: Dehydrogenase at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	622.6	311.3	6.42	
Block.*Units* stratum					
Treatments	5	815900.	163200.	3364.16	<.001
Sampling	1	12560000.	12560000.	258900.	<.001
Treatments.Sampling	5	65590.	13120.	270.44	<.001
Residual	22	1067.	48.51		
Total	35	13440000.			

Variate: Dehydrogenase at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	840.27	420.13	6.16	
Block.*Units* stratum					
Treatments	5	271497.07	54299.41	795.78	<.001
Sampling	1	3757858.82	3757858.82	55073.11	<.001
Treatments.Sampling	5	73761.43	14752.29	216.20	<.001
Residual	22	1501.15	68.23		
Total	35	4105458.74			

Variate: β-glucosidase at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1.0830	0.5415	4.68	
Block.*Units* stratum					
Treatments	5	526.9902	105.3980	910.51	<.001
Sampling	1	1645.0804	1645.0804	14211.43	<.001
Treatments.Sampling	5	67.5789	13.5158	116.76	<.001
Residual	22	2.5467	0.1158		
Total	35	2243.2791			

Variate: β-glucosidase at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	5.2097	2.6048	3.28	
Block.*Units* stratum					
Treatments	5	160.8521	32.1704	40.53	<.001
Sampling	1	112.7038	112.7038	141.98	<.001
Treatments.Sampling	5	33.5827	6.7165	8.46	<.001
Residual	22	17.4637	0.7938		
Total	35	329.8120			

Variate: Urease at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	10.582	5.291	1.13	
Block.*Units* stratum					
Treatments	5	924.165	184.833	39.60	<.001
Sampling	1	346.075	346.075	74.15	<.001
Treatments.Sampling	5	383.831	76.766	16.45	<.001
Residual	22	102.682	4.667		
Total	35	1767.335			

Variate: Urease at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	19.288	9.644	4.43	
Block.*Units* stratum					
Treatments	5	862.051	172.410	79.28	<.001
Sampling	1	145.750	145.750	67.02	<.001
Treatments.Sampling	5	383.408	76.682	35.26	<.001
Residual	22	47.843	2.175		
Total	35	1458.340			

Variate: Arylsulfatase at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	3.0458	1.5229	6.48	
Block.*Units* stratum					
Treatments	5	91.3111	18.2622	77.74	<.001
Sampling	1	2339.9922	2339.9922	9961.08	<.001
Treatments.Sampling	5	57.5026	11.5005	48.96	<.001
Residual	22	5.1681	0.2349		
Total	35	2497.0198			

Variate: Arylsulfatase at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.1604	0.0802	0.63	
Block.*Units* stratum					
Treatments	5	116.1204	23.2241	182.10	<.001
Sampling	1	1579.1121	1579.1121	12382.02	<.001
Treatments.Sampling	5	92.9292	18.5858	145.73	<.001
Residual	22	2.8057	0.1275		
Total	35	1791.1279			

Variate: Acid phosphatase at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	3.8099	1.9050	2.09	
Block.*Units* stratum					
Treatments	5	578.5344	115.7069	127.05	<.001
Sampling	1	27.6091	27.6091	30.32	<.001
Treatments.Sampling	5	477.5484	95.5097	104.88	<.001
Residual	22	20.0351	0.9107		
Total	35	1107.5370			

Variate: Acid phosphatase at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	7.2019	3.6009	5.68	
Block.*Units* stratum					
Treatments	5	403.5844	80.7169	127.29	<.001
Sampling	1	17.2851	17.2851	27.26	<.001
Treatments.Sampling	5	221.6428	44.3286	69.91	<.001
Residual	22	13.9507	0.6341		
Total	35	663.6649			

Variate: Alkaline phosphatase at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	4.5612	2.2806	3.38	
Block.*Units* stratum					
Treatments	5	1661.9978	332.3996	492.12	<.001
Sampling	1	406.3857	406.3857	601.65	<.001
Treatments.Sampling	5	336.0171	67.2034	99.49	<.001
Residual	22	14.8599	0.6755		
Total	35	2423.8217			

Variate: Alkaline phosphatase at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.7171	0.3585	1.84	
Block.*Units* stratum					
Treatments	5	459.5660	91.9132	472.86	<.001
Sampling	1	586.4938	586.4938	3017.27	<.001
Treatments.Sampling	5	148.6511	29.7302	152.95	<.001
Residual	22	4.2763	0.1944		
Total	35	1199.7043			

APPENDIX 3: Chapter five analysis of variance (ANOVA) tables

Variate: Total C % at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.01987	0.00994	0.57	
Block.*Units* stratum					
Treatments	5	0.20542	0.04108	2.36	0.074
Sampling	1	0.00134	0.00134	0.08	0.784
Treatments.Sampling	5	0.05496	0.01099	0.63	0.678
Residual	22	0.38319	0.01742		
Total 35	0.66479				

Variate: Total C at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.049400	0.024700	2.67	
Block.*Units* stratum					
Treatments	5	0.026258	0.005252	0.57	0.724
Sampling	1	0.000069	0.000069	0.01	0.932
Treatments.Sampling	5	0.019881	0.003976	0.43	0.823
Residual	22	0.203667	0.009258		
Total 35	0.299275				

Variate: N % at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.0024389	0.0012194	1.91	
Block.*Units* stratum					
Treatments	5	0.0043806	0.0008761	1.37	0.272
Sampling	1	0.0006250	0.0006250	0.98	0.333
Treatments.Sampling	5	0.0020917	0.0004183	0.66	0.660
Residual	22	0.0140278	0.0006376		

Total	35	0.0235639

Variate: C:N ratio at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	5.078	2.539	1.50	
Block.*Units* stratum					
Treatments	5	2.492	0.498	0.29	0.911
Sampling	1	0.788	0.788	0.46	0.503
Treatments.Sampling	5	5.003	1.001	0.59	0.708
Residual	22	37.360	1.698		
Total	35	50.720			

Variate: MBC at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	12205.	6103.	0.84	
Block.*Units* stratum					
Treatments	5	3071060.	614212.	84.71	<.001
Sampling	1	14524017.	14524017.	2003.04	<.001
Treatments.Sampling	5	27999.	5600.	0.77	0.580
Residual	22	159521.	7251.		
Total 35	17794802.				

Variate: MBC at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	27369.	13685.	4.06	
Block.*Units* stratum					
Treatments	5	3301151.	660230.	195.68	<.001
Sampling	1	15293224.	15293224.	4532.52	<.001
Treatments.Sampling	5	45.	9.	0.00	1.000
Residual	22	74230.	3374.		
Total 35	18696020.				

Variate: POM at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.008889	0.004444	0.56	
Block.*Units* stratum					
Treatments	5	6.448447	1.289689	163.78	<.001
Sampling	1	2.195336	2.195336	278.78	<.001
Treatments.Sampling	5	0.153247	0.030649	3.89	0.011
Residual	22	0.173244	0.007875		
Total	35	8.979164			

Variate: POM at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.005756	0.002878	2.69	
Block.*Units* stratum					
Treatments	5	5.020314	1.004063	936.87	<.001
Sampling	1	1.334025	1.334025	1244.75	<.001
Treatments.Sampling	5	0.041425	0.008285	7.73	<.001
Residual	22	0.023578	0.001072		
Total 35	6.425097				

Variate: WSC at 0-5 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.005419	0.002710	0.47	
Block.*Units* stratum					
Treatments	5	0.777278	0.155456	27.19	<.001
Sampling	1	0.809888	0.809888	141.68	<.001
Treatments.Sampling	5	0.064872	0.012974	2.27	0.083
Residual	22	0.125760	0.005716		
Total	35	1.783216			

Variate: WSC at 5-20 cm depth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.011302	0.005651	1.51	
Block.*Units* stratum					
Treatments	5	1.271678	0.254336	68.17	<.001
Sampling	1	0.535092	0.535092	143.42	<.001
Treatments.Sampling	5	0.081061	0.016212	4.35	0.007
Residual	22	0.082080	0.003731		
Total 35	1.981212				

Regression analysis

Response variate: MBC versus POM

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	5032443.	5032443.	12.52	0.001
Residual	34	13663577.	401870.		
Total	35	18696020.	534172.		

Response variate: MBC versus WSC

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	11379719.	11379719.	52.88	<.001
Residual	34	7316301.	215185.		
Total	35	18696020.	534172.		