Nitrogen utilization efficiency, growth and yield response of maize (*zea mays* l.) to integrated application of mineral nitrogen and cattle manure

BY

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KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

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DEDICATION

This work is first and foremost dedicated to Almighty God for seeing me through this program, to my late father Alhagie Bakary Sonko and my mother Fatou Drammeh for the sacrifices they made to see to it that I continued to go to school, to my wife Fatou Darboe, my daughter Bintou Sonko and my son Mustapha Sonko for their patience while I stayed away from them in pursuance of this degree.

DECLARATION

I hereby declare that this submission is my own work towards the award of a PhD in Crop Physiology. To the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except work which has been duly cited in the text.

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ABSTRACT

Numerous efforts have been put into maize research in Ghana over the years, however, productivity in farmers' fields generally remained low, averaging 1.6 tons/ha. This low productivity has been attributed among other factors, to inappropriate nutrient management practices. A study was conducted at the Plantation Crops Section of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Ghana to identify effective cattle manure and mineral nitrogen (N) combination practices that will improve nitrogen utilization and maize productivity.

Three field experiments were carried out in the study. The first was conducted in the 2014 major season, whilst the second and third were respectively conducted in the 2014 minor and 2015 major seasons. All three experiments were conducted within the same area but on different fields. The experiments were factorial in a randomized complete block design with four replications. Obatanpa was used as the test crop. The first two experiments had the same treatments with two factors i.e. cattle manure and time of nitrogen application. Cattle manure comprised four rates: 0, 2, 4 and 6 tons/ha. Nitrogen application times were as follows: 50% N at 2 weeks after planting and 50% at 4 WAP (NT1), 50% N at 2 WAP and 50% at 6 WAP (NT2), 50% N at 2 WAP and 50% at 8 WAP (NT3) and a control (0 kg N/ha). The third experiment comprised two factors i.e. cattle manure rates as in the previous experiments and mineral nitrogen rates as follows: 0, 30, 60 and 90 kg N/ha. Nitrogen uptake by aboveground plant parts was higher when inorganic N was applied in combination with cattle manure than when inorganic N or cattle manure alone was

applied. It was also higher in inorganic N treatments than manure treatments. Among the times of N application, N uptake was higher at NT2.

Nitrogen uptake increased with increase in N rate, but at a diminishing return. Remobilization of N from vegetative parts to the ears during grain filling was severe from leaves than culms; and in the control than in the mineral nitrogen and manure treatments. It was also relatively smaller at NT2 than at other N application times, and at higher N rates than lower rates. Applications of manure significantly ($P < 0.05$) reduced N remobilization in the 4 and 6 tons/ha manure treatments. Nitrogen use efficiency was higher at NT2 application time. Among the N rates, NUE was higher at 60 kg than at 30 and 90 kg N rates, but was also significantly higher ($P < 0.05$) at 30 kg than at 90 kg N rate. Crop growth rate and other growth parameters were mostly higher at NT2 than at other application times; and at higher than lower mineral nitrogen and manure rates. Dry matter accumulation was also greater at NT2 application time and increased with increase in N and manure rates. Leaf dry matter responded more to variations in nitrogen application, which makes leaves better indicators of nitrogen effect on maize dry matter partitioning than culms. Grain yield was higher in inorganic N and manure combined treatments than in sole inorganic N and sole manure treatments. Higher grain yields recorded in the nitrogen and manure combined treatments was as a result of higher N uptake, NUE, dry matter accumulation and growth parameters obtained in these treatments. The 60 kg N combined with 6 tons/ha cattle manure treatment effect on most of the parameters studied was higher than that of the 90 kg sole mineral nitrogen treatment effect and statistically the same as the 90 kg N combined with 6 tons/ha cattle manure treatment. Considering side effects of excessive N application on the environment and on production cost, application of 60 kg N/ha at NT2 in combination with 6 tons/ha cattle manure is a promising technology for greater nitrogen utilization and higher maize grain yield.

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LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization
SSA	Sub-Saharan Africa
IITA	International Institute for Tropical Agriculture
FYM	Farmyard manure
CIMMYT	International Maize and wheat improvement Center
WAP	Weeks after planting
NT1	Nitrogen application time 1
NT2	Nitrogen application time 2
NT3	Nitrogen application time 3
TNA	Time of N application
MR	Manure rate
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
QPM	Quality protein maize
N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
C	Carbon
Na	Sodium
OM	Organic matter

DM	Dry matter
NUE	Nitrogen use efficiency
NRE	Nitrogen recovery efficiency
NIE	Nitrogen internal efficiency
GLA	Green leaf area
LAI	Leaf area index
CGR	Crop growth rate
NO_3-N	Nitrate nitrogen
NH_4^+-N	Ammonium nitrogen
R2	Blister stage
R6	Physiological maturity stage
VT	Tasseling stage
LSD	Least significant difference
HI	Harvest index
SE	Standard error

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CHAPTER ONE

INTRODUCTION

Maize (*Zea mays* L.), along with wheat and rice are the three most traded cereals worldwide. The average world maize productivity of 5.22 tons/ha in year 2010 was higher than that of rice, 4.37 tons/ha and that of wheat, 2.99 tons/ha (FAOSTAT, 2011). Maize is one of the major food staples in Sub-Saharan Africa (SSA) and serves as a source of food and income for more than 300 million smallholder farmers in the region (La Rovere *et al*., 2010). In developed countries, an estimated 70% of maize produced is used for livestock feed; only 3% is consumed as food and the remainder is used for biofuels, industrial products and seed. In SSA, 77% of maize produced is used as food and only 13% is used for other purposes (Smale *et al*., 2011). This clearly signifies the important role it plays as a source of food for the many millions of people in this part of the world. Maize is an efficient crop when it comes to capturing the energy of the sun and converting it to food. Thus, maize more than any other crop, offers the promise of meeting Africa's food needs (Ado *et al*., 2007).

In Ghana, maize is the most important cereal crop in terms of production and consumption. Farmers grow it as a sole crop or intercropped with cassava for subsistence purposes and also because of the readily available market provided by the urban centers (Asare *et al*., 2012). To meet the needs of the farmers, several improved maize varieties of different maturity periods have been developed and released by the Crops Research Institute (CRI) of the Council for Scientific and Industrial Research of

Ghana (CSIR). Among these include Obatanpa noted for its high grain yield capabilities and improved nutritional status. Obatanpa is a white dent and flint endosperm quality protein maize (QPM) with elevated levels of lysine and tryptophan. It was first released by CRI in 1992 to help improve the protein nutritional status of a large population of low-income families who depend on maize as a major component of their dietary protein intake (Aflakpui *et al*., 2005). The variety is adopted extensively in Ghana and many other African countries (Sallah *et al*. 2003). It still by far remained the most popular maize variety in Ghana. From 2001 to 2011, it accounted for about 96% of certified seed production in the country (Ragasa *et al*., 2013).

Despite the achievements in maize research in Ghana, productivity in farmers' fields has been generally low, averaging 1.6 tons/ha (Oppong *et al*., 2014). This low productivity, among other factors, has been attributed to inappropriate nutrient management practices (Obeng-Bio *et al*., 2011). Nutrient management is an important factor for achieving the potential yield in maize production systems because mineral nutrients are the major contributors to increasing crop production (Khoshgoftarmanesh and Eshghizadeh, 2011). Finding the best approaches to achieve efficient nutrient management systems is, therefore, very essential both for economic and environmental reasons.

Nitrogen, either in organic or inorganic forms is universally accepted as a key component to high yield in maize production (Amanullah, 2007). Nitrogen is a component of a number of compounds, e.g. proteins, nucleic acids and chlorophyll; and plays important role in many plant physiological processes. In particular, it is important in the efficient capture and use of solar radiation (Amanullah *et al*., 2009). It mediates the utilization of potassium, phosphorus and other elements in the plant. The optimum amounts of these elements in the soil cannot be utilized efficiently if N is deficient in plants. Therefore, N deficiency can reduce maize yield substantially (Nemati and Sharifi, 2012). Nitrogen loss from cropping systems is, however, a major source of environmental pollution. Thus, N uptake and use by plants is of fundamental importance in crop production systems (Birch *et al*., 2008).

During the last century, increments in maize grain yield came as a result of improvements in both agronomic practices and conventional breeding. From a physiological perspective, this improvement can be attributed to several factors and among them is the maintenance of individual plant N uptake with extended reproductive stage accumulation (Ciampitti *et al*., 2013). The widespread availability of N fertilizers from the 1950s onward has enabled many farmers around the world to abandon exploitative, low-yielding agricultural practices that deplete soil nutrient without adequately improving crop yields. Maize grain yield is highly responsive to supplemental N; leading to annual applications of an estimated 10 million tons of N fertilizer to maize crops worldwide (FAO, 2004). Nearly half or more of the N applied to soil is, however, unutilized by plants and is lost through leaching into ground water or emission into the atmosphere (Xu *et al*., 2011).

The need to meet the increasing food demand and protect the environment remains a challenge in crop production systems. Synchronizing nitrogen supply with crop demand to avoid excess or deficiency is the key to optimizing tradeoffs amongst yield, profit, and environmental protection in both large-scale crop production systems in developed countries and small scale systems in developing countries (Cassman *et al*., 2002). Therefore, improving the nitrogen use efficiency of maize crops will result in significant economic and environmental benefits.

Nitrogen use efficiency (NUE) in maize is often defined as grain produced per unit of fertilizer N applied. Nitrogen use efficiency can be described in different ways depending on whether the focus is on grain only or on the total biomass N uptake (Bandyopadhyay and Sarkar, 2005). The NUE of maize on a global basis ranges from 25 to 50%, or an average of 33% (Xu *et al*., 2011). Ju *et al*. (2009) stated that if NUE of maize could be improved to 50%, the application rate of N fertilizer could be reduced to half without imposing any significant loss of yield or grain quality. World-wide nitrogen use efficiency reported for most cereal crops including maize is approximately 33% with estimated averages of 29% and 42% for the developing and the developed countries, respectively. Such a low NUE reflects ineffective nitrogen management in crop production systems and causes both great economic losses to producers and negative impact on the environment (Walsh *et al*., 2012). Therefore, creating effective N management systems and improving nitrogen use efficiency are critical issues which should be addressed to maintain and increase the sustainability of maize production.

Individual effects of chemical fertilizers and manure on maize growth and yield have been intensively investigated (Franke *et al*., 2008; Ayoola and Makinde, 2009). While inorganic N fertilizers can quickly increase crop yield, their use in crop production has been associated with increased soil acidity and nutrient imbalance (Ayoola and Makinde, 2007). On the other hand, the use of manure alone has been reported to be inadequate due to unavailability in the required quantities, their relatively low nutrient contents, and slow release of nutrients (Mugwe *et al*., 2007). Integrated nutrient management approaches, in which both manure and inorganic fertilizers are used, have been widely suggested as the most viable approach to maintain higher maize crop productivity (Ayoola and Makinde, 2008).

Working on the hypothesis that combining inorganic nitrogen at different application times and rates with cattle manure will lead to significantly different nitrogen utilization, growth and yield responses of maize, the objectives of the study were to:

I. determine the effect of integrated application of mineral nitrogen and cattle manure on nitrogen uptake, partitioning and remobilization in maize.
II. assess the effect of cattle manure and mineral nitrogen application times and rates on nitrogen use efficiency in maize.
III. evaluate the effect of mineral nitrogen and cattle manure on dry matter accumulation and partitioning in maize.
IV. determine the growth and yield responses of maize to time of nitrogen application and nitrogen rates, and their combinations with cattle manure.

CHAPTER TWO

LITERATURE REVIEW

2.1 Environmental requirements for maize crop production

Maize, also known as corn, is a very versatile crop, grows at all sorts of altitude and fertility conditions, which explains its global adaptability and its many types of varieties (Qi *et al.*, 2012). It is grown in climates ranging from temperate to tropic during the period when mean daily temperatures are above 15 °C (FAOSTAT, 2010). Although the minimum temperature for germination is about 10 ºC, germination will be faster and less variable at soil temperatures of 16 to 18 ºC. At 20 ºC, maize should emerge within five to six days (Plessis, 2003).

2.1.1 Climatic factors

Adaptability of maize varieties in different climates varies widely. Successful cultivation largely depends on the right choice of varieties so that the length of growing period of the crop matches the length of the growing season and the purpose for which the crop is being grown. The optimum temperature for maize growth and development is 18 to 32 °C and at tasselling 21 to 30 °C is ideal (Belfield and Brown, 2008). The critical temperature detrimental to yield is approximately 35 ºC and above. Temperatures below 8 ºC or above 40 ºC usually cause cessation of development (Birch *et al*., 2008). Different maize cultivars have different optimal temperature requirements, for example, tropical cultivars derived from 'highland' maize are able to grow and develop better at lower temperatures than those adapted to 'lowland' or 'mid-altitude' areas.

Temperatures that are outside the range of adaptation of a cultivar may impact negatively on factors such as photosynthesis, translocation, and pollen viability (Lafitte, 2000). Higher temperatures have a negative impact on kernel growth, kernel mass and protein accumulation (Monjardino *et al*., 2006).

Maize can grow and yield with as little as 300 mm of rainfall, which might result in 40% to 60% yield decline compared to optimal conditions; however, successful growth will be attained with a minimum annual rainfall of 600 mm. The preferred precipitation range for optimal growth is 500 to 1200 mm which should be well distributed throughout its growing stages (Belfield and Brown, 2008). Maize crop needs more than 50% of its total water requirement after tasseling and inadequate soil moisture at grain filling stage results in a poor yield and shriveled grains. Prolonged cloudy period is harmful for the crop but an intermittent sunlight and cloud of rain is the most ideal for its growth. It needs bright sunny days for its accelerated photosynthetic activity and rapid growth (Akmal *et al*., 2010).

2.1.2 Soil requirement

Maize plants grow well on most soils but less so on very heavy, dense clay and very sandy soils. The fertility demands for grain maize are relatively high. Up to about 200 kg N/ha, 50 to 80 kg P/ha and 60 to 100 kg K/ha are required by high yielding varieties (Sangoi *et al*., 2001). In general the crop can be grown continuously as long as soil fertility is maintained (Plessis, 2003). Maize does well, in terms of growth and yield on soils with a pH range of 5.5 to 8 (Bakht *et al*., 2006). The soil should preferably be well aerated and well drained as the crop is highly susceptible to water logging. Excess soil moisture causes major changes in physical and chemical properties in the rhizosphere; and under such condition, there is very little or no gaseous exchange between aboveground plant parts and inundated roots. Therefore, plant roots suffer from extreme oxygen stress which inhibits growth and development (Zaidi *et al*., 2003). The extent of damage due to excess moisture stress varies significantly with developmental stage, and past studies have shown that maize crop is comparatively more susceptible to excess moisture stress during the early seedling to tasseling stages (Zaidi *et al*., 2004).

2.2 Nitrogen application in maize production

Nitrogen is a vital plant nutrient and a major yield determining factor required for maize production. It is very essential for plant growth and makes up 1 to 4% of dry matter of the plants. Nitrogen is a component of protein and nucleic acids and when it becomes sub-optimal, growth is reduced. Its availability in sufficient quantities throughout the growing season is essential for optimum maize growth (Onasanya *et al*., 2009). Maize takes up nitrogen at a higher rate than many other crops. The crop removes from the soil between 20 and 25 kg of N per ton of grain produced (Sangoi *et al*., 2001). Depending on the growth stage, nitrogen absorption from the soil increases with the actual rates depending upon the soil type and previous cropping history. The maximum absorption per day is approximately 4.43 kg/ha at the silking stage (Boonlertnirun *et al*., 2010).

2.2.1 Effect of nitrogen on maize growth and yield parameters

Nitrogen plays a pivotal role in several physiological processes in maize plants. It is of fundamental importance to establishing the plant's photosynthetic capacity. Nitrogen is important for kernel initiation, contributes in determining maize sink capacity and helps to maintain functional kernels throughout grain filling. It also influences the number of developed kernels and final kernel size. However, nitrogen effects on kernel number per plant could be related primarily to traits responsible for plant biomass production (i.e., leaf area, light capture, and radiation use efficiency) rather than to the partitioning of biomass and N to the ear (D'Andrea *et al*., 2008).

Nitrogen deficiency promotes a reduction in maize crop growth rate and subsequently reduces grain yield (Andrade *et al*., 2002). Its deficiency in maize is often visually apparent through reductions in leaf area, leaf chlorophyll status, especially as leaves age and vegetative biomass. Such phenomenon decrease plant light interception, photoassimilate production, and final grain yield (Echarte *et al*., 2008). Nitrogen deficiency in maize could also be indicated by yellowing of mature leaves starting at the leaf tips and then extending along the mid-ribs, stunted plants, delayed flowering and short, poorly filled ears (Hughes, 2006).

Low nitrogen supply decreases grain yield by reducing grain number and individual grain weight (Hammad *et al*., 2011). The potential weight of individual grains is determined by two main factors: the number of endosperm cells which are formed within the first 2–3 weeks after pollination (i.e. during the lag phase of kernel development) and assimilate availability during grain filling (Paponov and Engels, 2005). Increase in N supply within limits has been associated with increase in leaf area, leaf weight and chlorophyll content, all of which determine the photosynthetic activity of the leaf and ultimately dry matter production and allocation to the various organs of plants. This shows that adequate N supply can be used to delay leaf senescence in maize thereby maintaining the leaves green and functional for a longer period. Photosynthetic rate, leaf surface area and size of the sink all increase with increase in nitrogen levels. Increase in leaf area and photosynthetic capacity with increased N levels is attributed to the effects of N on cell and tissue growth (Gungula *et al*., 2005). Availability of sufficient nitrogen to maize extends the periods of post-silking dry matter and N accumulation and this phenomenon has been associated with higher grain yields. However, increased N availability promotes greater yield responses with high yielding than with low yielding maize varieties (Ciampitti and Vyn, 2011).

2.2.2 Timing of nitrogen application

The dynamics of plant nutrient uptake is quite complex and a time lag always exists between when nutrients are available and when plant roots absorb them, during which the nutrients are vulnerable to losses. Nutrient loss potential is a function of nutrient type, soil type and weather conditions (Tarkalson *et al*., 2006). For instance, nitrogen and potassium behave quite differently in the soil environment; where N is biologically very dynamic and after conversion to nitrate (NO_3^-) becomes very mobile, P may quickly become inaccessible to crops due to chemical precipitation (Sogbedji *et al*., 2006). Timing of nitrogen fertilizer application is an important factor that affects the efficiency of crop nitrogen uptake. The time interval between application and crop uptake determines the length of exposure of nitrogen to loss processes such as leaching, denitrification and volatilization (Zebarth *et al*., 2007).

Nitrogen fertilizer is used more efficiently when the supply matches with the demand for nitrogen by the crop. Early nitrogen fertilizer application increases the risk of N loss from the root zone by leaching and denitrification, whereas N application after the period of rapid N uptake can reduce plant N uptake (Binder *et al*., 2000). Timing of nitrogen application to reduce the probability of N loss through leaching and denitrification can increase the efficiency of fertilizer N; therefore, improvement of N uptake by maize plants is of interest for both agronomic and environmental reasons (Howard *et al*., 2002).

Nitrogen uptake and utilization is maximized when N fertilizer is applied shortly before the period of most rapid crop N uptake. Delaying N application can lead to irreversible yield losses. However, maize yield remain responsive to nitrogen application until silking but full yield might not be achieved when applications are delayed until that stage (Ma *et al*., 2005). Many authors have emphasized the need for greater synchrony between maize crop N demand and N supply from all sources throughout the growing season (Cassman *et al*., 2002). Poor synchronization between N supply and crop demand is mainly due to large nitrogen fertilizer applications in the early growth stages, which result in high levels of soil inorganic nitrogen well before rapid crop N uptake occurs. Research findings have further demonstrated that in-season application of nitrogen considering site specific soil N supply and crop demand results in high N use efficiency (Chen *et al*., 2010).

2.2.3 Critical periods for nitrogen supply in maize production

Maize crop begins to rapidly take up nitrogen and other nutrients at the middle of vegetative growth stage, with the maximum rate of N uptake occurring near silking (Birch *et al*., 2008). The onset of grain filling has been identified as a critical phase for N supply because N uptake declines as the plant progresses to maturity, mainly due to reduced transport of carbohydrates to the roots. The ability of maize to maintain N uptake during grain filling could be related to the supply of photosynthates to the roots. Reduced N uptake during grain filling may enhance N remobilization from leaves and stem and may eventually lead to leaf senescence (Ciampitti and Vyn, 2011). If N uptake is maintained during grain filling, less N will be remobilized from the vegetative parts, which could result in increased leaf area duration, delayed leaf senescence and prolong dry matter accumulation. Prolong accumulation of dry matter and N by aboveground plant parts in maize during grain filling have been reported as an important characteristic associated with high grain yields (Rajcan and Tollenaar, 1999).

2.2.4 Nitrogen uptake, partitioning and remobilization of maize

Nitrogen mineralization is defined as the process by which soil organic N is transformed into inorganic forms (NO_3^- and NH_4^+) by microorganisms. The process in which inorganic nitrogen is transformed into organic nitrogen is defined as N immobilization. If environmental conditions are not limiting, ammonium nitrogen (NH_4^+-N) is oxidized to nitrate-nitrogen (NO_3^--N) almost as rapidly as it is formed (Ma *et al*., 1999). Maize can utilize nitrogen in both the ammonium and nitrate forms, but because of the ready conversion of ammonium to nitrate by soil microbes within hours or few days after application, most of the N is taken up as nitrate (Farnham *et al*., 2003; McCashin, 2000).

Nitrogen uptake and assimilation can be visualized as metabolic events mediated by carriers and enzymes that are interrelated and each of which is under genetic control (Barbieri *et al*., 2008). Maize varieties differ in their capabilities in terms of N uptake and assimilation even when they encounter similar or different levels of nitrogen (Machado *et al*., 2001). Increased productivity in maize genotypes is due to their ability to accumulate nitrate in their leaves during vegetative growth and to efficiently remobilize this stored nitrogen during grain filling. Maize hybrids which accumulate more N after silking tend to have higher grain yield (Hirel *et al*., 2001). Maize stalk plays a significant role in providing N for kernel development, and N fertility was found to influence the rate of N remobilization from vegetative to reproductive tissues (Subedi and Ma, 2007).

At about the 10th leaf stage, maize plant begins a rapid and steady increase in nutrient and dry matter accumulation, which continues into the reproductive stage (Cakir, 2004). Nitrogen taken up during the vegetative stage is used primarily for vegetative growth, and during late vegetative periods for reproductive organs initiation, whereas the N taken up after silking and tasseling is mainly directed towards the synthesis of grain proteins. Addition of N during the reproductive stage not only increase grain yield but substantially improves grain quality through increase partitioning of greater amounts of protein and carbohydrates to the grains (Amanullah, 2007).

Many studies have been conducted to explore the potential of several methods to indicate the N status of maize crops. Among these methods include the use of chlorophyll meter, remote-sensing techniques and plant tissue analysis. The chlorophyll meter and remote sensing technologies provide point measurements only, which limits their suitability for detecting luxury N uptake since maize plants achieve maximum chlorophyll content irrespective of the level of over fertilization. In-season plant tissue nitrogen test has proven effective for the assessment of the N status of maize plants (Herrmann and Taube, 2005).

2.3 Combined application of cattle manure and mineral nitrogen fertilizer

Soil nutrient management is an important factor for achieving the potential yield in maize production systems because mineral nutrients are the major contributors to increasing crop production and maintenance of soil productivity (Khoshgoftarmanesh and Eshghizadeh, 2011). Finding the best nutrient management approaches that promote efficient nutrient utilization is very essential both for economic and environmental reasons. Combination of chemical fertilizers with organic materials such as cattle manure is a recommended strategy to enhance efficient utilization of soil nutrients by crops (Yadav *et al*., 2000).

2.3.1 Mechanisms for interactive effect of manure and mineral fertilizers

The mechanisms for the interactive effect of inorganic and organic fertilizers when applied in combination have been categorized into three i.e., nutrient synchrony, general improvement in soil fertility and soil priming effect (Cassman *et al*., 2002; Kuzyakov *et al*., 2000).

2.3.1.1 Synchronization of nutrient supply

Manure nutrients are stored for a longer time in the soil, thereby supporting better root development, leading to higher soil microbial biomass and increased crop yields (Abou El-Magd *et al*., 2006). Cattle manure serve as a source of all necessary macro and micronutrients in available forms and, therefore, directly affect plant growth. Cattle manure nutrients are, however, released slowly during the course of the cropping season due to its high C/N ratio (Mugwe *et al*., 2007). To meet maize crops nutrient demand, supply of nutrients from the manure can be complemented by combining them with inorganic fertilizers that will release nutrients faster to compensate for the late release of mineral nutrients of the manure (Ayoola and Makinde, 2009).

Making most efficient use of animal manures depends critically on improving synchrony of mineralization with crop uptake (Rufino *et al*., 2006). Mineral N fertilizers applied along with cattle manure can provide sufficient N to crops early in the season, and when accompanied later in the season by a sustained release of N from mineralization of the cattle manure incorporated prior to seeding, the two sources can meet the peak of N demand of the crop (Kramer *et al*., 2002). Alemu and Bayu (2005) reported yield advantages from the integrated application of farmyard manure and mineral fertilizer on sorghum could possibly be attributed to the additive nutrient supply and to a better synchrony of nutrient availability with crop demand, i.e., the immediate availability of nutrients from mineral fertilizers and slow release from FYM.

2.3.1.2 General soil fertility improvement

The application of animal manure to agricultural land has been viewed as an excellent way to recycle nutrients and organic matter that can support crop production and improve soil quality (Bandyopadhyay and Sarkar, 2005). Manure application supplies organic matter which improves soil physical and chemical properties, thereby, increase plant nutrient concentration and nutrient uptake (Mugwe *et al*., 2007). Manure application also enhance soil moisture retention capacity, regularize soil pH and supply other soil macro and micro nutrients essential for effective crop growth and yield which all enhances nutrient uptake and efficient utilization (Azeez, 2009). Other than nitrogen, cattle manure application also provides most of the other essential macro and micro nutrients required for effective crop growth. It is, therefore, a safe and effective way of recovering lost plant nutrients.

Plant available phosphorus and potassium for example, are known to be quite high in manures and manure application is known to increase their levels in the soil (Zhou *et al*., 2012). The increase availability of the macro and micro nutrients ultimately enhance the crop uptake of these nutrients and thus grain yield. However, only a small fraction of cattle-manure nutrients are immediately available for plant uptake and use; thus, it is required to supply soil with both mineral fertilizers and cattle manure for high plant growth and maximum yields (Najm *et al*., 2012).

As a result of their low nutrient content, in particular nitrogen and slow release of nutrients, cattle manure alone cannot meet crop nutrient demand. The decomposition of cattle manure and the mineralization of nutrients contained in it can be fairly slow, thus, to enhance the quality and effectiveness of such organic materials, it will be necessary to apply them along with mineral fertilizers. Other studies have concluded that the combined application of mineral and organic fertilizers, using methods that best conserve organic matter may be the most promising strategy for improving soil fertility (Sogbedji *et al*., 2006).

2.3.1.3 Priming effect on soil

Priming effect is defined as the short-term change in the turnover of soil nutrients caused by treatments, usually due to addition of decomposable organic materials or inorganic fertilizers (Blagodatskaya and Kuzyakov, 2011). This effect would be positive, such as mineralization and release of the organic material nutrients for plant uptake and use; or immobilization and unavailability of the nutrients. In nitrogen studies, the priming effect is determined by N mineralization rates. Upon application of mineral nitrogen fertilizers in upland fields, the NH_4^+-N is quickly converted to NO_3-N which is the plant available form of N and increases the amount of N in the soil (Farnham *et al*., 2003). Increase in the amount of N present in the soil is known to have positive effect on the immediate N uptake rates in maize (Barbieri *et al*., 2008).

Supplementing manure with inorganic N fertilizers increase the supply of nitrogen for microorganisms involved in the decomposition of the manure and, therefore, limits the immobilization effect, speeds up the manure decomposition process and increases the availability and uptake of the nutrients (John *et al*., 2010). Ouédraogo *et al*. (2006) reported that fertilization of sorghum with organic materials reduced N uptake but it became greater when the organic fertilizer was supplemented with inorganic N fertilizer.

2.4 Availability of N from applied cattle manure and nitrogen fertilizer

The determination of crop available N from cattle manure is based on the contents of NH_4^+, NO_3^- and organic N in the manure. Cattle manure has a large proportion of the N in the organic fractions. The amount of N in such organic material that is available to plants is influenced by, among other factors, N mineralization, immobilization processes, soil type and soil properties (Materechera and Salagae, 2002).

Mineralization of organic N is a result of microbial decomposition of the organic material, which is influenced by the type of organic material, soil moisture, temperature, and oxygen content (Tarkalson *et al*., 2006). These factors vary, thus, making it difficult to determine exact availability factors from site to site. Past research has determined that the mineralization rates of organic N from cattle manure can range from 1 to 50%. Hartz *et al*. (2000) stated that over a 3 months period, approximately 13% of the organic N mineralized from non-composted aged cattle feedlot manure. In Nebraska, in the United States for instance, it is recommended that farmers assume that approximately 25% of the organic N in solid cattle manure will be available to maize in the first growing season after application (Koelsch and Shapiro, 2006). Several studies that focused on N recovery from applied cattle manure indicated that maize has a low N recovery of about 10-40% when 100 to 500 kg N/ha of cattle manure was applied (Cherney *et al*., 2002). The uncertainty and variability in N mineralization from organic N sources from site to site increases the risk of under or over application of nitrogen (Tarkalson *et al*., 2006). Cattle manure contains some mineral N ($NH_4^+$and NO_3^-) that may be immediately available for plant uptake, but majority of the N is organically bound and is available gradually because it needs to mineralize. However, N deficiency has been widely reported in maize when manure is used as the sole N source (Nyamangara *et al*., 2005).

Nitrogen in mineral fertilizers, unlike manure, is immediately available for plant uptake but it is susceptible to loss in gaseous forms or by leaching if added at the wrong time or in excess of plant demand. Availability of N from mineral as well as organic nitrogen sources is affected by several factors such as in-season N loss and soil properties such as organic matter content, pH¸ texture, structure, water content, and temperature (Nyiraneza *et al*., 2010). The soil N supply to maize crops may also vary due to climatic conditions. In a study on plant available N, Kay *et al*. (2006) found that soil N supply was mostly influenced by rainfall early in the growing season and that climatic conditions exert a more important control on soil N supply and crop response to N

fertilizer than soil properties.

2.5 Dry matter production and partitioning of maize

Research findings from the past years suggest that dry matter accumulation of maize follows a general pattern; a period of quick exponential growth, followed by a linear trend until late in the reproductive stages when dry matter reaches a maximum. Two peak periods have been found in the rate of dry matter accumulation, the first peak was during late vegetative growth stage and the other was during the latter part of the reproductive phases during grain filling (Boyer, 2013).

High rate of dry matter accumulation by a maize canopy at certain developmental growth stages is a prerequisite for higher grain yields. The most important stages are early growth stage, extending from fourth to sixth leaf stage when the number of leaves and ears are finally fixed, from tasselling up to blister stage where number of kernels per plant is determined and at ripening phases where kernel weight is decided (Grzebisz *et al*., 2010). The number of kernels per plant that a maize plant will set at a given plant growth rate depends upon the biomass partitioning to the developing ear. A slower rate of growth, associated with slow plant growth or less partitioning of assimilates to the ears, results in reduced kernel set (Severini *et al*., 2011). Increases in assimilate availability per kernel after flowering have resulted in minor increases in kernel weight at crop maturity, whereas reductions in the assimilate availability per kernel decrease kernel weight considerably. This finding suggests that kernel number and potential sink capacity are established early in the grain filling period and that further kernel growth is close to saturation in terms of assimilate availability (Borras and Westgate, 2006).

During grain filling, the developing kernels will be the primary sink for photosynthates produced by maize plants; hence, a greater proportion of the dry matter produced at this time will be partitioned to the kernels sometimes at the expense of other plant parts including stalks and leaves (Nielsen, 2013). The rate of partitioning of assimilates to grains depends on their sink strength and reproductive growth stage. In the first two weeks after completion of flowering i.e. the lag phase, the ear, husks, stem and roots could all be sinks and competing with the developing grains for assimilate supply (Amanullah, 2007). However, during linear grain filling stage, which begins from the second week after flowering upwards, the grains are the dominant sinks for the newly synthesized carbohydrates, nitrogen taken up through the roots and N compounds (Paponov and Engels, 2005). The two weeks after flowering marks the period when endosperm cell division occurs and when starch granules are initiated. These processes determine the number of sites for starch deposition, and thus the potential for kernel dry matter accumulation (Cazetta *et al*., 1999).

2.6 Nitrogen effect on dry matter production of maize

Dry matter production by crops is dependent on the rate of light interception and photosynthetic capacity of the vegetative parts of the plants. Dry matter yield response of maize to higher nitrogen supply is positive until factors other than nitrogen limit higher production (Worku *et al*., 2012). The positive response is brought about either by a larger amount of radiation intercepted over the crop growth period or a higher average daily rate of photosynthesis, or a combination of both. However, in N limited environments, leaf area and light interception are maintained to the detriment of the concentration of nitrogen and the photosynthetic capacity per unit leaf area (Vos *et al*., 2005). The difference in the dry matter accumulation in maize is attributed to postsilking N uptake and it improves with increase in nitrogen application rate. Differences in dry matter yield and N uptake vary partly due to decreased soil nitrogen mineralization and partly due to the drier weather conditions, and N uptake rate has been found to assist the improvement of dry matter yield in maize (Amanullah *et al*., 2009).

2.7 Concept and definition of nitrogen use efficiency

Nitrogen use efficiency (NUE) in maize is often defined as grain produced per unit of fertilizer N applied. The NUE concept commonly provides a quantitative measure of the effectiveness of plants to take up and convert available N into grain yield within a cropping system (Ciampitti and Vyn, 2011). To achieve a better understanding of the effects of different nutrient management practices on crop NUE, it is beneficial to examine the main components of NUE independently. Two important components of NUE are (i) nitrogen recovery efficiency (NRE), which refers to the ability of aboveground plant parts to recover N from the applied nitrogen fertilizer, and (ii) nitrogen internal efficiency (NIE), which refers to the capability of plants to transform the N taken up by the crop into grain yield (Salvagiotti *et al*., 2009). Individual evaluation of these NUE components is useful to advance understanding of the physiological mechanisms and processes (such as N uptake, assimilation, translocation, and remobilization) of the N within the plant.

To achieve higher nitrogen use efficiency, some authors have observed that the NRE component is more important under high N supply environments; whereas the NIE component becomes more essential in low N availability environments (Ma *et al*., 1998). With sufficient nitrogen supply in the field, variation in N use efficiency is due largely to differences in N uptake ability, whereas with deficient N supply, variation in N use efficiency is mainly due to differences in utilization of the accumulated N in plants (Peng *et al*., 2010).

2.8 Importance of N management on nitrogen use efficiency in maize

Ideal nitrogen management optimizes grain yield, farm profit and nitrogen use efficiency, while it minimizes the potential for leaching of N beyond the crop rooting zone. Excessive nitrogen fertilization may result in low nitrogen use efficiency and potentially exerts more pressure on the environment (El-Gizawy and Salem, 2010). World-wide nitrogen use efficiency reported for most cereal crops including maize is approximately 33% with estimated averages of 29% and 42% for the developing and the developed countries respectively (Walsh *et al*., 2012). Although it is impossible to achieve 100% efficiency for N fertilizer use in any crop production system, the low average nitrogen recovery efficiency for cereals including maize, suggests that a great opportunity exists for reducing N losses in current N management practices. Therefore, applying the optimum N level is the most important means for raising the yield of maize and improving efficiency in the use of nitrogen (Chen *et al*., 2010).

Nitrogen application in excess of crop requirements contributes to increased levels of nitrate in the soil, and a high concentration of nitrate increases the risk of leaching into ground water. Boonlertnirun *et al*. (2010) found that reducing N application rates to 5% less than that of the required rate to achieve maximum corn yield reduces nitrate leaching by 40% to 45%. The negative environmental impacts associating with maize production can therefore be minimized through efficient N management. Although grain yield would increase significantly in response to increased N application in areas where soil N is deficient, in areas with sufficient soil N, increased N fertilizer application does not result in increased yields and may even lead to yield reductions as well as further N loss. Therefore, proper N fertilizer management is essential in maintaining grain yield and N efficiency (Jin *et al*., 2012).

Use of optimum amounts of nitrogen fertilizer through a suitable application method at a time when it is most efficiently and effectively utilized is very essential. Plant N use efficiency can be improved by matching application rate and timing with plant demands (Ferguson *et al*., 2002). Nitrogen efficiency decreases with increasing N level, especially under dry soil condition but also the application of high N rates may result in poor N uptake and low nitrogen use efficiency due to excessive N losses. Therefore, the most logical approach to increasing N fertilizer use efficiency is to supply nitrogen when it is mostly needed by the crop (Nemati and Sharifi, 2012). Because of the need for continuous nutrient inputs to the soil, simply reducing the rates of N fertilizer application would obviously prevent maize producers from achieving their major goal which is higher yields; hence, creating an effective N management system, improving N recommendations, and increasing NUE are critical issues which should be addressed to maintain and increase the sustainability of crop production (Walsh *et al*., 2012).

2.9 Growth parameters of maize

Seedling emergence in maize usually occurs 6 to 10 days after planting (4-5 days under warm, moist soil conditions). If the seed is placed in a cool dry soil, it may take two weeks or longer for seedling emergence (Woltz *et al*., 2006). From breaking through the soil surface to maturity, maize plant will undergo several growth stages. These stages are separated into two distinct categories: vegetative and reproductive stages. The vegetative stage ranges from the time the first fully open leaf is visible to tasseling. Full development of the leaf is achieved when the collar is fully visible (Lee, 2011). The reproductive phase is categorized into the following stages: silking stage (R1), kernel blister stage (R2), milk stage (R3), dough stage (R4), dent stage (R5) and physiological maturity stage (R6).

Silk emergence is technically the first recognized stage of the reproductive period. The silks serve the purpose of capturing pollen grains that fall from the tassel and moves them down the silk to the ovule where fertilization occurs (O'Keeffe, 2009). At R2, kernels are very small and white in color. The fluid that fills the kernels at this stage is usually clear in color. The kernels at this stage consist of about 85% water and will gradually decline from this point until harvest. The milk stage occurs about 18 to 22 days after silking. The kernels at the stage contain mainly a white milky fluid. Dough stage occurs about 24 to 28 days after silking. At this stage, the kernel's milky inner fluid is becoming doughy as starch accumulation continues in the endosperm. At this time, the kernels have reached about 50% of their mature dry weight (Plessis, 2003). Dent stage occurs around 35 to 42 days after silking. The final stage is the physiological maturity stage at which the kernels have achieved peak dry matter accumulation. The hard starch layer has now reached the ear and a black abscission layer, called the black layer is now formed. This black layer signifies that the kernel is finished with its growth for the season. The kernel moisture content at this stage is around 30-35%, depending on the hybrid and environmental conditions (Nielsen, 2013).

2.9.1 Plant height and leaf number

Performance of a maize crop can be indicated by plant growth parameters such as plant height and number of leaves produced by plants at the point of assessment. Plant height measurements are easily accomplished, non-destructive and flexible in terms of the uniformity in the developmental stage of the crop. Plant height measurements can be accomplished with simple tools and quickly, so large number of plants and plots within a field can be measured within a short time (Tittonell *et al*., 2005). Plant height is a key indicator of plant growth and is linked to nitrogen nutrition status during vegetative development of maize (Yin *et al*., 2011). Provision of adequate nitrogen in split application extends vegetative growth period of maize and this increases the photosynthesis duration and partitioning of photoassimilates to stems which in turn positively impacts on maize plant heights (Amanullah *et al*., 2009).

2.9.2 Green leaf area and leaf area index

The physiological status of a crop is commonly characterized through the total leaf area index (LAI_{tot}) which is defined as the total leaf area (including both green and senesced leaf area) per unit area of ground beneath them, or the green leaf area index (LAI_g), defined as the green leaf area per unit area of ground beneath them. These biophysical characteristics have been considered basic to growth analysis and important in current estimates of crop canopies' potential photosynthesis (Ciganda *et al*., 2008).

For maize, leaf area influence the interception and utilization of solar radiation and consequently drive dry matter accumulation and grain yield (Valentinuz and Tollenaar, 2006). Both LAI_{tot} and LAI_g have some restrictions in characterizing crop physiological status or growth vigor. In the case of LAI_{tot}, it is not possible to differentiate among actual status of the leaves since all leaves, green or necrotic are considered. Due to this, LAI_{tot} can greatly overestimate the crops photosynthetic capability. Green leaf area index on the other hand distinguishes green from non-green leaves, so it is therefore a more accurate expression of the actual photosynthetic functional capacity of the crop (Ciganda *et al*., 2008).

Dry matter produced by maize crops is dependent on the amount of radiation absorbed by the canopy which also depends on green leaf area. Light interception by crop canopy is a function of dry matter productivity where leaf contributes more than 80%. Leaf area development is therefore an important parameter that affects maize grain yield and yield components (Akmal *et al*., 2010). Oscar and Tollenaar (2006) reported that breadth of the area per leaf profile decreases under high soil nitrogen level. They also reported that leaf area increased with higher rates of nitrogen. Mathematical relationships between length, width and area of maize leaf blades can serve as a basis for direct leaf area estimation. The area of maize leaf blade can be estimated as its length multiplied by its maximum width multiplied by 0.75 (Elings, 2000).

2.10 Maize grain yield determinants

Grain yield of a maize crop is a function of the number of ovules that are developed, the potential final size of each ovule and the efficiency and duration of grain filling. Out of these processes, kernel number and kernel size constitute kernel sink capacity, which is established early in kernel set and development (Cazetta *et al*., 1999). Grain yield of maize crop is mainly determined by the final number of kernels per unit area that reach maturity. This number is strongly related to crop growth rate during a critical period of about 30 days centered around silking and to biomass partitioning to the ear during this period (Rossini *et al*., 2011). Nitrogen availability can affect these physiological attributes and consequently final kernel number.

Variations in grain yield in maize have been related mainly to variations in kernel number and kernel size; however, among the two, maize grain yield is mainly dependent on kernel number per unit area (Andrade *et al*., 2002). Crop growth rate near flowering accounts for most of the variation in kernel number per plant (D'Andrea *et al*., 2008). Differences in number of kernels set per unit of crop growth rate may be attributed to variations in dry matter partitioned to the ear and number of kernels set per unit of ear growth rate during the critical period bracketing silking (Echarte *et al*., 2004).

Kernel number is strongly affected by environmental conditions. Severe water and nutrient stress can greatly reduce potential kernel number per row. Conversely, excellent growing conditions can encourage unusually high potential kernel number (Strachan, 2004).

Kernel set in maize has also been largely associated with intercepted radiation around silking. The position of the ear on the plant relative to the site of assimilate production has also been known to affect growth. The position of leaves influences the rate and direction of translocation of photoassimilates (Subedi and Ma, 2005a). Leaves above the ear, export principally to the ear during the post-silking period, while lower leaves export relatively less to the ear and more to the lower internodes and roots.

Kernel weight development during kernel filling period is usually described in terms of dry matter deposition through three phases which take place after flowering. During the first period of grain filling, called the lag phase, the number of starch deposition sites is established. Dry matter accumulation during this lag phase is almost zero, but water accumulation is rapid, driving endosperm expansion and increasing potential sink size. Kernels continue to accumulate water until about mid-grain fill, when kernel maximum water content is achieved (Severini *et al*., 2011). In the second phase, termed effective filling period, kernel weight increases linearly. During the last phase, kernel growth rate decreases and kernels reach their final kernel weight (Melchiori and Caviglia, 2008).

Kernel weight at physiological maturity depends on the potential kernel size established early in grain filling, and the plants' capacity to provide assimilates needed to fulfill this potential during grain filling (Borras and Westgate, 2006). Maize physiological traits that contribute to increased grain yield includes higher photosynthetic rate, leaf area duration, larger sink size, high leaf angle and decreased anthesis-silking interval (Qi *et al*., 2012).

2.11 Summary of literature review

The literature review revealed that nitrogen is one of the most required nutrients by maize crops for effective growth and yield. Nitrogen application in crop production was, however, widely reported to be a major source of environmental pollution due to high N loss. Rate and timing of N application were identified as key factors that determine efficient utilization of nitrogen. Cattle manure provides organic carbon which improves soil physical and chemical properties, and also supplies other plant nutrients. However, because of its slow release of nutrients, use of the manure as the sole fertilizer was mainly reported to be inadequate for maize. The integrated application of mineral nitrogen and manure was widely reported to increase crop growth, nitrogen utilization and grain yield of maize than the sole application of either type of fertilizers. This was due to the improvement in general soil fertility levels and the synchronization of nutrients released by the manure and mineral fertilizer.

There is, however, a prevailing gap in literature regarding the appropriate combinations of organic and inorganic fertilizers. The strategy in most of the studies was limited to combining different rates of organic and inorganic fertilizers with the aim of reducing the inorganic fertilizer rates. However, because of the difficulty in predicting nutrient mineralization rates from manure, higher reductions in inorganic fertilizer rates may not be compensated by the manure nutrients especially in the first season of manure application and this could lead to nutrient deficiencies. These observations necessitated a study of this kind and served as the basis for the formulation of the objectives.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the study area

3.1.1 Location and soil type

The study was conducted at the Plantation Crops Section of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi. The area is located within the semi-deciduous forest zone of Ghana (06^{0} 41.850’ N and 01^{0} 31.545’ W). The soils of the area were classified by Adu (1992) as Asuansi series or Orthi-Ferric Acrisol.

3.1.2 Climate

The study area is characterized by marked wet and dry seasons with a bimodal rainfall pattern. The two rainfall peaks make two growing seasons possible. There is heavy rainfall from May to July, which is interrupted by a dry period of about four weeks in August; this is followed by another period of heavy rainfall from September to October. Dry season length is between 120 – 130 days (Tuffour and Bonsu, 2014). The average temperature of the study area is around 26.5 °C (Agyare *et al.*, 2013).

3.2 Field experiments

Three field experiments were carried out in this study. The first experiment was conducted from April to August in the 2014 major season, the second was from September to December in the 2014 minor season, and the third was from April to August in the 2015 major season. All three experiments were conducted within the same area but on different fields. The 2014 major and minor season experiments were set up on plots adjacent to each other, but the 2015 experiment was carried out on a plot about 100 m away from them. On plots of the first and second experiments, egg plants were grown in the preceding season; whilst for the third experiment groundnut was grown in the field in the preceding season.

3.2.1 Maize variety

The test maize variety used in this study was Obatanpa. It is a tropically adapted, intermediate maturing, open pollinated maize (*Zea mays* L) cultivar developed by the Crops Research Institute (CRI) of Ghana in collaboration with the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria and The International Maize and Wheat Improvement Center (CIMMYT).

Obatanpa is a white dent and flint endosperm quality protein maize (QPM) with elevated levels of lysine. It has been widely adopted by farmers, covering more than 50% of maize acreage in Ghana and other West African countries including Benin, Nigeria and Togo (Dankyi *et al.*, 2005).

3.2.2 Experimental design

The experiments were factorial in a randomized complete block design and replicated 4 times. The first two experiments i.e. 2014 major and minor season experiments had exactly the same treatments with two factors: cattle manure and time of nitrogen application. Cattle manure comprised four different rates as follows: 0 tons per hectare for the control treatments, 2 tons per hectare, 4 tons per hectare and 6 tons per hectare for the plots receiving manure. Nitrogen application times were as follows: 0 N for the control plots, 50% N applied as basal at 2 weeks after planting and 50% top dressed at 4 weeks after planting, 50% at 2 weeks after planting as basal and 50% top-dressed at 6 weeks after planting, and 50% at 2 weeks after planting as basal and 50% top dressed at 8 weeks after planting.

Based on preliminary results obtained from the first two experiments, a third experiment was designed to test the effect of combination of the same manure rates in the previous experiments with different nitrogen rates. The 2015 major season experiment comprised two factors: cattle manure and mineral nitrogen rates. Cattle manure, as in the previous two experiments comprised four different rates: 0 tons per hectare for the control plots, 2 tons per hectare, 4 tons per hectare and 6 tons per hectare for the plots receiving manure. Nitrogen fertilizer rates were as follows: 0 N for the control plots, one third of the recommended rate (30 kg N/ha), two third of the recommended rate (60 kg N/ha) and recommended rate (90 kg N/ha) for plots receiving nitrogen fertilizer. The area of each plot was 14.56 m^2 and total experiment area was 1280.44 m^2.

For ease of referencing, nitrogen application times were referred to as: 0 N for the control, NT1 for ½ N at 2 WAP and ½ N at 4 WAP; NT2 for ½ N at 2 WAP and ½ N at 6 WAP; and NT3 for ½ N at 2 WAP and ½ N at 8 WAP. Nitrogen rates on the other hand, were referred to as: 0 N for control treatments, minimum, optimum and maximum for the 30, 60 and 90 kg/ha N rates, respectively.

3.2.3 Field preparation and planting

The experimental fields were ploughed using a tractor and the soil surface was leveled using long wooden handle hoes. The fields were then demarcated into plots and replicates using wooden pegs. For the all three experiments, cattle manure was applied at two weeks before the intended date of planting to enable decomposition and availability of nutrients during the crop growing period.

Seeds were planted two weeks after the application of cattle manure. Four seeds were sown in each planting hole at a depth of approximately 3-4 cm, and seedlings were later thinned to two per stand at twelve days after planting. The distance between rows and between plants was 80 cm and 40 cm, respectively. Each treatment had 7 rows and 16 plants per row. There were an estimated total of 112 plants in each treatment. Number of plants per meter square was 6.25 and the plant density was 62,500 plants/ha.

The three inner rows of each treatment were marked as net plots from where grain yield data were determined. The four outside rows were used for collection of plant samples for the purpose of plant tissue analysis, crop growth rate determination and dry matter accumulation.

3.2.4 Initial soil sampling

Soil samples were collected at 0-15 cm depth before planting of seeds using an auger. The samples were used to assess the initial physical and chemical soil properties of the experimental fields. Samples were tested at the Soil Science laboratory of the Department of Crop and Soil Sciences of Kwame Nkrumah University of Science and Technology in Kumasi.

3.2.5 Manure analysis

To analyze the nutrient content of the cattle manure, a handful of the manure was sampled from each bag which was bulked to form a single composite sample. The composite sample was thoroughly mixed and divided into 3 sub-samples as replicates. The samples were then oven dried at 70 ^0C for 24 hours after which they were analyzed using standard laboratory protocols. The moisture content of the manure was found to be 70%, calcium content was 0.81%, magnesium was 0.44%, available phosphorus was 0.18%, and potassium was 2.15%, whilst total nitrogen and organic carbon were 1.12% and 35.68% respectively with C/N of 32:1.

3.2.6 Soil analysis

Soil samples collected from the field experiments were analyzed to determine the mineral content, physical and chemical properties. Soil samples were air dried for 4 days after which they were ground to pass through a 2 mm sieve. The following determinations were made using the methodologies outlined below. The soils were defined as loamy fine sand. For the soil analysis data, see Table 3.1.

3.2.6.1 Organic carbon

Soil organic carbon was tested following Walkley and Black's wet oxidation method as described by Nelson and Sommers (1982). This procedure is based on the reduction of $Cr_2O_7^{2-}$ by organic matter. Oxidizable matter in a soil sample is oxidized by $Cr_2O_7^{2-}$ and the reaction is facilitated by the heat generated when 2 volumes of concentrated sulphuric acid (H_2SO_4) is mixed with 1 volume of 1.0 *N* (0.1667 M) $K_2Cr_2O_7$ solution.

The excess $Cr_2O_7^{2-}$ is determined by titration with standard ferrous sulphate solution.

The quantity of substances oxidized is then calculated from the amount of $Cr_2O_7^{2-}$ reduced. The procedure is as follows: 2.0 g of grinded soil was weighed and placed in a 500 ml Erlenmeyer flask. A burette was used to measure 10 ml of 1.0 *N* $K_2Cr_2O_7$ solution, followed by the addition of 20 ml of concentrated H_2SO_4. The mixture was swirled to ensure that the solution is in contact with all the soil particles. The flask with its contents was allowed to cool for about 30 minutes. Two hundred milliliters of distilled water was added followed by the addition of 10 ml of orthorphosphoric acid and 2.0 ml of diphenylamine indicator. The solution was titrated with 0.5 *N* ferrous sulphate solution until the color changed to dark blue and then to a green end–point. The titer value was recorded and blank solution was corrected at ≥ 10.5.

The formula below was used to calculate the organic carbon percentage in the soil samples:

$$\%C = \frac{N \times (V_{bl} - V_s) \times 0.003 \times 1.33 \times 100}{g}$$

Where: N is the normality of $FeSO_4$, V_{bl} = ml $FeSO_4$ of blank titration, V_s = ml $FeSO_4$ of soil sample titration, g= mass of soil taken in gram, 0.003 = milli-equivalent weight of C in grams (12/4000). 1.33 is the correction factor used to convert the Wet combustion C value to the true C value since the Wet combustion method is about 75 % efficient in estimating C value i.e. 100/75 = 1.33.

3.2.6.2 Total nitrogen

Soil total nitrogen content was tested using a modification of the micro-kjeldahl procedure as described by Persson *et al*. (2008). Most of the soil nitrogen is bound up in the organic matter (OM), and the basic principle involved in assessing or estimating the quantity held up in this manner is to boil a weighed quantity of the soil with concentrated sulphuric acid. The nitrogen is thus converted into sulphate of ammonia

[$(NH_4)_2SO_4$] and at the same time, the carbonaceous matter is oxidized to carbon dioxide (CO_2) with the sulphuric acid being reduced to sulphur dioxide (SO_2).

The micro-kjeldahl procedure involves three steps:

1. Digestion of the soil sample to convert organic nitrogen to ammonium nitrogen by sulphuric acid.
2. Distillation of ammonia.
3. Titration of ammonia back to ammonium to be able to measure the amount of total N in the digest.

The digestion is performed by heating the sample with H_2SO_4 containing substances which promote the oxidation of organic matter. The substances generally favored are salts such as K_2SO_4 or Na_2SO_4 which raise the temperature of digestion and catalysts such as selenium, mercury or copper sulphate which promote the rate of oxidation of organic matter by H_2SO_4.

The completion of the reaction is shown by the liquid becoming clear and colorless or light green. If the ammonium sulphate obtained as the product of the reaction of H_2SO_4 with nitrogen is then treated with excess caustic soda (40% NaOH), ammonia is liberated and may be distilled over and collected in 4% boric acid and titrated with standard HCl.

1. Digestion process

Ten grams of air dried soil was weighed and placed into a 500 ml long necked Kjeldahl flask. 10 ml of distilled water was added and allowed to stand for 10 minutes to moisten. One spatula full of Kjeldahl catalyst (mixture of l part Selenium + 10 parts $CuSO_4$ + 100 parts Na_2SO_4) was added. Thirty milliliters of concentrated H_2SO_4 was added after which the flasks were heated until the solution became clear and turned light green. This may take about 2 hours to complete. When samples turned light green, the flasks were cooled for 15-30 minutes and then rinsed with 10 ml of distilled water to collect all sample materials at the bottom of the flasks. One blank flask was used at every digestion as a standard for measuring the titration value of the samples. The blank contains all the substances added to the other flasks except the soil sample.

2. Distillation process

Ten milliliters of 4% Boric acid and 3 drops of mixed indicator were added into 50 mL Erlenmeyer flasks. Twenty milliliters of 40% sodium hydroxide was added into the kjeldahl flask. The distillate was collected in the Erlenmeyer flasks up to the 200 mL mark. Each distillation was started with distilled water, after which the blank sample was distilled, before starting the samples proper. A reverse distillation with distilled water was done after every distillation to cleanse the apparatus of the previous sample digest.

3. Titration process

The ammonia gas that was collected by adding an alkali substance (NaOH) needs to be reversed back to ammonium to be able to measure the amount of total N in the digest.
To achieve this, the Erlenmeyer flasks containing the collected ammonia gas were transferred to a titration tube containing HCl (0.01 *N*) as the titrant, where the ammonia was titrated back to ammonium. When titrating, the HCl was pumped to the 0 mark of the tube and was slowly dropped into the Erlenmeyer flask beginning with the blank sample. When the color of the liquid in the Erlenmeyer flask changed from green to pink (measuring it against the blank), the flask was removed and the titration value of the sample

was calculated by multiplying the number of scales by which the titrant (HCl) in the tube has dropped by 0.05. The percentage of total nitrogen in the sample was calculated using the formula below:

$$\% \text{ N in sample} = \frac{(\text{Sample titrant volume - Blank titrant volume}) \times \text{normality of HCl} \times 14 \times 100}{\text{Sample weight (g)} \times 1000}$$

3.2.6.3 Exchangeable cations

Exchangeable bases (calcium, magnesium potassium and sodium) on soil colloids were extracted with 1.0 *M* ammonium acetate (NH_4OAc) extract, exchangeable acidity (hydrogen and aluminum) determined in 1.0 *M* KCl extract and Na^+ and K^+ ions measured by flame photometry while Ca^{2+} and Mg^{2+} were determined by ethylenediamenetetraacetic acid (EDTA) titration. These analyses were carried out using the procedure described by Black (1986).

3.2.6.3.1 Exchangeable bases

A 5 g soil sample was transferred into a leaching tube and leached with 100 ml of buffered 1.0 *M* ammonium acetate solution at pH 7.

3.2.6.3.1.1 Determination of calcium and magnesium

In the determination of calcium and magnesium, a 25 ml of the extract was transferred into an Erlenmeyer flask. A 1.0 ml portion each of hydroxylamine hydrochloride, 2.0 % potassium cyanide buffer, 2.0 % potassium ferrocyanide, 10.0 ml ethanolamine buffer and, 0.2 ml Eriochrome Black T solution were added. The solution was titrated with 0.01 *M* EDTA to a pure turquoise blue color. A 20 ml 0.01 *M* magnesium chloride solution was also titrated with 0.01 *M* EDTA in the presence of 25 ml of 1.0 *M* ammonium acetate solution to provide a standard blue color for the titration.

For the determination of calcium, 1.0 ml of hydroxylamine hydrochloride and 1.0 ml each of 2% potassium cyanide and potassium ferrocyanide solutions were added to a 25 ml portion of the extract in a 250 ml Erlenmeyer flask. After a few minutes, 5 ml of 8 *M* potassium hydroxide solution and a spatula of murexide indicator were added. The solution obtained was titrated with 0.01 *M* EDTA solution to a pure blue color.

The concentrations of calcium + magnesium or calcium alone were calculated using the equation below:

$$Ca + Mg \text{ (or Ca)}(cmol^{+}Kg^{-1} \text{ soil}) = \frac{0.01}{W} \times (V_a - V_b) \times 100$$

Where w = weight (g) of air dried soil, V_a = ml of 0.01 *M* EDTA used in sample titration, V_b = ml of 0.01 *M* EDTA used in blank titration and 0.01= concentration of EDTA.

3.2.6.3.1.2 Determination of potassium and sodium

The flame photometry procedure was used in the determination of potassium (K) and sodium (Na) in the leachate. A standard series of potassium and sodium were prepared by diluting both 1000 mg/l potassium and sodium solutions to 100 mg/l. This was done by taking a 25 ml portion of each solution into 250 ml volumetric flasks and made to volume with water. Portions of 0, 5, 10, 15 and 20 ml of the 100 mg/l standard solution were put into 200 ml volumetric flasks. One hundred milliliters of 1.0 *M* NH_4OAc solution was added to each flask and made to volume with distilled water. The standard series obtained was 0, 2.5, 5.0, 7.5, 10.0 mg/l for potassium and sodium. Potassium and sodium were measured directly in the leachate by flame photometry at wavelengths of 766.5 and 589.0 nm, respectively. Exchangeable K and Na were obtained using the formulas below:

$$\text{Exchangeable K } (\text{cmol}_{+}\ \text{kg}^{-1}\ \text{soil}) = \frac{(a - b) \times 250 \times \text{mcf}}{10 \times 39.1 \times w}$$

$$\text{Exchangeable Na } (\text{cmol}_{+}\ \text{kg}^{-1}\ \text{soil}) = \frac{(a - b) \times 250 \times \text{mcf}}{10 \times 23 \times w}$$

Where a = mg/l K or Na in the diluted sample percolate, b = mg/l K or Na in the diluted blank percolate, w = weight (g) of air- dried sample, mcf = moisture correcting factor.

3.2.6.3.2 Exchangeable acidity

For exchangeable acidity, 3 g of air-dried soil was weighed and placed into folded filter papers, placed on a funnel and placed on the Erlenmeyer flask. Fifty milliliters of 1.0 *N* KCl solution was gently poured through the soil in the filter paper and the leachate was collected into the Erlenmeyer flask. Five drops of phenolphthalein indicator was added to the leachate. The leachate was titrated with 0.05 *N* NaOH to colorless end point and the volume (ml) of NaOH used (V) was recorded. For exchangeable aluminum, 4 ml of 3 *N* NaF was added to the titrated extract. The mixture was titrated with 0.05 *N* HCl to pink end point and the volume (ml) of HCl used (V) was recorded. Exchangeable acidity was calculated in (cmol kg^{-1} soil) with the formula below:

$$V \times \frac{0.05 \times 100}{} = V \times 1.67$$

W

Where V is the Titer volume of NaOH used, Normality of NaOH = 0.05 N and W is the weight of soil sample used.

3.2.6.4 Available phosphorus

Soil available phosphorus was determined using the Bray P_1 method (Nelson and Sommers, 1982). The method is based on the production of a blue complex of molybdate and orthophosphate in an acid solution. The procedure is as follows:

1. A standard series of 0, 0.8, 1.6, 2.4, 3.2, and 4.0 µg P/mL were prepared by diluting appropriate volumes of 10 µg P/mL standard sub-stock solution. These were subjected to color development and their respective absorbance values read using a spectronic 21D spectrophotometer at a wavelength of 660 nm. A standard line graph was constructed using the readings.

2. A 2.0 g of soil sample was then weighed into a 50 ml shaking bottle and 20 ml of Bray-1 extracting solution (0.03 *N* NH_4F + 0.025 *N* HCl) added. The sample was shaken for one minute and then filtered through a filter paper.

3. Ten milliliters of the filtrate was pipetted into a 25 ml volumetric flask and 1 ml each of molybdate reagent and reducing agent added for color development. The absorbance was measured at 660 nm wavelength on the spectrophotometer. The concentration of P in the extract was obtained by comparing the results with a standard curve.

Calculation: $$P\ (mg\ kg^{-1}) = \frac{(a - b) \times 35 \times 15 \times mcf}{w}$$

Where: a = mg/l P in sample extract; b = mg/l P in blank; w = sample weight in gram; mcf = moisture correcting factor; 35 = volume of extracting solution and 15 = final volume of sample solution

3.2.6.5 Soil pH

Soil pH was determined by putting 10 g of air dried soil into 10 mL of deionized water (1:1 ratio) in a 50 mL beaker. The mixture was stirred vigorously for 20 minutes and the soil-water suspension was allowed to stand for 30 minutes by which time most of the suspended clay had settled out from the suspension. pH meter was calibrated with a blank at pH 4 and 7. The electrode of the pH meter was inserted into the suspension and the pH value on the meter was recorded.

3.2.6.6 Effective cation exchange capacity

Effective cation exchange capacity (ECEC) was determined by summation of exchangeable bases (Ca^{2+}, Mg^{2+}, K^+ and Na^+) and exchangeable acidity (Al^{3+} + H^+).

3.2.6.7 Particle size analyses

The hydrometer method was used in the determination of the soil particle size. This method allows for the non-destructive sampling of suspensions undergoing settling and also provides for multiple measurements on the same suspension so that detailed particle size distribution can be obtained with minimum effort. Fifty one grams of air dried soil was weighed into 250 ml beakers. Ten milliliters of 5% Calgon (Sodium hexametaphosphate) alongside with 100 ml of distilled water were added to the soil. The Calgon served as a dispersing agent of the soil particles. The suspension was stirred vigorously for 1 minute using a glass rod and allowed to stand for 30 minutes. The mixture was shaken with a mechanical shaker for 20 minutes and the content was transferred into a sedimentation cylinder and made up to 1 liter with deionized water. The cylinder with the content was shaken to distribute the particles equally throughout the suspension and the first hydrometer and temperature readings were taken after 40 seconds. The suspension was left to stand for 3 hours to allow the soil particles to settle. Second hydrometer and temperature readings were taken after 3 hours and the percentage fractions of each soil component was calculated as follows:

$$\% \text{ Sand}=100-[H_1+0.2\ (T_1-20)-2]\times 2$$

$$\% \text{ Clay}=H_2+[0.2\ (T_2-20)-2]\times 2$$

$$\% \text{ Silt}=100-(\% \text{ Sand} + \% \text{ Clay})$$

where, H_1 is the first hydrometer reading after 40 seconds; H_2 is the second hydrometer reading after three hours, T_1 is the first temperature reading after 40 seconds and T_2 is the second temperature reading after three hours. The textural class was determined using the textural triangle.

3.2.6.8 Bulk density (ρ_b)

The dry bulk density was determined from soil cores collected at the field with core sampler. The core sampler with a diameter of 25 cm and a height of 30 cm was driven into the soil vertically with the aid of wooden plank and a mallet to fill the sampler. In order to prevent compression of the soil, another cylinder of equal diameter was placed directly on top of the sampling cylinder.

The sampler and its contents were then removed carefully to maintain the natural structure and packing of the soil. Soils that extended beyond the sampler were trimmed with a sharp knife and the volume of the soil was taken to be the same as the volume of the cylinder. The cylinders were covered and sent to the laboratory and oven dried at 105 ^{0}C for 24 hours to a constant mass. The oven dried soils were weighed and the dry bulk densities calculated by dividing the oven dry mass (M_s) by the total volume of the soil (V_t). Thus, the dry bulk density was calculated from the formula:

$$\rho_b = \left(\frac{M_s}{V_t}\right)$$

Table 3.1: Physical and chemical properties of field experiment soils at 0-15 cm prior to application of soil amendments

Soil property	2014 seasons fields	2015 season field
pH (1:1, H_2O)	6.15	5.92
Organic carbon (%)	1.40	1.59
Total N (%)	0.07	0.14
Available P (mg/kg soil)	6.09	5.92
Exchangeable bases (cmol$_+$/kg soil)		
K^+	0.12	0.17
Na^+	0.05	0.09
Mg^{2+}	1.68	2.12
Ca^{2+}	2.24	3.36
Exchangeable acidity (cmol$_+$/kg soil)		
Al^{3+}	0.76	0.52

H^+	1.68	1.30
ECEC ($cmol_+$/kg soil)	6.51	7.06
Soil particle size (%)		
Sand	84.80	67.32
Silt	8.56	13.63
Clay	6.64	19.05
Bulk density (g/cm^3)	1.45	1.40
Textural class	Loamy sand	

3.2.7 Manure application

Semi-decomposed cattle manure of about 70% moisture content was collected from a local cattle pen a few kilometers away from the research site. Manure application was carried out after land preparation two weeks prior to planting of seeds. The semidecomposed manure was broadcasted on the plots and incorporated immediately by turning the soil lightly.

3.2.8 Inorganic fertilizer application

Cattle manure was applied two weeks prior to planting. The semi-decomposed manure was broadcasted in the plots and incorporated immediately. Ammonium sulfate (21% N), Triple superphosphate (46% P_2O_5) and Muriate of potash (60% K_2O) were used as the sources of nitrogen, phosphorus and potassium, respectively. For the 2014 major and minor seasons experiments, nitrogen at the rate of 90 kg/ha was applied in two equal splits to all treatments except control. At 2 WAP, 50% N was applied to all plots as basal. The remaining N was applied at 4, 6 and 8 WAP for NT1, NT2 and NT3 application times respectively. In the 2015 experiment, with the exception of the control plots, N was applied in two equal splits to all plots. Half of the N rates were applied at 2 WAP to all treatments; the remaining half was applied at 6 WAP. Phosphorus and potassium were each applied in full at the rate of 60 kg/ha at 2 WAP along with the basal nitrogen. Fertilizer application at both basal and top dressing was done by banding the fertilizer along the rows, few centimeters away from the plants. After application, the soil was turned lightly to incorporate the fertilizer to avoid exposure to direct sunlight and surface runoff. The fertilizer rates used in this study followed the recommendations by Morris *et al*. (1999) reported by Ragasa *et al*. (2013) for lands in the forest and transitional zones of Ghana under continuous cultivation.

3.3 Plant nitrogen determination

Nitrogen content of plant tissues, nitrogen uptake, partitioning as well as remobilization were determined at two growth stages: The first sampling was done at 10 WAP which coincided with the second week after completion of silking, when the maize crop was at the blister stage. The second sampling was done at 14 WAP when the maize had reached physiological maturity.

3.3.1 Plant sampling for nitrogen analysis

To determine the plant nitrogen content, four whole maize plants were harvested from the outside rows of each plot by cutting them at the stem base and separated into leaves, culm (comprising the stalk, leaf sheath, husk and tassel), and ear (comprising the cob and kernels). The plant segments from the four plants were cut into smaller pieces and bulked to form a single sample. Samples were packed in paper envelops and dried in an oven at

70 ^{0}C to a constant weight. After drying, the plant materials were ground into powdered form, placed in air tied plastic bags and taken to the laboratory for N analysis.

3.3.2 Plant nitrogen analysis procedure

Plant nitrogen content was determined using the same micro-kjeldahl procedure used for testing soil total nitrogen content as described in section 3.2.6.3. The only difference between the soil and plant nitrogen analysis was the sample sizes. Two grams of plant material was used instead of 10 g used in testing soils because plant material is lighter than soil and therefore has more volume.

3.3.3 Nitrogen uptake measurement

Nitrogen uptake by maize plants was determined at two growth stages: two weeks after completion of silking and at physiological maturity (i.e. 10 and 14 WAP, respectively). Total nitrogen uptake of whole plants or plant parts was obtained by multiplying the % N concentration with the dry weight of that part. Nitrogen uptake of the different plant segments were summed up to obtain the total plant nitrogen uptake at every determination stage.

N uptake = Biomass (kg/ha) x % N of biomass

3.3.4 Nitrogen partitioning and remobilization

Nitrogen partitioning was calculated as the ratio of total nitrogen accumulated in a certain part of the plant over a certain period of time. An increment in partitioning ratio with growth was considered as a net accumulation whilst a reduction indicated remobilization.

$$\frac{\text{N content of plant part (R6)}}{\text{Shoot N content (R6)}} \times 100 - \frac{\text{N content of plant part (R2)}}{\text{Shoot N content (R2)}} \times 100$$

3.4 Determination of dry matter accumulation

Plant dry matter was measured at 5, 7, 10 and 14 weeks after planting. During each sampling, four whole maize plants were harvested from each plot and chopped into smaller pieces.

3.4.1 Plant sampling for dry matter assessment

The four maize plants harvested from each plot were separated into three segments: culm, leaf and ear and the segments from the four plants were bulked to form a single sample for each segment for each of the treatments. Samples were packed in paper envelops with appropriate treatment labels and dried in ovens at a temperature of 60 ^{0}C for three days. After removing samples from the oven, they were weighed immediately to determine their dry weights.

3.4.2 Dry matter partitioning

Plant parts were weighed separately and summed up to obtain the total dry weight per plant for each plot. Dry matter partitioning was measured as the ratio of biomass of the whole plant partitioned to a particular plant segment at a particular growth stage.

3.5 Determination of nitrogen use efficiency

Nitrogen use efficiency (NUE) was determined as kg grain produced per kg of nitrogen applied. It was calculated using the equation below.

$$NUE = \frac{\text{Grain yield}_{(fertilized\ plot)} - \text{Grain yield}_{(control)}}{\text{Rate of N Applied}}$$ (Salvagiotti *et al*., 2009)

Nitrogen use efficiency was further examined in two independent components as follows:

1. Nitrogen Recovery Efficiency (NRE) which refers to the capacity of the aboveground plant parts to recover nitrogen from the applied N fertilizer was calculated as follows:

$$NRE = \frac{\text{Nitrogen uptake}_{(fertilized\ plot)} - \text{Nitrogen uptake}_{(control)}}{\text{Rate of N Applied}}$$

2. Nitrogen Internal Efficiency (NIE) which refers to the capacity of the plants to convert uptake nitrogen into grain yield. It was calculated as follows:

$$NIE = \frac{\text{Grain Yield}_{(fertilized\ plot)} - \text{Grain Yield}_{(control)}}{\text{Nitrogen uptake}_{(fertilized\ plot)} - \text{Nitrogen uptake}_{(control)}}$$

Total nitrogen content of the aboveground plant parts (shoot) was used as an estimate for the total N uptake. Nitrogen recovery efficiency was measured as kg N uptake per kg N applied and nitrogen internal efficiency was measured as kg grain produced per kg N uptake.

3.6 Plant growth and grain yield assessment

At 4 weeks after planting, 6 maize plants were systematically selected from within the three inner rows of each plot and tagged. Plant growth parameters such as plant height, leaf number, green leaf area and leaf area index were measured from these tagged plants. Selection of sample plants at this early stage avoided possible bias in plant selection at later growth stages.

3.6.1 Plant height

In all three field experiments, plant heights were recorded at 5, 7 and 9 WAP. Heights of the 6 tagged plants in each plot were recorded and averaged to obtain the height per plant in each of the 64 plots during every recording stage. Plant heights were measured using portable tapelines from the stem base to the attachment of the flag leaf to the stalk.

3.6.2 Leaf number

Number of green leaves on each of the 6 tagged maize plants in each plot was counted at 5, 7 and 9 WAP. The average number of leaves for the 6 plants was used as the leaf number per plant for each of the plots. Lower leaves that were completely yellow or dried and newly formed leaves which were not fully opened were not counted.

3.6.3 Green leaf area

Green leaf area for all three field experiments was measured at 5, 7 and 9 WAP. The area of all green leaves on sample plants in each treatment was measured. The leaf area of the 6 tagged sample plants in each plot was used as the leaf area per plant for that plot at that particular growth stage. Leaves that were more than 50 % yellow or dried were not measured. Green leaf area was obtained as follows: Leaf area (cm^2) = leaf length x width x a constant (0.75).

3.6.4 Leaf area index

Leaf area index was determined at 5, 7 and 9 WAP. It was calculated as leaf area per meter square. Based on the spacing of 80 cm x 40 cm between rows and plants respectively and planting two maize plants per stand, the number of plants per meter square was 6.25. Leaf area per plant for each plot was multiplied by 6.25 to obtain the leaf area over a meter square. The leaf area over a meter square was converted from centimeters to meters and divided by 1 to obtain the leaf area index (Munaro *et al*., 2011).

3.6.5 Crop growth rate

Crop growth rate (CGR) was measured as the accumulated dry weight of maize plants over a certain period of time. Crop growth rate was determined for the periods between 5-7, 7-10 and 10-14 weeks after planting. To measure CGR, 4 whole plants were cut from each treatment and dried in an oven for three days at a temperature of 80 ^{0}C after which dry weights were recorded. Crop growth rate was measured using the following formula:

$$CGR = \frac{W2 - W1}{T2 - T1}$$ (Barbieri *et al*., 2008)

Where W1 is the dry biomass weight at T1, W2 is dry biomass weight at T2, T1 is the date of the previous plant harvest and T2 is the date of the later harvest.

3.6.6 Grain yield components

For grain yield determination, maize ears were harvested from a net plot area of 6.72 m^2 in each of the 64 plots. After harvesting, ears were oven dried at 70 ^{0}C to a constant weight after which dry ear weights were recorded. The maize was then shelled and the grain weights were recorded. The other grain yield determining factor measured was the 100 grain weight. Harvest index and shelling percentage were calculated using the formulas below:

$$\text{Harvest index (\%)} = \frac{\text{Grain}_{DW}}{\text{Whole plant}_{DW}} \times 100$$ (Ciampitti and Vyn, 2011)

Shelling percentage (%) $= \frac{\text{Grain}_{DW}}{\text{Ear}_{DW}} \times 100$ (Bakht *et al*., 2006)

3.7 Data analysis

The data collected from the field on soil, plant growth and yield, and from laboratory analysis were subjected to Analysis of Variance (ANOVA) using the GenStat statistical package (GenStat, 12^{th} Edition). The means were separated using the Least Significant Difference (LSD) at 5% level of probability. Regression and correlation analyses were carried out to establish relationships among the variables. Bar charts and line graphs were constructed using SigmaPlot version 12.2.

3.8 Weather data

Information on rainfall, temperature and relative humidity of the research site for the periods of the three cropping seasons was obtained from the Agrometeorology division of Ghana Meteorology Agency in Kumasi. These weather parameters were recorded at a satellite weather station which is located at the Animal Science Department of Kwame Nkrumah University of Science and Technology, less than 500 meters away from the experiment site. The rainfall data for the periods of the three experiments is presented in Figure 3.1, whilst temperature and relative humidity data are presented in appendix 1.

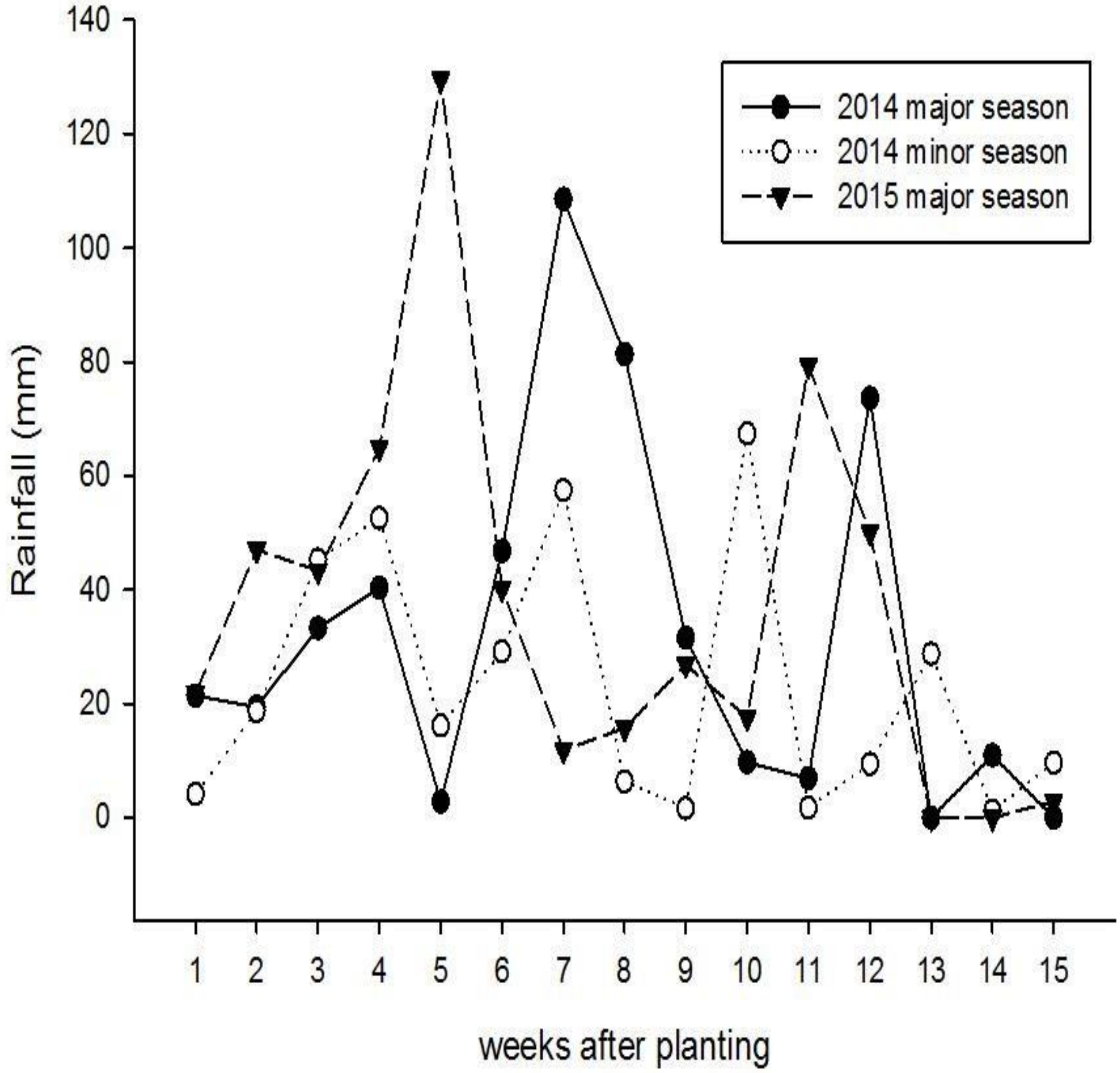


Figure 3.1: Rainfall recorded during each crop grown period at the experimental site

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Total aboveground plant nitrogen uptake at reproductive stages

In the 2014 major season, total aboveground nitrogen uptake at R2, as well as at R6 stages was greater at NT2 time of N application at all manure rates (Table 4.1). Application of mineral N along with cattle manure led to increments in aboveground plant nitrogen uptake. The increment was higher as the manure rate increased. However, at R6 stage, increments in N uptake as a result of application of manure were significant in the 6 tons/ha manure rate when the N was applied at NT1 or NT2; and in the 4 and 6 tons/ha manure rates when the N was applied at NT3. Comparing the times of N application, mean nitrogen uptake of 75.0 kg/ha at NT2 at R2 was not significantly different from the mean of 72.2 kg/ha at NT1 and mean of 67.8 kg/ha at NT3, all of them were, however, significantly higher than the mean of 36.4 kg/ha at 0 N. At R6 stage also, mean N uptake was highest at NT2 application time, but differences between times of N applications was not significant.

In the 2014 minor season, aboveground nitrogen uptake at R2 was highest at NT1 in all manure rates except at 4 tons/ha. At R6 stage, it was highest at NT2 in all manure rates. Addition of cattle manure to N fertilizer led to increments in aboveground plant nitrogen uptake. The increment was higher as the manure rate increased. At R6 stage, manure effect in all three manure applied rates did not result in significant increments in N uptake in comparison to N alone applied at NT1 and NT2. At NT3, however, the increments were significant in the 4 and 6 tons/ha manure rates (Table 4.2). Comparing the times of N application, mean nitrogen uptake at R2 was highest at NT1. At R6 stage, mean N uptake of 98.91 kg/ha at NT2 was not significantly different from the mean of 94.73 kg/ha at NT1, but was significantly higher than mean of 90.19 kg/ha at NT3 and
56.65 kg/ha at 0 N.

Table 4.1: Effect of cattle manure and time of nitrogen application on nitrogen uptake of maize plants at early and late reproductive stages in the 2014 major season

Manure rate (tons/ha)	10 WAP (R2)				14 WAP (R6)			
	Time of nitrogen application							
	0N	NT1	NT2	NT3	0N	NT1	NT2	NT3
	Total aboveground nitrogen uptake (kg/ha)							
0	28.5	65.2	69.6	56.8	45.24	89.40	90.04	71.69
2	35.8	69.1	69.9	66.4	55.91	90.39	95.64	84.91
4	37.5	73.8	76.1	74.3	65.515	101.43	105.45	91.19
6	43.8	80.8	84.3	73.6	69.22	107.14	110.22	92.97
LSD (0.05)	12.98				16.49			
CV (%)	12.4				11.6			

Means are the sum of culm, leaf and ear nitrogen contents at each growth stage. NT1: ½ N at 2 WAP and ½ at 4 WAP; NT2: ½ N at 2 WAP and ½ at 6 WAP; NT3: ½ N at 2 WAP and ½ at 8 WAP

Table 4.2: Effect of cattle manure and time of nitrogen application on nitrogen uptake of maize plants at early and late reproductive stages in the 2014 minor season

Manure rate (tons/ha)	10 WAP (R2)				14 WAP (R6)			
	Time of nitrogen application							
	0N	NT1	NT2	NT3	0N	NT1	NT2	NT3
	Total aboveground nitrogen uptake (kg/ha)							

0	38.15	71.570	68.84	60.96	49.77	87.20	92.42	73.91
2	44.83	80.44	77.055	71.26	52.58	91.36	93.64	86.41
4	43.07	79.54	82.071	73.93	57.80	100.11	106.56	97.82
6	50.21	92.67	91.761	74.33	66.45	100.24	103.02	102.63
LSD (0.05)	11.84				17.61			
CV (%)	10.3				12.4			

Means are the sum of culm, leaf and ear nitrogen uptake at each growth stage

Values obtained from using contrast to compare treatments in 2014 major season indicated that at R2 stage, nitrogen uptake at sole nitrogen treatments was significantly higher than at sole manure treatments. Addition of manure to inorganic N applied at NT1 and NT2, increased nitrogen uptake by 28.13 and 21.25 kg/ha respectively in comparison to applying N alone at NT1 and NT2. Nitrogen uptake at NT3 combined with manure was 43.75 kg/ha significantly higher than without manure. Total nitrogen uptake at manure combined with NT2 application time was only 6.56 kg/ha higher than manure with NT1 time of N application; while manure with NT3 application was 9.38 and 15.94 kg/ha lower than manure with NT1 and NT2 times of N application, respectively. At R6, when the maize had reached physiological maturity, total nitrogen uptake at sole nitrogen treatments was significantly higher than at sole manure treatments. When manure was combined with each of the times of N application, contrast differences in N uptake between them and when no manure was added was

30.63 kg/ha for NT1, 41.25 kg/ha for NT2 and 54.06 kg/ha for NT3. Nitrogen uptake at NT3 application time combined with manure was found to be significantly lower ($P > 0.05$) than NT1 combined with manure and NT2 combined with manure.

In the 2014 major season, out of the total aboveground plant nitrogen uptake at physiological maturity, a range of 63.4% to 81.5% across treatments was taken up at R2 stage. The percentage of total nitrogen at physiological maturity that was taken up between R2 and R6 stages was smaller with values ranging from 18.5% to 42.6%. In between these two reproductive stages, mean total nitrogen uptake at NT2 time of N application was 0.44 and 7.97 kg/ha higher than at NT1 and NT3 application times, respectively.

Contrast comparisons in 2014 minor season showed that at R2, plants in the sole nitrogen treatments had taken up 63.13 kg/ha significantly more N than those in the sole manure treatments. NT1 with manure treatment took up 37.81 kg/ha more N than NT1 without manure, NT2 with manure treatment took up 44.38 kg/ha more N than NT2 without manure and NT3 with manure treatment also took up 36.56 kg/ha more N than NT3 without manure. All of these differences were significant ($P < 0.05$). At manure combined with NT3 application time treatment, N uptake was 33.13 and 31.38 kg/ha significantly lower than at manure with NT1 application and manure with NT2 application times treatments respectively. However, when NT1 and NT2 were both combined with manure, the two treatments showed no significant differences in total N uptake (Table 4.2). At R6, plants in the sole N treatments took up 76.56 kg/ha more N than those in the sole manure treatments. This indicates an increment of 13.44 kg/ha in terms of differences in N uptake between the two treatments from R2 to R6 stages. NT3 combined with manure treatment led to N uptake of 65.0 kg/ha more than NT3 without manure.

In 2014 minor season, the proportion of total nitrogen taken up between early vegetative and R2 stages was even higher, with values ranging from 76.7% in the control treatment to 95.3% at NT1 in the 6 tons/ha manure rate. Contribution of N uptake between R2 and R6 to the final nitrogen uptake was as low as 4.7% at NT1 in the 6 tons/ha manure rate and as high as only 25.6% in the 4 tons/ha sole manure treatment.

Table 4.3: Effect of cattle manure rates and nitrogen rates on nitrogen uptake of maize plants at early and late reproductive stages in the 2015 major season

Manure rate (tons/ha)	10 WAP (R2)				14 WAP (R6)			
	Nitrogen fertilizer rate (kg/ha)							
	0	30	60	90	0	30	60	90
	Total aboveground nitrogen uptake (kg/ha)							
0	37.65	57.15	59.29	62.92	55.04	80.61	97.00	104.70
2	43.86	62.75	70.56	68.30	74.14	100.29	109.38	115.59
4	49.61	66.54	75.39	88.18	79.85	104.98	111.09	126.73
6	56.54	72.14	84.65	87.44	86.04	118.79	128.76	131.62
LSD (0.05)	13.50				21.39			
CV (%)	12.4				12.6			

Means are the sum of culm, leaf and ear nitrogen uptake at each growth stage

In the 2015 major season, nitrogen uptake at R2 and R6 stages at all N rates increased with application of manure. Mean aboveground nitrogen uptake of 119.66 kg/ha at 14 WAP at the maximum N rate was significantly higher than the mean of 73.77 kg/ha at 0 N and 101.17 kg/ha at minimum N rate. It was, however, not significantly different from the mean of 111.56 kg/ha at optimum N rate.

Contrast comparison showed no significant difference in N uptake between 30 kg N combined with manure and without manure. However, at 60 and 90 kg N rates combined with manure treatments, N uptake increased significantly by 52.81 and 55.31 kg/ha than at 60 and 90 kg N rates without manure respectively. Nitrogen uptake at the sole 90 kg N rate was 5.63 and 3.75 kg/ha higher than at the sole 30 and 60 kg N rates respectively. When the 30 kg N rate was combined with manure, nitrogen uptake was 12.81 kg/ha higher than at the sole 90 kg N rate. At 60 kg N rate combined with manure treatment, N uptake was 41.88 kg/ha significantly higher than at 90 kg/ha sole N treatment.

At 14 WAP, mean nitrogen uptake at 30, 60 and 90 kg N rates was significantly higher than at 0 N. The effect was significantly lower at 30 kg N rate than at 90 kg N rate but no significant differences existed between the 60 and the 90 kg N rates (Table 4.3). Nitrogen uptake at 90 kg sole N treatment was 24.06 and 7.81 kg/ha higher than at 30 kg sole N and 60 kg sole N treatments respectively; but when the 30 and 60 kg N rates were combined with manure, N uptake became 10.0 and 35.0 kg/ha respectively higher than at sole 90 kg N treatment. Mean N uptake at 30 kg N combined with manure treatment was 34% higher than at 30 kg N without manure, 60 kg N with manure treatment was 20%

higher than 60 kg N without manure and 90 kg N with manure treatment was 19% higher than 90 kg N without manure.

On an experiment basis, 58% to 72% of the total N uptake at physiological maturity had occurred between early vegetative and R2 stages. On average, plants in the minimum N rate treatment absorbed 63.9% of their total N at this stage, those in the optimum N rate took up 65% of their total N and those in the maximum N rate treatment took up 64% of their total nitrogen.

4.1.1 Discussion

Nitrogen is often the most limiting nutrient to maize growth and yield. Studies using Nlabeled fertilizers showed that current year fertilizer application contributes to only 10 to

50% of the total N uptake by maize, whereas the rest comes from the soil N reserve (Stevens *et al*., 2005). While N from inorganic sources are immediately available for plant uptake, N from organic sources such as manure has to go through a mineralization process which is influenced by factors such as soil moisture level, soil pH, total N and C content of the manure, soil microbial biomass among others (Nyiraneza *et al*., 2009).

From this study, it was observed that combining cattle manure with nitrogen fertilizer enabled more plant nitrogen uptake than when mineral nitrogen or manure was applied alone (Tables 4.1 - 4.3). Even though N released from cattle manure in the first season of application is relatively small due to lower rate of decomposition as a result of high C/N ratio which slows the release of nutrients (Mugwe *et al*., 2007), manure helps to improve soil physical and chemical properties, thereby, increasing plant nutrient concentration and nutrient uptake. These effects are, however, dependent on the amount of manure applied

(Hou *et al*., 2012). Nyamangara *et al*. (2005), in an experiment conducted in Zimbabwe reported an increment of 39.8% N uptake in maize when nitrogen fertilizer was combined with cattle manure in the first season of manure application. Similar results were also reported by Chikowo *et al*. (2004).

Nitrogen uptake from sole nitrogen treatments was higher than from sole manure treatments in all three seasons. In the 2014 major season, this difference in N uptake was greater at early reproductive stages and reduced at maturity. In the minor season, however, the difference was higher at crop maturity compared to early reproductive stages. Higher N uptake from inorganic nitrogen treatments than the manure treatments could be due to the fast release of N by inorganic fertilizer. The fact that N uptake difference between nitrogen and manure treatments in the major season reduced at 14 WAP showed that manure had released more of its N at later growth stages. Nitrogen uptake difference between the two been higher at physiological maturity compared to R2 stage in the minor season could be due to the low soil moisture condition at the time because adequate moisture is a prerequisite for mineralization. Hernandez-Ramirez *et al*. (2011) reported aboveground total nitrogen content of maize plants fertilized with ammonium sulphate to be significantly higher than manure fertilized plots.

Higher N uptake in inorganic N fertilizer than manure treatments could also be due to the slow release of N from the manure as a result of its higher C/N ratio. The C/N ratio of manure has an important influence on the mineralization and release of nutrients during decomposition (Gutser *et al*., 2005). The larger C/N ratio of cattle manure slowed its decomposition and release of nutrients. An initial immobilization of nitrogen in the first eight weeks of field grown maize that had received an application of 10 tons/ha of cattle manure was observed in South Africa (Materechera and Salagae, 2002). According to the authors this is an indication that organically bound nitrogen in manure with a large C/N ratio is released slowly and that it is not readily available to plants during the early growth stages. Combined application of mineral nitrogen with manure increase the supply of nitrogen for microorganisms involved in the decomposition of the manure and therefore, speeds up the manure decomposition process and increases the availability and uptake of the nutrients (John *et al*., 2010). Nitrogen mineralization in cattle manure could, therefore, be hastened by supplementing the manure with mineral nitrogen fertilizer.

Higher aboveground nitrogen uptakes recorded for the NT2 time of N application (Tables 4.1 and 4.2) than the other application times in 2014 major and minor seasons suggests that application of 50% N at 2 WAP and top dressing the remaining 50% at 6 WAP provided better opportunity for greater N uptake. The susceptibility of N to loss processes such as leaching, denitrification and volatilization requires that N be applied at a time when the plants will utilize it most. Nitrogen loss through these processes is greater when N is applied at a time when uptake rates are relatively lower. Rate of N uptake was found to be greatest between 8 leaf stage (35 days after plant emergence) and silking (Gadalla *et al*., 2007), a timing which is very similar to NT2 in this study. Binder *et al*. (2000) reported that early or delayed application of N can significantly reduce nitrogen recovery and maize yield. Application of N at the time it is most utilized by the crop is, therefore, the best approach to optimizing N use.

Nitrogen uptake in maize to a large extent, depends on the rate of N applied (Nsanzabaganwa *et al*., 2014; Yusuf *et al*., 2009). Results of the 2015 major season experiment showed that total aboveground nitrogen uptake at early and late reproductive stages was significantly higher at 90 kg N/ha rate than at 0 and 30 kg N/ha rates (Table 4.3), indicating that raising N rate increased nitrogen uptake. Contrast comparisons showed that total N uptake at 60 kg N combined with manure treatment was significantly ($P < 0.05$) higher than 90 kg N without manure; and 30, 60 and 90 kg N with manure were also significantly higher than without manure. These results showed that combining mineral nitrogen with cattle manure could greatly enhance maize plant nitrogen uptake. The lack of significant difference in total nitrogen uptake between 60 and 90 kg N rates in all the manure rates indicates that when cattle manure would be combined with N fertilizer, 60 kg N could be enough to enable sufficient N uptake.

Application of optimum inorganic N fertilizer can provide sufficient N to crops early in the season, and when accompanied later in the season by a sustained release of N from mineralized cattle manure incorporated prior to seeding, the two sources can meet the peak of N demand of the crop (Kramer *et al*., 2002). Combination of organic and inorganic N inputs, therefore, holds promise for reducing the use of inorganic fertilizers and possible N losses from agro-ecosystems without compromising crop yields.

Shoot growth rate has been identified as a driving force for N uptake in maize plants (Peng *et al*., 2010). The higher N uptake observed for the manure and mineral N combined treatments than the sole N and sole manure treatments, for NT2 than other application times, at higher N rates than lower rates and at N applied than 0 N treatment could all be related to the higher vegetative growth and dry matter accumulation obtained in these treatments than the others. Subedi and Ma (2005b) reported that the greater total N uptake by some maize hybrids under field conditions was possibly due to the fact that they maintained green leaves for a longer period of time and would have taken up N for a longer period of time than those that experienced leaf senescence earlier. Chen and Mi (2012) reported that high N accumulation of landrace maize varieties was closely related to their higher biomass, indicating that growth potential is the main driving force for N uptake and accumulation. Modupeola *et al*. (2011) also reported an increase in nitrogen uptake in maize as plant shoot dry weight increased.

4.2 Nitrogen partitioning and remobilization

4.2.1 Nitrogen partitioning

In the 2014 major season, the proportion of total aboveground N partitioned to either, the culms, leaves or ears at R2 and R6 stages was mainly influenced by the main effects of the two factors, i.e. cattle manure rates and times of nitrogen application, but not by the interaction between the factors (Appendix 2a). Two weeks after completion of flowering (10 WAP), percentage of total aboveground N partitioned to the culms across N application times ranged from 30 to 31%, with 40 to 43% and 26 to 29% partitioned to the leaves and developing maize ears, respectively. At R6 (14 WAP), 23 to 24% of the total aboveground nitrogen across N application times was partitioned to the culms, 15 to 16% to the leaves and 60 to 62% to the ears (Figure 4.1). At the manure rates, 29 to 30% of total shoot N at 10 WAP was partitioned to the culms, 39 to 44% to the leaves and 24 to 30% to the ears. At 14 WAP, 22 to 25% of total shoot nitrogen was partitioned to the culms, 14 to 17% to the leaves and 59 to 64% to the ears (Figure 4.2).

In the 2014 minor season, shoot nitrogen partitioning among plant parts at 10 and 14 WAP was mainly influenced by the main effects of cattle manure rates and times of nitrogen application, but not by the interaction between the factors (Appendix 2b). Two weeks after completion of flowering, percentage of total aboveground N partitioned to the culms across N application times ranged from 36 to 41%, with 36 to 38% and 23% to 26% partitioned to the leaves and developing maize ears respectively (Figure 4.3). For the manure rates, 37 to 40% of total aboveground N was partitioned to the culms, 35 to 39% to the leaves and 23 to 27% to the ears (Figure 4.4). At 14 WAP, 27 to 31% of the total aboveground nitrogen across N application times was partitioned to the culms, 19 to 21% to the leaves and 50 to 53% to the ears (Figure 4.3). At manure rates, 27 to 30% of total shoot N was partitioned to the culms, 19 to 21% to the leaves and 50 to 52% to the ears (Figure 4.4).

In the 2015 major season experiment, the proportion of total aboveground nitrogen partitioned to plant parts was affected by the main effects of cattle manure rates and nitrogen rates but not by the interaction between them (Appendix 2c). At R2 stage, percentage of total shoot nitrogen partitioned to maize culms across nitrogen rates ranged from 23 to 24%, 48 to 50% was partitioned to the leaves and 27 to 28% to the developing maize ears. At R6 stage, a range of 15 to 17% of total shoot N across nitrogen rates was partitioned to the culms, 27 to 29% to the leaves and 55 to 57% to the maize ears (Figure 4.5). Regarding the effect of manure rates, percentage of total shoot N partitioned to maize culms at R2 ranged from 22% to 24%, percentage partitioned to leaves ranged from 48% to 50% whilst 26% to 29% was partitioned to the ears. At R6, culm N percentage declined to a range of 15% to 17%, leaf N was between 27% and 29% whilst 54% to 57% of shoot N was partitioned to the ears (Figure 4.6).

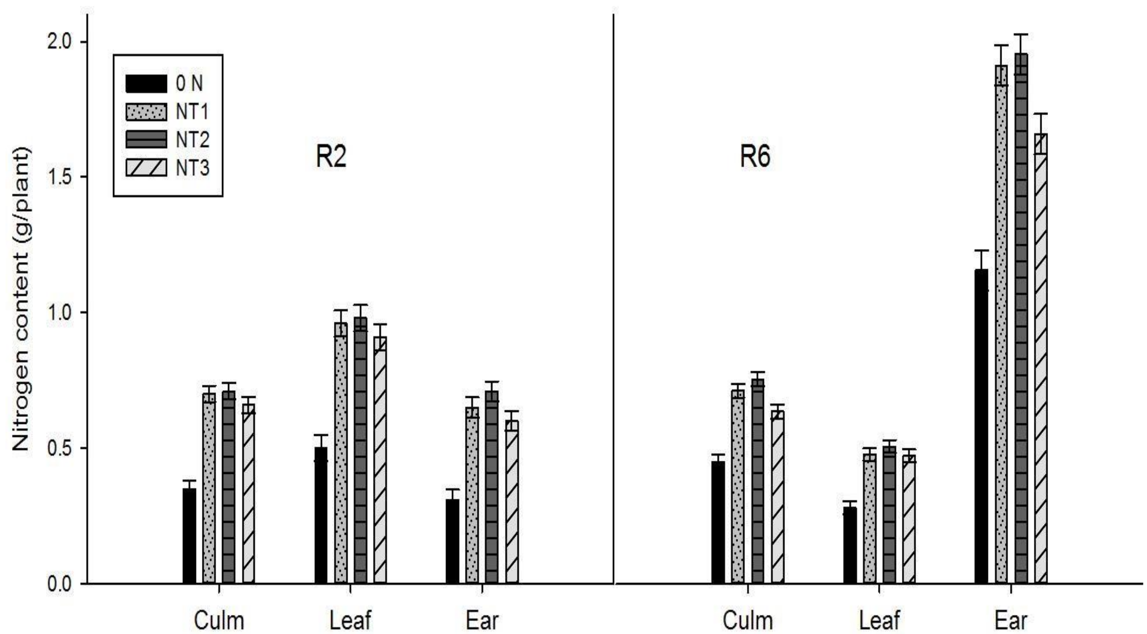


Figure 4.1: Effect of time of N application on nitrogen partitioning among plant parts at early and late reproductive stages in the 2014 major season. Bars represent SE.

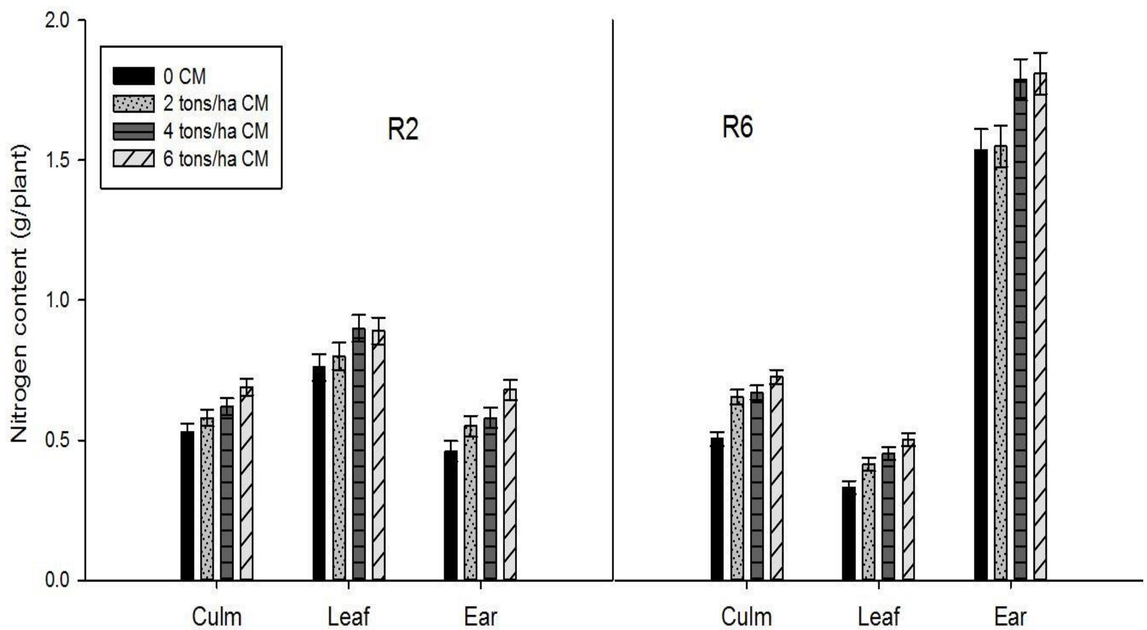


Figure 4.2: Effect of cattle manure rates on nitrogen partitioning among plant parts at early and late reproductive stages in the 2014 major season. Bars represent SE.

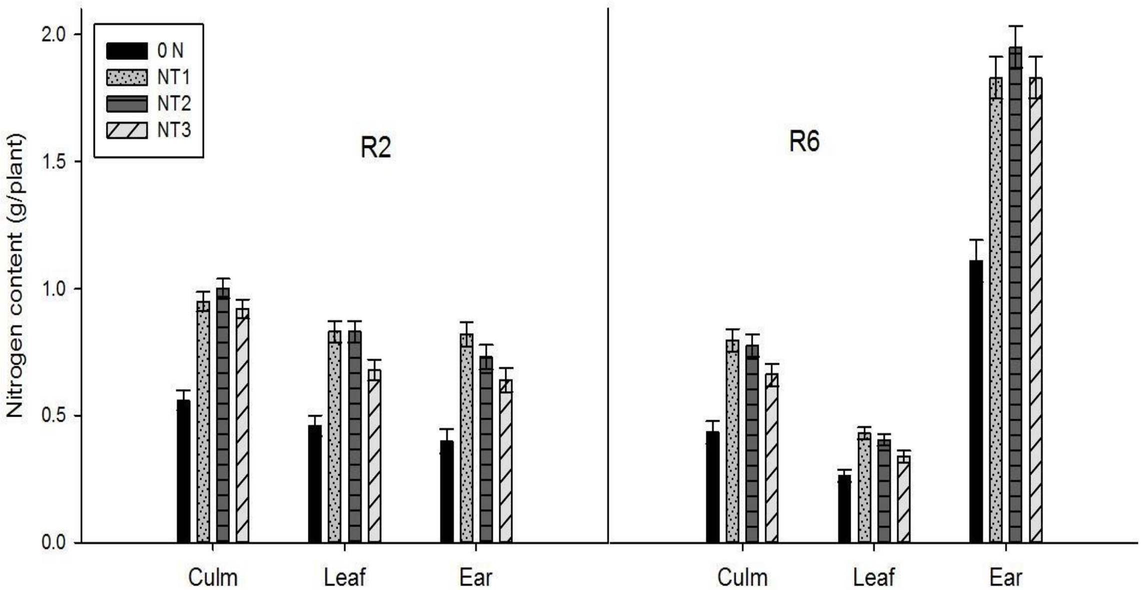


Figure 4.3: Effect of time of N application on nitrogen partitioning among plant parts at early and late reproductive stages in the 2014 minor season. Bars represent SE.

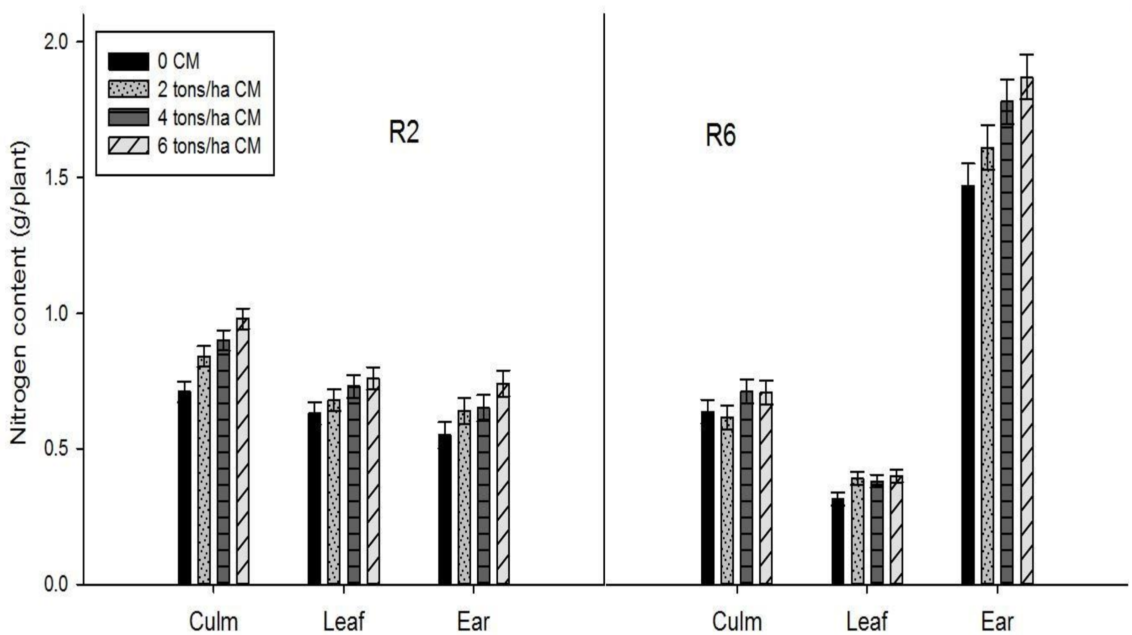


Figure 4.4: Effect of cattle manure rates on nitrogen partitioning among plant parts at early and late reproductive stages in the 2014 minor season. Bars represent SE.

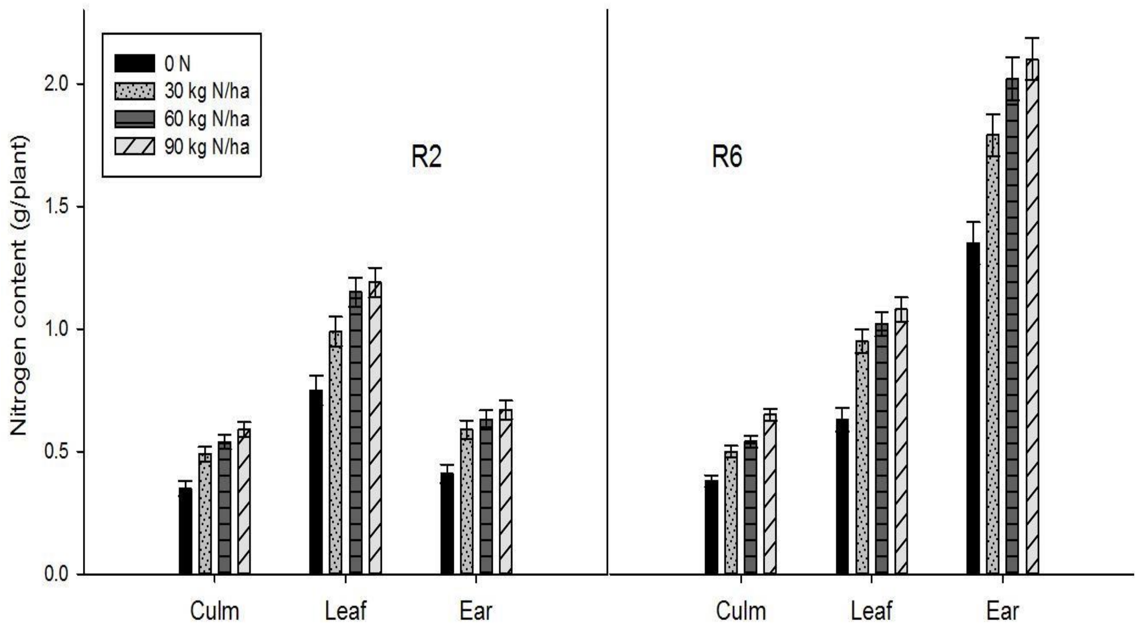


Figure 4.5: Effect of nitrogen rates on nitrogen partitioning among different plant parts at early and late reproductive stages in the 2015 major season. Bars represent SE.

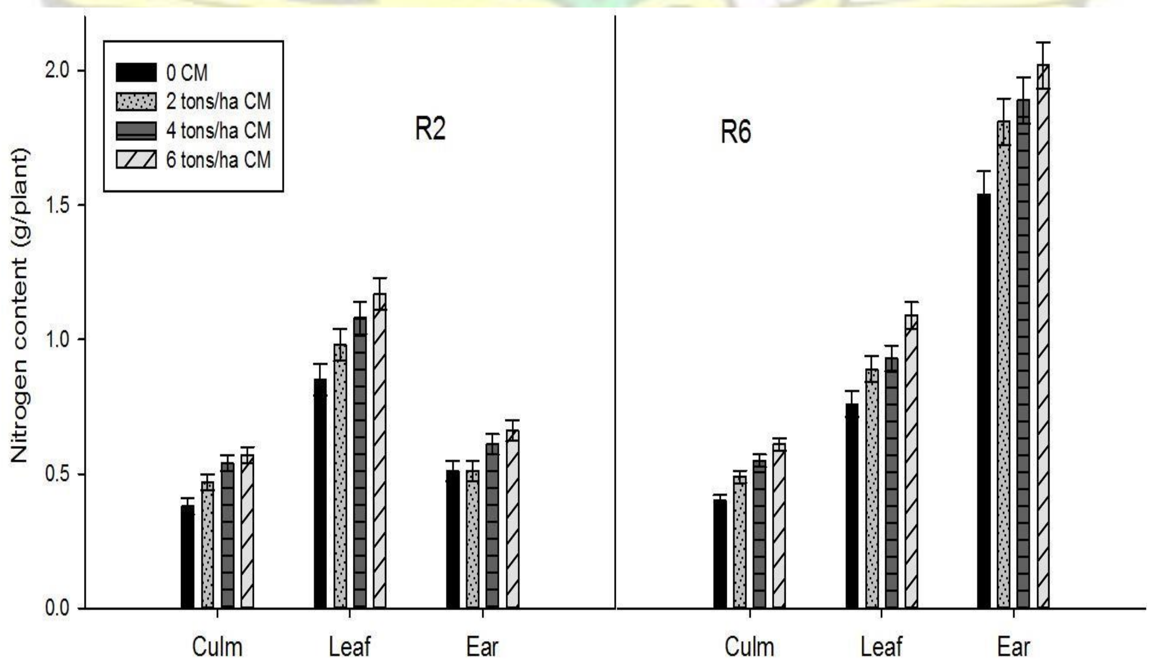


Figure 4.6: Effect of cattle manure rates on nitrogen partitioning among plant parts at early and late reproductive stages in the 2015 major season. Bars represent SE.

4.2.1.1 Culm nitrogen content

In the 2014 major season, differences in time of nitrogen application had no significant effect on maize culm nitrogen content at 10 WAP, but was significantly higher at all N applied treatments than at 0 N treatment. At 14 WAP, culm N at NT1 and NT2 application times was significantly higher than at NT3 application time and at 0 N (Figure 4.1). Cattle manure effect significantly increased culm N content in the 4 and 6 tons/ha manure treatments at 10 WAP, the effect at 2 tons/ha did not significantly increase culm N over the 0 manure rate. At 14 WAP, culm N content at all other manure rates was significantly higher than the 0 manure rate, but no significant difference existed among the three manure rates themselves (Figure 4.2).

In the 2014 minor season, Culm nitrogen content at 10 WAP was not significantly different between NT1, NT2 and NT3 application times, but was significantly higher at all N applied treatments than the 0 N treatment. At 14 WAP, culm N content at NT1 and NT2 application times showed no significant difference, but at NT1, it was significantly higher than at NT3 application time (Figure 4.3). Culm N content was not significant different among manure rates at R2; but at R6, manure at any of the three rates significantly increased culm N over the 0 manure rate. The 4 and 6 tons/ha manure rates showed no significant difference, but culm N at 6 tons was significantly higher than at 2 tons/ha rate (Figure 4.4).

At 10 WAP, in the 2015 major season, culm nitrogen content from 0 N was significantly lower than the N applied treatments. Culm N was also significantly higher at 90 kg N rate than the 30 kg N rate but 90 and 60 kg N rates showed no significant difference. At 14

WAP, culm nitrogen content was still significantly lower in 0 N compared to all other N rates. While 30 and 60 kg N rates showed no significant difference, both were significantly lower than the highest N rate (Figure 4.5). Application of cattle manure at any of the three rates significantly increased culm nitrogen in comparison to the nonapplication of manure at R2 stage. Culm N from 6 tons/ha manure rate was not significantly different from that of 4 tons/ha rate but was significantly higher than 2 tons/ha. The trend of manure effect on culm nitrogen content was the same at R6. The effect of no manure treatment was significantly lower than of all manure applied treatments, 6 tons/ha manure rate was not significantly different from 4 tons/ha rate but was significantly higher than 2 tons/ha rate (Figure 4.6).

4.2.1.2 Leaf nitrogen content

In the 2014 major season, leaf nitrogen content at R2 was significantly higher ($P < 0.05$) at all the N applied treatments than the 0 N treatment. Among the times of N application, NT1 and NT2 were not significantly different, but NT2 was significantly higher than NT3. At R6, no significant differences were found between NT1, NT2 and NT3 application times (Figure 4.1). Application of cattle manure at any of the rates used in this study had no significant effect on leaf N content at 10 WAP but at 14 WAP, all three manure rates had significantly increased leaf N over the no manure application. No significant difference existed between the 4 and 6 tons/ha manure rates (Figure 4.2).

In the 2014 minor season, leaf nitrogen content at 10 WAP was significantly higher at all N applied treatments than the 0 N treatment. NT1 and NT2 application times showed no significant difference but were both significantly higher than NT3. At 14 WAP, NT1, NT2 and NT3 application times showed no significant difference but were all significantly higher than 0 N (Figure 4.3). For the manure rates, leaf nitrogen content at 10 WAP only showed significant increment in comparison to non-addition of manure in the 6 tons/ha rate. At 14 WAP, manure applied treatments showed no significant difference; all of them were, however, significantly higher than the 0 manure rate (Figure 4.4).

Leaf nitrogen content at 10 WAP in the 2015 season was significantly higher ($P < 0.05$) in N treated plants than from 0 N treatment and increased with increase in N rate. The effect at 90 kg N rate was not significantly different from at 60 kg N rate, but was significantly higher than at 30 kg N rate. At 14 WAP, while leaf nitrogen content from 0 N was still significantly lower than from nitrogen applied treatments, no significant difference was obtained between the 30, 60 and 90 kg N rates (Figure 4.5). Leaf N content difference between manure rates at 10 and 14 WAP followed exactly the same trend. Significant increments were obtained when the manure application reached 4 and 6 tons/ha; but 2 tons/ha rate did not result in significant gains in comparison to the control (Figure 4.6).

4.2.1.3 Ear nitrogen content

In the 2014 major season, nitrogen content of maize ears at R2 stage was significantly higher at N applied treatments than the 0 N; and at NT2 than at NT3. At R6, mean ear N content of 1.91 g/plant at NT1 was not significantly different from the mean at NT2 (1.95), both were however, significantly higher than the mean at NT3 (Figure 4.1). Ear N content at 10 WAP at 0 manure rate was not significantly different from 2 tons/ha manure rate, but was significantly lower than at 4 and 6 tons/ha manure rates. At 14 WAP, while 0 and 2 tons/ha manure rates showed no significant difference, both were significantly lower than 4 and 6 tons/ha manure rates (Figure 4.2).

In the 2014 minor season, ear nitrogen content at R2 and R6 stages was significantly lower at 0 N than at any of the N application times. At 10 WAP, no significant difference was found between NT1 and NT2 application times, but ear N was significantly higher at NT1 than at NT3 application time. At 14 WAP, no significant difference was found between the times of N application (Figure 4.3). Manure effect on ear N content at 10 WAP was significant in the 6 tons/ha rate. At 14 WAP, manure effect significantly increased ear N content in the 4 and 6 tons/ha rates but not at 2 tons/ha rate (Figure 4.4).

In the 2015 season, ear nitrogen content at 10 WAP was significantly lower in 0 N than in any of the other three nitrogen rates; no significant difference, however, existed among the 30, 60 and 90 kg N rates. At 14 WAP, the effect at 0 N remained significantly lower than the N applied treatments. Minimum and optimum N rates had statistically the same effect on ear N content but maximum N rate was at this stage significantly higher than the minimum N rate (Figure 4.5). Effects of cattle manure on ear N content at 10 WAP increased significantly only in the 6 tons/ha manure rate; 2 and 4 tons/ha rates were not significantly different from the 0 rate. At 14 WAP, ear nitrogen content become significantly lower in the 0 manure rate than in any of the applied manure rates, on the other hand, 2, 4 and 6 tons/ha manure rates showed no significant difference (Figure 4.6).

4.2.2 Nitrogen remobilization

Table 4.4: Percentage of total nitrogen remobilized from vegetative parts between R2 and R6 at different manure rates and times of nitrogen application in the 2014 major season

Manure rate (tons/ha)	0	2	4	6	LSD (0.05)
Culm	8.96	6.98	6.72	5.53	2.133
Leaf	30.40	28.16	25.39	22.21	4.201
Nitrogen application time	0 N	NT1	NT2	NT3	
Culm	7.32	7.25	6.41	7.22	NS
Leaf	29.09	26.41	24.31	26.34	4.201

NS: not significant at P > 0.05. Means at manure rates were obtained across nitrogen application times including 0 N and means at nitrogen application times were obtained across the four manure rates.

Table 4.5: Percentage of total nitrogen remobilized from vegetative parts between R2 and R6 stages at manure rates and times of nitrogen application in the 2014 minor season

Manure rate (tons/ha)	0	2	4	6	LSD (0.05)
Culm	17.2	15.8	12.8	12.5	4.19
Leaf	21.61	18.49	17.04	15.89	4.138
Nitrogen application time	0 N	NT1	NT2	NT3	
Culm	18.2	13.7	12.6	13.9	4.19
Leaf	19.43	18.04	17.01	18.56	NS

NS: not significant at P > 0.05. Means at manure rates were obtained across nitrogen application times including 0 N and means at nitrogen application times were obtained across the four manure rates.

Table 4.6: Percentage of total nitrogen remobilized from vegetative parts between R2 and R6 at nitrogen and manure rates in the 2015 major season

Manure rate (tons/ha)	0	2	4	6	LSD (0.05)
Culm	9.13	8.51	6.90	6.22	2.658
Leaf	23.86	21.23	19.24	18.95	3.003
Nitrogen rates (kg/ha)	**0**	**30**	**60**	**90**	
Culm	9.97	9.12	5.85	5.78	2.658
Leaf	24.95	21.63	18.62	18.08	3.003

Means at manure rates were obtained across the four nitrogen rates and means at N rates were obtained across the four manure rates.

Percentage of total nitrogen remobilized from vegetative parts between R2 and R6 stages at manure rates and times of nitrogen application in 2014 major season was generally lower in the culms and higher in the leaves (Table 4.4). Nitrogen remobilizations from culms and leaves during this period were also severe at 0 and 2 tons/ha manure treatments than at the higher manure rates. Whilst differences in timing of nitrogen application did not significantly affect culm N remobilization, leaf nitrogen remobilization was significantly lower at NT2 time of N application than at 0 N.

In the 2014 minor season, percentage of total shoot nitrogen remobilized from culms and leaves between R2 and R6 stages decreased as manure rate increased. The rate of N remobilization from the three manure applied rates for both culms and leaves was not statistically different; but in the 4 and 6 tons/ha manure rates, N remobilization was significantly lower than the 0 manure rate. Application of nitrogen at NT1, NT2 or NT3 had no significant effect on rate of N remobilization from the culms in between these two reproductive stages, however, 0 N treatments had significantly higher N

remobilization than the nitrogen treatments (Table 4.5).

In the 2015 major season, the amount of nitrogen remobilized from culms and leaves between R2 and R6 stages at 60 and 90 kg N rates were significantly lower than from the 30 kg N rate and control (Table 4.6). Nitrogen remobilization from vegetative parts during this period was also seen to have decreased as cattle manure rate increased. Treatments that received 4 and 6 tons/ha manure rates experienced significantly less nitrogen remobilization from both leaves and culms than the control; nitrogen remobilization from 6 tons/ha manure rate was in fact significantly lower than that of 2 tons/ha manure rate.

Table 4.7: Correlation between nitrogen remobilization from culms and leaves and total shoot nitrogen uptake at two reproductive stages

Nitrogen remobilization	2014 major season		2014 minor season		2015 major season	
	Nitrogen uptake					
	R2	R6	R2	R6	R2	R6
Culm	-0.217	-0.170	-0.260	-0.342	-0.648**	-0.584*
Leaf	-0.256	-0.242	-0.234	-0.283	-0.500*	-0.643**

*, **: significant at $P < 0.05$, $P < 0.01$ respectively

4.2.3 Discussion

Results of the study showed a consistent nitrogen partitioning pattern in which the proportion of total aboveground N partitioned to maize ears was lower than the vegetative parts (culms and leaves) at R2 stage, but at physiological maturity, most of the N was partitioned to the ears (Figures 4.1 - 4.6). Average ratio of total aboveground N partitioned to ears at physiological maturity was 61% in 2014 major season, 62% in 2014 minor season and 56% in 2015 major season. This result is similar to the findings reported by Subedi and Ma (2007) that at physiological maturity more than 70% of the total N uptake was accumulated in maize ears. They associated the greater N content of the ears mainly to the higher dry matter partitioned to them.

Nitrogen partitioning to the vegetative parts on the other hand followed different patterns in the experiments. In the first two experiments where manure rates were combined with different times of N application, more of the shoot nitrogen at R2 was accumulated in the leaves than in the culms in the major season (Figures 4.1 and 4.2). In the minor season, culm N contents were slightly higher than that of the leaves at R2 stage (Figures 4.3 and 4.4). At R6, culm N was higher than that of the leaves in both seasons. For the 2015 experiment where manure rates were combined with nitrogen rates, leaf nitrogen content was higher than culm N content at both R2 and R6 stages (Figures 4.5 and 4.6).

These N partitioning pattern was not influenced by treatment effect, instead what might have happened was that, in the major seasons, maize plants tend to accumulate higher percentage of their shoot N in the leaves up to early reproductive stages which were subsequently translocated to the sink during active grain filling as indicated by the high rates of N remobilization from the leaves in Tables 4.4 and 4.6. In the minor season when soil moisture levels were lower, more of the shoot N were accumulated in the culms also for subsequent translocation to the sinks (ears) during active grain filling and this could be indicated by the culm N remobilization reaching double digits only in the minor season (Table 4.5). Apart from soil moisture level which seems to determine whether more of the shoot N was accumulated in leaves or culms in this study, other studies found pattern of N accumulation to be dependent on N availability. Paponov and Engels (2005) stated that at higher N supply, maize culms accumulated more N and became the most important net exporter of N to grains while at low N supply leaves accumulated more N and became the highest net exporters of N to the grains.

Nitrogen content of a whole plant or a plant part is determined by the concentration of N and dry biomass weight of the whole plant or its part (Subedi and Ma, 2005b). Treatment effects on N content of the plant parts discussed below were either due to differences in N concentration in the various plant parts or dry biomass weight or both.

4.2.3.1 Culm nitrogen content

Culm nitrogen content was consistently higher in nitrogen treatments than in the control treatment. The amount of aboveground plant nitrogen partitioned to maize culms at R2 stage was not significantly affected by the timing of N application. At R6 stage, culm N was significantly higher at NT2 application time in the major season (Figure 4.1), but in the minor season, NT1 application time was highest (Figure 4.3). While no significant differences existed between NT1 and NT2, both were significantly higher than 0 N and NT3 application time.

Results obtained from the 2015 major season experiment showed that at early and late reproductive stages, culm N content had increased with increase in N application rate (Figure 4.5). At R6 stage, mean culm N content in the 90 kg N rate was 62.5% significantly higher than in the 0 N, 30% significantly higher than in the 30 kg N rate and 20% significantly higher than in the 60 kg N rate. Hou *et al*. (2012) also reported an increase of 10.8-24.6% in N accumulation in maize culms in N fertilized treatments as compared to control treatment.

Differences in culm N contents between the N applied and 0 N treatments could be related to differences in both culm dry matter accumulation and N concentration. Culm N concentration at 0 N treatment of 7.05, 6.76 and 6.07 g N per kg culm biomass in 2014 major, 2014 minor, and 2015 major seasons respectively, were significantly lower than those in the N treatments. Differences in culm N content among times of N application and among N rates was mainly due to the significant differences in dry matter accumulation because in terms of N concentration, there were no significant differences among them.

Application of cattle manure in the major season significantly increased culm N content at R2 in the 4 tons/ha manure rate, additional gains were made from 6 tons/ha rate but those gains were not significant in comparison to the 4 tons/ha manure rate. At R6, culm N increased significantly at all manure applied rates than 0 manure rate (Figure 4.2). In the minor season, manure application had no significant effect on culm N up to R2 stage, but at R6, culm N content was significantly higher at all manure added treatments than 0 manure treatment (Figure 4.4). In the 2015 major season experiment, all manure applied rates significantly increased culm N over the 0 manure rate from R2 right up to physiological maturity. These results showed the slow but sure positive effect that manure application had on nitrogen accumulation in maize culms. Effective accumulation of N in culms provides assurances for adequate N supply to kernels during grain filling (Aflakpui *et al*., 2007).

4.2.3.2 Leaf nitrogen content

Photosynthesis is related to leaf N because it is dependent upon proteins in the mesophyll chloroplasts. As leaf N declines, there is a concomitant decrease in the majority of leaf proteins which diminishes the capacity of the leaf to absorb light for photosynthesis (Fernanda, 2005). The amount of N that accumulates in the leaves, therefore, affects the growth potential of the plants.

Results on partitioning of shoot N indicated that the ratio of shoot N partitioned to leaves at early and late reproductive stages was significantly higher at nitrogen treatments than control treatment. At R2 stage, leaf N content was significantly higher at NT2 than NT3 application times, but at R6 stage, leaf N content at the three N application times were not significantly different in the major season (Figure 4.1). In the minor season, leaf N content at NT3 application time at R6 was significantly lower than at NT1 and NT2 application times (Figure 4.3). Subedi and Ma (2005b) also reported N content of maize leaves to be higher when N was applied at late vegetative stage than when it was applied prior to, or later than that. Leaf N concentration of 20.13 g N per kg biomass at NT2 at R6 in the major season was significantly higher ($P < 0.05$) than the 17.88 g N per kg biomass at NT3. In the minor season, leaf N concentration at R6 was not significantly different among N application times. The lack of significant difference in leaf N content in the major season among N application times could be related mainly to the rapid dry matter accumulation observed in the NT3 time of N application during grain filling period which closes the significant DM gap between the times of N application at R6 (Figure 4.7), since the second N content determinant i.e. N concentration, was significantly differently among them. For the minor season, significant differences in leaf N content could be as a result of the significant difference in DM accumulation at R6 (Figure 4.9) since N concentration differences were insignificant.

In the 2015 major season experiment, leaf N content was found to increase at a diminishing return as nitrogen rate increased (Figure 4.5). Application of N at the minimum rate resulted in significantly less leaf N than the optimum and maximum N rates at reproductive stages. Leaf N content difference among N rates at R6 was due to the significant differences in dry matter accumulation between the higher and lower N rates (Figure 4.11) and N concentration. Leaf N concentration of 24.41 g N per kg leaf biomass at 90 kg N rate was the highest whilst 19.53 at 0 N was the lowest. Antonietta *et al*. (2015) also reported maize leaf concentration to have increased by 83-101% at higher nitrogen rate treatments than in the control treatment.

Application of cattle manure did not lead to significant increments in leaf N content at R2 in the 2014 major season (Figure 4.2); in the minor season, significant increments were obtained only when the manure rate reached 6 tons/ha (Figure 4.4). At physiological maturity stage, cattle manure effect became more pronounced with leaf N in all the manure applied treatments been significantly higher than 0 manure treatment in both seasons. In the 2015 major season experiment, application of manure also significantly increased leaf N at 4 and also at 6 tons/ha manure rates from R2 to R6 (Figure 4.6). This result showed that cattle manure at the above stated rates can significantly improve leaf nitrogen content.

The longevity and photosynthetic capacity of a leaf are related to its N status.

Maintenance of N supply to maize leaves increase leaf area duration and prolonged dry matter accumulation. Prolonged accumulation of dry matter and nitrogen by maize plants during grain filling has been reported as an important characteristic associated with higher grain yields (Valentinuz and Tollenaar, 2006).

4.2.3.3 Ear nitrogen content

In maize, partitioning of greater proportion of uptake nitrogen to ears during grain filling is a phenomenon that has been widely reported (Ciampitti and Vyn, 2011; HernandezRamirez *et al*., 2011; Paponov and Engels, 2005). In the 2014 major season, ear N content at R2 and R6 stages was consistently highest at NT2 application time (Table 4.1). In the minor season, NT1 time of N application had the highest ear N content at R2, but at R6, ear content in NT2 became higher (Figure 4.3). As stated earlier, N content of each plant segment is determined by the rate of N concentration and dry biomass weight. The higher ear N content in NT2 application time was due mainly to the higher ear dry weights (Figures 4.7 and 4.9) because ear N concentration at maturity was not significantly different among the N application times. The occurrence of significant differences in ear N content between N application times shows that the amount of N partitioned to maize ears is affected by not just the amount of nitrogen applied but also by the timing of N supply.

In the 2015 major season experiment, ear N content increased as N rate increased (Figure 4.5). The amount of total aboveground N partitioned to the ears at R2 and at R6 stages was significantly higher in the N applied treatments than 0 N. At R6, maximum N rate was significantly higher than not just the 0 N, but also the 30 kg N rate. Ear N content at 60 and 90 kg N rates were not significantly different. This result showed that the rate of N applied to maize crops affects the partitioning rate of N to the ears. Partitioning of N to maize ears increasing with increase in N rate applied was also previously reported by Gadalla *et al*. (2007) and Rozas *et al*. (2004).

The significant ear nitrogen content difference between the 90 kg N rate and the 0 and 30 kg N rates at R6 (Figure 4.5) could be related to the significant differences in ear DM (Figure 4.11) and also to N concentration for the 0 N rate. Ear N concentration of 13.60, 14.09 and 14.38 kg N per kg ear biomass for the 30, 60 and 90 kg N rates respectively were not significantly different ($P > 0.05$); hence, the significant ear N content difference between the 30 and 90 kg N rates was due to differences in ear dry weight. D'Andrea *et al*. (2008) attributed the effect of plant N content on grain yield to its effect on biomass production rather than partitioning of N to the ears. However, the amount of plant N that is partitioned to maize ears has been reported to have severe consequences on grain yield potential of the crop (Subedi and Ma, 2007).

Application of cattle manure also increased partitioning of N to maize ears. Manure effect on ear N content in the 2014 major season from R2 up to R6 stage was significant when the manure rate reached 4 tons/ha (Figures 4.2). In the minor season, manure effect on ear N content was significant at 6 tons/ha manure rate, but at R6, 4 tons/ha manure rate was also significantly higher than the 0 manure rate (Figure 4.4). In the 2015 major season experiment, manure effect on ear N content at R2 was significant only at 6 tons/ha manure rate, but at R6, it was significantly higher at all manure applied rates than the control (Figure 4.6). This result indicates that application of cattle manure at the above stated rates can significantly increase maize ear N content at physiological maturity. This result is in agreement with that of Kato (2012).

4.2.3.4 Nitrogen remobilization

Limiting nitrogen remobilization from vegetative parts of a plant is desirable for continued photosynthesis (Cirilo *et al*., 2009). High N remobilization from leaves to grains in maize decrease green leaf area duration (He *et al*., 2004). Decrease in leaf area duration lead to leaf senescence and declined photosynthesis which affects maize growth and yield.

Results of this study indicated that nitrogen remobilization from vegetative parts to maize ears at maturity was 3-4 times higher in the leaves than in the culms in the two major seasons (Tables 4.4 and 4.6). In the minor season, even though remobilization was higher in leaves than culms, the difference was relatively smaller compared to the major seasons (Table 4.5). Nitrogen remobilization from culms and leaves was found to be severe in the control than in the N treatments. Remobilization of N from vegetative parts during grain filling in maize has been widely reported (Tajul *et al*., 2013; Hammad *et al*., 2011; Herrmann and Taube, 2004). One common observation reported by these authors was that, the rate of N remobilization from vegetative to reproductive sinks was minimized when N was available in the right quantities at the right time.

Among the times of N application, vegetative N remobilization was relatively smaller at NT2 time of N application in both major and minor seasons (Tables 4.4 and 4.5), but the differences were not significant. For the N rates, N remobilization became less as N rate increased (Table 4.6). The difference between the highest N rate, and the lowest N rate and 0 N was significant ($P < 0.05$). Applications of cattle manure also reduced vegetative N remobilization significantly in the 4 and 6 tons/ha manure rates when compared to the 0 manure rate (Table 4.6). The N remobilization values obtained in this study in Tables

4.4, 4.5 and 4.6 are close to the estimation given by Ma *et al*. (1999) that, for highly fertilized maize crops, vegetative parts contribute to kernel N between 20 to 50%. Kivi *et al*. (2010) also reported that with increasing N levels, length of vegetative growth period in wheat increased and N remobilization into grains decreased.

The negative correlation found between N uptake at R2 and R6 stages and N remobilization from culms and leaves between the two stages even though not significant in some instances (Table 4.7), suggest that N remobilization from vegetative parts to the ears reduces as N uptake increased. The correlation between N uptake and N remobilization was stronger when different N rates were applied than when the same N rate was applied at different timings. Maintenance of N uptake during grain filling in maize is a critical aspect in minimizing the need for N remobilization from vegetative to reproductive sinks, which decreases green leaf area, and biomass accumulation (Rajcan and Tollenaar, 1999), hence, the need for application of N in the right quantities during growth stages considered critical for maximum nitrogen uptake (Gungula *et al*., 2005).

Nitrogen remobilization from vegetative parts to the ears in maize is a phenomenon that occurs even under sufficient nitrogen conditions. Yang *et al*. (2012) reported that after application of over 130 kg N/ha and confirmation of N status of maize plants to be above optimal with the aid of an N dilution curve, yet, N content of maize leaves and stems declined at reproductive stages as a result of remobilization to the ears. The target must, therefore, be to limit N remobilization from vegetative parts as much as possible but not to stop it all together.

4.3 Nitrogen utilization efficiency

In the 2014 major season, applications of inorganic nitrogen in combination with cattle manure significantly increased nitrogen recovery efficiency in the 4 and 6 tons/ha manure rates. Application of 2 tons/ha manure rate was insufficient to effect significant increment in nitrogen recovery in comparison to applying nitrogen fertilizer alone.

Table 4.8: Nitrogen utilization efficiency as affected by time of mineral nitrogen application at different cattle manure rates in the 2014 major season

Manure rates (tons/ha)	Time of nitrogen application								
	NT1	NT2	NT3	NT1	NT2	NT3	NT1	NT2	NT3
	NRE (kg N uptake/ kg N applied)			NIE (kg grain/ kg N uptake)			NUE (kg grain/ kg N applied)		
0	0.57	0.62	0.44	21.74	21.71	21.15	14.70	17.50	12.15
2	0.60	0.62	0.46	22.27	22.76	21.96	18.44	18.42	14.11
4	0.68	0.72	0.51	22.32	22.95	21.72	19.06	20.93	15.03
6	0.71	0.75	0.53	22.77	22.49	22.55	19.02	20.71	16.03
LSD (0.05)	0.07			NS			1.77		
CV (%)	7.1			9.3			6.1		

NRE: nitrogen recovery efficiency; NIE: nitrogen internal efficiency; NUE: nitrogen use efficiency. NS: not significant at $P > 0.05$.

Among the times of N application, mean nitrogen recovery efficiency at NT2 application time was significantly higher ($P < 0.05$) than at NT1 and NT3, whilst that of NT1 was also significantly higher than that of NT3 application time (Table 4.8).

Contrast comparison indicates that NT1 application time combined with manure recovered 0.29 kg/kg N applied more N than NT1 without manure, NT2 application time combined with manure recovered 0.23 kg more N than NT2 without manure whilst NT3 application time with manure recovered 0.17 kg more N than NT3 without manure. NT1 with manure and NT2 with manure showed no significant differences; however, NRE at NT3 combined with manure was 0.49 and 0.59 kg/kg N applied lower than at NT1 with manure and at NT2 with manure respectively (all significant at $P < 0.05$).

Nitrogen use efficiency was significantly higher at treatments where 4 or 6 ton/ha of manure were applied with nitrogen fertilizer than when N fertilizer was applied without manure or where only 2 tons/ha of manure was applied with the nitrogen. Contrary to NRE, application of 2 tons/ha of manure was sufficient to effect significant increment in NUE in comparison to the sole application of nitrogen fertilizer.

Mean NUE of 19.39 kg grain/kg N applied from NT2 application time was significantly higher than from NT1 and NT3 application times, at NT1 it was also significantly higher than at NT3 (Table 4.8). Contrast comparison indicated that NUE of NT1 application time combined with manure was 9.4 kg grain/kg N applied more than without manure; NT2 application time with manure was 7.6 kg more than without manure and NT3 application with manure was 8.7 kg more than without manure. Nitrogen use efficiency from NT2 application time combined with manure treatment was 3.5 kg grain/kg N applied significantly higher than at NT1 application time combined with manure. At NT3 application time combined with manure, it was 11.4 and 14.9 kg grain/kg N applied lower than at NT1 with manure and at NT2 with manure respectively.

Nitrogen internal efficiency on the other hand, followed no specific pattern and was not affected by manure rates, time of nitrogen application or their interaction (Table 4.8).

Table 4.9: Nitrogen utilization efficiency as affected time of mineral nitrogen application at different cattle manure rates in the 2014 minor season

Manure rates (tons/ha)	Time of nitrogen application								
	NT1	NT2	NT3	NT1	NT2	NT3	NT1	NT2	NT3
	NRE (kg N uptake/ kg N applied)			NIE (kg grain/ kg N uptake)			NUE (kg grain/ kg N applied)		
0	0.50	0.56	0.40	19.97	17.10	17.92	10.44	12.16	11.26
2	0.56	0.59	0.49	20.76	17.64	17.71	11.59	12.17	11.41
4	0.56	0.64	0.53	21.17	19.65	18.70	12.06	12.64	11.85
6	0.61	0.65	0.51	21.06	19.64	19.72	12.65	12.89	11.83
LSD (0.05)	0.053			NS			0.87		
CV (%)	5.7			7.5			4.3		

NRE: nitrogen recovery efficiency; NIE: nitrogen internal efficiency; NUE: nitrogen use efficiency. NS: not significant at P > 0.05.

In the 2014 minor season, application of 4 and 6 tons/ha manure rates with nitrogen fertilizer significantly increased nitrogen recovery efficiency by 0.089 and 0.102 kg/kg N applied more than at sole N application; but 2 tons/ha manure rate in most cases did not significantly increased NRE in comparison to sole N application.

Nitrogen recovery efficiency from NT2 application time was significantly higher than from NT1 and NT3 application times. At NT1 it was also significantly higher than at NT3 (Table 4.9). Contrast comparison showed differences of 0.23, 0.19 and 0.32 kg N uptake/kg N applied between NT1 with manure and without manure, NT2 with manure and without manure, and NT3 with manure and without manure respectively (all significant at $P < 0.05$). Nitrogen recovery efficiency from NT2 application time combined with manure was significantly higher than from NT1 combined with manure; whilst NRE from NT3 combined with manure was 0.19 and 0.35 kg N uptake/kg N applied significantly lower than at NT1 with manure and NT2 with manure respectively.

Nitrogen use efficiency also increased significantly when mineral N was applied along with 4 and 6 tons of manure than at sole mineral nitrogen applications. Application of 2 tons/ha manure rate with nitrogen fertilizer was insufficient to effect any significant increments over the application of mineral nitrogen alone.

Mean NUE from NT2 application time was significantly higher than at NT1 and NT3 application times, but that of NT1 and NT3 were not significantly different from each other (Table 4.9). Contrast comparison showed a significant difference of 5.0 kg grain/kg N applied between NT1 application time with manure and without manure; however, NT2 and NT3 application times with manure were not significant different from when no manure was added to them. NT1 application time with manure was not significantly different from NT2 application with manure, neither was it significantly different from NT3 application with manure. However, NT2 application time with manure was 2.6 kg grain/kg N applied significantly higher than NT3 with manure.

In the minor season, nitrogen internal efficiency followed no specific pattern and was not affected by manure rates, time of N application or their interaction (Table 4.9).

Table 4.10: Nitrogen utilization efficiency as affected by mineral nitrogen rates at different cattle manure rates in the 2015 major season

Manure rates (tons/ha)	Mineral nitrogen rates (kg/ha)								
	30	60	90	30	60	90	30	60	90
	NRE (kg N uptake/ kg N applied)			NIE (kg grain/ kg N uptake)			NUE (kg grain/ kg N applied)		
0	0.77	0.60	0.50	20.88	18.09	13.97	14.24	16.28	10.57
2	0.83	0.63	0.52	21.22	18.98	14.27	14.66	16.40	13.26
4	0.89	0.66	0.54	21.72	20.69	18.50	20.96	20.71	16.19
6	0.91	0.68	0.55	22.04	21.31	19.80	21.44	22.81	16.25
LSD (0.05)	0.08			3.49			1.28		
CV (%)	7.2			10.7			6.4		

NRE: nitrogen recovery efficiency; NIE: nitrogen internal efficiency; NUE: nitrogen use efficiency.

In the 2015 major season, mean nitrogen recovery efficiency of 0.85 kg N uptake/kg N applied at 30 kg N rate was significantly higher ($P < 0.05$) than the mean of 0.64 from 60 kg N rate and 0.53 from 90 kg N rate. The mean from 60 kg N rate was also significantly higher than the mean from 90 kg N rate (Table 4.10).

Application of mineral nitrogen with 2 tons/ha of cattle manure increased nitrogen recovery efficiency by 7.1% at the 30 kg N rate, 5.5% at the 60 kg N rate and 4.0% at the 90 kg N rate. Application of mineral N with 4 tons/ha manure rate increased NRE by 15.1% at the 30 kg N rate, 10.5% at the 60 kg N rate and 7.5% at the 90 kg N rate. Application of mineral N with 6 tons/ha manure rate also increased NRE by 18.6% at the 30 kg N rate, 13.4% at the 60 kg N rate and 9.2% at the 90 kg N rate.

Contrast comparison indicates that when no manure was added to either, NRE at 90 kg N rate was 0.27 kg N uptake/kg N significantly lower than at 30 kg N rate. The difference increased to 0.71 kg N uptake/kg N when manure was added to the 90 kg N rate. Nitrogen recovery efficiency at 60 kg sole N was also 0.17 kg N uptake/kg N applied significantly lower than at 30 kg sole N. The difference increased to 0.34 kg when manure was added to the 60 kg N rate. Nitrogen recovery efficiency at 30 kg N rate combined with manure was 0.36 kg/kg N applied significantly higher than when no manure was added to it. Nitrogen recovery efficiency at 60 and 90 kg N rates combined with manure were, however, not significantly different from without manure.

Mean nitrogen internal efficiency of 16.63 kg grain/kg N uptake at 90 kg N rate was significantly lower than the mean at 60 and 30 kg N rates. Mean NIE at 60 kg N rate of

19.77 was, however, not significantly different from the mean of 21.47 at 30 kg N rate (Table 4.10). Combination of mineral N with 2 tons/ha manure rate increased NIE by 2% at 30 kg N rate, 5% at 60 kg N rate and 2% at 90 kg N rate. When 4 tons/ha manure rate was applied, NIE at 30 kg N rate increased by 4%, at 60 kg N rate, it increased by 14.4% and at 90 kg N rate it increased by 32%. At this manure rate, NIE at 90 kg N rate increased by 8 folds more than the increment obtained at 30 kg N rate. When 6 tons/ha manure rate was applied, NIE at 30 kg N rate increased by 5.6%, at 60 kg N rate, it increased by 17.8% and at 90 kg N rate it increased by 42%. Nitrogen internal efficiency increment at this manure rate was 7.5 folds more at 90 kg N rate than at 30 kg N rate.

Mean nitrogen use efficiency of 19.05 kg grain/kg N applied at 60 kg N rate was significantly higher than the mean of 17.83 at 30 kg N rate and 14.07 at 90 kg N rate. At 30 kg N rate it was also significantly higher than at 90 kg N rate (Table 4.9). Application of mineral N in combination with 4 tons/ha manure rate increased NUE by 43.5% at 30 kg N rate, by 27% at 60 kg N rate and by 53.2% at 90 kg N rate. When 6 tons/ha manure rate was applied, NUE at 30 kg N rate increased by 50.6% in comparison to no manure addition, 40% at 60 kg N rate and 53.7% at 90 kg N rate. Nitrogen use efficiency at 30 kg sole N was 3.67 kg grain/kg N applied significantly higher than at 90 kg sole N. However, when manure was added to the 90 kg N, its NUE surpassed that of the 30 kg sole N by 3.0 kg grain/kg N applied. Nitrogen use efficiency at 30 kg N combined with manure was 14.3 kg grain/kg N more than when no manure was added, at 60 kg N with manure it was 11.1 kg grain/kg N more than without manure and at 90 kg N with manure it was 14.0 kg grain/kg N than without manure (all significant at $P < 0.05$).

4.3.1 Discussion

Improvement of nitrogen utilization efficiency in crop production can help greatly reduce the rate of nutrient loss, minimize cost of production and enhance crop yields (Cassman *et al*., 2003). Physiological and morphological components of plants have profound effects on their abilities to absorb and utilize nutrients under various environmental and ecological conditions. These plant traits and their interactions with external factors such as soil moisture, management practices, and fertilizer materials greatly influence nitrogen use efficiency (Baligar *et al*., 2001).

Results of the study showed that nitrogen recovery efficiency was higher at NT2 application than at NT1 and NT3 application times and this was consistent in both the major and minor seasons (Tables 4.8 and 4.9). Results also showed that when NT1 and NT2 application times were both combined with manure, their NRE was not significantly different; but NT3 application time combined with manure was significantly lower than the two. It can be stated from these results that NT2 time of N application provides the greatest opportunity of recovering more of the applied N than the other N application timings used in this study for this maize variety. Increase in N recovery has been stated by Barbieri *et al*. (2008) as one important way of increasing NUE of maize crops.

Results obtained from the 2015 experiment, where manure rates were applied in combination with nitrogen rates, showed that nitrogen recovery efficiency was higher at lower N rates and reduces as nitrogen rate becomes higher (Table 4.10). Dilallessa (2006), in a study conducted in South Africa, also found nitrogen recovery efficiency of maize to be higher at lower than higher N rates for the same tillage treatment though the differences were not always significant.

Application of mineral N fertilizer with cattle manure significantly increased nitrogen recovery efficiency at all N application times (Tables 4.8 and 4.9) and at all N rates (Table 4.10). Manure application increased N recovery because manure improves organic matter content of the soil thereby limiting N loss processes.

Nitrogen internal efficiency which refers to the ability of the plants to convert uptake N into grain yield was not significantly affected by the main effects of cattle manure rates and times of N application used in this study, or their interaction (Tables 4.8 and 4.9). On the other hand, N rates used and their interaction with manure rates produced significantly different NIE responses (Table 4.10).

In the 2014 major and minor season's experiments, all N treatments received equal amounts of N at the start of active grain filling, despite the different timings of N application. Therefore, there was no N insufficiency. In the 2015 experiment, differences in N rates applied was high, with some treatments receiving the recommended N rate (90 kg N/ha), some received two third the recommended rate (60 kg N/ha) and some received one third (30 kg N/ha). This difference in N rates might have induced N limitation in the lower N rate treatments, which was never the case in the N time, manure rate combined experiments in 2014. With sufficient N supply in the field, variation in N use efficiency is due largely to differences in N uptake ability of maize crops (NRE), whereas, when limited N supply exist, variation in N use efficiency is due mainly to differences in utilization of the uptake N in plants i.e. N internal efficiency (Peng *et al.*, 2010; Worku *et al.*, 2007). This N utilization dynamics of maize could explain the lack of treatment effect on NIE in the 2014 seasons' experiments.

Nitrogen internal efficiency was significantly higher at 30 and 60 kg N rates than at 90 kg N rate (Table 4.10). Ciampitti and Vyn (2011) also reported similar N rate effect on nitrogen internal efficiency of maize. The huge percentage increment in NIE at 90 and 60 kg N rates as manure rates added to nitrogen fertilizer increased is an indication that though NIE was higher at lower N rate than higher N rate, percentage increments obtained through addition of manure was far higher at higher N rates than lower rates.

Nitrogen use efficiency was found to be significantly higher when N was applied at NT2 in the major as well as in the minor seasons (Tables 4.8 and 4.9). Nitrogen use efficiency is determined by the ability of plants to recover N from the applied N fertilizer (NRE), and or ability to convert uptake N into grain yield (NIE). Since time of N application had no significant effect on NIE, the higher NUE at NT2 application time can, therefore, be attributed to its higher N recovery efficiency.

Nitrogen use efficiency increased with application of cattle manure at all N application times (Tables 4.8 and 4.9) and N rates (Table 4.10). The higher NUE differences among N application times in the first two experiments and among N rates in the final experiment at sole N applications reduced as manure rate increased. Nitrogen use efficiency had on average increased by up to 40% across N rates in the 4 and 6 tons/ha manure rates. Increased NUE as a result of combining manure with inorganic N fertilizer was most likely due to the contribution of manure in alleviating other crop growth constraints other than N. Manure application enhance soil moisture retention capacity, regularize soil pH and supply other soil macro and micro nutrients essential for effective maize growth and yield (Azeez, 2009).

Nitrogen use efficiency was also found to be significantly higher at 60 kg N rate than at 30 and 90 kg N rates. At 30 kg N rate, it was also significantly higher than at 90 kg N rate (Table 4.10). Nitrogen use efficiency would normally be expected to be higher at lower than at higher N rates. Jin *et al*. (2012) reported that NUE in maize increased with application of nitrogen, but decreased as the N rate becomes higher. The reason why NUE was higher at 60 kg N rate than at 30 kg N rate was that, even though NRE and NIE were higher at 30 than at 60 kg N rate, grain yield difference between the two was far higher in favor of the 60 kg N rate and superseded the NRE and NIE differences.

Nitrogen use efficiency been higher at lower N rates than at higher N rates is in line with the findings reported by Walsh *et al*. (2012); but in terms of effect of time of N application on NUE, results of this study which showed NT2 application time having significantly higher NUE than other N application times contradicts with their finding. They reported that top dressing of nitrogen at early or late vegetative stages in maize had no significant effect on NUE. The difference between this result and their findings could be due to differences in growing environment, maize varieties used or the additional advantage provided by the addition of cattle manure to N fertilizer in this study.

Nitrogen recovery, internal and use efficiencies were found to be significantly higher where nitrogen fertilizer was combined with cattle manure than where mineral nitrogen alone was applied irrespective of rate of N applied or time of application. Generally, under high N application, only 5–15% of N is transformed into grain yield (Erisman *et al*., 2007). The remaining N is lost as gaseous emissions or leached from the soil. Recording NUE values of 22.81 kg grain per kg N applied when 60 kg N was combined with 6 tons/ha manure rate (Table 4.10); and 20.93 kg grain per kg N applied at NT2 combined with 4 tons/ha manure rate (Table 4.8) showed that NUE of maize can be increased through combined application of manure and mineral N fertilizer.

Combined application of manure and chemical N fertilizer significantly increased maize crop biomass and total N concentration in the plant organs, thereby improving the N fertilizer utilization (Hou *et al*., 2012). Vanlauwe *et al*. (2011) also reported NUE in maize to be significantly higher at manure, mineral N fertilizer combined treatments than where mineral N alone was applied.

A decrease in NUE has been reported in other studies combining organic with inorganic sources of N due to increased immobilization, but not to a significant level in others. However, the immobilization of N associated with application of organic nitrogen sources was temporal and less severe in manure, most especially when the manure goes through a decomposition phase prior to planting (Kramer *et al*., 2002). Application of cattle manure alone was reported by Ouédraogo *et al*. (2006) to have reduced N use efficiency as a result of N immobilization, but when supplemented with inorganic N fertilizer, release of organically bound N was hastened and NUE increased.

4.4 Dry matter accumulation and partitioning among plant parts

4.4.1 Total shoots dry matter

In the 2014 major season, aboveground plant dry matter at all sampling periods was significantly higher in the nitrogen treatments than the control. Total shoot dry weight of plants at NT1 and NT2 application times showed no significant difference throughout; they were both, however, significantly higher ($P < 0.05$) than at NT3 application time at

7 and 10 WAP (Table 4.11). At 14 WAP, when the maize had reached physiological maturity, the shoot DM production gap between the times of N applications had closed and no significant differences existed among them. At late vegetative stage, cattle manure effect significantly increased aboveground plant DM in the 4 tons/ha manure treatment. Plant DM from the 6 tons/ha manure rate was higher than at 4 tons but the difference was not significant at ($P > 0.05$). At the reproductive stages, aboveground DM at all of the manure applied rates was significantly higher than the 0 manure rate.

In 2014 minor season, total aboveground plant dry matter was consistently significantly higher in the nitrogen applied treatments than the control treatment. While mean total aboveground DM at NT1 and NT2 application times showed no significant difference throughout; they were both significantly higher than NT3 application time at all three growth stages. Total aboveground DM at 7 and 10 WAP was not significantly different between 0 and 2 tons/ha manure rate, but was significantly lower in both than in 4 and 6 tons/ha rates. At 14 WAP, however, DM under 2 tons/ha manure treatment was still significantly lower than at 4 and 6 tons. All manure treatments at this stage produced significantly higher aboveground DM than the control treatment (Table 4.12).

Table 4.11: Effect of time of N application and cattle manure rate on total shoots dry matter at three growth stages in the 2014 major season

Treatment	Total aboveground plant dry weight (g/plant)		
Time of nitrogen application	7 WAP	10 WAP	14 WAP
0 N	66.19	138.97	180.93
NT1	113.07	216.13	304.97

NT2	111.33	226.64	320.36
NT3	92.75	196.45	291.95
LSD (0.05)	10.35	18.09	30.68
CV (%)	15.2	13.1	15.8
Manure rate (tons/ha)			
0	82.31	163.68	228.45
2	91.07	190.97	266.71
4	100.35	207.40	290.33
6	109.61	216.15	312.72
LSD (0.05)	10.95	18.08	32.96
CV (%)	16.0	13.0	16.9

Means of nitrogen application times were obtained across the four manure rates and means of manure rates were obtained across nitrogen application times including 0 N.

Table 4.12: Effect of time of nitrogen application and cattle manure rate on total shoots dry matter at three growth stages in the 2014 minor season

Treatment	Total aboveground plant dry weight (g/plant)		
Time of nitrogen application	7 WAP	10 WAP	14 WAP
0 N	52.01	134.09	169.81
NT1	78.08	210.16	270.31
NT2	77.88	215.43	280.01

NT3	66.49	198.57	244.98
LSD (0.05)	4.85	10.37	13.62
CV (%)	10.0	7.7	6.8
Manure rates (tons/ha)			
0	61.29	173.35	219.03
2	66.15	183.69	234.99
4	71.31	193.51	249.65
6	75.70	207.70	261.44
LSD (0.05)	5.10	11.12	13.60
CV (%)	10.4	8.2	6.8

Means of nitrogen application times were obtained across the four manure rates and means of manure rates were obtained across nitrogen application times including 0 N.

Table 4.13: Effect of nitrogen rates and cattle manure rates on total shoots dry matter at three growth stages in the 2015 major season

Treatment	Total aboveground plant dry weight (g/plant)		
Nitrogen rates (kg/ha)	7 WAP	10 WAP	14 WAP
0	57.05	145.56	210.43
30	75.19	189.68	268.36
60	80.00	201.60	288.42

90	89.40	210.37	299.37
LSD (0.05)	10.02	14.04	17.97
CV (%)	18.7	10.6	9.5
Manure rates (tons/ha)			
0	66.88	163.24	233.81
2	69.91	183.68	266.03
4	76.97	195.90	273.19
6	87.87	203.99	293.55
LSD (0.05)	10.81	15.06	18.80
CV (%)	19.1	11.3	9.9

Means of nitrogen rates were obtained across the four manure rates and means of manure rates were obtained across nitrogen rates including 0 N.

In the 2015 season, total shoots dry weight in all the nitrogen treatments was significantly higher than the 0 N at all measurement periods. Aboveground DM increased with increase in N rate at all three stages. From 7 up to 10 WAP, total aboveground DM was not significantly different (P > 0.05) between 30 and 60 kg N rates, but at 14 WAP, shoot dry matter in the 60 kg N treatment was significantly higher than in the 30 kg N treatment. While mean shoot DM in the highest N rate was consistently significantly higher than in the lowest N rate; it was not at any of the three stages significantly higher than the optimum N rate. By the 7th week after planting, significant increment in total shoot dry matter as a result of application of cattle manure was obtained in the 6 tons/ha treatment. At 10 and 14 WAP, application of manure at any of the rates significantly increased total aboveground DM (Table 4.13).

4.4.2 Dry matter partitioning among plant parts

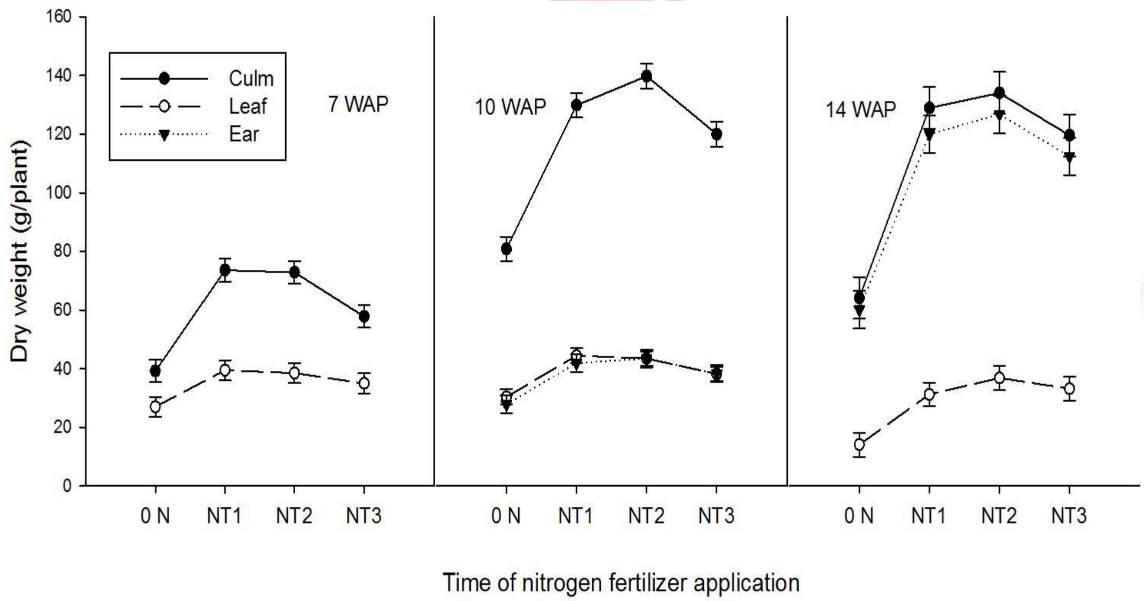


Figure 4.7: Effect of time of nitrogen application on dry matter partitioning to different plant parts at three growth stages in the 2014 major season. Bars represent SE.

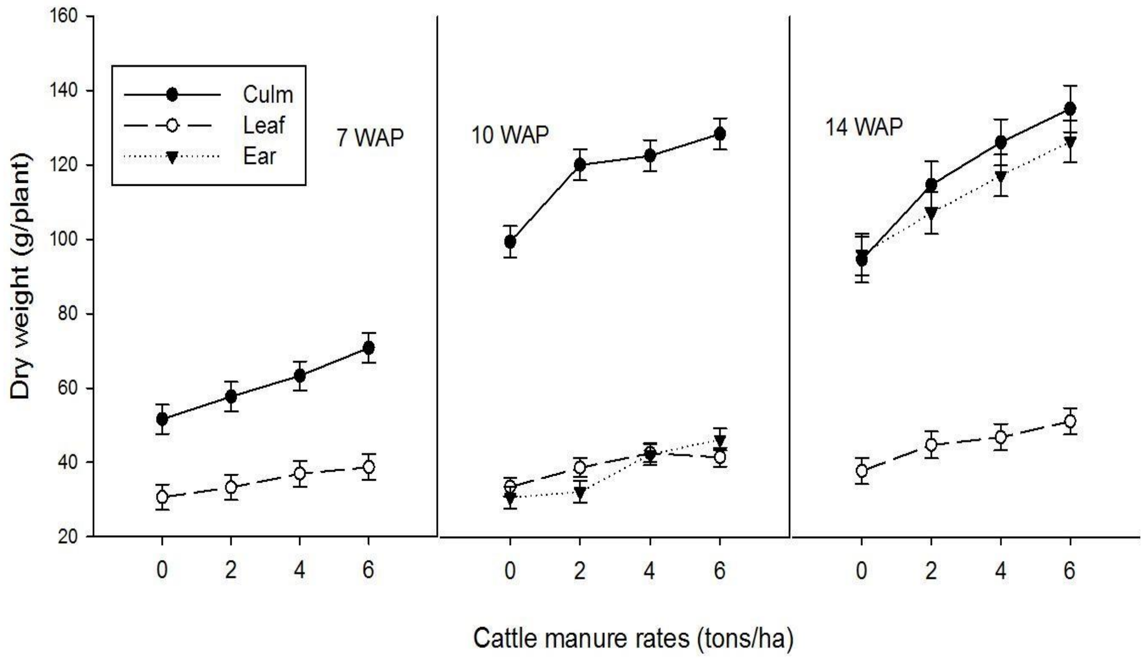


Figure 4.8: Effect of cattle manure rates on dry matter partitioning to different plant parts at three growth stages in the 2014 major season. Bars represent SE.

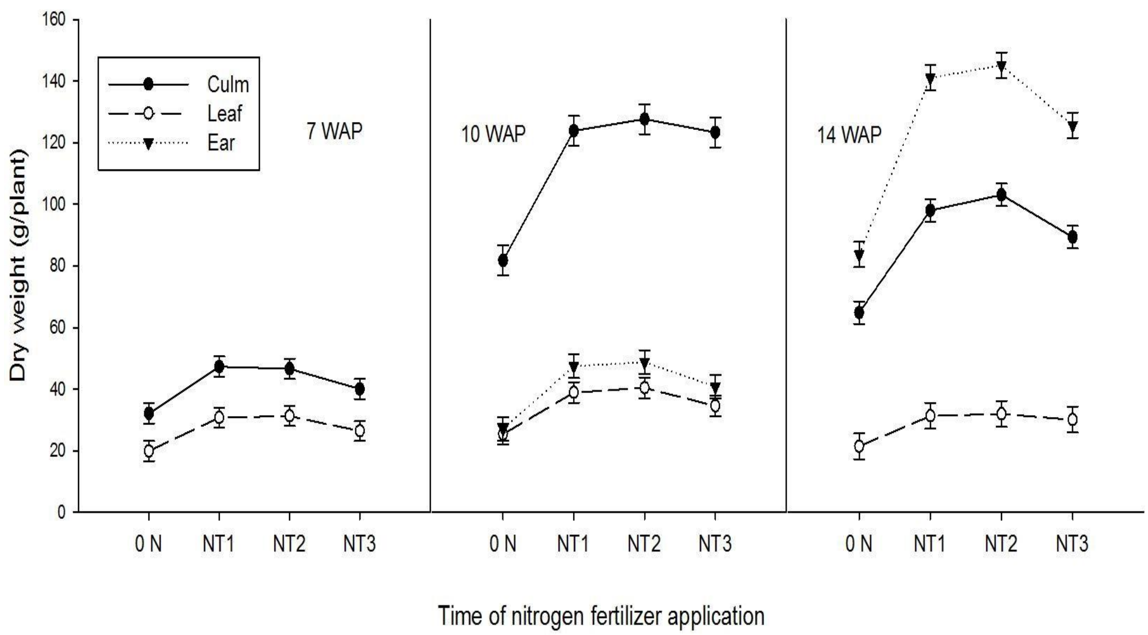


Figure 4.9: Effect of time of nitrogen application on dry matter partitioning to different plant parts at three growth stages in the 2014 minor season. Bars represent SE.

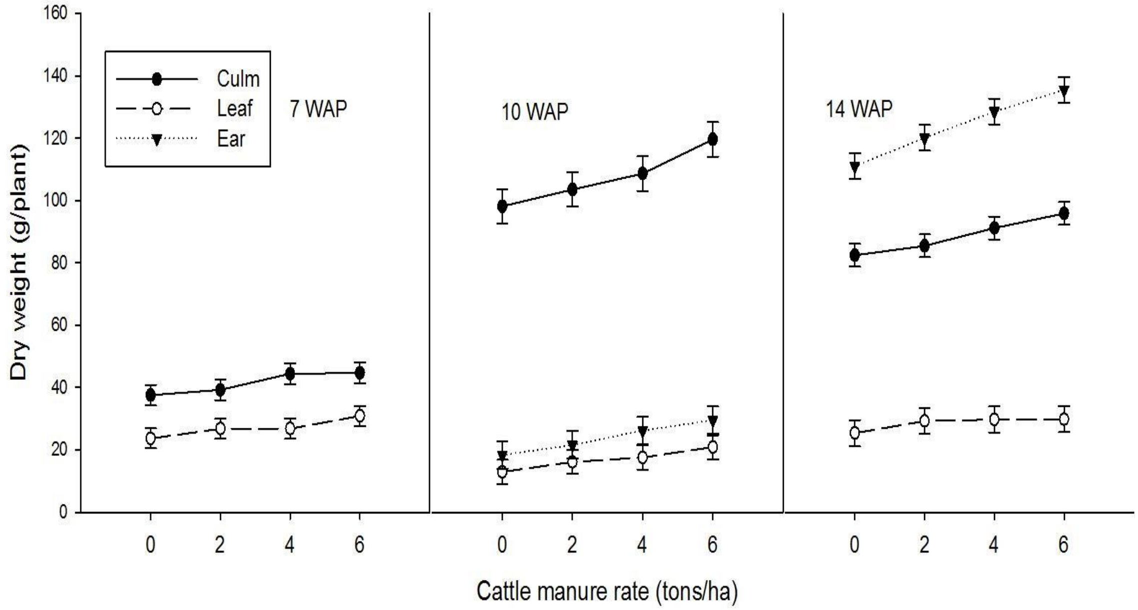


Figure 4.10: Effect of cattle manure rates on dry matter partitioning to different plant parts at three growth stages in the 2014 minor season. Bars represent SE.

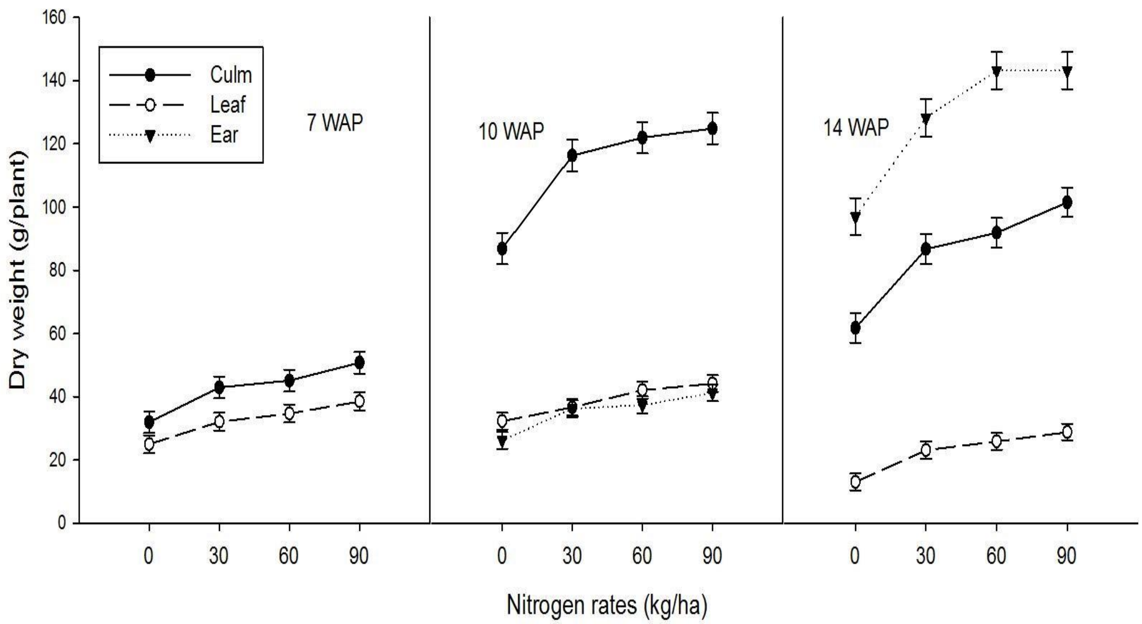


Figure 4.11: Effect of nitrogen rates on dry matter partitioning to different plant parts at three growth stages in the 2015 major season. Bars represent SE.

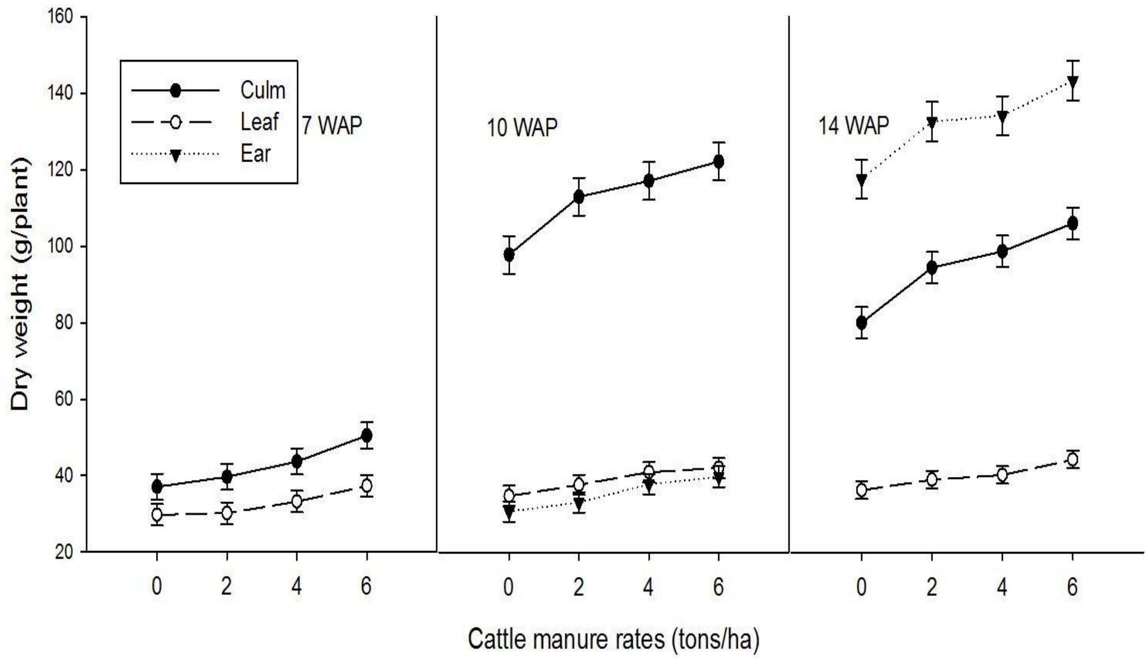


Figure 4.12: Effect of cattle manure rates on dry matter partitioning to different plant parts at three growth stages in the 2015 major season. Bars represent SE.

In 2014 major season, across manure rates and times of N application, an average of 62% of the total shoot DM produced at 7 WAP was accumulated in the culms and 38% in the leaves. Three weeks later when the maize had reached R2 (blister stage), 60% of total shoot DM was accumulated in the maize culms, 21% in the leaves and 19% in the maize ears. At R6, average culm DM declined to 43% of the total shoot DM, leaf DM also reduced to 17% and 40% was accumulated in the ears (Figures 4.7 and 4.8).

In the 2014 minor season, out of the total aboveground dry matter accumulated by plants at 7 WAP at manure rates and times of N application, an average of 61% was partitioned to the culms and 39% to the leaves. At 10 WAP, average culm DM remained at 61% of the total shoot DM, 18% was accumulated in the leaves and 21% in maize ears. At 14

WAP, culm DM declined to an average of 37%, leave DM declined to 12% on average, whilst ear dry matter increased to 51% on average (Figures 4.9 and 4.10).

In 2015 major season, an average of 56% of total aboveground plant DM accumulated at 7 WAP was partitioned to the culms and 44% to the leaves. At 10 WAP, culm DM on average increased to 60% of total accumulated DM, 21% was accumulated in leaves and 19% in the developing maize ears. At 14 WAP, the percentage of total shoot DM accumulated in maize culms declined to an average of 36%, leaves accumulated only 15% and an average of 49% of total aboveground DM was partitioned to the maize ears (Figures 4.11 and 4.12).

4.4.2.1 Culm dry matter

In 2014 major season, culm DM at 7 WAP was significantly higher at NT1 and NT2 than at NT3 and 0 N treatments. At 10 WAP, culm DM at NT1 and NT2 application times showed no significant difference, only NT2 was significantly higher than NT3 but all N treatments were significantly higher than the 0 N. At 14 WAP, culm dry weight of plants in control plots was significantly lower than in N treatments, but among the N application times, no significant differences existed (Figure 4.7). For manure effects, culm DM at 7 WAP increased significantly in the 4 tons/ha; 6 tons/ha resulted in additional increment but was not significantly different from the 4 tons/ha rate. At reproductive stages, culm dry matter in all manure added treatments was significantly higher than 0 manure rate treatment (Figure 4.8).

In the 2014 minor season, mean culm DM at 7 WAP at NT1 and NT2 application times were significantly higher than at NT3 application time. At 10 WAP, even though NT2 application time had numerically greater culm DM, no significant difference was found among the times of N application. At 14 WAP, culm DM was significantly higher at NT2 than at NT3 application time (Figure 4.9). Culm DM from 0 and 2 tons/ha manure rates showed no significant difference at all periods. At 7 WAP, 0 and 2 tons/ha rates were significantly lower than 4 and 6 tons/ha rates. At 10 and 14 WAP, 0 manure effect continued to be significantly lower than those of 4 and 6 tons/ha manure rates but 2 tons/ha manure rate was only significantly lower than 6 tons/ha rate (Figure 4.10).

In 2015 season, maize culm dry weights at late vegetative stage and reproductive stages were significantly higher at all N applied than 0 N treatments. Up to 10 WAP, culm DM at 30, 60 and 90 kg N/ha rates were not significantly different. At 14 WAP, culm dry weight at maximum N rate was significantly higher than minimum N rate but not significantly different from the optimum N rate (Figure 4.11). At 7 WAP, manure effect significantly increased culm dry weight at the 6 tons/ha rate; at reproductive stages, application of cattle manure at any of the three rates significantly increased culm dry weight over the control treatment (Figure 4.12).

4.4.2.2 Leaf dry matter

Leaf DM in 2014 major season was consistently significantly higher in nitrogen treated plants than 0 N treatment. NT1 and NT2 application times showed no significant difference throughout the three measurement stages. But at NT3, it was significantly lower than NT1 at 7 WAP, significantly lower than both NT1 and NT2 at 10 WAP; and at 14 WAP, no significant differences existed among the times of nitrogen application

(Figure 4.7). Cattle manure effect significantly increased leaf DM in the 4 and 6 tons/ha at 7 WAP. At reproductive stages, manure effect on leaf DM was significant in all manure treatments as compared to the control (Figure 4.8).

Leaf DM in 2014 minor season was also consistently higher in NT2 application time but was not significantly different from NT1 application time; however, at both NT1 and NT2 application times, leaf dry weights were significantly higher than NT3 application time at all three stages (Figure 4.9). At 7 WAP, leaf DM was significantly higher in the 6 tons/ha manure rate than the control; at physiological maturity, it was significantly higher in the 4 and 6 tons/ha rates than the control. At 14 WAP, leaf DM became significantly higher at all manure applied treatments than the control but among the three manure rates, no significant differences were observed (Figure 4.10).

In 2015 season, leaf dry weight was higher in the higher nitrogen rates than lower rates. At 7, 10 and 14 WAP, leaf dry weight from 0 and 30 kg N/ha rates was significantly lower than in the 90 kg N/ha rate. The 90 and 60 kg N rates were not significantly different at any of the DM measurement stages (Figure 4.11). Cattle manure significantly increase leaf dry weight in the 6 tons/ha rate at 7 WAP. During reproductive stages, 4 and 6 tons/ha manure applied treatments had significantly higher leaf dry matter than where no manure was applied (Figure 4.12).

4.4.2.3 Ear dry matter

In 2014 major season, ear dry weight at early and late reproductive stages was significantly higher in the nitrogen applied treatments than 0 N treatment. At reproductive stages, ear DM was always higher at NT2 application time, but the difference between it and the other N application times was not significant at R2 stage.

At physiological maturity stage, DM at both NT1 and NT2 application times were significantly higher than that of NT3 (Figure 4.7). Ear DM was significantly higher in the 4 and 6 tons/ha manure rates than in the control and 2 tons/ha rate (Figure 4.8).

In 2014 minor season, ear dry weight was also consistently highest at NT2 application time but was not significantly different from that of NT1 application time. Effects of NT1 and NT2 application times on ear DM were, however, significantly higher than that of NT3 application time (Figure 4.9). Manure effect on ear dry weight at R2 was significantly higher in the 4 and 6 tons/ha manure rates treatments than in the 0 control treatment; at physiological maturity, it was significantly higher at all manure applied treatments than the control (Figure 4.10).

In 2015 season, mean ear dry weights at R2 and R6 stages were significantly lower in 0 N than from any of the other N rate treatments. At R2, ear DM from 30 kg N rate was significantly lower than from 90 kg N rate. At R6, it was significantly lower in 30 kg N rate than both the 60 and 90 kg N rates. The 60 and 90 kg N rates, however, showed no significant difference at both stages (Figure 4.11). Manure effect on ear dry weight at R2 was significantly lower in the 0 and 2 tons/ha manure rates treatments than in the 4 and 6 tons/ha manure rates treatments. At R6, application of 2, 4 or 6 tons/ha manure rates did not result in significant differences in ear DM, however all manure applied treatments had significantly higher ear DM at physiological maturity than the control treatment (Figure 4.12).

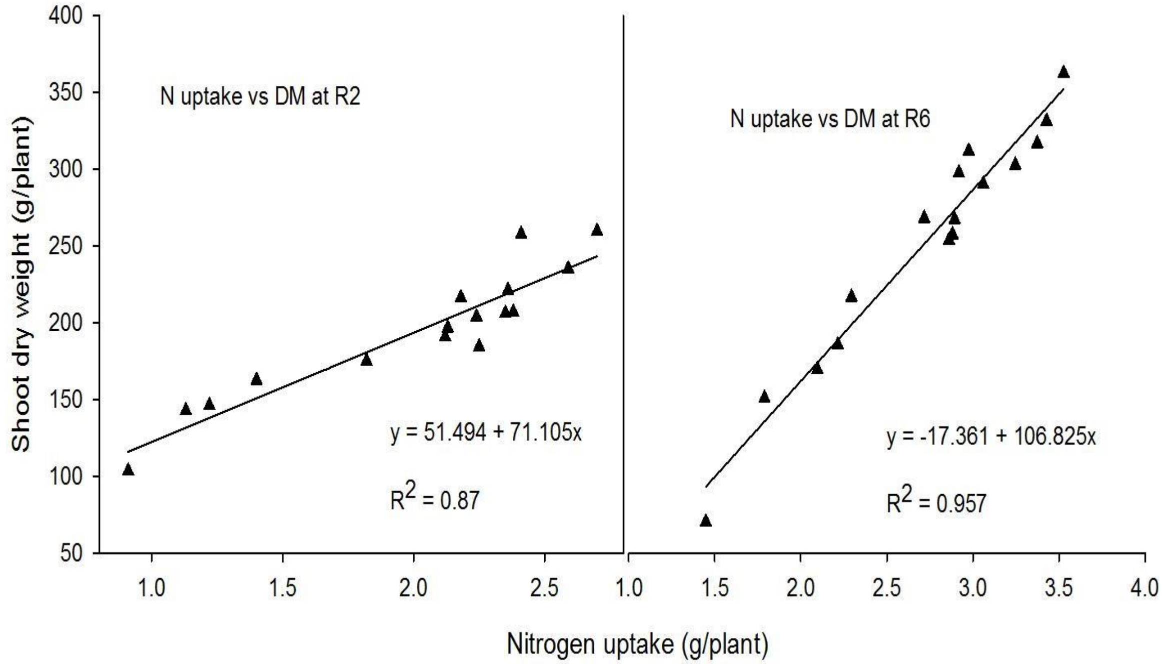


Figure 4.13: Relationship between aboveground plant nitrogen uptake and shoot dry matter at R2 and R6 stages in the 2014 major season

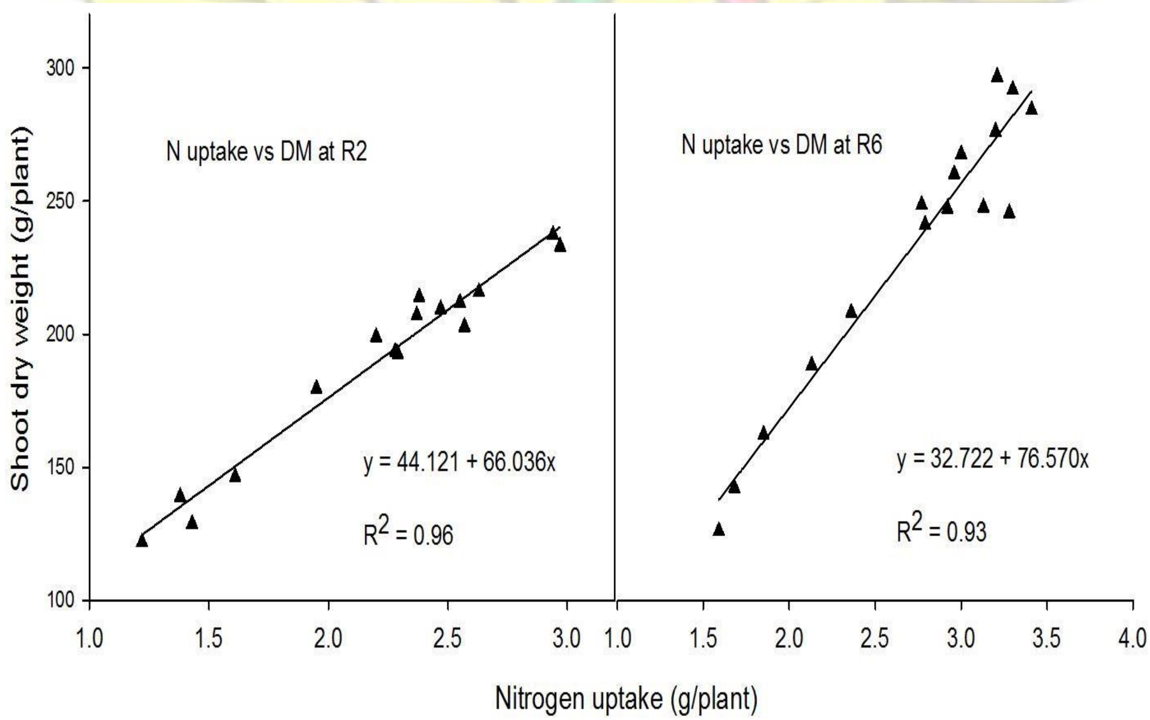


Figure 4.14: Relationship between aboveground plant nitrogen uptake and shoot dry matter at R2 and R6 stages in the 2014 minor season

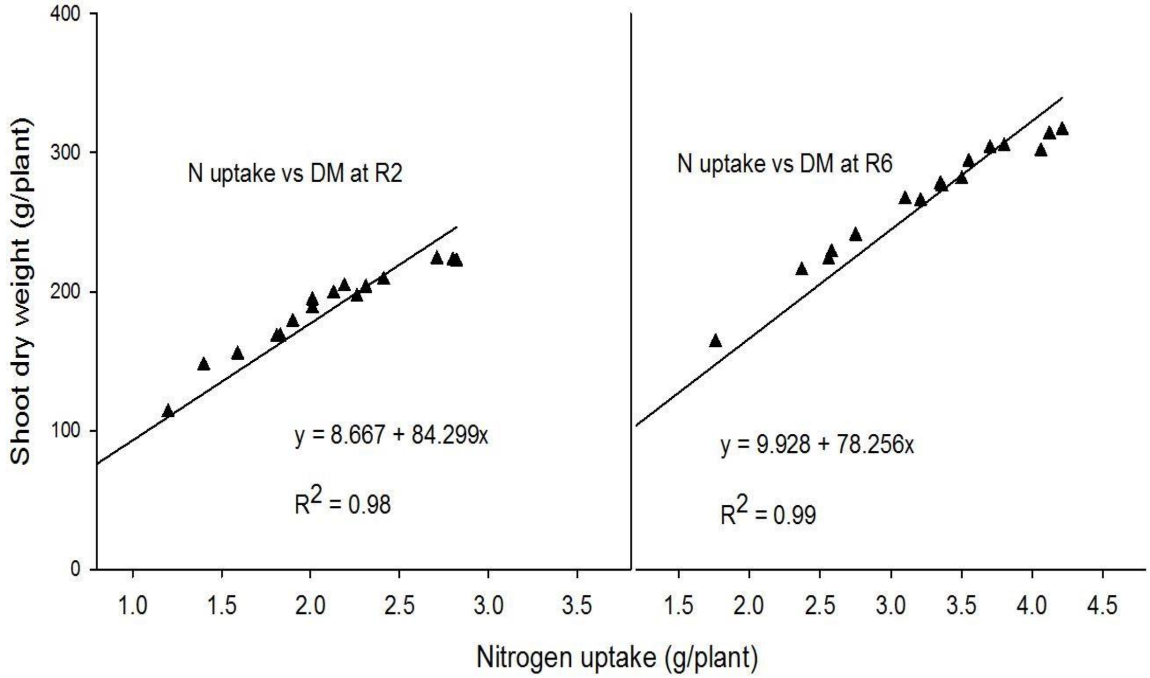


Figure 4.15: Relationship between aboveground plant nitrogen uptake and shoot dry matter at R2 and R6 stages in the 2015 major season

Table 4.14: Correlation between dry matter content of different plant parts and total N partitioned to those plant parts at two reproductive stages.

Dry matter	2014 major season		2014 minor season		2015 major season	
	Nitrogen content					
	R2	R6	R2	R6	R2	R6
Culm	0.616**	0.595*	0.811***	0.870***	0.835***	0.889***
Leaf	0.380	0.553*	0.786***	0.806***	0.802***	0.925***
Ear	0.767***	0.668**	0.884***	0.657**	0.961***	0.711***

*, **’ ***: significant at P < 0.05, P < 0.01 and P < 0.001 respectively

Dry matter production by maize plants from two weeks after completion of silking up to physiological maturity was highly dependent on the amount of nitrogen uptake and accumulation in shoots. This strong positive relationship is indicated by the high regression coefficients in Figures 4.13 to 4.15. Also, significant positive correlations were found between dry matter accumulation of plant parts and the amount of N partitioned to those parts at blister and at physiological maturity stages (Table 4.14)

4.4.3 Discussion

Shoot dry matter partitioning among plant parts followed a similar pattern in all the experiments. At late vegetative stage (7 WAP), 60% of the total shoot DM was accumulated in the culms. At R2 stage, the developing maize ears accumulated about 20% of the total aboveground DM mainly at the expense of leaves whose share of the shoot DM declined by almost the same percentage. At 14 WAP when the maize reached physiological maturity, the amount of shoot DM partitioned to vegetative parts continued to decline whilst more of the DM was partitioned to the ears. This DM partitioning pattern was not significantly affected by treatment differences. The partitioning followed a similar pattern as previously reported by Subedi and Ma (2007).

Aboveground plant dry matter accumulation data presented in Tables 4.11 and 4.12 showed that NT2 time of N application always resulted in greater plant DM at reproductive stages but was not significantly higher than at NT1. Therefore, application of the required N at NT2 period should enable better dry matter production. Application of N at NT3 which involves delaying top dressing of half of the N until 8 WAP i.e. after completion of silking, may still produce as much plant DM as NT1 and NT2 application times at plant maturity in the major season when moisture conditions are ideal (Table

4.11), but in the minor season when there is less moisture, plant growth ceases early so it will have little or no positive effect on total DM production.

The amount of total shoot DM partitioned to vegetative parts at late vegetative up to early reproductive stages was significantly higher at NT1 and NT2 application times than at NT3 application time, but at maturity, that gap was closed in the major season. The high DM accumulation between R2 and R6 stages observed at NT3 application time which helped it close the gap with NT1 and NT2 times did not, however, translate into higher DM partitioning to maize ears because the percentage of total aboveground DM partitioned to ears at R6 was significantly lower at NT3 than at NT1 and NT2.

In the 2015 major season, nitrogen effect, even at a minimum rate of one third of the N recommended rate (30 kg) produced significantly higher total aboveground plant dry matter than when no mineral N was applied. Shoot DM manifested a linear increment as N rate increased. This result is in agreement with that of Ziadi *et al*. (2008) who reported that maize shoot biomass generally increased with increasing N fertilization, although the effect was not always statistically significant. Similar findings were also reported by Rahman *et al*. (2008) and Lomer and Ali-zade (2013).

In terms of DM partitioning, 30, 60 and 90 kg N rates did not significantly differ in culm dry matter up to R2 stage, but at R6, 90 kg N rate effect was significantly higher than that of 30 kg N rate. Leaf DM on the other hand, showed significant differences as early as at 7 WAP when the highest N rate effect was significantly higher than that of lowest N rate. From this partitioning pattern, it could be stated that maize leaves are better indicators of effects of nitrogen rate on shoot dry matter partitioning than culms.

Dry matter production of a maize crop largely depends on the function of leaf area development and consequential photosynthetic activity. Leaf area development and photosynthetic rate are highly responsive to N fertilization (Tajul *et al*., 2013). If leaf number, green leaf area and leaf longevity are enhanced, vegetative growth period of maize can be improved thereby increasing photosynthetic production. Increase in photosynthetic rate and duration increase dry matter production (Amanullah, 2007). Variations in culm, leaf and total aboveground plant dry weights observed between different N application timings, N rates and manure rates could, therefore, be due to the varying effects on these leaf growth parameters. Rajcan and Tollenaar (1999) reported that increase in N rate increased leaf longevity and photosynthesis in maize which resulted in higher dry matter production. Amanullah and Shah (2011) also reported significant effect of rates and times of nitrogen application on maize leaf and stem dry weights at silking and physiological maturity stages.

The lack of significant difference between 60 and 90 kg N rates in terms of total shoot dry weight at all three growth stages (Table 4.13) as well as DM partitioning to culms, leaves and ears (Figure 4.11) indicates that application of 60 kg N rate would have been sufficient to produce as much aboveground plant DM as the 90 kg N rate.

At late vegetative stage, manure effect on total shoot dry matter accumulation was found to be significant only in the highest manure rate in most cases. At R6 stages, however, manure applied at any of the rates used in this study was able to produce significantly higher plant DM in comparison to no manure application (Tables 4.11, 4.12 and 4.13). Manure application not only adds N to the soil but also other mineral nutrients such as P, K, Ca, improves soil physical properties, raise soil pH and provide readily available C substrate for microbial growth (Khoshgoftarmanesh and Eshghizadeh, 2011). These factors aid the uptake of N and other nutrients which impact positively on biomass production. Zingore *et al*. (2008) reported that maize biomass yield showed no significant response to different N and P treatments, but when manure was added to them, the treatments showed significant differences, which, according to them indicates that manure alleviated other deficiencies that limited responses to N and P.

The positive relationships established between plant dry matter accumulation and nitrogen uptake and N partitioning in Figures 4.13, 4.14, 4.15 and in Table 4.14 shows that dry matter production in maize is strongly influenced by N availability. Therefore, nitrogen management practices that makes N available to plants when needed most are ideal to improve maize biomass production and subsequently grain yield. Among the times of N application, the superiority in shoot dry matter accumulation manifested at NT2 was due to the higher N uptake by plants at that application time (Table 4.1) which translated into higher vegetative growth. Higher rates of dry matter accumulation in maize during grain filling period have been associated with increased "stay green," and higher N uptake during this period (Echarte *et al*., 2008). Worku *et al*. (2007) also found a strong relationship between N uptake in maize and total aboveground biomass at physiological maturity with correlation coefficients ranging from 0.78 to 0.92
(significant at $P < 0.05$) in different environments.

4.5 Plant growth parameters

4.5.1 Leaf number, green leaf area and leaf area index

Leaf number, green leaf area and leaf area index were not significantly different among manure rates, times of nitrogen application or their interaction at 5 WAP; hence, data at this growth stage are not shown. Main effects of manure rates and times of nitrogen application at 7 and 9 WAP are presented in Tables 4.15 and 4.16 respectively.

With the exception of leaf number at 9 WAP in the minor season, leaf number, green leaf area and LAI at 7 and 9 WAP in the major and minor seasons were consistently significantly higher in the N applied treatments than the control treatment. Mean leaf number at NT3 time of N application at 7 WAP was significantly lower than at NT1 in the major season; in the minor season, it was significantly lower than the mean at both NT1 and NT2 application times (Table 4.15). At 9 WAP, the effect at NT3 was significantly lower than at NT1 and NT2 in the major season but in the minor season, only at NT2 was it significantly higher than at NT3 (Table 4.16). Leaf number at NT1 and NT2 application times showed no significant difference throughout in both seasons.

Manure effect on leaf number at 7 WAP was significant in the 6 tons/ha manure rate in the major season, in the minor season, there was significant effect in the 4 and 6 tons/ha rates (Table 4.15). At 9 WAP, 2, 4 and 6 tons/ha manure rates effect on leaf number was significantly higher than at the control treatment in the major season, but in the minor season, at 2 tons/ha manure rate, it was not significantly different from the control treatment (Table 4.16).

Table 4.15: Effect of time of nitrogen application and cattle manure rates on leaf number, green leaf area and leaf area index of maize at late vegetative stage (7 WAP) in a major and minor season.

Treatment	2014 major season			2014 minor season		
Time of N application	Leaf number plant^{-1}	Green leaf area plant^{-1} (cm^2)	Leaf area index	Leaf number plant^{-1}	Green leaf area plant^{-1} (cm^2)	Leaf area index

0 N	10.32	4383.48	2.74	10.19	3986.89	2.49
NT1	11.83	6260.11	3.91	11.53	5233.60	3.27
NT2	11.48	6175.75	3.86	11.59	5226.43	3.27
NT3	11.24	5894.27	3.68	11.18	4973.77	3.11
LSD (0.05)	0.45	348.30	0.22	0.36	317.96	0.20
CV (%)	5.6	8.7	8.7	4.5	9.2	9.2
Manure rate (tons/ha)						
0	10.92	5341.91	3.34	10.39	4476.35	2.80
2	11.05	5510.75	3.44	10.72	4804.95	3.00
4	11.38	5938.71	3.71	11.29	5102.05	3.19
6	11.51	5922.24	3.70	11.58	5037.35	3.15
LSD (0.05)	0.47	354.01	0.22	0.35	323.48	0.20
CV (%)	5.8	8.8	8.8	4.4	9.4	9.4
TNA x MR	NS	*	*	NS	*	*

TNA: time of nitrogen application; MR: manure rate; * significant at P<0.05, NS: not significant at P>0.05. Means of N application times were obtained across the 4 manure rates and means of manure rates were obtained across N application times including 0 N.

Table 4.16: Effect of time of nitrogen application and cattle manure rates on leaf number, green leaf area and leaf area index of maize at one week after completion of silking (9 WAP) in a major and minor season

Treatment	2014 major season			2014 minor season		
Time of N application	Leaf number plant^{-1}	Green leaf area plant^{-1} (cm^2)	Leaf area index	Leaf number plant^{-1}	Green leaf area plant^{-1} (cm^2)	Leaf area index
0 N	10.84	4845.92	3.03	11.06	4774.91	2.98
NT1	12.92	6465.54	4.04	11.66	6470.84	4.05

NT2	12.81	6539.51	4.09	11.89	6361.77	3.98
NT3	12.17	6377.08	3.99	11.41	6121.24	3.83
LSD (0.05)	0.39	580.01	0.36	0.36	351.22	0.22
CV (%)	4.6	13.5	13.5	4.4	8.4	8.4
Manure rate (tons/ha)						
0	11.61	5448.82	3.41	11.20	5555.84	3.47
2	12.22	5815.43	3.63	11.34	5925.80	3.68
4	12.43	6414.68	4.01	11.63	6202.00	3.88
6	12.48	6549.12	4.09	11.84	6083.13	3.80
LSD (0.05)	0.39	611.81	0.38	0.37	368.55	0.23
CV (%)	4.5	14.2	14.2	4.5	8.7	8.7
TNA x MR	*	NS	NS	*	NS	NS

TNA: time of nitrogen application; MR: manure rate; * significant at $P < 0.05$, NS: not significant at $P > 0.05$. Means of nitrogen application times were obtained across the 4 manure rates and means of manure rates were obtained across nitrogen application times including 0 N.

Leaf number was also affected by the interaction between manure rates and times of N application at 9 WAP in the major season and at 7 and 9 WAP in the minor season. In the major season, leaf number at 9 WAP was highest at NT2 application time in the 4 tons/ha manure rate. In the minor season, NT2 application time in the 6 tons/ha manure rate had the highest leaf number at 7 WAP which was significantly higher than most of the other treatments. At 9 WAP, NT1 and NT2 application times in the 6 tons/ha manure rate were significantly higher than any of the sole nitrogen and sole manure treatments, at NT3 application time in all manure rates and at NT1 in the 2 and 4 tons/ha manure rates.

Treatment effect on green leaf area (GLA) and leaf area index (LAI) followed exactly the same trend. At 7 WAP in the major season, GLA and LAI were not significantly different between NT2 and NT3 application times. The effect at NT3 was significantly lower than at NT1 which had the highest GLA and LAI. In the minor season, differences in time of N application had no significant effect on GLA and LAI at this stage (Table 4.15). At 9 WAP, green leaf area and LAI at NT1, NT2 and NT3 application times in the major as well as minor seasons showed no significant differences (Table 4.16).

Manure effect on green leaf area and leaf area index at 7 WAP was significantly higher in the 4 and 6 tons/ha manure rates treatments than the control treatment in the major season; in the minor season, it was significantly higher in all three manure applied rates than the control treatment (Table 4.15). At 9 WAP, control treatment effect on green leaf area and leaf area index was significantly lower than at any of the manure treatments in the two seasons (Table 4.16). While 4 and 6 tons/ha manure treatments showed no significant differences at the two growth stages in the two seasons, 2 tons/ha manure rate had significantly lower effect on the two parameters than the 6 tons/ha rate at 7 and 9 WAP in the major season, but in the minor season 2, 4 and 6 tons/ha manure rates showed no significant difference at both stages.

The interactions between cattle manure rates and times of nitrogen application had significantly influenced green leaf area and leaf area index at 7 WAP in both major and minor seasons. NT2 application time in the 6 tons/ha manure rate in the major season produced the highest green leaf area and leaf area index and these were significantly higher than all sole manure treatments, NT3 application time in the 0 manure treatment and NT3 in the 2 tons/ha manure rate treatment. No significant differences existed between the rests of the treatment combinations.

In the minor season also, NT2 application time in the 6 tons/ha manure rate produced the highest green leaf area and leaf area index at 7 WAP. This treatment combination was significantly higher than all manure rates at 0 N, sole nitrogen treatments and NT1 and NT3 application times in the 2 tons/ha manure rate.

Table 4.17: Effect of nitrogen rates and cattle manure rates on leaf number, green leaf area and leaf area index of maize at late vegetative stage and at one week after completion of silking in 2015 major season

Treatment	7 WAP			9 WAP		
Nitrogen rate (kg/ha)	Leaf number $plant^{-1}$	Green leaf area $plant^{-1}$ (cm^2)	Leaf area index	Leaf number $plant^{-1}$	Green leaf area $plant^{-1}$ (cm^2)	Leaf area index
0	10.30	4367.17	2.73	10.28	4659.37	2.91
30	11.20	5478.54	3.42	11.32	5875.79	3.67
60	11.71	5954.50	3.72	11.60	6081.15	3.80
90	11.77	6148.55	3.84	12.88	6912.54	4.32
LSD (0.05)	0.51	432.48	0.27	0.42	393.10	0.25
CV (%)	6.4	11.1	11.1	5.1	9.4	9.4
Manure rate (tons/ha)						
0	10.87	4937.46	3.09	11.10	5394.77	3.37
2	11.22	5433.03	3.40	11.39	5810.23	3.63

4	11.33	5713.26	3.57	11.54	5969.97	3.73
6	11.56	5865.02	3.67	12.05	6353.88	3.97
LSD (0.05)	0.435	455.15	0.28	0.43	406.45	0.25
CV (%)	6.7	11.6	11.6	5.3	9.7	9.7
NR x MR	NS	NS	NS	NS	NS	NS

NR: nitrogen rate; MR: manure rate; NS: not significant at $P > 0.05$. Means of nitrogen rates were obtained across the 4 manure rates and means of manure rates were obtained across nitrogen rates including 0 N.

At 7 WAP, leaf number at the minimum nitrogen rate was significantly lower than at both the optimum and maximum N rates, but at 9 WAP, minimum nitrogen rate effect was lower than only the maximum N rate effect. While minimum, optimum and maximum nitrogen rates all had significantly higher leaf numbers than the control at the two stages, minimum and optimum N rates were both significantly lower than the maximum N rate at 9 WAP (Table 4.17).

Manure application significantly increased leaf number at both stages in the 4 and 6 tons/ha manure rates. Mean leaf number in the 2 tons/ha rate treatment was not significantly different from the control treatment (Table 4.17).

Green leaf area and leaf area index were significantly lower in the control treatments than any of the other N rate treatments at 7 and 9 WAP (Table 4.17). Like leaf number, GLA and LAI at minimum N rate were significantly lower than both the optimum and maximum N rates at 7 WAP; at 9 WAP, the minimum and optimum N rates showed no significant difference but were both significantly lower than the maximum N rate.

At 7 WAP, effect of manure on green leaf area and leaf area index was significantly higher at all manure treatments than the control treatment. However, the three manure applied rates at this stage had statistically similar effect on these two leaf parameters. At 9 WAP, GLA and LAI in the 6 tons/ha manure rate treatment was significantly higher than in the 2 tons/ha rate but 2 and 4 tons/ha rates showed no significant differences (Table 4.17).

Table 4.18: Correlation between nitrogen uptake and nitrogen use efficiency; and leaf number, green leaf area and leaf area index

Nitrogen parameter	Leaf number	Green leaf area	Leaf area index
2014 major season			
N uptake	0.783***	0.821***	0.821***
NUE	0.741***	0.719***	0.719***
2014 minor season			
N uptake	0.371	0.547*	0.547*
NUE	0.489	0.544*	0.544*
2015 major season			
N uptake	0.706***	0.689**	0.689**
NUE	0.405	0.472	0.472

N uptake: total shoot nitrogen at physiological maturity; NUE: nitrogen use efficiency; Leaf number, Green leaf area and LAI at 9 WAP; *, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

Data presented in Table 4.18 showed significant positive correlations between leaf numbers, green leaf area and leaf area index at one week after completion of silking and nitrogen uptake and use efficiency in all three seasons. Even though both N parameters showed significant correlation with these growth parameters except for NUE in 2015 season, correlation coefficients indicated a stronger relationship between them and N uptake than NUE.

4.5.2 Crop growth rate

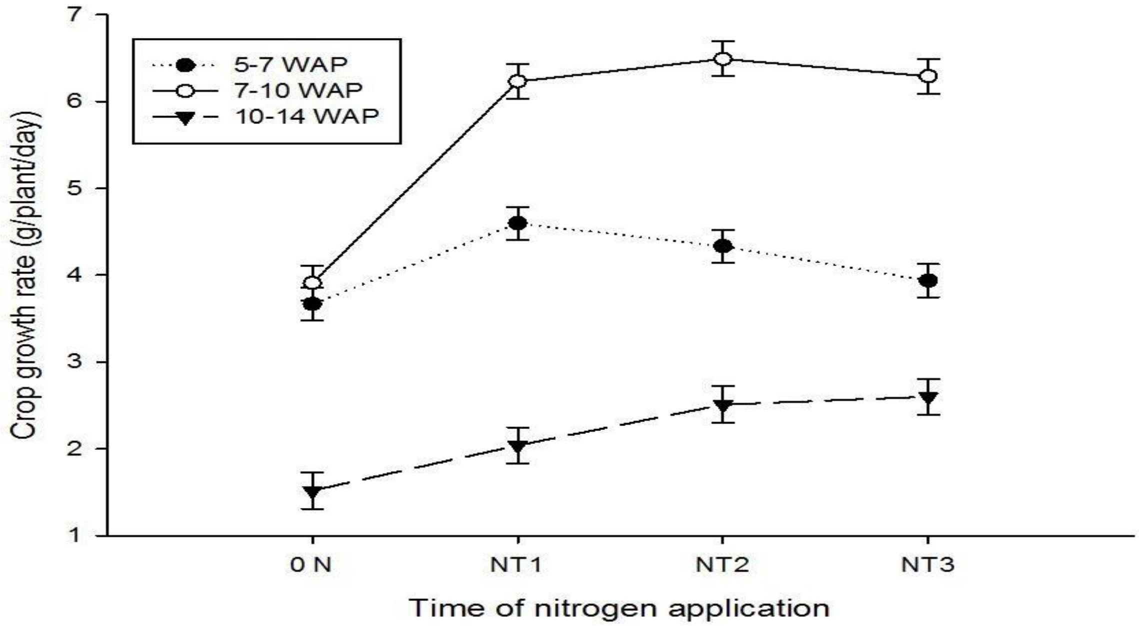
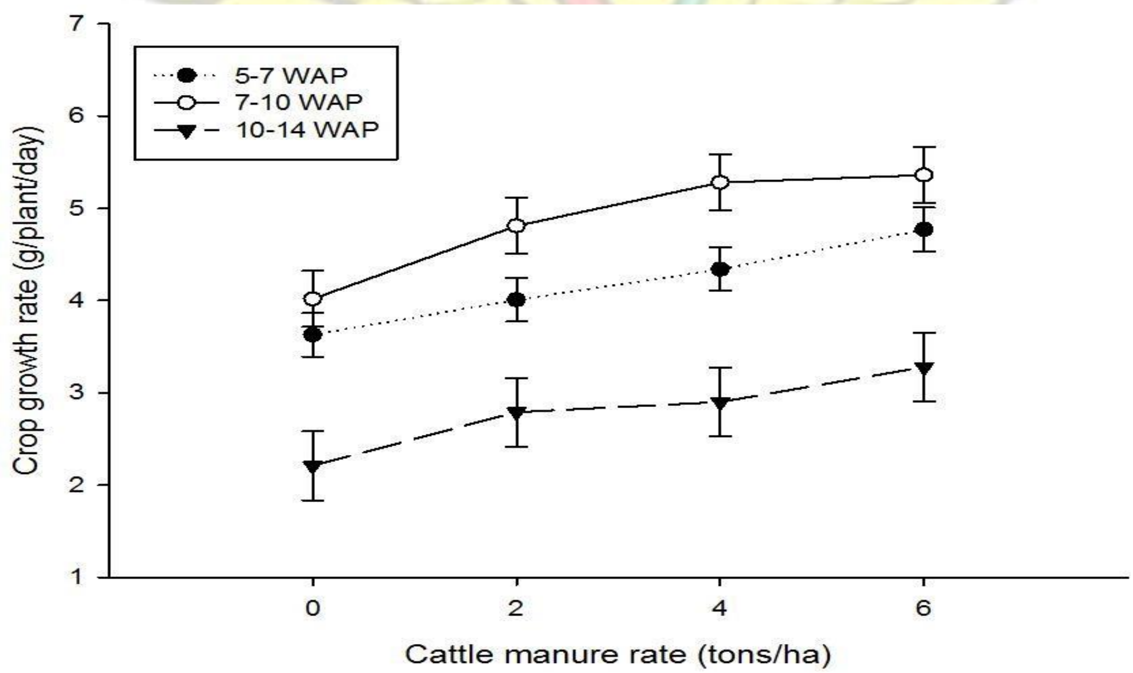


Figure 4.16: Effect of time of N application on maize crop growth rate during three growth periods in the 2014 major season. Bars represent SE.



Bars represent SE.

Figure 4.17: Effect of manure rate on maize crop growth rate during three growth periods in the 2014 major season.

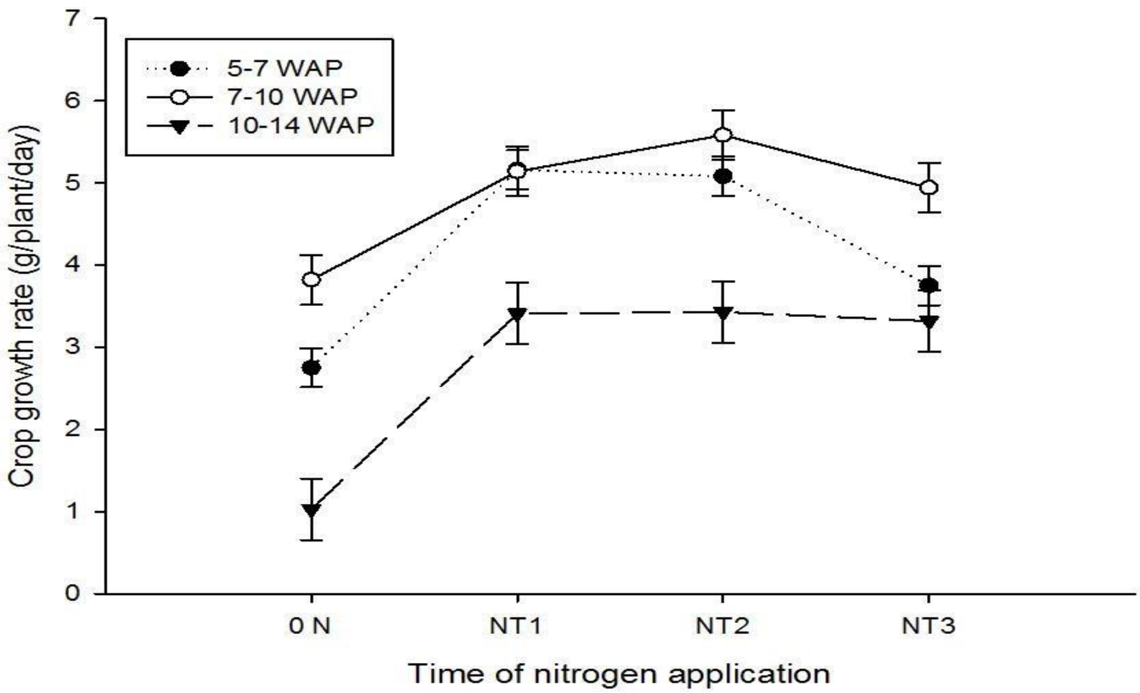


Figure 4.18: Effect of time of N application on maize crop growth rate during three growth periods in the 2014 minor season. Bars represent SE

Bars represent SE.

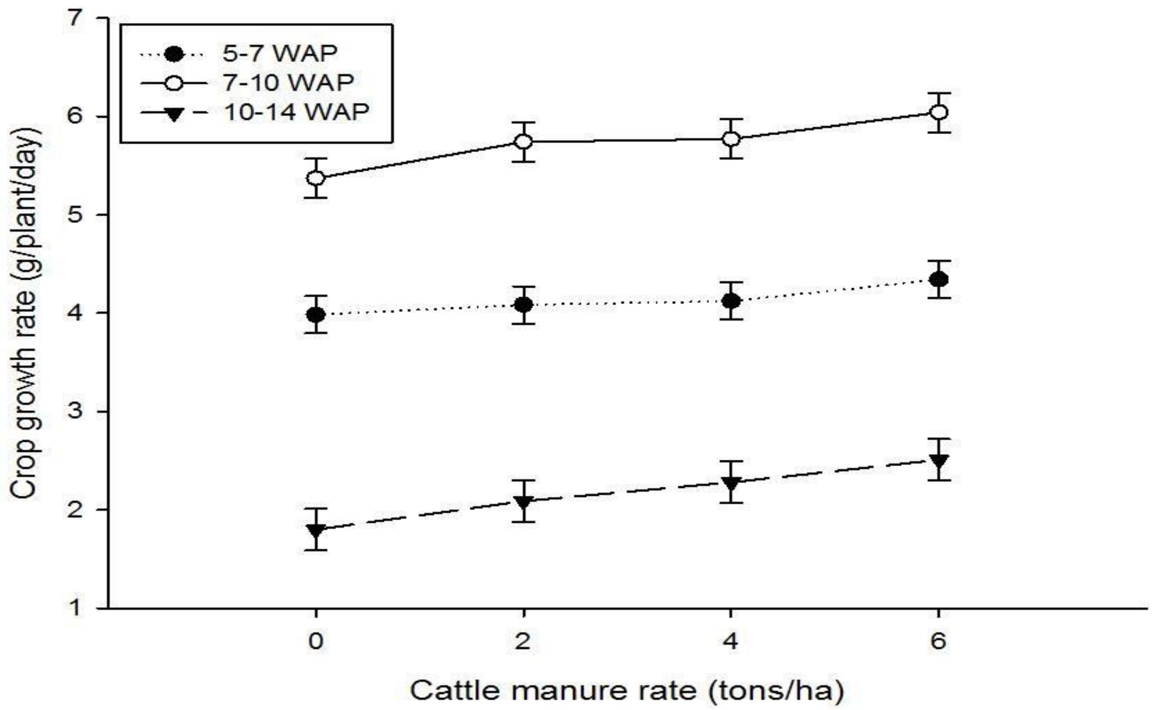
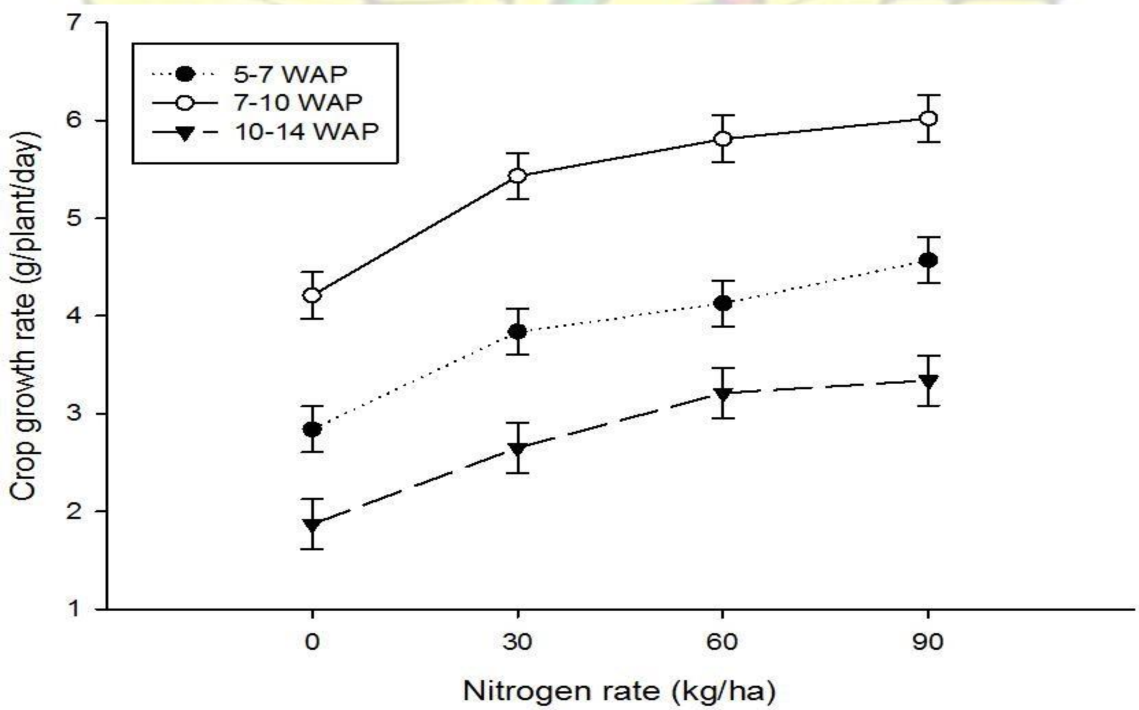


Figure 4.19: Effect of manure rates on maize crop growth rate during three growth periods in the 2014 minor season.



Bars represent SE.

Figure 4.20: Effect of nitrogen rates on maize crop growth rate during three growth periods in the 2015 major season. Bars represent SE.

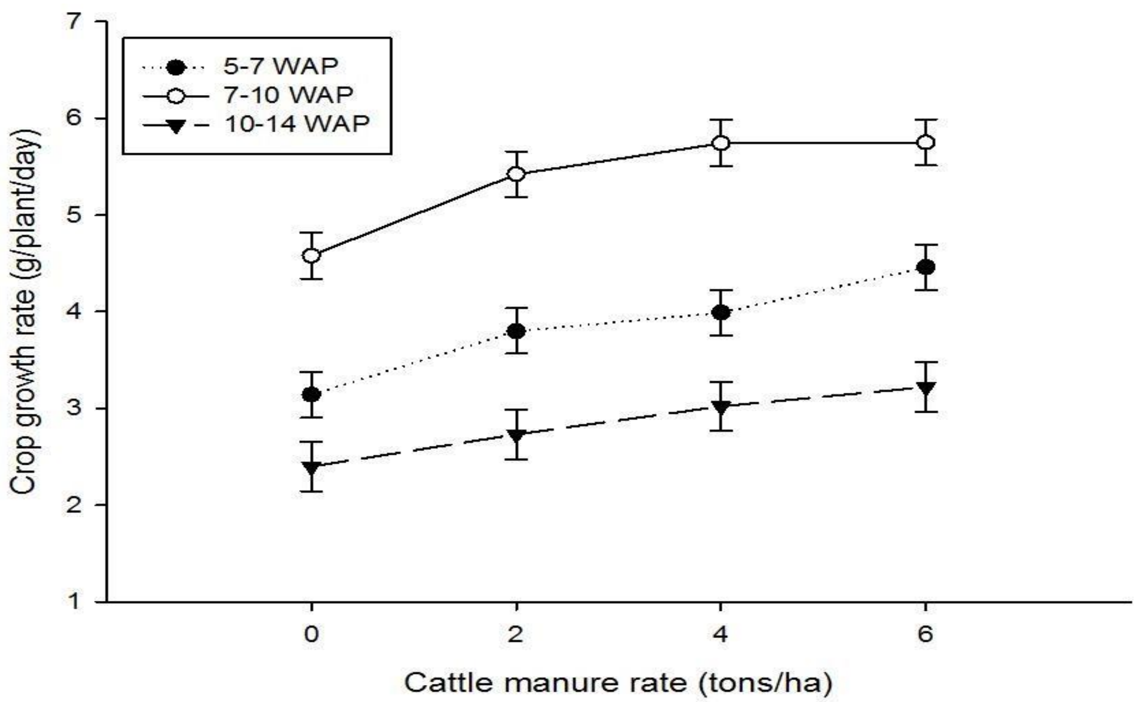


Figure 4.21: Effect of manure rates on maize crop growth rate during three growth periods in the 2015 major season.

Bars represent SE.

In the 2014 major season, among the two factors studied, crop growth rate was mainly influenced by differences in time of N application than by differences in manure rates applied. The interaction between manure rates and times of N application was not significant hence interaction data are not shown. Out of the three growth periods at which CGR was measured, highest growth took place at 7-10 WAP growth period in all treatments, followed by the 5-7 WAP period and the least CGR occurred during the 1014 WAP growth period (Figures 4.16 and 4.17).

During 5-7 WAP growth period, CGR was highest at NT1 application time but was not significantly different (P > 0.05) from the NT2 application time. During 7-10 WAP growth period, nitrogen applied at NT2 application time effected the most vigorous crop growth rate but was not significantly different from those of NT1 and NT3 application times. However, CGR was significantly higher at all N applied treatments than the control. During the 10-14 WAP growth period, NT3 application time produced the highest CGR which was significantly higher than at NT1, but not that of NT2 application time. Crop growth rate in the control treatment continued to be significantly lower than the nitrogen applied treatments. Crop growth rate during the 10-14 WAP growth period declined by 2.39 g/plant/day at the control treatment, 4.19 g/plant/day at NT1, 3.98 g/plant/day at NT2 and 3.69 g/plant/day at NT3 application times when compared to what was obtained during the 7-10 WAP period (Figure 4.16).

Cattle manure, like nitrogen, had greater effect on crop growth rate during 7-10 WAP growth period. Manure effect on CGR at 5-7 WAP growth period was significant when the manure was applied at 4 tons/ha. Crop growth rate from the 6 tons/ha manure treatment was not significantly different from that obtained from the 4 tons/ha rate but was significantly higher than the 2 tons/ha rate. At 7-10 WAP and 10-14 WAP growth periods, Control treatment effect was significantly lower than any of the manure treatments, but among the manure rates there was no significant difference (Figure 4.17).

In the 2014 minor season, among the three crop growth rate measurement periods, the highest growth rate occurred at 7-10 WAP growth period in all treatments followed by the 5-7 WAP growth period and the least growth rate was recorded at 10-14 WAP (Figures 4.18 and 4.19).

Crop growth rate in the control plots was consistently significantly lower than in the N treated plots. During 5-7 WAP growth period, mean CGR at NT1 application time was greater. It was not significantly different from the mean at NT2 application, but was significantly higher than that of the NT3 application time. At 7-10 WAP and 10-14 WAP growth periods, even though CGR was slightly higher at NT2, no significant difference existed between it and the other N application times (Figure 4.18). With the exception of NT1 application where no increments were observed, CGR values recorded for 0 N, NT2 and NT3 applications at 5-7 WAP increased by 1.07 g/plant/day, 0.5 g/plant/day and 1.19 g/plant/day respectively at 7-10 WAP. Crop growth rate during the 10-14 WAP growth period when compared to what was obtained at 7-10 WAP growth period declined by 73% in the 0 N treatment, 34% in NT1 application, 39% in NT2 application and 33% in NT3 application (Figure 4.18).

Manure effect on CGR during 5-7 WAP growth period in the minor season was not significantly different among manure rates. At 7-10 WAP and 10-14 WAP growth periods, manure effect was significantly higher in the 6 tons/ha manure rate. Application of manure at the rates of 2 and 4 tons/ha did not effect significant increment in CGR in comparison to no manure application (Figure 4.19).

In the 2015 major season experiment where nitrogen rates were combined with manure rates, only the main effects of the two factors affected crop growth rate. Out of the three growth periods at which CGR was determined, the highest growth rates were recorded at the 7-10 WAP period in all treatments, followed by the 5-7 WAP period and the least CGR was recorded during the 10-14 WAP growth period (Figures 4.20 and 4.21).

Crop growth rate at all three growth periods was significantly higher in all N applied rates than the control. Even though CGR increased slightly as nitrogen rate increased the differences between 30, 60 and 90 kg/ha N rates were not significant ($P > 0.05$) at all three determination periods (Figure 4.20). Crop growth rate at 10-14 WAP when compared to values obtained during the 7-10 WAP period had declined by 56% in the control plots, 51% in the 30 kg N rate, and by 45% in the 60 and 90 kg N rates.

Manure effect on CGR in the 2015 major season experiment, like in the 2014 major season experiment was significant in the 4 and 6 tons/ha manure rates treatments during the 5-7 WAP growth period. At later growth periods, however, the effect was significantly higher in all the manure applied rates than the control (Figure 4.21).

4.5.3 Discussion

Differences in time of nitrogen application, nitrogen rates, and cattle manure rates or the interaction between nitrogen and manure did not show significant effect on leaf number, green leaf area and leaf area index up to 5 WAP. Differences started to show at late vegetative stage upwards. However, the control treatment effect was significantly lower than the nitrogen treatments even at the mid vegetative stage. This observation is in agreement with the findings of Onasanya *et al*. (2009) that at 5 WAP, differences in rates of fertilizer application did not significantly affect maize leaf parameters, but significant differences started to show at 6, 7 and 8 WAP.

Number of green leaves on plants by the end of flowering, which in maize is known to be the stage by which leaf production ceases (Paponov and Engels, 2005) was similar between NT1 and NT2 application times. Delay of top dressing of half of the N as in the case of the NT3 application time, led to significantly lower leaf numbers as compared to the other two N application times (Table 4.16). In terms of N rate effect, leaf number was found to increase as N rate becomes higher, but maximum N rate produced statistically similar leaf numbers as the optimum N rate (Table 4.17). Leaf number in all N applied treatments was significantly higher than the control.

Number of green leaves retained on plants greatly affects photosynthetic capacity and dry matter production of the plant while early leaf shedding limits photosynthesis and dry matter production (Gungula *et al*., 2005). Therefore, if more leaves are retained on the plants by delaying senescence, dry matter production may be maintained for a longer period leading to higher biomass production and subsequently higher grain yield.

The significant differences observed in leaf number among N application times, N rates and manure rates at the various growth stages is an indication that the number of leaves produced by maize plants and leaf longevity are affected by these factors.

Treatment effect on green leaf area and leaf area index followed exactly the same pattern because LAI was calculated from the GLA, i.e. LAI was determined as green leaf area per unit ground area. Green leaf area and LAI showed a direct link to N availability such that at 7 WAP, highest GLA and LAI were obtained at NT1 application (Table 4.15). But at 9 WAP, when all N treatments had received complete N supply, the two parameters became statistically the same for all N application times (Table 4.16). Green leaf area and LAI were also found to increase as N rate increased. The difference between the lower and higher nitrogen rates was even higher at reproductive stages than at late vegetative stage. The differences in GLA and LAI could be attributed to the varying treatment effects on leaf number, leaf size and leaf longevity which are all traits that affect leaf area and leaf area index. Nitrogen rates and time of application having significantly different effects on maize leaf characteristics was also previously reported by Hammad *et al*. (2011).

At 7 WAP, leaf number per plant at 30, 60 and 90 kg N/ha rates increased by 8.7%, 13.7% and 14.3% respectively when compared to the 0 N. At the same time, GLA and LAI increased by 25.4%, 36.3% and 40.8%, respectively. At 9 WAP, leaf number had increased by 10.1%, 14.8% and 25.3% in the 30, 60 and 90 kg N rates treatments, respectively in comparison to 0 N. Green leaf area and LAI at the same time increased by 26.1%, 30.5% and 48.4% (Table 4.17). This trend indicates that GLA and LAI were more responsive to increments in nitrogen rate than leaf number. This is in agreement with the findings of Vos *et al*. (2005) who reported that maize leaf number was not affected by N supply, but a 30% difference in leaf area was found between lower and higher N rates.

Leaf number, green leaf area and leaf area index were all positively correlated with nitrogen uptake and nitrogen use efficiency (Table 4.18). This means that, the treatments that had higher N uptake and N use efficiency produced more leaf numbers, and developed larger leaf areas and leaf area index. These plant growth parameters determine maize crop photosynthetic capacity. Correlation between N uptake and leaf growth dynamics in maize is supported by the findings of Amanullah *et al*. (2009) who reported that increased nitrogen uptake by maize plants led to increased photosynthesis and partitioning of more dry matter to leaves and thus, resulted in higher leaf area per plant. Similar results were also reported by Azeez (2009).

Manure effect on leaf number, green leaf area and leaf area index was minimal at late vegetative stage with significant increments realized only in the highest manure rate; but by the start of grain formation, all three manure applied rates used in this study enabled the production of greater leaf numbers, GLA and LAI than the control (Tables 4.15, 4.16 and 4.17). Manure at the rates of 4 and 6 tons/ha had statistically the same effect on these leaf parameters at vegetative as well as reproductive stages. However, 2 tons/ha manure rate was in most instances significantly lower than 6 tons/ha rate. This shows that application of cattle manure at a rate of as low as 2 tons/ha can significantly increase these growth parameters, however, application of 6 tons/ha will provide additional significant increments.

The effect of interaction between N application times and manure rates on GLA and LAI were significant at 7 WAP, but not at 9 WAP. At 9 WAP only leaf number was affected by the interaction between N application times and manure rates. This was possible due to the fact that by 9 WAP, leaf growth differences between N application times had been closed due to the higher growth that took place in NT3 application plots between 10-14 WAP as result of the N top dressing they received at 8 WAP (Figures 4.16 and 4.18). In maize, leaf number, green leaf and LAI responding differently under different nitrogen management practices has been widely reported. Bakht *et al*. (2006) reported that various levels of N fertilizer had significantly ($p < 0.05$) affected the number of leaves produced by maize plants. Tollenaar and Lee (2002) reported that the appearance of leaves in maize was decreased with decreasing available nitrogen. Boonlertnirun *et al*. (2010) reported that different nitrogen rates had marked effects on leaf area in maize.

Leaf growth characteristics have a direct consequence on biomass production and grain yield of maize. Leaf area influences the interception and utilization of solar radiation which consequently drive dry matter accumulation and grain yield (Valentinuz and Tollenaar, 2006). Therefore, any effort geared towards improving maize growth and yield must include improving the crop's leaf number, green leaf area and leaf longevity.

Crop growth rate varied mainly between times of N application and N rates than between manure rates at all growth stages. Highest CGR occurred between 7-10 WAP and the period between 10-14 WAP witnessed the least CGR. This crop growth rate pattern was consistent throughout the study and was not affected by treatment differences. Tajul *et al*. (2013) also reported CGR, regardless of differences in N levels to have increased progressively and reaching peak at 65 days after sowing (9 WAP).

Crop growth rate was also found to be consistently higher in nitrogen treatments, irrespective of time of nitrogen application (Figures 4.16 and 4.18) and nitrogen rate (Figure 4.20) than at 0 N.

The ability of maize to maintain a substantial growth rate during reproductive stages is known to have positive effect on grain yield. Results of this study showed a general decline in CGR from R2 to R6 stage in comparison to earlier growth periods, which is a normal phenomenon in maize. The decline was, however, severe in 0 N plots (73%) than the 34%, 39% and 33% observed in NT1, NT2 and NT3 application times, respectively (Figures 4.16 and 4.18). For the N rates, 0 N experienced the highest decline of 56% compared to the 51% observed in the 30 kg N rate and 45% in the 60 and 90 kg N rates (Figure 4.20). These results indicate that the potential of maize crop to maintain a meaningful growth rate during grain filling depends on the quantity and timely availability of nitrogen.

Crop growth rate during 5-7 WAP which brackets mid to late vegetative growth stage was highest at NT1 application where the entire N was applied by 4 WAP; during 7-10 WAP which brackets late vegetative to R2 stage, CGR was highest in NT2 application where the entire N was applied by 6 WAP; and during the 10-14 WAP growth period which brackets R2 to R6 stage, CGR became higher in NT3 application where the entire N was applied by 8 WAP (Figures 4.16 and 4.18). This CGR pattern clearly shows that it was by and large dependent on the supply and availability of nitrogen. But the fact that plants in the NT3 application time were able to grow fast enough to close the CGR gap between them and the other nitrogen application times during 10-14 WAP shows the potential of this maize variety to continue growing right into reproductive stages when N was available.

The effect of cattle manure on maize crop growth rate was found to be different not just among manure rates but also in the different seasons of experimentation. In the 2014 and 2015 major seasons, manure effect on CGR was significant at higher manure rates than lower rates as early as between 5-7 WAP (Figures 4.17 and 4.21). But in the 2014 minor season, CGR difference among manure rates was not significant at 5-7 WAP (Figure 4.19). Also in the major seasons, between 7 WAP and physiological maturity, 2, 4 and 6 tons/ha manure rates all effected significantly higher CGR than 0 manure rate; but there were no significant differences among the three manure applied rates. In contrast, crop growth rate in the minor season, in comparison to non-application of manure was significant only in the 6 tons/ha manure rate. This trend in manure effect on CGR is an indication that when soil moisture levels are higher, decomposition of manure and release of nutrients and subsequent availability to crops becomes quicker and more effective. Similar effects of soil moisture levels on decomposition of cattle manure was reported by Tarkalson *et al*. (2006).

Based on the fact that 2, 4 and 6 tons/ha manure rates had statistically similar effect on CGR during later growth stages is an indication that manure rate somewhere in between the three, such as 4 tons/ha could be sufficient to enable adequate CGR for higher maize growth and yield.

4.6 Maize yield and yield components

Maize yield parameters that were assessed as part of this study included ear yield, grain yield, harvest index (HI), 100 grain weight and shelling percentage. In the 2014 major and minor seasons, main or interactive effects of treatment factors affected only ear yield, grain yield and harvest index. Main effects of manure rates and times of N application on ear yield, grain yield and HI are presented in Table 4.19. In the 2015 major season, all five yield parameters were either affected by main or interactive effects of the treatment factors. Main effects of manure rates and N rates on yield parameters are presented in

Table 4.20. The two parameters for which interactive effects were obtained i.e. ear and grain yield are presented in Figures 4.22 to 4.27.

4.6.1 Effect of manure rate, time of N application and N rate on maize yield

Ear yield in the 2014 major season was highest at NT2 application time. Its mean ear yield of 6.23 tons/ha was not significantly different from the mean at NT1 application time (5.98), but the two were significantly higher than the mean at 0 N and NT3 application time. Grain yield and HI followed the same pattern with NT1 and NT2 application times showing no significant difference but one or both of them being significantly higher than 0 N and NT3 application time. In the minor season, ear and grain yield at the 0 N treatment was significantly lower than the N applied treatments. Harvest index recorded on control plots was lower than on NT1 and NT2 applications, but not significantly different from that of NT3. Ear yield and HI from NT3 application time were significantly lower than from NT1 and NT2 application times. Grain yield was also significantly higher in NT2 time than 0 N and NT3 time (Table 4.19).

Table 4.19: Effect of time of N application and cattle manure rates on maize ear yield, grain yield and harvest index in 2014 major and minor seasons.

Treatment	2014 major season			2014 minor season		
Time of N application	Ear yield (tons/ha)	Grain yield (tons/ha)	Harvest index (%)	Ear yield (tons/ha)	Grain yield (tons/ha)	Harvest index (%)
0 N	4.02	2.485	46.05	2.155	1.300	36.83
NT1	5.98	3.937	50.13	3.885	2.447	40.22
NT2	6.23	4.015	51.30	4.002	2.459	40.73
NT3	5.17	3.454	48.08	2.983	2.127	37.51

LSD (0.05)	0.57	0.52	3.09	0.56	0.43	2.08
CV (%)	13.7	9.4	6.9	11.5	11.1	6.9
Manure rate (tons/ha)						
0	4.69	2.936	47.34	2.564	1.554	36.82
2	5.07	3.344	47.85	3.089	2.024	38.47
4	5.75	3.641	49.62	3.566	2.245	38.93
6	5.89	3.989	50.73	3.806	2.500	41.07
LSD (0.05)	0.60	0.55	3.15	0.58	0.44	2.12
CV (%)	14.7	10.0	7.3	13.0	11.8	7.1
TNA x MR	*	*	NS	*	*	NS

TNA: time of nitrogen application; MR: manure rate; * significant at P<0.05, NS: not significant at P>0.05. Means for nitrogen application times were obtained across manure rates and means for manure rates were obtained across nitrogen application times.

Table 4.20: Effect of nitrogen rates and cattle manure rates on maize yield and yield components in the 2015 major season

Nitrogen rate (kg/ha)	Ear yield (tons/ha)	Grain yield (tons/ha)	Harvest index (%)	100 grain weight (g)	Shelling percentage (%)
0	4.33	2.485	45.31	31.43	54.69
30	5.54	2.947	49.86	32.32	58.27
60	6.36	3.695	52.24	32.53	61.04
90	6.55	4.229	55.51	34.13	68.04
LSD (0.05)	0.75	0.60	3.01	2.11	6.71
CV (%)	13.8	13.0	11.0	9.0	17.9

Manure rate (tons/ha)					
0	5.12	2.853	47.30	31.49	56.71
2	5.54	3.261	49.19	32.13	59.49
4	5.95	3.495	51.90	32.87	60.81
6	6.18	3.748	54.52	33.91	65.02
LSD (0.05)	0.78	0.62	3.04	2.15	6.74
CV (%)	14.0	13.3	11.1	9.3	18.1
NR x MR	*	*	NS	NS	NS

NR: nitrogen rate; MR: manure rate; * significant at P = 0.05, NS: not significant at P = 0.05. Means for nitrogen application times were obtained across manure rates and means for manure rates were obtained across nitrogen application times.

In the 2015 experiment, all five yield parameters showed some level of increment with increase in N rate. Mean ear yield at 0 N treatment was significantly lower ($P < 0.05$) than all other N rates. Effect of the 30 kg N rate was also significantly lower than those of the 60 and 90 kg N rates. Effect of the 60 kg N rate was, however, not significantly different from that of the 90 kg N rate. Application of 30 kg N/ha increased maize ear yield by 1.22 tons/ha. Application of an additional 30 kg which raises the N rate to 60 kg/ha gave an additional 0.82 tons/ha of maize ears. Addition of an extra 30 kg which raises the N rate to 90 kg/ha gave only an additional 0.19 tons/ha. In comparison to nonapplication of nitrogen, ear yield at 30 kg N increased by 26%, at 60 kg N it increased by 43% and at 90 kg N it increased 47% (Table 4.20).

Grain yield from the 30 kg N plots was not significantly higher than the control, however, both were significantly lower than the 60 and 90 kg N rates (Table 4.20). In comparison to 0 N, grain yield at 30 kg N increased by 16%, whilst at 60 and 90 kg N rates, it increased by 42% and 59% respectively. Harvest index in the control was significantly lower than the N rates; 30 and 60 kg N rates showed no significant difference but were both significantly lower than 90 kg N rate. Hundred grain weight at 0 N was not statistically different from plots treated with 30 and 60 kg N rates. It was, however significantly lower than values recorded on the 90kg N plots. Application of N up to 60 kg/ha did not significantly increase shelling %, however, it was significantly higher at 90 kg N than at any of the other N rates.

Manure effect on ear and grain yields in the 2014 major and minor seasons was significant in the 4 and 6 tons/ha rates, but grain yield in the minor season was significant in the 2 tons/ha rate. Harvest index was significantly higher only in the 6 tons/ha manure rate (Table 4.19). In the 2015 experiment, ear and grain yield and HI significantly increased in the 4 and 6 tons/ha rates. Hundred grain weight and shelling % on the other hand, increased significantly only in the 6 tons/ha rate (Table 4.20).

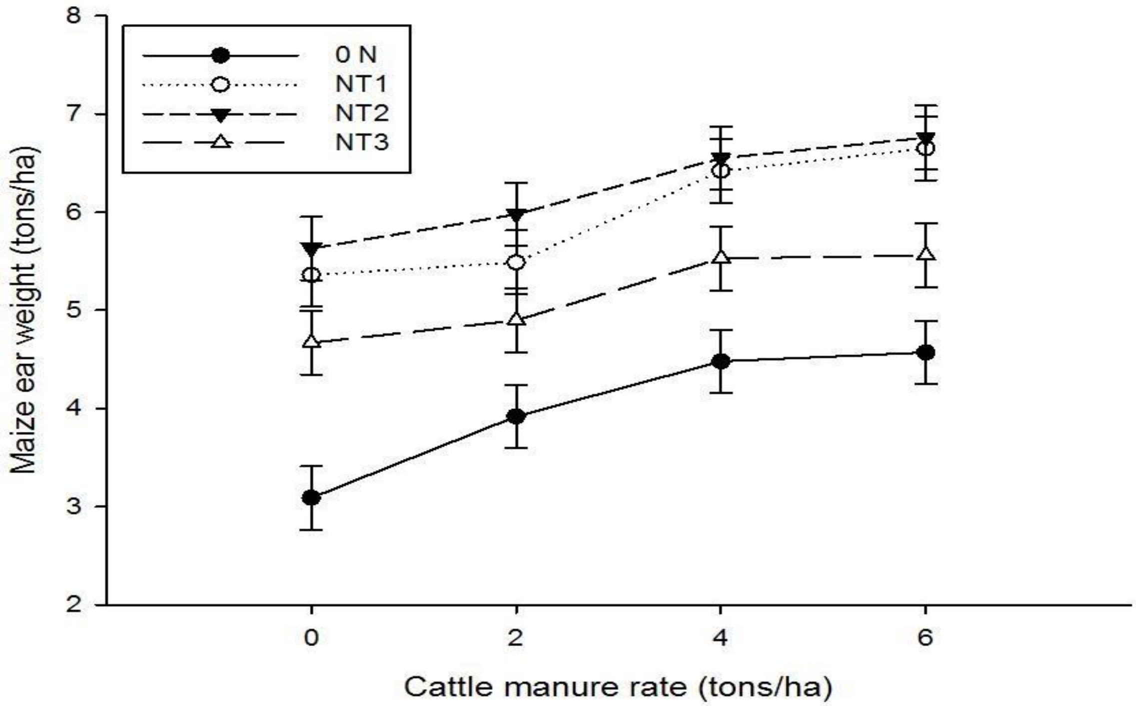


Figure 4.22: Effect of time of nitrogen application on maize ear yield at different cattle manure rates in the 2014 major season. Bars represent SE.

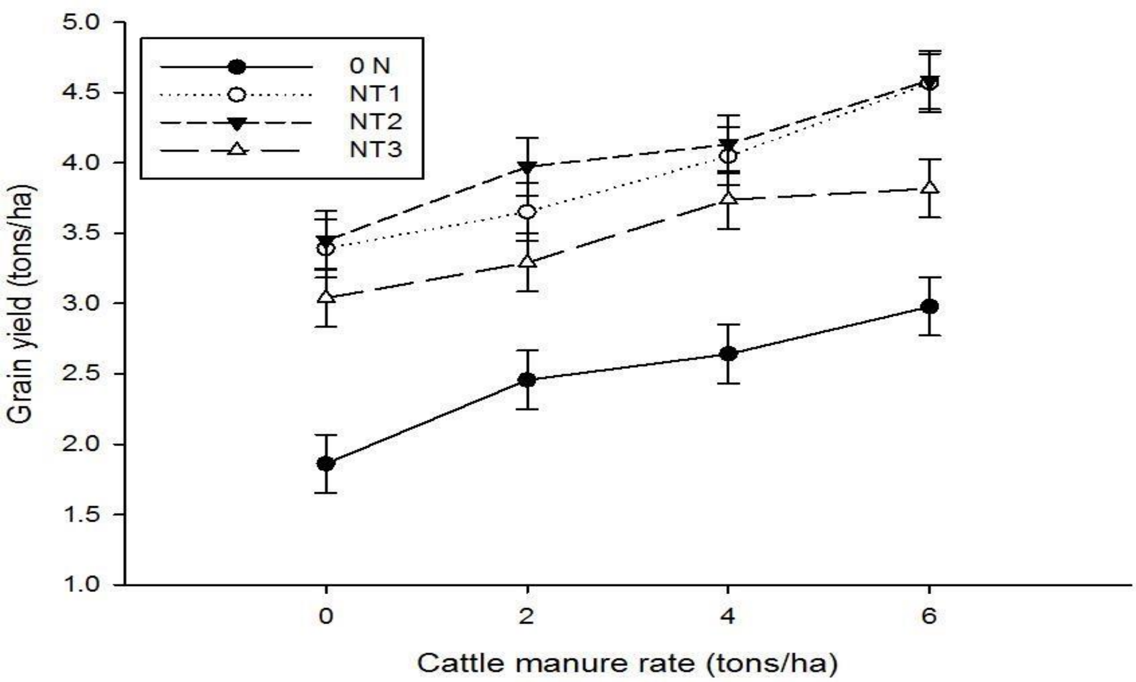


Figure 4.23: Effect of time of nitrogen application on maize grain yield at different cattle manure rates in the 2014 major season. Bars represent SE.

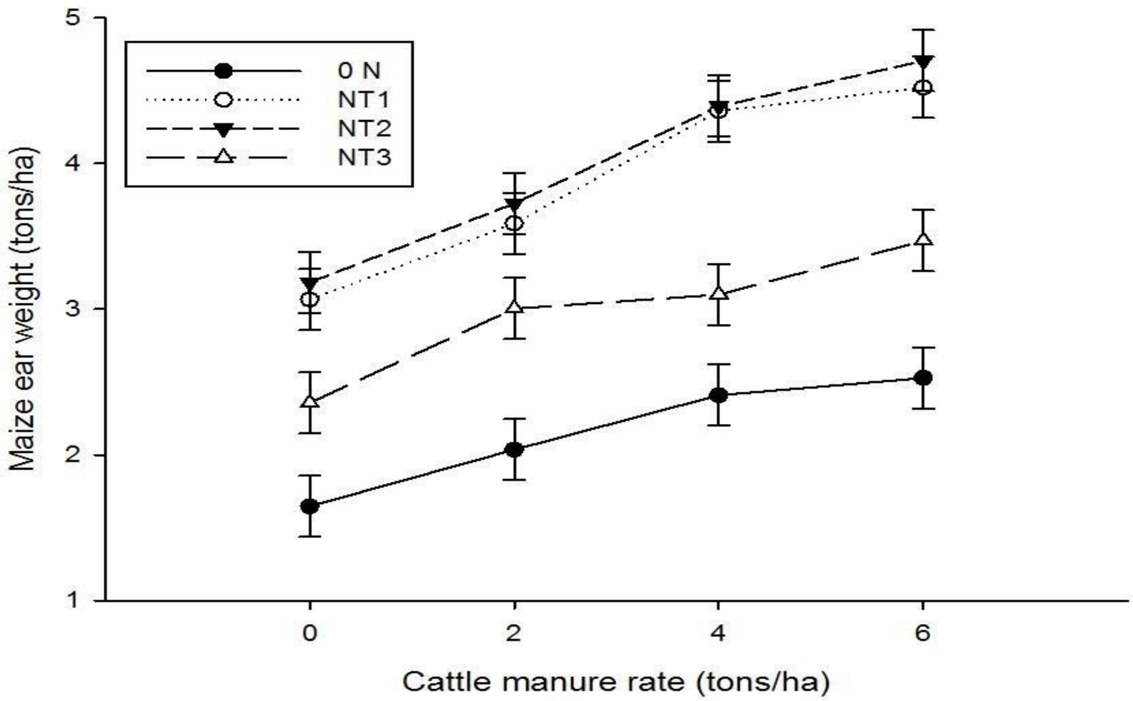


Figure 4.24: Effect of time of nitrogen application on maize ear yield at different cattle manure rates in the 2014 minor season. Bars represent SE.

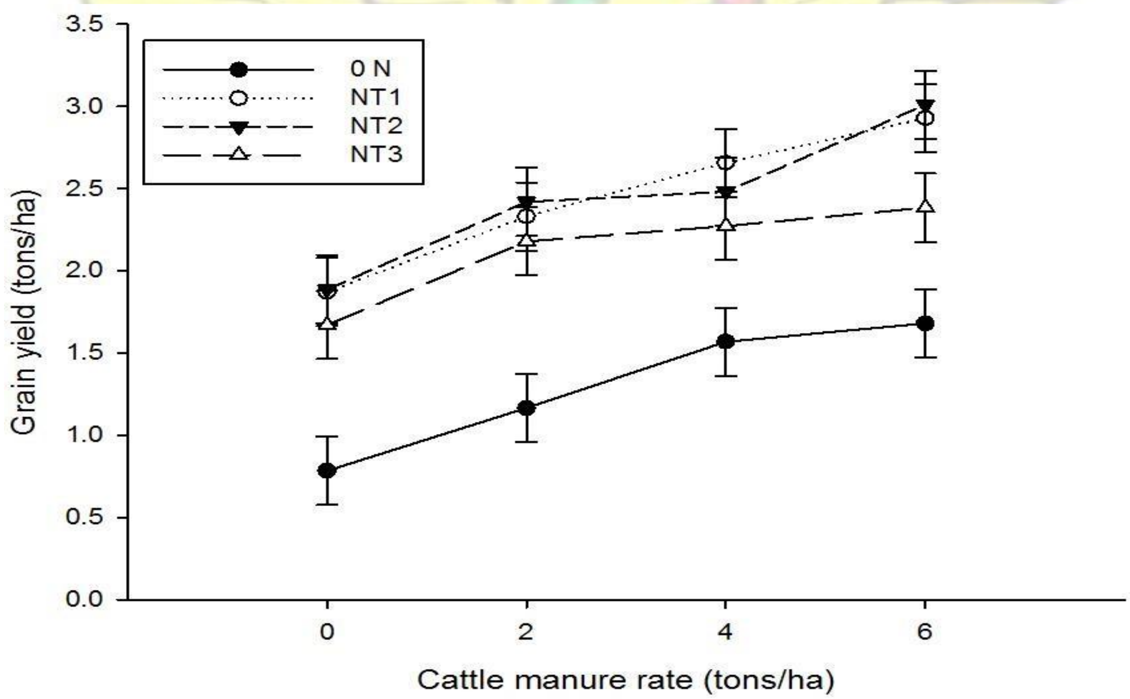


Figure 4.25: Effect of time of nitrogen application on maize grain yield at different cattle manure rates in the 2014 minor season. Bars represent SE.

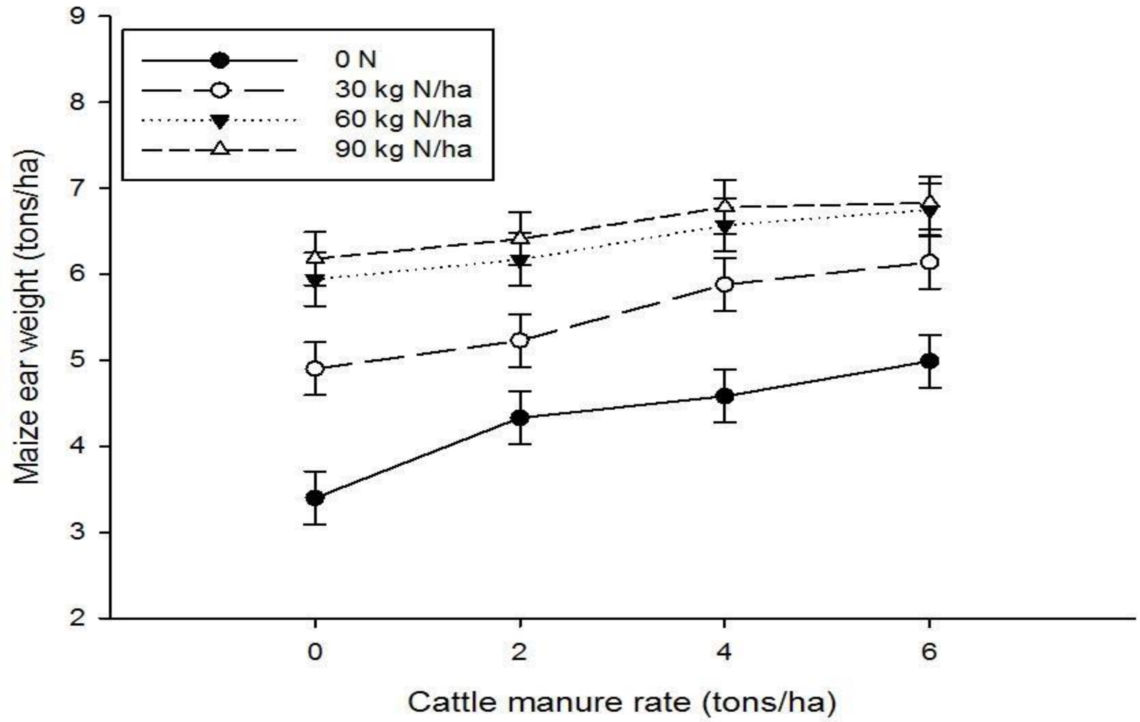


Figure 4.26: Effect of nitrogen rates on maize ear yield at different cattle manure rates in the 2015 major season. Bars represent SE.

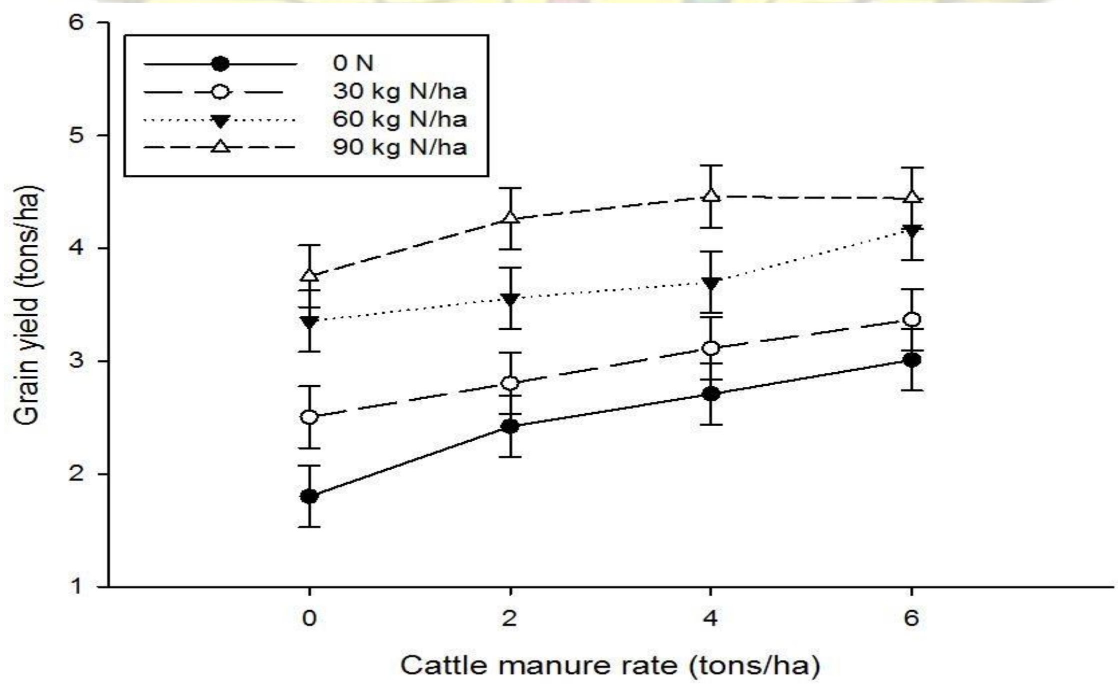


Figure 4.27: Effect of nitrogen rates on maize grain yield at different cattle manure rates in the 2015 major season. Bars represent SE.

In the 2014 major season, ear yield was highest at NT2 application time at all the manure rates. Mean ear yield of 6.76 tons/ha at NT2 at the 6 tons/ha manure rate was significantly higher ($P < 0.05$) than at NT1 time at the 0 and 2 tons/ha manure rates, and NT3 time in the 0, 2 and 4 tons/ha manure rates. Ear yield at NT1 at the 6 tons/ha manure rate was 1.29 tons/ha significantly higher than at NT1 without manure. For NT2 and NT3 application times, increments in ear yield were observed as manure rate increased; however, these increments were not significant. At the same manure rates, NT1, NT2 and NT3 application times showed no significant difference (Figure 4.22).

Grain yield in the 2014 major season was highest at NT2 application time in all the manure rates, but in the 4 and 6 tons/ha manure applications, it was almost exactly the same as NT1 application. Mean grain yield of 4.59 tons/ha at NT2 with 6 tons/ha manure treatment was significantly higher than at NT1, NT2 and NT3 applications without manure and NT3 with 2 tons/ha rate. Grain yield at NT1 application in the 6 tons/ha manure rate was 1.18 tons/ha significantly higher than at NT1 without manure; NT2 application in the 6 tons/ha manure rate was 1.14 tons/ha significantly higher than at NT2 without manure. At NT3 application, differences in manure rate did not result in significant differences in grain yield. At the same manure rates, grain yield differences between NT1, NT2 and NT3 application times was not significant (Figure 4.23).

In the 2014 minor season, maize ear yield was highest at NT2 application time in all the manure rates. Mean ear yield of 4.71 tons/ha at NT2 in the 6 tons/ha manure rate was significantly higher than at NT1, NT2 and NT3 application times without manure and NT3 in the 2, 4 and 6 tons/ha manure rates. Ear yield at NT1 in the 6 tons/ha manure rate was 1.46 tons/ha significantly higher than at NT1 without manure; NT2 in the 6 tons/ha manure rate was 1.52 tons/ha significantly higher than at NT2 without manure. At NT3, differences in manure rate did not result in significant differences in ear yield. In the 0 and 2 tons/ha manure rates, ear yield at NT1, NT2 and NT3 application times showed no significant difference. In the 4 tons/ha manure rate, NT3 application time was 1.26 and 1.29 tons/ha significantly lower than NT1 and NT2 times respectively. In the 6 tons/ha manure rate, NT3 was 1.24 tons/ha significantly lower than the mean at NT2 application, but was not significantly different from at NT1 application (Figure 4.24).

Grain yield in 2014 minor season was highest at NT2 application time in all the manure rates except in the 4 tons/ha rate where NT1 application was highest. Mean grain yield of 3.01 tons/ha at NT2 combined with 6 tons/ha manure treatment was significantly higher than at NT1, NT2 and NT3 applications without manure. Grain yield at NT1 with 6 tons/ha manure treatment was 1.06 tons/ha significantly higher than at NT1 without manure, and NT2 with 6 tons/ha manure treatment was 1.12 tons/ha significantly higher than NT2 without manure. At NT3 application, differences in manure rate did not result in significant differences in grain yield. At the same manure rates, NT1, NT2 and NT3 showed no significant differences (Figure 4.25).

In the 2015 major season, maize ear yield was highest at the maximum N rate in all the manure rates. However, mean ear yield at 90 kg N/ha rate in any of the manure rates was not significantly higher than 60 kg N/ha rate in the same manure rates. The highest mean ear yield of 6.75 tons/ha at 90 kg N in the 6 tons/ha manure rate was significantly higher than at 30 kg N in the 0 and 2 tons/ha manure rates.

At 30, 60 and 90 kg N rates, differences in manure rates applied did not result to significant differences in ear yield. In the control treatment, mean ear weight at 90 kg/ha N rate was 2.78 and 1.28 tons/ha significantly higher than at 0 and 30 kg N rates respectively, but was not significantly different from the mean at 60 kg N rate. In the 2 tons/ha manure rate, 90 kg N was 2.08 tons/ha significantly higher than 0 N, but was not significantly different from 30 and 60 kg N rates. In the 4 tons/ha manure rate, 90 kg N was 2.20 tons/ha significantly higher than at 0 N but was not significantly different from the minimum and optimum N rates. In the 6 tons/ha manure rate, 90 kg N was 1.84 tons/ha significantly higher than 0 N but was not significantly different from those of 30 and 60 kg N rates (Figure 4.26).

Grain yield in the 2015 major season experiment was highest at the 90 kg N rate in all the manure rates. The highest grain yield of 4.46 tons/ha at 90 kg N/ha combined with 4 tons/ha manure treatment was significantly higher than the 2.50, 2.80 and 3.11 tons/ha in the 30 kg N rate with 0, 2 and 4 tons/ha manure rates, respectively.

At all N rates, differences in manure rate did not result in significant differences in grain yield. However, at manure rates, differences in N rate did result in significant differences in grain yield. In the 2 tons/ha manure rate, grain yield at 90 kg N rate was 1.84 and 1.46 tons/ha higher than at 0 and 30 kg N rate respectively, but was not significantly different from the 60 kg N rate. In the 4 tons/ha manure rate, grain yield at 90 kg N rate was 1.75 and 1.35 tons/ha significantly higher than at 0 and 30 kg N, but was not significantly different from the 60 kg N rate. In the 6 tons/ha manure rate, grain yield at 90 kg N rate was 1.43 tons/ha significantly higher than at 0 N but was not significantly different from the 30 and 60 kg N rates (Figure 4.27).

4.6.2 Relationship between grain yield and nitrogen uptake, NUE and some growth parameters

Results presented in Table 4.21 below showed a significant positive correlation between maize grain yield and nitrogen uptake, nitrogen use efficiency and some plant growth parameters. Relationship between crop growth rate at 7-10 WAP and grain yield (r = 0.8156) and CGR at 10-14 WAP and grain yield (r = 0.8854) for the 2014 major season experiment was highly significant (Figure 4.28). Figure 4.29 below also showed a significant positive relationship between CGR at 7-10 WAP and grain yield (r = 0.8159) as well as at 10-14 WAP and grain yield (r = 0.8244) for the 2014 minor season. For the 2015 major season experiment, the relationship between crop growth rate at 7-10 WAP and 10-14 WAP growth durations and grain yield with coefficients of 0.7927 and 0.8222 respectively was also highly significant (Figure 4.30).

Table 4.21: Correlation between grain yield and N uptake, nitrogen use efficiency, dry matter accumulation, leaf number and green leaf area.

Grain yield	N uptake	NUE	Plant DM	Leaf number	Green leaf area
2014 major season	0.725***	0.622**	0.527*	0.638**	0.758***
2014 minor season	0.544*	0.563*	0.508*	0.722***	0.656**
2015 major season	0.559*	0.307	0.661**	0.568*	0.599*

NUE: nitrogen use efficiency; Plant DM: total shoot dry weight at maturity; *, **, ***: significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

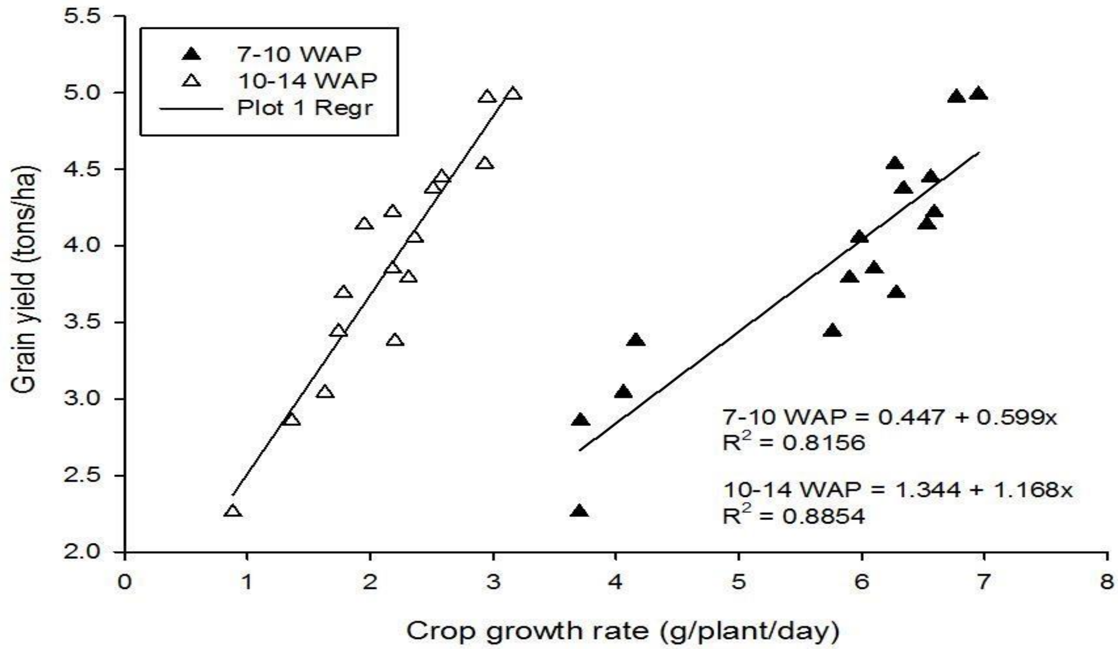


Figure 4.28: Relationship between crop growth rate at two growth periods and maize grain yield in the 2014 major season

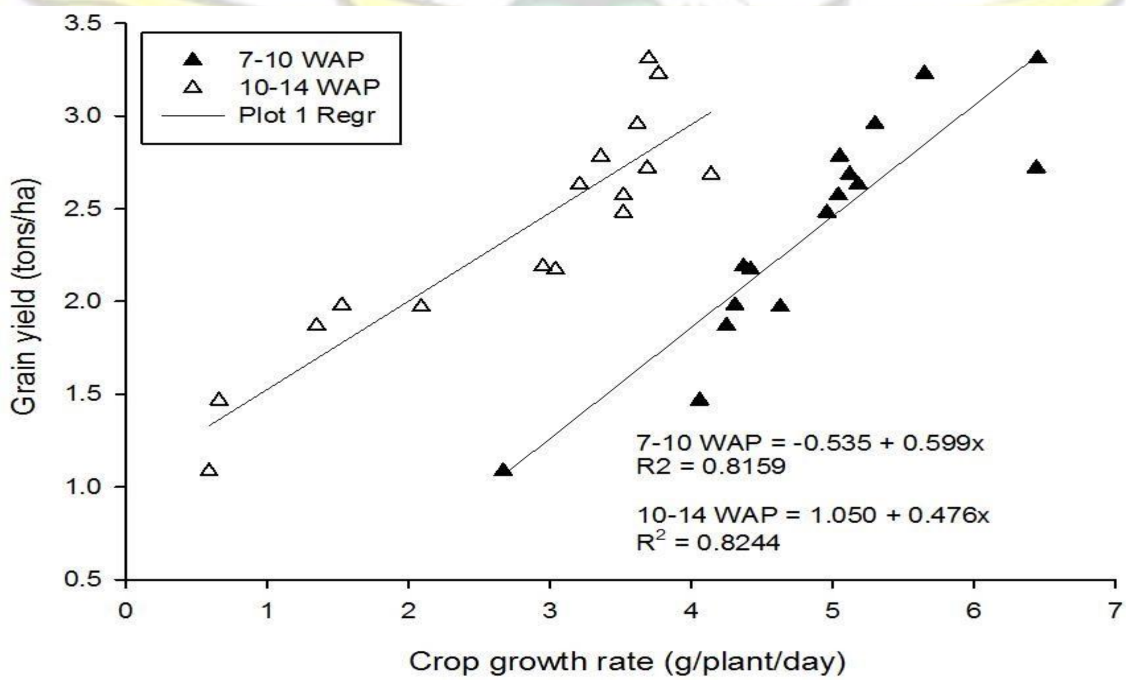


Figure 4.29: Relationship between crop growth rate at two growth periods and maize grain yield in the 2014 minor season

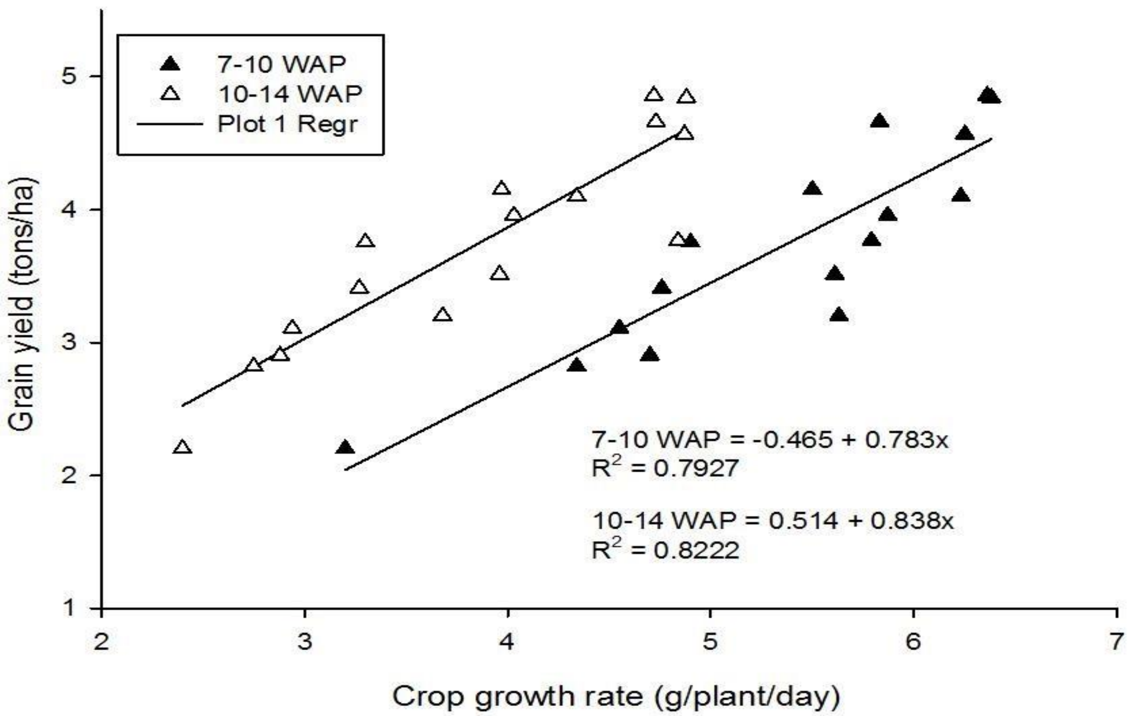


Figure 4.30: Relationship between crop growth rate at two growth periods and maize grain yield in the 2015 major season

4.6.3 Discussion

Results of the study showed that difference in times of nitrogen applications affected maize ear yield, grain yield and harvest index with nitrogen applied at NT2 producing highest yields (Table 4.19). In Ghana, the recommended time of nitrogen application according to Morris *et al.* (1999) is applying part of the N at 2 WAP and top dressing the other part at 4 WAP, which is similar to NT1 in this study. Maize varieties, based on growth duration are categorized as short, intermediate and long duration varieties. This recommendation may be ideal for short duration varieties. However, applying the whole nitrogen by 4 WAP, which is still early vegetative stage for intermediate and long duration varieties, could lead to high N losses and subsequent reduction in yield. A survey conducted by Ragasa *et al*. (2013) on adoption of improved maize technologies in Ghana indicated that, 53% of farmers in their study had in the major season of 2012 applied N at NT2 i.e. basal at 2 WAP and top dressing at 6 WAP. They could not tell if maize farmers do this out of ignorance of the recommended practice or based on their years of experience. Results of this study, however, showed that application of N at NT2 produced higher yields which concurred with the majority farmer practice. Significant difference in yield responses of maize to different timings of nitrogen application was also previously reported by Nemati and Sharifi (2012).

Differences in nitrogen rates applied also had an effect on maize yield and yield components with all yield parameters demonstrating a linear increment as N rate increased (Table 4.20). The 26%, 43% and 47% increase in ear yield and 16%, 42% and 59% rises in grain yield obtained through the application of 30, 60 and 90 kg N/ha, respectively is an indication of the importance of N in determining maize yield. Increase in maize yield with increase in N rate was also previously reported by Lin *et al*. (2012). It is, however, important to point out that the increase in yield associated with N application, decreased as N rate increased. Ear yield difference of 1.22 tons/ha between 30 and 0 kg N rates, 0.82 tons/ha between 60 and 30 kg N rates and 0.19 tons/ha between 90 and 60 kg N rates is a confirmation of this observation. Incremental maize grain yield of 54.1%, 60.0%, and 62.4% at 80, 160 and 240 kg/ha N rates in comparison to 0 N reported by Nsanzabaganwa *et al*. (2014) is similar to those observed in this study.

Application of 30 kg N/ha led to significant increments in some yield parameters but for most of the yield parameters, it was not significantly different from 0 N rate. It can, therefore, be stated that 30 kg N was insufficient to realize meaningful yields for Obatanpa maize variety. The fact that yield and yield components did not show any significant differences between 60 kg and 90 kg N rates is an indication that application of 60 kg N rate could produce as much yield as the 90 kg N rate.

Increasing partitioning of biomass to harvestable parts is one of the main targets of breeding and agronomic practices. In this study, HI was found to be affected by differences in time of nitrogen application, N rates and cattle manure rates. The HI range of 46.05% to 51.30%, 36.83% to 40.73% and 45.31% to 55.51% obtained across treatments in the 2014 major, 2014 minor and 2015 major seasons respectively were generally higher than the 38.8% HI reported by Asare *et al*. (2012) for the same maize variety and the 43% HI reported by Worku *et al*. (2012) for a tropical QPM maize variety in Zimbabwe. They were, however, similar to the 48% HI reported by Boateng *et al*. (2006) for Abeleehi which is another intermediate maize variety in Ghana with similar yield potential as Obatanpa. Abe *et al*. (2013) also reported HI to be significantly higher at 90 kg N/ha than at 30 kg N/ha for nine out of fourteen maize varieties in Nigeria. These variations indicate that harvest index of maize is influenced by differences in soil nutrient levels.

The decline in HI in the control treatment compared with NT2 application time (Table 4.19) and at 90 kg N/ha rate (Table 4.20) was much lower than the decline in total aboveground plant dry weight (Tables 4.11, 4.12 and 4.13), indicating that the dominant effect of treatments was on dry matter accumulation than on partitioning of DM to the maize ears. Similar effects were also reported by Ciampitti and Vyn (2011).

The study showed that ear and grain yield increased significantly on plots treated with 4 and 6 tons/ha manure rates as compared to the control. The 6 tons/ha manure application led to slightly higher yield than the 4 tons/ha rate, but the difference was not significant. Increase in maize yield with increase in cattle manure rate applied was reported in many previous studies (Eghball and Power, 1999; Zingore *et al*., 2008; Khoshgoftarmanesh and Eshghizadeh, 2011). Boateng *et al*. (2006) also reported that application of poultry manure at 8 tons/ha gave the highest maize grain yield of 3.1 tons/ha, but yields obtained with the application of 4 and 6 tons/ha manure rates were also significantly higher than the control, while at 2 tons/ha rate, grain yield was not significantly different from the control.

The significant manure rate x N application time interaction in the 2014 major and minor seasons (Figures 4.22 – 4.25) and manure rate x N rate in the 2015 major season (Figures 4.26 and 4.27) for ear and grain yields were mainly a consequence of the higher difference in ear and grain yield between N application times, N rates and manure rates at main factor level. For harvest index, 100 grain weight and shelling percentage, differences at main factor level were relatively smaller.

The effectiveness of the interaction between cattle manure and mineral nitrogen was more dependent on the nitrogen than the manure, because, results have shown that combining the same N rate with 2, 4 or 6 tons/ha manure rates did not result in significant increments in yields. However, when the same manure rate was combined with the different N rates, ear and grain yield were significantly higher at higher N rates than at lower rates. The difference in yield between the higher and lower N rates was bigger at sole mineral N applications and became smaller with increase in manure rate.

Inorganic N fertilizer having a dominant effect on maize yield when applied in combination with manure was also previously reported by Shafi *et al*. (2012).

Ear and grain yields were higher in the mineral nitrogen and cattle manure combined applications than in the sole nitrogen and manure applications. Highest grain yields at the sole N applications were obtained at NT2 in the major and minor seasons (Figures 4.23 and 4.25) and at the 90 kg/ha N rate in the 2015 season (Figure 4.27); the highest at sole manure applications were obtained at the highest manure rate in all seasons; and the highest at the interactions were obtained in NT2 combined with 6 tons/ha treatment in 2014 major and minor seasons and in the 90 kg N combined with 4 tons/ha manure treatment in 2015 season. These results compared very well to the 1.96 tons/ha and 3.44 tons/ha maize grain yields at 3 and 6 tons/ha cattle manure rates, and 4.28 tons/ha at 6 tons/ha manure plus the recommended N rate reported by Ncube *et al*. (2007) in Zimbabwe.

Higher maize yields from combined application of cattle manure and inorganic N fertilizer than in sole inorganic N fertilizer and manure treatments has also been reported by Mugwe *et al*. (2007) in Kenya, Ayoola and Makinde (2007) in Nigeria and Rusinamhodzi *et al*. (2013) in Zimbabwe.

CHAPTER FIVE

GENERAL DISCUSSION

Nitrogen is the mineral nutrient required in the largest quantity by cereal crops (Schulte auf'm Erley *et al*., 2007). Its deficiency constitutes one of the most limiting factors for maize. When water and temperature conditions are ideal, productivity of maize is mainly limited by availability of N (Birch *et al*., 2008). This important role of nitrogen in determining maize yield is dependent on its timely supply and rate. Application of adequate N rate at the appropriate time keeps N available within the plant root zone and, therefore, ensure efficient N utilization (Amanullah and Shah, 2011). On the other hand, too early or late application of N impact negatively on maize yield. Delay in top dressing of N until VT (tasseling) results in decreased grain yields (Walsh *et al*., 2012).

Among the times of N application in this study, N uptake was highest at NT2 because half of the N was applied a week before onset of tasseling (6 WAP) and that is a stage regarded in maize as active N uptake period (Birch, *et al*., 2008). Nitrogen uptake also increased with increase in N rate increased. This is in agreement with the findings reported by Barbieri *et al*. (2008). However, results have shown that the differences in nitrogen uptake that existed among N application times and N rates without manure became smaller when the mineral N was applied along with cattle manure, and in the highest manure rates, the differences were not significant ($P > 0.05$).

Higher N uptake by plants in the inorganic N and cattle manure combined treatments than in the sole inorganic N and sole manure treatments was due to the complementary effect of the two fertilizers. Manure application improves soil physical and chemical properties, thereby, limit nutrient losses, increase plant nutrient concentrations and nutrient uptake; whilst inorganic N increase the supply of N for microorganisms involved in the decomposition of the manure and, therefore, speeds up the manure decomposition process and increase the availability and uptake of manure nutrients including nitrogen (Butler and Muir, 2006). Zhou *et al*. (2012) reported an increase of 12-59% in N uptake by maize plants in different eroded soils when inorganic N fertilizer was combined with cattle manure than application of inorganic N alone. Ma *et al*. (1999) also reported that application of inorganic fertilizer in combination with cattle manure increased N uptake and grain yield of maize in eastern Canada.

Results on N partitioning have shown that before the start of grain filling, most of the uptake N was partitioned to maize culms and leaves. During grain filling stage, however, maize ears shown to be the dominant site for N accumulation and this was consistent throughout the study and not influenced by treatment differences. Maize ears being dominant sink for uptake nitrogen during reproductive stages was also previously reported by Hernandez-Ramirez *et al*. (2011).

Nitrogen use efficiency was highest at NT2 application time because application at this time might have enabled recovery of more N from the applied N fertilizer than the other application times. Nitrogen use efficiency was also found to be significantly higher at 60 kg N rate than at 30 and 90 kg N rates, whilst it was also higher in the 30 kg N rate than in the 90 kg N rate (Table 4.10). The higher NUE at 60 kg N than at 30 kg N rate was due to the higher grain yield difference between the two nitrogen rates in favor of the 60 kg N rate.

Application of inorganic N with cattle manure increased N use efficiency at all N application times and N rates, and NUE increased as the manure rate increased because manure alleviates other crop growth constraints other than N thereby increase N uptake and use efficiency (Azeez, 2009).

Aboveground plant dry matter accumulation was higher for N treatments than the control, at NT2 than other N application times, at higher N rates than lower rates and in the higher manure rates than lower rates because of the higher N uptake that took place in them. This higher N uptake triggered higher vegetative growths and subsequently higher DM accumulation. Higher rates of dry matter accumulation in maize had been associated with increased N uptake and leaf longevity (stay green) ability of maize (Echarte *et al*., 2008). Nitrogen management practices that make N available to crops in the right quantity and at the appropriate time promote development of early and larger surface leaf area which enhances biomass production and partitioning into grains (Gadalla *et al*., 2007).

Maize yield parameters as expected were significantly affected by differences in time of N application, N rate, manure rate and the interaction between N and manure in some cases. Regarding times of N application, ear yield, grain yield and harvest index were all significantly higher at NT2 application time in both major and minor seasons. For the N rates, all five yield parameters assessed in this study (i.e. ear yield, grain yield, HI, 100 grain weight and shelling percentage) demonstrated a linear increment with increase in nitrogen rate.

Since nitrogen has been widely identified as one of the most limiting factors to maize yield (Nemati and Sharifi, 2012), provision of adequate N should, therefore, enable maximum yields. From the results obtained in this study, however, application of the recommended N rate alone produced an average grain yield of 3.29 tons/ha which is 28.5% lower than the potential yield of 4.6 tons/ha for the maize variety used in this study. In contrast, average grain yield in the nitrogen combined with 4 tons/ha manure treatment was 3.97 tons/ha which was 13.6% lower than the potential yield. In the nitrogen combined with 6 tons/ha manure treatment, average grain yield obtained was 4.33 tons/ha which is only 6% lower than the potential yield.

The higher yields from manure plus inorganic N treatments than sole inorganic N and manure treatments is an indication that integrated application of manure and inorganic nitrogen sources is advantageous over the use of inorganic N or manure alone. Earlier studies demonstrated that use of manure could enhance efficiency of chemical fertilizers (Yadav *et al*., 2000; Nyamangara *et al*., 2005). Chivenge *et al*. (2011) reported that whilst application of sole organic and sole inorganic N fertilizers increased maize yield by 60% and 84% respectively over the control, combined application of organic and inorganic N fertilizer increased maize yield by 114% over the control, and 33% and 17% over the sole organic and inorganic fertilizers, respectively.

Combining mineral N fertilizer with cattle manure at the rates of 4 and 6 tons/ha nullified the ear and grain yield differences that existed among the different times of N application when no manure was added (Table 4.19). This means that, when mineral nitrogen fertilizer is combined with cattle manure at the above rates, applying the N at NT1, NT2 or NT3 application times will not result in significant differences in maize yield. Combined application of inorganic N with cattle manure could, therefore, help reduce maize yield decreases associated with late N application. The effectiveness of combining cattle manure with inorganic N also depends on the rate of manure applied and the timing of N application. When N was applied at NT1 or NT2, ear and grain yield in the higher manure rates were significantly higher than in the lower rates. But when N was applied at NT3, differences in manure rate did not lead to significant differences in ear or grain yield. Integrated application of inorganic N fertilizer and manure provide a more balanced supply of nutrients for better maize growth and yield (Mugwe *et al*., 2007).

Therefore, combining inorganic N fertilizer with organic material such as cattle manure could be considered as a better option to increase fertilizer use efficiency and crop yields.

Results presented in Figures 4.26 and 4.27 showed that combined application of cattle manure and mineral nitrogen could result in the reduction of the quantity of mineral N applied without compromising maize yields. Mean grain yield at 90 kg N without manure was 1.25 tons/ha more than at 30 kg N without manure; and 0.04 tons/ha more than at 60 kg N without manure. When the 30 kg N was combined with 6 tons/ha manure rate, its grain yield was 0.38 tons/ha lower than that of the 90 kg N without manure and that difference was not significant (P > 0.05). However, when grain yield at 60 kg N combined with 6 tons/ha manure rate was 11% significantly higher than at the 90 kg N without manure. Boateng *et al*. (2006) reported that 2 tons/ha poultry manure plus half rate of chemical N fertilizer produced significantly higher maize yield than the full dose of NPK alone. This enforces the suggestion that integrated application of chemical fertilizers with manure is more desirable than either type of fertilizers alone.

Maize grain yield is determined by many factors, among them, green leaf area and LAI which determines the photosynthetic capability of the plant, crop growth rate and dry matter production and partitioning to kernels at harvest (Cirilo *et al*., 2009). In Table 4.21, the positive significant correlation between grain yield and leaf number, green leaf area and shoot dry weight; and the positive relationship between grain yield and CGR in Figures 4.28 to 4.30 showed that grain yield was highly dependent on these growth parameters. Andrade *et al*. (2002) reported that variations in maize grain yield were explained mainly by variations in yield components such as kernel number, and this grain yield component was strongly related to crop growth rate around silking. Worku *et al*.

(2012) also reported significant positive correlation between grain yield and factors indicative of higher photosynthetic efficiency, such as, leaf area and leaf number. A close correlation between grain yield and biomass per plant in maize was also reported by Chen and Mi (2012). They suggested that increase in biomass production led to further increases in grain yield which is also in agreement with the findings reported by Lee and Tollenaar (2007).

Grain yield variations among treatments could also be attributed to the differences in nitrogen uptake and nitrogen use efficiency as indicated by the significant positive correlation between grain yield and these two nitrogen parameters (Table 4.21). The treatments that demonstrated superiority in N uptake and NUE also had higher grain yields. Maize grain yield was positively linked to both higher nitrogen uptake and ability to utilize accumulated N by the plants (Worku *et al*., 2007). Nyiraneza *et al*. (2009) also reported a significant positive correlation ($r > 0.80$***) between maize grain yield and nitrogen uptake.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study was conducted to investigate aboveground plant nitrogen uptake, nitrogen utilization efficiency and growth and yield responses of maize to time of nitrogen application, N rates, cattle manure rates and the interactions between mineral nitrogen and cattle manure. The following conclusions can be made from the results obtained.

Nitrogen uptake was higher from the inorganic N treatments than manure treatments. Among the times of N application, N uptake was higher at NT2. It was also higher at higher N rates than lower rates. It can, therefore, be concluded that N uptake of maize was very much dependent on the time and rate of N application. Nitrogen uptake was higher when inorganic N fertilizer was applied in combination with cattle manure than when inorganic N or cattle manure alone were applied. Remobilization of N from vegetative parts to ears during grain filling was severe in leaves than in culms and in 0 N than N treatments. It was also relatively smaller at NT2 than at other N application times, and at higher N rates than lower rates. Cattle manure effect significantly reduced nitrogen remobilization from culms and leaves in the 4 and 6 tons/ha manure treatments.

Nitrogen use efficiency was higher at NT2 application time because of the greater N recovery efficiency at this application time. Nitrogen use efficiency was also greater at lower than at higher N rates.

Dry matter accumulation was greater at NT2 application time and at higher N rates. Leaf dry matter responded more to variations in nitrogen supply, which makes leaves better indicators of nitrogen effect on maize dry matter partitioning than culms. Even though manure effect significantly increased shoot DM and DM partitioning to plant parts, the effect was minimal up to R2 stage, but at physical maturity, manure effect was greater.

Green leaf area and LAI were more responsive to N supply than leaf number. Manure application enhanced leaf number, GLA and LAI but the effect was minimal at vegetative stages and was more pronounced at reproductive stages. Manure effect also increased with increase in rate of application. The pattern of crop growth rate also showed that it was dependent on the time and rate of N application. Crop growth rate was also affected by manure rate and the effect was higher in the major season when soil moisture levels were higher than in the minor season when moisture levels were lower.

Ear yield, grain weight and HI were all higher at NT2 application time in both major and minor seasons. Yield parameters assessed in this study also increased with increase in N rate. The increase in yield associated with N, however, decreased at higher N rates. Grain yield was higher at inorganic N and manure combined treatments than at sole N and manure treatments. Grain yield differences that existed among N application times without manure were nullified when the N was applied with manure. It can, therefore, be concluded that integrated application of mineral N with cattle manure can reduce maize yield decreases associated with late application of nitrogen. From the results, it can also be concluded that, when inorganic N is applied in combination with cattle manure, the quantity of the inorganic N can be reduced without significantly reducing maize yields.

6.2 Recommendations

Application of half of the required nitrogen at two weeks after planting and the other half at six weeks after planting i.e. NT2 application time, enabled higher nitrogen uptake and N use efficiency which culminated into higher crop growth and grain yield. This time of nitrogen application is, therefore, recommended.

In comparison to application of inorganic N or cattle manure alone, combining the two resulted in higher N uptake, nitrogen use efficiency, dry matter production and grain yield of the maize crop. Therefore, integrated application of inorganic N with cattle manure is recommended.

Considering the side effects of excessive N application on the environment and production costs, application of 60 kg N/ha at NT2 in combination with 6 tons/ha manure rate is recommended since it produced significantly higher maize growth, grain yield and higher NUE than in the 90 kg/ha sole mineral N application and statistically the same effect as in the 90 kg/ha N combined with 6 tons/ha cattle manure rate.

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APPENDICES

Appendix 1: Temperature and relative humidity recorded for the experimental site during the three crop grown periods.

Weeks after planting	2014 major season			2014 minor season			2015 major season		
	Max. temp (^{0}C)	Min. temp (^{0}C)	Average RH (%)	Max. temp (^{0}C)	Min. temp (^{0}C)	Average RH (%)	Max. temp (^{0}C)	Min. temp (^{0}C)	Average RH (%)
1	32.71	22.81	72.36	29.36	21.16	78.21	33.04	23.00	71.64
2	31.90	22.56	72.50	29.00	21.31	78.93	33.36	23.43	68.86
3	31.90	22.39	74.00	29.21	21.31	79.86	33.04	22.74	71.29
4	32.87	22.81	76.21	29.79	21.49	78.43	32.43	22.63	70.71
5	32.77	23.17	70.57	29.56	21.27	78.86	31.29	22.37	72.86
6	31.69	22.83	72.79	31.47	21.69	74.29	29.84	21.21	76.21
7	30.51	22.36	76.79	31.33	21.70	75.93	31.50	21.47	74.07

8	31.09	22.29	74.64	31.30	21.76	73.71	30.26	21.44	74.36
9	30.79	22.54	76.29	32.16	22.21	71.14	30.14	21.29	77.07
10	30.14	22.36	77.93	31.86	22.21	73.71	30.87	21.56	74.07
11	29.64	21.51	75.29	32.36	22.29	72.14	30.71	21.87	77.21
12	26.36	21.43	77.57	32.01	22.43	71.57	30.21	21.16	76.36
13	27.86	21.43	81.07	31.80	22.43	72.36	28.36	20.70	78.57
14	28.14	21.26	83.57	31.80	22.97	70.93	28.07	21.40	79.57
15	26.86	21.06	83.79	32.07	22.80	70.00	28.96	21.94	79.36

Source: Agrometeorology Division of Ghana Meteorology Agency, Kumasi. Max temp: maximum temperature; Min temp: minimum temperature; RH: relative humidity

Appendix 2: ANOVA tables for nitrogen partitioning

Appendix 2a: ANOVA table for nitrogen partitioning at physiological maturity stage in the 2014 major season

Variate: Culm N content per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.001602	0.000801	0.10	
REP.*Units* stratum					
Manure levels	3	0.321029	0.107010	13.26	<.001
N Timings	3	0.654836	0.218279	27.05	<.001
Manure levels x N Timings	9	0.023141	0.002571	0.32	0.962
Residual	30	0.242106	0.008070		
Total	47	1.242714			

Variate: Leaf N content per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

REP stratum	2	0.036585	0.018293	2.70	
REP.*Units* stratum					
Manure levels	3	0.187834	0.062611	9.24	<.001
N Timings	3	0.364337	0.121446	17.92	<.001
Manure levels x N Timings	9	0.016873	0.001875	0.28	0.976
Residual	30	0.203336	0.006778		
Total	47	0.808965			

Variate: Ear N content per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.81947	0.40973	6.26	
REP.*Units* stratum					
Manure levels	3	0.78775	0.26258	4.01	0.016
N Timings	3	4.83876	1.61292	24.64	<.001
Manure levels x N Timings	9	0.21771	0.02419	0.37	0.941
Residual	30	1.96377	0.06546		
Total	47	8.62745			

Appendix 2b: ANOVA table for nitrogen partitioning at physiological maturity stage in the 2014 minor season

Variate: Culm N content per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.02652	0.01326	0.57	
REP.*Units* stratum					
Manure levels	3	0.08603	0.02868	1.23	0.315
N Timings	3	0.99676	0.33225	14.30	<.001
Manure levels x N Timings	9	0.04311	0.00479	0.21	0.991
Residual	30	0.69700	0.02323		
Total	47	1.84941			

Variate: Leaf N content per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

REP stratum	2	0.067400	0.033700	5.20	
REP.*Units* stratum					
Manure levels	3	0.053403	0.017801	2.75	0.060
N Timings	3	0.199227	0.066409	10.25	<.001
Manure levels x N Timings	9	0.032333	0.003593	0.55	0.823
Residual	30	0.194386	0.006480		
Total	47	0.546749			

Variate: Ear N content per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.00095	0.00047	0.01	
REP.*Units* stratum					
Manure levels	3	1.11637	0.37212	4.59	0.009
N Timings	3	5.29404	1.76468	21.77	<.001
Manure levels x N Timings	9	0.39426	0.04381	0.54	0.833
Residual	30	2.43213	0.08107		
Total 47		9.23776			

Appendix 2c: ANOVA table for nitrogen partitioning at physiological maturity stage in the 2015 season

Variate: Culm N content per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.017080	0.008540	1.36	
REP.*Units* stratum					
Manure levels	3	0.312639	0.104213	16.60	<.001
N Rates	3	0.435721	0.145240	23.13	<.001
M levels x N Rates	9	0.029987	0.003332	0.53	0.840

Residual	30	0.188344	0.006278		
Total 47	0.983771				

Variate: Leaf N content per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.10722	0.05361	1.86	
REP.*Units* stratum					
Manure levels	3	0.65059	0.21686	7.54	<.001
N Rates	3	1.41296	0.47099	16.38	<.001
Manure levels x N Rates	9	0.07637	0.00849	0.30	0.971
Residual	30	0.86267	0.02876		
Total 47	3.10980				

Variate: Ear N content per plant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.17420	0.08710	0.97	
REP.*Units* stratum					
Manure levels	3	1.47950	0.49317	5.51	0.004
N Rates	3	4.10828	1.36943	15.31	<.001
Manure levels x N Rates	9	0.21431	0.02381	0.27	0.979
Residual	30	2.68354	0.08945		
Total	47	8.65983			