# THE EFFECT OF INORGANIC FERTILIZER APPLICATION ON COMPOST AND CROP LITTER DECOMPOSITION DYNAMICS IN SANDY SOIL

By

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# ABSTRACT

Inorganic fertilizer applications are common practice in commercial agriculture, yet not much is known regarding their interaction with organic matter and soil biota. Much research has been done on the effect of inorganic N on forest litter decomposition, yet very little research has focused on the effect of inorganic fertilizers on crop litters and, to our knowledge, none on composted organic matter. Furthermore none of the research has been done in South Africa.

The main aim of this research project was to determine the effect of inorganic fertilizer applications on the decomposition of selected organic matter sources commonly used in South African agriculture and forestry. Two decomposition studies were conducted over a 3-month period, one on composts and the other on plant litters, using a local, sandy soil. In the first experiment a lower quality compost, compost A (C:N ratio, 17.67), and higher quality compost, compost B (C:N ratio, 4.92) was treated with three commercially used fertilizer treatments. Two were typical blends used for vegetable (tomato and cabbage) production: tomato fertilizer (10:2:15) (100 kg N, 20 kg P, 150 kg K per ha) and cabbage fertilizer (5:2:4) (250 kg N, 100 kg P, 200 kg K per ha). The third fertilizer blend, an equivalent mass application of N and P applied at 150 kg of each element per ha, is more commonly used in pastures.

In the second experiment, five commonly encountered crop and forestry litters, namely kikuyu grass, lucerne residues, pine needles, sugar cane trash and wheat straw, were selected to represent the labile organic matter sources. The litters were treated with the tomato and cabbage fertilizer applications rates. Both decomposition experiments were conducted under ambient laboratory conditions at field water capacity. Decomposition rates were monitored by determining $CO_2$ emissions, DOC production, β-glucosidase and polyphenol oxidase activity (PPO). At the start and end of decomposition study, loss on ignition was performed to assess the total loss of OM. Based on the results obtained from these two experiments, it was concluded that the addition of high N containing inorganic fertilizers enhanced the decomposition of both composted and labile organic matter. For both compost and plant litters, DOC production was greatly enhanced with the addition of inorganic fertilizers regardless of the organic matter quality. The conclusion can be made that inherent N in organic matter played a role in the response of decomposition to inorganic fertilizer application with organic matter low in inherent N showing greater responses in decomposition changes. For labile organic matter

polyphenol and cellulose content also played a role in the responses observed from inorganic fertilizer applications.

# OPSOMMING

Anorganiese kunsmis toedieningss is algemene praktyk in die kommersiële landbou sektor,maar nog min is bekend oor hul interaksie met organiese materiaal en grond biota. Baie navorsing is reeds oor die uitwerking van anorganiese N op woud en plantasiereste se ontbinding gedoen. Baie min navorsing het gefokus op die uitwerking van anorganiese kunsmis op die gewasreste en tot ons kennis, is daar geen navorsing gedoen op die invloed van anorganiese kunsmis op gekomposteer organiese material nie. Verder is geeneen van die navorsing studies is in Suid-Afrika gedoen nie.

Die hoofdoel van hierdie navorsingsprojek was om die effek van anorganiese kunsmis toedienings op die ontbinding van geselekteerde organiese materiaal bronne, wat algemeen gebruik word in die Suid-Afrikaanse landbou en bosbou, te bepaal. Twee ontbinding studies is gedoen oor 'n 3-maande-tydperk, een op kompos en die ander op die plantreste, met die gebruik van 'n plaaslike, sanderige grond. In die eerste eksperiment is ‘n laer gehalte kompos, kompos A (C: N verhouding, 17.67), en 'n hoër gehalte kompos, kompos B (C: N verhouding, 4.92) met drie kommersieel anorganiese bemesting behandelings behandel. Twee was tipiese versnitte gebruik vir die groente (tamatie en kool) produksie: tamatie kunsmis (10: 2:15) (100 kg N, 20 kg P, 150 kg K per ha) en kool kunsmis (5: 2: 4) (250 kg N, 100 kg P, 200 kg K per ha). Die derde kunsmis versnit was 'n ekwivalente massa toepassing van N en P van 150 kg van elke element per ha, wat meer algemeen gebruik word in weiding.

In die tweede eksperiment was vyf algemeen gewas en bosbou reste, naamlik kikoejoegras, lusern reste, dennenaalde, suikerriet reste en koring strooi, gekies om die labiele organiese materiaal bronne te verteenwoordig. Die reste is met die tamatie en kool kunsmis toedienings behandel. Beide ontbinding eksperimente is uitgevoer onder normale laboratorium toestande by veldwaterkapasiteit. Ontbinding tempo is deur die bepaling van die $CO_2$-vrystellings, opgelosde organiese koolstof (OOK) produksie, β-glukosidase en polifenol oksidase aktiwiteit (PPO) gemonitor. Aan die begin en einde van ontbinding studie, is verlies op ontbranding uitgevoer om die totale verlies van OM te evalueer. Gebaseer op die resultate van hierdie twee eksperimente, was die gevolgtrekking dat die toevoeging van hoë N bevattende anorganiese bemestingstowwe die ontbinding van beide komposte en plant reste verhoog. Vir beide kompos en plantreste word OOK produksie verhoog met die toevoeging van anorganiese bemesting, ongeag van die organiese materiaal gehalte. Die gevolgtrekking kan gemaak word dat die inherente N in organiese materiaal 'n rol gespeel het in die reaksie van

ontbinding op anorganiese bemesting toedienings met die grootste reaksie in organiese material laag in inherente N. Vir labiele organiese material het polifenol en sellulose inhoud ook 'n rol gespeel in die reaksie waargeneeming op anorganiese bemesting.

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# CHAPTER 1
# GENERAL INTRODUCTION AND RESEARCH AIMS

Increasing awareness of the importance of organic matter (OM) conservation in agricultural soils has led to much research into mechanisms of organic matter management that will decrease C losses through $CO_2$ emissions. Several very successful management practices have been developed such as minimal/no tillage, mulching and crop rotation systems. These all increase the amount of surface litter that is left on the soil which can aid in prevention of soil erosion and surface crusting and can also act as a source of plant nutrients if incorporated into the soil and decomposed during the crop growing season (Lal, 2009). Currently the main limitation in the utilization of organic matter as plant nutrient sources is the unpredictability of decomposition and subsequently nutrient mineralization. Much research has been done to establish predictive models for OM decomposition and several have proven to be relatively successful (Palm et al. 2000), however the added complexity of inorganic fertilizer applications to agricultural soils make these predictive models less dependable. Research into the effect of inorganic fertilizer, especially N, on the decomposition of OM has been performed recently, but due to the many factors influencing decomposition as well as the wide variety of biotic and abiotic interactions that take place in soils, these studies have not yet been able to establish consistent and conclusive patterns for the effect of fertilizers on OM decomposition. The majority of these research studies focused on the effect of atmospheric N deposition on decomposition of forest litters such as oak, maple, dogwood, spruce and pine tree litters (Carreiro et al. 2000). The current research available on the effect of fertilizer applications on decomposition monitored one or two decomposition parameters, predominantly being $CO_2$ evolution along with mass loss or enzyme activity. Very few studies monitored dissolved organic C (DOC) production which forms a major part of soil OM. No research has been done on the effect of fertilizer application to the decomposition of composted OM and very little research has been done on agricultural crop residues under commercially used fertilizer application rates. In addition none of this research has been conducted in South Africa.

Thus, the main objective of this study was to determine the effect of commercially used fertilizer rates on the decomposition dynamics of composted OM and plant litter in a local sandy soil. In order to evaluate the effect of substrate composition on the fertilizer effect

a selection of organic matters varying in their chemical properties were used. Based on current literature there appears to be different interactions and effects of inorganic fertilizer applications on labile and less-labile (stabilized) OM. Chapter 3 addressed the effect of inorganic fertilizer application on the decomposition dynamics of composted (stabilized) organic matter. In this study we selected two commercial composts of varying C:N ratios to determine that the fertilizer effect on decomposition changes were based on fertilizer effect and not substrate quality. In Chapter 4, the effect of inorganic fertilizer applications on the decomposition dynamics of labile (undecomposed) OM was investigated. We selected five crop litters with varying lignin and polyphenol compositions and C:N ratios thereby ensuring that changes in decomposition dynamics could be ascribed to either fertilizer applications or litter quality. The aim was to monitor the decomposition parameters that will allow for a more comprehensive understanding of the possible mechanisms through which C can be lost, as well as, the enzyme activities which are considered to be rate limiting to the decomposition of both labile and recalcitrant organic matter. For this reason, both $CO_2$ evolution and DOC production was monitored, which are currently considered reliable indicators of the rate of OM decomposition. The activity of two enzymes essential to the decomposition of cellulose (more labile) and lignin (less labile) (Huang and Hardie 2009) were also monitored. B-glucosidase is considered to be the rate limiting enzyme for cellulose degradation (Tabatabai, 1982) and is commonly used as an indication of cellulose decomposition and was therefore selected to monitor cellulose degradation. Polyphenol oxidase enzymes are considered the primary lignin degrading enzymes (Sinsabaugh 2010). They are easily measured in soils and were therefore selected to monitor the effect of fertilizer applications on lignin degradation. Finally, loss on ignition was performed at the start and end of decomposition period, in order to assess the extent of total decomposition for the various treatments. Soil pH was also measured at the start and end of the decomposition period. The above-mentioned analyses were done for both decomposition studies in order to compare the differences between the effects of inorganic fertilizers on labile and non-labile organic matter. The composts and plant litters were combined with a sandy soil low in organic matter to avoid artefacts due to soil organic matter content. The decomposition was conducted over three months to coincide with the growing period of most agricultural crops.

# CHAPTER 2
# LITERATURE REVIEW - SIGNIFICANCE AND FACTORS CONTROLLING ORGANIC MATTER DECOMPOSITION

## 2.1 Introduction

The following literature review highlights the most significant scientific literature on the decomposition of organic matter (OM) and the influence of mineral fertilizer application on decomposition dynamics. An overview of the importance of OM management to agriculture, environmental conservation and climate change highlights the need for focused research regarding factors influencing decomposition dynamics.

Soil organic matter (SOM) is essential for soil quality as it influences soil structure and density, water holding capacity and infiltration, as well as soil microbial activity and nutrient status (Sullivan, 1990; Tiessen et al. 1994; Wagner et al. 2007). Therefore poor management of OM will lead to decreased soil quality and subsequent decreased crop productivity.

Increasing awareness regarding the importance of OM in agriculture and environmental conservation has led to amplified interest in optimal management. Some of the practices receiving the most attention include minimal/no tillage, mulching and cover crop utilization as well as organic fertilizers and organic and biodynamic farming. All of these management practices increase the amount of OM that is either incorporated into the soil or left on the soil surface available as a source of plant nutrients. Optimal management of these organic matter sources can therefore lead to increased soil fertility at little to no extra cost.

## 2.2 Importance of organic matter management

The loss of organic matter from agricultural soils leads to decreased soil fertility as well as release of greenhouse gasses (Ghani et al. 2003; Lal, 2004; Parfitt et al. 2006). However, the effective management of crop residues and organic matter sources can lead to increased soil organic matter content which subsequently improves soil fertility and soil physical and chemical resilience, and ultimately acts as a sink for C thereby reducing greenhouse gas emissions (Lal, 2011). It is therefore important to develop a good understanding of the factors that contribute to the decomposition and stabilization of organic matter. Figure 2.1 depicts the various aspects of soil quality that are improved

with increased soil organic carbon content. Physical, chemical and microbial properties of the soil influence crop productivity as well as sustainability for agricultural production. Therefore increased efforts to preserve soil organic matter will in the long run decrease input costs as well as increase productivity of the soil (Mando, 1998; Ouédraogo et al. 2001).

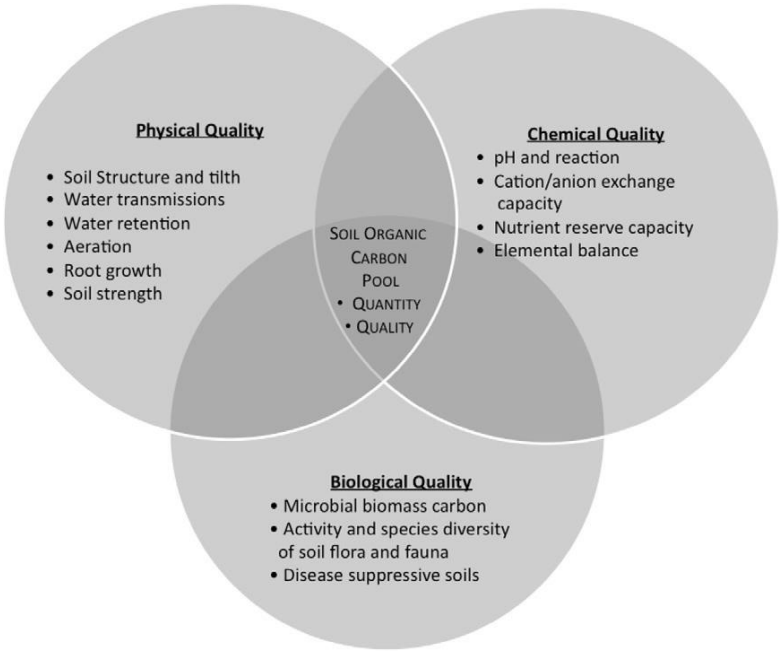


**Figure 2.1:** Agricultural soil quality improvement due to increased soil organic carbon: From Lal (2011). Reprint with permission from Elsevier

## 2.3 Importance of organic matter in soil quality

Organic matter plays a vital role in several soil factors which contribute to soil fertility and subsequent productivity of agricultural crops. Crop productivity is affected by several soil factors including soil structure, water retention, aeration and nutrient availability, all of which are improved with increased levels of soil organic matter and soil organic carbon levels. The productivity increase in crop production, with relation to increased soil organic carbon, can be easily noted on soils with less than 20% clay content, as well as soils with sandy-loam or loamy- sand texture (Lal, 2005). Data studies show that an increase of 20-70 kg per hectare of wheat, 10-50 kg per hectare of rice and a 30-300 kg per hectare of maize, per 1 Mg C increase per hectare, can be expected (Lal, 2005).

Soil structure is defined as the arrangement of particles and associated pores in soils across the size range from nanometres to centimetres (Oades, 1993). Soil structure has a direct influence on the rate of water infiltration, gas exchange, plant root penetration

and development, as well as water holding capacity, and is therefore of extreme importance for soil health and optimal crop production (Johnston et al. 2009). The development of soil structure is determined by the formation of soil aggregates which can be caused by several factors, physical, chemical and microbiological. Soils with a high clay fraction and/or high organic matter content have the ability to develop soil aggregates due to their physicochemical properties which lead to the flocculation of organic complexes (Semenov et al. 2009). During the process of aggregation, soil minerals are coated with plant debris and organic material which produce organic polymers when decomposed. These organic polymers can interact with silicate clays as well as iron- and aluminium oxides. This interaction binds clay minerals into domains, producing water-stable soil aggregates.

Soil humus is also able to form complexes with multi-valent cations, thereby allowing it to bind with clay mineral surfaces to form clay/humus domains. These domains have the ability to bind to each other, as well as silt particles, leading to the formation of the smallest groupings of soil aggregates and also providing long term stability for the micro-aggregates (Goh, 2004). Microbiological degradation of soil organic matter provides energy for biological activities which produce microbial exudates, such as polysaccharides and other organic compounds, which bind soil particles and soil aggregates to form micro- as well as macro-aggregates. Bacteria have also been found to produce glues through their decomposition of plant residues. These glues are known to be resistant to dissolution and therefore add to the stability of resulting soil aggregates (Czarnes et al. 2000).

### 2.3.1 Soil water

Soil organic matter has a direct and indirect effect on soil water holding capacity. The negative charge on soil organic matter, as well as, the large surface area of humus in soils, increases the soils ability to interact and bind with polar water molecules, allowing the water molecules to coat the organic matter. Organic matter has been found to be able to hold water up to 20 times its own weight (Sparks, 2003). Due to the increased surface area of a soil with high organic matter content, there is more space for these interactions which leads to an increase in the soil water holding capacity (Magdoff and Weil, 2004). These interactions also make it more difficult for soil water to evaporate as more energy is required to overcome the bonds formed between the soil organic matter and water, therefore decreasing water loss through evaporation. The indirect effect of organic matter on soil water is due to the effect of organic matter on soil structure as

discussed in section 2.3.1.. The increased structure leads to an increase in pore space (Magdoff and Weil, 2004). This leads to better water infiltration as well as increasing the water holding capacity due to the greater surface area within pores.

### 2.3.2 Soil nutrients

Soil organic matter is considered to be an immediate source of several essential plant nutrients such as N, P and S. It also holds the capacity to store these nutrients in the long term (Magdoff and Weil, 2004). The cation exchange capacity (CEC) of soil organic matter has been shown to be much greater than that of clay per unit mass, and for soils low in clay content, OM is primarily, if not the only source of CEC (Magdoff and Weil, 2004). Humus holds easily exchangeable cations which are available to plants yet are relatively resistant to leaching. Mineralisation of soil organic matter leads to the slow release of N, P, S and other micronutrients. Soil mineral decomposition is also known to be accelerated by humic acids, thereby releasing essential nutrients as exchangeable cations. Chelation of metal cations by organic acids, polysaccharides and fulvic acids make these metals more available to plants due to their solubility in the chelated state.

### 2.3.3 Soil buffer capacity

Humic substances, especially humic acids, are known to contain large quantities of acidic functional groups. The amounts of functional groups that can dissociate contribute significantly to soil fertility by providing the soil with a buffer capacity against pH change (Ceppi et al. 1999). Processes that determine the plant available nutrients in soil function under very narrow pH ranges, therefore soils with a low buffer capacity will have difficulty maintaining a constant pH within the required range for optimal nutrient availability (Garcia-Gil et al. 2004). Strong acidic functional groups such as carboxylic acids dissociate easily and will act as pH buffers at low pH levels. With increasing pH, weaker acidic functional groups, such as phenolic and amino groups, which do not dissociate as easily, will contribute to the buffer capacity of humic substances (Garcia-Gil et al. 2004). The contribution of humic substances to soil buffer capacity, even though it has not been quantified, makes it important for optimal nutrient availability and crop production.

### 2.3.4 Soil biota

The relationship between soil organic matter and soil biota is interdependent, whereby soil organic matter content of a soil can directly affect the ecology of soil biota and soil biota can both directly and indirectly influence the stability of soil organic matter (Oades, 1993). In arid soils studies have shown that moisture and soil organic matter act as

limiting factors for soil biotic activity (Steinberger et al. 1984). The low levels of soil organic matter in these soils limits the energy source available to the soil biota as soil micro- and macro- organisms utilize organic matter as a source of energy.

## 2.4 Organic matter decomposition

### 2.4.1 Organic matter pools

Considering the importance of organic matter to crop production and soil fertility it is essential that management of organic matter is understood and improved. Soil organic matter (SOM) consists of several pools from fresh undecomposed tissue, to partially decomposed organic matter to stable humic substances. Each fraction has specific characteristics and functions in soil ecosystems. Fresh tissue can further be divided into two C pools namely structural C and metabolic C. The metabolic pool consists of proteins, starches and sugars which are all easily metabolized by microorganisms whilst the structural pool consists of more resilient compounds such as lignin, cellulose and polyphenols which are less easily metabolized. The metabolic C pool has a short lifespan in soil (0.1-0.5 year) with a relatively narrow C:N ratio ranging between 10 and 25. The structural C pool on the other hand has a longer duration in soil (2-4 years) with a much wider C:N ratio of anything between 100-200. As these pools decompose they form part of C pools in soil organic matter (SOM). Three C pools exist in SOM namely i) Active, ii) Slow and iii) Passive SOM pools (Brady and Weil, 2008).

#### *2.4.1.1 Active organic matter pool*

This pool of organic matter consists of easily decomposable organic matter sources which contain polysaccharides and carbohydrates, amino compounds, litter fragments as well as some soluble organic acids (Wander, 2004). Even though this pool doesn't have a long lifespan in the soil ecosystem, it is an essential source of plant and microbial nutrients and aids in improving soil fertility through its mineralization (Brady and Weil, 2008).

#### *2.4.1.2 Slow organic matter pool*

The Slow organic matter pool consists of substrates that are less labile but still decomposable such as amino compounds and aggregate protected particulate organic matter(POM) as well as some humic substances and soluble humic acids (Wander, 2004). This pool doesn't have a primary function but appears to supplement the active and passive organic matter pools in soils (Brady and Weil, 2008). It can therefor provide mineralizable plant nutrients as well as form stabilized soil humus.

#### *2.4.1.3 Passive organic matter pool*

This pool consists of recalcitrant organic matter that has a very long lifespan in soils. Compounds such as lipids and cutans, charcoal, lignin and humic substances such as condensed SOM, humin and mineral bound SOM (Wander, 2004). These substances do not provide significant amounts of mineralizable plant nutrients but improve soil structure and aggregate stability as well as increasing the soil CEC thereby improving water infiltration and holding capacity as well as soil nutrient holding capacity (Brady and Weil, 2008).

### 2.4.2 Decomposition phases and processes

Organic matter decomposition in soils takes place in several phases that and is controlled by the decomposers present in soils. Primary decomposition is controlled by soil primary decomposers including mega-, macro-, and meso-fauna such as earthworms and mites. These fauna play a vital role in the initiation of litter decomposition. Leaf litter which is on top of the soil surface is not in contact with soil microorganisms and is exposed to sever climatic conditions making them slower to decompose. It is therefore essential that these litters are consumed by the above mentioned fauna in order to be incorporated into the soil where further decomposition can take place (Huang and Hardie, 2009). Some of these primary decomposers do not only chew up the litter and incorporate it into the soil, but contain decomposing microorganisms in their gut which is incorporated into the digested plant litters thereby enhancing decomposition in the gut as well as in the excreted organic matter which is then incorporated into the soil (Hammel, 1997). The primary decomposers are not able to decompose the more recalcitrant fractions such as lignin and cellulose. It is therefore necessary for secondary decomposers to continue the decomposition process in the soil. Microorganisms are the only secondary decomposers and continue the decomposition of organic matter with the use of extracellular enzymes specialized to the decomposition of the remaining fractions such as cellulose and lignin. All microorganisms are not suited to decompose the same fractions of organic matter. Two major distinctions in the microbial populations can be made based on their response to organic matter substrate quality. The two groups are known as K-strategists and R-strategists. K-strategists are microorganisms well adapted to survive in low organic matter conditions. They are specialized to feed on recalcitrant organic matter. R-strategists on the other hand cannot decompose recalcitrant substances but immediately respond to labile compounds such as soluble sugars and amino acids from fresh litter. Their rate of population growth is much greater than that of the K-strategists and with the

input of fresh litter these R-strategists quickly become the dominant populations in the soil. Once the labile organic matter is depleted these R-strategists begin to die off and the K-strategists once again become the primary microbial populations in the soil (Brady and Weil, 2008).

Understanding the factors influencing the decomposition processes for various organic matter sources under varying conditions has proven to be very complex and the development of predictive models for plant litter decomposition has been a focus point in decomposition research. However the major stumbling block in this area of research is a lack of understanding regarding the mechanisms behind decomposition dynamics. Many factors play a simultaneous role in the decomposition rates of organic matter, including i) plant litter composition, ii) soil pH and iii) soil texture and mineralogy.

### 2.4.4 Plant litter composition

Both chemical and physical compositions of plant litters have been found to be major controlling factors in litter decomposition rates. Structural composition of plant material can act as a barrier to decomposition (Wilson and Mertens, 1995). Lignified plant material has been found to decompose slowly, yet the decomposition can be enhanced by physical grinding/chewing of the plant material and thereby increasing the accessibility of plant material to enzyme degradation (Wilson and Mertens, 1995). Chemical composition such as N content, lignin and cellulose content as well as polyphenol content affect the decomposability of organic matter (Fog, 1988; Palm, 2000). Litter high in lignin and polyphenol content are considered to be chemically recalcitrant and will decompose at a slower rate that those high in cellulose. Palm (2000) suggested litter quality parameters based on N, lignin and polyphenol content with high quality litters containing greater that 2.5% N, less than 15% lignin and less that 4% polyphenol content. The litter quality as described by Palm (2000) can be directly correlated with predicted N mineralization and subsequent litter decomposition. As mentioned in section 2.4, not all microorganisms are able to decompose all fractions of organic matter. Specific enzymes are utilized for the breakdown of the different fractions of organic matter, therefore the composition of plant litters determine which microorganisms and enzymes will be present during decomposition. Polysaccharides and simple sugars are easily decomposed by both fauna and microbes and can be broken down by a broad class of hydrolytic enzymes due to their hydrolytic bonds (Huang and Hardie, 2009). Therefore litters high in sugars and polysaccharides will stimulate the growth of a wide range of microorganisms in the soil. Cellulose and lignin

on the other hand require more specialized enzymes to decompose. Both hydrolytic and oxidoreductase enzymes are required for the decomposition of cellulose. These enzymes are predominantly produced by white-, brown- and soft-rot fungi as well as a selected group of bacteria. Endoglucanases and cellobiohydrolases are responsible for the initial breakdown of cellulose into smaller molecules which can then undergo a final breakdown step into glucose molecules by β-glucosidase enzymes (Pérez et al. 2002). Lignin degradation is more complex as lignin contains no hydrolytic bonds. The initiation of lignin degradation takes place with the production of oxidoreducive enzymes. Further decomposition takes place through peroxidase and laccase activities. Peroxidases are able to degrade both phenolic and non-phenolic lignin molecules whereas laccase (blue-copper phenoloxidases) can only degrade phenolic lignin molecules (Martínez et al. 2005). These enzymes are predominantly produced by white-rot fungi which are considered to be the only microorganisms capable of fully decomposing lignin.

### 2.4.5 Soil pH

The effect of pH on decomposition is an indirect effect. Change in pH is associated with a shift in microbial community composition as well as the efficiency of individual microbial species and enzyme activities (Sinsabaugh, 2010; Rousk et al. 2010). Research has shown pH to be the principal soil function which controls enzyme activities and thereby biotic decomposition (Sinsabaugh et al. 2008). Rousk et al. (2010) showed that both bacterial abundance and diversity are positively correlated with soil pH. This trend was however not as prominent for fungal communities, which appear to be more negatively correlated with the activity and presence of bacteria. The change in microbial composition and abundance has a direct effect on the decomposition of organic matter. In addition to the alteration of abundance and diversity of microbes, pH also affects their functioning and enzyme activity. An example of this can be seen when considering laccase enzymes from various sources. Laccases of white rot fungi generally have lower pH optima (4-5) than the laccase of brown rot fungi and coprophilic fungi (6-7.5) (Sinsabaugh, 2010).

### 2.4.6 Soil texture and mineralogy

Soil texture is an important factor in the conservation of organic matter in soils as it can provide physical and chemical protection for soil organic matter against microbial decomposition. Several mechanisms can contribute to the conservation of organic matter. These include i) the physical protection of organic C within aggregates

(Christensen, 1996) and ii) the interaction with mineral surfaces (e.g. ligand exchange, cation bridging, weak interactions) (Torn et al. 1997).

#### *2.4.6.1 Physical protection*

Physical protection within aggregates decreases the susceptibility of organic matter to microbial breakdown as the organic matter is inaccessible to microbes and elements such as oxygen which are essential to decomposition (von Lützow et al. 2006). The degree of soil aggregate formation is directly associated with the soil texture classification. Soils high in clay and fine silt fractions show greater aggregation as well as aggregate stability along with increased aggregate size which all contributes to greater organic matter stabilization through physical protection.

#### *2.4.6.2 Interaction with mineral surfaces*

Mineral sorption provides one of the most effective and important organic matter stabilization mechanisms in soil (Kalbitz et al. 2005). This has been concluded due to the findings that show longer turnover times for OM associated with clay and silt compared to other particle fractions (Eusterhues et al. 2003; Kalbitz et al. 2005). Along with these findings, research has shown that OM sorption to subsoil materials coincided with a 20% decrease in OM mineralization (Kalbitz et al. 2005). Not only does this mechanism protect OM from decomposition, research has shown that this sorption is often irreversible and will therefore lead to permanent alteration to soil C content. The sorption to Fe oxides have shown that between 72-92% of the adsorbed DOC was irreversibly bound (Gu et al. 1994). It is, however, not only oxides and hydroxides that effectively sorb organic matter. Significant sorption is also associated with clay minerals and varies between minerals. Jardine et al. (1989) concluded that kaolinite is more effective than illite with regards to OM sorption.

## 2.5 Interaction between organic matter and inorganic fertilizer

Currently, very little is yet known about the mechanisms of interactions between inorganic fertilizer applications, organic matter and microbial populations and activities. Therefore the use of predictive models is limited for the prediction of decomposition dynamics under inorganic fertilizer applications. Even with the increased popularity of organic farming, inorganic fertilizers are still widely and extensively used in intensive crop production to meet the growing demand for high quality and high volume crops. Therefore in most cases organic matter and inorganic fertilizers will come in contact with

one another. A range of studies have shown that inorganic nitrogen additions decrease litter decomposition rates (Magill & Aber 1998; Hagedorn et al. 2003; Knorr et al. 2005; Rudrappa et al. 2006). The majority of these studies, however, do not exclude the effect of extra litter input due to increased crop residue production associated with the increased fertilizer application. In contrast, Alvarez (2005) states that mineral N addition only increased soil organic carbon levels if the crop residues were left in the field. Berg (2000) suggested that mineral N additions decrease litter decomposition due to the formation of more complex recalcitrant compounds as well as possibly repressing enzymes essential to lignin decomposition. Some research shows that N additions accelerate the decomposition of labile organic matter whilst stabilizing non-labile organic matter in alpine meadows under 10 yr N additions (Neff et al. 2002; Wang et al. 2004). Another study found that nitrogen additions accelerated decomposition of sugar maple litter but depressed the decomposition of soil organic matter (Saiya-cork et al. 2002). It is clear that there is much controversy with regards to the effect of N fertilizer and that the effects certainly vary based on litter quality (Köchy and Wilson, 1997). With so much inconsistency regarding the effects of N on decomposition (Hobbie 2005) it is difficult to predict how decomposition rates and nutrient cycles will be affected with fertilizer applications. Many factors contribute to this; however the main problem lies in the lack of understanding regarding the mechanisms with which inorganic fertilizer application interacts with SOM and microorganisms. The shift in decomposition dynamics due to the interactions between inorganic fertilizer application and organic matter may be due to several changes in i) nutrient availability, ii) microbial dynamics and iii) changes in dissolved organic matter production.

### 2.5.1 Changes in nutrient availability

Changes in nutrient availability and ratios such as C:N or lignin:N have been found to alter decomposition rates of organic matter. The optimal C:N ratio for microbial mineralization is less than 20:1 (Havlin et al. 2005). The alteration of C:N ratio is therefore expected to influence the decomposability of plant litters and organic matter, however research has shown that C:N ratio is not the only determinant factor in decomposition rates. Several authors have suggested alternative ratios to predict the effect of inorganic N addition to OM decomposition such as lignin:N ratio (Taylor et al. 1989), polyphenols:N ratio and (lignin+polyphenol)/N ratio (Wang et al. 2004). This means that the effect of inorganic N on decomposition of organic matter is dependent on the initial composition of the OM. Researchers have hypothesized that the addition of

$NH_4^+$ and $NO_3^-$ can lead to formation of covalent bonds through adsorption to quinones producing more recalcitrant organic matter and thereby decreasing decomposability (Nommik and Vantras 1982; Stevenson 1982), thereby implying that litter high in lignin and polyphenols would be subject to this adsorption and thereby show decreased decomposition rates with increased inorganic N application. This supports the observation that the addition of inorganic N accelerates early stage decomposition yet decreases cumulative decomposition of various plant litters including wheat, sugar cane, buffel grass, stylo and several other native Australian plant litters (Wang et al. 2004). These studies did not include composted organic matter and very few agricultural crop litters and may therefore be less relevant due to differences in composition and quality.

### 2.5.2 Microbial dynamics

There is much controversy regarding the effect of inorganic N on microbial population dynamics and activities. In a study on atmospheric N deposition on hardwood litters (Dogwood, Red Maple and Red oak), Carreiro et al. (2000) observed that cellulases were stimulated for all litter types; however, in terms of lignin degrading enzymes, a decreased activity was observed for phenol oxidases in high lignin containing litters. These findings were, however, contradicted in a study by Allison and Vitousek (2004) who observed no decrease in phenol oxidase activity for litters with high lignin content. In comparison with a study by Hobbie (2000), also conducted on Hawaiian plant material which also showed similar results for high lignin plant material, Allison and Vitousek suggested that fungi may not dominate the decomposer community in Hawaiian forests or that fungus response to N additions may be dependent on the ecosystem in which the study is conducted. Fog (1988) discussed the possibility that nitrogen additions may reduce the ability of basidomycetes to compete in soil environments, thereby reducing the rate of lignin decomposition. Wiemken et al. (2001), on the other hand, found no differences with increased nitrogen. The mechanisms behind PPO suppression have been observed in several decomposition studies and were found to be related to i) the polymerization and condensation reactions between low molecular N and organic matter, particularly lignin and polyphenols (Nommik and Vantras, 1982; Stevenson, 1982; Berg and Matzner, 1996), ii) the direct suppression of lignin degrading enzyme production (Keyser et al 1978; Eriksson et al 1990) and iii) the suppression of lignin degrading microorganisms (Keyser et al. 1978; Berg and Matzner, 1996). Whilst suppressing phenol oxidase enzyme activities, studies have shown stimulation of hydrolytic enzyme activity such as cellulase (Fog, 1988; Waldrop et al. 2004).

### 2.5.3 Dissolved organic carbon production

Dissolved organic carbon (DOC) is the carbon fraction of dissolved organic matter (DOM) which originates from plant litter, soil humus, microbial biomass and root exudates. DOM is composed of organic acids, sugars, amino acids and humic substances with humic substances forming the majority of DOM. For this reason a general chemical definition is impossible because humic substances are composed of randomised polymers of various constituents for which the origin can no longer be identified. The definition for DOM is therefore a continuum of organic molecules of different sizes and structures that pass through a filter of 0.45 µm pore size. Observed fluxes of DOM/DOC is net result of processes releasing DOM (leaching, desorption) and processes removing DOM (adsorption, decomposition). Both biotic and abiotic processes are involved in formation of potential and actual DOM (Kalbitz et al. 2000; Hernert andand Bertsch. 1995). Dissolved organic C forms a major part of SOC and can contribute to significant C losses from soil if allowed to leach. Research has shown that DOC can be irreversibly adsorbed onto soil minerals and Fe and Al oxides and hydroxides (Gu et al. 1994). Therefore increases in DOC production could lead to alteration in soil formation and organic carbon content of subsoils. According to research done on forest floor litter and DOC production, it has been found that the rate of DOC production is correlated to the amount of fresh litter present and not to the N content of the soil (Gundersen et al. 1998). The effect of inorganic fertilizer application on DOC production from compost has not been researched; research on inorganic fertilizer effect on DOC production from forest litter has been extensive yet inconclusive. McDowell et al. (1998) evaluated the effect of atmospheric N deposition on the production of DOC and dissolved organic nitrogen (DON). They observed statistically insignificant increases in DOC production of between 10-30%. Chemical structural studies suggest that increased mineral N will lead to greater DOC mobilization due to stimulated microbial activity and suppression of lignin degrading enzymes (Zech et al. 1994, 1996).

## 2.6 Conclusion and gaps in knowledge

From the current literature on organic matter and soils it can be concluded that organic matter is essential to soil fertility and successful crop production due to its influence on soil structure, water holding capacity, CEC and soil microbial activity. Without organic matter soil fertility is severely affected and can lead to unsuccessful crop production in soils low in clay content. Much research has been done on mechanisms of increasing SOM and preserving organic matter in agricultural soils. Practices such as minimal/no

tillage, mulching and crop rotation systems are currently being implemented in agriculture in South Africa and the rest of the world. These conservation practices also have an influence on greenhouse gas emissions from agricultural crop production, decreasing losses of C from soils as $CO_{2.}$ With increased crop production and increased residue conservation in soils these practices will lead to C sequestration whereby greenhouse gas emissions are not merely reduced but $CO_2$ is being removed from the atmosphere and stored in the soil. Most of the research has been done on physical soil preparation and utilization but not as much on the effect of fertilization on organic matter management.

Research has been done on the factors influencing and determining decomposition of plant litters in order to develop decomposition models. However the complexity of interactions between the factors makes it difficult to develop a predictive model that can be used universally. With the additional effect of fertilizer application to organic matter the complexity is further increased. The general conclusion based on current research is that the addition of inorganic fertilizers will accelerate decomposition of labile organic matter and suppress that of non-labile organic matter. Several reasons for this have been proposed and are related to the effect of inorganic fertilizer production on microbial dynamics and enzyme activities as well as to soil nutrient ratios. No conclusive pattern has been devised due to the complexity of the factors which all contribute to the rates of decomposition, namely nutrient status, plant litter structure and composition, amount of lignin and polyphenols, amount of fertilizer applied as well as ecosystem factors such as microbial population dynamics and soil type. For this reason it is not possible to predict how agricultural crop litters and compost decomposition will be affected with the application of commercially used fertilizer quantities and ratios under South African conditions. Currently no research has been done on this in South Africa and very little research has been done on agricultural crop litters and compost decomposition under inorganic fertilizer applications. Research which has been done on inorganic fertilizer applications to plant litters was predominantly done for forest litters such as Red and White oak, Red maple, Dogwood, pine and spruce trees and to some extent on grasslands. Majority of these decomposition studies focused on atmospheric N deposition which is significantly less than the inorganic N applied on agricultural soils. The studies also primarily looked at one or two decomposition parameters and seldom monitored DOC. For this reason valuable information on all aspects of decomposition were not monitored. Due to the lack of research done on fertilizer interaction with plant

litters and composts in South Africa, management of organic matter cannot be optimally implemented.

# CHAPTER 3
# THE EFFECT OF INORGANIC FERTILIZER APPLICATION ON COMPOST DECOMPOSITION DYNAMICS IN SANDY SOIL

## 3.1 Introduction

Crop productivity is affected by several soil factors including soil structure, water retention, aeration and nutrient availability, all of which are improved with increased levels of soil organic matter and soil organic carbon levels (Lal 2004; 2006; 2011). Composts are commonly used to amend soil organic matter, especially in organic and biodynamic farming, to increase nutrient availability for optimal crop production. In the majority of cases however, composts cannot provide sufficient rates of both macro and micro nutrients for this to be achieved (Evanylo et al. 2008). Commercial crop production requires provision of plant essential nutrients at various stages of crop production and at optimal rates. The primary concern regarding the use of compost as organic fertilizer is the difficulty in determining mineralization patterns and rates of these essential plant nutrients. For this reason an integrated system which makes use of both organic and inorganic sources of nutrients allows for better management of crop nutrients. Currently, there is very little knowledge regarding the interactions between composts and inorganic fertilizers. In order to efficiently manage both compost and inorganic fertilizer use a clear understanding into their interactions need to be established to eliminate potential negative interactions which may lead to poor crop production as well as adverse environmental effects.

To our knowledge, there is no research on the interaction of composts and inorganic fertilizers, however extensive research in the area of inorganic nitrogen application to forest litter and soils points to varying results. Even though these studies provide some insight into the possible interactions that can be expected, they were conducted on plant litter which is considered highly labile and decomposable. These results are therefore not reliable in predicting the effects of inorganic fertilizer applications on composts, which are substantially decomposed, stable organic matter sources. Composts are considered mature and ready for soil application when period of heat release from the compost has ceased, which corresponds with microbes having metabolized the most labile forms of C (Brady and Weil, 2008).

Thus far research indicates that the addition of inorganic N accelerates the decomposition of labile organic matter whilst promoting the stabilization of non-labile

organic matter (Neff et al. 2002; Wang et al. 2004). The mechanisms behind these results are unclear and based primarily on theoretical concepts, some of which include the formation of recalcitrant heterocyclic N organic matter, either directly or indirectly through the change in pH; the inhibition of enzyme production and activity, and the nitrogen mining theory (Berg, 2000;Moorhead and Sinsabaugh, 2006; Craine et al. 2007; Jung et al. 2011). Carreiro et al. (2000) looked at the effect of elevated atmospheric N deposition on the decomposition rates of three forest litter types of varying quality and carbon composition (Dogwood, Red Maple and Red oak), and investigated the effect on extracellular enzyme activity. It was observed that cellulases were stimulated for all litter types; however, in terms of lignin degrading enzymes, a decreased activity was observed for phenol oxidases in high lignin containing litters. The production of phenol oxidase enzymes is predominant in white rot fungi, even though some bacteria and macrofungi can produce other enzymes related to the partial decomposition of lignin. Therefore, Carreiro et al (2000) hypothesized that the observed decrease may be due to the suppression of phenol oxidase expression in white rot fungi under high N availability, and/or a reduction in the abundance of white rot fungi under these environments. These findings were however contradicted in a study by Allison and Vitousek (2004) who observed no decrease in phenol oxidase activity for litters with high lignin content.

Dissolved organic carbon (DOC) forms a significant portion of organic matter in soils and plays an essential role in pedogenesis, weathering of soil minerals and the transport of pollutants (Kalbitz et al. 1999). Research has shown that DOC can be irreversibly adsorbed onto soil minerals and Fe and Al oxides and hydroxides (Gu et al. 1994). Therefore increases in DOC production could lead to alteration in soil formation and organic carbon content of subsoils. According to research done on forest floor litter and DOC production, it has been found that the rate of DOC production is correlated to the amount of fresh litter present and not to the N content of the soil (Gundersen et al. 1998). The effect of inorganic fertilizer application on DOC production from compost has not been researched, while research on inorganic fertilizer effect on DOC production from forest litter has been extensive yet inconclusive. McDowell et al. (1998) evaluated the effect of atmospheric N deposition on the production of DOC and DON. They observed statistically insignificant increases in DOC production of between 10-30%. Chemical structural studies suggest that increased mineral N will lead to greater DOC mobilization due to stimulated microbial activity and suppression of lignin degrading enzymes (Zech et al. 1994, 1996).

As various aspects of decomposition may be affected by the interaction between mineral fertilizers and compost, it was the aim of this study to provide a comprehensive view on the decomposition process. As already shown, research regarding the effect of mineral N on respiration, enzyme activity and DOC has been done; however none of the research has looked at all three factors simultaneously. Therefore, $CO_2$ respiration, DOC production, β-glucosidase activity was monitored throughout the decomposition period. Respiration analysis has been one of the most frequently and easily used analyses to monitor decomposition rates. β-glucosidase enzymes are cellulose degrading enzymes and are considered to be the rate limiting enzymes in cellulose degradation (Alef and Nannipieri, 1995), therefore, the determination of β-glucosidase activity allows for a simple and relatively comprehensive analysis of overall cellulose decomposition.

The aim of this chapter is, therefore, to evaluate the effect of mineral fertilizer rates on the decomposition process of composts in soil, in order to better understand interactions involved and predict the final result.

## 3.2 Materials and Methods

### 3.2.1 Soil and composts

A laboratory decomposition study was conducted in order to allow for better control of environmental factors, and enable controlled monitoring of decomposition parameters. In order to avoid added complexity, a sandy soil inherently low in organic matter was used. The soil selected was acidic, sandy soil collected from a fallow field, partially covered with kikuyu grass and weeds, in Brackenfell, Western Cape (33˚53'42.67"S, 18˚43'26.982"E). The classification and characterisation of this soil has been previously reported in Sika and Hardie (2014). The soil was locally classified as Kroonstad form in the Morgendal family (Soil Classification Working Group, 1990) and in WRB classification systems as Haplic Stagnosol (Albic). The thin A horizon was removed and only sand from the E horizon was collected up to 1 meter in depth. The texture of the soil was classified as a pure sand of a medium sand grade, with 97.6% sand, 1.9% silt and 0.5% clay. The soil was air-dried and sieved (< 2 mm) prior to being characterised and used in the decomposition trials. This soil type is typically used for vegetable production in the Cape Town region.

A range of commercial composts were randomly selected from local gardening centres, air-dried and then chemically characterised by Bemlab, Pty Ltd., Somerset West. Based on the results obtained for the C and N content, two contrasting composts were

selected: a lower quality compost, Compost A (N content below 2.5%, C:N ratio = 17.67), and higher quality compost, Compost B (N content above 2.5%, C:N ratio = 4.92). The quality grading was based on a 2.5% N content threshold value above which organic matter can be considered as high quality (Palm, 2000). Fourier Transform Infrared (FTIR) spectroscopy was used to characterise the two composts according to their organic functional groups. The FTIR analysis was carried out on a 1% potassium bromide (KBr) pellet (Johnston 1996) using a Thermo Nicolet Nexus™ FTIR spectrophotometer (Thermoscientific, USA) with the OMNIC version 7.2 software. The composts were also sent to the Department of Forestry and Wood Science, Stellenbosch University, for lignin and polysaccharide analysis using the method for determination of structural carbohydrates and lignin in biomass described by Sluiter et al. (2008).

### 3.2.2 Fertilizer blends

Three mineral fertilizer blended mixtures were selected for the decomposition study. Two were typical blends used for vegetable (tomato and cabbage) production, which also commonly make use of sandy soil and compost additions. The tomato fertilizer (10:2:15) was applied at rate of 100 kg N, 20 kg P and 150 kg K per ha (100:20:150), providing a moderate application of mineral N and K and a relatively low application of P. The cabbage fertilizer (5:2:4) was applied at rate of 250 kg N, 100 kg P and 200 kg K per ha (250:100:200), providing a relatively high application of mineral N as well as a relatively high P and K application. The third fertilizer blend, an equivalent mass application of N and P applied at 150 kg of each element per ha (150:150). This blend was selected to investigate whether a high P content, typically used in pastures, affects decomposition. Table 3.1 provides the amount and type of mineral fertilizer sources which were used in the blends.

**Table 3.1: Constituents of fertilizer blends used in the compost decomposition trial (application rate per pot)**

| Fertilizer Applications | | | | | |
|---|---|---|---|---|---|
| | $NH_4NO_3$ (g) | $K_3PO_4$ (g) | $KH_2PO_4$ (g) | $KNO_3$ (g) | $Ca(H_2PO_4)_2$ (g) |
| Tomato (100:20:150) | 0.262 | 0.186 | 0 | 0.240 | 0 |
| Cabbage (250:100:200) | 0.799 | 0 | 0.549 | 0.238 | 0 |
| NP (150:150) | 0.536 | 0 | 0 | 0 | 0.708 |

### 3.2.3 Compost decomposition trial

Nursery pots with a top diameter of 18 cm, height of 15 cm and total volume of 2.4 L were used to carry out the incubation studies. Treatments consisted of 2 kg dried and sieved soil combined with Compost A and B to deliver a 0.5% increase in initial soil organic C content, thereby eliminating effects due to differences in total C content. This required the addition of 25 g, dry weight, Compost A and 50 g, dry weight, Compost B. The composts were air-dried and roughly ground with a pestle and mortar to homogenise their particle sizes before addition to the soil by thorough mixing in a plastic bag. There were also control soil treatments without any compost added. For each compost or soil only treatment, there were three fertilizer treatments (Tomato, Cabbage and NP) (Table 3.2). Three replicates were set up for each treatment. The fertilizer applications were done by dissolving the fertilizers in a pre-determined volume of water (530 g distilled water) to bring the soil to field capacity, and poured into each pot. The volume of water required for field capacity of the soil which was determined using a method described in Alef and Nannipieri (1995). Briefly, 20 g soil samples were weighed out and placed in a filtration system. Excess water was added to each soil sample and allowed to drain for 24 h whilst the sample was covered with aluminium foil to prevent evaporation. After 24 h the sample was weighed and the percentage gravimetric water content calculated to be 26.5%. Based on the mass of soil and compost in each pot the total gravimetric water content at field capacity was calculated to be approximately 530 g of water. The bulk density of the soil and soil-compost mixtures in each pot was determined to be 1.45 g $cm^{-3}$.

**Table 3.2: Experimental treatments in the compost decomposition trial**

| Treatments | No compost | Compost A | Compost B |
|---|---|---|---|
| No fertilizer | xxx | xxx | xxx |
| Tomato fertilizer | xxx | xxx | xxx |
| Cabbage fertilizer | xxx | xxx | xxx |
| NP fertilizer | xxx | xxx | xxx |

The decomposition trial was conducted for a period of three months at room temperature while the pots were kept at field water capacity by regular weighing of the pots. During the decomposition trial, the following decomposition indicators were periodically monitored: $CO_2$ respiration, DOC and β-glucosidase activity. Polyphenol oxidase activity was planned to be analysed in this experiment, but due to extensive delays in the delivery of required chemical standards it was not possible to determine its activity in the compost decomposition experiment.

At the end of the decomposition study, the soils and soil-compost mixtures were thoroughly mixed and subsamples were taken and air-dried for further analysis. The pH (water and 1M KCl), total C and N content (Eurovector Elemental Analyzer 3000), and Loss on ignition (LOI) of the soil and soil-compost mixtures was determined at the beginning and the end of the 3-month decomposition period.

#### *3.2.3.1 Respiration analyses*

The respiration rates were monitored on a weekly basis using a $CO_2$ trap of 1M NaOH solution. The respiration rates were analysed by pipetting 20 ml of 1M NaOH into 50 mL glass bottles which were placed on top of the soil in the pot and covered with a 0.5 L glass jar for 24 to 120 hours (Anderson, 1982). Blanks were prepared in the same manner and placed on the bench under 0.5 L glass jar for 24 to 120 hours to account for atmospheric $CO_2$. At the end of the collection period, the glass bottles were removed and immediately sealed with screw cap lids. A 10 ml aliquot of the NaOH from the respiration traps was added to 5 ml 1M $BaCl_2$ solution and centrifuged for 15 minutes at 6461 g and 7800 rpm. The centrifuged aliquot was then titrated with a 0.25M standardized HCl solution and phenolphthalein colour indicator until the endpoint was reached. The respired C-$CO_2$ was calculated using the following formula:

$$CO_2(mg) = (B-V)NE$$

Where B is the HCl (ml) needed to titrate the NaOH solution from the control, V is the HCl (ml) needed to titrate the NaOH solution from the respiration jar, N= 0.25 (HCl Normality) and E is the equivalent weight of C(6). The data was then expressed as mg C respired per $m^2$ per h.

#### *3.2.3.2 Dissolved organic carbon analyses*

Dissolved organic carbon (DOC) analyses were performed on leachate water collected from the soils. During the first three weeks of the incubation period leachate was

analysed every 7 days. Thereafter two analyses were done at 14 day intervals, and a final analysis was done after a 35 day interval. Collection of leachate was done by adding a fixed amount of water (18% higher than the FWC) to each soil treatment to allow for a volume of 50-100 ml to be collected from each treatment. The leachate water was filtered using 0.45 um membrane filters to remove any particulate matter from both the sand and organic matter fraction. A 50 ml, 5x dilution sample was prepared from each leachate sample. The diluted samples were sent to CSIR, Stellenbosch for DOC analyses by an Analytikjena multi N/C3100 TOC/TN analyser and the results were reported as mg C $L^{-1}$. Thereafter the results were corrected for the dilution factor and the total collected leachate volume was incorporated to express the results as mg DOC.

#### *3.2.3.3 β-Glucosidase activity*

β-Glucosidase activity was analysed according to the method described by Tabatabai (1982), and Eivazi and Tabatabai (1988). Analyses were done at the same time intervals as for the DOC analyses. One gram of soil sample was placed in a volumetric flask, followed by 0.25 ml of toluene and 4 ml modified universal buffer (MUB). The samples were then incubated with 1 ml of substrate, *p*-Nitrophenyl-β-D-glucopyranoside (PNG), for one hour at 37˚C to allow for the release of *p*-Nitrophenol. After the incubation an addition of $CaCl_2$ and Tris buffer is made to aid in the extraction of *p*-Nitrophenol. Blanks were prepared in the same manner but were incubated without substrate PNG which was added immediately after the incubation before the $CaCl_2$ addition. The samples were filtered through Whatman 2v filter paper and the absorbance was measured at 400 nm. The resultant absorbance readings were calibrated using a standard calibration curve, which was prepared using a standard *p*-Nitrophenol solution, and reported as µg *p*-Nitrophenol $g^{-1}$dwt $h^{-1}$ calculated as follows:

$$\frac{C \times v}{dwt \times SW \times t}$$

Where *C* is the measured concentration of p-*Nitrophenol, dwt* is the dry weight of 1 g moist soil sample, *v* is total volume of the soil suspension in millilitres, *SW* is the weight of the soil used (1 g) and *t* is the incubation time in hours.

### 3.2.4 Statistical analysis

Statistical analysis was performed for all decomposition parameters using SAS Enterprise Guide 5.1. A one way ANOVAs were fitted followed by Fishers LSD post- hoc multiples comparison tests to evaluate significant difference between treatments.

## 3.3 Results and Discussion

### 3.3.1 Soil and compost characterization

The C and N content of the sandy soil was very low with a 0.16% C content and 0.03% N content. The C:N ratio was therefore 5.33. The C and N content for the two composts differed greatly (Table 3.3); Compost A contained the most C (39.02% C) and the least N (2.21% N), with a C:N ratio of 17.66, while Compost B contained less C (18.85% C) and more N (3.83% N), with a C:N ratio of 4.92. Organic matter containing 2.5% N or more is considered to be high quality (Palm, 2000), therefore Compost A in this study is considered to be of low quality, whilst Compost B is considered to be high quality Even though the C:N ratios of the two composts differ significantly, they both fall in the range for net nitrogen mineralisation (Havlin et al. 2005). Compost B contained a higher amount of lignin in both the acid soluble and acid insoluble form, as well as having a greater polysaccharide percentage than Compost A (Table 3.4). The high C content of Compost A as well as the lower lignin content and high water extractives percentage all indicate that it underwent a less extensive composting process.


**Figure 3.1:** Digital photographic images of Compost A (left) and Compost B (right)

**Table 3.3: Soil and compost elemental characterisation.**

| Compost | %C | %N | %P | %K | %Ca | %Mg | C:N |
|---|---|---|---|---|---|---|---|
| Soil | 0.16 | 0.03 | | | | | 5.33 |
| Compost A | 39.02 | 2.21 | 0.61 | 1.20 | 5.76 | 0.43 | 17.67 |
| Compost B | 18.85 | 3.83 | 0.48 | 0.97 | 2.67 | 0.32 | 4.92 |

**Table 3.4: Lignin, polysaccharide and water extractives content of the composts.**

| | Lignin (%) | Polysaccharide (%) | Water extractives (%) |
|---|---|---|---|
| Compost A | 46.63 | 21.48 | 8.95 |
| Compost B | 51.16 | 25.01 | 4.49 |

FTIR spectroscopy of the two composts showed the compost composition to be similar in terms of the presence of carboxylic acids, aromatic rings and saccharide alcohols with Compost B showing greater absorbance for the above mentioned functional groups (Figures 3.2 and 3.3). The greater absorbance for saccharide alcohols coincides with the higher % polysaccharide content determined through carbohydrate and lignin analysis (Table 3.4). The very high absorbance peak at 1033 $cm^{-1}$ is associated with glucose (Chiş et al. 2010).

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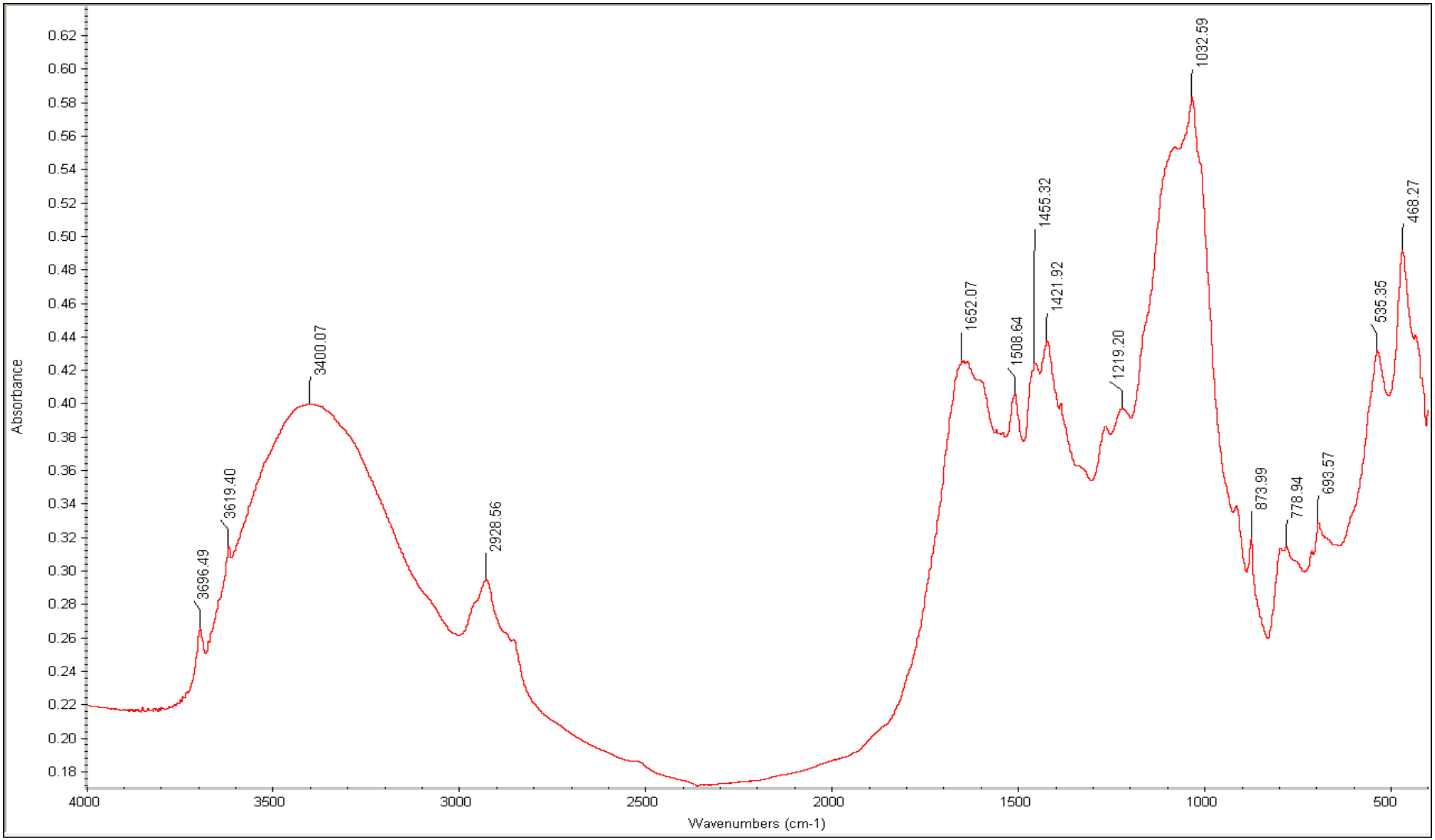


**Figure 3.2:** FTIR spectrum of Compost A

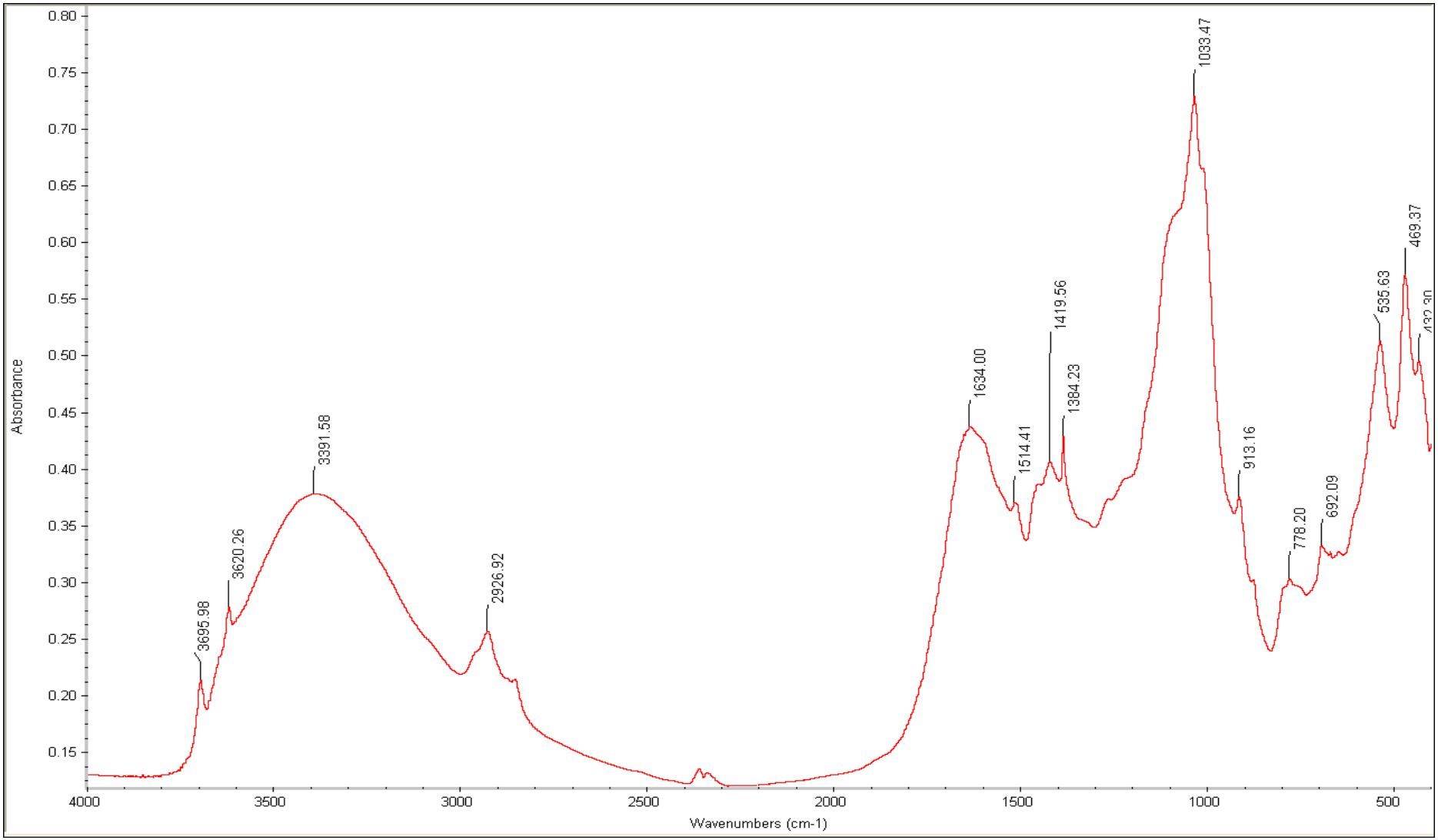


**Figure 3.3:** FTIR spectrum of Compost B

### 3.3.2 pH measurements

The addition of the composts to the acidic, sandy soil raised the soil pH for all fertilizer treatments by at least a pH unit (Figure 3.4). The fertilizers had an acidifying effect on the soil and compost treatments (Figure 3.4). The acidifying effect was more pronounced in the soil only treatments, which were poorly buffered compared to the compost treatments. The NP fertilizer had the most acidifying effect on all treatments due to the use of relatively high levels of both monocalcium phosphate and ammonium nitrate (Table 3.1). Monocalcium phosphate has a pH of 2.8 (10% slurry) and reacts with water to produce dicalcium phosphate and phosphoric acid (Harter, 2002) (Equation 3.1 and 3.2).

$$Ca(H_2PO_4)_2 + H_2O = CaHPO_4 + H_3PO_4 \qquad \text{(Equation 3.1)}$$

Phosphoric acid can undergo hydrolysis to produce 3 protons;

$$H_3PO_4 = H^+ + H_2PO_4^- = 2H^+ + HPO_4^{2-} = 3H^+ + PO_4^{3-} \qquad \text{(Equation 3.2)}$$

The cabbage fertilizer treatment was the second most acidifying treatment. This is attributed to the cabbage fertilizer's high ammonium content (Table 3.1) which, as shown in equation 3.3, is associated with acidification due to nitrification.

Ammonium nitrate undergoes nitrification associated with acidification of soil

$$NH_4^+ + 2O_2 = NO_3^- + 2H^+ + H_2O \qquad \text{(Equation 3.3)}$$

The tomato fertilizer treatment contained a smaller quantity of ammonium and no monocalcium phosphate, therefore being the least acidifying of the three fertilizer treatments (Figure 3.4). Compost B showed a greater buffering capacity, with smaller changes in pH over the range of fertilizer treatments as well as smaller pH differences between initial and final pH. This is probably due to better compost maturity. Extended composting time increases the degree of humification in the compost which is associated with increased carboxylic functional groups (Campetelli et al. 2003). This is supported for compost B by the greater absorbance around the 1600-1700 $cm^{-1}$ range (Figure 3.3).

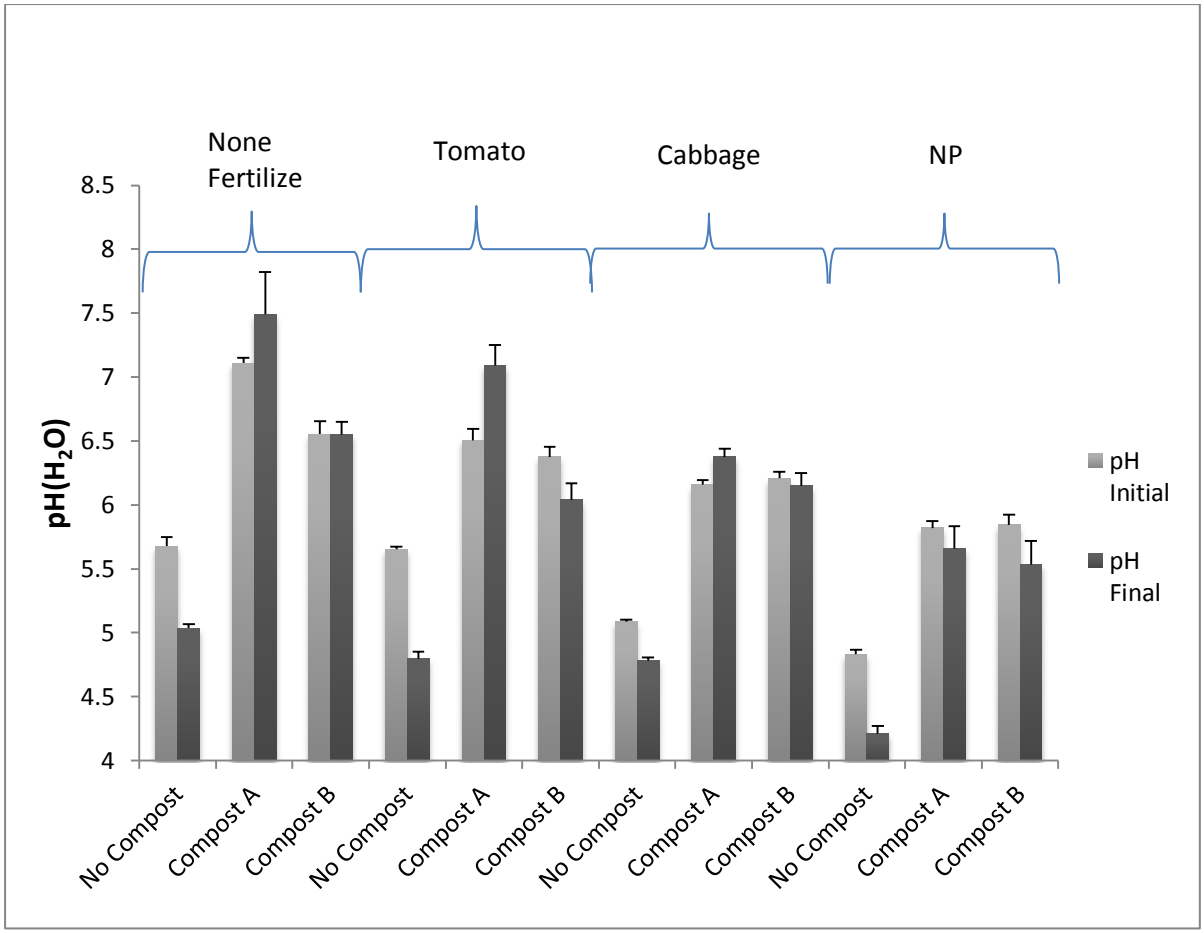


**Figure 3.4:** Initial and final (after 3 months) soil pH (water) values of the fertilized and unfertilized soil and compost-amended treatments. Standard Error bars are shown above each average bar.

### 3.3.3 Decomposition study results

#### *3.3.3.1 Respiration*

The respiration measurements varied over time and with ambient temperature which was not maintained constant for this study. This meant that linear regression modelling of the data would be complicated and speculative due to the relatively large time periods between measurements. Cumulative respiration over the incubation period gave clearer indications of the differences between respirations for the varying treatments (Figures. 3.5 and 3.6). Compost quality was found to be the predominant determining factor for respiration rates with Compost A consistently respiring more than Compost B for all fertilizer treatments (Figure 3.6). Though fertilizer rates showed no significant effect on respiration, the cabbage fertilizer consistently decreased respiration for both composts treatments (Compost A: -10%; Compost B: -11%) as well as slightly (-1%) in soil only treatment (Figure 3.6b-c). This response corresponds with decreased respiration rates observed in a previous study performed by Cleveland et al. (2006) of soils receiving dissolved organic matter as substrate along with various fertilizer treatments including 150 kg N $ha^{-1}$ $yr^{-1}$ and 150 kg N + P $ha^{-1}$ $yr^{-1}$ treatments. The NP fertilizer treatment resulted in a significant ($P = 0.019$) increase in respiration for the soil only treatment (+27%) and only slight increases in the compost-amended soils (Compost A: +4%; Compost B:+3%) (Figure 3.6a-c). The increased respiration observed for NP fertilizer could be attributed to both the decreased pH as well as the increased levels of $Ca^{2+}$. The optimum pH for lignin degradation is between pH 4-4.5 and is suppressed above pH 5.5. At higher pH levels $Ca^{2+}$, essential for the activity site integrity of lignin-degrading enzymes, dissociates from the distal and proximal sites of the enxyme complex. However under lowered pH levels $Ca^{2+}$ is reintroduced and reactivation of the enzyme complex takes place (Eriksson et al. 1990; Lindeberg, 1994; Singh andand Chen, 2008). Since the final pH of NP fertilized soil only treatment is 4.21 (Figure 3.4), it falls in the optimum pH range. Both compost-amended treatments under NP fertilizer have pH levels above 5.5 (Compost A: 5.66; Compost B: 5.53) (Figure 3.4) where enzyme suppression begins. Thus the lower pH of the NP soil only treatment could be the reason for the greater increase in respiration (Figure 3.6a). The added $Ca^{2+}$ due to the NP fertilizer (Table 3.1) is also likely to have a greater effect on the soil only treatment due to inherent low $Ca^{2+}$ content of acidic sandy soil (Sika 2012) compared to the two compost treatments which already contained high levels of $Ca^{2+}$ (Compost A: 5.8%; Compost B: 2.7%) (Table 3.3).

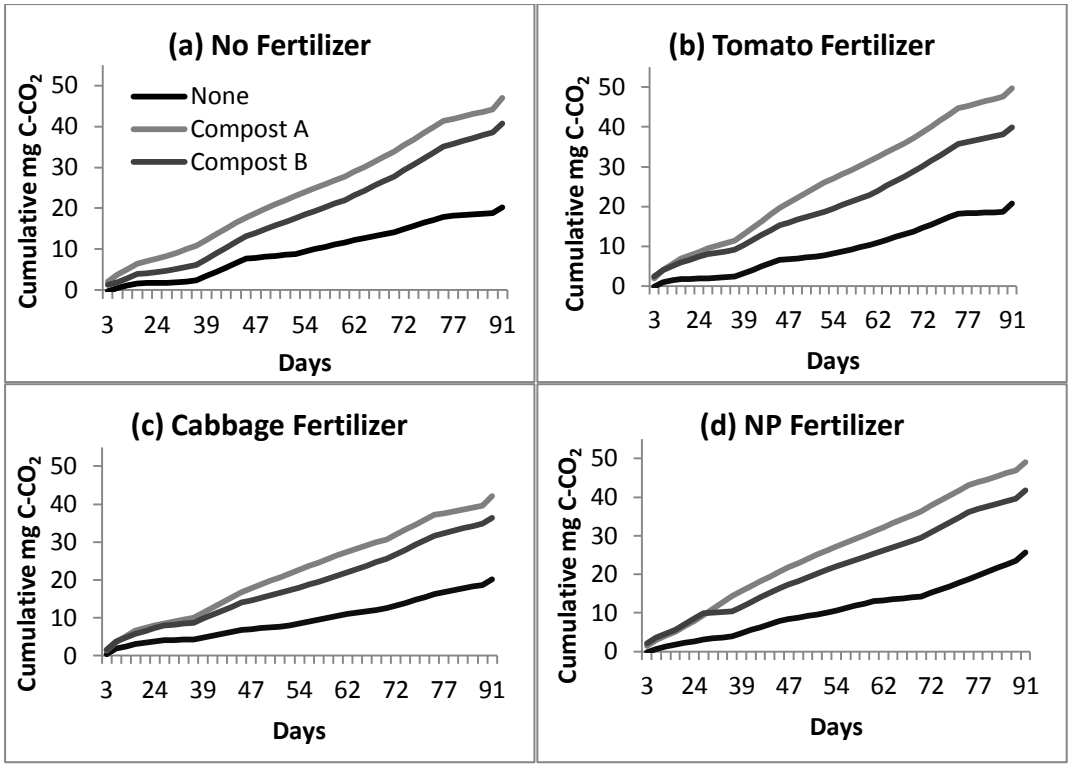


**Figure 3.5:** Cumulative respiration over time from the control and compost-amended treatments treated with: (a) no fertilizer, (b) Tomato fertilizer, (c) Cabbage fertilizer and (d) NP Fertilizer.

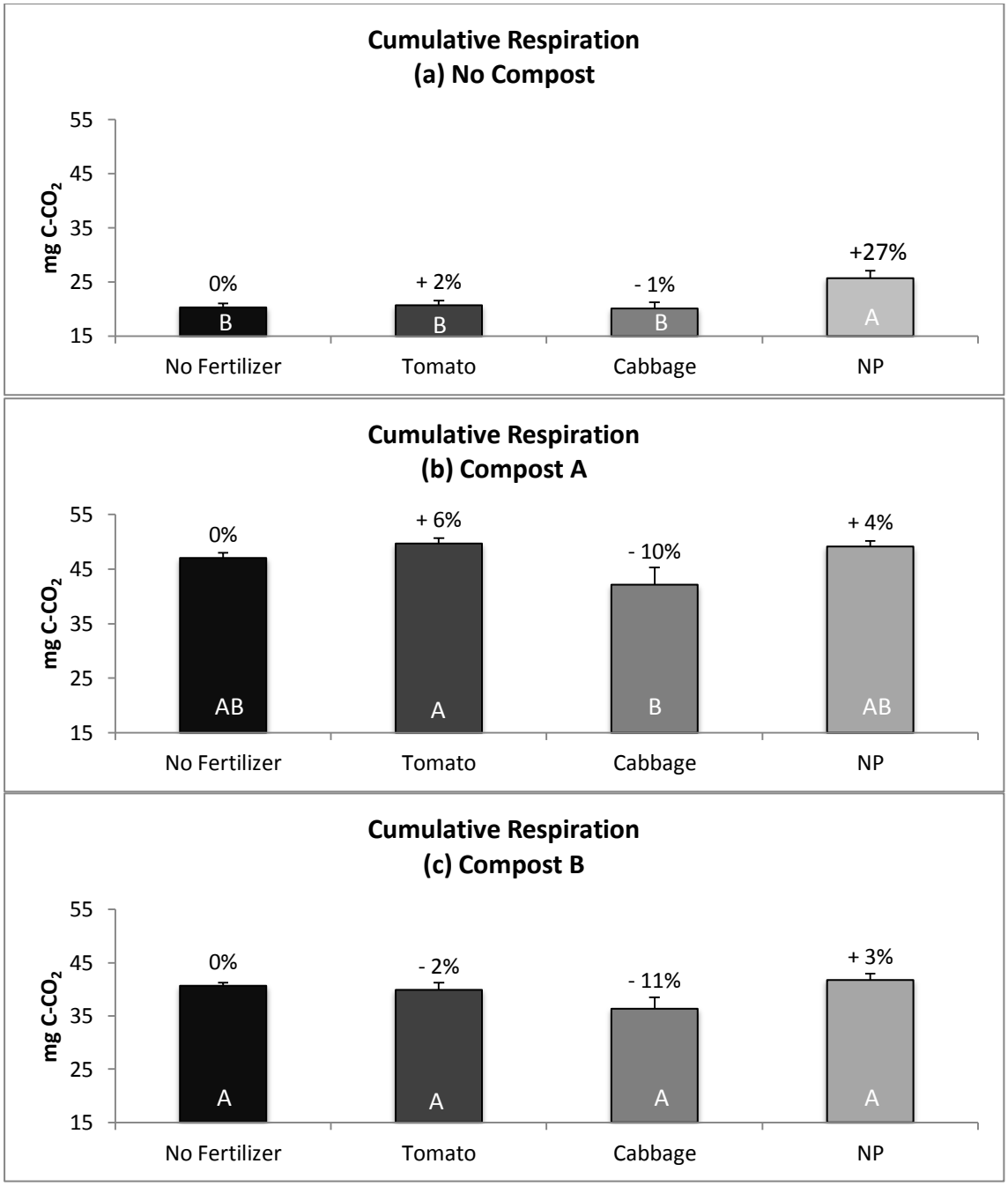


**Figure 3.6:** The effect of fertilizer treatments on cumulative respiration of (a) soil only, and soil amended with (b) Compost A and (c) Compost B. Standard error bars are shown above each average bar. Percentage change is indicated above each average bar. Alphabetic letters indicate significant difference according to Fisher's LSD test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

### *3.3.3.2 Dissolved Organic Carbon*

The production of DOC was at its peak in the first 21 days of decomposition, thereafter rapidly declining and stabilizing over the subsequent two months (Figure 3.7).

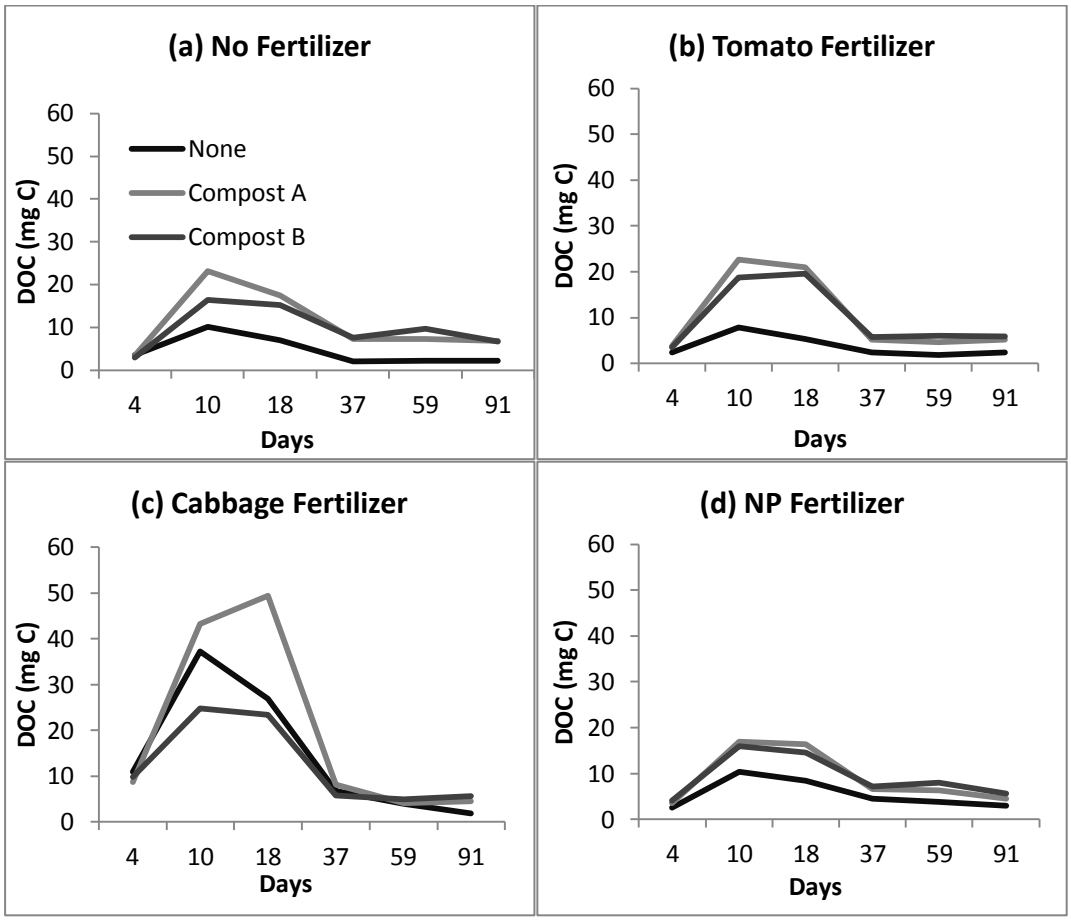


**Figure 3.7:** Dissolved organic carbon production over time from the soil and compost-amended treatments treated with: (a) no fertilizer, (b) tomato fertilizer, (c) cabbage fertilizer and (d) NP Fertilizer.

Total cumulative DOC clearly illustrates the effect of the fertilizer treatments on DOC production (Figure 3.8). The cabbage fertilizer treatment significantly increased the total DOC production from the soil only and compost-amended systems, compared to the other fertilizer treatments (Figure 3.8a-c). The relative increase in DOC production due to the cabbage fertilizer was greatest in the soil only treatment (225%) than two compost treatments. Compost A (81%) had a greater increase in DOC than Compost B (27%). This observation appears to correspond with the inherent N content of each of the systems; the lower the inherent N content of the soil system (Table 3.3), the greater the increase in DOC production due to the addition of cabbage fertilizer (Figure 3.8). Cabbage fertilizer contained the highest content of inorganic N (as both $NH_4^+$ and $NO_3^-$) compared to the other fertilizer treatments (Table 3.1). Several authors have previously shown that high levels of low molecular weight N, in the form of ammonium and nitrates

decreases $CO_2$ mineralisation rates of forest litters such as Scots pine, Norway spruce and Birch, promoting DOC production through the suppression of lignin degrading enzymes. Keyser at al., 1978 observed that some basidiomycetes only produced lignin degrading enzymes under low N environments and are suppressed in the presence of low molecular weight N. The suppression of these enzymes leads to increased levels of lignin and lignin like substances (Nömmik and Vahtras, 1982; Stevenson, 1982). Eriksson et al (1990) confirmed the suppression of lignin degrading enzyme production for several species of lignin degrading fungi. Furthermore, it has been suggested by various authors that DOC production, i.e., humification, is promoted by low molecular weight N through condensation reactions with lignin and phenolic components (Nömmik and Vahtras, 1982; Stevenson, 1982; Axelsson and Berg, 1988). Davidson et al. (2003) suggest that the reducing power of organic matter leads to nitrate being transformed into nitrite which is subsequently incorporated into DOM through nitrosation and nitration of aromatic rings in dissolved lignin and polyphenols. The resultant humification reaction products contain covalent bonds with N bridges that may be more resistant to decomposition than the starting materials due to their molecular complexity and types of chemical bonds (Huang and Hardie, 2008).

The addition of NP fertilizer led to a statistically significant decreases in DOC production in the compost A treatment (Figure 3.8b). These decreases could possibly be attributed to the pH decrease and $Ca^{2+}$ increase in the NP fertilizer treatments, leading to stimulated enzyme activity as observed by the increased $CO_2$ mineralisation in these treatments (Figure 3.6b and c) (Lindeberg, 1944; Eriksson et al. 1990; Dingh and Chen, 2008). The stimulation of the enzymatic activity could have been greater than the expected enzyme suppression through inorganic N addition. The addition of inorganic N in the NP fertilizer treatment is significantly less than that of cabbage fertilizer (150kg $ha^{-1}$ and 250kg $ha^{-1}$, resp.) which would therefore also induce less enzyme suppression than cabbage fertilizer. The tomato fertilizer had no significant effect on the production of DOC, and also contained a lower inorganic N content than the cabbage fertilizer (Table 3.1). In the absence of added fertilizer, it can be seen that compost quality had a relatively small effect on DOC production (Compost A: 65 mg C; Compost B: 58 mg C) (Figure 3.8b and c). Furthermore, for both the Tomato fertilizer and the NP fertilizer applications, differences between DOC production between Compost A and Compost B remain very small (Figure 3.8b and c).

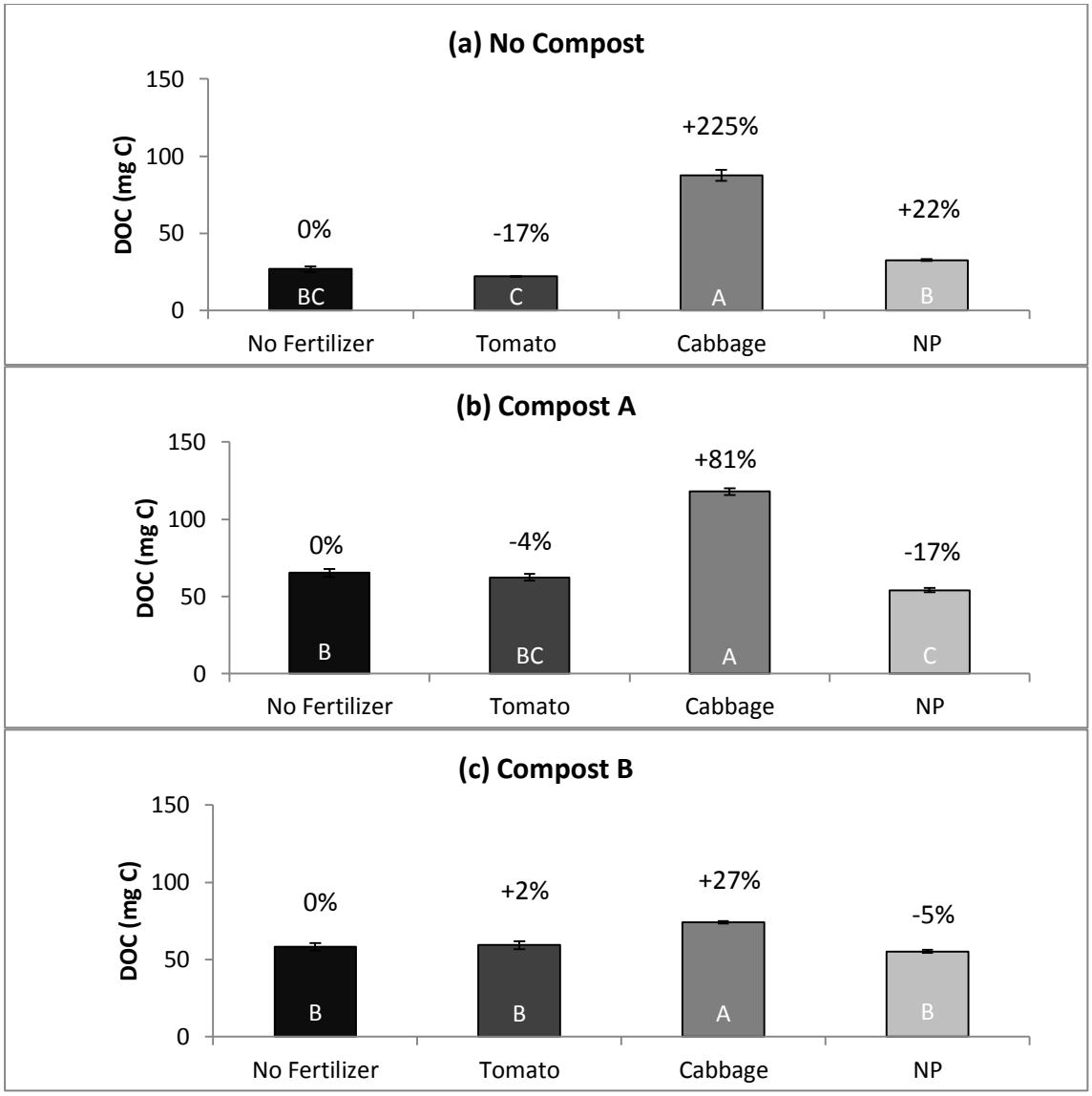


**Figure 3.8:** The effect of fertilizer treatments on cumulative DOC of (a) soil only, and soil amended with (b) Compost A and (c) Compost B. . Standard error bars are shown above each average bar. Percentage change is indicated above each average bar. Alphabetic letters indicate significant difference according to Fisher's LSD test at α = 0.05. Similar letters indicate lack of significant differences.

### *3.3.3.3 B-Glucosidase activity*

The effect of fertilizer application on β-glucosidase activity was statistically insignificant (Figure 3.9). However, it was found that NP fertilizer application consistently increased enzyme activity for both the soil only and compost treatments (Figure 3.9). This is attributed to the lower soil pH of the NP treatments (Figure 3.4), as the optimal pH for β-Glucosidase activity is around pH 5 (Hayano and Tubaki, 1985). From the total cumulative β-Glucosidase activity it can be concluded that β-Glucosidase activity is dependent on compost quality rather than fertilizer rates (Figure 3.9). Compost B showed greater activity for all fertilizer treatments than Compost A. This is contradictory

with the results found in a study by Carreiro et al (2000) where increased N deposition led to enhanced activity of cellulases. The primary difference in our study compared to theirs is the source of organic matter to which the N was added, theirs being labile, undecomposed plant litter. Therefore the difference in observation is likely due to the fact that the composts used in this study probably contain very low levels of polysaccharides compared to fresh residues. This is supported by the greater β-Glucosidase activity observed for Compost B which contained a greater amount of polysaccharides (Table 3.4 and Figure 3.3).

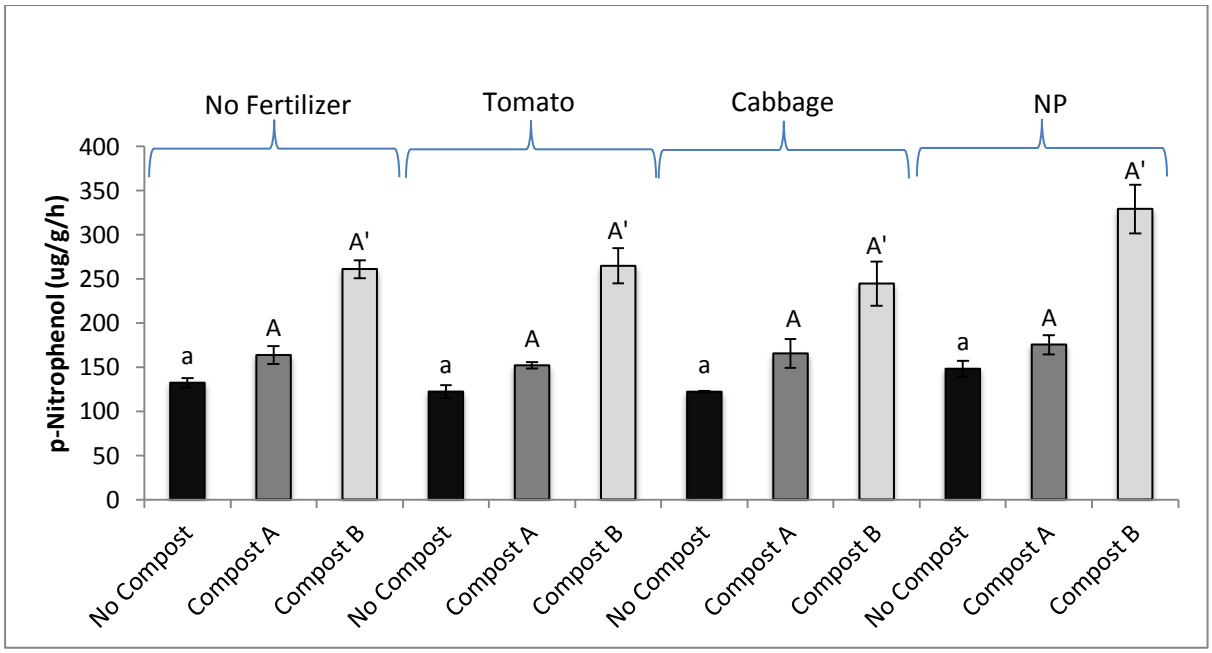


**Figure 3.9:** Total cumulative β-Glucosidase activity for fertilized and unfertilized soil only and compost-amended treatments. Standard error bars are shown above each average bar. Alphabetic letters indicate significant difference according to Fisher's LSD test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

### 3.3.4 Loss on Ignition

The cabbage fertilizer application resulted in the greatest % decrease of OM in the Compost A (13.64%) and Compost B treatments (18.18 %) compared to the other fertilizer treatments (Table 3.3 and 3.4). This correlates with the increased loss of organic matter in the form of DOC that was observed in the cabbage fertilizer treatments (Figure 3.8b and c). However, the NP fertilizer resulted in the greatest loss of OM (23.37%) in the soil only treatment (Table 3.5 and 3.6) even though the cabbage fertilizer treatment also resulted in significant increase in DOC production in the soil only treatment (Figure 3.8a). This loss correlates with significant 27% increase in respiration observed in the soil only treatment that received NP fertilizer (Figure 3.6a). Generally, the tomato fertilizer and NP fertilizer treatments (except the NP soil only treatment) showed similar OM losses to the treatments receiving no fertilizer (Table 3.3 and 3.4), which correspond with the minimal changes in respiration and DOC production observed in these treatments relative to the no fertilizer treatments (Figures. 3.6 and 3.8). It can be seen that in all treatments, especially the compost treatments, that DOC production was a major controlling factor in final OM content of the various treatments, thus emphasizing the importance of measuring this parameter in decomposition studies.

**Table 3.5: Percentage organic matter (determined by LOI) at start and end of decomposition trial for fertilized and unfertilized soil and compost-amended treatments.**

| | Initial %OM | Final %OM | | | |
|---|---|---|---|---|---|
| | No Fertilizer | No fertilizer | Tomato fertilizer | Cabbage fertilizer | NP fertilizer |
| Soil only | 0.87(0.027) | 0.73(0.019)(B) | 0.83(0.019)(A) | 0.70(0)(B) | 0.67(0.019)(B) |
| Compost A | 1.47 (0.072) | 1.40(0.033)(A) | 1.37(0.019)(A) | 1.27(0.087)(A) | 1.33(0.080)(A) |
| Compost B | 1.83(0.400) | 1.60(0.047)(A) | 1.63(0.134)(A) | 1.50(0.033)(A) | 1.67(0.019)(A) |

*Brackets represent Standard error for treatment replications.

**Table 3.6: The effect of fertilizer treatment on % change in organic matter content with reference to initial organic matter content before decomposition.**

| Organic matter % change | | | | |
|---|---|---|---|---|
| | No fertilizer | Tomato fertilizer | Cabbage fertilizer | NP fertilizer |
| Soil only | -15.70 | -4.21 | -19.50 | -23.37 |
| Compost A | -4.55 | -6.82 | -13.64 | -9.09 |
| Compost B | -12.73 | -10.91 | -18.18 | -9.09 |

## 3.4 Conclusion

In this study, the decomposition dynamics of composts in sandy soil were influenced by both compost quality as well as the addition of mineral fertilizers. Although slight decreases in respiration were observed for the cabbage fertilizer treatments, these decreases were not statistically significant. Therefore, it can be concluded that respiration rates of composted organic matter investigated in this study were primarily dependent on compost quality. Two factors which coincided with respiration changes were soil pH and polysaccharide content. The NP fertilizer, which had the greatest acidifying effect on the soil and composts, showed increased respiration, though not statistically significant. This is contradictory to the results observed by Cleveland et al. (2006) which found decreased respiration rates for treatments fertilized with N and NP fertilizers. The large decrease in pH for our NP fertilizer treatment can account for this different response due to increased microbial and enzyme activity at and around pH 5. Compost B containing the highest amount of polysaccharides consistently produced the highest respiration for all fertilizer treatments. This can be attributed to the greater content of labile substrate compared to that of Compost A.

Although respiration was insignificantly influenced by fertilizer treatments, DOC production was significantly altered by the addition of high levels of mineral fertilizer, especially for the cabbage fertilizer which delivered the greatest amount of mineral N. This response was consistent for all compost treatments. Compost quality did play a role in DOC production with Compost A producing slightly more DOC than Compost B. It is evident that the lower the inherent N content in the material, the greater the response in DOC production with the addition of mineral N. This increase in DOC production can be attributed to several factors which are altered by the addition of mineral N such as the suppression of lignin degrading enzyme production and functioning under high levels of low molecular weight N application (Keyser et al. 1987; Eriksson et al. 1990), incorporation of mineral N into dissolved organic matter through condensation and polymerization reactions with polyphenols and aromatic compounds (Nommik and Vahtras, 1982; Stevenson, 1982; Davidson et al 2003) and the abiotic immobilization of $NH_4^+$ and $NO_3^-$ and subsequent polymerization of amino nitrogen sources with saccharides to form recalcitrant organic matter (Nommik and Vahtras 1982; Johnson et al 2000).

In a substrate inherently low in polysaccharide content, β-Glucosidase activity is unaffected by the addition of mineral fertilizers, and predominantly affected by

differences in polysaccharide content as well as pH. The NP fertilizer enhanced β-Glucosidase activity for all compost treatments, yet the enzyme activity did not show a significant response to the high N containing cabbage fertilizer. This can be ascribed to the relatively lower pH of the NP treatments providing optimal conditions for β-Glucosidase activity. In terms of compost quality, Compost B consistently showed greater β-Glucosidase activity due to higher polysaccharide content than Compost A. β-Glucosidase activity will remain unaffected unless a change in pH takes place with the fertilizer applications.

The change in organic matter content as determined from the LOI results corresponded with the changes in DOC production indicating that cabbage fertilizer lead to the greatest increase in decomposition and subsequent loss of OM from the soil. Due to increased respiration and β-glucosidase activity for soil only treatments under NP fertilizer, this treatment showed the greatest % change in organic matter content between initial and final LOI results. Cabbage fertilizer was the only fertilizer to consistently result in OM losses greater than that of the unfertilized treatments, therefore it can be concluded that cabbage fertilizer enhanced decomposition for all compost treatments.

Based on these results the overall conclusion can be drawn that high levels of mineral N (±250 kg $ha^{-1}$) will lead to slight suppression of respiration and significant increases in DOC production regardless of the initial compost quality. This translates into greater losses of organic matter as confirmed by LOI results of this study. However, increased DOC production can also promote humification if it is not leached out of the soil, but rather binds to subsoil clay minerals and sesquioxides, thereby enhancing subsoil organic matter content and carbon stabilization.

# CHAPTER 4
# THE EFFECT OF INORGANIC FERTILIZER APPLICATION ON PLANT LITTER DECOMPOSITION DYNAMICS IN SANDY SOIL

## 4.1 Introduction

The global number of people falling under the food insecure population was estimated to be approximately 870 million (FAO, 2012). The continuous growing demand for quality and nutrient rich foods place agricultural sectors under severe pressures. Crop productivity is affected by several soil factors including soil structure, water retention, aeration and nutrient availability, all of which are improved with increased levels of soil organic matter and soil organic carbon levels.

Several studies have looked into mechanisms of carbon sequestration in agricultural soils(Lal 2011; Ogle et al. 2005; Manna et al. 2005), emphasizing the importance of proper soil management to enable carbon sequestration and sustainable crop production. In order to properly manage soil organic matter, a proper understanding with regards to the factor affecting its decomposition is essential. Much research has been done in the general area of forest and grassland litter decomposition and the effect of nitrogen on decomposition rates. However very little has been done on agricultural crop residues and no research has been done on this in South Africa.

A range of studies have shown that inorganic nitrogen additions decrease litter decomposition rates (Magill and Aber 1998; Hagedorn et al. 2003; Knorr et al. 2005; Rudrappa et al. 2006). The majority of these studies, however, do not exclude the effect of extra litter input due to increased crop residue production associated with the increased fertilizer application. In contrast, Alvarez (2005) states that nitrogen addition only increased soil organic carbon levels if the crop residues were left in the field. Berg (2000) suggested that nitrogen additions decrease litter decomposition due to the formation of more complex recalcitrant compounds as well as possibly repressing enzymes essential to lignin decomposition. Some research shows that nitrogen additions accelerate the decomposition of labile organic matter whilst stabilizing non-labile organic matter (Neff et al. 2002; Wang et al. 2004).

Another study found that nitrogen additions accelerated decomposition of litter but suppressed the decomposition of soil organic matter (Saiya-cork et al. 2002). It is clear that there is much controversy with regards to the effect of nitrogen fertilizer and that the effects certainly vary based on litter quality (Köchy and Wilson., 1997).With so much inconsistency regarding the effects of nitrogen on decomposition (Hobbie 2005) it is

difficult to predict how decomposition rates and nutrient cycles will be affected with fertilizer applications. Many factors contribute to this; however the main problem lies in the lack of understanding regarding the mechanism with which inorganic fertilizer application interacts with SOM and microorganisms.

Several studies have hypothesized about the mechanisms of interaction; however little scientific proof has been established to support these theories. Jung et al (2011) suggested that inorganic nitrogen either directly or indirectly, through a soil pH decrease, mediates the formation of recalcitrant carbon forms, whilst several authors have suggested the nitrogen mining theory(Moorhead and Sinsabaugh, 2006; Craine et al. 2007).There is much debate regarding the effect of inorganic fertilizer on microbial activity. Fog (1988) discussed the possibility that nitrogen additions may reduce the ability of basidomycetes to compete in soil environments, thereby reducing the rate of lignin decomposition. Wiemken et al. (2001), on the other hand, found no differences with increased nitrogen. Recent research done on the influence of litter and organic matter quality and rates of decomposition have shown that the relationship between N addition and decomposition rates is much more complex than initially expected, and that several factors contribute to the variation observed in the above mentioned studies. Studies evaluating the relationship between cellulose and lignin degrading enzymes, and organic matter mass loss have found a positive correlation (Sinsabaugh and Linkins 1993; Sinsabaugh and Moorhead 1994). Carreiro et al. (2000) looked at the effect of elevated atmospheric N deposition on the decomposition rates of three litter types of varying quality and carbon composition (Dogwood, Red Maple and Red oak), and investigated the effect on extracellular enzyme activity. From their results it was understood that decomposition rates varied depending on initial lignin content and the litter lignin-cellulose index (LCI) (Melillo et al. 1989). Litter with low lignin content showed an increase in the rate of decomposition with both low and high N additions, whilst high lignin litters showed decreased decay rates. In terms of enzyme activities they observed that cellulases were stimulated for all litter types, concluding that litter with a low LCI, which already decompose relatively quickly, will decompose at an even greater rate under N additions. However, in terms of lignin degrading enzymes, a decreased activity was observed for phenol oxidases in high lignin containing litters. The production of phenol oxidase enzymes is predominant in white rot fungi, even though some bacteria and macrofungi can produce other enzymes related to the partial decomposition of lignin. Therefore, Carreiro et al (2000) hypothesised that the observed decrease may be due to the suppression of phenol oxidase expression in white rot fungi under high N

availability, and/or a reduction in the abundance of white rot fungi under these environments. These findings were however contradicted in a study by Allison and Vitousek (2004) who observed no decrease in phenol oxidase activity for litters with high lignin content. In comparison with a study by Hobbie (2000), also conducted on Hawaiian plant material which also showed similar results for high lignin plant material, Allison and Vitousek suggested that fungi may not dominate the decomposer community in Hawaiian forests or that fungus response to N additions may be dependent on the ecosystem in which the study is conducted.

In addition to C, N and lignin content, other chemical properties of litter quality, such as polyphenol content have also been found to play a role in enzyme activity and decomposition rates. It has long been known that polyphenols act as microbial inhibitors and can, at high levels, be toxic to bacteria and fungi. Appel (1993) concluded that polyphenols in organic matter have the ability to bind to proteins and thereby decrease the enzyme activity of litter degrading microorganisms. Allison and Vitousek (2004) further suggested that not only the chemical properties but also the physical properties can influence the relationship between enzyme activities and litter decay rates. Cornelissen (1996) observed that litter surface area and toughness affected both microbial access as well as enzyme diffusion, making the litter less decomposable. A litter decomposition model developed by Sinsabaugh and Moorhead (1994) represented a linear relationship between decay rates and activity of cellobiohydrolase and polyphenol oxidase enzymes, however in the study done by Allison and Vitousek (2004) the decay rates correlated more strongly with water soluble carbon content than enzyme activity. They also found that water soluble carbon was more important in the determination of the decay constants than the commonly used quality parameters of lignin and N content.

In addition to the lack of mechanistic understanding, very few studies that evaluated decomposition rates took into account the influence of fertilizers on dissolved organic matter (DOM). Most decomposition models were solely based on $CO_2$ respiration rates. Under high rainfall or irrigation DOM can contribute significantly to mass loss unrelated to enzyme activity, and can change decomposition dynamics (Schofield et al. 1998; Cleveland et al. 2004). Increases in soil DOC have been observed for soils amended with composts and manures. The initial increase in DOC can be attributed to the DOM of the compost or manure, however desorption of soil DOM may occur, altering the quantity and quality of soil DOC ( Wright et al. 2008).

Due to the controversy as well as the lack of research on agricultural crop residues, the objectives of this chapter was to evaluate the effect of varying levels of inorganic fertilizer application rates on the decomposition dynamics of crop litters commonly encountered in South African agriculture and forestry. The plant litters were selected to represent a wide range of quality parameters such as C:N ratio, lignin content and polyphenol content. Respiration, DOC, β-glucosidase and polyphenol oxidase activity were analysed during the decomposition period to evaluate the effect of inorganic fertilizer application on all major decomposition aspects, thereby providing a comprehensive view of the effects which can be expected from litter decomposition.

## 4.2 Materials and methods

### 4.2.1 Soil and plant litter

The same sandy soil was used for the litter decomposition studies, as was used in the previous compost decomposition study. For soil description and characterisation refer to Section 3.2.1**.** A range of five different plant litters were selected based on their expected C:N ratios. The plant litters were kikuyu grass, lucerne, sugar cane trash, wheat straw and pine needles. All of the litters were collected in and around the Stellenbosch area, except for the sugar cane trash, which came from the Greytown area in KwaZulu-Natal. The selected litters were air-dried, finely ground and then analysed for total C and N, lignin and polysaccharide content as previously described in Section 3.2.1. The soluble polyphenol content of the litters was determined using the Folin-Ciocalteau method described in Yu and Dahlgren (2000).

### 4.2.2 Fertilizer blends

Two mineral fertilizer blended mixtures were selected for the decomposition study. These were typical blends used for vegetable (tomato and cabbage) production, which also commonly make use of sandy soil. The tomato fertilizer application rate had a NPK ratio of 100:20:150 providing a moderate application of mineral N and K and a relatively low application of P. The cabbage fertilizer had a NPK ratio of 250:100:200 providing a relatively high application of mineral N as well as a relatively high P and K application. Table 3.1 provides the amount and type of mineral fertilizer sources which were used in the blends.

**Table 4.1: Constituents of fertilizer blends used in the litter decomposition trial.**

| Fertilizer Applications | | | | |
|---|---|---|---|---|
| | $NH_4NO_3$ (g) | $K_3PO_4$ (g) | $KH_2PO_4$ (g) | $KNO_3$ (g) |
| Tomato (100:20:150) | 0.262 | 0.186 | 0 | 0.240 |
| Cabbage (250:100:200) | 0.799 | 0 | 0.549 | 0.238 |

### 4.2.3 Litter decomposition trial

The litter decomposition trial was conducted in the same manner as the compost decomposition trial. For experimental design and methodology refer to chapter 3 section 3.2.3**.** The plant litters were homogenized to 0.5 mm in length in a blender and then added to the soil to the equivalent of 0.5% C input to ensure that differences in total C would not affect the decompositions.

**Table 4.2:** Experimental treatments in the litter decomposition trial.

| Treatments | No litter | Kikuyu | Lucerne | Pine needles | Sugar cane | Wheat |
|---|---|---|---|---|---|---|
| No fertilizer | xxx | xxx | xxx | xxx | xxx | Xxx |
| Tomato fertilizer | xxx | xxx | xxx | xxx | xxx | Xxx |
| Cabbage fertilizer | xxx | xxx | xxx | xxx | xxx | Xxx |

During the decomposition trial, the following decomposition indicators were periodically monitored: $CO_2$ respiration, DOC, β-glucosidase activity and polyphenol oxidase activity.

At the end of the decomposition study, the soils and soil-litter mixtures were thoroughly mixed and subsamples were taken and air-dried for further analysis. The pH (water and 1M KCl), total C and N content (Eurovector Elemental Analyzer 3000), and Loss on ignition (LOI) of the soil and soil-litter mixtures was determined at the beginning and the end of the 3-month decomposition period.

For methodology on respiration, DOC and β-Glucosidase activity refer to Chapter 3 Sections 3.2.3.1, 3.2.3.2 and 3.2.3.3.

#### *4.2.3.1 Polyphenol oxidase activity*

Polyphenol oxidase activity was analysed according to a modified method described by Allison and Vitousek (2004). Analyses were done at the same time intervals as for DOC and β-Glucosidase activity. One gram of soil was placed in plastic bottles with 4 ml 50mM sodium acetate buffer, pH 5, and 1 ml 5mM L-dihydroxyphenylalanine [L-DOPA] substrate. Blanks consisted of 1 g soil with 5 ml 50mM sodium acetate buffer. The samples were vigorously shaken for 1 h, then the supernatant was pipetted into centrifuge tubes and centrifuged for 5 min at 7800 rpm and the absorbance was subsequently measured at 450 nm. The readings were calibrated using a standard calibration curve which was prepared by oxidising 10 ml 8mM L-DOPA to Dopachrome with mushroom tyrosinase (Sigma T7755) until all the L-DOPA was converted to the orange coloured dopachrome (Figure 4.1). A dilution series was prepared with the dopachrome and the absorbance's measured.

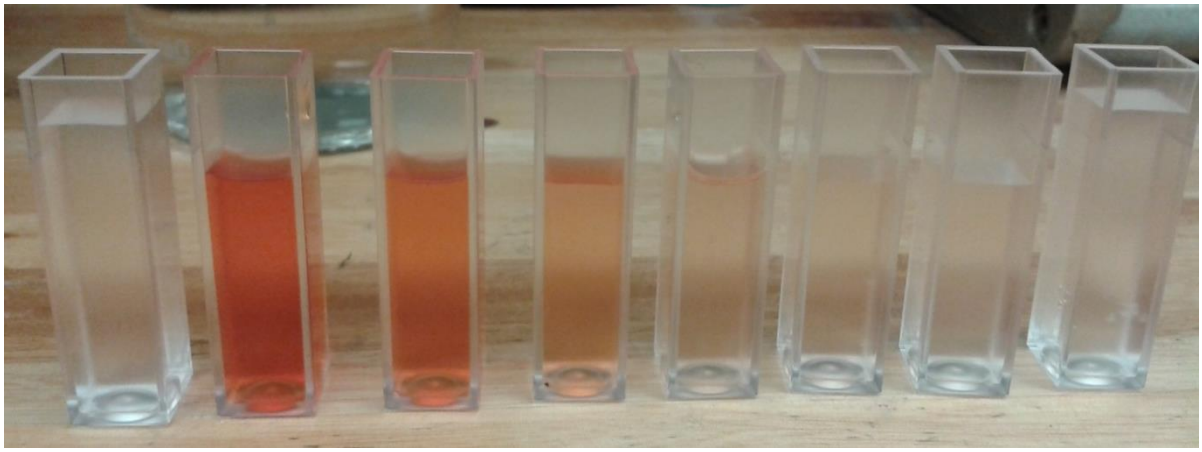

**Figure 4.1:** Digital image of Dopachrome dilution series for PPO standard curve.

The dopachrome concentration was determined using the standard curve and expressed as μmol $g^{-1}$ dwt $h^{-1}$ using the calculation shown in Chapter 3 section 3.2.3.2

## 4.3 Results and Discussion

### 4.3.1 Soil and Plant litter characterization

The C and N content of the sandy soil was very low with a 0.16% C content and 0.03% N content. The C:N ratio was therefore 5.33 (Table 4.3). For the plant litters, the C content differed very little between all five litters, however the N content differed significantly, with kikuyu grass containing the most N and wheat the least (Table 4.3). A

wide range of C:N ratios was therefore observed between the plant litters and was ascribed solely to the differences in N content.

**Table 4.3: Total C and N content of soil and plant litters, and calculated C:N ratios of soil, plant litters and fertilizer-amended treatments.**

| Substrate | %N | %C | C:N | Tomato fertilizer soil C:N | Cabbage fertilizer soil C:N |
|---|---|---|---|---|---|
| Soil | 0.03 | 0.16 | 5.33 | 4.41 | 3.51 |
| Kikuyu | 2.77 | 39.21 | 14.15 | 12.03 | 9.81 |
| Lucerne | 2.41 | 39.81 | 16.50 | 13.69 | 10.89 |
| Pine needles | 1.53 | 43.54 | 28.43 | 20.98 | 15.06 |
| Sugar cane | 0.58 | 39.82 | 68.60 | 36.93 | 21.82 |
| Wheat | 0.46 | 40.51 | 88.04 | 41.94 | 23.49 |

The pine needles contained the highest levels of soluble polyphenols, containing more than 4 times that of the other plant litters (Table 4.4). Kikuyu grass contained the second highest levels, with wheat containing the least (Table 4.4).

**Table 4.4**: Plant litter soluble polyphenol content.

| Plant litter | Polyphenol content (mg $ml^{-1}$) |
|---|---|
| Kikuyu | 110.08 |
| Lucerne | 74.05 |
| Pine needles | 468.87 |
| Sugar cane | 62.73 |
| Wheat | 19.42 |

Pine needles contained the highest % lignin of all plant litters, kikuyu the second highest, and insignificant differences between lucerne and sugar cane, while wheat contained the least (Table 4.5). Wheat contained the highest percentage cellulose and polysaccharides, whilst pine needles and kikuyu grass contained the least polysaccharides (Table 4.5). Pine needles and lucerne contained the highest % total

extractives followed closely by kikuyu grass whilst wheat contained the lowest % total extractives.

**Table 4.5: Plant litter cellulose, lignin, polysaccharide and water extractives composition.**

| Plant litter | Cellulose(%) | Lignin(%) | Polysaccharides(%) | Extractives(%) |
|---|---|---|---|---|
| Kikuyu | 24.36 | 27.92 | 50.22 | 27.41 |
| Lucerne | 27.56 | 22.75 | 45.99 | 29.89 |
| Pine needles | 24.88 | 37.40 | 36.04 | 29.69 |
| Sugar cane | 35.38 | 23.37 | 58.49 | 18.83 |
| Wheat | 40.59 | 22.02 | 58.47 | 12.83 |

From these results it can be seen that pine needles contained the most lignin and polyphenols with the lowest levels of cellulose and polysaccharides however its C:N ratio falls in that which can be considered optimal for microbial decomposition (C:N = 28.43) (Magdoff and Weil, 2004). Wheat contained the least lignin and polyphenols and the most cellulose and polysaccharides yet had the widest C:N ratio (C:N = 88.04).

### 4.3.2 Soil pH measurements

Final soil pH measured for all litter treatments after the decomposition period were higher than the initial pH (Figure 4.2). This could be due to the leaching of plant litter-derived acids and acidic functional groups as DOC from the soil. It could also be due to the decomposition process of ammonification of organic N which increases soil pH (Havlin et al. 2005).

Ammonification:

$$RNH_2 + H_2O + H^+ = ROH + NH_4^+ \qquad \text{(Equation 4.1)}$$

The increased pH may also be due to the addition of more organic functional groups from the fresh litters which can accept protons. This is supported by the observed raised initial pH's after litter additions. The soil only treatment showed a large decrease in final pH after the decomposition, which is possibly due to favourable conditions for excessive nitrogen mineralization (C:N< 20:1)(Table 4.3) and subsequent nitrification of organic matter and fertilizer-derived ammonium which has an acidifying effect on the soil (Havlin

et al,. 2005). The sandy soil only treatment is also much less buffered to pH changes than the litter treatments, and thus more dramatic pH changes are expected.

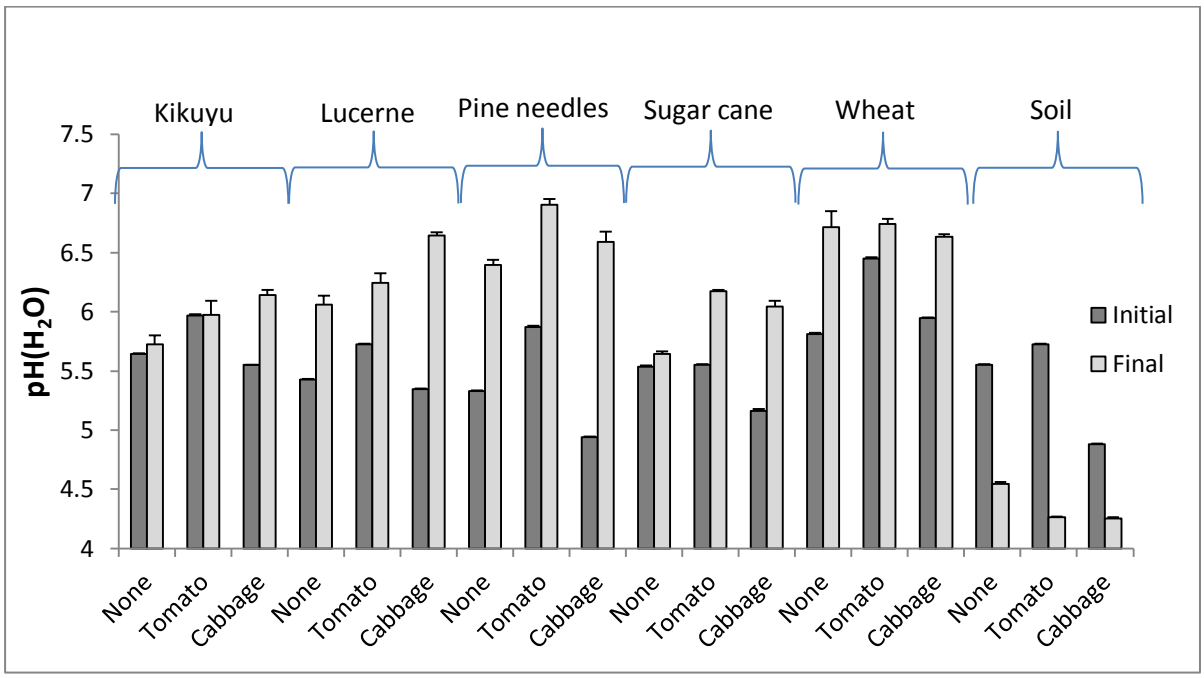


**Figure 4.2:** Initial and final pH in water for all fertilized and unfertilized soil and litter-amended treatments. Standard error bars are shown above each average bar.

### 4.3.3 Decomposition study results

#### *4.3.3.1 Respiration*

The respiration measurements varied over time and with temperature which was not maintained constant for this study. This meant that linear regression modelling of the data would be complicated and speculative due to the relatively large time periods between measurements. Cumulative respiration over the incubation period gave clearer indications of the differences between respirations for the varying treatments. Statistically significant respiration changes, as a result of fertilizer treatments were observed for all litter treatments (Figure 4.4). For the plant litters it can be seen that the lower the inherent initial N content of the plant litter the greater the positive response in respiration to inorganic fertilizer applications (Figure 4.3). Wheat contained the lowest inherent N content (0.46%) and the widest C:N ratio (88.04) of all litter treatments (Table 4.3). The total cumulative respiration for wheat with no fertilizer application was the lowest of all the plant litters. The addition of inorganic fertilizer increased the respiration rate for wheat to 149.2 mg C-$CO_2$ under cabbage fertilizer which is the equivalent of 48% increase in respiration compared to the no fertilizer treatment (Figure 4.4f).This

resulted in wheat producing the highest respiration of all litters under cabbage fertilizer (Figure 4.3c). Lucerne produced the greatest respiration under the no fertilizer treatment, indicating its inherent lability (Figure 4.4c). However, both fertilizer treatments resulted in a significant decrease in the lucerne treatment's respiration (Figure 4.4c), indicating that increased levels of inorganic N actually suppressed decomposition of inherent high N lucerne litter (2.41% N)(Table 4.3). The same effect would therefore be expected for kikuyu, which contained the highest inherent N% (2.77%)(Table 4.3); however an increase in respiration was observed for both fertilizer treatments, though only the tomato fertilizer effect was statistically significant (Figure 4.4b). This indicated that kikuyu decomposition was stimulated under tomato fertilizer. The observation could be explained by the unavailability of the inherent N due to the high polyphenol content of kikuyu (Table 4.4) (Palm and Sanchez, 1991), therefore increased availability of inorganic N from the mineral fertilizer led to increased decomposition and subsequent increased respiration.

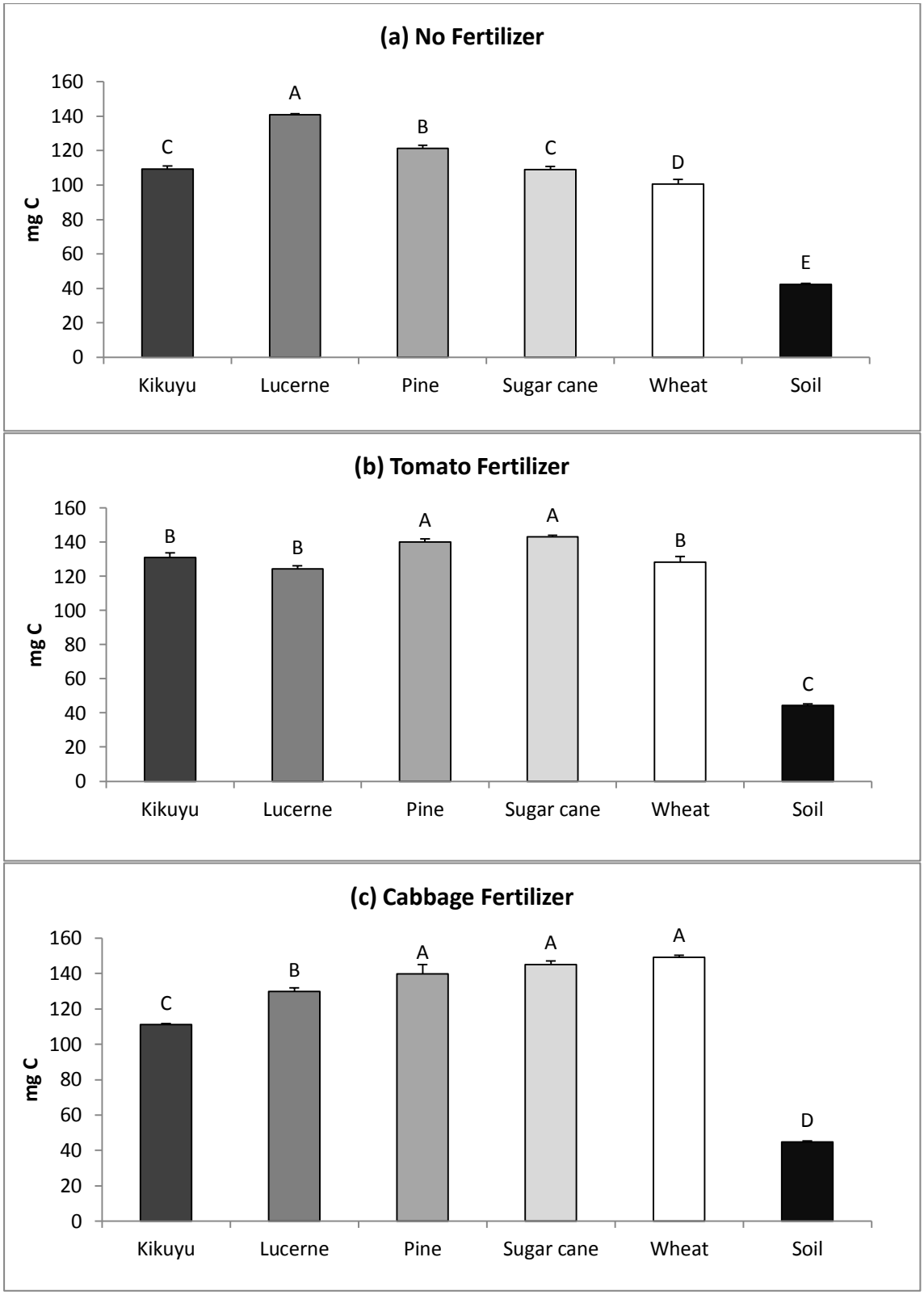


**Figure 4.3:** The effect of plant litter on cumulative respiration for treatments with (a) no fertilizer application, (b) tomato fertilizer application and (c) cabbage fertilizer application. Standard error bars are shown above each average bar. Alphabetic letters indicate significant difference according to Fisher's LSD test at $\alpha = 0.05$. Similar letters indicate lack of significant differences

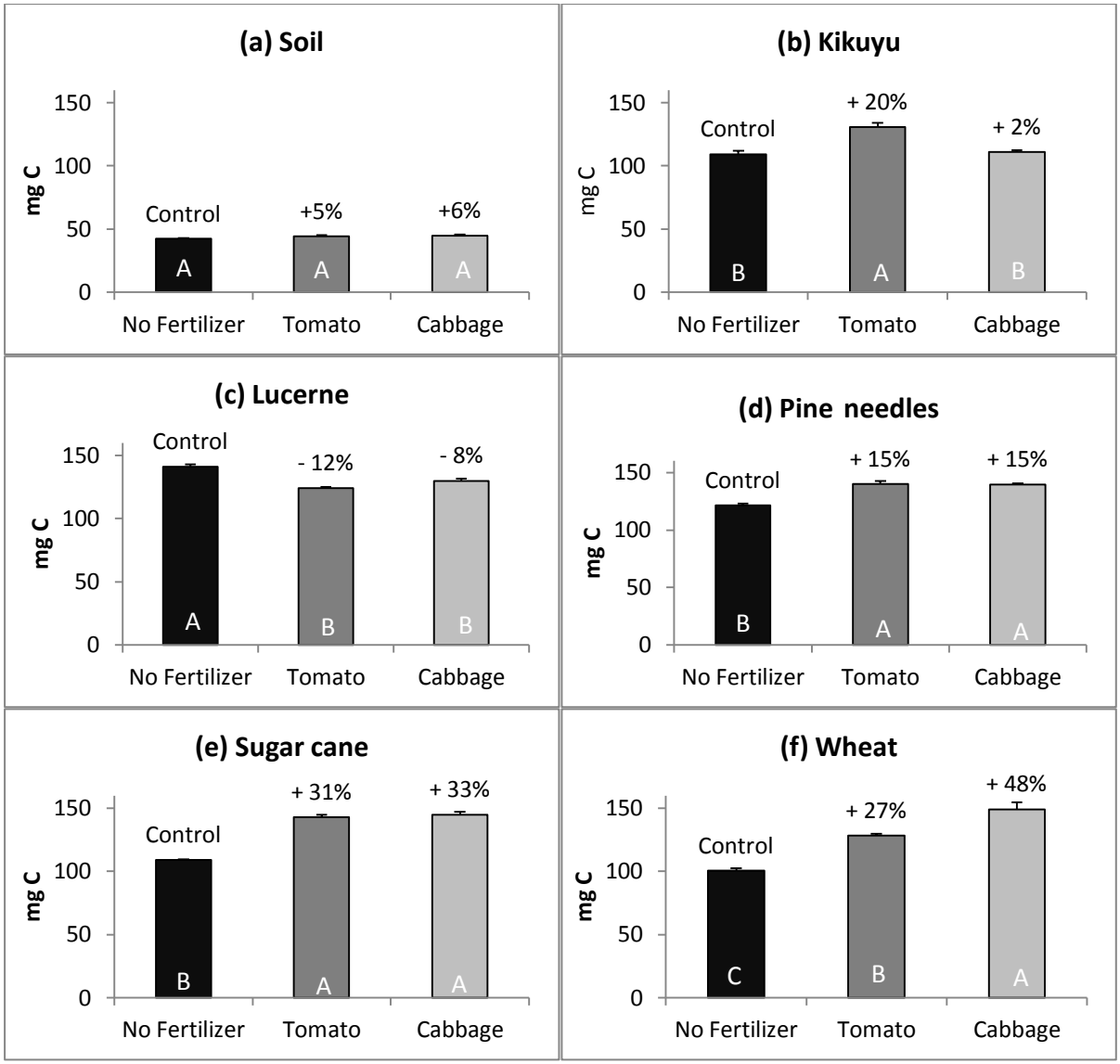


**Figure 4.4:** The effect of fertilizer treatments on total cumulative respiration of (a) soil, (b) kikuyu grass, (c) lucerne, (d) pine needles, (e) sugar cane and (f) wheat plant litter. Standard error bars are shown above each average bar. Percentage change relative to the control is indicated above each average bar. Alphabetic letters indicate significant difference according to Fisher's LSD test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

#### *4.3.3.2 Dissolved Organic Carbon*

The greatest production of DOC was derived from plant litter inherently high in N content with a low C:N ratio; the kikuyu grass and lucerne produced up to 6 times greater cumulative DOC than the other plant litters (Figure 4.5). Pine needle DOC was insignificantly affected by fertilizer application with a 14% increase for tomato fertilizer and a 0% change for cabbage fertilizer compared to the No fertilizer treatment (Figure 4.6d). Sugar cane showed the greatest DOC response to fertilizer treatments with a percentage change of +78% under tomato fertilizer and +186% under cabbage fertilizer (Figure 4.6e), whilst wheat showed the second highest % change under both fertilizer treatments (Figure 4.6f). In terms of relative percentage changes in cumulative DOC, it can be seen that plant litters lowest in inherent N (sugar cane and wheat)(Table 4.3) showed the greatest positive response to fertilizer treatment even though the production in DOC (mg) is significantly lower than for plant litters high in organic N (Figure 4.5). A large change in DOC production was observed for the soil alone treatment with both fertilizer applications (Figure 4.6a), and the change was statistically significant. From the results for pine needles and kikuyu grass it can be seen that fertilizer applications appear to have little significant effect on DOC production. This is likely due to the very high levels of soluble polyphenols (Table 4.4) in these litter sources since kikuyu contained the most inherent N (Table 4.3) yet produced less DOC and smaller percentage change than lucerne (Figure 4.6) which contained less inherent N but also less soluble polyphenols. Research has suggested that soluble polyphenols and lignin polymerize and condense with inorganic N making it more recalcitrant and unavailable for microbial decomposition (Nommik and Vahtras 1982; Johnson et al 2000). Therefore the high polyphenol content in kikuyu grass could have led to increased polymerization reactions with inorganic N, thereby decreasing the effect of N on decomposition.

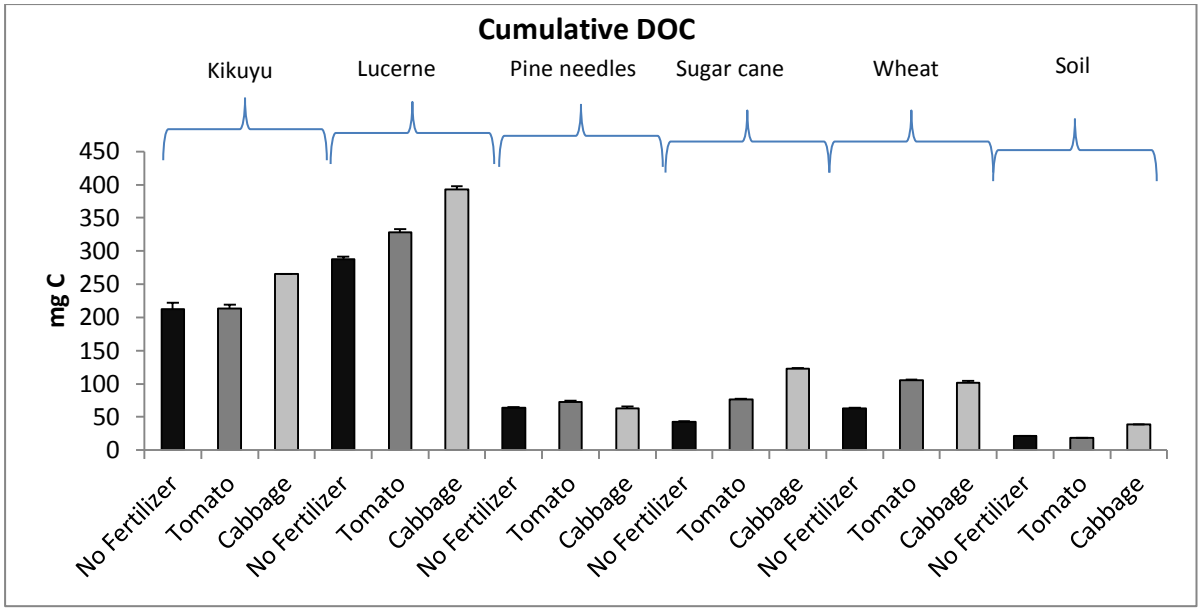


**Figure 4.5:** Total cumulative DOC for fertilized and unfertilized soil and litter-amended treatments indicating the difference in DOC production between plant litter sources. Standard error bars are shown above each average bar.

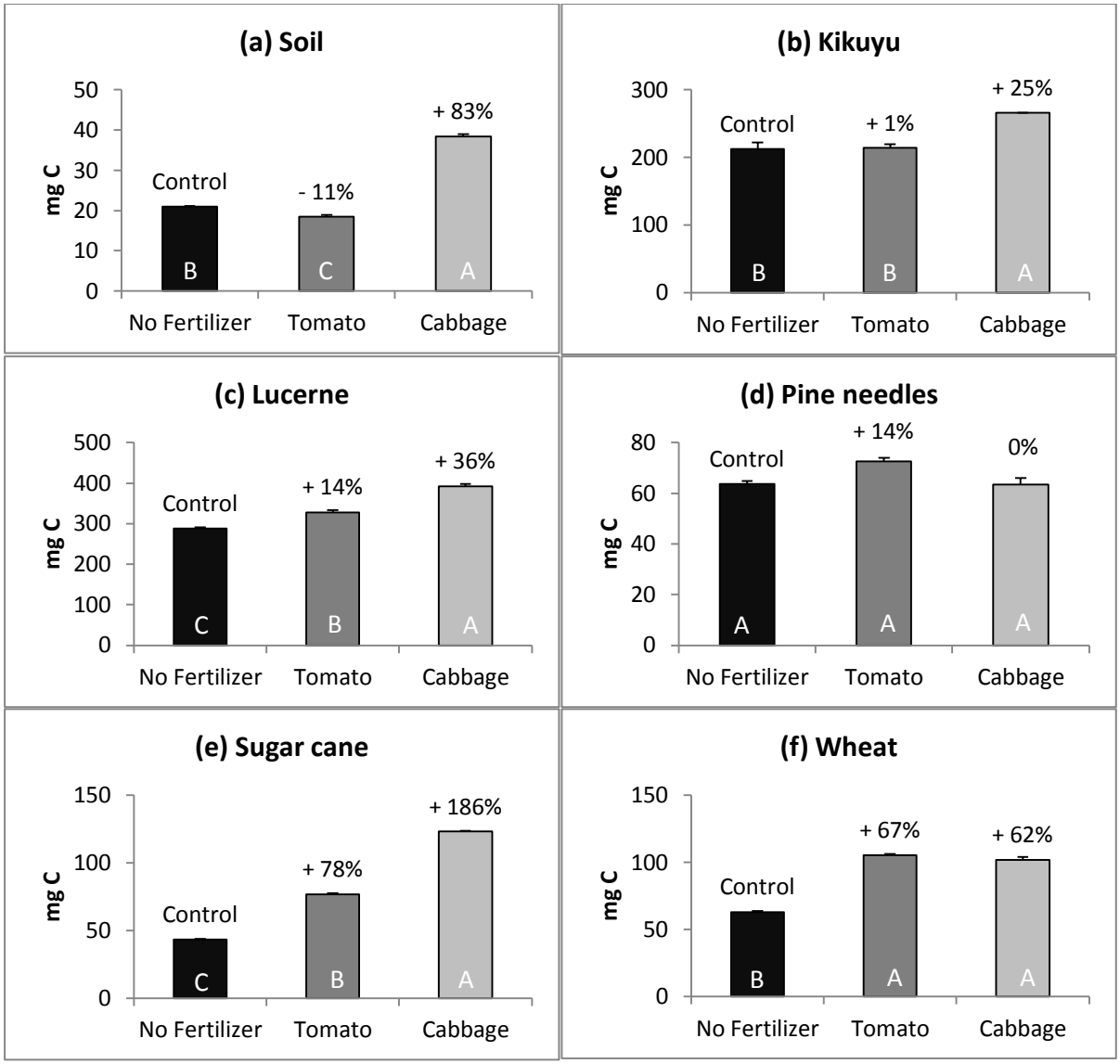


**Figure 4.6:** The effect of fertilizer treatments on total cumulative DOC for plant litters (a) soil, (b) kikuyu grass, (c) lucerne, (d) pine needles, (e) sugar cane and (f) wheat. Standard error bars are shown above each average bar. Percentage change relative to the control is indicated above each average bar. Alphabetic letters indicate significant difference according to Fisher's LSD test at α = 0.05. Similar letters indicate lack of significant differences.

#### *4.3.3.3 B-Glucosidase activity*

The inherent β-glucosidase activities indicate the natural decomposition reactivity of the plant litter. For the selected plant litters the inherent activity from highest to lowest were as follows: lucerne > kikuyu > sugar cane > pine needles > wheat > soil (Figure 4.7a-f). This ratio does not correlate with the cellulose contents of the plant litters but appears to be based on the inherent N content of the plant litters (Table 4.3). Even though Kikuyu had a greater N content, it also contained more soluble polyphenols which may have

caused the β-glucosidase activity to be slightly suppressed. The same can be concluded for the pine needle litters which contained more N than sugar cane.

Cabbage fertilizer application increased β-glucosidase activity for all plant litter sources (Figure 4.7). However, the increase in activity was only statistically significant for pine needles, sugar cane and wheat (Figure 4.7d, e and f), which all had initial C:N ratios above 20:1 and N% well below 2.5% (Table 4.3), which is considered the %N baseline threshold for high quality organic matter that will result in short-term N mineralisation in soil (Palm, 2000). Therefore all three these litters are considered to be low quality in terms of soil fertility purposes. This once again demonstrates that the lower the inherent N content, the greater the response of decomposition indicators to inorganic fertilizer applications. For the inherently high N kikuyu and lucerne litters, the cabbage fertilizer effect on β-glucosidase activity was positive but insignificant, whilst tomato fertilizer led to a significant decrease in activity for both litters (Figure 4.7b and c). This could be due to the very high inherent activity for both kikuyu (637 µg p-Nitrophenol) and lucerne (752 µg p-Nitrophenol) treatments in the absence of fertilizer, potentially indicating sufficient supply of nutrients for microbial degradation. Thus it is possible that the addition of tomato fertilizer could have led to β-glucosidase inhibition which contained very little P compared to cabbage fertilizer, therefore causing a P shortage for microbial activity and subsequently leading to decreased β-glucosidase activity under tomato fertilizer (Aerts and de Caluwe, 1997). Due to the inherent high N content of both litters, cabbage fertilizer containing high N and high P (Table 4.1) showed insignificant effect on β-glucosidase activity.

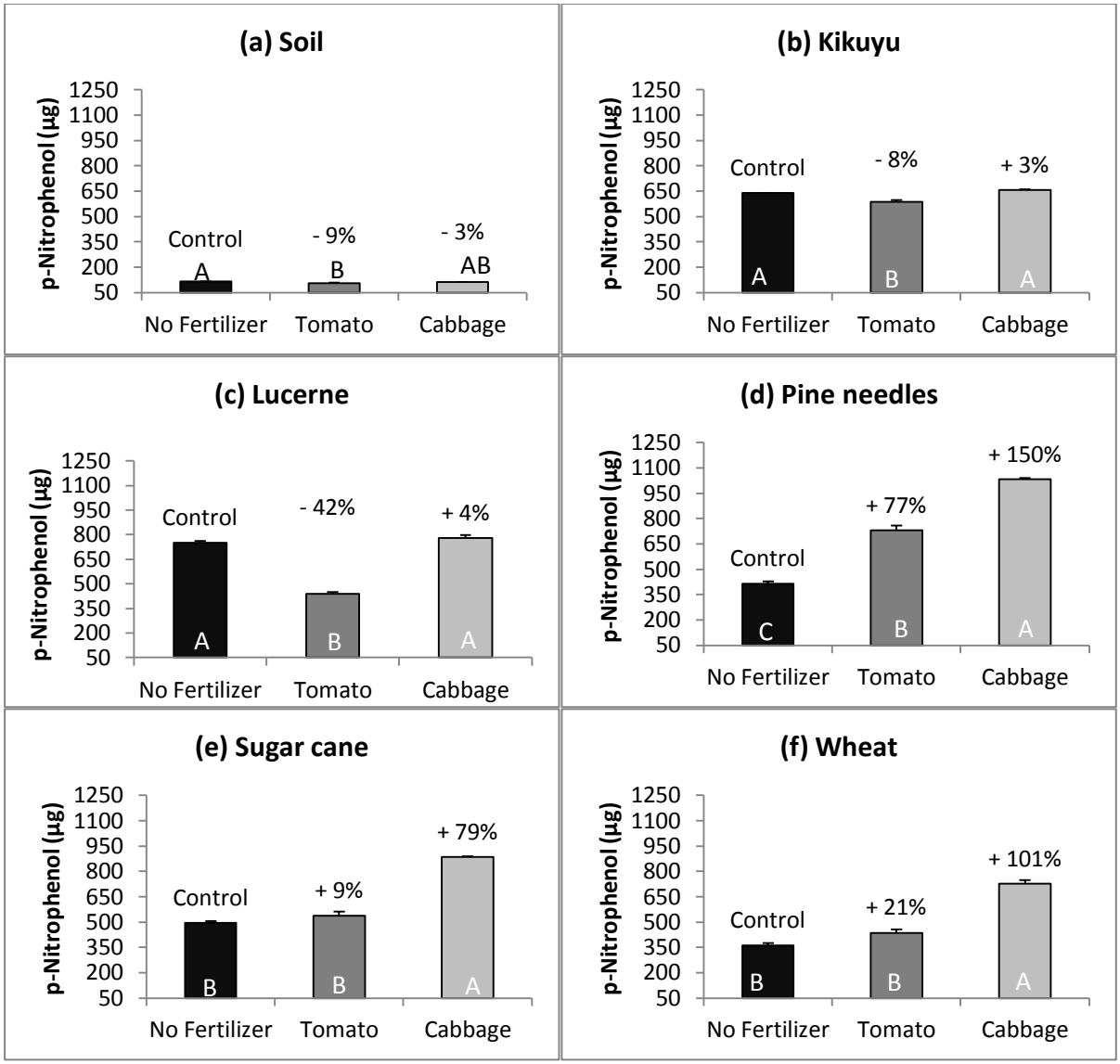


**Figure 4.7:** The effect of fertilizer treatments on total cumulative β-Glucosidase activity for plant litter treatments (a) Soil, (b) Kikuyu, (c) Lucerne, (d) Pine needles, (e) Sugar cane and (f) Wheat. Standard error bars are shown above each average bar. Percentage change is indicated above each average bar. Alphabetic letters indicate significant difference according to Fisher's LSD test at α = 0.05. Similar letters indicate lack of significant differences

#### *4.3.3.4Polyphenol oxidase activity*

The inherent PPO activity for plant litters without fertilizer application was as follows: Wheat < lucerne < kikuyu < pine needles < sugar cane (Figure 4.8a). This holds a weakly positively correlation with cellulose content, except for sugar cane (35.38% cellulose)(Figure 4.10a). The reason for the sugar cane response may be due to physical inaccessibility due to its fibrous nature. With the addition of inorganic fertilizer application PPO activity showed very strong positive correlations with cellulose content.

Cumulative polyphenol oxidase (PPO) activity showed a general trend throughout all plant litters whereby PPO activity was suppressed with increased inorganic N application

(Figure 4.9). Greatest % suppression was observed for kikuyu, lucerne and pine needle litters, even though these litters contained the greatest amount of lignin (Table 4.5). This is probably due to their low cellulose content (Table 4.5). Berg et al. (1986) observed that lignin degradation increased with high levels of cellulose and decreased with high levels of N content. Wheat contained the highest levels of cellulose whilst pine needles and kikuyu, shortly followed by lucerne, contained the least cellulose (Table 4.5). Sugar cane also contained higher levels of cellulose compared to pine needles, kikuyu and lucerne and also showed less suppression in lignin degrading enzyme activity under high N application. The mechanisms behind PPO suppression have been observed in several decomposition studies and were found to be related to i) the polymerization and condensation reactions between low molecular N and organic matter, particularly lignin and polyphenols (Nommik and Vantras, 1982; Stevenson, 1982; Berg and Matzner, 1996), ii) the direct suppression of lignin degrading enzyme production (Keyser et al 1978; Eriksson et al 1990) and iii) the suppression of lignin degrading microorganisms (Keyser et al 1978; Berg and Matzner, 1996). The soil alone treatment showed the greatest suppression of PPO activity under increasing inorganic N application. This demonstrates that PPO suppression is primarily a direct effect of fertilizer on PPO activity rather than an indirect effect due to substrate-fertilizer interactions which is enhanced due to the soil's inherent low N content and low organic matter content which would subsequently lead to low cellulose content.

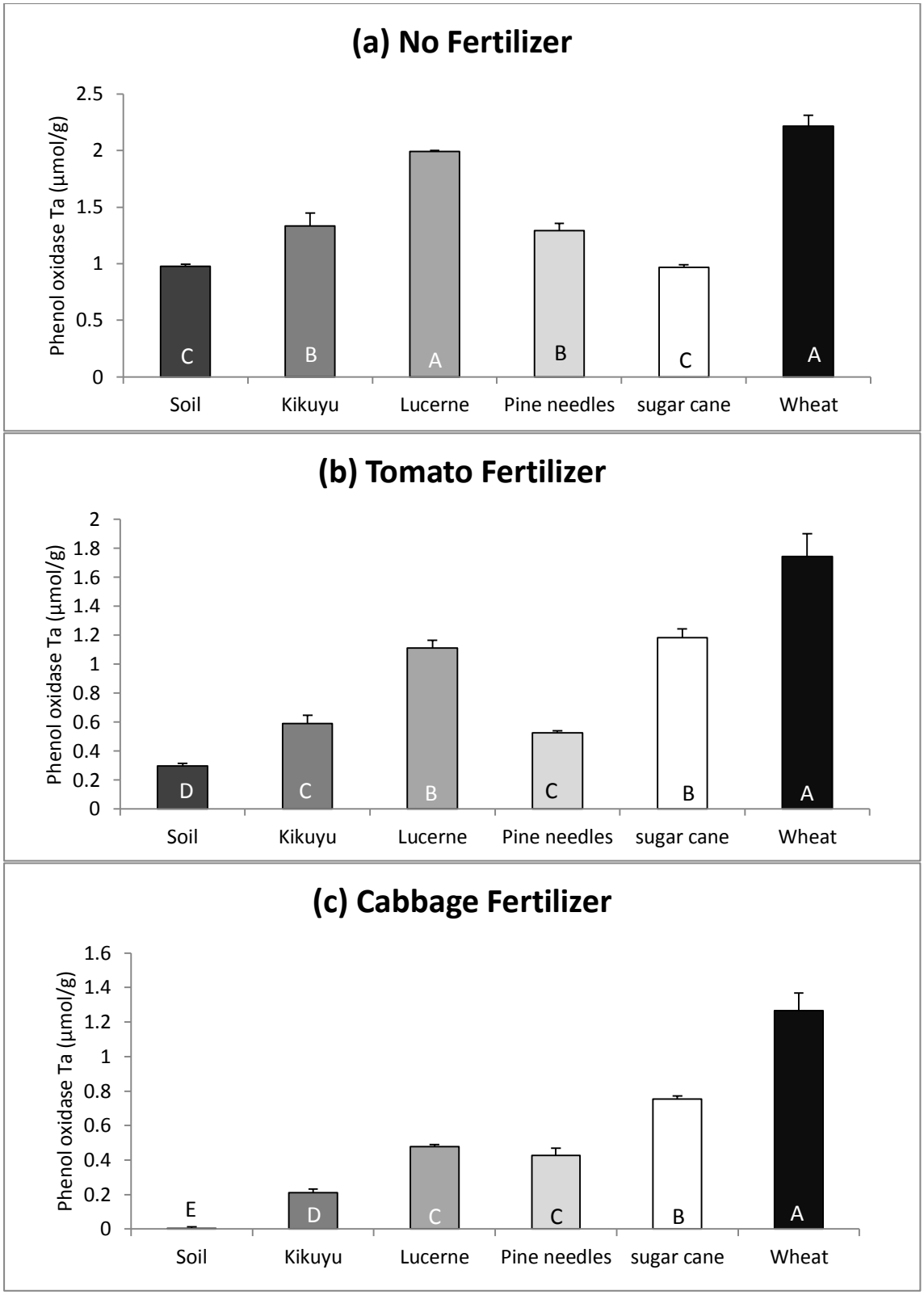


**Figure 4.8:**The effect of plant litter on total cumulative polyphenol oxidase turnover activity (Ta, µmol/g) for fertilizer treatments (a) no fertilizer, (b) tomato fertilizer and (c) cabbage fertilizer. Standard error bars are shown above each average bar. Alphabetic letters indicate significant difference according to Fisher's LSD test at α = 0.05. Similar letters indicate lack of significant differences

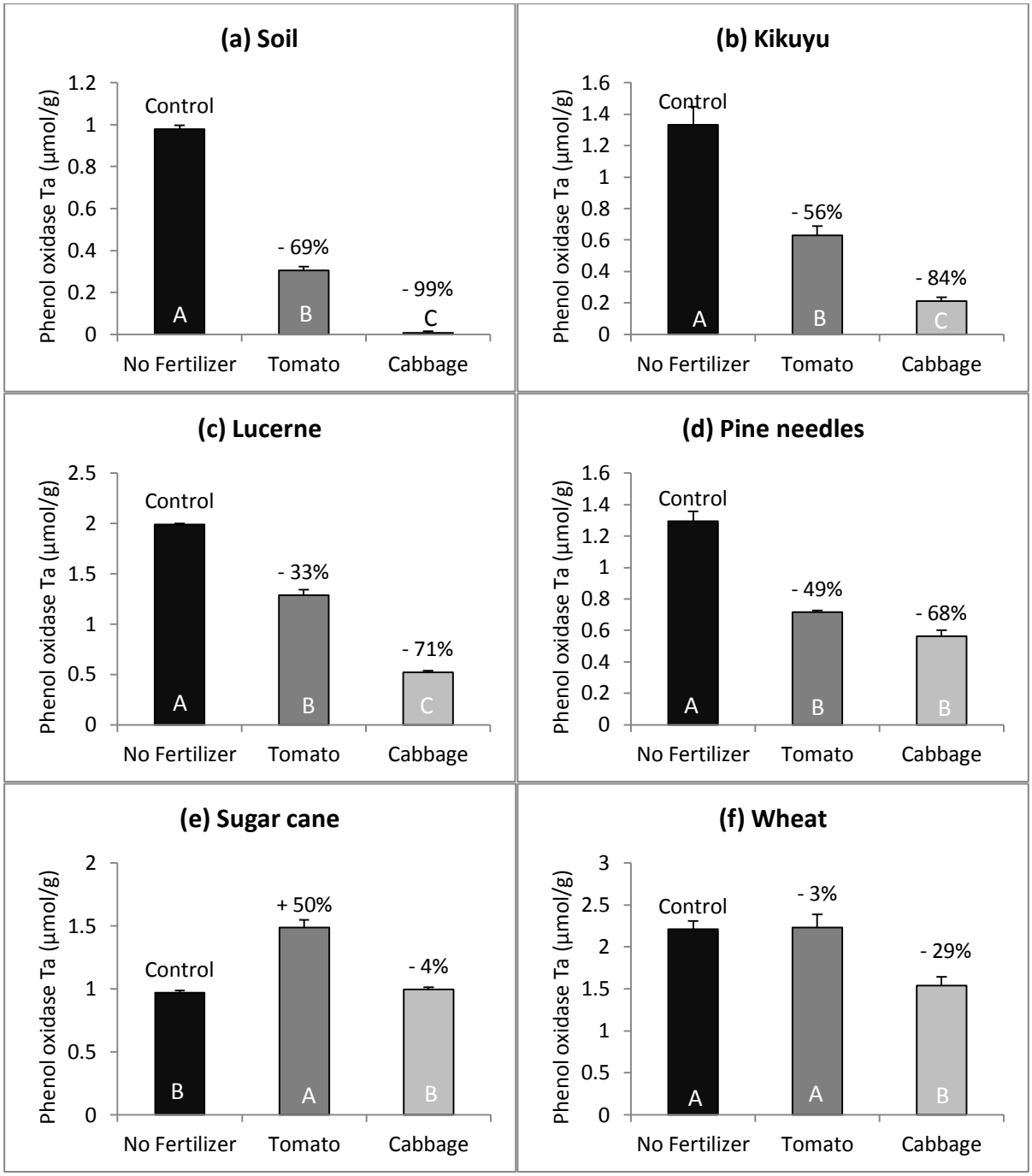


**Figure 4.9:** The effect of fertilizer treatment on total cumulative polyphenol oxidase turnover activity (Ta, µmol/g) for plant litter treatments (a) soil, (b) kikuyu grass, (c) lucerne, (d) Ppne needles, (e) sugar cane and (f) wheat. Stander error bars are shown above each average bar. Percentage change relative to the control is indicated above each average bar. Alphabetic letters indicate significant difference according to Fisher's LSD test at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

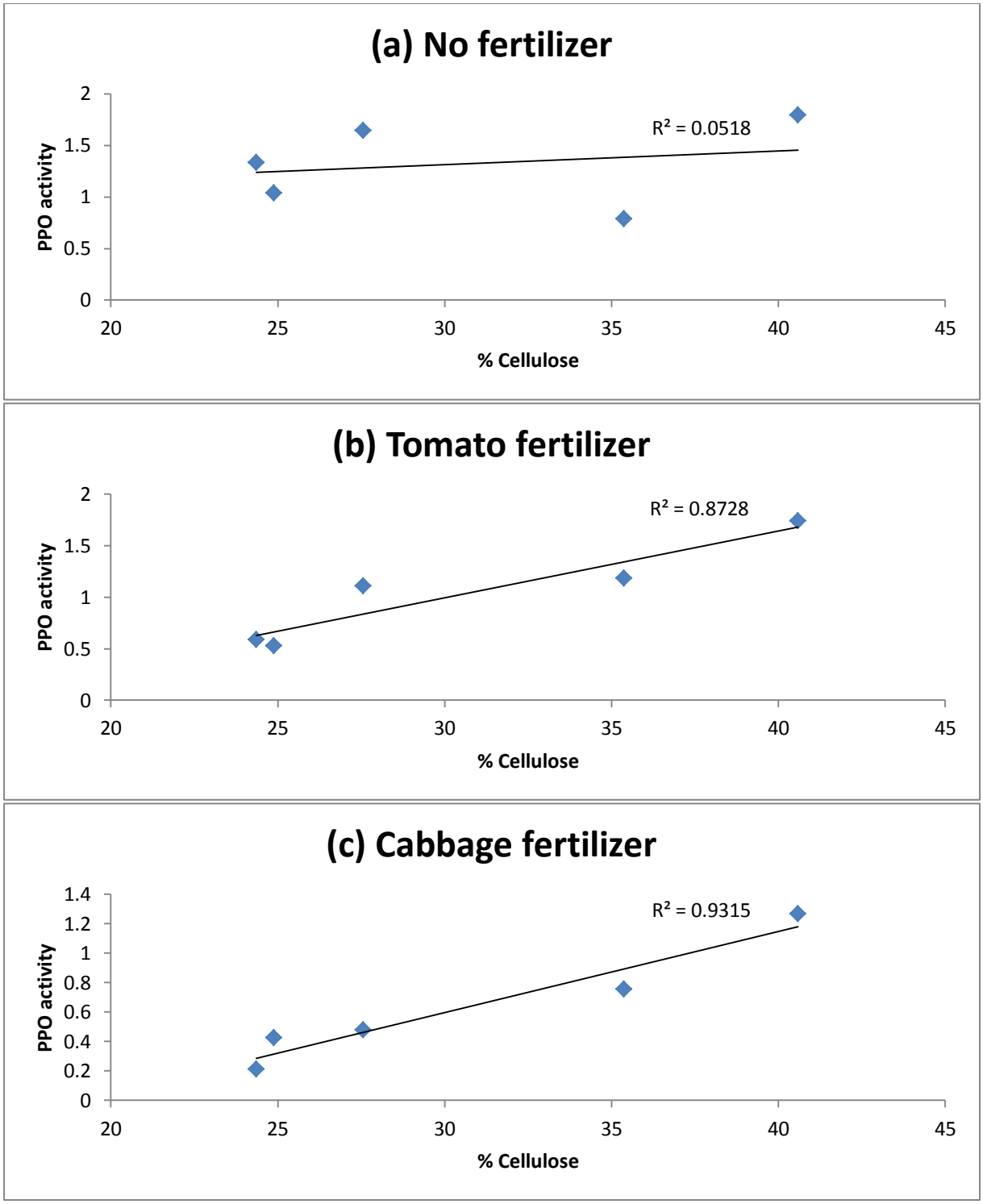


**Figure 4.10:** Linear relationship between cellulose content and polyphenol oxidase activity under fertilizer treatments.

### *4.3.3.5Loss on Ignition*

The loss on ignition results (Tables 4.6 and 4.7) for the unfertilized plant litters represent the inherent decomposability of the plant litters and can be linked to substrate quality

and composition. For the plant litters without fertilizer applications the greatest loss in OM was observed for lucerne (-35.19%) (Table 4.7) followed by kikuyu and pine needles. This inherent decomposability can be associated with the inherently high N content of all three litter sources, enabling microbes to easily decompose the organic matter without N limitations. After pine needles, the soil alone treatment showed the greatest organic matter loss followed by wheat and finally sugar cane. Even though the soil OM in the soil alone treatment is already partially decomposed and no longer fresh litter, the very narrow C:N ratio led to it decomposing more than that of both wheat and sugar cane litter. Sugar cane contained more N than wheat yet decomposed less. This is likely due to its higher polyphenol content (Table 4.4) and lower cellulose than that of wheat (Table 4.5). From the unfertilized litter decomposition results we can conclude that inherent N content is the primary controlling factor, with polyphenol content and cellulose content acting as secondary controlling factors in litter decomposability.

The LOI results for the fertilized treatments (Table 4.6 and 4.7) show the addition of high N-containing fertilizers to suppress organic matter decomposition in the soil without litter and soil amended with kikuyu grass litter. This doesn't coincide with some of the decomposition indicator analyses which indicated an increase in decomposition (increased respiration (Figure 4.4a and b) increased DOC (Figure 4.6a and b)). However it does correspond with decreased β-glucosidase activity (Figure 4.7a and b) and significant suppression in PPO activity (Figure 4.10a and b). The decrease in organic matter decomposition is therefore likely due to the suppression of both β-Glucosidase and PPO activity, being greater than the slightly increased respiration and DOC production. In the case of the kikuyu, this is likely due to the high polysaccharide and water extractives % (Table 4.5) which may have stimulated other hydrolytic enzymes not measured in this study. The soil OM most likely contained less labile C, therefore decomposition would be reliant on lignin degradation which is significantly suppressed under inorganic N applications. Therefore the addition of inorganic fertilizer to soil only treatments would lead to decreased decomposition due to the suppression of lignin decomposition.

For pine needles, sugar cane and wheat treatments, final OM content decreased with increasing N fertilizer application (Table 4.6 and 4.7). This indicated that increased mineral N stimulated decomposition of these plant litters and coincides with the results obtained through respiration, DOC and β-Glucosidase analyses (Figures 4.4, 4.6, 4.7 and 4.9). Cabbage fertilizer enhanced OM decomposition to a greater extent than the

tomato fertilizer in the sugar cane treatment and the wheat treatments (Table 4.7). This is due to the inherently low content of N for both litter sources leading to a greater response in decomposition. Both litters contained low amounts of soluble polyphenols and greater amounts of cellulose and polysaccharides which are labile sources of C and easily decomposed. It can therefore be concluded that N content was a rate limiting factor in the decomposition of both sugar cane and wheat which explains why the increase in decomposition response correlates with greater inorganic N additions. Lucerne showed no significant differences in final organic matter content between the treatments (Table 4.6). This may be due to the decreased respiration rates under fertilizer application (Figure 4.4c) negating the effect of the increased DOC production under fertilizer application (Figure 4.6c) thereby resulting in insignificant net change between treatments. In summary, the fertilizer applications slightly suppressed OM decomposition in the soil only and kikuyu treatments, and had no effect on the lucerne treatment. Thus, in the treatments with lowest C:N ratio (Table 4.3), fertilizer application resulted in slight decrease or no change in the net OM decomposition. However, in the litter treatments with the widest C:N ratio, i.e., pine needles, sugar cane and wheat, the addition of fertilizers enhanced net organic matter decomposition.

**Table 4.6: Percentage of organic matter (determined by LOI) at start and end of decomposition study for all fertilizer soil and litter-amended treatments.**

| Plant litter | Initial %OM | Final % OM | | |
|---|---|---|---|---|
| | No Fertilizer | No Fertilizer | Tomato Fertilizer | Cabbage Fertilizer |
| Soil | 0.77(0.027)* | 0.67(0.027)(A) | 0.70(0.047)(A) | 0.70(0.047)(A) |
| Kikuyu | 1.73(0.027) | 1.17(0.054)(A) | 1.20(0.098)(A) | 1.20(0.047)(A) |
| Lucerne | 1.73(0.027) | 1.17(0.027)(A) | 1.17(0.082)(A) | 1.17(0.027)(A) |
| Pine needles | 1.67(0.027) | 1.37(0.072)(A) | 1.13(0.082)(A) | 1.20(0)(A) |
| Sugar cane | 1.77(0.027) | 1.60(0.027)(A) | 1.47(0.054)(AB) | 1.20(0.027)(B) |
| Wheat | 1.77(0.027) | 1.53(0.082)(A) | 1.30(0.098)(B) | 1.23(0.082)(B) |

*Brackets represent standard error for the sample replications. Alphabetic letters indicate significant difference according to Fisher's LSD test at $\alpha = 0.05$. Similar letters indicate lack of significant differences

**Table 4.7: Percentage change in organic matter content with reference to initial organic matter content before decomposition for fertilized and unfertilized soil and litter amended treatments.**

| Organic matter % change | | | |
|---|---|---|---|
| Plant litter | Fertilizer Treatment | | |
| | No fertilizer | Tomato Fertilizer | Cabbage Fertilizer |
| Soil | -16.67 | -12.50 | -12.50 |
| Kikuyu | -31.37 | -29.41 | -29.41 |
| Lucerne | -35.19 | -35.19 | -35.19 |
| Pine needles | -19.61 | -33.33 | -29.41 |
| Sugar cane | -11.11 | -18.51 | -33.33 |
| Wheat | -14.81 | -27.78 | -31.48 |

## 4.4 Conclusions

In this chapter, the decomposition dynamics of plant litters in sandy soil were influenced by both fertilizer treatment and plant litter composition and quality. Inherent N content was found to play a major role in the decomposability of the various plant litters as well as cellulose and polyphenol content. The addition of inorganic fertilizers high in N proved to enhance respiration and DOC as well as β-Glucosidase activity whilst suppressing PPO activity for all litter sources. However, plant litters low in inherent N (Pine needles, sugar cane and wheat) showed greater response to inorganic fertilizer applications. This shows that N was a limiting factor for the decomposition of these plant litters. Plant litters with high inherent N produced the most DOC, however, the greater the polyphenol content the lower the DOC production and respiration. This was observed for kikuyu grass which contained the greatest % N yet respired less and had lower DOC released than that of lucerne which had less N. The reason for this can be ascribed to the much greater polyphenol content found in kikuyu than in Lucerne which has been shown to polymerize with N making it unavailable for microbial use as well as forming more recalcitrant organic matter (Nommik and Vahtras 1982; Johnson et al 2000). For the soil alone treatments under both tomato and cabbage fertilizer treatments, Respiration, DOC and β-Glucosidase were insignificantly affected by the addition of mineral fertilizers. Only polyphenol oxidase activity showed significant negative changes under increasing fertilizer application indicating that polyphenol oxidase suppression was primarily a direct effect from fertilizer addition rather than an indirect effect on substrate leading to decreased PPO activity, since the soil contained very little organic matter. For plant litters high in inherent N and low in cellulose, PPO suppression was most pronounced under mineral fertilizer application with suppression increasing as N content increased. In plant litters lower in N with greater cellulose content, the effect of mineral fertilizer was less pronounced and even negated for sugar cane treated with tomato fertilizer. This is supported by research done by Berg (1986) which found that lignin degradation was enhanced with higher cellulose content and suppressed with high levels of N. For this reason the sugar cane and wheat plant litters (Figure 4.10 e and f) showed the least PPO suppression under mineral fertilizer application due to the high cellulose content which stimulated lignin degradation. From the high PPO activity that was observed for pine needles without fertilizer application it can be concluded that high polyphenol content will lead to enhanced activity due to the need for polyphenol polymerization to reduce its toxicity to the microbes (Sinsabaugh, 2010).

Finally the loss on ignition results show that mineral fertilizer application led to increased decomposition for plant litters low in inherent N content however it appears that decomposition was reduced for plant litters with already high inherent N. This Is likely due to the suppression of PPO activity under additional N application as well as a possible imbalance between N and P leading to decreased β-Glucosidase activity (Aerts and de Caluwe, 1997).

Based on the results of this study, it can be concluded that the addition of mineral N to fresh crop litters will enhance decomposition of plant litters inherently low in N content. The greater the application of inorganic N the greater the response in decomposition that is observed in terms of increased respiration, DOC and β-Glucosidase activity with suppressed PPO activity. This suppression of PPO and enhanced activity of β-Glucosidase may lead to the build-up of recalcitrant soil organic matter. If the increased DOC due to polymerization and condensation with mineral N is not leached from the soil and allowed to sorb to clay minerals and sesquioxides, the combination with PPO suppression will lead to the promotion of humification thereby increasing soil organic matter content and carbon sequestration in soils.

The findings of the study can have significant influence on management of agricultural and forestry soil and litters. Though long term decomposition falls outside the scope of this research, the period for which the study was done (3 months) coincides with the annual growing season of most crops and is therefore of great importance for optimal organic matter management. With the enhanced decomposition observed under fertilizer applications, farmers applying fertilizer to soils containing crop litter from a previous rotation can expect increased decomposition of the plant litters and thereby also increased nutrient supply from the organic matter. This can aid in effective crop nutrient management during the growing season as organic matter mineralization rates are altered to take place at a greater rate enabling a farmer to better utilize the nutrient resource that is otherwise slower to become available for crop use. Since organic matter mineralization patterns and rates are difficult to determine and therefore can lead to insufficient nutrient provision for the growing crop during the growth season, the application of inorganic fertilizer may be a solution to the problem. Due to the enhanced DOC production consideration should be given to irrigation timing and water management to limit loss of DOC through leaching. For this reason the use of inorganic fertilizers to enhance decomposition will likely have the best results under dry land crop production due to a smaller chance of DOM leaching from the soil. The increased DOC

that is allowed to remain in the soil through effective water management will subsequently interact with soil minerals and sesquioxides further stabilizing and thereby increasing the C content of the subsoil and improving soil fertility. Further research should be done to establish that the same results will be observed in field and for various soil types under different climatic conditions; however the results from this study provide a good steppingstone toward optimal crop litter management in South Africa.

# CHAPTER 5
# GENERAL CONCLUSIONS AND FUTURE RESEARCH

Organic matter management has become a focus point of much research due to its important role is soil fertility and crop production as well as its effect on greenhouse gas emissions. Much emphasis has been placed on soil management practices to maintain and increase soil organic matter. Practices such as minimal/no tillage, mulching and crop rotations has shown to be highly effective in decreasing organic matter losses and all of these practices lead to increased residue that is left on the surface of the soil. These residues are of great value for several reasons. They protect the soil for erosion, surface crusting, decrease evaporation and can provide plant nutrients to the next growing crop. However in order to utilize these benefits optimally an understanding into the decomposition of these litters as well as factors which influence their decomposition are needed. Inorganic fertilizer applications are common practice in commercial agriculture, yet not much is known regarding their interaction with organic matter and soil biota. It is therefore essential that research is done to develop an understanding as to how inorganic fertilizers will affect organic matter decomposition in order to prevent unwanted losses of organic matter. Much research has been done on the effect of inorganic N on forest litter decomposition and to some extent on grasslands and most of the research done was done in field. Very little research has focused on crop litters and none, to our knowledge, on composted organic matter. Furthermore none of the research has been done in South Africa.

The aim of the first research chapter, Chapter 3, was to elucidate the effect of inorganic fertilizers on composted organic matter. Due to the extent to which composts have been decomposed, these sources of organic matter can be described as more stable and non-labile organic matter. By using two composts of different quality, we were able to establish which decomposition parameters were affected by fertilizer applications and which were due to organic matter quality. Form the results we were able to conclude that respiration and β-glucosidase activity were substrate dependant for composted organic matter, whilst DOC production was significantly affected by the addition of fertilizers. It appears that the lower the inherent N content the greater the effect of inorganic N on the production of DOC. This increase in DOC production can be attributed to several factors which are altered by the addition of mineral N. It has been shown that lignin degrading

enzyme production and functioning is suppressed under high levels of low molecular weight N application (Keyser et al. 1987; Eriksson et al. 1990). Furthermore, incorporation of mineral N into dissolved organic matter through condensation and polymerization reactions with polyphenols and aromatic compounds has also been observed (Nommik and Vahtras, 1982; Stevenson, 1982; Davidson et al 2003) which could promote DOC production. Abiotic immobilization of $NH_4^+$ and $NO_3^-$ and subsequent polymerization of amino nitrogen sources with saccharides may lead to the formation of recalcitrant organic matter (Nommik and Vahtras, 1982; Johnson et al. 2000) further enhancing DOC stabilization. Based on the observations for the compost decomposition study it can be said that respiration is not a sufficient indicator of decomposition for non-labile organic matter and that DOC appears to be a much better correlated parameter to monitor since the changes in DOC production correspond with the final LOI results for the compost decomposition study. It is therefore important that DOC be taken into consideration when evaluating decomposition of various organic matter sources.

The aim of the second research chapter, Chapter 4, was to evaluate the effect of inorganic fertilizer applications on labile organic matter from crop and forestry litters commonly found in South African agriculture. In order to determine which decomposition parameters were affected by fertilizer application and which by organic matter quality we selected five plant litters with varying composition. Based on the results from this study we were able to conclude that respiration, DOC and β-glucosidase activity are enhanced by inorganic fertilizer additions. PPO activity was suppressed with increasing inorganic N application for all litter sources. Once again the lower the inherent N contents of the litters the greater the response in decomposition parameters. Even though inherent N content was the primary influential factor from plant litter quality, polyphenol content and cellulose content played secondary roles in determining the response in decomposition to inorganic fertilizer application. It can be concluded that the addition of mineral N to fresh crop litters will enhance decomposition of plant litters inherently low in N content. The greater the application of inorganic N the greater the response in decomposition that is observed in terms of increased respiration, DOC and β-glucosidase activity with suppressed PPO activity. This suppression of PPO and enhanced activity of β-glucosidase may lead to the build-up of recalcitrant soil organic matter. If the increased DOC due to polymerization and condensation with mineral N is not leached from the soil and allowed to sorb to clay minerals and sesquioxides, the combination with PPO suppression will lead to the promotion of humification thereby increasing soil organic matter content and carbon sequestration in soils.

The overall conclusion is that the application of inorganic NPK fertilizer will significantly stimulate decomposition of labile organic matter fractions but suppress the activity of lignin degrading enzymes for fresh litter sources under short term decomposition. The application of inorganic fertilizer has little effect on the respiration and cellulose degrading enzyme activities for non-labile composted organic matter fractions and significantly increased DOC production. Organic matter low in inherent N will show greater responses to inorganic fertilizer applications. This response is further enhanced with higher inorganic N application.

When looking at the study as a whole the research indicates that the addition of high N containing inorganic fertilizer applications will lead to enhanced decomposition in both composted and fresh organic matter sources. Even though the response in fresh litter is greater both composts and fresh litters increased decomposition with greatest responses for organic sources inherently low in N. DOC production is enhanced significantly for both composted and fresh organic matter under inorganic fertilizer applications.

This research is the first to show the effect of inorganic fertilizer applications on compost decomposition and the findings for both compost and crop litter decomposition can have a significant effect on organic matter management in South Africa. By applying inorganic fertilizers to soils containing fresh crop litter as well as composted organic matter, decomposition will be enhanced and nutrient mineralization will increase making more plant nutrients available for the current growing crop. This may serves as a solution to the slow nutrient release encountered when applying composts to soil, thereby enhancing nutrient mineralization during the crop growing season and increasing the efficiency of composts as a source of crop nutrients. If water management is correctly implemented the increased DOC will remain in the soil and will interact with soil minerals and sesquioxides further stabilizing and leading to humification in soils. This will ultimately lead to increased SOC and soil fertility.

This research provides a good foundation for further research into the effect of inorganic fertilizer applications on organic matter decomposition in South Africa. Further laboratory research should be done on different soil types and a greater variety of organic matter sources to establish whether the results from this study will hold for different soil texture classes and organic matter qualities. The evaluation of nitrogen and phosphorous mineralization would also further aid in better understanding the role of phosphorous limitations on the decomposition of the treated organic matter sources. The extent of humification should also be evaluated to determine whether DOC produced is further

stabilized by the addition of inorganic fertilizer applications. Field studies will aid in understanding how climate and ecosystems affect these responses observed in this laboratory study. It will also be of great importance to evaluate the changes in SOM pools, with the use of density fractionation, during the decomposition process to establish whether the mineral bound passive pools increase over time. This will confirm the stabilization of DOC in subsoils. Further research can also look at the effect of inorganic fertilizer application on the mineralization patterns of plant nutrients. All of this research will aid farmers in managing organic matter sources to provide improved nutrient availability for growing crops as well as improve their soil quality and fertility which will in the long term decrease input costs and enhance crop production without negatively impacting the soil and environment.

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