

**Tillage management impact on greenhouse gas emissions and soil health on
a maize long-term trial in KwaZulu-Natal**

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DECLARATION

I, **Bonginkosi Samuel Vilakazi**, declare that;

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ABSTRACT

Population growth has prompted expansion of arable land, intensification of cropping and fertilizer usage to increase crop production in order to meet increasing food demand. These agricultural activities have a great impact on greenhouse gases (GHGs) emissions, global warming and changes in global climate patterns. Therefore emission of GHGs such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) have increased. Better methods of agriculture must be employed to sustain ecosystem, crop productivity and still allow farmers to obtain profit. Conservation agriculture practices such as no-till (NT) and minimum tillage has great potential to improve soil fertility and reduce GHG emissions, while sustaining crop productivity. The objectives of the study were to; (1) assess selected soil physicochemical properties under different tillage techniques and fertilizer application rates; (2) assess the impact of tillage and N fertilizer application on soil C sequestration; (3) assess the effect of tillage and urea fertilizer application on N and P mineralisation patterns in the soil; (4) assess soil enzymes activity under different tillage and N fertilizer management and; (5) compare emissions of GHGs between conventional tillage (CT-ANNUAL) and NT at different N rates. The study was done in a randomized split-plot experimental design, under dry-land maize monocrop. Tillage treatments included no-till (NT), annual conventional till (CT-ANNUAL), and conventional tillage every 5th year (CT-Y5). In each tillage treatment urea fertilizer was applied at four application rates of 0, 60, 120 and 240 kg N ha⁻¹. Soil samples were collected at 0-10, 10-20 and 20-30 cm depth and analysed for soil properties whereas permanent PVC chambers were installed for GHGs sampling.

Soil physicochemical properties were obtained through laboratory analysis of pH, EC, exchangeable acidity, bases, total C and N, bulk density and texture. Carbon (C) pools such as organic C, particulate organic C (POC), permanganate oxidizable C (POXC) and microbial biomass C (MBC) were analysed. Mineralisation patterns of N and P together with organic P were assessed using anaerobic N mineralisation and ascorbic acid reducing agent, respectively. Biological soil properties such as activities of urease, invertase and acid phosphatase enzymes were determined, while seasonal (summer and winter) fluxes of CO₂, CH₄, N₂O, soil moisture and temperature were monitored. Results showed that NT had higher bases, soil moisture, C pools, N pools, P pools and enzyme activities in most treatments at 0-10 cm depth compared to CT-Y5 and CT-ANNUAL (p<0.05). This was attributed to accumulation of crop residues and less soil disturbance under NT. However, these parameters decreased with depth under NT

and CT-Y5 while opposite was observed under CT-ANNUAL. The incorporation of crop residues through ploughing increased distribution of soil parameters with depth in CT-Y5 and CT-ANNUAL compared to NT. NT had the highest bulk density, with the control treatment of NT at 20-30 cm having as high as 1983 kg m^{-3} , across most depths than CT-Y5 and CT-ANNUAL.

CT-ANNUAL had larger emissions of N_2O and CO_2 compared to NT ($p < 0.05$), whereas NT had higher fluxes of CH_4 ($p < 0.05$). Generally, most gas effluxes were higher in summer than winter, which was attributed to higher summer soil temperatures and moisture. High soil moisture and temperature particularly accelerated CH_4 emissions, while drier winter soil moisture suppressed emissions of CO_2 . Both CO_2 and N_2O emissions positively responded to tillage while CH_4 negatively correlated with it. Thus greater CO_2 emissions from CT-ANNUAL were attributed to increased rates of OM turnover during ploughing. Whereas higher N_2O emissions from CT-ANNUAL were mostly due to improved soil aeration thus increase in diffusivity and better nitrification. Furthermore, greater emission of CH_4 ($0.15 \text{ mg CH}_4\text{-C ha}^{-1} \text{ day}^{-1}$) under NT were attributed to the higher soil moisture and temperature in this technique that favoured methanogenic bacterial activity. Employing appropriate tillage practices and N fertilizer, in this case NT at 120 kg N ha^{-1} , can be recommended because it resulted in less effluxes especially during summer season. In addition, NT at 120 kg N ha^{-1} had mostly high C, P, N pools, enzymes activities and physicochemical properties compared to other treatments thus making it a better treatment in terms soil fertility and productivity.

Key Words: C sequestration; Carbon dioxide; Methane; Microbial activity; Nitrous oxide; Tillage system; Urea fertilizer.

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CHAPTER 1

1. GENERAL INTRODUCTION

1.1 Background

The growth of the world's population during the forthcoming decades will prompt a need for more agricultural production. This large increase of food demand in Africa and South America, 127% and 67%, respectively will result in more greenhouse gases (GHGs) released into the atmosphere (Verge et al., 2007). In Africa, the fertilizer application rate may be expected to grow in order to achieve higher crop production, consequently GHG emissions will likely increase. In recent decades, crop production has increased as a result of expansion of arable land, increase in cropping intensity and improvement in crop yield (Verge et al., 2007). These activities will result in increased fertilizer usage that will indefinitely raise GHG emissions (Nawaz et al., 2017). Agriculture influences global warming due to related direct and indirect GHG emissions from carbon (C) and nitrogen (N) dynamics (Hellebrand et al., 2003). Intensive tillage, use of agricultural chemicals, livestock farming and the burning of crop residues are major farm activities that enhance GHG emissions (Nawaz et al., 2017). Thus, agriculture is an important contributor of methane (CH₄), nitrous oxide (N₂O) and carbon dioxide (CO₂) emissions into the atmosphere (Decock et al., 2015; Muñoz-Rojas et al., 2015). Though agriculture can benefit from a warmer climate in some parts of the world through longer growing seasons, it could also negatively affect many regions of the world through drought, flooding, pests and/or disease outbreaks (Verge et al., 2007). Therefore, GHG emissions represent an indirect economic loss to farmers in most parts of the world. Strategies to mitigate emissions of GHGs through agriculture are urgently needed.

Emissions of GHGs from agricultural activities depend on several soil and climatic factors including the soil organic carbon (SOC) pool, microbial biomass and activity, pH, texture, bulk density, temperature and moisture regimes, tillage, fertilizer management and land use type (Hüppi et al., 2015). Furthermore the choice of tillage technique plays an essential role in GHG emissions. Thus, converting from conventional till (CT) to no-till (NT) will improve soil properties such as structure, infiltration rate and nutrient retention thereby indirectly reducing GHG emissions. However, the net effects of these are difficult to quantify. Therefore the study

aimed to assess the effects of tillage on selected soil properties and GHG fluxes. Emission of N₂O under NT may be less (Mutegi et al., 2010) or more than from CT (Six et al., 2004), depending on soil type, cropping system and other site-specific conditions. It is well documented that long-term use of NT enhances SOC storage in the surface layers (Laudicina et al., 2015). Changing soil physicochemical properties as a result of tillage will alter GHG fluxes in the soil. Adoption of NT improves soil structure, infiltration rate and water holding capacity and reduces emissions of N₂O. This is because soil moisture content remains less than 62 % making it insufficient for denitrification to occur (Oorts et al., 2007). Ploughing increases oxidation of soil organic matter (SOM) and soil-crop residue contact, thereby exposing aggregate-protected SOM to microbial attack and enhancing emission of CO₂ in CT systems (Jacinthe & Lal, 2005). In that regard, NT may decrease CO₂ emission by reducing oxidation of SOM. However, with the adoption of NT, application of mineral fertilizers may play a significant role in GHG emissions.

The adoption of NT in South Africa has been very slow, especially by small-scale farmers who constitute the majority of the population. A study by Vilakazi et al., (2019) in South Africa, KwaZulu Natal, found that small-scale farmers resisted adopting new unfamiliar farming techniques such as NT, instead preferring the more familiar CT. Farmers, however acknowledge that there is a massive reduction in rainfall and increase in air temperature, which are enhanced by deforestation, bad agricultural practices and industrial pollution (Vilakazi et al., 2019). Agriculture and land use change emitted an estimated 4.9% of South Africa's GHG emissions in 2004 (Du Toit et al., 2013). According to Du Toit et al., (2013) agriculture is the third largest GHG contributor in South Africa after the energy and industrial sectors, which contribute 78.9% and 14.1% to GHG emissions, respectively. Otter et al., (2010) reported that in South Africa livestock farming contributed 98% of the agricultural sector's CH₄ emissions. Furthermore, in South Africa, Eastern Cape Province has the highest CH₄ emissions profile originating from extensive cattle ranching, followed by KwaZulu-Natal, Free State and the North West, reflecting to a large extent the population numbers of cattle (Du Toit et al., 2013). Improper irrigation techniques, such as excessive application of water, use of poor quality water and poor irrigation design, inefficient N fertilizer use (excessive application) and burning of biomass in South Africa increase GHG emissions (Pryor et al., 2017). In arid areas of KwaZulu Natal province, South Africa, greater CO₂ emissions from CT than NT was observed because of adverse climatic conditions such as high rainfall intensities. These in turn cause soil erosion and aggregate breakdown, making SOM more accessible to microbial attack, more

especially under CT compared to NT with soil cover (Chaplot et al., 2012). KwaZulu Natal province, where this study was done, is one of the wettest parts of the country, and hence has more intense and diverse cropping systems (sugarcane, maize and soybeans), so it is more likely to contribute a lot to GHG emissions. In a 20 year assessment (1991 to 2011) of rainfall in South Africa, Ratna et al., (2014) observed that Kwazulu-Natal (3.97 mm day^{-1}), followed by Mpumalanga (3.88 mm day^{-1}) and Gauteng (3.76 mm day^{-1}) received better rains than other provinces, and would be more likely to have great agricultural activities which emit GHGs. Therefore KwaZulu Natal with its unique climatic conditions of warm temperatures and higher rainfalls, soil types which ranges from shallow to very deep and well-drained as well as diverse cropping systems necessitated this study. The role of agriculture and land use in mitigating GHG emissions in KwaZulu Natal and South Africa as a whole has to be investigated so that policy makers can devise more informed mitigatory measures.

Fertilizer application also has an impact on GHG emissions of agricultural soils. In a study by Eggington & Smith, (1986), amendment with ammonium nitrate fertilizer led to significant increases in N_2O concentrations in soil air compared with unfertilized treatments, which lasted for several months or even longer than a year (Cates & Keeney, 1987). According to Heincke & Kaupenjohann, (1999), the extent of N_2O accumulation in soil is partly a function of the quantity of mineral N fertilizer applied. Therefore an ideal fertilizer application rate is required in order to decrease emission of gases through over application. Furthermore, fertilizer application and ploughing will intensify GHG emissions during summer because of higher temperature and water availability for decomposition. This is because decomposition and most soil biochemical reactions are enhanced with availability of moisture and warmer soil temperatures, therefore during winter, there will be lower GHG effluxes. According to Huang et al., (2015), a large percentage of applied N was lost to ammonia volatilization and nitrate leaching following high N input. Therefore, use of NT and appropriate application of N fertilizer at reasonable rates, should be promoted as this reduces N_2O emissions without affecting crop yield. Reduced GHG emissions will result from improved land management practices, better fertilizer management and other practices that will take advantage of existing organic matter supplies, such as retained crop residues (Verge et al., 2007). In this regard, crop residue retention together with appropriate fertilizer application under NT could mitigate GHGs emissions, while sustaining crop yields and reducing production costs. Such practices will also improve ecosystem viability and environmental sustainability.

Soil respiration and chemical decay processes produce GHGs in soils (Chapuis-Lardy et al., 2007). The rate of decomposition, mineralisation, nitrification and other biochemical processes are dependent on the type of management and physicochemical condition of the soil. Thus sufficient supply of substrates make it easy for microbes to function. Both crop residue and fertilizer application provide soil microbes with substrates (nutrient and energy sources) thereby enhancing microbial activity. It is therefore important to avoid deficient or excessive supply of substrates to maintain ecosystem stability. Low microbial activity will be detrimental to soil productivity, while high activity may lead to excessive effluxes of GHGs. According to Oertel et al., (2016), N₂O is formed in the soil where N sources are applied frequently or produced during organic matter decomposition. Again, N₂O emissions negatively correlate with the C/N-ratio of decomposing material (Pilegaard et al., 2006). While flooding of soil leads to GHG such as N₂O and CH₄ emissions as a result of fermentation of decomposing SOM (Verge et al., 2007). On the other hand, CO₂ emission from agricultural soils is mainly due to land use change, e.g., when forests are cleared for agricultural land development (Verge et al., 2007).

Soil cultivation and annual crop production often accelerate the conversion of organic C to CO₂ by microbes. According to Janušauskaite et al., (2013), soil biological properties such as microbial biomass and enzyme activity, may respond to soil disturbances over a shorter period of time compared to physical or chemical properties. As a consequence, the microbiological properties have been used as good indicators of soil quality because of their rapid response to changes in soil management. Again labile C can be used to check the response of tillage and fertilizer application to the soil as it is more sensitive to management. The availability of C has a salient effect on microbial activity which in turn with other factors such as pH, C: N and soil moisture determine phosphorus (P) availability. Furthermore, tillage, N fertilizer application and soil depth, determine both distribution and type of the P pool in the soil. According to Romanya et al., (2017) soil P can occur in organic and inorganic forms, which can be strongly retained or mineralized in soil. Soil organic P can be transformed to its inorganic form via mineralization by root or microbial-released phosphatase enzymes, while inorganic P can be immobilized to organic forms by microbes (Chang, 2007). O'Halloran, (1993), reported that soil inorganic P increased near the bottom of the plough layer presumably due to the incorporation of crop residues. While Weill et al., (1990) observed that increase in the concentration of inorganic P in surface soil layers was due to fertilizer placement and lack of soil mixing under NT.

Tillage has influence on biochemical processes occurring in soil in the short-term. Research by Fenster & Peterson, (1979) showed that lower soil nitrate levels with NT during the first 8 to 10 years after changing from CT systems was due to continuous immobilization of N in residues of NT. The initial changes in soil microbial activity and N cycling may account, in part, to lower than expected initial crop yields with NT systems (Broder et al., 1984). However a study by Doran et al., (1998), showed that soil microbial populations and enzyme activity were greater with NT, making the amount of potentially mineralizable N in the surface 7.5 cm of NT soils 35% greater than in CT, thereby indicating greater conservation of N in organic forms. Increased soil microbial populations, stimulated by greater organic matter and water content with reduced tillage, could however compete with the crop for available N. There are few studies that have been conducted in South Africa, particularly in KwaZulu Natal on the emission of GHGs from arable lands under dryland agriculture. Thus the aim of this study was to assess the effectiveness of conservation tillage to minimise GHG emissions and improve soil productivity compared with CT under N fertilizer management. This was done under rain-fed maize mono-cropping during summer, with winter fallowing. Treatments included conventional annual tillage (CT-ANNUAL), conventional tillage every 5th year (CT-Y5), and no-till (NT) with various urea fertilizer application rates at different soil depths. The study looked at the impact of tillage and N fertilization on selected soil biological and physicochemical properties as well as effluxes of GHGs in soil. The null hypothesis of the study was that conservation tillage would increase fluxes of GHGs compared to CT-ANNUAL.

Specific objectives

1. To assess selected soil physicochemical properties under different tillage techniques and fertilizer application rates.
2. To assess the impact of tillage and N fertilizer application on soil C sequestering.
3. To assess the effect of tillage and urea fertilizer application on N and P mineralisation patterns in the soil.
4. To assess soil enzymes activity under different tillage techniques and urea fertilizer application rates.
5. To compare emissions of GHGs between CT and NT at different N fertilizer application rates.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The impact of human activity on escalating GHG emissions, global warming and changes in global climate patterns is an important 21st century issue (Verge et al., 2006). Anthropogenic GHG emissions have increased since the pre-industrial era, driven largely by economic and population growth (Mangalassery et al., 2014). This has led to an increase in atmospheric concentrations of CO₂, CH₄ and N₂O (Mangalassery et al., 2014). According to Paustian et al., (2016), agriculture accounts for 10–12% of total anthropogenic GHGs emissions globally, with CO₂ emissions alone estimated to be 5.4–6.1 Gt yr⁻¹ in 2012. The main drivers of GHG emissions in soils are root respiration, chemical decay processes, as well as heterotrophic respiration of soil fauna and microbes (Ortel et al., 2016). Emission flux rates largely depend on soil water content, temperature, nutrient availability and pH (Ludwig et al., 2001), as well as land-cover-related parameters. Dutta et al., (2015) elucidated that the emission of GHGs from agricultural activities depends on several soil and climatic factors including amount and time of precipitation, SOC pool, texture, bulk density, temperature and moisture regimes, and tillage and fertilizer management. Therefore adoption of proper land management systems, which minimise emissions, will be a good step to mitigate GHG emissions.

Conservation tillage is among many options to reduce GHG emissions from agricultural soils (Mangalassery et al., 2014). However, its effects are sometimes contradictory and depend on the soil type, climate and management history (Prasad & Power, 1991). NT has been found to sometimes increase N₂O loss from soils compared with CT (Mackenzie et al., 1998). This is because N₂O (like CH₄) is produced under reducing conditions of waterlogged or poorly aerated soils. Therefore increased potential of N₂O emissions from NT soils is attributed to wetter and denser soil conditions found under this regime. Mangalassery et al., (2014) also reported 21 to 86% higher N₂O flux in NT soils compared to tilled soils. Although NT may sometimes have negative impacts, overall it helps in sustaining soil quality and should be promoted. This is because conservation tillage protects soil against erosion and degradation,

creates greater aggregate stability, increases soil organic matter (SOM) content, hence enhances C sequestration and eventually mitigates GHG emissions (Mangalassery et al., 2014).

Tilling the soil breaks down aggregates, reduces compaction and allows roots to penetrate easily. It modifies the distribution of organic matter (OM) in the soil profile. In CT, OM is distributed down the profile unlike in NT where crop residues and OM are concentrated near the soil surface (Arshad et al., 1990). Therefore unlike NT which has higher nutrient retention in the top layer, CT will generally have nutrients distributed down the profile. Logsdon et al., (1990) reported greater bulk density, soil penetration resistance and lower total porosity in NT compared with CT in the top layer. While organic material buried after ploughing is responsible for the higher rate of soil microbial activity in deeper soil layers of CT (Angers et al., 1993). Thus, Kandeler & Böhm, (1996) observed that microbial biomass and activity were greater in the surface of NT than CT soil, while the reverse was observed in deeper soil layers of around 20-30 cm soil depth. Cereti & Rossini, (1995) also found that soils under NT generally contained greater concentrations of organic C and microbial biomass, especially in the upper layer. Aerobic conditions and distribution of nutrients down the profile under CT will increase microbial biomass than under NT, which may be attributed to the availability of substrate and oxygen in the soil. A study by Kandeler et al., (1998), showed that the microbial biomass C turnover of NT was significantly lower (0.99 year^{-1}) than that of CT (1.5 year^{-1}) in the surface layer due to limited oxygen for aerobic reactions. According to Kladivko, (2001) there was higher urease activity under CT than NT at 10-20 cm depth, due to a flush of microbial activity directly after tillage and residue incorporation in CT as a result of increased substrate and oxygen availability. Hence CT can lead to soil microbial community structures dominated by aerobes, while NT increases microbial population and activity (Balota et al., 2003). The aerobic microorganisms under CT will then increase OM decomposition thus releasing GHGs. According to Mangalassery et al., (2014), CT produced 20% greater net global warming than NT soil, indicating a potential for NT systems to mitigate GHG emissions.

With all the benefits that NT offers to the soil, it is yet to be well adopted and implemented on a global scale especially by communal farmers. According to Janušauskaite et al., (2013), the reason for low adoption of NT is that it often results in lower crop yields than CT. This is caused by soil compaction, germination problems, and higher weed and pest incidence under NT (Janušauskaite et al., 2013). Higher soil moisture in NT soils results in greater denitrification rates as compared with CT (Aulakh et al., 1992), which could also result in loss

of plant available N. On the other hand, Janušauskaite et al., (2013) discovered that long-term NT increased organic C content, positively affecting not only soil structure, but also microbiota activity. Lal & Kimble, (1997) emphasised that NT improves soil C and N sequestration and accentuates soil biodiversity. There is therefore a need to identify the best management options employed by farmers, land users and environmentalist to reduce emission of GHGs and ensure ecosystem sustainability. The aim of this review was to assess the impact of conservation tillage on GHG emissions and soil physicochemical properties, C, P and N pools as well as enzyme activity in comparison with CT.

2.2 Greenhouse gases fluxes

GHGs are gaseous components of the atmosphere that absorb energy reflected from the earth's surface as infrared radiation (de Klein et al., 2008). They are critical for regulating the earth's surface temperatures, as without them, the average temperature would be -6 °C, instead of 15 °C (Steinfeld et al., 2006). The main anthropogenic GHGs are CO₂, CH₄ and N₂O. According to de Klein et al., (2008) each of these gases has a different global warming potential (GWP), which is based on its ability to absorb solar energy and on its half-life. Solomon et al., (2007) reported the GWP for CO₂, CH₄ and N₂O to be 1, 25 and 298 respectively, based on GWP per molecule of CO₂.

2.2.1 Carbon dioxide effluxes

Soils provide the largest terrestrial store for C in litter and soil humic C fractions (Lal et al., 1998). In several areas, agricultural land offers the possibility to sequester CO₂ as SOM (Six et al., 2004). Cultivation and annual crop production often accelerate the conversion of soil C to CO₂ by microbes. According to Houghton et al., (1983), two major agricultural sources that have contributed to the rise in CO₂ are SOM decomposition and plant biomass burning. After soil has been cultivated for a few decades, the loss of soil C usually slows down or ceases completely, and its level becomes stable again but lower (Hutchinson et al., 2007).

2.2.2 Nitrous Oxide effluxes

N₂O emissions from agriculture are estimated to account for more than 75% of the total global anthropogenic emission (Isermann, 1994). Most N₂O is produced in soils as an intermediate during nitrification and denitrification (Hutchinson & Davidson, 1993). Emissions of N₂O in agriculture are predominantly from soils amended with N-rich amendments e.g fertilizers, manure, and compost (U.S. Environmental Protection Agency, 2010). In general, N₂O emissions are directly related to the type, quantity and method of fertilizer placement, as well as other factors such as soil type, tillage method and weather patterns (U.S. Environmental Protection Agency, 2010). Though weather patterns are difficult to control, tillage can be controlled, therefore tillage types that emit less GHGs must be employed (Beauchamp, 1997). N fertilizer inputs are required in order to maximize crop yields (Murungu & Madanzi, 2010), but fertilizer application generally results in leaching, GHG emission and eutrophication. However, de Maria et al., (1999) reported that application of N-based fertilizers increases SOM, particularly on lands that have already experienced significant OM loss as a result of cultivation. However, the appropriate fertilizer application rate and tillage type must be used to avoid GHG emissions.

2.2.3 Methane effluxes

Emissions of CH₄ from agriculture are linked to bacterial processes in anaerobic conditions created by flooded soils, and from enteric fermentation that occurs in the digestive tracts of ruminant livestock (cattle, sheep, and goats). CH₄ emissions also occur during decomposition of animal manure in uncovered lagoons and from crop residues under very wet field conditions (Greenhouse Gas Working Group, 2010). According to Bousquet et al., (2006) soils are a major source of CH₄, through anaerobic activity of methanogenic bacteria under waterlogged conditions. However, well drained soils, especially forest soils can provide an important CH₄ sink through the activity of methanotrophic bacteria (Dalal et al., 2008), contributing as much as 6% to the overall global atmospheric CH₄ sink. A review by Bodelier & Laanbroek (2004), showed that N can stimulate both emissions and consumption of CH₄ in soil, with mineral-N being a prerequisite for CH₄ consumption. Consumption and emission by soil balances CH₄ concentrations in the atmosphere. In an area with soils having reduced CH₄ sink ability, the concentration of CH₄ will increase in the atmosphere. Therefore employing conservation tillage such as NT can assist in reducing emission of CH₄ by increasing soil's sinking ability through improved soil properties such as aggregation and organic C. Tillage disturbs the ecological niche that allows gas sinking, restricting methanotrophic bacteria, and accentuating CH₄

emissions (Hutsch, 1998). According to Nawaz et al., (2017), the highest flux of CH₄ was recorded with CT than NT, with the reduction in CH₄ flux under NT being as much as 90.5%.

The tillage type plays an integral role in emission and consumption of CH₄, however other factors such as moisture content and fertilizer type also contributes to CH₄ dynamics. An increase in CH₄ emission by soil with less moisture and N added were noted by Mosier et al., (2003). This was attributed to CH₄ emission mediated by ammonium-oxidizing bacteria which reduces CH₄ oxidation in the soil. Knief et al., (2005), who reported high CH₄ emissions from N fertilized soils, argued that NH₄⁺-N inhibits CH₄ consumption. This is because NH₄⁺-N and CH₄ compete for CH₄-monooxygenase, the enzyme which catalyses the first step in the oxidation of CH₄ (Bédard & Knowles, 1989). In addition, high soil water content can limit O₂ and CH₄ diffusion in the soil, thus, reducing CH₄ consumption.

2.3 Sources of greenhouse gases

2.3.1 Environmental sources of GHGs

The main natural sources of GHGs are soils, wetlands, lightning, grasslands, forests and oceans (IPCC, 1990). However climatic conditions, land use change and management may alter emissions. Biochemical processes such as enzyme activity and nitrification occurring in the soil enhance warming of the earth as a result of increased emissions of GHGs (Smith et al., 2003).

2.3.1.1 Soils

Soil, depending with type and its state, acts as a sink and/or source of GHGs. A study by Martikainen et al., (2002) found that effluxes of GHGs differ with soil type. The aeration status of soil determines the direction and magnitude of gas flux. Hütsch, (2001) observed that sandy soil had lower oxidation rate of organic material similar to that of forest soils hence poor effluxes of CH₄, while peat soil with a high water table had higher CH₄ emissions. According to Smith et al., (2003), soil, particularly in its undisturbed natural state acts as a sink for CH₄ because CH₄ can be oxidized to CO₂. It is however rare for soil to be undisturbed as it provides food for survival. However, the size of gas effluxes between the soil and the atmosphere depends heavily on soil physical, chemical and biological factors. Soil temperature and

moisture directly affect production and consumption of GHGs because they influence soil microbial processes and root respiration (Smith et al., 2003). Moisture content and soil temperature can be altered by the method of tillage as a result of disturbing or sustaining soil aggregate stability and soil cover. Tilling the soil increases gas diffusivity from the soil to the atmosphere because of open soil pores thus increases effluxes.

There is high concentration of CO₂ in soil compared to the atmosphere. It is produced by root, microbial and faunal respiration, while fixed by vegetation; giving a positive or negative net effect (Martikainen et al., 2002). According to Prather et al., (1995) about 30 teragrams of atmospheric CH₄ per year is oxidized to CO₂ by aerobic soil bacteria which are adapted to living on this very small concentration of substrate. Oxidation is most rapid in coarse-textured forest soils with well-developed soil structure and a surface organic layer through which gases can readily diffuse (Smith et al., 2000). However, Brumme & Borken (1999) showed that the inhibition of litter decomposition in acid conditions in some forest soils can substantially reduce the entry of atmospheric CH₄ into the soil. CH₄ is consumed by bacteria in the top layer of agricultural soils, usually resulting in its flux from the atmosphere to the soil. However, compared to pristine ecosystems, the capacity of agricultural soils to consume CH₄ is lowered by N fertilizers, especially NH₄⁺ fertilizers because NH₄⁺ has an inhibitory effect on CH₄ oxidation (Hütsch, 2001). Implementing better, less emitting methods of tillage such as NT must be emphasized to mitigate GHG emissions from agricultural activities. In a study by Pipatti, (2001) cultivation of organic soils caused most of the reported agricultural CO₂ emissions, meaning NT should be employed as a solution to mitigate emissions.

2.3.1.2 Forestry and pasture

Conversion of forests and pastures to cropland leads to a paradigm shift of natural ecosystems. Such land cover change often alters C storage; e.g. converting arable cropland to grassland results in the accrual of soil C owing to lower soil disturbance and reduced C removal in harvested products. Compared to cultivated lands, pastures may also have reduced N₂O emissions from lower N inputs and higher rates of CH₄ oxidation (Paustian et al., 2004). According to Smith et al., (2008) planting trees can reduce emissions of GHGs. However, tropical forest soils because of high denitrification due to wet soil and accumulation of organic material throughout the year are the largest single natural source of N₂O to the atmosphere, (Smith & Conen, 2004). Therefore it is important to know the effect of land use change on

emissions from these ecosystems. Several comparisons of N₂O emissions from forest and pastures have been carried out with widely varying results. Verchot et al., (1999) recorded emissions from pastures to be only one-eighth to one-third of those from primary forest. While Melillo et al., (2001), found pasture emissions to be two-and-a half times those from the forest soils for the first two years, but decreasing to below those in forest in pastures three or more years old. A study by Robertson et al., (2000), showed that poplar and alfalfa systems added 32 to 44 g C m⁻² year⁻¹ to the soil C pool, similar to that added by the NT system. Therefore forests and pastures can be utilised to mitigate GHG emissions global. Fertile soils under forests would store high concentrations of soil C which is attributed to leaves that are always added to the soil surface. Boreal forests (covering 22.6% of terrestrial area) constitute 10–13% of the organic C stocks in the world (McGuire et al., 1997), with more than half of this C stock stored in soil (McGuire et al., 1997).

2.3.2 Agricultural activities causing GHGs emission

At the farm level, the dominant GHGs are N₂O from soil and livestock (manure, urine and applications of N fertilisers) and CH₄ from ruminant digestion, rice cultivation and anaerobic respiration (Garnett, 2011). CO₂ emissions arising from fossil fuel combustion, manufacture of synthetic fertilisers and burning of biomass also significantly contribute (Garnett, 2011). Agricultural activities control the physicochemical properties of soil. Any change in these would affect the rate and extent of microbial processes, which in turn control the stabilization of C and N in soil. Farmed organic soils are a large source of both CO₂ and N₂O due to net degradation (oxidation) of organic material (Eriksson, 1991). According to Kasimir-Klmedtsson et al., (1997) cultivation of peat soils increases soil aeration and reverses the C flux favouring net CO₂ emission into the atmosphere. Tillage, legume cropping, crop residue management, the type and rate of mineral N fertilizer application all contribute to N₂O emission (Gregorich et al., 2005). An estimated 45% of agricultural N₂O emission in Canada originates from collection, storage, and application of animal manure (Desjardins & Riznek, 2000). In cool temperate regions, N₂O comprises the majority of GHG emissions associated with crop production (Robertson et al., 2000). Agricultural activities may influence CH₄ fluxes by affecting either its consumption in aerated soils or emission in waterlogged areas. Biological CH₄ production in anaerobic environments (e.g. enteric fermentation in ruminant animals, flooded rice fields, and animal waste processing) is the principle source of CH₄ from agriculture

(Flessa et al., 2002). Well aerated arable land is usually a sink for atmospheric CH₄, because soil methanotrophs use CH₄ as a source of energy and C (Topp & Pattey, 1997).

2.3.3 Manure management

Animal manure can either be stored wet (slurry) or dry (farm yard manure). Generally, intensive livestock systems use liquid manure management due to the large volumes of manure produced and the method of collection (Reid et al., 2004). According to Linquist et al., (2012) farmyard manure (FYM) is an important nutrient source for many plants. Thus with application of manure, net CH₄ uptake by soil is reduced relative to un-manured soil (Rochette & Co^{te}, 2000). Emission rates are mostly influenced by the type of manure, handling and storage methods. They also depend on the animal's diet, with higher energy feeds producing manure with more volatile solids that may emit more GHGs depending on surrounding environmental conditions (Bellarby et al., 2008). Emissions from manure storage are affected by the type of waste management and the storage duration (Woodbury & Hashimoto, 1993). Thus, substantial CH₄ emission may occur when manure decomposes in an anaerobic environment. CH₄ emissions occur mainly when the manure is managed in liquid forms (lagoon or holding tanks) or remains wet (Bellarby et al., 2008). While manure deposited on fields and pastures or otherwise handled in dry form do not produce significant amounts of CH₄ (Reid et al., 2004). Chen et al., (2011) showed that composting FYM lead to a 75% reduction in CH₄ emissions relative to un-composted manure. Hence solid manure may be a potential mitigation strategy to GHGs emission from croplands (Desjardins et al., 2005). This option can be improved by manure pre-treatment through composting (Petersen, 1999), although the compost itself may be an important source of GHGs (Hao et al., 2001).

Studies that have examined N₂O emission during storage of animal wastes indicated that in contrast to CH₄ emissions, release of N₂O tends to increase with increasing manure aeration (Flessa et al., 2002). Heinemeyer et al., (1997) observed N₂O emissions from slurry storage that ranged between 0.01 to 0.08% of the slurry N. They concluded that losses of N₂O from dung heaps were about 0.1-0.8% of the manure N (Heinemeyer et al., 1997). Though liquid manure can be easily stored and transported than dry manure, it increases the emission rate of GHGs. This is because it contains labile soluble organic C that can stimulate N₂O production where C availability limits denitrification (Gregorich et al., 2005). According to Gregorich et

al., (2005) application of liquid manure results in higher soil moisture, lower oxygen availability and a relatively large amount of labile C, all of which promote denitrification. Thus in soils with initial low C content, liquid manure has often resulted in greater N₂O emission than mineral fertilizer (Rochette et al., 2000). A better understanding of how storing, processing and application of manure can affect GHG emissions is needed in order to avoid mitigating a certain gas at the expense of another.

2.4 Impact of climatic conditions on the emission of greenhouse gases

Climatic conditions are responsible for biochemical reactions in the soil, therefore have a huge role on the fluxes of GHGs. Emissions of CO₂ and N₂O for example are significantly influenced by temperature and rainfall (Li et al., 2010). This is because soil temperature and moisture content directly affect production and consumption of GHGs, through influencing microbial breakdown of organic compounds and root respiration (Smith et al., 2003). Gas diffusivity, which depends on the air-filled porosity (and thus varies inversely with water content), also controls the movement of the gases to and from the atmosphere. While moisture content affects soil aeration, with saturated soil inhibiting aerobic microbial activity and root respiration due to poor aeration (Smith et al., 2003). According to Martikainen et al., (2002) several climatic conditions affect effluxes of gases from agricultural soils, however, there is lack of data on the magnitude of these. Seasonal climate patterns also alter effluxes of GHGs due to temperature and precipitation variabilities. As such, climate policies are needed to device strategies on GHG emissions.

2.4.1 Temperature variability on soil GHGs emission.

An increase in soil temperature leads to higher respiration rates as a positive feedback of increased microbial metabolism (Ortel et al., 2016). This leads to decreasing O₂ concentrations in the soil, which in turn reduces CH₄ oxidation and nitrification of NH₄⁺ thereby enhancing CH₄ and N₂O emissions (Butterbach-Bahl et al., 2013). Again, CO₂ emissions increase exponentially with increase in soil temperature (Ludwig et al., 2001; Tang et al., 2003). Several studies have shown increased production of CO₂ and CH₄ when temperature was raised (Liikanen et al., 2003). Stadmark & Leonardson, (2005) found that temperature and NO₃⁻ availability increased the amount of CH₄ produced as they enhance the functionality of methanotrophic (CH₄ consumer/oxidizer) and methanogens (CH₄ producer) bacteria. These

results agree with a field study by Stadmark & Leonardson, (2005) where CH₄ emissions increased remarkably when temperature exceeded 15 °C. The release of CO₂ from SOM by heterotrophic and autotrophic root respiration, also generally increases exponentially with temperature (Smith et al., 2003). However high temperature, from 27 °C and above, may reduce soil moisture content through evaporation making it impossible for root and microbial respiration to continue thus reducing emissions. In this regard, the positive effects of temperature may be overlain by soil water stress, since water is needed as a transport medium for nutrients required by microbes (Fowler et al., 2009).

N₂O emissions increase with soil temperature up to about 37 °C; thereafter denitrification decreases because denitrifying bacteria will be denatured (Abdalla et al., 2009). However, Huang et al., (2015) observed that N₂O flux rates were negatively correlated with air temperature, with peak N₂O emissions occurring at lower air temperatures of 3.7–8.4 °C while lower N₂O emissions were observed with higher air temperatures (14.0–25.8 °C). There was evidence that at low soil temperatures N₂O was produced not only from the nitrate pool added to the soil but also from the mineralised N (Martikainen et al., 2002). Soil freezing-thawing generally enhances N₂O production but the mechanism for this is still poorly understood (Martikainen, 2002). A study by Martikainen et al., (2002), revealed N₂O production at temperatures as low as -6 °C. While Martikainen et al., (2002) observed that N₂O production was higher when increasing soil temperature from freezing (i.e. during soil thawing), with peak N₂O production close to temperatures of 0 °C.

2.4.2 Effect of precipitation on the fluxes of GHGs.

The amount and intensity of rainfall has a direct impact on the functioning of the soil. Fluxes of GHGs are controlled by soil pores hence water saturated soil tends to have limited air movement. According to Livesley et al., (2008) there was a positive relationship between soil water filled pores and N₂O flux in soil, although the intensity and longevity of rainfall response flux differed between months. This agreed with the results by Jacinthe & Lal, (2004) where the frequency of soil wet-and-dry cycles in response to rainfall frequency was more critical to N₂O flux in unfertilised soils than temperature, surface soil conditions or vegetation. CH₄ uptake in soils also decreases with increasing precipitation (Drewer et al., 2012). This is because precipitation increases soil moisture content, filling up pores with water therefore making it impossible for oxidizing methanotrophic bacteria to function. Only anaerobic microbes, such

as methanogenic bacteria, can function under waterlogged conditions. In a study by Kirschbaum (2006), there was no consistent relationship between soil CO₂ flux and temperature, but a positive relationship ($r > 0.5$) between soil CO₂ flux and soil water. Again tillage affect gas effluxes, with higher soil moisture as a result of NT causing higher N₂O emissions. Unfortunately higher N₂O emissions under NT can sometimes not be balanced by higher C sequestration and CH₄-uptake rates (Li et al., 2005).

Excess emission of N₂O after rainfall is normally attributed to high denitrification because of soil water saturation. This is because the water filled pores tend to stimulate microbial denitrifiers (Davidson, 1991). In addition, soils dominated by fine pores promote emission of CH₄ and N₂O produced under anaerobic conditions (Gu et al., 2013). On the other hand, Dilustro et al., (2005) observed higher CO₂ emissions in fine textured soils compared to sandy soils during warm dry periods. Sandy soils suppress emission because of excessive draining whereas finer soil texture buffer soil moisture and enhances CO₂ efflux even during warm dry periods (Dilustro et al., 2005). Sponseller, (2007) observed that CO₂ emissions increased within minutes or hours after the onset of precipitation before returning to normal levels in a few days.

2.4.3 Effect of seasonal changes on effluxes of GHGs.

In a study by Jin et al., (2009), there was a clear seasonal pattern of CO₂ emissions, with the summer daily CO₂ effluxes higher than those in winter. Temperature variation and soil moisture distribution, which influence enzyme activity are aligned with seasonal changes. Winter is associated with low soil temperatures and moisture whereas the opposite is true in summer. Thus the summer season is associated with higher enzyme activity than winter (Kladivko, 2001). Field experiments by Johansson et al., (2004) also showed seasonal variation in GHG emissions, which may be caused by the amount and quality of organic material (Stadmark & Leonardson, 2007). It was observed that there was high amount of fresh organic material with low C: N ratio (high quality) in summer which increased mineralization and effluxes of GHGs, whereas winter was characterised by low quantity humified material with higher C: N ratio. Thus, winter CO₂ emissions are considered less important for the annual emission budget since root and soil respiration is relatively low when compared to summer (Groffman et al., 2006). During freeze-thaw cycles, additional nutrients are released for microbial metabolism through disaggregation of soil particles (Christensen & Christensen, 1991), thereby inducing effluxes of GHGs.

Husted, (1994) discovered that CH₄ emissions from pig and cattle slurry were low during winter and high during summer (Figures 2.1 & 2.2); with CH₄ emission rates from cattle slurry ranging from 17.5 to 34.5 g m⁻³ d⁻¹ in summer and 0.02 to 1.4 g m⁻³ d⁻¹ in winter (Figure 2.2). Significant CH₄ emission during the winter months was not expected, but due to heat from the storage tanks, slurry temperature never dropped below 4 °C even when atmospheric air temperatures went down to -4.2 °C, (Husted, 1994). According to Husted, (1994), CH₄ emission from pig slurry did not differ across seasons during most months except January, April, May and December because of a surface crust that formed on top of the slurry, which reduced diffusivity of air (Figure 2.1). In a study by Smith et al., (2007) they deduced that surface crusting reduces exposure of liquid slurry and was effective in decreasing emissions. Furthermore, Smith et al., (2007) explained that slurry solids content (dry matter content >1%), continuous undisturbing and cumulative evaporation of water from the surface of the slurry had an important role on the formation of the crust. Summer months (in northern hemisphere) in Figure 2.1 experienced higher slurry crusting due to increased cumulative evaporation because of higher temperatures. CH₄ emissions in January and December, in which slurry was without a surface crust were relatively high compared with November, February & March periods with low porosity and an intact surface crust (Figure 2.1). The interaction between slurry temperature and crust formation was not clear for both pig slurry (Fig 2.1) and cattle slurry (Fig 2.2). However, it was observed that surface crusts were dry and compact at low slurry temperatures of November, February and March but wet and porous during summer months (June, July, August and September). Again summer months with average slurry temperature of 15-20 °C and air temperature of 12.5-18 °C had the highest crusting and emissions showing the salient effect of temperature on emission. Crust permeability to CH₄ rose with increasing crust porosity, explaining the reduced effect of crusting at high slurry temperatures (Husted, 1994). Figure 2.2 shows that cattle slurry did not form a surface crust in May and from July to December; this may be attributed to precipitation which washed away the crust, leading to higher CH₄ emission rates in these months. Temperature of both air and slurry resulted in seasonal differences in gaseous emission with both pig and cattle slurry having peak emissions at their highest temperatures.

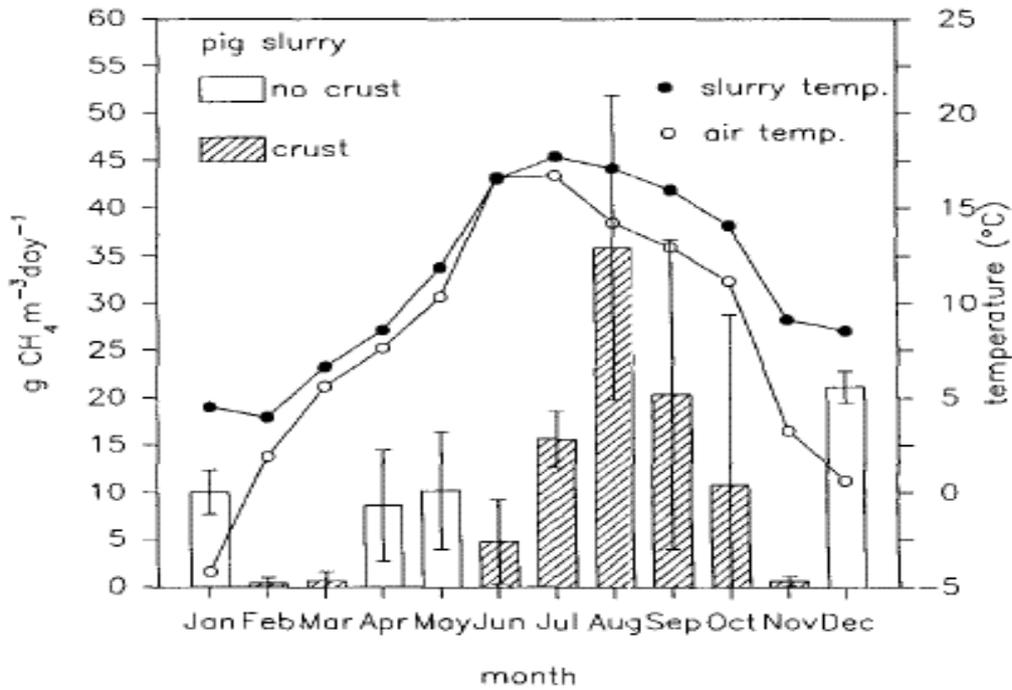


Figure 2. 1: Seasonal variation of CH₄ emission rates with pig slurry temperature and natural crust on slurry surface (Husted, 1994).

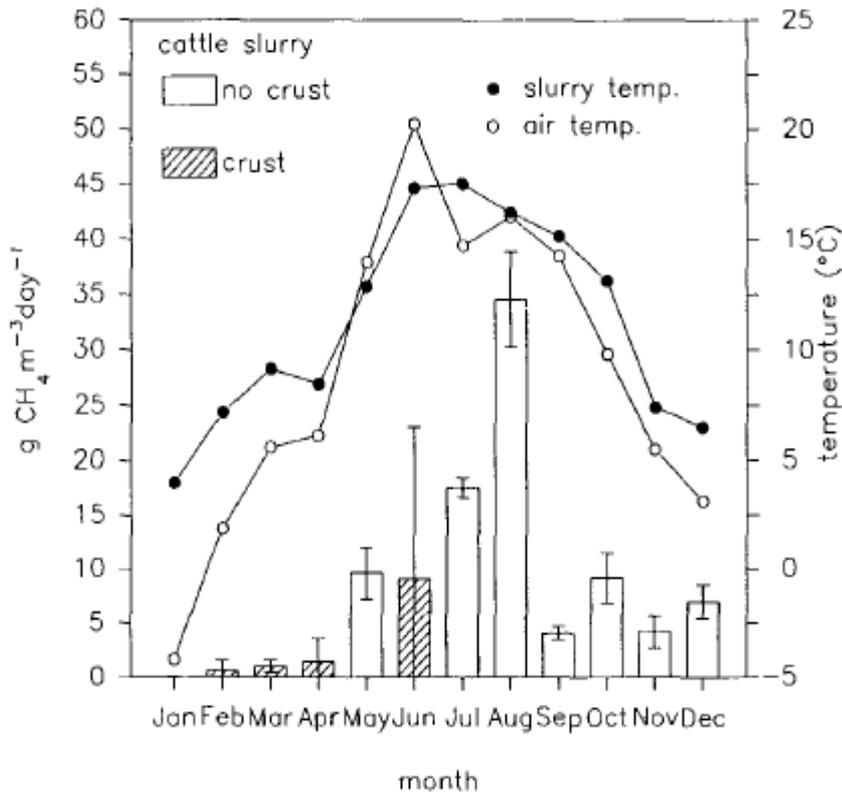


Figure 2. 2: Seasonal variation of CH₄ emission rates with cattle slurry temperature and natural crust on slurry surface (Husted, 1994).

2.5. Effect of soil physicochemical properties on the emission of GHGs

The size of GHG effluxes between the soil and atmosphere also depends on soil physicochemical factors (Smith et al., 2003). According to Konnerup et al., (2014), soils with higher OM content have higher CH₄ uptake (1.49 mol CH₄ m⁻² h⁻¹) because of higher methanotrophic bacteria populations due to availability of substrate than those with lower OM content (1.0 mol CH₄ m⁻² h⁻¹) which have less methanotrophic bacteria populations. Most researchers have correlated N₂O fluxes from the soil surface with biological, chemical and physical soil parameters (Clemens et al., 1997). High soil moisture combined with an unfavourable soil structure for gas transport can cause N₂O to be entrapped for several weeks especially in watered puddled soils or those with a hardpan (Heincke & Kaupenjohann, 1999). Different tillage practices also alter soil properties thereby affecting effluxes of GHGs. Thus, Nawaz et al., (2017) concluded that adoption of long-term NT reduces GHG (CO₂, CH₄, N₂O) emissions by increasing soil organic C, moisture and porosity.

2.5.1 Soil pH

Soil pH and its variability affects effluxes of GHGs in the soil. Although biological processes dominate N₂O production in most environments, chemo-denitrification, an abiotic process where inorganic N species are reduced to gaseous species has been reported in soils with high concentrations of Fe (II) or humic acid extracts with low pH by means of coupled oxidation (Samarkin et al., 2010). It appears that nitrate reduction is catalyzed by Fe (III)-rich precipitates formed during dissolution of the Fe (II). The reaction between nitrate and iron may either proceed as an iron dissolution process subsequently followed by an independent nitrate reduction reaction with Fe²⁺ in solution at low pH (Postma, 1990). According to Ortel et al., (2016) microbial activity, which dominate N₂O production, is influenced by soil pH. Increased pH, improved nutrient availability, and additional C may increase microbial activity and the release of GHGs (Saarnio, 2016). The optimal pH for methanogenesis lies between pH 4 and 7 (Dalal et al., 2008). Cuhel et al., (2010) observed that CO₂ emissions were observed to be highest at neutral pH where oxidation reaction is prominent. N₂O emissions decrease under acidic soil conditions because nitrification increases with higher pH, since higher pH shifts the equilibrium between NH₃ and NH₄⁺ more toward NH₃, thus increasing NH₃ availability (Nugroho et al., 2007). NH₃ is the substrate for ammonium oxidizing bacteria which enhances nitrification thus emitting N₂O in the process (Burton & Prosser, 2001). However, in a study

by Pilegaard et al., (2006) there was no significant correlation between N₂O emissions and pH. Biochars with pH reduced to 5.6 had higher N₂O emissions than when not adjusted (Cayuela et al., 2013). When pH was not adjusted (remained higher than pH 5.6), however, most biochars decrease the total amount of N₂O emitted by 41-72 % (Cayuela et al., 2013). On the other hand, However, Burford & Bremner, (1975) found out that N₂O mitigation was also positively correlated with soil dissolved organic C, and less strongly with soil pH. According to Cayuela et al., (2013) a shift in soil pH (either higher or lower) does not mitigate N₂O emissions because change in soil pH does not by itself induce N₂O reductions. These finding were in agreement with those by Yanai et al., (2007), who after increasing soil pH with ash applications did not observe any reductions in N₂O emissions.

2.5.2 Soil organic C and N

Alleviating nutrient deficiencies by fertilizer or organic amendments increases plant litter returns and hence, soil C storage (Schnabel et al., 2001). Adding N, however, may stimulate N₂O emissions (Conant et al., 2005) thereby offsetting some of the benefits. Increasing crop residue incorporation elevates SOC accumulation rates due to direct addition of OM. An increase in the initial SOC from 1 to 2% elevated SOC decomposition rate, which led to more CO₂ emitted (Li et al., 2009). Conversely, a decrease in the initial SOC content from 1 to 0.25% converted the soil from a source (231 kg C ha⁻¹ y⁻¹) to a sink (-1104 kg C ha⁻¹ y⁻¹) of atmospheric CO₂ (Li et al., 2009). The environmental control of SOM mineralisation is gaining attention because of its importance to global C cycling (Kirschbaum, 2006). However more can be done by sustaining OM in the soil thereby increasing both soil organic C and N while simultaneously reducing GHGs emissions.

Huang et al., (2015) found that it was necessary to add N by straw and manure application to reduce the use of mineral N fertilizer in NT systems. According to Huang et al., (2015) the average N₂O emission under NT was significantly greater than that of CT, likely due to organic C stimulating denitrification. Research suggests that more N₂O is emitted from organic sources than inorganic ones, but there is little evidence to prove this (Frenay, 1997). The use of inorganic N-fertilizers has been alluded by Mulvaney et al., (2009) to loss of soil organic C through increased respiration and GHG emissions. In addition, this loss would also decrease soil productivity and agronomic efficiency of applied-N, thereby shifting the N and C balance in favour of GHG emissions (Mapanda et al., 2011). According to Li et al., (2009) results from

a sensitivity test indicated that among the tested factors, SOC content showed the greatest impact on N₂O fluxes. Thus when SOC increased from 0.25% to 2%, the annual N₂O emission rate increased from <1 to 22 kg N ha⁻¹ yr⁻¹ (Li et al., 2009).

2.5.3 C: N ratio

Addition of organic material such as straw, plant litter and compost to soil helps in C accumulation and in reducing emissions of GHGs especially CO₂. However, increase of organic material might increase emission of GHGs depending on the rate of decomposition of that material, influenced by its C: N ratio. Several studies have reported that addition of composted straw reduced CH₄ emissions relative to fresh straw (Yagi & Minami, 1990). Corton et al., (2000) attributed this to lower C: N ratio in the composted straw (6–10) than in fresh straw (25–45). However, an assessment of the effect of composted materials must also consider the GHG generated during the composting process which can be highly variable depending upon the material and how it is composted (Brown et al., 2008). N₂O flux in temperate forest soils has also been shown to positively correlate with gross nitrification rates and negatively to soil or litter C: N ratios (Ambus et al., 2006). Thus in forest systems, there were high C: N ratios of between 20 and 32, inducing low net nitrification rates, whereas in pasture systems lower C: N ratio of 14 induced higher net nitrification and greater N₂O flux (Livesley et al., 2008).

According to Stevenson, (1994) cereal plants (wheat, rice and rye) with higher lignin contents (16–24%) than maize and millet (11–16%), had slow OM decomposition rates which reduce GHG emissions. A study by Cayuela et al., (2013) discovered that most biochars used immobilized NO₃⁻, since their C/N ratios were greater than that of the soil (C/N = 18.7). Thus N₂O emissions negatively correlated with the C/N-ratio (Pilegaard et al., 2006), with N₂O emissions being lowest at C/N-ratios ≥30 (limited disintegration of organic material) and highest at a C/N-value of 11 (optimum disintegration and humus build-up). However, soils with low soil moisture combined with low pH-values, can significantly suppress N₂O emissions even though the C/N-ratios would be <20 due to less microbial activity (Gundersen et al., 2012). While emissions of CH₄ positively correlated with the C/N-ratio (Shi et al., 2014) due to accumulation of organic material without decomposition which creates anaerobic conditions and high temperatures.

2.5.4 Soil moisture

Soil moisture content influences gas effluxes through the number of pores filled with water, to allow air movement. According to Livesley et al., (2008) soil moisture is the most important environmental variable influencing CO₂ flux from soils, which probably reflects the larger CO₂ flux when soil was wetter. The soil water regime also plays a key role in the dynamics of N₂O production, its reduction and transport (Heincke & Kaupenjohann, 1999). Thus high soil moisture stimulates denitrification and production of N₂O, while it also restricts its diffusion (Heincke & Kaupenjohann, 1999). Therefore the residence time of N₂O in the soil increases and microorganisms gain time to reduce it to N₂. In many studies there is a positive correlation between increasing water content and rising concentrations of N₂O in the soil solution (Davidson & Swank, 1990; Schnabel & Stout, 1994) and soil air (Benckiser et al., 1986;). Elevated nitrification also increases N₂O flux in the soil when soil water filled pore space is lower (Livesley et al., 2008). As a result, higher soil water content in NT soils results in greater denitrification rates as compared with CT (Aulakh et al., 1992), which could also result in a loss of plant available NO₃⁻-N.

In forest soils, nitrification is often the dominant microbial process producing N₂O, as soil water content rarely increases to produce anaerobic conditions that initiate and favour microbial denitrification processes (Stevens et al., 1997). Nawaz et al., (2017), noticed that N₂O flux was negatively correlated with soil temperature, probably because of denitrifying bacteria functioning well under low temperatures of between 10-14 °C thus increasing N₂O effluxes but positively with soil moisture (r =0.99). This may be attributed to the presence of mulch that had moderated high temperatures but generating higher N₂O emissions. Kader et al., (2017) explained that mulch protected the soil from hot summer temperatures by lowering the daily maximum soil temperature by 1-2 °C compared to bare soil which exhibited greater fluctuation of temperature between 21.6 °C and 30.8 °C. Furthermore, unlike covered soil, bare soil increased penetration resistance and soil compaction hence decreasing N₂O effluxes due to reduced gas diffusivity (Ball et al., 2008). On the other hand, Neue et al., (1997) reported maximum CH₄ flux with CT when the soil was saturated with water after a heavy rainfall event. This was attributed to anaerobic decomposition of SOM accentuated methanogenesis. Leip et al., (2002) also noted that the use of one or more brief drainage episodes during rice growing, or delaying flooding after sowing, produced a beneficial trade-off with reduced CH₄ emissions. This is because under high soil water filled pore space, anoxic conditions are created in the

subsurface and the diffusion of CH₄ to methanotrophs bacteria is restricted (Ball et al., 1997). Though higher soil moisture leads to anaerobic conditions that promote N₂O and CH₄ flux, adequate soil moisture will increase decomposition therefore high emissions of CO₂ will be experienced.

2.5.5 Bulk density

Soil structural degradation, particularly through compaction, which is a common problem in poorly drained soil with fine texture, can adversely affect CH₄ consumption (Ball et al., 1999). This is because soil compaction increases bulk density and reduces soil pores, which in turn hinder fluxes of GHGs through impeding diffusion of air and movement of water. Soil bulk density has a strong negative correlation with N₂O emission, because high bulk density intensifies accumulation of N₂O in soil air reducing N₂O diffusivity to the atmosphere. According to Hansen et al., (1993) in compacted soils, N₂O concentrations in the soil air are significantly higher than in the non-compacted soils. This is because soil compaction reduces the total pore volume, thereby creating more anaerobic sites than in non-compacted soil (Sitaula et al, 2000). In parallel, Beare et al., (2009) found 2.3 times more CO₂ production under low bulk density than in high bulk density soil because of high total soil pore volume for aerobic oxidation. In addition, increased bulk density can prevent flow of CH₄ in soil and enhance its retention which may improve its oxidation by methanotrophs (Smith et al., 2001) resulting in lower CH₄ emissions. Furthermore, Nazaries et al., (2011), discovered that the development of methanotrophic populations in the topsoil was negatively affected by tillage because of reduced soil moisture and temperature. Therefore NT soils with high soil moisture content may accumulate more methanotrophic organisms than CT and would experience more CH₄ sink than emissions.

2.6 Effect of tillage on biochemical processes in the soil

Research on conservation tillage (zero and minimum tillage) in farming systems has demonstrated an opportunity to increase SOC, microbial biomass C, total N, and extractable P due to accumulation of crop residues at the soil surface compared with CT (Mathers et al., 2007). According to Doran, (1980) the biological environment of soil is greatly modified by type and degree of tillage. NT resulted in a cooler and wetter environment for biological activity

near the soil surface than CT (Doran et al., 1998). Consequently, biological activity and organic C reserves were also concentrated near the soil surface of NT and there was greater potential for immobilization of plant available N in organic forms (Doran et al., 1998). Although, potential exists for less aerobic microbial activity with NT, this is rarely expressed in a semi-arid environment.

In a study by Doran, (1987), soil microbial populations and enzyme activity were greater with NT, with the amount of potentially mineralizable N in the surface 7.5 cm depth being 35% greater than in CT soils, thereby indicating a greater conservation of N in organic forms. Many studies have examined the long-term (10 years or more) effects of tillage on soil microbial biomass and N mineralisation (Dalal et al., 1991), but less information is available on the temporal dynamics for more than one vegetation period (Franzluebbbers et al., 1995). Obtaining knowledge of the magnitude of changes in microbial biomass and soil microbial processes is important to understand how tillage can be better managed to increase C and N sequestered into SOM to improve the long-term productivity of soil (Salinas-Garcia et al., 1997).

2.6.1 Impact of tillage on N mineralization

Tillage has huge influence on organic N mineralization as it facilitates decomposition by increasing porosity and faunal activity. Doran et al., (1998), observed that the levels of potentially mineralizable N (PMN) in the 0-15 cm depth of NT and minimally tilled soils were 20-39% and 12-33% greater, respectively than in CT. This was attributed to more OM in reduced till. Greater levels of PMN with reduced tillage reflected either greater immobilization or less mineralization compared with CT. On the same site the NO_3^- -N concentrations of NT were 20-22% less than those in CT, reflecting differences in nitrifier and denitrifier populations, with greater denitrifier population in NT and possible immobilization. Figure 2.3 shows that a reduction in tillage intensity resulted in more N mineralisation at 0-10 cm depth, which may be attributed to higher microbial activity compared to CT (Kandeler et al., 1998). However a different trend was observed at 20-30 cm depth with CT having higher N mineralisation due to incorporation of organic material into the subsoil during tillage thus providing organic substrates to microbes at lower depth. Overall, the 0-10 cm depth had higher N mineralization than 20-30 cm in all three tillage systems which may be due to higher microbial activity and OM in the topsoil. According to Kandeler et al., (1998) soil organic N

was significantly greater with reduced tillage than CT, which could have masked differences in net mineralization of N among tillage practices.

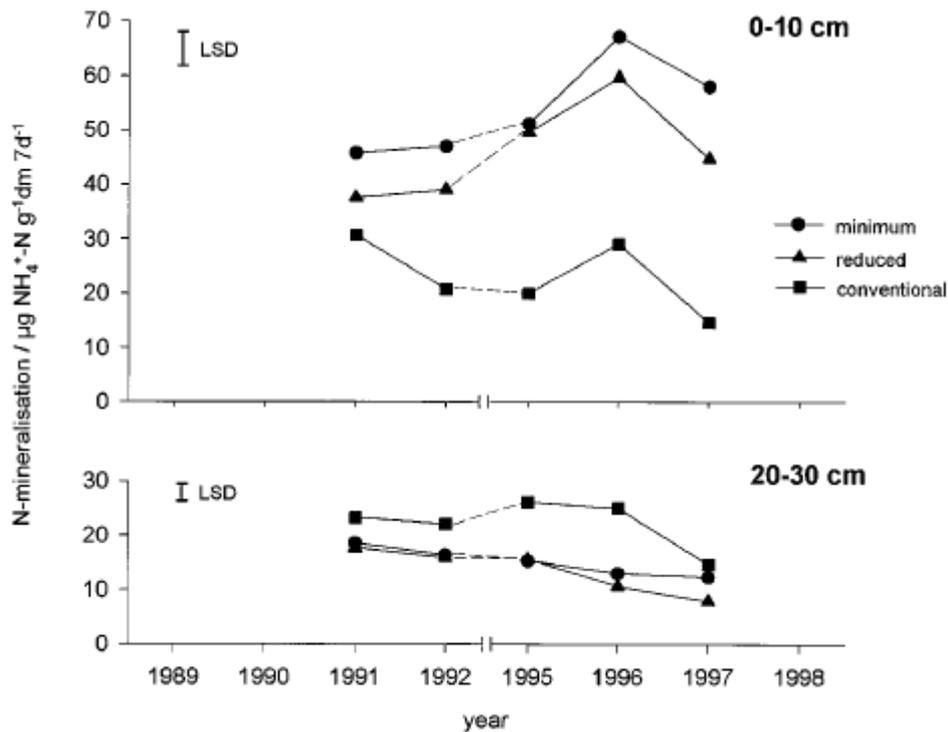


Figure 2. 3: N mineralisation at two depths under minimum, reduced and conventional tillage between 1991 and 1997 (Kandeler et al., 1998).

2.6.2 Effect of tillage on P mineralization

The accumulation of crop residues in the surface soils of different tillage systems was reported to be closely related to plant available pools of inorganic P (Pi) and organic P (P_o) (Bunemann et al., 2006). When comparing farming systems with widely differing P input–output budgets, changes in available Pi as well as P_o have to be seen in relation to P budgets or changes in total P. Oehl et al., (2002) noticed that changes in available Pi and total P were positively related to the P budget in conservation and conventional farming systems. Extractable P in the 0-25 cm soil layer ranged from 68.0 to 89.6 mg kg⁻¹ in NT, and was 5.3 times higher than under CT (Bertol et al., 2007). The accumulation of crop residues in the soil surface of NT and low P mobility downwards also contributed to this response (Bertol et al., 2007). Dick, (1983) observed greater organic P concentration under NT in Wooster soil compared to MT and CT

at 0-7.5 cm depth due to accumulation of residues (Figure 2.4). Whereas in the Hoytville soil profile, differences were only observed among the three tillage treatments in the 22.5-30 cm depth where the organic P in NT was lower than in MT or CT plots. Figure 2.4 indicates that unlike organic C and N, the greatest organic P concentrations under NT did not occur at the soil surface but in the soil increments between 2.5-15 cm depth. This may be due to movement of organic P compounds down the soil profile. Pinck et al., (1941) showed that organic P compounds are more mobile in the soil than inorganic P because they are sorbed less strongly to soil than inorganic P.

Bunemann et al., (2006), observed that soil P dynamics within the profile were affected by crop rotation, residue management and tillage giving changes in P_o pools. Thus, cultivated soil would have less of the plant-available forms of soil P, less extractable and residual P due to exposure to microbial attack (Hedley et al., 1982). In a study by Andraski et al., (2003), long-term NT produced significantly higher concentrations of soil P in the surface 0-5 cm, whereas it decreased at 5–15 cm depth compared with CT. The accumulation of P in surface soil under NT was attributed to lack of physical disturbance and accumulation of residues (Selles et al., 1997). Furthermore, Andraski et al., (2003) discovered that the accumulation of labile P in the sub-surface under CT was probably due to the greater mineralisation of P_o and incorporation of crop residues into the subsoil. P solubility also increased under conservation tillage, and this was attributed to greater microbial activity during the decomposition of SOM (Zibilske & Bradford, 2003). However, Bolland & Brennan, (2006) postulated that tillage practices which mix the topsoil such as CT would also mix previously applied P, and thus improve the availability of P to subsequent crops.

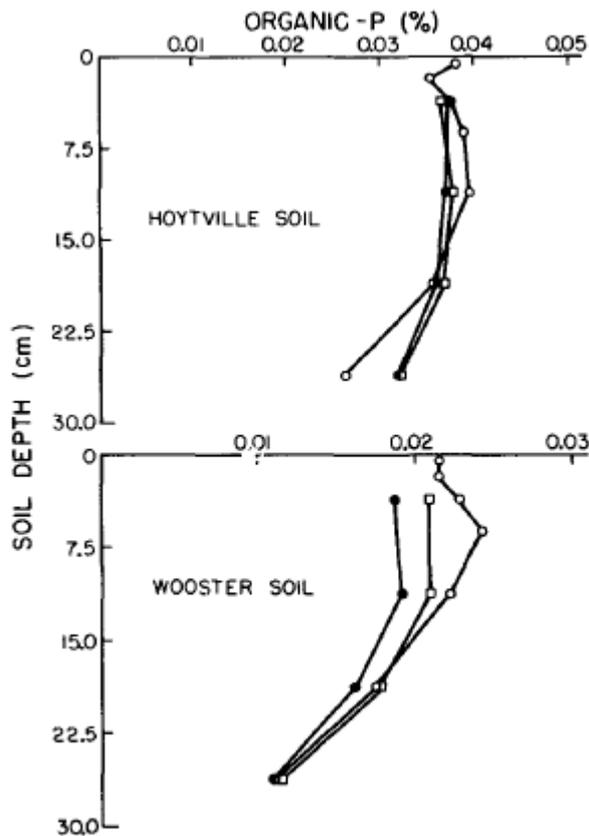


Figure 2. 4: Effect of tillage intensity on organic P concentrations at different soil depths (□, MT; ○, NT, and ●, CT) (Dick, 1983).

2.6.3 Tillage influence on microbial activity

Generally NT increases microbial activity in the upper soil horizon but this decreases with increase in soil depth, contrary to CT. Janušauskaite et al., (2013), found decreasing bacterial and fungal populations under NT by 3.49% and 11.83% respectively, as well as decreasing enzyme activity at 10-20 cm compared with that at 0-10 cm. Angers et al., (1993) also found evidence of more biological activity in the upper soil layer of NT systems than at 10-20 cm. According to Doran et al., (1998), soil respiration was generally greater with NT at 0-10 cm compared with CT. This resulted from more optimal moisture for microbial activity and greater amounts of available substrate for microbial activity in NT compared with CT at this depth. Thus, microbial biomass and enzyme activity can be used as early indicators of changes in soil properties induced by tillage (Powlson et al., 1987).

2.6.3.1 Microbial biomass

Unlike SOM, the influence of tillage may be detectable within short period of time using microbial biomass (Janušauskaite et al., 2013). This is because it responds to soil disturbances in a shorter period of time than those based on soil physical or chemical changes. Nitrifier-denitrifier populations and available N levels in soil were influenced by tillage practices set up 8-12 months earlier (Doran et al., 1998), with populations of nitrifiers in NT and sub-till soils ranging from 22-56% and 16-35% respectively, which was lower than in CT. Kruglov et al., (1979) attributed these lower nitrifier populations to reduced mixing of residues with soil and inhibition of nitrifiers by herbicides used in NT. However denitrifier populations tended to increase as degree of tillage was reduced (Doran et al., 1998). This was attributed to higher soil moisture of NT soils, which created anaerobic conditions that favoured greater denitrification rates compared with CT (Aulakh et al., 1992). Denitrification results in great loss of plant available NO_3^- -N. Several studies found that in the surface layer, NT results in high microbial biomass (Franzluebbers et al., 1995), fungal and bacterial populations than CT (Govaerts et al., 2008). The opposite is true at lower horizons however as Janušauskaite et al., (2013), observed decreases in bacterial and fungal populations of NT subsoil by 25.5 and 22.7%, respectively. This was attributed to CT stimulating microbial growth due to uniformly distributed residues in the arable layer (Salinas-Garcia et al., 2002), and increased rate of oxygen supplied to soil microsites. Soils subjected to reduced tillage accumulate crop residues and organic C which are substrates for microorganisms near the surface (Kandeler & Böhm 1996). As a consequence, soil microbial biomass and various soil microbial processes tend to increase in surface soils after a change from CT to NT.

Kandeler et al., (1998), discovered that NT provided an environment for soil microorganisms which was characterised by slightly higher soil bulk density and reduced air circulation compared with CT. However in the long run (after 4 years of different tillage treatments), the microbial biomass and the rates of soil microbial processes increased in the conservation tillage treatments. This was due to accumulation of soil microbial biomass within the profile of conservation tillage, which is largely responsible for higher microbial activity near the soil surface compared with that of CT. However CT had increased microbial activity in the 20-30 cm soil layer. These results confirmed observations that organic material buried after ploughing is responsible for higher microbial activity in deeper soil layers of CT (Kandeler & Böhm,

1996). However, increased soil microbial populations stimulated by greater OM and water contents of NT, could compete with the crop for available N.

2.6.3.2 Enzyme activity

Since soils managed through reduced tillage generally have more surface plant residues, higher moisture content, better structure and aggregation compared to CT soils, they will have higher enzyme activity (Janušauskaite et al., 2013). Jin et al., (2009) showed that throughout the growing season, soil enzyme activity responded to tillage, residue management and sampling time for urease and invertase, with increases in soil enzyme activity being associated with decrease in tillage intensity. Janušauskaite et al., (2013) also noticed that enzyme activity decreased with soil depth, especially urease by 5.13%. According to Doran et al., (1998) soil phosphatases are indices of the levels of activity of microbial populations, with greater microbial activity observed in the surface of NT compared with CT. Kandeler et al., (1999) observed a strong dependence of enzyme activity on SOC. However, Gianfreda et al., (2005) when investigating the activity of different enzymes in CT and NT soils, found that high SOC did not necessarily reflect corresponding increases of enzymatic activity. The higher enzyme activity, especially of urease in CT compared to NT was partially explained by a flush of microbial activity directly after tillage and residue incorporation as a result of an increase in substrate and oxygen availability (Kladivko, 2001). This effect was however temporary, as enzyme activity fell back to lower levels again at subsequent sampling stages (Jin et al., 2009). Kandeler et al., (1998) observed that phosphatase showed higher activity in reduced and minimum tillage treatments within the second year compared to CT. This may be attributed to shallow incorporation of residues in the top layer of minimum tillage (Saffigna et al., 1989), which induced higher enzyme production by soil microorganisms, and provided a higher surface area of particles to bind this enzyme.

2.7. Strategies for mitigating soil GHG emissions.

The way soils are managed can influence the flux of GHGs by changing one or more of the following: the soil climate (i.e., temperature and water content), the physical/chemical environment of the soil, and the amount and chemical composition of organic residues applied to soil (Gregorich et al., 2005). Changes in these variables control the rate and extent of

microbial processes, which in turn control the stabilization of C in soil and production of GHGs (Gregorich et al., 2006). Conservation agriculture, proper fertilizer management, good irrigation design, appropriate land use and management are few of the mitigatory strategies to reduce emissions of GHGs. Considerable emissions originate from soils, livestock, animal wastes, consumption of fossil fuels and production of fertilizers (Kramer et al., 1999). Thus practices such as NT, reduction of bare fallow and residue retention to land have proven to minimise GHGs emissions. However, any management option should maintain high crop yields, conserve diminishing natural resources, and minimize environmental damage (Lal, 2003).

According to Garnett, (2011) some sequestration activities can undermine agricultural production. If, for instance arable land is converted to grass or forestry for sequestering purposes, this may require more intensive cultivation in other areas to compensate for production losses. This could possibly trigger land clearance to grow food elsewhere, with possible GHG emissions in these new areas (Garnett, 2011). Again, applying more OM to one land at the expense of another results in zero net C gain, making C sequestration ineffective (Smith et al., 2007). Garnett, (2011) further emphasized that soil C sequestration is not only reversible but time bound; so once equilibrium is reached, no further sequestration occurs and the agricultural land may again become a net emitter. What is clear is that avoiding further soil C losses is as important as sequestering it (Garnett, 2011). It may be helpful to consider soil C sequestration as an outcome of good agricultural management rather than a primary goal (Kibblewhite et al., 2008).

To minimize N₂O emissions from agricultural lands, N fertilizer application rates need to be controlled to meet plant needs since not all applied N can be taken up by plants (Ortel et al., 2016). Using controlled-release fertilizers or denitrification inhibitors prevents increased N₂O losses (Shoji et al., 2001). In a study by Malhi et al., (2006), the amount of N lost through N₂O-N emissions increased with N fertilization and was greater in CT. Gregorich et al., (2005) also recommended that solid manure was a better mitigation strategy for N₂O loss compared to liquid manure. The lower N₂O emission following application of solid manure may be gradual release of mineralized N to the soil solution resulting in the uptake of available N by growing plants, which precludes a large build-up of mineral N (Gregorich et al., 2006). Liquid manure also contains labile soluble organic C that can stimulate N₂O production in areas where soil C availability limits denitrification.

Enteric fermentation emits high amounts of CH₄ and it should be well managed. Beauchemin and McGinn, (2005), explained that CH₄ emissions can be reduced by feeding livestock more concentrates in place of forages. Although concentrates may increase daily CH₄ emissions, emissions per kilogram feed intake and per kilogram product are invariably reduced (Lovett et al., 2006). The net benefit, however, depends on reduced animal numbers or younger age slaughter for beef animals and on how the practice affects emissions of other GHGs especially CO₂ when producing and transporting the concentrates (Phetteplace et al., 2001). Other practices that can reduce CH₄ emissions include: adding oils to the animal diet (Jordan et al., 2004); improving pasture quality, especially in less developed regions, because it improves animal productivity and reduces the proportion of energy lost as CH₄ (Alcock & Hegarty, 2011). Optimizing protein intake by animals also reduces N excretion and N₂O emissions (Clark et al., 2005). A portion of CH₄ emitted to the atmosphere can be sequestered by aerobic soils (Stolze et al., 2000). Again land application of manure could significantly decrease the net CH₄ emitted to the atmosphere compared with stockpiling or long-term lagoon storage of manure. According to Chadwick et al., (2000) manure applied to pasture land did not appear to impact CH₄ emission. In contrast, net uptake of CH₄ by CT soils under corn amended with manure was reduced relative to soils without manure (Rochette & Cote, 2000).

The protection of existing peat-wetlands and restoration/creation of other wetlands to sequester C may be considered for C credits in the near future (Whiting & Chanton, 2001). These wetlands should be carefully designed to curtail the emission of CH₄ while sequestering soil C (Whiting & Chanton, 2001). This is because C fixation in flooded wetlands is strongly coupled to CH₄ production and emission to the atmosphere due to reducing conditions conducive for methanogens bacteria (Whiting & Chanton, 1993). Land use and its management also affect the production, fixation and emission of GHGs. Thus conversion of natural ecosystems to croplands often results in loss of soil organic C due to increased mineralization and lower C inputs (Carter et al., 1998). Land use (pasture, cropping, forest or plantation), soil type and management determines the overall GHGs balance of that landscape (Hall et al., 2004). In most cases, forests are important terrestrial C sinks (Dalal et al., 2008) but may also contribute considerably to global N₂O emissions (Butterbach-Bahl & Kiese, 2005). Pastures can also support a large soil C store, but when forest systems are converted to pastures, there is often a decrease in soil organic C (Guo & Gifford, 2002). However, practices such as afforestation of

agricultural land can be more beneficial to the net GHG balance of that landscape than a change in agricultural management, such as conversion from to NT (Jacinthe & Lal, 2004).

2.8. Conclusion

Agriculture has the potential to reduce its environmental footprint and offset GHG emissions. Efficient crop and animal production systems are required to achieve better crop productivity, soil C sequestration, and reduce GHG emissions. Enhancing crop yields is not incompatible with a reduction of agricultural inputs in many circumstances. To the contrary, careful and efficient management of nutrients and water by proper farming methods such as crop residue incorporation and less intensive tillage are critical to sustainable and increased agricultural output. Furthermore, it has been shown in several cases that yield gains alone do not necessarily preclude expansion of cropland, suggesting that intensification must be coupled with conservation and development efforts. Many farmers have to shift from CT to NT as it assists in mitigating GHGs. NT results in conservation of SOM and reduces loss of GHGs. NT also enhances high retention of nutrients and soil moisture thus facilitating higher microbial activity. Though NT will mostly have higher C and N content in the surface when compared to CT, the distribution with depth is poor due to accumulation of residues on the surface and lack of mixing with subsoil. Tillage with residue incorporation enhances mineralization of organic C, P and N, therefore CT increases cumulative inorganic P and N in soils. Thus, depending on soil type and climatic conditions, conservation agricultural systems can sequester CO₂, enhance CH₄ consumption and reduce N₂O emissions.

Practices such as afforestation lead to increased C sequestration through tree biomass growth. In addition, it decreases N₂O emissions because of lower N inputs, compared to crop farming, and ensures tighter nutrient cycling. While management practices such as manure or crop residue incorporation instead of increased mineral fertilizer application would more efficiently mitigate GHG emissions from arable land. NT usually experiences wet and cool conditions which trigger denitrification processes that might induce N₂O emissions. Though denitrification and nitrification are both controlled by availability of soil moisture, the major driver of these process are microbial biomass and enzyme activity, which are in turn controlled by tillage. Soil enzyme activity is mostly higher in NT than CT, especially in the upper soil due to availability of organic substrates. Thus the tillage type and soil depth are two important factors affecting soil microbial communities. Due to high soil C and N contents in the upper

depth of NT, microbial populations and activity are higher when compared to CT. Whereas soil C and N are better distributed with depth under CT therefore increasing microbial activity in subsoils when compared to NT. Tillage also has a significant impact on the physicochemical characteristics of soil in the long-term, while its influence on biological soil properties is more immediate. Though NT is more effective at mitigating GHG emissions and improving soil quality than CT, its continuous practise using mechanisation increases penetration resistance, while reducing porosity and soil air circulation. Many researchers have tried to emphasize on both the benefits and detriments of NT and CT on GHGs emissions, hence more studies are needed to develop specific strategies to mitigate these while increasing crop production. Policies attempting to curb GHG emissions have to start at regional level then developed globally.

CHAPTER 3

CHANGES IN SELECTED SOIL PHYSICOCHEMICAL PROPERTIES UNDER DIFFERENT TILLAGE PRACTICES AND N FERTILIZER APPLICATION IN A DRY LAND MAIZE MONO-CROP

3.1 Introduction

The maintenance of optimum soil physical and chemical characteristics is integral to soil fertility management. This can be achieved through the application of mineral fertilizers, retaining crop residues to soil and reduced tillage. The improvement of physicochemical characteristics of soil would also lead to reduced erosion, improved soil water and nutrient retention as well as increased crop productivity. The high plant biomass produced by both retaining of residues and fertilizers results in increased returns of organic material to the soil in the form of decaying roots, litter and crop residues. However, threshold application must be determined to sustain soil quality, since excess fertilizer application may be detrimental to soil physicochemical properties. Land management practices such as tillage affect soil physicochemical and biological properties (Ishaq et al., 2002). Tillage-induced changes in these properties depend on antecedent soil properties, type of tillage, and climate (Rasmussen, 1999). Research has shown that crop residues under NT conserve soil moisture, maintain or increase OM and improve crop production (Halvorson et al., 2002). According to Lal, (1993) improper tillage such as CT leads to a range of degradative processes such as decline in soil structure, accelerated erosion, depletion of SOM and fertility, disruption in cycles of water, organic C and plant nutrients. Tillage, residue retention and N fertilization also influence aeration and available C and subsequently affects gaseous N loss (Baggs et al., 2003).

According to Malhi & Lemke, (2007), the benefits of NT and residue retention on crop yield is through conservation of soil moisture and improvement in soil properties (which is a slow process). Maurya, (1988) reported that NT could increase bulk density as a result of soil compaction. Contrary, Ishaq et al., (2002) reported no significant effect of tillage method on soil bulk density which was due to soil type of sandy clay loam in texture, less organic material and short duration of experiment. Unger, (1984) also did not observe a significant effect of tillage on soil bulk density. Hamblin, (1986) reported that tillage increased total porosity by increasing the number of pores and pore size distribution. These contradictory findings by researchers may be attributed to different climatic conditions, soil types, cropping and

experiment duration. Malhi & Lemke, (2007) demonstrated that NT enhanced soil C storage and soil aggregation.

CT practices involving soil turnover usually decrease SOC concentration (Balesdent et al., 2000). Franzluebbers & Hons, (1996) reported that soil under NT had greater micronutrient concentrations in the 0-5 cm layer than with CT. Malhi & Lemke, (2007) observed that after 8 seasons, tillage had no significant effect on total organic C and N in the top 15 cm of soil. However, Halvorson et al., (2002) observed substantial increase of total organic C in soil under NT compared to CT. Malhi & Lemke, (2007) argued that the build-up of OM is a slow process and it takes many years to accumulate significant amounts of total organic C and N. Lal et al., (1990) reported higher concentrations of OM in the surface layer with NT compared to CT soils. Higher OM under NT would enhance soil nutrients thus indirectly improving soil physicochemical properties. According to Ishaq et al., (2002) tillage did not affect soil properties while fertilizer application significantly improved soil chemical properties.

Increase in N fertilization has generated concern about its possible negative influence on soil properties, particularly when long-term use of NH_4^+ is involved (Stone et al., 1991). Addition of fertilizers to soil will influence the chemical composition of soil solution pH, ionic strength and ionic composition which in turn can induce dispersion or flocculation of clay particles and thus affect soil aggregation (Haynes & Naidu, 1998). Application of N fertilizers has sometimes been shown to have an adverse effect on soil aggregation (Haynes & Naidu, 1998). This is because when NH_4^+ ion accumulates in soils in large amounts, it can become a dominant exchangeable cation, and like Na^+ , it favours dispersion of soil colloids. Chawla & Chabra, (1991) found that annual applications of N fertilizer resulted in increased infiltration rate, hydraulic conductivity and percent water stable aggregates, and decreased bulk density and water-dispersible silt or clay thus positively improving soil properties.

The effect of fertilizer application and tillage is also dependent on soil depth. According to Stone et al., (1991) in the upper 0-15 cm soil layer, N-fertilized plots had reduced pH, available P and exchangeable bases, but increased micronutrient concentration compared with the unfertilized plots. In the lower 21-29 cm soil layer, N-fertilization resulted in reduced pH and exchangeable Na compared with unfertilized plots, (Stone et al., 1991). Blevins et al., (1977) reported high exchangeable Ca and Al under NT than CT and they increased with increasing N. Further, soil pH in the 0-5 cm depth dropped from 5.2 to 4.1 when $330 \text{ kg N ha}^{-1}\text{year}^{-1}$ was

applied to NT for 5 years (Blevins et al., 1977). This was attributed to nitrification which caused soil acidification, increased extractable micronutrients (Fe, Cu, and Mn) and decreased available P as well as exchangeable bases.

The application of optimum fertilizer rates may lead to both crop and OM enhancement, which may directly or indirectly stimulate soil biological activity. This is because OM or fertilizer nutrients provide substrates (and energy) to microbes thus inducing microbial activity. The OM input and increased soil microbial or faunal activity will favour soil aggregation and increase soil porosity (Haynes & Naidu, 1998). Generally, increasing soil OM content characteristically leads to a decrease in bulk density or surface crusting, increased water holding capacity, macroporosity, infiltration capacity, hydraulic conductivity due to improved soil aggregation (Haynes & Naidu, 1998). Depending on the type of fertilizer, their application can have an effect on soil physicochemical properties and biological activities. Fertilizers such as phosphatic fertilizer can flocculate soil particles by acting as a cementing agent, but it can also disperse soil particles especially NH_4^+ fertilizers such as $(\text{NH}_4)_2\text{SO}_4$ and NH_4NO_3 (Haynes & Naidu, 1998). In semi-arid regions where conditions are torrid, it is necessary to make sure that there is exhaustive dissolving of fertilizer by adding water through supplementary irrigation. Henceforth the fertilizer threshold under irrigated conditions is different from that under dry land conditions when all other conditions are maintained constant. Many studies on tillage and N fertilization effects on soil properties have been under irrigation, crop rotation, annual and pasture cropping systems. Very few studies have explored the effect of these under dry land agriculture with mono-cropping, especially in South Africa. The aim of this study was to assess the effect of tillage and N fertilizer application on selected physicochemical soil properties under rain-fed maize mono-cropping.

3.2 Material and methods

The study was done in Loskop, KwaZulu Natal Province, South Africa, located at latitude $28^\circ 55' 26.83''$ S and longitude $29^\circ 33' 38.64''$ E. The trial was set up in 1990, and had been previously utilised for dryland maize-soybean rotation, under No-Till. As from the 2003/2004 season to date, the trial was changed to a dryland maize monocrop in summer with winter following under zero tillage (NT), annual conventional till (CT-ANNUAL), and conventional tillage every 5th year (CT-Y5). However the experimental trial for the current study begun in February 2018 and was terminated in December 2019. Appendix A.1 depicts field experimental trial layout, while Figure 3.1, picture taken on the 5th year of CT-Y5, shows plants growing in

the different tillage systems. The soil was classified as Hutton (Soil Classification Working Group, 1991) with a clay loam texture. The area receives approximately 643 mm of rainfall per annum which occurs mostly during summer (November-January), and has mean average midday temperature ranging between 19.3 °C in June and 28 °C in January (Vilakazi, 2017).

The field trial was arranged as a randomized split plot design, with tillage forming whole plots and fertilizer application rates being sub-plots. In each treatment urea fertilizer was applied at four application rates of 0, 60, 120 and 240 kg N ha⁻¹. All tillage treatments were replicated in the experiment (Appendix A.1). Furthermore, the tillage technique was used as the blocking factor in the experimental trial. The NT was characterised by no soil disturbance (no ploughing) with crop residues from previous season left on the surface, while a planter was utilized for planting. CT-Y5 comprised leaving the soil unploughed for four years, then tilling every 5th year. Soil sampling in CT-Y5 was done in the 5th year. In CT-ANNUAL there was annual disturbance of soil by tilling using a tractor to a depth of 30 cm.



Figure 3. 1: Maize plants growing in no-till (NT), CT-ANNUAL (annual ploughing) and CT-Y5 at 120 kg N ha⁻¹ of urea.

3.2.1 Sampling procedure

Soil was sampled in year 2018 after harvest to avoid plant disturbance at three depths of 0-10, 10-20 and 20-30 cm. Sampling was done during the ploughing cycle of CT-Y5. Each tillage treatment had three tillage blocks sampled making nine blocks in total, with each block having four N fertilizer application rates which were all replicated three times. Therefore 108 samples were sampled for each tillage treatment thus making 324 samples altogether (3 tillage * 4 application rates * 9 blocks * 3 replicates in each application rate). Collected soil samples were spread out to air dry under the shed, then gently crushed with a mortar to pass through a 2 mm sieve prior to analysis. Cores for bulk density were sampled using core sampler at three depth of 0-10, 10-20 and 20-30 cm.

3.2.2 Laboratory Analyses

3.2.2.1 pH and electrical conductivity (EC)

About 10 g of soil was weighed into a 50 mL beaker. Both distilled water and 1M KCl were used to determine pH at a ratio of 1: 2.5, (Diez et al., 2004). Samples were allowed to stand for 30 minutes with occasional stirring using glass rods. An electrode pH meter (PHM 210) was used to measure the pH of the supernatant liquid. The samples with water added were also used to measure electrical conductivity (EC) using an EC meter (CDM 210).

3.2.2.2 Total N and Carbon

Total N and C were analyzed using LECO TruMac CNS/NS Carbon/Nitrogen/Sulfur Determinator (Leco Corporation, 2012). Air dried soil was passed through a 0.5 mm sieve, then a 0.2 g sample was put into the LECO for analysis of C and N. The procedure is based on dry combustion of air dried samples in crucibles, subjected to a 1450 °C furnace temperature for about 6 minutes. The C: N ratios of the soils were also calculated.

3.2.2.3 Exchangeable acidity

About 10 g of soil was weighed into a 100 mL plastic centrifuge tube, then 50 mL of 1M KCl was added before shaking for 4 minutes (Lourenzi et al., 2011). The sample was centrifuged for 2 minutes at 400 rpm, and the solution filtered with Whatman No 41 filter paper into a glass bottle. A 25 mL aliquot was extracted into a 100 mL conical flask, while another 25 mL of 1M

KCI only served as a blank. About 5 drops of phenolphthalein indicator were added to each sample then titrated with 0.01M NaOH until it remained pink for at least 30 seconds. The blank was titrated first following steps by Ndayegamiye and Cote, (1989). Exchangeable acidity was calculated in cmol_c/kg using the formulae by (Smith & Hughes, 2002; Edwards & Duncan, 2009):

$$\text{Exchangeable acidity (mg/L)} = \frac{v_{\text{NaOH}} * c_{\text{NaOH}}}{v_{\text{H}^+}}$$

$$\text{Exchangeable Acidity (cmol}_c\text{/kg)} = \text{mg/L} * \frac{50 \text{ mL}}{1000} * \frac{1000}{10\text{g}} * 1 * 1000.$$

Where 50 mL is the volume of solution and 10 g is the mass of soil used.

3.2.2.4 Exchangeable bases

Air dried soil (5 g) was weighed into a clean plastic bottle with a stopper then 100 mL of 1 M ammonium acetate (NH₄OAc) solution (pH 7) was added. The contents were shaken for 30 minutes and filtered through Whatman No. 41 paper (Okaleb et al., 2002). According to Anderson & Ingram, (1993) this is the soil extract that was used for Na, K, Ca and Mg determinations. About 5 mL of soil extract was pipetted into a 50 mL volumetric flask then 1 mL of 26.8 % lanthanum chloride solution was added, and the contents were diluted (10 times) to the mark with 1M NH₄OAc extraction solution (Okaleb et al., 2002; Anderson & Ingram, 1993). In the determination of Mg, the soil extract was diluted 25-fold. Then 5 mL of 5000 ppm strontium chloride was added into the soil extract and filled up to the mark with the 1 M NH₄OAc extracting solution. All elements were analysed by an atomic absorption spectrophotometer (AAS 280 FS, fast sequential). The concentrations of Ca, Mg, K and Na in the soil sample were calculated in mg kg⁻¹ (Okaleb et al., 2002; Anderson & Ingram, 1993) then finally expressed in cmol_c/kg:

$$\text{mg kg}^{-1} \text{ K, Ca, Na and Mg in soil} = \frac{(a-b)*v*f*1000}{1000*w}$$

Where a = concentration of K, Na, Ca, and Mg in the sample extract; b = concentration of analyte in the blank extract; v = volume of the extract; w = weight of the soil sample; f = dilution factor.

3.2.2.5 Bulk density

The whole soil core was placed in a weighing boat and weighed. The weights of the boat and plastic bag were recorded as W_1 and W_2 , respectively. W_1 and W_2 were measured before sampling and after drying according to Blake, (1965). The samples were then dried in an oven at 105 °C, with the drying time varying with the amount of soil present. For larger cores, (7.62 cm diameter x 7.62 cm length), 72 hours was used, while for smaller cores 48 hours was required to dry; (Blake, 1965). The combined weight of the oven dry sample, boat and plastic bag were recorded as W_3 .

$$\text{Bulk density (kg m}^{-3}\text{)} = \frac{W_3 - W_1 - W_2}{\text{volume of cylinder}}$$

3.2.2.6 Particle size distribution

Particle-size analysis was done using the sieve and double pipette method (Walter et al., 1978). Calgon solution was prepared with 35.7 g sodium hexamataphosphate and 7.9 g sodium carbonate in de-ionized water and was made-up to 1 L. Soil (50 g) was weighed into a 100 mL beaker and transferred to a stirrer cup then 50 mL of calgon solution (dispersing agent) was added. About 500 mL of distilled water was added and placed on a stirrer for 5 minutes. Dispersed samples were washed through the 0.053 mm sieve into 1 L measuring cylinder using distilled water. The soil fraction coarser than 0.053 mm was transferred into 250 mL beaker and dried in an oven at 105 °C overnight. This was the sand fraction, and it was transferred to a nest of sieves. Sieves were arranged in apertures of 0.500 mm, 0.250 mm, 0.106 mm and pan, and these were shaken for 5 minutes. The mass of empty sieves were firstly recorded accurately after which it was again recorded with the soil fraction. The clay and silt fraction were analyzed in the sedimentation column, using different settling times guided by the temperature of sedimentation (Walter et al., 1978). After the appropriate settling time, 20 mL each of fine silt was sampled at 100 mm and clay was sampled at 75 mm below the surface using a double pipette (Walter et al., 1978). Each pipetted soil suspension was placed into a pre-weighed 50 mL beaker and dried in an oven at 105 °C overnight. After 24 hours beakers were removed from the oven and allowed to cool in a desiccator and re-weighed. Proportions of sand, silt and

clay were calculated and expressed as percentages of the total then used to determine soil textural class with a textural triangle.

3.2.2.6 Soil moisture content

Freshly sampled field-moist soils (10.0 g) were weighed and dried overnight at 105 °C in an oven, after which the oven-dry samples were weighed to determine dry mass (van Reeuwijk, 2002). Moisture content was calculated using the formula by van Reeuwijk, (2002):

$$\text{Moisture (wt\%)} = \frac{\text{wet soil weight} - \text{oven dry soil weight}}{\text{oven dry soil weight}} (100)$$

Furthermore the moisture correction factor was calculated using the formula:

$$\text{Moisture correction factor} = \frac{100 + \% \text{ Moisture}}{100}$$

3.2.3 Statistical analyses

An analysis of variance (ANOVA) test was done to determine the effect of tillage and fertilizer application on soil physicochemical properties. Treatment factors were tillage practice, N fertilizer application rate and soil depth. The Fisher's protected LSD test was used as a post-hoc test to compare treatment means and their interactions at $p < 0.05$. All tests were performed with GenStat 14.1 for Windows software (VSN international, 2011).

3.3 Results

3.3.1 Soil properties at 0-10 cm depth.

At the 0-10 cm depth, the soil texture of all treatments was clay loam (Table 3.1). The bulk density of NT and CT-ANNUAL was higher (1294 and 1529 kg m⁻³, respectively), than that of CT-Y5 (940 kg m⁻³) in the control ($p < 0.05$). Soil moisture content was highest at 0-10 cm depths in NT at all application rates serve for 240 kg N ha⁻¹, while it was highest in CT-ANNUAL at this application rate ($p < 0.05$). The 240 kg N ha⁻¹ rate at 0-10 cm had the lowest ($p < 0.05$) soil moisture content under all tillage techniques (Table 3.1). In the case of bases, NT had the highest while CT-Y5 mostly had the lowest concentration of exchangeable bases than other tillage treatments at all N application rates, while the control had highest base concentrations across all tillage treatments than the other N rates ($p < 0.05$). Exchangeable acidity was highest under CT-Y5 at 240 kg N ha⁻¹ (3.9 cmol_c/kg), while it did not differ in all the other treatments ($p < 0.05$). NT at 0 and 60 kg N ha⁻¹ had higher electrical conductivity (172.9 mS m⁻¹ and 130.6 mS m⁻¹ respectively), than all other treatments ($p < 0.05$). However, at higher application rates of 120 and 240 kg N ha⁻¹, CT-ANNUAL had the highest EC, (93.9 and 72.5 mS m⁻¹, respectively), ($p < 0.05$). Soil pH was highest at 60 kg N ha⁻¹ in NT (6.4 pH_{water}), while it was lower in CT-Y5 at 240 kg N ha⁻¹ (4.4.pH_{water}), ($p < 0.05$). Meanwhile at 120 kg N ha⁻¹ CT-ANNUAL had the highest C: N ratio ($p < 0.05$) than NT in 0-10 cm depth (Table 3.1).

Table 3. 1: Soil characteristics under different tillage systems and fertilizer application rates at 0-10 cm depth.

Rates (kg N ha ⁻¹)	Tillage	pH_w	pH_{KCl}	EC (mSm ⁻¹)	Acidity (cmolc/kg ⁻¹)	Bases (cmolc/kg ⁻¹)	C:N	Soil moisture (%)	Bulk Density (kg m ⁻³)	Textural Class
0	NT	5.6 ^{bc}	4.7 ^{bcd}	172.9 ^d	0.1 ^a	13.9 ^g	18.3 ^a	17.7 ^{fg}	1294 ^{abc}	Clay Loam
	CT-Y5	6.0 ^{bc}	4.7 ^{bcd}	32.9 ^{ab}	0.1 ^a	5.1 ^{bc}	40.4 ^{ab}	16.5 ^{efg}	940 ^a	Clay Loam
	CT-A	6.3 ^c	5.2 ^{cd}	69.5 ^{abc}	0.3 ^a	7.4 ^{cde}	32.7 ^{ab}	14.0 ^{cd}	1529 ^{bc}	Clay Loam
60	NT	6.4 ^c	5.31 ^d	130.6 ^{cd}	0.1 ^a	11.4 ^f	16.5 ^a	17.4 ^{fg}	1448 ^{abc}	Clay Loam
	CT-Y5	6.0 ^{bc}	4.7 ^{bcd}	56.8 ^{abc}	0.1 ^a	5.8 ^{bcd}	22.3 ^a	15.4 ^{de}	1846 ^c	Clay Loam
	CT-A	5.9 ^{bc}	4.60 ^{bcd}	39.8 ^{ab}	0.3 ^a	5.0 ^b	27.9 ^{ab}	14.9 ^{cde}	1307 ^{abc}	Clay Loam
120	NT	6.3 ^c	4.7 ^{bcd}	19.2 ^a	0.1 ^a	8.5 ^c	19.7 ^a	15.8 ^{ef}	1360 ^{abc}	Clay Loam
	CT-Y5	5.4 ^b	4.6 ^{bcd}	32.6 ^{ab}	0.1 ^a	4.6 ^b	32.5 ^{ab}	18.3 ^g	1422 ^{abc}	Clay Loam
	CT-A	6.4 ^c	5.2 ^{cd}	93.9 ^{bc}	0.1 ^a	6.8 ^{bcde}	51.2 ^b	15.2 ^{de}	1339 ^{abc}	Clay Loam
240	NT	5.4 ^b	4.3 ^{abc}	43.6 ^{ab}	0.1 ^a	8.0 ^{de}	17.8 ^a	11.9 ^{ab}	1398 ^{abc}	Clay Loam
	CT-Y5	4.4 ^a	3.4 ^a	24.0 ^{ab}	3.9 ^b	0.7 ^a	24.8 ^a	10.9 ^a	1338 ^{abc}	Clay Loam
	CT-A	5.9 ^{bc}	4.2 ^{ab}	72.5 ^{abc}	0.2 ^a	4.8 ^b	36.4 ^{ab}	13.1 ^{bc}	1187 ^{ab}	Clay Loam

Rates- different UREA fertilizer application rates; NT-Zero tillage; CT-Y5-Ploughing on the 5th year; CT-A-ploughing annual; EC-Electrical conductivity; Similar letters on the same columnar there is no significance (P<0.05) whereas different letters indicates significant difference (P<0.05).

3.3.2 Soil properties at 10-20 cm depth

All the treatments at 10-20 cm soil depth had a clay loam soil texture (Table 3.2). On the other hand, NT had higher bulk density than the other tillage treatments in the control and at 240 kg N ha⁻¹, ($p < 0.05$). In the control and at 120 kg N ha⁻¹, CT-Y5 had higher soil moisture, (about 17.0 % in both), while NT in the control had the lowest soil moisture than other tillage treatments ($p < 0.05$). The C: N ratio however was highest in NT at 240 kg N ha⁻¹ ($p < 0.05$) while it did not significantly differ in all the other treatments at 10-20 cm soil depth (Table 3.2). However, NT had the highest amount of exchangeable bases in all treatments, while CT-Y5 had a lower base concentration than the other tillage treatments at most N application rates ($p < 0.05$). The exchangeable acidity on the other hand was highest in CT-ANNUAL (1.0 cmol/kg) at 120 kg N ha⁻¹ ($p < 0.05$), while it did not significantly differ in all the other tillage treatments (Table 3.2). NT had the highest EC in the control (124.1 mS m⁻¹) and at 120 kg N ha⁻¹ (199.5 mS m⁻¹), while CT-Y5 had lower EC at these same application rates ($p < 0.05$). Soil pH was lowest in CT-ANNUAL at 120 kg N ha⁻¹ ($p < 0.05$), while it did not significantly differ in all the other treatments (Table 3.2).

Table 3. 2: Soil characteristics under different tillage systems and fertilizer application rates at 10-20 cm depth.

Rates (kg N ha ⁻¹)	Tillage	pH _w	pH _{KCl}	EC (mSm ⁻¹)	Acidity (cmol _c kg ⁻¹)	Bases (cmol _c kg ⁻¹)	C:N	Soil moisture (%)	Bulk Density (kg m ⁻³)	Textural Class
0	NT	6.4 ^{bc}	5.1 ^{bc}	124.1 ^{bc}	0.1 ^a	7.8 ^{bcd}	24.2 ^a	11.4 ^a	1860 ^b	Clay Loam
	CT-Y5	6.1 ^{bc}	4.72 ^{abc}	46.5 ^a	0.2 ^a	5.0 ^{abc}	35.4 ^a	17.0 ^b	1416 ^{ab}	Clay Loam
	CT-A	6.4 ^{bc}	5.2 ^{bc}	88.7 ^{ab}	0.3 ^a	7.2 ^{bcd}	32.2 ^a	13.2 ^{ab}	1772 ^{ab}	Clay Loam
60	NT	6.4 ^{bc}	5.3 ^{bc}	48.1 ^{ab}	0.1 ^a	9.1 ^d	25.2 ^a	16.3 ^b	1560 ^{ab}	Clay Loam
	CT-Y5	6.4 ^{bc}	5.2 ^b	81.7 ^{ab}	0.1 ^a	8.4 ^{cd}	28.0 ^a	16.0 ^{ab}	1626 ^{ab}	Clay Loam
	CT-A	5.8 ^{bc}	4.6 ^{abc}	103.9 ^{ab}	0.1 ^a	6.5 ^{abcd}	35.1 ^a	14.9 ^{ab}	1704 ^{ab}	Clay Loam
120	NT	6.2 ^{bc}	5.0 ^{bc}	199.5 ^c	0.1 ^a	7.3 ^{bcd}	25.3 ^a	15.4 ^{ab}	1633 ^{ab}	Clay Loam
	CT-Y5	5.7 ^{bc}	4.6 ^{abc}	38.1 ^a	0.1 ^a	4.9 ^{ab}	31.0 ^a	16.8 ^b	1663 ^{ab}	Clay Loam
	CT-A	4.8 ^a	3.9 ^a	45.1 ^a	1.0 ^b	5.1 ^{abc}	26.8 ^a	15.3 ^{ab}	1526 ^{ab}	Clay Loam
240	NT	6.3 ^{bc}	5.3 ^{bc}	68.0 ^{ab}	0.1 ^a	7.8 ^{bcd}	80.8 ^b	14.0 ^{ab}	1706 ^{ab}	Clay Loam
	CT-Y5	5.6 ^{ab}	4.4 ^{ab}	63.7 ^{ab}	0.2 ^a	3.3 ^a	34.0 ^a	15.3 ^{ab}	1343 ^a	Clay Loam
	CT-A	5.9 ^{bc}	4.8 ^{bc}	51.3 ^{ab}	0.1 ^a	5.8 ^{abcd}	35.9 ^a	13.7 ^{ab}	1415 ^{ab}	Clay Loam

Rates- different UREA fertilizer application rates; NT-Zero tillage; CT-Y5-Ploughing on the 5th year; CT-A-ploughing annual; EC-Electrical conductivity; Similar letters on the same columnar there is no significance (P<0.05) whereas different letters indicatessignificant difference (P<0.05).

3.3.3 Soil properties at 20-30 cm depth

Soil moisture was also highest in NT in the control and at 60 kg N ha⁻¹, while at 120 and 240 kg N ha⁻¹, it was highest in CT-Y5, ($p < 0.05$). Exchangeable bases were highest at 60 N ha⁻¹ in NT (6.45 cmol_c/kg) and CT-ANNUAL (6.11 cmol_c/kg) treatments, while they were lowest in all CT-Y5 treatments ($p < 0.05$). Table 3.3 shows that exchangeable acidity was highest in NT and CT-ANNUAL in the control as well as in CT-ANNUAL at 120 kg N ha⁻¹, while it was lowest in NT at 120 kg N ha⁻¹ than all the other treatments ($p < 0.05$). Soil pH_{water} was highest in NT (6.46) at 60 kg N ha⁻¹ compared to CT-ANNUAL (5.15) at 120 kg N ha⁻¹ ($p < 0.05$).

Table 3. 3: Soil characteristics under different tillage systems and fertilizer application rates at 20-30 cm depth.

Rates (kg N ha ⁻¹)	Tillage	pH_w	pH_{KCl}	EC (mSm ⁻¹)	Acidity (cmolc kg ⁻¹)	Bases (cmolc kg ⁻¹)	C:N	Soil moisture (%)	Bulk Density (kg m ⁻³)	Textural Class
0	NT	5.3 ^{ab}	4.1 ^{ab}	76.1 ^a	1.0 ^f	4.3 ^{abcde}	60.5 ^a	17.4 ^d	1918 ^{ab}	Clay Loam
	CT-Y5	5.5 ^{abc}	4.3 ^c	38.5 ^a	0.3 ^{abcd}	3.6 ^{abcd}	46.4 ^a	16.6 ^{cd}	1762 ^{ab}	Clay Loam
	CT-A	5.9 ^{abcd}	4.6 ^d	139.2 ^a	0.9 ^{ef}	4.7 ^{bcde}	57.8 ^a	16.6 ^{cd}	1602 ^{ab}	Clay Loam
60	NT	6.5 ^d	5.0 ^f	39.9 ^a	0.1 ^a	6.5 ^e	27.8 ^a	16.6 ^{cd}	1994 ^b	Clay Loam
	CT-Y5	5.6 ^{abc}	4.3 ^c	62.5 ^a	0.4 ^{bcd}	2.8 ^{ab}	33.3 ^a	15.2 ^{abc}	1779 ^{ab}	Clay Loam
	CT-A	5.6 ^{abc}	4.7 ^e	44.4 ^a	0.4 ^{bcd}	6.1 ^e	50.6 ^a	15.6 ^{bcd}	1734 ^{ab}	Clay Loam
120	NT	5.9 ^{bcd}	4.9 ^e	72.8 ^a	0.1 ^a	5.8 ^{de}	24.1 ^a	15.5 ^{abcd}	1878 ^{ab}	Clay Loam
	CT-Y5	5.3 ^{ab}	3.9 ^a	38.2 ^a	0.6 ^{de}	1.9 ^a	77.2 ^a	16.7 ^{cd}	1516 ^a	Clay Loam
	CT-A	5.2 ^a	4.2 ^{bc}	47.8 ^a	0.9 ^{ef}	2.9 ^{ab}	81.4 ^a	15.3 ^{abc}	1523 ^a	Clay Loam
240	NT	5.5 ^{abc}	4.6 ^d	85.9 ^a	0.1 ^a	5.3 ^{cde}	104.3 ^a	13.6 ^a	1746 ^{ab}	Clay Loam
	CT-Y5	5.3 ^{abc}	4.3 ^c	41.2 ^a	0.5 ^{cde}	2.3 ^a	37.1 ^a	15.7 ^{bcd}	1648 ^{ab}	Clay Loam
	CT-A	6.1 ^{bcd}	5.0 ^f	136.7 ^a	0.1 ^a	3.2 ^{abc}	73.0 ^a	13.8 ^{ab}	1737 ^{ab}	Clay Loam

Rates- different UREA fertilizer application rates; NT-Zero tillage; CT-Y5-Ploughing on the 5th year; CT-A-ploughing annual; EC-Electrical conductivity; Similar letters on the same columnar there is no significance (P<0.05) whereas different letters indicates significant difference (P<0.05).

3.4. Discussion

The soil texture in all treatments was clay loam. This is attributed to the fact that texture is an inherent soil property that does not change much. However, in a study by Tollner et al., (1984), CT produced a texturally homogeneous soil on a sandy loam only in the upper soil, while NT preserved the soil's texture in the whole profile. Soil texture affects aggregation of particles and pore-size distribution, ultimately controlling microbial activity and organic matter mineralization in surface soil layers (Six et al., 2004). Thus aggregates are less vulnerable to breakdown when the soil has higher amounts of clay (Edwards & Bremner, 1967) and/or SOM (Lehrsch et al., 1991). Lehrsch, (1998) observed the highest macro aggregate stability and pore size distribution in the soil with highest clay than sand content. According to Hill, (1990), the changes in soil physical properties as a result of shifting from mouldboard ploughing to conservation tillage might be expected to develop slowly after the initiation of conservation tillage. In the present study tillage, fertilizer application and soil depth significantly affected bulk density, soil moisture, bases, exchangeable acidity and pH. Thus soil bulk density generally increased with depth in most treatments. Higher bulk density values are normally expected at lower layers of the soil profile due to the overburden pressure of the upper layer (Bronick & Lal, 2005). Hill, (1990) also observed similar increases of bulk density with increasing depth for NT, while the opposite was true for CT, and this was attributed to higher soil strength for NT compared to CT.

Low soil bulk density under CT-Y5 treatment in 0-10 cm depth may be attributed to the increase of OM. Unlike NT and CT-ANNUAL which may have high penetration resistance and less organic material accumulation, respectively, in CT-Y5 there was incorporation of plant residues during tillage on the 5th year hence soil physical characteristics improves. Bulk density of the soil in NT may increase due to the repeated passes of planters and the lack of the loosening action of tillage (Lampurlane' s & Cantero-Martí'nez, 2003). Hill, (1990) also reported that continuous conservation tillage may increase bulk density and soil strength, thereby inhibiting plant root growth. In the current study the bulk density under CT-Y5 at 0-10 cm depth was lower in the control treatment compared to fertilized treatment. This concurs with the findings of Celik et al., (2010) where mineral fertilizer application resulted in greater bulk density than that of unfertilized soil because fertilizers can cement soil particles. Low bulk density under CT-Y5 maybe attributed to organic components which have a dilution effect in

lowering bulk density. The mixing of organic materials with more dense mineral fractions of soils causes a decrease in bulk density (Bronick & Lal, 2005). The increase in OM content results in greater total porosity and lowers soil bulk density (Tejada et al., 2008). The bulk density results of this experiment are similar to other findings where bulk density of sub-surface layers of 10-20 and 20-30 cm soil depths of NT were higher compared to CT in some treatments (Yang & Kay, 2001). According to Varsa et al., (1997) bulk density was greatly reduced in deep tilled plots compared with NT treatments due to diminishing of penetration resistance.

According to Celik et al., (2010) since organic materials have low bulk density and higher porosity, addition of OM to soil through residue incorporation improves soil physical properties. Again fertilizer application may induce mineralization of OM and subsequently acidification of soil thus higher fertilizer application rates may result in acidic soils with less cationic bases. The bases under NT and exchangeable acidity under CT-Y5, in Table 3.1, decrease and increase, respectively, with the increase in fertilizer application. High mineralization of OM leads to lower bases and higher exchangeable acidity because OM serves as the reservoir for plant nutrients. Contrary, Celik et al., (2010) observed that OM in the 0-15 cm depth slightly increased with mineral fertilizer application as compared to the control, due to increased plant biomass production with the addition of plant nutrition. OM addition in the soil is a favourable way of improving soil properties by providing a favourable soil structure, enhancing soil cation exchange capacity, increasing the quantity and availability of plant nutrients, and providing the substrate for microbial activities (Miransari, 2011).

Residue accumulation under NT increased soil moisture content, which is in association with improved pore spaces and reduced water evaporation from the soil surface. NT in the control and at 60 kg N ha⁻¹, as well as CT-Y5 in the control and at 120 kg N ha⁻¹ had higher soil moisture in the 0-10 cm soil depth. Both NT and CT-Y5 had residue cover, which encourages infiltration thus reducing runoff and evaporation from the soil surface. NT, had greater soil water storage (by reducing run-off and evapotranspiration, and consequently leaving more water in soil for plant use) relative to CT due to crop residue retention (Malhi & Lemke, 2007). The highest fertilizer application rate (240 kg N ha⁻¹), had the lowest soil moisture in all tillage treatments at 0-10 cm soil depth. High soil moisture content in the control treatment compared to fertilized treatments may be due to better crop biomass under fertilized treatments which extract more water for its biochemical processes. High fertilizer application rates result in high

exchangeable acidity and low bases which may have a detrimental effect on crop productivity. Research has shown that crop residue under NT, conserve soil moisture, maintain or increase OM and improve crop production (Halvorson et al., 2002). A similar trend was observed in the 20-30 cm soil depth under NT treatment, where soil moisture decreased with increasing fertilizer application rate. According to Malhi & Lemke, (2007) root mass and straw yield increases with the first and second increment of 40 kg N ha⁻¹, this indicated that the major response of straw and root mass parameters to applied N was with the initial increment rate. This elucidate high soil moisture retention in the current study which can be due to increased crop residue retention under low N application rates. Furthermore, NT can increase water storage replenishment of deeper layers compared with CT (Bonfil et al., 1999), which was observed between NT and CT-ANNUAL in the present study in 20-30 cm depth. Unlike CT-ANNUAL, soil moisture of CT-Y5 at 0 and 240 kg N ha⁻¹ in 10-20 cm, and CT-Y5 at 240 kg N ha⁻¹ in 20-30 were higher than NT. This agrees with the findings of Malhi et al., (2006) which shown that minimum tillage and retaining straw often improved the capacity of soil to store water. Gantzer & Blacke, (1978) reported an increase of earthworm population and biochannels in NT system compared with CT, resulting in higher infiltration of water. Continuous practise of NT may have a detrimental effect on soil infiltration however, as penetration resistance might increase. Hence tilling after few seasons may enhance great soil moisture content as was the case under CT-Y5 at higher N rates.

N fertilizers have generally been known to progressively acidify soil especially where N sources are eventually nitrified in the soil subsequently soil acidity develop. There was a general decrease in pH and bases with the increase in fertilizer application rate and depth. This was similar to the findings by Matowo et al., (1997) who observe that bases decreased as the N rate increased for samples from 5-25 cm depth, which was likely a result of increased crop uptake. The higher concentration of bases of NT at 0-10 cm, may be attributed to the accumulation of residue, less soil disturbance and less decomposition of organic material. Ismail et al., (1994) also found decreases in soil pH as N rate increased for soil samples collected down to 30 cm in NT and CT which was attributed to nitrification of NH₄⁺ fertilizer. Similarly, CT-Y5 at 240 kg N ha⁻¹ in 0-10 cm had significant higher exchangeable acidity than other treatments. CT-Y5 at higher fertilizer application rate may have stimulated mineralization and translocation of bases, due to availability of crop residues, thus in the process increasing exchangeable acidity and lowering pH. According to Matowo et al., (1999) lack of incorporating

fertilizer and crop residue under NT may have caused significant difference of bases with minimum tillage, in this instance CT-Y5. This is likely to reflect incorporation of the fertilizer and subsequent acidification of the soil layers near the surface under minimum tillage as compared to NT where no such mixing occurs (Matowo et al., 1999).

Higher fertilizer rates under CT-Y5 intensify crop residue decomposition, depleting base concentration at 0-10 cm. Decomposition increases aluminium saturation thus decreasing pH (van Breemen et al., 1983). The pH decreases with increasing N rates because of the acidity produced from nitrification (Ismail et al., 1994). High exchangeable acidity and low pH under CT-Y5 at 240 kg N ha⁻¹ in 0-10 cm depth may be attributed to decomposition of residues during ploughing which subsequently releases organic acids. CT-ANNUAL at 10-20 cm depth and 120 kg N ha⁻¹ had the highest exchangeable acidity and lowest pH. This may be attributed to ploughing and supply of optimum fertilizer of 120 kg N ha⁻¹ compared to lower rates (0 and 60 kg N ha⁻¹) and higher rate (240 kg N ha⁻¹) for microbes to function well, therefore intensification of nitrification. Meta-analyses by Lu et al., (2011) suggest that both decreasing and increasing N inputs suppress soil microorganisms. When urea fertilizer is applied at high rates can inhibit soil microorganisms due to toxicity of ammonia, decreases in pH and increases in ionic strength (Omar & Ismail, 1999), which is similar to the trend of the current results. Barabasz et al., (2002) reported that mineral N fertilization at rates exceeding 120 kg N ha⁻¹ reduced the number of bacteria by 50% on average and complete eradication of bacterial function. Contrary, Lupwayi et al., (2011) reported that application of 120 kg N ha⁻¹, tended to decrease microbial biomass and functional diversity.

According to Blevins et al., (1977) ploughing aerates the soil causing oxidation of OM thus releasing H⁺ ions. Whereas, Matowo et al., (1999) observed that the effects of N rate diminished with depth for both NT and CT treatments, with the greatest decreases in pH at 10-15 cm for CT and at 15-20 cm for NT. These corresponded to the zone with the lowest extractable bases, suggesting that the zone of maximum root activity was slightly deeper for NT than CT (Matowo et al., 1999). Tillage and N fertilization is associated with a decrease in soil pH and depletion of bases in the current study. Lower soil pH under CT-Y5 especially at 0-10 cm depth may be attributed to the formation of organic acids during decomposition of organic material, nitrification of NH₄⁺ fertilizer and mineralization of crop residues (Ismail et al., 1994). At the 0-10 cm depth, EC was higher in NT in the control and at 60 kg N ha⁻¹

compared to higher N application rates. EC trend is similar to soil moisture on the surface whereas on lower depth the trend is different with soil moisture. This may be due to high concentration of soluble ions on the surface which water can not all dilute because of abundance of organic compounds, whereas at 10-20 cm depth soluble ions were diluted by water. Adviento-Borbe et al., (2006) reported that application of high amounts of fertilizers may cause an accumulation of soluble salts in agricultural soils. In the 10-20 cm depth EC of all the application rates, except for 240 kg N ha⁻¹, had an inverse concentration with soil moisture content. This concurs with the findings of Adviento-Borbe et al., (2006) that soil water content influences soil EC through the concentration of dissolved ions in the soil, when soil water content is high, dissolved ions (solutes) are diluted; when soil water content is low, dissolved ions are concentrated. Also significant lower EC under CT-ANNUAL compared to NT at both the control and 60 kg N ha⁻¹ in 0-10 cm may be attributed to the increase in drainage due to ploughing therefore most of the salts may have leached to subsoil. According to Eigenberg et al., (2002) N fertilizers, unlike manure and composts, tended to have the lowest soil conductivity and least residual effect after application. Generally, soil EC becomes lower with NT due to increased water movement and higher soil aggregation (Dalal, 1989), these findings were contrary to the current study especially in the control treatment where CT-ANNUAL had lower EC at both 0-10 and 20-30 cm depth.

3.5 Conclusion

The soils in the area were from the same parent material and under the same climatic conditions, hence the similarity. The lower bulk density of CT-Y5 in the control at 0-10 and 10-20 cm depth than NT and CT-ANNUAL, may be due to incorporation and intensifying decomposition of crop residue during tillage after four seasons of omitting tillage. However, bulk density of CT-ANNUAL decreased with the increase of depth especially at 20-30 cm depth. Again, bulk density of CT-ANNUAL decreased with the increase of N fertilizer rates at both both 0-10 and 10-20 cm depth. This may be attributed to the incorporation of fertilizer with soil thus improving pore space which directly lowers bulk density. Mixing of organic material with soil during ploughing might have played a salient role in lowering bulk density under CT-Y5 at 0-10 and 10-20 cm depths and under CT-ANNUAL at 20-30 cm. The lack of soil loosening under NT increases bulk density, soil resistivity and penetration at 10-20 and 20-30

cm in some treatments. This may be attributed to continuous accumulation of residual load and use of heavy machineries in planting without loosening the soil.

Bases were higher under NT across all application rates and soil depths because of accumulation of crop residues. While the control treatment under NT had high soil moisture at all depths (except at 10-20 cm), than CT-Y5 and CT-ANNUAL which may be attributed to reduced mineralization of organic compounds compared to fertilized treatments henceforth it has better surface cover by crop residue which in turn reduces evaporation and increases infiltration. High rate of N fertilization mostly led to decrease in pH and reduction in bases. The pH decrease could be a result of acidification created during N fertilizer nitrification. Higher exchangeable acidity and low pH in 240 kg N ha⁻¹ under CT-Y5 at 0-10 cm depth may be due to both decomposition of organic compounds during ploughing which releases organic acids and nitrification of high amount of N fertilizer because of high quantity application. Further, higher exchangeable acidity and low pH in 10-20 cm depth under CT-ANNUAL is attributed to ploughing and incorporation of organic material to the subsoil. It is recommended to practise NT and apply an optimum fertilizer rate of 60 kg N ha⁻¹ to avoid the depletion of soil physicochemical characteristics especially in the 0-10 cm depth. This is because soil moisture, bases, exchangeable acidity, EC and pH of this combination treatment (NT at 60 kg ha⁻¹) had better results at 0-10 cm depth. However because of site-specificity, tillage and N fertilizer investigations need to be carried out under different soil and climatic conditions on a long-term basis.

CHAPTER 4

TILLAGE AND UREA FERTILIZER APPLICATION IMPACTS ON SOIL C FRACTIONS AND SEQUESTRATION

4.1 Introduction

Soil can function as a source or sink of atmospheric C, and may have an important role in decreasing the build-up of atmospheric GHGs, thereby aiding in mitigating global climate change (Johnson & Kern, 1991). Farming practices such as conservation tillage, which allow soil to be a sink of atmospheric C must be intensified on agricultural land. Intensive and continual ploughing have caused enormous losses of SOC and N pools as GHGs such as CO₂, CH₄ and N₂O to the atmosphere (Blanco-Canqui & Lal, 2008). It is estimated that as much as 60% of SOC in temperate and 75% in the tropical regions has been depleted by CT, contributing about 23% of the total GHGs concentration in the atmosphere (Lal, 2004). Tillage is generally considered to increase SOC mineralization due to the mechanical and rain-induced disruption of soil aggregates and the consequent release of CO₂ (Chenu et al., 2019). Therefore NT has been considered more suitable to maintaining SOC stocks compared with CT.

The results of recent global meta-analyses confirm that SOC stocks increase in the upper soil layers (0-15 or 0-20 cm) of NT, but has low to no effect on SOC stocks below the 30 cm depth (Haddaway et al., 2017; Meurer et al., 2018). Aguilera et al., (2013) suggested that NT practices resulted in net accumulation of C in the surface layers, while there was net loss in deeper layers. Therefore, shallow sampling under NT may imply an overestimation of SOC gains in those treatments, given that losses occurring at deeper soil layers are not accounted for. Unlike topsoil organic C which is prone to rapid perturbations and decomposition by increased near-surface microbial activity, and high fluctuations in soil temperature or moisture regimes, subsoil organic C is protected inside aggregates and has lower turnover rates (Lorenz & Lal, 2005). The amount of SOC stored in deeper layers is the most important fraction for long-term soil C sequestration (Blanco-Canqui & Lal, 2008). Thus Chenu et al., (2019) postulated that under NT, there may be additional SOC storage in superficial soil layers, but little to no SOC sequestration for the land unit if the whole soil profile is considered. This seems to be particularly the case in humid and temperate regions (Dimassi et al., 2014), as opposed to drier

semi-arid climates, where significant benefits of NT relative to CT are observed. Helgason et al., (2014) observed that under temperate and tropical climatic conditions the decomposition of C residues was the same whether the residues were incorporated or not, whereas under semi-arid conditions decomposition was greater when residues were incorporated than left on the surface. Dimassi et al., (2014) also showed that the response of SOC to NT is dependent on climate, in particular precipitation, with a greater response in drier conditions. This is because dry years allow C sequestration in NT system due to a greater reduction in SOC mineralization compared to CT (Dimassi et al., 2014). Therefore in dryland agriculture, especially in semi-arid regions of Sub-Saharan Africa, conservational tillage can be practised as low cost agriculture which will assist in soil C sequestration.

Carbon sequestration can be defined as increase in C storage in soil or plant material (Kern & Johnson, 1993). Whereas, the C sequestration potential of a given soil would be the maximum gain in SOC allowing a net removal of CO₂ from the atmosphere under a given climate and for a specified timeline (Chenu et al., 2019). Some argue that only very recalcitrant C should be regarded as sequestered C; however, soil C varies in degree of permanence (or in its residence time). Furthermore different C fractions such as particulate organic C (POC), microbial biomass C (MBC), soil organic C (SOC) and permanganate oxidizable C (POXC) have preference for various soil characteristics showing different residence time in the soil. The labile soil organic C fraction is a relatively small fraction of total C that has a short half-life, and responds quickly to changes in soil tillage and fertilization practices (Magdoff & Weil, 2004). It is an important component of soil quality because of its soil aggregate stabilization effect and direct link to soil C or N mineralization (Gunapala & Scow, 1998). An ability to predict or detect soil organic C changes at early stages of land-use or management is important to allow land managers to make informed decisions that reduce soil fertility decline, erosion, and GHG emissions (Skjemstad et al., 2006).

According to Chenu et al., (2019) increasing SOC stocks is not necessarily simple or even possible at all locations. Thus, it can be more difficult to increase C inputs at some places due to limited access to necessary resources such as fertilizer or water. In a meta-analysis of the effects of NT on SOC stocks, increased organic C inputs were the only factor explaining additional SOC storage under NT (Virto et al., 2012). Adding N fertilizer usually results in increased crop production, C inputs, and may therefore increase C sequestration in soils

(Hutchinson et al., 2007). Lu et al., (2011) observed a 3.48% relative increase of SOC stocks with N fertilization in agricultural soils in a meta-analysis with 340 paired observations. Little work has been documented on the labile C fractions of Sub-Saharan and South Africa to be specific, hence this work focused on C fractions under different management systems in a dryland maize monocrop in KwaZulu Natal, South Africa. The null hypothesis was that an increase in fertilizer application rate and minimisation of tillage will decrease soil C pools. The objectives of the study were to:

1. Assess the impact of different tillage techniques and fertilizer application on soil C fractions.
2. To assess impact of soil depth on C pools under different tillage systems.

4.2 Material and methods

The study site located in Loskop area, Figure 4.1, was described in detail in the previous chapter (Section 3.2).

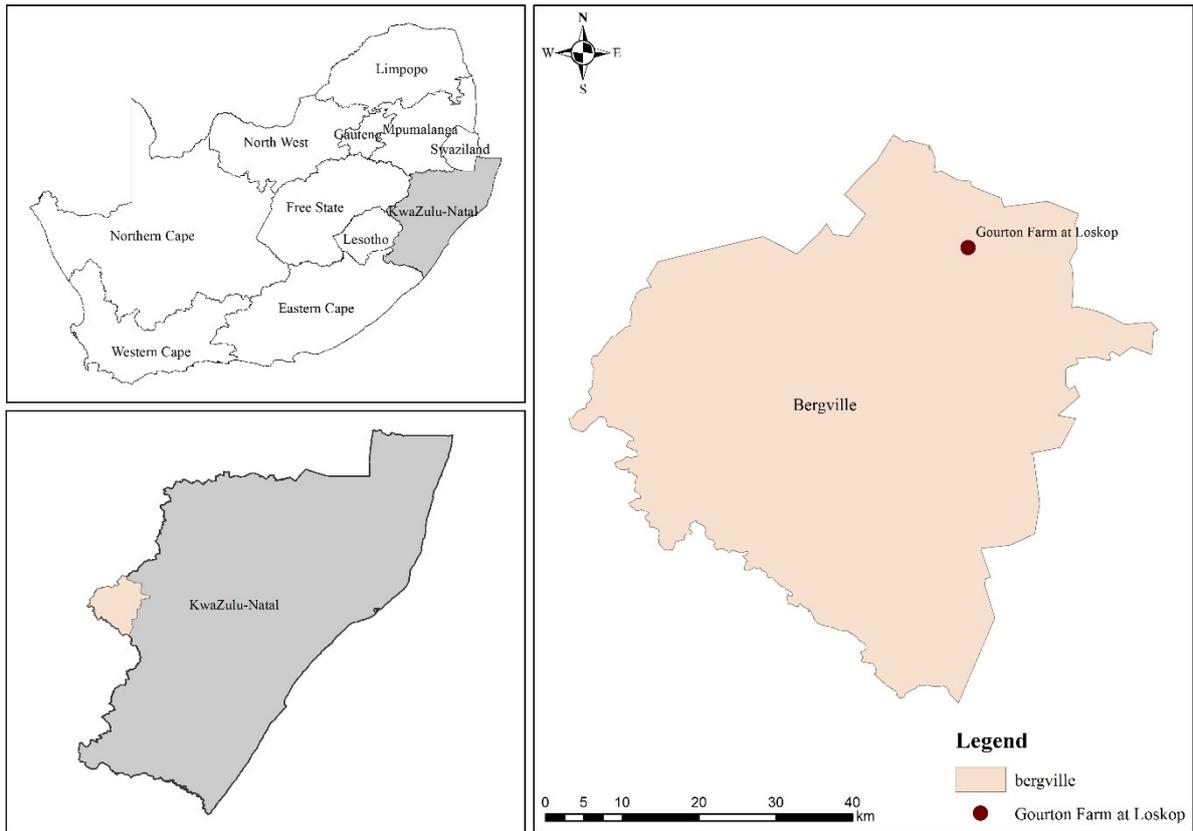


Figure 4. 1: The experimental site at Loskop in Estcourt, KwaZulu Natal Province, South Africa.

4.2.1 Sampling procedure

The soil sampling procedure was described in detail in the previous chapter (Section 3.2.1).

4.2.2 Laboratory Analyses

4.2.2.1 Total carbon

Analysis of total C was described in detail in Chapter 3, Section 3.2.2.2.

4.2.2.2 Soil organic carbon (SOC)

SOC was determined using the Walkey-Black method (van Reeuwijk, 2002). Finely grounded soil (1.0 g) was weighed into a 500 mL Erlenmeyer flask, and 10 mL of 0.167 M $K_2Cr_2O_7$ solution was added. The flask was gently swirled, then 20 mL of H_2SO_4 was added rapidly with continuous swirling and allowed to cool for 30 minutes. De-ionized water (150 mL), 10 mL of concentrated ortho-phosphoric acid and 1 mL of barium diphenylamine sulphonate indicator were also added. The dichromate was back titrated with 0.5 M ferrous ammonium sulphate solution. At the end point the colour changed sharply to green (van Reeuwijk, 2002). The formula below by van Reeuwijk, (2002) was used to calculate organic C.

$$\text{Organic C (\%)} = \frac{0.003g * N * 10 \text{ mL} * \left(1 - \frac{T}{S}\right) * 100}{ODW}$$
$$= \frac{3 \left(1 - \frac{T}{S}\right)}{ODW}$$

Where;

N = Normality of $K_2Cr_2O_7$ solution

T = Volume of $FeSO_4$ used in sample titration (mL)

S = Volume of $FeSO_4$ used in blank titration (mL)

ODW = Oven-dry sample weight (g)

Then C stock was calculated from organic C using soil bulk density and sampling depth:

$$\text{Soil C stock} = \text{Organic C (g C kg}^{-1} \text{ soil)} * \text{bulk density (kg m}^{-3}) * \text{soil depth (m)}$$

The Soil C stocks was finally obtained in $kg \text{ ha}^{-1}$ for the whole total profile depth of 0.3 m.

4.2.2.3 Particulate organic carbon (POC)

Wet sieving was used to determine POC, with a sieve set of 2 mm, 250 and 53 μm . Sodium hexametaphosphate was used as a dispersal agent to dispense 50 g of fresh soil. According to Elliot et al., (1991) wet soil is recommended in order to reduce changes in soil structural properties resulting from air drying the soil. Dispersed soil was wet sieved with distilled water, then after 20 minutes sample fractions from the 250 and 50 μm sieves were collected (Elliot et al., 1991), for oven drying for 24 hours at 65 $^{\circ}\text{C}$. The mass of oven dried samples was weighed and total C determined using LECO TruMac CNS/NS Carbon/Nitrogen/Sulfur Determinator (LECO Corporation, 2012). The POC was calculated according to Elliot et al., (1991) as follows:

$$\text{POC} = (DW_{53\mu\text{m}-250\mu\text{m}}) * \left(\frac{\% \text{C}}{100}\right)$$

Where;

DW- weight of the fresh soil used (50 g)

53- 53 μm sieve

250- 250 μm sieve

4.2.2.3 Permanganate oxidizable carbon (POXC)

Air-dried soil (2.5 g), was weighed into a 50 mL centrifuge tube and 2 mL of 0.2 M KMnO_4 and 18 mL of distilled water were added. The centrifuge tube was tightly closed and hand shaken vigorously for 2 minutes to ensure soil dispersion within the solution. After that, the tubes were put on a shaker and shaken at 240 oscillations for another 2 minutes. Samples were then removed from the shaker and swirled to ensure that there was no soil clinging to the sides or cap of the tube. Caps were removed to avoid further soil disturbance after settling. Samples were placed in a dark area to allow soil to settle for ten minutes. After settling, about 0.5 mL of supernatant was extracted and mixed with 49.5 mL deionized water in a 50 mL centrifuge tube. This step was performed as quickly as possible as the permanganate will continue to react with soil as long as it remains in contact. The centrifuge tube was capped and inverted to mix. The standard concentrations of 0, 0.005, 0.01, 0.015 and 0.02 M were prepared from the KMnO_4 stock solution and standard curve was constructed using values of concentration of standards and absorbance values from spectrophotometer. The sample solution absorbance was

then read using a spectrophotometer set at 550 nm. According to Weil et al., (2003) the amount of C oxidized is a function of the quantity of permanganate reduced. Consequently, the higher the POXC values the lower the absorbance (intensity of the colour of the solution). The equation below was used to determine POXC, after Weil et al., (2003):

$$\text{POXC (mg kg}^{-1}\text{ soil)} = [0.02 \text{ mol/L} - (a + b \times \text{Abs})] \times (9000 \text{ mg C/ mol}) \times (0.02 \text{ L solution/ Wt)}$$

Where:

0.02 mol/L = initial solution concentration

a = intercept of the standard curve

b = slope of the standard curve

Abs = absorbance of sample

9000 = milligrams of carbon oxidized by 1 mole of MnO_4 changing from $\text{Mn}^{7+} \rightarrow \text{Mn}^{4+}$

0.02 L = volume of stock solution reacted

Wt = weight of air-dried soil sample in kg

4.2.2.4 Microbial biomass carbon (MBC)

Two 50 g soil samples (one to be fumigated and another an unfumigated control) that have been passed through a 2 mm sieve were weighed into 100 mL glass vials. Each sample was adjusted to 40% water holding capacity with distilled water. The sample to be fumigated was put together with a beaker containing 25 mL ethanol-free chloroform in a desiccator. The desiccator was evacuated until chloroform boiled vigorously for 2 minutes, then incubated in the dark for 24 hours. After incubation, the chloroform was removed by repeated evacuation about six times. Each of the fumigated and unfumigated glass vials were placed into separate 1L jars each containing 20 mL of 1M NaOH in a 50 mL beaker and 20 mL water at the bottom of the jar. The samples were then incubated in the dark for 10 days at 25 °C. The evolved CO_2 , from the incubation, was adsorbed by NaOH to produce Na_2CO_3 (Alef & Nannipieri, 1995). A 5 mL aliquot of the 1 M NaOH, from each incubation jar, was placed into a conical flask and then 1 mL of 1.5 M BaCl_2 solution was added to precipitate the carbonate as insoluble BaCO_3 , and a few drops of phenolphthalein indicator were added. The unreacted NaOH was slowly

titrated with 0.1 M HCl under a magnetic stirrer until the endpoint where the colour changes from pink to colourless. According to Alef & Nannipieri (1995) at this stage acid must be added slowly to prevent possible dissolution of the precipitated BaCO₃. Thereafter evolved CO₂ was calculated as follows;

$$\text{CO}_2\text{-C } (\mu\text{g g}^{-1} \text{ soil}) = (\text{B-S}) / [(M * E * A) / (\text{DW})]$$

Where:

B = Volume of acid needed to titrate the blank

S= Volume of acid needed to titrate the soil sample

M= Molarity of HCl

E= Equivalent weight to express the data as carbon

A= The ratio volume of NaOH to the NaOH aliquot.

DW= Dry weight of the soil sample (g) after moisture correction.

This was done for both fumigated and unfumigated samples.

Furthermore Biomass C was calculated according to Alef & Nannipieri (1995) using the formula below:

$$\text{Biomass C} = F_C / K_C$$

Where;

F_C = [CO₂- C evolved from fumigated] - [CO₂- C evolved from unfumigated soil]

K_C = 0.45 - A constant representing the fraction of the killed biomass mineralized to CO₂ over the incubation period.

Thereafter the microbial quotient (qMic) in the soil was calculated as ratios of MBC to SOC (Makova et al., 2011).

4.2.3 Statistical analyses

An analysis of variance (ANOVA) test was done to determine the effect of tillage and urea fertilizer application on soil C pools at various soil depths. The Fisher's protected LSD test was used as a post-hoc test for multiple comparison to compare treatment means and their interactions. The p value (<0.05) was used to test for significant differences between treatment factors. All tests were performed with GenStat 14.1 for Windows software (VSN international, 2011).

4.3 Results

4.3.1 Soil total C variations

Figure 4.2 shows that at 0-10 cm soil depth NT had higher total C ($p < 0.05$) for all application rates compared to CT-Y5 and CT-ANNUAL treatments. At 10-20 cm depth, the control (0 kg N ha^{-1}) of NT and CT-ANNUAL had higher total C i.e. 15.1 and 15.2 g C kg^{-1} , respectively, compared with CT-Y5 that had 13.7 g C kg^{-1} ($p < 0.05$). NT at 60 kg N ha^{-1} in 10-20 cm soil depth had higher ($p < 0.05$) total C (15.3 g C kg^{-1}) than CT-ANNUAL (13.8 g C kg^{-1}). While at 120 and 240 kg N ha^{-1} , CT-ANNUAL had higher ($p < 0.05$) total C than NT and CT-Y5 (Figure 4.2). In the 20-30 cm soil depth, NT had higher ($p < 0.05$) total C at 60 and 120 kg N ha^{-1} compared to CT-ANNUAL. Furthermore at 120 kg N ha^{-1} , CT-Y5 had higher total C ($p < 0.05$) of 11.2 g C kg^{-1} than CT-ANNUAL which had 9.9 g C kg^{-1} .

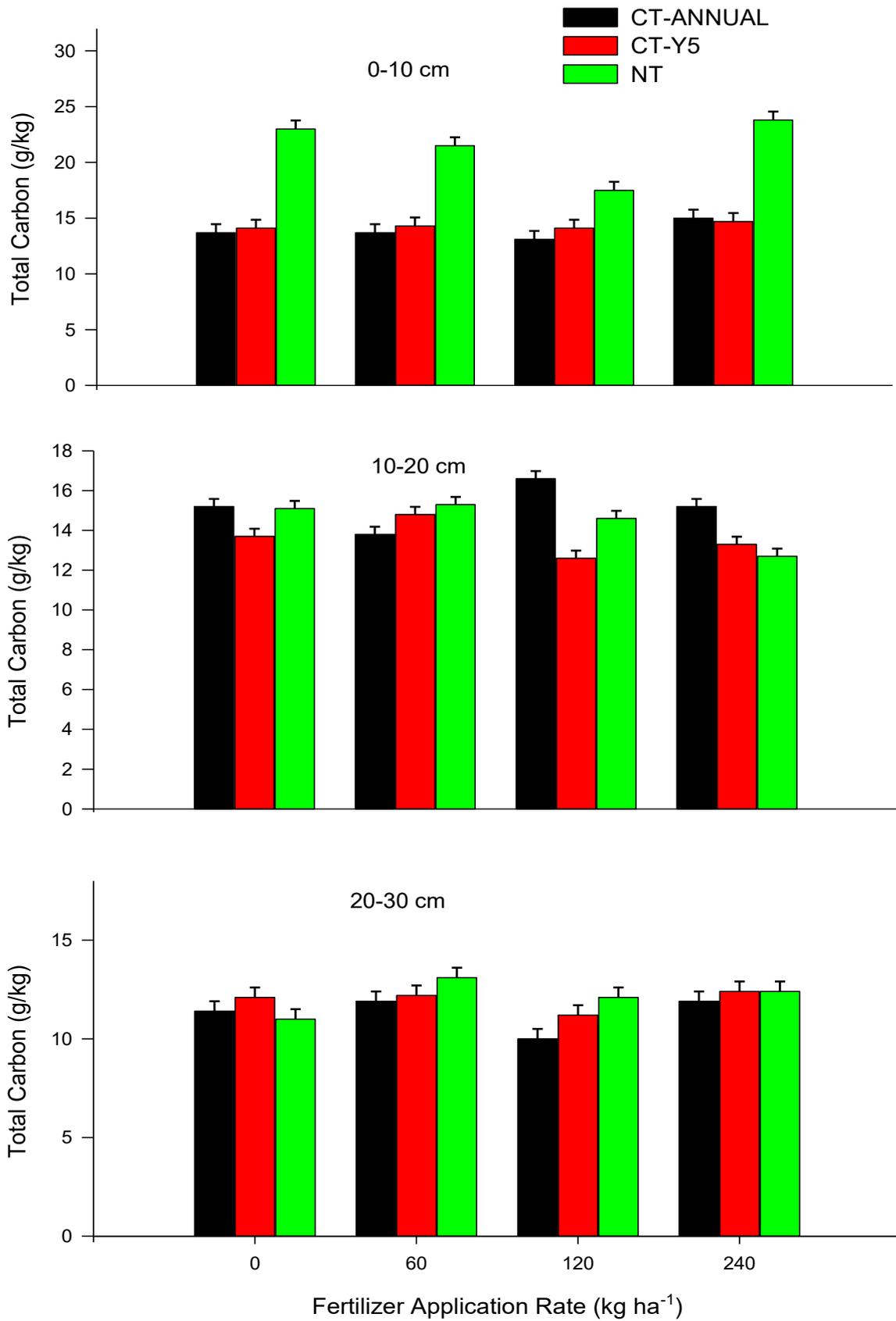


Figure 4. 2: Total carbon variation with soil depths for three tillage techniques.

4.3.2 Organic C variation in different treatments

Figure 4.3 shows that at 0-10 cm soil depth, NT had higher organic C than both CT-Y5 and CT-ANNUAL in all fertilizer application rates ($p < 0.05$). Both NT and CT-ANNUAL at 240 kg N ha⁻¹ in 20-30 cm soil depth, had higher organic C levels of 7.9 and 8.1 g C kg⁻¹ respectively, compared with CT-Y5 which had 2.4 g C kg⁻¹; while at 120 kg N ha⁻¹, CT-ANNUAL had the lowest organic C ($p < 0.05$).

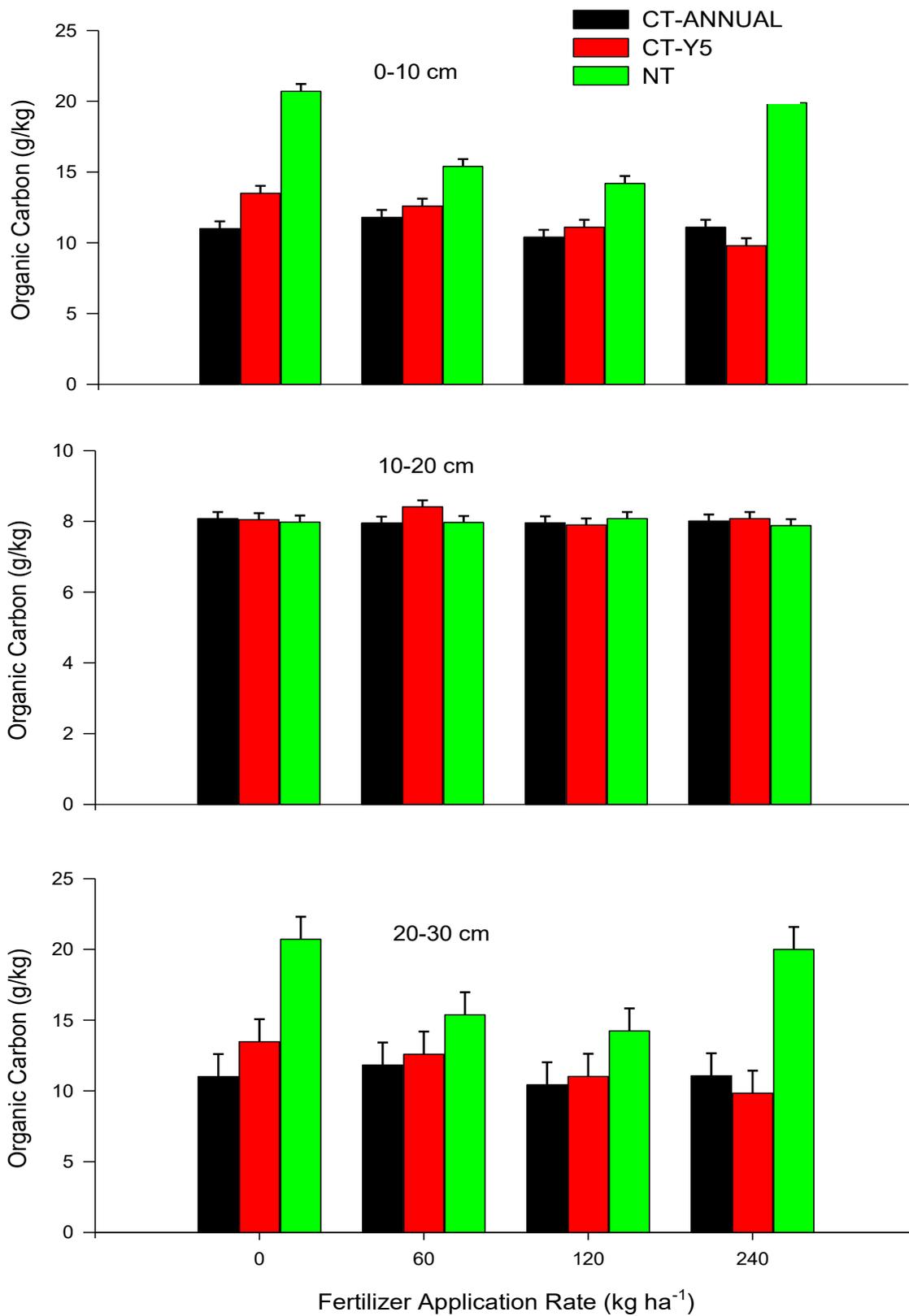


Figure 4. 3: Organic carbon with depths and urea fertilizer application (kg N ha⁻¹) for three tillage techniques.

4.3.3 POC variations in different treatments.

NT had the highest POC for all application rates ($p < 0.05$) at 0-10 cm soil depth (Figure 4.4). While at 120 and 240 kg N ha⁻¹, CT-Y5 had higher POC than CT-ANNUAL in the 0-10 cm soil depth ($p < 0.05$). At 10-20 cm soil depth, CT-ANNUAL had the lowest POC at 60 kg N ha⁻¹ ($p < 0.05$). On the contrary at 120 and 240 kg N ha⁻¹, CT-ANNUAL had the highest POC at this depth ($p < 0.05$). Figure 4.4 shows that in 20-30 cm soil depth, NT had the lowest POC at 60 kg N ha⁻¹ ($p < 0.05$).

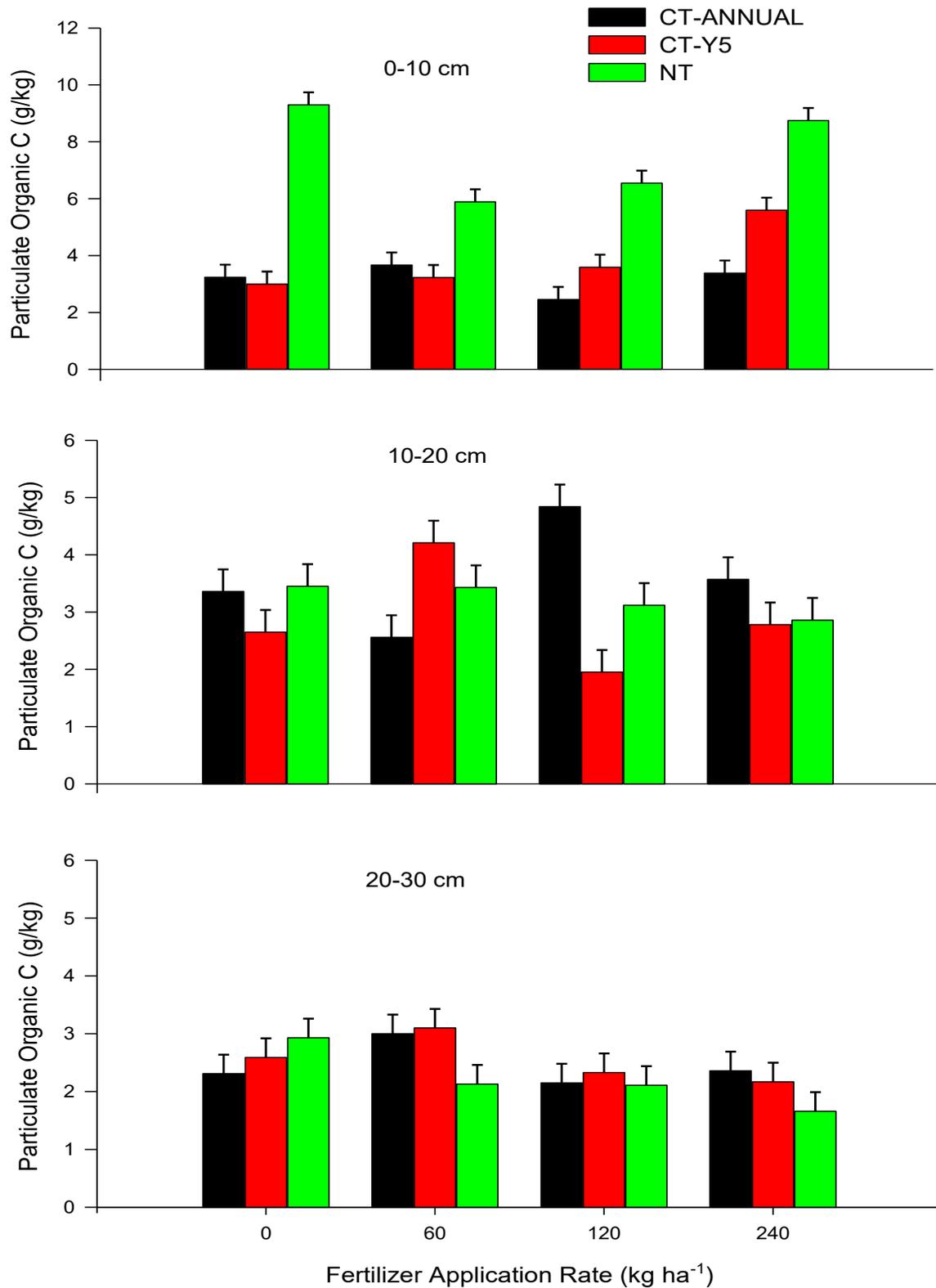


Figure 4. 4: Particulate organic carbon with soil depths and fertilizer application (kg N ha⁻¹) for three tillage techniques.

4.3.4 POXC on different tillage and N rates

Figure 4.5 shows that in the 0-10 cm soil depth, NT had higher POXC than CT-ANNUAL at 0, 60 and 120 kg N ha⁻¹ ($p < 0.05$). At 60 and 240 kg N ha⁻¹ application rates in 0-10 cm soil depth CT-Y5 had higher ($p < 0.05$) POXC than CT-ANNUAL but again it had lower POXC than NT. At 10-20 cm soil depth, NT had the highest POXC in the control, while at 60 kg N ha⁻¹, CT-Y5 (which was not different from NT) had higher POXC of 1.68 g C kg⁻¹ than CT-ANNUAL which had 1.60 g C kg⁻¹ ($p < 0.05$). Figure 4.5 shows that both 120 and 240 kg N ha⁻¹ application rates of CT-ANNUAL had higher POXC than CT-Y5 at 10-20 cm soil depth ($p < 0.05$). However at 20-30 cm soil depth, CT-Y5 had higher ($p < 0.05$) POXC (1.56 g C kg⁻¹) than CT-ANNUAL (1.51 g C kg⁻¹) in the control (Figure 4.5). Whereas at 120 kg N ha⁻¹ NT had higher POXC than CT-ANNUAL in the 20-30 cm soil depth ($p < 0.05$).

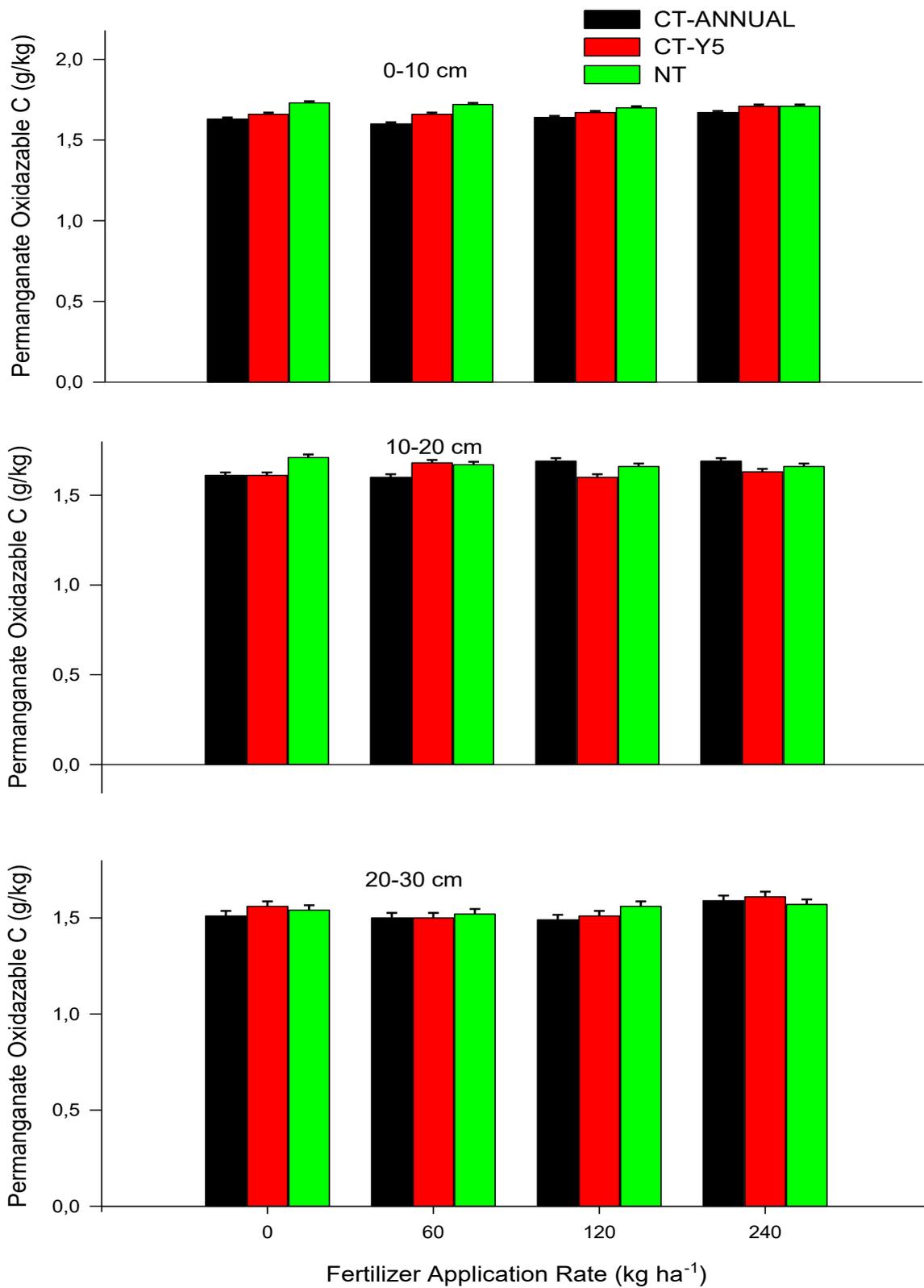


Figure 4. 5: Permanganate Oxidizable C with soil depths and urea fertilizer application (kg N ha⁻¹) for three tillage techniques.

4.3.5 Microbial biomass carbon (MBC) variations

CT-ANNUAL generally had the least microbial biomass C (MBC) at both 0-10 and 10-20 cm soil depths for most N application rates (Figure 4.6). For the most part NT and CT-Y5 did not significantly differ in MBC at these depths, with the exception of 240 kg N ha⁻¹ application rate at 0-10 cm where CT-Y5 recorded the highest MBC, as well as in the control at 10-20 cm depth where NT had higher MBC (0.46 g C kg⁻¹) than CT-ANNUAL (0.26 g C kg⁻¹) ($p < 0.05$).

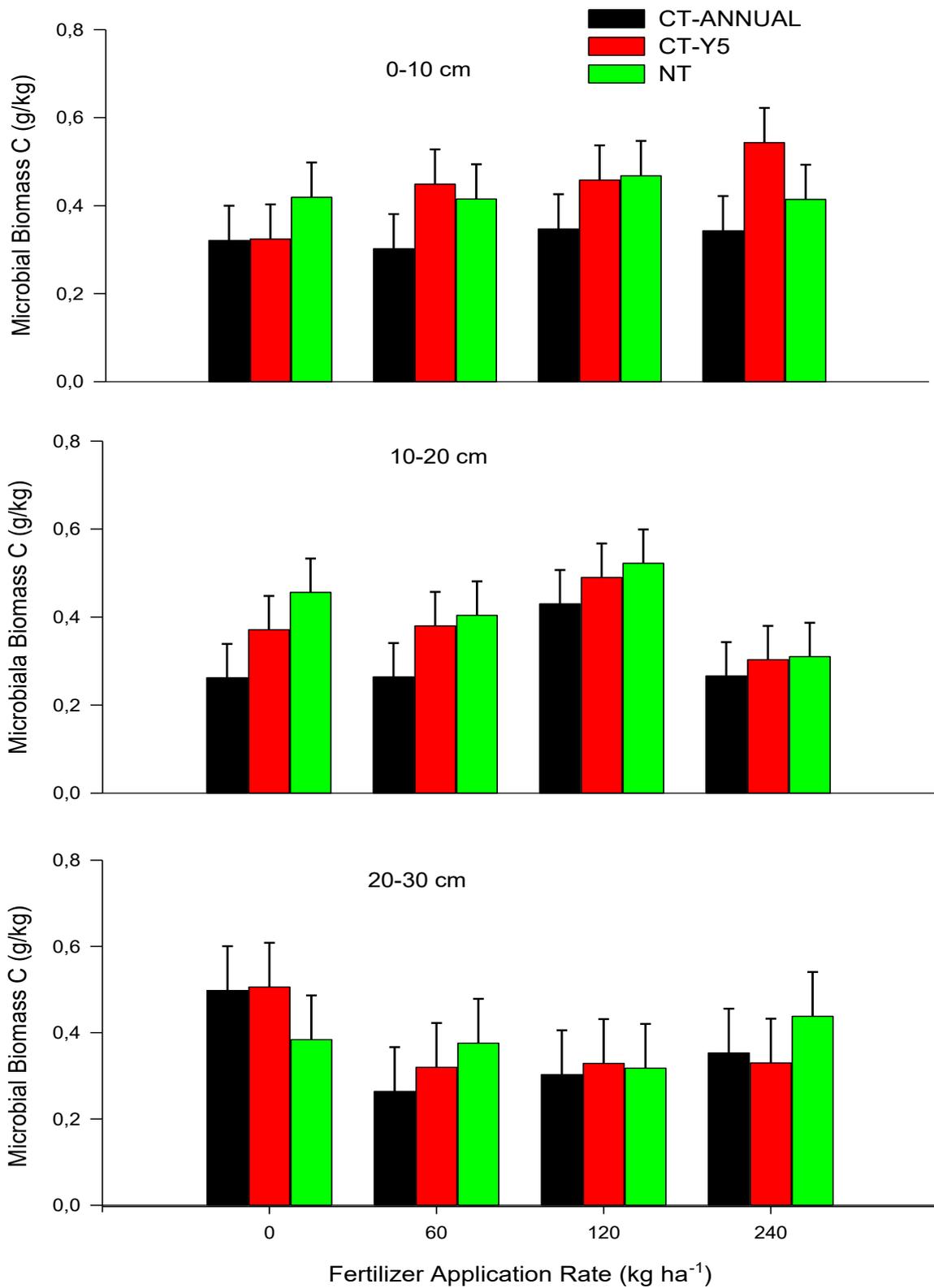


Figure 4. 6: Microbial Biomass C with soil depths and urea application (kg N ha⁻¹) for three tillage techniques.

4.3.6 Microbial quotient (qMic) variations

Figure 4.7 shows that qMic at 240 kg N ha⁻¹ in CT-Y5 was higher in all treatments in the 0-10 cm soil depth ($p < 0.05$). Further, in 0-10 cm depth CT-Y5 had higher qMic compared to NT at 120 kg N ha⁻¹ and CT-ANNUAL at 60 kg N ha⁻¹ ($p < 0.05$). In the control treatment of 0-10 cm depth CT-ANNUAL had higher qMic than NT ($p < 0.05$). Figure 4.7 shows that in 10-20 cm soil depth, NT had the highest qMic than both CT-Y5 and CT-ANNUAL at all application rates. In the control treatment of 10-20 cm depth CT-Y5 had lower qMic than NT but higher qMic than CT-ANNUAL ($p < 0.05$). Figure 4.7 shows that CT-Y5 at 240 kg N ha⁻¹ had highest qMic than all the treatments in 20-30 cm soil depth ($p < 0.05$).

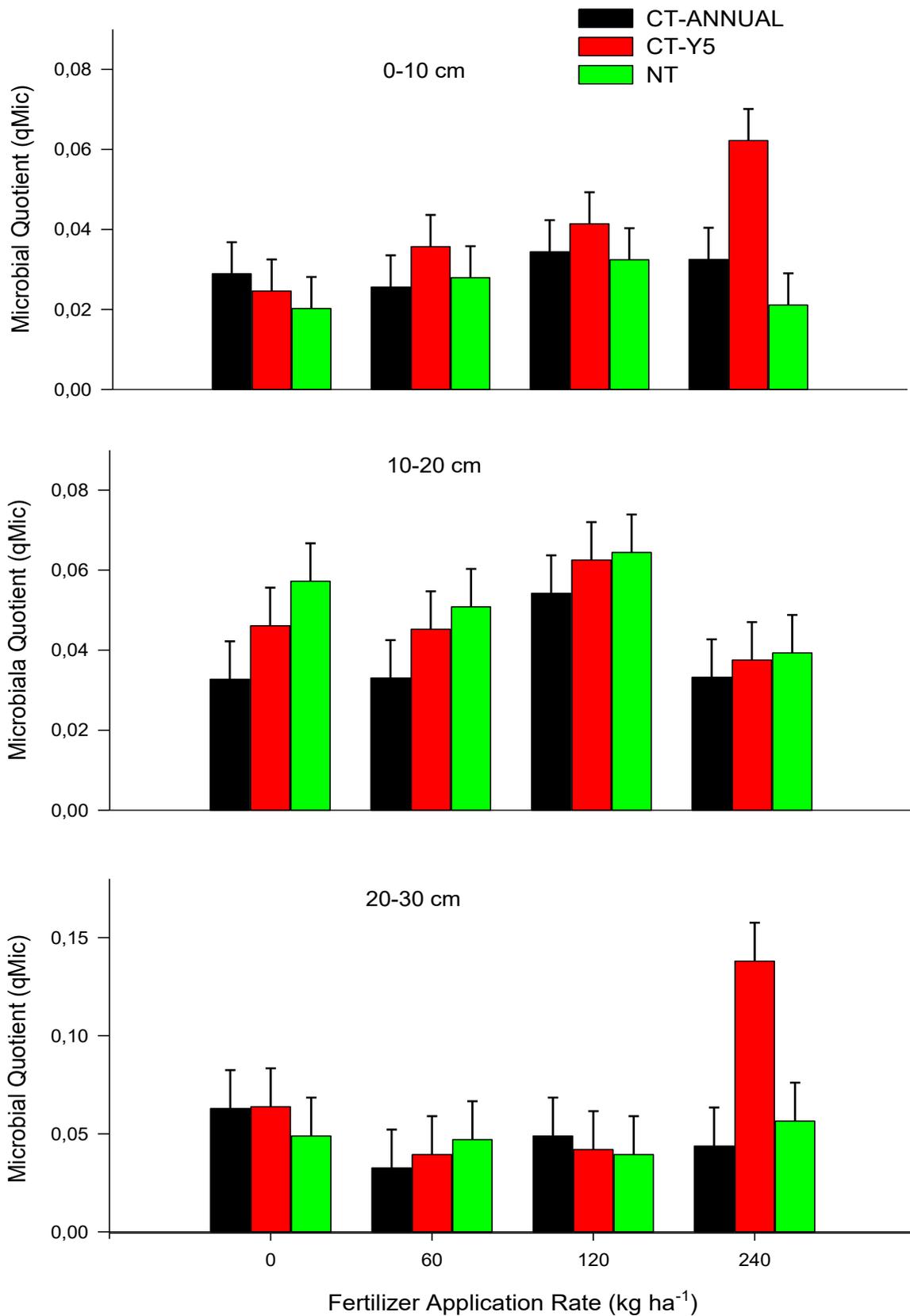


Figure 4. 7: Variation of microbial quotient of different soil depths.

4.3.6 Total soil carbon stocks

Total soil C stocks, depth 0-30 cm, of NT were higher ($p < 0.05$) in all treatments, except for CT-Y5 at 60 kg N ha⁻¹ (Table 4.1). At 120 kg N ha⁻¹ total C stocks of CT-Y5 in 0-30 cm was higher ($p < 0.05$) than of CT-ANNUAL, however at 240 kg N ha⁻¹ CT-ANNUAL had higher ($p < 0.05$) C stocks compared to CT-Y5. In NT the control treatment at 0-30 cm had higher total C stocks than at 120 kg N ha⁻¹ ($p < 0.05$). Table 4.1 shows that the total C stocks in 0-30 cm depth were higher at 60 kg N ha⁻¹ in CT-Y5 compared to other 3 application rates ($p < 0.05$). Also both 0 and 120 kg N ha⁻¹ application rate of CT-Y5 at 0-30 cm had higher ($p < 0.05$) total C stocks than 240 kg N ha⁻¹. There was higher total C stocks at both 0 and 60 kg N ha⁻¹ than at 120 kg N ha⁻¹ in CT-ANNUAL treatment ($p < 0.05$).

The soil C stocks at 0-10 cm under NT treatment was higher than CT-Y5 and CT-ANNUAL in all application rates except for CT-Y5 at 60 kg N ha⁻¹ ($p < 0.05$). Whereas at 10-20 cm depth, the 60 kg N ha⁻¹ of CT-Y5 had higher soil C stocks compared to 240 kg N ha⁻¹ ($p < 0.05$). Soil C stocks of NT in 20-30 cm was higher ($p < 0.05$) at 60 kg N ha⁻¹ compared to 240 kg N ha⁻¹ (Table 4.1). At 0 and 60 kg N ha⁻¹ CT-Y5 had higher C stocks than at 120 and 240 kg N ha⁻¹ in 20-30 cm soil depth, with 120 kg N ha⁻¹ further having higher C stocks than 240 kg N ha⁻¹ in the same depth ($p < 0.05$). Table 4.1 shows that in 20-30 cm of CT-ANNUAL, 120 kg N ha⁻¹ had lower ($p < 0.05$) C stocks than all other application rates.

Table 4. 1: Soil carbon stocks per soil layer and total depth for different tillage systems.

Tillage treatment	Application rate (kg N ha ⁻¹)	SOC per soil layer (kg C ha ⁻¹)			Total SOC stocks
		0-10 cm	10-20 cm	20-30 cm	0-30 cm
NT	0	26890 ^g	29650 ^d	45160 ^{ef}	101700 ^g
	60	22218 ^{ef}	24872 ^{abcd}	48101 ^f	95191 ^{fg}
	120	19258 ^{cdef}	26407 ^{abcd}	45706 ^{ef}	91370 ^{ef}
	240	27745 ^g	26888 ^{bcd}	41214 ^{de}	95848 ^{fg}
CT-Y5	0	12742 ^a	22799 ^{ab}	41716 ^{de}	77258 ^{bc}
	60	23260 ^{fg}	27345 ^{bcd}	43570 ^{ef}	94175 ^{fg}
	120	15708 ^{abc}	26245 ^{abcd}	35632 ^c	77586 ^{bcd}
	240	13481 ^{ab}	21716 ^a	11738 ^a	46935 ^a
CT-ANNUAL	0	16815 ^{abcd}	28623 ^{bcd}	38147 ^{cd}	83585 ^{cd}
	60	15424 ^{abc}	27080 ^{bcd}	42389 ^{de}	84893 ^{de}
	120	18089 ^{bcd}	24254 ^{abc}	28732 ^b	71076 ^b
	240	13105 ^{ab}	22748 ^{ab}	42179 ^{de}	78033 ^{bcd}

NT-no-till; CT-Y5- conventional tillage on the 5th year; CT-ANNUAL-annual ploughing; SOC-soil organic carbon Similar letters on the columnar it means there is no significant difference, whereas difference shows significance difference (P<0.05)

4.4 Discussion

Before the inception of the experiment crop rotation of maize and soya beans under NT, were practiced on the site. Therefore any differences obtained on the experimental site after may have been influenced by management changes namely tillage type and N application rates. Soil organic C plays an important role in long-term ecosystem productivity in the global C cycle, by maintaining a soil nutrient pool and improving their availability for uptake. Fertilizer application resulted in increased in various C pools which may have been influenced by an increase in crop biomass. Distribution of C pools were not only dependent on N fertilizer but also the type of tillage technique had a salient role. Changes in the amount of total soil C generally occur slowly (of the order of several decades) unless the soil is subject to severe disturbance such as intensive tillage or erosion (Mann, 1986). According to Benbi et al., (2015) SOC is a heterogeneous mixture of organic substances, with different fractions or pools of SOC exhibiting different sensitivity to management. Earlier studies have also shown that some biologically active or labile SOC pools respond to changes in management to a greater extent than total C (Gong et al., 2009).

Total C had a similar trend to SOC, thus in the top 0-10 cm, NT had higher total C than CT-Y5 and CT-ANNUAL. According to Mrabet et al., (2001) elimination of soil mixing in NT leads to concentration of OM at the soil surface. Previous research has shown substantial increase of total C in soil under NT compared to CT (Halvorson et al., 2002). However, at 10-20 cm depth CT-ANNUAL at 120 and 240 kg N ha⁻¹ had higher total C than both NT and CT-Y5. This may be attributed to the vertical distribution of soil C under CT-ANNUAL with increasing fertilizer rates allowing higher amount of total C to accumulate in subsoil. Peculiar, at 20-30 cm soil depth at both 60 and 120 kg N ha⁻¹ NT had higher total C than CT-ANNUAL again. Baker et al., (2007) found that NT increased soil C in surface soils at the expense of the lower plough layer, resulting in no net increase in total C storage within the soil profile. It is reasonable to believe that soil C depletion at depth found under NT mainly results from reduced burial of crop residues and less root inputs compared to CT (Yang et al., 2008). Again, low C storage under CT-ANNUAL at 0-10 cm was probably due to high oxidation rates, release of organic compounds to the soluble form, and greater microbial activity. Mrabet et al., (2001) elucidated that soil C is higher in the entire profile under NT, and concluded that there is a stratification of soil C in surface horizons without any depletion of it at deeper horizon

compared to treatments receiving tillage. Therefore the higher C in NT at 20-30 cm soil depth is because of high inputs of C in the surface and less turnover of OM at lower depths. In a study by Six et al., (1999) the turnover soil C in aggregates was faster in CT than in NT, resulting in a greater loss of 53 to 250 μm intra-macro-aggregate POC in CT.

In the current study the soil surface (0-10 cm), had the highest concentration of organic C compared to other depths in all tillage treatments and fertilizer rates, with NT having higher organic C. This may be related to the severe moist anaerobic conditions under NT, since it had higher soil moisture (Chapter 3, Table 3.1), resulting in slower oxidation of OM. Tilling mixed the profile soil, thus OM on the surface was able to migrate into deeper soil layers thereby reducing organic C in the surface under CT-ANNUAL. Luo et al., (2010) explained that less subsoil organic C under NT was a result of lack of redistribution of surface soil C into deeper soil layers by ploughing. In addition, the restricted root growth due to soil compaction, caused by using heavy machinery and lack of soil loosening under NT may limit root penetration into deeper soil layers. Usually roots respire, exude C and during senescence decompose thus assist in adding organic C on the subsoil. Deen & Kataki, (2003) also found that under reduced tillage; C was higher in the 0-10 cm soil layer compared to CT. According to Kern & Johnson, (1993) soil organic C tends to increase with NT on the surface layer because less OM is lost to oxidation from mixing of the soil.

It is worth noting that at 10-20 cm soil depth, there were no significant differences in SOC for all fertilizer application rates or tillage treatments. This was similar to the findings of Deen & Kataki, (2003) who noted that non-significant differences in SOC at 10-20 cm depth between different tillage treatments. Contrary, previous research has shown that CT significantly affects vertical distribution of SOC with the concentration of SOC increasing with both depth and tillage (Angers et al., 1997). Deen & Kataki, (2003) further argued that the influence of tillage on SOC depends on the depth to which the tillage operation incorporated plant material. In the current study the influence of NT on SOC, with significantly higher organic C compared to CT-Y5 and CT-ANNUAL, was observed especially at 0-10 cm. One potential method for increasing the amount of C held in agricultural soil is to convert CT practices to conservation tillage practices that reduce tillage and retain crop residues (Kern & Johnson, 1993).

NT leads to increased soil cover and reduced soil disturbance which increases soil strength (compaction). The increased soil strength under NT not only discourages root growth into

deeper soil layers (Martínez et al., 2008), but also reduces downward movement of surface soil C. According to Luo et al., (2010) NT can also increase soil moisture through reduced evaporation in the top soil, leading to high crop root growth and biological processes related to SOC decomposition in the top soil layer. Conservation tillage may increase the amount of SOC by providing an environment where fungal decomposition is greater than bacterial decomposition (Kern & Johnson, 1993). This may be attributed to reducing conditions which are better tolerated by fungi compared to bacteria. In NT systems, fungal hyphal networks are left intact by the elimination of mechanical mixing that occurs during tillage events (Helgason et al., 2009). According to Holland & Coleman, (1987) fungal decomposition results in more recalcitrant decomposition products than bacterial decomposition because fungal biomass has a higher proportion of cell-wall material than bacterial biomass. According to Kassim et al., (1981) cell-wall material decomposes more slowly and the decomposed material is stabilized as biomass end products more quickly than cytoplasmic material.

The tillage effect at 20-30 cm soil depth was observed as CT-ANNUAL had the highest SOC at 240 kg N ha⁻¹ compared to all the tillage treatments. Tillage-induced change in soil C distribution with soil depth is likely a result of redistribution of surface soil C (Luo et al., 2010). CT also loosens the soil down to the depth of 15-35cm, thus changing the soil physical conditions, thereby promoting crop root growth which exude C and increase C input through root senescence at deeper soil layers (Luo et al., 2010). Notwithstanding the movement of C down the profile under CT-ANNUAL, however, higher concentration of organic C was observed in upper compared to lower soil depths in all tillage techniques. Minimum tillage apparently does not lead to additional C being sequestered into soil, but prevents net loss of SOC as CO₂ during decomposition (Kern & Johnson, 1993). According to Kern & Johnson, (1993) C released during minimum tillage are not less than those of CT whereas NT systems increase the amount of C sequestered in soil.

POC (readily decomposable C for microbes), was higher under CT-ANNUAL at both 120 kg N ha⁻¹ and 240 kg N ha⁻¹ compared to NT and CT-Y5 in the 10-20 cm soil depth. This was probably a result of ploughing and crop residue incorporation into soil under CT-ANNUAL, whereas plant residues accumulate on the surface under NT and CT-Y5. Again POC was highest under CT-Y5 at 60 kg N ha⁻¹ at 10-20 cm, while CT-Y5 and CT-ANNUAL at 60 kg N ha⁻¹ were significant higher than NT in the 20-30 cm soil depth. According to Angers et al.,

(1993), NT sequesters more POC in the soil than CT. This was evident in our study at 0-10 cm depth, where NT had higher POC at all application rates compared to CT-Y5 and CT-ANNUAL. Higher accumulation of organic material under NT than other treatments may be the cause of this. POC can account for over 10% of the soil C (Carter et al., 1994). This was supported by our findings where it was between 20-48 % of total C.

This POC pool is important to SOM turnover because it serves as readily decomposable substrate for soil microorganisms, and short-term reservoir of plant nutrients (Mrabet et al., 2001). It has been suggested as a sensitive indicator of changes of SOM because of its responsiveness to management practices (Gregorich & Carter, 1997). SOC had no observable differences with tillage at 10-20 cm depth, however major differences were observed in POC. This shows that POC is more sensitive to management than SOC. Tillage impacts on POC were consistent with those of total C, but were still more pronounced with POC. Chan, (2001) also found that changes in POC at different sites followed the trend of total C.

Wander et al., (1998) observed that NT increased POC by up to 70% at the surface but was lower by 18% in subsoil when compared with CT. Six et al., (1999) attributed this increase to a combination of reduced litter decomposition and less soil disturbance under NT. Reduced rates of litter decomposition may be due to a microclimate which hinder microbial activity in the surface residue layer. The CT-Y5 treatments had intermediate POC fractions of both NT and CT-ANNUAL. Six et al., (1998) explained that a fraction of SOM is lost due to tillage and this explains low POC under CT in the topsoil, which concurs with our results. On the other hand, Mrabet et al., (2001) noted that though NT had higher POC concentrated in surface horizons, there was no depletion of this fraction in deeper horizons, making magnitude of differences between tillage systems is more important at 0-2.5 cm depth. According to Six et al., (1999) in the subsurface layer (5-20 cm), POC was greater in CT than in NT. Wander et al., (1998) found differences in POC at the 5-17.5 and 17.5-30 cm depths to be greater in CT than NT soils. Many studies credit soil mixing with more even distribution of SOM with depth in CT soils (Collins et al., 1992).

Differences in POC concentrations at depth could also be a result of root productivity. Though crop productivity was not quantified, data from previous years showed that NT had a bumper harvest than CT-Y5 and CT-ANNUAL. According to Wander et al., (1998) NT had greater

yields than CT which suggested accumulation of SOM by NT surface soils, which may be a result of reduced mineralization and erosion rates. Fertilizer application and tillage exposed C to microbial attack due to high contact between plant residues and the soil mineral matrix, and POC pool with its high sensitivity get easily mineralized. CT treatments have less stable aggregates compared to NT as these are constantly disrupted through tillage (Six et al., 1999). Disrupted aggregates release higher amount of C compared to stable aggregates which help in C sequestration. When POC is released from aggregates, it becomes exposed to microbial decay, which leads to its loss and increased CO₂ flux in CT compared with NT (Six et al., 1999). Doran et al., (1998) reported that the biochemical environment of NT soils is less oxidative than CT, especially at lower depths. Franzluebbers & Stuedemann, (2003) explained that greater POC under NT at 15-30 cm might be explained by more anaerobic conditions in deeper soil layers and contributions from crop roots.

Culman et al., (2012) observed that POC comprised roughly 20-40% of the total C in the soil, while POXC and MBC made up only 4 and 2% of the total C, respectively. These findings were contrast with our data where POXC comprised 8-14%, POC 20-48% and MBC had 0.4-1-1.0% of the total C. Thus, POC can be regarded as the most labile C sensitive to management such as tillage and fertilizer application, followed by POXC and then MBC. Awale et al., (2013) also observed a greater response to tillage by POC compared to the POXC fraction, while Gregorich et al., (1996) observed a higher increase in POC than bulk SOC in response to long-term fertilization of maize. Contrary, Culman et al., (2012), found POXC to be a more sensitive indicator of differences in management (tillage and fertilizer application) than other measured C fractions. They explained that POXC was more strongly related to heavier and smaller than lighter and larger POC fractions, indicating that it reflects a more processed, stabilized pool of labile soil C (Culman et al., (2012). In a study by Romero et al., (2018), it was evident however that total C exhibited greater sensitivity than POXC to changes in land management or soil depth. Skjemstad et al., (2006) also observed that POXC did not provide a clear advantage over SOC when considering major land use alterations such as pasture vs agriculture.

The difference in concentration and sensitivity of labile C fractions may be due to climate, soil type, cropping system and laboratory procedures utilized in different analyses. Unlike, MBC and POXC which utilized chemical composition, POC relied on physical fractionation to

determine its concentration which can respond in less short space of time to management. POC represents a partially decomposed C fraction with a short turnover time, while POXC reflects a more processed fraction of soil C (Cambardella and Elliot, 1992). This was demonstrated by Tirol-Padre & Ladha, (2004) who observed a significant relationship between POXC and SOC but none between POXC and labile C fractions such as MBC. Plaza-Bonilla et al., (2014) got similar findings to ours with POC showing greater sensitivity to N fertilization than SOC and POXC for the three soil depths studied (0-5, 5-20 and 20-40 cm), and greater sensitivity to tillage management at 5-20 and 20-40 cm depth. POC presents the highest response to changes in agricultural management and can be used as an early indicator of optimized practices to sequester soil C (Plaza-Bonilla et al., 2014). However, it does not necessary disqualify POXC and MBC as good soil quality indicators.

Though, SOC showed no significance difference of tillage and application rates at 10-20 and 20-30 cm, the trend was different with POXC. However, significant positive correlations between total C and POXC at 0-5 and 5-10 cm depth showed that total C is a major determinant of the quantity of labile organic C fractions (Yang et al., 2012). A strong positive relationship between POXC and SOC has been reported previously which was attributed to similar methodology, both rely on soil C oxidation (Culman et al., 2012). The POXC fractionation is based on its oxidation with $KMnO_4$, on the premise that microbiological decomposition of SOM is largely associated with an enzymatic oxidation (Loginow et al., 1987). SOC is determined by complete oxidation of all soil C, while POXC relies on partial oxidation of the more easily oxidized C pool (Wang et al., 2017). According to Wang et al., (2017) the POXC fraction is a pool of labile soil C that has greater sensitivity to changes in management or environmental variation than other commonly measured pools e.g. POC, MBC or SOC. Weil et al., (2003) stated that compared to total C, POXC measured a C pool more closely associated with soil biological functions.

A decline of POXC with depth in this study reflects decreased root density and residue accumulation. Decreased root density with depth is thought to be associated with a parallel decline in sub-surface rhizodeposition (Petersen et al., 2008). With higher microbial populations and respiration activity nearer the soil surface, POXC in surface soil will be more rapidly oxidized or converted into protected soil total C than in the deeper soil (Wang et al., 2017). According to Wang et al., (2014) frequent tillage under CT may break down aggregates

and expose protected OM to microbial decomposition, thereby increasing loss of POXC which is evident in the current study. Plant residue might enter the labile organic C pools, provide substrate for soil microorganisms, and contribute to the accumulation of POXC (Wang et al., 2014). In addition, residue in conservation tillage system can improve soil physical and chemical quality through increased infiltration, aggregate stability and enhancing POXC fractions (Govaerts et al., 2007). At all soil depths, an increase in fertilizer application did not have an impact on POXC pools. This may be attributed to soil acidification, with the application of N fertilizer, which impedes enzymatic oxidation. Mi et al., (2016) found that organic mulches generally increased POXC contents in the upper soil profile compared with inorganic fertilizer alone, with cattle manure being the most effective. According to Shashidhar et al., (2009) application of organic mulches on soil increase soil pH, which may be attributed to liberation of bases during the decay process. Contrary, Wang et al., (2017) stated that POXC was higher in high N soil especially in the lower subsoil, suggesting that N-rich soil stimulated more root growth and rhizodeposition in the fertilized crop.

POXC oxidation simulates microbial decomposition, and therefore reflects in-situ enzymatic decomposition of labile SOM (Loginow et al., 1987). MBC, as the living component of SOM, plays a critical role in nutrient cycling, OM decomposition and transformation (Wang et al., 2014). MBC responded differently from total or SOC. MBC had lower concentrations than both POC and POXC labile fractions. The lower sensitivity of MBC compared to POC might be due to their more existence in micro aggregate sizes and highly labile nature (Chen et al., 2009). The increase of MBC with increased N application rate at 0-10 cm depth can be explained by the increase of root biomass, crop productivity, residue accumulation and henceforth decomposable organic material. Reported effects of chemical fertilizer on MBC are inconsistent. Omay et al., (1997) reported that chemical fertilizers decreased MBC, because severe N resulted in a microbial population with a comparatively high proportion of dormant cells. Grego et al., (1998) also noted low MBC with chemical fertilizer use that was comparable with that of the control. However in a study by Gong et al., (2009), chemical fertilizer increased MBC because it resulted in higher organic C input into soil, implying that microbial biomass is more controlled by substrate supply and less by chemical fertilizer. Mi et al., (2016) found that chemical fertilizer increased MBC by 63.2% compared to the control after 37 years.

In a study by Kushwaha et al., (2000), the highest MBC was associated with the N highest rate of 225 kg N ha⁻¹. This is similar to our study which had the highest MBC at 240 kg N ha⁻¹ in the 0-10 and 20-30 cm depths, whereas at 10-20 cm depth 120 kg N ha⁻¹ gave the highest MBC. Dou et al., (2008) observed greater MBC in surface soil under NT, and at deeper depths under CT, which was explained by the effects of litter availability on the surface under NT and incorporation to the subsoil in CT. Balota et al., (2004) suggested that crop residue accumulation provides substrate for microorganisms, which accounts for higher MBC in surface soil. Furthermore, soil under NT was wetter and had conducive high temperature, favourable for soil microbial activity (Franzluebbers et al., 1995). CT-Y5 have likely similar soil properties such as soil moisture and pH (Chapter 3, Table 1.1) with NT, with a better advantage of mixing of crop residues after four seasons thereby enhancing decomposition, giving it the highest MBC at 240 kg N ha⁻¹ in 0-10 cm depth. Kushwaha et al., (2000) noticed that the combined effect of residue retention and minimum tillage increased MBC by 82% over control. However in NT, surface application of retained residues increased MBC only by 36% over the control (Kushwaha et al., 2000). CT-Y5 is a proxy of minimum tillage therefore our findings were in line with the above study.

The MBC decreased with depth but 20-30 cm soil depth had on total average almost same concentration as at 10-20 cm. This may be attributed to less OM in subsoil. Dou et al., (2008) also observed decreased MBC with depth. In general, situations favouring accumulation of OM increase MBC (Jenkinson & Ladd, 1981). At 0-10 and 20-30 cm soil depths, CT-Y5 had greater average MBC than NT and CT-ANNUAL. According to Wright et al., (2005) MBC was highest under minimum tillage at 2.5–7.5 cm soil depth. Most impacts of NT on enhancing C sequestration have been observed in surface soils near the rooting zone and residue litter (Paustian et al., 1997). However, long-term increases in SOM have been observed in subsurface soils after 20 years of NT (Wright & Hons, 2004). The current experiment has been running for more than 20 years, hence observations were made of higher MBC values under NT than CT-ANNUAL (particularly in the control) at 10-20 cm soil depth. The living fraction of OM, i.e. microbial biomass, rather than total organic C has been suggested as a useful and more sensitive measure of change in OM status (Powlson & Jenkinson 1981). These changes can be monitored by qMic ratio which is the ratio of MBC to SOC. According to Makova et al., (2011) qMic ratio represents the amount of metabolic active C in the total SOM. The qMic ratio could serve to indicate if soil C is in equilibrium, accumulating or decreasing from the threshold of

2.3 under monoculture and 2.5 under rotations with fertilizer (Anderson & Domsch, 1989). Though, in our case sampling was only done once making it difficult to assess if it was decreasing, increasing or in equilibrium. However, using qMic ratios from the literature the study area had very low qMic. The qMic ratio is generally considered as sensitive change indicator of SOM quality (Sparling, 1992). Our findings, with low values of qMic, suggest that a larger proportion of non-microbial C was contained in the SOC rather than MBC.

Generally, the lowest qMic was in CT-ANNUAL, with CT-Y5 having higher qMic at 0-10 and 20-30 cm while NT was predominantly higher at 10-20 cm. Anderson & Domsch, (1990) suggested that the higher qMic under conservation tillage rather than CT was caused by the quality of the OM input which was more suitable for microbial growth and survival. Our results shows that CT-ANNUAL had continuous reduction in SOC and MBC and eventually qMic. In the study by Jiang et al., (2011), the lowest qMic in CT and NT indicated the lowest substrate availability to microorganisms. Both NT and CT-Y5 resulted in increase of SOM, from crop residues, compared to CT-ANNUAL which eventually resulted in higher MBC. The 240 kg N ha⁻¹ of CT-Y5 had the highest qMic ratio at the 0-10 and 20-30 cm depth whereas at 10-20 cm the highest qMic ratio was observed under NT at 120 kg N ha⁻¹ which was similar to the trend of MBC. The qMic generally increased with increase in N application. In contrast, Sparling, (1992) reported that unfertilized soil increased qMic ratio, while increasing total soil N did not increase qMic ratio. Consequently, it can be inferred that the increase in qMic ratio is from the organic C inputs and not necessarily related to N status of the soil (Sparling, 1992). The significance effect of both tillage and N application on qMic shows that C is not stabilized in the soil. Consistence of the qMic ratio is thus an indication of a system at a new equilibrium (Anderson & Domsch, 1989).

Differences in SOC stocks are a result of an imbalance between C inputs, mainly in the form of dead plant material and outputs, caused by decomposition, leaching and erosion losses (Poeplau & Don, 2014). Total soil C stocks were higher in NT at all application rates, while the 60 kg N ha⁻¹ of CT-Y5 also recorded high total SOC stocks. This trend is similar to both total and SOC trends and to previous findings by Krauss et al., (2017). According to Luo et al., (2010) conversion from CT to NT did not increase the overall SOC stock in most cases, except for those with double cropping systems. Baker et al., (2007) reported that conservation tillage may sequester C only for the upper depth. In single cropping systems, the crop type affects

vertical distribution of soil C (Luo et al., 2010). Thus in our study maize with root depth of almost 30 cm enhanced vertical distribution of C to subsoil through decomposition of root at senescence. According to Krauss et al., (2017) reducing tillage intensity and fertilizer application increased SOC stock after thirteen years. This is in conjunction with our findings only for tillage because both NT and CT-Y5 had higher SOC stocks compared to CT-ANNUAL, whereas SOC stock did not significant increase with the increase in N fertilizer. Contrary, Crittenden et al., (2015) did not find significant differences in SOC stocks (0–0.5 m) between CT and NT after three years.

In all the treatments soil C stocks trends with depth followed the order; 20-30 cm > 10-20 cm > 0-10 cm, thus inversely related to the trend for organic C. The increase in soil C stocks may be attributed to higher soil bulk density with increasing depth. Schulz et al., (2014) only reported SOC stocks per soil layer, with higher SOC stocks in 0-30 cm in the NT compared to CT treatment after 11 years, and lower SOC stocks at lower depth (30-90 cm). Whereas, Zikeli et al., (2013) found higher SOC stock concentrations in reduced tillage compared to CT in the topsoil (0-20 cm) and no changes below (40-60 cm) after 12 years. Contrary, Krauss et al., (2017) reported an accumulation of SOC stocks by conversion of CT to NT to be mostly restricted to topsoil whilst in lower horizons, a decrease in SOC stocks occurred. Although tillage increases residue incorporation and moves surface soil C into deeper layers, it also stimulates SOC oxidation. Tillage fragments macro-aggregates and increases the surface area for soil microbes to attack and decompose the originally physically aggregate-protected soil C (Six et al., 1999). Incorporation of crop residues through tillage also leads to more favourable soil moisture and thermal conditions that accelerates their decomposition in deeper layers than on the soil surface (Coppens et al., 2007). This provides nutrients and energy for microbial growth, further enhancing decomposition of soil C, including inert C (Fontaine et al., 2007).

4.5 Conclusions

Tillage and fertilizer application had an effect on soil C fractions at different depths. NT increased most C fractions, (except MBC) in the upper soil layer, which could lead to higher soil productivity. Thus C sequestration is greater in NT than in CT-Y5 and CT-ANNUAL. This was because of slower OM turnover in NT leading to formation and stabilization of C. Whereas

under CT-ANNUAL, SOM turnover was easily intensified by tillage which breaks down aggregates protecting C, thus releasing it into the atmosphere as CO₂ or its consumed by microbes. Tillage, fertilizer application and depth had significant effects on labile C fractions, showing a greater sensitivity compared to both SOC and total C. POC in particular exhibited a relatively greater response to tillage, fertilizer application and depth than MBC and POXC, while POXC showed more sensitivity to management than MBC labile fraction. The MBC responded more to CT-Y5 at 0-10 cm, with significantly higher values at 240 kg N ha⁻¹, compared to other tillage techniques. CT-Y5 at 240 kg N ha⁻¹ (0-10 cm) and NT at 120 kg N ha⁻¹ (10-20 cm) had the highest qMic which was in similar trend with MBC. The significance difference in qMic ratio among both tillage and fertilizer rates explicitly clarifies that there is no C balance in the soil. Though CT-Y5 did not always have the highest C fractions, it maintained intermediate concentrations compared with other tillage techniques. The 120 kg N ha⁻¹ of all tillage treatments had intermediate C fractions in all depths when compared to 0, 60 and 240 kg N ha⁻¹ which had either very low or higher fractions. C sequestration will not automatically increase with increase of C input, but will depend on the rate of C output from the soils, which is affected by the management type employed. A larger input over output of OM is the reason for the increase of total C and its various fractions in the fertilized treatments. This implies that a high amount of organic material input through better crop production and N fertilizer application is required for increasing the SOC pools. Therefore using conservation agriculture, particular NT or minimum tillage (CT-Y5) at 0-10 cm, with 120 kg N ha⁻¹ application rate in dryland agriculture is well recommended. The null hypothesis of the study stating that increase in fertilizer application rate and minimising tillage will decrease the C pools of soil was therefore rejected.

CHAPTER 5

PHOSPHORUS AND NITROGEN POOLS UNDER DIFFERENT TILLAGE TECHNIQUES AND N FERTILIZER RATES IN DRY LAND AGRICULTURE.

5.1 Introduction

Nitrogen (N) and phosphorus (P) are major elements essential for plant growth and development. N is a constituent of proteins and nucleic acids, with most soil N found in OM, where it is continuously mineralised into ammonium (NH_4^+) and nitrate (NO_3^-), the mineral available forms. According to Bremner & Keeney, (1965) there is need to assess the amounts and mineralisation patterns of NH_4^+ and NO_3^- in soils during cropping in order to make informed recommendations on application rates of N fertilizers and organic inputs. P in soil is limited hence needs to be supplemented to sustain crop productivity (Khan et al., 2018). Tillage and fertilizer application alter soil profile nutrient distribution thus impacting availability, adsorption, leaching, decomposition and mineralization of nutrients. The above factors are dependent on climatic and soil conditions of that particular area. Sharifi et al., (2008) found increased N mineralization in CT compared to NT. This is because ploughing of soil increases aeration and water movement thereby enhancing decomposition, mineralization and nitrification.

According to Dowdell & Cannell, (1975) both CT and NT affect the N cycle with CT influencing N mineralization while NT favours denitrification due to increased stored soil water. Increases in N mineralization following soil disturbance by CT are largely due to more organic N substrates becoming available to support greater microbial activity (Campbell et al., 1971). Franzluebbers et al., (1994) explained that although the rate constant for organic N mineralization is often greater in CT systems, gradual or long-term accumulation of a larger organic N pool in NT systems may compensate for this. NT has an abundance of crop residues which keep on adding organic N after mineralization. N fertilizer application on the other hand changes both N pools and mineralization patterns by providing microbes with substrates. N fertilizers are needed by both plants and microbes, however excessive application may have a detrimental effect on microbial activity. According to Salinas-Garcia et al., (1997) N

fertilization had little effect on N mineralization, while changes in active N pools of SOM depended more on tillage for distribution within surface soil than upon N fertilization. However, Salinas-Garcia et al., (1997) realised that N mineralization at flowering and harvest was increased by greater N fertilization, possibly because fertilizer N that was immobilized into soil microbial biomass early in the season was later mineralized. NO_3^- can be easily leached because it is an anion while NH_4^+ is adsorbed by negative soil colloids, but both have high potential to be extracted by crops. Hence, the decrease of organic C decomposition and mineralization lowers NH_4^+ and NO_3^- concentration.

P remains a major limitation to agricultural productivity (Nziguheba et al., 2016). Residue accumulation and less soil disturbance under NT will sustain both N and P. Again, slower decomposition of surface-placed residues may prevent rapid leaching of nutrients through the soil profile, which is more when residues are incorporated into the soil (Franzluebbers & Hons, 1996). Unger, (1991) reported more extractable P in NT compared with CT below the till-zone, rather than only near the surface as reported in other studies, and suggested it may be due to accumulation of P in senescent roots. Globally critical P values have been reported on different soils from 5 to 26 mg kg^{-1} for wheat (Singh et al., 2016) and 6.9 to 28 mg kg^{-1} for maize (Singh et al., 2016). Since P dynamics differ between soil types and climate, application requirements will differ from one environment to another (Blake et al., 2000). Therefore, it is crucial to determine P availability, organic and total P for each specific environment and cropping system. Compared to other major nutrients, P is firmly bound in soils, due to precipitation of P with calcium ions in calcareous soil, and adsorption of P by Fe and Al- oxides in acidic soils (Hinsinger, 2001). However, this fixation is largely reversible over time as plant roots can take up P accumulated in soil from applications of fertilizers and organic sources over many years (Khan et al., 2018). By differing fertilizer application rates, one can determine its suitability for crop uptake. Fudge, (1928) found that in acid soils, the use of basic N fertilizers such as sodium nitrate and calcium nitrate increased solubility and plant utilization of P, while acidic fertilizers such as ammonium sulphate and urea had the opposite effect.

Though P is immobile in soil, its profile distribution may be influenced by the type of tillage applied. The P cycle, from total to organic P and inorganic P should be well researched, especially in poor resourced arable farms, in order to sustain crop and soil productivity. Furthermore, many studies have demonstrated P leaching to subsoil in both coarse-and fine-

textured soils (Wang et al., 2015). In a study by Franzluebbbers & Hons, (1996), NT had greater concentration of P at 0-5 and 15-30 cm soil depths compared to CT. Therefore tillage systems which improve P availability for uptake through elimination of leaching, adsorption and precipitation should be promoted. According to Margenot et al., (2017) improving P availability may be an added advantage of conservation agriculture in weathered soils, since reduced tillage and residue retention could reduce P fixation, increase labile P, and increase organic P accumulation and its mineralization by phosphatases. Residue retention can reduce P fixation by increasing organic anion competition for P binding sites (Palm et al., 2007). Thus, knowledge of the magnitude of changes in the active soil N and P pools is very crucial for understanding how tillage systems can be better managed to increase N and P sequestration of soil. The processes governing SOM decomposition and mineralization are important to soil fertility, GHGs and ecosystem sustainability, however they are not fully understood. Thus studies on N and P pools are highly essential. The objectives of this study was to determine P and N dynamics in soil under different tillage and fertilizer management practices.

5.2 Material and Methods

The study site with maize mono-cropping, Figure 5.1, was described in detail in the previous chapter (Chapter 3, Section 3.2).

5.2.1 Sampling procedure

The soil sampling procedure was described in detail in Chapter 3, Section 3.2.1.



Figure 5. 1: Different tillage techniques, NT (Zero tillage), CT-Y5 and CT-ANNUAL (annual ploughing), in the control.

5.2.2 Laboratory Analyses

5.2.2.1 Total Nitrogen (N)

Analysis of total N was described in detail in Chapter 3, Section 3.2.2.2.

5.2.2.2 Ammonium (NH_4^+)

Colorimetry was used through use of N1 and N2 reagents to extract NH_4^+ from the soil, (Freney & Wetselaar, 1969 and; Okalebo, 1993). N1 was made from 34 g sodium salicylate, 25 g sodium citrate and 25 g sodium tartrate dissolved in 750mL distilled water. Thereafter 0.12g of sodium nitroprusside was added and made to 1 litre with distilled water. N2 was made from 30 g sodium hydroxide dissolved in 750 mL distilled water, which was allowed to cool and 10 mL sodium hypochlorite added, and made up to 1 litre with distilled water (Okalebo, 1993). About 10.0 g of fresh soil sample (kept in a refrigerator) was weighed into a plastic shaking bottle. Thereafter, 100 mL of 0.5 M K_2SO_4 extracting solution was added. The contents were shaken with a stopper for 1 hour and filtered through Whatman No. 42 filter paper. Using a micro-pipette, 0.2 mL of the sample extract was pipetted into a test tube with blanks and standards undergoing the same procedure. About 5.0 mL of reagent N1 was then added and allowed to stand for at least 15 minutes with continuous mixing of the contents (Okalebo, 1993). Furthermore 5.0 mL of reagent N2 was added, and continuously mixed (Okalebo, 1993). The contents were allowed to stand for 1 hour, then absorbance was measured at 655nm using a spectrophotometer (SPECTRO UV-11). A calibration curve was plotted and the concentration of the sample was determined. The concentration of ammonium in $\mu gNH_4^+-N kg^{-1}$ was calculated according to Freney & Wetselaar, (1969) and Okalebo, (1993) as follows:

$$NH_4^+-N (\mu g/kg^{-1}) = \frac{(a-b)*v*MCF*f*1000}{w}$$

Where:

a = concentration of N in the solution,

b = concentration of N the blank,

v = volume of the extract;

w = weight of fresh soil;

MCF = moisture correction factor in percentage;

f = multiplication factor of the sample which was one since similar quantity was extracted for samples, blanks and standards.

5.2.2.3 Nitrate (NO_3^-)

NO_3^- was determined calorimetrically by weighing 10.0 g of soil into a plastic shaking bottle, then adding 100 mL of 0.5 M K_2SO_4 extracting solution and shaking well. Thereafter, 0.5 mL of the sample extract was transferred into a test tube (the same procedure was done for blanks and standards), before adding 1.0 mL of 5% salicylic acid and shaking the mixture. After waiting for 30 minutes, 10.0 mL of 4 M sodium hydroxide was added to each test tube. The contents were mixed well and left for 1 hour for a full yellow colour to develop (Bremner & Keeney, 1965). The absorbance was measured at 419 nm wavelength (Okalebo, 1993), using a spectrophotometer (SPECTRO UV-11). The calculation of NO_3^- was done using the formula below (Okalebo, 1993; Bremner & Keeney, 1965):

$$\text{NO}_3^- \text{-N } (\mu\text{g}/\text{kg}^{-1}) = \frac{(a-b) \cdot v \cdot \text{MCF} \cdot 1000}{w}$$

Where;

a = concentration of NO_3^- -N in the solution,

b = concentration of NO_3^- -N in the blank,

v = volume of the extract;

w = weight of fresh soil;

MCF = moisture correction factor.

The aliquot used for both the standards and the samples were of the same volume therefore no multiplication factor was required within the calculations (Bremner & Keeney, 1965).

5.2.2.4 Nitrogen mineralisation

Airtight bottles of 100 mL and 15 mL volume were weighed, then 10 and 5 g of fresh soil was added into the bottles respectively, (Anderson & Ingram, 1994). Thereafter, 50 mL 1 M KCl was added into the 100 mL bottle before shaking on a shaker for 20 minutes at about 60 Hz (Anderson & Ingram, 1994). Using a micropipette, 0.1 mL of sample, standard and blank was

transferred into suitably marked test tubes. In addition, 5.0 mL of reagent NI (used in NH_4^+ extraction above) was added to each test tube, mixed and left for 15 minutes. Also 5.0 mL of reagent N2 (used in NH_4^+ extraction above) was added to each test tube, mixed before adding 2 mL 5M NaOH.

After 20 minutes NH_4^+ -N was determined using the spectrophotometer (SPECTRO UV-11) at wavelength of 655 nm and referred to as time_0 NH_4^+ -N (Anderson & Ingram, 1994). Meanwhile into the 15 mL bottle with 5 g of fresh soil, 12.5 mL distilled water was added with gentle swirling to remove air bubbles (Anderson & Ingram, 1994). The bottle (with a stopper) was then placed in an incubator for 7 days at 40 °C. After incubation, the contents were filtered using Whatman No. 42 filter paper, transferred into a clean 50 mL bottle, then 25 mL of 2 M KCl was added. Thereafter, 0.1 mL of sample was extracted using a micropipette and transferred to a test tube, with the same procedure done for blanks and standards. About 5.0 mL of reagent NI (used above in NH_4^+ extraction) was then added to each test tube, mixed and left for 15 minutes. Another 5.0 mL of reagent N2 (also used above in NH_4^+ extraction) was added to each test tube, then mixed well before adding 2 mL 5M NaOH. After 20 minutes, NH_4^+ -N was determined and referred to as time_1 NH_4^+ -N (Anderson & Ingram, 1994). The time_0 NH_4^+ -N was obtained immediately on the day of starting the experiment whereas time_1 NH_4^+ -N was obtained after 7 days of incubation. Using the spectrophotometer (SPECTRO UV-11) the absorbance was read at 655 nm. The calculation of anaerobic N mineralisation was done as follows (Anderson & Ingram, 1994):

$$\text{Anaerobic N mineralisation rate (ugN/g soil/day)} = [(\text{time}_1 \text{ NH}_4^+ \text{ -N}) - (\text{time}_0 \text{ NH}_4^+ \text{ -N})] / 7$$

5.2.2.5 Total Phosphorus

Digestion of soil for total P was done using an electric hot plate with 5 N H_2SO_4 as a digesting reagent and 0.057 M ascorbic acid as a reducing agent. In a separate container 12 g of ammonium molybdate was dissolved in 250 mL of warm (50 °C) distilled water, while 0.291 g antimony potassium tartrate was dissolved in 100 mL distilled water. Both solutions were added to 1000 mL of 5 N H_2SO_4 (above) to make ammonium molybdate/antimony potassium tartrate solution (Novosamsky et al., 1983). The solution was thoroughly mixed and diluted

with distilled water to 2 litres. It was then transferred to a reagent bottle and stored in a cool dark place. Ascorbic acid reducing agent was developed from this solution by dissolving 2.108 g ascorbic acid in 400 mL of ammonium molybdate/antimony potassium tartrate solution mixed well.

About 0.3 g of air-dried soil was weighed into a 125 mL Pyrex conical flask, then 4.0 mL concentrated H₂SO₄ was added with careful swelling, (Anderson & Ingram, 1989). The mixture was heated in a fume hood on an electric hot plate set at medium heating. The flask was then removed from the heater, allowed to cool and 10 drops of H₂O₂ were added. The flask was swirled, keeping contents at the bottom of the flask, then reheated while avoiding excessive heating that causes spattering (Novosamsky et al., 1983). Contents were allowed to cool then 6 drops of H₂O₂ were carefully added before reheating again. There was continuous reheating, cooling and adding 6 drops of H₂O₂ until the colour changed from black to dark brown (Novosamsky et al., 1983). After this, the heater was turned to high setting on the hot plate before re-heating, cooling and adding 6 drops of H₂O₂ again. Thereafter, contents turned colourless on cooling then H₂O₂ was added and left for 12 minutes. The contents were cooled and transferred quantitatively into a 50 mL volumetric flask, using distilled water to bring to the mark, before cooling and thorough mixing. About 5.0 mL of the supernatant, clear and wet-ashed with H₂SO₄ digest solution was pipetted into a 50 mL volumetric flask, then 20 mL distilled water and 10 mL of ascorbic acid reducing agent was added (including to the blanks and standards). Distilled water was used to make to the mark and the flask was shaken well with a stopper on. The contents were allowed to stand for 1 hour to permit full colour development (Novosamsky et al., 1983), and absorbance was measured using a spectrophotometer (SPECTRO UV-11) set at 880nm wavelength. A graph of absorbance was plotted against standard concentration of P in ppm, and solution concentrations for each sample and the blanks were determined. The blank value was subtracted from the sample, hence a value for corrected concentration was obtained (Anderson & Ingram, 1989). The equation below by Novosamsky et al., (1983) was used to calculate total P:

$$P \text{ in sample (\%)} = \frac{C*V*F}{W}$$

Where:

C = concentration of P in the sample;

V = volume of the digest;

F = dilution factor;

W = weight of the sample.

5.2.2.6 Organic phosphorus determination

Organic P was analysed from two samples of the same soil, with one ignited on the furnace and the other unignited. About 1.0 g of air-dried soil was weighed into a porcelain crucible, then placed in a cool muffle furnace. The temperature of the furnace was slowly raised to 550 °C over a period of 1 to 2 hours (Olsen & Sommers, 1982). Thereafter the soil was ashed at 550 °C for 1 hour, allowed to cool then transferred to a 100 mL polypropylene centrifuge tube (Bowman, 1989). In another separate 100 mL polypropylene centrifuge tube, 1.0 g of unignited air-dried soil was weighed. In both centrifuge tubes with ignited and unignited soil, 50 mL of 1 M H₂SO₄ was added before shaking overnight (Olsen & Sommers, 1982). After centrifuging, the contents were filtered using Whatman No. 42 filter paper. A 1.0 mL aliquot of either standard, blank or sample was then pipetted into a test tube, to which 4.0 mL of 0.057M ascorbic acid solution and 3.0 mL molybdate reagent were added and mixed well. The contents were left for an hour for a blue colour to develop (Watanabe & Olsen, 1965). The absorbance was read using a spectrophotometer (SPECTRO UV-11) set at 880 nm wavelength, and organic P calculated as follows (Olsen & Sommers, 1982):

$$P_{\text{ignited}} (\%) = \frac{c \cdot 0.2}{w}$$

$$P_{\text{unignited}} (\%) = \frac{c \cdot 0.2}{w}$$

$$\text{Organic Phosphorus (\%)} = P_{\text{ignited}} (\%) - P_{\text{unignited}} (\%)$$

Where;

W= weight of sample;

c = P concentration for sample solution.

5.2.2.7 Extractable phosphorus determination

The 0.057 M ascorbic acid and molybdate reagent used in the analysis of organic P above were again used on bicarbonate extractable P determination. The 0.5 M of Na₂CO₃ solution was transferred to a 2000 mL volumetric flask, adjusted to pH 8.5 with 10 % NaOH and made up

to the mark with distilled water. Therefore this solution was used to extract available P in the soil (Watanabe & Olsen, 1965). Air-dried soil, 2.5 g, was weighed into a polyethylene bottle and 50.0 mL extracting solution was added. The polyethylene bottle was shaken for 30 minutes then filtered through Whatman No. 42. A 1.0 mL aliquot of either standard, blank and sample were pipetted into a test tube, then 4.0 mL ascorbic acid solution and 3.0 mL molybdate reagent were added and mixed well. The contents were left for an hour for the colour to develop (Watanabe & Olsen, 1965). The absorbance was read using a SPECTRO UV-11 spectrophotometer set at 880 nm wavelength, and the following equation by Olsen & Sommers, (1982) was used to calculate extractable P:

$$\text{Bicarbonate extractable phosphate } (\mu\text{g g}^{-1}) = \frac{c*20}{w}$$

Where;

w = weight of sample;

c = P concentration for sample solution.

5. 2.3 Statistical Analyses

An analysis of variance (ANOVA) test was done to determine the effect of tillage and fertilizer application rate on soil P and N availability at different soil depths. Treatment factors were tillage practices, N fertilizer application rate and soil depth. The Fisher's protected LSD test was used as a post-hoc test for multiple comparison to compare treatment means and their interactions. The P value (<0.05) was used to determine significant differences between treatment factors. All tests were performed with GenStat 14.1 for Windows software (VSN international, 2011).

5.3 Results

5.3.1. Total N variations

Total N of NT was higher ($p < 0.05$) than CT-Y5 and CT-ANNUAL at all application rates in the 0-10 cm soil depth (Figure 5.2). At 240 kg N ha⁻¹, total N of CT-Y5 (0.46 g kg⁻¹) was higher than that of CT-ANNUAL (0.42 g kg⁻¹) in the 0-10 cm depth, while 60 and 240 kg N ha⁻¹ rates generally had higher total N than the control and 120 kg N ha⁻¹ rate for most tillage techniques ($p < 0.05$). At 10-20 cm soil depth, NT had higher total N (0.64 g kg⁻¹) than CT-Y5 and CT-ANNUAL (0.48 and 0.43 g kg⁻¹, respectively) in the control, while at 60 kg N ha⁻¹, NT had higher total N, (0.61 g kg⁻¹) than CT-ANNUAL (0.42 g kg⁻¹) ($p < 0.05$). However at 120 kg N ha⁻¹ in 10-20 cm depth, CT-ANNUAL (0.62 g kg⁻¹) had higher total N than CT-Y5 (0.45 g kg⁻¹), while at 240 kg N ha⁻¹, CT-ANNUAL had more total N (0.18 g kg⁻¹) than NT (0.43 g kg⁻¹). In the 20-30 cm depth at 60 and 120 kg N ha⁻¹, NT had higher total N than CT-ANNUAL, whereas CT-Y5 had more total N than NT at 240 kg N ha⁻¹ application rate ($p < 0.05$).

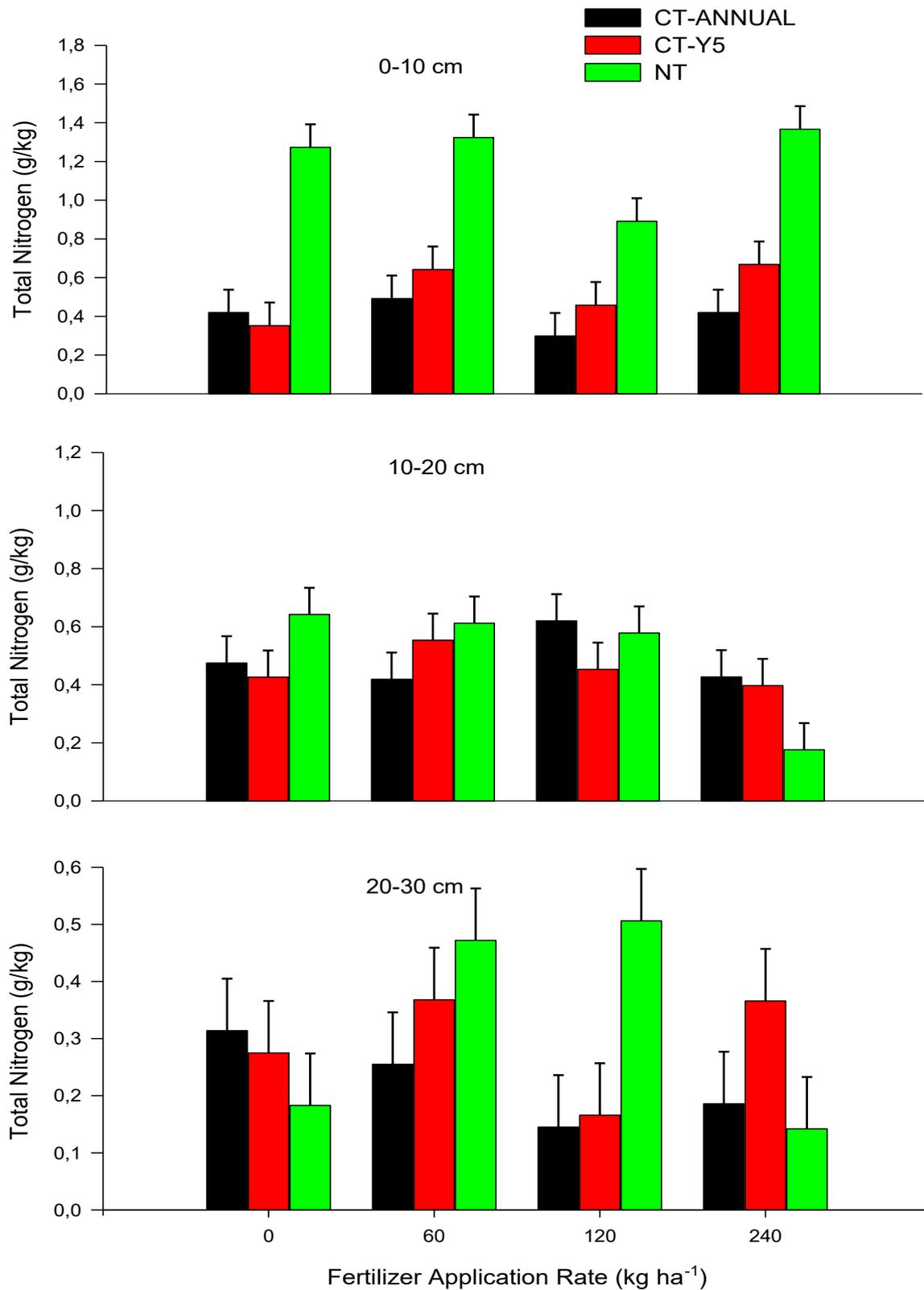


Figure 5. 2: Variation of total N with depth under different tillage techniques and urea fertilizer application rates.

5.3.2 Nitrate variation.

Figure 5.3 shows that at 0, 60 and 120 kg N ha⁻¹, CT-Y5 had the lowest nitrate-N in the 0-10 cm soil depth, while at 120 kg N ha⁻¹ CT-ANNUAL had higher nitrate-N than NT ($p < 0.05$). However at 240 kg N ha⁻¹, NT (9.96 mg kg⁻¹) had higher ($p < 0.05$) nitrate-N, than CT-ANNUAL (5.27 mg kg⁻¹) and CT-Y5 (5.22 mg kg⁻¹). In the 10-20 cm soil depth, CT-ANNUAL had higher ($p < 0.05$) nitrate-N (9.48 mg kg⁻¹) than NT (6.71 mg kg⁻¹) and CT-Y5 (4.52 mg kg⁻¹) at 60 kg N ha⁻¹, while at 120 and 240 kg N ha⁻¹, NT had the highest nitrate-N ($p < 0.05$) (Figure 5.3). In the 20-30 cm soil depth, CT-Y5 had the lowest nitrate-N than CT-ANNUAL at 0 and 60 kg N ha⁻¹, however at 60 kg N ha⁻¹, CT-ANNUAL (9.33 mg kg⁻¹) had higher nitrate-N than NT (6.0 mg kg⁻¹) ($p < 0.05$). Again, at 120 and 240 kg N ha⁻¹, NT had higher nitrate-N than CT-Y5 in the 20-30 cm soil depth, while at 120 kg N ha⁻¹, NT was also higher than CT-ANNUAL ($p < 0.05$).

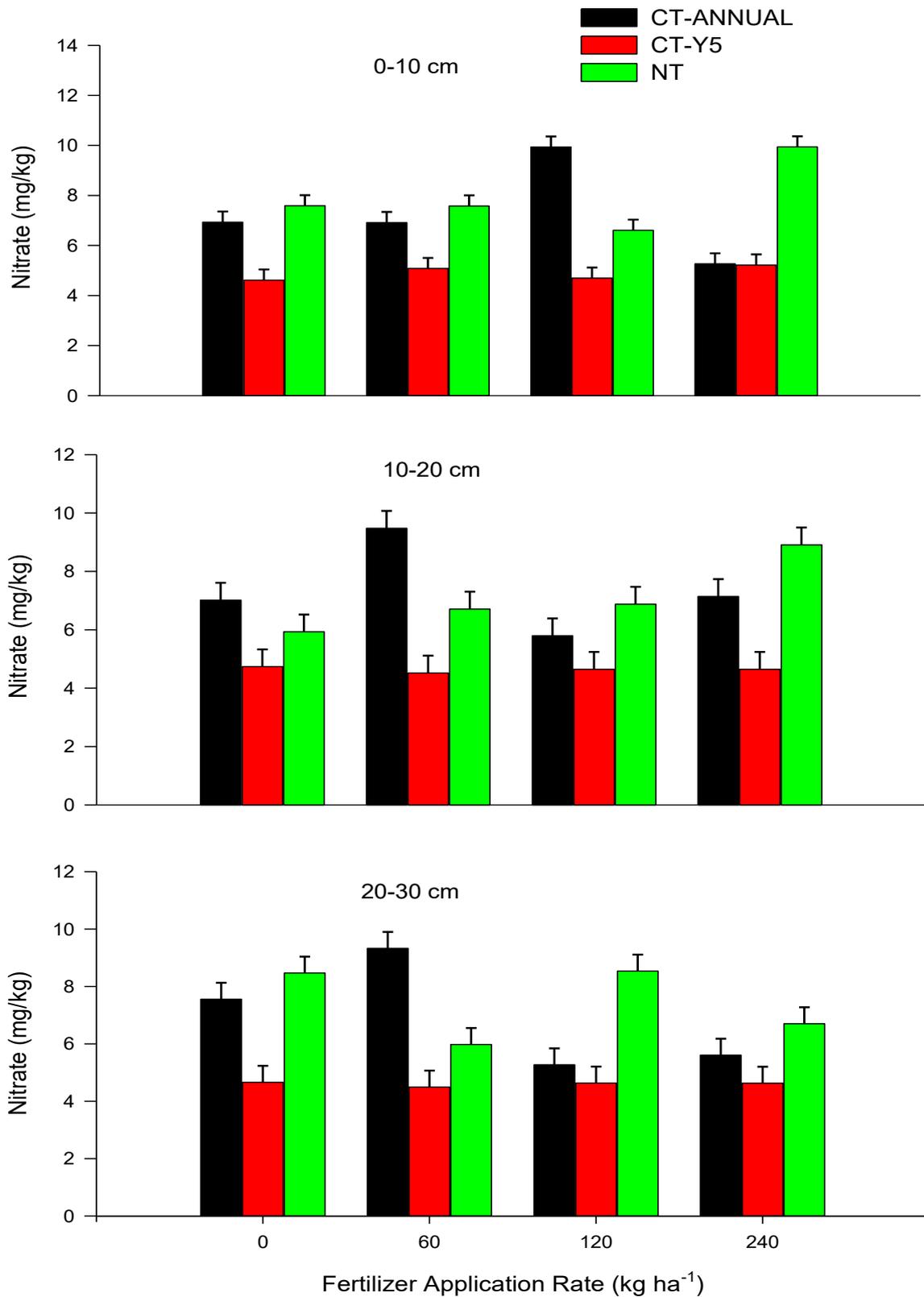


Figure 5. 3: Variation of nitrate-N with depth under different tillage techniques and urea fertilizer application rates.

5.3.3 Ammonium concentration at different depths.

Ammonium-N was highest in NT at 0 and 60 kg N ha⁻¹ in the 0-10 cm soil depth ($p < 0.05$), while no notable differences were recorded at the other application rates (Figure 5.4). In the 10-20 cm depth, NT also had higher ($p < 0.05$) ammonium-N ($1.77 \mu\text{g kg}^{-1}$), than CT-Y5 ($0.58 \mu\text{g kg}^{-1}$) and CT-ANNUAL ($0.41 \mu\text{g kg}^{-1}$) at 60 kg N ha⁻¹. The last 20-30 cm depth saw CT-Y5 having higher ($p < 0.05$) ammonium-N ($1.55 \mu\text{g kg}^{-1}$) than CT-ANNUAL ($0.32 \mu\text{g kg}^{-1}$) and NT ($0.26 \mu\text{g kg}^{-1}$) at 120 kg N ha⁻¹.

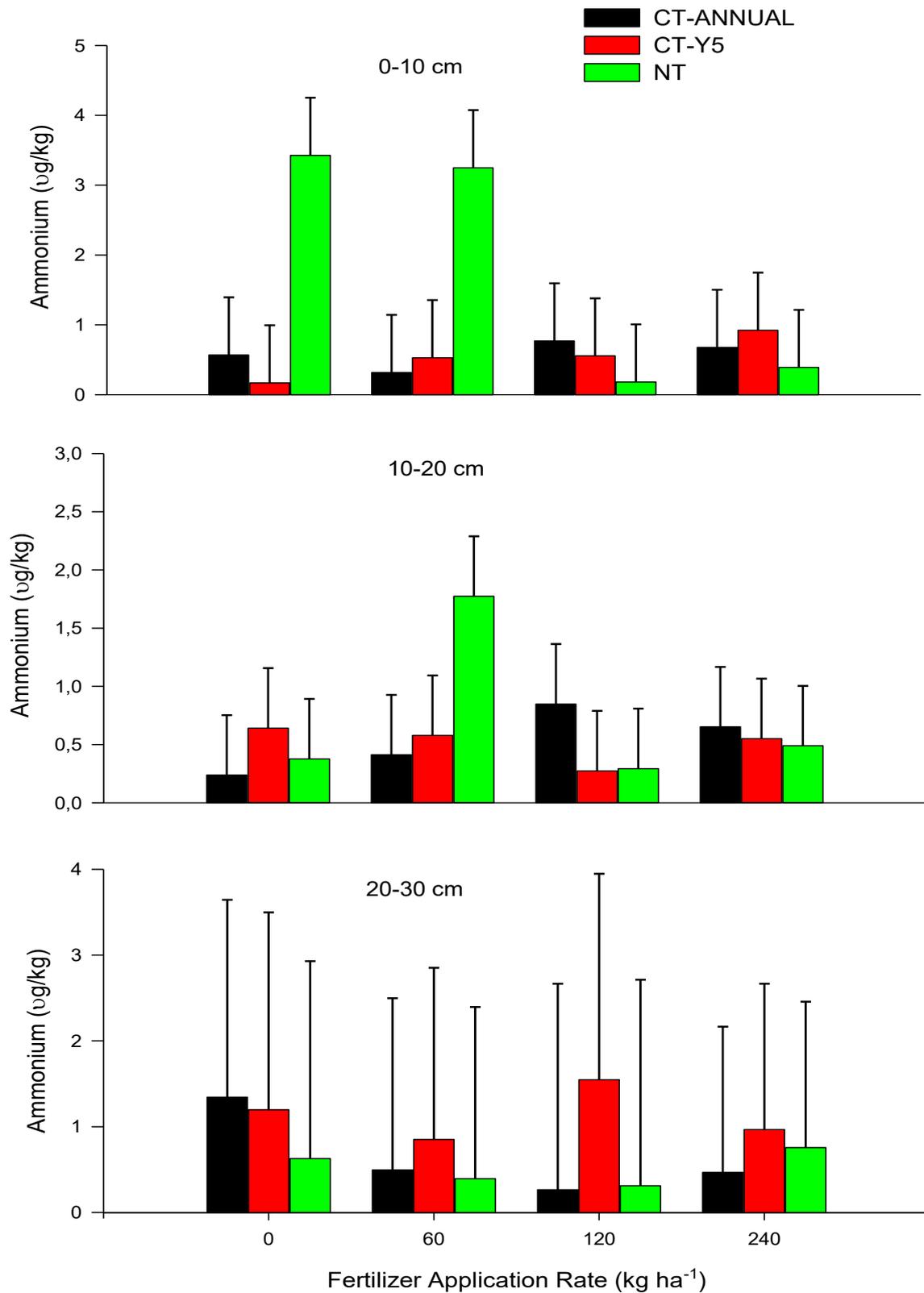


Figure 5. 4: Variation of ammonium-N with depth under different tillage techniques and urea fertilizer application rates.

5.3.4 N mineralisation in different treatments.

N mineralisation was highest in NT in the control at 0-10 cm soil depth ($p < 0.05$), but did not vary at other N rates (Figure 5.5). In the 10-20 cm soil depth, CT-Y5 had higher N mineralisation than NT at 120 and 240 kg N ha⁻¹ ($p < 0.05$). Furthermore CT-Y5 at 120 kg N ha⁻¹ had higher N mineralisation, 0.044 $\mu\text{g N kg}^{-1}$ soil, compared to CT-ANNUAL which had 0.017 $\mu\text{g N kg}^{-1}$ soil in 10-20 cm ($p < 0.05$). Moreover in the 20-30 cm soil depth, CT-ANNUAL had higher N mineralisation than NT at 60 and 240 kg N ha⁻¹, while CT-Y5 at 240 kg N ha⁻¹ had higher mineral N than NT ($p < 0.05$).

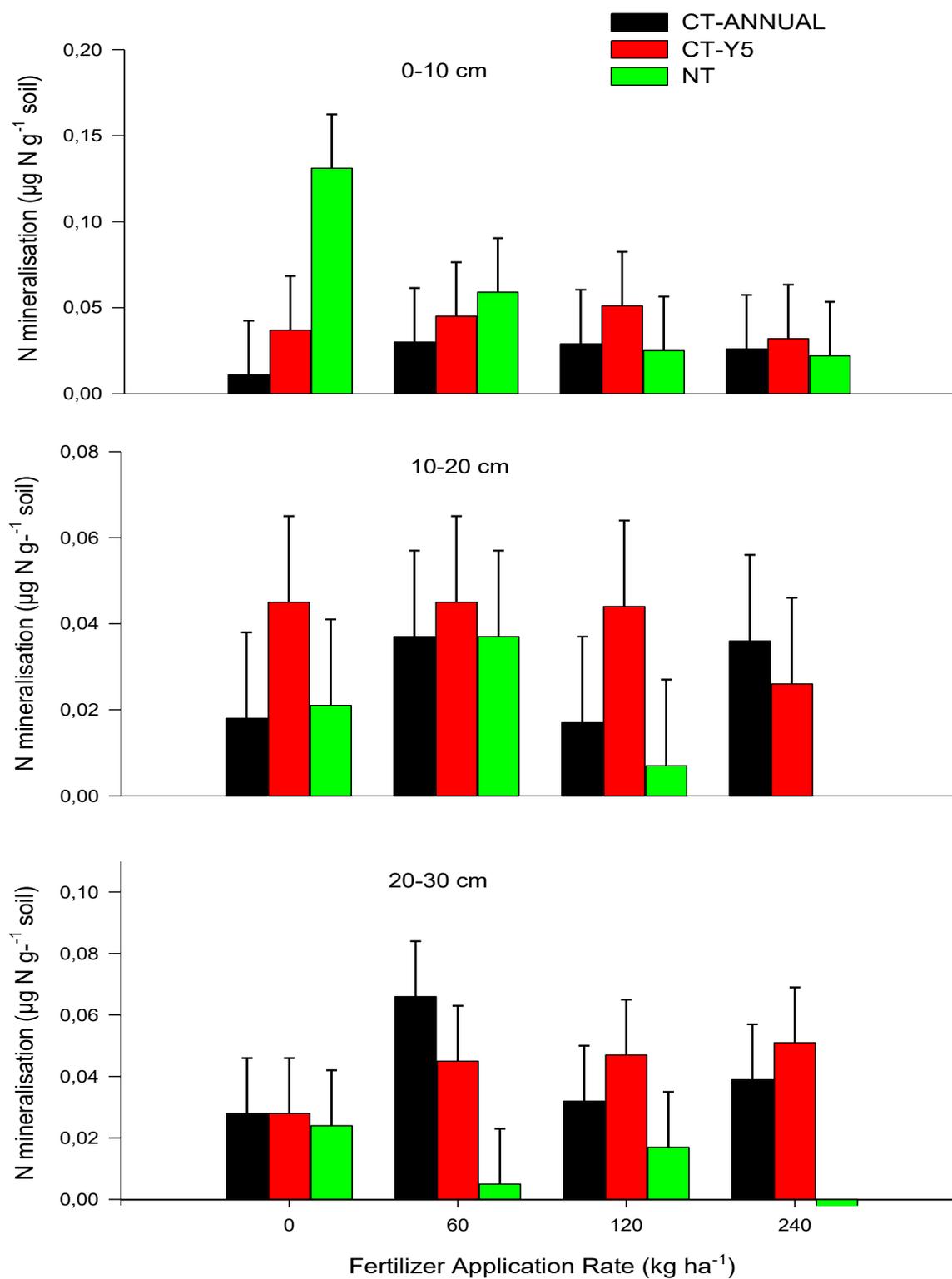


Figure 5. 5: Variation of anaerobic N mineralization with depth under different tillage techniques and urea fertilizer application rates.

5.3.5 Total P variations.

Total P in the 0-10 cm soil depth was higher ($p < 0.05$) under NT in all treatments (Figure 5.6). At 120 kg N ha^{-1} , CT-ANNUAL had higher total P (1.13 g kg^{-1}) than NT (0.49 g kg^{-1}) in the 10-20 cm soil depth, whereas at 240 kg N ha^{-1} , CT-Y5 had higher total P than NT ($p < 0.05$). In the 20-30 cm soil depth, total P was highest under NT at all application rates ($p < 0.05$).

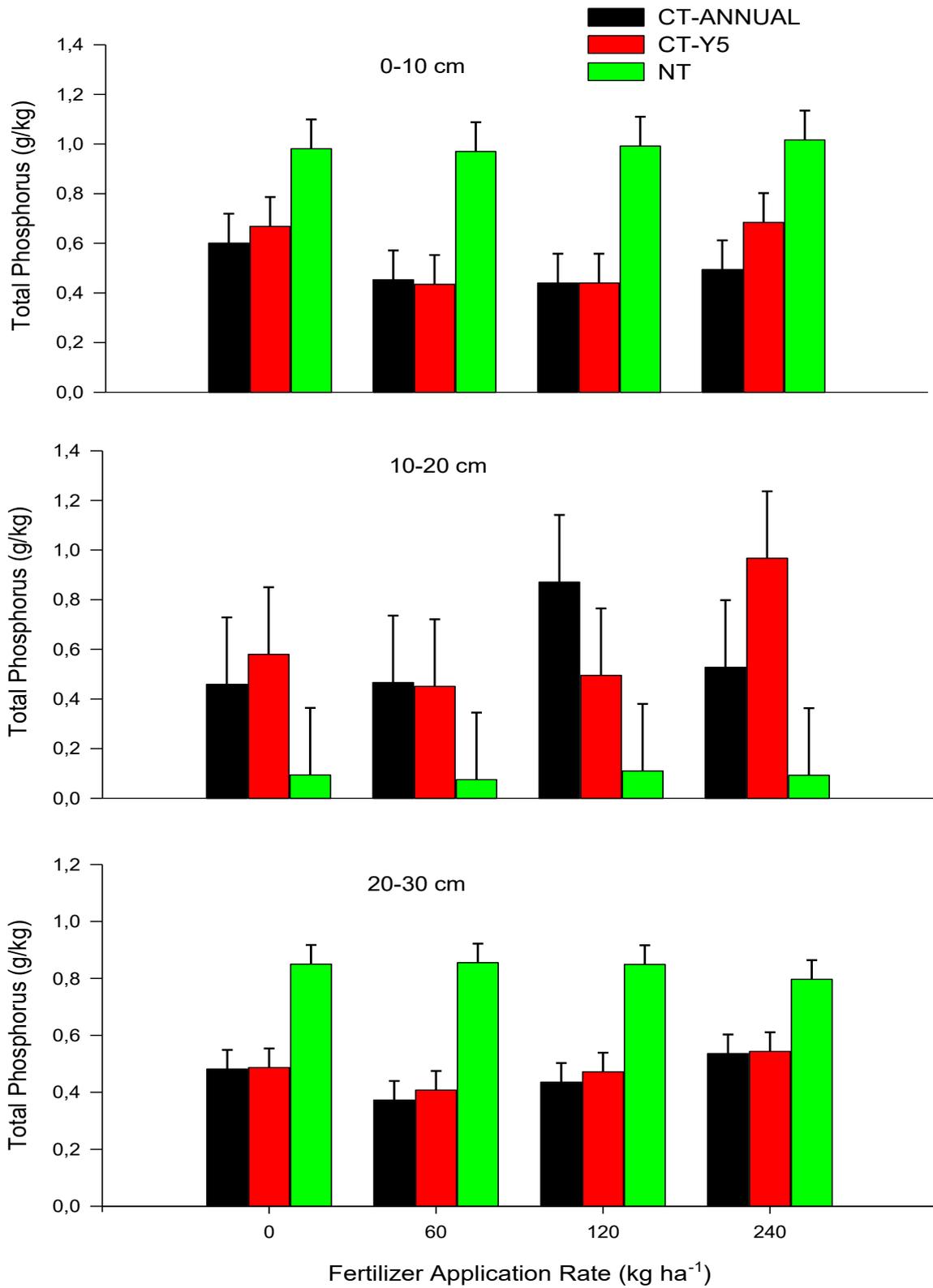


Figure 5. 6: Variation of total P with depth under different tillage techniques and urea fertilizer application rates.

5.3.6 Organic P variation in different treatments

Figure 5.7 shows that organic P was generally higher under NT compared to other tillage techniques at all application rates in the 0-10 cm soil depth, ($p < 0.05$). CT-ANNUAL at 0 and 240 kg N ha^{-1} also had higher organic P than CT-Y5 in this depth ($p < 0.05$). At 10-20 cm soil depth, NT had higher organic P than CT-ANNUAL in the control ($p < 0.05$). While at 60 kg N ha^{-1} both NT and CT-ANNUAL had higher organic P than CT-Y5, with NT being also higher in organic P (110 mg kg^{-1}) than CT-ANNUAL (70 mg kg^{-1}) in the 10-20 cm depth ($p < 0.05$). Again at 60 kg N ha^{-1} in the 20-30 cm soil depth, NT had higher organic P than CT-Y5 ($p < 0.05$).

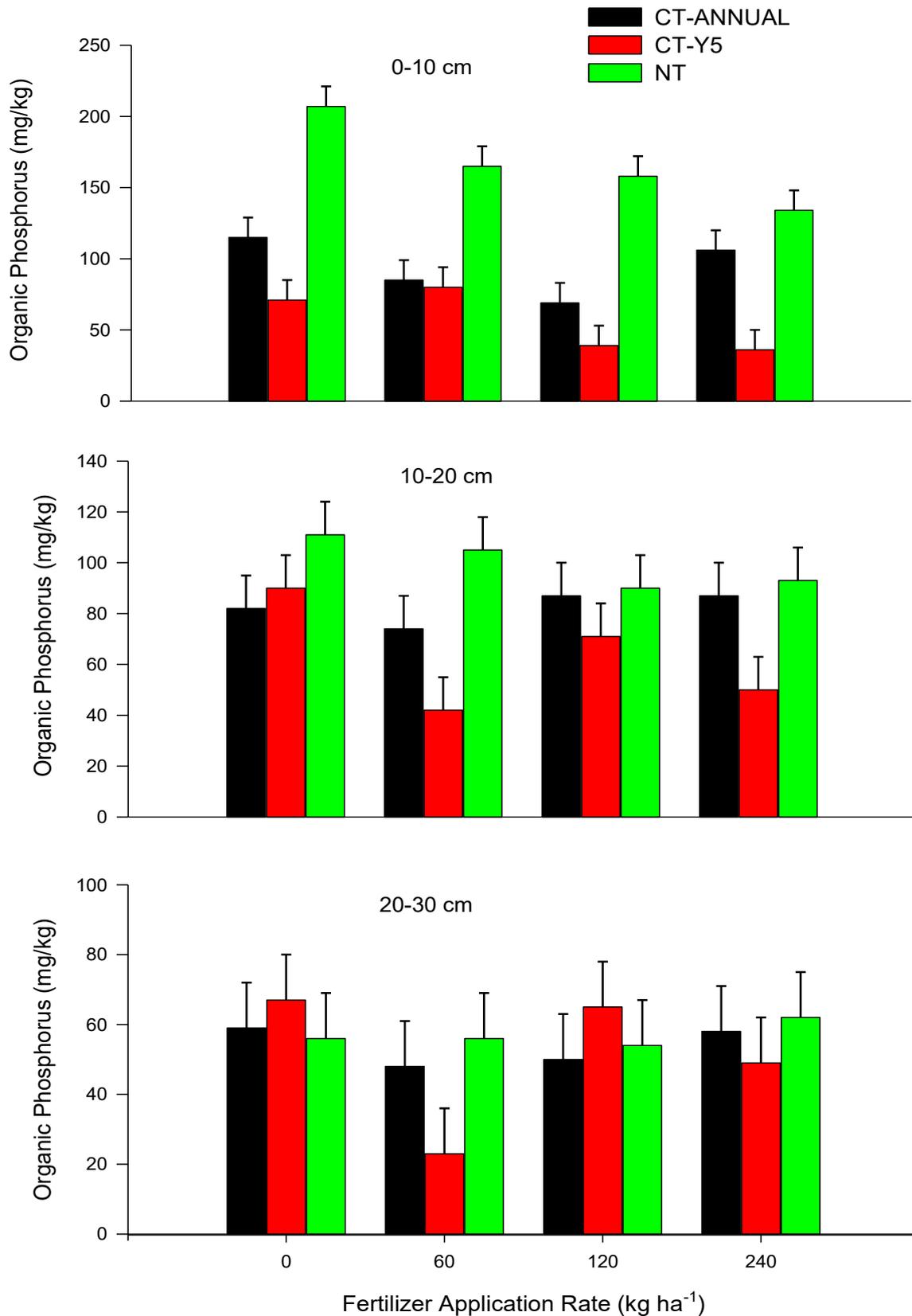


Figure 5. 7: Variation of Organic P with depth under different tillage techniques and urea fertilizer application rates.

5.3.7 Extractable phosphorus variations

Figure 5.8 shows that extractable P in the 0-10 cm soil depth was higher under NT in all treatments ($p < 0.05$). At 60 kg N ha⁻¹ in the 10-20 cm soil depth, CT-Y5 had higher extractable P (0.008 $\mu\text{g g}^{-1}$) than CT-ANNUAL (0.001 $\mu\text{g g}^{-1}$), while at 120 kg N ha⁻¹, NT (0.007 $\mu\text{g g}^{-1}$) had higher extractable P than CT-Y5 (0.003 $\mu\text{g g}^{-1}$), and at 240 kg N ha⁻¹ CT-ANNUAL had higher extractable P than CT-Y5 ($p < 0.05$). In the control at 20-30 cm depth, CT-Y5 and CT-ANNUAL had higher extractable P than NT, while the other N rates did not differ in extractable P ($p < 0.05$).

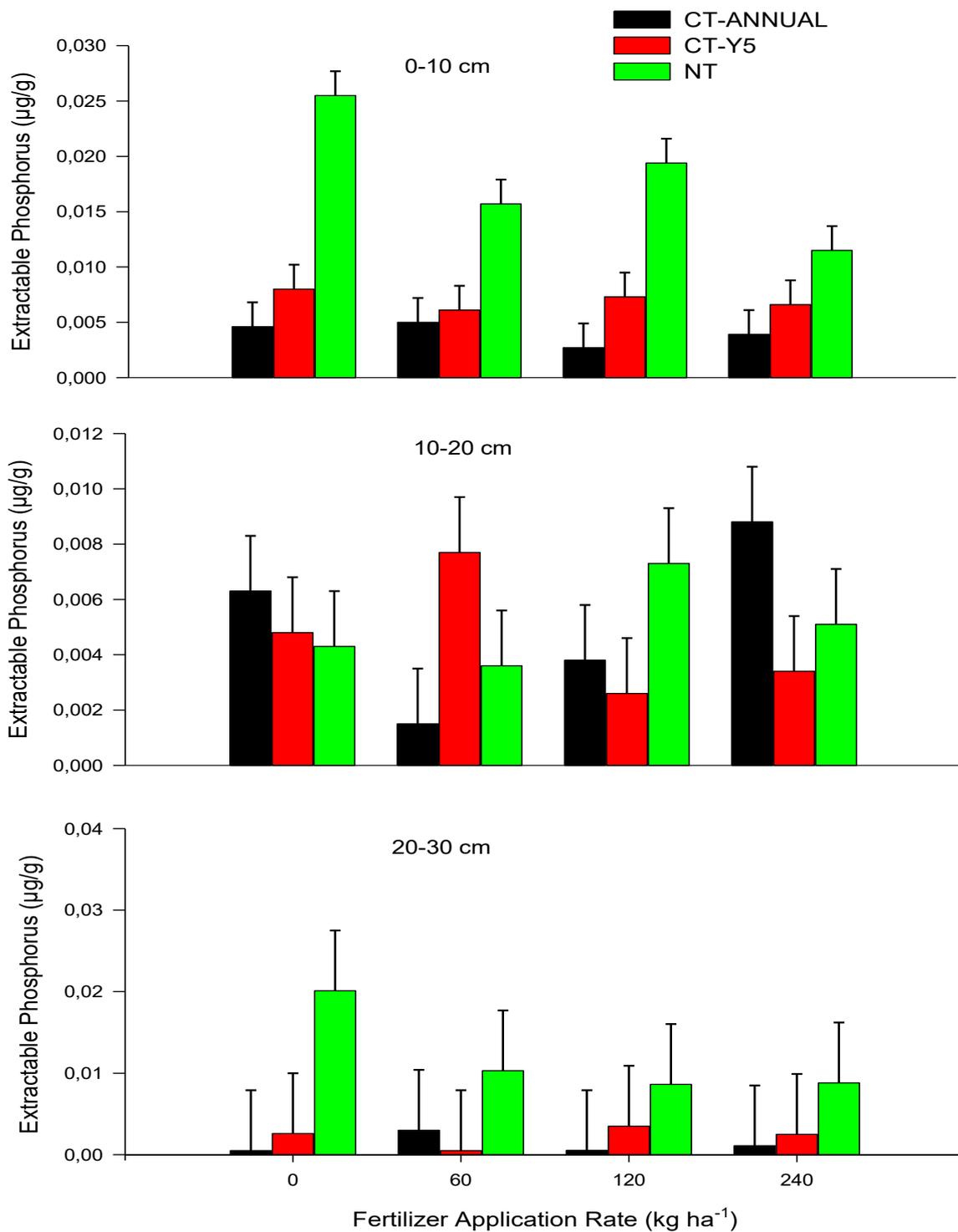


Figure 5. 8: Variation of extractable P with depth under different tillage techniques and urea fertilizer application rates.

5.4 Discussion

The high levels of total N under NT than CT-Y5 and CT-ANNUAL in the 0-10 cm depth may be attributed to accumulation of crop residues on the surface and less soil disturbance. While ploughing of soil under CT-ANNUAL improves aeration that accelerates decomposition of organic material and mineralization of organic N into soluble forms that can be easily lost through leaching. The reduced oxygen availability below the surface of NT systems also reduces decomposition rates causing SOM to be retained in conservation-tillage systems (Wershaw, 1993). While buried residues in CT also decompose 3.4 times the rate of residues left on the soil surface (Beare et al., 1994). Zibilske et al., (2002) observed that NT and minimum tillage promoted greater concentrations of N at the soil surface, but was uniformly distributed with depth under CT. This explains the observed increased total N under CT-ANNUAL at 120 and 240 kg N ha⁻¹ due to incorporation of mineral N fertilizer to deeper soil layers at 10-20 cm depth. Havlin et al., (1990) determined that reducing tillage and maintaining surface residues in a long-term study increased soil N in the surface 2.5 cm depth. While Ghidey & Alberts, (1993) also observed that buried residues and roots experienced more extensive rapid decomposition than surface residues, causing the more slowly decomposed surface residues to supply nutrients to crops in the long-term.

In our study tillage solely determined variations of N concentrations in the surface layer, whereas in the subsoil, N fertilization also had a salient effect. Thus high total N at 20-30 cm depth and 240 kg N ha⁻¹ observed in CT-Y5 may be attributed to both increased decomposition of organic material, through residue incorporation during tillage and high amount of N fertilizer. McCarty & Meisinger, (1997) observed that N fertilization at rates in excess of crop requirements tended to decrease amounts of OM in these soils. Green & Blackmer, (1995) explained that NO₃⁻ stimulated decomposition of residues, which accounted for decreased SOM due to excessive N fertilization in their study. In contrast, other researchers argued that although N fertilizer addition often accelerates initial decomposition of crop residue it has little effect on amounts of C ultimately retained in soil (Parr & Papendick, 1978).

Soil NO₃⁻ and NH₄⁺ concentration were inversely related in all treatments. Thus NO₃⁻ had its peak in 0-10 cm under CT-ANNUAL at 120 kg N ha⁻¹, whereas the NH₄⁺ peaked in NT at 0 kg N ha⁻¹ for this depth. Moreover, NO₃⁻ was the more predominant available N ion in all

treatments. This can be attributed to clay loam texture of the soil with good drainage and aeration which enhances nitrification of NH_4^+ to NO_3^- . The soil characteristics (shown in Chapter 3) had low exchangeable acidity ($0.03\text{-}3.9 \text{ cmol}_c \text{ kg}^{-1}$), slightly acidic pH ($3.44\text{-}5.28$) and adequate soil moisture for nitrification to occur. According to Li et al., (2018) NH_4^+ levels are usually relatively low compared to NO_3^- in arable soils as nitrification is predominant. This is contrary to the findings by Silgram & Shepherd, (1999) who observed that NH_4^+ was more predominant than NO_3^- and attributed this to the high activity of the ammonifying bacteria in contrast to nitrifiers which can be retarded by low water potential of dryland soil. In addition, environmental concerns have arisen in recent years regarding the susceptibility of soils to NO_3^- leaching (Silgram & Shepherd, 1999). Loss of NO_3^- from soil systems via leaching depletes soil N reserves over the long term, and can carry an agronomic cost as a result of declining soil fertility. Leached NO_3^- also contributes to eutrophication of freshwater bodies which have been linked to human health issues (Silgram & Shepherd, 1999).

Minimum tillage (CT-Y5), had the lowest NO_3^- concentration at all depths. This was similar to findings by Dowdell & Cannell, (1975), who observed lower soil NO_3^- in minimum tillage than ploughed treatments, due to decreased mineralization of organic N under minimum tillage than CT, and reduced accumulation of residues compared to NT. Cameira et al., (1996) also reported lower soil NO_3^- under minimum tillage than CT, and attributed this to lower nitrification and mineralization, as well as larger loss of NO_3^- through denitrification due to less aerobic soil conditions under minimum tillage than under CT (Doran, 1980). The high NO_3^- in CT-ANNUAL at 0-10 cm may be attributed to increased mineralisation of organic N during ploughing, while at 60 kg N ha^{-1} in sub-surface may be attributed to enhanced mineralisation of immobilized N as a result of incorporation of surface material. High N application rates of 120 and 240 kg N ha^{-1} were not beneficial as they had reduced NO_3^- since they stimulate increased microbial population which would assimilate and immobilize N, and also excessive NO_3^- is susceptible to leaching losses. Goss et al., (1993) found that CT at 20 cm soil depth increased NO_3^- leaching losses by 21% mainly as a result of the enhanced mineralization of SOM. However, minimum tillage increased NO_3^- leaching losses following fertilizer application (Goss et al., 1988), probably as a result of greater bypass (macropore) flow in minimum tillage compared to CT, because ploughing would have disrupted the continuity and connectivity of macropore channels involved in solute transport. El-Haris et al., (1983) reported

that CT resulted in higher mineral N, lower organic C, and a narrower C/N ratio in the top 15 cm relative to NT, which was likely caused by rapid decomposition during ploughing.

The high NO_3^- levels in the surface of NT (with the exception of CT-ANNUAL at 120 kg N ha^{-1}) were expected, because microbial activity is higher due to organic surface residues of NT. This was contrary to findings by Rees et al., (1996), who found no observable differences in NO_3^- accumulation at any depth when N rates were <90 kg N ha^{-1} . According to Rees et al., (1996) excess NO_3^- not consumed by microbes is assimilated by the crop, fixed on soil exchange sites, denitrified, volatilized and/or immobilized via other pathways. In our study, the control was characterised by low NO_3^- levels compared to 60 kg N ha^{-1} in most treatments, and this may be attributed to less biomass production due to low N supply. Singh & Singh, (1993) also observed greater NO_3^- (by 45-66 %) in the N fertilized plots. At a dryland long-term research site, El-Haris et al., (1983) reported an 88% increase in inorganic N after 67 kg N ha^{-1} fertilizer application. Again, the disturbance caused by cultivation has not always increased soil mineral N status (Soon & Clayton, 2002), and the fate of any additional mineral N will depend on soil texture, which will control its susceptibility to leaching.

The higher NH_4^+ in NT (at 0 and 60 kg N ha^{-1} in 0-10 cm) and CT-Y5 (at 120 kg N ha^{-1} in 20-30 cm) may be attributed to high ammonification due to abundant OM substrate, and high adsorption of NH_4^+ by organic soil colloids. Das et al., (2009) found that the application of N fertilizers contributed to large amounts of NH_4^+ , which will eventually be converted into ammonia that volatilises in gaseous form. However, we cannot explicitly verify the concentration of ammonia that volatilized since there were no measurements done but the soil conditions such as high soil moisture and temperature, and accumulation of soil residues favoured volatilisation under NT. A study by Rees et al., (1996), found no treatment differences in NH_4^+ accumulation at any depth or N application rate. In our study, the higher NH_4^+ levels under NT at low application rates in 0-10 cm depth is because of high organic N that can be easily decomposed to available N, whereas at higher application rates ammonia will be volatilized. NH_4^+ is normally generated by the hydrolysis of inorganic fertilizers and is either adsorbed on clay surfaces or nitrified (Singh et al., 2002).

N mineralisation generally decreased with fertilizer application. Again, it was higher at 0-10 cm depth compared to subsurface soil, especially in the control of NT. El-Haris et al., (1983)

also observed high N mineralization at 0-10 cm in NT soil, whereas for CT, it was greater at 10-20 cm depth which was similar with the current study at 240 kg N ha⁻¹. Long-term residue retention in NT systems can increase the size and turnover of microbial biomass, and hence OM mineralization compared with CT, due to improved soil moisture retention induced by surface residues (El-Haris et al., 1983). However, Silgram & Shepherd, (1999) reported that practices that disrupt soil structure such as cultivation, may accelerate N mineralization and nitrification. This is due to their positive effects on soil porosity, aeration, hydraulic conductivity, and because the physical disruption may bring microbial populations into contact with fresh, previously unavailable organic substrates (Silgram & Shepherd, 1999).

CT-Y5 generally had higher N mineralisation for most treatments at 10-20 cm. Combined residue retention and ploughing after 4 seasons might be attributed to this. El-Haris et al., (1983) also observed that ploughing after 4-5 years of NT enhanced N mineralization over NT and CT. Results also showed that NT recorded the least N mineralisation at 20-30 cm depth. According to Doran, (1987), tillage effect differs with depth, with N mineralisation of NT soils being 34% higher in the surface layer (0-7.5 cm) than those of ploughed soils, while the opposite was observed at 7.5-15 cm depth. Cultivation generally leads to a temporary increase in soil mineral N and availability of a larger pool of C substrates that supports greater microbial activity (Li et al., 2018). Without CT, OM and nutrients such as N tend to accumulate at or near the surface, and this may restrict mineralization in the soil beneath (Chamen & Parkin, 1995). Over supply of N, 240 kg N ha⁻¹, under NT may lead to immobilisation, N assimilation and reduction in N mineralisation of subsurface soil. This is because microbes will assimilate the excess N thus immobilizing and fixing it. Thus, excessive N fertilization causes a shift in the decomposer-community composition, where microbes with high N-assimilation efficiency outcompete decomposers (Couteaux et al., 1995). Of particular importance to agriculture is the question of whether soil disturbance alters net N mineralization, or simply modifies the temporal dynamics of N release into plant-available forms (Silgram & Shepherd, 1999).

NT had higher total P at 0-10 and 20-30 cm soil depths. This may be attributed to high OM under NT due to accumulation of crop residues and lack of soil disturbance. The low P of NT at 10-20 cm may be due to accumulation of P from senescent roots under CT-Y5 and CT-ANNUAL because roots can be easily distributed to open pores from tillage. Similar findings

were observed by Franzluebbers & Hons, (1996) where soil under NT had greater P at 0-5 cm and 15-30 cm depths compared to CT which had higher P at 5-15 cm depth. This was due to surface accumulation of residues and increased OM in NT whereas in CT residues accumulated mainly in the root zone (5-15 cm depth). Bertol et al., (2007) observed accumulations of total P at the surface three to five times greater in NT than CT. According to Khan et al., (2018), the rate of translocation of P down the soil profile was much less than that of N and K, as P was fixed onto mineral and organic compounds, which resulted in its accumulation in the plough layer. However, many field studies have demonstrated P leaching to subsoil in both coarse and fine-textured soils (Wang et al., 2015). Indeed, continuous, long-term fertilization in excess of crop requirements has been shown to result in P leaching in many soils (Khan et al., 2018). Redel et al., (2007) highlighted that greater total soil P at 0-10 but not 15-30 cm under reduced tillage reflected a lower degree of mixing and distribution of P compared to CT. In a study by Selles et al., (1997), P was distributed uniformly throughout the depth of sampling under minimum tillage and CT, whereas under NT, its distribution reflected accumulation of crop residues in the soil surface layer. The significant drop in P with depth under NT was attributed to lack of soil disturbance which fosters accumulation of organic material at or near the surface and has the potential to affect bio-cycling of P (Selles et al., 1997).

An increase in P availability under NT could be attributed to its release from crop residues (Guppy et al., 2005). Damon et al., (2014) however observed that crop residues with low P concentration (as a result of translocation of most stubble P into grain), such as cereal stubble, will not make a significant contribution to soil P. Selles et al., (1997) highlighted that the difference in total P could also be attributed to higher erosive losses under CT than NT. In our current study NT had higher total P in all application rates in all sampled depth except at 10-20 cm depth which may be attributed to higher bases and ammonium, which fixed P, on the same depths relative to other tillage systems. P fixation by acidic ions is high at lower soil pH, particularly in weathered soils with high Fe and Al oxides and pH-dependent charge minerals such as kaolinite (Bolan et al., 2003). Therefore, CT-Y5 and CT-ANNUAL with lower pH than NT especially at 0-10 cm depth, (Chapter 3, Table 3.1), had its P fixed. According to Barber et al., (1963) P is absorbed from a small cylinder of soil surrounding the root, because it diffuses slowly through the soil with high pH favouring P availability compared to soil with low pH. NH_4^+ is often superior to NO_3^- in stimulating P uptake, because of its effect on transferring P across the root symplast (Riley & Barber, 1971). The uptake of NO_3^- on the other hand causes

plant roots to exude anions into the soil solution to balance excess negative charge, which can displace P adsorbed onto soil colloids therefore enhancing P desorption. P sorption capacity of soils is governed by the concentration, types and surfaces of Al and iron oxides, even in calcareous soils (Frossard et al., 1995). While P removal from the soil solution results from its adsorption, precipitation and immobilization (Bünemann, 2015). However, P replenishment results from a combination of desorption, dissolution and mineralization (Bünemann, 2015). Immobilisation of soil P occurs when total P content of residues is insufficient to meet the P requirement of microbial biomass as it proliferates in response to new C substrates (Damon et al., 2014). While availability of adsorbed organic P to microorganisms or enzymes can increase with increasing residue cover of the soil (Olsson et al., 2012) as well as with exudation of organic anions (Giles et al., 2012).

Slower decomposition of plant residues may prevent rapid mineralisation of organic P and its translocation through the soil profile, which is more likely to occur when plant residues are accumulating on the soil surface under NT, because of less aeration and high soil moisture content. However, the higher organic P under CT-ANNUAL relative to CT-Y5 at 10-20 cm depth may be attributed to lower pH and higher exchangeable acidity especially at 60 and 120 kg N ha⁻¹, (Chapter 3, Table 2) under CT-ANNUAL which will intensify immobilisation of organic P. Reed et al., (2011) highlighted that sorption and stabilisation of organic P is greatest in highly weathered soils with high P fixing capacities because weathering results in the prevalence of 1:1 clays (e.g. kaolinite) and in Al and Fe sesquioxides that effectively sorb P. This agrees with the current study since annual ploughing breaks down soil aggregates and increase soil pores which in turn may accelerate chemical weathering making CT-ANNUAL to sorb high organic P compared to CT-Y5. Sorption and subsequent stabilisation of organic P in soil remains a major cause for the dynamics of P release from crop residues to deviate from that of C and N (Damon et al., 2014). Furthermore, under CT there is rapid mineralization of organic P to supply plant and microbial P compared to conservation tillage. On the other hand under NT, organic P in plant residues is decomposed slowly by microbes and would have less opportunity to be fixed by soil colloids compared to CT (Thibaud et al., 1988). Thus high organic P under NT compared to CT-Y5 and CT-ANNUAL were observed in the current study especially in surface soil. Under NT organic P remain protected against fixation whereas in CT, residues are decomposed and organic P get adsorbed immediately (Thibaud et al., 1988).

In the current study extractable P was less than both total and organic P which concurs with many previous literature. Again the effect of N application on extractable P at 0-10 and 20-30 cm soil depth was minimum with fluctuations at 10-20 cm depth, however extractable P decreased with depth. This was similar to findings by Franzluebbers & Hons, (1996) who observed no effect of N fertilization on extractable P. The high extractable P in NT at 0-10 cm depth can also be attributed to residue accumulation and low soil disturbance compared to CT-Y5 and CT-ANNUAL. This is because higher total and organic P under NT at 0-10 cm allowed sufficient plant available P within the soil solution. According to Margenot et al., (2017), reduced tillage appeared to favour P immobilization, while CT favoured its mineralization, which was contrary this study. Higher mineralisation under CT than NT was not evident in the current study because of high OM under NT which compensated for higher concentration of mineralized P. Thus over a longer time period there was more P mineralised under NT than CT due to sufficient OM supply. In CT, ploughing exposes adsorption sites thereby increasing adsorption of P by ligand exchange in comparison to NT (Fink et al., 2016). Soluble P accumulation in NT is attributed to a decrease in the adsorption of P onto mineral surfaces due to less adsorption sites and to the release of P from mineralization of OM (Guppy et al. 2005). Franzluebbers & Hons, (1996) reported similar findings of greater extractable P under NT in surface soil, before decreasing rapidly with depth. This agrees with current findings especially at 20-30 cm depth in the control treatment where extractable P was extremely low under NT.

Decrease of extractable P with depth in NT compared with CT is probably a direct result of surface-placement of crop residues that leads to accumulation of SOM and microbial biomass near the surface (Franzluebbers et al., 1995). OM additions under residue retention can reduce P fixation by increasing organic anion competition for P binding sites (Palm et al., 2007). Furthermore, NT improves soil aggregation, which can reduce P fixation by decreasing outer soil surface area and therefore potential sorption sites (Zhang et al., 2004). This shows that P availability is influenced by the physicochemical properties of soil such as moisture, organic C, bases and pH which change with depth and tillage technique. Bases, soil moisture and soil organic C were higher at the surface of NT relative to CT-ANNUAL, however, in the subsurface the opposite was true. This was contrary to findings of Franzluebbers & Hons, (1996) who reported that more extractable P with NT than CT was also present below the till-zone, which could be due to accumulation of P in senescent roots (Unger, 1991). In a study by Edwards et al., (1992), there were no differences of extractable P between tillage systems in

the subsoil. The elongation of plant roots under CT-ANNUAL and their decomposition later may increase plant available P in subsoil through P release from roots. Rubaek et al., (2013) proposed that the transfer of P can be caused by leaching of dissolved and colloidal P from top to subsoil, bioturbation by soil-dwelling animals and redistribution due to root activity.

5.5 Conclusion

Soil tillage influences the magnitude and dynamics of mineralization by altering the distribution of OM with depth. Total P and N, as well as organic P were greater under NT at 0-10 cm depth. This was due to the accumulation of crop residues and minimal soil disturbance. Moreover total P was higher in NT at 20-30 cm, while total N also was higher at 60 and 120 kg N ha⁻¹ at this depth this may be due to high ability to replenish adsorbed and extracted P from the solution. Further, NT caused greater N mineralization in the control at 0-10 cm depth which eventually decreased with depth. The NO₃⁻ and NH₄⁺ trends were inversely related, with NO₃⁻ having higher concentrations in all treatments. NO₃⁻ was higher under CT-ANNUAL at 10-20 and 20-30 cm depth in 0 and 60 kg N ha⁻¹, which was attributed to great mineralization due to ploughing and mixing of soil with organic materials and fertilizer, respectively. However, CT-Y5 had lower NO₃⁻ concentrations than CT-ANNUAL and NT due to lower nitrification, mineralization of OM and residue accumulation, respectively under CT-Y5. Leaching and sorption of extractable P may have contributed to its low levels under CT-ANNUAL at 0-10 cm depth especially in lower application rates. While conservation tillage tended to have enough buffer to be able to replenish diminished P by plants or microbes. NT was great in nutrient (N and P) retention and distribution especially on the surface. NT at 60 kg N ha⁻¹ in 0-10 cm soil depth had an optimum total N, NO₃⁻, NH₄⁺, total P and organic P thus showing its salient impact on the sustenance of soil fertility. Therefore NT at 60 kg N ha⁻¹ would be mostly recommended in sustaining soil productivity.

CHAPTER 6

ENZYME ACTIVITY UNDER DIFFERENT TILLAGE TECHNIQUES AND N FERTILIZER MANAGEMENT IN DRY LAND AGRICULTURE

6.1 Introduction

Soil is a habitat of a wide range of microorganisms, which help in the decomposition of OM and immobilization of heavy metals. Microorganisms together with plants are major sources of soil enzymes. They play an important role in ecosystem function because they contribute to the cycling of key nutrients such as C and N (Alster et al., 2013). Soil enzymes influence the release of nutrients for plant and microbial growth, and regulate gas exchange between soils and the atmosphere (Conrad et al., 1983). As such enzyme activity can be used as an index of soil microbial activity (Benitez et al., 2000). Again enzymes provide a useful tool for long-term monitoring of changes in soil health and quality and they have been proposed as soil fertility indicators (Frankenberger & Dick, 1983).

Climate, especially rainfall and temperature play a significant role on enzyme activity. In the study area, precipitation is mostly predominant in summer with high temperatures, while the winter season has low temperatures and less precipitation. Therefore under these conditions enzymes activity would be reduced in winter and high in summer due to more conducive climatic conditions. According to Alster et al., (2013), a decrease in enzyme activity with drier conditions could be due to lower microbial biomass or adsorption of enzymes to soil particles in drier conditions, that limit catalytic rates while reducing enzyme turnover (Steinweg et al., 2012). Furthermore, thinner water films could increase contact between enzymes and insoluble OM, leading to enzyme immobilization and protection from degradation (Nannipieri et al., 2002). Thus the activity of soil enzymes is sensitive to the environment in which they are located (Dick, 1984). Dick & Tabatabai, (1984) observed that acid phosphatase activity was predominant in acid soils, while alkaline phosphatase activity was high in neutral to alkaline soils.

Management practises such as tillage and fertilizer application can also alter spatial and profile distribution of soil enzymes. Dick, (1984) showed that the activity of some enzymes was higher in NT than CT in the top 7.5 cm layer. In essence, long-term tillage can alter soil structure and increase OM losses, since it disrupts soil aggregates exposing more OM to microbial attack (Beare et al., 1994). Considering that soil enzymes are produced by microbes and plants, their activity would be strongly influenced by the microbial biomass pool. There is also a strong relationship between SOM content and enzyme activity, with both parameters influenced by cultivation (Kennedy & Smith, 1995). Enzyme activity would increase with decreased tillage in a manner similar to SOM content (Alvarez et al., 1995). Dick, (1984) found soil urease, acid phosphatase and invertase activity in the 0-10 cm surface layer to be higher in NT than CT fields. According to Dick, (1984) the higher enzyme activity in the surface profile of NT compared to CT plots indicate greater biological activity near the soil surface due to higher organic residues of NT.

Soil physical and chemical properties are important factors affecting microbial growth and activity, as a consequence, they may determine changes in enzymatic activity (Curci et al., 1997). N fertilization enhances physicochemical and biological characteristics of the soil through cementing aggregates together and providing substrates to the microbes, thereby affecting enzymatic activity. Long-term N addition resulted in reduced C versus N availability for microbial growth, thus stimulating the production of C-acquiring rather than N-acquiring enzymes (Cenini et al., 2016). Inorganic N availability generally has an inhibitory effect on the activity of N-acquiring enzymes (Allison et al., 2010). Jian et al., (2016) found that N fertilization stimulated hydrolases associated with C and P acquisition, depressed oxidase activity and had no effect on hydrolases associated with N acquisition. In contrast to the P-acquiring enzymes with a demonstrated robust link to microbial P demand, the correspondence between N-acquiring enzyme activity and N demand is weak because mineralization of organic N compounds is often coupled to energy acquisition (Sullivan et al., 2014). Thus unlike P-acquiring phosphatase enzymes which have wide substrate affinities, each N compound is decomposed by a distinct enzyme system (Fujita et al., 2017). Because of its sensitivity, ease and low cost of measurement, soil enzyme activity may be key for soil assessments related to sustainability (Park & Seaton, 1996). Enzyme activity may be useful for validation of changes in soil quality predicted by simulation models from existing data bases (Acton, 1994). Again it may be useful to verify effectiveness of management practices in sustainable crop production

systems (Kennedy & Papendick, 1995). Soil enzyme activity responds to tillage and N fertilizer input, but it is important to assay their activity at different depths. Therefore the objective of this study was to assay the activities of urease, invertase and acid phosphatase under different tillage and urea fertilizer management. The criteria for choosing the types of enzyme assays was based on their importance in major nutrient cycling processes.

6.2 Material and Methods

The study site with maize mono-cropping, was described in detail in Chapter 3, (Section 3.2).

6.2.1 Soil sampling procedure

The soil sampling procedure was also described in Chapter 3, (Section 3.2.1).

6.2.2 Urease enzyme assay

Fresh soil samples, refrigerated at 4 °C for 2 weeks, were ground and sieved to pass through a 2 mm sieve. Five grams of soil was placed in 100 mL Erlenmeyer flask and wetted with 2.5 mL of 0.08M aqueous urea solution (Kandeler & Gerber, 1988). The flask was stoppered and placed in an incubator at 37 °C for 2 hours. After 2 hours the stopper was removed and 30 mL of 1M KCl and 20 mL 0.01M HCl were added, then the mixture was shaken for 30 minutes. After centrifuging, the contents were filtered using Whatman No. 42 filter paper, so that a clear suspension could be extracted. One millilitre of filtrate was diluted with 10 mL of distilled water, then 5.0 mL of sodium salicylate solution and 2.0 mL of 0.1% sodium dichlorisocynurate were added. The diluted filtrate was then analysed for ammonium after 30 minutes using a SPECTRO UV-11 spectrophotometer set at 690 nm (Kandeler & Gerber, 1988). Standards and blanks containing 0, 0.5, 1.0, 1.5 and 2.0 $\mu\text{g mL}^{-1}$ $\text{NH}_4^+\text{-N}$ were prepared and also analysed as above. The NH_4^+ content was calculated by reference to a calibration graph plotted from the results obtained with diluted standards (Kandeler & Gerber, 1988). Urease activity was then expressed as $\text{mg NH}_4^+ \text{g}^{-1}$ soil, (Kandeler & Gerber, 1988).

6.2.3 Invertase enzyme assay

Sieved soil (<2 mm) of about 3.0 g was placed into a 50 mL Erlenmeyer flask, then 0.2 mL of toluene and 5.0 mL of 0.17 M modified universal buffer (MUB) was added, and the contents swirled for a few seconds to mix well (Frankeberger & Johanson, 1983). Furthermore 5.0 mL of 10% sucrose solution with a final concentration of 0.29 M was added, and the flask swirled again for a few seconds before placing it in an incubator set at 37 °C for 24 hours. After incubation, the contents were filtered using Watman No. 42 filter paper. A 1.0 mL aliquot of filtrate was pipetted into a 50 mL test tube then 5.0 mL of deionized water, 2.0 mL of 2 M NaOH and 2.0 mL of colour reagent (a mixture of 0.2 g of 3, 5-dinitrosalicylic acid monohydrate, 0.025 g of sodium carbonate and 0.005 g of EDTA (ethylenedinitrilo) - tetra acetic acid disodium salt) were added. After vortexing, the test tube was placed into a boiling water bath for 5 minutes then allowed to cool at room temperature. A similar procedure was conducted on the blanks and standards. The colour intensity was measured using a spectrophotometer (SPECTRO UV-11) set at wavelength 540 nm. The reducing sugar content of the extracts was calculated by reference to a calibration graph plotted from results obtained with glucose.

6.2.4 Acid phosphatase enzyme assay

Considering that soil pH was mostly slightly acidic, acid phosphatase activity was determined by incubation of soil with p-nitrophenol phosphatase (Alef & Nanniepieri, 1995). Sieved soil (<2 mm) of 1.0 g mass was placed into a 50 mL Erlenmeyer flask, then 0.25 mL of toluene, 4.0 mL of MUB (adjusted to pH 6.5) and 1.0 mL of 0.025 M p-nitrophenyl phosphate solution were added. The contents, with a stopper, were swirled for few seconds then incubated for an hour at 37 °C. The stopper was removed after incubation and 1.0 mL of 0.5 M CaCl₂ and 4.0 mL of 0.5 M NaOH were added. The flask was swirled for a few seconds then the suspension filtered through Whatman no. 42 filter paper. According to Alef & Nanniepieri, (1995) the filtrate is supposed to have a yellow colour. All the steps done on the samples were also done on the blanks and standards. The absorbance of the filtrate was measured using a spectrophotometer (SPECTRO UV-11) set at wavelength 400 nm. The results were corrected for the blank, and p-nitrophenol per 1.0 mL of the filtrate then calculated by reference to the calibration curve as follows (Alef & Nanniepieri, 1995);

$$\text{p-nitophenol } (\mu\text{g g}^{-1} \text{ dwt hr}^{-1}) = \frac{(C*v)}{\text{dwt}*SW*t}$$

Where:

C = measured concentration of sample in $\mu\text{g g}^{-1}$ mL filtrate;

Dwt = dry weight of 1.0 g soil;

V = total volume of soil suspension in mL;

SW = weight of soil sample used which (i.e. 1.0 g) and

T= incubation time which was 1 hour.

6.2.5 Statistical Analyses

An analysis of variance (ANOVA) test was done to determine the effect of tillage and fertilizer rates on soil enzyme activity at different depths. Treatment factors were tillage practice, N fertilizer application rate and soil depth. The Fisher's protected LSD was used as a post-hoc test for multiple comparison between treatment means and their interactions. The p value (<0.05) was used to determine the level of significant difference between treatment means. All tests were performed with GenStat 14.1 for Windows software (VSN international, 2011).

6.3 Results

6.3.1. Urease activity

Urease activity at 0-10 cm soil depth was higher ($p < 0.05$) under NT in all treatments, except for CT-Y5 at 240 kg N ha⁻¹ (Figure 6.1). While CT-Y5 at 0 and 240 kg N ha⁻¹ had higher urease activity than CT-ANNUAL at this depth ($p < 0.05$). In the 10-20 cm soil depth, urease activity was higher under CT-Y5 at 60 kg N ha⁻¹ ($p < 0.05$). Furthermore, at 120 kg N ha⁻¹, CT-Y5 (8.38 mg NH₄⁺ g⁻¹) had higher urease activity ($p < 0.05$) than NT (6.87 mg NH₄⁺ g⁻¹ soil) in this depth (Figure 6.1). Moreover NT in 10-20 cm soil depth had higher urease activity than CT-ANNUAL at 60 kg N ha⁻¹, as it also had higher urease activity than CT-Y5 and CT-ANNUAL at 240 kg N ha⁻¹ ($p < 0.05$). Figure 6.1 shows that CT-ANNUAL at 20-30 cm depth had higher ($p < 0.05$) urease activity than NT in the control and CT-Y5 at 240 kg N ha⁻¹. While at 60 and 120 kg N ha⁻¹ urease activity was higher under NT compared to both CT-ANNUAL and CT-Y5 ($p < 0.05$). In addition at 240 kg N ha⁻¹, urease activity under NT (13.27 mg NH₄⁺ g⁻¹ soil) was higher than under CT-Y5 (8.37 mg NH₄⁺ g⁻¹ soil) in the 20-30 cm soil depth ($p < 0.05$).

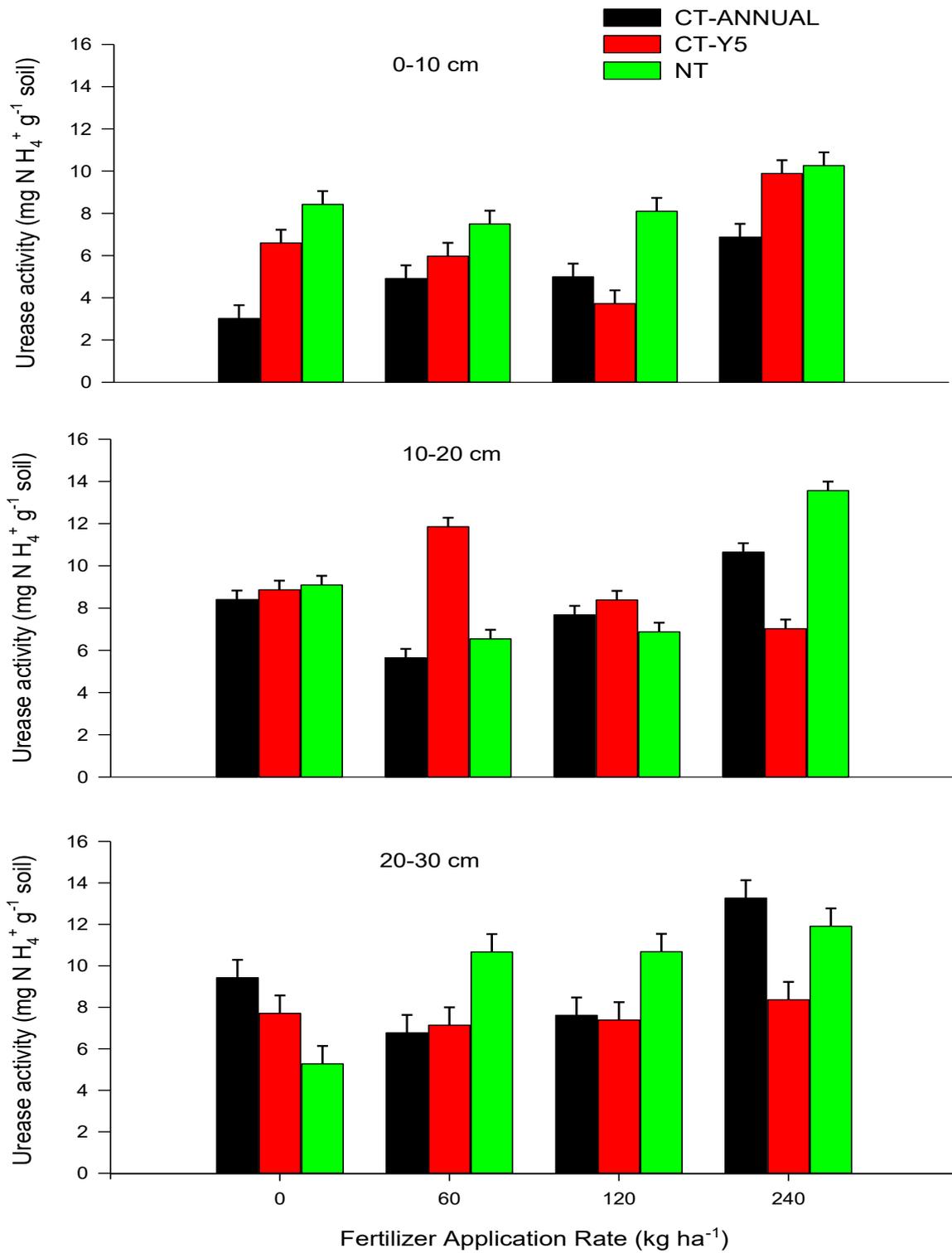


Figure 6. 1: Urease activity at different soil depths, N application rates and tillage techniques.

6.3.2. Invertase activity

Figure 6.2 shows that in the 0-10 cm soil depth, NT at 120 kg N ha⁻¹ had higher invertase activity than CT-Y5 and CT-ANNUAL ($p < 0.05$). However at 10-20 cm depth, NT had the lowest invertase activity in the control, while CT-Y5 was lowest at 60 kg N ha⁻¹, ($p < 0.05$). However, at 120 kg N ha⁻¹ in 10-20 cm soil depth, NT had the highest invertase activity ($p < 0.05$). In the 20-30 cm depth, CT-ANNUAL had the highest invertase activity at 0 and 240 kg N ha⁻¹ ($p < 0.05$). While at 60 and 120 kg N ha⁻¹, NT had the highest invertase activity ($p < 0.05$), and CT-ANNUAL (1.68 mg glucose g⁻¹ soil) had the lowest activity at 120 kg N ha⁻¹, (Figure 6.2).

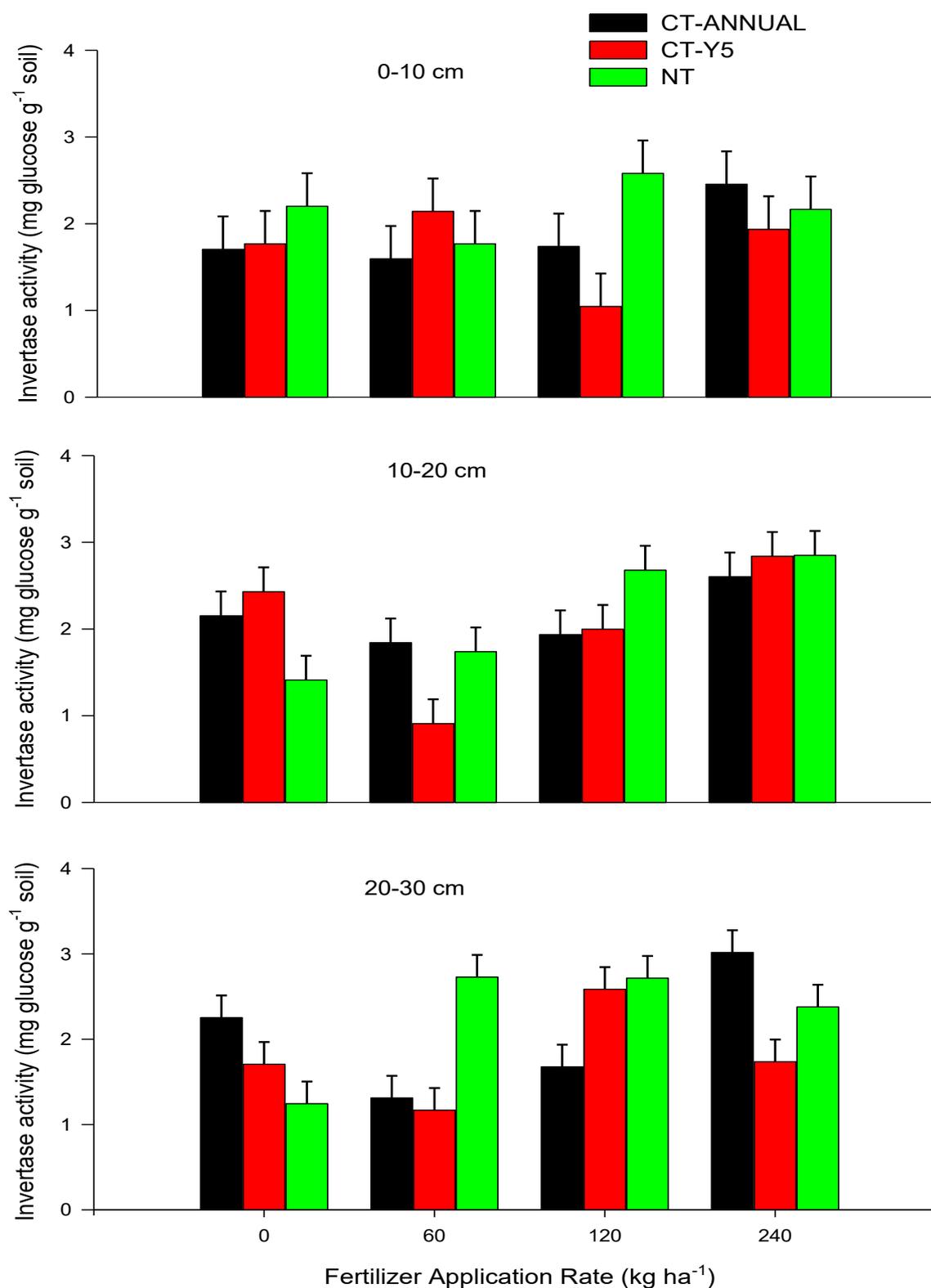


Figure 6. 2: Invertase activity at different soil depths, N application rates and tillage techniques.

6.3.1. Acid phosphatase activity

Acid phosphatase activity was highest under NT ($p < 0.05$) at 0 and 240 kg N ha⁻¹ in the 0-10 cm soil depth (Figure 6.3). However, in the 10-20 cm depth CT-ANNUAL had the highest phosphatase activity at 0 and 240 kg N ha⁻¹, whereas at 60 and 120 kg N ha⁻¹, CT-Y5 had higher phosphatase activity than CT-ANNUAL ($p < 0.05$). However, NT actually recorded the highest phosphatase activity (7062 $\mu\text{g g}^{-1} \text{dwt}^{-1} \text{hr}^{-1}$) at 60 kg N ha⁻¹ for this depth ($p < 0.05$). In the 20-30 cm depth, CT-Y5 at 0 and 60 kg N ha⁻¹ had the highest phosphatase activity ($p < 0.05$). While at 120 and 240 kg N ha⁻¹, CT-ANNUAL had the highest activity of this depth ($p < 0.05$).

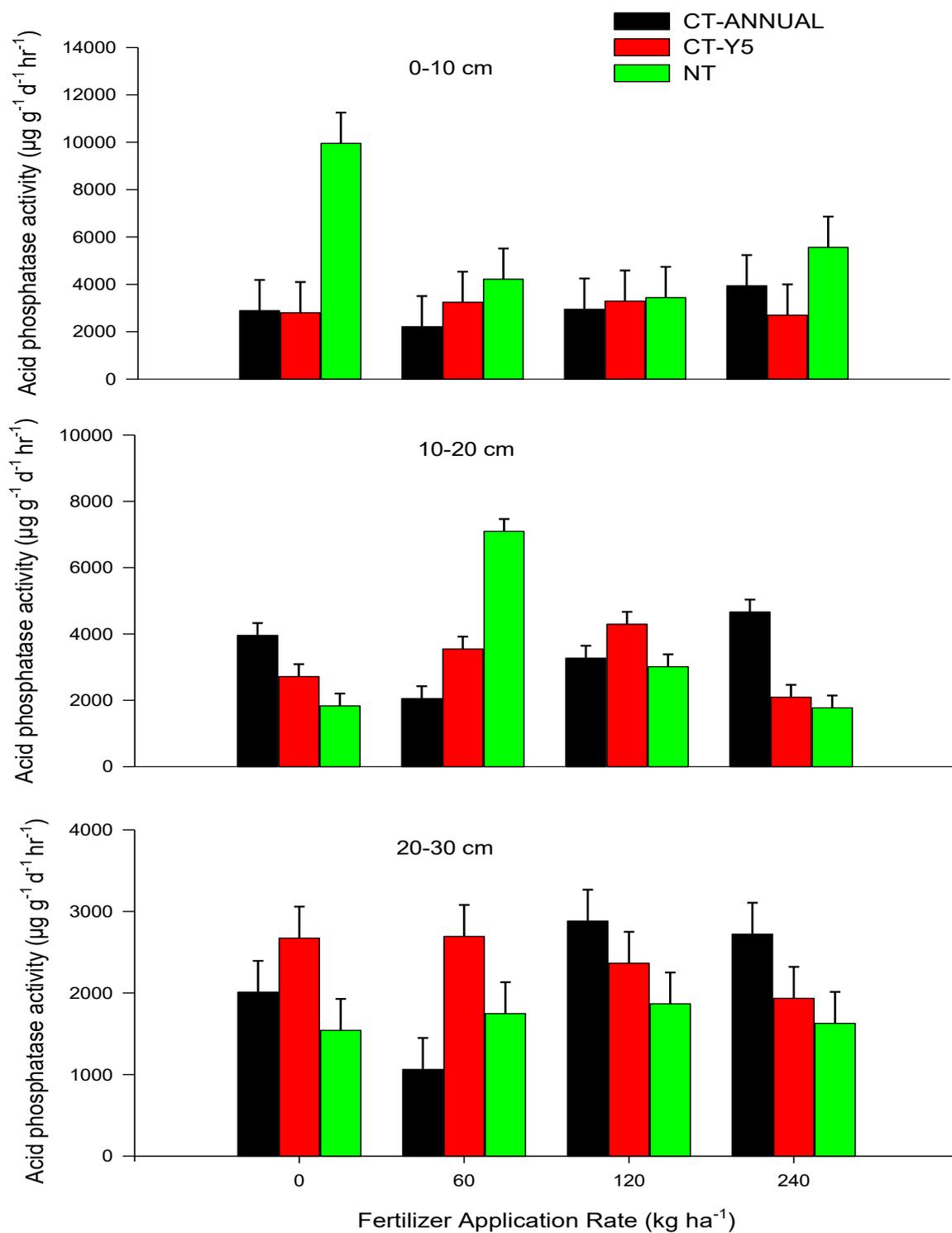


Figure 6. 3: Acid phosphatase activity at different soil depths, N application rates and tillage techniques.

6.4 Discussion

Enzyme activity has often been found to be directly related to the organic C content of soil (Tabatabai & Dick, 1979). The enzyme urease in particular is central to N cycling by microbial cells (Rotini 1935); and produces mineral N during decomposition of aliphatic and aromatic N compounds in SOM (Monreal & Bergstrom, 2000). Thus, higher urease activity in NT in surface soil was similar to total C and N trends shown in chapters 4 and 5, respectively. Frankenberger & Dick, (1983) also reported direct relationships between organic C and total N with activity of urease. According to Deng & Tabatabai, (1996) urease is of microbial origin therefore tillage practices with sufficient microorganisms would secrete more urease. Monreal & Bergstrom, (2000) also observed the lowest urease activity in CT, indicating a more limited supply of energy and C sources for protein synthesis than under conservation tillage. Increased urease activity under conservation tillage systems may also be related to increased functional diversity of soils (Kandeler et al., 1999). Findings by Kabiri et al., (2016) also concur that the activity of urease was generally greater in conservation tillage than CT. Kladvik, (2001) explained that observations of higher urease activity in CT compared to NT that are sometimes made could be explained by a flush of microbial activity directly after tillage and straw incorporation as a result of an increase in substrate and oxygen availability. Jin et al., (2009) highlighted that this effect was, however temporary as enzyme activity fell back to lower levels again in CT.

However, the high urease activity under CT-Y5 at 10-20 cm depth may be attributed to availability of substrates in the subsurface due to mixing of residue with soil during tilling. OM, which has been accumulating on the surface of CT-Y5 during no-till seasons is ploughed into lower horizons in the 5th year, and will stimulate the activity of enzymes. Beare et al., (1993) reported that buried residues would have higher densities of fungal hyphae than surface residues of NT. In a study by Dick (1984), contrary results were observed as there was a rapid decrease in urease activity with increase in soil depth while in our study we observed an increase in urease activity with soil depth in all tillage techniques. Tabatabai & Bremner, (1972) observed that acidity generated by repeated surface application of N caused soil pH to decrease which decreased urease activity while acid phosphatase activity increased. However, in our study there was a general increase of urease activity with N application in all treatments. This may be attributed to the type of fertilizer used (urea), which may increase the production

demand of urease enzyme to catalyse the substrate urea. Contrary, McCarty et al., (1992) reported suppression of microbial urease production through addition of inorganic N, in NH_4^+ form, or amino acids to soils. Suppression of microbial urease production by NH_4^+ has been linked to the activity of glutamine synthetase, the enzyme primarily responsible for inorganic N assimilation by microorganism in N-limited environments (Reitzer & Magasanik 1987). Jian et al., (2016) reported that urease activity showed significant suppression when the urea N load was high ($>150 \text{ kg N ha}^{-1}$), this is contrary to our findings since 240 kg N ha^{-1} induced higher urease activity. In a study by Sinsabaugh et al., (2008) they demonstrated that a large quantity of available N in soil could have substantially relieved N limitations for microbes and caused more conservative production of N-associated enzymes. According to Mobley & Hausinger, (1989), microbial regulation of urease production is induced by the presence of the substrate urea, and the enzyme is synthesized constitutively. Readily available N forms e.g. NO_3^- would be more favourable for production of hydrolytic enzymes such as phosphatase (Hobbie et al., 2012; Talbot & Treseder, 2012). According to Cenini et al., (2016) this mismatch between low activity of N-acquiring enzymes and high soil N may be due to the fact that N is found in a diversity of compounds such as amino sugars, polypeptides, humus, which are degraded by a diverse range of N-acquiring enzymes. The addition of inorganic forms of N to soils through fertilization may as a consequence provide N forms available to be incorporated into SOM (Cenini et al., 2016), thus explaining the positive relationship between increase in N rates with increase in urease activity in our study.

High concentration of crop residue under NT can have a tremendous impact on microbial activity and vertical distribution of soil enzymes. One of the benefits of NT may be the rhizosphere effect, which probably contributes to higher enzyme activity than in CT systems (Bandick & Dick, 1999). There is growing evidence that NT improves biological activity in the rhizosphere, which is often associated with accumulation of microorganisms responsible for producing enzymes (Souza et al., 2015). Invertase activity, for most treatments, generally increased with depth and N application rate which may be attributed to the low C: N ratio of subsoil organic material compared to the high C: N ratio of fresh surface organic material, and availability of sucrose substrate from urea fertilizer. Its activity is more influenced by the type of organic material rather than its quantity (Pancholy & Rice, 1973). This is because of different C: N ratio, lignin, polyphenol and silica content of different plant residues (Tian et al., 1992). According to Jin et al., (2009) invertase catalyses the hydrolysis of sucrose to D-glucose and

D-fructose, and is widely distributed in microorganisms, where it releases low molecular weight sugars that are important as energy sources for microorganisms. Dick, (1984) observed a rapid decrease in invertase activity in NT with increasing soil depth, while below the 7.5 cm depth its activity in CT was generally higher than in NT plots. However these findings contradicted our results, since invertase activity was generally higher under NT at 60 and 120 kg N ha⁻¹ in the deeper depths. This can be attributed to sufficient sucrose substrate supply from both organic residues and fertilizer to microbes under NT thus inducing highly populated microorganisms on the surface level down the profile. Since organic residues are concentrated in the surface in NT supplying microbes with substrate N makes it possible for fertilizer added N to move down the profile without being immobilised by microbes. According to Jian et al., (2016), N fertilization sustains soil microbes to produce more extracellular enzymes associated with hydrolytic C-acquisition, resulting in overall lower energy acquisition costs. However, in our study it can be observed that at deeper depths CT-Y5 and CT-ANNUAL treatments had lower invertase activities compared to NT treatment of similar N application rates which can be attributed to low pH levels, higher exchangeable acidity and higher C: N ratios under tilled soils (Chapter 3, Table 2 & 3).

Considering that soil pH ranged from 3.4 to 5.28 (Chapter 3; Table 3.1, 3.2 & 3.3), soil acid phosphatase was assayed instead of alkaline phosphatase. Phosphatase enzyme is predominantly secreted by plant roots and associated mycorrhiza (Joner et al. 2000). Acidic phosphatase enzyme is associated with P acquisition that cleave PO₄³⁻ from P-containing organic compounds (Jian et al., 2016). Therefore depending on the tillage system, phosphatase activity will dominate at different depths of distinct tillage techniques. Very high phosphatase activity on the surface of NT may be due to conducive soil biological and physicochemical conditions of the surface compared to lower depths. This is because NT with residue cover can supply more substrate for phosphatases to act on (Wei et al., 2014). The control treatment of NT at 0-10 cm depth had the highest phosphatase activity. However, in lower depths, activity patterns fluctuated and they were mostly higher than in the control treatment. According to Jian et al., (2016) under NT and CT systems of deeper soils, phosphatase had relatively less activity because it responded less to other factors, such as climate, compared to surface horizon. These current findings concurred with previous work by Dick et al., (1988) who reported higher acid phosphatase activity in the 0-7.5 cm surface of NT compared to CT. Whereas Bergstrom et al., (1998) found that in the surface layer (0-7.5 cm) phosphatase enzyme activity was greater in

NT soil, while below this depth it was equal to or greater in CT soil. In our case, phosphatase activity was also high at higher N rates (120 and 240 kg N ha⁻¹) of CT-ANNUAL compared to NT at both 10-20 and 20-30 cm soil depth. This was probably because the quantity of potentially mineralizable organic P in surface soil of NT was higher than that for CT, while the opposite was true at lower depth because of incorporation of organic residues. Furthermore, the higher phosphatase activity under CT systems at lower soil depths may be attributed to mixing of fertilizer substrate making it more available to microbes. According to Bergstrom et al., (1998), tillage influences vertical distribution of crop residues and SOM which accumulate at the surface of reduced or NT where it enhances phosphatase activity.

Geisseler & Scow, (2014) after a meta-analysis of 26 agricultural sites revealed that urea and ammonia fertilizer had negative effect on phosphatase activity because they reduced soil pH below a certain threshold which inhibit microbial communities to function. However, Jian et al., (2016) observed that acidic phosphatase activity was stimulated under urea fertilization because urea fertilizer amendments increased those microorganisms that produces phosphatase enzymes (Bayer et al., 2006). This agreed with the current findings especially under NT at 60 kg N ha⁻¹ (10-20 cm) and CT-Y5 (20-30 cm) where acid phosphatase activity escalated with the application of urea fertilizer. In contrast, CT-ANNUAL at 60 kg N ha⁻¹ generally had the lowest phosphatase activity in all treatments. This may be attributed to high N demand from microbes and roots stimulated by limited fertilizer application. Again this may be attributed to an increase of crop productivity with N fertilizer which would extract all N, if less is applied, for their productivity hence leaving enzyme producing microorganisms without substrate. An overall decrease in microbial activity with NH₄NO₃ fertilizer was suggested to be due to increase of copiotrophic microorganisms that rely on more labile C sources, and less on the need for extracellular enzyme secretion (Ramirez et al., 2012). Meanwhile, Marklein & Houlton, (2012) revealed that phosphatase activity increased significantly by 10.6% as a result of N fertilization (though they did not specify which one), and attributed this to sufficient N supply which sustains soil microbes to produce more phosphatase enzymes. Furthermore a study by Keeler et al., (2009) found that N addition increased the activity of phosphatase by 17%.

In the current study the practice of maize monoculture could had have a detrimental effect on phosphatase activity since it reduces soil microbial diversity (Xue et al., 2006). Again mono-

cropping may have negative effects on soil physical properties by reducing aggregation and increasing soil erosion. In a previous study on the same site by Basset, (2010), there was higher micro-aggregate stability under NT than CT-ANNUAL in the top horizon, but no differences in the subsoil. Differences in micro-aggregates among tillage systems may affect the distribution of microorganisms which in turn affect the distribution of enzymes. Gupta & Germida, (1988) observed that CT reduced the microbial population of all soil aggregate size fractions, however, lower populations microorganisms were observed in macro aggregates. The activity of phosphatases among different aggregate size fractions can also be affected by tillage (Qin et al., 2010). According to Wei et al., (2014) acidic phosphatase activity differed among tillage systems only for micro-aggregates, with CT having higher activity followed by minimum tillage and NT having the least. This suggests that the response of phosphatase activity to tillage may be more sensitive in the micro-aggregate size fraction.

6.5 Conclusion

Enzymatic responses to tillage and N fertilization influences ecosystem function and nutrient dynamics. The three assayed enzymes, (urease, invertase and acid phosphatase) responded differently to different tillage techniques and N fertilization in different depths. Urease activity was higher for NT and CT-Y5 in the top 0-10 cm but fluctuated at 10-20 cm. However at 20-30 cm, NT had higher urease activity again in the fertilized treatments. Accumulation of crop residues under conservation tillage may have contributed to higher urease activity compared to CT-ANNUAL. Urease activity was higher at the highest N fertilizer application rate (240 kg N ha⁻¹), because it is mainly produced to hydrolyse urea. On the other hand, invertase activity was higher in NT at 120 kg N ha⁻¹ for all 3 depths. Again invertase activity showed a general increase with increase in N fertilizer rate. The very high acid phosphatase activity in the control of NT at 0-10 cm depth was attributed to high OM accumulation and more microbes, with higher N rates suppressing this because of increasing exchangeable acidity and low pH. In deeper horizons acid phosphatase activity generally increased with N fertilization for CT-ANNUAL. This may be attributed to N amendment increasing crop productivity and consequently root distribution. Thus in the subsurface, roots decompose providing organic substrate to microbes thereby more phosphatase production. Amongst the three enzymes assayed, urease and phosphatase responded more to tillage and fertilizer application than

invertase, and can therefore be useful indicators of soil quality. It is worthwhile to note that long term NT practice may have an adverse effect on enzyme activity, especially under monoculture, due to continuous use of the same herbicides, pesticides and fertilizers which may accumulate and disturb microbial activity.

CHAPTER 7

Seasonal greenhouse gas fluxes under different tillage and N fertilizer management in a dryland maize mono-crop.

7.1 Introduction

Soil and land use contribute substantially to greenhouse gas (GHG) emissions. Tillage increases soil aeration, mixes the topsoil with plant residue and enhances the decomposition of OM. Mineralized organic C and N in the soil can be easily lost through leaching, runoff, precipitation, volatilization and emission, depending on their form. GHGs are a major problem leading to global warming, therefore their emission from the soil to the atmosphere should be mitigated. Agricultural crop production systems are a major contributor to anthropogenic emissions which must be reduced in arable soils (Regina & Alakukku, 2010). The global demand, scale and intensity of agriculture might be the reason for high emissions of GHGs. However, because farmlands are intensively managed, farmers can to some extent, control the amounts of these gases (Kern & Johnson, 1993). Implementing better management and farming practices may assist in mitigating emissions.

Conversion from conventional tillage (CT) to no-tillage (NT) was considered to be one of the potentially efficient strategies to reduce GHGs emission (Six et al., 2004). Lal (2004) found NT to enhance C sequestration by 100–1000 kg ha⁻¹ per year. However, there are inconsistencies in the effect of NT, which vary from significant increase to significant decline in soil C (Christopher et al., 2009). According to Luo et al., (2010), these inconsistencies result from differences in environmental or management factors, sampling strategies or methodology used. Regina & Alakukku, (2010) reported CO₂ flux from NT to be lower, attributing this to a slowing down of mineralization in the topsoil, while losses of OM from a CT topsoil are generally high (Maljanen et al., 2007). Almost all trace GHGs must be considered in mitigation strategies to avoid mitigating one gas at the expense of the other. This is because N₂O is 300 times more potent than CO₂, and it has been estimated that its increase could offset 75-310% of the advantage gained from C sequestration (Li et al., 2005). Nevertheless, field

measurements have shown both decreased and increased emissions of N₂O under NT management (Kaharabata et al., 2003; Gregorich et al., 2008).

There are few studies that have assessed GHG effluxes under dry-land agriculture, especially in South Africa. Therefore the need to study effluxes of CO₂, N₂O and CH₄ in a dryland semi-arid agro-ecological zone over two seasons (summer and winter) was salient. Von Arnold et al., (2005) observed strong seasonal variations in emissions, with the highest emissions of CO₂, CH₄ and N₂O occurring during summer, as a result of the high temperature and moisture characteristic of this time, which intensify soil biochemical processes. GHG production, transport and emission in soil will generally depend on environmental factors such as soil aeration, temperature, moisture, supply of organic C and fertilization (Janzen et al., 1998). Thus, N input has been regarded as a significant determinant for N₂O losses from agricultural soils (Petersen et al., 2006). Zhai et al., (2011) observed that application of N fertilizers at a rate of 300 kg N ha⁻¹ yr⁻¹ significantly increased N₂O emissions. While relatively high N₂O efflux was observed when soil moisture content and temperature were higher than 65% and 4.5°C, respectively, (Dobbie & Smith 2003).

The type of tillage, fertilizer application and seasonal variation of soil temperature and moisture also have an effect on CH₄ emission. Generally CH₄ emissions decrease with decreasing soil moisture, with soils eventually becoming net sinks for CH₄ depending on the prevailing environmental conditions (Danevcic, et al., 2010). Low temperature, however, triggers methanogenesis (Duddleston et al., 2002). According to Nozhevnikova et al., (1994) acetate accumulated at low temperature with methanogens developing subsequent to acetogens. These organisms favour low temperatures due to excessive supplements of saccharides and cellulose because of less bacterial decomposition at low temperatures. According to Regina & Alakukku, (2010), CH₄ is oxidized by bacteria in the aerobic surface layer of soils. Since these bacteria are sensitive to disturbance, CT may experience a huge increase of oxidizing bacterial populations, thereby decreasing CH₄ emissions. However, Ussiri et al., (2009) reported an increased CH₄ consumption (thus decreased emissions) in NT soils by CH₄ bacteria due to reducing conditions. According to Regina & Alakukku, (2010) there were no clear differences in CH₄ flux rates between NT and CT sites. Adoption of a tillage practice which will assist in mitigating GHGs without compromising soil productivity is required. An effective way to sequester C and reduce organic N mineralization may differ with soil type, N fertilizer use and

prevailing environmental soil conditions. Therefore the objective of the study was to assess GHG effluxes across seasons in CT-ANNUAL and NT systems under N fertilizer management.

7.2 Material and methods

7.2.1 Study site

The study was conducted at an experimental site described in Chapter 3. However, in this section only NT and CT-ANNUAL (annual ploughing) in the 0, 120 and 240 kg N ha⁻¹ plots were sampled.

7.2.2 Soil GHGs sampling

Fluxes of CO₂, CH₄ and N₂O were sampled in the summer and winter seasons. The sampling was mostly projected, using South African weather service predictions, to be done on rain days. Sometimes the predictions were incorrect but sampling was still conducted. Summer was from December 2018 to February 2019, while winter was from May 2019 to July 2019. In each month of the season sampling was done daily for 3 weeks in a month thus making 9 sampling times per season and 18 altogether. Gases were sampled using static PVC chambers (16 cm in diameter and 25 cm in height) installed 8 cm into the soil, 24 hours prior to the first measurement in order to minimise soil disturbance (Hutchinson & Mosier, 1981; Aulakah et al., 1991).

The experiment included 2 tillage treatments (NT and CT-ANNUAL) and 3 urea fertiliser application rates (0, 120 and 240 kg N ha⁻¹) arranged in 3 blocks (or reps). Two chambers were installed per plot, giving 6 chambers per tillage treatment block, thus making 36 chambers altogether. Gas sampling was done between 10:00 am to 12:30 pm of each sampling day to minimise bias associated with diurnal temperature fluctuations (Aulakah et al., 1991). Sampling was done by inserting a 25 mL polypropylene syringe into the chamber septa, then slowly removing the gas at 0, 30 and 60 minute intervals, thus giving 3 replicates at each sampling point (Arnold et al., 2001), with atmospheric air sampled first. The gas sample was

then transferred to a 12 mL pre-evacuated glass vial that was sealed with a grey butyl rubber septum before transportation to the laboratory for analysis. Samples were analysed for CO₂, CH₄ and N₂O using Gas chromatography (GC), CP7539 S111coStel, Porapak Q80/100 GC, within a week of sampling.

7.2.3 Gas measurement

The temperature of the GC during analysis was 80 °C, with a return time of 5 minutes, and both helium and hydrogen were used as pushing gases on the columns. The columns used were packed columns (NBR: 80486-800 C58867) with shin carbon ST 80/100 2 mm 1/8 OD silico made in USA. A gas sample injector was utilized to inject gases into an inert valve of the GC. The concentration of each gas was measured using a GC with an Electron Capture Detector (ECD) and Flame Ionization Detector (FID). The GC was fitted with a sample valve to minimize injection error. To account for problems associated with GC drift, samples from individual chambers were run in sequence (t₀, t₃₀, t₆₀,) rather than segregating them by time, and standards were run periodically for every 20 sample batch (Aulakah et al., 1991). FID for hydro-carbon was used in the analysis of both CO₂ and CH₄, while ECD was used in the analysis of N₂O. The data was then transferred into an excel data sheet where gas effluxes were calculated. A positive value represented gas emission from the soil, while a negative value represented gas uptake by the soil. Effluxes were calculated using the following equation by Ginting et al., (2003).

$$F = k \times d \times \frac{273}{T} \frac{\Delta C}{\Delta t} \times \frac{V}{A}$$

Where:

F = the rate of gas emission (mass ha⁻¹ day⁻¹);

k= is unit of conversion (1.44 × 10⁶ for CO₂-C and 144 for both CH₄-C and N₂O-N);

d= is gas density (g cm⁻³) at 273 K and 0.101 MPa pressure (5.36 × 10⁻⁴ g cm⁻³ for CO₂-C and CH₄-C, and 1.25 × 10⁻³ g cm⁻³ for N₂O-N);

T = is the air temperature (Kelvin) within the chamber;

V = is the volume of the PVC chamber (cm³);

A= is the area of the PVC chamber (cm²);

Δt= is 30 minutes (time interval between sampling periods)

ΔC = is the average rate of change of concentration between $C_{30}-C_0$ and $C_{60}-C_{30}$ (C_0 - is gas concentration at time 0, C_{30} - concentration at 30 minutes and C_{60} - concentration at 60 minutes).

7.2.4 Soil temperature and moisture measurements

Soil temperature and moisture were measured *in-situ* in each chamber using a temperature probe (CR10X) and soil moisture sensor (ML3), respectively. The probes were inserted inside the PVC chamber into a 7.5 cm soil depth and left for 5 minutes before taking readings. They were measured at every gas sampling exercise.

7.2.5 Statistical Analysis

An analysis of variance (ANOVA) for split plots was done to determine the effect of tillage and N fertilizer application on seasonal GHG effluxes. The treatment factors were tillage technique, season and fertilizer application rate. Fisher's protected LSD was used as a post-hoc test for multiple comparisons to compare treatment means and their interactions. The P value ($p < 0.05$) was also used to determine the significant difference between treatment means. All tests were performed with GenStat 14.1 for Windows software (VSN international, 2011).

7.3 Results

7.3.1. Soil temperatures variations in different treatments

Table 7.1 shows that soil temperature was highest for NT in the control during summer months (December, January and February) and lowest for CT-ANNUAL in the control in winter months (June and July) ($p < 0.05$). NT also recorded higher ($p < 0.05$) temperatures at 0 and 240 kg N ha⁻¹ than CT-ANNUAL in both seasons, (except at 240 kg N ha⁻¹ in December and February). However the opposite was true at 120 kg N ha⁻¹, with CT-ANNUAL having higher temperature than NT in summer (December, January and February) ($p < 0.05$).

Table 7. 1: Soil temperature variations in different treatments across sampling periods.

N rate (kg N ha ⁻¹)	Tillage	Soil summer temperature (° C)			Soil winter temperature (° C)		
		Dec	Jan	Feb	May	Jun	Jul
0	NT	29.99 ^j	29.98 ^j	29.82 ^j	26.75 ^{gh}	26.95 ^h	26.84 ^{gh}
	CT-AN	24.89 ^e	24.98 ^{ef}	24.98 ^{ef}	17.37 ^{ab}	17.18 ^a	17.23 ^a
120	NT	26.25 ^f	25.98 ^f	24.99 ^{ef}	18.53 ^b	18.37 ^b	18.20 ^b
	CT-AN	28.96 ⁱ	28.94 ⁱ	28.57 ⁱ	19.88 ^{bc}	18.92 ^b	19.25 ^{bc}
240	NT	26.05 ^f	25.64 ^{fg}	25.43 ^{fg}	22.07 ^d	22.31 ^d	22.51 ^d
	CT-AN	24.93 ^{ef}	24.75 ^e	25.09 ^f	19.04 ^{bc}	18.27 ^b	18.45 ^b

CT-AN- Annual Conventional tillage; Dec-December; Jan-January; Feb-February; Jun-June; Jul-July

7.3.2 Soil moisture variations in different treatments

Soil moisture was higher in summer than winter months in all treatments ($p < 0.05$, Figure 7.1). In summer, it was also consistently higher for NT than CT-ANNUAL at all N rates ($p < 0.05$). However, summer soil moisture variations in NT showed that both 120 and 240 kg N ha⁻¹ rate having the lowest, while the control had the highest soil moisture ($p < 0.05$). Figure 7.1 shows CT-ANNUAL at 120 kg N ha⁻¹ had the lowest soil moisture in summer. ($p < 0.05$).

In the winter season, soil moisture decreased with increase in N application rate under NT, while CT-ANNUAL recorded the highest soil moisture at 120 kg N ha⁻¹ (p<0.05). A comparison between treatments saw NT in the control recording the highest winter soil moisture followed by NT at 120 kg N ha⁻¹ while NT at 240 kg N ha⁻¹ had the lowest soil moisture (p<0.05; Figure 7.1). Both December and February month in NT at 120 kg N ha⁻¹ had higher (p<0.05) soil moisture compared to January. The control of CT-ANNUAL in winter had higher soil moisture compared to 240 kg N ha⁻¹ treatment, with both May and June months having higher soil moisture compared to July month in the control treatment (p<0.05).

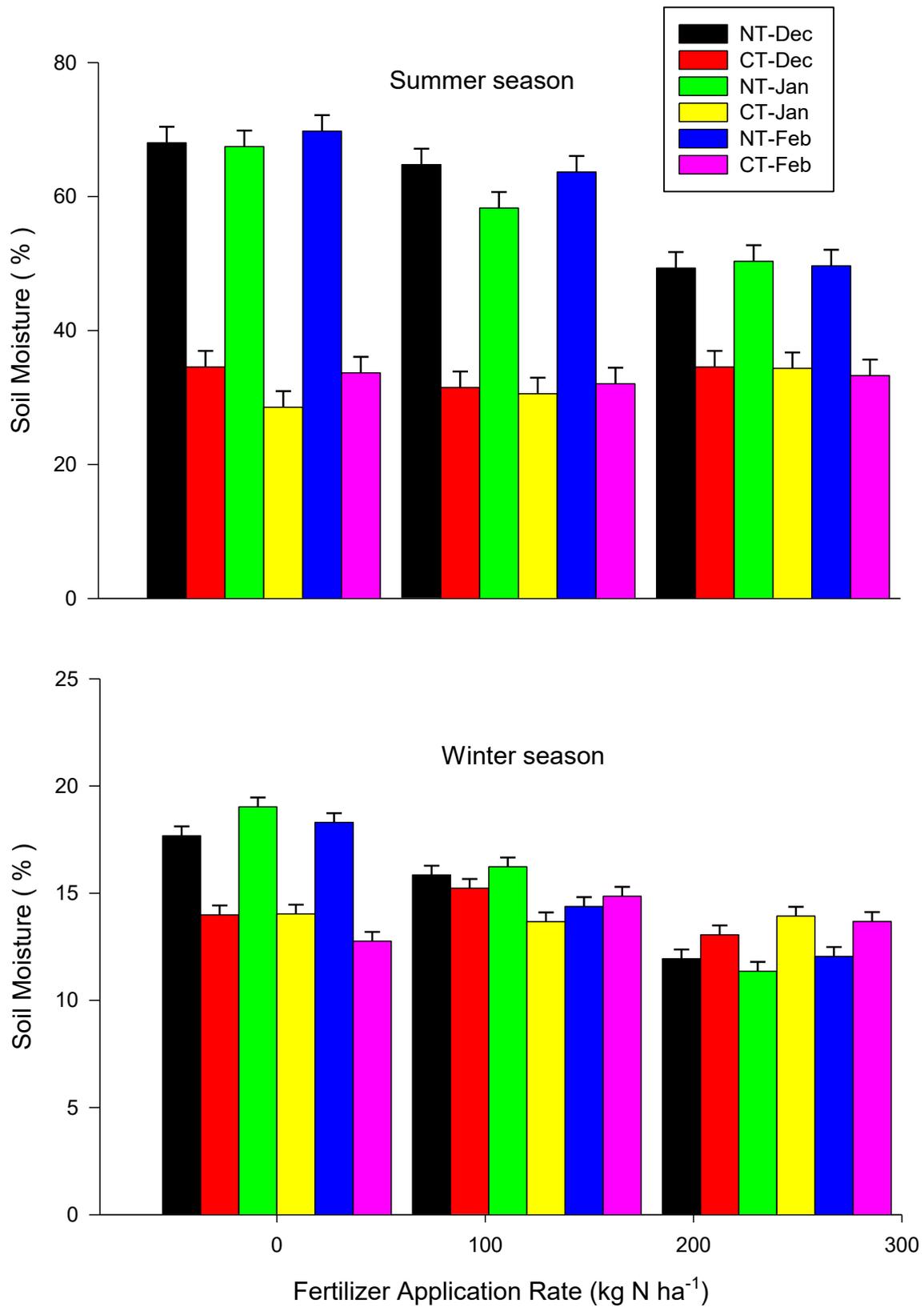


Figure 7. 1: Seasonal soil moisture variations in different N fertilizer and tillage treatments.

7.3.3. Soil CH₄ effluxes across sampling periods.

Fluxes of CH₄ were highest in NT at 240 kg N ha⁻¹ but lowest in CT-ANNUAL (with a negative efflux ranging from -0.033 mg CH₄-C ha⁻¹ day⁻¹ in January to -0.037 mg CH₄-C ha⁻¹ day⁻¹ in December) at the same N rate (p<0.05) in summer (December, January and February), while the other treatments did not significantly differ (Figure 7.2). However, winter (May, June and July) CH₄ effluxes were higher under NT than CT-ANNUAL for each application rate (p<0.05). NT at 120 kg N ha⁻¹ had the highest (p<0.05) CH₄ effluxes while CT-ANNUAL at the same rate had the most negative effluxes in winter. Figure 7.2 shows that CT-ANNUAL had negative CH₄ effluxes at all N application rates, indicating uptake rather than emission of this gas, with the control being a poorer sink of CH₄ compared to other treatments (p<0.05).

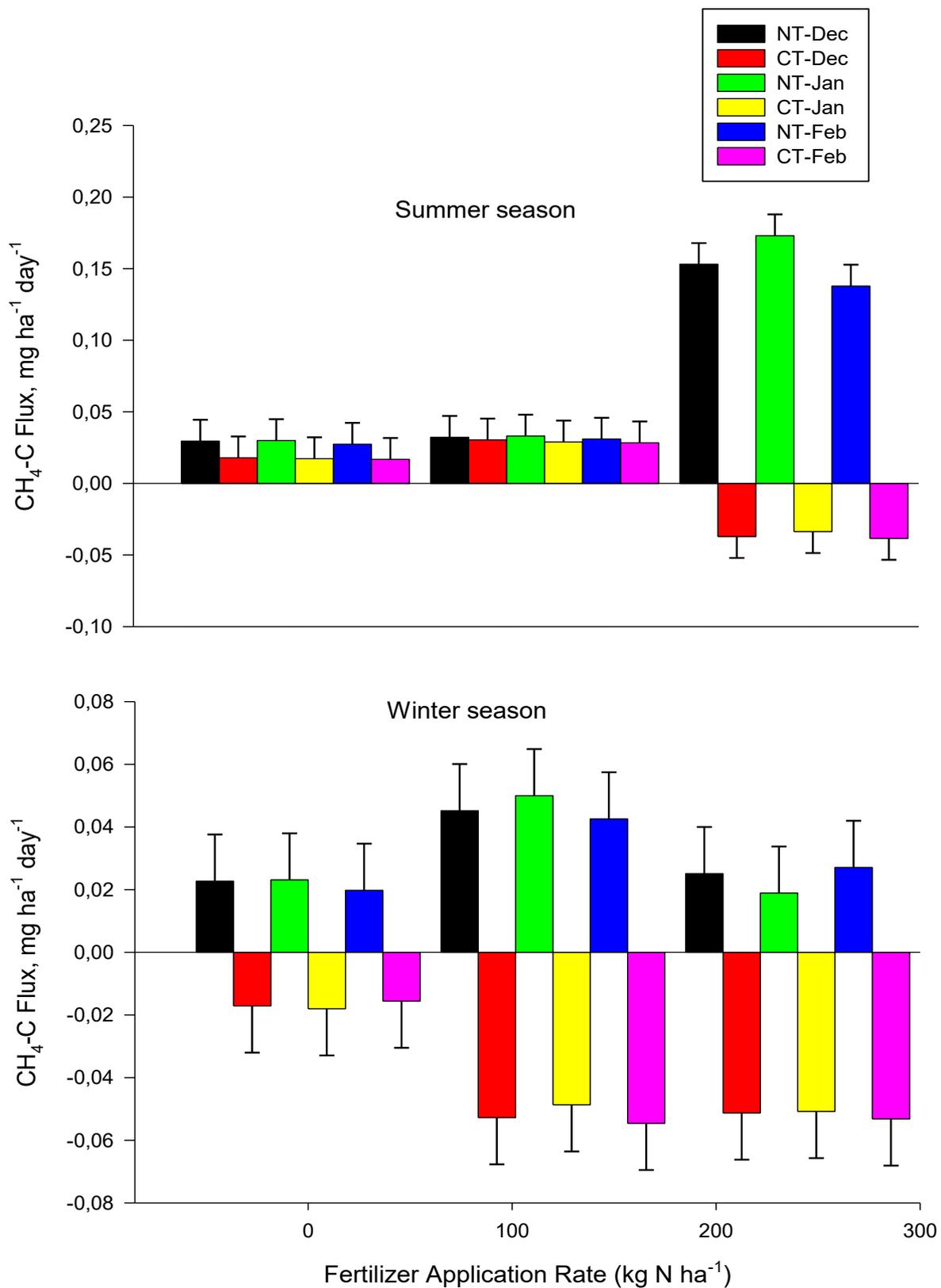


Figure 7. 2: Soil CH₄ effluxes in different N fertilizer and tillage treatments across sampling periods.

7.3.4. Soil N₂O effluxes across sampling periods

During summer (December, January and February), N₂O efflux was higher ($p < 0.05$) under CT-ANNUAL at 120 and 240 kg N ha⁻¹ compared to NT, with December and January having highest efflux (Figure 7.3). NT at 240 kg N ha⁻¹ actually served as a sink of N₂O since it recorded negative emissions of this gas in summer. In winter however, positive N₂O emissions were recorded in all CT-ANNUAL treatments with the control being the highest emitter ($p < 0.05$), while all NT treatments were net sinks of N₂O especially the control (Figure 7.3).

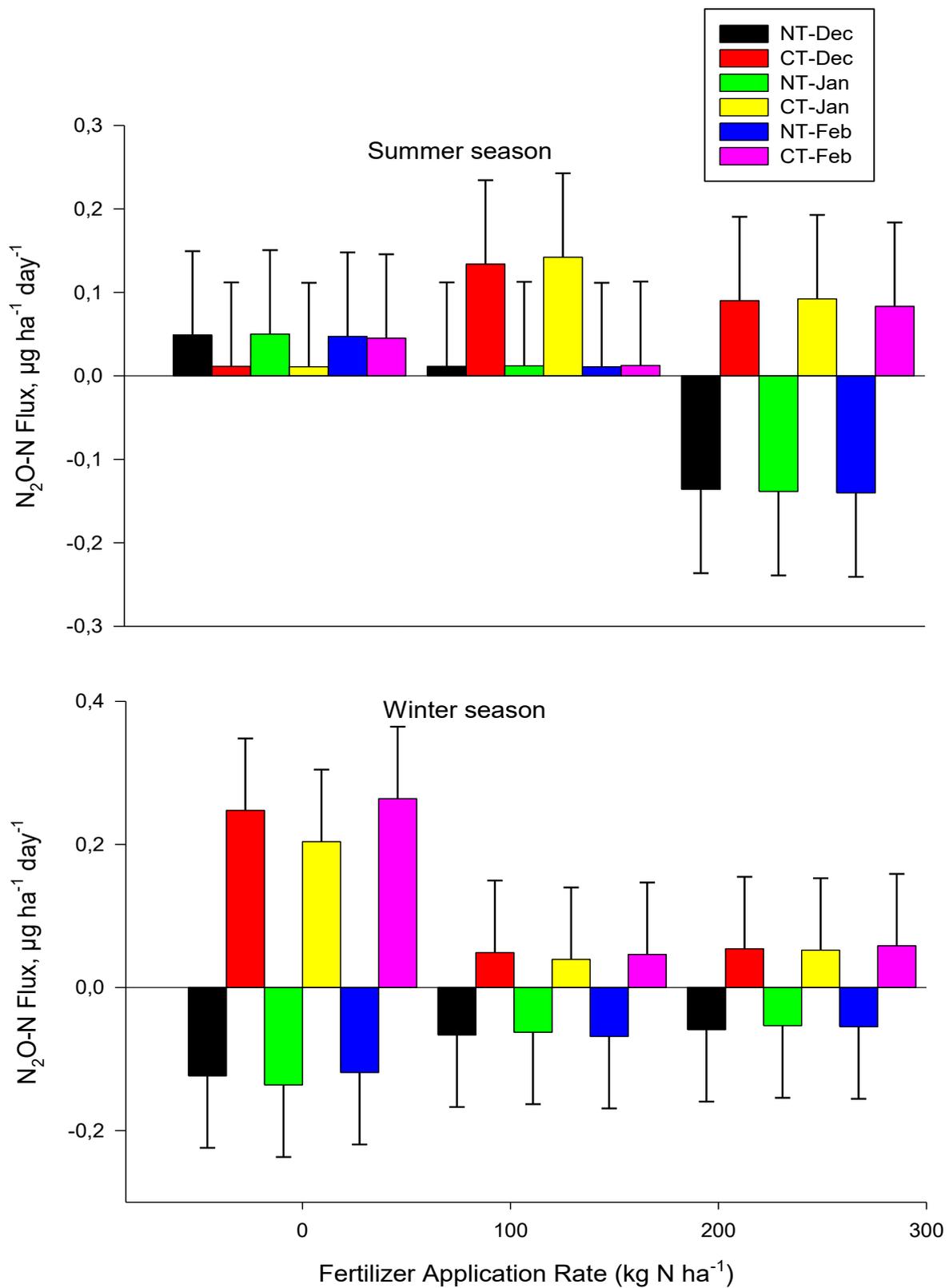


Figure 7. 3: N₂O effluxes in different N fertilizer and tillage treatments across sampling periods.

7.3.3. Soil CO₂ effluxes across sampling periods

The CO₂ effluxes of CT-ANNUAL were higher ($p < 0.05$) than NT at all N rates and seasons, with most NT treatments being net sinks of this gas in both seasons (Figure 7.4). Furthermore, most CO₂ effluxes of CT-ANNUAL in summer were higher ($p < 0.05$) than those in winter. The CO₂ efflux under CT-ANNUAL increased with increased N application rate in summer, but there was a decrease in CT-ANNUAL effluxes in winter at 240 kg N ha⁻¹ (Figure 7.4). Also December and February months had higher ($p < 0.05$) CO₂ efflux than January at 240 kg N ha⁻¹. All NT treatments were net sinks of CO₂ during summer, especially the 120 kg N ha⁻¹ rate, whilst the 240 kg N ha⁻¹ treatment had the least CO₂ uptake ($p < 0.05$).

In winter (May, June and July) the 120 kg N ha⁻¹ treatment under CT-ANNUAL had the highest while the 240 kg N ha⁻¹ had the lowest CO₂ efflux ($p < 0.05$, Figure 7.4). In the case of NT, only the control was a net emitter of CO₂ during winter, with both 120 and 240 kg N ha⁻¹ being net sinks of CO₂ into the soil.

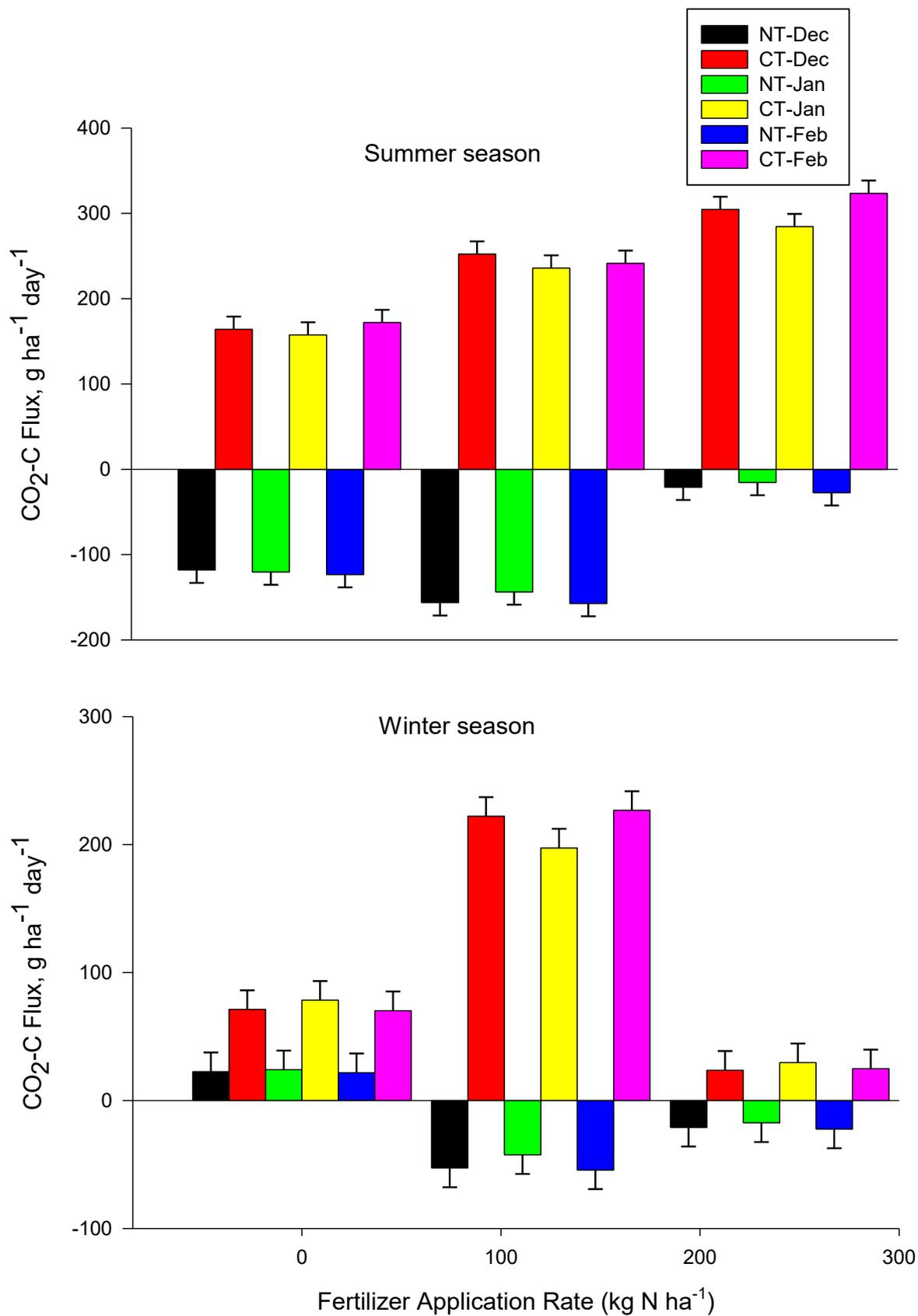


Figure 7. 4: Seasonal CO₂ effluxes in different N fertilizer and tillage treatments.

7.3.5 Effect of soil variables on GHG effluxes under different treatments

Table 7.2 shows that tillage induced CO₂ (r = 0.81) and N₂O (r = 0.41) emissions but was negatively related with CH₄ emissions (r = -0.54), soil moisture (r = -0.36) and temperature (r = -0.28). High soil temperatures induced CH₄ emissions (r=0.40), and also had positive correlations with soil moisture (r = 0.71). While high soil moisture also induced CH₄ emissions (r = 0.38) but suppressed the release of CO₂ (r = -0.38), (Table 7.2). As a result, soils that experienced high emissions of CH₄, had suppressed CO₂ release (r = - 0.36). N fertilizer application did not seem to have an effect on any of the measured soil variables or tillage techniques (Table 7.2).

Table 7. 2: Seasonal Pearson’s correlation analysis of soil variables and GHG effluxes with N fertilization and tillage.

Variables	Fertilizer	CO ₂	CH ₄	Moisture	N ₂ O	Temperature	Tillage
Fertilizer	-						
CO₂	0.08	-					
CH₄	0.01	-0.36**	-				
Moisture	-0.14	-	0.38***	-			
		0.38***					
N₂O	0.04	0.19	-0.16	0.097	-		
Temperature	-0.16	-0.03	0.40***	0.710***	-0.15	-	
Tillage	0.00	0.81***	-	-0.36***	0.41***	-0.28*	-
			0.54***				

Each * signifies significant difference; where * p <0.05, ** p <0.01 and *** p <0.001.

7.4 Discussion

The higher soil temperature and moisture under NT than CT-ANNUAL in both seasons may be attributed to more crop residues in NT, which act as mulch by absorbing more solar radiation during the day and emitting less from the soil to the atmosphere during the night. An exception was CT-ANNUAL at 120 kg N ha⁻¹ that had higher soil temperature than NT in both seasons. According to Franzluebbers et al., (1995) soil temperature under NT in the upper depth was also greater than CT during summer. Crop residue cover may have reduced the amplitude of the diurnal soil temperature cycle in NT. Furthermore, crop residue cover under NT may reduce evapotranspiration, runoff and increase soil infiltration rate thereby inducing higher soil moisture retention compared to CT-ANNUAL. Higher temperatures under NT may have been due to the capacity of crop residues to reduce heat loss from the soil surface to the atmosphere (Franzluebbers et al., 1995). This was similar to our findings in both seasons at 0 and 240 kg N ha⁻¹. However, Alvarez et al., (1995) reported no significant difference between tillage systems on soil temperatures in all monthly measurements.

Greater soil temperatures under CT compared with NT have been measured by Johnson & Lowery, (1985), which was similar to our findings at 120 kg N ha⁻¹ in both seasons. Fluctuations of seasonal soil temperature among treatments had an influence on the variation of effluxes of GHG, with CT-ANNUAL at 120 kg N ha⁻¹ having higher N₂O effluxes in summer and CO₂ in winter due to higher temperatures. Also NT at 240 kg N ha⁻¹ in summer induced higher CH₄ effluxes due to higher soil temperatures. In a study by Dobbie & Smith, (2003) high GHGs fluxes were associated with increase in soil temperatures. Generally, the rate of CO₂ released from the soil depends on the biological activity which is affected by soil temperature variations. The higher CO₂ emissions in summer compared to winter observed under CT-ANNUAL in the current study may be attributed to higher soil temperature in summer than winter. This was similar to findings by Buyanovsky & Wagner, (1983) who observed higher emissions of CO₂ in summer than winter due to maximum soil microbial activity as well as soil respiration. Again, in our study, CO₂ emission increased with crop development in summer because it was strongly influenced by respiration of the roots and associated microorganisms. However, although summer with higher temperatures induced higher CO₂ emissions in CT-ANNUAL than winter, but NT with higher temperatures than CT-ANNUAL was mostly a net sink for CO₂ in both seasons. This can be attributed to the effect

of annual ploughing on decomposition and breaking down of aggregates thus releasing entrapped CO₂. Tillage facilitated a high rate of soil CO₂ emission due to increased porosity (Buyanovsky & Wagner, 1987). Franzluebbers et al., (1995) reported that small differences in soil temperature between tillage regimes had only a minor effect on soil CO₂ evolution compared with larger seasonal temperature fluctuations.

According to Zengeni et al., (2016) soil moisture had a positive effect on the CO₂ emissions, thus as soil moisture increased, soil penetration resistance decreased resulting in an increase in seasonal soil CO₂ effluxes. This is similar to our findings where summer, with higher soil temperature and moisture had higher CO₂ emissions compared to winter under CT-ANNUAL. It is worthwhile to note that though NT had higher soil moisture than CT-ANNUAL, it experienced less CO₂ emissions which may be attributed to soil loosening during ploughing in CT-ANNUAL, which improves aeration thus enhancing soil respiration. According to Feiza et al., (2015) soil CO₂ efflux was reduced in NT because of limited diffusion of oxygen. Van Straaten et al., (2009) observed that CO₂ effluxes peaked in summer at intermediate soil moisture, and decreased under drier conditions of winter, but also decreased when soils became water saturated. In the present study, unlike CO₂ efflux, N₂O under CT-ANNUAL had the highest emission in the control in winter. On the contrary, Dobbie & Smith, (2003) reported that summer conditions tend to be warm and wet with soil pores saturated, thereby allowing denitrification to occur, thus inducing high effluxes of N₂O. In all fertilizer application rates of both seasons (except the control in summer), N₂O effluxes were higher under CT-ANNUAL than NT, which may be attributed to more aeration for nitrifying bacteria under CT-ANNUAL. These findings agree with Chirinda et al., (2010) who observed a stimulation of N₂O emissions by fertilization and soil cultivation. Again in our study NT had higher bulk density, Chapter 3, meaning the soil was denser leading to poor aeration hence N₂O diffusivity would be limited. Mutegi et al., (2010) revealed that CT plots in their study were characterized by higher total porosity and gas diffusivity, which facilitated the escape of N₂O at the soil-atmosphere interface. Six et al., (2004) also reported that N₂O fluxes tended to increase during the first 10 years after adopting NT due to greater soil moisture and water-filled pore space in recently established NT which stimulate denitrification resulting in N₂O emissions. This agrees with our results since the experiment site was way beyond 10 years henceforth N₂O effluxes may have decreased under NT.

Chatskikh & Olesen, (2007) observed higher N₂O emissions from CT than NT plots in summer and winter, which is similar to our findings. High N₂O emissions in CT during summer were most likely a function of soil disturbance during ploughing and planting under wet warm conditions, which increased SOM and N mineralization and hence nitrification due to elevated oxygen consumption rates (Mutegei et al., 2010). The large stimulation of N₂O emissions in CT during summer was therefore very likely a direct or indirect result of nitrification activity following rapid mineralization of residue-N after soil disturbance (Mutegei et al., 2010). Production of N₂O through nitrification is favoured by low O₂ partial pressures which are not adequate for complete nitrification of NH₄⁺-N to NO₃⁻-N (Bollmann & Conrad, 1998). Nitrification which induces N₂O effluxes could have been more prominent under CT-ANNUAL in both seasons. However, due to CT-ANNUAL having high porosity, aeration may have been sufficient enough to allow nitrification to complete resulting in less N₂O production compared when O₂ is limited. The summer increase of N₂O effluxes with fertilization under CT-ANNUAL maybe attributed to hydrolysis of urea into ammonium, and its subsequent nitrification to NO₃⁻ due to adequate aeration, high soil temperatures and moisture thereby inducing N₂O release in the process. Six et al., (2002) reported N₂O emissions whenever N fertilizers are added to the soil.

In winter, there was high uptake of N₂O into NT treatments. This may be attributed to oxidizing soil conditions under NT due to low winter temperatures and soil moisture, which hinder functionality of denitrifying bacteria. Usually NT experiences N₂O emission through denitrification in areas with saturated soil pores spaces. This corresponds with the findings by Davidson et al., (2000) that higher soil N₂O emissions under NT are related to anaerobic conditions where soil moisture is higher than 60%. Current results showed that in winter and at 240 kg N ha⁻¹ during summer, NT had <50 % soil moisture which would limit denitrification. In a study by Cruvinel et al., (2011), N₂O peaks were observed after N-fertilization and irrigation that resulted in increased N availability and favourable water filled pores (denitrifying conditions). Soils are usually considered a net source of atmospheric N₂O, however, Cruvinel et al., (2011) explained that well-drained or N-limited soils acted as sinks that gave negative effluxes of N₂O especially in the post-harvest stage. In general after harvesting, soil are well-drained and relatively N-limited, thus exhibiting low nitrification rates which reduce N₂O production (Nardoto & Bustamante, 2003). This agreed with present results since winter effluxes were measured after harvest. The CT-ANNUAL treatments, however,

still experienced N₂O emissions during winter which may be attributed to gradual nitrification of the incorporated organic N during ploughing that will produce N₂O.

Drainage and better aeration under CT, increase organic C and N availability, due to increased mineralization, which also affects gas emissions through increase in gas diffusivity (Silvola et al., 1996). Lowest N₂O emissions were observed during the winter, when the temperatures had decreased nitrification rates (Mørkved et al., 2006). NT had higher soil moisture content, although it was not saturated to allow denitrification, limiting N₂O diffusivity because most of the pores were filled with water therefore contributing less fluxes compared to CT-ANNUAL. According to Chapuis-Lardy et al., (2007) soil with moderate to high soil moisture and temperature promote N₂O uptake. Oxygen concentration also becomes low following soil compaction, which concurs with high bulk density observed in the previous chapter under NT (Chapter 3 on Table 3.1, 3.2 & 3.3). Yamulki et al., (1995) linked low emission rates, as well as net negative effluxes to high temperatures and moderate soil moisture contents which is similar to conditions observed under NT in the current study. An increase in soil temperature positively influences microbiological activity and gas diffusion, while it negatively affects solubility of N₂O (Heincke & Kaupenjohann, 1999). In a study by Chapuis-Lardy et al., (2007), N₂O sink was recorded at soil moisture content of 56% and at relatively high soil temperatures > 23 ° C, which is similar with soil conditions of NT in the current study.

The effect of fertilization changed with season and tillage technique. Thus, in summer N₂O emission generally increased with increased N application under CT-ANNUAL, while the opposite was true under NT. Whereas during winter N₂O efflux decreased with increase in N fertilizer rates under CT-ANNUAL, and there was net N₂O uptake in NT. The increase of soil N₂O emission with increase in N fertilization in summer compared to winter may be attributed to high soil moisture and temperature (more favourable conditions) in summer. Carvalho et al., (2006) found higher N₂O fluxes immediately after N fertilization and irrigation in maize fields under CT which decreased with time. This is similar to present findings because in winter the control had higher N₂O emission compared to N fertilized treatment (fertilization was done in summer). According to Liu et al., (2011) after fertilization, N₂O emissions were immediately increased but this only lasted for short period of time. High soil moisture and temperature in summer increases nitrification, thus induces N₂O efflux.

A linear relationship between fertilizer N applied and N₂O emission was found by Bouwman, (1996). This agrees with current results where a great positive relationship, under CT-ANNUAL in both seasons, was observed between N fertilizer and N₂O fluxes. The addition of N fertilizers to agricultural soils increases the potential for N₂O emissions (Granli & Bøckman, 1994). Previous results (Chapter 5, Fig 5. 3) show that NO₃⁻ was higher under CT-ANNUAL at 120 kg N ha⁻¹ compared to NT, which is in accordance with higher N₂O effluxes in summer. Higher N₂O emission in the control treatment of CT-ANNUAL relative to N fertilized treatments during winter may be due to higher populations of microbes under fertilized plots, because of N substrate, which immobilized organic N hindering nitrification. Denitrification, which results in release of N₂O and NO may happen on anaerobic microsites. In a study by Dobbie & Smith, (2003), soil NO₃⁻ concentration was the main factor affecting N₂O emission. After hydrolysis of urea, nitrification produces NO₃⁻ in the soil, and this can subsequently be denitrified causing larger N₂O effluxes. Lemke et al., (1998) also reported that higher N₂O emission from the soil was related to higher NO₃⁻ content. Davidson, (1991) also confirmed that the rate of N₂O production depends on the amount of NH₄⁺ and NO₃⁻ in the soil and soil temperature. This is similar to current findings as there was higher N₂O in summer under CT-ANNUAL at 120 kg N ha⁻¹ as NO₃⁻ and NH₄⁺ were also higher in this treatment (Chapter 5, Fig 5. 3 & 5.4).

CH₄ fluxes showed a different trend with N₂O, with effluxes being higher in summer especially at 240 kg N ha⁻¹ of NT. This may be attributed to anaerobic conditions created as crop residues enhance more moisture retention in NT. These reducing conditions stimulate more CH₄ production in NT than CT-ANNUAL. This resulted in CT-ANNUAL being more of a sink (especially in winter) while NT, with high soil moisture and temperature, was an emitter of CH₄ in all treatments. According to Whalen & Reeburgh, (1990) higher oxygen availability in the soil profile reduces CH₄ production and favours its oxidation to CO₂. This concurs with current findings because CT-ANNUAL (which was more aerated due to ploughing) emitted greater amounts of CO₂, but was mostly a sink on CH₄ compared with NT. As a result of this, well drained and aerated soils under CT are generally considered to contribute to the removal of CH₄ from the atmosphere (Smith et al., 2000). Thus, several studies have also shown that drained agricultural soils act as CH₄ sinks (Flessa et al., 1998; Le Mer & Roger, 2001), although the magnitude of the sink depends on soil moisture content. Hence soils with water saturated pores have less or no air movement and less CH₄ sink ability (Maljanen et al., 2003). The

present findings also supported this notion as CT-ANNUAL was mostly well drained and aerated due to drier soil conditions in winter making it a net CH₄ sink. However, in a study by Robertson et al., (2000), higher CH₄ uptake rates by soil was observed under NT in fertilized plots. This was attributed to a more stable and porous soil structure under NT that facilitated CH₄ diffusion into oxidizing zones, and activated methanotrophic bacteria responsible for CH₄ consumption (Ball et al., 1997), while reducing methanogen activity, i.e. bacteria responsible for CH₄ production (Hutsch, 1998).

Soil moisture content (<65 %) did not reach saturation levels (90-100 %) in both seasons, which may have led to low CH₄ production. Thus CH₄ production positively correlated with soil temperature and moisture but was negatively related to tillage especially CT-ANNUAL. However, NT favoured CH₄ production in relative to CT-ANNUAL. This is because methanogenic bacteria need high soil temperature and moisture to be able to produce CH₄. According to Topp & Pattey, (1997) methanogens function in a temperature range of 20-40°C and under high soil moisture content between 90 to 100 %. This concurs with all measured summer soil temperatures and NT temperatures in winter. However, although soil moisture of the current study did not reach 90-100 %, NT summer moisture was around 65 % thus allowing some methanogenesis compared to CT-ANNUAL. Under highly reducing conditions, and in the absence of other potential electron acceptors such as NO₃⁻, organic substrates e.g. organic acids, alcohols and methyl amines can be converted to CH₄ (Topp & Pattey, 1997). Methanogens are CH₄ producing bacteria sensitive to oxygen and thus only occur under anaerobic conditions, whereas methanotrophs are CH₄ oxidizing bacteria that require oxygen as a terminal electron acceptor (Woese et al., 1990). According to Topp & Pattey, (1997) soils generally harbour both methanogens (under reducing conditions) and methanotrophs (under oxidizing conditions). We would thus assume that in the present study, methanogens were prominent under NT due to higher emissions while methanotrophs were more under CT-ANNUAL because of CH₄ consumption. Generally, soil temperature, moisture, appropriate N fertilizer application rate and availability of C and N determine which bacteria will be predominant in situ. CH₄ effluxes showed that although methanogens were more predominant in summer, both bacteria had a role in the production and consumption of CH₄ during winter. In their measurements, Edwards, (1992) also found CH₄ consumption at dry sites while emissions were at wet sites. Differences in temperature sensitivity would result in CH₄ emissions decreasing at lower temperatures, but since CH₄-consuming bacteria are relatively

insensitive to temperature fluctuations in the mesophilic range, there is little diurnal variation in soils that are CH₄ sinks (Topp & Pattey, 1997). This is similar the current findings under NT since soil temperatures fluctuated between 17 to 29 °C ranges therefore making it a great CH₄ sinks especially in winter with low temperatures.

CO₂ showed significantly higher effluxes compared to the other two trace GHGs and had high effluxes in CT-ANNUAL than NT in all treatments. It has been widely stated that conversion from CT to reduced tillage could have a favourable impact on atmospheric concentrations of GHGs by promoting the storage of soil C (Lal et al., 1998). Ploughing has been found to enhance CO₂ flux and this increase typically lasts for some hours immediately after tilling (Reicosky et al., 1997). The positive correlation between tillage and CO₂ effluxes may be attributed to higher CO₂ emissions as a result of ploughing under CT-ANNUAL. The higher summer CO₂ effluxes were due to warmer and moistier soils that favoured faster SOM decomposition than in colder, drier winter soils (Triberti et al., 2008). The residence time of C in soil should therefore be longer in colder and wetter regions, thanks to a slower mineralization of OM (Freibauer et al., 2004). Eriksen & Jensen, (2001) reported that CO₂ emissions showed emissions to peak in coincident with the highest soil temperatures which was contrary to current findings in both summer and winter season. Crop residues left from the previous season can also provide ready C substrate for decomposition during summer, (Baggs et al., 2000). In a study by Baggs et al., (2006) lower emission of CO₂ was attributed to greater storage of soil C under NT. In the present study NT showed higher total C at 0-10 cm soil depth compared to CT-ANNUAL (Chapter 4, Fig 4.2) therefore indicating less turnover of OM under NT. Hungria et al., (2009) observed that CO₂ effluxes positively correlated with total N, C and mineral N in the topsoil indicating higher microbial activity in the presence of high amounts of OM. In a study by Regina & Alakukku, (2010) they observed a positive correlation between soil temperature at 5 cm and the daytime CO₂ emissions, which was similar to the current findings under CT-ANNUAL.

N fertilizer application also had an impact on CO₂ effluxes of both seasons. In CT-ANNUAL, CO₂ emission generally increased with increased N application rate. This may be attributed to the presence of N substrate from urea fertilizer which increased microbial decomposition. Again, though not measured, fertilization increases crop productivity thus roots respire releasing CO₂, also more crop residues will be available for decomposition. Chatskikh &

Olesen, (2007) however noticed that responses of CO₂ emissions to tillage disappeared after fertilisation, showing that microbiologically producing emissions were hindered by fertilizer since fertilizer increased exchangeable Al in the soil. According to Oorts et al., (2007) increased emissions of CO₂ induced by tillage indicate a higher turnover rate of OM after tillage, which partly may originate from plant residue incorporation between treatments. CO₂ emissions are closely connected to the microbial turnover and the physical accessibility of OM to microbes and extracellular enzymes (Paustian et al., 2000). Thus, tilling breaks up soil aggregates and exposes once physically protected SOM particles to microbial decay processes (Follett, 2001). Passianoto et al., (2003) observed a close relationship between physical disturbance and elevated CO₂ emissions in the tilled treatment. Hence tilling accelerates the decay of SOM and CO₂ efflux to the atmosphere (Brady, 1974).

Fertilizer application is required to increase productivity however it must be applied in consideration to the negative effect it can bring to the environment, in this case accelerated GHG emission. Results in the current study shows that excessive application of N, 240 kg N ha⁻¹, should be avoided because it increased the emissions especially of CO₂ and CH₄ during summer. The Kyoto Protocol emphasizes C sequestration in agricultural soils and advocates sustainable cropping techniques such as crop rotation (Triberti et al., 2008). Less soil disturbance, optimization of water use efficiency and use of organic materials such as farmyard manure, animal slurries, crop residues and N fertilization can influence C dynamics as well (Triberti et al., 2008). A study by Zhai et al., (2011), revealed that average CO₂ emissions from soils in a fertilized treatment during the maize growing season were higher than in the control. This trend mostly agreed with current results for CT-ANNUAL, which can be attributed to increase in organic C with N fertilizer increase. According to Triberti et al., (2008) the use of high rates of mineral N (>200 kg ha⁻¹) proved useless, not only from a productive standpoint since it did not significantly increase maize yield, but also environmentally damaging, because it did not substantially modify SOC content. Such an increase of CO₂ emission with N application occurred in the current study at 240 kg N ha⁻¹ in summer. Sustainable application of N fertilizer conserves ecosystems and improves climate, water and soil quality through mitigation of emission and high crop productivity. Crop biomass may be another factor influencing CO₂ emission, thus high crop productivity alleviates CO₂ release to the atmosphere through capturing it and using it for photosynthesis (Cavazzoni & Volk, 1996). Optimizing N supply means producing plenty of crop biomass per unit area through photosynthesis and this

implies a capture of great amounts of atmospheric CO₂, which can be partially transferred into the pedosphere (Triberti et al., 2008).

7.5 Conclusion

CO₂ effluxes were much higher than those of N₂O and CH₄, in all treatments. CT-ANNUAL also had higher emissions of N₂O and CO₂ while NT was mostly a sink for these two gases, and the opposite was true for CH₄. Generally, most gas effluxes were higher in summer than winter, which was attributed to higher summer soil temperatures and moisture. High soil moisture and temperature particularly accelerated CH₄ emissions, while drier winter soil moisture suppressed emissions of CO₂. Both CO₂ and N₂O emissions positively responded to tillage while CH₄ negatively correlated with it. Thus greater CO₂ emissions from CT-ANNUAL were attributed to increased rates of OM turnover due to ploughing. Whereas higher N₂O emissions from CT-ANNUAL were mostly due to improved soil aeration thus increase in diffusivity and better nitrification. Furthermore, greater emission of CH₄ under NT were attributed to the higher soil moisture and temperature in this technique that favoured methanogenic bacterial activity. Though correlation analysis did not show a clear effect of N fertilization on gas emissions, there was a general increase of CO₂ emissions with increased N rate in CT-ANNUAL (especially in summer). On the other hand, NT at 240 kg N ha⁻¹ which was the biggest emitter of CH₄ in summer, was also the greatest sink of N₂O in this treatment. In all, farmers should practice conservation tillage (NT) since it is an effective sink of CO₂, especially during summer where high soil moisture, high temperatures (as well as high N rates) would accelerate emissions of this gas under CT-ANNUAL. NT is also a good sink for N₂O, especially in cool dry winter soils. However, NT is not an ideal CH₄ sink since it enhances soil moisture and temperature, both of which favour methanogenic activity. Thus CT-ANNUAL would be a better sink for CH₄ in cool dry winter soils. Thus with careful monitoring of soil moisture and temperature fluctuations, which ultimately affect soil bulk density and gas diffusivity; emissions can be curbed through choosing the right tillage and fertilizer management for each gas.

CHAPTER 8

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General Discussion

This chapter synergizes all the chapters of the dissertation, by elucidating responses to the initially set objectives of the study, and major findings from them. Thus, effluxes of GHGs and soil properties analysed in conservation -versus- conventional tillage systems are highlighted, then conclusions, recommendations and research gaps drawn from the study are given.

8.2 Soil properties under different tillage and fertilizer management

The NT treatment at 0-10 cm depth had higher bases, pH, EC, bulk density, soil moisture (only at 0 and 60 kg N ha⁻¹) and low C: N in all N application rates than CT-Y5 and CT-ANNUAL; this may be due to accumulation of crop residues and lack of soil disturbance. Blevins et al., (1977) suggested instant soil surface acidification in NT was due to mineralization of crop residue on the soil surface, that release organic compounds which bind Al³⁺, thus raising and stabilizing soil pH with time. Since the experimental has been running for 16 years, this has been a long enough time to raise soil pH under NT. However, in the 10-20 cm depth CT-Y5 had higher soil moisture. The subsurface soils had better properties such as bulk density under CT-ANNUAL compared to the conservation tillage treatments, which may be attributed to incorporation of organic material to deeper layers under CT-ANNUAL. The higher bulk density of NT (caused by compaction due to lack of tillage and residue incorporation) had a negative effect of lowering moisture content of subsoils. According to Dalal et al., (2011) there is a strong link between SOM content, bulk density and aggregation, with high OM and good aggregation observed more in soils with low bulk density. This concurs with the current study since both NT and CT-Y5 with higher OM in the surface had low bulk density which it increased with depth as OM content also decreased. OM observed through soil properties such as available N, labile C and enzyme activity was lower in surface soils of CT-ANNUAL then increased with depth. SOM is negatively correlated with tillage and bulk density especially in

the surface because minimum or no tillage improves soil aggregation, lowers bulk density and increases SOM (Bronick & Lal, 2005). This is similar to the present findings where high labile organic C was observed under NT in 0-10 cm than CT-Y5 and CT-ANNUAL but the opposite was observed in lower horizons. Furthermore high bases under NT which form cationic bridges with SOC help to sustain high soil fertility. According to Cusack et al., (2018) basic cations provide important nutrients to root biomass production and assist in improving soil properties.

C: N ratio was lowest for NT for the top 2 depths making nutrients accessible to both plants roots and microorganisms. This may be attributed to higher substrate availability from both N fertilizer and crop residues for microbes which will then help to decompose OM in surface soils of NT. This is accordance with Yamashita et al., (2006) who found that decomposed OM had lower C: N ratio. In a study by Diekow et al., (2005) an increase in the soil C: N ratio with depth under NT was observed which was attributed to some high C: N soluble organic acids that leached from the surface into deeper layers. According to McGroddy & Silver, (2000) decomposers may be inhibited by wet soil conditions due to unavailability of oxygen, however in the current study NT with high soil moisture had higher enzymatic activities and labile C which shows high decomposition compared to other tillage treatments.

8.3 The impact of tillage and N fertilizer application on soil C sequestration

Total C, organic C, POC and POXC were all higher in surface soil of NT than CT-Y5 and CT-ANNUAL; as a result of residue accumulation and lack of soil disturbance. However, in the 10-20 cm depth at higher N rates (120 and 240 kg N ha⁻¹), CT-ANNUAL had more total C, POC and POXC than the other tillage treatments. Incorporation of residues during ploughing influenced these high fractions of C in CT-ANNUAL and CT-Y5 with increasing soil depth. Gong et al., (2009) observed that application of N fertilizers resulted in significant increases in SOC and its fractions due to the positive effects of N on crop biomass accumulation. This concurred with current findings especially at 0-10 cm since NT had higher C pools at 240 kg N ha⁻¹ rate. Contrary, Bhattacharyya et al., (2012) argued that soil treated with residues would be characterized by the high mineralization potential of its OM, which in turn, corresponds to high gaseous C efflux from the soil.

The loss of SOM because of poor soil management practices such as over grazing and monoculture depletes soil C and increases GHG emissions (Bronick & Lal, 2005). While conservation tillage can increase soil C sequestration which consequently enhances nutrient availability to crops (Roldan et al., 2007). Since maize monocropping was practiced in this study, CT-ANNUAL with poor soil management (annual ploughing) had higher emissions of CO₂ & N₂O. Similarly, SOC stocks of NT were higher than CT-Y5 and CT-ANNUAL at all depths indicating more potential to sequester C. The findings indicate that NT with higher crop residue accumulation resulted in more C storage, while both CT-Y5 and CT-ANNUAL experienced losses of C from leaching and decomposition during ploughing.

8.4. Effect of tillage and N fertilizer application on N and P mineralisation.

The total N and P, then organic and extractable P were all higher under NT in the 0-10 cm depth due to crop residues accumulation and lack of soil disturbance. This is similar to the study by de Moraes Sa et al., (2009) where they found higher P pools under NT due to fresh organic material which decomposed into OM and these changes were most evident in the surface layers. Whereas NH₄⁺ and N mineralisation were higher at lower N rates (0 and 60 kg N ha⁻¹) but reduced at higher rates of 120 and 240 kg N ha⁻¹ under NT. This was because high N rates reduced soil pH which possibly inhibited microbial OM decomposition. Soils with low pH and high exchangeable acidity, Al and Fe, sorb organic P and NO₃⁻ making it inaccessible to plants and microbes (Coward et al., 2017). These findings correlate with current results in the 0-10 cm depth under CT-Y5 at 240 kg N ha⁻¹ where NO₃⁻, organic P and pH were all very low whereas exchangeable acidity was higher. Furthermore, CT-Y5 had the lowest nitrate in all treatments. Also NT had the lowest total P at 10-20cm depth.

The trends of N mineralisation, POC, available and organic P were inversely related with C: N ratio. NT had the lowest C: N ratios in all treatments at 0-10 cm depth, then most treatments at lower depths than CT. This agrees with findings of Dalal et al., (2011) who observed higher N mineralisation in soils that had lower C: N ratio, indicating that low C: N ratio and high N mineralisation reflect improved SOM quality. In all, CT reduces extractable P, organic P, total P, total and NH₄⁺ fractions in surface soil due to ploughing breaking down soil aggregates and

incorporating organic compounds down the profile, which concurs with current findings (Kahlon et al., 2013).

8.5. Soil enzyme activity under different tillage and N fertilizer management

The surface soil had higher urease activity under NT, whereas acid phosphatase activity was higher under NT at 0 and 240 kg N ha⁻¹. The higher urease and phosphatase activity in top soil was attributed to crop residue accumulation on the surface and less soil disturbance. This was similar to findings by Deng & Tabatabai, (1996) who observed increased enzyme activity with accumulation of organic N and C on the surface under NT system involving crop residue placement. Long-term intensive monoculture usually supplies lower amounts and diversity of OM thus suppressing microbial and enzyme activity (Klose & Tabatabai, 2000). This concurs with current findings since CT-ANNUAL, with its intensive ploughing had reduced enzyme activity, especially at 0-10 cm depth, compared to NT and CT-Y5 which had no and little ploughing, respectively. In lower depths CT-ANNUAL had higher enzyme activity especially acid phosphatase at 20-30 cm depth. Further, urease activity was higher in CT-ANNUAL in some treatments at lower depth. Higher enzyme activity under CT-ANNUAL than NT at lower depths was attributed to incorporation of organic N, P and C down the profile during ploughing. In a study by Taylor et al., (2002) they noticed that soil enzyme activity usually positively correlated with SOC and total N contents since organic C is a substrate for enzyme degradation. This is similar to current findings since treatments with higher SOC and total N had also higher enzyme activities. Urease and acid phosphatase activities had almost similar trend whereas invertase activities was opposite which may be due of their functionality in the soil. Gianfreda et al., (2005) highlighted that invertase catalyse hydrolytic OM breakdown, while urease and phosphatase play a role in the mineralization of N, P and sulphur compounds, respectively.

8.6. Seasonal effluxes of GHG under different tillage and N fertilizer application rates

The response of soil nutrient cycles to management is closely linked to effluxes of gases from the soil. In the current study N, C and P pools, soil physicochemical properties and the activity of different enzymes influenced effluxes of GHGs. CT-ANNUAL had higher CO₂ and N₂O

effluxes than NT in both summer and winter, whereas the opposite was true for CH₄ effluxes. CH₄ appeared to follow the same trend as labile C pools of POC, POXC and MBC which were higher under NT especially at 0-10 cm depth compared to CT-ANNUAL. According to Bhattacharyya et al., (2012) high labile C fractions promoted the growth of methanogens thereby inducing higher CH₄ emissions. Crop residues decompose to produce acetate, which is essential for the growth of methanogens. Thus NT experienced higher CH₄ effluxes compared to CT-ANNUAL in both seasons due to presence of residues. Observations were also made of higher CH₄ effluxes at higher N rates. According to Zheng et al., (2007), this is because N fertilization especially with crop residues increases the bioavailable pool of organic C for microbes and, in turn, promotes CH₄ production by utilization of bioavailable organic C by methanogenic microbes.

N₂O on the other hand had higher effluxes under CT-ANNUAL compared to NT. This was attributed to opening of air spaces during ploughing in CT-ANNUAL that accelerates nitrification leading to high NO₃⁻ production. According to Bhattacharyya et al., (2013), NO₃⁻ content showed a positive correlation with N₂O emission ($r = 0.96$), because denitrification requires an organic C source (for energy) and the substrate NO₃⁻. Relatively higher N₂O emissions during winter (in the control treatment) than summer was concurrent with findings of Del Grosso et al., (2002), who attributed this to little N crop uptake in winter after harvest. Furthermore Holtan-Hartwig et al., (2002) elucidated that the production of N₂O exceeds its reduction N₂ at low temperature, thus contributing to high N₂O emission during winter, which concurs with current findings only in the control treatment.

Soil moisture and degradation of organic material increases GHGs emissions, especially CO₂ and N₂O (Favoino & Hogg, 2008), this concurs with present findings since well-draining CT-ANNUAL resulted in high decomposition of the residues, releasing GHGs such as CO₂ and N₂O to the atmosphere. During summer, decomposition and mineralization of OM significantly increased under CT-ANNUAL at both 120 and 240 kg N ha⁻¹, thus releasing more GHGs, CO₂ and N₂O, in the process compared to winter. This was because soil moisture and temperature were high in summer, favouring accelerated decomposition hence emission of gases. Again ploughing and N fertilization were done in summer, which created well aerated conditions and provided substrates that induced enzyme activity thereby increasing available N, P and labile C. According to Holtgrieve et al., (2006) soil moisture regulates the cycling of C, N, P and

trace gas production through stimulation of microbial activity and the diffusion GHGs from soils. In the present study high soil moisture and temperature in summer than winter induced higher GHGs effluxes especially CO₂ under CT-ANNUAL and CH₄ under NT. Furthermore higher soil moisture under NT especially at 0-10 cm intensified N, P and C mineralisation and enzyme activity thereby inducing more CH₄ production (due to the anaerobic conditions created). However, less soil moisture under CT-ANNUAL compared to NT created aerobic conditions that stimulated oxidation of organic P, N and C and diffusivity of CO₂ and N₂O. This is supported by findings of Vitousek, (2004), who illustrated that less soil moisture resulted in enough oxygen within soil pores to allow nitrification to occur, which in turn induces N₂O effluxes.

8.7 Conclusions and Recommendations

Conservation tillage improved soil physicochemical and biological properties. C, P, N and enzyme activities were higher under NT on the surface compared to CT-Y5 and CT-ANNUAL. CT-Y5, although it had less soil physicochemical and biological properties than NT, had higher C, N, P and enzymes activities compared to CT-ANNUAL on the surface. In contrast, CT-ANNUAL showed an increase of soil properties with depth, although it had higher NO₃⁻ on the surface. Variation of soil properties with depth indicates that tillage and N fertilizer application stimulate leaching and mineralisation. Improving C sequestration can be done by increasing SOM through minimizing soil disturbance and returning crop residues as it was done under NT and CT-Y5. Proper C sequestration can reduce global warming because less CO₂ will be emitted to the atmosphere. CT-ANNUAL tends to deplete soil C because of its high OM oxidation reactions. Moreover, the activity of enzymes such as urease and acid phosphatase were mostly higher under NT in the 0-10 cm depth which may be due to accumulation of organic N from crop residues. During ploughing crop residues are incorporated into the soil bringing them into contact with microbes for decomposition thus depleting soil C. CT-ANNUAL experienced higher CO₂ emission in both seasons due to availability of oxygen and improved gas diffusivity as pores are opened during ploughing. However, the accumulation of crop residues under NT increased soil bulk density which reduced the movement of air thus lowering CO₂ effluxes. Moreover the high soil moisture under NT compared to CT-ANNUAL also led to less soil pores for gases to flow. Fertilizer application also had some impact on gas effluxes. Thus the highest N fertilizer, 240 kg N ha⁻¹, resulted in the highest summer CO₂

emission, under CT-ANNUAL, showing the detrimental effect of excessive fertilizer application. NT with its high C pools had a far less CO₂ effluxes compared to CT-ANNUAL, making it effective at sequestering soil C.

Higher N mineralization in the control of NT at 0-10 cm depth led to high N₂O effluxes during summer, which eventually decreased as N rate increased which may be attributed to fertilizer acidification which hinders microbial activities. Since the experiment was located in a semi-arid zone, soils were not fully saturated, hence the main mechanism driving N₂O production was nitrification. Aerobic conditions under CT-ANNUAL as a result of ploughing favoured nitrification which stimulated higher N₂O effluxes compared to NT. Moreover increased N fertilizer under CT-ANNUAL also increased N₂O during summer, which may be attributed to high soil moisture and temperature to enhance nitrification, whereas in winter the opposite was observed. The control had the highest N₂O emission under CT-ANNUAL in winter which may be due acidification of soil in fertilized treatments reducing nitrification processes. Thus improving N-use efficiency especially by minimising ploughing and applying the appropriate amount of N in a timely manner (in summer) to enhance crop uptake must be advocated. Conservation tillage, both NT and CT-Y5 had higher total N, NH₄⁺ and N mineralization compared to CT-ANNUAL which had higher NO₃⁻ and N₂O effluxes. This implies that conservation tillage must be employed in order to sustain soil N and reduces N₂O emissions from the soil.

NT and CT-Y5 promoted good soil properties such as C, N and P pools and minimized effluxes of CO₂ and N₂O compared to CT-ANNUAL, while increasing effluxes of CH₄. Thus NT had higher effluxes of CH₄ in both seasons, which may be attributed to reducing conditions and high bulk density, both of which favour methanogenic activity. Again higher bulk density under NT compared to CT-ANNUAL reduced methanotrophs, which oxidise CH₄ in the soil. However, N fertilization increased effluxes of CH₄ under NT with the highest rate, 240 kg N ha⁻¹, having higher effluxes. GHG effluxes were higher in summer than winter, which was attributed to higher summer soil temperatures and moisture. Drier winter soil moisture reduced CO₂ emissions whereas high soil moisture and temperature induced CH₄ emissions. Further, CT-ANNUAL induced effluxes of CO₂ and N₂O, whereas CH₄ was induced by NT. Moreover greater CO₂ emissions from CT-ANNUAL were attributed to increased rates of OM turnover during ploughing. Whereas higher N₂O emissions from CT-ANNUAL were mostly due to

improved soil aeration thus increase in diffusivity and better nitrification. Furthermore, greater emission of CH₄ under NT was attributed to the higher soil moisture and temperature in this technique that favoured methanogenic bacterial activity. Though CH₄ and N₂O have higher global warming potential, their effluxes were much less than those of CO₂. Thus, optimizing N fertilization is necessary to mitigate GHGs effluxes from the soil by maintaining high C, P and N pools in the soil. The best treatment to reduce emissions was NT in summer (except for CH₄ at 240 kg N ha⁻¹), since it had far less effluxes of all the GHGs measured compared to CT-ANNUAL. Again, NT at 120 kg N ha⁻¹ in 0-10 cm depth generally had high C, P, N pools, enzymes activity (invertase and urease activities) and physicochemical properties compared to other treatments. Therefore conservation tillage especially NT was more effective to minimise GHG emissions compared to CT. The null hypothesis that conservation tillage would increase fluxes of GHGs was rejected. However, more needs to be done to extrapolate results to different agro-ecological zones, soil types, fertilizer sources and other management regimes e.g. irrigation. It is worth to note that CT-Y5, though had less physicochemical, P and C pools and enzyme activities than NT, had better properties compared to CT-ANNUAL henceforth it can be recommended to farmers.

9. References

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10. Appendices

Appendix A. 1: Layout of experiment site at Loskop area.

REP 1			REP 2			REP 3		
NT	CT- ANNUAL	CT- Y5	CT- ANNUAL	NT	CT- Y5	CT- Y5	NT	CT- ANNUAL
60 UREA	60 UREA	60 UREA	0 UREA	240 UREA	0 UREA	60 UREA	240 UREA	60 UREA
120 UREA	0 UREA	240 UREA	120 UREA	0 UREA	120 UREA	120 UREA	60 UREA	240 UREA
240 UREA	120 UREA	120 UREA	60 UREA	120 UREA	60 UREA	240 UREA	120 UREA	0 UREA
0 UREA	240 UREA	0 UREA	240 UREA	60 UREA	240 UREA	0 UREA	0 UREA	120 UREA

NT = No-Till

CT-ANNUAL = Conventional till annually

CT-Y5 = Conventional till every 5th year