LONG-TERM EFFECTS OF TILLAGE PRACTICES ON BIOLOGICAL INDICATORS OF A SOIL CROPPED ANNUALLY TO WHEAT

by

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DECLARATION

I declare that the dissertation hereby submitted by me for the Magister Scientiae degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I furthermore cede copyright of the dissertation in favor of the University of the Free State.

Signature: ______________________________ Date: ______________________

ABSTRACT

Long-term effects of tillage practices on biological indicators of
a soil cropped annually to wheat

Soil sustainability is a long-term goal. Although physical and chemical properties of soil have been utilized extensively to evaluate soil quality, the application of biological indicators is becoming more important. In order to assess soil quality, soil enzymes and other biological parameters need to be considered.

In semi-arid Bethlehem, South Africa, samples were taken at a wheat (*Triticum aestivum* L.) monoculture trial which was established in 1979 by the Agricultural Research Council-Small Grain Institute. The treatments were: no-tillage (NT), stubble-mulch (SM), and conventional tillage (CT); all paired with chemical weed control, the absence of burning residues, and 40 kg nitrogen ha^{-1} as limestone ammonium nitrate with single superphosphate as the fertilizer sources. The study period lasted from October 2010 to October 2011 with eight sampling times conducted over this year and two depths sampled (0-5 cm, 5-10 cm). Oat (*Avena sativa* L.) was growing in the plots from the start of the study until December 2010 when it was harvested. A fallow period then lasted until the planting of wheat in August 2011 which was harvested after the end of the study period.

Potential enzyme activities were assayed for β-glucosidase, urease, acid- and alkaline-phosphatase, and dehydrogenase at all eight sampling times, along with soil texture, total carbon, total nitrogen, Olsen-extractable phosphorus, and pH. Whole microbial community profiling using BIOLOG EcoPlates™ was employed at the first sampling time and phospholipid fatty acid (PLFA) analysis for the first, third, and fifth sampling times.

It was found that NT and SM had higher values than CT across all enzymes except alkaline phosphatase, which ranked NT higher than both SM and CT. BIOLOG EcoPlates™ and PLFA showed similar results across tillage treatments. Microbial biomass, estimated from both potential dehydrogenase activities and PLFA values, was higher in NT and SM than in CT.

Over the study period the values for all parameters varied but the average ranking of tillage treatments stayed consistent. In comparing the two soil depths, soil quality was easily shown to be higher in NT and SM in the 0-5 cm depth, but often in the 5-10 cm depth the differences faded. Potential acid phosphatase activity was the only measured parameter which was consistently higher in the 5-10 cm depth.

If the parameters can be used as an index of soil quality, then it can be accepted that NT has higher quality than CT and often SM has higher quality than CT, but is not at the same level as NT; it can then be recommended that in semi-arid South Africa, NT will enhance soil quality under a monoculture cropping practice.

Keywords: crop residues, enzyme activities, organic matter, soil quality

UITTREKSEL

Lang-termyn effekte van bewerkingspraktyke op biologiese
indikatore van 'n grond wat jaarliks met koring geplant word

Die volhoubare gebruik van grond is 'n lang-termyn doel. Alhoewel fisiese en chemiese grondeienskappe omvattend gebruik word om grondkwaliteit te evalueer, word die toepassing van biologiese indikatore al hoe meer belangrik. Om grondwaliteit te evalueer, moet grondensiemes en ander biologiese parameters ook oorweeg word.

In semi-ariede Bethlehem, Suid-Afrika, is monsters geneem by 'n koring (*Triticum aestivum* L.) monokultuur proef wat al reeds in 1979 deur die Landbounavorsingsraad-Kleingraaninstituut begin is. Die behandelings was: geen-bewerking (NT), stoppellaagbewerking (SM), en konvensionele bewerking (CT); almal gekoppel met chemiese onkruidbeheer, geen brand van reste, en 40 kg stikstof ha^{-1} as kalksteenammoniumnitraat met enkel superfosfaat as die kunsmisbronne. Die studietydperk het gestrek vanaf Oktober 2010 tot Oktober 2011 met agt tye van monsterneming oor hierdie jaar, met twee dieptes (0-5 cm, 5-10 cm) wat gemonster is. Hawer (*Avena sativa* L.) het in die persele gegroei vanaf die begin van die studietydperk tot en met Desember 2011 toe dit geoes is. 'n Braakperiode het toe gevolg totdat koring geplant is in Augustus 2011, wat geoes is na die einde van die studietydperk.

Potensiële ensiemaktiwiteite is bepaal vir β-glukosidase, urease, suur- en alkaliese-fosfatase, en dehidrogenase vir al agt monsternemingstye, tesame met grondtekstuur, totale koolstof, totale stikstof, Olsen-ekstraheerbare fosfor, en pH. Algehele mikrobiese gemeenskapsprofilering is toegepas deur gebruik te maak van BIOLOG EcoPlates™ tydens die eerste monsternemingtyd en fosfolipied vetsuur (PLFA) ontledings is gedoen vir die eerste, derde en vyfde monsternemingstye.

Daar is gevind dat NT en SM hoër waardes as CT getoon het vir al die ensiemes behalwe vir alkaliese fosfatase, waar NT hoër as beide SM en CT was. BIOLOG EcoPlates™ en PLFA het

soortgelyke resultate getoon regoor al die bewerkingsbehandelings. Mikrobiese biomassa, afgelei vanaf beide potensiële dehidrogenase aktiwiteit en PLFA waardes, was hoër in NT en SM as in CT. Oor die studietydperk het die waardes vir al die parameters verskil, maar die gemiddelde rangorde van die bewerkingsbehandelings het konstant gebly. Deur die twee gronddieptes met mekaar te vergelyk, was grondkwaliteit die hoogste vir NT en SM in die 0-5 cm diepte, maar die verskille het dikwels vervaag in die 5-10 cm diepte. Potensiële suur fosfatase aktiwiteit was die enigste gemete parameter wat konstant hoër was in die 5-10 cm diepte.

As die parameters gebruik kan word as ‘n indeks vir grondkwaliteit, dan kan aanvaar word dat NT ‘n hoër kwaliteit as CT en SM dikwels ‘n hoër kwaliteit as CT het, alhoewel dit nie op dieselfde vlak as NT is nie; daar kan ook aanbeveel word dat in semi-ariede Suid-Afrika, grondkwaliteit sal verbeter met NT onder ‘n monokultuur gewasbestuurspraktyk.

Sleutelwoorde: ensiemaktiwiteite, gewasreste, grondkwaliteit, organiese materiaal

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1 MOTIVATION AND OBJECTIVES

1.1 Motivation

Soil sustainability is a long-term goal. In the United States (US), the Dustbowl of the 1930's highlighted how mismanagement of soil quality negatively impacts highly productive soils through wind erosion and loss of topsoil and nutrients. Currently, the US government has conservation measures in place to reduce the risk of a repeated Dustbowl event (Carpenter-Boggs *et al.,* 2003). Some soils, like in the Loess Plateau of China, are naturally erosive and require strict measures to protect soil for agricultural purposes or even just as a habitat. After abandonment of farming in the loess, a soil consisting mainly of silt weakly cemented together by calcium carbonate, then microbial biomass C (MBC), microbial biomass N (MBN), microbial biomass P (MBP), substrate induced respiration (SIR), total organic carbon (TOC), and water-stable aggregates (WSA) increased. The erodibility of the soil also decreased, possibly as a result of the other factors' improvement (Zhu *et al*., 2010).

Soil management also affects water quality. Nutrients, particularly N, can migrate away from the crop rhizosphere through leaching, volatilization, and transference into non-available forms, and is a problem with over-fertilization, over-irrigation, or misunderstanding the soil type. In the Chesapeake Bay, which is the largest and most productive estuary in North America (Prasad *et al*., 2011), water quality has been greatly harmed by the release of nutrients into the watershed from piggeries, crop production, and private land use (Morgan & Owens, 2001), leading to eutrophication. Currently, the summer anoxic zone spreads annually. This not only affects marine life, but also the livelihood of small-scale fishermen who make their living from the Bay's formerly robust fishing areas (Prasad *et al*., 2011).

With one quarter of the Earth's arable land qualifying as degraded, ways of restoring previously degraded soils and preventing further decreases of soil quality and health are necessary to farm sustainably (Uphoff *et al*., 2006). In South Africa specifically, the FAO category "human-induced degradation due to agricultural activities" defines 16% of the

country's total 1,219,000 km^2 area (FAO, 2005). However, for sub-Saharan Africa, reliable data is difficult to obtain (FAO, 1995).

Soil quality, soil health, and soil sustainability are all used nearly interchangeably in the literature. Soil quality is a scientific measurement of a specific time the soil is sampled and has been defined as "the capacity of a specific kind of soil to function" (Gil-Sotres *et al*., 2005), an example of which is a soil's ability to support high-yielding crops. Soil health refers to a less-defined holistic approach to soil conservation. It aims to identify the momentary peaks of activity within the soil itself, which reflect on yield and performance of the agro-ecosystem. Doran and Parkin (1994) have stated that soil health is "the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health." Soil sustainability is assessed by evaluating the soil quality or soil health and its change over time (Laudicina *et al*., 2012).

Being able to assess soil health is vitally important to the continuation of agriculture. A healthy soil might be defined as one which conserves soil organic matter, does not easily erode, is managed with renewable resources instead of synthetic chemicals, and coexists with surrounding ecosystems rather than dominating their functions (Doran *et al*., 1996).

While one can measure conventional indicators, *e.g.,* organic C levels, soil pH, soil texture, and N mineralization rates, soil enzyme levels can track plant-available nutrient levels and are also very sensitive to disturbances of the soil microbial community (Acosta-Martínez & Tabatabai, 2000; Gil-Sotres *et al*., 2005). However, Gil-Sotres *et al*. (2005) mention that while enzymes show potential as indicators of soil health, there are caveats associated with their use. For instance, there are no reference values, some enzymes have been reported to act inconsistently in some situations, *e.g.,* after herbicide application (Mahía *et al*., 2007), and maximum expression levels vary by region.

To date, very little research has been conducted utilizing biological indicators to assess soil health. Enzymes show promise, but there are no threshold levels available. In the past, articles focused on the rate constants of enzyme-catalyzed reactions (Tabatabai, 1994), but now most studies concentrate on the significant difference of the enzyme levels between

treatments. Despite this progress, there is a lack of studies defining “healthy” soils and “unhealthy” soils with data that can be used in other contexts (Gil-Sotres *et al.*, 2005).

Since different management practices influence the state of the soil microbial community (van Groenigen *et al.,* 2010), tracking indicators such as enzymes in soil between treatments over time gives a more complete picture on the soil health as it relates to agricultural yield and sustainability (Verbruggen *et al.*, 2012). It had been determined that urease is important to the N-cycle as it hydrolyzes urea from organic matter, making it plant available. β-glucosidase does the same for C, providing glucose for growing plants and microbe populations. Phosphatases catalyze the formation of phosphates from an organic source. By tracking soil enzyme levels, one can see how the nutrient cycles work. Without these enzymes produced mainly by microorganisms, soil would not contain enough plant-available forms of nutrients needed for crop growth (Tabatabai, 1994).

Microbial biomass C and N define how large the soil microbial population is, which therefore limits the amount of enzymes produced. MB can be measured and linked to the enzyme dehydrogenase, specifically, as well as to the relative levels of the other enzymes (Bandick & Dick, 1999). Analyzing glomalin reflects a longer term approach to soil health and is closely related with soil C levels and fungal biomass (Bedini *et al*., 2010). Fatty acid analysis can give the relative biomass of different functional groups, such as fungi (Baumann *et al.,* 2011). BIOLOG shows general diversity of organisms through C utilization (Bonanomi *et al.,* 2011).

Enzyme and other biological indicators, when used to differentiate between treatments, needs to be studied in an area where those treatments have been applied consistently for an appropriate length of time, such as the experimental site in Stromberger *et al*. (2007) and the Willamette Research and Extension Center in Oregon, USA (Bandick & Dick, 1999), both of which have been in operation since the 1980s. A long-term trial provides for certainty in the treatment effects. Such a trial was found at the Agricultural Research Council (ARC)-Small Grain Institute near Bethlehem. Wiltshire and du Preez (1993) published a report on a study conducted at the monoculture wheat trial, followed by du Preez *et al*. (2001), and Kotzé and du Preez (2007; 2008). Loke (2012) with his research followed upon the previous work and, like this study, falls three decades after the commencement of the trial. No soil

samples earlier than 1989 exist for testing though the trial was established in 1979; however, yield has been measured and recorded since the trial's beginning. All of the studies have focused on soil fertility parameters with respect to wheat residue management. As of yet, none of the studies from the ARC-Small Grain Institute wheat trial have investigated the microbial, or soil biological, parameters.

The earliest study done at the above-mentioned trial was by Wiltshire and du Preez (1993). They used soil samples from a decade after the establishment of the continuous wheat trial, and focused on the N status of a soil under long-term conservation practices, with samples taken in the years 1989 and 1990. It was found that conservational practices, including no-tillage (NT) and non-burning of residue, reduced the rate of soil fertility loss, and that fungal diseases of wheat inhibited yield enough that wheat monoculture should be avoided. It is assumed that "soil health" could still be measured between the treatments, even if the values won't be applicable to fields experiencing the benefits of crop rotation.

Certain macronutrients were assessed by du Preez *et al*. (2001). They tested for pH, Zn, K, and P and looked at their relationships with residue management in soil samples from ten years after the trial began. It was found that straw burning and conservational tillage (as opposed to non-burning and conventional tillage) increased pH and the concentrations of Zn, P, and K. However, despite the accumulation of nutrients, there was no evidence to support that straw burning or conservational tillage had negative effects on the uptake of these nutrients.

The most recent investigation prior to this dissertation focused on nutrients and organic matter (OM). Kotzé and du Preez (2007) found that OM was mainly affected by tillage practice, with straw burning or weeding method having a small to negligible effect on the OM status of the treatments. In the 0-10 cm layer, tillage practices affected soil OM most significantly, whereas weeding practices had a small effect. Organic C and total N of the chemically weeded treatments was higher than in the mechanically weeded treatments. Kotzé and du Preez (2008) reported on the influence of long-term residue management on pH and cation concentrations (*i.e.,* P, K, Ca, Mg, and Na). According to their research the pH was significantly lower in unburned plots compared to burned plots and acidification

increased when wheat residues were incorporated into the soil instead of being left on top for eventual decay. The levels of P, K, Ca, and Mg increased with burning, chemical weeding, and NT practices.

1.2 Objectives

Based on the above as background, the objectives are as follows:

(a) To investigate how tillage practices (NT, stubble mulch, or conventional tillage) influence soil health by measuring biological indicators: five soil enzyme levels which represent key chemical transformations for nutrient availability (urease, β-glucosidase, acid- and alkaline-phosphatase, and dehydrogenase), a soil protein (glomalin), BIOLOG community profiling, and fatty acid analysis.

(b) To measure the effect of tillage practices on soil enzyme levels, and therefore soil health, over a full crop cycle, including fallow periods.

(c) To further investigate soil enzyme levels by sampling two different depths (0-5 cm and 5-10 cm) in order to show potential stratification of soil microbial enzymes, particularly in the NT treatment, in which residue is left on the surface to decompose.

(d) To establish a connection with the four previous studies done on the ARC-Small Grain Institute wheat trials, by analyzing soil nutrient levels and basic parameters such as pH and clay content.

1.3 Hypotheses

The hypotheses are as follows:

(a) Soil enzyme levels (urease, β-glucosidase, acid- and alkaline-phosphatase, and dehydrogenase) will be highest in the NT plots, followed by the stubble mulch and the conventional tillage, in decreasing order.

(b) Dehydrogenase will correlate to soil MB, which will again be highest in the NT plots.

(c) Soil pH will be directly proportional to the levels of alkaline phosphatase and inversely proportional to acid phosphatase.

(d) Soil enzyme levels will correlate to the concentration of available nutrient levels.

(e) Glomalin will correspond to tillage intensity. Soils with higher rates of disturbance will have lower levels of arbuscular-mycorrhizal fungi (AMF) produced proteins, as tillage disturbs the hyphae and favors the development of bacteria.

2 LITERATURE REVIEW

2.1 Introduction

Soil microbial communities are affected by agricultural practices, resulting in long-term changes in nutrient and OM processing (Carpenter-Boggs *et al*., 2003; Caesar-TonThat *et al*., 2010). In order to better elucidate the relationships between agricultural practice, soil activity, and soil quality, enzymes, phospholipid fatty acids (PLFA), and BIOLOG EcoPlates^{TM} were used as indirect indicators of patterns in soil microbial activity, biomass, and community composition in response to different tillage in a long-term experimental plot.

Soil enzymes were considered and chosen as a proxy for bacterial and fungal cell culture counts because they concern the nutrient cycling and reflect the specific efficiency and activity of the whole community of microorganisms, not just the gene products of a few species or classes (Sardans *et al*., 2008). Due to the need to produce a comprehensive representation of the soil health, whole community profiling with BIOLOG EcoPlates^{TM}, PLFA, easily extracted - glomalin related soil protein (EE-GRSP), and MBC and MBN were also used. BIOLOG EcoPlates^{TM} focuses on the broad C-profile of the whole community, though it also has biases and representation flaws to consider. EE-GRSP, which measures the soil protein glomalin, is thought to represent the ability of the physical soil to withstand perturbation, and is a product of fungal activity (Bedini *et al.*, 2010). PLFA can be utilized to determine microbial community structure as well as measure total MB (Elgersma *et al.*, 2011), though this study will tentatively use it for fungal and bacterial markers to determine relative percentages. MBC and MBN evaluate the amount of C and N in the cells of microorganisms and is a widely accepted form of biomass determination (Zhu *et al.*, 2010).

Enzymes may be good indicators of soil health because they integrate information from microorganisms and soil physico-chemical conditions (Bandick & Dick, 1999; Aon & Colaneri, 2001). The factors which influence enzyme activity include concentration of the enzyme and substrate, pH, temperature, enzyme inhibition, and biochemical factors (Tabatabai, 1994).

2.2 Effect of tillage on biological soil properties

Tillage disturbs the soil body. As the soil structure is degraded by tillage, aggregates break down and hence habitat for microorganisms is lost (Wang *et al.,* 2010). Van Groenigen *et al*. (2010) compared the effect of reduced tillage and CT systems on soil biological characteristics and found reduced tillage increased both bacterial and fungal biomass throughout the tillage layer, though particularly in the 0-5 cm depth. NT systems can therefore increase C sequestration even more (Abreu *et al*., 2011). If C and other nutrients are conserved in the soil, then microbial activity increases as microorganisms thrive on this energy. Microbial exudates, excreted from both fungi and bacteria, increase aggregation, improve the water holding capacity of the soil, and in general make the soil more sustainable for long-term agriculture (González-Chávez *et al*., 2010).

Soil health can be indicated by soil enzymes (Gil-Sotres *et al.,* 2005). In Aon *et al*. (2001a), enzyme levels were analyzed in an agricultural soil that was either conventionally tilled or under NT. Acid- and alkaline-phosphatase, dehydrogenase, FDA hydrolysis, β-glucosidase, and urease were the enzymes quantified (Table 2.1). It was found that at the time before planting (T_0), and at the pre-harvest period (T_2), soil enzymes were highly stratified when testing the 0-5 and 5-20 cm depths. Not only were enzyme levels at a peak of activity at the flowering stage (T_1), they also exhibited less stratification. Tillage was not used as a factor in analyzing the data. Instead, the study was a search for an index across seasons and depths to find a reliable soil quality indicator. It was found that certain enzyme activities strongly correlate to groups of bacteria and fungi across seasons, but no index was suggested at the end of their study (Aon *et al.,* 2001a).

Table 2.1 Enzyme activities (g substrate m^{-3} soil incubation $time^{-1}$) in a soil cropped to soybean (*Glycine max* L.) before planting (T_0), at the flowering stage (T_1), and at the pre-harvest period (T_2). Data adapted from Aon and Colaneri (2001)

Enzyme	**T_0**	**T_1**	**T_2**
Acid phosphatase	380 ± 26	798 ± 25	420 ± 7
Alkaline phosphatase	325 ± 25	554 ± 16	103 ± 16
Urease	185 ± 5	120 ± 5	81 ± 6
β-glucosidase	169 ± 15	282 ± 9.7	262 ± 8

More recently, Bastida *et al*. (2008) published a review on soil quality indices, stating that it is more difficult and controversial to maintain a soil quality index and that there still exists a lack of consensus on how to use any of the indices that have been proposed. For instance, papers focusing on farmers in developing regions of the world put forward “simple” measurements, such as salinity (by taste), color, landscape position, and native vegetation (Mairura *et al*., 2007; Ali, 2003). On the other hand, expensive measurements, such as that concerning soil biology, but which are highly specialized due to type of soil tested and geographical region, are recommended (Bastida *et al*., 2008).

Roldán *et al*. (2005) studied a trial with NT and CT for three years. It was found that dehydrogenase, acid-phosphatase, urease, and glomalin levels, as well as aggregate stability, were higher in the NT system, especially in the 0-5 cm layer.

Furthermore, Wang *et al*. (2010) found that species richness and diversity of fungi were significantly higher in NT treatments as opposed to tillage treatments, and the difference increased with depth of tillage. Also, WSA (those aggregates which can withstand the force of a raindrop) increased in quantity in NT fields.

The sensitivity of soil enzyme levels to changes in the environment make it an ideal biological indicator, but also create difficulties for creating a global, or even land-wide, standard (Tabatabai, 1994). For example, soil texture affects microbial activity (Taylor *et al*., 2002) and for this reason must be considered when comparing “soil health levels” between agricultural and native soils. In most instances, an increase in clay content spiked soil enzyme levels (Table 2.2). Some agricultural soils may have changed clay content during cultivation, and a perceived difference in soil quality levels could simply be from a textural shift. Another important confounding variable is climate. In southern Spain, climate is seen as the limiting factor in accumulating soil organic C; however, CT will have lower soil organic C than NT fields (Melero *et al.,* 2009) and those fields will therefore show differences in soil enzyme levels. The question is, should a norm be widely applied or should every climate zone or soil pedon have a minimum level for healthy soil biological activity, and how should this information be found and implemented. Given the confounding effects of

environmental variability, extensive research is necessary to develop a standard for assessing soil quality using soil enzyme assays.

Table 2.2 Effect of soil texture on activities of five enzymes. Data adapted from Taylor *et al*. (2002)

Enzyme	Silty clay loam soil	Loamy sand soil
β-glucosidase (μg ρNP g^{-1} SDW $2h^{-1}$)	25	15
Alkaline phosphatase(μg ρNP g^{-1} SDW $2h^{-1}$)	100	100
Acid phosphatase(μg ρNP g^{-1} SDW $2h^{-1}$)	80	80
Urease (μg NH_4-N g^{-1} SDW $4h^{-1}$)	35	7
Dehydrogenase (μg INF g^{-1} SDW $24h^{-1}$)	10	9

2.2.1 Soil enzymes

The biochemical component of nutrient cycling in soils is mediated by enzymes produced by soil microorganisms, plants, and soil fauna, of which microorganisms are the primary source (Tabatabai, 1994). Enzymes cause reactions, such as the mineralization of important nutrients, to proceed at much faster rates but are extremely specific in the substrate they affect (Das & Varma, 2011). Due to this relationship between soil enzymes and nutrients (or substrates) it can be assumed that by studying a specific enzyme, the dynamics of nutrient cycling would be understood better (Tabatabai, 1994).

For instance, extensive research has been done on N mineralization in soil and on the enzymes governing the process. Tabatabai *et al*. (2010) have concluded that NAGase (N-acetyl-beta-D-glucosaminidase; EC 3.2.1.30) has the highest correlation to cumulative N mineralization at the enzyme's optimum pH value, with a significance of r = 0.87 at 20°C and r = 0.95 at 30°C. However, urease will be studied instead, as it is also a relevant predictor of N mineralization processes (Klose & Tabatabai, 1999).

Carpenter-Boggs *et al.* (2003) studied acid- and alkaline- phosphatase and dehydrogenase to determine the difference, if any, between pastured fields and CT and NT crop fields. Only alkaline phosphatase differed significantly between NT and CT fields, with NT being higher in quantity. However, a small shift could be seen with the other indicators favoring NT, and a clear difference could be seen with respect to the pastures. This indicates that the microbial

community either has access to more nutrients in the pasture system, though generally agricultural systems have higher levels of nutrients.

Soil microbial indicators are most often analyzed from bulk soil samples. Most of the bulk soil samples are actually composites from a site, which averages the local heterogeneity in order to correctly compare with completely different sites. This method imparts little insight into the spatial orientations of microbes, but due to the wide variation in microhabitats it is the only way one can compare sites. Due to microbes using aggregates as habitat, different water potentials, pH, and pore sizes lead to micro-sites with higher diversity and quantity of microbial activity. This is evidence that soils which lack these aggregates have a dearth of habitat and will, on average, have a lower total microbial activity than a composite sample from a site with good soil structure (Mummey *et al*., 2006).

2.2.1.1 Enzyme assays

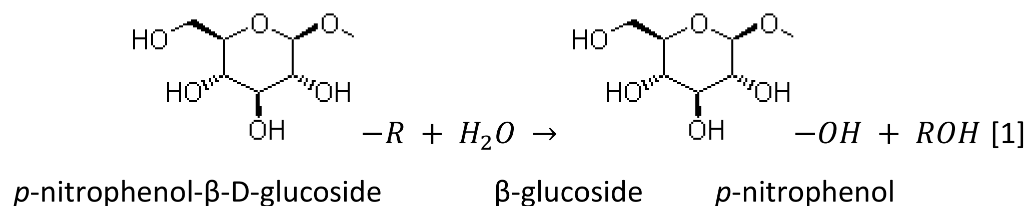
Enzymes can exist outside of the cell and may still be active after the responsible microorganism has been decomposed. It has been proven that after autoclaving soil, the enzymes are still present and are still active (Carter *et al.,* 2007). As such, enzymatic assays are considered to be representative not of the active MB but of the functional component of microorganisms. One enzyme which has been connected to the active-only biomass is dehydrogenase, which degrades quickly after the death of the responsible cell (Tan *et al*., 2008). Therefore, dehydrogenase is used not as a predictor of a specific nutrient cycle, but as an overall indicator of MB activity.

Enzymes are chosen specific to the study done. In Wang *et al*. (2009) enzymes were chosen based on laboratory simplicity and importance in the nutrient cycles. As such, urease, acid phosphatase, and invertase were assayed. In Zeglin *et al*. (2009) enzyme assays were done to measure geochemical restraints on productivity in a desert ecosystem; by measuring the enzymes they linked productivity to the nutrients those enzymes work on. Three hydrolytic enzymes were assayed along with MBC. In this study, the enzymes were chosen based on relevance to nutrient cycling and sensitivity to change.

Before scheduling an enzyme assay, many considerations must be taken into account. Incubation time, type of microbial inhibitory agent, and the specific reaction the enzyme catalyzes may all differ. For this reason it is difficult to pinpoint which available enzyme assay will give the best results. The definitive literature on this subject is that of Tabatabai (1994). According to him, several methods exist and have been tested in literature. In particular, the advantages of various microbial inhibitory agents are discussed. Toluene, ethanol, and Triton X-100 all serve to suppress the microorganisms' ability to produce enzymes and therefore alter the results. Toluene serves as a plasmolytic agent and as an antiseptic. Even though it has been shown to have little effect on acid and alkaline phosphatases, α-glucosidase, and invertase, and a severely inhibiting effect on catalase and dehydrogenase, in most assays toluene is the preferred agent.

2.2.1.2 β-glucosidase enzyme

β-glucosidase (formerly known as gentobiase or cellobiase, EC 3.2.1.21) provides an important energy source for microorganisms, *e.g.,* glucose, by hydrolyzing β-D-glucopyranosides (Eq. 1; Tabatabai, 1994).



$$-R + H_2O \rightarrow \quad -OH + ROH \quad [1]$$

p-nitrophenol-β-D-glucoside β-glucoside *p*-nitrophenol

β-glucosidase is a very common enzyme that works with the C cycle. A soil with higher levels of this enzyme has been found to have increased soil suppression of different plant diseases, such as Take-all of wheat. This is accomplished with the help of inoculated *Pseudomonas* spp. that serve as biocontrol agents (Borrero *et al*., 2004). Much of the β-glucosidase activity can be assumed to come from abiontic enzymes (those no longer associated with living cells) and those now associated with organo-mineral complexes in soil. This enzyme is particularly sensitive to changes in the soil system such as soil pH and management practices (Bandick & Dick, 1999; Makoi & Ndakidemi, 2008).

2.2.1.3 Acid- and alkaline-phosphatase enzyme

Acid (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) and alkaline (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) phosphatase are both phosphomonoesterases. This class of enzymes has also been widely studied due to its importance in agriculture. It has been proven that acid phosphatases are more prevalent in acid soil, and alkaline phosphatases are more prevalent in alkaline soils (Eivazi &Tabatabai, 1977). Alkaline phosphatase activity is completely derived from microorganisms as it does not exist in higher plants (Dick & Tabatabai, 1983), whereas acid phosphatases can come from both plants and microorganisms; plant roots excrete acid phosphatase as a mechanism for P uptake from soils. Legumes which have N-fixer associations excrete larger amounts of phosphatase, probably due to the increased requirements of P for the symbiosis. Phosphatase enzymes are a good indicator of soil fertility and soils with low amounts tend to show nutrient deficiency symptoms. Phosphomonoesterases hydrolyze many types of phosphomonoesters, according to the general equation below (Eq. 2; Tabatabai, 1994):

$$R - PO_4 + H_2O \rightarrow R - OH + PO_4H \qquad [2]$$

Phosphatases have been correlated to P stress and plant growth, showing that they are good indicators of the P supply in a soil system. A major influence of phosphatase activity is soil pH. Exudation of this enzyme by plants and microorganisms is influenced by the need for orthophosphate, which is in turn influenced by soil pH. However, it is acid, and not alkaline phosphatases, which correspond best to organic C and possibly other indicators in soil (Dodor & Tabatabai, 2003).

2.2.1.4 Urease enzyme

Urease (urea amidohydrolase, EC 3.5.1.5) is important in the N cycle as it catalyzes the hydrolysis of urea (Eq. 3; Krajewska, 2009) and has a high correlation to N in soil. It originates from both plants and microorganisms and exists both inside and outside the cell. Due to the instability of intracellular urease, it is assumed that most ureolytic activity is carried out by extracellular urease, which has the side benefit of being stabilized by organo-mineral complexes in soil (Makoi & Ndakidemi, 2008; Krajewska, 2009).

$$NH_2 - CO - NH_2 + H_2O \rightarrow H_2N - COOH + NH_3 \rightarrow H_2CO_3 + 2NH_3 \qquad [3]$$

Urease has been widely studied due to the importance of urea fertilizer to agriculture. Urea has been shown to be the main source of N to flooded rice paddies and corn in Africa and Asia (Makoi & Ndakidemi, 2008). An increase in urease activity may result in accelerated loss of N through ammonia volatilization (Krajewska, 2009). Urease activity in soils is influenced by cropping history, SOM, soil depth, soil amendments, and presence of heavy metals (Makoi & Ndakidemi, 2008).

2.2.1.5 Dehydrogenase enzyme

Unlike the specific enzymes, dehydrogenase is actually a composite enzyme class. It represents all the specific enzymes which assist in biological oxidation of organic compounds (Tabatabai, 1994). Dehydrogenase oxidizes organic matter in soil by transferring protons and electrons. Dehydrogenase only comes from microorganisms and does not exist outside of the cell. Therefore, dehydrogenase is a good proxy for total microbiological activity of a soil. The general reaction is shown below (Eq. 4; Tabatabai, 1994):

$$XH_2 + A \rightarrow X + AH_2 \quad [4]$$

The enzyme assay for dehydrogenase tracks all of the dehydrogenases in soils. A large volume of research has been done on this enzyme due to a correlation between oxygen uptake and enzyme activity. This relationship may be tenuous because all studies refer only to the work done by Stevenson (1959). Dehydrogenase is higher in flooded systems, like rice paddies (Makoi & Ndakidemi, 2008), and if it is assumed dehydrogenase represents active MB, then it can be said that dry conditions in soil lower this enzyme (Hueso *et al*., 2011). Pascaud *et al.* (2012) found that cell counts derived from dehydrogenase activity were much higher than counts from epifluorescence microscopy, which indicates that enzyme activity can access a greater portion of the microbial community than traditional cell culture techniques.

2.2.2 Other biological indicators

2.2.2.1 Microbial biomass via fumigation-extraction

Microbial biomass via fumigation-extraction is one of the ways to get a proxy for real MB. The C and N of the soil are measured before and after fumigation, and the difference yields the microbially associated C and N.

Biomass changes can precede the changes in traditional methods, like measuring soil OM. When mixing in straw into a soil, MBC increased before any corresponding increase in OM could be seen (Powlson *et al*., 1987). In Jacinthe *et al*. (2011) MBC was significantly higher in the organic farming treatments as compared to conventional farming treatments, even though the organic farming treatment had no reliable effect on the various soil properties evaluated. MB changes under different cropping systems; Acosta-Martínez *et al.* (2010) found that MB was highest in cropping systems which included the most diversity of crops in rotation. A rotation with cotton, rye, and sorghum as well as one with just cotton and sorghum improved the MB in comparison to non-cotton containing rotations (sorghum and rye only), and in cotton monoculture plots the MB took five years to improve to the level which a rotational cotton system already had. Thus, MB is generally higher with more diverse agricultural practices.

2.2.2.2 Glomalin-related soil protein

Arbuscular-mycorrhizae fungi (AMF) hold a key place in ecosystem function. They interact with plants, providing essential nutrients. AMF are widely distributed in soil (Rillig & Mummey, 2006) and it is from this that GRSP derives (Preger *et al.,* 2007). However, mycorrhizae are seldom important in agricultural crops (Ryan & Graham, 2002). AMF enhance the uptake of P and Zn for their host plants and require in return up to 20% of the host’s energy from photosynthesis. In studying the production of glomalin by hyphae, a hydrophobic scum was noted on the water removed from sand cultures of the AMF hyphae. This indicated that large amounts of glomalin can be produced by AMF hyphae and also that glomalin might be useful in the stabilization of WSA in soils (Bird *et al*., 2002; Wright *et al*.,

2007; Kumar *et al*., 2010). Immuno-reactive material on AMF hyphae is the actual definition of glomalin, though it varies in the operational definitions due to the ease and expense of extraction procedures. Glomalin or GRSP can also be referred to as Bradford-related soil protein (BRSP) due to the method of extraction and subsequent assay used. Different times of extraction and methods used for quantification lead to easily extractable Bradford-reactive soil protein, EE-BRSP; Bradford-reactive soil protein, BRSP; easily extractable immuno-reactive soil protein, EE-IRSP; and immuno-reactive soil protein, IRSP.

Glomalin has an unstable terminology and mostly non-standard procedures for its extraction (Janos *et al*., 2008). GRSP is that portion of soil protein which is extractable only under extreme conditions. Glomalin has been shown to be on AMF hyphae (Wright *et al.,* 2000) and it is assumed that nearly all of the protein extracted by the method of citrate solution and autoclaving is glomalin. It may have a retention time in soils of between 6-42 years (Janos *et al*., 2008). One cycle of 30 min of autoclaving with a citrate solution is necessary for the EE fraction, while several 60 min cycles is needed for the total glomalin fraction of BRSP. Autoclaving the extract continues until the supernatant runs yellow, indicating a lack of the red-brown color that indicates glomalin (Preger *et al.,* 2007). A further method of glomalin measurement is by taking the extracts needed for a Bradford assay and instead running an enzyme-linked immuno-sorbent assay (ELISA) using the monoclonal antibody MAb32B11 (Wright & Upadhyaya, 1998). Immuno-fluorescence is then assessed.

No-tillage systems favor the development of fungi, including AMF, as CT systems lean towards bacteria dominating the microbial communities (Caesar-TonThat *et al*., 2010). However, in a contrasting opinion, Amelung *et al*. (2002) states that after cropping ended and the soil use was converted to grassland, fungal biomarkers made way for those of bacterial origin. In this study, amino sugars were used as an indicator. It was shown that prolonged tillage in the South African Highveld resulted in the decrease of living and dead MB and in the decrease of microbial residues.

Spohn and Giani (2010) state that aggregation is a process that integrates soil organic matter (SOM), soil biota, ionic bridging between particles, and carbonates. GRSP is

correlated with the labile SOM fraction, including fungal exudates, which act as a glue for aggregates. In Wright *et al*. (2007) they proposed that glomalin be officially recognized as microbial glue for aggregates. In their study, GRSP increased with aggregate size fractions. In disturbed treatments, GRSP was lower on average, as was the proportion of larger aggregate classes. With good soil structure and good aggregation of soil particles the appropriate water infiltration properties, habitat for microbial communities, and good soil "health" are established (Wick *et al*., 2009). Similarly, in a winter wheat-summer fallow in Oregon, USA, Wuest *et al*. (2005) measured glomalin fractions, basidiomycetes, earthworm density, water percolation, and aggregate size and stability on an intensively-tilled silt-loam soil with treatments of burning after harvest and unburned stubble. Immuno-reactive EE-GRSP was found to have a good relationship with total N (r = 0.88), water stability of whole soil (r = 0.87), and percolation (r = 0.85). Since glomalin is noted for its hydrophobic properties (being insoluble in water), recalcitrance in soil, and wide distribution in soils, it is a good candidate for aggregate stabilization (Wright & Upadhyaya, 1998). Hontoria *et al*. (2009) found that EE-BRSP did not show a significant difference in unstable and WSA under the same treatment, but increases of both easily extractable and total extractable BRSP occurred under abandoned olive groves compared to groves still under management.

Halvorson and Gonzalez (2008) found that Bradford assays were affected by tannic acid additions, changing the results for BRSP. It was concluded that the Bradford assay is unpredictable and not a good procedure for assessing soil glomalin concentrations. Steinberg and Rillig (2003) also studied glomalin using the Bradford assay. They found that total glomalin can be regarded as a long-term indicator of C and soil health, but that easily-extractable glomalin (EEG) is least bound to organo-mineral complexes and more immunoreactive (showing more in the ELISA assay compared to the Bradford assay) and is more recently produced by the fungi. Since EEG is more recent, it cannot be used as a reliable indicator for long-term soil health, but like some enzymes, is a better short-term soil health indicator.

2.2.2.3 BIOLOG whole community profiling

BIOLOG measurements are considered a measure of community-level physiological profiling (Pascaud *et al.*, 2012). BIOLOG EcoPlates^{TM} consists of 31 C sources with three replicates in a 96-well microplate. A soil dilution is pipetted into the wells and left to incubate at 37°C. As the soil microorganisms synthesize the C source a purple product is formed. By measuring the presence and intensity of this purple color, a conclusion can be reached regarding the general diversity of the soil microbial population.

Oksinska *et al*. (2011) used this C utilization analysis to identify good and weak colonizers from different strains of *Pseudomonas* spp., a known biocontrol agent group, and then, once identified, colonized wheat (*Triticum aestivum* L.) seedlings. Soils with higher levels of disease suppression, *i.e.,* those with good colonizers of biocontrol agents, showed a resistance to soil pathogens such as *Fusarium* and *Rhizoctonia*. Zhang *et al.* (2012) demonstrated microbial community differences in soils with various water saturation levels in a soil aquifer.

2.2.2.4 Phospholipid fatty acids

Fatty acid analysis provides an insight into the soil microbial community profile. With the proper identification system in place, the peaks from a gas chromatograph (GC) chromatogram can be identified as specific fatty acids (*e.g.,* cy18:0). Signature fatty acids are known for Gram negative bacteria, Gram positive bacteria, fungi, and actinomycetes (Frostegård *et al*., 1993; Baumann *et al*., 2011; see Table 2.3). The software run to identify the peaks, *i.e.,* the amount of time it takes to run through a column under a ramped temperature system, is commonly MIDI Sherlock, though other software packages can be used. PLFA results can also be run through statistical software in order to identify changes over sampling time, rather than identifying any particular groups (Frostegård *et al*., 2011).

In Helgason *et al.* (2010), CT was shown to cause a shift in the microbial community and a decrease in MB as estimated from PLFA. Diedhiou *et al.* (2009) mentioned different PLFA ratios of concern. The fungal:bacterial ratio indicates general changes in the microbial

community and is indicated by the branched acids for fungi and the other bacterial fatty acids identified (Table 2.3). Fungi are sensitive to tillage practices and dry soil conditions (Potthoff *et al*., 2006). Other ratios are the saturated : monosaturated fatty acid ratio which, if decreasing over time, indicates a shift to anaerobic conditions and/or decreasing substrate availability and cy19:0 : 18:1ω7, which is used as an indicator of ecological stress. The fatty acid 16:1ω5 belongs only to the Glomus group of fungi and ergosterol (a branched fatty acid) only belongs to non-AMF groups (*i.e.,* ones that presumably don't produce glomalin).

Table 2.3 Summary of signature fatty acids from Frostegård *et al*. (1993) and Baumann *et al.* (2011)

Biomass group	**Signature fatty acids**	**Technical name**
Bacteria	C14:0, C15:0, C16:0, C16:1, C17:0	Saturated and monoenoic acids
Gram +	iC14:0, iC15:0, aC15:0, iC16:0, iC17:0	
Gram -	C16:1ω7t, cyC17:0	
Actinomycetes	10Me18:0	Tuberculostearic acid
Fungi	16:1ω5, 18:2ω6	Branched acids, Ergosterol

PLFA are sensitive to changes in land use overall, and the bacterial and fungal ratios also change with land use and plant cover type (Hossain & Sugiyama, 2011). In their study they chose 32 sites across a geographical gradient with different land uses (bare, agricultural, grassland, and forest) and found differences in total PLFA, bacterial PLFA, and fungal PLFA markers. The variance was high but could be explained by differences caused by spatial variations including soil pH, texture, and specific assemblages of plants.

Naming for fatty acids is straightforward. For instance, a straight chain of 14 C without any double bonds would be C14:0. The little i denotes iso-branching and a anteiso-branching. The prefix 10Me shows a methyl group on the tenth C from the carboxyl end and *cis* and *trans* configurations of the molecule are shortened as c and t, respectively. Cyclopropane fatty acids have a cy prefix (Frostegård *et al*., 1993).

2.3 Conclusion

Although physical and chemical properties of soil have been utilized extensively to evaluate soil quality, the application of biological indicators is becoming more important. In order to assess soil quality, soil enzymes and other biological parameters need to be considered.

3 MATERIALS AND METHODS

3.1 Experimental layout and treatments

The experimental site is located in Bethlehem, Free State, South Africa. The site was established in 1979 by the ARC-Small Grain Institute. The soil is of the Avalon form and Mafikeng family in the South African soil classification system (Soil Classification Working Group, 1991) and a Plinthustalf in the USDA soil taxonomic system (and therefore a Typic Plinthustalf; Soil Survey Staff, 2010). This Plinthosol (FAO, 1978) contains the following diagnostic horizons: from 0-30 cm, an orthic Ap; at 30-65 cm, a yellow-brown apedal B1; followed by a soft plinthic B2 at the depth greater than 65 cm. The profile shows a transition from sandy loam (0-45 cm depth) to clay (140-180 cm depth) and throughout contains few concretions and rocks. The soil structure is apedal to massive and the parent material is aeolian and colluvial materials on shale of the Tarkastad formation (Hoffman, 1990); it is found in land type Ca6n and occupies 420,000 ha locally (Land Type Survey Staff, 2001).

Though it was subjected to tillage for at least 20 years prior to the experimental set-up, the specifics of land management are unknown prior to acquisition by the ARC. The plot is located at approximately 28°13'S and 28°18'E at 1680 m above sea level. Long-term climatic data and specific weather data from the sampling period are presented in Table 3.1 and 3.2, respectively. See Figure 4.3 for detailed temperature and rainfall data during the study period.

This site is a monoculture wheat trial (*Triticum aestivum* L.) with no cover crop or rotation built in. However, when signs of soil-borne diseases (Take-all) are found in some plots, a growing season of oat (*Avena sativa* L.) is substituted for wheat and the harvest is not recorded. This situation applied for 1990, 2004, and 2010, while 1992 was a drought year. Most of the rain falls between harvesting and seeding, which follows a five month fallow period to conserve soil water levels (Loke, 2012). The wheat cultivar used is Elands, though before 2005 the now-obsolete Betta was used.

Table 3.1 Long-term climatic data from weather station 19833 near the experimental site (ARC-ISCW, 2002)

Parameter	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Annual
Rain (mm)	115.8	91.1	74.2	49.6	24.2	9.8	9.8	17.4	32.2	77.5	94.0	99.6	695.2
E_0 (mm)	214.1	179.4	164.9	122.0	103.4	82.5	93.7	129.1	172.7	195.7	201.2	223.9	1882.8
AI	0.54	0.51	0.45	0.41	0.23	0.12	0.11	0.13	0.19	0.40	0.47	0.45	0.37
Tmax (°C)	26.7	25.9	24.5	21.4	18.7	15.7	16.1	18.7	22.3	23.5	24.6	26.1	22.0
Tmin (°C)	13.4	13.0	11.2	6.7	1.8	-2.4	-2.5	0.0	4.6	8.1	10.5	12.3	6.4
Tm (°C)	20.1	19.5	17.9	14.1	10.2	6.7	6.8	9.4	13.5	15.8	17.6	19.2	14.2

E_0 = class-A pan evaporation
AI = Aridity Index = rainfall / class-A pan evaporation
Tmax = Mean daily maximum temperature
Tmin = Mean daily minimum temperature
Tm = Mean daily temperature = (Tmax + Tmin) / 2

Table 3.2 Short-term weather data for the study period between September 2010 and October 2011 from the weather station 19833 (ARC-ISCW, 2011)

Month	Tmax (°C)	Tmin (°C)	Rainfall (mm)
SEP 2010	25.95	5.00	0.0
OCT 2010	25.37	8.42	42.4
NOV 2010	25.49	11.24	91.2
DEC 2010	24.94	12.87	193.3
JAN 2011	24.40	14.46	180.4
FEB 2011	24.91	13.68	102.4
MAR 2011	25.79	12.84	24.1
APR 2011	19.70	7.92	66.8
MAY 2011	18.06	3.58	30.5
JUN 2011	15.54	-1.03	24.4
JUL 2011	14.47	-3.18	14.2
AUG 2011	19.08	-0.09	3.6
SEP 2011	23.79	4.57	5.8
OCT 2011	24.56	7.73	32.8

The site is a randomized complete block design with three blocks, which serve as replicates. It includes 36 field treatments, which was a full factorial combination, consisting of two methods of straw disposal (burned versus non-burned), three tillage methods (no-tillage: NT, conventional tillage: CT, and stubble mulch: SM), and two methods of weed control (mechanical versus chemical). It also includes three applications of N fertilizer. The levels 20, 30, and 40 kg N ha^{-1} were used until 2003 and currently 20, 40, and 60 kg N ha^{-1} is used. The current 40 kg N ha^{-1} level was used for this study. Each plot is 6 x 30 m with 10 m borders.

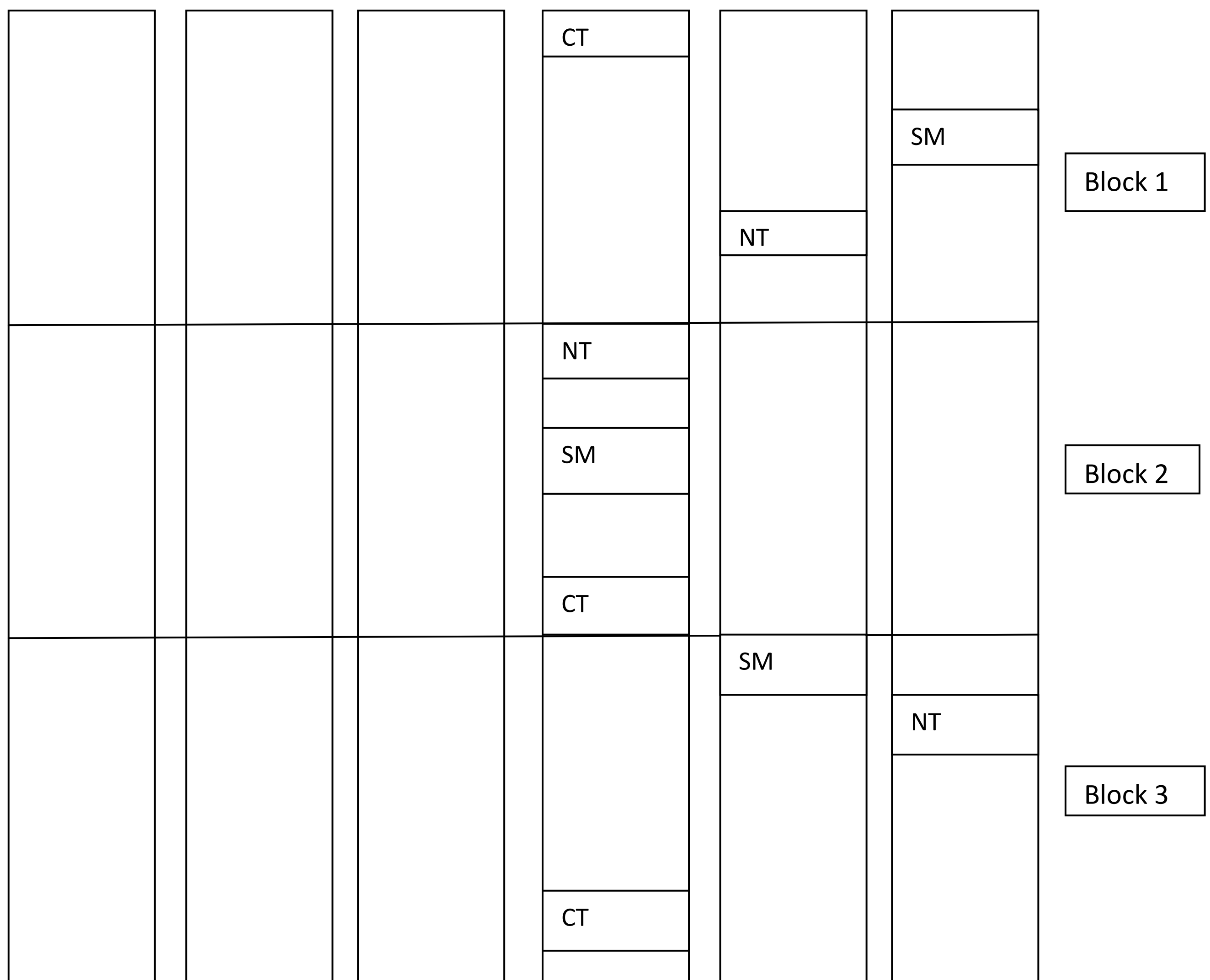


Figure 3.1 Experimental layout represented, showing the blocks and general position of sampled plots with the tillage method denoted.

The plots are harvested in December. The remaining wheat straw post-harvest is either burned or left unburned. For the CT treatments, a two-way offset disk incorporates either the left-over residues or the residue ashes to a depth of 150 mm. In February, after the soil

has become moist and easier to work, a mouldboard plow is set to a depth of 250 mm. At the same times as the CT treatment, the SM treatment is first cut using a v-blade at 100-150 mm depth and then ripped to a depth of 250 mm with a 50 mm-wide chisel plow at a spacing of 300 mm. No-tillage treatments are left completely unplowed.

Mechanical weeding has been conducted with a light tiller during the fallow period since 2003, and before that with a rod-weeder or V-blade, as according to soil water levels. Chemical weeding is done by spraying herbicides. First, Round-up, a broad-spectrum herbicide containing glyphosate, was used. Then, glyphosate and paraquat were alternated to avoid the development of herbicide resistance. All plots were planted with a combined seeder-fertilizer drill used for sowing the wheat seed and 3:2:0 (25) + 0.75% Zn fertilizer; this resulted in a rate of 20 N, 13 P, 0 K, and 1 Zn in kg ha^{-1}, respectively. A thoroughly mixed limestone ammonium nitrate (LAN; 28% N) was added to the fertilizer mixture to change the N application rate for the other N levels. Since 2003, a more advanced planter was used that allows for computer-applied mixing rates for N and P (DBS No-tillage Planter). Currently, the sources changed to only LAN (28) and single superphosphate (10%).

The sampling design has been restricted to the tillage treatments (CT, SM, and NT) that coincide with chemical weeding and non-burning of residues and two depths (0-5 and 5-10 cm) due to the logistical and time constraints of biological sampling. In the worst case, enzyme assays were optimally completed within two weeks of sampling as long as they were kept at 4°C. It has been recorded that soil enzymes react differently to soil storage time (*e.g.,* phosphatases were more sensitive to storage length than β-glucosidase) and freezing serves better than air-drying (DeForest, 2009). The conservative estimate is completing assays within 10 days of sampling, which has been adhered to during this study.

3.2 Soil sampling, preparation, and storage

Samples were taken with a soil auger (5 cm dia.) at the depths 0-5 cm and 5-10 cm to investigate stratification, and also to mimic the sampling styles of previous research done at the ARC-Small Grain Institute wheat trials (Wiltshire & du Preez, 1993; du Preez *et al*., 2001; Kotzé & du Preez, 2007; Kotzé & du Preez, 2008; Loke, 2012).

Table 3.3 shows a full schedule of the sampling times during the study period. The first sampling was done in October 2010 during flowering, representing the peak of microbial activity. The year 2010, however, was unusually dry and no rain had yet fallen for the rainy season. The next sampling date was in November 2010. Though herbicide had been sprayed on the oats to keep them from using scarce water supplies after the kernels had grown fully, this sampling date was scheduled because the rainy season had commenced. Rain continued to fall during the next two sampling dates, in February 2011 and April 2011, showing a prolonged wet period. The former date was directly after the tillage treatments were applied and counted as the first fallow sampling. The latter was the second fallow period sampling. In May 2011, the fifth sampling was scheduled directly after herbicide was sprayed to control the weeds. By then, rain events had mostly halted. The sixth sampling period was scheduled for mid-August 2011. It was during a dry period and occurred after planting and another herbicide round. The seventh sampling date was in September 2011, during the growth of the wheat and the final samples were collected in October 2011 at the flagleaf stage of the wheat.

Table 3.3 Sampling times during the study period

Sampling time	Date	Conditions
1	11 October 2010	Oat: flowering; Dry
2	24 November 2010	Oat; herbicide sprayed recently; After first rains
3	28 February 2011	Fallow; After treatments
4	11 April 2011	Falllow
5	30 May 2011	Fallow; herbicide sprayed recently
6	15 August 2011	Wheat: after planting; herbicide sprayed recently
7	19 September 2011	Wheat: during growth
8	25 October 2011	Wheat: flagleaf stage

Five samples were randomly taken from each plot and these samples were combined into one composite sample for each treatment. There were three replications of each sample from each of the three experimental blocks on the trial. Microbial samples were bagged and put on ice (approximately 4°C) before sieving (<2 mm) and processing, whereas soil used for the physical parameters was dried and sieved (<2 mm) as soon as possible. Wallenius *et al*. (2010) notes the difficulty of preparing soil samples for microbial analyses, and shows that even small storage times can affect the results. Because of this, we used the soil as soon as

possible and completed the enzyme assays in a certain order so the deviance from the original sample would be similar across the study period.

3.3 Soil analyses

3.3.1 Physical soil parameters

The following tests were completed on the soil samples: particle size distribution and gravimetric moisture content. Particle size distribution was determined through the pipette-sieve method (Kilmer & Alexander, 1949) in order to see the effect clay percentage has on enzymes, and to determine the accuracy of sampling itself. Moisture content was done during the enzyme assays on the samples in order to correct the values found from the spectrophotometer, but not necessarily at the same moisture as when it was taken out of the field. These analyses were completed for each sampling time.

3.3.2 Chemical soil parameters

For each sampling time, pH (H_2O), total C, total N, and extractable P were analyzed for in the soil samples. The soil pH method used a 1:2.5 soil:water suspension. Total C and total N was estimated by combustion using a Leco Truspec CNS analyzer (Leco Corp., St. Joseph, MI, USA). An adapted Olsen method for P determination was used, to mimic previous studies (Kotzé and du Preez, 2008). All chemical data was done in duplicate. Full methods can be found in The Non-Affiliated Soil Analysis Work Committee (1990). Hereafter in this dissertation, total C, total N, and extractable P are referred to as C, N, and P, respectively.

3.3.3 Biological soil parameters

For all eight sampling times, enzymes were determined colorimetrically using enzyme-specific procedures (described below). Once the color was developed, a spectrophotometer or microplate reader, ELx800 (BioTek Instruments Inc., USA), was used in determining the absorbance at the specified wavelength. Soils were sieved (<2 mm) and kept at 4°C. Enzyme assays were done in duplicate, with a control for each sample. Dehydrogenase is the only

method which mandated keeping the soil moist for the assay; however, this was done for all the assays for standardization of results (Tabatabai, 1994). Due to this, all data was corrected for moisture content during the data analysis.

β-glucosidase was determined through an adaptation of Dick *et al*. (1996). One g of field-moist soil was incubated at 37°C for 1 h with toluene, modified universal buffer pH 6.0, and *p*-nitrophenol-β-D-glucosidase (ρNG) then shaken with calcium chloride and tris(hydroxymethyl) aminomethane before filtering through a Whatman no. 2v filter paper. Absorbance of released *p*-nitrophenol (ρNP) was tested with a microplate reader at 405 nm.

Kandeler and Gerber (1988) described the method for urease enzyme determination. The non-buffered method was used, where 5 g of field-moist soil was incubated at 37°C for 2 h with a urea solution and then shaken with potassium chloride. The resulting suspension was filtered through Whatman no. 2v filter paper. Before the absorbance was measured with a microplate reader at 690 nm, the filtrate was prepared with sodium salicylate/sodium hydroxide solution and sodium dichloroisocyanide solution for the color development.

The assay for both acid- and alkaline-phosphatase was adapted from Tabatabai (1994). One g of field-moist soil was incubated at 37°C for 1 h with toluene, modified universal buffer (pH 6.5 for acid phosphatase and 11 for alkaline), and ρNP. Then, after adding calcium chloride and sodium hydroxide, it was filtered immediately through Whatman no. 2v filter paper. Absorbance, also of ρNP, was measured with a microplate reader at 405 nm.

The dehydrogenase assay from von Mersi and Schinner (1991) was used. One g of field-moist soil was mixed with THAM and iodonitrotetrazolium violet-formazan (INT) solution. This enzyme assay required incubation at 40°C in the dark for 2 h. Then the samples were mixed with an extraction solution and kept in the dark for another 30 min. Absorbance of the reaction product INT was tested with a glass cuvette on the spectrophotometer at 464 nm.

Soil MBC and MBN were also measured for all eight sampling times. A modification of the chloroform-fumigation method was used (Coleman *et al.,* 2004; von Lützow *et al.,* 2007) by

analyzing the C and N percentages with Leco combustion. These values were compared with the normal C and N values also obtained through Leco combustion and the difference was, therefore, soil MB activity. Because chloroform-fumigation must be done on field-moist soils, they were dried out directly following the procedure for analysis on the Leco machine. Equations 5 and 6 (Acosta-Martínez *et al.*, 2010) were followed to obtain the values. The k factor is included to account for C and N in gaseous form escaping during the fumigation.

$$MBC = \frac{(C\ in\ fumigated\ soil - C\ in\ unfumigated\ soil)}{k_{EC}} \text{ where } k_{EC} = 0.45 \quad [5]$$

$$MBN = \frac{(N\ in\ fumigated\ soil - N\ in\ unfumigated\ soil)}{k_{EN}} \text{ where } k_{EN} = 0.54 \quad [6]$$

Easily extracted-GRSP was measured through a procedure used by Wright *et al*. (1996), Wright and Upadhyaya (1996), and Wright and Upadhyaya (1998) for all eight sampling times. Only the easily extractable protein was extracted. One g of soil was mixed with a sodium citrate solution and autoclaved at 121°C for 30 min. After centrifugation, 1 mL of the supernatant was decanted into mini centrifuge tubes and centrifuged again. This was the sample that the Bradford assay was done on in duplicate, while using Bradford dye (Sigma-Aldrich) and bovine serum albumin (BSA; Sigma-Aldrich) as the standard. The standard curve was expressed by $y = 0.2233x + 0.0097$. The assay was done on a microplate and read by a microplate reader at 590 nm. It was possible to store the supernatant for 2-4 weeks at 4°C.

The PLFA method from Marschner (2007) was modified by Prof A. Hugo and Mr. T. Bambo (UFS; unpublished). PLFA were only analyzed for the first, third, and fifth sampling times. Briefly, 2 g of frozen field-moist soil was mixed with a citrate buffer, chloroform, methanol, and Bligh and Dyer reagent. The samples were shaken for 2 h, then vortexed and centrifuged at 2500 g. The soil-free supernatant was moved to a new tube and the soil was washed with more Bligh and Dyer reagent and once it was vortexed and centrifuged at 2500 g again, more supernatant was transferred to the new tube. Chloroform and citrate buffer were added and then the organic phase was removed. The samples were dried under a N_2 stream, conditioned with chloroform, and run through an elution chamber. The phospholipids were collected and dried again under a N_2 stream. An internal standard was added along with methanol:toluol, hexane:chloroform, acetic acid, and deionized water. The

organic phase was collected and dried under a N_2 stream. Then the purified PLFA, which had been methanolyzed into fatty acid methyl esters, were read on a Varian 430-GC gas chromatograph (Agilent Technologies, USA).

BIOLOG EcoPlates^{TM} were used for one of the sampling times only (October 2010). 10 g of soil was added to 90 mL of sterilized water and shaken for 50 min. After settling for 2 h, this soil dilution was pippetted into the 96 wells of the plate. Color was noted by eye and from a microplate reading after 36 h of incubation at 25°C. The results were interpreted by eye with corresponding data from the microplate reader printouts. No change in color was given a value of 1 while a slight change was 2 and a dark purple color was a 3.

3.4 Data processing and analysis

The experimental design compared enzyme activity and nutrients and pH between 3 treatments at 2 depths over 12 months. Three-way analysis of variance (ANOVA) was conducted using the general linear model available in SAS software. An alpha of 0.05 was chosen and the Tukey-Kramer test was used as a post-hoc test using Tukey's honest significant difference (HSD). For BIOLOG community profiling and fatty acid results, no statistical software was used; instead, bar graphs were created with Microsoft Excel and trends were noted.

4 ENZYME ACTIVITIES AND NUTRIENT LEVELS

4.1 Introduction

The purpose behind having so many parameters in this and the next chapter is because, as de Varennes & Torres (2011) state, one soil property on its own cannot determine changes or overall quality of the soil. This section will focus on the enzymes β-glucosidase, acid- and alkaline-phosphatase, urease, and dehydrogenase. Enzymes are simply proteins which are biological catalysts, causing the reaction to be provided with an alternate path with a lower energy of activation (E_A), as shown in Figure 4.1.

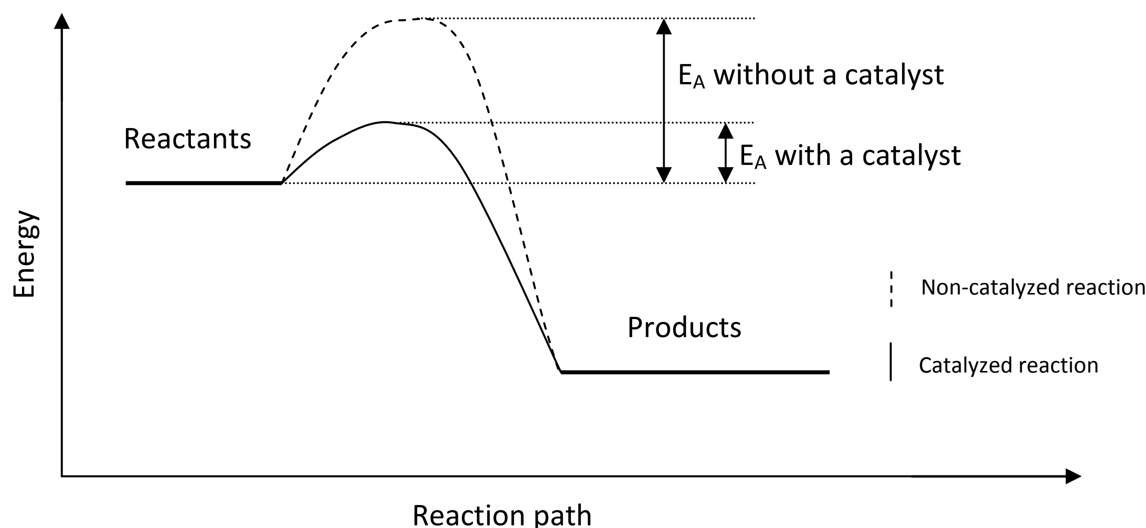


Figure 4.1 An illustration of the amount of activation energy (E_A) required for a reaction to start with and without a biological catalyst.

β-glucosidase provides energy for microorganisms by hydrolyzing β-D-glucopyranosides, which is a step in the hydrolysis of cellobiose, a disaccharide. Only microorganisms produce this enzyme, as the product is an energy source only usable by them. There are four known hydrolase enzymes in soil which act upon glycosyl compounds (α-glucosidase, β-glucosidase, α-galactosidase, and β-galactosidase); of which β-glucosidase is the most common in soils (Tabatabai, 1994).

The phosphatase enzymes are more appropriately defined as phosphomonoesterases, as they cleave phosphate compounds to produce a single phosphate (one P paired with three

O and one hydroxyl group). Acid and alkaline phosphatase is not specific to the reactant. In nature, the hydrolysis of phenylphosphate, ρ-nitrophenyl phosphate (ρNP) and others has been reported (Tabatabai, 1994). In the laboratory assay, the substrate chosen is ρNP. In soils, orthophosphate acts as an inhibitor of this group of enzymes. The more plant available P in the soil, the less phosphatases which will be active; however, this might not affect their concentration, just whether the enzymes are able to work or not. As the names suggest, acid phosphatase is active in more acidic soils, and alkaline phosphatase thrives in high pH soils.

Urease hydrolyzes the reaction of turning urea (NH_2CONH_2) to ammonia (NH_3). This is a two-step reaction (Reithel, 1971) and produces carbamate as a substrate for the second step. It is a popular enzyme to study simply because it is widely distributed in nature and because, in 1926, it happened to be the first enzyme protein crystallized (Tabatabai, 1994). They require Nickel (Ni) in their activation site, which is unusual compared to other enzymes, and are inhibited by the presence of phosphate (Krajewska, 2009; see Figure 4.2).

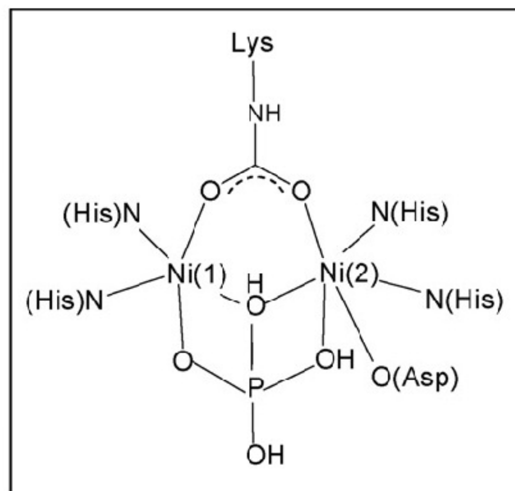


Figure 4.2 A schematic representation of urease with its co-factor (Ni) in the activation site and connected to a phosphate, which is inhibiting the use of the enzyme (from Krajewska, 2009).

Dehydrogenase is not connected to any nutrient cycle, but instead to the presence of the microorganisms themselves. Theoretically, dehydrogenase cannot exist outside of its host cell, and as such is considered as a proxy for active MB. Dehydrogenase oxidizes SOM as the reaction shifts H ions from substrates to acceptors (or the product; Tabatabai, 1994).

In this chapter, results on the activity of the five preceding enzymes and their associated nutrients are presented and discussed.

4.2 Results and discussion

Sampling was conducted across parts of two growing seasons. The first growing season was planted with oat due to disease found in the usual wheat, followed by a fallow period and another cropping period, this time with wheat (Figure 4.3). Table 4.1 shows a summary of water content of the soil measured for enzyme analysis throughout the study period. This corresponds well with the rainfall measured, showing higher water content in the deeper soil layer, as could be expected. As compared to Table 3.1, the maximum and minimum temperatures agreed well with the long-term averages. However, the precipitation changed slightly (Table 3.1). The majority of the precipitation fell in the summer months (October to February) and in general, more than the usual amount of rainfall fell in summer but less than usual rainfall fell in winter. This was the case particularly at the beginning of the study when only 0.25 mm of rain was experienced in the month and a half before the first sampling date (Figure 4.3, Table 4.1).

Table 4.1 Summary of gravimetric water content measured for enzyme analysis throughout the study period, with the sampling times corresponding with those in Figure 4.2

	0-5 cm soil depth	**5-10 cm soil depth**	
Sampling time	**Water content (%)**		**Average**
1	1.6	1.7	**1.6**
2	4.0	6.2	**5.1**
3	11.0	11.0	**11.0**
4	8.1	9.3	**8.7**
5	10.3	17.7	**14.0**
6	8.3	11.2	**9.8**
7	4.6	9.0	**6.8**
8	2.0	4.6	**3.3**
Average	**6.2**	**8.8**	

During the first sampling date, due to the dryness and hardness of the soil, the two layers of soil (0-5 cm and 5-10 cm) are suspected to not be properly separated. As such, the values for the 5-10 cm soil layer may not be accurate. However, the values do not appear to

destroy the trends between sampling times or depths. Only for the fourth sampling time are data from a third soil depth, 10-15 cm, available. Its trends are noted in chapter six.

For all five enzymes analyzed, there existed significant differences amongst sampling times, tillage treatments (Figure 4.2), and soil depths at a 95% confidence interval. In the nutrients, only P did not differ significantly with depth, but significant differences showed in all other categories. pH differed only across the eight sampling times and the three tillage treatments. Interaction effects existed, but only as a two-way interaction (Table 4.2).

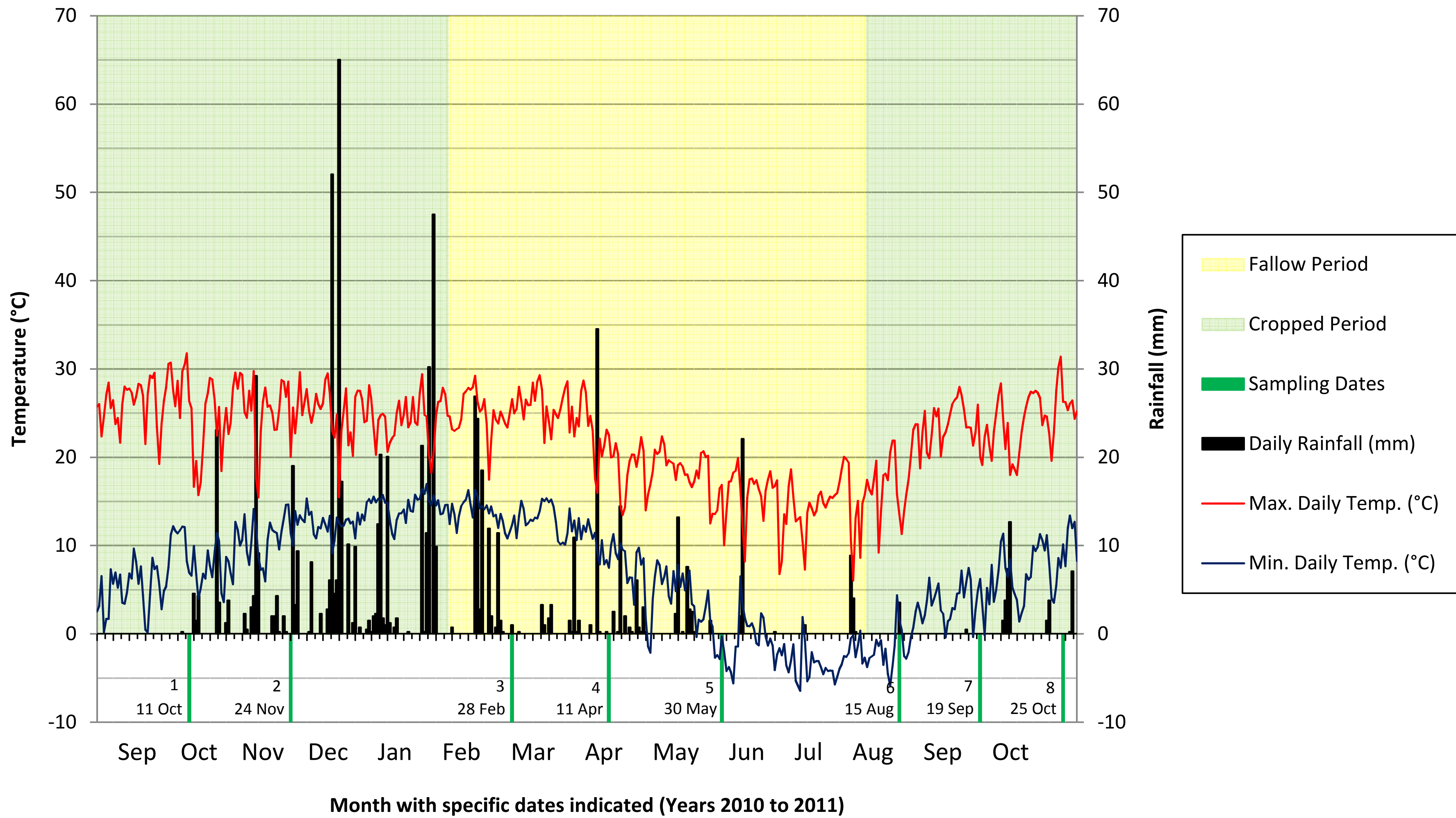


Figure 4.3 This graph shows the two cropped periods, starting with oats (*Avena sativa* L.) and ending with wheat (*Triticum aestivum* L.), with the fallow period in the middle. The sampling dates (corresponding to Table 3.3), temperatures, and rainfall are shown for every day and are presented for the study period at the long-term monoculture wheat trial near Bethlehem.

Table 4.2 Summary of the analysis of variance (ANOVA) indicating significant effects at a 95% confidence interval (α = 0.05)

	β-glucosidase	Acid phosphatase	Alkaline phosphatase	Urease	Carbon	Dehydrogenase	Nitrogen	Phosphorus	pH (H_2O)
Replication			*	*					
Sampling time (A)	*	*	*	*	*	*	*	*	*
Tillage treatment (B)	*	*	*	*	*	*	*	*	*
Depth (C)	*	*	*	*	*	*	*		
A*B			*	*					*
A*C				*		*	*		*
B*C	*		*	*	*	*			*
A*B*C									

Table 4.3 Summary of averages with ranking of the three tillage treatments according to Tukey's HSD for measured parameters

	Tillage treatments		
Parameters	**NT**	**SM**	**CT**
β-glucosidase (µg ρNP g^{-1} SDW $1h^{-1}$)	711.1^{a}	727.3^{a}	471.8^{b}
Acid phosphatase (µg ρNP g^{-1} SDW $1h^{-1}$)	2646.2^{a}	2573.8^{ab}	2334.7^{b}
Alkaline phosphatase (µg ρNP g^{-1} SDW $1h^{-1}$)	936.8^{a}	640.6^{b}	503.4^{c}
Urease (µg NH_4-N g^{-1} SDW $2h^{-1}$)	21.8^{a}	21.9^{a}	14.8^{b}
Dehydrogenase (µg INF g^{-1} SDW $2h^{-1}$)	125.7^{a}	120.4^{a}	89.1^{b}
Carbon (mg kg^{-1})	6004.6^{a}	5539.0^{b}	5232.5^{b}
Nitrogen (mg kg^{-1})	860.8^{a}	764.5^{b}	705.3^{c}
Phosphorus (mg kg^{-1})	39.6^{a}	31.3^{b}	19.7^{c}
pH (H_2O)	6.4^{a}	6.1^{b}	6.2^{b}

4.2.1 β-glucosidase and carbon

The correlation between this enzyme and its corresponding nutrient was very low (r = 0.13 for the 0-5 cm depth, r = 0.24 for the 5-10 cm depth; Figure 4.4). This low correlation could be due to one of two reasons:

1. As enzymes change faster in soils than their respective nutrients, it could be showing micro-changes in the soil which are not indicated properly in bulk C stocks (de Varennes & Torres, 2011).
2. The C parameter was not measured by a method sensitive enough to the changes in organic C.

Main effects

Across sampling times, β-glucosidase did not follow a clear trend with regard to cropping cycles, unless the results include a rather long lag time, as the values are the highest in the beginning of the study period (between 783.2 µg ρNP g^{-1} SDW $1h^{-1}$ and 692.7 µg ρNP g^{-1} SDW $1h^{-1}$), then declines during dates 5 and 6 (values at 363.9 µg ρNP g^{-1} SDW $1h^{-1}$ and 377.3 µg ρNP g^{-1} SDW $1h^{-1}$) and generally recover for the last two sampling dates (567.8 µg ρNP g^{-1} SDW $1h^{-1}$ to 807.8 µg ρNP g^{-1} SDW $1h^{-1}$). However, if the values are superimposed with the temperature, it is seen that the low values occur during the time of the year when the minimum temperatures go below 0°C at night (Figure 4.3; Figure 4.5). De Varrenes & Torres (2011) found the β-glucosidase activity was more linked to water availability than temperature; however our data do not suggest a similar link, as the first sampling time held the least soil moisture content, but the values were not similarly lower than at other times.

Carbon levels only changed across the season by increasing for the last three sampling dates, all of which fell in the last wheat cropping period. It averaged 4530.2 mg kg^{-1} in the beginning, and increased to an average of 7361.7 mg kg^{-1} in the end. This could be due to the amount of residues on the soil surface. The first cropping season had very little rainfall, which led to low levels of biomass, and was followed by a fallow period. The second cropping season had much more water available to the plants, which could have led to an increase in SOM, hence higher total C.

Interaction effects

The enzyme had interaction effects between tillage treatment and depth. The trends are shown in Figure 4.6, with the rankings for tillage treatments in Table 4.3. For the 0-5 cm depth only, NT had the highest potential enzyme activity (900.9 µg ρNP g^{-1} SDW $1h^{-1}$), followed by SM (784.2 µg ρNP g^{-1}SDW $1h^{-1}$) and CT (445.5 µg ρNP g^{-1}SDW $1h^{-1}$). The difference between the two conservation tillage treatments, NT and SM, however, did not significantly differ as with CT. For the lower depth, none of the tillage treatments differed with depth.

At each sampling treatment, the potential β-glucosidase activity was higher in the 0-5 cm depth and the CT treatment was lowest.

C had interactions between tillage treatment and depth. The interaction shows that the more conservational the tillage is, the higher C it will be, but also that the gradient is more pronounced in the surface soil than in the lower depth. The C decreased from 6548.3 mg kg^{-1} in NT to 5390.4 mg kg^{-1} in CT for the top 5 cm, but only from 5460.8 mg kg^{-1} in NT to 5074.6 mg kg^{-1} in CT for the 5-10 cm depth. This should be due to the higher amount of residues available at the surface in NT as compared to SM and then CT.

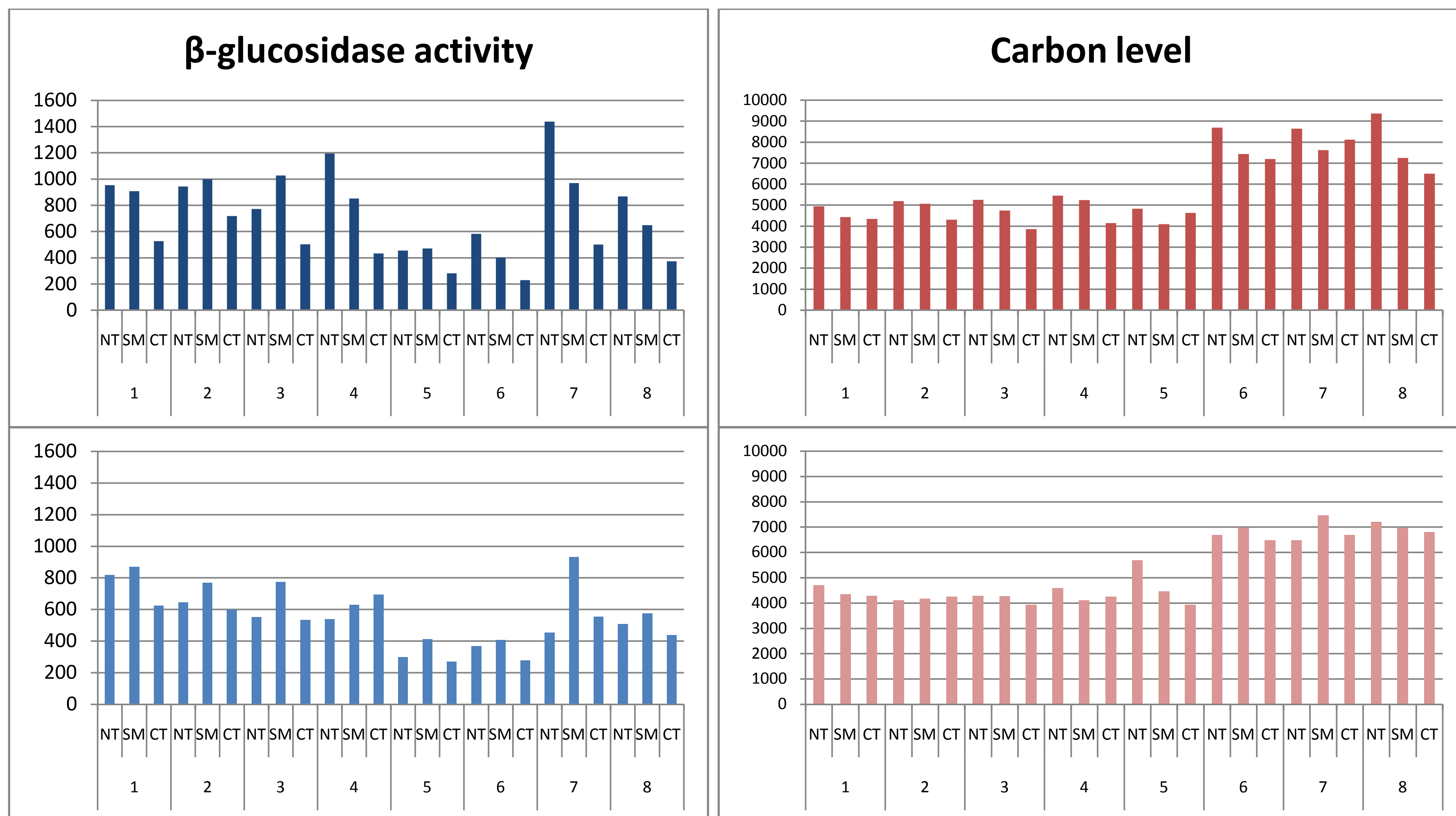


Figure 4.4 All values for potential β-glucosidase activities (µg ρNP g^{-1} SDW $1h^{-1}$) and carbon contents (mg kg^{-1}). The top figures are from the 0-5 cm depth and the bottom figures are from the 5-10 cm depth.

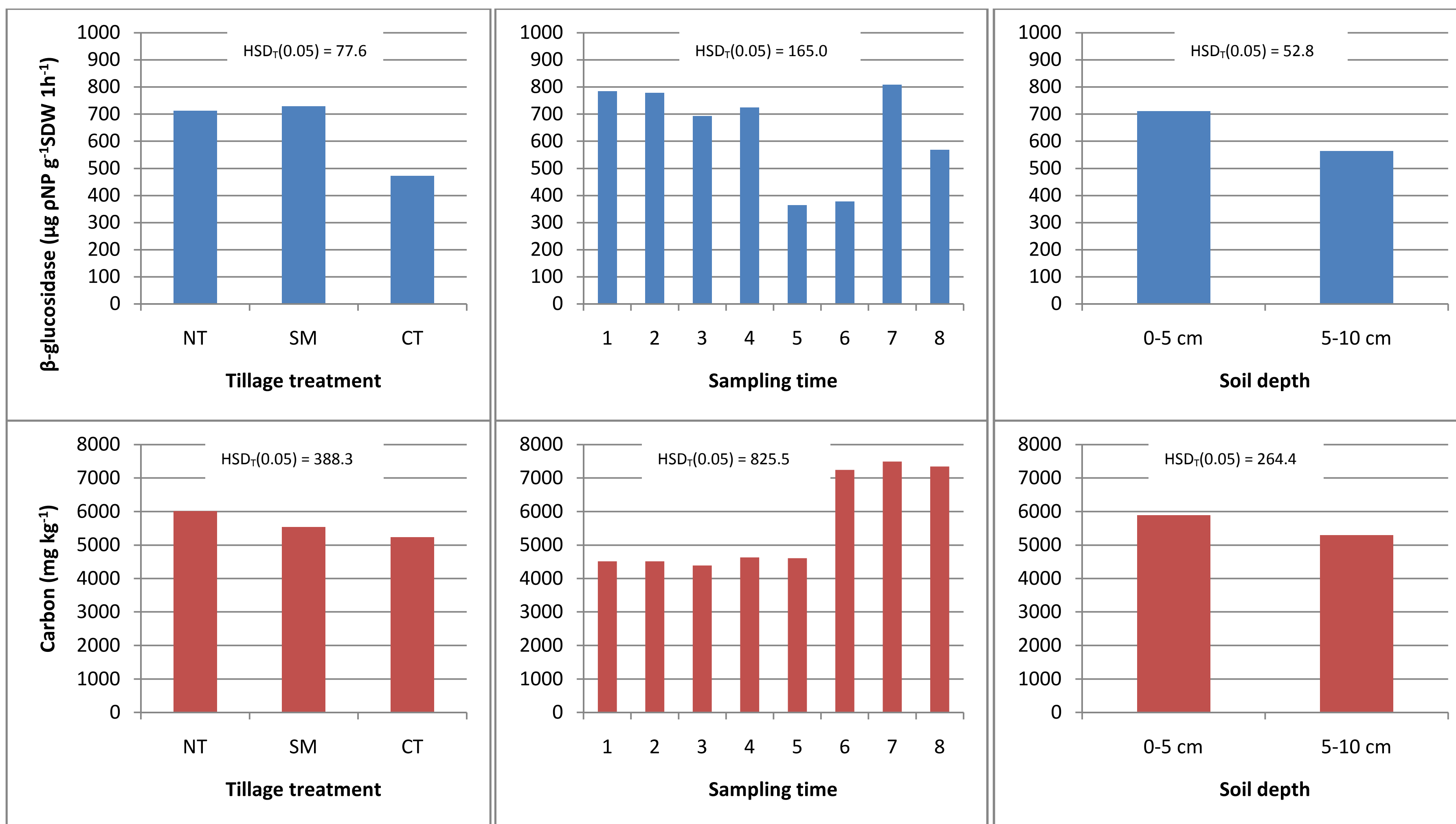


Figure 4.5 Main effects of potential β-glucosidase activities and carbon contents across tillage treatment, sampling time, and soil depth. Tukey values are included for each.

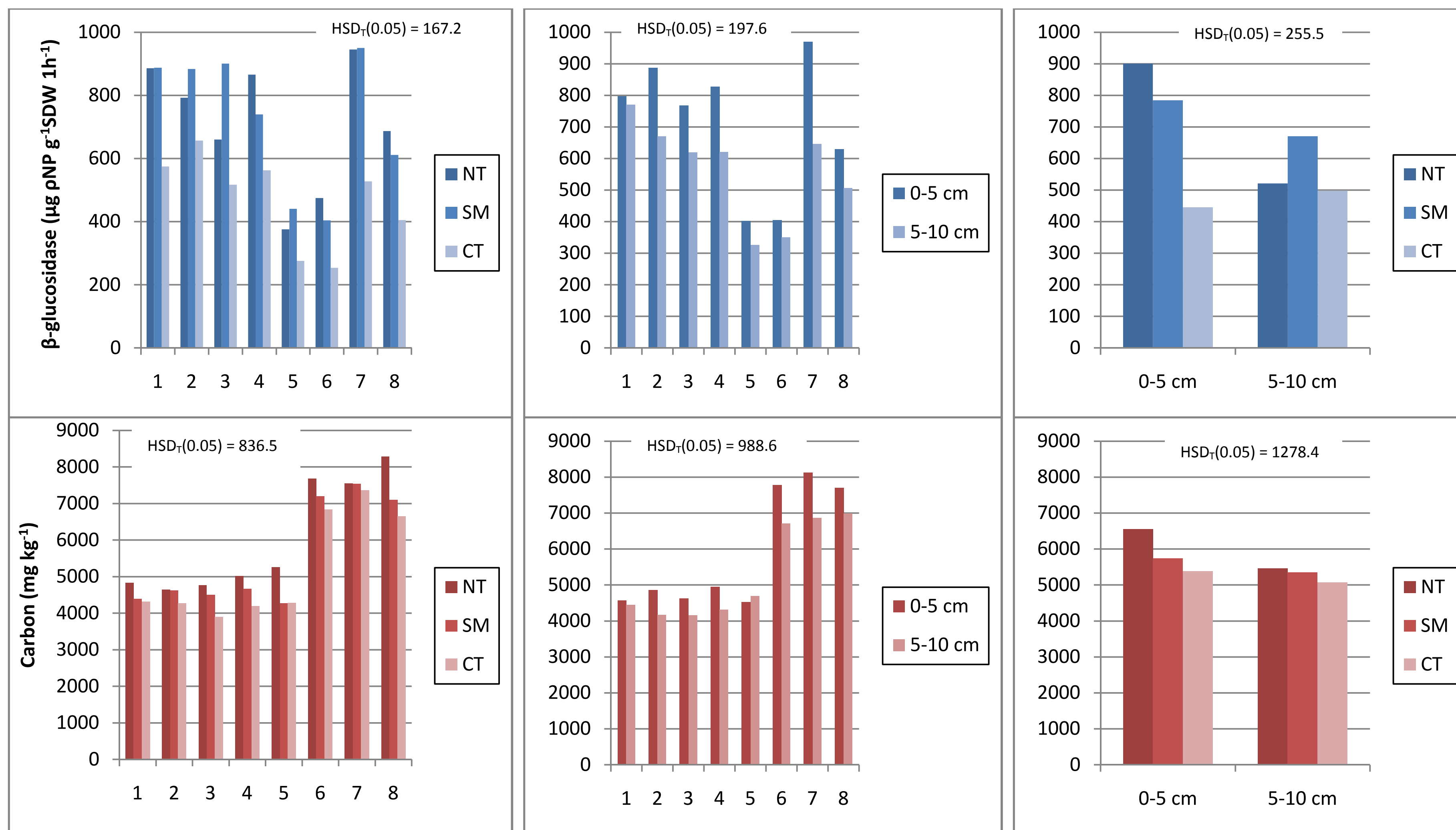


Figure 4.6 Interaction effects shown for potential β-glucosidase activities and carbon contents. Tukey values are included for each.

4.2.2 Acid- and alkaline-phosphatase and phosphorus

Acid- and alkaline phosphatase showed different trends and were present in different amounts (Figure 4.7; Figure 4.10). Acid phosphatase was present in quantities more than doubling those of alkaline phosphatase; the soil pH averaged 6.2 across all treatments, depths, and sampling dates, and since the soil was more acidic this backs up the previous finding of the dominance of acid phosphatase.

The correlation between acid- and alkaline- phosphatase enzymes, P, and pH were all poor. Acid phosphatase activity and P had r of 0.00 at the top depth and 0.08 at the lower depth, while alkaline phosphatase was slightly higher at r = 0.37 and 0.32, respectively. Acid phosphatase and pH did not show any relationship (r = 0.53 and 0.00, respectively) and alkaline phosphatase fared slightly better at r = 0.51 and 0.50, respectively. Dick *et al.* (2000) found that the ratio between acid phosphatase and alkaline phosphatase activities was very sensitive to the pH of the soil and predicted changes in pH better than the traditional method with a pH meter. Since the pH values of the soils in this study were all close to each other, this ratio could not apply.

Main effects

Acid phosphatase activity showed a clear trend with regards to tillage treatment, favoring the conservation tillage methods. NT was the highest with 2646.2 µg ρNP g^{-1} SDW $1h^{-1}$, followed by SM 2573.8 µg ρNP g^{-1} SDW $1h^{-1}$ and the lowest at CT with 2334.7 µg ρNP g^{-1} SDW $1h^{-1}$. NT was significantly different from CT, but SM differed from neither. The top depth was lower in value at 2329.5 µg ρNP g^{-1} SDW $1h^{-1}$ than the 5-10 cm depth at 2706.9 µg ρNP g^{-1} SDW $1h^{-1}$ and differed from each other significantly.

The sampling times showed an inverse relationship with P in soil. In Figure 4.8, it was noticed that as the acid phosphatase activity increased over time, the soil P decreased. This suggests that as more P was available in soil, less enzymes were needed (Tabatabai, 1994; Balota *et al.*, 2004). Alkaline phosphatase shares this trend (Figure 4.11), though more clearly. The time when there is less P in the soil also lines up with the fallow period. After

the fertilizer applications, when the crop has utilized it before harvest, there should be less P, and with no crop on the soil, there shouldn't be a large need for P, hence no enzymes. However, the microbial component of phosphatase production is high; alkaline phosphatase only comes from microorganisms (Dick & Tabatabai, 1983) and so when there is less P in the soils, microorganisms will still produce this enzyme to extract available P from the soil, and acid phosphatase comes from both plants and microorganisms, which explains why the trend was less clear. Also, the fallow period had many weeds and an abundance of algae on the surface from the heavy rainy season, and these could also produce acid phosphatase.

P only differed significantly for tillage treatment and sampling date. Tillage treatment showed a clear difference between all three treatments, again favoring the conservation treatments. NT was the highest at 39.6 mg kg^{-1}, SM intermediate at 31.3 mg kg^{-1}, and CT the lowest at 19.7 mg kg^{-1}. Sampling dates showed a clear fertilization bias. During the fallow period, the P content was significantly lower (average of 21.9 mg kg^{-1}) than during the cropped seasons (averages of 36.5 mg kg^{-1} for oat and 34.4 mg kg^{-1} for the wheat growing seasons). P exhibited no significant difference and no trend between depths.

Alkaline phosphatase activity showed differences in tillage treatment, sampling date, and depth, and besides urease was the only parameter to show differences in the replications (blocks) (see Table 4.2 and Figure 4.11). It is interesting to note that as acid phosphatase was the only parameter to increase with depth, its sister enzyme, alkaline phosphatase, decreased as per usual (Figure 4.8, 4.11). This could be due to the presence of plant roots which produce only acid, not alkaline, phosphatase, and are more common just below the soil surface. In Aon and Colaneri (2001) the enzymes all decreased with depth, except for dehydrogenase, which defied expectations only once during their study.

Interaction effects

Acid phosphatase and P did not have any interaction effects (Figure 4.9).

For the alkaline phosphatase parameter (Figure 4.12), the combined treatment of sampling date and tillage treatment and that of tillage treatment and depth were both significant. NT

led in all sampling times and CT was always lowest. NT was significantly higher than CT during the sampling dates 4 through 8, but the sampling dates that fell in the first cropping period were much closer in value across the tillage treatments. With the tillage treatment across depths, no difference was found at the 5-10 cm layer, but NT was significantly higher than SM and CT at the 0-5 cm layer, which also showed a better trend across tillage treatments.

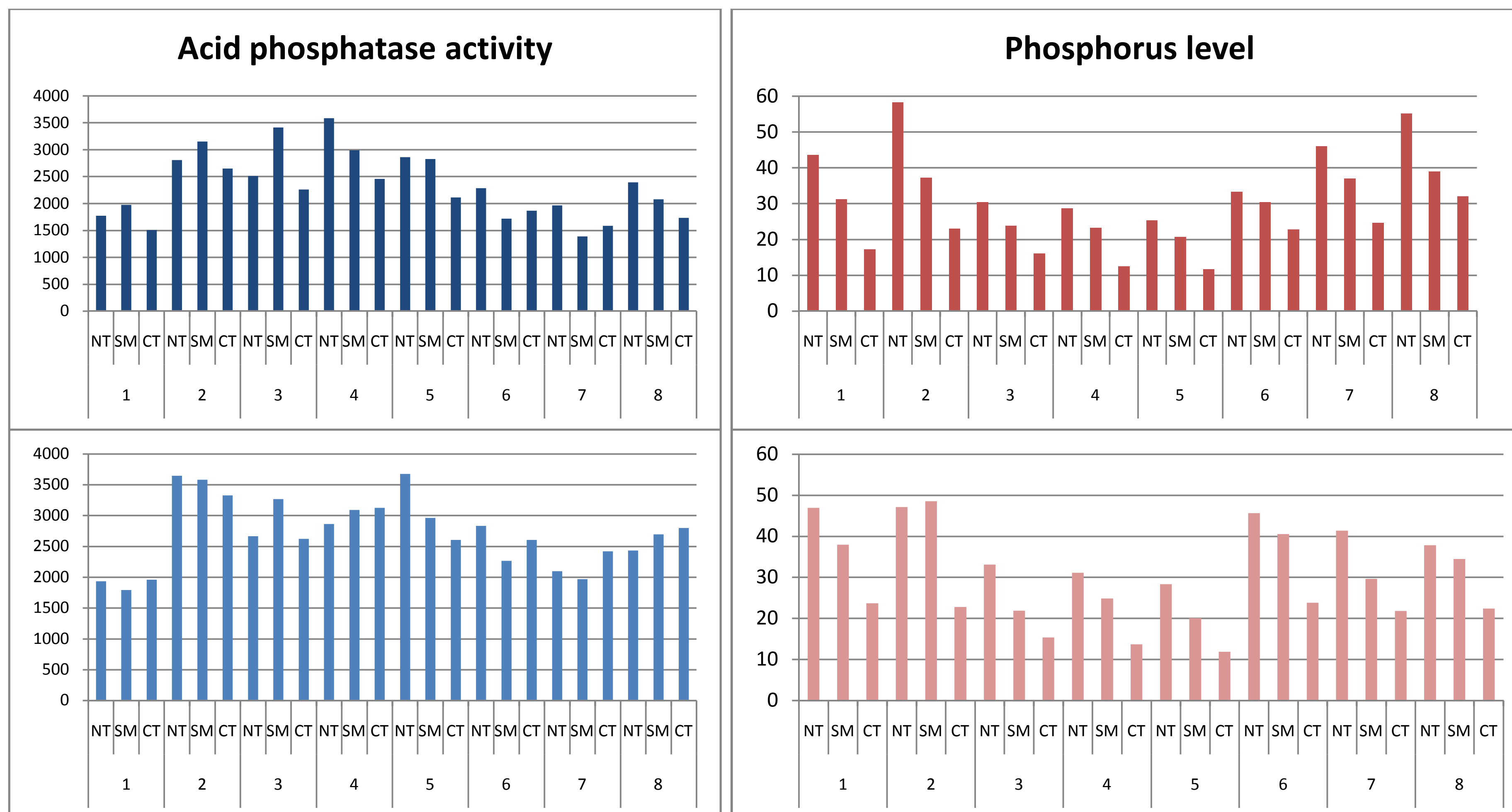


Figure 4.7 All values for potential acid phosphatase activities (µg ρNP g^{-1} SDW $1h^{-1}$) and phosphorus contents (mg kg^{-1}). The top figures are from the 0-5 cm depth and the bottom figures are from the 5-10 cm depth.

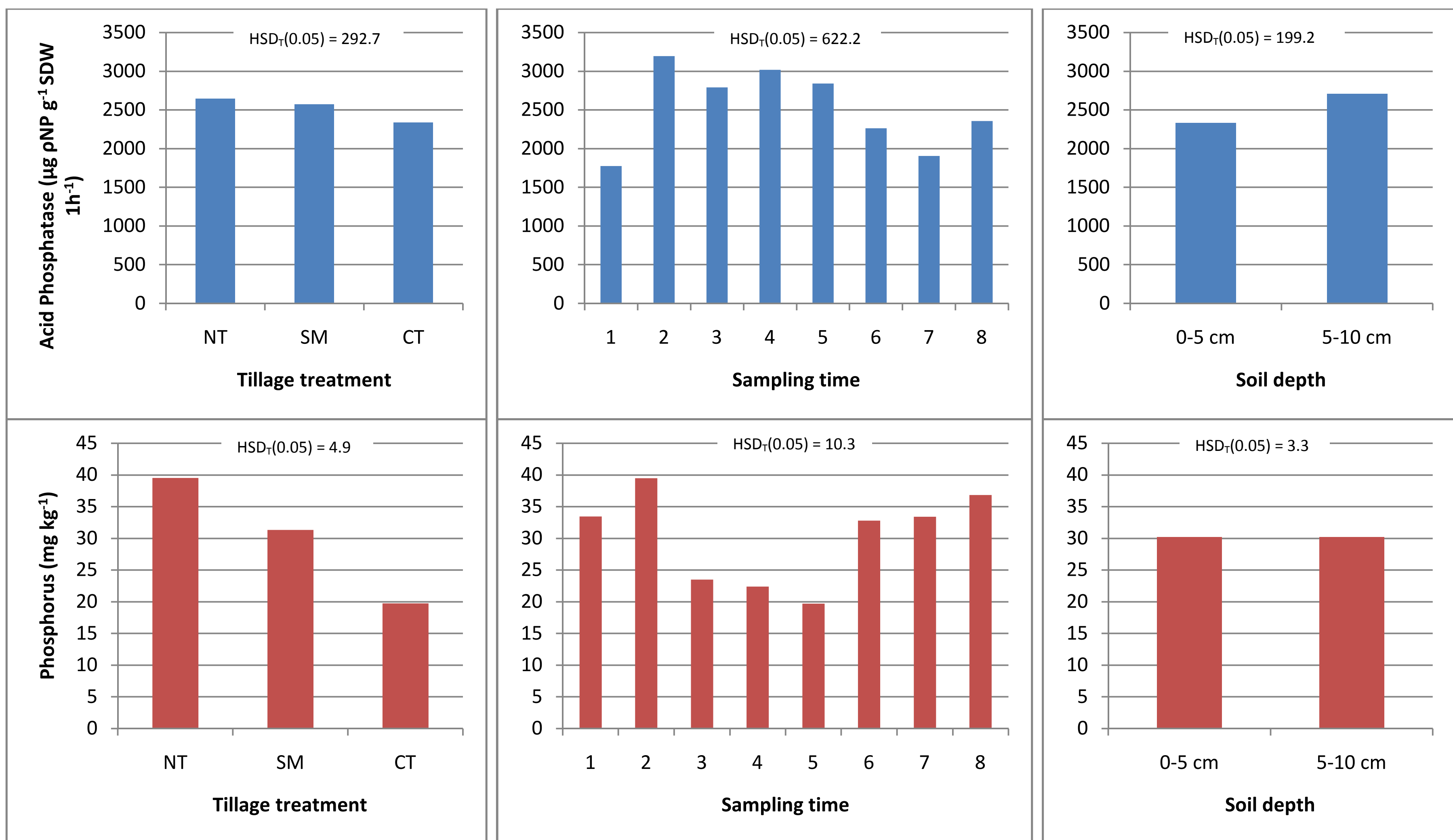


Figure 4.8 Main effects for potential acid phosphatase activities and phosphorus contents, across tillage treatments, sampling time, and soil depth. Tukey values are included for each.

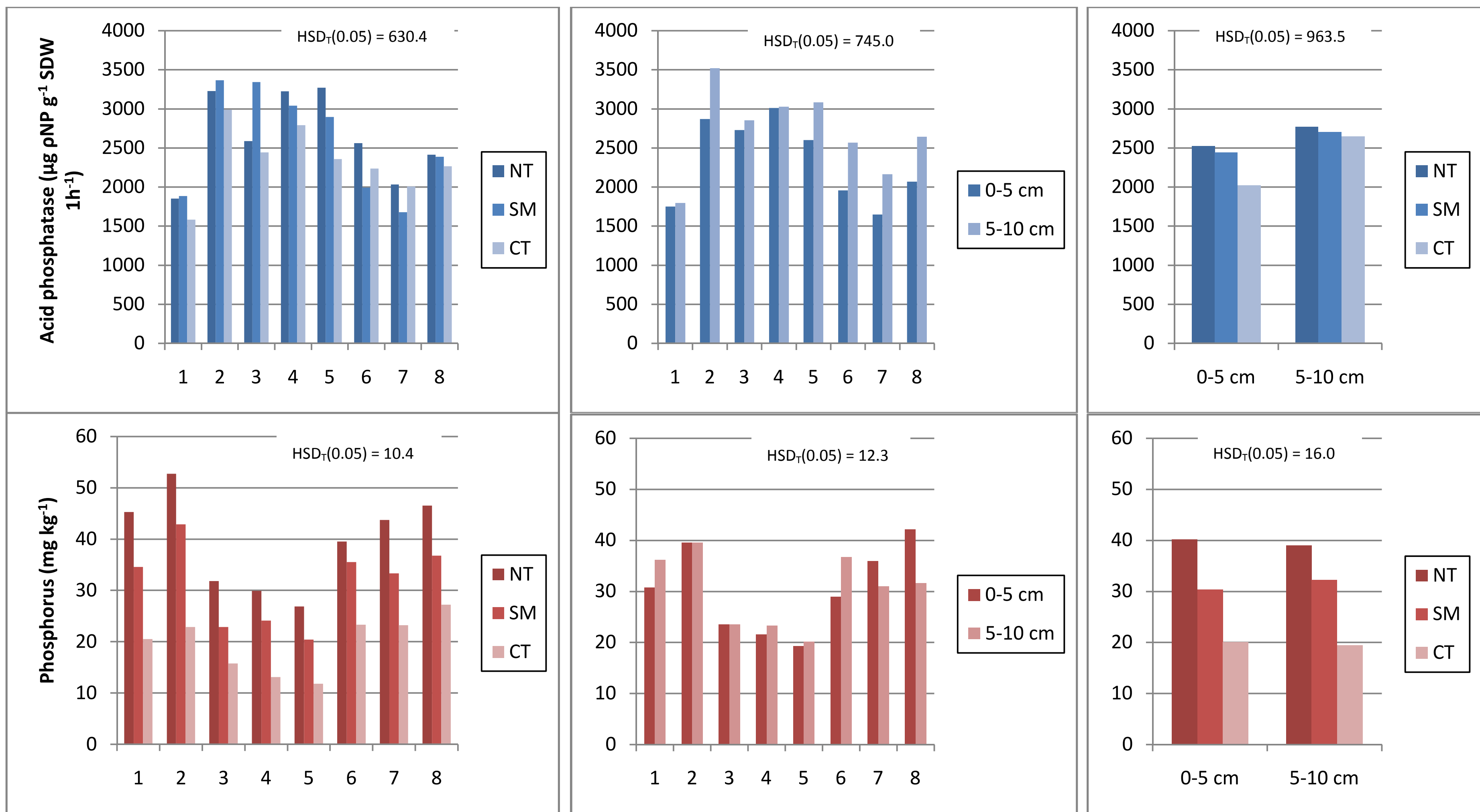


Figure 4.9 Interaction effects for potential acid phosphatase activities and phosphorus contents. Tukey values are included for each.

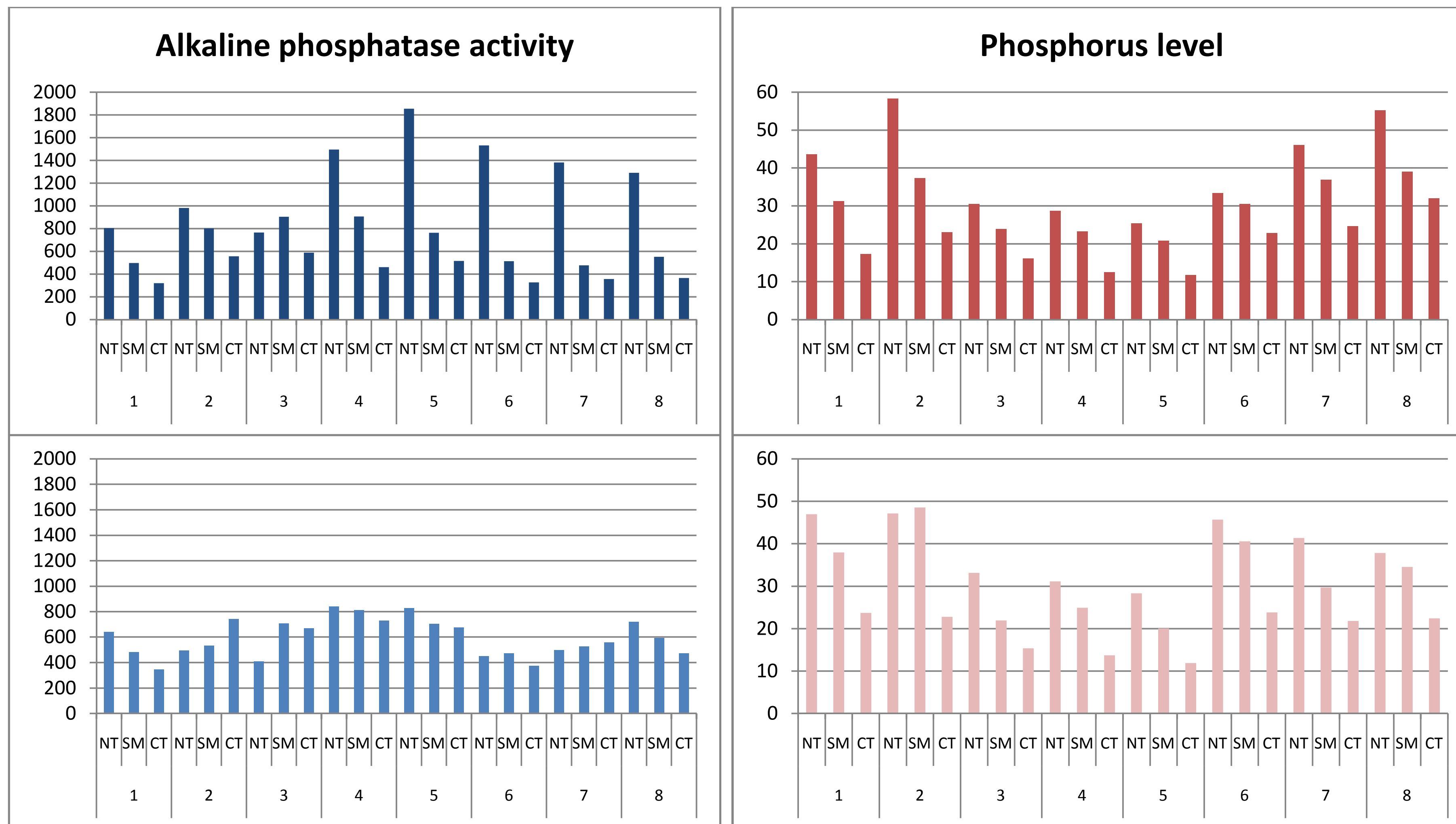


Figure 4.10 All values for potential alkaline phosphatase activities (µg ρNP g^{-1} SDW $1h^{-1}$) and phosphorus contents (mg kg^{-1}). The top figures are from the 0-5 cm depth and the bottom figures are from the 5-10 cm depth.

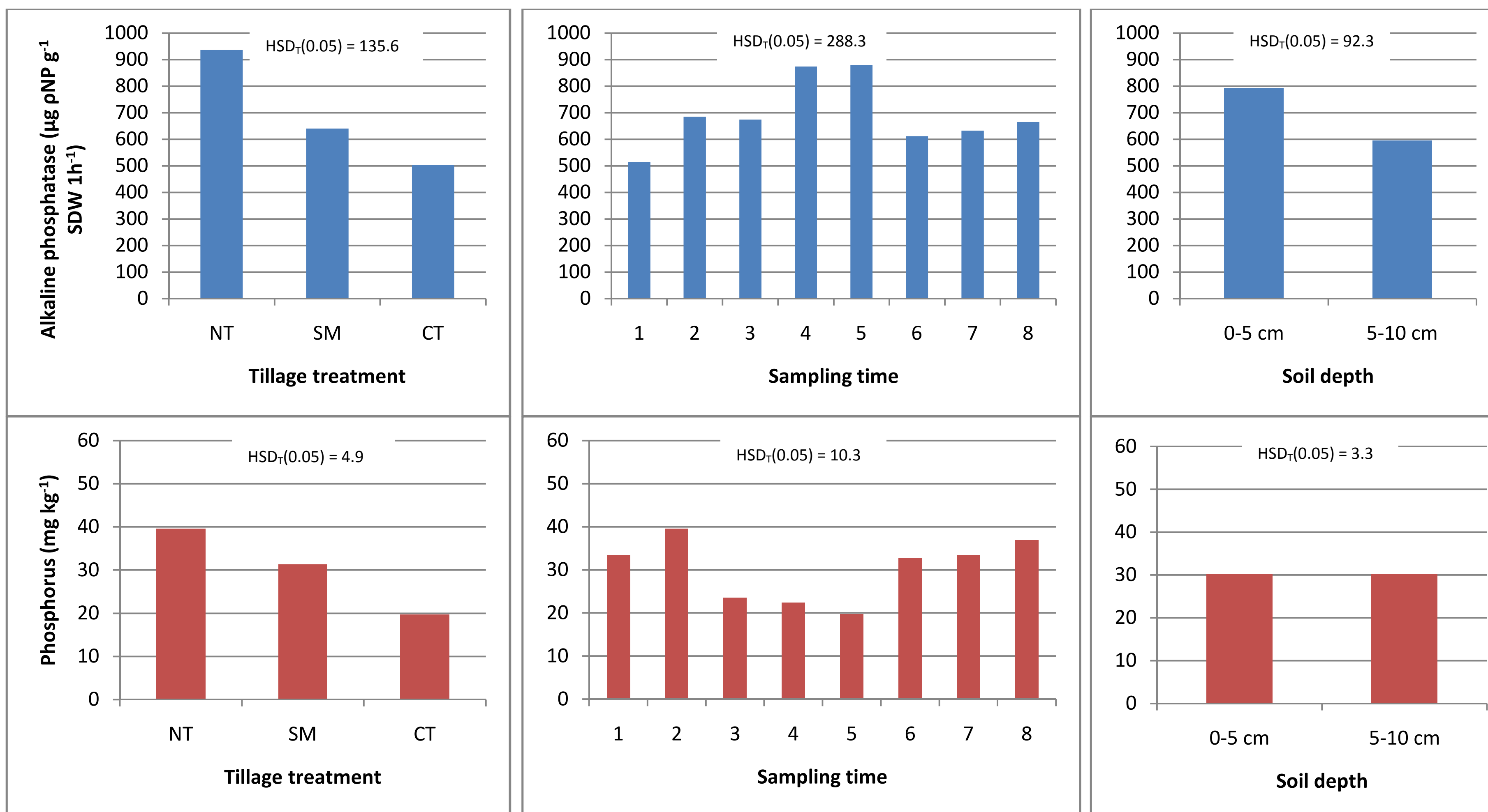


Figure 4.11 Main effects for potential alkaline phosphatase activities and phosphorus contents, across tillage treatments, sampling time, and soil depth. Tukey values are included for each.

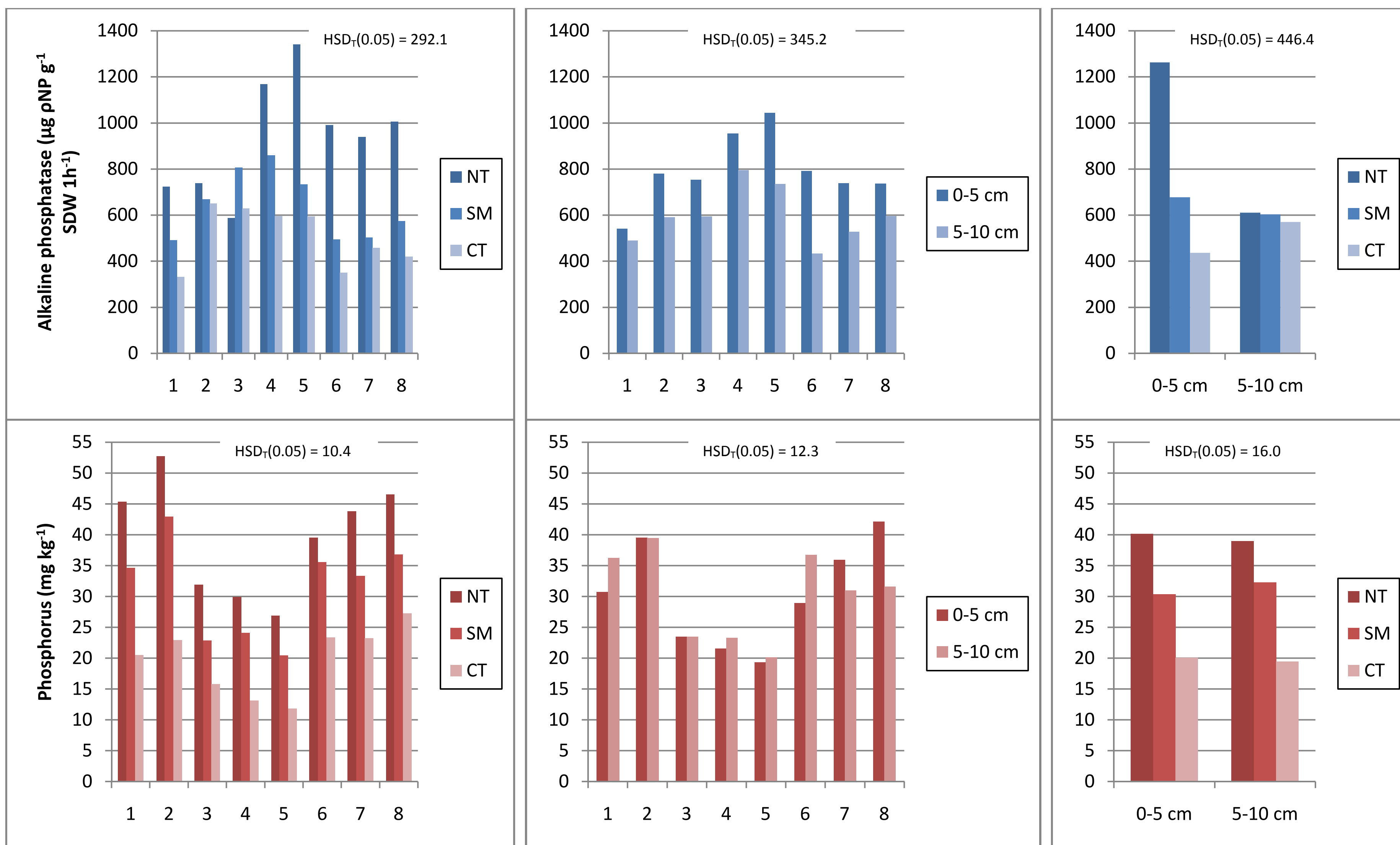


Figure 4.12 Interaction effects for potential alkaline phosphatase activities and phosphorus contents. Tukey values are included for each.

4.2.3 Urease and nitrogen

The correlation between urease and N was very low at both soil depths (r = 0.13 and 0.04, respectively; see Figure 4.13).

Main effects

Urease had significant differences in all of the main effects, as well as across replications (blocks). Nitrogen also had significant effects in all treatments (tillage, depth, and sampling time; Figure 4.14). Amongst tillage treatments, N was highest in NT (860.8 mg kg^{-1}), which was significantly different from SM (764.5 mg kg^{-1}), which was again significantly higher than CT (705.3 mg kg^{-1}). N was higher in the top soil layer (825.4 mg kg^{-1} versus 738.3 mg kg^{-1}).

Interaction effects

Urease showed sensitivity to the combinations of all treatments in the two-way interaction (Table 4.2; Figure 4.15). In the sampling dates and tillage treatment combination, seven out of eight sampling dates showed that NT and SM were significantly higher. For the second sampling date, which occurred right at the start of the rainy season, the SM and the CT treatment were significantly higher than the NT. Across the sampling dates and divided by depth, only the first sampling date did not agree with the trend of the 0-5 cm depth significantly higher than the 5-10 cm layer, though due to the dryness of the soil, that sampling date has concerns about the sampling process.

Across tillage and depth, NT and SM (27.2 and 25.0 µg NH_4-N g^{-1} SDW $2h^{-1}$, respectively) showed a good trend favoring urease activity in the 0-5 cm layer with CT significantly lower at 15.0 µg NH_4-N g^{-1} SDW $2h^{-1}$. The 5-10 cm layer, however, did not differ significantly. In Bonanomi *et al*. (2011) the only soil depth analyzed was 0-20 cm, and urease activities were significantly higher in less intensive agricultural treatments as opposed to high intensity tillage. As in our study, when the two depths are put together, the less intensive tillage practices (NT and SM) exhibited significantly higher urease activities than the high intensity tillage practice (CT).

Nitrogen had a significant interaction between sampling dates and depths. Only sampling dates one, four, and six were statistically significant, each with the 0-5 cm depth higher than the 5-10 cm depth, though that is the trend shown in the other dates as well. The differences in soil depth and tillage treatment could be due to the availability of crop residues on the soil in NT and SM treatments. The difference over time could be a function of crop demand of N during the cropping period and leftover fertilizer and crop residues during the fallow period.

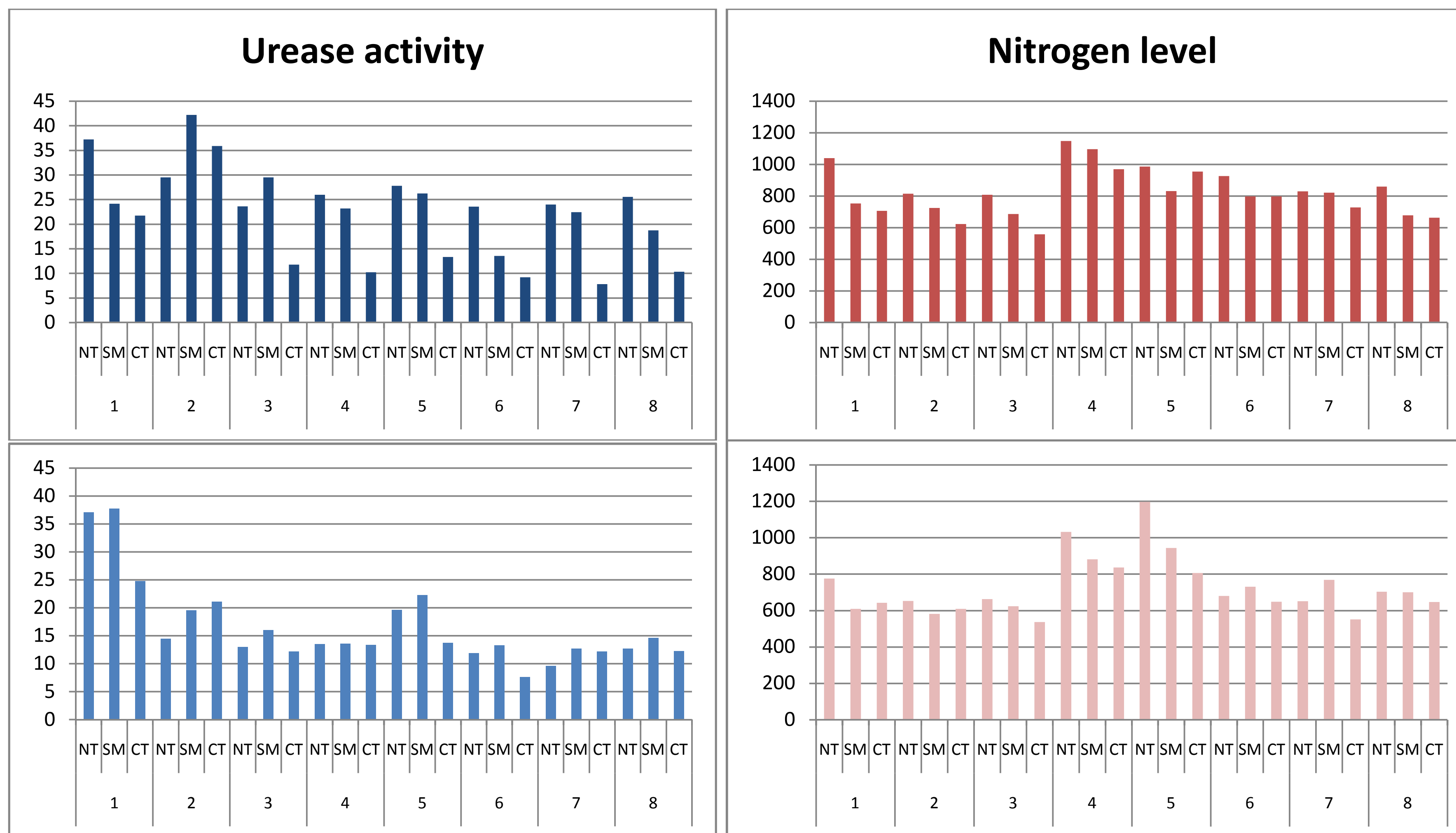


Figure 4.13 All values for potential urease activities (µg NH_4-N g^{-1} SDW $2h^{-1}$) and nitrogen contents (mg kg^{-1}). The top figures are from the 0-5 cm depth and the bottom figures are from the 5-10 cm depth.

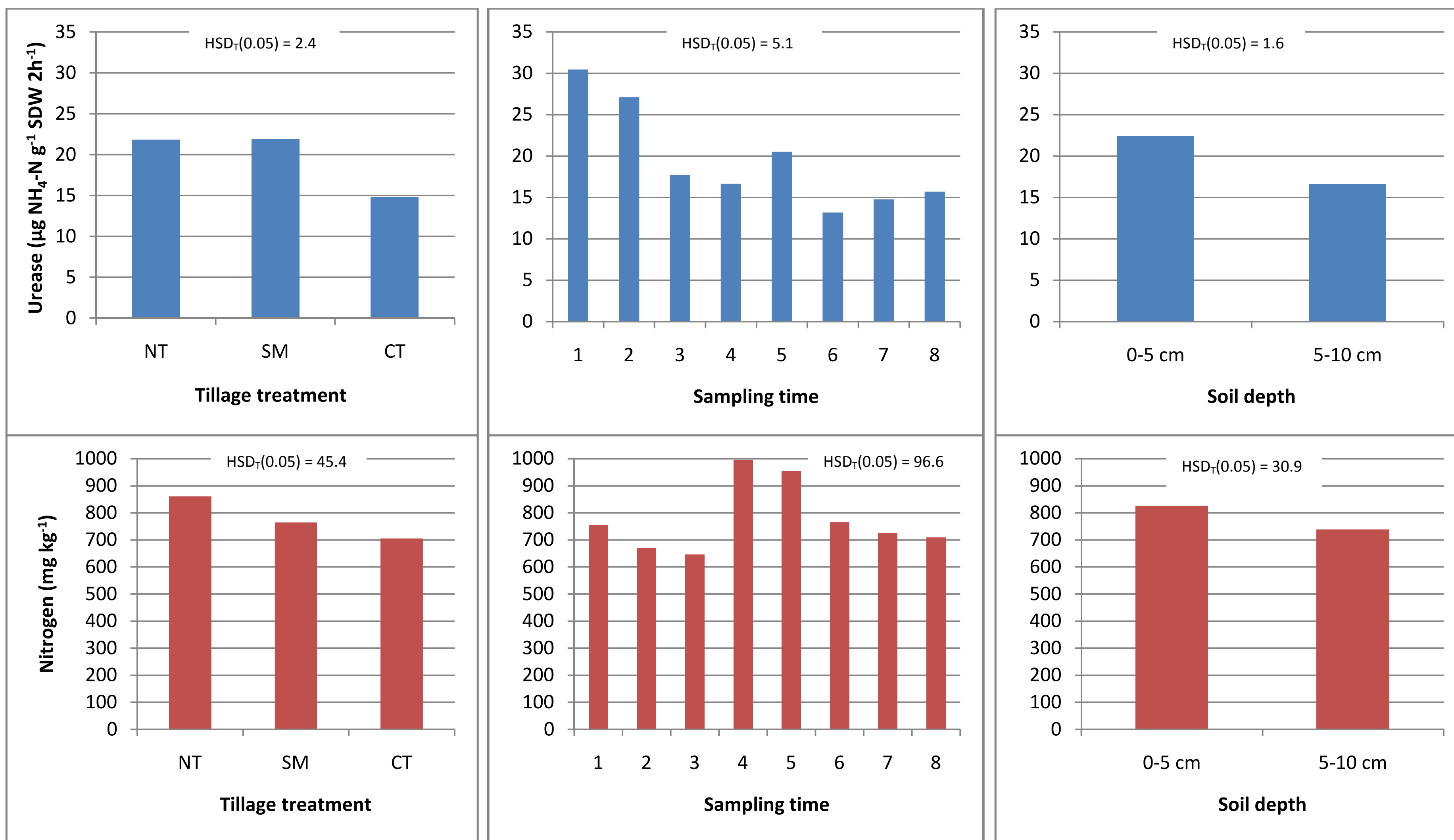


Figure 4.14 Main effects for potential urease activities and nitrogen contents, across tillage treatments, sampling time, and soil depth. Tukey values are included for each.

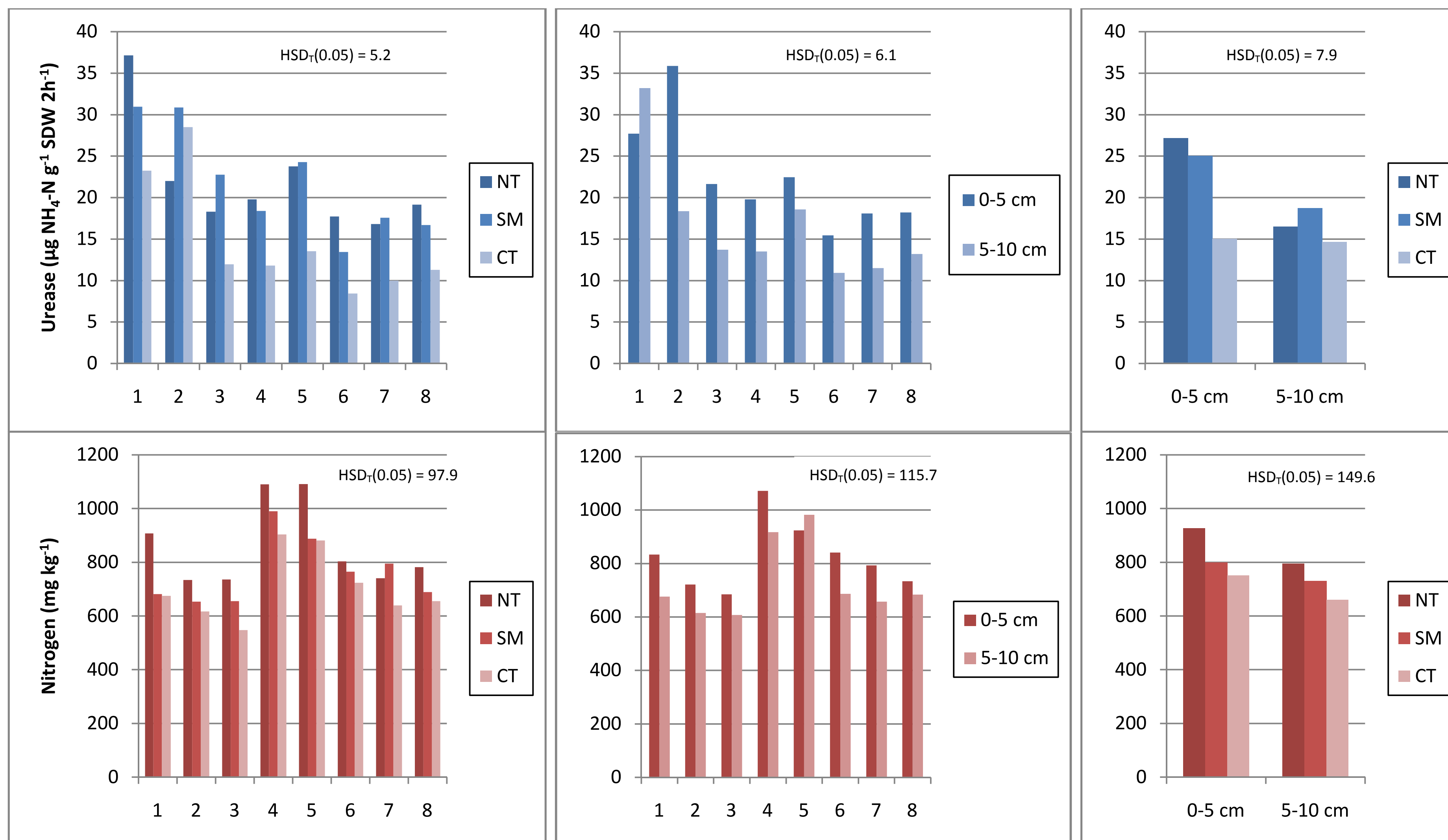


Figure 4.15 Interaction effects for potential urease activities and nitrogen contents. Tukey values are included for each.

4.2.4 Dehydrogenase

Main effects

All values for dehydrogenase activity are shown in Figure 4.16. Dehydrogenase activity had significant effects with sampling dates, tillage treatments, and soil depths. Since dehydrogenase is the enzyme which represents the active MB, following the main effects shows most closely where and when the largest microbial community was thriving (Figure 4.17).

Interaction effects

Potential dehydrogenase activity had sensitivity to sampling dates and depth as well as tillage treatments and depths together, but not across time and tillage treatments.

The climate in the beginning of the study was very dry, as the rainy season had been delayed. In Figure 4.18, this is reflected in the first sampling time separated by depth; it disturbs the general trend of the 0-5 cm layer having a higher potential dehydrogenase activity. For all other sampling dates, the top soil layer is significantly higher in activity.

For the 0-5 cm soil depth, NT and SM (172.4 and 142.8 µg INF g^{-1} SDW $2h^{-1}$, respectively) were significantly higher than CT (95.5 µg INF g^{-1} SDW $2h^{-1}$), but at the 5-10 cm layer, no treatment differs from the other. In Pascaud *et al*. (2012) dehydrogenase activity was the preferred soil quality measurement, above PLFA and others, due to its simple relationship with MB instead of looking at different microorganism categories, and ability to summarize the total oxidative capacity of the soil. Therefore, the MB was of a larger size, and the soil had higher quality, in NT and SM treatments, but the difference is only significant in the top 0-5 cm of soil.

In general, research has found that enzyme activities increase following NT systems. Balota *et al.* (2004) found a large improvement in enzyme activities in NT as compared to CT in a long-term crop rotation trial in Brazil. The results showed an increase of 46% for acid

phosphatase and 61% for alkaline phosphatase in the 0-5 cm soil layer. Correlations were found to be high between the phosphatase enzymes and P, which was not the case for this study. However, the long-term site in Brazil consisted of an Oxisol with 85% clay content. It is known that differences in clay content change microbial properties while other factors stay constant. Types of CT also affect the microbial community. If the tillage was less intense, then differences between CT and NT plots were not as large as in studies which had high intensity CT (Carpenter-Boggs *et al.*, 2003).

Chaer *et al.* (2009) found high correlations between enzyme activities and nutrient levels, particularly SOC. The soil texture ranged from silty loam to clay loam in an experimental forest site. Another study found that OM and dehydrogenase activity had a low correlation. This was attributed to the degraded nature of the soils, resulting in a uniformly low OM content. It was also noted that dehydrogenase would be a more sensitive indicator of change, and as such would change more rapidly than traditional parameters like OM (García *et al*., 1997).

Temporal changes in soil enzymes are another confounding factor in assessing soil quality. However, it has been recorded that despite changes in temperature, rainfall, and season, the inherent quality of certain treatments still demonstrated an increase over other treatments. An example was NT and unburned plots continually having a higher amount of soil quality than CT and burned plots (Aon *et al.*, 2001b; Boerner *et al.*, 2005).

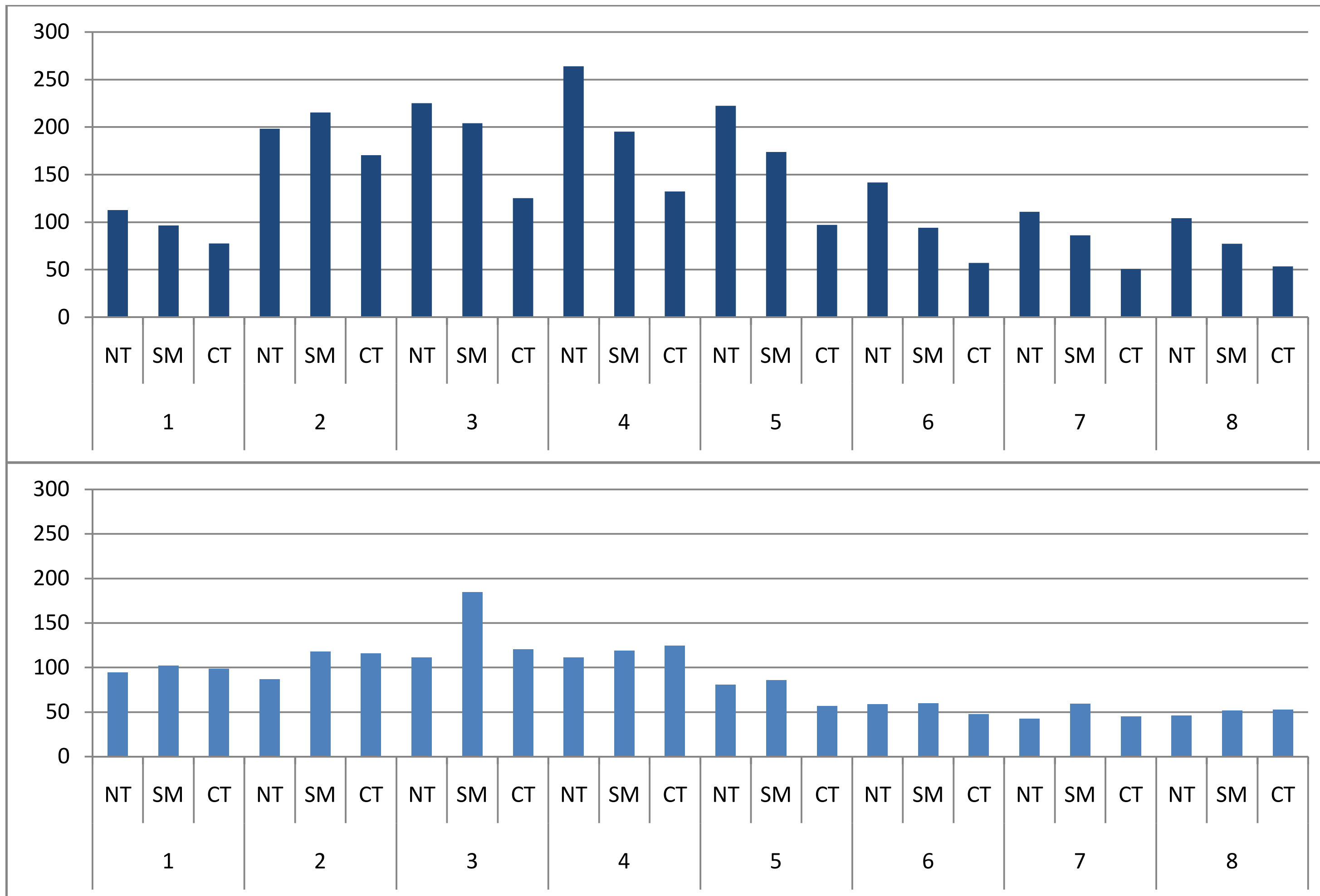


Figure 4.16 All values for potential dehydrogenase activities (µg INF g^{-1} SDW $2h^{-1}$). The top figure is from the 0-5 cm depth and the bottom figure is from 5-10 cm.

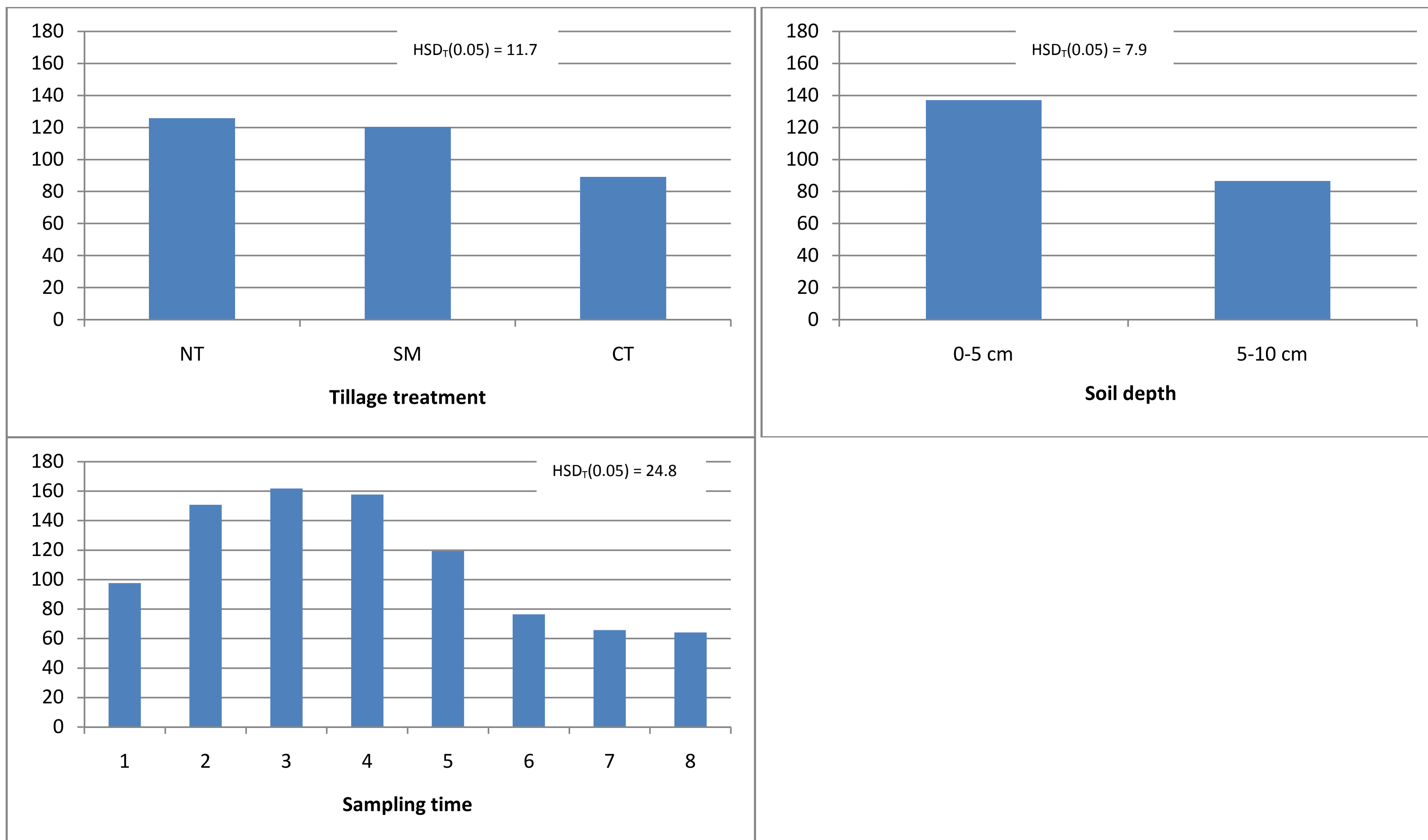


Figure 4.17 Main effects for potential dehydrogenase activities (µg INF g^{-1} SDW $2h^{-1}$) across tillage treatment, sampling time, and soil depth. Tukey values are included for each.

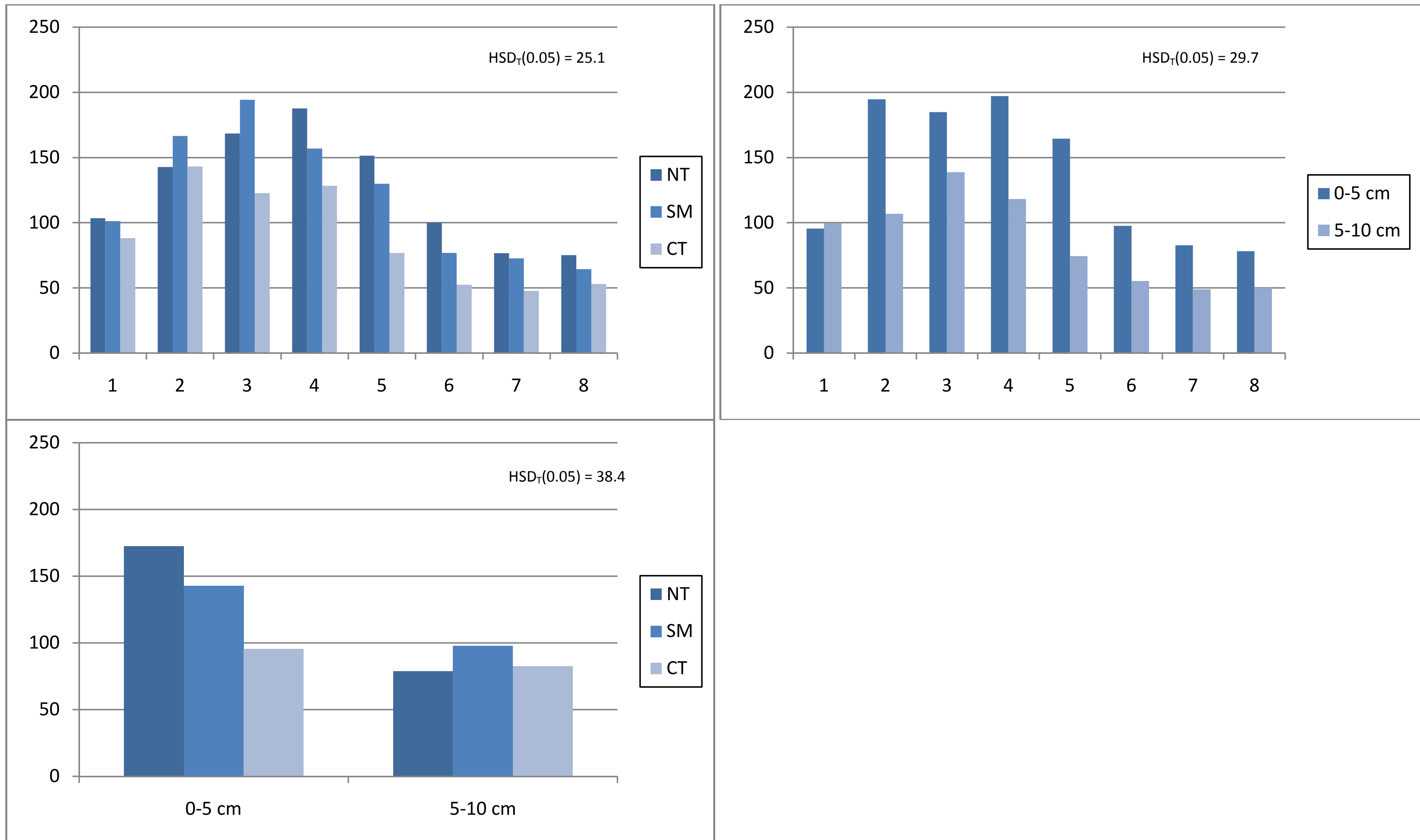


Figure 4.18 Interaction effects for potential dehydrogenase activities (µg INF g^{-1} SDW $2h^{-1}$). Tukey values are included for each.

4.3 Conclusion

Analyzing the different enzymes illustrates which conditions certain enzymes can predict and also demonstrates how the microbial community works better under certain treatments. β-glucosidase seems most sensitive to soil temperature and performed similar in NT and SM, showing higher activity than CT. It also performed best in the 0-5 cm depth.

Acid phosphatase activities also didn't differentiate between NT and SM and placed them higher than CT. Oddly, it had a higher activity in the 5-10 cm depth. The enzyme itself seemed to be sensitive to the presence of its product, phosphate, and the activity mostly followed the fallow and cropping periods, which supported the hypothesis of higher activity in the NT followed by the SM and CT treatments.

Urease showed sensitivity to seasonal and agricultural activity, as it changed the most rapidly and followed no clear trend across time. However, it was higher in the top soil layer, and ranked both NT and SM above CT.

Dehydrogenase also had higher activities in NT and SM together above CT and the 0-5 cm depth above the 5-10 cm depth. Temporally, it was sensitive but also followed trends with known events. The activity was lower in dry periods and also didn't perform well at colder times. It was the highest directly after the rainy season started, when the temperatures were still mild. None of the enzymes correlated to the nutrient it affects or pH.

5 OTHER BIOLOGICAL INDICATORS

5.1 Introduction

Sampling times had to be carefully considered for the other biological indicators, because of constraints which disallowed these indicators to be measured at all eight times. Microbial biomass via fumigation and EE-GRSP were the exception to that, but as explained below, the values did not come through.

BIOLOG community profiling highlighted the general community diversity by reporting how many C sources the microbial community can easily and difficultly use and those they cannot utilize at all. The first sampling time was chosen (Figure 4.3) on advice that during time of unfavorable climatic conditions, the differences between treatments would be more visible.

The PLFA results can indicate many characteristics of the microbial community in the soil. Thus samples from only the first, third, and fifth sampling dates were chosen to represent different moisture and temperature regimes within the study period.

Furthermore, results which evolved from the mentioned biological indicators are dealt with in this chapter.

5.2 Results and discussion

5.2.1 Microbial biomass via fumigation-extraction

There are no MBC and MBN results for this study. A modified method was used to determine MBC and MBN, and unfortunately did not prove effective. It is not known whether the fault was in the chloroform fumigation step, as the vacuum available was never tight enough to bring the chloroform to boiling; or if the Leco analyzer was not sensitive enough to pick up the generally minute difference in MB from the normal C and N sample. It

is recommended for any future users to not go by this method, but to use a TOC analyzer on the extracts of the soil, as is described in Acosta-Martínez *et al*. (2010).

5.2.2 EE-GRSP

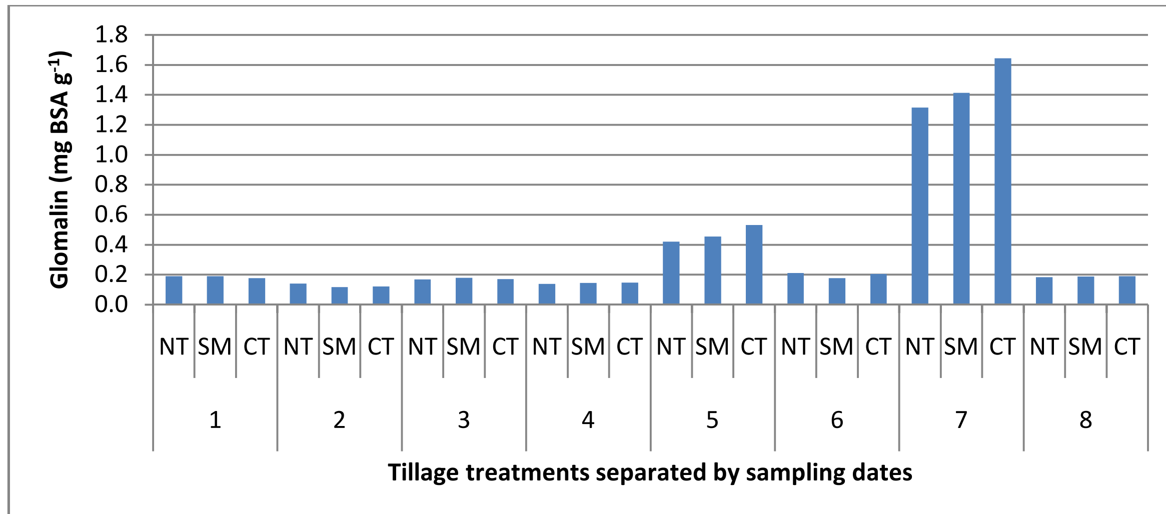


Figure 5.1 Glomalin levels shown across the tillage treatments and sampling times. It was chosen to combine depths due to the low variance between the two.

The results for EE-GRSP (easily extractable – glomalin related soil protein) in this study were considered under the detectable limits. This is because the laboratory assay showed a constantly low amount for all sampling times except for five and seven (Figure 5.1). The higher amounts of bovine standard albumin (BSA) detected by the coloring reagent in the assay were considered to be a false positive. Instead, it was noticed that the longer the delay before the autoclaved citrate buffer solution was centrifuged and the solution was separated from the soil pellet, the higher the color response would be. Given that laboratory assays should not be sensitive to a small amount of time, waiting for the centrifuge to be available or waiting for the autoclave to cool down and continuing on with another assay during the wait, the results were considered to be the figment of one of two scenarios:

1. The amount of glomalin was undetectable and only a product of the dye and how well it bonded with unknown compounds in the soil solution.

2. The glomalin levels were unknown due to the unreliability of the assay.

However, given the lack of PLFA signatures from the fungal species thought to produce glomalin, the first scenario will be provisionally accepted.

5.2.3 BIOLOG whole community profiling

Figure 5.2 shows the averages of the three replications for sampling time one (October 2010) at two depths from a 96-well microplate with 31 individual C sources (BIOLOG EcoPlates^{TM}). All results are tabulated in the Appendix (Table 1, 2, and 3). At the 0-5 cm depth, NT and SM appear nearly the same with regards to positive, late positive, and negative results. CT has fewer positive results, though the negative results again do not change. Instead, the bulk of CT can be described as a late positive reaction. This means that microorganisms present in the solution can make use of the C source, but only as a last resort. Further, it refers to the assumption that the microorganisms needed to adjust to the unfamiliar C sources it found in the plate and therefore the C sources in the CT plots are less diverse than in the NT and SM plots.

At the 5-10 cm depth, a more clear distinction is seen in the results. NT has the highest amount of positive results, followed by SM, and then lastly CT. The negative results again stay the same. This, again, indicates that there is greater diversity of microorganisms in the NT, and to some extent the SM, plots are familiar with a diversity of C sources, and those plots quite possibly hold a more diverse community of microorganisms to begin with. The microbial community in the CT plots are not used to certain C sources, but that does not mean they have lost the ability to quickly adapt.

Since the crop is the same and only tillage treatment differs for this part of the study, it is possible that the microbial communities are composed of nearly the same members, but that those in more undisturbed conditions (*i.e.,* NT and SM) are more robust in their activities.

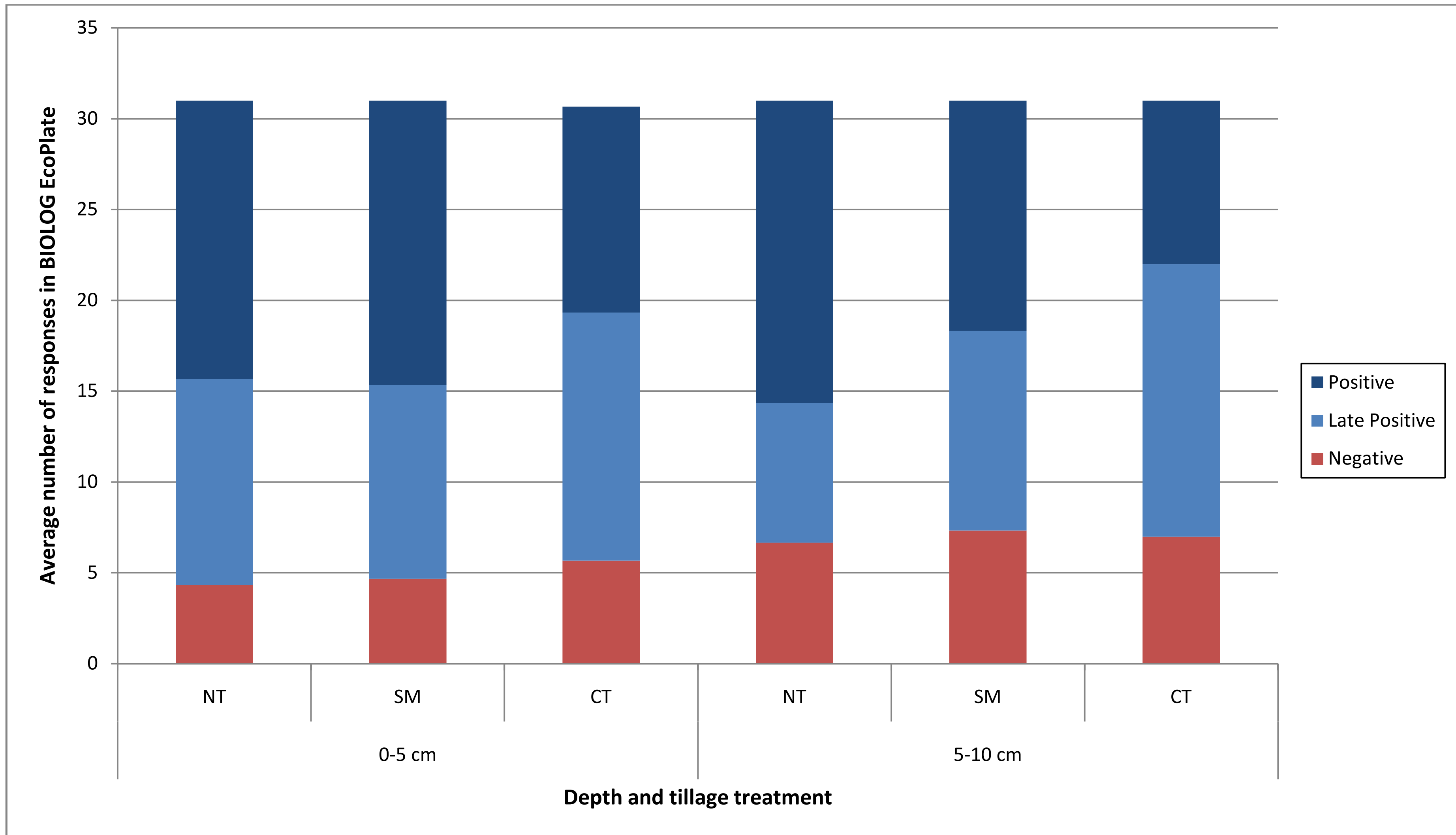


Figure 5.2 Results from the BIOLOG EcoPlates^{TM}, averaged across three replications, from the first sampling date (October 2010).

5.2.4 PLFA

Figure 5.3 shows a stacked bar graph with amounts of each PLFA identified (PLFA which could not be identified were left out) and the total amounts of PLFA for each tillage treatment at three different sampling dates (Table 4, 5, and 6 in the Appendix). Only the top 0-2 cm of soil was collected. All soil samples contained the same identified PLFA signatures in about the same ratios of the total. However, total PLFA (nmol g^{-1}) changed across tillage treatments and season.

From the soil collected in October, 2010, a clear trend was noted, echoing the general trend seen throughout this study, that the NT and SM had higher amounts of PLFA than CT, although the community composition did not change. The climatic conditions which characterized the first sampling date were dry with temperatures at the maximum in the 20's and the minimum fell below 10°C. In February 2011, the maximum temperature was in the 20's and the minimum near 10°C, and a rainfall of 102.4 mm in the month of February alone. The trends were not very clear at this sampling date, with CT increasing to the values of PLFA in NT and passing SM. This may be indicative of a phenomena pointed out for choosing the sampling time for BIOLOG profiling; that when nutrients and soil moisture are not a limiting factor, it is harder to see the difference between treatments which encourage an improvement in soil quality and those that do not. In this case, it appeared that CT had a microbial community just as diverse as that of the NT and SM plots.

However, sampling time proved important, because that was only the case for this sampling time; if this was the only sampling period used, then no difference would have been noted in PLFA. At the fifth sampling date, less precipitation fell with only 66.8 mm in April. The highest temperature was at 10-20°C and the lowest temperature below 5°C. Once again, the trend favoring NT and SM was observed (Figure 5.3). Across all times, NT steadily increased in total amount of PLFA, as did SM, though less dramatically. CT showed both an increase and a decrease across time.

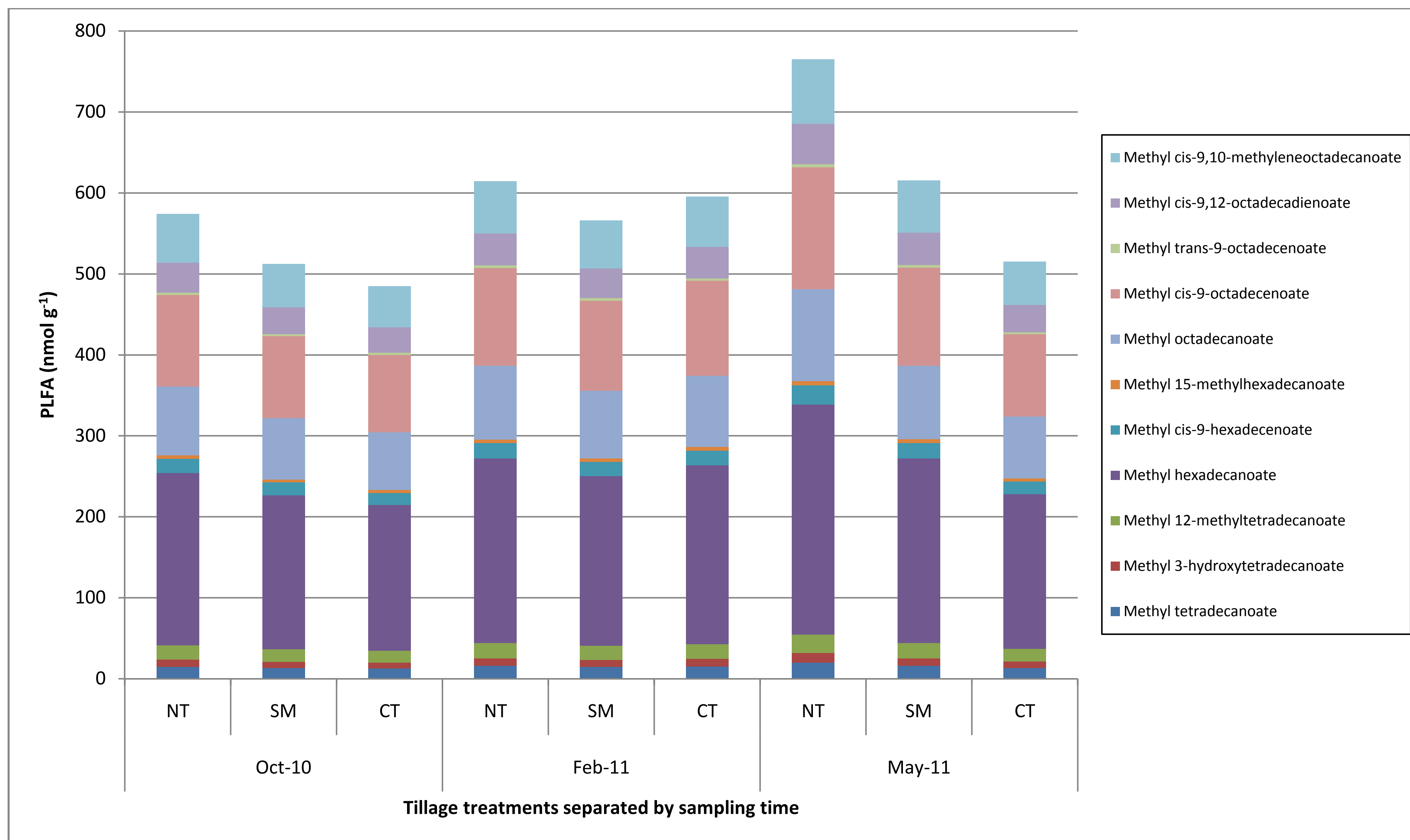


Figure 5.3 The amounts of PLFA compounds detected in each sample, averaged across the three replications.

It has been noted that the total moles of PLFA is proportional to the active MB. In Hedrick *et al*. (2005) 2.5 x 10^4 cells is assigned for every pmol PLFA per gram soil. The calculated biomass (Table 5.1) based on this assumption follows the same trends as above. The ratios for determining different conditions in the soil put forth by Diedhiou *et al.* (2009) cannot be calculated due to lack of certain PLFA signatures, including fungi and actinomycetes.

Table 5.1 A summary of the MB estimated from the PLFA results

	October 2010			February 2011			May 2011		
Treatment	**NT**	**SM**	**CT**	**NT**	**SM**	**CT**	**NT**	**SM**	**CT**
Cells g^{-1}	13 772	12 288	11 629	14 744	13 574	14 283	18 358	14 757	12 358

The signature for *Glomus* species of fungi, which is thought to produce glomalin, was absent. It could still be present in another, unknown, PLFA or present in amounts lower than the detection limit for the method. Diedhiou *et al.* (2009) sampled from the Ap horizon (0-10 cm) and ran all the analyses on this sample size, including PLFA, which indicates that some PLFA signatures may be located at a lower depth than the 0-2 cm depth used in this study. However, given the modifications made to the laboratory assay in order to catch any PLFA signatures, *e.g.,* increasing the grams of soil used per the same amount of chemicals and pulling out as much extract as possible, instead of the standard 1 mL, suggest that the soil would not have contained any significant amount of PLFA at a lower depth.

The soils contained only bacterial signatures, including C14:0 and C16:0, which are general bacteria, and i-C17:0, which is gram positive bacteria. None of the other compounds matched known PLFA signatures, so it is only known that bacteria were present in the soil.

In general, research has found that microbial community structure, measured via BIOLOG community profiling and PLFA analysis, changes over time and with different treatments (Frostegård *et al.*, 1993; Berg & Smalla, 2009; Baumann *et al.*, 2011; Bonanomi *et al.*, 2011; Hossain & Sugiyama, 2011; Jindo *et al.*, 2012). Lupwayi *et al.* (2001) analyzed soil quality using BIOLOG community profiling. They found no significant difference between NT and CT treatments for any index of diversity. However, the treatments did show changes in which C sources were utilized by the communities.

Fatty acid analysis usually yields a large number of different identified PLFA signatures. Feng *et al.* (2003) found 66 different PLFA in all their samples, 46 of which were consistent throughout. There was a difference between the NT and CT treatments. Different PLFA dominated the treatments, though overall, the NT had a higher total amount of PLFA. It was concluded that NT improved soil quality, particularly in the surface soil layer.

5.3 Conclusion

Of the other biological indicators tested, only PLFA and BIOLOG profiling provided sensitive detection of soil biological activities. Both illustrate the relative diversity of the soil microbial community. The BIOLOG EcoPlates™ results indicated that the NT and SM treatments had a more robust and diverse microbial community than the CT. PLFA added to these results and suggested that while the microbial community is made out of similar, if not the same, organisms, the NT and SM yet again carried an advantage by having a larger community. Microbial biomass acts as a nutrient reservoir and produces a large amount of the enzymes which convert these compounds into plant available nutrients, so a larger community is considered a characteristic of a soil with a higher quality (Jacinthe *et al.*, 2011).

6 SUMMARY AND RECOMMENDATIONS

The results of this dissertation came out in favor of conservation tillage practices according to soil enzyme, BIOLOG, PLFA, and nutrient values. Therefore, some of the postulated hypotheses (Section 1.3) for this dissertation were not supported. In hypothesis (a) it was put forth that NT would have the highest of all enzyme results, followed by SM and lastly by CT. Instead, it was found that generally NT did not differ significantly from SM but both were higher than CT. That was the case for β-glucosidase, acid phosphatase, urease, and dehydrogenase. Only alkaline phosphatase grouped NT above SM and CT and the latter treatments did not differ.

Dehydrogenase represents the active MB and was assumed to be able to correlate to MBC and MBN; since MB via fumigation was unsuccessful, the MB from PLFA was used. The results supported the finding that NT and SM have higher biomass than CT. It was found that pH maintained a very low correlation for both acid and alkaline phosphatase, which was stipulated in hypothesis (c), and also with the correlation between enzyme activities and nutrient levels from (d).

According to the PLFA results, no glomalin-producing fungi were present in the soil samples, or possibly that the concentration was under the detection limits, which explains why the glomalin assay gave unverifiable information. So hypothesis (e) could not be affirmed nor denied with regards to the correlation of glomalin and tillage practices.

Tillage disturbs the natural state of soil (Laudicina *et al*., 2012). The microbial community in the conservation tillage treatments, NT and SM, showed improved potential soil enzyme activities, enhanced ability to immediately utilize different C sources, a larger MB (based on both PLFA and dehydrogenase), and higher amounts of nutrients (C, N, and P). During sample preparation for the laboratory, a note was made that NT and SM samples held up

better to mixing than the CT samples, importantly when soil water was higher. This is an unverified instance of the presence of WSA in conservation tillage treatments, and a lower amount of stable aggregation indicates poorer microbial habitat and therefore lower microbial activities and biomass.

Aggregation was originally measured with glomalin, the soil protein which acts as glue between soil particles, but since neither glomalin-producing fungi were found in the PLFA output nor was the EE-GRSP method considered functional, the above impression on WSA was the only information available to presume on the microbial habitat quality. Clay content stayed constant during the study period (see Table 7 in the Appendix) and could not have produced any of the variation between treatments. Considering the 5-10 cm depth contained a slightly larger quantity of clay, but generally had a lower microbial activity, this is simply a basic relationship with C and other nutrients and not a function of texture.

The yield of the different tillage treatments, within the plots sampled in this study, are not consistent (Figure 1 in the Appendix). In the last ten years of the records, only six times was NT higher in yield than CT. These differences did not follow the trends in soil microbial enzyme activity and nutrients.

Sampling times are very vulnerable to known and unknown factors causing sudden changes in results, which may or may not be representative of the actual average. The sampling times chosen for this dissertation provided for relatively consistent rankings between the tillage treatments, but with drastically different values which could be traced back to certain conditions prevalent at the study site which varied depending on the sensitivity of the parameters, including temperature influencing β-glucosidase and P fertilizer influencing acid- and alkaline-phosphatase.

Soil depth continued the potential enzyme activity trends when further studied at the 10-15 cm layer for one sampling time. Acid phosphatase continued to increase, though not significantly. The other four enzymes all decreased, though most of them were not significantly different from the 5-10 cm soil layer.

Soil quality is the sum of many variables, including crop yield, nutrient status, enzyme activities, and whole community profiling. However, since there is no formula widely available concerning a soil quality index, the results of this study will be considered as a soil health indication. As such, it can be proposed that conservation tillage does improve soil health under monoculture wheat production. Both NT and SM showed improved soil health indicators as compared to CT, which was always lowest.

For soils with clay contents of 15-20% in the semi-arid summer rainfall region of South Africa, NT and SM agricultural practices are recommended to increase soil health.

Recommendations for future work on soil biological indicators

- Future work must pinpoint either certain culturable microorganisms or provide a more detailed formula for the evaluation of soil quality using selected soil biological indicators.
- A study covering more than one year at a long-term study site would be advantageous in averaging out the effects of sampling time and providing a more constant definition of soil quality.
- The trial used for this dissertation was a monoculture wheat trial, and adding in rotations would provide a higher and more measurable amount of soil health values.

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APPENDIX

Table 1 Complete table of results from the no-tillage treatment for BIOLOG EcoPlates^{TM} from the first sampling period. A (-) indicates a negative result (no growth), a (+) indicates that the C source was utilized by the microbial community, but only as a second choice resulting in a light purple color, and a (++) indicates a strong preference of the microbial community for the present C source

	No Tillage Treatment (NT)					
	0-5 cm depth			5-10 cm depth		
Nutrients	**NT1a**	**NT2a**	**NT3a**	**NT1b**	**NT2b**	**NT3b**
pyruvic acid methyl ester	++	+	+	++	++	++
Tween 40	++	+	+	+	+	+
Tween 80	++	+	+	+	+	+
a-cyclodextrin	++	+	+	++	++	+
glycogen	++	++	+	++	++	+
D-cellobiose	++	++	++	++	++	++
a-D-lactose	++	+	+	+	+	+
ß-methyl-D-glucoside	++	++	++	++	++	++
D-xylose	++	+	+	++	++	+
i-erythritol	+	-	+	-	-	-
D-mannitol	++	++	++	++	++	++
N-acetyl-D-glucosamine	++	++	++	++	++	++
D-glucosaminic acid	++	+	+	+	+	+
glucose-1-phosphate	++	++	++	++	++	++
D,L-a-glycerol phosphate	+	+	+	+	-	-
D-galactonic acid y-lactone	++	++	++	++	++	++
D-galacturonic acid	++	++	++	++	++	++
2-hydroxy-benzoic acid	-	-	-	-	-	-
4-hydroxy-benzoic acid	++	++	++	++	++	++
y-hydroxybutyric acid	-	-	-	-	+	-
itaconic acid	+	+	++	+	++	++
a-ketobutyric-acid	-	-	-	-	-	-
D-malic acid	+	+	+	-	++	-
L-arginine	++	++	++	++	++	++
L-asparagine	++	++	++	++	++	++
L-phenyl-alanine	++	+	++	++	++	+
L-serine	++	++	++	++	++	++
L-threonine	-	+	-	-	-	+
glycyl-L-glutamic-acid	+	+	-	-	-	+
phenyl-ethyl-amine	+	+	+	+	++	-
putrescine	++	+	+	++	++	+

Table 2 Complete table of results from the stubble-mulch treatment for BIOLOG EcoPlates™ from the first sampling period. A (-) indicates a negative result (no growth), a (+) indicates that the C source was utilized by the microbial community, but only as a second choice resulting in a light purple color, and a (++) indicates a strong preference of the microbial community for the present C source

	Stubble Mulch Treatment (SM)					
	0-5 cm depth			5-10 cm depth		
Nutrients	**SM1a**	**SM2a**	**SM3a**	**SM1b**	**SM2b**	**SM3b**
pyruvic acid methyl ester	++	++	+	+	++	+
Tween 40	+	+	+	+	+	+
Tween 80	+	+	+	+	+	+
a-cyclodextrin	+	+	+	++	+	+
glycogen	+	++	++	+	+	+
D-cellobiose	++	++	++	++	++	++
a-D-lactose	-	+	+	+	+	-
ß-methyl-D-glucoside	++	++	++	++	++	++
D-xylose	++	++	+	+	++	+
i-erythritol	+	+	+	+	-	-
D-mannitol	++	++	+	++	++	+
N-acetyl-D-glucosamine	++	++	++	++	++	++
D-glucosaminic acid	++	+	+	+	-	-
glucose-1-phosphate	++	++	++	++	++	++
D,L-a-glycerol phosphate	-	+	-	-	+	-
D-galactonic acid y-lactone	++	++	++	++	++	++
D-galacturonic acid	++	++	++	++	++	++
2-hydroxy-benzoic acid	-	-	-	-	-	-
4-hydroxy-benzoic acid	++	++	++	++	++	+
y-hydroxybutyric acid	+	++	+	-	++	-
itaconic acid	++	-	+	++	++	+
a-ketobutyric-acid	-	-	-	-	-	-
D-malic acid	+	+	++	-	+	+
L-arginine	++	++	+	++	+	+
L-asparagine	++	++	+	++	++	++
L-phenyl-alanine	+	+	++	+	+	-
L-serine	++	++	++	++	++	++
L-threonine	-	-	-	-	-	-
glycyl-L-glutamic-acid	+	+	-	-	-	-
phenyl-ethyl-amine	++	++	++	+	++	+
putrescine	++	++	++	+	++	+

Table 3 Complete table of results from the conventional tillage treatment for BIOLOG EcoPlates^{TM} from the first sampling period. A (-) indicates a negative result (no growth), a (+) indicates that the C source was utilized by the microbial community, but only as a second choice resulting in a light purple color, and a (++) indicates a strong preference of the microbial community for the present C source

	Conventional Tillage Treatment (CT)					
	0-5 cm depth			5-10 cm depth		
Nutrients	**P1a**	**P2a**	**P3a**	**P1b**	**P2b**	**P3b**
pyruvic acid methyl ester	++	++	+	+	+	+
Tween 40	+	+	+	+	+	+
Tween 80	+	+	+	+	+	+
a-cyclodextrin	++	+	+	+	+	+
glycogen	++	+	+	+	+	+
D-cellobiose	++	++	++	++	++	++
a-D-lactose	++	-	+	-	+	+
ß-methyl-D-glucoside	++	++	++	++	++	++
D-xylose	++	+	-	+	+	+
i-erythritol	+	-	-	-	-	-
D-mannitol	++	++	++	+	++	++
N-acetyl-D-glucosamine	+	++	++	++	++	++
D-glucosaminic acid	+	+	+	+	+	+
glucose-1-phosphate	++	++	++	+	+	+
D,L-a-glycerol phosphate	-	+	-	-	-	-
D-galactonic acid y-lactone	++	++	++	++	++	++
D-galacturonic acid	++	++	++	++	++	++
2-hydroxy-benzoic acid	-	-	-	-	-	-
4-hydroxy-benzoic acid	+	+	+	+	+	+
y-hydroxybutyric acid	+	+	+	+	-	+
itaconic acid	+	+	+	+	+	+
a-ketobutyric-acid	-	-	-	-	-	-
D-malic acid	+	+	+	+	+	+
L-arginine	++	+	+	++	++	+
L-asparagine	++	++	++	++	++	++
L-phenyl-alanine	+	+	-	+	++	+
L-serine	++	++	++	++	++	++
L-threonine	+	-	-	-	-	-
glycyl-L-glutamic-acid	+	+	-	-	-	-
phenyl-ethyl-amine	++	-	+	+	-	+
putrescine	+	+	+	+	+	++

Table 4 Full results for PLFA analysis for the first sampling date

		October 2010 (1st sampling date)								
Component name	**Formula**	**NT1**	**NT2**	**NT3**	**SM1**	**SM2**	**SM3**	**CT1**	**CT2**	**CT3**
Methyl tetradecanoate	**C14:0**	14.64	14.89	15.00	14.75	12.91	12.06	13.94	10.98	12.67
Methyl 3-hydroxytetradecanoate	**3-OH-C14:0**	8.58	8.73	8.79	8.65	7.57	7.07	8.17	6.43	7.43
Methyl 12-methyltetradecanoate	**a-C15:0**	17.31	17.60	17.72	17.44	15.26	14.25	16.48	12.97	14.98
Methyl hexadecanoate	**C16:0**	210.05	213.57	215.14	211.65	185.26	172.99	200.03	157.49	181.84
Methyl cis-9-hexadecenoate	**C16:19**	17.52	17.81	17.94	17.65	15.45	14.43	16.68	13.13	15.16
Methyl 15-methylhexadecanoate	**i-C17:0**	4.12	4.19	4.22	4.15	3.63	3.39	3.92	3.09	3.57
Methyl octadecanoate	**C18:0**	83.72	85.12	85.74	84.35	73.84	68.95	79.72	62.77	72.48
Methyl cis-9-octadecenoate	**C18:19**	111.54	113.41	114.25	112.40	98.38	91.87	106.23	83.63	96.57
Methyl trans-9-octadecenoate	**C18:19**	3.01	3.07	3.09	3.04	2.66	2.48	2.87	2.26	2.61
Methyl cis-9,12-octadecadienoate	**C18:29,12**	36.67	37.29	37.56	36.95	32.34	30.20	34.92	27.50	31.75
Methyl cis-9,10-methyleneoctadecanoate	**C19:0Δ**	58.98	59.97	60.41	59.43	52.02	48.57	56.17	44.22	51.06
Total PLFA (nmol g^{-1})		566.13	575.63	579.85	570.45	499.33	466.27	539.14	424.47	490.12

Table 5 Full results for PLFA analysis for the third sampling date

		February 2011 (3rd sampling date)								
Component name	**Formula**	**NT1**	**NT2**	**NT3**	**SM1**	**SM2**	**SM3**	**CT1**	**CT2**	**CT3**
Methyl tetradecanoate	**C14:0**	12.65	18.97	16.04	14.47	15.69	13.72	15.03	15.95	15.19
Methyl 3-hydroxytetradecanoate	**3-OH-C14:0**	7.42	11.12	9.40	8.48	9.20	8.04	8.81	9.35	8.91
Methyl 12-methyltetradecanoate	**a-C15:0**	14.96	22.43	18.96	17.11	18.55	16.22	17.76	18.85	17.96
Methyl hexadecanoate	**C16:0**	181.54	272.21	230.07	207.62	225.10	196.82	215.62	228.83	217.97
Methyl cis-9-hexadecenoate	**C16:19**	15.14	22.70	19.19	17.31	18.77	16.41	17.98	19.08	18.18
Methyl 15-methylhexadecanoate	**i-C17:0**	3.56	5.34	4.51	4.07	4.41	3.86	4.23	4.49	4.27
Methyl octadecanoate	**C18:0**	72.35	108.49	91.70	82.75	89.71	78.44	85.94	91.20	86.87
Methyl cis-9-octadecenoate	**C18:19**	96.41	144.55	122.18	110.26	119.54	104.52	114.51	121.52	115.75
Methyl trans-9-octadecenoate	**C18:19**	2.61	3.91	3.30	2.98	3.23	2.82	3.09	3.28	3.13
Methyl cis-9,12-octadecadienoate	**C18:29,12**	31.69	47.52	40.17	36.25	39.30	34.36	37.65	39.95	38.06
Methyl cis-9,10-methyleneoctadecanoate	**C19:0Δ**	50.97	76.43	64.60	58.30	63.20	55.26	60.54	64.25	61.20
Total PLFA (nmol g^{-1})		489.30	733.67	620.11	559.60	606.70	530.48	581.16	616.77	587.49

Table 6 Full results for PLFA analysis for the fifth sampling date

		May 2011 (5th sampling date)								
Component name	**Formula**	**NT1**	**NT2**	**NT3**	**SM1**	**SM2**	**SM3**	**CT1**	**CT2**	**CT3**
Methyl tetradecanoate	**C14:0**	11.60	21.58	26.17	11.14	21.11	15.45	13.95	9.99	16.02
Methyl 3-hydroxytetradecanoate	**3-OH-C14:0**	6.80	12.65	15.34	6.53	12.37	9.06	8.17	5.85	9.39
Methyl 12-methyltetradecanoate	**a-C15:0**	13.71	25.50	30.93	13.17	24.95	18.27	16.48	11.81	18.93
Methyl hexadecanoate	**C16:0**	166.44	309.57	375.40	159.84	302.84	221.73	200.07	143.29	229.80
Methyl cis-9-hexadecenoate	**C16:19**	13.88	25.81	31.30	13.33	25.25	18.49	16.68	11.95	19.16
Methyl 15-methylhexadecanoate	**i-C17:0**	3.26	6.07	7.36	3.13	5.94	4.35	3.92	2.81	4.51
Methyl octadecanoate	**C18:0**	66.33	123.38	149.62	63.71	120.70	88.37	79.74	57.11	91.59
Methyl cis-9-octadecenoate	**C18:19**	88.38	164.40	199.35	84.88	160.82	117.75	106.25	76.09	122.03
Methyl trans-9-octadecenoate	**C18:19**	2.39	4.44	5.39	2.29	4.35	3.18	2.87	2.06	3.30
Methyl cis-9,12-octadecadienoate	**C18:29,12**	29.06	54.05	65.54	27.91	52.87	38.71	34.93	25.02	40.12
Methyl cis-9,10-methyleneoctadecanoate	**C19:0Δ**	46.73	86.92	105.40	44.88	85.03	62.26	56.18	40.23	64.52
Total PLFA (nmol g^{-1})		448.59	834.37	1011.80	430.82	816.24	597.61	539.24	386.20	619.37

Table 7 Clay content values separated by sampling time

1		2		3		4		5		6		7		8	
Lab ID	**Clay %**	**Lab ID**	**Clay %**	**Lab ID**	**Clay %**	**Lab ID**	**Clay %**	**Lab ID**	**Clay %**	**Lab ID**	**Clay %**	**Lab ID**	**Clay %**	**Lab ID**	**Clay %**
NT1a	16	NT1a	15	NT1a	16	NT1a	14	NT1a	16	NT1a	17	NT1a	16	NT1a	15
NT1b	17	NT1b	18	NT1b	18	NT1b	16	NT1b	17	NT1b	17	NT1b	17	NT1b	16
NT2a	14	NT2a	13	NT2a	14	NT1c	17	NT2a	14	NT2a	17	NT2a	14	NT2a	13
NT2b	15	NT2b	15	NT2b	15	NT2a	14	NT2b	15	NT2b	18	NT2b	15	NT2b	16
NT3a	17	NT3a	17	NT3a	17	NT2b	15	NT3a	17	NT3a	17	NT3a	17	NT3a	17
NT3b	17	NT3b	19	NT3b	19	NT2c	16	NT3b	17	NT3b	19	NT3b	17	NT3b	17
SM1a	18	SM1a	16	SM1a	16	NT3a	16	SM1a	18	SM1a	19	SM1a	18	SM1a	18
SM1b	17	SM1b	18	SM1b	17	NT3b	16	SM1b	17	SM1b	17	SM1b	17	SM1b	17
SM2a	16	SM2a	17	SM2a	18	NT3c	17	SM2a	16	SM2a	16	SM2a	15	SM2a	16
SM2b	16	SM2b	16	SM2b	16	SM1a	18	SM2b	16	SM2b	15	SM2b	16	SM2b	16
SM3a	16	SM3a	15	SM3a	15	SM1b	16	SM3a	15	SM3a	16	SM3a	16	SM3a	16
SM3b	18	SM3b	17	SM3b	17	SM1c	16	SM3b	18	SM3b	17	SM3b	18	SM3b	18
CT1a	18	CT1a	17	CT1a	18	SM2a	15	CT1a	18	CT1a	17	CT1a	18	CT1a	17
CT1b	17	CT1b	17	CT1b	17	SM2b	16	CT1b	17	CT1b	17	CT1b	17	CT1b	16
CT2a	19	CT2a	19	CT2a	19	SM2c	17	CT2a	19	CT2a	19	CT2a	18	CT2a	18
CT2b	18	CT2b	17	CT2b	15	SM3a	16	CT2b	18	CT2b	18	CT2b	19	CT2b	18
CT3a	18	CT3a	19	CT3a	18	SM3b	17	CT3a	17	CT3a	18	CT3a	17	CT3a	18
CT3b	19	CT3b	19	CT3b	19	SM3c	18	CT3b	18	CT3b	19	CT3b	18	CT3b	19
						CT1a	18								
						CT1b	17								
						CT1c	17								
						CT2a	19								
						CT2b	18								
						CT2c	17								
						CT3a	18								
						CT3b	19								
						CT3c	19								

a = 0-5 cm soil depth
b = 5-10 cm soil depth
c = 10-15 cm soil depth
1, 2, and 3 refer to blocks

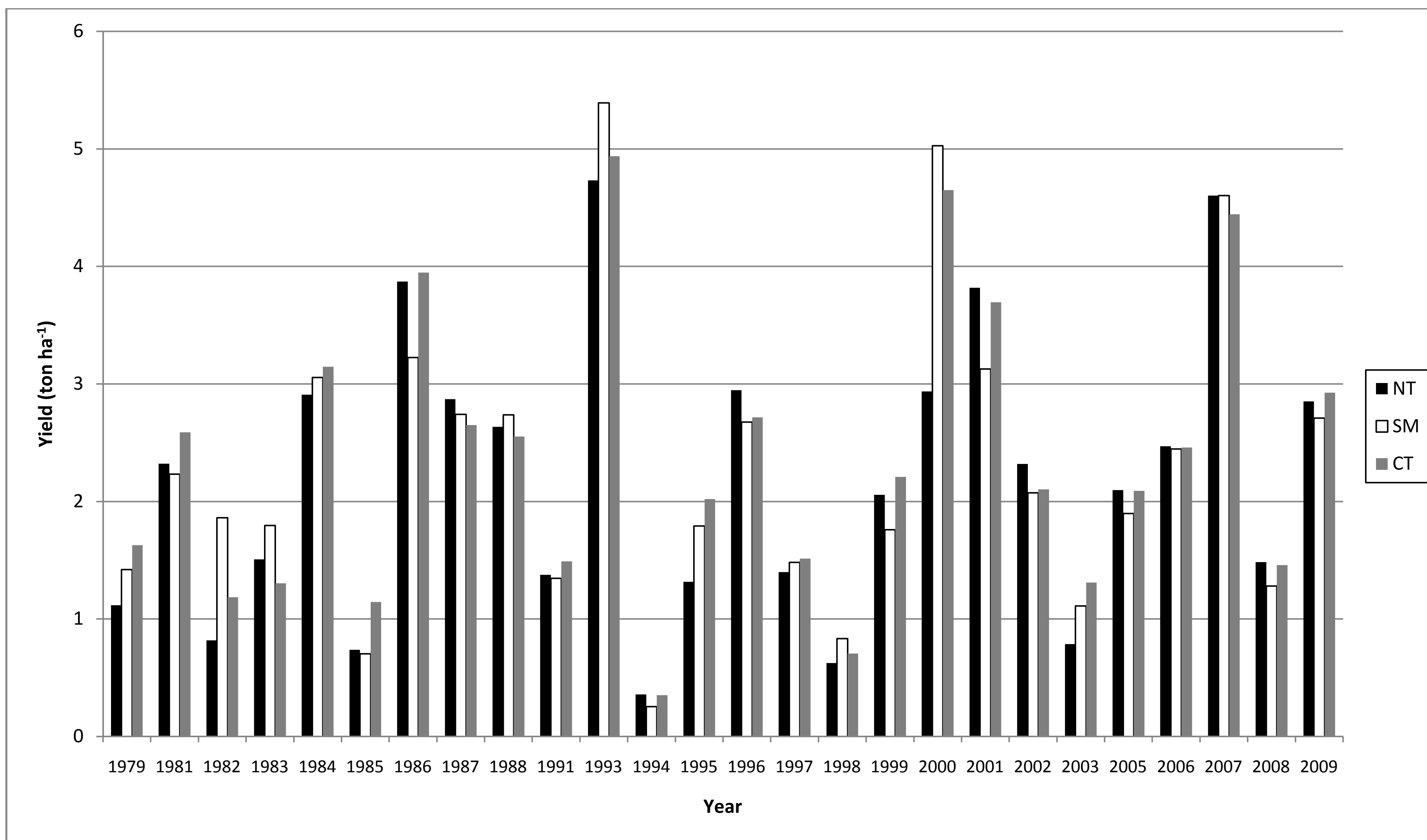

Figure 1 Harvest at the monoculture wheat trial at the ARC-Small Grain Institute near Bethlehem since inception. Some years are skipped due to drought or oat instead of wheat. Values only come from plots with no burning of residues, chemical weeding, and the appropriate level of N.