EFFECTS OF BT MAIZE (MON810) CROP AND ITS RESIDUES ON SELECTED SOIL BIOLOGICAL PROPERTIES AND N AND P RELEASE IN A SANDY LOAM SOIL FROM ALICE, EASTERN CAPE, SOUTH AFRICA

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DECLARATION

I, Besule Landzela, declare that the content of this dissertation being submitted to the University of Fort Hare for the degree of Master of Science in Agriculture (Crop Science) is my own work and that information from other sources used herein has been acknowledged. Furthermore, I declare that this dissertation has never been submitted in this or any other form to another institution for the award of any degree.

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PREFACE

This study evaluated the effects of growing Bt maize and residues amended with soil on soil MBC, enzyme activities, vesicular arbuscular mycorrhizal fungi and N and P release in a sandy loam soil from Alice, Eastern Cape, South Africa. It is composed of four chapters. Chapter one consists of the general introduction, justification of the study and the literature review which basically evaluates what other researchers have found out about Bt maize. Chapter two deals the effects of growing Bt maize and soil amended with residues on selected biochemical and biological properties. Chapter three explores the N and P mineralisation release from different parts of maize residues (i.e. leaf, stem and root). Chapter four includes the general discussion, conclusion and recommendations for further studies.

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ABSTRACT

There are apprehensions that genetic modification of maize with *Bacillus thuringiensis* (Bt) may have negative effects on soil biodiversity, ecosystem processes and functions. This study aimed at determining the effect of Bt maize crop, Bt maize residues and its genetic modification on microbial biomass carbon (MBC), selected enzyme activities, vesicular arbuscular mycorrhizal (VAM) fungi and N and P release patterns. The study was conducted under field, glasshouse and laboratory conditions.

In 2010/2011 season, four maize cultivars; DKC 61-25B (Bt), DKC 61-24 (non-Bt), PAN 6Q-321B (Bt) and PAN6777 (non-Bt) were planted. Determination of MBC, enzyme activities and fungal spore count was done at 42, 70, and 105 days after planting (DAP). A loam soil amended with Bt or non-Bt maize leaf residues from a study of 2009/2010 season was incubated to investigate effects of Bt maize residues on MBC and soil enzyme activities. Leaf residues of Bt and non-Bt maize cultivars (DKC 61-25B, DKC 61-24, PAN 6Q-321B and PAN6777) were used and soil without residues was used as a control. Samples were collected at 7, 28 and 56 days of incubation (DOI).

An incubation study was also carried out in the laboratory to determine the effect of Bt maize residues (i.e. leaf, stem and root) and its genetic modification on N and P release patterns. Residues of DKC 61-25B, DKC 61-24, PAN 6Q-321B and PAN6777and soil without residues as a control were incubated in the

laboratory. After destructive sampling at 0, 7, 14, 28, and 56 DOI, N in the form of NH_4-N and NO_3-N and P mineralisation were determined.

Amendment of soil with residues enhanced MBC ($p < 0.05$) at all the sampling dates. For example MBC increased from 95 in the control to 146.3 mg/kg in the DKC 61-25B treatment at the end of the glasshouse trial. In the field DKC 61-25B had 9.1 mg/kg greater MBC than DKC 61-24, while PAN 6Q-321B had 23.9 mg/kg more MBC than PAN6777 at the end of the trial. However, no differences ($p < 0.05$) were observed in enzyme activities under field and glasshouse conditions except for dehydrogenase that had greater activity where DKC 61-25B and PAN 6777 were grown. There were no differences between the type of residues (Bt and non-Bt) on enzyme activities tested. However, differences were observed among the sampling dates. No effects of Bt maize crop on fungal spore count were observed. Similarly no differences were observed in leaf, stem and root tissues composition between Bt and non-Bt maize cultivars. Net N and P mineralisation from Bt maize cultivars did not differ from that of non-Bt maize cultivars. However, differences were observed among the cultivars. The results of this study suggested that Bt maize with Bt MON810 event can be grown in the central region of the Eastern Cape (EC), South Africa without affecting MBC, soil enzyme activities, VAM, and release of N and P nutrients from its residues.

Keywords: Bt maize, residues, MBC, enzyme activities, mycorrhizae, N and P mineralisation

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LIST OF ABBREVIATIONS AND ACRONYMS

AC PH	Acid phosphatase
ALK PH	Alkaline Phosphatase
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of Variance
Bt	*Bacillus thuringiensis*
C	Carbon
Cd	cadmium
cm	centimetre
C: N	Carbon to nitrogen ratio
Cry1Ab	Crystal proteins from Bt subspecies *kurstaki*
Cu	Copper
d	Day
DOI	Days of incubation
DAP	Days after planting
DEHYD	Dehydrogenase
EC	Eastern Cape

Fe	Iron
g	grams
GM	Genetically modified
ha	hectares
H	Heated
L	liters
LAN	Lime ammonium nitrate
LSD	Least significant difference
K	Potassium
KMD	KeMingDao
kg	kilogram
m	meter
mg	milligram
ml	millilitre
MBC	microbial biomass carbon
MON810	Monsanto transformation event of Bt maize
MUB	Modified universal buffer
MYCO	Mycorrhizae

N	Nitrogen
NASAWC	NON-AFFILIATED SOIL ANALYSIS WORK COMMITTEE
NRF	National Research Foundation
OM	Organic matter
p	probability
P	Phosphorus
PAN	Pannar
PNP	*p*-nitrophenyl
SA	South Africa
SANBI	South African National Biodiversity Institute
t	tonne
TPF	Triphenyl formazan
TTC	Triphenyltetrazolium chloride
UFH	University of Fort Hare
UH	Unheated
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1. 1 GENERAL INTRODUCTION

An increase in the world population and its consequences on food security around the globe, particularly in developing countries, is a major concern (Pinstrup-Andersen *et al*., 1999). The current status of food security is uncertain as a result of challenges like global warming, climate change, losses of productive land to other uses and, soil erosion and land degradation among other problems (Ehrlich *et al*., 1993). Therefore, there is a need to increase food production. Approaches to achieve this objective include improved soil fertility management, improved cultivars, and weed and pest control, among other things (Jung and Sheaffer, 2004; Fanadzo, 2007). Genetic engineering of crop plants has also played a crucial role in food production increase (Keetch *et al.*, 2005). Genetically modified (GM) crops are the crops that possess a gene or genes that have been transferred from different species (Sanvido *et al*., 2006).

These crops have been developed for longer shelf life, tolerance to herbicides, improved nutritional value, resistance to pests, diseases and drought among other challenges (Keetch *et al*., 2005; Icoz and Stotzky, 2008). Genetically modified crops like soybean, canola, cotton, maize, and alfalfa are currently grown worldwide, particularly in United States of America (USA),

Argentina, Brazil, Canada, India, South Africa (SA) and China (James, 2005). In South Africa GM crops including cotton (pest control), soyabean (herbicide resistance) and maize (pest control and herbicide resistance) are commercially grown (Keetch *et al*., 2005; Marx, 2010).

A number of maize cultivars have been modified to express the Cry1Ab protein from the bacterium *Bacillus thuringiensis* (Bt) in plant tissue. Some transformation events produce the protein in some parts of the plant (Dutton *et al*., 2003). Transformation events like MON810, Bt11, and Bt176 express Cry1Ab protein to kill lepidopteran pests e.g. stem borer (Saxena *et al*., 2002). Control of these insect pests could result in increased crop yields. Although the adoption of GM crops is slow in parts of South Africa (Gouse *et al*., 2006), an increase of 6% was observed between 2009 and 2011 (James, 2009; Kumwenda and Agbroko, 2011).

In Mpumalanga and North West provinces, large-scale yellow Bt maize farmers achieved yield increase of 11 % in 2001/2 season both under irrigation and dryland production (Gouse *et al*., 2006). This increase could be due to resistance of the crop to stem borer (Keetch *et al*., 2005; Mungai *et al*., 2005). However, slow adoption of these crops by some farmers is related to lack of adaptation of some Bt maize cultivars to local agricultural conditions, while other farmers prefer to address the stem borer problem by managing planting dates (Gouse *et al*., 2006). Moreover, feared technological fees, yearly purchase cost of Bt maize seed and restriction on saving Bt maize seed, as return seed, seem to hinder adoption of Bt maize by smallholder farmers (Gouse *et al*., 2006; Mushunje *et al*., 2011). Besides economic issues

there are also concerns that the cultivation of GM crops might have negative impacts on non-target soil organisms, and microbial processes and functions as a result of the protein and changes in chemical composition of the plant material (Motavalli *et al.*, 2004; Mungai *et al.,* 2005; Icoz and Stotzky, 2008).

Crecchio and Stotzky (2001) and Muchaonyerwa *et al*. (2004), found that Cry1Ab protein persists for several months in soil, which could affect soil environment. In their study, Saxena and Stotzky (2001) did not find any deleterious effect of the Cry1Ab protein on nematodes, protozoa, culturable bacteria, fungi, and earthworms, though earthworm casts and their guts contained the Bt toxin. However, Hilbeck *et al.* (1998) reported mortality of the *Chrysoperla carnea* (lacewing) larvae using artificial diet treated with the Cry1Ab protein.

There are also conflicting findings in literature on effects of genetic modification on chemical composition of maize plants and their residues. Mungai *et al*. (2005) found no differences in chemical composition, decomposition and N mineralization of stem and leaves of Bt (M00112Bt) and non Bt (M-00110) maize, whereas N mineralized 2.7 times more in non-Bt maize roots in field and laboratory experiments. However, Saxena and Stotzky (2001), Stotzky (2004), Poerschmann *et al*. (2005), and Daudu *et al*. (2009) reported that Bt maize cultivars containing Cry1Ab protein had higher lignin content than their near isogenic lines, which could slow decomposition. Bt maize residues were reported to decompose less in soil, possibly as a result of their higher lignin content (Flores *et al*., 2005). This modification

could cause a reduction in microbial activity and nutrient cycling (Motavalli *et al.*, 2004; Flores *et al*., 2005; Mungai *et al.,* 2005).

Commonly grown Bt maize cultivars are of transformation events MON810, Bt11 and 176 which represent different truncated forms of the Cry1Ab protein (Nguyen and Jehle, 2007). These transformation events express the Cry1Ab protein, which is toxic to the European maize stalk borer (*Osrrina nubilalis*). The expression of Bt protein amongst these transformation events is what makes them differ. In the MON810 and Bt11 events the protein expression is higher in leaves and roots compared to pollen while in the event 176 expression of the Bt toxin is highest in leaves and pollen, but low in roots (Dutton *et al*., 2003). The difference between the MON810 and Bt11 is the level of the Cry1Ab in their leaf residues which is high in Bt11 than in MON810 (USEPA, 2007).

Nitrogen and phosphorus are essential nutrients required in large quantities for crop growth and decomposition of crop residues is an important source of these nutrients (Mafongoya *et al*., 2000). Bt maize (events M00112Bt, M9114Bt, H9247Bt, G8484Bt and 33P67) has been reported to have no effect on the mineralisation of N (Mungai *et al*., 2005; Devare *et al*., 2007). Microbial biomass and the associated enzymes are involved in nutrient cycling through decomposition of organic residues in the soil. Any changes in chemical composition of Bt maize and any substance (i.e. Cry1Ab protein) that affect MBC could influence nutrient cycling (Mungai *et al*., 2005). The adverse effect on microbial biomass leads to the reduction in soil nutrients.

Arbuscular vesicular mycorrhizal fungi establish mutualistic symbioses with roots of most plant species, including maize, which improves uptake of P, Zn and Fe (Smith and Read, 1997). Mycorrhizal fungi are strongly affected by agricultural practices e.g. mechanical tillage and by changes in plant and soil characteristics and are key non-target soil organisms to be monitored when studying the impact of GM crops (Fontanet *et al*., 1998; Giovannetti and Avio, 2002). Turrini *et al.* (2004) found that root exudates of Bt maize (event 176) significantly reduced pre-symbiotic hyphal growth of the arbuscular mycorrhizal fungi, compared to those of another Bt maize hybrid (event Bt11) and non-Bt maize. Lower mycorrhizal fungi colonization in pre-symbiotic mycelium of *G. mosseae* was reported after incorporation of Bt11 compared to non-Bt maize plants (Castaldini *et al*., 2005). It is, therefore, important to study the effect of Bt maize on mycorrhizae particularly in the Eastern Cape where soil P and Zn is inherently low (Mandiringana *et al*., 2005).

Soil enzyme activities are closely related to biochemical processes involved in nutrient cycling and are therefore sensitive biological indicators of soil quality, particularly soil fertility (Cookson and Lepiece, 1996; Huang, 2000). Wu *et al*. (2004) reported no effect of the incorporation of Bt (KMD) rice straw on dehydrogenase activity in paddy soils but increased in flooded soils under laboratory conditions. Soil urease, acid phosphomonoesterase, invertase, and cellulase were stimulated by the addition of Bt cotton compared to non-Bt cotton tissue, whereas activity of arylsulfatase was inhibited in an incubation experiment of a silty loam (Sun *et al*., 2006). These enzymes play an important role in soil microbial activity, being related to some important

reactions such as C, N, P and S cycles (Nannipieri *et al*., 2002). These effects suggest that Bt maize enhance N and P mineralisation and reduce S mineralisation when incorporated in soil.

The hypothesis of this study is that modification of chemical composition of Bt maize (MON810) residues and the component Bt protein (Cry1Ab) could negatively affect soil biological function. The aim of this study was to determine effects of Bt maize (MON810) cultivars commercially grown in South Africa and their decomposing residues on selected biological properties and N and P release patterns in soil.

Hypothesis

i. A growing Bt maize (event MON810) crop has no effect on soil MBC.

ii. A growing Bt maize (event MON810) crop has no effect on activities of selected soil enzyme.

iii. A growing Bt maize (event MON810) crop has no effect on soil VAM fungal spore counts.

iv. Decomposition of Bt maize (MON 810) residues does not affect soil MBC

v. Decomposition of Bt maize (MON 810) residues does not affect activities of selected soil enzymes

vi. Nitrogen and P release patterns are not affected by the genetic modification of maize (event MON810).

Objectives

The specific objectives of the study were therefore to determine:

i. effects of a growing Bt maize (event MON810) crop on soil MBC

ii. effects of a growing Bt maize (event MON810) crop on activities of selected soil enzymes

iii. effects of a growing Bt maize (event MON810) crop on soil VAM fungal spore counts

iv. the effects of Bt maize (event MON810) residues on soil MBC

v. the effects of Bt maize (event MON810) residues on activities of selected soil enzyme

vi. the effects of Bt maize (event MON810) residues on soil N and P release patterns.

1.2 LITERATURE REVIEW

1.2.1 Introduction

Transgenic crops, especially those with genes from bacterium *Bucillus thuringiensis*, are increasingly becoming popular for their ability to reduce problems associated with synthetic pesticides because they continuously produce active toxins within the plants (Saxena *et al*., 2002). Crop plants that have been genetically modified include soybean and maize for herbicide tolerance and maize and cotton for pest resistance (Keetch *et al*., 2005).

In the case of maize, damaging pests like the stem borers are continually exposed to the toxin throughout their life cycles (Schnepf *et al*., 1998) and at stages they are most susceptible (Mazier *et al.*, 1997). Unlike Bt commercial formulations (Xentari^{TM}), Bt maize may not require the pests to possess specific proteolytic enzymes and a high midgut pH to solubilize and activate the Cry toxins (Stotzky, 2004; Hernández-Martínez *et al*., 2009). While there are a number of positive effects on food production increase from this technology, it may have its own challenges.

1.2.2 Genetic modification and use of modified crops in agriculture

Bucillus thuringiensis (Bt) is a ubiquitous spore forming, gram positive soil bacterium that produce insecticidal crystal proteins during sporulation protecting maize against stem borer pest (Höfte and Whiteley, 1989; Addison, 1993; Schnepf *et al*., 1998). This bacterium has been commercially used in formulations for the control of these insect pests. Commercial formulations of

Bt have been used as an alternative to chemical pesticides as a result of challenges associated with the use of these pesticides. The challenges include pest and disease resistance to pesticides, contamination of soil and water bodies, air pollution and decimation of beneficial non-target organisms e.g. the monarch butterfly (Ehrlich *et al.*, 1993; Pinstrup-Andersen *et al.*, 1999; Bertoni and Marsan, 2005). Commercial formulations also presented problems ranging from limited stability (Höfte and Whiteley, 1989) and low remainence in fields (Mazier *et al*., 1997) requiring numerous applications.

Developments of genetic engineering have offered opportunity to incorporate the *cry* genes into the plant genome to exhibit active toxins within the plant matrix. These engineered crops carry traits that are different from those found in their conventional crops (Saxena *et al*., 2002). Differences in traits of these engineered crops from those of their conventional crops result in several advantages such as pest resistance, herbicide tolerance and longer shelf life (Keetch *et al*., 2005; Icoz and Stotzky, 2008).

The first GM crop to be approved for commercial use was tomato (U.S. Food and Drug Administration, 1994), which was engineered to ripen on the vine, have a longer shelf life by having delayed ripening, softening, and rotting processes. Herbicide tolerant soybean is tolerant to Round-Up; a non-selective herbicide which acts by entering the plant and inhibiting an enzyme necessary for building aromatic amino acids, and lack of these amino acids kills the plant (van Wyk *et al*., 2009). Herbicide tolerant crops are considered not to have a direct effect on non-target organisms because the enzymes that

code for the herbicide tolerance are normally available in plants and not have any toxic properties (Icoz and Stotzky, 2008).

Some GM crops have been modified to control pests therefore, giving them an economic benefit of less (money spent) on pesticides and fewer chemicals are released to the environment (Bennett *et al*., 2006). Cultivation of some of these GM crops reduces labour costs in the application of pesticides and herbicides. Keetch *et al*. (2005) found that GM maize cultivars increased yields by 11 % and decreased stalk borer damage by 34.6 % than the comparable non-GM maize cultivars. As a result of these advantages GM crops have increasingly been grown worldwide (Icoz and Stotzky, 2008).

1.2.3 Effects of GM maize on other non-GM crops (gene-flow) and on non-targetinsects

Despite the benefits reported over use of Bt maize, there are concerns that transgenic maize may pose negative effects on other non-GM crops. Pollen has been proven to travel long distances in favourable conditions (Brookes and Barfoot, 2006), therefore, coexistence of the GM and non-GM plants might cross pollinate organic plants and that might also cause problems in agricultural practices.

There are also concerns that the cultivation of GM crops might have a negative impact on the non-target soil organisms which will in-turn disturb soil functions and processes (Motavalli *et al.*, 2004). These could be affected, for example, by the presence of Cry proteins in soils through cultivation of Bt

crops (Holst-Jensen, 2009). Saxena and Stotzky (2002) showed that insect resistant GM crops, such as Bt maize, potato, and rice, contributes to the presence and persistence of Cry proteins in soil via root exudation as compared to Bt cotton and canola.

The release of these proteins as root exudates could lead to higher concentrations of Cry protein in rhizosphere soil and possible accumulation and persistence of the protein in the soil (Saxena and Stotzky, 2001). Degradation of the Cry protein in the soil could be by microbial decomposition or exposure to light (Saxena and Stotzky, 2001).

1.2.4 Effects of growing Bt maize on soil microbial biomass and diversity, vulnerable microbial groups and enzyme activities

All agricultural crops that are planted in the soil interact with the soil ecosystem and the effects of these interactions have a major influence on the microbial diversity. Microorganisms are responsible for the normal functioning of the soil as they are responsible for the soil processes such as the cycling of nitrogen, decomposition of waste and the distribution of nutrients in the soil. The disturbance of these processes will affect the number of organisms and their diversity.

Soil microbial biomass is the living component of soil organic matter (OM) (Zhang and Zhang, 2003; Gil-Sotres *et al.*, 2005); and it generally comprises 1-5% of total OM content (Anderson and Domsch, 1989; Sparling, 1992;

Nsabimana *et al.*, 2004). It is used as an indicator of the fertility status of soil. Because of its higher turnover rate, MBC could respond more rapidly to changes of soil environment than OM. It has a fast turnover rate so it plays a key role in controlling nutrient cycling and energy flow in soils (Li and Chen, 2004). The close relationship between soil enzyme activities and biochemical processes make soil enzyme activities sensitive biological indicators for soil quality.

There are concerns that transgenic crops in general may have negative effects on soil ecosystem, microbial processes and functions, and soil biodiversity (Zwahlen *et al.*, 2003; Motavalli *et al.*, 2004; Mungai *et al.*, 2005).The toxin from Bt maize is introduced into soil primarily in root exudates and by incorporation of plant residues after harvest of the crop (Tapp and Stotzky, 1998), with probably some input from pollen during tasseling (Losey *et al.*, 1999). Bt proteins have been found to be present in the rhizosphere soil during the growth of the plant and months after harvest (Saxena and Stotzky, 2001). Although they are present in soil after the harvest, they had no effects on Collembolans (Heckmann *et al*., 2006), nematodes, algae, fungi and earthworms (Koskella and Stotzky, 2002).

Saxena and Stotzky (2001) reported that there were no differences in the colony-forming units of culturable bacteria, actinomycetes, and fungi and in the numbers of protozoa and nematodes between rhizosphere soil of Bt (NK4640Bt) and non Bt maize or between soil amended with biomass of Bt and non-Bt maize. These findings came from studies conducted under field

and plant-growth room incubation conditions for 40 and 45 days, respectively. Koskella and Stotzky (2002) observed no effects of the insecticidal toxin from Btk, Bti, and Btt on the growth of bacteria, fungi, and algae in mixed and pure cultures.

Mycorrhizal fungi consist of a network of filaments that grow in and around the plant root cell. As these mycorrhizal fungi form, they form a mass that extends beyond the root system of the plant (Ardakani, 2009). Mycorrhizal fungi facilitate the uptake of zinc (Zn), and phosphorus (P), as P does not move towards plant roots easily. In turn, the plant provides the mycorrhizal fungi with the energy in the form of sugars. In order to complete their life cycle and produce spores, arbuscular mycorrhizal fungi needs a compatible plant host; and maize has been found to be an appropriate host for them (Wenke and Lianfeng, 2008).

However, Turrini *et al*. (2004) found that exudates of Bt (event 176) reduced pre-symbiotic hyphal growth when compared with Bt 11 and non-transgenic plants. Bt transgenic crops affected the colonization and symbiotic development of AMF in Bt176 as there were differences between Bt and non-Bt treatments. There is limited information on the effects of Bt maize on vesicular mycorrhizal fungi. There is therefore, a need to determine effects of Bt maize, with MON810 event, grown in South Africa on soil microbial functional diversity, in order to get an understanding on nutrient cycling and soil health.

No significant differences on microbial biomass and enzyme activities were observed by Lang *et al*. (2006) between soil with Bt and non-Bt maize. These findings agreed with Icoz *et al*. (2007), who also reported no consistent effects of Bt maize on microbial populations and activity of arylsulfatases, acid and alkaline phosphatases, dehydrogenases, and proteases. Devare *et al.* (2007) reported that there were no adverse effects of Cry3Bb1 from Bt maize on MBC and N mineralisation over a 3-year cropping cycle under field conditions. Although Bt maize with different transformation events was used in these studies, the findings suggest that of Bt maize (MON810) is not likely to have a negative effect on soil microbial biomass and enzyme activities, but may affect mycorrhizae. Such effects may also be affected by local environmental conditions.

1.2.5 Effects of incorporation of Bt maize residual material on microbial biomass, diversity, and enzyme activities

The challenges faced by farmers in SA are many and varied. In most parts of the Eastern Cape (EC) province, rain is low and poorly distributed; soil fertility is a constraint whilst use of both organic and inorganic fertilizers is low. Weeds compete with crops for limited moisture and nutrients negatively impacting maize yield (Mandiringana *et al*., 2005; Fanadzo *et al*., 2010).

Phosphorus (P) is among the nutrient factors limiting crop production (Mandiringana *et al.,* 2005). It has a vital role in plant growth, maturity and in

quality and quantity of crop yields. Resource poor farmers depend on addition of organic amendments to the soil. Any negative effects of these amendments on soil MBC could result in negative effects on decomposition and nutrient release and availability. Residues of Bt maize could be a source of plant nutrients for resource poor farmers in SA.

Several studies have reported that genetic modification of maize may result in possible unintended effects on plant structure and chemical compositions, which may have implications on decomposition processes resulting in nutrient recycling being affected (Poerschmann *et al.*, 2005). Soil enzymes are important for catalysing a significant amount of reactions necessary for life processes of microorganisms in soil, decomposition of organic residues, cycling of nutrients, and formation of organic and soil structure (Bandick and Dick, 1999). Moreover, soil enzyme activities such as acid and alkaline phosphatases, dehydrogenase, urease and arylsulfatase have a significant function in some microbial activities as they are involved in P, C, N and S reactions, respectively.

The effects of Bt maize residue decomposition on soil enzyme activities has brought about a number of mixed results. Flores *et al.*, (2005) reported that there were no consistent statistical differences on activity of proteases, acid and alkaline phosphatases, arylsulfatases, and dehydrogenases enzymes between soil amended or unamended with biomass of Bt maize (MON810 and Bt11) and non-Bt maize under incubation. Icoz *et al.*, (2007) found out that after 4 consecutive years of maize growing (2003-2006), numbers and types of microorganisms and enzyme activities differed with season and with

the cultivars of maize, but these differences were not related to the presence of Cry1Ab and Cry3Bb1 proteins of Bt maize (events Bt 11 and MON810 and MON 863) in soil. In contrast, Bt rice straw expressing Cry1Ab were found to increase activities of phosphatases and dehydrogenases in soil as well as increase in methanogenesis, after the addition to flooded soil.

1.2.6 Decomposition of Bt maize residues and nutrient release

Decomposition is the breakdown of large organic molecules into smaller components (Brady and Weil, 2008), which result from complex microbial processes controlled by several factors (Swift *et al.*, 1979). Among these factors, biochemical composition of residues exerts an important influence (Heal *et al.*, 1997). There are conflicting results in the literature on the effects of genetic modification on chemical composition of plant materials. There were reports that Bt maize and non-Bt maize are chemically different with some findings saying that Bt has higher lignin content than the non-Bt maize (Lehman *et al*., 2008), which could affect decomposition. Yanni *et al*. (2010) reported that Bt maize has more lignin than the non-Bt maize and could take more time to decompose in soil. Residues that have higher lignin content have a tendency of decomposing slowly resulting in longer persistence in soil than residues with low lignin content (Poerschmann *et al*., 2005).

The decomposition of residues might disturb soil functioning by affecting the most valuable enzymes and microorganisms in the soil (Austin and Ballare, 2010). Masoero (1999) reported that Bt maize had higher content of starch

and lignin and lower content of protein and soluble N than non-Bt maize. Flores *et al*. (2005) also found that Bt maize had higher lignin content than non Bt maize and that it took longer to decompose in soil. Saxena and Stotzky (2001) and Daudu *et al*. (2009) also reported elevated lignin content in Bt maize residues. In addition to that Daudu *et al*. (2009) reported that Bt maize residues have higher total polyphenols and lower C: N ratio compared to the residues of near isolines. In contrast, Escher *et al*. (2000) reported lower lignin content in Bt maize (Bt11) than in non-Bt maize.

Several studies comparing Bt and non-Bt maize residue composition and evaluating residue decomposition under field and laboratory conditions have produced mixed results. Hopkins and Gregorich (2003) did not observe any detectable difference in the decomposition of plant material from Bt and non-Bt maize. Similar findings were reported by Lehman *et al*. (2008); Tarkalson *et al*., (2008) and Daudu *et al.*, (2009). Forlmer *et al*. (2002) and Mungai (2005) reported no differences between Bt and non-Bt maize in their effect on N dynamics in a laboratory and field studies.

Mineralisation of soil organic P plays an important role in phosphorus cycling of a farming system. There is limited information about P-mineralisation of Bt maize, however, phosphatases enhances the potential for almost 50% of the plant roots and soil microorganisms for P-mineralisation (Tarafdar *et al*., 1988). Therefore any disturbance to phosphatases may result in low P mineralization. Macronutrients are important for the growth of plants especially, N, P, K and sulphur, therefore any effects on mineralisation as a

result of Bt maize (MON810) modification may limit the release of these nutrients (Belfield and Brown, 2008).

Incorporation of crop residues in soil increases the populations of macro and micro organisms, and the new cells require all the essential nutrients for their growth and activity. Mineral availability in soil is an important factor controlling decomposition under field conditions (Mary *et al.*, 1996). It is unusual for nutrients other than N to limit the decomposition of plant materials in normal soils. Within the context of nitrogen turn over, soil quality is significantly affected by N-mineralisation (Maly *et al.*, 2002). The rate of supply of available N generated by N-mineralisation involves the microbial conversion of more complex organic nitrogen into simpler available mineral nitrogen (NH^{+}_{4} – N + NO^{-}_{3} – N) (Singh and Kashyap, 2007).

The rate of N-mineralisation not only governs the availability of mineral N for plant growth but also involve the ability to retain N, especially after disturbances (Haynes, 1986). Since the mineralisation of organic materials and the release of mineral N, from either native soil or decaying litter is the result of complex interaction between microbial population and their activities. The major factors that limit N-mineralisation are environmental parameters like temperature, aeration, soil moisture, soil organic matter (quantity and quality) and soil type (Arunachalam and Arunachalam, 1999; Banerjee *et al.*, 1999; Kiese *et al.*, 2002; Owen *et al.*, 2003).

Studies on N-mineralisation of Bt maize residues have indicated that Bt maize mineralises almost the same as its near-isogenic crop. Mungai *et al.* (2005)

observed no differences in cumulative N-mineralisation from Bt and non-Bt leaf and stem residues, however, non-Bt maize roots mineralised 2.7 times more than Bt maize roots in silt loam soils in an aerobic incubation study. Cortet *et al.* (2006), after 4 months incubation in the field, reported no differences in decomposition and N-mineralisation dynamics of wheat straw and Bt maize. Devare *et al.* (2007) also reported no differences in potential aerobic N-mineralisation from Bt and non-Bt maize expressing Cry 3Bb1 under field conditions.

The literature has reported several studies on the effects of Bt maize on soil biodiversity and soil functions. Other studies have reported effects on the chemical composition of Bt maize which could affect non-target organisms. Effectiveness of plant residues depends mainly on the litter quality and extent of its degradation (Majumder *et al*., 2010). There is therefore, a need to understand the effects of Bt maize and its residues on non-target organisms which contribute to the nutrient status of the soil through decomposition of residues especially in the EC region where the nutrient status of soils is poor.

CHAPTER 2

EFFECTS OF BT (MON810) MAIZE CROP AND INCORPORATION OF ITS RESIDUES ON SELECTED SOIL BIOLOGICAL PROPERTIES

2.1 ABSTRACT

An increase in the cultivation of genetically modified (GM) crops has led to concerns over their possible effects on non-target organisms. A field study was conducted at the University of Fort Hare Research Farm to determine the effect of growing Bt maize on microbial biomass carbon (MBC), enzyme activities and spore counts of VAM fungi. The trial was designed as a randomized complete block design with four maize cultivars (DKC 61-25B, PAN 6Q-321B, DKC 61-24, PAN 6777) as the treatments and replicated three times. Determination of MBC, enzyme activities and fungal spore counts in rhizosphere soil was done at 42, 70 and 105 days after planting (DAP). In a follow-up experiment, 15 g samples of shredded Bt or non-Bt maize leaf residues (DKC 61-25B, PAN 6Q-321B, DKC 61-24, and PAN 6777) collected after harvesting the 2009/2010 season crop, were incubated in 10 kg pots to determine effects of Bt maize residues on MBC and soil enzyme activities. Soil without residues was used as a control. This experiment was laid out as a factorial in a randomized complete block design in a glasshouse with three replicates for each sampling date. Samples for analysis of MBC and enzyme activities were collected from each pot after 7, 28, and 56 days. Cultivation of Bt maize enhanced MBC ($p < 0.05$) in rhizosphere soil when compared to non

Bt maize near isogenic lines. For example, for DKC cultivars the Bt maize cultivar had 9.1 mg/kg more MBC than the near-isogenic line while for PAN cultivars the increase was 23.9 mg/kg. Activities of dehydrogenase, acid and alkaline phosphatases were not influenced by Bt maize. No differences ($p < 0.05$) were observed in the fungal spore counts among Bt maize and non Bt maize treatments. Incorporation of Bt and non-Bt maize residues resulted in similar levels of MBC and activities of acid and alkaline phosphatases and dehydrogenase. The findings suggested that genetic modification of maize with the MON810 event does not have negative effects on MBC, activities of the selected enzymes, and VAM.

Keywords: Bt maize, maize leaf residues, MBC, enzyme activities, mycorrhizal fungi**.**

2.2 INTRODUCTION

Improvements in agricultural biotechnology have resulted in increased use of genetically modified (GM) crops, like Bt maize, worldwide (James, 2007). The expression of Cry1Ab protein from *Bucillus thuringiensis* (Bt) to kill stem borer in Bt maize, and changes in chemical composition of the plant material, has led to concerns on possible effect on non-target beneficial soil organisms that are important for soil function. Bt maize has been reported to release Cry1Ab protein with root exudates deposited into the rhizosphere (Saxena and Stotzky, 2001). Soil organisms that feed on root debris and exudates, like

earthworms, bacteria and mycorrhizal fungi could be exposed to the protein during maize growth.

In most agricultural systems, crop residues are either incorporated into soil to recycle nutrients or left on the soil surface as mulch (Flores *et al*., 2005). The continued release of Bt proteins and their stabilization in the soil may lead to their accumulation, which will increase their exposure to non-target soil organisms (Koskella and Stotzky, 2002).

Cultivation of GM crops could result in addition to the soil of large amounts of the GM products and plant residues with modified chemical composition (Icoz and Stotzky, 2008), which could affect soil organisms and interfere with microbe-mediated processes and soil fertility. Release of Cry proteins into the rhizosphere of Bt maize could affect microbial diversity and function (Icoz and Stotzky, 2008). Changes in chemical composition of the Bt maize residues could also alter soil microbial composition and activity (Stotzky, 2004).

Soil organisms are important for decomposition of organic material, mineralization of nutrients and supporting uptake of nutrients (Motavalli *et al*., 2004) by plants. Negative effects on such organisms could therefore affect soil quality and productivity. Microbial biomass C is an important indicator of soil quality and it is affected by soil organic matter levels (Wardle, 1992). Soil microbial biomass together with microbial diversity and enzyme activities, could be useful in understanding effects of Bt maize on soil function. Anderson and Domsch (1989) and Sparling (1992) reported that MBC comprises only 1 to 4 % of organic carbon, but due to its fast turnover time, it

plays a crucial role in nutrient cycling and energy flow (Li and Chen, 2004). Soil MBC is made up of different bacteria, fungi, and actinomycetes etc, which are important for different soil functions.

Vesicular-arbuscular mycorrhizal (VAM) fungi play an important role in uptake of some nutrients, notably P, Zn and Fe, from the soil by plant roots as it extends the plant roots through its hyphae (Smith and Reed, 1997). Information on effect of growing Bt maize plants and their residues containing Cry1Ab protein on soil microbial biomass, different microbial functional groups and enzyme activities, is scanty and in some cases conflicting.

Soil enzyme activities are strongly affected by agricultural practices and by changes in plant and soil characteristics. Activity of soil enzymes could affect soil biological processes, such as decomposition of residues, recycling of nutrients, formation of organic matter and soil structure (Jepson *et al*., 1994; Dadenko, 2006), and soil quality especially soil fertility (Huang, 2000). Martens *et al*. (1992) reported an increase in activity of soil enzymes responsible for cycling of carbon, nitrogen, phosphorus and sulphur when organic residues of poultry manure, sewage sludge and plant residues were applied to soil. Wu *et al*. (2004) reported no effect of incorporating of Bt-transgenic rice straw into paddy soils, while dehydrogenase activity increased. Under field conditions, Devare *et al. (*2007*)* found no effects of Bt maize expressing Cry3Bb1 on MBC. Flores *et al*. (2005) reported that there were no effects of Bt maize (MON810 and Bt11) on microbial biomass and activities of

some enzymes (arylsulfatases, phosphatases, dehydrogenases and proteases).

Phosphatases catalyse soil organic phosphorus decomposition and improve soil phosphorus bio-availability (Pascual *et al*., 2002). Dehydrogenase is produced by living microorganisms, promoting soil organic matter mineralization (Brzezinaska *et al*., 1998).

No effects of Bt maize expressing Cry1Ab protein were found on total soil bacteria, fungi, earthworms and nematodes in an experiment conducted under controlled conditions (Saxena and Stotzky, 2001). de Vaufleury *et al*. (2007) reported that Bt protein expressed in Bt maize (MON810) is not toxic, either directly or indirectly, to mycorrhizal fungi, snails and collembola in a 4-month microcosm study. Icoz *et al*. (2007) reported no consistent significant effect of Bt MON810 and Bt11 on activities of soil enzymes. While most studies suggested no effects of Bt maize and other Bt crops on soil microorganisms and enzyme activities, Icoz and Stotzky (2008) reported that mycorrhizal fungi, nitrifying and nitrogen fixing bacteria, and nematodes were susceptible to the Cry1Ab protein in Bt maize. Sun *et al*. (2006) also reported that activities of soil urease, acid phosphomonoesterase, invertase, and cellulase were stimulated by the addition of Bt cotton tissues, whereas activity of arylsulfatase was inhibited. The stimulation of phosphonoesterase could result in increased levels of soil P while the inhibition of arylsulfatase could cause decreased amounts of S release to the soil. Activities of enzymes differ with seasons (climatic conditions), cultivars, and the type of protein exposed

to them (Icoz *et al*., 2007) and it is important to investigate effects of different Bt maize cultivars commercially available in South Africa on soil biological properties under local climatic conditions and soils. The main objective of this study was to determine the effects of growing Bt maize crop and incorporation of its residues on MBC, selected enzyme activities and VAM fungi.

Hypothesis

i. Soil MBC is not affected by growing Bt maize.
ii. Enzyme activities are not affected by growing Bt maize.
iii. VAM fungi are not affected by growing Bt maize.
iv. Soil incorporation of Bt maize (MON 810) residual material does not affect MBC.
v. Soil incorporation of Bt maize (MON 810) residual material does not affect enzyme activities.

Objectives

The specific objectives were to determine the effects of:

i. growing Bt maize on MBC
ii. growing Bt maize on acid and alkaline phosphatases, and dehydrogenase
iii. growing Bt maize on VAM fungi
iv. incorporated Bt maize residual material on MBC
v. incorporated Bt maize residual material on acid and alkaline phosphatases, and dehydrogenase.

2.3 MATERIALS AND METHODS

This study consisted of field and glasshouse pot experiments. The field experiment was conducted at the University of Fort Hare (UFH) Research Farm, Eastern Cape, South Africa (SA) (32^{o}48 S; 26^{o}51 E; altitude 509 m above sea level) on a soil derived from alluvial material and classified as the Ritchie family of the Oakleaf form in the South African classification system (Soil classification Working Group, 1991), and as a Eutric Cambisol according to the World Reference Base (WRB) for Soil Resources system (IUSS Working Group WRB, 2006). The soil contains high content of mica (2:1 non-expanding clay mineral) and low contents of kaolinite and hematite (Mandiringana *et al*., 2005).

2.3.1 Characterisation of the experimental soil

Soil samples (1 kg) were collected from the top soil (0-20 cm) using an auger, air-dried and sieved (< 4 mm). The hydrometer method, as described by Gee and Or (2002), was used for the determination of soil texture after oxidizing soil organic matter with hydrogen peroxide. Soil pH was measured at a soil: water ratio of 1:2.5. The soil suspension was shaken for 30 min and allowed to equilibrate for 10 min as described by Okalebo *et al*. (2002). The same suspension was used to measure electrical conductivity (EC) using an EC meter (model CM 35, Crison Instruments, South Africa) after settling for 1 hr. Total C and N were determined using a LECO C & N auto-analyzer (LECO Corporation, 2003) and total P was determined following wet digestion with H_2O_2/H_2SO_4 (Okalebo *et al.,* 2002)**.**

2.3.2 Field study

The field study was initiated in the 2009/10 season and repeated in the 2010/11 season. Data for effects of growing Bt maize on soil microorganisms were collected in the 2010/11 season only, whereas residues of the maize from the 2009/10 season were used in a follow up glasshouse incubation study.

The field experiment was laid out in a randomized complete block design with four maize cultivars commercially grown in South Africa (DKC 61-25B, PAN 6Q-321B, DKC 61-24, PAN 6777) as the treatments, which were replicated three times. DKC 61-25B is Bt maize (MON810) cultivar (a yellow hybrid) from Monsanto, whereas DKC 61-24 is the corresponding near-isogenic line, PAN 6Q-321B is a Bt maize (MON810) cultivar (a white hybrid) from Pannar while PAN6777 is the near-isogenic. The near-isogenic lines contain the background genetic material of Bt cultivars. The maize crops were planted on 7th December 2010, with intra-row spacing of 0.27 m and inter-row spacing of 0.90 m. The plot size was 12 m × 7.2 m, and plots within a block were separated from each other by 1 m. The distance between blocks (replicates) was 2 m.

Before planting, the land was bush-cut followed by application of glyphosate (360g/L) at a rate of 5L/ha immediately, to kill the weeds. Planting of maize was done under no till and hoes were used to open rows two weeks after application of glyphosate. Marked reeds were used to mark the planting stations, where more than three seeds were planted to avoid poor

emergence. Basal fertilizer used at planting was 2:3:2 (22) (N: P: K), applied at a rate of 400 kg/ha to supply 25 kg N ha^{-1}, 38 kg P ha^{-1} and 25 kg K ha^{-1}. Topdressing with LAN (28 % N) was done at a rate of 50 kg N ha^{-1} at six weeks after planting. Fertilizer application was done for a target a yield of 5 t/ha which, according to Manson *et al*., (2004), is realizable by small holder farmers with medium resources and have access to irrigation. The field was irrigated immediately after planting to facilitate seed germination. The seedlings were thinned to two per station at three weeks after planting. Weed control was done whenever there was need, using Basagran® (bentazon) and Atrazine® at 2 L ha^{-1} applied post emergence for sedges and broad leaved weeds. Supplementary irrigation was applied when required using a sprinkler system. Sampling was done three times at 42, 70 and 105 days. At each sampling the whole plant was uprooted, gently shaken and brushes used to collect rhizosphere soil for the analysis of MBC, enzyme activities and fungal spore count.

At each sampling time, gravimetric moisture content was determined by drying soil samples (0- 20 cm) at 105^{o} C for 24 h. Maize grain yield at the end of the study could not be measured because the study focussed on soil biological function during the growth of maize and on the decomposition of maize residues.

2.3.3 Glasshouse experiment

Leaf residues collected after harvest at the end of the 2009/2010 season were used in this glasshouse incubation study. The residues were air-dried in the glasshouse and cut into 3-4 cm pieces using scissors. They were then incorporated into soil sampled from plots on which the maize was grown (section 2.3.1) using spade. The incubation was at 28^{o} C and the moisture content was kept at 50 % water holding capacity for 56 days.

Experimental design

The experiment was laid out as a factorial in a randomized complete block design in a glasshouse with temperature control (28^{o} C), wet wall and fans at UFH School of Agriculture. The treatments were (i) no residues (control), (ii) DKC 61-25B residues, (iii) PAN 6Q-321B residues, (iv) DKC 61-24 residues and (v) PAN 6777 residues, with three replicates for each of the three samplings. Ten kg plastic pots 30 cm in diameter were used. Fifteen grams of residues were incorporated into 10 kg of soil to approximate 3-6 t (dry matter)/ha, an average maize dry matter expected for smallholder irrigation in South Africa. The soil and residues were mixed and moisture content adjusted to 50 % of water holding capacity, and incubated at 28^{o} C. The pots were placed on top of wire mesh tables. Water was added to replace losses due to evaporation on weekly basis.

Sampling was done using a 300 mm hand digging spade at a depth of 15 cm after 7, 28, and 56 days of incubation and stored in a cold room at 4^{o} C before analysis of MBC and activities of dehydrogenase, and phosphatase (acid and alkaline) enzymes. Dehydrogenase is related to MBC and phosphatases are responsible for P release.

2.3.4 Analyses

Microbial biomass carbon

The fumigation-extraction method was used to measure MBC (Okalebo *et al*., 2002). Soil samples, previously stored at 4^{o} C, were pre-incubated at 37^{o} C for 10 days before fumigation in a desiccator for 3 days. Samples (15 g) were placed into 50 ml beakers and placed into two paired desiccators. In one of the desiccators 100 ml beaker containing 25 ml of chloroform (alcohol-free) was placed. After closing the lids of desiccators, a vacuum was applied to the fumigated treatments until the chloroform rapidly boiled. The desiccator was closed and stored under dark conditions for 72 hours at room temperature. Fumigated treatments were evacuated using vacuum pump repeatedly (8-12 times). After opening the desiccators and transferring soil samples to flasks (250 ml), 50 ml of 0.5 M K_2SO_4 was added and shaken at 200 rpm for 25 minutes. Whatman No. 42 filter paper was used to filter the soil suspension. The concentration of organic C in the extract was determined by oxidation with potassium dichromate in sulphuric acid followed by back titration with ferrous ammonium sulphate as described by Anderson and Ingram (1993).

Briefly, 4 ml of sample extracts were transferred into digestion tubes, after addition of 1 ml 0.0667 M potassium dichromate and 5 ml concentrated sulphuric acid were thoroughly mixed. Two blank tubes (i.e. with reagents but without extracts) were also prepared. Sample tubes and one blank were placed in a preheated block digester at 150^{o} C for 30 minutes. After removal and cooling, they were quantitatively transferred to labelled conical flasks (250 ml), and 3-4 drops of ferroin indicator solution were added. Using a magnetic stirrer, all samples and blanks were titrated with acidified ferrous ammonium sulphate solution; the endpoint with a colour change from green/violet to red was reached. The titres of samples (ml_{sample}), heated (ml_{HB}) and unheated (ml_{UB}) blanks were recorded. Organic carbon percentage was calculated as:

Organic carbon (%) = {(A x M x0.003) / g} x (E x S) x 100

Where: T= standardisation titre, M= molarity of ferrous ammonium sulphate (≈ 0.033 M), A = ($ml_{HB} - ml_{sample}$) x [($ml_{UB} - ml_{HB}$) / ml_{UB}] + ($ml_{HB} - ml_{sample}$), g = dry soil mass (g), E = extraction volume (ml), S = digest sample volume (ml)

The calculation of MBC was done as:

MBC = ($C_{fumigated} - C_{control}$)

Enzyme activity analysis

Acid and alkaline phosphatase activities in the soil were measured following a method used by Icoz *et al*. (2007). One gram of soil (< 2 mm) was placed in a

50-ml Erlenmeyer flask, and 0.2 ml of toluene, 4 ml of Modified universal buffer (MUB) (pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase), 1 ml of *p*-nitrophenyl phosphate solution were added and swirled for a few seconds. The flasks were stoppered, incubated at 37^{o} C for 1 hour, and 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added. After swirling for a few seconds, the soil suspension was filtered through Whatman no. 2 filter paper and the intensity of the yellow colour of the filtrate was measured with a Helios Delta Thermo Spectrometer (Thermo Fisher Scientific, England). Controls with 1 ml of PNP solution after the additions of 0.5M $CaCl_2$ and 0.5 M NaOH (immediately before filtration of the soil suspension), were included.

Dehydrogenase activity was measured as described by Wei *et al*. (2003). Soil (6.7 g) was thoroughly mixed with 0.07 g of $CaCO_3$ in a test tube. One millilitre of 3 % aqueous solution of 2, 3, 5, Triphenyltetrazolium chloride (TTC) and 2.5 ml of distilled water were added and the contents were thoroughly mixed with a glass rod, and incubated for 24 hours at 37^{o} C. After incubation, 10 ml of methanol were added and the tube was stoppered and shaken for 1 minute, before filtration through a plastic funnel plugged with absorbent cotton into a 100ml volumetric flask. The tubes were washed with methanol and the soil was quantitatively transferred to the funnel. Additional methanol was added until the reddish colour disappeared from the cotton plug and the filtrate was diluted with methanol to a 100 ml volume. The intensity of the reddish colour was measured at a wavelength of 485 nm with methanol as a blank.

Mycorrhizal fungi spore count

Mycorrhizal spore counts were determined by wet sieving and decantation as described by Sylvia (1994). Fifty grams of soil was weighed into a 2-L container and 1 L of water was added. This mixture was thoroughly mixed for 1 minute, using a blender, to free spores from soil. The suspension was left to settle for 30 minutes and decanted through a series of standard sieves (450, 250, 150, and 45 µm) and the material collected on the 45 µm sieve were transferred to 50-ml centrifuge tubes with 35 ml of water and centrifuged at 1300 g for 3 minutes. The supernatant was carefully removed without disturbing pellets and 60 % sucrose solution (60 g sugar diluted to 100 ml with water) was added, mixed with a spatula and centrifuged at 1300 g for 1.5 minutes. The spores were transferred onto a Whatman no. 1 filter paper on the Buchner filter apparatus and counted using a dissecting microscope (motic camera-moticam No. 352, motic image + 2.0) and JEOL JSM-6390LV Scanning electron microscope (JOEL stands 4 Japanese electronic optical laboratory, Japan).

2.3.5 Data analysis

The data for MBC, enzyme activities and VAM spore counts (field experiment), for MBC and enzyme activities (glasshouse experiment) were subjected to analysis of variance (ANOVA) and mean separation was done using least significant differences (LSD) at $p \leq 0.05$ using GENSTAT Release 7.22 DE statistical package (Lawes Agricultural Trust, 2008).

2.4 RESULTS

2.4.1 Physicochemical properties of the soil

The soil was sandy loam (15.5 % clay) with pH 6.1 and electrical conductivity of 0.15 dS/m. The soil contained an average C of 0.72 % with lower levels of total N (0.047% and P (0.025%) (Table 2.1).

Table 2.1: Selected chemical and physical properties of soil used in the study

Sand	Silt	Clay	pH	EC	C	N	C: N	P	C:P
_____%_____			H_2O	dS/m	____%____			g/kg	
51.5	33	15.5	6.1	0.15	0.72	0.047	15.3	0.025	288

2.4.2 Total rainfall and temperature at sampling times

Temperatures during the season were comparable to the long-term (30 years) averages (Table 2.2). A total rainfall of 329.5 mm was received during the growing period of December 2010 to March 2011 (Table 2.2). Most of the rainfall was received during the vegetative stage of growth and it decreased during flowering and grain filling stages. Seasonal rainfall was higher in December and January than the 30-year average while it was lower in February and March (Table 2.2).

Table 2.2: Mean monthly temperatures, rainfall and irrigation in the 2010/2011 summer season.

Month	Temperature		Rainfall		Irrigation
	_____(^{o}C)_____		_____mm_____		mm
		30 year (mean)		30 year (mean)	
December	20.58	21.03	122.6	73.5	15
January	22.54	22.24	103.2	67.3	15
February	23.15	22.58	40.2	66.9	30
March	20.70	21.05	63.5	63.5	-

- No irrigation applied

2.4.3 Effects of growing Bt maize plants on MBC, enzyme activities and VAM spores under field conditions

2.4.3.1 Microbial biomass carbon

There was a significant ($p < 0.001$) interaction between maize cultivars and sampling time on MBC (Table 2.3). At 42 DAP, the rhizosphere of DKC 61-25B had significantly ($p < 0.001$) higher MBC than PAN6Q-321B and DKC61-24 while PAN6777 had the least. At 70 and 105 DAP; PAN 6Q-321B had significantly ($p < 0.001$) higher MBC than DKC61-25B and PAN6777 and DKC61-24 had the least (Table 2.3). At all the sampling dates Bt maize treatments had higher MBC than their corresponding near-isogenic treatments. At 70 DAP; MBC was lower than at 42 and 105 DAP.

Table 2.3: MBC in soil grown with Bt maize and their near-isogenic lines

Treatment	MBC (mg/kg)		
	42 DAP	70 DAP	105 DAP
DKC 61-25B[1]	131.0	69.8	116.3
DKC 61-24	103.5	59.1	107.2
PAN 6Q-321B[1]	105.3	88.4	133.9
PAN6777	90.96	65.4	110.6
LSD (p=0.05)		8.1	

B[1] Bt maize cultivars, MBC- microbial biomass carbon

2.4.3.2 VAM spore counts

There was no significant ($p < 0.05$) interaction effect between maize cultivars and sampling time on VAM spore counts. All maize treatments had similar VAM spore counts (Table 2.4), whereas sampling time had a significant effect ($p < 0.001$) on VAM spore counts (Table 2.5). At 70 DAP VAM spore counts were higher than at 42 and 105 DAP.

2.4.3.3 Soil enzyme activities

There was no significant ($p < 0.05$) interaction effects between maize cultivars and sampling time on activities of the selected enzymes (acid phosphatases, alkaline phosphatases, and dehydrogenase). Maize hybrid treatments did not have any effects of both acid and alkaline phosphatase activities, while

sampling time significantly ($p < 0.001$) affected acid phosphatase and alkaline phosphatase activities (Table 2.5). Acid phosphatase activity was lower at 42 than at 70 and 105 days whereas the lowest alkaline phosphatase activity was at 70 days. Both sampling time and maize cultivar treatments, as main factors, had significant effects on dehydrogenase activity (Table 2.4 and 2.5). At 42 DAP; activity of dehydrogenase was higher than at 70 and 105 DAP (Table 2.5). All maize treatments had similar activities of acid and alkaline phosphatases. The activity of dehydrogenase was higher in the PAN6777 treatment than in the DKC 61-25B and PAN 6Q-321B, whereas the DKC 61-24 treatment had the least activity (Table 2.4). The effect of Bt maize, compared to the corresponding near-isogenic line on dehydrogenase activity was inconsistent, in that whereas the DKC61-25B treatment had higher dehydrogenase activity than that of its near-isogenic line, that in the PAN6Q-321B treatment was lower than the corresponding near-isogenic maize treatment.

Table 2.4: Selected soil enzyme activities and fungal spore counts as affected by growing Bt maize cultivars and their isogenic lines

	Enzyme activity (mg/g of soil)			VAM
Treatment	DEHYD	AC PH	ALK PH	(spores kg^{-1} soil)
DKC 61-25B[1]	104.16	40.96	28.24	31
DKC 61-24	83.51	38.41	32.52	34
PAN 6Q-321B[1]	92.99	40.32	30.08	31
PAN 6777	118.35	39.08	27.43	30
LSD (p= 0.05)	11.8	2.5	6.8	5.1

B[1]- Bt maize cultivars, AC PH- acid phosphatase, ALK PH- alkaline phosphatase, DEHYD- dehydrogenase

Table 2.5: Selected soil enzyme activities and fungal spore counts as affected by sampling time in soil grown with Bt maize and their near-isogenic lines

	Enzyme activity (mg/g of soil)			VAM
Sampling time (days)	DEHYD	AC PH	ALK PH	(spores kg^{-1} soil)
42 DAP	120.69	36.99	33.28	31
70 DAP	91.27	41.68	24.70	36
105 DAP	87.30	40.41	30.71	27
LSD (p= 0.05)	10.2	2.2	5.9	4.4

DAHYD- dehydrogenase, AC PH- acid phosphatase, ALK PH- alkaline phosphatase

2.4.4 Effects of soil incorporation of Bt maize leaf residues on MBC and selected enzyme activities under glasshouse conditions

2.4.4.1 Microbial biomass carbon

There was no significant ($p < 0.05$) interaction effects between residue treatments and sampling time on MBC. The main effects of maize residue and sampling time on MBC were significant ($p < 0.001$). Soils amended with the different maize leaf residues had similar MBC levels, which were higher than that of the unamended control (Table 2.7). MBC increased with time, the least and highest values were obtained after 7 and 56 days of incubation (DOI), respectively (Table 2.7).

2.4.4.2 Enzyme activities

There was no significant ($p < 0.05$) interaction between maize leaf treatments and sampling time on acid and alkaline phosphatases and dehydrogenase activities. Activities of soil enzymes studied did not respond to amendment of the soil with any of the maize residues (Table 2.6). Activities of all the three enzymes tested increased with sampling dates ($p < 0.001$) (Table 2.7).

Table 2.6: MBC and selected enzyme activities in soil amended with leaf residues of Bt maize and their near-isogenic lines

Treatments	MBC	Enzyme activity (mg/g of soil)		
	(mg/kg)	DEHYD	AC PH	ALK PH
CONTROL	95.0	118.5	538.4	78.1
DKC 61-25B[1]	132.2	129.5	532.7	85.8
DKC 61-24	128.8	130.3	520.5	83.4
PAN 6Q-321B[1]	146.3	123.5	496.5	83.4
PAN 6777	131.7	121.2	510.5	86.3
LSD (p= 0.05)	20.1	11.6	53.7	12.8

B[1]- Bt maize cultivars, AC PH- acid phosphatase, ALK PH- alkaline phosphatase, DAHYD- dehydrogenase, MBC- microbial biomass carbon

Table 2.7: Effect of sampling time on MBC and selected enzyme activities in soil amended with leaf residues of Bt maize and their near-isogenic lines

Sampling time (days)	MBC (mg/kg)	Enzyme activity (mg/g of soil) DEHYD	AC PH	ALK PH
7 DOI	87.8	85.4	301.5	46.2
28 DOI	128.5	129.3	501.3	83.7
56 DOI	164.2	159.1	756.4	120.3
LSD (p= 0.05)	15.6	9.0	41.6	9.9

2.5 DISCUSSION

A combined increase in rainfall and temperature could have increased soil MBC, fungal spore numbers and enzyme activities (Yang *et al*., 2010) under field conditions. Any decrease in one of these factors would result in decrease in these parameters. Several studies have reported decreased MBC as a result of drought (Willson *et al*., 2001; Li *et al*., 2004). Diaz-Ravine *et al*. (1995) suggested that soil moisture is important in determining MBC of temperate forest soils and is a major factor controlling microbial biomass. In this study, decline in MBC at 42 DAP (February) may be related to decreased rainfall and soil moisture content during this period. An increase in MBC at flowering, 70 DAP, could be explained by elevated rainfall at that period.

Differences in composition among different cultivars may also affect MBC (Hunt *et al*., 1993). The higher MBC in soil from rhizosphere of Bt maize cultivars than their near isogenic lines could be a result of microorganisms utilising root exudates containing the Cry1Ab as a substrate. The composition of such exudates was not determined. The Cry1Ab protein has been reported to be released into the rhizosphere as part of root exudates (Saxena and Stotzky, 2001). No effect was found on phosphatases both in field and glasshouse suggesting lack of effects on microbes important for P-cycling. However, the glasshouse study resulted in similar MBC levels in all the treatments. Amendments of soil with Bt and non-Bt maize residues had no significant effects on MBC. The significantly lower MBC in the control than the other treatments at 28 and 56 days of incubation could be attributed to a decrease in numbers of microbes with time due to depletion of food since there were no residues added to this treatment.

There were inconsistent variations in the activities of soil enzymes over time under field conditions making it difficult to detect treatment effects. It is not clear what caused higher activities of dehydrogenase where DKC 61-25B and PAN6777 were grown as these effects were not related to Bt maize modification. The results of dehydrogenase activity were closely related to those of MBC in the field study possibly because dehydrogenase enzyme is associated with living cells.

No effects of Bt maize on VAM were observed in this experiment, which was in agreement with findings reported by Donegan *et al*. (1995), Saxena and

Stotzky (2001b), and de Vaufleury *et al*. (2007), who reported no effects of Bt toxin (Cry1Ab protein) on earthworms, nematodes, protozoa, bacteria, and fungi. Koskella and Stotzky (2002) also found no effects of Cry1Ab on growth of bacteria, fungi and algae. These results differ from those of Turrini *et al*. (2004) and Castaldini *et al*. (2005), who reported effects of plant residues of transgenic plants, ploughed under at harvest, and kept mixed for 28 days, on soil respiration, bacterial communities, and mycorrhizal establishment by indigenous endophytes.

Mycorrhizal fungi play a very crucial role in a growing maize plant as it improves P and micronutrient uptake by the roots, plant growth, and reproductive responses (Subramanian and Charest, 1997; Sylvia *et al*., 1993; Jeffries, 1987). The critical stage for P requirement of a maize crop is at early stages of growing period (40 to 50 days) and even more at flowering stage (Belfield and Brown, 2008).

The decline in VAM spore numbers at 70 DAP could not be associated with tillage practices as the soil was not disturbed. At the same time, during this period, temperature, rainfall and soil moisture were increased. This decrease could be as a result of the growth stage of the maize since mycorrhizae fungi colonize young roots of a growing crop (Belfield and Brown, 2008).

In the present study, selected enzymes involved in the bio-degradation of plant residues were studied but no significant differences were observed between soil amendments of Bt and non-Bt maize residues, whereas slight inconsistent effects were observed on dehydrogenase activity.

The results of this study agree with the findings of Flores *et al*. (2005), who reported no significant differences in the activities of proteases, acid and alkaline phosphatases, arylsulfatases, and dehydrogenase between soil amended with biomass of Bt (MON810 and Bt11 events) and non-Bt maize in an incubation study. Shen *et al*. (2006) also reported no consistent significant differences of urease, alkaline phosphatase, dehydrogenase, phenol oxidase and proteases between Bt and non-Bt cotton biomass amended with soil in an incubation study that lasted 90 days. However, the findings of this study disagree with those of Wu *et al*. (2004), who reported increased activities of phosphatases and dehydrogenases, after the addition of Bt rice straw to flooded soil.

Soil enzyme activities are strongly related to organic matter content and the type of residues amended or added to the soil (Garcia-Gil *et al*., 2000). Organic matter modifies the development of the enzyme producing microbial population (Browman and Tabatabai, 1978). Madejon *et al.* (2001) reported that amendments of two soils with organic materials increased soil enzyme activities in an incubation study that lasted 280 days. The increased enzyme activities observed in the present study could be as a result of growth of microorganisms during incubation period caused by the addition of residues which increased the substrate for the microbes.

2.6 CONCLUSIONS

Microbial biomass carbon and dehydrogenase activity was enhanced by Bt maize (MON810) relative to non Bt maize in the field study but no effects were observed where leaf residues were incorporated into the soil. Activities of acid and alkaline phosphatases and spore counts of VAM fungi were not affected by growing Bt maize or incorporation of its residues into soil.

Growing Bt maize (MON810) and incorporation of its residues in soils in the Central Region of Eastern Cape would not have negative effects on MBC, enzyme activities and VAM fungal spores. It is suggested that further study should be carried out focusing on effects of Bt maize on different mycorrhizal fungi species and soil enzyme activities in a long-term study. Whereas, there were no effects of Bt residues amendments on MBC and enzyme activities, it would be important to understand the N and P mineralisation from these residues in the Central Region of Eastern Cape.

CHAPTER 3

EVALUATION OF NITROGEN AND PHOSPHORUS RELEASE PATTERNS OF SOIL INCORPORATED BT MAIZE AND NON BT MAIZE RESIDUES

3.1 ABSTRACT

The cultivation of genetically modified crops may have negative effects on ecosystem processes including nutrient cycling. The objective of this study was to evaluate the release of N and P from Bt maize residues incorporated in soil. Leaf, stem, and root residues of Bt maize cultivars PAN 6Q-321B and DKC61-25B and their near-isolines PAN6777, and DKC61-24, were incorporated into soil and incubated in the laboratory at 25^{o} C for 56 days. Soil without residues was included as control. Generally, no differences ($p < 0.05$) in leaf, stem and root tissue characteristics (C, N, P, C: P and C: N) composition were observed between Bt and non-Bt maize cultivars. In addition, there were no differences in net N and P mineralisation from Bt and non Bt maize residues were observed. Differences ($p < 0.05$) were observed between DKC and PAN cultivars on total N and C: N ratio. DKC cultivars (e.g. leaves) had more N (3.15 - 3.32 %) than PAN cultivars (1.30 – 1.50 %) which also had wider C: N ratio (30. 3 – 47.1) in all plant parts. The results suggested that genetic modification of maize (event MON810) did not affect the mineralization of N and P from its residues.

Key words: Bt maize, residues, N and P mineralisation,

3.2 INTRODUCTION

Recycling of nutrients is one of the important practices that can be used to improve the status of soil fertility in the Eastern Cape especially for N and P. Plant nutrients are released from litter either by leaching or breakdown of structural organic components by soil organisms (Berg and Staaf, 1981). Nutrient release and litter quality depends mainly on the litter type and its chemical composition (Majumder *et al*., 2010). A lot of research has been done on genetically modified maize expressing Cry1Ab protein from *Bacillus thuringiensis* (Bt) to address a number of concerns on non-targeted organisms.

The expression of Cry1Ab protein and lignin content in a maize plant differs with plant parts, which makes it important to understand decomposition of different plant tissues (leaf, stem and root) for the release of nutrients (Nguyen and Jehle, 2007; Daudu *et al*., 2009). Moreover, roots have higher lignin content than other plant parts (Yanni *et al*., 2010). Lignin content is an important factor affecting decomposition of residues. Roots would be expected to release N and P at a slower rate than leaves and stems.

The literature, on composition of Bt and non-Bt maize residues reveal conflicting results. No differences were found in chemical composition of Bt (event MON810 and Bt 11) and isogenic non-Bt maize tissues by Forlmer *et al*. (2002), Jung and Sheaffer (2004) and Mungai *et al*. (2005). In contrast, Masoero *et al*. (1999) reported higher lignin content and starch and lower protein and soluble N in Bt maize than in non-Bt. Saxena and Stotzky (2001),

Stotzky (2004), Poerschmann *et al.* (2005) and Daudu *et al.* (2009) reported higher lignin content in Bt maize (MON810) than in non-Bt maize. Modification of chemical composition could reduce rates of decomposition by microorganisms resulting in alteration of nutrient release patterns. Some studies have reported on effects of Bt maize on ecosystem functions, like decomposition and chemical composition of residues such as the proportion of lignin. These effects have direct influence on soil fertility (Eijsackers and Zehnder, 1990).

Based on a litterbag study, Daudu *et al*. (2009) reported that differences in lignin content did not result in a difference in the decomposition of the residues they studied. No differences were reported on decomposition of Bt and non-Bt maize by Hopkins and Gregorich (2003) while Gupta and Watson (2004) reported some differences. However, Saxena and Stotzky (2001) reported elevated lignin content in Bt maize residues which could slow their decomposition. A few studies have reported no differences on the N mineralization from Bt and non-Bt maize.

Mungai *et al*. (2005) reported that incorporation of Bt (Merschman-00112Bt) and non-Bt (M-00110) maize residues in the field and laboratory did not differ in their N dynamics. Cortet *et al*. (2006) found no effects of Bt toxin on decomposition and N dynamics in a litter-bag study after 4 months incubation in the field. Results reported in Chapter 2 indicated that incorporation of Bt maize residues did not affect MBC, phosphatase and dehydrogenase activities, which are involved in decomposition of organic residues. It is

therefore, essential to establish effects of Bt maize cultivars grown in South Africa on nutrient release patterns of different maize plant parts.

Hypothesis

Nitrogen and P release patterns of maize residues are not affected by the genetic modification (MON810).

Objectives

The objective of the study was to determine the effects of genetic modification on N and P release patterns of Bt maize (MON810) in soil.

3.3 MATERIALS AND METHODS

This was a laboratory incubation study using the same soil described in Chapter 2 of this dissertation.

3.3.1 Maize Residues

Leaf, stem and root residues of the maize in Chapter 2 were used in this incubation study. Separately collected leaf, stem and root residues of DKC61-25B, DKC61-24, PAN6Q-321B and PAN6777, were air-dried and ground to < 2 mm. Portions of the residues were ground to ≤ 0.1 mm before determination

of total carbon and nitrogen by digestion method as described by Okalebo *et al*. (2002). Total N and P of the residues were analysed before setting up the incubation study. Total N was determined colorimetrically by diluting the digest a ratio 1:9 (v/v) with distilled water to match the standards. Sample digest and blanks (0.2 ml) were taken into a clear labelled test tube. Five millilitres of the reagent N1 and 5 ml of reagent N2 were added. After standing for 2 h, the absorbance was measured at 650 nm and the calculation of the N concentration in the sample material, expressed in % N, was done as:

$$N\ \% = [(a-b) \times v \times 100] / [1000 \times w \times al \times 1000]$$

Where a = concentration of N in the solution, b = concentration of N in the blank, v = total volume at the end of analysis procedure, w = weight of the dry sample and al = aliquot of the solution taken.

Phosphorus concentration was determined by digesting the plant material in sulphuric acid-selenium digestion mixture followed by a colorimetric determination described by Okalebo *et al*. (2002). Briefly, a clear wet-ashed digest solution (5 ml) was pipetted into a 50 ml volumetric flask to which 20 ml of distilled water and 10 ml of ascorbic acid reducing agent were added. After making to the mark with water, the flasks were shaken well and allowed to stand for 1 hr for full colour development. Absorbance (blue colour) of the sample was measured at 880 nm and the calculation of the P concentration in the sample material expressed in % P was done as:

$$P\ \% = [c \times v \times f] / w$$

Where c = concentration of P in the sample, v = volume of the digest, f = dilution factor, w = weight of the sample.

3.3.2 Experimental setup

Fifty grams of soil, in 250 ml plastic bottles, was amended with 1 g of either leaf, stem or root residues equivalent to 40 t/ha. Soil without residues was also included as a control. The treatments were amendments of soil with (i) leaf (ii) stem (iii) root of PAN 6Q-321B, DKC 61-25B, PAN 6777, DKC 61-24 cultivars and (iv) a control. Each treatment was replicated three times for each sampling of 5 dates to allow for destructive sampling. The soil and residues were thoroughly mixed and soil moisture content maintained at 80 % of water holding capacity. The field capacity moisture content was determined as described by Okalebo *et al*. (2002). The incubation temperature was maintained at 25^{o} C throughout the incubation period of 56 days as suggested by Trinsoutrot *et al*. (2000). Soil samples were collected destructively at 0, 7, 14, 28, and 56 days and stored at 4^{o} C, before analysis of mineral N and P.

3.3.3 Analysis of mineral N and P

Ammonium-N and nitrate-N were extracted from the soil using distilled water at a ratio of 1:5 as described for a Continuous Flow Analyzer method (Skalar Analytical B.V. Breda, Netherlands). Ten grams of soil was weighed into a 100 ml plastic bottle and 50 ml of diluted water was added and shaken for 60 minutes. The samples were filtered on a Whatman no. 42 filter paper and

analysed for ammonium- and nitrate-N using a SKALAR Continuous Flow Analyzer (Skalar Analytical B.V. Breda, Netherlands).

Extractable P was determined following Bray 1 extraction method using a mixture of ammonium fluoride and hydrochloric acid and colour development using ascorbic acid mixture as described by NASAWC (1990). Five grams of sample was weighed into a 50 ml plastic shaking bottle and one standard sample and two blanks were included. Thirty-five millilitres of Bray 1 extracting solution (30 ml of 1.0 M ammonium fluoride, and 50 ml of 0.5 M hydrochloric acid made up to 1L with distilled water) was added shaken for one minute by hand and immediately filtered through a Whatman no. 5 filter paper. The mineral P was analysed using a SKALAR Continuous Flow Analyzer (Skalar Analytical B.V. Breda, Netherlands). Mineral N and P contents in control soil (without residues) were also determined.

3.3.4 Data analysis

Nitrate- and ammonium-N data for each sampling time were added to obtain mineral-N. Data for N and P were subjected to analysis of variance (ANOVA) and mean separation was done using least significant differences (LSD) at $p < 0.05$ using GENSTAT Release 7.22 DE statistical package (Lawes Agricultural Trust, 2008).

3.4 RESULTS

3.4.1 Chemical composition of maize residues

Leaf residues of DKC 61-25B and DKC 61-24 had higher carbon, nitrogen and phosphorus content than PAN 6Q-321B and PAN6777 (Table 3.1). The magnitude of this difference was much greater for N and P than for plant C hence PAN 6Q-321B and PAN6777 had higher C: N and C: P ratios than DKC 61-25B and DKC 61-24 (Table 3.1). Similar pattern was also observed in the stem residue composition except that P was almost similar in all the treatments resulting in similar C: P ratios. Root residues had similar trend with that of stem composition (Table 3.1). Plant parts had similar carbon content, however, on average stems had higher carbon % compared to leaf and root residues, while leaf residues had higher N and P % compared to stem and root residues. In general, Bt maize cultivars had higher C, N, and P content than the near-isogenic cultivars (Table 3.1). DKC 61-25B had higher C: N ratio in leaf and stem but lower in roots than DKC 61-24 while PAN 6Q-321B had lower C: N and C: P ratios in leaf and root residues compared to DKC 61-24.

Table 3.1: Characteristics for Bt (DKC 61-25B and PAN 6Q-321B) and non-Bt (DKC 61-24 and PAN6777) maize residues used in the incubation study (Means±SEM).

Parameter	Total C (%)	Total N (%)	Total P (%)	C:N	C:P
Leaf					
DKC 61-25B[1]	50.1±0.50	3.15±0.11	1.09±0.09	15.9±0.72	45.9±4.18
DKC 61-24	49.2±1.04	3.32±0.21	1.08±0.13	14.8±0.71	45.5±6.52
PAN 6Q-321B[1]	45.4±0.99	1.50±0.15	0.98±0.04	30.3±3.38	46.3±2.56
PAN 6777	44.9±2.21	1.30±0.23	0.95±0.20	34.5±5.66	47.3±9.81
Stem					
DKC 61-25B[1]	51.9±0.58	2.94±0.32	1.02±0.07	17.7±2.03	50.9±4.16
DKC 61-24	49.7±1.00	2.72±0.23	0.90±0.24	16.3±1.08	55.2±15.9
PAN 6Q-321B[1]	46.2±0.54	0.98±0.03	0.82±0.10	47.1±0.70	56.3±7.96
PAN 6777	45.2±0.36	1.32±0.06	0.81±0.14	34.2±1.54	55.8±9.81
Root					
DKC 61-25B[1]	46.8±0.78	3.70±0.42	0.90±0.03	12.6±1.59	52.0±2.24
DKC 61-24	45.0±0.68	2.90±0.27	0.70±0.12	15.5±1.81	64.2±12.2
PAN 6Q-321B[1]	42.5±1.07	1.20±0.09	0.70±0.06	35.1±1.38	60.7±7.44
PAN 6777	40.0±1.04	1.10±0.02	0.60±0.13	35.7±0.60	66.7±18.2

B[1]- Bt maize cultivars

3.4.2 Nitrogen mineralisation

There were no differences ($p < 0.05$) in net mineral N from Bt (DKC 61-25 and PAN 6Q-321B) and non-Bt (DKC 61-24 and PAN6777) maize leaf, stem and root residues incubated in a loam soil for 56 days. Generally, mineral N was higher in the root residues of DKC (DKC 61-25B and DKC 61-24) cultivars compared to PAN (PAN 6Q-321B and PAN6777) cultivars (Figure 3.1, 3.2 and 3.3). Incorporation of maize residues increased mineral N in the soil, more N was mineralised from root residues compared to leaves, and stem residues at 28 DO). General trends for the treatments on N release were in the orders; DKC 61-24, stem > roots > leaves, DKC 61-25B, stem = root = leaves, PAN 6777 (leaves > root = stem) and PAN 6Q- 321B (root > stem > leaves).

3.4.3 Phosphorus mineralisation

No differences ($p < 0.05$) were observed in net mineralised P from Bt (DKC 61-25 and PAN 6Q-321B) and non-Bt (DKC 61-24 and PAN6777) maize leaf, stem and root residues incubated in a loam soil for 56 days (Figure 3.4, 3.5 and 3.6). There was not much increase in extractable P up to 7 DOI, after which, an increase in P mineralisation was observed at 14 DOI. However, P mineralisation declined after 14 DOI up to the end of the experiment (Figure 3.4, 3.5 and 3.6). Net P mineralisation from the stem was in the order DKC 61-24 = DKC 61-25B > PAN6777 = PAN 6Q-321B. Stem residues had greater mineral P at the end of the study than roots and leaf residues.

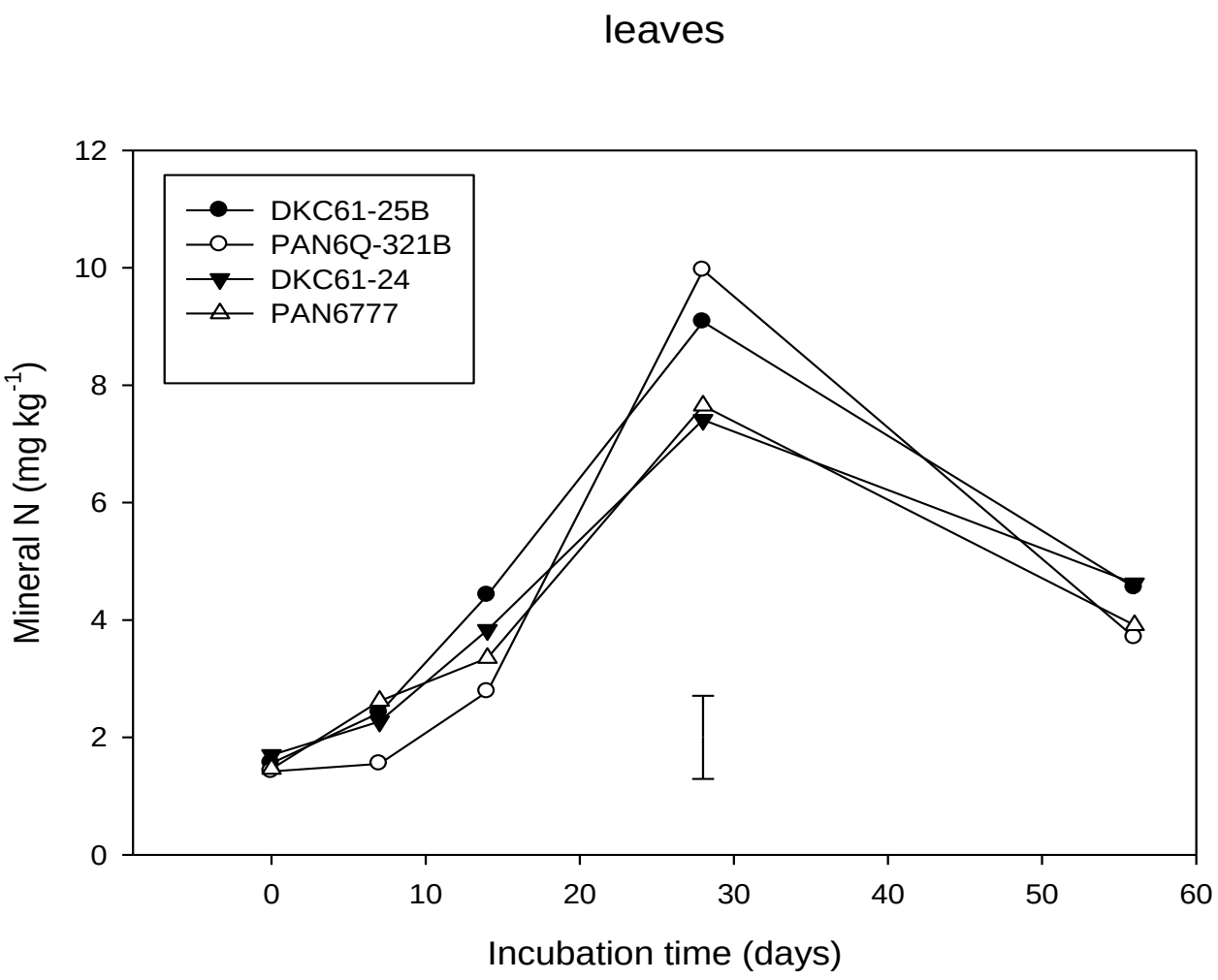

Figure 3.1: Net mineralized inorganic N (NH_4-N + NO_3-N) from Bt (DKC 61-25B and PAN 6Q-321B) and Non-Bt (DKC 61-24 and PAN6777) maize leaf residues incubated with soil under laboratory conditions. Error bar represents least significant differences ($p < 0.05$).

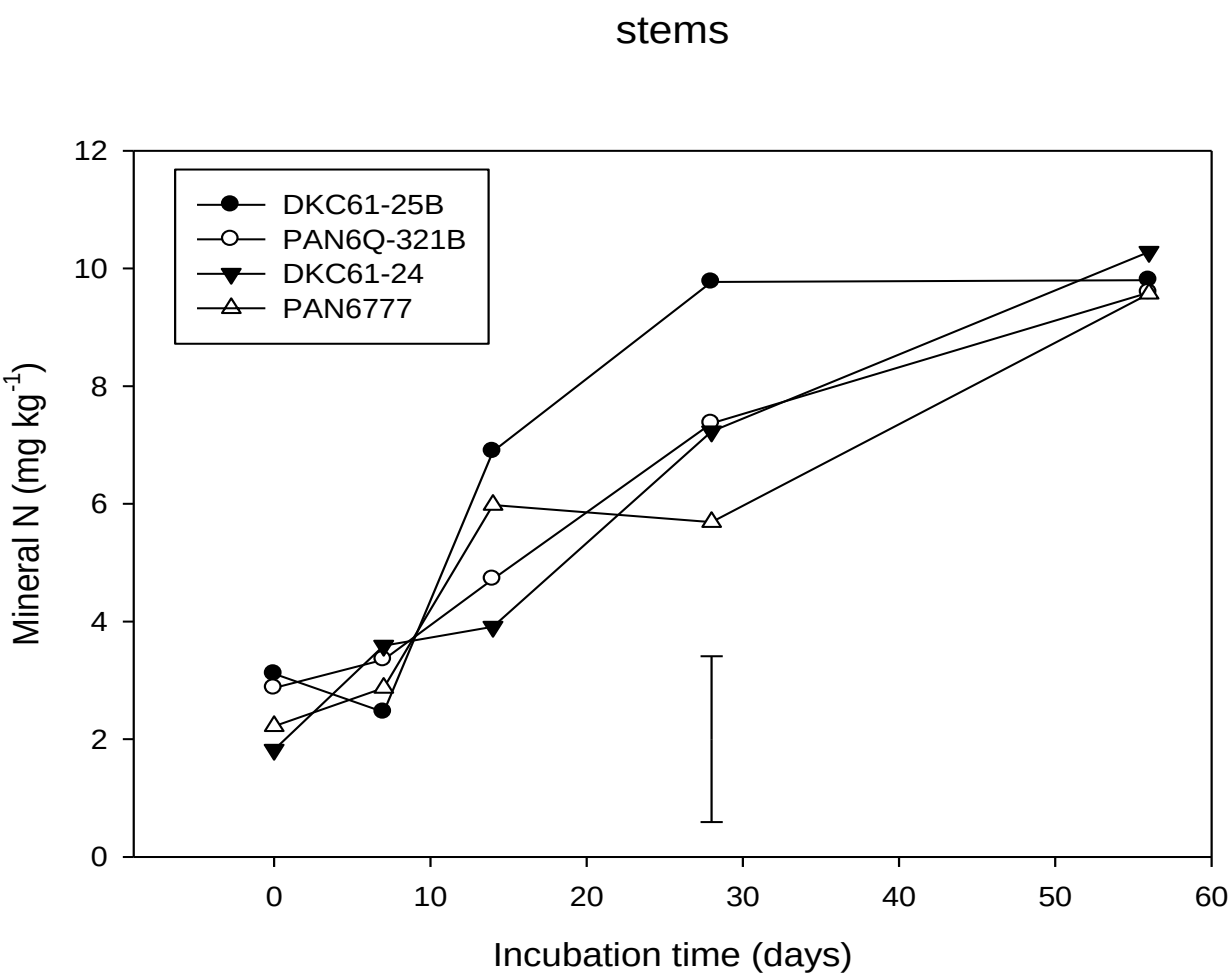

Figure 3.2: Net mineralized inorganic N (NH_4-N + NO_3-N) from Bt (DKC 61-25B and PAN 6Q-321B) and Non-Bt (DKC 61-24 and PAN6777) maize stem residues incubated with soil under laboratory conditions. Error bars represents least significant differences ($p < 0.05$).

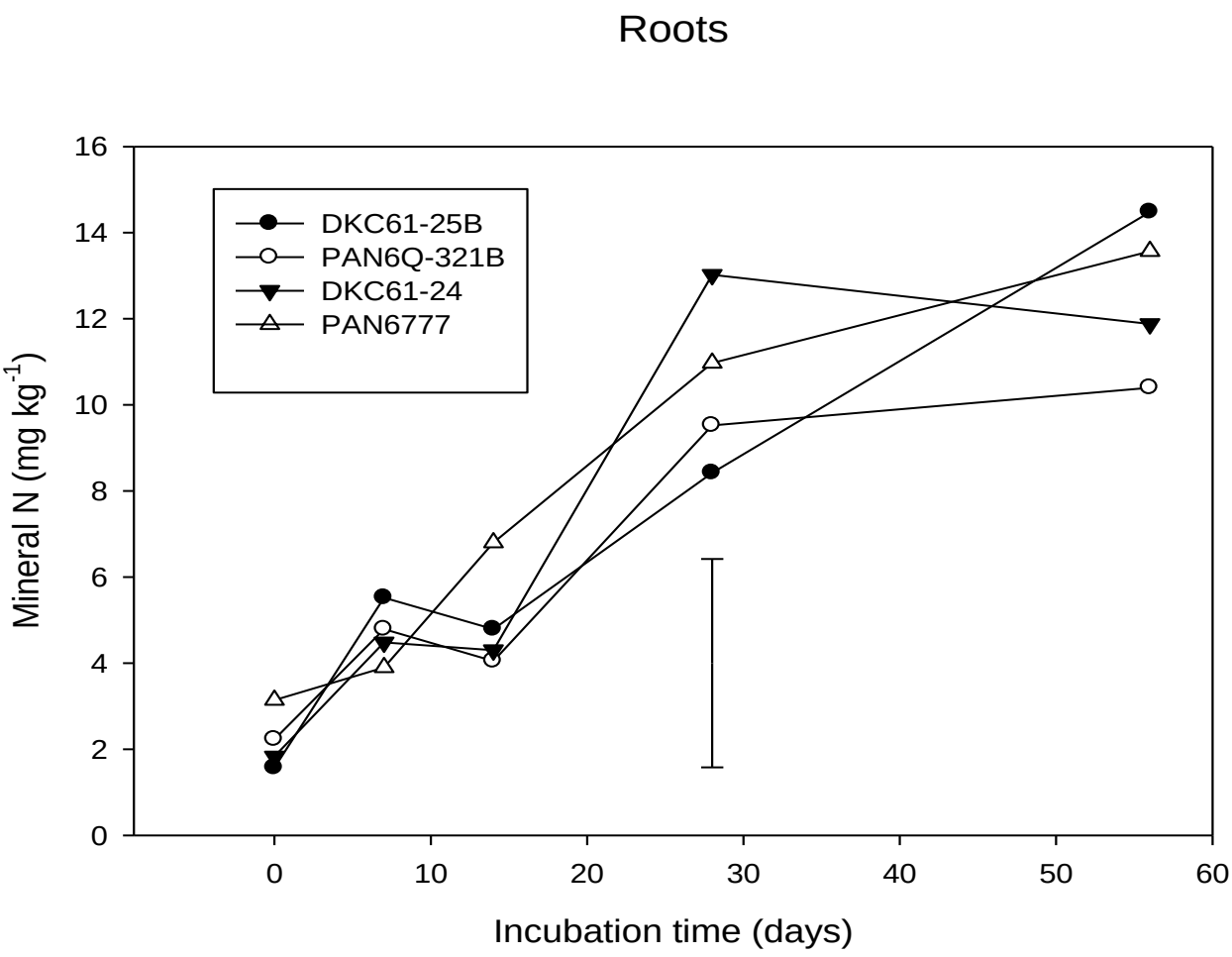

Figure 3.3: Net mineralized inorganic N (NH_4-N + NO_3-N) from Bt (DKC 61-25B and PAN 6Q-321B) and Non-Bt (DKC 61-24 and PAN6777) maize root residues incubated with soil under laboratory conditions. Error bar represents least significant differences ($p < 0.05$).

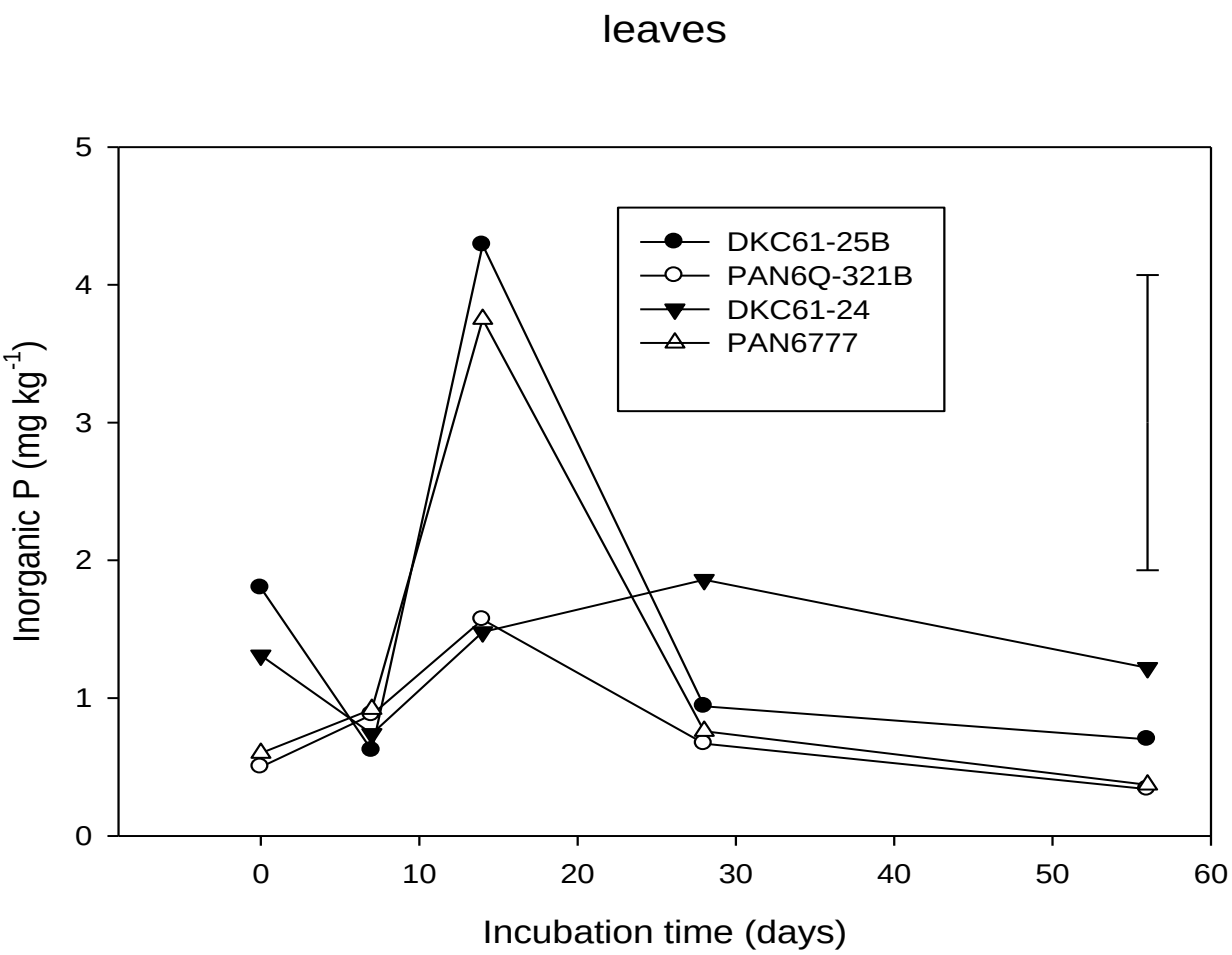

Figure 3.4: Net mineralized extractable P from Bt (DKC 61-25B and PAN 6Q-321B) and Non-Bt (DKC 61-24 and PAN6777) maize leaf residues incubated with soil under laboratory conditions. Error bar represents least significant differences ($p < 0.05$).

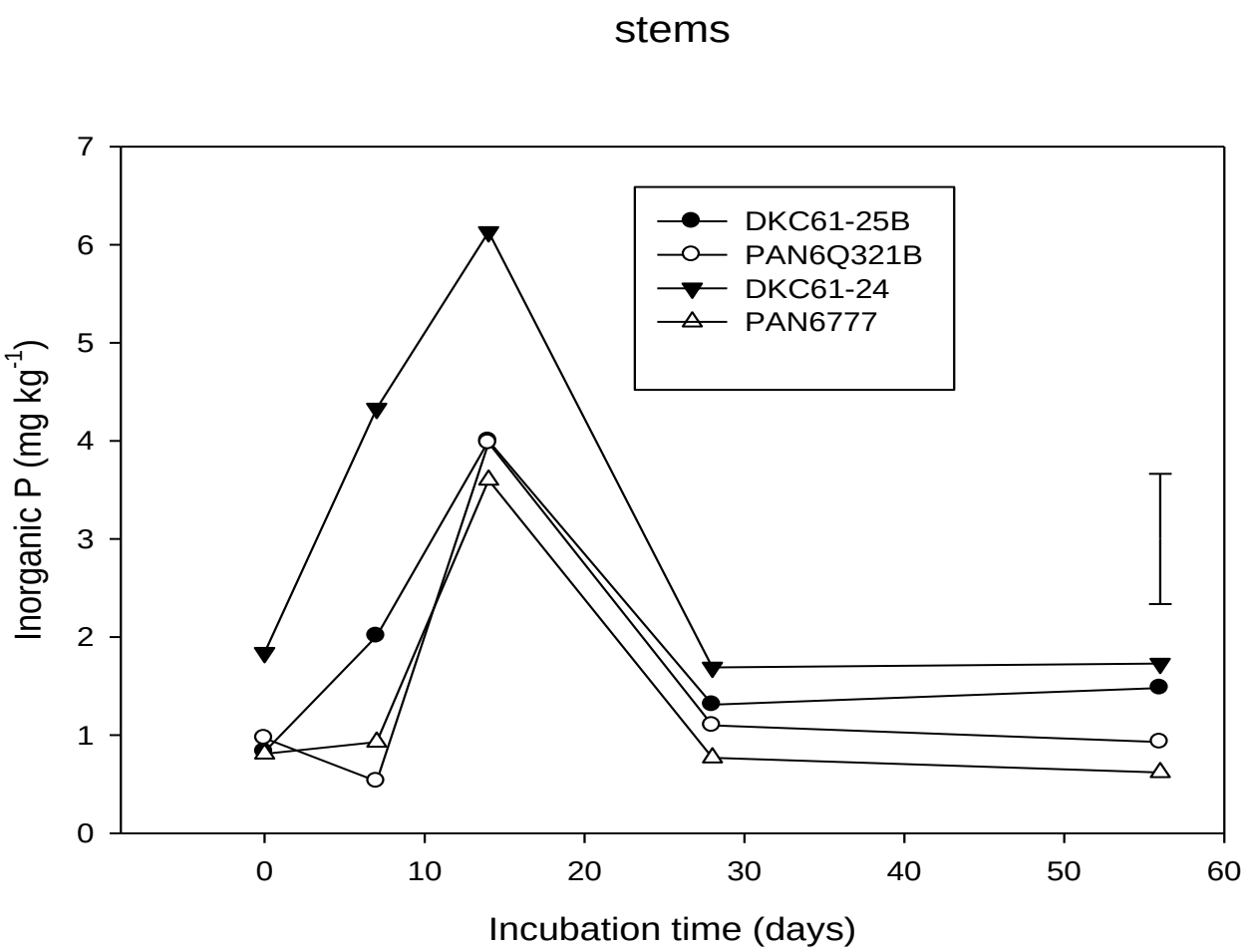

Figure 3.5: Net mineralized extractable P from Bt (DKC 61-25B and PAN 6Q-321B) and Non-Bt (DKC 61-24 and PAN6777) maize stem residues incubated with soil under laboratory conditions. Error bar represents least significant differences ($p < 0.05$).

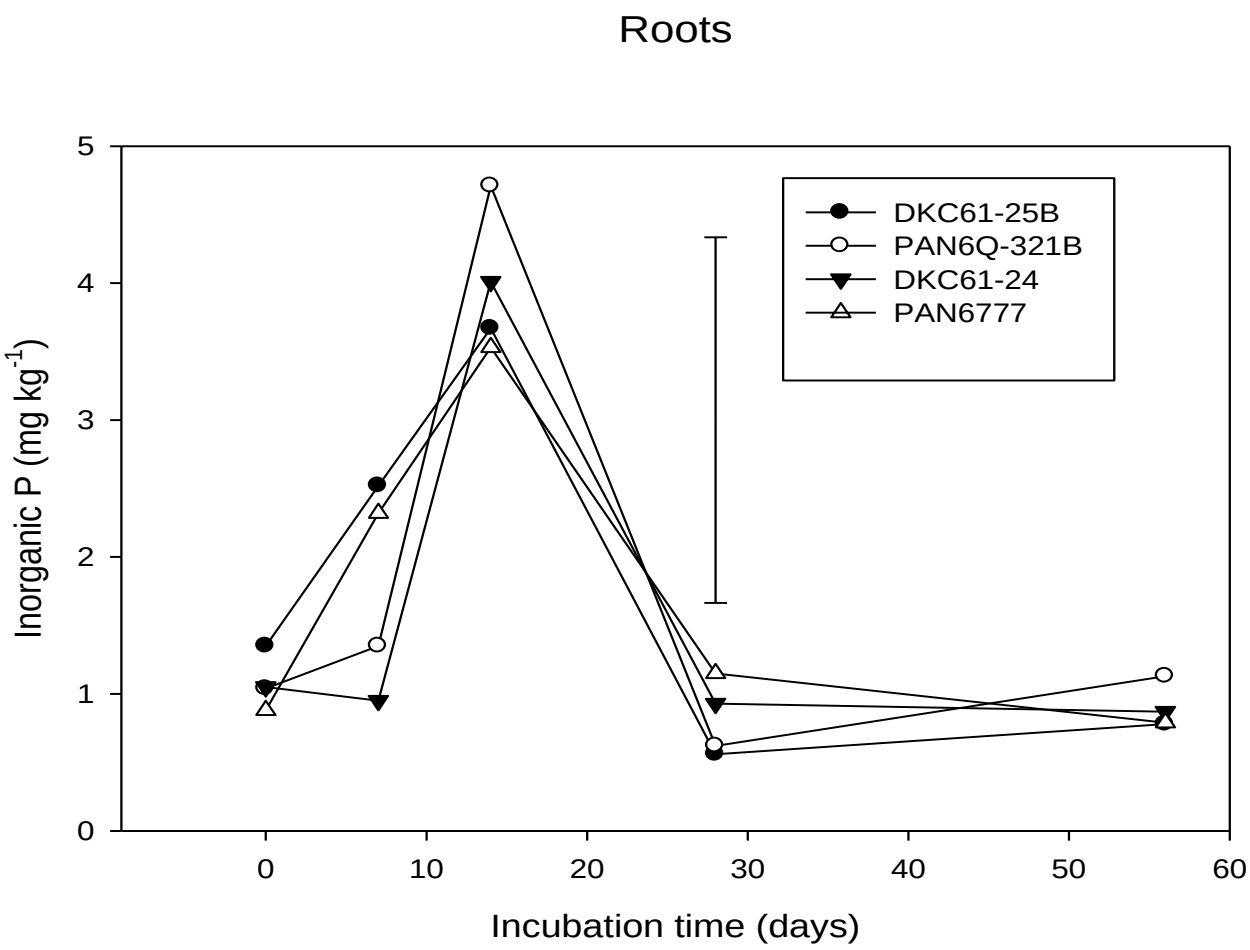

Figure 3.6: Net mineralized extractable P from Bt (DKC 61-25B and PAN 6Q-321B) and Non-Bt (DKC 61-24 and PAN6777) maize root residues incubated with soil under laboratory conditions. Error bar represents least significant differences ($p < 0.05$).

3.5 DISCUSSION

The higher N and lower C: N ratio in DKC cultivars than PAN cultivars were likely to be related to genetic differences of the cultivars. This difference could affect N mineralisation due to reduced decomposition rate of the material with higher C: N ratio (Motavalli *et al*. 2004).

Similarities in leaf, stem and root tissue characteristics of Bt and non-Bt maize cultivars with respect to total C, N and P and C: N and C: P ratios, are in contrast to findings by Hopkins and Gregorich (2003), and Mungai *et al*. (2005), who reported a higher N and C: N ratio in a Bt isoline (Pioneer 38W36) compared with the non-Bt (P3893) maize line. However, Escher *et al*. (2000) reported a lower C: N ratio in the leaves of one Bt maize line compared with the corresponding non-Bt isoline. Differences in these studies could be as a result of the transformation events and background genetic material used.

The C: N ratio is used to predict N mineralisation during crop residue decomposition. A C: N ratio above 30 is known to increase potential for N immobilisation in the soil and N mineralisation occur if the C: N ratio is less than 30 (Trinsoutrot *et al*., 2000; Sainju *et al*., 2005). Generally, all incorporated residues had similar N-mineralisation patterns. Overall, DKC (DKC 61-25B and DKC 61-24) cultivars had more mineral N than PAN cultivars (PAN 6Q-321B and PAN6777), which could be explained by the lower C: N ratio in DKC maize cultivars than in PAN cultivars. Similarities of N-mineralisation from Bt cultivars and non Bt cultivars could likely be related

to similarities in chemical composition due to similar background genetic material (Flores *et al*., 2005; Poerschmann *et al*., 2005).

Net P mineralisation occurred to the same extent in all treatments. Net mineralisation of P depends on the initial P content and C: P ratio of the plant material. Floate (1970) reported that residues with P value < 0.2 % show little or no net P mineralisation. Mafongoya *et al*. (2000) also observed net P immobilisation when leaves of agroforestry tree species (*Gliricidia sepium*, *Acacia nilotica*), with total P content of < 0.2 % were incubated with soil. In this study P was greater than the limit. In general, residues high in P decompose faster and release more P within a shorter period (Tian *et al*., 1992) because these residues contain sufficient P (and N) for the survival of the microbes which have low C: N and C: P ratios. The C: P ratio above 250 is known to increase P immobilisation (Clark and Woodmansee, 1992). In this study the C: P ratio for all the tested residues was lower than this critical value.

3.6 CONCLUSION

Soil incorporated Bt maize and non Bt maize residues had similar mineralisation patterns for N and P in the laboratory incubation study reported herein. Therefore, the genetic modification of maize (MON810) did not affect the mineralisation of N and P from the residues (leaf, stem, or root) of the modified maize cultivars studied. Confirmation of these results under field conditions is recommended.

CHAPTER 4

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 GENERAL DISCUSSION

Genetically modified maize expressing Cry1Ab protein from *Bucillus thuringiensis* (Bt) is among the major GM crops commercially grown in SA. An increase in the production of Bt crops has generated concerns on their environmental effects. Several studies to address some of the concerns were focusing on possible effects on chemical and structural component of the Bt maize crop (Saxena and Stotzky, 2001a; Folmer *et al*., 2002; Jung and Sheaffer, 2004; Flores *et al*., 2005; Mungai *et al*., 2005; Poerschmann *et al*., 2005; Daudu *et al*., 2009). It has been reported that Bt maize has higher lignin content which could have unintended effects on decomposers, soil functions and decomposition of the residues (Poerschmann *et al*., 2005). The Cry1Ab protein released by Bt maize in the rhizosphere could also have negative effects on soil biodiversity. The objective of this study was to determine the effects of a growing Bt maize crop and its residues on MBC, soil enzyme activities, VAM fungi under field and glasshouse conditions and N and P release patterns under laboratory conditions.

Microbial biomass carbon plays an important role in nutrient cycling as a result of its faster turnover rate (Li and Chen, 2004). Any effect on the MBC could affect nutrient status of the soil.

Differences observed in MBC between Bt and non Bt maize under field conditions could be a result of root exudates of Bt maize being better substrates than their near-isolines. In this study Bt maize cultivars were similar in their chemical composition to their near-isolines. Differences existing in the chemical composition of DKC and PAN cultivars could explain the difference in MBC in their rhizosphere (Grayston *et al*., 1998; Icoz and Stotzky 2008). Observed differences in MBC at different sampling times could be explained by the variations in temperature and rainfall. Microbial biomass decreases as a result of drought conditions (Willson *et al*., 2001; Li *et al*., 2004), and the decreased MBC 42 DAP could be associated with lower rainfall and increased temperatures, during that period.

The toxin from Bt maize is not only introduced into soil by root exudates but also by incorporation of plant residues after harvest of the crop (Tapp and Stotzky, 1998). In this study it was essential to incorporate residues in the soil so as to understand the effects of Bt maize residues on MBC and enzyme activities. No differences were, however, observed between the amendments of soil with Bt and non-Bt maize residues, which agreed with Muchaonyerwa *et al*. (2004) who reported no effects of Bt proteins on MBC and bacterial and fungal populations in vertisol, alfisol and oxisol. These findings imply that Bt maize may improve microbial biomass carbon in the rhizosphere under field conditions, but would not negatively affect this parameter when the residues are incorporated into the soil.

Microbial activities include enzyme activities, like phosphatases, which are responsible for the degradation of P compounds in soil. Dehydrogenase which is related to living cells is involved in C reactions for the benefit of microbes responsible for nutrient cycling.

Bt maize had no effect on enzyme activities under field conditions except for dehydrogenase, which had more activity where DKC 61-25B and PAN6777 were grown. These effects were not related to the modification of Bt maize. Whereas dehydrogenase is closely associated with living microbial cells there was no direct correlation between activity of the enzyme and MBC under field conditions. There were no differences in phosphatase activity in rhizosphere of Bt maize and its isoline and this was in agreement with findings in soils amended with residues of the corresponding maize cultivars. The findings are in agreement with Flores *et al*. (2005), and Shen *et al*. (2006), who found no effects of Bt maize residues in incubation studies.

These findings appear to suggest that root exudates of Cry1Ab and the chemical composition of residues have no effect on soil phosphatase activity associated with P cycles. Enzyme activities in this study are closely related to MBC with the C inputs (Mohammadi, 2011). The similarities on enzyme activities that were observed in this study were supported by similarities in release patterns of P and N.

Lack of effects of Bt maize on MBC and enzyme activities observed under glasshouse conditions in this study can be used to explain similarities in the N and P release patterns.

Although, decomposition and mineralisation of plant material is influenced by other plant components, such as lignin content and polyphenols (Flores *et al*. (2005); Daudu *et al*. (2009)) which were not tested in this study; these parameters appear to have not caused any effects on MBC and enzyme activities and mineralisation of N and P.

No evident effects on N and P release patterns from leaf, stem and root tissues were observed. These findings suggested that N and P mineralised to the same extent from Bt or non-Bt maize. There were no negative effects of Bt maize on mycorrhizal fungi. These findings agreed with de Vaufleury *et al*. (2007), who demonstrated that mycorrhizal fungi colonization was not affected by Bt maize. Lack of effects of Bt maize on VAM fungi under field conditions, suggest that uptake of mineralised P may not be curtailed by Bt maize.

Based on the findings of this work it can be summarized that: (i) a growing Bt maize crop would not affect MBC, enzyme activities (acid and alkaline phosphatases and dehydrogenase) and VAM fungi, (ii) decomposition of Bt maize residues has no effect on MBC and enzyme activities, and (iii) genetic modification of maize does not affect the mineralisation of N and P from its residues incorporated in soil.

4.2 CONCLUSIONS

Growing Bt maize (event MON810) does not impact negatively on MBC, soil enzyme activities (acid and alkaline phosphatases and dehydrogenase) and mycorrhizal fungi. Genetic modification of maize (event MON810) did not affect N and P mineralisation from leaf, stem and root residues. Growing Bt maize (MON810) and incorporation of its residues into soil may not have negative effects on soil biological function and nutrient release.

4.3 RECOMMENDATIONS

i. A long term study is required to establish effects of Bt maize on the soil biochemical properties.

ii. Further research to investigate the effect of a growing Bt maize on different species of mycorrhizal fungi is necessary as fungi could differ with regions.

iii. Nutrient mineralisation need to be studied in field soils where Bt maize residues have been incorporated in the medium to long term.

iv. Different transformation events, including stacks in Bt maize need to be studied in terms of enzyme activities, mycorrhizae and their nutrient mineralisation.

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